

Full Length Research Paper

Genetic characterization of the indigenous Landim and Pafuri goat breeds from Mozambique

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This paper describes genetic diversity between and within the indigenous Landim and Pafuri goat breeds from Mozambique, with reference to the unimproved South African Boer goat, the paternal ancestor of the Pafuri breed. Microsatellite polymorphism was used to quantify genetic diversity and drift. Heterozygosity (Hz) in three populations of the Landim breed were closely comparable (Hz = 0.588 - 0.623), with a higher Hz value of 0.672 in Pafuri, the latter most likely reflecting the influence of the Boer goat. Allelic richness values supported this trend. Drift among individual Landim populations was low ($F_{st} = 0.046-0.059$) compared to $F_{st} = 0.077-0.091$ between the Pafuri breed and individual Landim populations, again reflecting introgression of Boer goat genetic material. The Landim populations also clustered together in a consensus neighbour-joining tree, with the Pafuri breed slightly distant and the Boer goat most distant from the Landim breed. A Bayesian assignment test placed the majority of Pafuri and Boer goat animals in a common cluster, with the Landim individuals spread over three additional clusters. The results are discussed with reference to the importance of conserving local breeds and the influence of marker sample size on statistical analysis.

Key words: Landim, Pafuri, genetic structure, Mozambique, goat.

INTRODUCTION

Indigenous breeds of small ruminants are important for subsistence and socio-economic livelihoods of communities in developing countries (Kunene et al., 2009; Qi et al., 2009). These breeds may also be important reservoirs of adaptation to specific local conditions. In Africa, south of the Zambezi River, goats were introduced shortly before and after the arrival of

Europeans. The southern African goat population is therefore a combination of types derived from various breeds that were brought from tribes to the north and introduced types. In this regard, the southern African population has been influenced by the Boer type goat, which was developed from the 18th century onwards (Gall, 1996).

In Mozambique, livestock and agriculture are major sources of income and employment for over 85% of the population who dwell in the rural areas. Goats are generally used for meat production, for festive occasions and are sometimes milked for home consumption. Two main types of goat are found in Mozambique. The Landim breed is the predominant breed distributed throughout the country. It is also known as the

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Abbreviations: AMOVA, analysis of molecular variance; **Ds**, standard genetic distance; **FST**, inbreeding due to population sub-division; **Ho**, observed heterozygosity; **H_z**, unbiased heterozygosity; **Rs**, Allelic richness.

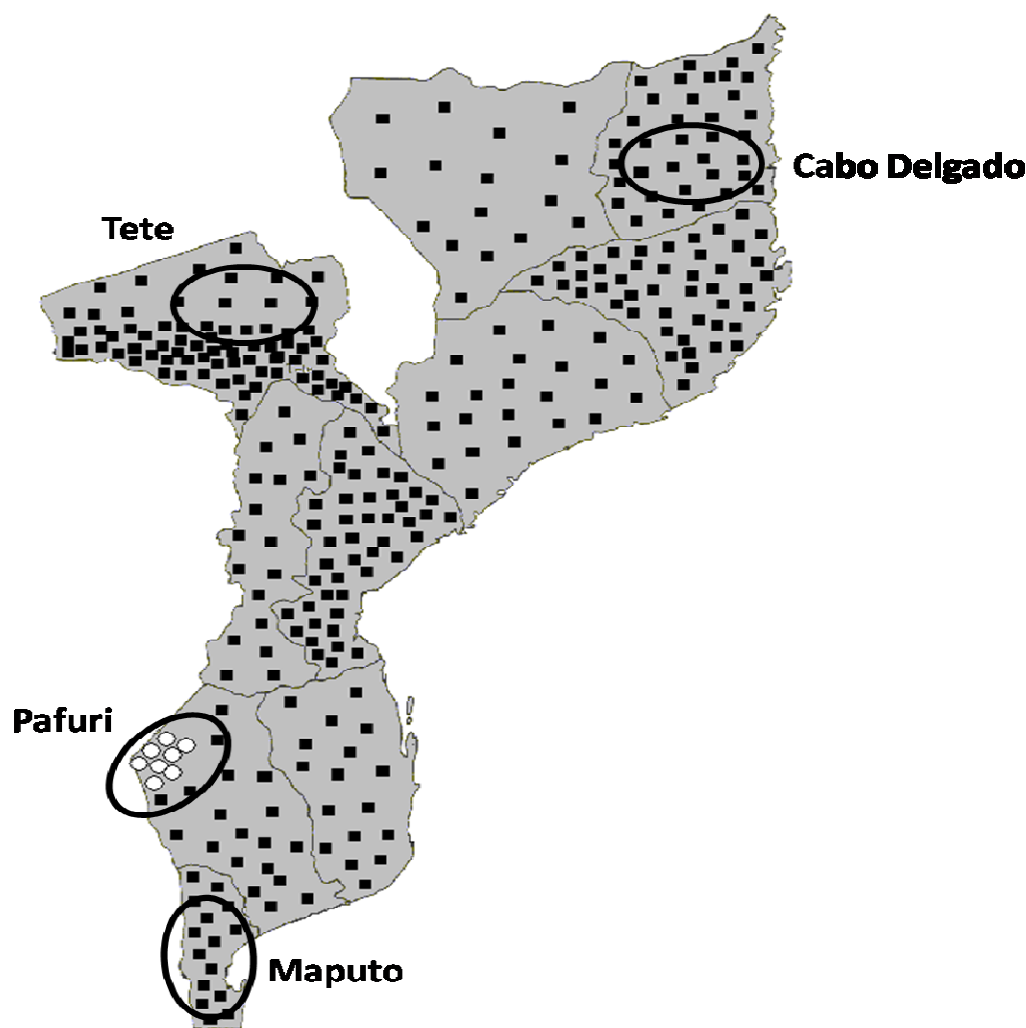


Figure 1. Distribution of Landim ■ and Pafuri ○ goats in Mozambique, and location of sampling sites.

Portuguese Landrace (Gall, 1996). There is little variation in size and adaptation in the breed. The Pafuri breed is a result from crossing Boer goat males with Landim females. The Boer goats were introduced from the northern Transvaal (now Limpopo Province) in South Africa in 1928 into the Pafuri area in south-west Mozambique (Figure 1). The breed is kept under an agro-pastoral to pastoral management system generally in a semi-arid to arid environment (Gall, 1996). Its occurrence is spreading to other southern provinces such as Maputo and Gaza.

The conservation of unique local populations of farm animals is a common theme in animal genetic resources management and this also applies to indigenous goat populations (Ouafi et al., 2002; Kotze et al., 2004; Gour et al., 2006; Fan et al., 2007; Qi et al., 2009). The replacement and uncontrolled crossbreeding of indigenous animals with “improved” breeds may lead to

the replacement of indigenous populations and the subsequent loss of their unique genetic traits (Visser et al., 2004). Iamartino et al. (2005) stated that the first step for the conservation and use of domestic animal biodiversity is a comprehensive knowledge of the existing genetic variability, and of the partitioning of this variability among breeds. Microsatellite markers are an accepted marker for characterization that have been used by several authors to quantify the uniqueness of indigenous goat populations, including Kotze et al. (2004), Iamartino et al. (2005), Fan et al. (2008) and Qi et al. (2009). In this paper, we report on a micro-satellite-based investigation that describes genetic diversity between and within the indigenous Landim goat populations from Mozambique, a comparison with the closely related Pafuri indigenous breed, and we also provide data on the relationship of these Mozambican breeds with the unimproved South African Boer goat, the paternal

Table 1. Numbers of goats from each breed and population sampled in Mozambique.

Breed and Province	District/ Locality	n	Remarks
Pafuri – Gaza:	Pafuri / Sede	1	
	Pafuri/ Chicumba	16	4 farms
	Pafuri/ Mbuzi	11	2 farms
	Pafuri/ Mungaban	3	
	Pafuri/Salane	4	
	Pafuri / unknown	5	
Landim - Tete	Changara	4	
	Marara/ PFP	11	
	Marara/ Centro	9	2 farms
	Marara/ P8	8	2 farms
	Matambo	8	
Landim - Maputo	Magude	12	2 farms
	Chobela	17	2 farms
	Impauto	11	
Landim - Cabo Delgado	Miesi	11	2 farms
	Pemba Metuge	12	4 farms
	Pemba Metuge/sede	6	
	Mecufi	11	3 farms

ancestor of the Pafuri breed.

MATERIALS AND METHODS

Populations sampled and sample collection

Individuals of the Landim breed were collected from three areas of Mozambique: Maputo (southern region), Tete (central region) and Cabo Delgado (northern region) (Figure 1). Individuals of the Pafuri breed were sampled from Gaza, in the south-western region of the country. Forty unrelated animals were sampled from each breed (10 males and 30 females). To ensure that individuals sampled were not closely related, different flocks were identified within the districts within each province (Table 1). Hairs with visible roots were collected from the tail of each animal. For DNA isolation, approximately eight hair roots from each animal were used, with a modified Proteinase K digestion method (Higuchi et al., 1988). To measure the influence of introgression from the Boer goat, we used 11 genotypes previously analyzed by Pieters et al. (2009).

Genetic analysis

Goats from the Mozambican indigenous populations were genotyped at 17 loci: BM1258, BM1329, CsRd247, INRA006, INRA023, INRA63, ILSTS002, ILSTS087, MAF65, MCM527, OARFCB11, OARFCB20, RM004, SrCrSp5, SrCrSp8, SrCrSp23 and SrCrSp24. These markers were selected from a panel of markers recommended by the International Society for Animal Genetics (ISAG) for application in genetic diversity studies. Approximately 100 ng of genomic DNA was used as a template for

amplification, with the rest of the reaction mixture containing 2.5 mM dNTPs, 1.5 mM MgCl₂, 1.25 U Supertherm Gold Taq polymerase (Southern Cross Biotechnology ©), 1x buffer, approximately 50 pmol/μL of each primer (varying for specific primers) and 1.28 μL Tween.

Reactions were conducted in a total volume of 7.5 μL. Forward primers were labelled with fluorescent labels. PCR conditions consisted of the following steps: an initial step of 15 min at 95°C, followed by 35 cycles of a step consisting of 45 s at 94°C, 45 s at 59°C and one minute at 72°C, and with a final extension step of 60 min at 72°C. Amplified fragments were separated on an ABI377 sequencer, with Genescan™ and Genotyper™ software (Applied Biosystems) used to size fragments. Eight of the 17 loci listed above were previously genotyped in Boer goat by Pieters et al. (2009). These Boer goat profiles were used as a reference group for some analyses, based on the reduced number of loci. The loci for which genotypes were available in Boer goat were: BM1258, BM1329, ILSTS002, ILSTS087, MAF65, OARFCB11, RM004 and SrCrSp5.

Statistical analyses

MSToolkit software (Park, 2001) was used to quantify genetic diversity within populations as unbiased heterozygosity (H_z: Nei, 1987) and observed heterozygosity (H_o). Allelic richness (R_s) was used to measure allele diversity, using FSTAT software (Goudet, 2001). All calculations involving genetic diversity was performed twice, first using only the Mozambican populations and the full complement of 17 loci, followed by analyses that also included the Boer goat but with the number of loci reduced to the eight shared loci. The MSToolkit software was also used to create input files for

Table 2. Genetic diversity in goat breeds and populations from Mozambique and South Africa, based on 17 and eight loci respectively. Parameters are sample size (n), unbiased heterozygosity (Hz), observed heterozygosity (Ho), and allelic richness (Rs). The abbreviation s.d. denotes standard deviation.

Population:	n	Hz	Hz s.d.	Ho	Ho s.d.	Rs	Rs s.d.
Diversity based on 17 loci:							
Pafuri	38	0.672	0.036	0.586	0.020	6.508	2.507
Landim -Tete	40	0.599	0.046	0.549	0.019	5.251	2.073
Landim -Maputo	40	0.623	0.047	0.529	0.019	5.911	2.258
Landim -Cabo Delgado	40	0.588	0.043	0.556	0.019	5.621	2.044
Diversity based on eight loci shared with Boer goat study:							
Pafuri	38	0.618	0.062	0.604	0.028	4.385	2.001
Landim -Tete	40	0.562	0.086	0.565	0.028	3.711	1.756
Landim -Maputo	40	0.587	0.085	0.482	0.028	4.202	1.721
Landim -Cabo Delgado	40	0.515	0.064	0.491	0.028	3.632	1.313
Boer goat	11	0.604	0.095	0.634	0.052	3.930	2.061

Table 3. Genetic differentiation between five goat populations and breeds, with Fst values based on 17 loci below the diagonal and Fst from eight loci above the diagonal. Differentiation between all pair-wise combinations of populations was significant at the 95% level.

	Pafuri	L-Tete	L-Maputo	L-Cabo D	Boer goat
Pafuri	-	0.052	0.082	0.075	0.110
L-Tete	0.077	-	0.030	0.052	0.102
L-Maputo	0.087	0.046	-	0.049	0.102
L-Cabo D	0.091	0.059	0.056	-	0.162

other software used for statistical analyses. Hardy-Weinberg equilibrium of genotypes at specific loci were not tested for since the nominal populations studied do not represent true panmictic populations but rather consists of numerous local populations that would likely contribute to a Wahlund effect.

Relationships among populations were studied using measures describing both drift and genetic distance. Firstly, genetic divergence among population pairs was determined using Fst (Wright, 1965), as implemented in ARLEQUIN software (Excoffier et al., 2005). A sequential Bonferroni correction (Rice, 1989) was applied to all P values from Fst analysis, to prevent Type I errors resulting from multiple pair-wise comparisons. Genetic distance was also calculated, using Nei's standard genetic distance (Ds: Nei, 1978) from DISPAN software (Ota, 1993). A neighbour-joining tree based on genetic distances was also created using the DISPAN software, with 1,000 replications to determine bootstrap support for the final consensus tree. A hierarchical analysis of the distribution of total genetic diversity was performed using an analysis of molecular variance (AMOVA: Michalakis and Excoffier, 1996) and ARLEQUIN software. As for genetic diversity, all calculations involving FST, Ds and AMOVA was first performed using only the Mozambican populations and the full set of loci, followed by analyses that also included the Boer goat individuals and the reduced locus set.

STRUCTURE software (Pritchard et al., 2000; Falush et al., 2003) and the Bayesian approach of Pritchard et al. (2000) was used to determine the true number of populations (clusters) and to assign individuals probabilistically to identified clusters. A model with assumption of admixed ancestry and correlated allele

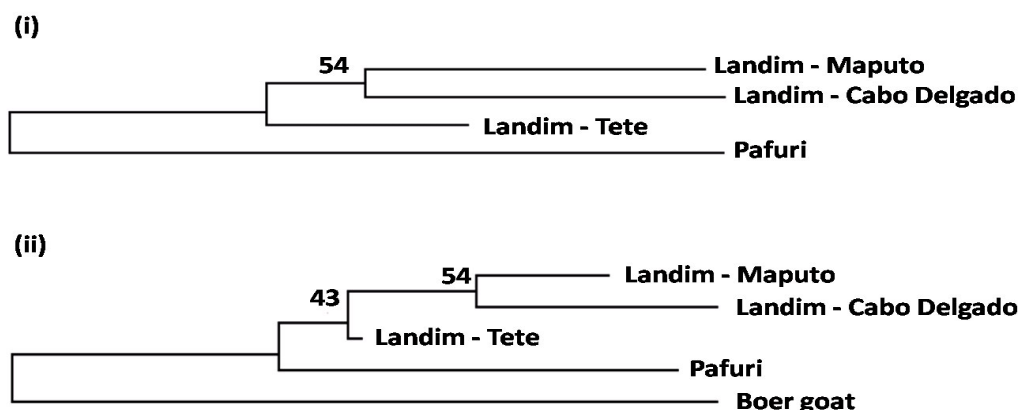
frequencies was used. To estimate the true number of populations, the parameter $-\ln Pr(X|K)$ was calculated five times for each of five possible K values ranging from K=1 to K=5. All simulations consisted of a burn-in period of 100,000 steps, followed by 200,000 MCMC iterations.

RESULTS

Values describing genetic diversity within individual populations are presented in Table 2. Among the indigenous Mozambican goat populations, the highest genetic diversity was observed in the Pafuri breed, with Hz=0.672 compared to Hz values of 0.588-0.623 in the Landim populations. Based on the reduced set of eight loci, the trend among indigenous Mozambican populations remained the same, with Hz=0.618 in the Pafuri breed compared to 0.515-0.587 in the Landim populations, and with Hz=0.604 in the Boer goat breed. Similar trends were evident from the Ho and Rs values, using both 17 and eight loci (Table 2). The Fst values showed more drift between the Pafuri breed and individual Landim populations (FST=0.077-0.091) compared to drift among pairs of Landim populations (FST=0.046-0.059) (Table 3). With the addition of the Boer goat and the use of the reduced number of loci, the

Table 4. Standard genetic distance between five goat populations and breeds, with Ds values based on 17 loci below the diagonal and Ds from eight loci above the diagonal.

	Pafuri	L-Tete	L-Maputo	L-Cabo D	Boer goat
Pafuri	-	0.080	0.147	0.105	0.223
L-Tete	0.158	-	0.042	0.066	0.171
L-Maputo	0.197	0.077	-	0.061	0.187
L-Cabo D	0.184	0.095	0.093	-	0.260

**Figure 2.** Consensus neighbour-joining trees depicting standard genetic distances among goat populations. Trees indicate genetic distances (i) among Mozambican Landim and Pafuri populations, based on 17 microsatellite loci; and (ii) among Mozambican indigenous populations and South African Boer goat, based on 8 shared loci. Bootstrap values are based on 1000 iterations.

nature of relationships between the Pafuri and Landim breeds is preserved, with $F_{ST}=0.052-0.082$ between Pafuri and Landim populations, compared to $F_{ST}=0.030-0.052$ among Landim populations (Table 3). The F_{ST} value between the Pafuri breed and the partially ancestral Boer goat breed was 0.110, compared to $F_{ST}=0.102-0.162$ between the Boer goat and individual Landim populations.

Clustering based on genetic distance values mirrored the results from F_{ST} (Table 4). There was more separation between the Pafuri breed and Landim populations ($D_s=0.158-0.197$) compared to distances among Landim populations (0.077-0.095). With the smaller set of loci and the addition of the Boer goat breed, D_s values between Pafuri and Landim (0.080-0.147) was larger than D_s values among Landim populations (0.042-0.064). The distance between the Pafuri and Boer goat breeds was 0.223, compared to D_s values of 0.171-0.260 between the Boer goat and specific Landim populations. The clustering of populations in consensus neighbour-joining trees followed known relationships among groups (Figure 2). The three Landim populations consistently appeared in a clade not containing other breeds. The Pafuri breed was placed

more distant from the Landim clade, with the Boer goat most distant.

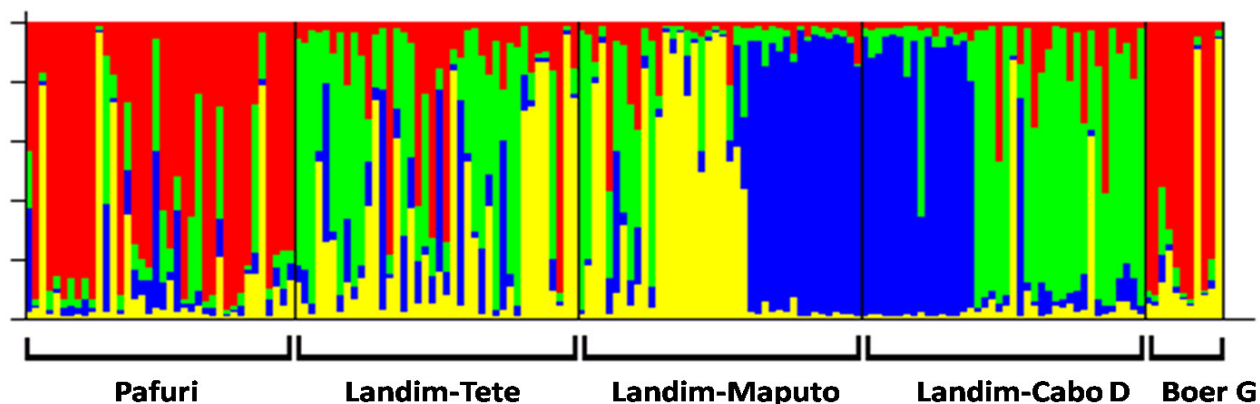
The hierarchical analysis of total genetic diversity in Mozambican groups showed that 91.029% of variation exists within individual populations, with 4.034% between the Landim and Pafuri breeds, and 4.936 among the three Landim breeds (Table 5). With the addition of the Boer goat individuals and the use of the smaller marker set, the biggest component of total diversity remained with individual populations, at 91.390%. Differences between the Boer goat, Landim and Pafuri breeds contributed 4.624% of total diversity, with 3.986% attributed to the differences among Landim populations. Results from the Bayesian Assignment test showed the highest probability for a real structure consisting of four populations. The average $-\ln$ probability for $K=4$ over five runs was -3183.8 compared to values of -3255.6 to -3461.6 for $K=1-3$ and $K=5$. Also, the standard deviation (SD) of the average $-\ln$ probability values for $K=4$ was only 1.304 compared to SD values of 2.074-21.142 for $K=2,3$ and 5. Standard deviation for multiple runs of $K=1$ was low at 0.894 but this K value yielded the largest $-\ln$ probability values recorded and $K=1$ was therefore not considered a possible real value of K . A high proportion

Table 5. Relative contributions of different hierarchical levels of diversity to total genetic diversity in goat breeds (AMOVA). (i) Over all indigenous Mozambican populations; (ii) over Landim, Pafuri and Boer goat.

Source of variation	Sum of squares	Variance components	Percentage of variation
(i) In Indigenous Mozambican groups:			
Among Landim and Pafuri:	51.143	0.233	4.034
Among Landim populations:	56.606	0.285	4.937
Within populations:	1621.985	5.261	91.029
Total:	1729.134	5.779	
(ii) In all populations studied:			
Among Landim, Pafuri and Boer goat:	31.831	0.116	4.624
Among Landim populations:	20.433	0.099	3.986
Within populations:	754.606	2.288	91.390
Total:	806.869	2.504	

Table 6. Proportion of membership of 5 breeds and populations of goats to 4 nominal clusters, based on Bayesian analysis.

	Cluster:			
	1	2	3	4
Pafuri	0.650	0.124	0.145	0.080
Landim - Tete	0.183	0.386	0.288	0.143
Landim -Maputo	0.073	0.171	0.341	0.415
Landim -Cabo Delgado	0.092	0.453	0.061	0.395
Boer goat	0.637	0.049	0.290	0.023

**Figure 3.** Bar plot showing individual goats by breed and population. Differently coloured zones on each vertical bar show the proportion of membership of an individual to each of one or more of 4 real clusters identified from Bayesian analysis.

of Pafuri (65.0%) and Boer goat (63.7%) individuals were assigned to a common cluster (Cluster 1 - Table 6), a cluster that did not contain a significant component of Landim individuals (7.3-18.3%). The bulk of animals from the Landim populations were spread over Clusters 2 - 4. The bar plot based on a real population structure of 4 populations, with individuals in original order, is

presented in Figure 3.

DISCUSSION

The levels of genetic diversity within the three Landim populations are very similar, with H_z ranging from 0.588-

0.623 and with a similar trend from H_o and R_s values. The similarity between the three indigenous Mozambican populations is indicative of a breed that is widely farmed across the country. The high sample size of 40 animals used for each population probably also minimized sampling error among populations. The levels of diversity observed are also comparable to that of other local populations in southern Africa and further afield. The heterozygosity values estimated for the Landim breed are comparable to the value of 0.630 published for another southern African local goat breed, the Kalahari red, by Kotze et al. (2004). In comparison to local breeds on other continents, the heterozygosity values observed in the Landim falls within the range reported for a range of breeds, being slightly higher than the value of 0.526 published for indigenous Indian goat populations by Gour et al. (2006), but lower than values of 0.710-0.839 reported in Italian (Iamartino et al., 2005) and Chinese (Qi et al., 2009) local goat breeds. The higher levels of genetic diversity in Italian and Chinese breeds may reflect larger ancestral populations compared to the introduced southern African groups, but this hypothesis cannot be tested from the available data. Nevertheless, the level of genetic diversity retained in the Landim breed appears to be satisfactory and this will facilitate the conservation of the breed as a distinct genetic unit, since it will not be necessary to induce gene flow with other breeds to raise levels of genetic diversity.

Compared to the three Landim populations, a higher heterozygosity value was observed in the Pafuri breed, with $H_z=0.672$ based on 17 microsatellite markers. This increased level of diversity most likely reflects the influence of the Boer goat as the paternal ancestor of the Pafuri breed. Reference to the values based on eight shared markers shows that heterozygosity in the Boer goat (0.604) is higher than in any Landim population (0.515-0.587). Similarly, allelic richness in the Boer goat (3.930) is higher than values recorded in two of the Landim populations studied (3.632-3.711). The use of Boer goat rams thus probably resulted in an increase of genetic diversity when the new Pafuri breed was established. Overall, the greatest component of genetic diversity (91%) was found within individual populations. This is comparable to the within-population component of 92.7% reported by Iamartino et al. (2004) in Italian goat populations but lower than the within-population components of 94.3-94.8% reported for Chinese goat breeds by Fan et al. (2008) and Qi et al. (2009). This suggests some structure among the goat populations studied, despite the high within-population component. In fact, significance values calculated supported the hypothesis of significant (0.05) drift between all population pairs. The actual F_{st} values reflect the known history of the populations and breeds studied accurately: F_{ST} values among Landim populations (0.046-0.059) were consistently smaller than values between Landim

and Pafuri (0.077-0.091). This pattern of closer identity between Landim populations compared to Landim/ Pafuri was also found when using the reduced marker set and is in line with the founding history of the Pafuri breed, that is, this breed contains a significant component of non-Landim genetic material since Boer goat was used as the paternal ancestor. Drift values between the Boer goat and populations of other breeds, based on eight shared loci, mostly corresponded well with known relationships. The F_{st} value between Pafuri and the Boer goat was 0.110, which is lower than the average F_{st} value of 0.122 observed between Boer goats and the Landim populations.

Tendencies from genetic distance values are similar to those observed from F_{st} and the known relationships among breeds are well represented in the N-J trees in Figure 2. In all cases, Landim populations formed a recognizable cluster, with the Pafuri breed somewhat distant from Landim populations. Where included, the Boer goat individuals grouped separate from all the Mozambican populations as expected considering known breeding histories, though with low bootstrap support which suggests limited genetic separation. A notable observation from the results of the current study is that the estimates used to characterize breeds and populations were only slightly influenced by the number of loci used. For all values of genetic diversity and drift, absolute values were different when eight loci were used compared to 17, but patterns of differentiation among populations remained remarkable constant irrespective of the number of loci used. For example, the Pafuri breed consistently showed more diversity than Landim, and genetic drift among Landim populations and Pafuri showed the same uniform pattern. While we do not advocate a general scaling down of the number of markers used to characterize breeds; our results demonstrate convincingly that smaller studies based on a low number of loci can add valuable data for the genetic characterization of breeds in the absence of more elaborate datasets, provided that highly polymorphic loci are used.

Results from the Bayesian assignment test showed most support for a real population structure consisting of four clusters, based on eight shared loci. The assignment of populations and breeds to the four clusters identified corresponds well with founding histories. A high proportion of individuals from the Pafuri (65.0%) and Boer goat (63.7%) breeds were assigned to a common cluster (Cluster 1). This cluster however contained only a limited portion of the Landim animals, at 7.3 - 18.3% of given populations. The greater part of Landim animals were assigned to Clusters 2 - 4. With the individuals in at least two of the Landim populations (Maputo and Cabo Delgado) distributed more or less evenly over more than one cluster, it could be argued that support for Clusters 2 - 4 is weak and that the Landim populations share high

similarity. The only exception to this strong identity is possibly the 18.3% of Landim goats from the Tete region that clustered with Pafuri and Boer goat.

In conclusion, our results provide new data on the genetic characteristics of two indigenous breeds of domestic goat from Mozambique. The results confirm high overall similarity among goat populations in that country but nevertheless confirm the distinctiveness of the Pafuri breed relative to the Landim, reflecting the introgression of genetic material from the Boer goat breed in the 1920's. The genetic distinction between the Landim and Pafuri breeds should be taken into account in future management plans aimed at the conservation of indigenous Mozambican goats. The results reported here should preferably be followed by a full analysis of the influence of the Boer goat on genetic structure of the Mozambican Pafuri breed, based on a larger set of markers. Conversely, our data demonstrate that a good indication of genetic relationships among populations can be made using a limited set of markers.

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