Phenotypic and whole-genome single nucleotide polymorphisms (SNP) characterization of Mozambican indigenous cattle breeds

By

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Dedication

To my daughters Tiffany and Kenisha King and my wife Clara King

In memory of my mother and my father

Declaration

I, Félix João Manuel King, declare that the thesis which I hereby submit for the degree PhD Animal Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other tertiary institution.

Félix João Manuel King

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Abstract

Mozambican (MZ) indigenous cattle are generally reared in resource-poor management systems and are well adapted to harsh environments. Despite their significance in food security and improved livelihoods, a limited number of studies have attempted to investigate the genetic variation of these genetic resources or their genetic relationships with other cattle breeds and ecotypes in the region. The current study aimed to characterize three Mozambican indigenous cattle breeds on both phenotypic and genomic levels, in four provinces of Mozambique, namely Maputo, Gaza, Inhambane, and Tete. Firstly, a total of 614 cattle from three indigenous populations including Angone (n=140), Landim (n=292), and Tete (n=182), were sampled in twenty-three villages in four provinces to collect body measurements and qualitative data. Findings showed significant morphological differentiation between the populations. Landim males and females were significantly (p<0.05) heavier than their Tete and Angone counterparts. A combination of six morphometric traits (Body length, horn length, rump width, height at withers, height at the rump, and top-line length) could be used for an individual assignment using discriminant function analysis of SPSS, with a success rate exceeding 70% for both Angone and Landim cattle. Tail hair samples from 228 animals (consisting of the three indigenous populations) were genotyped using the International Dairy and Beef SNP BeadChip array (IDBV3). Population genetic parameters showed a moderate level of variation, with estimates of expected heterozygosities ranging between 0.304 ± 0.166 (Angone) to 0.329 ± 0.148 (Tete). The three indigenous populations all had low positive (across-population average of 0.065±0.109) inbreeding rates. Both principal component (PCA) and admixture analyses indicated poor between-breed differentiation. A downward trend was observed in the effective population sizes of all three populations over time, indicating a narrowing genetic pool for the genetic resources. South African genotypes which included SA Nguni (n=150), SA Tuli (n=150), and SA Boran (n=150) were included in a between-population analysis. These genotypes were generated using the GeneSeek Genomic Profiler (GGP) 80K panel and compared with the Mozambican Nguni (MZ Nguni) cattle. Medium levels of genetic diversity, measured as expected heterozygosity, were observed, ranging from 0.284±0.158 (SA Boran) to 0.324±0.153 (SA Tuli). Runs of homozygosity (ROH) analysis revealed low inbreeding rates with the average F_{ROH} ranging from 0.003 (SA Nguni) to 0.006 (SA Tuli). A high frequency of short (ROH ≤ 5Mb) ROH segments suggested ancient inbreeding in all populations. PCA and Admixture analyses revealed a tight cluster of the two Nguni populations, while SA Tuli and SA Boran diverged, as expected, into two distinct clusters. Genome-wide F_{ST} and Rsb analyses identified 229 differentiated SNP potentially under selection in the MZ Nguni cattle breed compared to SA cattle populations. These regions were enriched with genes

implicated in several metabolic processes essential for adaptation and production traits. This study indicate that Mozambican indigenous cattle populations have a high level of genetic diversity; and Mozambican Nguni and South African Nguni have similar genetic ancestry. A joint regional strategy for the preservation and sustainable use of indigenous animal resources will improve regional food security and the livelihood of farming communities.

Keywords: Mozambique, Genetic diversity, Indigenous cattle, Production system, Morphological traits, Smallholder

Thesis outputs

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Abbreviations

AMOVA	Analysis of molecular variance
AnGR	Animal genetic resource
BL	Body Length
Bp	Base pairs
BW	Body weight
CC	Coat color
CHIP	Chromatin Immuno-precipitation
CP	Coat pattern
CV	Cross-validation
DNA	Deoxyribo Nucleic Acid
EHH	Extended haplotype homozygosity
EO	Ear orientation
FAO	Food and Agriculture Organization of United Nation
F_{IS}	Inbreeding coefficients of an individual
F_{ST}	Inbreeding coefficient of sub-population
g	Grams
GDP	Gross Domestic Product
GLM	Generalized Linear Model
ha	Hectares
HC	Hock circumference
H_E	Expected heterozygosity
HG	Heart Girth
HL	horn length
HO	Horn orientation
H_O	Observed heterozygosity
HP	Horn presence
HS	Horn shape
HWE	Hardy Weinberg Equilibrium
iHS	Integrated Haplotype Score
KB	Kilobytes
Kg	Kilogramme
l	Liters
LD	Linkage disequilibrium
LSD	Least significant differences
MAF	Minor Allele Frequency
Mb	Megabytes
MC	Muzzle circumference
mtDNA	Mitochondrial Deoxyribucleic Acid
MZ	Mozambican
N_e	Effective population size
PCA	Principal Component Analysis
RH	Height at rump
ROH	Runs of homozygosity
Rsb	Ratio of EHHS between populations
RW	Rump width
SA	South African
SADC	South African Development Countries
SNP	Single Nucleotide Polymorphism

SPSS	Statistical package for social sciences
TL	top line length
WH	Height at withers

General Introduction

1.1 Overview

The local and indigenous animal genetic resources of Mozambique can serve as an important platform for poverty alleviation, with its greatest impact in rural areas where most of the Mozambican population lives. More than 80% of the Mozambican population finds their source of employment and income from agriculture (INE, 2006; Vernooij *et al*., 2016) and livestock is closely linked to the cultural and economic structure of their lives. Almost 50% of the smallholder farmers in Mozambique raise livestock of some kind (Andersen & Learch, 2016). According to national survey figures, 65% of rural households have chickens, 25% have goats or sheep, 12% have pigs and 6% have cattle (Vernooij *et al*., 2016).

Animal production is a main attribute of the Mozambican agricultural sector and contributes widely towards combating food insecurity, as well as providing draft animals, manure to fertilize the soil, transport and cash income (MASA, 2011). This is particularly true in the southern region of the country, which has a long history of raising livestock thanks to favorable agroecological conditions (Vernooij *et al*., 2016). Ruminant animals in rural communities act as a partial determinant of wealth, being used for dowry payments and as a bank and insurance in difficult times.

Various estimates show that the livestock sub-sector contributes approximately 2% to the total and between six and 15% to the agricultural Mozambican gross domestic product (GDP), respectively (FAO, 2005; Nhlengethwa *et al*., 2014; INE, 2016). Despite favourable agricultural conditions, Mozambique does not produce enough food for domestic consumption, remaining a permanent livestock products importer (Ross, 2014). In 2020, Mozambique produced 15 101 tons of beef, 2 811 tons of pork, 120 137 tons of chicken meat, and 2 771 thousand tons of milk (INE, 2020), in the formal sector. Even though such production, the national products do not meet the demand and, as a consequence, per capita consumption remains low. The per capita consumption of livestock products in Mozambique is 9.64 kg for meat, 5.14 l of milk and 1.77 kg of eggs per year. These levels of consumption are below the averages for Africa, which are 17.82 kg, 28.8 l and 2.36 kg respectively (FAOSTAT, 2020). These records reveal alarming deficiencies in the recommended daily intake of 20 g of animal protein per capita, which can be achieved with an annual per capita consumption of 33 kg of meat or 230 kg of milk or 60 kg of eggs (FAO, 2014). Food demand exceeds the supply, forcing the

country to import large amounts of food, especially from South Africa and Europe. Approximately 30% of meat, 80% of milk, and 90% of eggs consumed in the country are imported (Vernooij *et al*., 2016).

Cattle is one of the livestock species whose production is to be promoted to achieve food self-sufficiency in Mozambique (MASA, 2011). There is an estimated 2.02 million head of cattle in Mozambique (FAOSTAT, 2020), however, the uneven dispersion of the cattle population across the country, poses a problem. Cattle farming varies significantly from one geographic region to another and is mostly confined to the central and southern regions, where more than 50% of the national cattle population can be found, due to the low incidence of the tsetse fly (INE, 2015).

Similar to most African countries, Mozambique has two main cattle production systems. These include a small-holder cattle production system and a commercial cattle production system (Maciel *et al*., 2004; MASA, 2015; Van Marle-Köster & Visser, 2018). Most cattle are kept under the small-holder production system using local natural veld (Vernooij *et al*., 2016). This sector generally keeps indigenous breeds and is characterized by limited financial resources and uninformed management decisions (MASA, 2015).

Both indigenous and exotic breeds contribute to the Mozambican national herd. Exotic cattle breeds imported from other African and European countries include the Afrikaner, Brahman, Bonsmara, Holstein, Hereford, Jersey, Simmental, and Brown Swiss (Maciel, 2001), which are used in pure breeding and crossbreeding systems. The indigenous cattle breeds of Mozambique can be classified into two main groups including the Sanga group (i.e., Landim and Tete breeds) and the Zebu group (i.e., Angone breed) (Kotze *et al*., 2000; Bessa *et al*., 2009). Indigenous cattle, such as the Landim, are typically highly fertile and well adapted to the harsh local environmental conditions that are characterized by a high prevalence of tick-borne diseases and droughts (Bessa *et al*., 2009; Maciel *et al*., 2013). The adaptive traits of these animals can make a valuable contribution to selection and breeding programs, in times of biological stress, such as famine, drought or disease epidemics (Nyamushamba *et al*., 2017).

In Mozambique, social such as civil war and natural such as floods and cyclones events contributed to the current limited pool of genetic diversity in livestock species. The civil war, which lasted from 1977 to 1992, killed approximately 80% of cattle in Mozambique (Bessa *et al*., 2009), thus severely affecting people's lives and the genetic diversity of cattle. The civil war persisted for almost 16 years in Mozambique and its influence on agriculture and livelihoods was exacerbated by extensive droughts and flooding (Maciel, 2001). After the considerable loss of the national cattle herd due to the war, the introduction of new cattle breeds was prioritized (Bessa *et al*., 2009) through a national

Livestock Restocking Programme. Nguni animals were imported from South Africa and other neighboring countries to grow the national herd's numbers. In 1994, the cattle population was estimated at 300 thousand heads and by the year 2004, thanks to the livestock restocking program and improved animal health, the national herd had increased to approximately 1 million (MASA, 2011).

In the past, Mozambican indigenous cattle were used in crossbreeding projects to improve cattle productivity. For instance, between 1951 and 1987, Landim cattle were intensively used in artificial insemination programs with semen of exotic breeds, both for milk and meat improvement (Maciel, 2001). These projects and programs were discontinued and/or had no follow-up due to intermittent funding, lack of appropriate support policies and limited involvement of farmers in the design of the interventions. Furthermore, the resulting offspring were used indiscriminately for reproduction, and thus contributed to the dilution of indigenous breeds (Cumbula & Taela, 2020).

For the effective conservation and utilization of indigenous breeds, it is necessary to characterize and evaluate the genetic variation that exist within and among breeds (Maciel *et al*., 2013; FAO, 2015). Apart from preventing extinction, it is important to preserve genetic variability in the population, since without variation animals will be unable to adapt to environmental changes nor to artificial selection for genetic improvement (Nyamushamba *et al*., 2017).

The preservation of genetic diversity in cattle involves comprehensive knowledge about the structure of these populations, as well as the source of genetic variability between and within breeds (Ebrahimi *et al*., 2017). Moreover, information on the effective population size, its structure, as well as its geographic distribution and production environment is a prerequisite to control inbreeding and effectively utilize breed specific characteristics (Mapiye *et al*., 2019). Up to now, research related to nutrition, health and production of the Landim and Angone cattle breeds has been carried out (Catalão & Syrstad, 1990; Otto *et al*., 2000; Maciel *et al*., 2013). However, comprehensive research into the nature and extent of the genetic variation underlying these breeds is limited.

1.2 Aim and specific objectives

The aim of this study was to assess the level of phenotypic and genetic variation within and among indigenous cattle breeds in Mozambique.

The specific objectives included to attain the overarching aim, were:

1. To perform phenotypic characterization of three Mozambican indigenous cattle populations.
2. To assess the level of genetic diversity, structure of population, breed relationships and breed admixture within and between three Mozambican indigenous breeds.
3. To investigate the genetic relatedness and admixture of indigenous Mozambican and South African cattle breeds.

1.3 Thesis outline

This thesis has been prepared under the supervision of Prof. Carina Visser from the Department of Animal Science at the Faculty of Natural and Agricultural Science, University of Pretoria and Prof. Cuthbert Banga, formerly from the Animal Production Institute, Agricultural Research Council of South Africa. It comprises six chapters including an introduction and literature review as well as scientific manuscripts (chapters 3, 4 and 5) either published or submitted to peer-reviewed journals for publication.

Chapter one describes the livestock industry in Mozambique and concludes with a problem statement, and aims and objectives of the study. Chapter two presents a review of literature on various aspects of cattle domestication and genetic diversity of indigenous cattle. Genomic tools as well as statistical measures to investigate genetic diversity and population structure were also reviewed. Chapters three, four and five were prepared in manuscript format according to the specifications of each targeted journal.

The manuscript titled “Morphological characterization of three indigenous Mozambican cattle populations” was prepared using morphometric data collected during sampling of four Mozambican provinces, and submitted to the Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS) where it is currently under review. In this Chapter, a morphometric study was conducted to determine the extent of phenotypic diversity among three indigenous cattle populations using sixteen morphometric variables.

Chapter four consists of a published scientific paper titled “Genetic diversity and population structure of three native cattle populations in Mozambique” (Tropical Animal Health and Production, 2021). This chapter performs a genome-wide analysis using the International Dairy and Beef SNP BeadChip (IDBV3) to assess genetic diversity, population structure and admixture in Mozambican cattle breeds.

The genetic relationship between Mozambican Nguni cattle and three other indigenous South African breeds was investigated in Chapter five, using both IDBV3 and GeneSeek Genomic Profiler (GGP) 80K SNP panels. Population structures of the breeds and patterns of admixture were assessed using the complementary approaches Principal Component Analysis and ADMIXTURE. Additionally, putative selection signatures across these cattle breeds, were identified using two inter-population-based approaches (F_{ST} and Rsb statistics). This manuscript was published in the Livestock Science journal. The thesis ends with a chapter that incorporates a critical review, conclusions and recommendations based on of the research findings.

References

Andersen, J. & Learch, C., 2016. National survey and segmentation of smallholder households in Mozambique-understanding their demand for financial, agricultural, and digital solutions. Available at: http://www.cgap.org/publications/national-survey-segmentation-smallholder-households-mozambique.

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. & Alexandrino, P., 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African Journal of Animal Science, 39, 61-72.

Catalão, D., & Syrstad, O. (1990). Productivity of Nguni and Africander cattle in Mozambique. Livestock Production Science, 24(1), 29–36.

Cumbula, D. & Taela, M., 2020. Animal genetic resources. (AnGR) in Mozambique. IOP Conference Series. Earth and Environmental Science, 482, 012045.

Ebrahimi, M.T.V., Mohammadabadi, M. & Esmailizadeh, A., 2017. Using microsatellite markers to analyze genetic diversity in 14 sheep types in Iran. Archives Animal Breeding, 60, 183-189.

FAO (Food and Agriculture Organization), 2005. Livestock sector brief Mozambique. Livestock Information, sector analysis and policy branch. AGAL.

FAO (Food and Agriculture Organization), 2014. Food outlook – Biannual report on global food markets. Rome, Italy.

FAO (Food and Agriculture Organization), 2015. The second report on the State of the World's Animal Genetic Resources for Food and Agriculture. Rome, Italy.

FAOSTAT (Food and Agriculture Organization Statistics), 2020. Available at: http/www.fao.org/faostat.

INE (National Institute of Statistics), 2006. Integrated survey of the workforce. Final report. Maputo, Mozambique.

INE (National Institute of Statistics), 2015. Statistical Yearbook 2014 Mozambique. Maputo, Mozambique

INE (National Institute of Statistics), 2016. Statistical Yearbook 2015 Mozambique. Maputo, Mozambique.

INE (National Institute of Statistics), 2020. Statistical Yearbook 2019 Mozambique. Maputo, Mozambique.

Kotze, A., Harun, M., Otto, F. & Van der Bank, F.H., 2000. Genetic relationship between three indigenous cattle breeds in Mozambique. South African Journal of Animal Science, 30(2), 92-97.

Maciel, S., 2001. National strategies for the conservation, improvement and utilization of animal genetic resources in smallholder systems. Proceedings of the Planning and Priority Setting Workshop on Animal Genetic Resources in the SADC region. SACCAR, CTA, ILRI, Gaborone, Botswana.

Maciel, S., Harun, M., & Capece, B., 2004. First national country report on the status of farm animal genetic resources in Mozambique. MADER, Maputo, Mozambique, and DAD-IS, FAO (Food and Agriculture Organization of the United Nations), Rome, Italy.

Maciel, S., Okeyo, A.M., Amimo, J., Scholtz, M.M., Neser, F.W.C., & Mart, M., 2013. The effect of geographical region of birth on the reproductive performance of the Nguni in southern Mozambique. South African Journal of Animal Science, 43 (1), 60-63.

Mapiye, C., Chikwanha, O.C., Chimonyo, M. & Dzama, K., 2019. Strategies for Sustainable Use of Indigenous Cattle Genetic Resources in Southern Africa. Diversity, 11, 214.

MASA (Ministry of Agriculture and food security of Mozambique), 2011. National Agriculture Plan 2013–2017.

MASA (Ministry of Agriculture and food security of Mozambique), 2015. Programa de intensificação da produção pecuária 2015–2019. Maputo, Mozambique.

Nhlengethwa, S., Matchaya, G. & Chilonda, P., 2014. The agriculture sector performance in Mozambique. Economic review Galicia. 23(4). Available online at: https://ideas.repec.org/s/sdo/regaec.html.

Nyamushamba, G., Mapiye, C., Tada, O., Halimani, T. & Muchenje, V., 2017. Conservation of indigenous cattle genetic resources in Southern Africa's smallholder areas: Turning threats into opportunities – A review. Asian-Australasian Journal Animal Science, 30, 603-622.

Otto, F., Vilela, F., Harun, M., Taylor, G., Baggasse, P. & Bogin, E., 2000. Biochemical blood profile of Angoni cattle in Mozambique, Israel Journal Veterinary Medicine, 55 (3), 1-9.

Ross, D. R., 2014. Mozambique Rising: Building a New Tomorrow. Washington, DC: IMF. Available at: www.imf.org/external/pubs/ft/dp/2014/afr1404.pdf (accessed 20 January 2023).

Van Marle-Köster, E. & Visser, C., 2018. Genetic Improvement in South African Livestock: Can Genomics Bridge the Gap Between the Developed and Developing Sectors? Frontiers in Genetics, 9, 331.

Vernooij, A., dos Anjos, M. & van Mierlo, J., 2016. Livestock Development in the Zambezi Valley, Mozambique: Poultry, Dairy and Beef Production. Wageningen University and Research Centre for Development Innovation. Report CDI-16-027, Wageningen.

Literature review

2.1 Introduction

Cattle numbers in the world are estimated at 1.5 billion, most of which are concentrated in the Americas (34.9%), Asia (30.6%), and Africa (23.9%) (FAO, 2019). Africa is known to possess around 180 breeds of cattle, with most of them (approximately 98%) being native and commonly found in smallholder areas (Mwai *et al*., 2015). The numerous breeds that have evolved over time in various environmental conditions may share a large fraction of their genetic material, but each breed also represents unique genetic diversity (Hanotte *et al.,* 2002).

Reports of a continuous decline in indigenous animal genetic resources, coupled with declining genetic diversity within indigenous livestock breeds, are a major concern (Nyamushamba *et al*., 2017). An alarming 35% of these breeds are reported to be facing the risk of extinction, in addition to 7% that are already extinct (FAO, 2015). According to FAO (2016), 559 mammalian breeds were recorded as extinct and 16% are at risk of extinction. Among the mammalian species, cattle are the most affected in terms of number of breeds in such dire situations (FAO, 2015). Several factors, including indiscriminate crossbreeding, substitution of natural breeds with exotic ones, as well as societal and environmental disasters, have been linked to the rapid loss of genetic diversity among Africa's indigenous cattle populations (Nyamushamba *et al*., 2017). As a result, there have been extensive efforts to estimate the degree of genetic diversity in livestock species, with a view to developing well-structured programs for conserving these important genetic resources. To enable their sustainable utilization, indigenous breeds must be genetically characterized in order to develop sound breeding programs.

This chapter presents a review of the characteristics of Mozambican indigenous cattle breeds and their value as animal genetic resources. The different approaches applied in assessing genetic diversity, with special reference to the application of genomics tools, are discussed as well.

2.2 Cattle domestication and formation of Southern African breeds

Humans have purposefully bred domestic animals to have desirable characteristics that suit human society. Genetic drift, founder effects, mutations, and migration, contributed to create breeds specifically adapted to the various environments they live in (Lenstra *et al*., 2014) and that provide

humans with specific products. The progenitor of domestic cattle was earlier postulated to be the wild auroch (*Bos taurus primigenius*) (Epstein, 1971). However, subsequent research has shown that the hump-less taurine cattle (*Bos taurus*) originated from a domestication event independent from that of the humped Zebu cattle (*Bos indicus*), with the progenitors being *Bos primigenius* and *Bos namadicus,* respectively (Mwai *et al*., 2015). Domesticated cattle appeared about 10 000 years ago in the Fertile Crescent (northern Iraq and western Iran) (Ajmone-Marsan *et al.,* 2010). Other putative domestication regions include Pakistan's Indus Valley, which dates back 8500 years (Loftus *et al*., 1994) and north-east Africa about 8000 to 9000 years ago (Pitt *et al*., 2018).

Analysis of mitochondrial deoxyribonucleic acid (mtDNA) later confirmed these domestication sites. An analysis of the mtDNA of Zebu cattle collected from several Asian regions revealed that populations on the Indian subcontinent are more likely to have haplogroups I1 and I2 than those in other Asian regions. (Chen *et al*., 2010). This finding, when used in conjunction with existing archaeological findings (Meadow, 1993), suggests that modern Zebu cattle may have been descended from wild Zebu cattle from the *Bos primigenius namadicus* in the subcontinental regions of India, with the Indus Valley being the most probable region. Furthermore, taurine cattle found in the Near East have a larger mtDNA diversity, with multiple haplogroups (T, T2, T3, and, seldomly, T1), compared to cattle from Europe (haplogroup T3) and Africa (haplogroup T1) (Troy *et al*., 2001). These findings support the hypothesis that taurine cattle originated in the Near East from the wild aurochs *Bos primigenius* and then spread throughout Europe and Africa. It has been speculated that a second taurine domestication event occurred in North Africa due to the absence of the African mitochondrial haplotype (T1) in Europe. An analysis of maternal mitochondrial DNA has revealed that African cattle have a higher prevalence of the mitochondria haplogroup T1 compared to cattle from other regions (Bradley *et al*., 1996).

The first domestic cattle introduced to Africa were taurine cattle (*Bos taurus*), followed by Indicine cattle (*Bos indicus*) (Mwai *et al*., 2015). In accordance with archaeological evidence and pictorial representations, taurine populations migrated from the Near East domestication center to Northeast Africa via Egypt approximately 5000 years ago (Gifford-González & Hanotte, 2011). Some 2500 years later, humpless shorthorn cattle may have followed the introduction of humpless longhorns (Mwai *et al*., 2015). From this point, taurine cattle moved west and south over Africa and spread inland (Hanotte *et al*., 2002).

Zebu cattle arrived in Africa in two different waves following the first dispersal of taurine cattle (Hanotte *et al*., 2002). Zebu cattle migrated from the Indian subcontinent, its center of domestication,

and around 4000 years ago reached East Africa via the Horn of Africa (Mwai *et al*., 2015). However, their expansion to North and East Africa only occurred around 1300 years ago, with Arab migrations (Utsunomiya *et al*., 2019). Africa's cattle types are dominated by Zebu cattle (*Bos indicus*). Studies analyzing the Y chromosome, autosomal DNA, and mitochondrial DNA show evidence that Zebu introgression on the African continent took place mostly among male lines (Bradley *et al*., 1994; Hanotte *et al*., 2002; Porto-Neto *et al*., 2013). Morphologically, *Bos indicus* cattle can be differentiated from *Bos taurus* cattle by the presence of a thoracic hump and dewlap in the former.

In addition, the African continent is populated with Sanga cattle, the product of crossbreeding taurine and Zebu cattle (Mapiye *et al*., 2019). This type of cattle breed which includes the Mozambican Tete, Tuli, and Nguni cattle breeds, are distinguished by its short cervico-thoracic hump (Rege & Tawah, 1999; Lashmar *et al*., 2018). The Sanga breed is thought to be descended from the *Bos taurus* animals that were domesticated in Northern Africa 8000 years ago and which were later crossbred with Zebu cattle (Bester *et al*., 2003). Through the tsetse-free Mozambique corridor, Sanga cattle originated in Ethiopia and spread southward with tribesmen and formed many different cattle breeds and types (Felius *et al*., 2014). Mwai *et al*. (2015) reported that Southern African Sanga cattle have unique traits including resistance to diseases and heat tolerance that set them apart from other cattle throughout the world. These genetic pools represent mainly indigenous breeds from South Africa, such as the Nguni, Drakensburger, and Afrikaner, the Landim, Sanga, and Mashona from Mozambique, Swaziland, and Zambia, and Mashona, Nkone, and Tuli from Zimbabwe. (Pienaar *et al*., 2014; Nyamushamba *et al*., 2017; Mapiye *et al*., 2019).

2.3 Cattle distribution and production in Mozambique

There are an estimated 2.02 million heads of cattle in Mozambique (FAO, 2019); however, inequalities in cattle distribution across the country pose a challenge. There is a large variation in cattle population density among the country's regions (Figure 2.1). The country's southern region has the highest livestock density, with more than 9 cattle units per km^2. This region has a low incidence of tsetse fly infestation (Maciel *et al*., 2013; Vernooij *et al*., 2016) and a long-standing tradition of keeping livestock. The three southern provinces of Maputo, Inhambane, and Gaza account for approximately 57.6% of the national cattle population, and only about 20% of the total area of the country (INE, 2015).

Due to the high prevalence of tsetse flies (*Glossina spp*) and trypanosomiasis, cattle production has not been as prosperous in the northern region of Mozambique (Maciel *et al*., 2013).

2.3.1 Cattle production systems in Mozambique

Cattle farming in Mozambique can broadly be divided into two production systems, namely a commercial system and a smallholder system (or family sector) (Vernooij *et al*., 2016). The smallholder cattle production system comprises approximately 85% of the total Mozambican livestock, distributed throughout the country (Maciel *et al*., 2004; FAO, 2019). Low investment and limited animal husbandry inputs are typical of this production system. Although there is no formal mating season, mating often occurs during the summer months, with the majority of calves being born between September and November when the climate is hot and dry and parasites are less prevalent (Carvalheira *et al*., 1995). Smallholder farmers mostly rear indigenous cattle (98% of the national head), with herd sizes ranging from five to ten cattle (Maciel *et al*., 2004; INE, 2014). There is generally no separation of different cattle classes (cows, bulls and calves graze in the same area). Around the villages, cattle are kept communally, forming herds owned and managed typically by more than one individual. Animals from different farmers are taken to these communal grazing areas for daily grazing, and are collected at the end of the day and taken to the respective owners' corrals (Vernooij *et al*., 2016).

The profit-oriented commercial sector typically has high-level management and clearly defined breeding objectives. Approximately 15% of the national livestock population is accounted for by this sector, comprising mostly of exotic livestock breeds which are crossbred with the indigenous local breeds (Maciel *et al*., 2004). Animal productivity in this system is well documented and the herd size typically ranges from ten to 100 cattle (medium scale) and sometimes exceeds 100 animals (large scale) (INE, 2014). Commercial farmers generally have better infrastructure including water troughs, dip tanks and fencing. Restricted breeding seasons, which could be summer (between January/February and late March/April) or winter (from August to October) are part of their general management (Carvalheira *et al*., 1995). Breeding animals are selected within the farm based on phenotypic and pedigree data.

The natural pastures and grasslands of Mozambique are suitable for raising cattle on over 44 million ha (Vernooij *et al*., 2016). Cattle are typically raised in an extensive management system that allows them to graze on natural grassland, and occasionally on crop residues as well (Bessa *et al*., 2009). However, most cattle farmers face considerable challenges due to the country's extended dry

season and droughts, which result in a lack of feed and water. Furthermore, the shortage of fodder for cattle is exacerbated by the decline of available grazing for ruminants, uncontrolled fires, pasture degradation, and lack of pasture management that contributed to this degradation (Timberlake & Jordão, 1985; Nyamushamba *et al*., 2017).

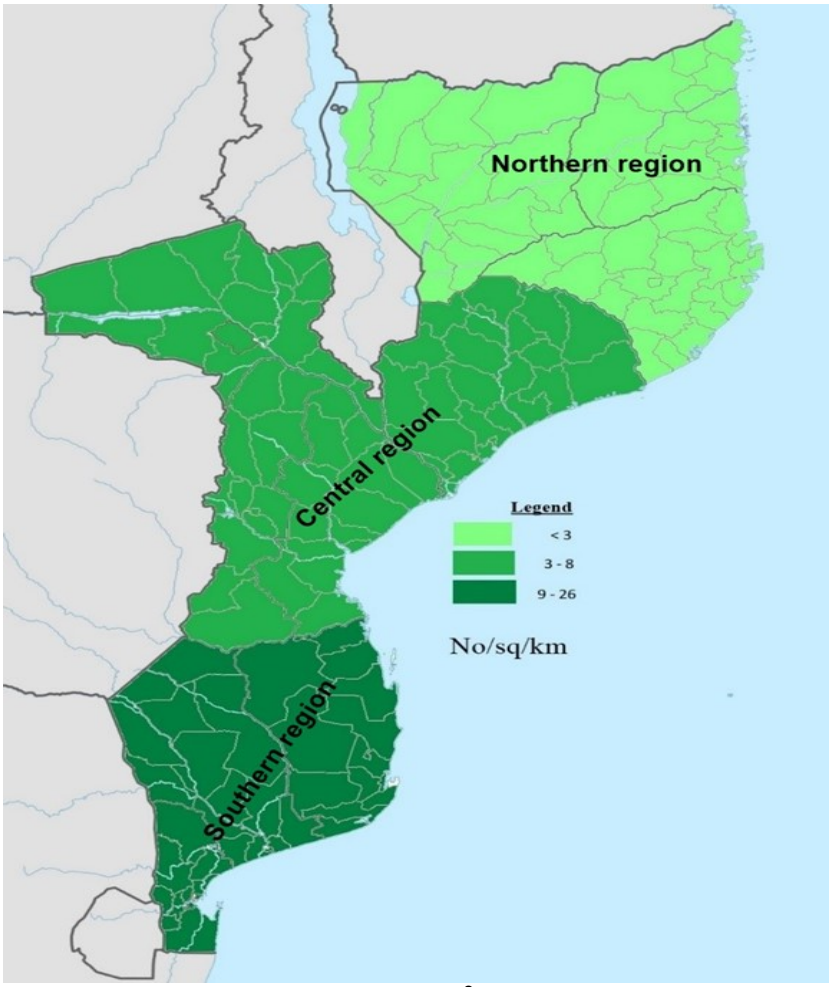

Figure 0.1 Number of cattle per km^2 in Mozambique; Source: INE, 2011

2.4 Indigenous cattle breeds of Mozambique

Mozambique's three indigenous cattle breeds are Landim, Tete and Angone (Alberro, 1983). The country's national herd consists of more than 70% Landim cattle, followed by the Tete (20%) and Angone (8%) (Maciel, 2001). These breeds play an important socio-economic role.

Landim cattle breed

The Landim cattle breed (Figure 2.2), is the most abundant beef cattle breed in Mozambique. Mostly found in the south of the country, it is the most widely used breed by smallholder farmers (Maciel, 2001). The coat colour of Landim cattle varies and sometimes presents a mixture of colors, where the dominant pattern distinguishes different groups. Characteristics such as high fertility (83%), low mortality (4%), and compensatory growth during the rainy season (Dionisio & Syrstad, 1990; Carvalheira *et al*., 1995) are typical of Landim cattle. Although the cows are medium in size (225 – 450 kg) and maintenance requirements are low, this breed is the largest when compared to the other indigenous cattle breeds (Maciel, 2001). Mozambique's southern region mainly uses Landim cattle for ploughing because they are suitable for draught power. Means for reproductive, growth and carcass traits of Landim cattle reported by Carvalheira *et al*. (1995) are summarized in Table 2.1. The Landim is resistant to ticks and internal parasites (Table 2.2), and has good maternal ability (Nyamushamba *et al*., 2017). It is highly productive under good management conditions (Carvalheira *et al*., 1995).

Table 0.1 Performance parameters for Landim cattle in the commercial sector (Carvalheira et al., 1995)

Parameters	Mean values
Calving rate	86%
Age at first calving	41.2 months
Birth weight	32.6 kg
Weaning weight	149.5 kg
18-month weight	237.1 kg
Calving interval	445.8 days

Figure 0.2 Landim cows with their calves in an overnight corral at Chobela Research Station (Picture taken by the author)

Tete Cattle breed

Tete cattle (Figure 2.3), morphologically resemble the Landim, but are relatively smaller and have a larger hump (Tomo & Macamo, 2000). In the Tete province, the cattle are located between the western and southeast ends, bordering Malawi (Morgado, 2004). Their origin is unclear, but they are considered part of the Sanga group.

It has been suggested that the Tete breed was a result of crossbreeding the Landim and Angone breeds (Rege & Tawah, 1999), while other researchers postulated that it is derived from the Mashona breed (Sanga type), which is found in the region bordering Zimbabwe and Mozambique (Morgado, 2000). The Tete is adapted to low rainfall areas (less than 500 mm) with high temperatures (above 27°C) and can produce good quality meat (Rocha, 1985) and can also be used for draught power (Maciel, 2001).

Figure 0.3 Typical Tete cattle bull (Picture taken by the author)

Angone cattle breed

Mozambique has only one indigenous Zebu cattle breed, namely the Angone, which is also found in Zambia, Malawi, and Madagascar (Figure 2.4) (Otto *et al*., 2000). The Angónia plateau in the Tete province in Mozambique is home to Angone cattle. It is located near the Malawi border in the northeast corner, which has an average altitude of 1300 m. Morphologically, Angone fits into the East African short-horned Zebu group, to which the Zebu from Southern Malawi and Angoni from Zambia also belongs (Alberro, 1983). A typical Angone female weighs 230 kg and has a well-developed thoracic hump. There is a significant difference in the coat color pattern of Angone cattle, ranging from mainly black with white spots to red, white, and spotted patterns. However, the majority of the animals (60.5%) are red-coated (Tomo, 1997). Studies in eastern Africa have shown that Angone cattle have a higher frequency of taurine alleles than other Zebu breeds, such as the Tanzanian Iringa Red (Hanotte *et al*., 2000). A variety of diseases such as theileriosis, fascioliapsis and gastrointestinal parasites (Table 2.2) and adverse climatic conditions can be tolerated by these animals (Otto *et al*., 2000; Mwai *et al*., 2015).

Figure 0.4 Angone cattle herd grazing (Picture taken by the author)

Table 0.2 Adaptive characteristics of Landim, Tete and Angone cattle breeds of Mozambique

Breed Name	Geographical distribution	Description	Adaptive characteristics	Source
Landim	Southern region of Mozambique	Coat color: black, white, dark brown, brown and white; Horns: normally heavier in males than females, grow in an upward and backward curve.	Heat and humidity-tolerant as well as drought-resistant; Exceptionally resistant to foot and mouth disease	1, 2, 3
Tete	Western and southeast parts of the Tete province	Coat color: cream and gray; Hump: larger hump than Landim; Horns: lateral and black.	Tolerant to trypanosomosis, disease resistant, heat tolerant	1, 2, 3
Angone	Northeast part of the Tete Province	Coat color: vary considerably, ranging from black to brown with a mix of colours in between; Hump: (and dewlap) well developed; Horns: short and thick and lateral rather than upright.	Tolerant to theileriosis, fascioliapsis and gastrointestinal parasites. Adapted to browsing during dry season.	2, 3, 4

[1]Maciel, 2001; [2]Mwai *et al*., 2015; [3]Nyamushamba *et al*., 2017; [4]Otto *et al*., 2000

2.5 The importance of genetic diversity

Genetic variation derives from both natural and artificial selection (Ceriotti *et al*., 2003; Groeneveld *et al*., 2010) and has been shaping domesticated species through processes such as mutation, fixation and genetic drift (FAO, 2015). There are a variety of farm animal species on the planet which reflect the conditions they adapt to, ranging from climatic to ecological, as well as the diversity of human needs. Without genetic variation, breeds will not survive adverse environmental conditions, such as those caused by climatic change, as well as disease epidemics (FAO, 2010; Nyamushamba *et al*., 2017). Moderate and high genetic diversity assist populations to adapt to adverse environmental effects (Pienaar *et al*., 2014). Loss of genetic diversity in the absence of migration is irreversible and results in reduced survival and reproductive performance of animals (FAO, 2000).

Globally, livestock populations are losing genetic diversity and facing an increased threat of extinction (FAO, 2007). There are several factors that threaten livestock breeds, resulting in declining genetic diversity. These factors include assisted reproduction technologies (AI and MOET), and inadequate design of breeding systems such as indiscriminate crossbreeding and upgrading (Bett *et al*., 2013). Genetic diversity of cattle is also lost when there are high levels of inbreeding along with inbreeding depression, and an increased expression of deleterious genes (Szpiech *et al*., 2013). Maintenance of genetic diversity is essential for breed survival as it reduces the chances of inbreeding depression (Hlophe, 2011).

The importance of genetic differentiation between breeds can be seen in the ability of rare and locally adapted breeds to meet future demands such as those brought on by future stresses like famine, drought or epidemics (FAO, 2010; Engelsma, 2012). Additionally, our cultural heritage is shaped by the diversity of breeds and lines available today, and food security and cultural ceremonies are dependent on local breeds, specifically in communities with limited resources (Gandini & Villa, 2003). It is therefore important that these indigenous lines and breeds be preserved (Chagunda & Wollny, 2003).

2.6 Characterization of animal genetic resources

As explained by the FAO (2012), the classification of animal genetic resources (AnGR) entails identifying, quantifying and qualitatively describing livestock populations, as well as describing their habitats and production systems. The overarching objective of characterization is to provide an overview of AnGR abundance and potential to ensure their management and conservation (Boettcher *et al*., 2010; Groeneveld *et al*., 2010; Gamaniel & Gwaza, 2017). AnGR for food and agriculture must be characterized based on the animals' morphology, genetic makeup, and history (FAO, 2012).

2.6.1 Phenotypic characterization

Effective assessment of the status of genetic resources requires phenotypic characterization (Hailu & Getu, 2015). FAO (2012) defined phenotypic characterization as the description of the external and productive characteristics of AnGR in a given environment. Phenotypic characterization comprises three main categories: (1) morphological description; (2) parameters of performance; and (3) adaptation to the environment (FAO, 2011b). The morphological characteristics of a breed, such as its coat color, body size, and horn shape are essential in describing and establishing breed standards (McManus *et al*., 2010). Examples of morphological characteristics commonly measured in cattle are listed in Table 2.3.

Table 0.3 Standard breed descriptors for quantitative traits of cattle (FAO, 2012)

Trait	Definitions
Heart girth	Circumference of the body (cm) measured immediately behind the front leg
Body length	Horizontal length (cm) from the point of shoulder to the pin bone
Height at withers	The height (cm) from the platform on which the animal stands to the highest point of the shoulder
Ear length	Length (cm) of extreme part of ear from its roots to tip
Horn length	Distance from the base of the horn to tip horn
Muzzle circumference	Circumference (cm) measured immediately posterior to the muzzle
Pelvic width	The horizontal distance (cm) between the extreme later points of the hook bone (tuber coxae) of the pelvis
Hock circumference	Circumference (cm) measured just above the hock joint

Southern African cattle present distinctive morphological traits which differentiate them from other cattle. African indigenous cattle have generally been characterized as horned, humped, small framed and multi-colored (Table 2.4). However, there is still a need to define a sizable number of the indigenous cattle populations in the sub-region (Nyamushamba *et al*., 2017). The majority of African livestock populations, including those in Mozambique, are categorized based on ethnicity or historical

and anthropological data (Mammo *et al.*, 2017). The majority of indigenous cattle species in Mozambique go by the term Landim, which in Changana language (spoken in southern Mozambique), means "Local" or "Indigenous". Depending on the regional language used throughout the country, indigenous cattle can be named in different ways (Maciel, 2001).

Table 0.4 Phenotypic description of some indigenous cattle breeds in Southern Africa (DAGRIS, 2007)

Breed	**Country**	**Phenotypic description**	**Mature body weight (kg)**	
			Male	**Female**
Nguni	South Africa	Variable coat color; Short haired; Horns are typically lyre shaped, twisted in males	500-700	320-440
Barotse	Angola, Namibia Botswana Zambia	Usually multi-colored; animals with black, white or brown patches on the sides are also common; Horns are long and curved backwards; Small hump, often barely visible in females	630	450
Tonga	Zambia	Multi-colored ranging from black to white, (brown tend to dominate); Small body size; Medium sized horns that tend to spread outwards	560	360
Afrikaner	Namibia, Botswana, Swaziland, Zimbabwe, Zambia, Malawi, South Africa	Coat color ranges from dark to light red; Cervico-thoracic hump (prominent but not large); Long horns, growing downward and backward	745	525
Tuli	Zimbabwe, Botswana, South Africa	Color is mostly red, red-and-white and golden brown; Long wide-spreading horns; Heavy boned with long strong legs	750	400
Boran	South Africa	Usually white or grey fawn coats; Small ears, which are occasionally pendulous Deep chest, slightly slopping rump, well-developed hindquarters.	550-850	400-550
Mashona	Zimbabwe	Mostly black coat color, followed by red; Short, straight, glossy hair; Long and heavy horns (45 to 60 cm); Compact body conformation; Long tail	350-635	260-410
Angoni	Zambia, Mozambique	Variable coat color (red, brown, black, sometimes with white, or brindle); Short and thick lateral horns; Hump and dewlap well developed	550-650	175-480

2.6.2 Molecular characterization and genomic tools

Molecular characterization is a procedure used to disentangle the genetic makeup of phenotypes and their inheritance patterns across generations and establish relationships between breeds (FAO, 2011a). Genetic characterization involves a description of the breeds with a focus on the level of polymorphism, allele frequencies and genetic distance between the breeds (Solomon *et al*., 2011). Identifying the mechanisms that control gene flow, that is the movement of alleles across and within populations of a species, and its effects are possible thanks to molecular characterization (Toro *et al*., 2009).

Evaluation of the genetic composition and diversity of diverse animal populations has changed over time. Initially, scientists relied on phenotypic data for selection and livestock improvement (Teneva, 2009). In previous studies, morphological markers were used to detect genomic variation, then the karyotype was developed (Walsh, 2000). Proteins and iso-enzymes were introduced as biochemical markers with the development of contemporary biotechnology. Over time, they were, however, superseded by DNA-based technologies due to their incapacity to adequately explain the degree of genetic variation due to the few loci available and their low level of polymorphism (Lenstra *et al*., 2012; Hailu & Getu, 2015).

Molecular markers are segments of DNA exhibiting distinct variants, and are located at specific points along the genome (Dekkers, 2004; Toro *et al*., 2009). It can also measure the genetic diversity of a population, divide it into subpopulations, and estimate gene flow and genetic ancestry (Falush *et al*., 2007; Hailu & Getu, 2015). Many genetic characterization studies based on microsatellite markers have been conducted globally on livestock. Table 2.5 summarizes some studies on the genetic characteristics of indigenous African cattle breeds. From the values presented in this table it can be concluded that, judging by the expected heterozygosity, cattle from Africa generally have high genetic diversity (H_e = 0.57 to 0.74) based on microsatellite-based studies (Mwai *et al*., 2015).

Table 0.5 Summary of genetic characterization studies on indigenous African cattle populations using microsatellite markers

Cattle population	Markers	MNA	H_o	H_e	F_{ST}	References
Afrikaner (South Africa)	11	5.18	-	0.57	-	Pienaar *et al*., 2014
Cameroonian indigenous cattle (Cameroon)	13	6.10	0.76	0.71	0.07	Ngono Ema *et al*., 2014
Angone, Landim and Bovino de Tete (Mozambique)	13	6.23	0.60	0.67	-	Bessa *et al*., 2009
Ankole cattle (Uganda)	19	6.36	0.70	0.73	-	Kugonza *et al*., 2011
Ankole Longhorn cattle (Kenya)	15	7.54	0.70	0.74	0.03	Ndumu *et al*., 2008
East African cattle breeds	18	6.63	0.62	0.61	-	Adhiambo, 2002
Nguni cattle ecotypes(South Africa)	22	6.47	0.69	0.70	0.05	Sanarana, 2015
Nguni cattle (Southern Africa)	25	7.20	0.66	0.72	-	Madilindi *et al*., 2020
Zimbabwean Sanga cattle populations	16	5.17	0.74	0.71	0.08	Gororo *et al*., 2018
South African cattle breeds	11	10.48	-	0.70	-	Van der Westhuizen *et al*., 2019

MNA mean number of alleles, H_o observed heterozgosity, H_e expected heterozygosity, F_{ST} Inbreeding coefficient of sub-populations.

Microsatellites, despite being widely used to investigate genetic diversity in many animal species, have the disadvantage that they are not directly linked to genes influencing phenotypes due to their position in non-coding regions of the genome (Lenstra *et al*., 2012). Furthermore, the use of microsatellite markers has limitations due to complex mutation models, high incidence of homoplasia and low genotypic throughput (Hailu & Getu, 2015). These shortcomings have motivated scientists to look for alternative types of markers.

When one nucleotide (A, T, C, or G) is changed for another, the result is a difference in the DNA sequence known as a single nucleotide polymorphism (SNP). These SNPs have evolved into the preferred markers for livestock diversity research due to their abundance in the genome, genetic stability, and suitability for high throughput automated investigations (Vignal *et al*., 2002). With the use of SNP technology, genomic data can now be produced quickly and automatically on a massive scale (Matukumalli *et al*., 2009). Commercial SNP array panels have been used in numerous countries, including Ethiopia (Edea *et al*., 2013), India (Sharma *et al*., 2016) and South Africa (Makina *et al*., 2014) to analyse the distribution of several indigenous cattle breeds, their diversity, and their admixture patterns.

According to studies utilizing SNP arrays, South African Afrikaner, Bonsmara, Drakensberger and Nguni cattle all have moderate expected heterozygosity levels ranging between 0.22 and 0.36 (Makina *et al*., 2014; Zwane *et al*., 2016; Bosman *et al*., 2017). Similar levels of heterozygosity were reported for Ethiopian indigenous cattle populations (0.30–0.32; Edea *et al*., 2012). However, a study by Msalya *et al*. (2017) using the BovineSNP80 assay, reported relatively higher values of H_e (0.40) in four indigenous Tanzanian cattle breeds. Using SNP data, moderate levels of heterozygosity were also observed for the Boran cattle breed in South Africa (0.32) and Tanzania (0.40) (Msalya *et al*., 2017; Van Marle-Köster *et al*., 2021).

In order to examine the population structure and genetic diversity of South African Drakensberger cattle, SNP arrays were used (Lashmar *et al*., 2018), and similar analysis was also carried out for Tanzanian indigenous cattle (Msalya *et al*., 2017). Studies in South Africa examined genetic diversity (Makina *et al*., 2014; Makina *et al*., 2016), linkage disequilibrium (LD) and effective population size (N_e) (Makina *et al*., 2015a) of indigenous cattle breeds. Edea *et al*. (2014) reported genome wide genetic diversity of African cattle breeds, while population structure (Mbole-Kariuki *et al*., 2014) and inbreeding depression (Murray *et al*., 2013) were described in the East African Shorthorn Zebu cattle population. Single Nucleotide Polymorphism (SNP) panels can also be used to identify selection signatures; however, Makina *et al*. (2015b) reported limitations in investigating indigenous South African cattle due to SNP ascertainment bias and small sample sizes. The SNP50 bead chip was additionally utilized to identify copy number variations (CNVs) in South African Nguni cattle (Wang *et al*., 2015).

2.7 Estimation of genetic diversity and population structure

2.7.1 Genetic diversity

It was historically the morphology, physiology, and behaviour of individuals within a population that distinguished them from each other (Meuwissen, 2009). Currently, the genotypes and alleles of a population can be used to describe a population's genetic diversity. As a major component of evolutionary and adaptive processes, genetic diversity plays a crucial role in conservation programs (Frankham *et al*., 2002). Gene frequency distributions, polymorphic loci, and observed and expected heterozygosity are all ways that molecular data can be applied to estimate genetic diversity (Toro *et al*., 2009; Peakall & Smouse, 2012).

2.7.2 Heterozygosity levels

Gene diversity or expected heterozygosity (H_e) describes a population's genetic variation (Nei, *et al*., 1983). The expected heterozygosity is the most widely used parameter and for a specific locus, and it can be calculated as follows:

$$H_E = \frac{(N(NM) - E(\mathrm{Hom}))}{N(NM)}$$

where: N(NM) represents the number of non-missing genotypes,
O(Hom) represents the observed number of heterozygotes and
E(Hom) represents the expected number of heterozygotes

A population's heterozygosity can range from zero to one, with one indicating greater genetic diversity and zero indicating inbreeding and high selection pressure (Mburu & Hanotte, 2005). The expected heterozygosity is higher than that observed ($H_e > H_o$), in the presence of forces such as inbreeding, whereas when two previously isolated populations merge, heterozygotes are increased. In a population with random mating, the expected heterozygosity is the same as that observed ($H_e = H_o$) (Mburu & Hanotte, 2005).

2.7.3 Inbreeding and Effective population size

Breeding in which related individuals are mated more closely than the average relationship in a population, is known as inbreeding (Falconer & Mackay, 1996). A population's genetic diversity can also be estimated using this parameter. High inbreeding rates lead to loss of genetic variation among livestock populations, which results in deleterious alleles being expressed more frequently and the development of inbreeding depression (Szpiech *et al*., 2013). The level of inbreeding is assessed by the coefficient of inbreeding (F_{IS}), which measures the lack of heterozygosity within the population due to non-random mating (Dorji & Daugjinda, 2014). F_{IS} values range from -1 (outbreeding) to a maximum of 1 (inbreeding) (Paiva *et al*., 2011).

The F statistic are defined as follows:

$F_{IS}=f=(H_e-H_o)/H_e$

Where H_e is the expected heterozygosity and H_o is the observed heterozygosity

Before the advent of DNA technology, inbreeding was estimated based on pedigree information. Inbreeding estimated via pedigree (F_{PED}) is based on Mendelian sampling and does not include recombination events during meiosis. In addition, pedigree-based inbreeding (F_{PED}) estimates may be biased due to inconsistent pedigree recording and pedigree errors (Purfield *et al*., 2012). By using genetic markers such as microsatellite markers and SNPs, genomic tools can be used to more accurately estimate inbreeding (Lenstra *et al*., 2012). One of the genomics approaches is using Runs of Homozygosity (ROH) to estimate genome-based inbreeding (F_{ROH}) (Bjelland *et al*., 2013; Purfield *et al*., 2017).

Runs of Homozygosity are uninterrupted homozygous segments that arise in an individual's genome when identical haplotypes are inherited from each parent and, therefore, the haplotype formed is homozygous (Ceballos *et al*., 2018). Researchers have used these ROH to infer the history of a population and inbreeding depression in humans and livestock. The length and distribution of ROH categories can provide details regarding the history of a population or how inbred it is (Ferenčaković *et al*., 2011; Bjelland *et al*., 2013; Purfield *et al*., 2017).

Inbreeding estimated from ROH (F_{ROH}) is closely correlated with homozygosity, which makes it more preferable than F_{PED} (Keller *et al*., 2011). It has been demonstrated that PLINK is a suitable software package for estimating ROH (Purcell *et al*., 2007). The F_{ROH} can be calculated as follows (McQuillan *et al*., 2008):

$$F_{ROH} = \Sigma L_{ROH} / \Sigma L_{AUTO}$$

where: L_{ROH} = the length of ROH in one individual

L_{AUTO} = the length of the genome covered by SNPs, excluding the centromeres

Worldwide, cattle populations are showing an increase in long ROH segments due to genetic selection, and these segments have been associated with increased inbreeding and negative effects on livestock (Szpiech *et al*., 2013). Msalya *et al*. (2017) reported that the highest average ROH length in cattle populations from Tanzania was identified for the Maasai breed (17.5 Mb), whereas in other breeds such as Tarime, Sukuma, and Boran, the average ROH length ranged from 9.5 to 13.1 Mb. ROH has been used to estimate cattle inbreeding in a number of studies on South African cattle (Lashmar *et al*., 2018; Van Marle-Köster *et al*., 2021). There is considerable evidence that inbreeding estimates based on ROH are more accurate than those based on pedigree. Other studies described the correlation between F_{ROH} and inbreeding coefficients. For instance, Lashmar *et al*. (2018) reported

a correlation of 0.64 between F_{ROH} and F_{PED} in the South African Drakensberger cattle breed, while Msalya *et al*. (2017) reported a strong correlation of 0.75 between these two parameters in various Tanzanian cattle populations.

Effective population size (N_e) is based on the number of breeding animals in an idealized population that would result in the same levels of inbreeding as in a real population (Hayes *et al*., 2003). In population genetics and AnGR management, N_e is a crucial parameter. Inferring the inbreeding rate from this parameter allows us to estimate the population's genetic diversity loss (Groeneveld *et al*., 2010). Past estimates of N_e were based on pedigree records (Falconer & Mackay, 1996). However, due to its dependence on integrity and accuracy of pedigree information, this method of estimating N_e is unreliable (Barbato *et al*., 2015). Effective population size can be estimated using the Linkage disequilibrium (LD) between markers in the genome using the marker-based method (Saura *et al*., 2015). Barbato *et al*. (2015) designed an SNP-based tool (SNeP) for estimating N_e between generations. As well as phasing, recombination rate and sample size can all be corrected by using this tool, by applying the following formula:

$N_{T(T)} = (4f\,(c_{(t)})^{-1}(E[r^2_{adj}|^{ct}]^{-1} - \alpha)$,
Where *NT* is the effective population size T generations ago;
T is expressed as (2f (ct)) −1 according to Hayes *et al*. (2003);
ct is the recombination rate for specific physical distance;
$r^{2adj} = r^2 - (\beta n)$, $^{-1}$ where r^{2adj} is the LD adjusted for sample size (n = sample size, β = 2 if gametic phase is known or β = 1 if unknown);
α = correction factor for mutations.

A downward trend in N_e has been observed in various breeds of cattle, mainly caused by the use of artificial insemination and increased selection pressure (Hayes *et al*., 2008). Van Marle-Köster *et al*. (2021) estimated the effective population size of eight South African cattle populations and found that N_e for these populations has declined over the past 13 generations with the lowest current N_e being seen in the Tuli breed (147 individuals). Similarly, Makina *et al*. (2015a) estimated N_e for South African Nguni, Drakensberger, Bonsmara and Afrikaner cattle breeds and found that N_e has been decreasing over the past 100 generations. In addition, Msalya *et al*. (2017) estimated N_e in Tanzanian indigenous cattle breeds using Genome-Wide SNP data and revealed N_e values of 67 (Sukuma), 119 (Tarime) and 96 (Maasai) over the last 10 generations.

2.7.4 Linkage disequilibrium

Linkage disequilibrium (LD) is another important factor in assessing genetic diversity (McKay *et al*., 2007). Linkage disequilibrium exists when alleles at two or more loci are associated non-randomly (Hayes *et al*., 2003). Most often, it occurs as a result of migration, mutation, selection, or other demographic events (Slatkin, 2008). Measurements of LD based on the D' statistic are often inflated by small sample sizes, so large datasets are necessary to avoid this problem (Lee *et al*., 2011). Linkage disequilibrium is commonly measured by r^2 in livestock studies. This parameter measures the correlation between two loci. For a pair of biallelic loci, r^2 corresponds to 1 (known as perfect LD) if only two haplotypes are available in the population (Khatkar *et al*., 2008). Linkage disequilibrium as expressed by r^2 can be calculated based on the following equation:

$r^2 = D^2 \div f(Ai)f(Bi)f(Aj)f(Bj)$ where;

D^2 = difference between the observed and the expected haplotype frequencies

f (Ai)f(Bi)f(Aj)and f(Bj) are observed frequencies of alleles Ai, Bi, Aj and Bj respectively. i and j are markers. (The frequencies of allele A or B at the i or/and j markers)

It has been proposed that cattle populations will exhibit greater LD than human populations, which is explained by the higher selection pressure and smaller effective population size typical for livestock populations (McRae *et al*., 2002; Tenesa *et al*., 2007). Previous reports have demonstrated that moderate LD (r^2 ≥0.2) extends up to 100 kb in cattle whereas in humans it does not go beyond 5 kb. However, very high LD (r^2 ≥0.8) only stretches a relatively small distance (1 kb) in both cattle and humans (Dunning *et al*., 2000; Reich *et al*., 2001; Tenesa *et al*., 2007).

Studies on indigenous SA breeds utilizing the BovineSNP50 test have revealed much lower levels of linkage disequilibrium (LD) (0.18) than those seen in European taurine breeds (0.5) (McKay *et al*., 2007; Edea *et al*., 2013; Makina *et al*., 2014). Average r^2 values ranging from 0.17 (SA Nguni) to 0.19 (SA Tuli) were reported by Van Marle-Köster *et al*. (2021) in four South African indigenous cattle breeds at < 100 kb distances between markers. Moreover, Lashmar *et al*. (2018) found estimated LD of 0.32, 0.24 and 0.21 at marker distances of 0-10kb, 10-20kb and 20-30kb, respectively, for the South African Drakensberger cattle breed.

The LD for SNPs at an average marker distance of 40-60 kb in South African cattle breeds, have been reported to be equal to 0.23 (Afrikaner), 0.15 (Nguni), 0.14 (Drakensberger), 0.16 (Bonsmara), 0.21 (Angus) and 0.21 (Holstein) (Makina *et al*., 2015a). In several cattle populations, linkage disequilibrium has been exploited to identify genes responsible for genetic variation (Saravanan *et al*., 2020).

2.7.5 Population differentiation (F_{ST})

The F_{ST} parameter which was developed by Wright (1951, 1965) is used to divide genetic variance among populations. This parameter uses a set of fixation indices known as F coefficients to characterize the distribution of genetic variation. F_{ST} gauges the degree of genetic difference across subpopulations based on allele frequency (Balloux & Goudet, 2002; Kalinowski, 2002), and ranges between 0 and 1 (Norberg & Sørensen, 2007). AMOVA (Analysis of Molecular Variance), which depicts the division of gene diversity into hierarchical distribution, may be used to quantify genetic divergence within and between subdivided populations (Excoffier *et al*., 2005).

2.7.6 Population structure

Population structure refers to the pattern of genetic makeup that exists in a population as a whole. By analyzing population structure, we are better able to understand differences between groups and admixture between groups (Patterson *et al*., 2006). Principal component analysis (PCA) as well as model-based clustering approaches such as STRUCTURE (Pritchard *et al*., 2000) and ADMIXTURE (Alexander *et al*., 2009) may all be used to examine the extent of admixture and population structure (Patterson *et al*., 2006).

For assessing variance and to better understand how different cattle breeds are structurally connected, principal component analysis (PCA) can be used (Lewis *et al*., 2011). In contrast to regression analysis, where variables are divided into dependent and independent categories, principal component analysis treats all variables equally. The initial variables are converted into primary components (PC), which are new uncorrelated variables (Ojango *et al*., 2011). The PCs are arranged in priority order, with the most informative one at the top and the least informative one at the bottom (Suhr, 2005).

Admixture occurs when individuals from two or more different populations interbreed and form a hybrid population (Shriver *et al*., 2003). Admixture analysis is extremely important for estimating the genetic diversity of populations using genetic markers. In this analysis DNA from several genetically distinct populations is used for the study at the individual or population level (Frkonja *et al*., 2012).

The ADMIXURE (Alexander *et al*., 2009) or STRUCTURE (Pritchard *et al*., 2000) computer software may be used to describe population structure. Since they both detect the genetic organization of a population, their results are comparable. STRUCTURE uses an iterative Bayesian system where populations are allocated to a cluster taking into account similar patterns of variation (Porras-Hurtado *et al*., 2013) while ADMIXTURE has higher computational speed than STRUCTURE because it has an optimized numerical algorithm (Alexander *et al*., 2009). Distinguishing between different breeds and populations is difficult when based solely on morphology or when there is no genealogical information available; however, STRUCTURE can be used to appropriately connect individuals to a population or a breed (Alexander *et al*., 2009). Linkage disequilibrium is not fully accounted for by ADMIXTURE, which is one of its disadvantages (Porras-Hurtado *et al*., 2013). Through the statistical methods described above, it is possible to study the genome and thus unravel the historical events that shaped cattle populations in the process of their domestication. Such knowledge may be essential for the protection of animal genetic resources and, as a result, for the survival of the animals' adaptive features (Fan *et al*., 2010).

A study using SNP data from 134 domesticated cattle breeds worldwide was conducted by Decker *et al*. (2014). The study discovered distinctive autosomal backgrounds in African cattle, which may have resulted from auroch introgression in the region. Makina *et al*. (2014) examined the genomes of six South African cattle breeds and found significant genetic differences between them and *Bos taurus*. In addition, the genetic makeup and population dynamics of East African short-horned Zebu cattle populations detailed in Mbole-Kariuki *et al*. (2014) and Weerasinghe (2014) revealed all native cattle breeds in Tanzania, Kenya, Uganda and Ethiopia as a mixture of *Bos indicus* and African *Bos taurus*.

2.7.7 Signatures of selection

Since domestication, cattle species have been subjected to natural and artificial selection. (Ajmone-Marsan *et al*., 2010). When selection acts on a particular trait, the frequency of alleles of that locus will change over time (Simianer *et al*., 2014). This can result in highly differentiated genomic regions among breeds and certain haplotypes with increased frequencies. These regions can therefore be referred to as "selection signatures" (Nielsen *et al*., 2005). When selection effects beneficial mutations over many generations, it can also reduce genetic variation at neutral sites adjacent to the mutation (Zhao *et al*., 2015). The phenomenon where a desirable mutation increases in frequency with a resultant reduction of variation in the neutral sites adjacent to it is referred as “hitchhiking effect” or “selective sweep” (Fay & Wu, 2000; Akey *et al*., 2002; Gouveia *et al*., 2014).

The search for selection signatures in the genome is crucial as it can help in the identification of beneficial genes and mutations in cattle populations (Zhao *et al*., 2015). Moreover, selection signatures can provide necessary information attributed to the origins and expansion of the animal husbandry industry (Otto, 2000). The advent of the genomic era and new computational biology tools, have significantly enhanced the identification of genomic regions of importance (Qanbari & Simianer, 2014).

Several approaches have been proposed to identify selection signatures, mostly based on differences in demographic processes (Stella *et al*., 2010; Vitti *et al*., 2013). These approaches were categorized by Saravanan *et al*. (2020) into intra-population and inter-populations methodologies.

2.7.7.1 Intra-population statistics

Intra-population methods aim to detect selection footprints by comparing genetic data in the population. These methodologies are based on three theories:

1) Site/allele frequency spectrum (SFS) is a statistic that evaluates how alleles at a given genomic region vary in their frequency distribution.
   Tajima's D (Tajima, 1989), Fay and Wu's H (Fay & Wu, 2000) and composite likelihood ratio (CLR) (Nielsen *et al*., 2005) are some examples of this class.
2) Linkage disequilibrium-based methods identify selection signatures by evaluating the relationship between LD and allele frequencies (Sabeti *et al*., 2002). Haplotypes that have high levels of LD with a selection signature have an increased frequency; however, this LD breaks

down immediately when the locus under selection reaches fixation (Nielsen *et al*., 2005). Commonly used LD-based tests include integrated haplotype score (iHS) (Voight *et al*., 2006), linkage disequilibrium decay test (LDD) (Wang *et al*., 2006) and relative extended haplotype homozygosity (rEHH) (Sabeti *et al*., 2002).

LD-based methods including integrated haplotype scoring (iHS) are particularly useful in identifying variants under partial or soft selective scans. However, this method requires that the haplotypes are phased and that the ancestral and derived alleles are known for each central SNP (Sabeti *et al*., 2002). Because it is minimally influenced by demographic factors, the iHS is less likely to give rise to false-positive results (Voight *et al*., 2006). The iHS statistic is not able to identify a selective scan when the allele under selection is in complete fixation or has a high allelic frequency (Tang *et al*., 2007; Gautier & Naves, 2011).

3) Genomic regions exhibiting lower variation compared to the average of the genome can be identified by a statistic known as reduced local variability. Examples of these types of tests are pooled heterozygosity (Hp) (Rubin *et al*., 2010) and ROH method (McQuillan *et al*., 2008).

2.7.7.2 Inter-population statistics

Inter-populations statistics identify regions with selection signatures between two or more populations. Assuming that all populations show some degree of structure, inter-population analysis of molecular data can identify regions that have undergone selection across populations. These statistics focus on differentiation among populations and can be categorized as differentiation based on:

a) Single site differentiation, which can be regarded as the easiest and most widely used approach.

Single-site inter-population differentiation-based statistics include F_{ST} (Wright, 1949), which identify increased local levels of the loci that are being selected, and the FLK test (Bonhomme *et al*., 2010).

Most selection signature detection tests are based on estimating F_{ST} at various loci (Akey *et al*., 2002). The F_{ST} test identifies strongly differentiated alleles in regions of the genome where selection has led to excessive frequency differences among populations. F_{ST} values appear when the selection is positive, while low values indicate that a negative or even neutral selection has occurred. Single-site differentiation approaches, such as F_{ST}, are designed for unlimited population sample sizes, and can give rise to bias when used in small population samples (Wright, 1949). Unlike LD or SFS- based methods, the F_{ST} is SNP-specific with the ability to

identify the real variants that are being selected for (Fariello *et al*., 2014). Bonhomme *et al*. (2010) suggested the FLK statistic that takes into account the historical ramifications of populations and deals with the variation of N_e over time.

b) Haplotype-based differentiation (HBD). These statistics combine haplotype information from multiple populations, thus minimizing the effect of ascertainment bias (Sabeti *et al*., 2007). HBD methods include cross-population extended haplotype homozygosity (XP-EHH; Sabeti *et al*., 2007), Rsb statistic (Tang *et al*., 2007) and hapFLK (Fariello *et al*., 2013).

Haplotype based differentiation methods use haplotypes from different populations to detect signatures of selection (Sabeti *et al*., 2007). Browning & Weir (2010) indicated that haplotype-based differentiation methods are less influenced by SNP bias than single-site-based ones. Haplotype-based differentiation statistics do not consider the structure of populations from a hierarchical perspective. Hence the hapFLK method (Fariello *et al*., 2013), which is an extended form of the FLK statistic, was designed and incorporate information on haplotypes as well as hierarchical structure. When haplotype information is used to analyse signatures of selection, the detection power of F_{ST}-like statistics improves significantly (Sabeti *et al*., 2007; Fariello *et al*., 2013).

One of the haplotype-based differentiation approaches is the Rsb statistic. This approach computes the EHH in both alleles for each specific SNP in each population (Gautier & Naves, 2011). Then, the standardized log-ratio of the integrated EHHS (iES) distributed among population pairs (known as Rsb), is used to identify genomic regions with the highest Rsb scores. The advantage of the Rsb statistic is that it does not partition EHH into ancestral and derived alleles but computes it for the entire population. Furthermore, the Rsb statistic is similar to XP-EHH with the exception that the former uses each central SNP allele to calculate the EHH while the latter uses the central SNP site (Sabeti *et al*., 2007; Tang *et al*., 2007).

There have been several approaches in identifying the signatures of selection in many livestock species. Table 2.6 presents a summary of some studies carried out to identify selection signatures in different African cattle breeds.

Table 0.6 Summary of studies accomplished to identify selection signatures in African cattle breeds using different methods

Breed	Methodology	Associated trait	Reference
Beninese indigenous cattle	XP-EHH	Immunity, feed intake, carcass traits, efficiency and body weight	Vanvanhossou *et al*., 2021
East African cattle (Boran, Ogaden and Kenana)	XP-EHH and XP-CLR	feeding and metabolism, thermotolerance, immune system response	Taye *et al*., 2018
South African indigenous cattle	F_{ST}	immune response, adaptation to tropical environments, production and reproductive performance	Makina *et al*. 2015b
EASZ cattle from Kenya	iHS, Rsb and F_{ST}	immune system, reproductive system, heat tolerance, and development	Bahbahani *et al*., 2015
Tanzanian Maasai, Boran and Friesian	iHS	immune system, growth and development and metabolism	Msalya *et al*., 2017
South African Cattle	Hp	walking ability, heat tolerance, meat production, reproduction, and bone and muscle development	Zwane *et al*., 2021
North African cattle	iHS, Rsb and XP-EHH	Adaptation, Disease resistance, adaptive immune response	Ben-Jemaa *et al*., 2020

2.8 Conclusion

The Mozambican indigenous cattle breeds have adaptive characteristics that make them a valuable animal genetic resource, especially for small farmers whose production is characterized by low inputs. These adaptive traits are essential to breeding programs and must be preserved for present and future generations. Characterization of Mozambique's indigenous animals provides relevant information on population structure and genetic diversity, which are important for the sustainable use and conservation of these valuable genetic resources. Utilization of genomic tools for genetic characterisation of Mozambican indigenous cattle present opportunities to unravel their unique genetic structure.

References

Adhiambo, M., 2002. Characterization of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques. Animal Genetics Training Resource. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Ajmone-Marsan, P., Garcia, J.F. & Lenstra, J.A., 2010. On the origin of cattle: How aurochs became cattle and colonized the world. Evolutionary Anthropology,19, 148-157.

Akey, J.M., Zhang, G., Zhang, K., Jin, L. & Shriver, M.D., 2002. Interrogating a high-density SNP Map for signatures of natural selection. Genome Research, 12, 1805-1814.

Alberro, M., 1983. The indigenous cattle of Mozambique. World animal review, 48, 12-17.

Alexander, D.H., Novembre, J. & Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research, 19, 1655-1664.

Bahbahani, H., Clifford, H., Wragg, D., Mbole-Kariuki, M.N., Van Tassell, C., Sonstegard, T., Woolhouse, M. & Hanotte, O., 2015. Signatures of positive selection in East African Shorthorn Zebu: A genome-wide single nucleotide polymorphism analysis. Scientific Reports, 5, 11729, 1-13.

Balloux, F. & Goudet, J., 2002. Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. Molecular Ecology, 11, 771-783.

Barbato, M., Orozco-terWengel, P., Tapio, M. & Bruford, M.W., 2015. SNeP: A tool to estimate trends in recent effective population size trajectories using genome-wide SNP data. Frontiers in Genetics, 6, 109-122.

Ben-Jemaa, S., Mastrangelo, S., Lee, S.H., Lee, J.H., & Boussaha, M., 2020. Genome-wide Scan for Selection Signatures Reveals Novel Insights into the Adaptive Capacity in Local North African Cattle. Scientific Reports, 10 (1), 1-14. doi:10. 1038/s41598-020-76576-3

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. & Alexandrino, P., 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African Journal of Animal Science, 39, 61-72.

Bester, J., Matjuda, L.E., Rust, J M. & Fourie, H.J., 2003. The Nguni: case study, in: FAO Community-based management of animal genetic resources. Rome: UNDP, GTZ, CTA, FAO. pp. 45-68.

Bett, R.C., Okeyo, M.A., Malmfors, B., Johansson, K., Agaba, M., Kugonza, D.R., Bhuiyan, A., Filho, A.E.V., Mariante, A.S., Mujibi, F.D. & Philipsson, J., 2013. Cattle breeds: extinction or quasi-extant? Resources, 2, 335–357. doi: 10.3390/resources2030335.

Bjelland, D.W., Weigel, K.A., Vukasinovic, N. & Nkrumah, J.D., 2013. Evaluation of inbreeding depression in Holstein cattle using whole genome SNP markers and alternative measures of genomic inbreeding. Journal of Dairy Science, 96, 4697-4706.

Boettcher, P.J., Tixier-Boichard, M., Toro, M.A., Simianer, H., Eding, H., Gandini, G., Joost, S., Garcia, D., Colli, L., Ajmone-Marsan, P. & the GLOBALDIV Consortium., 2010. Objectives, criteria, and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. Animal Genetics, 41(1), 64-77.

Bonhomme, M., Chevalet, C., Servin, B., Boitard, S., Abdallah, J., Blott, S. & SanCristobal, M., 2010. Detecting selection in population trees: the Lewontin and Krakauer test extended. Genetics, 186, 241-262.

Bosman, L., Van Marle-Köster, E., Van der Westhuizen, R.R., Visser, C. & Berry, D.P., 2017. Population structure of the South African Bonsmara beef breed using high density single nucleotide polymorphism genotypes. Livestock Science, 197, 102-105.

Bradley, D.G., Machugh, D.E., Cunningham, P. & Loftus, R.T., 1996. Mitochondrial diversity and the origins of African and European cattle. Proceedings of the National Academy of Sciences of the United States of America, 93, 5131-5135.

Bradley, D.G., Machugh, D.E., Loftus, R.T., Sow, R.S., Hoste, C.H. & Cunningham, E.P., 1994. Zebu-taurine variation in Y chromosomal DNA: a sensitive assay for genetic introgression in West African trypanotolerant cattle populations. Animal Genetics, 25, 7-12.

Browning, S.R. & Weir, B.S., 2010. Population structure with localized haplotype clusters. Genetics, 185, 1337–1344. doi:10.1534/genetics.110.116681.

Carvalheira, J.G.V., Blake, R.W., Pollak, E.J. & Van Soest, P.J., 1995. Comparison of Landim and Africander cattle in southern Mozambique: II. Female fertility, reproduction and beef off-take. Journal of Animal Science, 73, 3527-3533.

Ceballos, F.C., Joshi, P.K., Clark, D.W., Ramsay, M. & Wilson, J.F., 2018. Runs of homozygosity: windows into population history and trait architecture. Nature Reviews Genetics, 19, 220-234. Available online at: https://www.nature.com/articles/nrg.2017.109.

Ceriotti, G., Caroli, A., Rizzi, R. & Crimella, C., 2003. Genetic relationships among taurine (*Bos taurus*) and zebu (*Bos indicus*) populations as revealed by blood groups and blood proteins. Journal of Animal Breeding and Genetics, 120, 57-67.

Chagunda, M.H.G., & Wollny, C.B., 2003. Conserving and managing the biodiversity of Malawian farm animal genetic resources: A case of Malawi zebu cattle. University of Malawi. IN: Abdi-Khalil, E. (ed.) The dynamics of food production systems and adoption of technologies in a village economy. Lilongwe, Malawi: University of Malawi.

Chen, H., Patterson, N., & Reich, D., 2010. Population differentiation as a test for selective sweeps. Genome Research, 20, 393-402.

DAGRIS, 2007. Domestic Animal Genetic Resources Information System (DAGRIS). (eds. Kemp S, Mamo Y, Asrat B and Dessie T). International Livestock Research Institute, Addis Ababa, Ethiopia (available at http://dagris.ilri.cgiar.org).

Decker, J.E., McKay, S.D., Rolf, M.M., Kim, J., Alcalá, A.M., Sonstegard, T.S., Hanotte, O. *et al*., 2014. Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. PLOS Genetics, 10 (3), e1004254.

Dekkers, J.C., 2004. Commercial application of marker and gene-assisted selection in livestock: Strategies and lessons. Journal of Animal Science. 82(E.Suppl.), E313–E328.

Dionisio, A.C. & Syrstad, O., 1990. Productivity of Nguni and Africander cattle in Mozambique. Livestock Production Science, 24, 29.

Dorji, N. & Daugjinda, M., 2014. A fundamental statistical tools application for livestock diversity studies from microsatellite data - mini review. Open access library journal, 1, 1-8.

Dunning, A.M., Durocher, F., Healey, C.S., Teare, M.D., McBride, S.E., Carlomagno, F., Xu, C.F., Dawson, E., Rhodes, S., Ueda, S., Lai, E., Luben, R.N., Van Rensburg, E.J., Mannermaa, A., Kataja, V., Rennart, G., Dunham, I., Purvis, I., Easton, D. & Ponder, B.A.J., 2000. The extent of linkage disequilibrium in four populations with distinct demographic histories. American Journal of Human Genetics, 67, 1544-1554.

Edea, Z., Bhuiyan, M., Dessie, T., Rothschild, M.F., Dadi, H. & Kim, K.S., 2014. Genome-wide genetic diversity, population structure and admixture analysis in African and Asian cattle breeds. Animal, 9, 1-9.

Edea, Z., Dadi, H., Kim, S.W., Dessie, T. & Kim, K.S., 2012. Comparison of SNP variation and distribution in indigenous Ethiopian and Korean Cattle (Hanwoo) populations. Genomics Informatics, 10(3), 200-205.

Edea, Z., Dadi, H., Kim., S.W., Dessie, T., Lee, T., Kim, H., Kim, J.J. & Kim, K.S., 2013. Genetic diversity, population structure and relationships in indigenous cattle populations of Ethiopia and Korean Hanwoo breeds using SNP markers. Frontiers in Genetics, 4, 35. doi:10.3389/fgene. 2013.00035

Engelsma, K.A., 2012. Use of SNP markers to conserve genome-wide genetic diversity in livestock. PhD Thesis, Graduate School of Wageningen Institute of Animal Sciences. Netherlands.

Epstein, H., 1971. The origin of the domestic animals of Africa, Africana publishing corporation. Vol.1.

Excoffier, L., Laval, G. & Schneider, S., 2005. Arlequin version 3.1: an integrated software package for population. genetics data analysis. Evolutionary Bioinformatics, 1, 47–50 http://cmpg.unib.ch/software//arlequin3

Falconer, D.S., Mackay, T.F. & Frankham, R., 1996. Introduction to quantitative genetics (4th edn). Trends in Genetics, 12(7), 280.

Falush, D., Stephens, M. & Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes, 7, 574-578.

Fan, B., Du, Z-Q., Gorbach, D.M. & Rothschild, M.F., 2010. Development and application of high-density SNP arrays in genomic studies of domestic animals. Asian-Australasian Journal of Animal Sciences, 23, 833-847.

FAO (Food and Agriculture Organization), 2000. World Watch List for Domestic Animal Diversity, 3rd Ed., FAO, Rome, Italy.

FAO (Food and Agriculture Organization), 2007. Global Plan of Action for animal genetic resources and the Interlaken declaration, Rome, Italy.

FAO (Food and Agriculture Organization), 2010. Progress report of the FAO on selected activities related to agricultural biodiversity. http://www.fao.org/docrep/meeting/018/k5700e.pdf

FAO (Food and Agriculture Organization), 2011a. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No.9. In: Commission on Genetic Resources for Food and Agriculture Assessments. FAO, Rome, Italy.

FAO (Food and Agriculture Organization), 2011b. Draft Guidelines on Developing the Institutional Framework for the Management of Animal Genetic Resources. Commission on Genetic Resources for Food and Agriculture, FAO, Rome.

FAO (Food and Agriculture Organization), 2012. Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines 11. Rome, Italy.

FAO (Food and Agriculture Organization), 2015. The second report on the State of the World's Animal Genetic Resources for Food and Agriculture, Rome, Italy.

FAO (Food and Agriculture Organization), 2016. Status of animal genetic resource. Intergovernmental technical working group on animal genetic resources for food and agriculture, ninth session, Rome, 6-8 July 2016., Rome, Italy.

FAO (Food and Agriculture Organization), 2019. World Food and Agriculture – Statistical pocketbook 2019, Rome.

Fariello, M.I., Boitard, S., Naya, H., SanCristobal, M. & Servin, B., 2013. Detecting signatures of selection through haplotype differentiation among hierarchically structured populations. Genetics, 193, 929-941.

Fariello, M.I., Servin, B., Tosser-Klopp, G., Rupp, R., Moreno, C., International Sheep Genomics Consortium, San Cristobal, M. & Boitard, S., 2014. Selection signatures in worldwide sheep populations. PLOS ONE, 9, e103813.

Fay, J.C. & Wu, C., 2000. Hitchhiking under positive Darwinian selection. Genetics, 155, 1405-1413.

Felius, M., Beerling, M.L., Buchanan, D.S., Theunissen, B., Koolmees, P.A. & Lenstra, J.A., 2014. On the history of cattle genetic resources. Diversity, 6, 705–750. doi: 10.3390/d6040705.

Ferenčaković, M., Hamzic, E., Gredler, B., Curik, I. & Sölkner, J., 2011. Runs of homozygosity reveal genome-wide autozygosity in the Austrian Fleckvieh cattle. Agriculturae Conspectus Scientificus, 76, 325-328.

Frankham, R., Ballou, J.D. & Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge, UK: Cambridge University Press.

Frkonja, A., Gredler, B., Schnyder, U., Curik, I. & Sölkner, J., 2012. Prediction of breed composition in an admixed cattle population. Animal Genetics, 43, 696–703.

Gamaniel, I.B. & Gwaza, D.S., 2017. Molecular Characterization of Animal Genetics Resources, its Potential for use in Developing Countries. Journal of Genetics and Genetic Engineering,1 (1), 43-57.

Gandini, G. & Villa, E., 2003. Analysis of the cultural value of local livestock breeds: a methodology. Journal of Animal Breeding and Genetics, 120, 1–11.

Gautier, M. & Naves, M., 2011. Footprints of selection in the ancestral admixture of a New World Creole cattle breed. Molecular Ecology, 20, 3128-3143.

Gifford-Gonzalez, D. & Hanotte, O., 2011. Domesticating animals in Africa: implications of genetic and archaeological findings. Journal of World Prehistory, 24, 1–23.

Gororo, E., Makuza, S.M., Chatiza, F.P., Chidzwondo, F. & Sanyika, T.W., 2018. Genetic diversity in Zimbabwean Sanga cattle breeds using microsatellite markers. South African Journal of Animal Science, 48(1), 128-141.

Gouveia, J.J.D.S., Silva, M.V.G.B.D., Paiva, S.R. & Oliveira, S.M.P.D., 2014. Identification of selection signatures in livestock species. Genetics and Molecular Biology, 37, 330-342.

Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B. & Pilling, D., 2010. Genetic diversity in farm animals – a review. Animal Genetics, 41, (1), 6-31.

Hailu, A. & Getu, A., 2015. Breed Characterization: Tools and Their Applications. Open Access Library Journal, 2, e1438. http://dx.doi.org/10.4236/oalib.1101438.

Hanotte, O., Bradley, D.G., Ochieng, J.W., Verjee, Y., Hill, E.W. & Rege, J.E., 2002. African Pastoralism: Genetic Imprints of Origins and Migrations. Science, 296, 336-339.

Hanotte, O., Tawah, C.L., Bradley, D., Okomo, M., Verjee, Y., Ochieng, J. & Regé, J.E.O., 2000. Geographic distribution and frequency of a taurine Bos taurus and an indicine Bos indicus Y specific allele amongst sub-Saharan African cattle breeds. Molecular Ecology, 9, 387-396.

Hayes, B.J., Lien, S., Nilsen, H., Oslen, H.G., Berg, P., MacEachern, S., Potter, S. & Meuwissen, T.H.E., 2008. The origin of selection signatures on bovine chromosome six. Animal Genetics 39(2), 105-111.

Hayes, B.J., Visscher, P.M., McPartlan, H.C. & Goddard, M.E., 2003. Novel multi locus measure of linkage disequilibrium to estimate past effective population size. Genome Research, 13, 635–643.

Hlophe, R.S., 2011. Genetic variation between and within six selected South African sheep breeds using random amplified polymorphic DNA and protein markers. MSc dissertation. University of Zululand, South Africa.

INE (National Institute of Statistics), 2011. Censo Agro-Pecuário, 2009-2010. Available online at: http://mozdata.microdatahub.com/index.php/catalog/37

INE (National Institute of Statistics), 2014. Anuario estatístico 2013, Maputo: Available online at: http://www.ine.gov.mz/estatisticas/publicacoes/anuario/nacionais/anuario_2013.pdf/view

INE (National Institute of Statistics), 2015. Anuario estatístico 2014, Maputo: Available online at: http://www.ine.gov.mz/estatisticas/publicacoes/anuario/nacionais/anuario-estatistico-2014/view

Kalinowski, S.T., 2002. Evolutionary and Statistical Properties of Three Genetic Distances. Molecular Ecology, 11, 1263-1273.

Keller, M.C., Visscher, P.M., & Goddard, M.E., 2011. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. Genetics. 189, 237–249. doi: 10.1534/ genetics.111.130922.

Khatkar, M.S., Nicholas, F.W., Collins, A.R., Zenger, K.R., Cavanagh, J.A., Barris, W., Schnabel, R.D., Taylor, J.F. & Raadsma, H.W., 2008. Extent of genome-wide linkage dis-equilibrium in Australian Holstein-Friesian cattle based on a high- density SNP panel. BMC Genomics 9, 187.

Kugonza, D.R., Jianlin, H., Nabasirye, M., Mpairwe, D., Kiwuwa, G.H., Okeyo, A.M. & Hanotte, O., 2011. Genetic diversity and differentiation of Ankole cattle populations in Uganda inferred from microsatellite data. Livestock Science, 135, 140–147.

Lashmar, S., Visser, C., Van Marle-Koster, E. & Muchadeyi, F., 2018. Genomic diversity and autozygosity within the SA Drakensberger beef cattle breed. Livestock Science, 212, 111-119.

Lee, S.H., Cho, Y.M., Lim, D., Kim, H.C., Choi, B.H., Park, H.S., Kim, O.H., Kim, S., Kim, T.H., Yoon, D. & Hong, S.K., 2011. Linkage Disequilibrium and Effective Population Size in Hanwoo Korean Cattle. Asian Australasian Journal of Animal Sciences, 24 (12), 1660-1665.

Lenstra, J., Ajmone-Marsan, P., Beja-Pereira, A., Bollongino, R., Bradley, D., Colli, L., De Gaetano, A., Edwaeds, C., Felius, M., Ferretti, L., Ginja, C., Hristov, P., Kantanen, J., Lirón, J., Magee, D., Negrini, R. & Radoslavov, G., 2014. Meta-Analysis of Mitochondrial DNA Reveals Several Population Bottlenecks during Worldwide Migrations of Cattle. Diversity, 6, 178-187.

Lenstra, J.A., Groeneveld, L.F., Eding, H., Kantanen, J., Williams, J.L., Taberlet, P., Nicolazzi, E.L., Sölkner, J., Simianer, H., Ciani, E., Garcia, J.F., Bruford, M.W., Ajmone-Marsan, P. & Weigend, S. 2012. Molecular tools and analytical approaches for the characterization of farm animal diversity. Animal Genetics, 43, 483–502.

Lewis, J., Abas, Z., Dadousis, C., Lykidis, D., Paschou, P. & Drineas, P., 2011. Tracing cattle breeds with principal components analysis ancestry informative SNP. PLOS ONE, 6 (4),1-8.

Loftus, R.T., Machugh, D.E., Bradley, D.G., Sharp, P.M. & Cunningham, P., 1994. Evidence for two independent domestications of cattle. Proceedings of the National Academy of Sciences of the United States of America, 91, 2757-2761.

Maciel, S., 2001. National strategies for the conservation, improvement and utilization of animal genetic resources in smallholder systems. Proceedings of the Planning and Priority Setting Workshop on Animal Genetic Resources in the SADC region. SACCAR, CTA, ILRI, Gaborone, Botswana.

Maciel, S., Harun, M., & Capece, B., 2004. First national country report on the status of farm animal genetic resources in Mozambique. MADER, Maputo, Mozambique, and DAD-IS, FAO (Foodand Agriculture Organization of the United Nations), Rome, Italy.

Maciel, S., Okeyo, A.M., Amimo, J., Scholtz, M.M., Neser, F.W.C., & Mart, M., 2013. The effect of geographical region of birth on the reproductive performance of the Nguni in southern Mozambique. South African Journal of Animal Sience, 43 (1), 60-63.

Madilindi, M.A., Banga, C.B., Bhebhe, E., Sanarana, Y.P., Nxumalo, K.S., Taela, M.G., Magagula, B.S. & Mapholi, N.O., 2020. Genetic diversity and relationships among three Southern African Nguni cattle populations. Tropical Animal Health and Production, 52, 753–762.

Makina, S.O., Muchadeyi, F.C., Van Marle-Köster, E., MacNeil, M.D., Maiwashe, A., 2014. Genetic diversity and population structure among six cattle breeds in South Africa using a whole-genome SNP panel. Frontiers in Genetics, 5, 333.

Makina, S.O., Taylor, J.F., Van Marle-Köster, E., Muchadeyi, F.C., Makgahlela, M.L., MacNeil, M.D. & Maiwashe, A., 2015a. Extent of linkage disequilibrium and effective population size in four South African Sanga cattle breeds. Frontiers in Genetics, 6, 1-12.

Makina, S.O., Muchadeyi, F.C., Van Marle-Köster, E., Taylor, J.F., Makgahlela, M.L., & Maiwashe, A., 2015b. Genome-wide scan for selection signatures in six cattle breeds in South Africa. Genetics Selection Evolution, 47, 92.

Makina, S.O., Whitacre, L.K., Decker, J.E., Taylor, J.F., MacNeil, M.D., Scholtz, M.M., Van Marle-Köster, E., Muchadeyi, F.C., Makgahlela, M.L. & Maiwashe, A., 2016. Insight into the genetic composition of South African Sanga cattle using SNP data from cattle breeds worldwide. Genetics Selection Evolution, 48, 88.

Mammo, M., Alemayehu, K. & Tassew, A., 2017. Conformation traits of dairy cattle populations in selected Districts of Northwestern Amhara. Ethiopia. Biodiversitas Journal of Biological Diversity, 18, 1669–1679. doi:10.13057/biodiv/d180447

Mapiye, C., Chikwanha, O.C., Chimonyo, M. & Dzama, K., 2019. Strategies for Sustainable Use of Indigenous Cattle Genetic Resources in Southern Africa. Diversity, 11, 214.

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'Connell, J., Moore, S.S., Smith, T.P., Sonstegard, T.S. &. Van Tassell., C.P., 2009. Development and characterization of a high-density SNP genotyping assay for cattle. PLOS ONE, 4(4), e5350

Mbole-Kariuki, M. N., Sonstegard, T., Orth, A., Thumbi, S. M., Bronsvoort, B. M., Kiara, H., Toye, P., Conradie, I., Jennings, A., Coetzer, K., Woolhouse, M. E., Hanotte, O. & Tapio, M., 2014. Genome-wide analysis reveals the ancient and recent admixture history of East African Shorthorn Zebu from Western Kenya. Heredity, 113, 297-305.

Mburu, D. & Hanotte, O., 2005. A practical approach to microsatellite genotyping with special reference to livestock population genetics. ILRI biodiversity project. Available online at: http//www-naweb.iaea.org/naf a/aph/stories/dna-manual.pdf

McKay, S.D., Schnabel, R.D., Murdoch, B.M., Matukumalli, L.K., Aerts, J., Coppieters, W., Crews, D., Dias-Neto, E, Gill, C.A., Gao, C. *et al.*, 2007. Whole genome linkage disequilibrium maps in cattle. BMC Genetics, 8, 74.

McManus, C., Paiva, S.R. & Araújo, R.O., 2010. Genetics and breeding of sheep in Brazil. Revista Brasileira de Zootecnia, 39, 236–246.

McQuillan, R., Leutenegger, A-L., Abdel-Rahman, R., Franklin, C.S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., MacLeod, A.K., Farrington, S.M., Rudan, P., Hayward, C., Vitart, V., Rudan, I., Wild, S.H., Dunlop, M.G., Wright, A.F., Campbell, H. & Wilson, J.F., 2008. Runs of homozygosity in European populations. American Journal of Human Genetics, 83, 359-372.

McRae, A.F., McEwan, J.C., Dodds, K.G., Wilson, T., Crawford, A.M. & Slate, J., 2002. Linkage disequilibrium in domestic sheep. Genetics, 160, 1113–1122.

Meadow, R.H., 1993. Animal domestication in the Middle East: a revised view from the Eastern Margin. In: Harappan Civilisation, New Delhi, Oxford & IBH.

Meuwissen, T.H.E., 2009. Towards consensus on how to measure neutral genetic diversity? Journal of Animal Breeding and Genetics, 126, 333-334.

Morgado, F.P., 2000. A pecuaria no norte de Moçambique. Vega: Portugal.

Morgado, F.P., 2004. A Pecuária no Centro de Moçambique. Vega: Portugal.

Msalya, G., Kim, E. S., Laisser, E.L., Kipanyula, M.J., Karimuribo, E.D., Kusiluka, L.J., et al., 2017. Determination of genetic structure and signatures of selection in three strains of Tanzania shorthorn zebu, Boran and Friesian cattle by genome-wide SNP analyses. PLOS ONE, 12, e0171088. doi: 10.1371/journal.pone. 0171088.

Murray, G., Woolhouse, M., Tapio, M., Mbole-Kariuki, M., Sonstegard, T., Thumbi, S., Jennings, A., van Wyk, I., Chase-Topping, M., Kiara, H., Toye, P., Coetzer, K., Bronsvoort, B. & Hanotte, O., 2013. Genetic susceptibility to infectious disease in East African Shorthorn Zebu: a genome-wide analysis of the effect of heterozygosity and exotic introgression. BMC Evolutionary Biology, 13, 246.

Mwai, O., Hanotte, O., Kwon, Y.J. & Cho, S., 2015. African indigenous cattle: unique genetic resources in a rapidly changing world. Asian-Australasian Journal of Animal Sciences, 28, 911-21.

Ndumu, D.B., Baumung, R., Hanotte, O., Wurzinger, M., Okeyo, M.A., Jianlin, H., Kibogo, H. & Solkner, J., 2008. Genetic and morphological characterization of the Ankole Longhorn cattle in the African Great Lakes region. Genetics Selection Evolution, 40, 467- 490.

Nei, M., Tajima, F. & Tateno, Y., 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. Journal of Molecular Evolution, 19, 153-170.

Ngono Ema, P.J., Manjeli, Y., Meutchieyié, F., Keambou, C., Wanjala, B., Desta. A.F., Ommeh, S., Skilton, R. & Djikeng, A., 2014. Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers. Journal of Livestock Sciences, 5, 9-17.

Nielsen, R., Williamson, S., Kim, Y., Hubisz, M.J., Clark, A.G. & Bustamante, C., 2005. Genomic scan for selective sweeps using SNP data. Genome Research, 15, 1566-1575.

Norberg, E. & Sørensen, A.C., 2007. Inbreeding Trend and Inbreeding Depression in the Danish Populations of Texel, Shropshire, and Oxford Down. Journal of Animal Science, 85,299-304. http://dx.doi.org/10.2527/jas.2006-257.

Nyamushamba, G., Mapiye, C., Tada, O., Halimani, T. & Muchenje, V., 2017. Conservation of indigenous cattle genetic resources in Southern Africa's smallholder areas: Turning threats into opportunities – A review. Asian-Australasian Journal Animal Sciences, 30, 603-622.

Ojango, J.M., Mpofu, N., Marshall, K. & Andersson-Eklund, L., 2011. Quantitative methods to improve the understanding and utilization of animal genetic resources. In: Animal Genetics Training Resources, version 3. Ojango, J.M., Malmfors, B & Okeyo, A.M (Eds). International Livestock

Research Institute, Nairobi, Kenya and Swedish University of Agricultural Science, Uppsala, Sweden.

Otto, F., Vilela, F., Harun, M., Taylor, G., Bagasse, P. & Bogin, E., 2000. Biochemical blood profile of Angoni cattle in Mozambique. Israel Veterinary Medicine Association, 55 (3).

Otto, S.P., 2000. Detecting the form of selection from DNA sequence data. Trends Genetics, 16, 526-529.

Paiva, S.R., Facó, O., Faria, D.A., Lacerda, T., Barretto, G.B., Carneiro, P.L.S., Lobo, R.N.B. & McManus, C., 2011. Molecular and pedigree analysis applied to conservation of animal genetic resources: The case of Brazilian Somali hair sheep. Tropical Animal Health and Production, 43,1449-1457.

Patterson, N., Price, A.L. & Reich, D., 2006. Population structure and eigen analysis. PLOS genetics, 2(12),190.

Peakall, R. & Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28, 2537-2539.

Pienaar, L., Grobler, J.P., Neser, F.W.C., Scholtz, M.M. & Swart, H., 2014. Genetic diversity in selected stud and commercial herds of the Afrikaner cattle breed. South African Journal of Animal Science, 44, 80-84.

Pitt, D., Sevane, N., Nicolazzi, E.L., MacHugh, D.E., Park, S.D.E., Colli, L. & Orozco-terWengel, P., 2018. Domestication of cattle: Two or three events? Evolutionary Applications, 12, 123–136. https://doi. org/10.1111/eva.12674.

Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, A. & Lareu, M.V., 2013. An overview of STRUCTURE: applications, parameter settings, and supporting software. Frontiers in Genetics, 4, 98.

Porto-Neto, L.R., Lee, S.H., Sonstegard, T.S., Van Tassell, C.P., Lee, H.K., Gibson, J.P. & Gondro, C., 2013. Genome-wide detection of signatures of selection in Koren Hanwoo cattle. Animal Genetics, 45, 180-190.

Pritchard, J.K., Stephens, M. & Donnelly, P., 2000. Inference of population structure using multi-locus genotype data. Genetics, 155, 945-959.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J. & Sham, P.C., 2007. PLINK: A toolset for whole-genome association and population-based linkage analyses. American Journal Human Genetics, 81, 559-575.

Purfield, D.C., McParland, S., Wall, E. & Berry, D.P., 2017. The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. PLOS ONE, 12, e0176780. doi: 10.1371/journal.pone.0176780.

Purfield, D.C., Berry, D.P., McParland, S., Bradley, D.G., 2012. Runs of homozygosity and population history in cattle. BMC Genetics, 13, 70.

Qanbari, S. & Simianer, H., 2014. Mapping signatures of positive selection in the genome of livestock. Livestock Science, 166 133-143.

Rege, J.E.O. & Tawah, C.L., 1999. The state of African cattle genetic resources. II. Geographical distribution, characteristics and uses of present-day breeds and strains. Animal Genetic

Resources Information Bulletin, 26, 1-25.

Reich, D.E., Cargill, M., Bolk, S., Ireland, J., Sabeti, P.C., Richter, D.J., Lavery, T., Kouyoumjlan, R., Farhadian, S.F., Ward, R. & Lander, E.S., 2001. Linkage disequilibrium in the human genome. Nature, 411, 199-204.

Rocha, A., 1985. Revisão da investigação sobre a avaliação de raças e melhoramento animal em Moçambique. Seminário de Produção Animal. Ministério da Agricultura, 2-7 Dezembro, pp. 86-103.

Rubin, C.J., Zody, M.C., Eriksson, J., Meadows, J.R.S., Sherwood, E., Webster, M.T., Jiang, L., Ingman, M., Sharpe, T., Ka, S., Hallböök, F., Besnier, F., Carlborg, O., Bed'hom, B., Tixier-Boichard, M., Jensen, P., Siegel, P.,Lindblad-Toh, K. & Andersson, L., 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. Nature, 464, 587-591.

Sabeti, P.C., Reich, D.E., Higgins, J.M., Levine, H.Z.P., Richter, D.J., Schaffner, S.F., Gabriel, S.B., Platko, J.V., Patterson, N.J., McDonald, G.J., Ackerman, H.C., Campbell, S.J., Altshuler, D., Cooper, R., Kwiatkowski, D., Ward, R. & Lander, E.S., 2002. Detecting recent positive selection in the human genome from haplotype structure. Nature, 419, 832-837.

Sabeti, P.C., Varilly, P., Fry, B., Lohmueller, J., Hostetter, E., Cotsapas, C., Xie, X., Byrne, E.H., McCarroll, S.A., Gaudet, R., Schaffner, S.F. & Lander, E.S., 2007. Genome-wide detection and characterization of positive selection in human populations. Nature 449, 913–918. doi:10.1038/nature06250

Sanarana, Y.P., 2015. Genetic characterization of South African Nguni cattle ecotypes using microsatellites markers. MSc dissertation. University of Pretoria, South Africa.

Saravanan, K.A., Panigrahi, M., Kumar, H., Bhushan, B., Dutt, T. & Mishra, B.P., 2020 Selection signatures in livestock genome: a review of concepts, approaches and applications. Livestock Science, 241, 104-257.

Saura, M., Fernández, A., Varona, L., Fernández, A.I., de Cara, M.Á.R., Barragán, C. & Villanueva, B., 2015. Detecting inbreeding depression for reproductive traits in Iberian pigs using genome-wide data. Genetics Selection Evolution, 47, 1.

Sharma, R., Kumar, B., Arora, R., Ahlawat, S., Mishra, A.K. & Tantia, M.S., 2016. Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. Meta Gene, 8, 14–20.

Shriver, M.D., Parra, E.J., Dios, S., Bonilla, C., Norton, H., Jovel, C., Pfaff, C., Jones, C., Massac, A., Cameron, N., Baron, A., Jackson, T., Argyropoulos, G., Jin, L., Hoggart, C.J., McKeigue, P.M. & Kittles, R., 2003. Skin pigmentation, biogeographical ancestry and admixture mapping. Human Genetics, 112, 387–99.

Simianer, H. M., Y. & Qanbari, S., 2014. Statistical Problems in Livestock Population Genomics. Proceedings of the 10th World Congress of Genetics Applied to Livestock Production. Vancouver, BC, Canada, 17-22 August 2014.

Slatkin, M., 2008. Linkage disequilibrium--understanding the evolutionary past and mapping the medical future. Nature Reviews Genetics, 9, 477- 485.

Solomon Gizaw, Komen, H., Hanote, O., van Arendonk, J.A.M., Kemp, S., Aynalem Haile, Mwai, O. & Tadelle, D., 2011. Characterization and conservation of indigenous sheep genetic resources: A practical framework for developing countries. ILRI Research Report No. 27. Nairobi, Kenya, ILRI.

Stella, A., Ajmone-Marsan, P., Lazzari, B. & Boettcher, P., 2010. Identification of selection signatures in cattle breeds selected for dairy production. Genetics, 185, 1451-1461.

Suhr, D.D., 2005. Principal component analysis vs. exploratory factor analysis. SUGI 30 proceedings, 203, p.230.

Szpiech, Z.A., Xu, J., Pemberton, T.J., Peng, W., Zoellner, S., Rosenberg, N.A. & Li, L., 2013. Long runs of homozygosity are enriched for deleterious variation. American Journal of Human Genetics, 93, 90-102.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123, 585-595.

Tang, K., Thornton, K.R. & Stoneking, M., 2007. A new approach for using genome scans to detect recent positive selection in the human genome. PLOS Biology, 5 (7), e171.

Taye, M., Lee, W., Caetano-Anolles, K., Dessie, T., Cho, S., Jong Oh, S., Lee, H.-K. & Kim, H., 2018. Exploring the genomes of East African Indicine cattle breeds reveals signature of selection for tropical environmental adaptation traits. Cogent Food. Agric. 4, 1552552. 10.1080/23311932 .2018.1552552.

Tenesa, A., Navarro, P., Hayes, B.J., Duffy, D.L., Clarke, G.M., Goddard, M.E. & Visscher, P.M., 2007. Recent human effective population size estimated from linkage disequilibrium. Genome Research, 17(4), 520-526.

Teneva, A., 2009. Molecular markers in animal genome Analysis. Biotechnology in Animal Husbandry. Institute for Animal Husbandry, Belgrade-Zemun. pp. 1267-1284.

Timberlake, J. & Jordão, C., 1985. Inventory of feed resources for small scale livestock production in Mozambique. In: Kategile, J. A., Said, A. N. & Dzowela, B. H. (eds.), Animal feed resources for small-scale livestock producers-Proceedings of the second PANESA workshop, held in Nairobi, Kenya, 11–15 November 1985. International Development Research Centre.

Tomo, P., 1997. Characterization of Angoni cattle in Mozambique. MSc Thesis. Universtity of Orange Free State, Bloemfontein, South Africa.

Tomo, P. & Macamo, V., 2000. Field Guide on Farm Animal Genetic Resources. DINAP and IPA. Maputo, Mozambique.

Toro, M.A., Fernandez, J. & Caballero, A., 2009. Molecular characterization of breeds and its use in conservation. Livestock Science, 120, 174-195.

Troy, C.S., Machugh, D.E., Bailey, J.F., Magee, D.A., Loftus, R.T., Cunningham, P., Chamberlain, A.T., Sykes, B.C. & Bradley, D.G., 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature, 410, 1088-91.

Utsunomiya, Y.T., Milanesi, M., Fortes, M.R.S., Porto-Neto, L.R., Utsunomiya, A.T.H., Silva, M.V.G.B., Garcia, J.F. & Ajmone-Marsan, P., 2019. Genomic clues of the evolutionary history of *Bos indicus* cattle. Animal genetics, 50 (6), 557-568.

Van der Westhuizen, L., MacNeil, M.D., Scholtz, M.M., Neser, F.W.C., Makgahlela, M.L. & Van Wyk, J.B., 2019. Genetic variability and relationships in nine South African cattle breeds using microsatellite markers. Tropical Animal Health and Production, 52, 177–184

Van Marle-Köster, E., Visser, C., Sealy, J. & Frantz, L., 2021. Capitalizing on the Potential of South African Indigenous Beef Cattle Breeds: A Review. Sustainability, 13(8), 4388.

Vanvanhossou, S.F.U., Scheper, T.Y.C., Fries, R., Dossa, L.H. & König, S., 2021. Unraveling Admixture, Inbreeding, and Recent Selection Signatures in West African Indigenous Cattle Populations in Benin. Frontiers in Genetics, 12. DOI:10.3389/fgene.2021.657282

Vernooij, A., dos Anjos, M. & Van Mierlo, J., 2016. Livestock Development in the Zambezi Valley, Mozambique: Poultry, Dairy and Beef Production. Wageningen University and Research Centre for Development Innovation. Report CDI-16-027. Wageningen.

Vignal, A., Milan, D., Sancristobal M. & Eggen A., 2002. SNP and other types of molecular markers & their use in animal genetics-review. Genetics Selection Evolution, 34, 275-305.

Vitti, J.J., Grossman, S.R. & Sabeti, P.C., 2013. Detecting natural selection in genomic data. Annual Review of Genetics, 47, 97–120.

Voight, B.F., Kudaravalli, S., Wen, X. & Pritchard, J.K., 2006. A map of recent positive selection in the human genome. PLOS Biology, 4, e72, 1-13.

Walsh, B., 2000. Quantitative genetics in the age of genomics. Theoretical Population Biology, 59, 175-184.

Wang, E.T., Kodama, G., Baldi, P. & Moyzis, R.K., 2006. Global landscape of recent inferred Darwinian selection for Homo sapiens. Proceedings of the National Academy of Sciences of the United States of America, 103, 135–140.

Wang, M.D., Dzama, K., Hefer, C.A. & Muchadeyi, F.C., 2015. Genomic population structure and prevalence of copy number variations in South African Nguni cattle. BMC Genomics, 16, 894.

Weerasinghe, S.P., 2014. Use of Genetic Polymorphisms to Assess the Genetic Structure and Breed Composition of Crossbred Animals. PhD Thesis. University of New England, Australia.

Wright, S., 1949. The genetical structure of populations. Annals of Eugenics, 15, 323–354. doi: 10.1111/j.1469-1809. 1949.tb02451.x

Wright, S., 1951. The genetical structure of populations. Annals of Eugenics, 1, 323-354.

Wright, S., 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution, 19, 395-420.

Zhao, F., McParland, S., Kearney, F., Du, L. & Donagh, P.B., 2015. Detection of selection signatures in dairy and beef cattle using high-density genomic information. Genetics Selection Evolution, 47, 92, 1-12.

Zwane, A.A., Nxumalo, K.S., Makgahlela, M.L., Van Marle-Koster, E. & Maiwashe, N., 2021. Gene-set enrichment analysis of selective sweeps reveals phenotypic traits in Nguni cattle. South African Journal of Animal Science, 51 (6).

Zwane, A.A., Maiwashe, A., Makgahlela, M.L., Choudhury, A., Taylor, J.F. & Van Marle-Köster, E., 2016. Genome-wide identification of breed-informative single nucleotide polymorphisms in three South African indigenous cattle breeds. South African Journal of Animal Science, 46, 302-312.

Morphological characterization of three indigenous Mozambican cattle populations

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Abstract

Information on phenotypic and morphometric variation is important in the characterization of indigenous cattle breeds. The objective of this study was to assess the morphological variation of the indigenous Angone, Landim, and Tete cattle breeds of Mozambique, kept under traditional management systems. These cattle are farmed mainly for meat and draught power, although they can produce some milk, especially the Landim. Data were collected through a survey of twenty-three villages in Maputo, Gaza, Inhambane (southern Mozambique), and Tete (central Mozambique) provinces. A total of 614 heads of adult animals including 140 Angone, 292 Landim, and 182 Tete were sampled. The collected qualitative and quantitative data were examined using SPSS version 16. The dominant coat colour pattern was even (no spotting) (59.5%), with black being the most common coat colour (51.5%), followed by light brown (26.5%). The three cattle populations showed morphological differentiation based on size, body weight, and horn shape. Landim males (346.22 kg) and females (309.99 kg) were significantly ($p<0.05$) heavier than their Tete (males: 316.38 kg; females: 265.67 kg) and Angone (males: 256.90 kg; females: 243.34 kg) counterparts. Across populations, the majority of cattle (95.2%) had horns, and 66.9% of these horns were curved. Individual assignment using discriminant function analysis revealed that 73.0% of Landim, 77.4% of Angone, and 59.9% of Tete cattle were correctly assigned to their respective populations. Results from this study indicate a considerable phenotypic variation of Mozambican indigenous cattle and will assist in future improvement and conservation programs.

Keywords: Body measurements, Livestock, Morphometric Qualitative traits, Smallholder

3.1 Introduction

Livestock plays an essential role in the social, economic and cultural stability at households and national levels. It contributes significantly to livelihoods by providing meat, milk, draught power, and transport (INE, 2006; MASA, 2011) and is also used for cultural purposes and to improve social status (Morgado, 2000; Swanepoel *et al.,* 2010). The majority of Mozambique's cattle population of 2.02 million heads consists of indigenous breeds that are distributed across the country (FAO, 2019a), of which 85% are owned by smallholder farmers (MASA, 2011). Three native cattle breeds are recognized in Mozambique, namely the Landim, Tete, and Angone (Alberro, 1983; Kotze *et al*., 2000; Bessa *et al*., 2009).

Indigenous Mozambican cattle are characterized by their adaptive traits including disease resistance, heat tolerance, walking ability, and ability to use poor quality grazing (Maciel *et al*., 2013; Matjuda *et al*., 2014; Nyamushamba *et al*., 2017). These cattle breeds thrive on very little human inputs compared to exotic ones. A major advantage of their adaptive traits is that they are useful for the development of climate-smart cattle production, particularly in smallholder farming systems, which are characterized by low-cost inputs (Meissner *et al*., 2013a; 2013b). Indigenous breeds are thus preferable for resource-poor smallholder farmers who cannot afford the high demanding exotic cattle breeds (Maciel, 2001; MASA, 2011).

Despite their socio-economic importance, indigenous cattle genetic resources are in an advanced state of dilution and/or extinction (Bessa *et al*., 2009; FAO, 2015), due to several factors. Nyamushamba *et al*. (2017), noted that these factors include indiscriminate crossbreeding, breed substitution, and social and environmental disasters. Indigenous populations of cattle in Mozambique experienced a serious genetic bottleneck owing to a civil war that lasted 16 years (1977-1992), which reduced the national herd by approximately 80%. After this period, there was an introgression due to the livestock re-stocking program, where several breeds of cattle were imported, with emphasis on Nguni cattle from Zimbabwe and South Africa (Bessa *et al*., 2009; Maciel *et al*., 2013). Another force that may have shaped the genetic diversity of indigenous cattle in Mozambique is their crossbreeding with exotic breeds for improvement purposes, especially in smallholder production systems.

Loss of genetic diversity in native livestock breeds is a major concern worldwide. It is estimated that 7% of known livestock breeds have already become extinct and another 24% are at different stages of risk (FAO, 2019b). Among mammal species, cattle are one of those with the largest number of extinct breeds reported worldwide (FAO, 2015). Because of this, the Food and Agriculture Organization of the United Nations (FAO) has highlighted the importance of characterizing all Animal

Genetic Resources (AnGR) to determine the current genetic status within and among indigenous livestock populations (FAO, 2011).

Comprehensive studies have been conducted on aspects related to the health, nutrition and production systems of indigenous Mozambican cattle breeds (Catalão & Syrstad, 1990; Otto *et al*., 2000; Maciel *et al*., 2013). However, little effort has been made to identify, characterize and conserve the genetic diversity of these livestock species, and there is little information available on their genetic characteristics (Maciel *et al.*, 2004). Consequently, the implementation of rational and effective conservation and utilization strategies for these genetic resources is difficult (FAO, 2012). Thus, this study aimed to describe the quantitative and qualitative morphological characteristics of the three major indigenous Mozambican cattle breeds, as the first step towards their genetic characterization.

3.2 Materials and Methods

The University of Pretoria Ethics Committee (ECO25-18) and the Institutional Committee of bioethics in Health of the Eduardo Mondlane University (CIBS FM&HCM/18/2018) have approved the research.

Sampling was conducted in Maputo, Gaza, Inhambane, and Tete provinces of Mozambique, which represent five of the ten agro-ecological regions of the country (R1, R2, R3, R6 and R7). The five agro-ecological zones vary in geographical landscapes, which have differences in rainfall, temperature and vegetation (Table 1, supplementary material). Mozambique has a tropical humid climate with two distinct seasons, dry winter and wet summer. The average annual precipitation is approximately 1200 mm and occurs mostly during the summer months of November to April. The average temperature ranges from 24°C to 40°C in summer (October to April) and from 18 to 23°C in winter (May to September) (INE, 2015). The vegetation is characterized by Miombo and Mopane forest with an herbaceous layer of grasses including mainly *Setaria sp.*, *Themeda triandra, Urochloa sp., Panicum maximum, Digitaria sp.* and *Cenchrus sp*. (Timberlake & Jordão, 1985). Based on livestock availability and road accessibility, three districts per province were identified and using purposeful sampling techniques two rural areas were chosen in each district. Cattle sampled for the study were Landim (n=292) from Magude, Namaacha, Chibuto, Chokwé, Guija, Vilankulo, Zavala, and Inharrime districts; Tete (n=182) from Marara and Changara districts, and Angone (n=140) from Angonia district (King *et al*., 2021). A total of 132 males and 482 females of adult (4 pairs of permanent incisors) cattle were randomly selected from the identified populations (Table 3.1). Cows in late pregnancy were excluded from sampling. Quantitative, as well as qualitative, data collection was done according to FAO guide for phenotypic characterization of AnGR (FAO, 2012). Qualitative traits recorded included coat colour

(CC), sex, coat pattern (CP), horn presence (HP), horn shape (HS), horn orientation (HO), and ear orientation (EO). Quantitative traits such as heart girth (HG), muzzle circumference (MC), body length (BL), horn length (HL), top line length (TL), height at withers (WH), height at rump (RH), rump width (RW), hock circumference (HC), and body weight (BW) were taken using a tape measure.

Table 2.1 Number of male and female animals sampled per population and location

Locations	Populations	Females	Males
Tete	**Angone**	99	41
Tete	**Tete**	152	30
Maputo	**Landim**	95	15
Gaza	**Landim**	23	17
Inhambane	**Landim**	113	29
	Total	482	132

Data analyses were performed with SPSS software v16.0 (Statistical Package for the Social Science). Initially, descriptive statistics were performed to test for statistical differences between the three cattle populations. Frequency distributions were determined for classes of qualitative traits and the Chi-square test was performed to assess any statistical significance. Quantitative traits were subjected to an analysis of variance to test for statistically significant differences between cattle populations, using the GLM (General Linear Model) approach. Comparisons of means between cattle populations were performed using the least significant differences (LSD) method at a 5% level of significance. In addition, stepwise discriminant analysis and canonical discriminant analysis were used to identify any highly discriminant variables and their level of differences. In this study, the 10 quantitative variables were introduced in a stepwise fashion as independent variables in the discriminant function analysis. The relative importance of these functions in differentiating the three populations was assessed by F-to-remove statistics, under default settings. The discriminating ability of these functions was indicated as the percentage of assignment of individuals to their populations of origin. Genetic distance was evaluated by SPSS hierarchical cluster analysis, from which a dendrogram was constructed showing the relationship between the three populations under study.

3.3 Results

The distribution of coat colour and patterns varied among the three populations (Figure 3.1). The majority of cattle in each population had plain (non-spotted) coat patterns (Figure 3.1A). Black was the dominant coat colour in all three populations (Figure 3.1B). However, the Landim population presented a higher percentage of black coat colour compared to Angone and Tete (Table 3.2). Other coat colours observed were light brown, white, dark brown, grey, and fawn, with fawn being found only in the Angone population (Figure 3.1B).

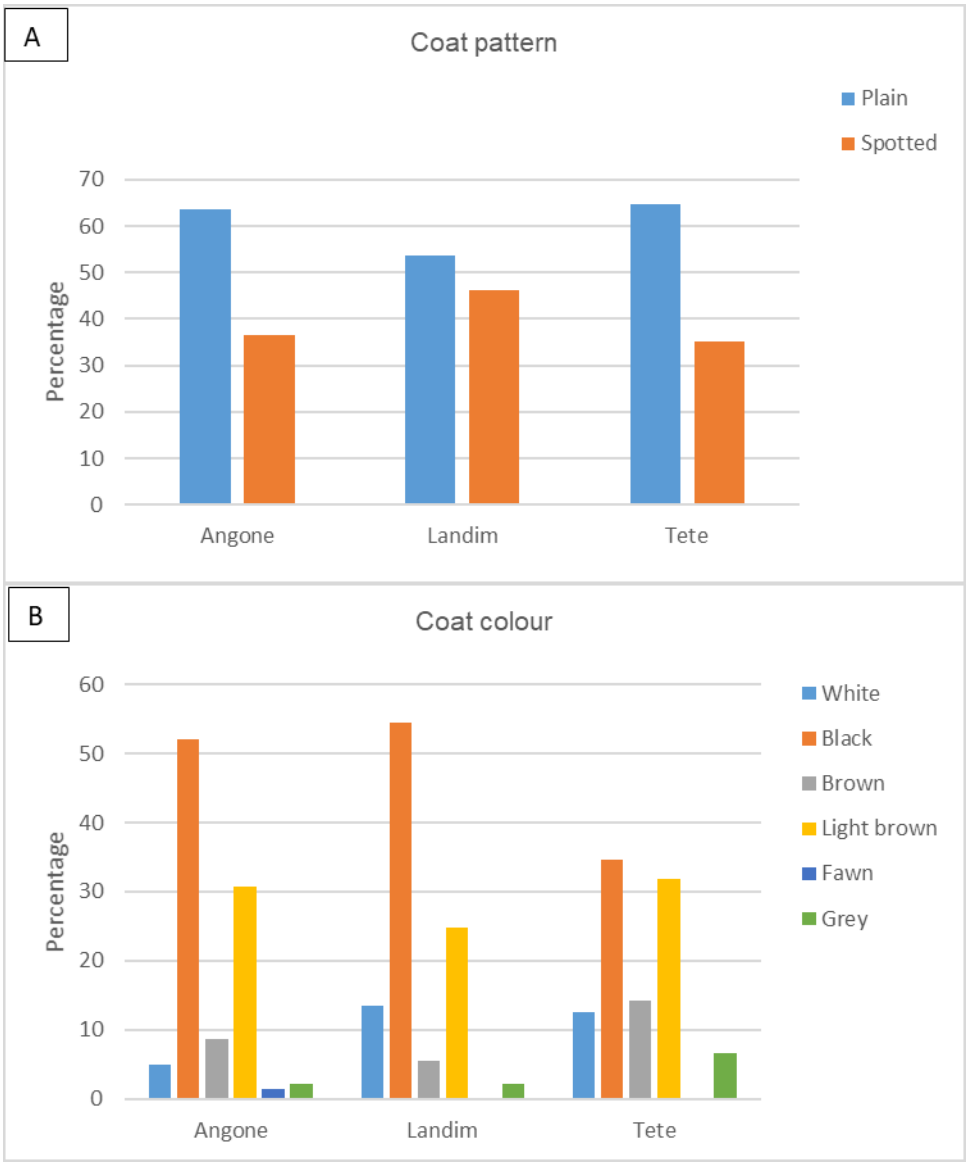

Figure 2.1 Distribution of coat pattern (A) and coat color (B) in three indigenous Mozambican cattle populations

Substantial variation among the three populations was also observed in the distribution of horn presence, horn shape, horn and ear orientation (Table 3.2). Nearly all animals (> 90%) were horned, of which most horns were curved and upward oriented. More than 70% of Angone cattle had straight horns; however, most of the Landim (>80%) and Tete (>70%) cattle had curved horns. Horns with upward, forward, or lateral orientation were observed in all the studied populations. Most animals in the three populations had laterally oriented ears, with very few drooping ears found only in the Angone. The distribution of classes of qualitative traits by sex is shown in Table 3.2. There were no significant differences between sexes in the parameters studied except for horn shape and horn orientation.

Table 2.2 Distribution of external characteristics of three Mozambican cattle populations

Parameter	Angone (%)		Landim (%)		Tete (%)		Overall (%)	
	Female	Male	Female	Male	Female	Male	Female	Male
Coat color pattern								
Plain	65.7	58.5	52.8	57.4	64.5	66.7	59.1	59.8
Patchy	34.3	41.5	47.2	42.6	35.5	33.3	40.9	40.2
Coat color type								
White	6.1	2.4	15.2	6.6	12.5	13.3	12.4	6.8
Black	47.5	63.4	53.2	59.0	32.2	46.7	45.4	57.6
Dark Brown	10.1	4.9	4.3	9.8	15.1	10.0	8.9	8.3
Light Brown	34.3	22.0	24.7	24.6	33.6	23.3	29.5	23.5
Fawn	0.0	4.9	0.0	0.0	0.0	0.0	0.0	1.5
Grey	2.0	2.4	2.6	0.0	6.6	6.7	3.7	2.3
Horn presence								
Present	97.0	100.0	91.3	95.1	96.7	93.3	94.2	96.2
Absent	3.0	0.0	8.7	4.9	3.3	6.7	5.8	3.8
Horn shape	***		*		**		***	
Straight	59.4	95.1	5.2	13.8	14.3	39.3	19.6	45.7
Curved	40.6	4.9	92.9	86.2	85.7	60.7	79.5	54.3
Lyre-shape	0.0	0.0	1.9	0.0	0.0	0.0	0.9	0.0
Horn orientation					*		***	
Lateral	41.7	61.0	9.0	19.0	10.2	28.6	16.3	34.6
Upward	52.1	36.6	61.6	53.4	66.0	60.7	61	49.6
Downward	0.0	0.0	0.9	0.0	1.4	0.0	0.9	0.0
Forward	6.2	2.4	27.5	25.9	22.4	10.7	21.4	15
Other	0.0	0.0	0.9	1.7	0.0	0.0	0.4	0.8
Ear orientation								
Erect	45.8	14.6	8.7	9.8	9.2	16.7	16.4	12.9
Lateral	52.1	82.9	91.3	90.2	90.8	83.3	83.2	86.4
Drooping	2.1	2.4	0.0	0.0	0.0	0.0	0.4	0.8

* significant (p<0.05); ** significant (p<0.001); *** significant (p<0.0001); F = female; M = male.

Tables 3.3 and 3.4 present within sex comparisons of means for the morphological variables and body weight among the three Mozambican cattle populations. Females of the three populations differed significantly ($p<0.05$) in all measurements, except muzzle circumference. Landim cows had significantly ($p<0.05$) higher measurements in all parameters, compared to the other two populations.

Table 2.3 Adult female body measurements of three indigenous Mozambican cattle populations

Parameter	Angone	CV	Landim	CV	Tete	CV	Average	CV
MC (cm)	39.60^{a}±0.30	6.1	42.15^{b}±0.20	8.6	40.18^{a}±0.24	4.9	40.65±0.14	7.7
HL (cm)	17.26^{a}±1.07	38.3	29.95^{b}±0.70	42.1	24.65^{c}±0.86	37.6	23.95±0.51	45.3
TL (cm)	158.43^{a}±1.29	7.7	167.56^{b}±0.85	8.7	163.23^{c}±1.04	6.2	163.07±0.62	8.1
BL (cm)	116.31^{a}±1.09	7.4	134.20^{b}±0.71	9.7	123.55^{c}±0.88	6.3	124.68±0.52	10.2
WH (cm)	116.78^{a}±0.80	7.9	123.38^{b}±0.53	7.0	119.48^{c}±0.64	4.7	119.88±0.39	6.9
HG (cm)	147.93^{a}±1.22	10.6	161.28^{b}±0.79	7.7	152.56^{c}±0.97	4.9	153.92±0.58	8.4
RW (cm)	37.15^{a}±0.25	7.2	40.58^{b}±0.16	6.2	39.16^{c}±0.20	5.5	38.96±0.12	7.0
RH (cm)	113.92^{a}±0.60	4.3	123.98^{b}±0.40	5.5	119.65^{c}±0.48	4.2	119.18±0.29	5.9
HC (cm)	32.08^{a}±0.29	9.4	34.55^{b}±0.20	8.4	33.28^{c}±0.23	7.8	33.31±0.14	8.9
BW (kg)	243.34^{a}±5.51	17.5	309.99^{b}±3.54	21.6	265.67^{c}±4.36	12.8	273.00±2.62	21.3

MC Muzzle circumference; **HL** Horn length; **TL** Top line length; **BL** Body length; **WH** Height at withers; **HG** Heart girth; **RW** Rump width; **RH** Height at rump; **HC** Hock circumference; **BW** Body weight; **Kg** Kilogramme; cm Centimeter; CV coefficient of variation; Means that have different superscripts in the same row differ statistically ($p<0.05$)

Angone males were significantly ($p<0.05$) lighter and generally smaller than those of the other two breeds. Landim and Tete males were similar in most body measurements and differed significantly ($p<0.05$) for only three (horn length, body length, and height at rump) characteristics.

Table 2.4 Adult male body measurements of three indigenous Mozambican cattle populations

Parameter	Angone	CV	Landim	CV	Tete	CV	Average	CV
MC (cm)	41.34^{a}±0.55	6.4	45.17^{b}±0.46	9.0	44.27^{b}±0.65	7.8	43.59±0.32	8.9
HL (cm)	15.95^{a}±1.55	37.8	31.05^{b}±1.28	35.9	20.83^{c}±1.81	55.3	22.61±0.90	49.8
TL (cm)	161.00^{a}±2.47	7.1	173.61^{b}±2.06	9.2	169.07^{b}±2.89	11.8	167.89±1.44	9.9
BL (cm)	114.66^{a}±1.70	5.9	137.87^{b}±1.40	8.1	128.63^{c}±1.99	11.1	127.05±0.99	11.5
WH (cm)	124.81^{a}±1.45	6.0	132.14^{b}±1.20	8.4	129.53^{b}±1.69	5.7	128.83±0.84	7.5
HG (cm)	148.49^{a}±2.63	13.4	166.58^{b}±2.19	8.9	160.07^{b}±3.08	10.0	158.38±1.54	11.6
RW (cm)	38.34^{a}±0.54	6.8	42.39^{b}±0.45	8.7	41.70^{b}±0.63	9.4	40.81±0.31	9.4
RH (cm)	119.24^{a}±1.06	5.3	128.27^{b}±0.88	5.8	125.13^{c}±1.23	4.7	124.22±0.62	6.2
HC (cm)	34.05^{a}±0.46	7.7	36.39^{b}±0.46	9.2	35.77^{b}±0.53	7.4	35.40±0.28	8.7
BW (Kg)	256.90^{a}±11.1	18.9	346.22^{b}±9.18	24.0	316.38^{b}±12.9	22.4	306.50±6.46	25.8

MC Muzlle circumference; **HL** Horn length; **TL** Top line length; **BL** Body length; **WH** Height at withers; **HG** Heart girth; **RW** Rump width; **RH** Height at rump; **HC** Hock circumference; **BW** Body weight; **Kg** Kilogramme; **cm** Centimeter; **CV** coefficient of variation; Means that have different superscripts in the same row differ statistically (p<0.05)

Out of 10 variables subjected to the stepwise discriminant analysis, six (BL, HL, RW, WH, RH, and TL) showed significant (p<0.001) discriminatory power (Table 3.5). MC, HG, HC and BW were excluded from the analysis as they did not meet the minimum partial F to enter of 3.84 (default settings).

Table 2.5 Stepwise selection of different traits in the Landim, Angone, and Tete cattle populations

Step	Trait	F-value	*p* - value	Wilks' Lambda
1	BL	252.364	***	0.600
2	HL	166.188	***	0.532
3	RW	125.551	***	0.500
4	WH	103.877	***	0.475
5	RH	100.181	***	0.428
6	TL	88.645	***	0.413

BL Body length; **HL** Horn length; **RH** Height at rump; **WH** Height at wither; **RW** Rump width; **TL** Top line length; and *** (p<0.001).

To assess the performance of the discriminant function in discriminating the three cattle populations, the percentage of misclassification for each of the respective populations (Table 3.6), was estimated. Thus, approximately 70% of individuals were correctly allocated to their source population. More than 70% of Angone and Landim cattle were correctly assigned to their respective source populations. The Tete population had poorer differentiation, as 23,6% of the individuals were allocated to the Angone and another 16.5% to Landim cattle populations.

Table 2.6 Percentage (%) of individual cattle (mis-)assigned to the respective populations

Source population	Angone	Landim	Tete
Angone (N=137)	**77.3**	1.5	21.2
Landim (N=281)	6.0	**73.0**	21.0
Tete (N=182)	23.6	16.5	**59.9**
Priors	0.333	0.333	0.333

The mahalanobis distances between the three cattle populations are shown in Table 3.7. The greatest distance was observed between Landim and Angone (6.45) and the smallest distance between Landim and Tete (1.28).

Table 2.7 Squared mahalanobis distances between three Mozambican cattle populations

Subpopulation	**Angone**	**Landim**	**Tete**
Angone	0		
Landim	6.446	0	
Tete	2.316	1.277	0

The relatedness among the populations was also studied by cluster analysis based on body measurements. The three populations under study formed two main clusters as shown in the dendrogram (Figure 3.2). The first cluster was composed exclusively of Landim cattle while the second comprises the two remaining populations of indigenous cattle (Angone and Tete).

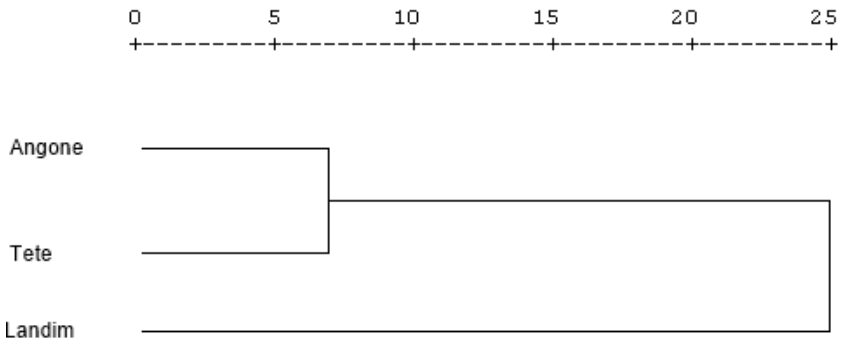

Figure 2.2 Dendrogram showing the relationship among Mozambican indigenous cattle

The canonical analysis performed on the quantitative variables identified two statistically significant canonical functions (p<0.01) that explained 97.4% and 2.6% of the total variation, respectively (Table 3.8). The combination of variables that best discriminates the three cattle populations is canonical function 1. BL and RH were strongly correlated to function 1 while WH was more associated with function 2.

Function 1 = -0.204MC + 0.372HL - 0.249TL+ 0.648BL - 0.62WH + 0.123HG + 0.326RW + 0.651RH - 0.03HC + 0.087BW; Function 2 = 0.211MC - 0.115HL - 0.371TL+ 0.588BL + 0.666WH + 0.251HG - 0.317RW - 0.94RH - 0.109HC + 0.33BW.

Table 2.8 Standardized coefficients for the canonical discriminant function, the canonical correlation, the eigenvalue and the percentage of total variance accounted for

Variables	Function 1	Function 2
MC	-0.20	0.21
HL	0.37	-0.12
TL	-0.25	-0.37
BL	0.65	0.59
WH	-0.62	0.67
HG	0.12	0.25
RW	0.33	-0.32
RH	0.65	-0.94
HC	-0.03	-0.11
BW	0.09	0.33
Canonical correlation	0.71	0.16
Approximate standard error	0.06	0.04
Eigenvalue	1.01	0.03
Variance accounted for (%)	97.40	2.60
Cumulative variance (%)	97.40	100.00

MC Muzzle circumference; **HL** Horn length; **TL** Top line length; **BL** Body length; **WH** Height at withers; **HG** Heart girth; **RW** Rump width; **RH** Height at rump; **HC** Hock circumference; **BW** Body weight

Figure 3.3 shows the spatial distribution of the three populations in the current study, as determined by canonical discriminant functions. Function1 differentiated Landim from Angone cattle populations, with limited overlapping. Some individuals belonging to the Tete population were detected as outliers in the Angone and Landim populations.

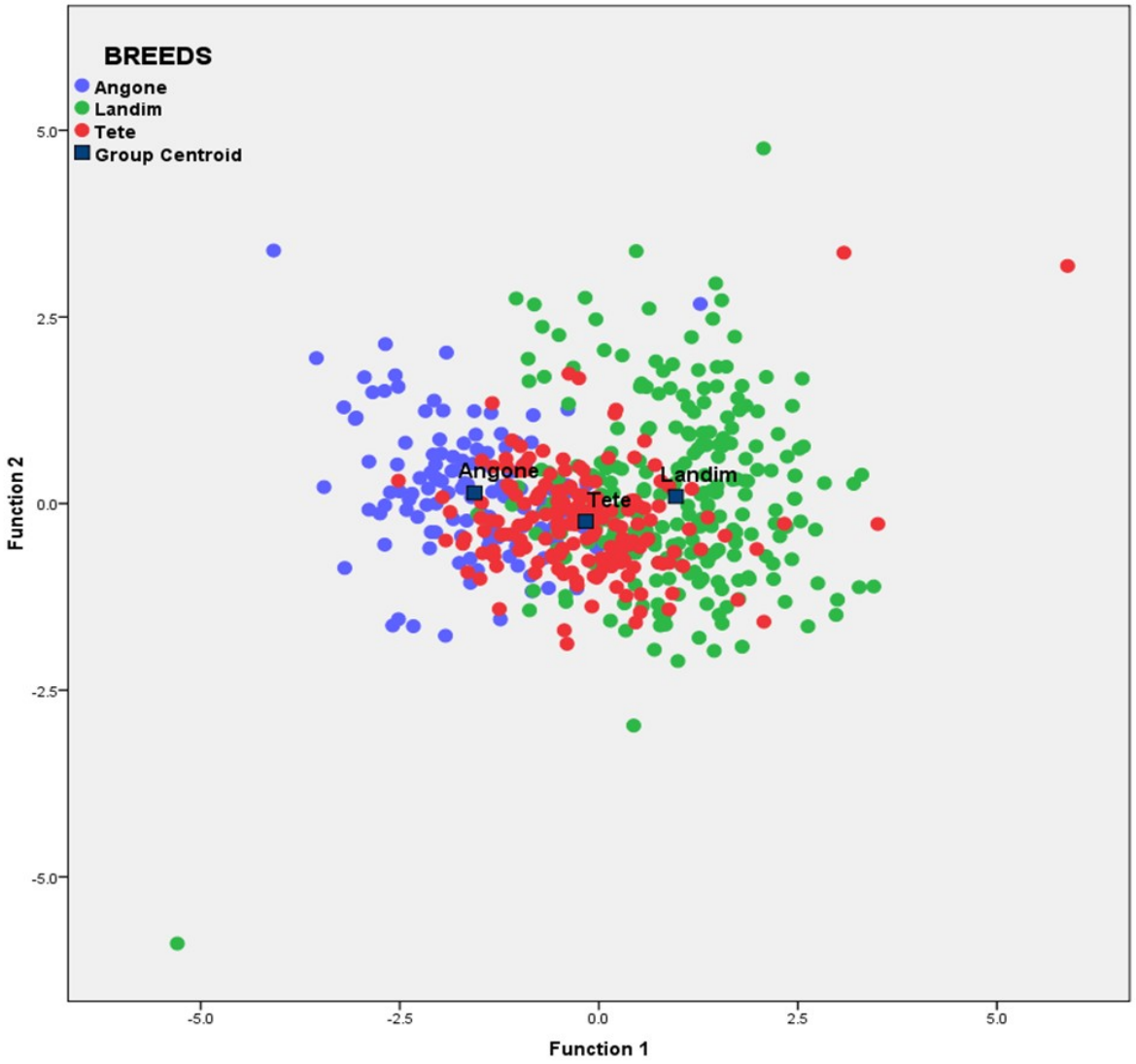

Figure 2.3 Canonical analysis for individual body measurements in three studied cattle populations.

3.4 Discussion

Morphological variation among cattle breeds may be a result of distinctions between their genetic makeup, geographical distributions, agro-ecological conditions, or production systems (Desta *et al.* 2011; Mekonne & Meseret, 2020). Physical characteristics of a breed such as coat colour, body size, and shape of horns assist in the definition of the breed and the setting of breed standards (McManus *et al*., 2010). The current study attempted to describe the morphological features of three indigenous Mozambican cattle populations. Our findings showed substantial morphological variation among these populations, which can further be utilized for the design of rational breed improvement, conservation, and utilization strategies.

Results indicated a wide range of coat colours, with black being the predominant one. In a previous study, Kotze *et al*. (2000) also reported high coat colour variability for relatively small sampling groups of the same breeds. The observed wide variation in coat colours among Mozambican cattle breeds might be an adaptation mechanism to the different local environments, as postulated by Yougbaré *et al*. (2020) in cattle from Burkina Faso and Mani *et al*. (2014), in Niger's goat populations. Mixed coat colour works as camouflage and cattle with such coat pattern better defend themselves from predators and insect bites (Hagan *et al*., 2012; Kojima *et al*., 2019). For instance, Saini *et al*. (2017) reported lower trypanosome infections in white and brownish-red-coloured animals compared to black-coloured animals. Black-coated cattle seem to have lower food intake as these animals have higher body temperatures. Therefore, the predominance of black colour may be an adaptive response of these animals to withstand food shortages (Baenyi *et al*., 2020).

In Tanzania, Chasama (2013), found more black-coated animals in the district of Ukweru where cattle are tethered under trees than in the Bunda district where cattle graze openly and extensively. Black-coated cattle are thought to be more adapted to seasonal cold weather, as the dark pigment they have helps them to absorb heat and warm them up faster than light-coated Kenyan Borana cattle (Abdurehman, 2019).

Horn shape and size are important characteristics and help cattle to graze in thickets as well as in struggles for dominance and hierarchy, especially in bulls (Kugonza *et al*., 2011; Hirwa *et al*., 2017). The horns of Angone cattle were short to medium (16 cm) and mainly straight and laterally oriented. This finding is in agreement with Kotze *et al*. (2000) who observed that Angone cattle had short horns, suggesting that this breed originated from the East African Zebu, which has short horns (Otto *et al*., 2000). Most Landim (57.5%) and Tete (63.4%) cattle had upward oriented horns, which is similar to Eritrean Arado (60%), Ethiopian Begait (62.2%), and Ethiopian Malle (63.3%) cattle (Gebrul *et al*.,

2017; Goitom *et al*., 2019; Getaneh *et al*., 2020). The observed phenotypic similarity may be due to shared ancestry as well as selective pressures.

Body weight and morphological measurements values obtained in the current study are lower compared to those obtained in earlier studies on the same breeds. Catalão & Syrstad (1990), for example, reported an average body weight of 384-416 kg for mature female Landim breed. Tomo (1997) reported an average hearth girth of 157.8 cm for bulls and 148.1 cm for cows of the Angone breed, indicating that animals of this breed some two decades ago were somewhat bigger than the present ones (present hearth girth 148.49 cm in male and 147.93 cm in female). The size reduction could be a result of inbreeding within the population and/or deteriorating environmental conditions and decreasing feed resources as reported by Genzebu *et al*. (2012) in the Ethiopian Arado cattle breed and by Nguluma *et al*. (2016) in the Tanzanian SAE goats. Indigenous cattle in Southern Africa have been naturally selected to adapt to the food shortage that is prevalent in the region (Nyamushamba *et al*., 2017). For example, to cope with drought and forage seasonality these animals are small to medium-sized, which makes them less demanding in terms of maintenance requirements (Bester *et al*., 2003; Matjuda *et al*., 2014).

Variations in morphometric measurements between cattle breeds may be caused by differences in genetics, climate, management systems, or feed quality (Ftiwi & Tamir, 2015). These variations may be useful for distinguishing different breeds (Gatesy & Arctander, 2000), evaluating breeding goals (Zechner *et al*., 2001), and comparing feeding and production systems. Discriminant analysis revealed that 6 of the 10 body measurements were significant in distinguishing between the three populations of cattle. Body length and height at wither were among the most discriminating traits for the three populations, in agreement with a previous study by Pundir *et al*. (2015) in Indian indigenous cattle. Thus, the six morphometric variables are sufficiently informative and can be reliably used to assign individual cattle to one of the three populations.

Multivariate analysis of morphological traits has been found to reliably discriminate populations of cattle (Pundir *et al*., 2015), goats (Mdladla *et al*., 2017), sheep (Wagari *et al*., 2020), and horses (Rezende *et al*., 2021). Assessment of the relationship among the three cattle populations using canonical discriminant functions (Function 1 and 2) revealed little differentiation between Angone and Tete. On the other hand, Landim cattle were generally assigned to a distinct cluster, with little overlapping. The proportion of individuals correctly allocated to their source population indicates the level of differentiation of that population. Canonical discriminant analysis assigned approximately 70% of the individuals to their populations of origin. This study is in agreement with previous reports where the correct classification ranged from 62.6% to 96.55% for indigenous cattle populations (Yakubu *et al*.,

2010; Chencha *et al*., 2013; Pundir *et al*., 2015). Hirbo *et al*. (2006) postulated that high misclassification rates could indicate high gene flow or low assignment power due to the small number of variables used in the analysis. The classification errors also depend on the method and variables used in the analysis (Yakubu et al., 2012; Correa *et al*., 2013).

Tete cattle had the greatest percentage of individuals miss-assigned to other populations, with the majority (23.6 %) of these being wrongly assigned to Angone. The reason for this high misclassification between Angone and Tete breeds may be attributed to crossbreeding, as these are the predominant breeds in the central province of Tete. The high number of Tete individuals being miss-assigned to Landim (16.5 %) cattle show a relatively low morphometric differentiation between these cattle suggesting shared genetic identities. These results corroborate with those from a population structure analysis study, using microsatellite markers, reported by Madilindi *et al*. (2019).

3.5 Conclusions

The present study has shown substantial phenotypic heterogeneity in qualitative traits of Mozambican indigenous cattle populations. The coat colour, as well as the shape and orientation of the horn, showed variation in the three populations, indicating a lack of strong selection in these traits. Significant differences were observed for most of the morphometric traits, suggesting differences in size between the three studied cattle populations. The three cattle populations are best differentiated by measuring body length. The assessed morphological characteristics, together with genetic information, could be a valuable tool for the design of breed improvement programs, conservation, and utilization of genetic resources of indigenous cattle.

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References

Abdurehman, A., 2019. Physiological and anatomical adaptation characteristics of Borana cattle to pastoralist lowland environments. Asian Journal of Biological Sciences, 12, 364-372.

Alberro, M., 1983. The indigenous cattle of Mozambique. World Animal Review, 48, 12-17.

Baenyi, S.P., Birindwa, A.B., Mutwedu, V.B., Mugumaarhahama, Y., Munga, A., Mitima, B., Kamgang, V.W. & Ayagirwe, R.B.B., 2020. Effects of coat color pattern and sex on physiological traits and heat tolerance of indigenous goats exposed to solar radiation. Journal of Animal Behaviour and Biometeorology, 8, 142-151.

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. & Alexandrino, P., 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African Journal of Animal Science, 39(1), 61-72.

Bester, J., Matjuda, I.E., Rust, J.M. & Fourie, H.J., 2003. The Nguni: a case study. In FAO Community-based management of animal genetic resources. Food and Agriculture Organization of the United Nations, Rome, 45-68.

Catalão, D. & Syrstad, O., 1990. Productivity of Nguni and Africander cattle in Mozambique. Livestock Production Science, 24(1), 29-36.

Chasama, G.L., 2013. Phenotypic characterisation of Ukerewe and Bunda Tanganyika shorthorn Zebu Cattle in the Lake Victoria basin. MSc Thesis; Sokoine University of Agriculture. Morogoro, Tanzania.

Chencha, C., Workneh, A. & Zewdu, W., 2013. On-farm phenotypic characterization of indigenous cattle populations of Gomo Goffa zone, Southern Ethiopia. Animal Genetic Resources, (52), 71-82.

Correa, M.P.C., Dallago, B.S.L., Paiva, S.R., Canozzi, M.E.A., Louvandini, H., Barcellos, J.J. & McManus, C., 2013. Multivariate analysis of heat tolerance characteristics in Santa Inês and crossbred lambs in the Federal District of Brazil. Tropical Animal Health and Production, 45, 1407-1414.

Desta, T.T., Ayalew, W. & Hedge, B.P., 2011. Breed and trait preferences of Sheko cattle keeper in southern Ethiopia. Tropical Animal Health and Production, 43, 851–856.

FAO (Food and Agriculture Organization), 2011. Draft Guidelines on Developing the Institutional Framework for the Management of Animal Genetic Resources. Commission on Genetic Resources for Food and Agriculture, FAO, Rome.

FAO (Food and Agriculture Organization), 2012. Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines 11. Rome, Italy. Available at: https://www.fao.org/3/i2686e/I2686E.pdf

FAO (Food and Agriculture Organization), 2015. The second report on the State of the World's Animal Genetic Resources for Food and Agriculture, Rome, Italy.Available at: https://www.fao.org/3/i4787e/I4787E.pdf

FAO (Food and Agriculture Organization), 2019a. World Food and Agriculture – Statistical pocketbook 2019. Rome.

FAO (Food and Agriculture Organization), 2019b. Status and trends of Animal Genetic Resources. Commission on Genetic Resources for Food and Agriculture, Rome, Italy. Available online at: http://www.fao.org/3/my867en/my867en.pdf.

Ftiwi, M. & Tamir, B., 2015. Phenotypic characterization of indigenous cattle in Western Tigray, Northern Ethiopia. Indian Journal of Dairy Science, 68(2).

Gatesy, J. & Arctander, P., 2000. Hidden morphological support for the phylogenetic placement of Pseudoryx ngetinhensis with bovine bovids: a combined analysis of gross anatomical evidence and DNA sequences from five genes. systems biology, 49(3), 515-538.

Gebrul, T., Yigrem, S. & Banerjee, S., 2017. Some morphometrical, production and reproduction traits of begait cattle reared in Tigray region of Ethiopia, Wayamba Journal of Animal Science, 75(9), 1571-1585.

Genzebu, D., Hailemariam, M. & Belihu, K., 2012. Morphometric characteristics and livestock keeper perceptions of Arado cattle breed in Northern Tigray, Ethiopia. Livestock Research for Rural Development, 24(6).

Getaneh, D., Banerjee, S. & Taye, M., 2020. Morphometrical Traits and Structural Indices of Malle Cattle Reared in the South Omo Zone of Southwest Ethiopia. Research Journal of Animal Sciences, 14, 1-11.

Goitom, S., Gicheha, M.G., Ngeno, K. & Njonge, F.K., 2019. Morphological characterisation of indigenous cattle breeds in Eritrea. Advances in Animal and Veterinary Sciences, 7(10), 848-857.

Hagan, J.K., Apori, S.O., Bosompem, M., Ankobea, G. & Mawuli, A., 2012. Morphological characteristics of indigenous goats in the coastal savannah and forest eco-zones of Ghana. Journal of Animal Science Advances, 2, 813-821.

Hirbo, J., Muigai, A., Naqvi, A.N., Rege, E.D. & Hanotte, O., 2006. The genetic diversity of trans-Caucasian native sheep breeds. Asian-Australasian Journal of Animal Sciences, 19, 943-952.

Hirwa, C.D.A., Kugonza, D.R., Murekezi, T., Rwemarika, J.D., Kayitesi, A., Musemakweri, A., Shabayiro, J.P., Shumbusho, F., Manzi, M. & Safari, T., 2017. Management and phenotypic features of indigenous cattle in Rwanda. International Journal of Livestock Production, 8(7), 95-112.

INE (National Institute of Statistics), 2006. Integrated survey of the workforce. Final report, Maputo: National Institute of Statistics of Mozambique. Maputo, Mozambique.

INE (National Institute of Statistics), 2015. Statistical Yearbook 2014, Maputo: National Institute of Statistics of Mozambique.

King, F.J.M., Banga, C.B. & Visser, C., 2021. Genetic diversity and population structure of three native cattle populations in Mozambique. Tropical Animal Health and Production, 53, 117.

Kojima, T., Oish, K., Matsubara, Y., Uchiyama, Y., Fukushima, Y., Aoki, N., Sato, S., Masuda, T., Ueda, J., Hirooka, H. & Kino, K., 2019. Cows painted with zebra-like striping can avoid biting fly attack. PLOS ONE, 14(10), e0223447. https://doi.org/10.1371/journal.pone.0223447.

Kotze, A., Harun, M., Otto, F. & Van der Bank, F.H., 2000. Genetic relationship between three indigenous cattle breeds in Mozambique. South African Journal of Animal Science, 30(2), 92-97.

Kugonza, D.R., Nabasirye, M., Hanotte, O., Mpairwe, D. & Okeyo, A.M., 2011. Pastoralists' indigenous selection criteria and other breeding practices of the long-horned Ankole cattle in Uganda. Tropical Animal Health and Production, 44(3), 557-565.

Maciel, S., 2001. National strategies for the conservation, improvement and utilization of animal genetic resources in smallholder systems. Proceedings of the Planning and Priority Setting Workshop on Animal Genetic Resources in the SADC region. SACCAR, CTA, ILRI, Gaborone, Botswana.

Maciel, S., Harun, M. & Capece, B., 2004. First national country report on the status of farm animal genetic resources in Mozambique. Relatório sobre o workshop–FAO, pp1-64. Available online at:http://www.fao.org/tempref/docrep/fao/010/a1250e/annexes/CountryReports/Mozambique.pdf

Maciel, S., Okeyo, A.M., Amimo, J., Scholtz, M.M., Neser, F.W.C. & Mart, M., 2013. The effect of geographical region of birth on the reproductive performance of the Nguni in southern Mozambique. South African Journal of Animal Science, 43(1), 60-63.

Madilindi, M.A., Banga, C.B., Bhebhe, E., Sanarana, Y.P., Nxumalo, K.S., Taela, M.G., Mapholi, N.O., 2019. Genetic differentiation and population structure of four Mozambican indigenous cattle populations, Livestock Research for Rural Development, 31 (47).

Mani, M., Marichatou, H., Issa, M., Chaïbou, I., Sow, A., Chaibou, M. & Sawadogo, J.G., 2014. Caractéristiques phénotypiques de la chèvre du sahel au Niger par analyse des indices de

primarité et des paramètres qualitatifs. Animal Genetic Resources Information Bulletin, 54, 11-19.

MASA (Ministry of Agriculture and food security of Mozambique), 2011. National Agriculture Investment Plan 2013–2017. Available online at: https://www.agricultura.gov.mz/wp-content/uploads/2018/05/PNISAmoz.pdf.

Matjuda, L.E., MacNeil, M.D., Maiwashe, A., Leesberg, V.R. & Malatje, M., 2014. Index-in-retrospect and breeding objectives characterizing genetic improvement programmes for South African Nguni cattle. South African Journal of Animal Science, 44 (2), 161-172.

McManus, C., Pavia, S.R., Rezende, A.V., Murata, L.S., Louvandin, H., Cubillos, G.P., Martinez, R. A., Dellacasa, M.S. & Perez, J.E., 2010. Phenotypic characterisation of Naturalized swine breeds in Brazil, Uruguary and Columbia. Journal of Biology and Technolgy, 53, 583-591.

Mdladla, K., Dzomba, E.F. & Muchadeyi, F.C., 2017. Characterization of the village goat production systems in the rural communities of the Eastern Cape, KwaZulu-Natal, Limpopo and North West Provinces of South Africa. Tropical Animal Health and Production, 49, 515–527.

Meissner, H.H., Scholtz, M.M. & Palmer, A.R., 2013a. Sustainability of the South African livestock sector towards2050. Part 1: Worth and impact of the sector. South African Journal of Animal Science, 43, 282-297.

Meissner, H.H., Scholtz, M.M. & Engelbrecht, F.A., 2013b. Sustainability of the South African livestock sector towards 2050. Part 2: Challenges, changes and required implementations. South African Journal of Animal Science, 43, 298-319.

Mekonne, T. & Meseret, S., 2020. Characterization of Begait cattle using morphometric and qualitative traits in Western Zone of Tigray, Ethiopia. International Journal of Livestock Production. 11(1), 21-33.

Morgado, F.P., 2000. The Livestock production in Northern Mozambique. Vega: Portugal.

Nguluma, A.S., Msalya, G. & Chenyambuga, S.W., 2016. Phenotypic variation among four populations of small East African goats of Tanzania. Livestock Research. Rural Development, 28 (136).

Nyamushamba, G., Mapiye, C., Tada, O., Halimani, T. & Muchenje, V., 2017. Conservation of indigenous cattle genetic resources in Southern Africa's smallholder areas: Turning threats into opportunities – A review. Asian-Australasian Journal Animal Science, 30, 603-622.

Otto, F., Vilela, F., Harun, M., Taylor, G., Bagasse, P. & Bogin, E., 2000. Biochemical blood profile of Angoni cattle in Mozambique. Israel Veterinary Medicine Association, 55 (3).

Pundir, R.K., Singh, P.K. & Sadana, D.K., 2015. Multivariate analysis of morphometric traits of three different indigenous cattle populations from North east states of India. Indonesian Journal of Animal and Veterinary Sciences, 20(2), 79-86.

Rezende, M.P.G., Souza, J.C., Malhado, C.H.M., Carneiro, P.L.S., Araujo, J.I.M., Sitorski, L.G., Moretti, R. & Bozzi, R., 2021. Phenotypic diversity of horse breeds used in sports activities, employing multivariate analysis. Spanish Journal of Agricultural Research, 19 (1).

Saini, R.K., Orindi, B.O., Mbahin, N., Andoke, J.A., Muasa, P.N., Mbuvi, D.M., Muya, C.M., Pickett, J.A., & Borgemeister, C.W., 2017. Protecting cows in small holder farms in East Africa from tsetse flies by mimicking the odor profile of a non-host bovid. PLOS Neglected Tropical Diseases, 11(10): e0005977.

Swanepoel, F., Stroebel, A. & Moyo, S., 2010. The Role of Livestock in Developing Communities: Enhancing Multifunctionality. Available at: https://www.researchgate.net/publication/265348731.

Timberlake, J. & Jordão, C., 1985. Inventory of feed resources for small scale livestock production in Mozambique. In: Kategile, J.A., Said, A.N. & Dzowela, B.H. (eds.), Animal feed resources for small-scale livestock producers - Proceedings of the second PANESA workshop, held in Nairobi, Kenya, 11–15 November 1985. International Development Research Centre.

Tomo, P., 1997. Characterization of Angoni cattle in Mozambique. MSc Thesis. Universtity of Orange Free State, Bloemfontein, South Africa.

Wagari, G., Getachew, T. & Bayou, E., 2020. Multivariate analysis of phenotypic traits of indigenous sheep revealed new population in western part of Ethiopia, International Journal of Agricultural Science and Food Technology, 6(1), 050-057.

Yakubu, A., Idahor, K.O., Haruna, H.S., Wheto, M. & Amusan, S., 2010. Multivariate analysis of phenotypic differentiation in Bunaji and Sokoto Gudali cattle. Acta agriculturae Slovenica, 96(2), 75-80.

Yakubu, A., Peters, S.O., Ilori, B.M., Imumorin, I.G., Adeleke, M.A., Takeet, M.I., Ozoje, M.O., Ikeobi, C.O.N. & Adebambo, O.A., 2012. Multifactorial discriminant analysis of morphological and heat-tolerant variables in indigenous, exotic and cross-bred turkeys in Nigeria. Animal Genetic Resources, 50, 21–27.

Yougbaré, B., Soudré, A., Ouédraogo, D., Zoma, B.L., Tapsoba, A.S.R., Sanou, M., Ouédraogo-Koné, S., Burger, P., Wurzinger, M., Khayatzadeh, N., Tamboura, H.H., Traoré, A., Sölkner, J. & Mészáros, G., 2020. Morphometric characterization of purebred and crossbred Baoulé cattle in Burkina Faso, Acta Agriculturae Scandinavica, 69:4, 193-202

Zechner, P., Zohman, F., So¨lkner, J., Bodo, I., Habe, F., Marti, E. & Brem, G., 2001. Morphological description of the Lipizzan horse population. Livestock Production Science, 69(2): 163–177.

Genetic diversity and population structure of three indigenous cattle populations from Mozambique

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Abstract

In the present work, the population diversity and structure of three populations of native Mozambican cattle were studied, to develop knowledge that is required for sound conservation and genetic improvement programs of these genetic resources. A total of 228 animals (Landim, Angone, and Tete) were genotyped using the International Dairy and Beef version three (IDBV3) SNP BeadChip array. Population parameters varied within a limited scope, with the average minor allele frequency (MAF) ranging from 0.228 ± 0.154 in the Angone to 0.245 ± 0.145 in the Tete population, while estimates of expected heterozygosities varied from 0.304 ± 0.166 in the Angone to 0.329 ± 0.148 in the Tete population. Low positive (0.065 ± 0.109) inbreeding rates were detected in the three cattle groups. Population structure and admixture analyses indicated low genetic differentiation and various degrees of admixture among the populations. The effective population size has decreased over time and at 12 generations ago ranged between 349 (Tete) and 929 (Landim). The average linkage disequilibrium (LD) of the studied populations ranged from 0.400 ± 0.213 (Tete) to 0.434 ± 0.232 (Landim). The findings of this study will be valuable for formulating management and conservation strategies for indigenous Mozambican cattle populations.

Keywords: Admixture, Angone, Landim, Smallholder, Tete

4.1 Introduction

Livestock production is a major feature of Mozambique's agriculture and contributes largely to improving food security (Vernooij *et al*., 2016). More than 70% of Mozambicans rely on agriculture and livestock for employment and subsistence (INE, 2016). Currently, the cattle population in Mozambique is estimated at 2.02 million heads, of which 98% are indigenous (FAO, 2019). Alberro (1983) classified indigenous Mozambican cattle breeds into two groups, namely Sanga (including Landim and Tete cattle) and Zebu (Angone cattle).

Landim cattle, commonly known as Nguni in the Southern African region, represents about 70% of the total national cattle herd. These cattle are widely spread in the southern provinces of the country, including Gaza, Inhambane, and Maputo (Maciel, 2001; Maciel *et al*. 2013). Small cattle populations can also be observed along the banks of the Limpopo River, in the area bordering Zimbabwe, where they regularly breed with Mashona cattle (Alberro, 1983).

The Tete cattle breed represents roughly 20% of the Mozambican cattle population. These cattle are found in the area between the western and southeast ends of the Tete province, bordering Malawi (Alberro, 1983; Morgado, 2000; Maciel, 2001). Tete cattle are similar to the Sanga type breed; however, their genesis is still unclear. Some research speculates that this breed is a result of the crossbreeding of the Landim with Angone cattle (Rege & Tawah, 1999), while others associate its origin to the Mashona breed (Alberro, 1983; Morgado, 2004). The Angone is the only indigenous Zebu cattle breed in Mozambique and can also be found in Zambia, Malawi, and Madagascar (Otto *et al*., 2000). Angone cattle are scattered along the Mozambican Angónia plateau, which is located in the northeast corner of Tete Province, on the border with Malawi (Alberro, 1983; Maciel, 2001).

Indigenous cattle in Mozambique are an important genetic resource due to their adaptive traits, their capability to convert low-quality pasture into an animal protein of high biologic value, and their resistance to a variety of endemic subtropical diseases (Bessa *et al*., 2009; Maciel *et al*., 2013; Mwai *et al*., 2015). Cattle production in Mozambique is mostly communal, resulting in random mating and indiscriminate crossbreeding (Bessa *et al*., 2009). This poses a threat to indigenous populations as it results in the erosion of unique genetic resources (FAO, 2015).

An important prerequisite for the formulation and implementation of a comprehensive conservation program is knowledge of the population genetic structure of the available livestock in a given country (Maciel *et al*., 2013; FAO, 2015). To date, studies concerning managerial aspects such as nutrition, health, and production systems of Mozambican livestock breeds have been undertaken

(Van Niekerk & Pimentel, 2000; Otto *et al*., 2000; Maciel *et al*., 2013); however, genetic aspects have not been fully investigated. Kotze *et al*. (2000) and Bessa *et al*. (2009) found Angone, Landim, and Tete to share some ancestry, with the Tete being an admixture of the Angone and Landim breeds. Madilindi *et al*. (2019) reported a high level of diversity among Mozambican indigenous cattle populations and observed distinct clustering of the four populations studied, despite some evidence of admixture. These studies used low-density microsatellites (Bessa *et al*., 2009; Madilindi *et al*., 2019), protein markers (Kotze *et al*., 2000; Bessa *et al*., 2009), or focused on the Y chromosome (Bessa *et al*., 2009). Single-nucleotide polymorphism (SNP) arrays are suitable for the study of population diversity and structure in livestock (Makina *et al*., 2014; Onzima *et al*., 2018). The SNP panels have been applied to study population structure, diversity, and admixture patterns of diverse indigenous cattle breeds in several countries, such as Ethiopia (Edea *et al*., 2014), India (Sharma *et al*., 2016), and South Africa (Makina *et al*., 2014).

The objectives of this study were to investigate (i) population genetic diversity and structure, (ii) linkage disequilibrium, and (iii) effective population size in three indigenous Mozambican cattle populations using genome-wide SNP markers. This information will shed some light on the level of crossbreeding and/or inbreeding in the indigenous cattle populations. It can serve as a useful management tool for sound conservation and improvement programs of these indigenous cattle genetic resources in Mozambique.

4.2 Materials and Methods

Animals and sampling

All animals were handled in compliance with the rules of the Ethics Committee (ECO25–18) of the Faculty of Natural and Agricultural Sciences, University of Pretoria. A total of 228 animals from three cattle populations, including Landim (n=119), Tete (n=61), and Angone (n=48), were included in the study. Twenty-one samples of the Angone and nineteen of the Landim cattle were collected from Angonia and Chobela Research Stations respectively, while the remaining was sampled from smallholder farms where cattle are kept under extensive systems in communal areas. Hair collection took place in readily available animal handling facilities that are also used by the extension officers of the Ministry of Agriculture (Mozambique). Sampling was carried out in regions where each population is predominantly found, namely the provinces of Maputo, Gaza, Inhambane, and Tete. Two to three districts in each province were identified based on livestock availability and district accessibility, after

which two rural areas were selected in each district (Figure 4.1). To assure that only non-related individuals were selected, no more than three animals were taken per herd, and sampling animals that graze in the same fields were avoided. Approximately 50 to 100 tail hairs from each animal were collected, ensuring intact follicles. The hair samples were kept in labeled envelopes and sent to Weatherbys Scientific Laboratory (Ireland), for DNA extraction and genotyping.

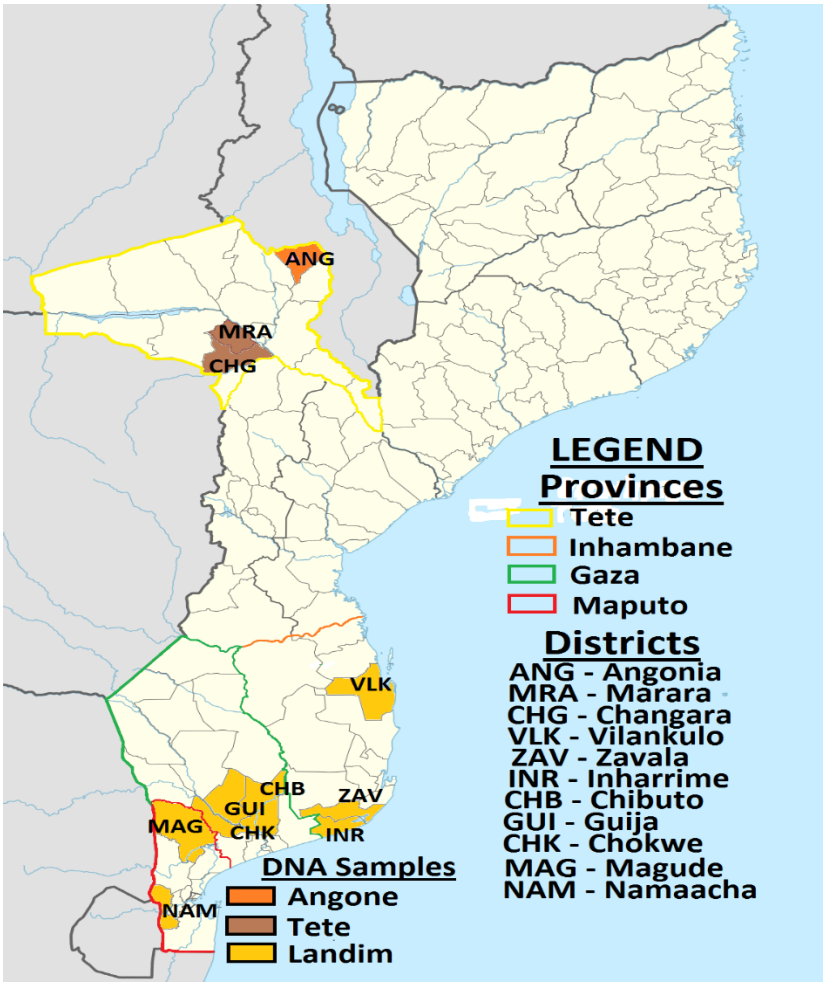

Figure 3.1 Map of Mozambique showing sampling locations**.**

Genotyping and quality control

The genotyping of the 228 individuals was performed with the IDBV3 SNP array, which comprises 53 450 SNPs located along the bovine genome (Twomery *et al*. 2019). All non-autosomal SNPs and duplicate variants were eliminated, while a set of 49 996 SNPs remained for subsequent analysis. Quality control was carried out within the breed, to eliminate SNPs and/or individuals that could skew the estimates of population genetic parameters. Basic data filtering was conducted with

standard thresholds, where samples missing more than 10% of the genotypes, and SNP call rate under 95% (Landim and Tete) or 90% (Angone, due to poor DNA quality) were excluded. Uninformative SNPs with MAF below 0.01 and SNPs that diverged considerably from Hardy Weinberg Equilibrium (p<0.001) were also discarded.

Data analysis

To assess genetic diversity in the population, we computed the observed (H_o) and expected (H_e) heterozygosities, as well as inbreeding rates (F_{IS}), using PLINK v1.09 (Purcell *et al*., 2007). The principal component analysis was performed to assess population structure and relationships using the GCTA version 1.24 software (Yang *et al*., 2011). To estimate the most probable number of ancestral populations, ADMIXTURE version 1.23 (Alexander *et al*., 2009) was used. The software uses a cross-validation (CV) system and was performed from K=2 to K=8. The cluster with the lowest CV error (K=6) was considered the most accurate. Linkage disequilibrium (LD) was assessed computing pairwise r^2 values per chromosome in each population using PLINK (Purcell *et al*., 2007). The LD SNP pairs were computed running the command "–r^2 –ld-windowkb 2000 –ld-window-r^2 0" up to a distance of 2000 Kb. The SNPs were then grouped into ten categories according to the distance of the pairwise, and the mean distance was calculated in each group. The effective population size (N_e) was estimated according to Corbin *et al*. (2012). SNP-marker distances between 0 and 1000 Mb were used with 30 distance bins of 50 kb each. The r^2 values at various distances were used to estimate N_e at different time points using SNeP version 1.1 (Barbato *et al*., 2015).

4.3 Results

During quality control, one Tete individual was excluded due to missing genotypes (−mind < 0.1). A common subset of 39 436 SNPs (98.67%) from 227 individuals remained for downstream analysis (Table 4.1).

Table 3.1 Individuals and SNPs removed in the quality control process

Population	N	Excluded Individuals	SNP Call rate	Excluded SNPs MAF	HWE	Total	Remaining SNPs (%)
Angone	48	0	2525	8241	241	11007	38989 (98.36)
Landim	119	0	3452	7621	399	11472	38524 (98.73)
Tete	61	1	2774	6416	294	9484	40512 (98.79)
Merged	228	1	3362	6566	632	10560	39436 (98.67)

MAF Minor Allele Frequency; **HWE** Hardy Weinberg Equilibrium (p<0.001)

Average MAF varied from 0.228 in the Angone to 0.245 in the Tete population. The levels of genetic diversity as indicated by observed and expected heterozygosities were similar in the three populations, with the Tete showing marginal superiority. The average inbreeding coefficients differed slightly among populations, but were all low and positive, with the highest level being observed in the Tete population (Table 4.2).

Table 3.2 Genetic diversity parameters and inbreeding rates of three cattle populations

Population	N	MAF ±SD	H_o ±SD	H_e±SD	F_{IS} ±SD
Angone	48	0.228±0.154	0.288±0.163	0.304±0.166	0.054±0.085
Landim	119	0.233±0.150	0.298±0.154	0.312±0.159	0.046±0.091
Tete	61	0.245±0.145	0.303±0.144	0.329±0.148	0.078±0.138
Merged	270	0.232±0.150	0.291±0.034	0.312±0.160	0.065±0.109

MAF Minor Allele Frequency; **H_o** Observed Heterozygosity; **H_e** Expected Heterozygosity; **F_{IS}** Inbreeding rate

Principal component analysis (PCA) separated Landim from the other two populations, with limited overlapping. Animals belonging to the Landim cattle were generally allocated to a distinct cluster, with a few outliers (Figure 4.2). However, the Angone and Tete showed very little differentiation and could almost be perceived as one population.

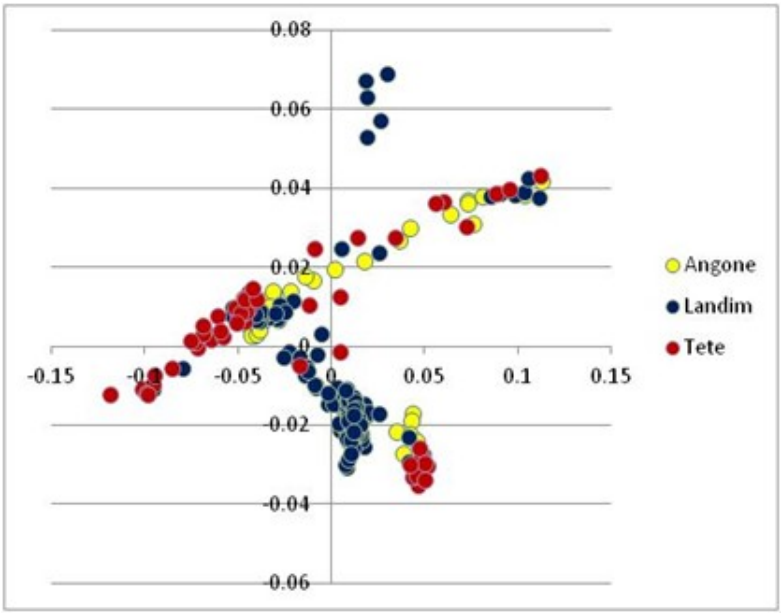

Figure 3.2 PCA1 against PCA3 plot for 3 cattle populations

ADMIXTURE was performed from K=2 to K=8, with K=6 being the most likely cluster at an inflection point of 0.509. Cross-validation errors (CV) were plotted (Figure 4.3) for comparison purposes. When K=6, none of the three populations were well-differentiated indicating a strong similarity between the populations and suggesting a certain degree of genetic admixture. Angone was subdivided into two groups, comprising individuals sampled at the Angonia research station (dark blue) and those derived from small-scale community farmers (light blue). All three populations showed evidence of admixture, and a highly heterogeneous genetic background was observed (Figure 4.4).

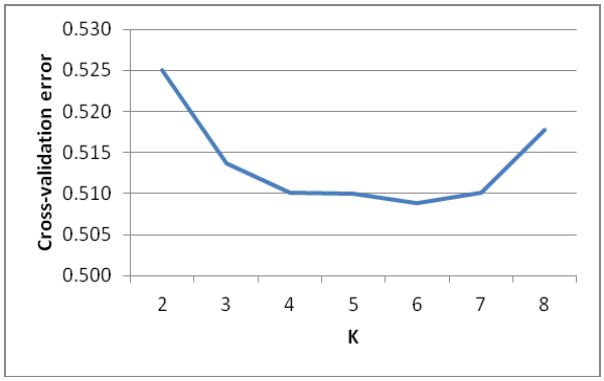

Figure 3.3 Cross-validation error of three populations of cattle

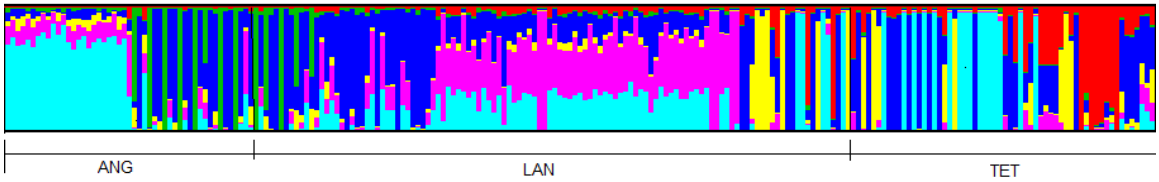

Figure 3.4 Admixture plot showing the clustering of three cattle populations

The proportions of individuals per population allocated to each of the six inferred clusters are presented in Table 4.3. Landim and Tete were clustered in cluster 3 while Angone was clustered in cluster 6.

Table 3.3 Breed proportion of three cattle populations given six clusters

Population	**N**	**Inferred clusters**					
		K1	K2	K3	K4	K5	K6
Angone	48	0.006	0.161	0.266	0.043	0.100	*0.425*
Landim	119	0.045	0.045	*0.335*	0.080	0.283	0.213
Tete	60	0.229	0.004	*0.322*	0.127	0.036	0.282

Italics indicate inferred cluster

The distribution of SNPs varied among chromosomes according to size, from 2434 on BTA1 to 730 on BTA25. The smallest average r^2 was noticed in the Tete population (0.400 ± 0.213) while the Landim population generally had the highest (0.434 ± 0.232) r^2 values across all chromosomes. The highest r^2 for an individual chromosome across all populations was observed for BTA20 (0.512 ± 0.272), whereas BTA14 had the lowest r^2 value (0.385 ± 0.197) (Figure 4.5).

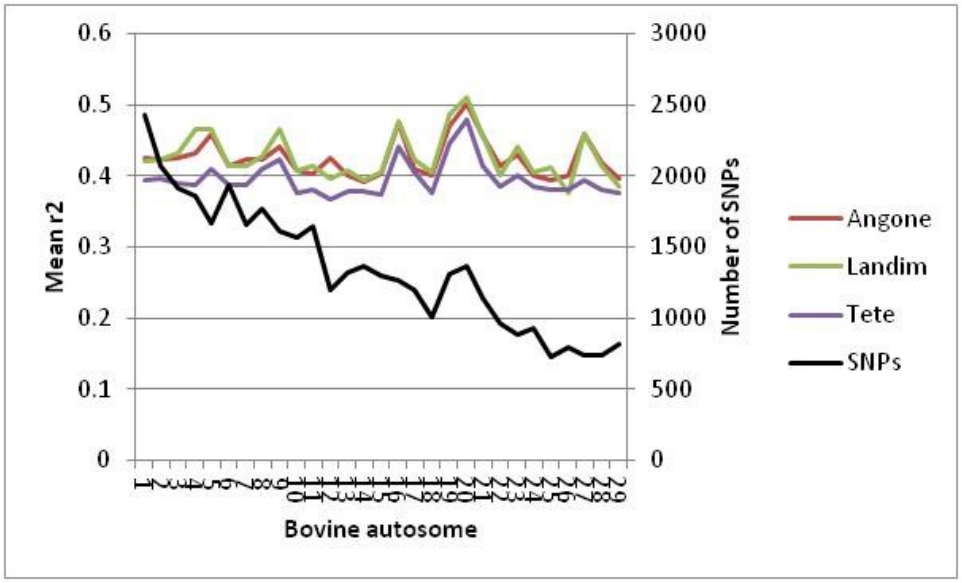

Figure 3.5 Distribution of autosomal SNPs and average r^2 values per population across the bovine genome

The greatest mean r^2 was seen at 0–10 kb in all populations. Linkage disequilibrium (LD) decreased as the distance between SNP pairs increased. The decrease of LD with increasing physical inter-marker distance was progressively slower for distances above 60 kb (Table 4.4).

Table 3.4 Average r^2 and SNP pairs over different distances across all autosomes, in three cattle populations

Distance	Angone		Landim		Tete	
(unit)	SNP pairs	r^2	SNP pairs	r^2	SNP pairs	r^2
< 10	5916	0.339	5837	0.351	6162	0.335
10-20	9865	0.291	9691	0.299	10308	0.285
20-40	27414	0.223	26945	0.217	28971	0.212
40-60	41091	0.198	40445	0.189	43582	0.187
60-80	54129	0.180	53245	0.170	57567	0.169
80-100	66929	0.166	65748	0.155	71291	0.156
100-200	127821	0.128	125431	0.114	136856	0.119
200-500	280403	0.091	275446	0.076	297553	0.086
500-1000	342069	0.083	338007	0.068	356144	0.080
1000-2000	347994	0.083	344216	0.068	361773	0.079

Effective population size (N_e) decreased over time across all populations. At 12 generations ago, the effective N_e was 627, 929, and 349 animals for the Angone, Landim, and Tete populations, respectively (Figure 4.6).

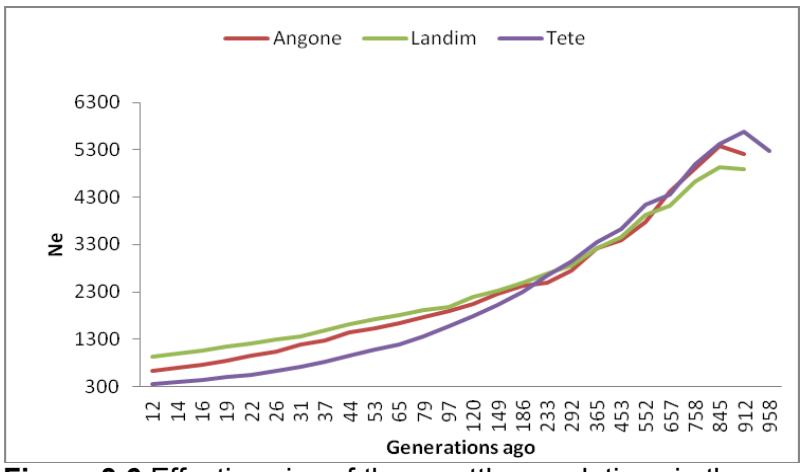

Figure 3.6 Effective size of three cattle populations in the past 12 generations

4.4 Discussion

Indigenous cattle are a precious genetic resource, essentially for smallholder farmers, due to their low maintenance input and ability to survive in a variety of agroecological environments (Morgado, 2000). Knowledge of population structure and genetic diversity plays a key role in planning genetic improvement programs for indigenous cattle, as well as in the conservation and effective use of genetic resources (Maciel *et al*., 2013; Mwai *et al*., 2015).

Average MAF (0.23) in the current study is higher than that estimated in the South African *Bos indicus* breeds (Qwabe *et al*., 2013), but lower than the mean values reported for most *Bos taurus* breeds (Edea *et al*., 2015; Zwane *et al*., 2016). The comparatively lower MAF in *Bos indicus* cattle may indicate the low representativeness of this population in the design of the assay (Edea *et al*., 2012; Lashmar *et al*., 2018). Makina *et al*.,2015, for instance, reported lower average MAF in South African indigenous cattle than in European taurine breeds.

Expected heterozygosities compared to those reported in the present study have previously been reported in South African Sanga cattle breeds (Makina *et al*., 2014) as well as in Ethiopian indigenous cattle breeds (Edea *et al*., 2012). These relatively high values may be related to long-term natural selection for adaption in an environment where the admixing of different populations occurred (Maciel, 2001; Ojango *et al*., 2011), as well as the lower levels of sustained artificial selection in

smallholder populations. The Mozambican indigenous cattle populations have been kept in communal systems since their origin and were mainly exposed to natural selection. Therefore, these cattle have become genetically adapted to their natural environment, while maintaining high genetic variability (Morgado, 2000; Mwai *et al*., 2015). This variability may be due to the wide gene flow between the local populations and imported breeds from neighboring countries, at the time of the livestock restocking program that took place after a massive loss of cattle due to the civil war (Bessa *et al*., 2009).

The diversity of these populations may have been affected by bottleneck effects (scarcity, epidemics, and civil war), founder events of breed formation, and the use of exotic germplasm among others (Maciel, 2001; FAO, 2015). Mozambican indigenous cattle populations experienced a significant genetic bottleneck due to the civil war (1977–1992), which decimated nearly 80% of the national herd. This period was followed by introgression, as the government imported several breeds, mainly from South Africa and Zimbabwe for restocking (Bessa *et al*., 2009; Maciel *et al*., 2013). Other forces that may have shaped the current status of diversity is the importation of exotic breeds for upgrading purposes. The use of exotic animals has been a common practice to increase livestock production and productivity in the tropics.

The average individual inbreeding (0.065) in this study was slightly higher than in previous reports (0.053), using microsatellite markers (Madilindi *et al*., 2019). Tete cattle were relatively more inbred (0.078) compared with the other two breeds and, consequently, presented the smallest effective population size (N_e). The relatively low inbreeding rate in the current study should be maintained to avoid harmful effects, such as loss of genetic variation and inbreeding depression (Szpiech *et al*., 2013).

The principal component analysis showed that most animals clustered relatively close to each other, revealing significant genetic relatedness among them. The PCA also demonstrated limited differentiation between the Angone and Tete cattle, proposing a historic genetic relatedness between them. This concurred with previous studies (Kotze *et al*., 2000; Bessa *et al*., 2009), and these authors concluded that the observed admixture maybe because the two populations share common genes from Zebu cattle (Bessa *et al*., 2009). Furthermore, the close relationship between Angone and Tete breeds is consistent with their geographical proximity, as these populations are located close together (Alberro, 1983).

Admixture analysis supported the PCA analysis, indicating poor between-breed differentiation. This observation was consistent with earlier reports based on microsatellite marker analysis (Bessa *et al*., 2009). However, Madilindi *et al*. (2019) using microsatellite markers reported moderate differentiation among the same populations sampled from three research stations. The lack of variability among Mozambican cattle populations may be related to common ancestry, a short domestication history, and low-intensity selection (Bessa *et al*., 2009; Edea *et al*., 2014). Admixture and indiscriminate crossbreeding that are common in the communal management systems stimulate the level of gene flow among the populations, which results in a low level of variability (Maciel, 2001; Bessa *et al*., 2009). A large portion of the Tete cattle genome is shared with Landim cattle. Thirty-four percent of the genetic links of the Tete cattle is derived from Landim while only thirty come from the Angone cattle. These results could support the theory that Tete cattle were developed from the crossbreeding between Landim and Angone cattle, defended by some authors. Although it was originated from two different bovine groups, Tete cattle are commonly classified as Sanga breed comparable to Landim (Rege & Tawah, 1999; Bessa *et al*., 2009). LD decay progressed slower in the Landim population than in the SA Nguni population studied by Makina *et al*. (2015). The low level of LD over long inter-marker distances indicates a lack of selection in these populations or a greater population size in the very recent past (Brito *et al*., 2015). The lowest mean r^2 was detected in the Tete population (0.400) while the Landim population had the highest (0.434) value. These results suggest that in studies of genomic association Landim will require a slightly lower marker density than Angone and Tete cattle.

We found a downward tendency in effective population size (N_e) over time, probably due to bottleneck effects such as natural disasters and civil war (Maciel, 2001; Bessa *et al*., 2009). Previous studies in Mozambique demonstrated that a large number of indigenous cattle are facing genetic erosion owing to inter-breeding with exotic breeds, particularly in the smallholder sector (FAO, 2015). This practice can contribute to a decrease in the population size of purebred indigenous populations. To ensure sufficient genetic variability, the Organization for Agriculture and Food (FAO) (FAO, 2013) suggested a N_e of 50 per generation. In our research, the estimated population size (N_e) (twelve generations ago) for the studied populations is above 50. Nevertheless, attention should be paid to keep N_e in these populations over the suggested threshold. The high effective population sizes observed in the current study indicate that there is a potential for appropriate selection and preservation of the studied cattle populations. However, the downward trend in N_e represents a narrow genetic pool for these Mozambican genetic resources. It is important to manage the effective population size to preserve genetic diversity in the indigenous Mozambican cattle populations for optimal utilization and sustainable development programs.

4.5 Conclusion

The present study aimed to evaluate the population structure and diversity of three Mozambican indigenous cattle populations. The results revealed moderate genetic variability and limited genetic differentiation among Mozambican indigenous cattle, but also indicated some genetic erosion, probably due to indiscriminate crossbreeding between the populations. Although poorly differentiated, Mozambican indigenous cattle still retain their genetic identity that could be exploited for further genetic improvement of especially adaptive traits, to face the future challenges of climate change. To maintain the high genetic diversity in Mozambican indigenous cattle, comprehensive conservation programs are needed. These could include the development of structured breeding schemes that incorporates the "in-situ" conservation centers existing in the country (such as Chobela, Angonia, and Impaputo breeding Stations) as well as the smallholder farmers (Maciel *et al*., 2013; Mwai *et al*., 2015).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alberro, M., 1983. The indigenous cattle of Mozambique. World Animal Review, 48, 12-17.

Alexander, D.H., Novembre, J. & Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research, 19, 1655-1664.

Barbato, M., Orozco-terWengel, P., Tapio, M. & Bruford, M.W., 2015. SNeP: A tool to estimate trends in recent effective population size trajectories using genome-wide SNP data. Frontiers in Genetics, 6,109-122.

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. & Alexandrino, P., 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African Journal of Animal Sciences, 39(1), 61-72.

Brito, L.F., Jafarikia, M., Grossi, D.A., Kijas, J.W., Porto-Neto, L.R., Ventura, R.V., Salgorzaei, M. & Schenkel, F.S., 2015. Characterization of linkage disequilibrium, consistency of gametic phase and admixture in Australian and Canadian goats. BMC Genetics, 16, 67-82.

Corbin, L.J., Liu, A., Bishop, S. & Woolliams, J., 2012. Estimation of historical effective population size using linkage disequilibria with marker data. Journal of Animal Breeding and Genetics, 129(4), 257–270.

Edea, Z., Bhuiyan, M., Dessie, T., Rothschild, M.F., Dadi, H. & Kim, K.S., 2014. Genome-wide genetic diversity, population structure and admixture analysis in African and Asian cattle breeds. Animal, 9, 1-9.

Edea, Z., Dadi, H., Dessie, T. & Lee, S., 2015. Genome-wide linkage disequilibrium analysis of indigenous cattle breeds of Ethiopia and Korea using different SNP genotyping Bead Chips. Genes and Genomics, 37, 759–765.

Edea, Z., Dadi, H., Kim, S.W., Dessie, T. & Kim, K.S., 2012. Comparison of SNP variation and distribution in indigenous Ethiopian and Korean Cattle (Hanwoo) populations. Genomics Informatics, 10(3), 200–205.

FAO (Food and Agriculture Organization), 2013. In vivo conservation of animal genetic resources. Rome, Italy.

FAO (Food and Agriculture Organization), 2015. The second report on the State of the World's Animal Genetic Resources for Food and Agriculture. Rome, Italy.

FAO (Food and Agriculture Organization), 2019. World Food and Agriculture – Statistical pocketbook 2019. Rome, Italy.

INE (National Institute of Statistics), 2016. Statistical Yearbook 2015 Mozambique. Maputo, Mozambique.

Kotze, A., Harun, M., Otto, F. & Van der Bank, F.H., 2000. Genetic relationship between three indigenous cattle breeds in Mozambique. South African Journal of Animal Science, 30(2), 92-97.

Lashmar, S., Visser, C., Van Marle-Koster, E. & Muchadeyi, F., 2018. Genomic diversity and autozygosity within the SA Drakensberger beef cattle breed. Livestock Science, 212, 111-119.

Maciel, S., 2001. National strategies for the conservation, improvement and utilization of animal genetic resources in smallholder systems. Proceedings of the Planning and Priority Setting Workshop on Animal Genetic Resources in the SADC region. SACCAR, CTA, ILRI, Gaborone, Botswana.

Maciel, S., Okeyo, A.M., Amimo, J., Scholtz, M.M., Neser, F.W.C., & Mart, M., 2013. The effect of geographical region of birth on the reproductive performance of the Nguni in southern Mozambique. South African Journal of Animal Science, 43 (1), 60-63.

Madilindi, M.A., Banga, C.B., Bhebhe, E., Sanarana, Y.P., Nxumalo, K.S., Taela, M.G., Mapholi, N.O., 2019. Genetic differentiation and population structure of four Mozambican indigenous cattle populations. Livestock Research for Rural Development, 31 (47).

Makina, S.O., Muchadeyi, F. C., Van Marle-Köster, E., MacNeil, M. D., Maiwashe, A., 2014. Genetic diversity and population structure among six cattle breeds in South Africa using a whole genome SNP panel. Frontiers in Genetics, 5, 333.

Makina, S.O., Taylor, J., Van Marle-Köster, E., Muchadeyi, F.C., Makgahlela, M., MacNeil, M.D. & Maiwashe, A., 2015. Extent of linkage disequilibrium and effective population size in four South African Sanga cattle breeds. Frontiers in Genetics, 6, 1-12.

Morgado, F.P., 2000. A pecuaria no norte de Moçambique. Vega: Portugal.

Morgado, F.P., 2004. A Pecuária no Centro de Moçambique. Vega: Portugal.

Mwai, O., Hanotte, O., Kwon, Y.J. & Cho, S., 2015. African indigenous cattle: unique genetic resources in a rapidly changing world. Asian-Australasian Journal Animal Sciences, 28, 911–21.

Ojango, M.P., Mpofu, N., Marshall, K. & Anderson-Eklund, L., 2011. Quantitative methods to improve the understanding and utilisation of animal genetic resources. International Livestock Research Institute, Nairobi, Kenya.

Onzima, R.B., Upadhyay, M.R., Mukiibi, R., Kanis, E., Groenen, M.A.M. & Crooijmans, R.P.M.A., 2018. Genome-wide population structure and admixture analysis reveals weak differentiation among Ugandan goat breeds. Animal Genetics, 49, 59–70.

Otto, F., Vilela, F., Harun, M., Taylor, G., Baggasse, P. & Bogin, E., 2000. Biochemical blood profile of Angoni cattle in Mozambique. Israel Journal Veterinary Medicine, 55 (3), 1-9.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J. & Sham, P.C., 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. American Journal Human Genetics, 81, 559-575.

Qwabe, S.O., Van Marle-Köster, E., Maiwashe, A. & Muchadeyi, F.C., 2013. Evaluation of the BovineSNP50 genotyping array in four South African cattle populations. South African Journal of Animal Science, 43, 64-67.

Rege, J.E.O. & Tawah, C.L.,1999. The state of African cattle genetic resources. II. Geographical distribution, characteristics and uses of present-day breeds and strains. Animal Genetic Resources Information, 26, 1-25.

Sharma, R., Kumar, B., Arora, R., Ahlawat, S., Mishra, A.K. & Tantia, M.S., 2016. Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. Meta Gene, 8, 14–20.

Szpiech, Z., Xu, J., Pemberton, T.J., Peng, W., Zoellener, S., Rosenburg, N.A. & Li, L., 2013. Long runs of homozygosity are enriched for deleterious variations. American Journal of Human Genetics, 93, 90- 102.

Twomery, A.J., Berry, D.P., Evans, R.D., Doherty, M.L., Graham, D.A. & Purfiled, D.C., 2019. Genome-wide association study of endo-parasite phenotypes using imputed whole-genome sequence data in dairy and beef cattle. Genetics Selection Evolution, 51, 15.

Van Niekerk, W. & Pimentel, P., 2000. Goat production in the smallholder section in the Boane district in Southern Mozambique, South African Journal of Animal Science, 34.

Vernooij, A., dos Anjos, M. & van Mierlo, J., 2016. Livestock Development in the Zambezi Valley, Mozambique: Poultry, Dairy and Beef Production. Wageningen University and Research Centre for Development Innovation. Report CDI-16-027. Wageningen.

Yang, J., Lee, S.H., Goddard, M.E. & Visscher, P.M., 2011. GCTA: A tool for genome-wide complex trait analysis. American Journal Human Genetics, 88, 76-82.

Zwane, A.A., Maiwashe, A., Makgahlela, M.L., Choudhury, A., Taylor, J.F. & Van Marle-Köster, E., 2016. Genome-wide identification of breed-informative single nucleotide polymorphisms in three South African indigenous cattle breeds. South African Journal Animal Science, 46, 302-312.

Genetic characterization of Mozambican Nguni cattle and their relationship with indigenous populations of South Africa

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Abstract

Knowledge of genetic variability among cattle populations is essential to gain insight into the adaptation mechanisms to different environments and to support the conservation of genetic resources. Individuals from Mozambican Nguni (MZ Nguni; n=119), South African Nguni (SA Nguni; n=150), South African Tuli (SA Tuli; n=150), and South African Boran (SA Boran; n=150) cattle populations were genotyped using the International Dairy and Beef SNP BeadChip version three (IDB) and the GeneSeek Genomic Profiler (GGP 80k) assays, to investigate their levels of genetic diversity and the relationships between these indigenous breeds. Levels of genetic diversity, assessed by expected heterozygosity (H_e), varied from 0.284 (SA Boran) to 0.324 (SA Tuli). Population structure, as well as principal component analysis (PCA), revealed tight clustering of the two Nguni populations, while the SA Tuli and SA Boran populations diverged, as expected, into two distinct clusters. Little genetic distance (0.031) was observed among MZ Nguni and SA Nguni, while SA Boran (a Zebu breed) was further removed from SA Tuli than from the other Sanga cattle populations. Runs of homozygosity (ROH) analysis revealed low inbreeding rates (with the average F_{ROH} per population ranging from 0.003 to 0.006). Short ROH segments (ROH ≤ 5 Mb) were more frequent in all four populations than longer segments, suggesting more ancient inbreeding in these populations. The highest number of ROH (303) was observed in SA Tuli, while the lowest (56) was detected in SA Nguni. Analysis of both Wright's fixation index (F_{ST}) and ratio of extended haplotype homozygosity (Rsb) identified a total of 229 differentiated single nucleotide polymorphisms (SNP) to be under selection, in a comparison between the MZ Nguni cattle and South African cattle populations. Highly differentiated SNP (F_{ST} ≥ 0.26 or *p*Rsb ≥ 3) indicated genes including *KLHL29*, *ZEB2*, *LAMC1*, *MYLK3*, and *KCNK5* that are implicated in several metabolic processes essential for adaptation and production traits.

Keywords: Boran, Admixture, SNPs, Dendrogram, Selection Signatures, Tuli

5.1 Introduction

Livestock is an important contributor to rural livelihoods in Southern Africa (Nyamushamba *et al*., 2017). Cattle provide a wide range of benefits including economic security through income from the sale of hides, milk, and meat (i.e., food security) and are commonly used in festivities and cultural rituals such as marriage and initiation ceremonies (Ibeagha-Awemu *et al*., 2019).

Archaeological and molecular evidence indicate that all modern breeds of cattle (indicine and taurine) originated from two subspecies of aurochs in two independent domestication events (Mwai *et al.,* 2015). Taurine cattle (*Bos taurus*) are presumed to have been domesticated in the Near East over 10,000 years ago and then brought to Africa through successive migrations (Van Marle-Köster *et al*., 2021). Zebu cattle (*Bos indicus*) emerged from the Indian subcontinent and reached the African continent around 4000 years ago. However, its expansion to North and East Africa only occurred around 1300 years ago, accompanying Arab migrations (Utsunomiya *et al*., 2019).

Recent genomic work suggests that taurine and indicine cattle were probably first hybridized 4500 years ago in the Near East and then dispersed to West and East Africa (Van Marle-Köster *et al*., 2021). These highly resilient livestock populations were kept in what is now known as the Sahara and reached East Africa between approximately 4000 and 3000 years ago (Horsburgh *et al*., 2013) and Southern Africa about 2000 years ago (Robbins *et al*., 2005; Robbins *et al*., 2008). Nowadays, the African continent is mainly inhabited by Zebu and taurine cattle, as well as their various derivatives (hybrids) known as Sanga (*Bos taurus africanus*) cattle (Mwai *et al*., 2015).

The Southern region of Africa (SADC) has more than 64 million head of cattle, distributed across various agroecological zones and included in different production systems and this has resulted in considerable phenotypic variation. Many of these cattle populations are named according to their origin and physical characteristics (Mapiye *et al*., 2019). For instance, Mozambique (MZ) and South Africa (SA) share transboundary indigenous Sanga cattle, including the Mozambican Nguni (MZ Nguni; known as Landim) and South African Nguni (SA Nguni) breeds. However, their names may not be an indication of any genetic differentiation among them.

Indigenous cattle, including the South African Boran (SA Boran), South African Tuli (SA Tuli), South African Nguni (SA Nguni), and Mozambican Nguni (MZ Nguni) have been naturally selected to withstand food shortages, high temperatures, and high incidence of parasites and diseases characteristic of the Southern African region (Nyamushamba *et al*., 2017).

Even though the important role of these indigenous populations, their large phenotypic diversity, and superior adaptive capacity to harsh environments have been acknowledged (Mwai *et al*., 2015; Mapiye *et al*., 2019), knowledge of their genetic diversity and the relationships among them is still lacking.

Genetic markers including single nucleotide polymorphisms (SNP), by means of genotyping panels, have been successfully used in Spain (Cañas-Álvarez *et al*., 2015), Sudan (Bahbahani *et al*., 2018), Ireland (Kelleher *et al*., 2017), and Mozambique (King *et al*., 2021) to examine patterns of genome-wide genetic relatedness, population structure (and admixture), and genetic diversity parameters within and among several indigenous cattle populations. The genetic relationship between indigenous breeds of cattle from Mozambique and South Africa has, however, received limited research attention. Genetic characterization of SA Boran, SA Tuli, SA Nguni, and MZ Nguni has been carried out previously, but was limited to within country studies (Bessa *et al*., 2009; Makina *et al*., 2016; Van der Westhuizen *et al*., 2019; Mamogobo *et al*., 2020). The only known molecular study investigating the genetic relationship among indigenous breeds of cattle in Mozambique and South Africa is by Madilindi *et al.* (2020), using microsatellite markers and small sample sizes (n=30).

The purpose of this study was to investigate the genetic diversity and relationships of four indigenous cattle populations from Mozambique and South Africa using genome-wide SNP markers. This information could be beneficial in the joint planning of conservation programs in both countries, as their indigenous cattle populations are in decline due to indiscriminate cross-breeding with imported exotic breeds (Mapiye *et al*., 2019).

5.2 Materials and Methods

Animals and sampling

Ethical approval for the sampling of the Mozambican Nguni (MZ Nguni) cattle population was obtained from the Ethics Committee of the University of Pretoria, Faculty of Natural and Agricultural Sciences, University of Pretoria (ECO25–18). Tail hairs were collected in the Gaza (n=33), Inhambane (n=60), and Maputo (n=26) provinces where the Nguni predominates. Permission to use the South African genotypic data was granted by the relevant breeder associations. Data included the genotypes of South African Nguni (SA Nguni; n=150), South African Tuli (SA Tuli; n=150) and South African Boran (SA Boran; n=150).

Genotyping and quality control

The 119 MZ Nguni individuals were genotyped at the Weatherbys Scientific Laboratory (Ireland) using the International Dairy and Beef BeadChip version three (IDB) SNP panel, composed of 53 450 genome-wide SNP (Twomery *et al*., 2019). Genotypes for the three South African cattle populations were generated from the GeneSeek® Genomic Profiler™ (GGP) 80K SNP genotyping panel, at the Agricultural Research Council's Biotechnology Platform (ARC-BTP). A set of 37 069 common SNPs were identified, and merged using PLINK v1.09 (Purcell *et al*., 2007). After excluding individuals exceeding 10% missing genotypes, removing SNP with minor allele frequencies (MAF) below 0.01 and a call rate of less than 0.90, 29 010 SNP and 559 individuals remained for further analysis.

Data analysis

Expected and observed heterozygosity (H_e and H_o) values and inbreeding rates (F_{IS}) were estimated with PLINK software (Purcell *et al*., 2007). Analysis of the principal components (PCA) was used to evaluate the within- and between-population diversity using GCTA version 1.24 (Yang *et al*., 2011). For the PCA, a genomic relationship matrix was created, from which the eigenvectors and eigenvalues were calculated. The eigenvectors for principal components 1 and 2 were then plotted against each other.

Genetic structure among the analyzed populations was confirmed by the 'find.clusters' function performing principal components discriminant analysis (DAPC) with Adegenet (Jombart and Collins, 2015), an R package version 3.3.2. This analysis ran consecutive clustering of K-means from K=1 to K=5 and the optimal cluster was chosen as the one with the lowest Bayesian Information Criterion (BIC) value (Jombart *et al*., 2010). Ancestry coefficients were calculated from K=2 through K=5 and, for each analysis, 100 iterations were performed and summarized using the snmf function of the LEA package in R. A graphical representation of the admixture patterns was depicted with GENESIS version 0.2.6.

Genetic distance between the populations was computed following the method proposed by Nei (1972) and implemented in StAMPP version 1.6.1 software (Pembleton *et al*., 2013). The matrix of genetic distances based on pairwise F_{ST} values was utilized to calculate the neighbour-joining dendrogram via the neighbor-joining method. The dendrogram was then plotted using the APE package in R version 3.3.2 (Paradis *et al*., 2004).

Estimates of effective population size (N_e) were performed according to Corbin *et al*. (2012) with SNeP version 1.1 (Barbato *et al*., 2015). Values from 0 to 1000 Mb were used for minimum and maximum inter-SNP distances, respectively. Data from each population were grouped into 30 bins of 50 kb distances. The N_e was estimated from the average r^2 value in each of these bins.

For each of the four populations, runs of homozygosity (ROH) were defined in PLINK v1.9 (Purcell *et al*., 2007) by: (1) using 50 SNP for each sliding window, (2) including a maximum of one heterozygous SNP, and a maximum of two missing SNP, (3) allowing 1 SNP every 75 kb as minimum density and, (4) allowing up to 1 Mb between successive homozygous SNP. Runs of homozygosity-based inbreeding (F_{ROH}) was computed as a fraction of the total ROH length (L_{ROH}) to the overall autosomal length covered by SNPs.

To detect regions that have undergone positive selection in the genome of the four cattle breeds, two inter-population-based statistics, namely Wright's fixation index (F_{ST}) and ratio of extended haplotype homozygosity (Rsb), were used. Genetic differentiation (F_{ST}) was computed using an unbiased estimator proposed by Weir & Cockerham (1984). Pairwise F_{ST} was computed using the $–F_{ST}$ functionality in PLINK v1.09 (Purcell *et al*., 2007) for each SNP comparing the MZ Nguni to each of the other populations in this study. Genomic regions representing the top 2 % of SNP ($F_{ST} \geq 0.25$), were considered as being under selection. To detect selection signals, Bonferroni corrected F_{ST} *P*-values were $–\log_{10}$ transformed and Manhattan plots were drawn using the qqman R package (Turner, 2014) with default settings. Bonferroni correction consisted of adjusting the critical significance level of 0.05, dividing it by the number of SNPs tested. This procedure was done with the STAT package in R (Bolar, 2019).

The ratio of Extended Haplotype Homozygosity (EHH) between populations (Rsb) approach was employed for a pairwise comparative analysis of EHH measures in each SNP between the Mozambican Nguni and each of the three South African populations, using the rehh R package version 1.11. To detect loci that were under selection, the values of Rsb were converted into *p*Rsb (*p*Rsb = $-\log[1 - 2 \times (\Phi_{(Rsb)} - 0, 5)]$), where the function $\Phi(x)$ represents the Gaussian cumulative distribution (Gautier & Vitalis, 2012). The Rsb scores were computed for each pairwise comparison of the studied breeds, and the SNPs for which *p*Rsb ≥3 (p − value = 0.001) were investigated.

Genes within putative selection signature regions were identified in the National Center for Biotechnology Information (NCBI), using the Bovine reference assembly ARS-UCD1.2. The function and metabolic processes in which these genes are implicated were identified through Panther (Mi *et al*., 2013).

5.3 Results

Quality control statistics

Quality control (Table 5.1) was performed on the common extracted SNP, and 8059 SNP were excluded. Of these, 25% had a call rate below 0.90, 20% had MAF under 0.01 and 55% deviated considerably from Hardy-Weinberg equilibrium (p<0.001). The highest number of excluded SNP (6043) was observed in the SA Boran population, whereas the lowest number (2697) was observed in SA Tuli cattle. Overall, 29 010 SNP (96.10%) and 559 individuals remained for downstream analyses.

Table 4.1 Animals and SNP excluded in the process of quality control for each studied cattle populations

Population	N	Omitted Individuals	Omitted SNP				Remaining SNP (%)
			CR	MAF	HWE	Total	
SA Boran	150	2	1 191	4 443	409	6 043	29 720 (98.25)
SA Nguni	150	7	894	2 464	318	3 676	33 393 (98.63)
SA Tuli	150	1	522	1 848	327	2 697	34 372 (99.16)
MZ Nguni	119	0	1 016	2 335	434	3 785	33 284 (99.02)
Merged	569	10	2 001	1 592	4 466	8 059	29 010 (96.10)

SNP Single nucleotide polymorphism**; CR** Rate of calling; **MAF** Minor Allele Frequency; **HWE** Hardy Weinberg Equilibrium (p<0.001); **SA Boran** South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

The mean MAF varied between 0.205 (±0.145) in the SA Boran and 0.242 (±0.147) in the SA Tuli. The observed heterozygosity value (H_o=0.298±0.153) was lower than the expected heterozygosity (H_e=0.313±0.158) value in the MZ Nguni, which also had the highest level of inbreeding (0.047±0.092). The SA Tuli population revealed the greatest levels of genetic variability (0.326±0.158). Negative (-0.025±0.053) to low positive (0.047±0.092) rates of inbreeding were detected among the four cattle populations (Table 5.2).

Table 4.2 Mean (±SD) estimated genetic diversity parameters and rates of inbreeding in the cattle populations

Studied cattle	N	MAF	H_e	H_o	F_{IS}
SA Boran	148	0.205±0.145	0.284±0.158	0.291±0.166	-0.025±0.053
SA Nguni	143	0.233±0.151	0.312±0.160	0.315±0.165	-0.007±0.044
SA Tuli	149	0.242±0.147	0.324±0.153	0.326±0.158	-0.009±0.052
MZ Nguni	119	0.234±0.150	0.313±0.158	0.298±0.153	0.047±0.092
Merged	559	0.226±0.147	0.306±0.157	0.293±0.150	-0.011±0.087

SD Standard deviation; **MAF** Minor Allele Frequency; **SA Boran** South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

The average MAF for the studied cattle populations showed little variation, with a range from 0.205±0.145 for SA Boran cattle to 0.242±0.147 for SA Tuli cattle. In all four cattle populations, the proportion of SNP in the lower MAF categories were higher compared to the higher MAF categories (Figure 5.1). The SA Boran had the least SNP with MAF exceeding 0.3.

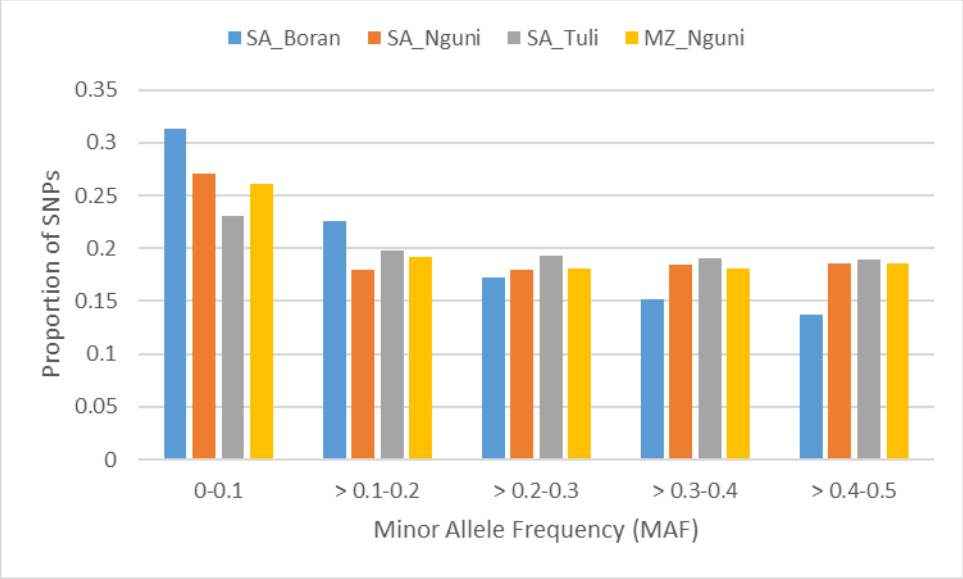

Figure 4.1 Distribution of SNP across MAF categories in each cattle population.

MAF Minor Allele Frequency**; SA Boran** South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

Runs of homozygosity

The overall number of ROH identified (Table 5.3) varied among populations, with the SA Tuli and SA Nguni populations displaying the highest (303) and lowest (56) number of ROH segments, respectively. Shorter ROHs were more abundant than longer ROH in all populations, suggesting more ancient inbreeding. The longest ROH segment (28.1 Mb) was found in the MZ Nguni population, which also had the largest mean number of SNP per ROH (93.92±50.87). The majority of ROH in all populations fell within the 1–5 Mb length category. The SA Tuli, followed by MZ Nguni and SA Boran, had the highest number of ROH segments in the largest length category (i.e., >15Mb). The estimated inbreeding coefficients were fairly uniform among the populations; SA Tuli exhibited the highest average F_{ROH} (0.006±0.003), whereas the lowest value was observed in the SA Nguni (0.003±0.003).

Table 4.3 Statistical parameters of ROH analyses in the four cattle populations

	SA Boran	**SA Nguni**	**SA Tuli**	**MZ Nguni**
Average length (Mb)	6.14±3.28	5.84±2.72	6.28±3.06	6.44±3.76
F_{ROH} (±SD)	0.004±0.003	0.003±0.003	0.006±0.003	0.004±0.004
F_{IS} (±SD)	-0.025±0.053	-0.007±0.044	-0.009±0.052	0.041±0.092
r (F_{ROH} – F_{IS})	0.430**	0.422**	0.462**	0.538**
Average number of SNP per ROH	88.56±43.76	85.71±40.32	92.07±42.80	93.92±50.87
N_e (12)	226	466	193	914
Maximum ROH length	24.52	15.77	19.93	28.10
Minimum ROH length	2.69	2.90	2.82	2.62
Number of ROH				
1–5 Mb	68	30	140	54
> 5–10 Mb	66	21	123	43
> 10-15 Mb	9	4	35	12
>15 Mb	4	1	5	4
Total	147	56	303	113

F_{ROH} ROH based inbreeding; **F_{IS}** Genomic based inbreeding; **r** Correlation; **N_e (12)** effective population size 12 generations ago; ****** (p<0.01); **ROH** Runs of Homozygosity; **Mb** Megabyte; **SA Boran** South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

Admixture and population structure analyses

Principal component analysis (PCA) was performed to examine genetic relationships between the four populations of cattle (Figure 5.2). The first and second principal components explained 5.32% and 2.46% of the variability, respectively. The animals investigated in this study were allocated to three clusters. The first cluster comprised the two Nguni populations (MZ, and SA). The second and third clusters consisted of the SA Boran and SA Tuli populations, respectively. The SA Tuli and SA Boran populations were the most genetically distant (F_{ST} = 0.027). Some individuals belonging to the MZ Nguni population were detected as outliers towards the SA Tuli and SA Boran populations, implying some genetic admixture with these populations or incorrect breed allocation.

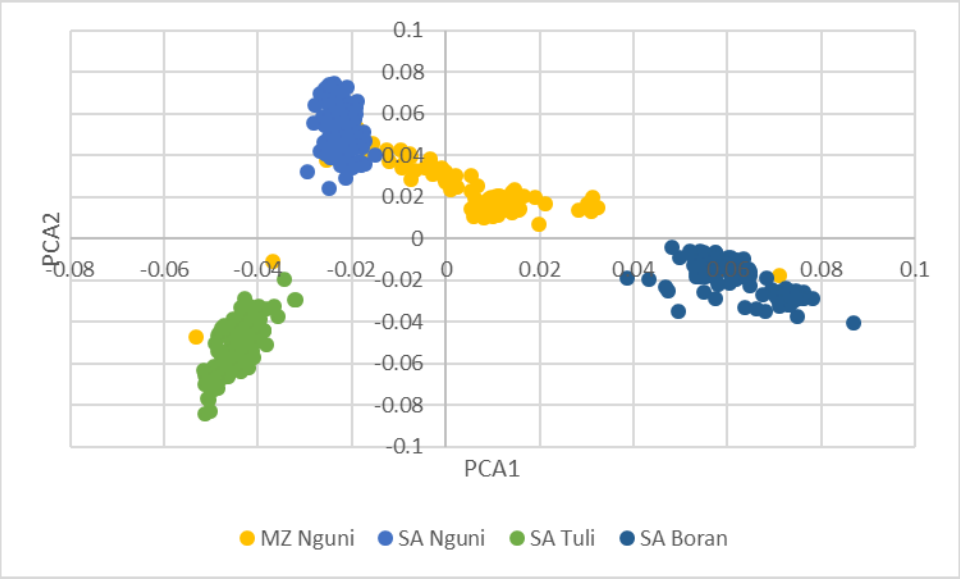

Figure 4.2 Genetic relationships between the four cattle populations

SA Boran South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni; **PCA** Principal component analysis

The most likely number of ancestral populations was estimated as three (K=3) based on the smallest Bayesian Information Criterion (BIC) value (Figure 5.3).

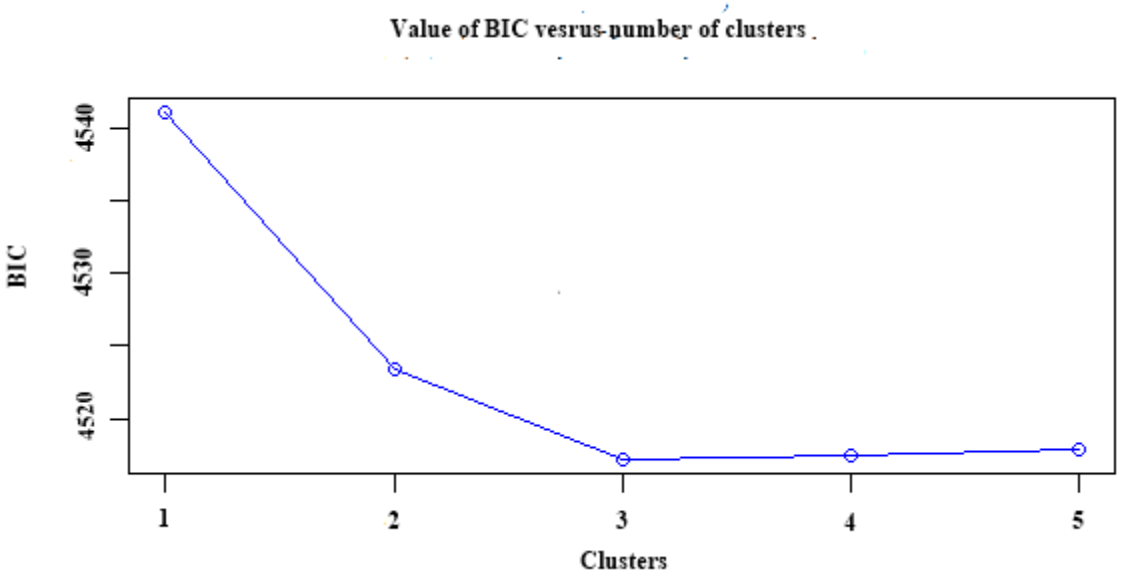

Figure 4.3 Bayesian Information Criterion (BIC) values versus number of clusters in the four cattle populations

The ADMIXTURE analysis supplied further evidence of the genomic similarity between the two Nguni populations, and the distinctness of this breed from the other breeds. At K=3, which was proposed to be the most probable number of clusters, the SA Boran and SA Tuli populations formed distinct clusters. The MZ Nguni and SA Nguni populations shared a common ancestor (Figure 5.4).

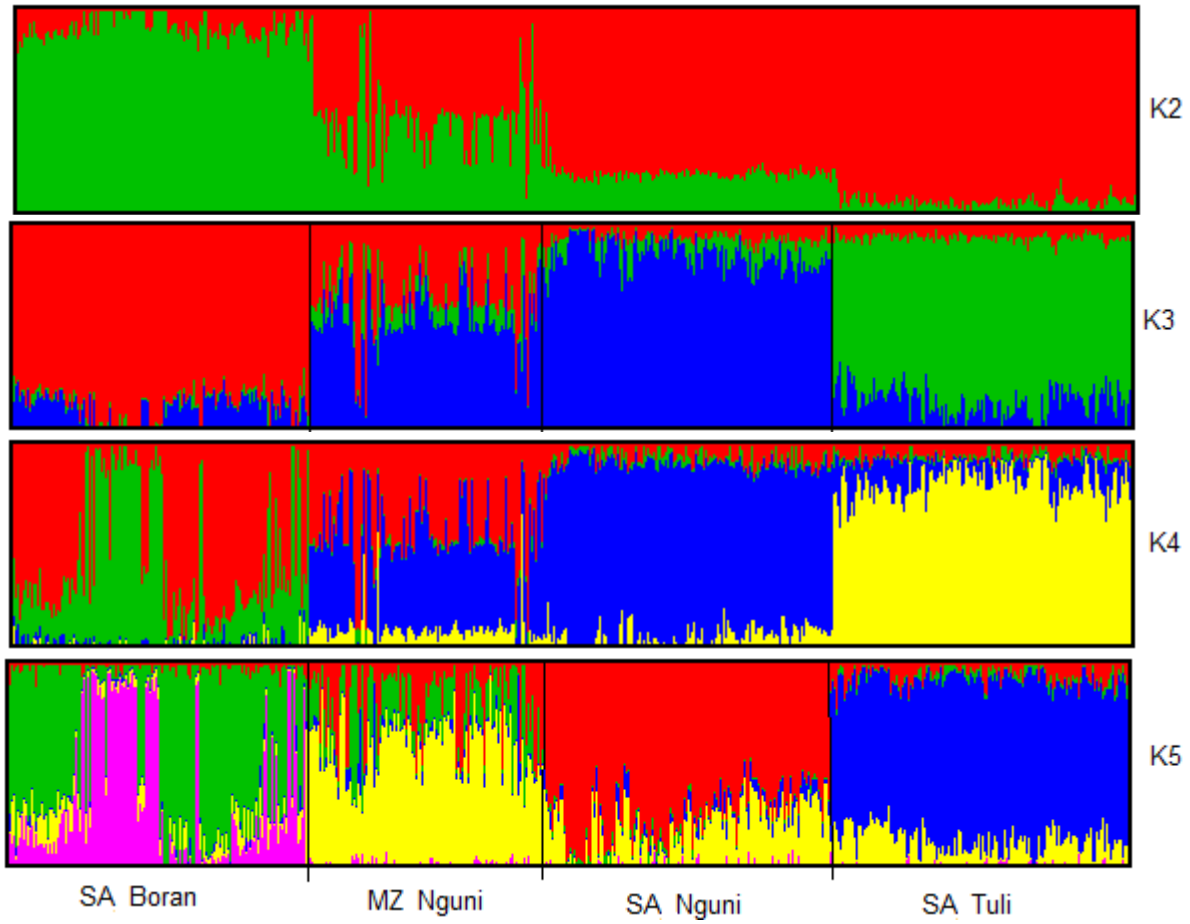

Figure 4.4 Admixture plot depicting the cluster allocation for the four cattle populations.
SA Boran South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

The SA populations were more uniform, with the within-population shared ancestry ranging between 81 % and 88 %, whereas the MZ Nguni population was more admixed and shared 55%, 35%, and 10% of their genome with SA Nguni, SA Boran, and SA Tuli populations, respectively (Table 5.4).

Table 4.4 Membership probability estimates for four studied cattle populations

	Main genetic clusters (K=3)		
Population	Cluster 1	Cluster 2	Cluster 3
SA Boran	**0.881**	0.019	0.100
SA Nguni	0.057	0.080	**0.863**
SA Tuli	0.058	**0.811**	0.131
MZ Nguni	0.351	0.103	**0.546**

SA Boran South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

Pairwise F_{ST} estimates revealed less differentiation between populations than expected. The smallest genetic distance was observed among the SA Nguni and MZ Nguni cattle and the largest between the SA Boran and SA Tuli populations. Of the SA breeds, the SA Nguni and SA Tuli Sanga breeds were the least related, with the indicine SA Boran being more related to the SA Tuli than the SA Nguni (Table 5.5).

Table 4.5 F_{ST}-based genetic differentiation among Mozambican and South African cattle populations

Population	**SA Boran**	**MZ Nguni**	**SA Nguni**	**SA Tuli**
SA Boran	***			
MZ Nguni	0.051	***		
SA Nguni	0.081	0.031	***	
SA Tuli	0.088	0.049	0.062	***

SA Boran South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli**; MZ Nguni** Mozambican Nguni

Figure 5.5 shows an individual-animal-based Neighbor-Joining (NJ) dendrogram for the 559 individuals. The groups formed in the NJ dendrogram were, in general, in agreement with the ADMIXTURE and PCA analyses. Most of the animals were grouped within their population in the dendrogram, although some of the MZ Nguni individuals were dispersed. Some MZ Nguni animals clustered with or towards the SA Nguni, SA Boran, and SA Tuli populations, indicating some genetic relationship.

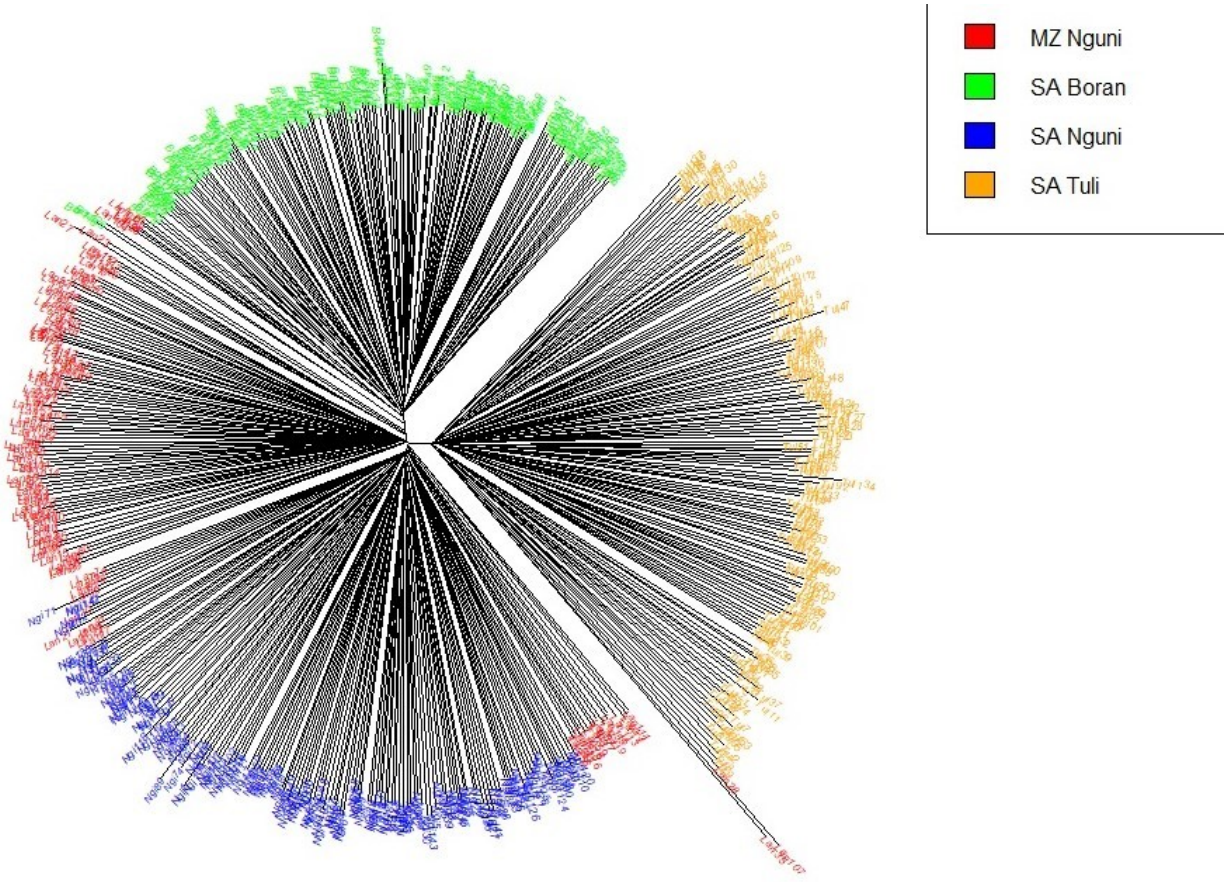

Figure 4.5 Unrooted NJ dendrogram depicting the relationships between animals from four indigenous Southern African cattle populations based on pairwise genetic distances.
SA Boran South African Boran; **SA Nguni** South African Nguni; **SA Tuli** South African Tuli; **MZ Nguni** Mozambican Nguni

Signatures of selection identified using F_{ST}

Figure 5.6 shows Manhattan plots of 72, 31, and 20 highly significantly differentiated SNP ($F_{ST} \geq 0.25$) between the MZ Nguni population and the SA Boran, SA Nguni, and SA Tuli populations, respectively. These SNP were located throughout the genome except for chromosomes (BTA) 15, 16, 20, 21, 26, 27, 28, and 29, which did not contain any selection signatures. One of the differentiated regions (on BTA4) was shared between the MZ Nguni vs SA Nguni and MZ Nguni vs SA Tuli breed comparisons. The MZ Nguni vs SA Boran pair had the most differentiated regions (72) while the MZ Nguni vs SA Tuli pair had the least (20). The most differentiated region (F_{ST} = 0.47) was detected between the MZ Nguni and SA Boran breeds on BTA11.

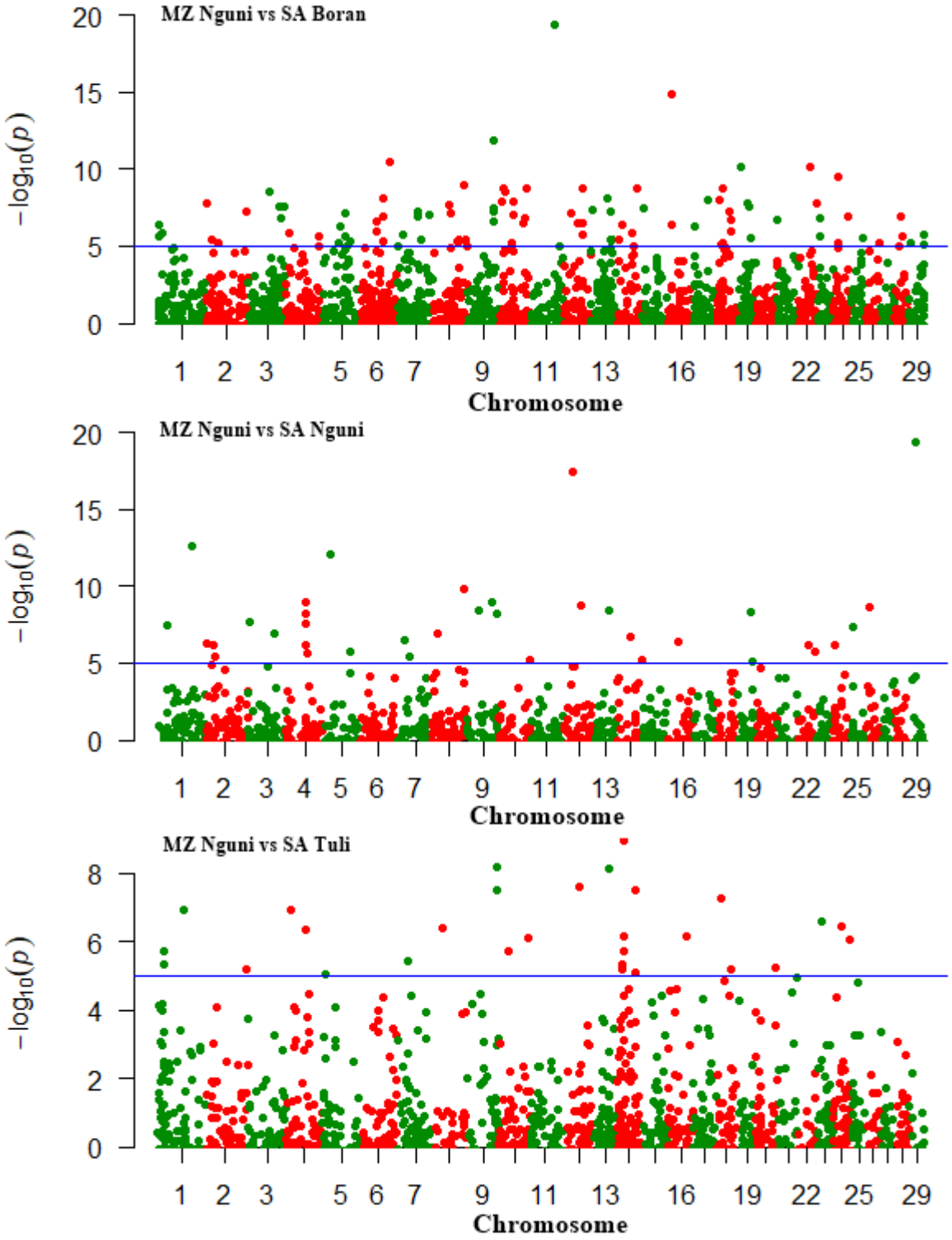

Figure 4.6 Manhattan plot of FST p-values in three pairwise cattle comparisons across the genome;

Blue line Suggestive significance line. **SA Boran** South African Boran**; SA Nguni** South African Nguni**; SA Tuli** South African Tuli; **MZ Nguni** Mozambican Nguni

Signatures of selection detected with Rsb approach

The Rsb analysis found 21 significant SNP (*p*Rsb ≥ 3) across ten chromosomes in the MZ Nguni vs SA Boran comparison. In the MZ Nguni vs SA Nguni comparison, a total of 36 SNPs located across eighteen chromosomes were significantly differentiated. Finally, in the MZ Nguni vs SA Tuli comparison, 49 significant SNPs were found spread over twenty-one chromosomes. One of these candidate regions was shared between the MZ Nguni vs SA Nguni and MZ Nguni vs SA Boran breed comparisons. SNP with scores above the mean (-log10 *p*-value ≥ 4) were found on BTA2 and BTA20 in the MZ Nguni vs SA Boran comparison, on BTA10 for the MZ Nguni vs SA Nguni comparison, and on BTA14 for the MZ Nguni vs SA Tuli comparison (Figure 5.7).

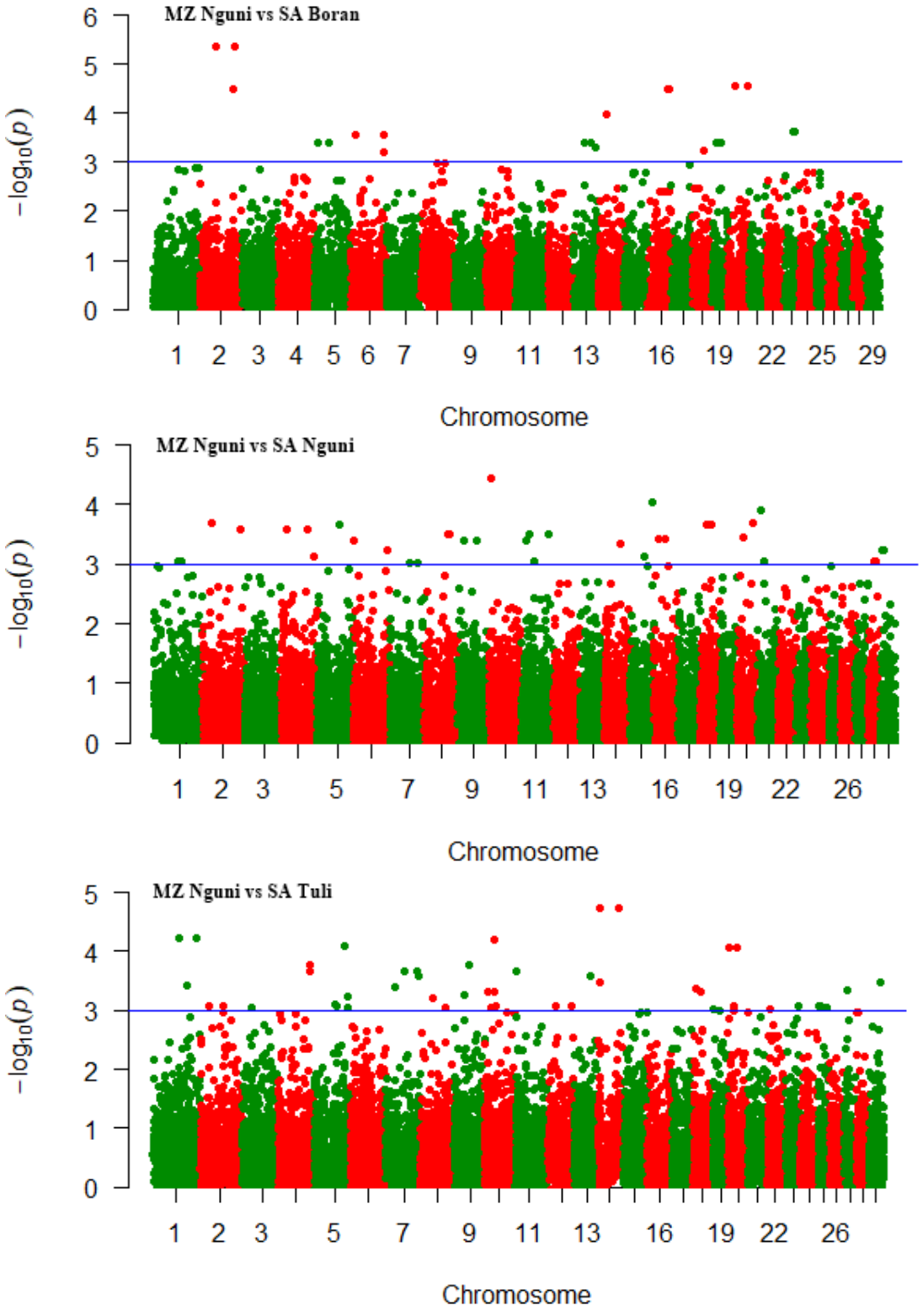

Figure 4.7 Manhattan plot of Rsb p-values in three pairwise cattle comparisons across the genome; Blue line, Suggestive significance line.

SA Boran South African Boran**; SA Nguni** South African Nguni**; SA Tuli** South African Tuli; **MZ Nguni** Mozambican Nguni

Using Panther analysis (Mi *et al*., 2013), a number of genes associated with important functions and pathways in cattle were identified. Corresponding genes were identified by both Rsb and F_{ST} analyses on chromosomes BTA1, BTA4, BTA8, BTA9, and BTA18. These genes are related to several biological functions, such as growth and feed efficiency (*TRNAC-GCA*), muscle movements and calving ease (*KCNMB2* and *MYLK3*), as well as oxytocin signaling pathway, and milking speed (*MYLK3*) (Table 5.6).

Other genes that warrant further investigation include *ZEB2*, which has been associated with polledness and congenital malformations, and *LAMC1*, which has been associated with parasites resistance in cattle. Genes related to riboflavin transport (e.g., *SLC52A3*), lipid metabolism and adipose tissue development (e.g., *NGFR*), cell differentiation and embryo development (e.g., *NGFR*, *FGD5*), renal potassium transport (e.g., *KCNK5*) and ciliogenesis (e.g., *CEP83*) were also identified.

Table 4.6 Genes under selection in three pairwise comparisons between Southern African cattle populations

Breed pair	BTA	Position (bp)		Method	Gene name
		Start	End		
MZ Nguni vs SA Boran	2	52174189	52264481	Rsb	*ZEB2*
	4	13061908	13441077	F_{ST}	*DYNC1I1*
	11	75240502	75572709	F_{ST}	*KLHL29*
	16	64062643	64183739	Rsb	*LAMC1*
	18	9138128	10154233	F_{ST}, Rsb	***CDH13***
	19	37093168	37112123	F_{ST}	*NGFR*
	22	57381762	57505298	F_{ST}	*FGD5*
MZ Nguni vs SA Nguni	1	88158068	88671406	F_{ST}, Rsb	***KCNMB2***
	4	17065366	17065438	F_{ST}, Rsb	***TRNAC-GCA***
	5	24070150	24216286	F_{ST}	*CEP83*
	8	33330545	33331036	F_{ST}, Rsb	***LOC782926***
	8	33969443	33971875	F_{ST}, Rsb	***LOC100141071***
	15	76852567	77063233	Rsb	*CSTPP1*
	18	15032402	15089534	F_{ST}, Rsb	***MYLK3***
	21	12595555	12855213	Rsb	*MCTP2*
MZ Nguni vs SA Tuli	4	17065366	17065438	F_{ST}, Rsb	***TRNAC-GCA***
	8	33330545	33331036	F_{ST}, Rsb	***LOC782926***
	8	33969443	33971875	F_{ST}, Rsb	***LOC100141071***
	9	17257479	17257550	F_{ST}, Rsb	***TRNAA-UGC***
	10	29455840	29970835	F_{ST}	*FMN1*
	13	60290424	60307559	F_{ST}	*SLC52A3*
	14	69265272	69301597	Rsb	*NDUFAF6*

	18	15032402	15089534	F_{ST}, Rsb	***MYLK3***
	23	13060758	13101202	F_{ST}	*KCNK5*

Bold indicate coincident genes; **SA Boran** South African Boran; **SA Nguni** South African Nguni; **SA Tuli**; **MZ Nguni** Mozambican Nguni

5.4 Discussion

Knowledge of genetic diversity and relationships amongst indigenous cattle populations is crucial for successful animal production, especially in the challenging environments of Southern Africa, and in the face of climate change (Nyamushamba *et al*., 2017). The genetic diversity of a population is essential for its genetic improvement, particularly for adaptive traits and those associated with sustainable production (Groeneveld *et al*., 2010; Mapiye *et al*., 2019). This work examined the genetic relationships and diversity between four cattle populations from Mozambique and South Africa.

The average MAF for the four populations was 0.226±0.147, with the highest proportion of SNP showing low MAF values (≤ 0.1). These findings correspond with values found in other indicine and indicine-hybrid (Sanga) breeds (Qwabe *et al*., 2013; Perez O'Brien *et al*., 2014; Lashmar *et al*., 2018; Gebrehiwot *et al*., 2021). Unlike indicine cattle (*Bos indicus*), taurine breeds (*Bos taurus*) generally have a higher percentage of SNP in the higher MAF categories as they are discovery and international transboundary breeds (Qwabe *et al*., 2013; Zwane *et al*., 2016; Lashmar *et al*., 2018). The lower values reported in indicine cattle have been associated with possible ascertainment bias due to the design and development process of commercial SNP panels (Perez O'Brien *et al*., 2014; Bejarano *et al*., 2018).

Heterozygosity is an indicator of genetic diversity within and between populations, which is important in the design of breeding programs and conservation strategies for indigenous cattle populations. Moderate genetic diversity was detected in the four studied cattle populations. These values were higher than those observed in South African Nguni cattle populations (0.23–0.24) by Zwane *et al*. (2016). They were, however, lower than expected heterozygosity values (0.40) reported in Sukuma, Tarime, and Maasai in Tanzanian indigenous Zebu populations (Msalya *et al*., 2017).

Similar estimates of observed and expected heterozygosity were reported for Sanga cattle from South Africa (Makina *et al*., 2014) and for indigenous Ethiopian cattle (Edea *et al*., 2012). Among the four studied populations, SA Tuli cattle showed the highest degree of genetic variability (H_e = 0.32) whereas SA Boran revealed the lowest. The lower H_e values of SA Boran cattle could reflect its smaller population size due to its late introduction to the country (Abin *et al*., 2016). High heterozygosity levels are usually related to long-term natural selection for adaptation and the admixture history of different populations (Gororo *et al*., 2018). However, uncontrolled breeding practiced in pastoral and agro-

pastoral production systems was reported to be responsible for the high genetic variability present in Barentu and Awgaro indigenous cattle populations of Eritrea (Goitom *et al*., 2019), and this could also be a contributing factor in the current findings.

The principal component analysis (PCA) indicated a strong genetic relationship between MZ Nguni and SA Nguni, as opposed to the SA Tuli and SA Boran. The close relationship between the MZ Nguni and SA Nguni could be explained by the common origin of these two cattle populations. Nguni cattle originated from North Africa and migrated southwards, settling in different areas including South Africa and Mozambique where distinctive cattle ecotypes were developed (Bester *et al*., 2003; Mwai *et al*., 2015). Furthermore, SA Nguni cattle were re-introduced to Mozambique after the civil war which had greatly reduced the national herd (King *et al*., 2021). The neighbor-joining dendrogram supported the PCA results as many MZ Nguni animals were blended with the SA Nguni, SA Boran, and SA Tuli populations, indicating some genetic proximity.

The results of the Admixture analyses supported the strong genetic relationship between the MZ Nguni and the SA populations. The MZ Nguni's genetic material is shared with all three South African populations, with the highest levels of similarity observed with the SA Nguni population. The genetic links observed among the populations suggest common genetic origin and a high degree of gene flow among populations (Radhika *et al*., 2018). MZ Nguni cattle have undergone a significant genetic bottleneck owing to the civil war (1977–1992), which significantly reduced their numbers. This period was followed by introgressions, as the Mozambican government imported cattle from neighboring countries, especially Zimbabwe and South Africa (King *et al*., 2021). Furthermore, gene flow may have been possible as a result of cattle migrations and trading, as well as indiscriminate cross-breeding practiced in communal management systems in both countries.

Generally, effective population size estimates in the present study are comparable with those of other indigenous cattle breeds in South Africa (Makina *et al*., 2015; Abin *et al*., 2016). The small effective population size of the SA Tuli could reflect the small founding population of this breed. The SA Tuli, which was developed and improved from Tswana cattle in Zimbabwe, was only introduced to South Africa in 1976 (Glennels, 2019). Among the studied populations, SA Tuli cattle had a relatively higher genomic inbreeding coefficient compared to the other three populations. Additionally, the SA Tuli has a relatively small population size of approximately 7000 animals, and might have undergone bottlenecks as suggested by the high number of ROH segments in the genome. The small number of long ROH segments found in this study indicates that none of the populations were subjected to recent inbreeding. The lower ROH abundance found in Nguni populations suggests that a relatively larger effective population size was preserved over generations.

Signatures of selection containing genes with important biological functions were identified using both Rsb and F_{ST} approaches. Overall, this study detected 229 signatures of selection, most of which were identified by a single methodology. Only eleven signatures of selection were identified by both the Rsb and F_{ST} approach. This may be due to differences in the parameters used in each methodology, thus detecting distinct traces left in the genome over time (Tang *et al*., 2007).

The *LAMC1* gene was found to be significantly differentiated in the MZ Nguni vs SA Boran comparison by Rsb analysis (Table 5.5). This gene was previously associated with resistance to *H. contortus* infection in Red Maasai and Dorper sheep breeds under natural infection conditions (Benavides *et al*., 2015). A region identified by Rsb analysis in the MZ Nguni vs SA Boran comparison (Figure 5.7) included the *ZEB2* gene. Recent studies indicate the association of this gene with polledness and congenital malformations in French Charolais cattle (Wiedemar *et al*., 2014).

Other important genes in the selected regions are directly or indirectly involved in reproduction, including fertilization and calving ease in cattle. These genes were identified by both Rsb and F_{ST} analyses and included *KCNMB2* and *MYLK3* in the MZ Nguni vs SA Nguni comparison (Buzanskas *et al*., 2017; Fraser *et al*., 2020). Indigenous Southern African cattle breeds are adapted to survive and reproduce without assistance in the extensive, harsh environments found in the region (Mwai *et al*., 2015). These findings support the existence of selection signatures at loci involved in reproduction which likely occurred in the course of adaptation of these cattle populations to southern African conditions.

5.5 Conclusions

Genomic studies examining the population structure of Mozambican indigenous cattle are scarce. This work is the first extensive study aiming to evaluate the genetic structure of MZ Nguni and its relationships with South African indigenous cattle using genome-wide SNP markers. The results of this research indicate low genetic differentiation among the four populations, which could be a consequence of both common ancestry and high gene flow rates. Most importantly, the study confirmed that the MZ Nguni and the SA Nguni have similar genetic ancestry. Furthermore, candidate genes were detected which may contribute to a better understanding of the genetic adaptation to various selection pressures. Finally, these findings point to the need for a common genetic resource management program between Mozambique and South Africa to conserve indigenous livestock in the region.

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References

Abin, S., Theron, H.E. & Van Marle-Köster, E., 2016. Short communication: Population structure and genetic trends for indigenous African beef cattle breeds in South Africa. South African Journal of Animal Science, 46, 152-156.

Bahbahani, H., Salim, B., Almathen, F., Al Enezi, F., Mwacharo, J.M. & Hanotte, O., 2018. Signatures of positive selection in African Butana and Kenana dairy zebu cattle. PLOS ONE, 13, e0190446. https://doi.org/10.1371/journal.pone.0190446.

Barbato, M., Orozco-terWengel, P., Tapio, M. & Bruford, M.W., 2015. SNeP: A tool to estimate trends in recent effective population size trajectories using genome-wide SNP data. Frontiers in Genetics. 6,109-122.

Bejarano, D., Martínez, R., Manrique, C., Parra, L.M., Rocha, J.F., Gómez, Y., Abuabara, Y. & Gallego, J., 2018. Linkage disequilibrium levels and allele frequency distribution in Blanco Orejinegro and Romosinuano Creole cattle using medium density SNP chip data. Genetics and Molecular Biology, 41, 2, 426-433.

Benavides, M.V., Sonstegard, T.S., Kemp, S., Mugambi, J.M., Gibson, J.P., Baker, R.L., Hanotte, O., Marshall, K. & Van Tassell, C., 2015. Identification of Novel Loci Associated with Gastrointestinal Parasite Resistance in a Red Maasai x Dorper Backcross Population. PLOS ONE, 10(4), e0122797.http://dx.doi.org/10.1371/journal.pone.0122797

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. & Alexandrino, P., 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African Journal of Animal Science, 39, 61-72.

Bester, J., Matjuda, I.E., Rust, J.M. & Fourie, H.J. 2003. The Nguni: a case study. In FAO Community based management of animal genetic resources. Rome: FAO:45-68.

Bolar, K., 2019. Interactive document for working with basic statistical analysis. Available at: http s://cran.r-project.org/web/packages/STAT/STAT.pdf.

Buzanskas, M.E., Grossi, D.A., Ventura, R.V., Flavio Schramm Schenkel, F.S., Chud, T.C.S., Stafuzza, N.B., Rola, L.D., Meirelles, S.L.C., *et al*., 2017. Candidate genes for male and female reproductive traits in Canchim beef cattle. Journal of Animal Science and Biotechnology, 8, 67.

Cañas-Álvarez, J.J., González-Rodríguez, A., Munilla, S., Varona, L., Díaz, C., Baro, J.A., Altarriba, J., Molina, A.A. & Piedrafita, J., 2015. Genetic diversity and divergence among Spanish beef cattle breeds assessed by a bovine high-density SNP chip. Journal of Animal Science, 93, 5164–74.

Corbin, L.J., Liu, A., Bishop S. & Woolliams, J., 2012. Estimation of historical effective population size using linkage disequilibria with marker data. Journal of Animal Breeding and Genetics, 129(4), 257–270.

Edea, Z., Dadi, H., Kim, S.W., Dessie, T. & Kim, K.S., 2012. Comparison of SNP variation and distribution in indigenous Ethiopian and Korean Cattle (Hanwoo) populations. Genomics Informatics, 10(3), 200–205.

Fraser, L., Paukszto, Ł., Mańkowska, A., Brym, P., Gilun, P., Jastrzębski, J.P., Pareek, C.S., Kumar, D. & Pierzchała, M., 2020. Regulatory Potential of Long Non-Coding RNAs (lncRNAs) in Boar Spermatozoa with Good and Poor Freezability. Life (Basel). 21, 10(11):300. doi: 10.3390/life10110300.

Gautier, M. & Vitalis, R., 2012. rehh: An R package to detect footprints of selection in genome-wide SNP data from haplotype structure. Bioinformatics, 28, 1176–1177. https: / / doi .org/ 10 .1093/ bioinformatics/ bts115.

Gebrehiwot, N.Z., Strucken, E.M., Marshall, K., Aliloo, H. & Gibson, J.P., 2021. SNP panels for the estimation of dairy breed proportion and parentage assignment in African crossbred dairy cattle. Genetics Selection Evolution, 53, 21.

Glennels, K., 2019. Tuli Cattle, Beef production in South Africa. Retrieved from South Africa's showcase of our collective heritage. Available online at: http://southafrica.co.za/tuli-cattle.html.

Goitom, S., Gicheha, M.G., Njonge, F.K. & Kiplangat, N., 2019. Genome-wide genetic diversity, population structure and admixture analysis in Eritrean Indigenous Cattle. South African Journal of Animal Science, 49 (6).

Gororo, E., Makuza, S.M., Chatiza, F.P., Chidzwondo, F. & Sanyika, T.W., 2018. Genetic diversity in Zimbabwean Sanga cattle breeds using microsatellite markers. South African Journal of Animal Science, 48(1), 128-141.

Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B. & Pilling, D., 2010. Genetic diversity in farm animals – a review. Animal genetics, 41(1), 6-31.

Horsburgh, K. A., Prost, S., Gosling, A., Stanton, J-A., Rand, C. & Matisoo-Smith, E.A., 2013. The Genetic Diversity of the Nguni Breed of African Cattle (*Bos spp*.): Complete Mitochondrial Genomes of Haplogroup T1. PLOS ONE, 8(8), e71956.

Ibeagha-Awemu, E.M., Peters, S.O., Bemji, M.N., Adeleke, M.A. & Do, D.N., 2019. Leveraging Available Resources and Stakeholder Involvement for Improved Productivity of African Livestock in the Era of Genomic Breeding. Frontiers in Genetics, 10, 357.

Jombart, T. & Collins, C., 2015. A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0.0. London: Imperial College London, MRC Centre for Outbreak Analysis and Modelling. Available online at: http://adegenet.r-forge.r-project.org/files/ tutorial-dapc.pdf.

Jombart, T., Devillard, S. & Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetic, 11, 94.

Kelleher, M.M., Berry, D.P., Kearney, J.F., Mcparland, S., Buckley, F. & Purfield, D.C., 2017. Inference of population structure of purebred dairy and beef cattle using high-density genotype data. Animal, 11,15-23. https://doi.org/10.1017/S1751731116001099.

King, F.J.M., Banga, C.B. & Visser, C., 2021. Genetic diversity and population structure of three native cattle populations in Mozambique. Tropical Animal Health and Production, 53, 117.

Lashmar, S., Visser, C., Van Marle-Koster, E. & Muchadeyi, F., 2018. Genomic diversity and autozygosity within the SA Drakensberger beef cattle breed. Livestock Science, 212, 111–119.

Madilindi, M.A., Banga, C.B., Bhebhe, E., Sanarana, Y.P., Nxumalo, K.S., Taela, M.G., Magagula, B.S. & Mapholi, N.O., 2020. Genetic diversity and relationships among three Southern African Nguni cattle populations. Tropical Animal Health and Production, 52, 753–762.

Makina, S.O., Muchadeyi, F.C., Van Marle-Köster, E., MacNeil, M.D. & Maiwashe, A., 2014. Genetic diversity and population structure among six cattle breeds in South Africa using a whole genome SNP panel. Frontiers in Genetics, 5, 333.

Makina, S.O., Taylor, J.F., Van Marle-Köster, E., Muchadeyi, FC., Makgahlela, M., MacNeil, M.D. & Maiwashe, A., 2015. Extent of linkage disequilibrium and effective population size in four South African Sanga cattle breeds. Frontiers in Genetics, 6, 1–12.

Makina, S.O., Whitacre, L.K., Decker, J.E., Taylor, J.F., MacNeil, M.D., Scholtz, M.M., Van Marle-Köster, E., Muchadeyi, F.C., Makgahlela, M.L. & Maiwashe, A., 2016. Insight into the genetic composition of South African Sanga cattle using SNP data from cattle breeds worldwide. Genetics Selection Evolution, 48, 88.

Mamogobo, M.D., Mapholi, N.O., Nephawe, K.A., Nedambale, T.L., Mpofu, T.J., Sanarana, Y.P. & Mtileni, B.J., 2020. Genetic characterisation of non-descript cattle populations in communal areas of South Africa. Animal Production Science, 61, 84–91.

Mapiye, C., Chikwanha, O.C., Chimonyo, M. & Dzama, K., 2019. Strategies for Sustainable Use of Indigenous Cattle Genetic Resources in Southern Africa. Diversity, 11, 214.

Mi, H., Muruganujan, A. & Thomas, P.D., 2013. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Research, 41, D377–86.

Msalya, G., Kim, E.S., Laisser, E.L., Kipanyula, M.J., Karimuribo, E.D., Kusiluka, L.J., *et al*., 2017. Determination of genetic structure and signatures of selection in three strains of tanzania shorthorn zebu, boran and Friesian cattle by genome-wide SNP analyses. PLOS ONE, 12, e0171088. doi: 10.1371/journal.pone. 0171088.

Mwai, O., Hanotte, O., Kwon, Y.J. & Cho, S., 2015. African indigenous cattle: unique genetic resources in a rapidly changing world. Asian-Australasian Journal of Animal Sciences, 28, 911–921

Nei, M., 1972. Genetic distance between populations. The American Naturalist, 106, 283-292.

Nyamushamba, G., Mapiye, C., Tada, O., Halimani, T. & Muchenje, V., 2017. Conservation of indigenous cattle genetic resources in Southern Africa's smallholder areas: Turning threats into opportunities – A review. Asian-Australasian Journal of Animal Sciences, 30, 603–622.

Paradis, E., Claude, J. & Strimmer, K., 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289–290. doi:10.1093/bioinformatics/btg412.

Pembleton, L.W., Cogan, N.O.I. & Forster, J.W., 2013. StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Molecular Ecology Resources, 13, 946-952.

Perez O'Brien, A.M., Utsunomiya, Y.T., Meszaros, G., Bickhart, D.M., Liu, G.E., Van Tassell, C.P., Sonstegard, T.S., Da Silva, M.V., Garcia, J.F. & Solkner, J., 2014. Assessing signatures of selection through variation in linkage disequilibrium between taurine and indicine cattle. Genetics Selection Evolution, 46, 19.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J. & Sham, P.C., 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics, 81, 559–575.

Qwabe, S.O., Van Marle-Köster, E., Maiwashe, A. & Muchadeyi, F.C., 2013. Evaluation of the BovineSNP50 genotyping array in four South African cattle populations. South African Journal of Animal Science, 43, 64-67.

Radhika, G., Aravindakshan, T.V., Jinty, S. & Ramya, K., 2018. Evaluation of genetic diversity, population structure, and relationship between legendary vechur cattle and crossbred cattle of Kerala State, India. Animal Biotechnology, 29, 50–58. doi:10.1080/10495398.2017.1297719.

Robbins, L.H., Campbell, A.C., Murphy, M.L., Brook, G.A., Liang, F., Skaggs, S.A., Srivastava, P., Mabuse, A.A. & Badenhorst, S., 2008. Recent archaeological research at Toteng, Botswana: Early domesticated livestock in the Kalahari. Journal of African Archaeology, 6, 131–149.

Robbins, L.H., Campbell, A.C., Murphy, M.L., Brook, G.A., Srivastava, P. & Badenhorst, S., 2005. The Advent of Herding in Southern Africa: Early AMS Dates on Domestic Livestock from the Kalahari Desert. Current Anthropology, 46, 671–677.

Tang, K., Thornton, K.R. & Stoneking, M., 2007. A new approach for using genome scans to detect recent positive selection in the human genome. PLOS Biology, 5 (7), e171.

Turner, S.D., 2014. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. BiorXiv, pp 005165, 1-2.

Twomery, A.J., Berry, D.P., Evans, R.D., Doherty, M.L., Graham, D.A. & Purfiled, D.C., 2019. Genome-wideassociation study of endo-parasite phenotypes using imputed whole-genome sequence data in dairy and beef cattle. Genetics Selection Evolution, 51, 15.

Utsunomiya, Y.T., Milanesi, M., Fortes, M.R.S., Porto-Neto, L.R., Utsunomiya, A.T.H., Silva, M.V.G.B., Garcia,J.F. & Ajmone-Marsan, P., 2019. Genomic clues of the evolutionary history of *Bos indicus* cattle. Animal genetics, 50 (6), 557-568.

Van der Westhuizen, L., MacNeil, M. D., Scholtz, M.M., Neser, F.W.C., Makgahlela, M.L. & Van Wyk, J.B., 2019. Genetic variability and relationships in nine South African cattle breeds using microsatellite markers. Tropical Animal Health and Production, 52, 177–184

Van Marle-Köster, E., Visser, C., Sealy, J. & Frantz, L., 2021. Capitalizing on the Potential of South African Indigenous Beef Cattle Breeds: A Review. Sustainability, 13(8), 4388.

Weir, B.S. & Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38, 1358-70.

Wiedemar, N., Tetens, J., Jagannathan, V., Menoud, A., Neuenschwander, S., Bruggmann, R., Thaller, G. & Drogemuller, C., 2014. Independent Polled Mutations Leading to Complex Gene Expression Differences in Cattle. PLOS ONE, 9(3), e93435. doi: 10.1371/journal.pone.0093435

Yang, J., Lee, S.H., Goddard, M.E. & Visscher, P.M., 2011. GCTA: A tool for genome-wide complex trait analysis. American Journal of Human Genetics, 88, 76–82.

Zwane, A.A., Maiwashe, A., Makgahlela, M.L., Choudhury, A. & Taylor, J.F., Van Marle-Köster, E., 2016. Genome-wide identification of breed-informative single nucleotide polymorphisms in three South African indigenous cattle breeds. South African Journal of Animal Science, 46, 302-312.

Critical review, conclusions and recommendations

6.1 Critical review

Introduction

Millions of smallholder farmers in Mozambique depend on indigenous cattle breeds for their livelihoods. Despite facing challenges such as feed scarcity, endemic diseases, and poor husbandry (Vernooij *et al*., 2016; Cambaza, 2018), these breeds are endowed with adaptive traits such as tolerance to drought, heat and poor-quality feed (Maciel *et al*., 2013; Mwai *et al*., 2015). These and other adaptive characteristics allow indigenous cattle to maintain productivity and survival under diverse agro-ecological conditions, and their low-input requirements make them suitable for communal farming (Nyamushamba *et al*., 2017). In spite of their valuable adaptive traits, the unique genetic composition of these populations is being diluted due to poor animal recording, uncontrolled crossbreeding and poor genetic resource utilization and management (Rischkowsky *et al*., 2007). Therefore, there is a need to develop strategies to preserve this genetic pool in Mozambique. Breed characterization is a prerequisite to developing sound strategies for sustainable utilization and conservation of animal genetic resources (AnGR).

The development of highly informative genetic markers and high throughput genotyping methods, in recent times, has provided efficient tools for the genetic characterization of livestock breeds. It has been strongly recommended that the use of phenotypic data should be complemented with genotypic information for successful conservation programs (Ali, 2003; Galal *et al*., 2008). In terms of morphology, the physical attributes of indigenous cattle may reflect adaptation to harsh environments. In this thesis, a morphometric study was first carried out to determine the level of phenotypic variation among indigenous cattle populations employing sixteen morphometric characteristics (Chapter 3). In Mozambique, information on genetic diversity and/or the extent to which the gene pool of native cattle breeds has been disturbed by migration and introgression, particularly after restocking programs, is scarce. Thus, the next step was to evaluate genetic variability, population structure as well as admixture levels in Mozambican native cattle populations using genome-wide SNP panels (Chapter 4). Lastly, the genetic relationship of Mozambican Nguni cattle with three South African indigenous cattle breeds was studied. This is important as it provides information on the regional genetic diversity of these cattle populations, in order to develop a joint plan for their sustainable management and use (Chapter 5).

Morphological Characterisation

Morphological characterization assists in the description and differentiation of breeds and is used by smallholder farmers more often than other means of characterization (Sheriff *et al*., 2021). The patterns of morphological diversity of indigenous cattle populations in Mozambique have not been fully studied, and remain unknown. Some studies have been carried out globally to relate morphological variations to environmental adaptation (Saleem *et al*., 2013; Khan *et al*., 2013; Mani *et al*., 2014); however, similar research has not been conducted in Mozambican indigenous cattle.

Yougbaré *et al*. (2020) observed that the variation of linear body measurements in cattle is a consequence of both genetic and environmental effects and that its magnitude depends on management and environmental conditions. The current study identified six morphological measures (BL, HL, RW, WH, RH, and TL) to be important in differentiating the three Mozambican cattle populations studied, with body length (BL) having the highest discriminant power. The Landim had higher morphometric measurements than Tete and Angone cattle. This variation could be due to the breeds adapting to different agro-ecological regions. The Landim is predominantly found in the lowland regions, whereas the Tete and Angone cattle are mainly from the highland areas where feed is scarce. The smaller body size of these cattle (as compared to Landim cattle) is recognized as being valuable for surviving in harsh environments, due in part to the lower maintenance requirements.

Indigenous Mozambican cattle are widely known and identified based on their morphological characteristics (Alberro, 1983; Tomo, 1997). However, dependence on phenotypic data as the basis for designing conservation programs is generally subjective (Okomo-Adhiambo, 2002). The characterization of these cattle breeds using DNA information is more reliable and necessary, as it is based on accurate genomic information (Ajmone-Marsan *et al*., 2014). It was therefore necessary, in the current study, to conduct further characterization of the three breeds using DNA markers.

Genetic diversity and population structure

Successful conservation of the biodiversity of native livestock breeds largely depends on a thorough understanding of the genetic variability in the population under study. Genetic characterization of indigenous breeds of Mozambican cattle can provide insights into their adaptation to tropical regions and contribute to their development and improvement.

Mozambican cattle breeds were previously characterized using low-density microsatellites and small sample sizes (Bessa *et al*., 2009; Madilindi *et al*., 2019), which revealed low genetic distinctiveness among the breeds. Microsatellites, although widely used in biodiversity studies, have the disadvantage of being located in non-coding areas of the genome and not being directly related to the genes responsible for the phenotypes (FAO, 2011).

The current study used medium-density SNP markers, which provide consistent genomic information, for analyzing the structure and genetic diversity of the populations (Kijas *et al*., 2013), and are therefore preferred in conservation programs. Genetic diversity and linkage disequilibrium analyses indicated that indigenous Mozambican cattle breeds have maintained high genetic diversity levels and low inbreeding rates. The high levels of diversity observed suggest that these populations are able to respond positively to selection for production traits and that genetic improvement can be attained. However, their small frame size and capacity to survive and reproduce in harsh low-input habitats (especially Angone) suggest that they may carry genes for adaptation to harsh and low-input situations. These animals seem to possess a superior balance between production and adaptation, relative to exotic cattle. Furthermore, this study confirmed significant differences between the populations in the extent of LD. The Landim cattle had the highest proportion of LD in comparison with other indigenous breeds from Mozambique. It would appear that Landim cattle were subject to stronger selection pressures (since they are the most abundant and utilized) than the other indigenous breeds, and fewer SNPs would be required to conduct genomic studies.

Population structure analysis revealed some overlap between the breeds and limited genetic differentiation. The three breeds (Landim, Angone and Tete) were allocated into two rather than three main clusters. This might be the result of admixture between breeds or can result from the breeds being descendants of a common ancestral population. The results reported here suggest that Tete cattle were derived from the Landim breed, as it shares more of its genome with the latter than it does with the Angone. This is a significant finding that needs to be considered in improvement and conservation programs. To determine the exact cause of the low differentiation, it would be necessary to carry out further analyses, such as mitochondrial DNA (mtDNA) sequencing.

The effective population size (N_e) tended to decrease over time in the three populations studied, which increases the risk of inbreeding and genetic drift that can lead to biological bottlenecks (Rischkowsky *et al*., 2007). Mozambican cattle populations were affected by a significant genetic bottleneck. As a result of the civil war (1977 to 1992), nearly 80% of the national herd was decimated. A lack of record keeping and indiscriminate crossbreeding were contributing factors, as these are common practices among smallholder communal systems. Adapting to new environments requires genetic diversity of animals (Nyamushamba *et al*., 2017). Therefore, the decrease in diversity of genetic resources could jeopardize the adaptation of livestock to climate change, thus compromising their improvement and, consequently, food security for the smallholder farmers.

In recognition of the diversity of indigenous cattle, the government of Mozambique has established livestock research and conservation centers to maintain and improve these unique animal genetic resources. For instance, Angone and Landim cattle are kept at the Angonia and Chobela research stations, respectively. Even though these centers are not fully functional, they offer opportunities for the utilization and development of indigenous breeds (Nyamushamba *et al*., 2017). Steps toward increasing diversity, such as the development of structured breeding schemes (Maciel *et al*., 2013; Mwai *et al*., 2015) should be prioritized.

Genetic relationship among indigenous cattle populations of Mozambique and South Africa

The last chapter of this thesis investigated the genetic variability and relationships of four indigenous cattle populations from Mozambique and South Africa through genome-wide SNP panels. Information pertaining to the amount of genetic variation amongst breeds in the whole Southern Africa Development Community (SADC) region is of utmost importance (Mapiye *et al*., 2019) and more data will be needed for this purpose. This study, however, is a valuable starting point in developing sound regional AnGRs conservation strategies, as the knowledge generated can be used as baseline information. Most indigenous cattle breeds in the region look similar; however, they have different names and geographical habitats. It was, therefore, necessary to ascertain whether they might be regarded as distinct populations, for conservation purposes.

The Nguni (Landim) populations of Mozambique and South Africa are genetically linked and share the same ancestry. The cattle followed the same migratory route with nomadic people who migrated from North Africa and settled in different parts of Southern Africa (Mwai *et al*., 2015). These populations may also have undergone similar selection pressures, although they are geographically

separated. They are mostly kept in extensive breeding systems, with low levels of artificial selection. As a consequence, these breeds have become adapted to a wide range of environments and are heat tolerant and resistant to local disease epidemics. A significant finding of the study was confirmation that the Landim and the SA Nguni are both ecotypes of the Nguni group.

Domestication of cattle shaped the pattern of genetic variation through natural and artificial selection (Van Marle-Köster *et al*., 2021). This research investigated whether the genome targeted by selection showed signs of positive selection. A total of 123 fragments that are potentially under selection between three pairwise cattle populations in the two countries were detected. One of these differentiated regions (BTA4) was shared between the MZ Nguni vs SA Nguni and MZ Nguni vs SA Tuli breed pairs. This shared region between these breeds could be because they have been exposed to similar selection pressures.

Indigenous breeds are generally kept in environments that are less favorable to their exotic counterparts who lack adaptive traits (Rothschild & Plastow, 2014). Due to continuous exposure to adverse environments, indigenous breeds have adapted over generations, making them more tolerant to tropical diseases (Porto-Neto *et al*., 2014). Indigenous cattle, including the SA Boran, SA Tuli, SA Nguni, and MZ Nguni have been naturally selected to withstand the harsh agro-ecological conditions of Southern Africa (Nyamushamba *et al*., 2017). Based on our analysis of four cattle populations from Southern Africa, genomic regions/genes associated with adaptation were identified. Selection signature analysis identified genes associated with adaptation including *KCNMB2* and *MYLK3* which are involved in muscle movements and calving ease, *TRNAC-GCA* involved in growth and feed efficiency and *LAMC1*, associated with parasites resistance in cattle.

In the current study, strong evidence suggests genetic similarities between the Mozambican Nguni cattle and South African indigenous cattle as a result of common ancestry as well as high gene flow rates. Interestingly, the results support the presumption that the Mozambican Nguni and South African Nguni belong to the same population. In the identified genomic regions, there are several candidate genes associated with numerous biological functions that are fundamental to improved survival of these cattle in their natural environment. Finally, these findings point to the need for the establishment of a common genetic management program between Mozambique and South Africa, aimed at safeguarding the indigenous Southern African cattle.

6.2 Study limitations and Challenges

This study employed a sampling strategy designed to capture the diversity of indigenous breeds across the country's agro-ecological regions. Sampling was conducted in the main cattle producing regions of the country and targeted the provinces where each breed is predominantly found (Maputo, Gaza, Inhambane and Tete). These cattle populations represented the main indigenous breeds raised extensively on smallholder farms, as well as those that are conserved in some research stations.

As in all research, in this study there were also constraints in the course of data collection. One of the constraints found by the sampling team was the lack of handling facilities and the communal nature of the farming system. Animals were generally released early for communal grazing, which left the sampling team with a very short window of opportunity for handling the animals. Added to this, is the fact that morphometric data and hair collection had to take place without the assistance of head clamps or animal crushes. The team had to collect data from a considerable number of farms in a very short period of time. In addition, the data collection was interrupted several times due to heavy rains that fell during the sampling period. Furthermore, some cattle keepers were reluctant to take part in the study for various reasons. Regardless of these challenges, the results presented in this study provide sound baseline information for conservation and improvement programs, taking into account the adaptive attributes and available genetic diversity in these indigenous cattle.

Although the genetic diversity results are comparable to those from other studies, more work is still needed in characterizing indigenous Mozambican cattle based on SNP array genotyping, including much larger and balanced populations with more representatives of each breed at each location. It is sometimes prohibitively expensive to collect comprehensive data, which puts a limit on research, particularly in developing countries such as Mozambique. Some important rare variants may not have been detected due to the limitations of the medium density panel, specifically due to ascertainment bias. Using a higher density panel (at least 300 000 SNPs) has been suggested for cattle, particularly for between breed analyses and selection signatures (Goddard & Hayes, 2009). However, the high cost of high-density assays often prohibits their use in developing countries.

6.3 Conclusions and recommendations

The results of this study provide new insights into genetic and phenotypic variation that can serve as guidelines in conservation programs for indigenous Mozambican cattle breeds. Since sampling was carried out in only 4 of the country's 11 provinces, complementary studies covering other provinces will be needed to describe the national genetic diversity of Mozambican indigenous cattle.

In Africa, studies on genetic diversity have recommended conserving and utilizing indigenous livestock genetic resources. However, many of the conservation programs that have been initiated in Mozambique have achieved limited success, due to reasons such as political instability and lack of funds, viable agricultural policy or farmer cooperation. Successful conservation programs are highly dependent on government support. Raising awareness among policy-makers regarding the importance of animal genetic resources (AnGR) could further encourage government to allocate more resources to their conservation.

Judging by the high genetic diversity reported in this study, it is confirmed that indigenous Mozambican cattle are an important genetic resource with the potential to increase productivity for food security and the livelihoods of small holder farmers. These genetic resources are of even greater importance in a world undergoing constant environmental changes. The phenotypic and genetic diversity reported in the current investigation provide a good basis for genetic improvement through selection. However, given the financial fragility that characterizes the smallholder farming system, where indigenous cattle are mostly raised, government support is essential for these improvement and conservation programs to succeed.

Future attention should focus on expanding this study to more agro-ecological areas, for example the northern regions of the country, which were not included in this study. As a result, Mozambican cattle breeds will be represented by their genetic diversity nationwide. An additional step would be to associate these genes with phenotypes. The use of panels with high density in native cattle would allow us to examine their genome with high-definition. With a denser panel of SNP markers, linkage disequilibrium (LD) between markers and functional genes will be increased, which will facilitate the identification of marker-trait associations in genome-wide association studies (GWAS). Sound animal recording and national livestock improvement programs are also necessary to build datasets with accurate pedigree and phenotypic data.

Association studies based on linkage disequilibrium, are essential for identifying genetic variants in livestock species that are related to trait attributes such as adaptability, disease resistance, heat resistance and drug response. The selection sweeps and genes identified in the current study should be validated using other methodologies which are more accurate such as GWAS, candidate gene approach and gene expression analysis, or whole genome sequencing.

The success of an *in-situ* conservation program for indigenous breeds depends on the active participation, not only of government and other institutional stakeholders, but also of the communities that keep these breeds. In the long-term interest of these populations in their natural habitat, breeders should take measures to conserve genetic diversity in these livestock populations.

As a starting point, it is necessary to establish breeders' societies to maintain and genetically improve the breeds, while conserving their genetic diversity with good management and selection programs. Additionally, a registration authority responsible for registering these livestock populations should be set up. In Mozambique, record keeping is almost non-existent and the transmission of information depends mainly on hearsay. Therefore, in order to implement a successful conservation program, it is proposed that an efficient national animal recording and improvement scheme should be started. The scheme has to be based on sound animal husbandry principles and should concentrate on effective identification of animals and the recording of basic information on births, birth weights, weaning and post weaning weights. This scheme should be backed up with an effective extension service for maximum effect.

It is recommended that the results of this thesis be applied in the formulation of a joint regional strategy for indigenous cattle breeds in the region to be conserved and sustained appropriately. This will contribute towards improving regional food security, farming communities' livelihoods and economic growth.

References

Ajmone-Marsan, P., Colli, L., Han, J.L., Achilli, A., Lancioni, H., Joost, S., Crepaldi, P., Pilla, F., Stella, A., Taberlet, P. & Boettcher, P., 2014. The characterization of goat genetic diversity: Towards a genomic approach. Small Ruminant Research, 121(1), 58-72.

Alberro, M., 1983. The indigenous cattle of Mozambique. World animal review, 48, 12-17.

Ali, B.A., 2003. Genetic similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers. African Journal of Biotechnology, 2(7), 194-197.

Bessa, I., Pinheiro, I., Matola, M., Dzama, K., Rocha, A. & Alexandrino, P., 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African Journal of Animal Science, 39, 61-72.

Cambaza, E., 2018. A glance at Mozambican dairy research. African Journal of Agricultural Research, 13(53), 2945-2956.

FAO (Food and Agriculture Organization), 2011. Draft Guidelines on Developing the Institutional Framework for the Management of Animal Genetic Resources. Commission on Genetic Resources for Food and Agriculture, FAO, Rome.

Galal, S., Gürsoy, O. & Shaat, I., 2008. Awassi sheep as a genetic resource and efforts for their genetic improvement - A review. Small Ruminant Research, 79, 99-108.

Goddard, M. E. & Hayes, B. J., 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics, 10, 381-91.

Khan, M., Rhahim, H., Rueff, S., Jalali, S., Saleem, M., Maselli, D., Muhammad, S. & Wiesmann, U., 2013. Morphological characterization of the Azikheli buffalo in Pakistan. Animal Genetic Resources information, 52, 65-70.

Kijas, J.W., Ortiz, J.S., McCulloch, R., James A., Brice, B., Swain, B. & Tosser-Klopp, G., 2013. Genetic diversity and investigation of polledness in divergent goat populations using 52 088 SNPs. Animal Genetics, 44(3), 325-335.

Maciel, S., 2001. National strategies for the conservation, improvement and utilization of animal genetic resources in smallholder systems. Proceedings of the Planning and Priority Setting Workshop on Animal Genetic Resources in the SADC region. SACCAR, CTA, ILRI, Gaborone, Botswana.

Maciel, S., Okeyo, A.M., Amimo, J., Scholtz, M.M., Neser, F.W.C., & Mart, M., 2013. The effect of geographical region of birth on the reproductive performance of the Nguni in southern Mozambique. South African Journal of Animal Sience, 43 (1): 60-63.

Madilindi, M.A., Banga, C.B., Bhebhe, E., Sanarana, Y.P., Nxumalo, K.S., Taela, M.G. & Mapholi, N.O., 2019. Genetic differentiation and population structure of four Mozambican indigenous cattle populations, Livestock Research for Rural Development, 31 (47).

Mani, M., Marichatou, H., Issa, M., Chaïbou, I., Sow, A., Chaibou, M. & Sawadogo, J. G., 2014. Caractéristiques phénotypiques de la chèvre du sahel au Niger par analyse des indices de primarité et des paramètres qualitatifs. Animal Genetic Resources Information, Bulletin de information sur les ressources génétiques animales, 54, 11–19.

Mapiye, C., Chikwanha, O.C., Chimonyo, M. & Dzama, K., 2019. Strategies for Sustainable Use of Indigenous Cattle Genetic Resources in Southern Africa. Diversity, 11, 214.

Mdladla, K., Dzomba, E.F. & Muchadeyi, F.C., 2018. Landscape genomics and pathway analysis to understand genetic adaptation of South African indigenous goat populations. Heredity, 369–378.

Mohlatlole, R.P., Dzomba, E.F. & Muchadeyi, F.C., 2015. Addressing production challenges in goat production systems of South Africa: the genomics approach. Small Ruminant Research, 131, 43-49.

Mwai, O., Hanotte, O., Kwon, Y.J. & Cho, S., 2015. African indigenous cattle: unique genetic resources in a rapidly changing world. Asian-Australasian Journal of Animal Sciences; 28, 911-21.

Nyamushamba, G., Mapiye, C., Tada, O., Halimani, T. & Muchenje, V., 2017. Conservation of indigenous cattle genetic resources in Southern Africa's smallholder areas: Turning threats into opportunities – A review. Asian-Australasian Journal Animal Science, 30, 603-622.

Okomo-Adhiambo, M., 2002. Characterization of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques. International Livestock Research Institute (ILRI), Nairobi, Kenya, 1-10.

Porto-Neto, L.R., Lee, S.H., Sonstegard, T.S., Tassell, C.P., Lee, H.K. & Gibson, J.P., 2014. Genome-wide detection of signatures of selection in Korean Hanwoo cattle. Animal Genetics, 45, 180 - 190.

Rischkowsky, B., Pilling, D., & Scherf, B., 2007. Status of animal genetic resources. In B. Rischkowsky, & D. Pilling (Eds.), Part 1: The state of agricultural biodiversity in the livestock sector — The state of the world's animal genetic resources for food and agriculture (pp. 23–49). Rome: FAO, Commission on Genetic Resources for Food & Agriculture.

Rothschild, M.F. & Plastow, G.S., 2014. Applications of genomics to improve livestock in the developing world. Livestock Science, 166, 76–83.

Saleem, M., Rahim, I., Jalali, S., Rueff, H., Khan, M., Maselli, D., U. Wiesmann, U. & Muhammad, S., 2013. Morphological characterization of Achai cattle in sedentary and transhumant systems in Pakistan. Animal Genetic Resources, 52, 83–90.

Sheriff, O., Alemayehu, K., Haile, A., 2021. Phenotypic ranking experiments in identifying breedingobjective traits of smallholder farmers in northwestern Ethiopia. PLOS ONE,16(3).

Tomo, P. 1997. Characterization of Angoni cattle in Mozambique. MSc Thesis. Universtity of Orange Free State, Bloemfontein, South Africa.

Van Marle-Köster, E, Visser, C., Sealy, J. & Frantz, L., 2021. Capitalizing on the Potential of South African Indigenous Beef Cattle Breeds: A Review. Sustainability, 13 (8), 4388.

Vernooij, A., dos Anjos, M. & van Mierlo, J., 2016. Livestock Development in the Zambezi Valley, Mozambique: Poultry, Dairy and Beef Production. Wageningen University and Research Centre for Development Innovation. Report CDI-16-027, Wageningen.

Yougbaré, B., Soudré, A., Ouédraogo, D., Zoma, B.L., Tapsoba, A.S.R., Sanou, M., Ouédraogo-Koné, S., Burger, P., Wurzinger, M., Khayatzadeh, N., Tamboura, H.H., Traoré, A., Sölkner, J. & Mészáros, G., 2020. Morphometric characterization of purebred and crossbred Baoulé cattle in Burkina Faso, Acta Agriculturae Scandinavica, Section A — Animal Science, DOI: 10.1080 /09064702. 2020. 1825785.

Addendum

Supplementary material - Chapter 4

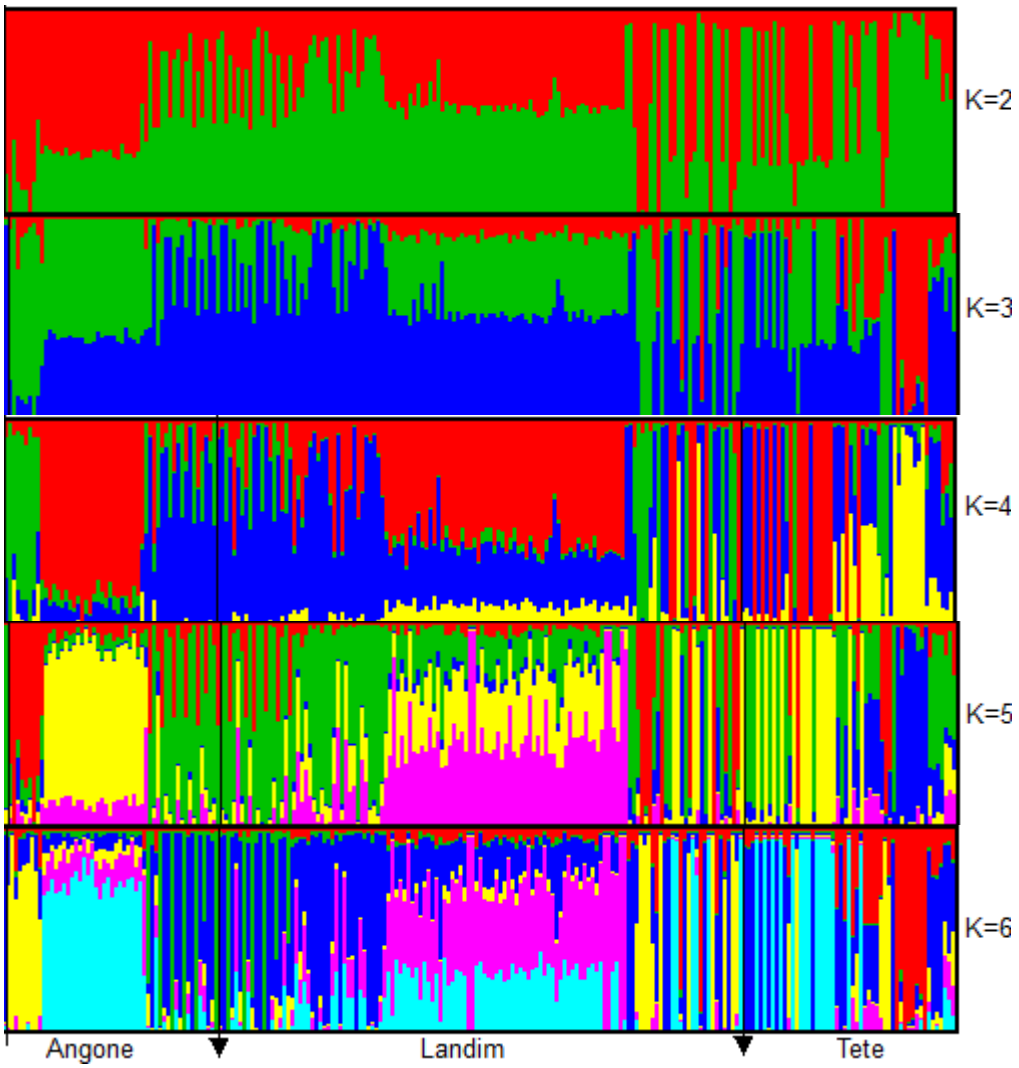

Figure 5.1 Admixture plot showing the clustering of three cattle populations ranging from K = 2 to 6

Supplementary material - Chapter 3

Table 5.1 Agro-ecological regions of Mozambique

Agro-ecological zones	Province	Altitude (m)	Temperature (°C)	Rainfall (mm)	Humidity Index[1]
R1	Inland Maputo & South Gaza	Major part under 200	20-25	800-1000	Dry Semi-arid, with small humid semi-arid spots in the Libombos heights
R2	Southern Maputo to Northern Inhambane	0-200	20-25	800-1000	Humid Semi-arid, with some sub-humid spots in the littoral
R3	Centre and North of Gaza and West of Inhambane	100-200	22-26	400-600	Semi-arid and arid
R4	Sofala and Manica	200-1000	17.5-22.5	1000-1200	Sub-humid with
R5	Sofala and Zambezia	0-200	24-28	1000-1400	Humid semi-arid
R6	Zambezia valley and Southern Tete	0-200	20-25	Mostly 500-800 with one area 1200 – 1400 and another with water deficit	Dry Semi -arid
R7	Zambezia, Nampula, Tete, Niassa and Cabo Delgado	200-1000	20-25	1000-1400	Humid Semi- arid , with sub-humid
R8	Coastal Littoral of Zambezia, Nampula and Cabo Delgado	0-200	Above 25	800-1200	Humid Semi-arid with spots of sub- humid and an extensive dry and semi-arid area
R9	North of Cabo Delgado	200-1500	20-26	1000-1200	Humid and semi-arid.
R10	Zambezia, Niassa,Tete and Manica	Above 1000	15-22.5	Above 1200	Sub-humid and humid

[1]Humidity index: Arid: < 500 mm of precipitation; Dry semi-arid: 500-800 mm, Humid semi-arid 800-1000 mm, Sub-humid, 1000-1400 mm.