GENETIC DIVERSITY AND POPULATION STRUCTURE OF THREE NATIVE CATTLE POPULATIONS IN 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 amongst 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

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 and 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 and 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.

MATERIALS AND METHODS

Animals and sampling

All animals were handled in compliance with the rule 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 were 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 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.

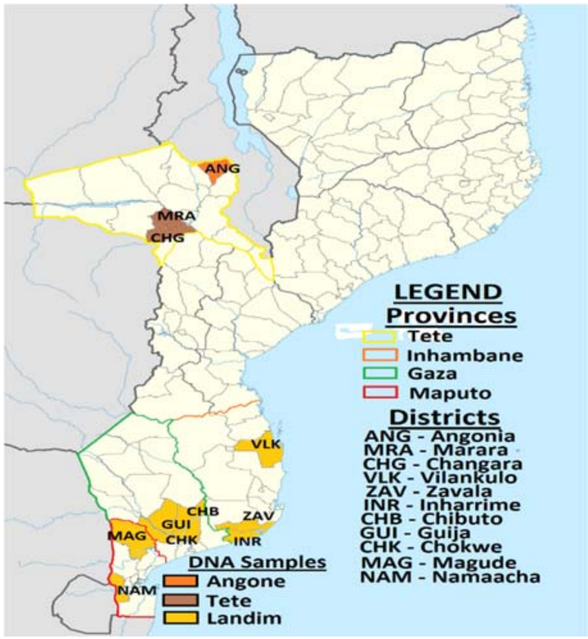

Figure 1 Map of Mozambique showing sampling locations

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 (Twomey *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 (HO) and expected (HE) heterozygosities, as well as inbreeding rates (Fis), using PLINK v1.09 (Purcell et al., 2007).

The Principal components analysis was performed to assess population structure and relationships using 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 pair-wise r^2 values per chromosome in each population using PLINK (Purcell *et al*., 2007). The LD SNP pairs were computed running the command "–r2 –ld-window-kb 2000 –ld-window-r2 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 (Ne) 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 Ne at different time points using SNeP version 1.1 (Barbato *et al*., 2015).

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 1).

Table 1 Individuals and SNPs removed in the quality control process

Population	N	Excluded Individuals	Excluded SNPs				Remaining SNPs (%)
			SNP Call rate	MAF	HWE	Total	
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-value < 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 2).

Table 2 Genetic diversity parameters and inbreeding rates of three cattle populations

Population	N	MAF±SD	HO±SD	HE±SD	Fis±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; HO = observed heterozygosity; HE = expected heterozygosity; Fis = 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 2). However, the Angone and Tete showed very little differentiation and could almost be perceived as one population.

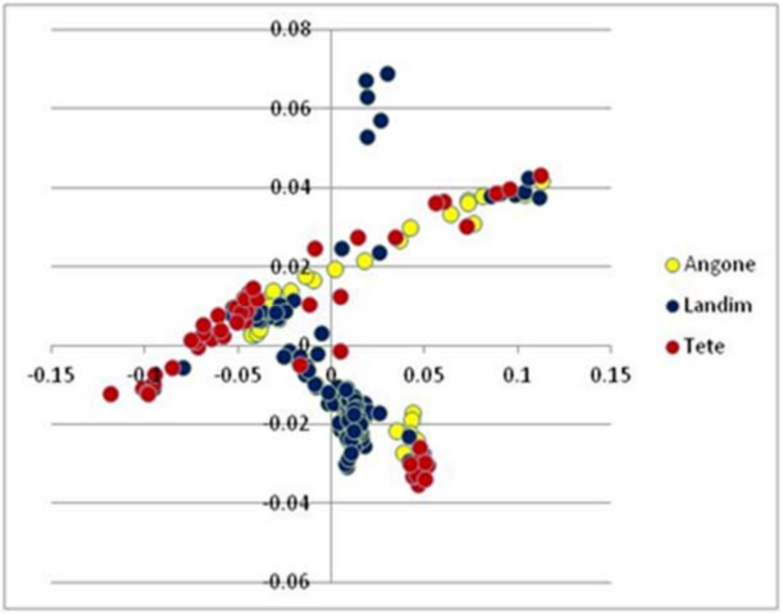

Figure 2 PCA1 against PCA3 plot for 3 cattle populations

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 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).

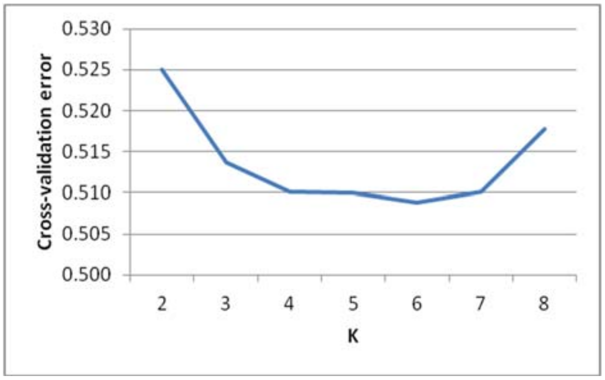

Figure 3 Cross-validation error of three populations of cattle

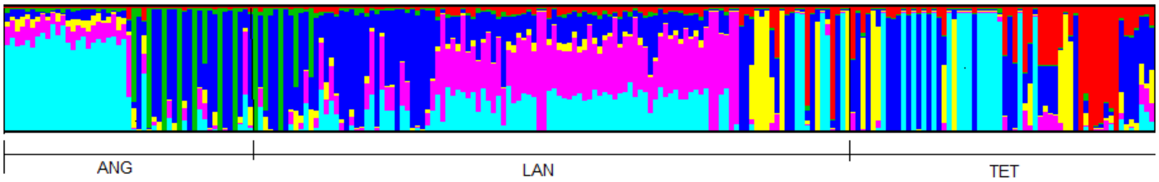

Figure 4 Admixture plot showing the clustering of three cattle populations

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

Table 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

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 5).

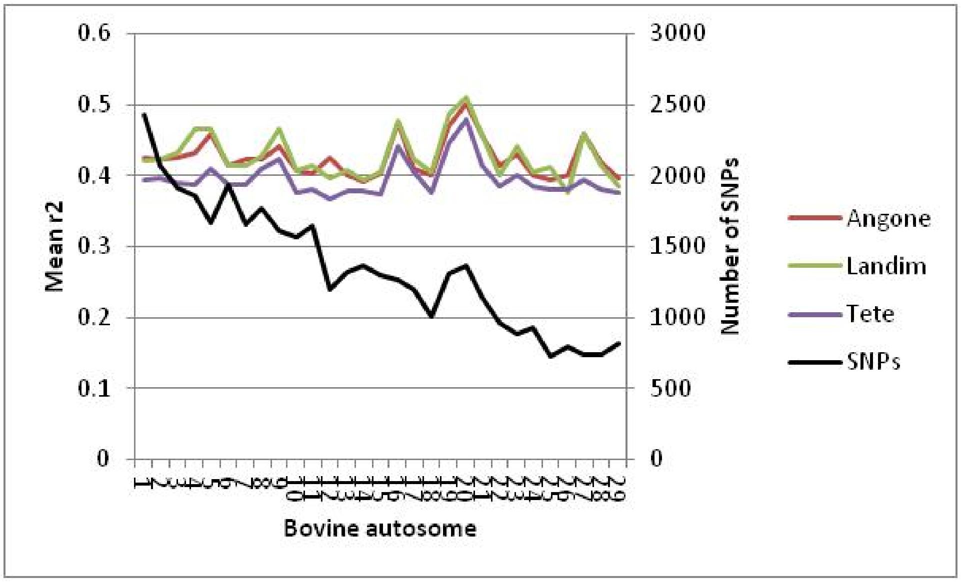

Figure 5 Distribution of autosomal SNPs and average r^2 values per population across the bovine genome

The greatest mean r^2 was seen at 0–10 kb in all populations. Linkage disequilibrium (LD) decreased as the distance between SNPs pairs increased. The decrease of LD with increasing physical inter-marker distance was progressively slower for distances above 60 kb (Tabel 4).

Table 4 Average r^2 and SNP pairs over different distances across all autosomes, in three cattle populations

Distance (unit)	**Angone**		**Landim**		**Tete**	
	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 (Ne) decreased over time across all populations. At 12 generations ago, the effective Ne was 627, 929, and 349 animals for the Angone, Landim, and Tete populations, respectively (Figure 6).

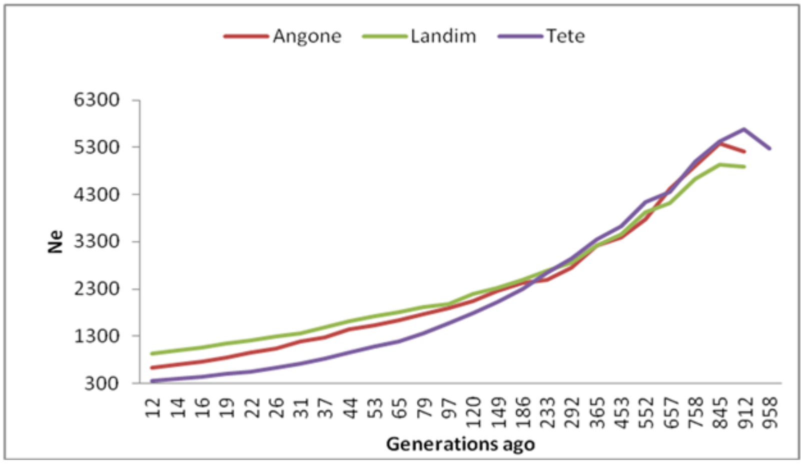

Figure 6 Effective size of three cattle populations in the past 12 generations

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 re-stocking (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 (Ne). 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 markers 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 cross-breeding that are common in the communal management systems stimulate the level of gene flow amongst the populations, which results in a low level of variability (Maciel *et al*., 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 derive 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 and 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 indicate 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 (Ne) 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 Ne of 50 per generation. In our research, the estimated population size (Ne) (twelve generations ago) for the studied populations is above 50. Nevertheless, attention should be paid to keep Ne 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 Ne 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.

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).

Acknowledgments Many thanks are due to the Ministry of Science, Technology and Higher Education of Mozambique for their financial support. Likewise, we thank the Ministry of Agriculture and Rural Development of Mozambique and all the breeders covered in this study, for their collaboration and patience.

Conflict of interest The authors would like to unanimously inform everyone that there is no conflict of interest related to this study.

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. and 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, 2013. In vivo conservation of animal genetic resources, Rome, Italy.

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

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

INE, 2016. Anuário de Estatísticas Agrárias 2015, Maputo: Instituto Nacional de Estatística de Moçambique. https://www.masa.gov.mz/wp-content/uploads/2017/12/Anuario_Estatistico2016.pdf. Accessed 5 October 2020.

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. and 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., and 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: as províncias do norte: Cabo Delgado, Niassa, Nampula Nova Vega Lda., Lisboa.

Morgado, F.P., 2004. A Pecuária no Centro de Moçambique, Nova Vega Lda, Lisboa.

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 utilization 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 reveal 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 toolset 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.and 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.