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# Polymorphism of mitochondrial DNA in the Brazilian Canindé goat breed

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**ABSTRACT.** The success of the geographical distribution of goat populations around the world is a consequence of the adaptive potential of these breeds. Several relevant traits to the success of the species in colonizing different ecosystems (and use by man) evolved before domestication. These features were relevant for the selection of different breeds. Each breed represents a genetic heritage that may be unique and essential for maintaining the species. The objective of this study was to catalog the mtDNA haplotypes of the Brazilian autochthonous Canindé goat breed and to characterize the genetic diversity observed in subpopulations by sequencing a 481-bp fragment corresponding to the first portion of the control region in 178 individuals from 10 herds, sampled in six Brazilian states. The global population displays a total

of 29 haplotypes and 56 polymorphic sites. About one-third (10) of the haplotypes were common to all subpopulations while the remaining (19) were exclusive to a single subpopulation. The population exhibited high average haplotype diversity (0.82), with maximum and minimum values of 0.90 and 0.56 in individual subpopulations, respectively. In contrast, nucleotide diversity was 0.014, with maximum and minimum values of 0.020 and 0.004, respectively. The spatial analysis of molecular variance did not detect structure within the Canindé goat breed, and analysis of molecular variance revealed that 88.4% of the variation observed in the population was due to differences among individuals in the same subpopulation. Only 11.4% of the genetic variation referred to differences among subpopulations. About one-third (33.1%) of the individuals within population shared the same haplotype, which may be due not only to the breed developing from a small number of matrilines. The Brazilian autochthonous Canindé breed was classified as haplogroup A, a haplotype predominant in the Europe region.

**Key words:** *Capra hircus*; Hypervariable region; D-loop control region; Matrilineal marker

## INTRODUCTION

Goats (*Capra hircus*) were introduced to the Americas in the century XVI, during the first decades of Portuguese and Spanish colonization (Primo, 2004). A wide diversity of local goat genetic groups developed along the path of the colonizers through Brazil. However, only the Canindé and Moxotó breeds are officially recognized. Ribeiro et al. (2004) cited Canindé as one of the main local breeds in northeastern Brazil, predominantly reared in extensive and multiple-purpose systems (meat, milk, and leather). The remaining populations are distributed among 12 herds from different Brazilian states (Bahia, Pernambuco, Paraíba, Rio Grande do Norte, Ceará, and Piauí) in the northeastern region, with higher density in the Rio Grande do Norte.

Some studies on the genetic diversity of the Canindé breed have been undertaken to examine the intra- and interpopulation variability in public and private herds (Menezes et al., 2006; Araújo et al., 2010). Ribeiro et al. (2012) studied the structure, diversity, and genetic relations between the Canindé breed and other Brazilian and Portuguese breeds using microsatellite markers. These authors found that the Canindé breed has specific alleles, but shares some alleles with other Portuguese breeds. However, these studies were not sufficient to infer the genetic origin of the Canindé breed, essential to infer the breed's maternal lineages. Genetic origin is usually inferred based on phylogeographic studies that discriminate the evolutionary history of maternal (Avise, 2000) and/or paternal lineages (Pérez et al., 2011), relating it to the geographical distribution mainly on the basis of the differences between the mitochondrial DNA (mtDNA) and Y chromosome sequences.

The first studies with mtDNA in goats were conducted by Luikart et al. (2001), Sultana et al. (2003), and Naderi et al. (2007), who identified four, six, and seven maternal lineages, respectively. These early studies constituted the basis for more specific regional studies. Oliveira (2007) analyzed a fragment of the D-loop region in goats from northeastern Brazil and Lopes et al. (2016) performed studies on Crespa ecotypes in southern Brazil, and the

haplogroup A was predominant in the studied ecotypes. Haplotypes from Brazilian goat breeds are phylogenetically close to haplogroup A, which represents more than 90% of haplotypes worldwide (Taberlet et al., 2011) and has been widespread across the globe.

The largest portion of genetic diversity occurs in breeds with weak geographic structure due to the heavy transit of goats between continents, which is related to human migration and trade. This has taken place since ancient times, mainly because of the animals' ability to adapt and the ease with which they can be transported because of their small size (Pereira and Amorim, 2010).

Understanding breed development is crucial as it may help in choosing conservation and breeding strategies. In turn, cataloging a breed's mitochondrial variability is important, especially when considering the remaining genetic diversity, because of the potential use of a genetic source for the conservation of local genetic resources and the protection of the genetic resources of the country. Thus, the objective of this study was to catalog the mtDNA haplotypes of the Canindé breed and characterize the breed's genetic diversity by analyzing a fragment of the control region (D-loop) of the DNA.

## MATERIAL AND METHODS

### Sampling and biological material collection sites

The Ethics Committee approved the procedures used in the current experiment (Uso de Animais da Universidade Federal da Paraíba (CEUA-UFPB) No. 135/2015).

We sampled 178 Canindé goats (CAN), belonging to 10 herds (subpopulations) covering a vast geographic area in northeastern Brazil (**Table S1**). The number of sampled herds varied from state to state, according to goat density. Therefore, more herds were sampled in Rio Grande do Norte and Paraíba States.

### DNA extraction, amplification of the D-loop region, and sequencing

Goat DNA was isolated from hair samples through alkaline extraction (Coelho et al., 2004) and was quantified using a spectrophotometer (NanoDrop®, Thermo Scientific).

A 481-bp fragment of the first hypervariable segment (HVR1) of the control region (CR), corresponding to the region between 15,707 and 16,187 bp of the mitochondrial DNA, was amplified from the 178 extracted DNA samples. Amplification was performed via polymerase chain reaction (PCR) in 25-µL reactions, containing 2.5 µL reaction buffer (2.5X stock solution, 1X final buffer concentration), 2.5 µL BSA, 0.5 µL dNTPs (10 mM of each), 1 µL of each primer (10 µM), 0.2 µL (1 U) Taq DNA polymerase, and 0.2 µL genomic DNA (50 ng/µL). PCR amplification was performed according to Pereira et al. (2004), with some adaptations to the PCR conditions, and using the same forward (5'-CGCTCGCCTACACACAAATA-3') and reverse (5'-AAGAGTGGGCGATTTTAGG-3') primers. Amplification cycles were as follows: initial denaturation at 94°C for 3 min; 38 cycles at 94°C for 30 s, at 60°C for 45 s, and at 72°C for 1 min; and a final extension at 72°C for 10 min.

The products obtained were purified using a QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA, USA), following the protocols recommended by the manufacturers. The fragment of interest was sequenced in an ABI PRISM® 3730-XL DNA Analyzer automatic sequencer (Applied Biosystems™), following the manufacturer's instructions and using the same PCR primers.

## Data analysis

Goat D-loop sequences were also retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) were aligned and compared using MEGA® 6 (Tamura et al., 2013). The sequences obtained were compared by alignment to the goat reference sequence AF533441 (Parma et al., 2003), following the criteria described in Pereira et al. (2004). Analysis involved 178 sequences from Brazilian Canindé goats, including 14 additional sequences of different goat haplogroups obtained from GenBank under the following accession numbers: A (AJ317736, AJ317661, and AJ317778; Luikart et al., 2001); B1 (AJ317826; Luikart et al., 2001) and (EF618355 and EF617850.1; Naderi et al., 2007); B2 (AJ317833; Luikart et al., 2001); C (AJ317835 Luikart et al., 2001) and (AB110559; Sultana et al., 2003); D (AB110587; Sultana et al., 2003) and (EF617701; Naderi et al., 2007); F (DQ241349; Sardina et al., 2006); and G (EF617728; Naderi et al., 2007) and (AF533441; Parma et al., 2003), and *Capra pyrenaica* (FJ207528; Hassanin et al., 2009) as outgroup. To include all the available sequence data in the phylogenetic analysis, sequences generated in this study were trimmed and only a 447-bp fragment, between 15,735 and 16,187, was used in this analysis. The polymorphic sites, the number of haplotypes, haplotype diversity (h), and nucleotide diversity (p) were calculated using DnaSP 5.0 (Librado and Rozas, 2009), based on the total 481-bp fragment.

Analysis of molecular variance (AMOVA) was performed using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). The statistical significance of the $F_{ST}$ values was estimated by permutation analysis, using 10,000 permutations. Spatial analysis of molecular variance (SAMOVA) was performed with SAMOVA 1.0 (Dupanloup et al., 2002) to investigate the distribution of genetic diversity throughout the entire dataset, using 100,000 permutations. Because three herds were located nearby in the municipality of Lajes, the three subpopulations were considered as one in SAMOVA.

Sequences were grouped by haplotype, and neighbor-joining tree (NJ) (Saitou and Nei, 1987) and maximum parsimony (MP) trees were generated using MEGA 6 package (Tamura et al., 2013) based on haplotypes, using a single sample by haplotype. The significance of the branches was evaluated after 1000 bootstrap replicates. The NJ tree of the subpopulations was edited using FigTree 1.4.2 (Rambaut, 2014). A median-joining network (Bandelt et al., 1999) of the haplotypes was generated using NETWORK 4.6.1 program (available at http://www.fluxus-engineer-ing.com/sharenet.htm).

# RESULTS

## Polymorphism analysis

The Canindé breed population exhibited many haplotypes (H = 29), with 56 variable sites (Table 1 and **Table S2**). Oliveira (2007) analyzed a 130-bp fragment of the same mitochondrial region in 1531 sequences and found 55 polymorphic sites in goats from northeastern Brazil. Studies on goats from the Brazilian Crespa ecotype (Lopes et al., 2016), Portuguese goats (Pereira et al., 2005), Mediterranean goats (Hughes et al., 2012), Chinese goats (Zhong et al., 2013; Zhao et al., 2014), African goats (Kibegwa et al., 2016; Kadowaki et al., 2016), and goats of different regions worldwide (Naderi et al., 2007) were presented. The fragments of the D-loop region utilized in these studies varied from 130 to 696 bp, with 24 to 118 polymorphic sites.

Our sample of 179 goats included 29 distinct haplotypes, with a haplotype diversity of 0.820, a value higher than the values obtained for Crespa goats (0.740) studied by Lopes et al. (2016) and lower than that observed by Liu et al. (2007) in Chinese goats (0.930). Haplotype diversity was high for each subpopulation (Table 1), ranging from 0.562 (Boa Vista-PB, PBB) to 0.900 (Lajes-K, RNL1).

**Table 1.** Genetic variability in the 10 Canindé goat subpopulations based on 481-bp fragment sequences from the D-loop region of the mtDNA.

| Subpopulation | N | mtDNA H | h | π |
|---|---|---|---|---|
| BA | 18 | 4 | 0.627 | 0.0045 |
| PBT | 19 | 4 | 0.695 | 0.0090 |
| PE | 10 | 6 | 0.888 | 0.0056 |
| PBB | 18 | 6 | 0.562 | 0.0130 |
| PI | 15 | 7 | 0.781 | 0.0102 |
| CE | 18 | 6 | 0.849 | 0.0169 |
| RNP | 20 | 6 | 0.810 | 0.0168 |
| RNL1 | 20 | 11 | 0.900 | 0.0186 |
| RNL2 | 20 | 7 | 0.789 | 0.0129 |
| RNL3 | 20 | 7 | 0.857 | 0.0139 |

Jeremoabo-BA (BA); Taperoá-PB (PBT); Floresta-PE (PE); Boa Vista-PB (PBB); Barro Duro-PI (PI); Sobral-CE (CE); Pedro Avelino-RN (RNP); Lajes-K (RNL1); Lajes-N (RNL2); Lajes-A (RNL3). N: sample size; H: number of haplotypes; h: haplotype diversity; and π: nucleotide diversity.

The nucleotide diversity (π) of the population was 0.0140. The Jeremoabo-BA (BA) subpopulation exhibited the lowest nucleotide diversity (π = 0.0045), whereas the RNL1 population exhibited the highest value (π = 0.0186).

The nucleotide diversity observed in this study was lower than that found by Paiva et al. (2008), 0.0216 ± 0.01146, also in Canindé goats, at the Embrapa Sheep and Goat (Embrapa Caprinos) facility located in Sobral, Ceará State, Brazil.

The RNL1 subpopulation exhibited high h values and low π (Table 1), whereas the others exhibited a combination of high haplotype diversity (Hd) and π values, which may have been due to the sharing of females from different maternal lineages among the subpopulations. The nucleotide diversity was not directly proportional to the haplotype diversity since it is influenced by the haplotype frequencies.

## Distribution, frequency, and haplotype network

Only 34.0% of the haplotypes was shared by the total population while a significant quantity of them (65.51%) was exclusive to specific subpopulations (Figures 1 and 2). RNL2 and PI presented the greatest number of private haplotypes (six and four, respectively). The majority subpopulation showed at least one private haplotype, but the PBT subpopulation did not have exclusive haplotypes (Figures 1 and 2).

Haplotypes H_1 (33.13%), H_2 (19.66%), H_5 (11.79%), and H_6 (10.11%) were the most frequent. The H_3, H_12, H_17, H_18, H_21, and H_28 haplotypes showed ratios ranging from 1.68 to 3.37%. The other haplotypes exhibited a frequency of 0.56%, occurring in only a single individual and accounting for 10.74% of all the haplotypes obtained.

Only the H_1 haplotype was common to all of the subpopulations, present in 59 individuals. The H_2 haplotype appeared in 35 individuals as the second most prevalent haplotype in the population, absent only in the PBB subpopulation. The H_18 was exclusive to

CE subpopulation, present in three individuals. The H_21 was exclusive to both the RNL1 and RNL3 subpopulations. On the other hand, even belonging to the same state of the Federation, the subpopulation RNL2 showed a private haplotype (H_28) in five individuals. All of the haplotypes found belong to the A haplogroup, which is predominant across all continents (Figure 2).

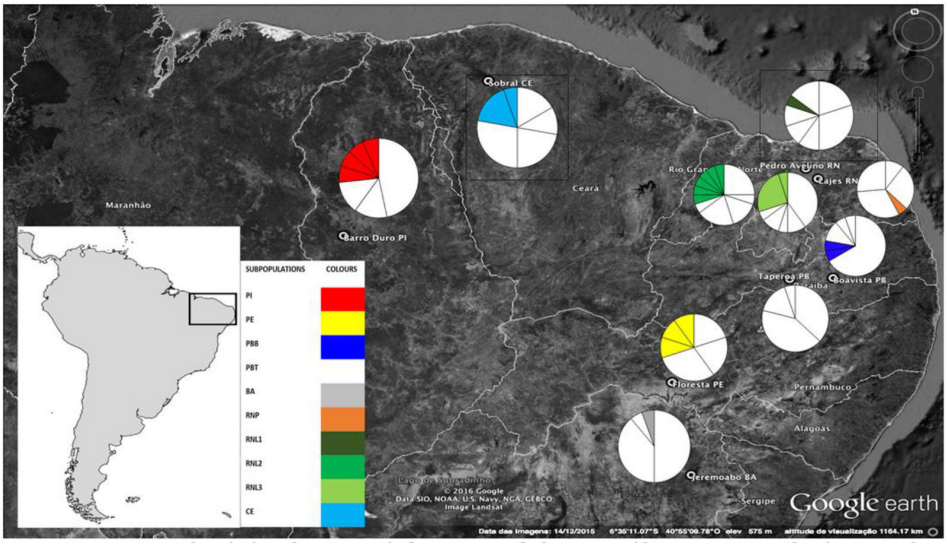


**Figure 1.** Geographical distribution and frequency of the Canindé goat mtDNA haplotype. The different colors are related to the geographical origin represents each exclusive haplotype of the subpopulation.

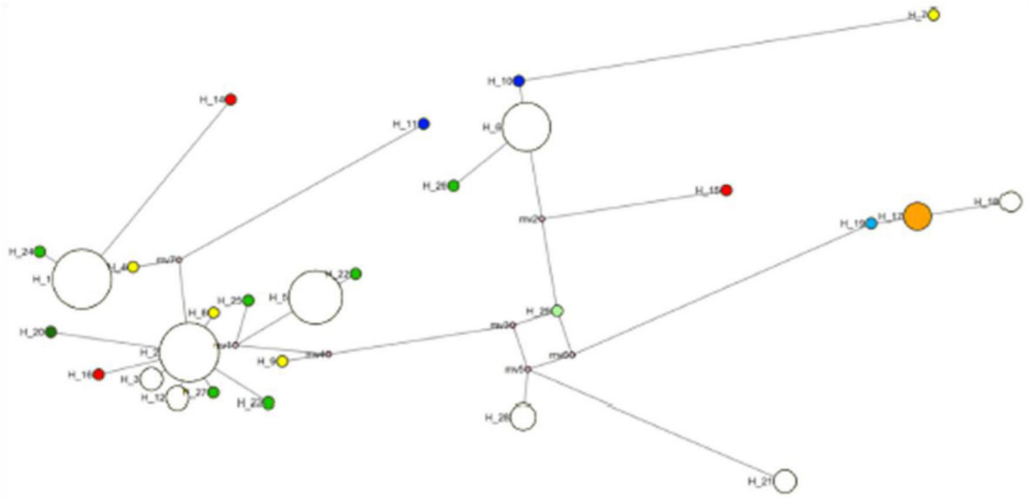

**Figure 2.** Relations among the Canindé goat haplotypes. The size of the circle is proportional to the number of individuals that have the respective haplotype. The small red circles indicate intermediate vectors, introduced by the algorithm executed.

According to the haplotype network, it was possible to show that there was no trend of geographic or subpopulation structure, considering that the haplotypes of the different subpopulations were mixed (Figure 2).

The H_7, H_11, and H_14 haplotypes occur less frequently, occupying a peripheral position in the network, a characteristic that suggests a recent origin of these haplotypes in the population. Seven mean vectors were also identified in the haplotype network; these vectors suggest the possible existence of unsampled haplotypes or haplotypes corresponding to already extinct ancestral haplotypes.

## Genetic differentiation

The estimated genetic differentiation among the 10 Canindé goat subpopulations, based on the $F_{ST}$ fixation index for mtDNA, indicated less genetic differentiation between the RNL3 and BA populations, whereas animals from CE and BA exhibited higher genetic differentiation ($F_{ST}$ = 0.329) (Table 2).

**Table 2.** Pairwise genetic distance estimates between 10 Canindé goat subpopulations based on $F_{ST}$ measurements.

| Pop. | BA | PBT | PE | PBB | PI | CE | RNP | NRL1 | NRL2 | RNL3 |
|---|---|---|---|---|---|---|---|---|---|---|
| BA | 0 | | | | | | | | | |
| PBT | 0.023 | 0 | | | | | | | | |
| PE | 0.322* | 0.191 | 0 | | | | | | | |
| PBB | 0.071 | 0.079 | 0.142 | 0 | | | | | | |
| PI | -0.009 | -0.009 | 0.169 | -0.009 | 0 | | | | | |
| CE | 0.328* | 0.237* | 0.220* | 0.265* | 0.250* | 0 | | | | |
| RNP | 0.239* | 0.085 | 0.015 | 0.161 | 0.132 | 0.162* | 0 | | | |
| RNL1 | 0.134* | 0.023 | 0.059 | 0.102 | 0.047 | 0.203* | 0.013 | 0 | | |
| RNL2 | 0.164* | 0.055 | -0.009 | 0.063 | 0.057 | 0.181* | -0.019 | -0.008 | 0 | |
| RNL3 | 0.135 | 0.062 | 0.102 | 0.050 | 0.046 | 0.210* | 0.068 | 0.038 | 0.018 | 0 |

Jeremoabo-BA (BA); Taperoá-PB (PBT); Floresta-PE (PE); Boa Vista-PB (PBB); Barro Duro-PI (PI); Sobral-CE (CE); Pedro Avelino-RN (RNP); Lajes-K (RNL1); Lajes-N (RNL2); Lajes-A (RNL3). *Significant at $P < 0.05$.

Most divergence ($F_{ST}$) values found among the subpopulation pairs were low to moderate, and not significant, revealing little genetic differentiation (Table 2). According to Wright (1931), $F_{ST}$ values above 0.25 are indicators of strong differentiation. The higher significant genetic difference found in the CE subpopulation compared to the others may be explained by the fact that it is a relatively closed subpopulation, with a limited exchange.

The genetic differentiation of the subpopulations was also assessed using AMOVA, which also revealed a weak but significant genetic differentiation among the subpopulations, a finding strongly supported by the fixation index ($F_{ST}$ = 0.083; $P < 0.05$). Around 11.4% of the variation found in the sequences corresponds to the variation among the subpopulations, whereas 88.6% of the variation was assigned to the variation within subpopulations (Table 3). SAMOVA did not reveal any structure among subpopulations.

## Population dynamics

The neutrality test ($F_S$) was significant when applied to each subpopulation and the entire population ($F_S$ = -2.019; $P < 0.05$). In contrast, the Tajima index revealed significant differences only during the subpopulation analysis.

**Table 3.** AMOVA among the Canindé goat subpopulations.

| Source of variation | Sum of squares | Variance components | Percentage variation |
|---|---|---|---|
| Among subpopulations | 95.950 | 0.41745 | 11.4% |
| Within subpopulations | 547.186 | 3.25907 | 88.6% |
| Total | 643.136 | 3.67652 | 100.0% |

## Phylogenetic tree construction

A dendrogram was constructed with the 477 bp of the 178 Canindé individuals belonging to the 10 subpopulations sampled, using *C. pyrenaica* as the outgroup (GenBank accession number FJ20528.1; Parma et al., 2003). The dendrogram shows that all of the Canindé goat samples belong to the A haplogroup, separate from the other haplogroups. Sequences referring to the C, F, and *C. pyrenaica* haplogroups appear at the base of the tree, followed by the clade with both B subgroups (B1 and B2). Next, the G haplogroup appears, and finally, the D haplogroup appears, more closely related to all of the other sequences, including the four sequences representative of the A haplogroup (Figure 3).

European haplotypes appear in the studied Canindé subpopulations, as shown in the haplotypes H17, H18, and H19, which belong to the A haplogroup (Figure 3). This is observed throughout the world, always at high frequencies (Sultana et al., 2003).

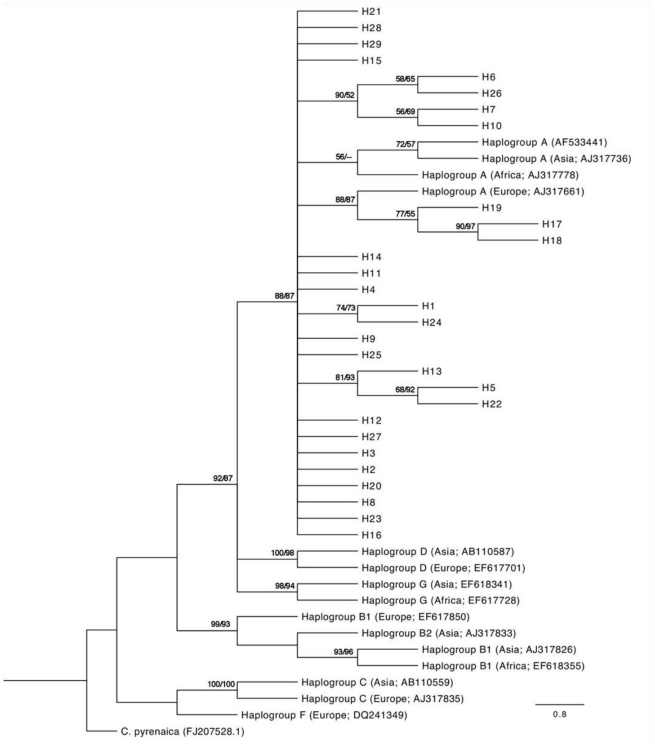


**Figure 3.** Dendrogram constructed based on 481-bp fragments from the HVR1 region of 178 Canindé goat mtDNA sequences, and representative of the six mtDNA haplogroups for goats (A; B1 and B2; C; D; F; and G) and *C. pyrenaica*. Values above the branches stand for the bootstrap support for NJ/MP phylogenies.

## DISCUSSION

The low nucleotide diversity and high haplotype diversity observed in this study may be evidence that the population underwent a genetic bottleneck followed by rapid growth, thereby accumulating mutations (Grant and Bowen, 1998). This diversity could be the result of the diverse goat groups introduced in northeastern Brazil during the colonization period.

According to breeders interviewed during sample collection, the genetic base of the Canindé breed came from several locations in northeastern Brazil, mainly from Bahia State. Thus, the subpopulations had the same genetic origin and were divided for anthropogenic reasons. Although most subpopulations exhibited numerous private haplotypes and lived hundreds of kilometers apart from each other, the lack of differentiation among them could be the result of original stock with a common origin. According to Luikart et al. (2001), weak geographic differentiation is common in livestock breeds due to the heavy flow of animals after domestication. Goats are easily transportable and manageable; therefore, these animals were commonly used as currency for exchange (Wang et al., 2015).

The diversity found in the major haplogroup A is the result of the initial domestication in the Fertile Crescent region, followed by an expansion throughout the world (Luikart et al., 2001). The European haplotypes appear in the Canindé subpopulations studied. This result is attributed to commercial trade that occurred during the colonial period and several animal introductions in Brazil over the years (Ribeiro et al., 2004). The results of the Canindé analyses are supported by the broad distribution of the A lineage, which is dominant throughout the world (Sultana et al., 2003; Naderi et al., 2007; Benjelloun et al., 2011; Zhong et al., 2013; Wang et al., 2015; Lopes et al., 2016; Kadowaki, et al., 2016; Kibegwa et al., 2016).

A consistent finding with all molecular markers is that genetic variability declines with increasing distance from the domestication centers. This has been shown for pigs, sheep, goats, cattle, and chickens (Groeneveld et al., 2010).

A large proportion of genetic variations (88.6%) were distributed within the breed, and the differences among subpopulations within states were 11.4%, indicating a weak phylogeographic structure in Canindé goats. Similar results were reported by Lopes (2012), who found 10.6% variation among Crespa goat populations, and by Oliveira (2007), who found 11.5% variation when comparing goats from northeastern Brazil with those from the Old World. Naderi et al. (2007) also reported that 77% of mtDNA variation is distributed within breeds. In African indigenous goats, very high percentage (99.9%) of the total molecular variance was included in the within-breed component (Kibegwa et al., 2016).

While working with several Brazilian local goat breeds, Oliveira (2007) found a strong participation of European and African breeds in the formation of the Canindé populations. Lima and Loures (2010) point out that the European breeds, which came with the colonizers, were probably also influenced by African breeds brought on slave ships. Several routes were equally important for the introduction of animals to the Americas.

In the 19th century, modern standard breeds began to be introduced in Brazil. From 1925 until 1937, there were 387 animals of Toggenburg breeds from Switzerland, Murciana breeds from Spain, and Angora and Nubian breeds from South Africa (Ribeiro et al., 2004). Therefore, all these different influences, as well as the probable existence of a very distinct haplotype in the founding stock initially introduced in the region, contributed to the genetic diversity remaining in the Canindé goat breed.

## CONCLUSIONS

Based on analyses of the mtDNA D-loop region, no genetic or geographic structure was observed in the sample. The H1, H2, H5, and H6 haplotypes were the most frequent. Even with a reduced effective number, the Canindé breed exhibited mitochondrial variability, with higher variation among individuals than among subpopulations, which makes it possible to maintain the maternal lineages that contributed to forming the breed. The Brazilian Canindé population was classified as haplogroup A, with haplotypes predominantly of European descent. The Brazilian Canindé breed exhibited high haplotype diversity and low nucleotide diversity.

### Conflicts of interest

The authors declare no conflict of interest.

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## Supplementary material

**Table S1.** Geographical location of the Canindé subpopulations used in this study

**Table S2.** Polymorphic sites nucleotide (SNP) observed in the D-loop region of mtDNA between 178 Canindé goats in Northeast Brazil. Nucleotide positions are numbered according to the reference sequence, GenBank AF533441 (Parma et al., 2003). Identical sequences and deletions are represented by dots and dashes, respectively.