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RESEARCH ARTICLE

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Genetic relationships between local Brazilian goat breeds based on mtDNA D-loop region similarity

Núbia M. V. Silva (Silva, NMV)[1], Edgard C. Pimenta-Filho (Pimenta-Filho, EC)[1], Janaina K. G. Arandas (Arandas, JKG)[2], Rosália B. N. Medeiros (Medeiros, RBN)[2], Aderbal Cavalcante-Neto (Cavalcante-Neto, A)[3], Carlos Fonseca (Fonseca, C)[4] and Maria N. Ribeiro (Ribeiro, MN)[2]

[1]Federal University of Paraiba, Animal Science Dept., Areia, Paraiba State, Brazil. [2]Federal Rural University of Pernambuco, Animal Science Dept., Recife, Pernambuco State, Brazil. [3]Federal University of Pará, Animal Science Dept., Guamá, Belém, Pará State, Brazil. [4]Aveiro University, Biology Dept. & CESAM, Aveiro, Portugal.

Abstract

Aim of study: Our objective was to investigate the mitochondrial DNA of local Brazilian goats to gain insights into the genetic composition of this precious genetic resource.

Area of study: The study was developed in Brazil.

Material and methods: We analyzed a hypervariable region of the mitochondrial DNA of 83 goats belonging to four local Brazilian breeds, including Canindé (CAN-RN), Moxotó (MOX-CE), Marota (MAR-PI) and Azul (AZU-PE) as well as of exotic breeds raised in different states of the Federation. Sequences related to local Brazilian goats showed a dispersed distribution throughout the median-joining network, and clustering with sequences of exotic breeds occurred in some haplotypes. The obtained sequences were analyzed and compared with different haplogroups (A, B1, B2, C, D, F, and G) available on GenBank.

Main results: The local Brazilian goat breeds showed significant diversity, with 16 (0.8240) haplotypes. Population structure analysis revealed substantial differences among breeds ($p < 0.05$). Mitochondrial lineage A was observed in Brazilian goats. Phylogeny showed European goats as the dominant stock for Brazilian goats, but there weare some haplotypes within haplogroup A, clustering with African and Asian haplotypes.

Research highlights: These results could be suitable for creating a strategic conservation program, potentially benefitting future breeding programs.

Additional keywords: Brazilian goats; *Capra hircus*, mitochondrial lineages; phylogenetic analyses.

Abbreviations used: ALP-BA (British Alpine breed in Bahia state); AZU-PE (Azul breed in Pernambuco state); CAN-RN (Canindé breed in Rio Grande do Norte state); CRE (Crespa breed); MOX-CE (Moxotó breed in the Ceará state); MAR-PI (Marotá breed in Piaui state); SAA-PE (Saanen breed in Pernambuco).

Authors' contributions: NMVS: Responsible for the execution of the experiment and scientific writing. Work is part of the doctoral thesis. Technical support: JKGA and RBNM. Data analysis: CF. Revision of the manuscript: JKGA. Critical review of the manuscript regarding intellectual content: ECPF, CAN, CF, and MNR. Supervision of the work and coordination of the research Project: MNR.

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Correspondence should be addressed to Maria N. Ribeiro: normaribeiro70@gmail.com; maria.nribeiro@ufrpe.br

Introduction

Goats are vital to the livelihoods of many Brazilians and the broader Brazilian economy. They are especially vital in the semiarid region, which raises 7.6 million heads, representing more than 90% of the national goat herds (IBGE, 2018). There are ten local goat breeds in the country (Moxotó, Canindé, Marota, Repartida, Gurguéia, Azul, Crespa, Graúna, Parda Sertaneja, and Nambi) with the effective number below 50 (Lima *et al*., 2007). The Moxotó and Canindé are the only officially recognized breeds and the most widespread ones in the country.

The large quantity of goat breeds in Brazil indicates substantial genetic diversity, but previous studies on the ancestry of Brazilian goats have proved controversial. Recently, Ginja *et al*. (2017) have verified that the level of genetic diversity in our creole goats is lower than that observed in other parts of the world, possibly reflexing the effect of genetic introduction from the domestication center, agreeing with Sevane *et al*. (2018), who observed a significant contribution of African breeds to Brazilian goat breeds. Ribeiro *et al*. (2012) observed high genetic diversity among Portuguese and Brazilian goat populations. On the other hand, Amills *et al*. (2009) could not find a clear Iberian signature.

In the Americas, especially Brazil and the United States, there has been a large gene flow of animals from continents such as Europe, Africa, and Asia due to the constant importation of specialized meat-, milk- and skin-producing animals (Carvalho *et al*., 2015). Goat imports, over time, have been made without planning, which has put the available genetic heritage of the animals at risk.

The diversity of Brazilian goat genetic resources reflects the adaptation to different production systems, with a predominance of local breeds, which are in several levels of threat. Little knowledge exists regarding the formation, migration, and evolution of the genetic status of Brazilian local goat breeds, and more technical information on the genetic diversity of Brazilian goats is therefore necessary (Câmara *et al*., 2017). This study used the mtDNA control region (D-loop) to determine the diversity and genetic structure of goat populations in the northeast of Brazil and to investigate the presence of non-European mitochondrial haplotypes in Brazilian goats.

Material and methods

Sampling

The procedures employed here were approved by the Ethics Committee for the usage of Animals in experiments of the Federal University of Paraiba (CEUA-UFPB) nº 135/2015.

Hair samples from 113 goats were collected, corresponding to four Brazilian local and two exotic breeds. Data were obtained from Canindé, n = 20, raised in Rio Grande do Norte state (CAN-RN); Moxotó, n = 19, raised in the Ceará state (MOX-CE); Marota, n = 24, raised in Piaui state (MAR-PI); Azul breed, n = 20, raised in Pernambuco state (AZU-PE) (Table 1). Two cosmopolitan exotic breeds were used in this study: Saanen, n = 20, raised in Pernambuco State (SAA-PE), and British Alpine, n = 10, raised in Bahia state (ALP-BA). Care was taken to distinguish pure native breeds from crossed or commercial breeds (*e.g*., cosmopolitan breeds) and non-descript breeds through accessing indigenous knowledge shared by breeders.

DNA extraction and D-loop region amplification and sequencing

The DNA of the 113 individuals was isolated from hair samples via alkaline extraction, according to Coelho *et al*. (2004), and quantified using a spectrophotometer (NanoDrop®, Thermo Scientific).

The PCRs were performed according to Pereira *et al*. (2004), with some modifications, using the forward primer 5'-CGCTCGCCTACACACAAATA-3' and the reverse primer 5'-GAAGAGTGGGCGATTTTAGG-3'. The PCR program consisted of an initial denaturation at 94°C for 3 min, followed by 38 cycles of 94°C for 30 s, 60°C for 45 s and 72°C for 1 min, with a final extension step at 72°C for 10 min. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's recommendations. The amplified fragments were sequenced using the same primers used for the PCRs and an automatic ABI Prism 3730XL DNA analyzer (Applied Biosystems), according to the manufacturer's recommendations.

Data analysis

The sequences were edited, aligned, and compared using the Clustal W algorithm and MEGA® v. 7 software (Tamura *et al*., 2013). For the Brazilian local

Table 1. Key features of the analyzed sequences from populations of local Brazilian goats.

Breed	Nº. of individuals	No. of polymorphic sites	No. of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's D	Fu's FS
Canindé	20	27	10	0.8842	0.0188	0.4981	1.060
Azul	20	19	5	0.7368	0.0165	1.7299	6.463
Moxotó	19	22	5	0.6842	0.0185	1.5409	6.934
British Alpine	10	10	5	0.8222	0.0083	0.5275	0.977
Saanen	20	20	8	0.8947	0.0166	1.4448	2.184
Marota	24	25	8	0.8478	0.0164	0.5902	3.175

and exotic goat analyses, a fragment between the nucleotide positions 15,707 and 16,187 was taken into consideration, with 481 bp of the control region referring to 113 sequences. The concatenated sequences were aligned against the reference sequence AF533441 (Parma *et al*., 2003) and further trimmed using Clustal W implemented in the MEGA7 software.

To provide more complete information on the origin of Brazilian goats, new mtDNA sequences of both wild and domestic goats were included: haplotypes A (accessions 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), plus those of the Crespa breed (CRE) submitted to Genbank by Lopes *et al*. (2016), (Crespa, n = 10, KM260525.1-KM260536.1; Anglo-Nubian, n = 6, KM260504.1-KM260509.1; and Boer, n = 6, KM260519.1- KM260524.1).

The comparison was performed according to Pereira *et al*. (2004), using *Capra pyrenaica* as an outgroup (FJ207528; Hassanin *et al*., 2009). As many published goat mtDNA sequences are substantially shorter or longer than our 481 bp sequence, fragments of 464 bp instead of 481 bp were considered to allow a comparison of sequences of the same size. These sequences were included to facilitate the recognition of haplogroups and to determine phylogenetic relationships. All fragments from the three datasets were aligned and trimmed using Clustal W in the MEGA7 software.

Polymorphic sites, number of haplotypes, haplotype diversity (h), and nucleotide diversity (p) were calculated using DnaSP 5.0 (Librado & Rozas, 2009). The haplotypes found in each breed were deposited in GenBank under the accession numbers KM500901-KM500921. The molecular variance was analyzed (AMOVA), along with diversity measures, F_{ST} (estimates of genetic differentiation among populations) distances and Fu's Fs (test for an excess of rare alleles) values, using the Arlequin v. 3.5 software (Excoffier & Lischer, 2010). We constructed a median-joining network for all sequences using the Network v. 4.6.1 software (http://www.fluxus-engineering.com/sharenet.htm). The demographic expansion was estimated using Fu's F_S neutrality test.

The MEGA v.7 software (Tamura *et al*., 2013) was also used to generate a Maximum Likelihood tree to identify relationships between the sampled populations, using the Kimura 2-parameter model with 1,000 bootstrapping replicates. All positions containing gaps and missing data were deleted.

Results and discussion

Variation of mtDNA in the most representative local and exotic goat breeds raised in Brazil

Alignment of 481 bp of the hypervariable region, with 83 control region sequences (15,707-16,188) from local Brazilian goats, showed 33 polymorphic sites. The fragment showed a high-frequency polymorphism in the Brazilian goats, with a haplotype diversity (h) of 0.8240, revealing 16 different haplotypes. Of these, 88% were different from each other. The most frequent sequence appeared 27 times, while 11 sequences occurred only once, signifying that they were unique to a breed (Canindé, n = 6; Marota, n = 3; Azul, n = 1; and Moxotó, n = 1) (Fig. 1). The most frequent haplotype (H2) was shared among all local Brazilian breeds, while the second most shared haplotype (H5) was also common to all local breeds studied here. The haplotypic diversity index (Hd) for each population was high (above 0.6), and previous studies have verified that the index ranges from 0.6842 (Moxotó) to 0.8842 (Canindé) (Table 1). Nucleotide diversity ranged from 0.0164 (Marota) to 0.0188 (Canindé); the lowest nucleotide diversity was observed in the Marota goat breed.

The neutrality test was not statistically significant for the populations analyzed (Table 1). Table 1 shows the number of polymorphic sites and Hd, π, Tajima´s D and Fu's F_S values for all breeding populations. We observed no tendency toward breed structuring.

The high degree of diversity found in Brazilian caprine breeds, with some haplotypes sharing lineage

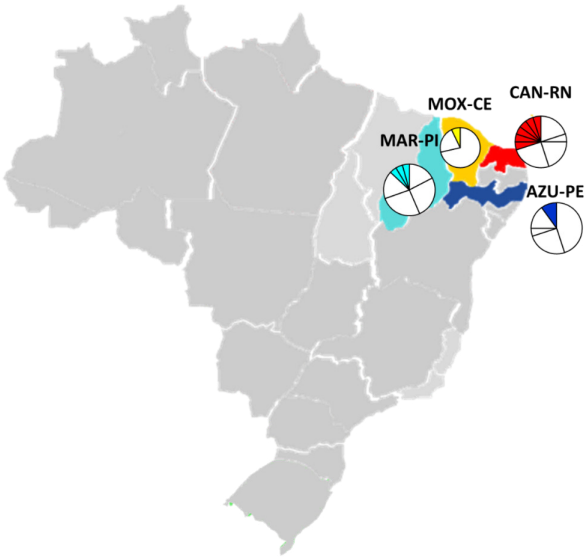

Figure 1. Geographical and mtDNA haplotype distribution of local Brazilian goat breeds: Marota (MAR-PI), Moxotó (MOX-CE), Canindé (CAN-RN) and Azul (AZU-PE). The different colors are related to the geographic origin and represent the particular haplotype of each population.

Table 2. Fixation index (F_{ST}) for the nine populations evaluated.

Breeds	1	2	3	4	5	6	7	8	9
1	0.00000								
2	0.24227*	0.00000							
3	0.05970	0.01415	0.00000						
4	0.02295	0.07034*	-0.02197	0.00000					
5	0.40220*	0.05955	0.16445*	0.20296*	0.00000				
6	0.12985*	0.02248	-0.01465	-0.00997	0.15115*	0.00000			
7	0.15107*	0.15743*	0.05320	0.05690	0.23832*	0.10453	0.00000		
8	0.22942*	0.10394	0.07272	0.08189	0.13954	0.09583	-0.03299	0.00000	
9	0.11183*	0.24721*	0.12020*	0.07998*	0.34304*	0.15183*	0.02524	0.09678	0.00000

Breeds: 1, British Alpine; 2, Azul; 3, Canindé; 4, Marota; 5, Moxotó; 6, Saanen; 7, Anglo-Nubian; 8, Boer; 9, Crespa.
*Significant at $p<0.05$.

with foreign breeds, is consistent with our knowledge about the initial formation of the national herd. Specifically, it seems to be the result of European/African animals that were introduced into the country. This argument is consistent with historical accounts of the introduction of domestic goats into Brazil by Portuguese settlers in 1515 (Simonsen, 1937).

In addition to the colonial history of Brazil, Saanen, British Alpine, Anglo-Nubian, Boer, and other breeds were introduced over the decades to increase production. Many breeders used them in crosses to improve the production of local breeds, and this inclusion of exotic, non-targeted material culminates in genetic dilution.

The analysis revealed 61 polymorphic sites with 40 different haplotypes, with 75% of all haplotypes different from one another. The most common sequence appeared 31 times, reaching a frequency of 22.79%, while 30 sequences occurred only once. Of these 40 identified haplotypes, 11 were unique to northeastern Brazilian breeds, including Canindé (H26, H27, H28, H29, H30, and H31), Marota (H32, H33, and H35), Azul (H13) and Moxotó (H36) (Figs. 1 and 2).

The most common haplotype (H12) was shared among 31 individuals of the Canindé, Azul, Moxotó, Marota, and Saanen breeds. The second most shared haplotype (H2) was absent only in the ANG breed (Fig. 2). There were no haplotypes common to all local and exotic breeds studied here.

The values of F_{ST} and numbers of haplotypes shared among all breed pairs were used to establish the genetic distances among all studied groups (Table 2). The most notable example of genetic differentiation was observed between ALP-BA and MOX-CE breeds ($F_{ST} = 0.4022$), while the most compelling example of significant weak differentiation was observed between MAR-PI (Population 4) and AZU-PE (Population 2) breeds.

Comparative analysis of Canindé with other goat breed populations

A phylogenetic tree was constructed for the available mtDNA control region sequences to analyze the relationship between the goat breeds raised in Brazil (local and exotics) and those from other world regions. Additionally, it was used to compare the local goat diversity, allowing researchers to compare local Brazilian breeds with other domestic and wild goats.

We identified some haplotypes that are present in the Iberian goat breeds (Parma *et al*., 2003). The local Brazilian goat breeds presented specific haplotypes in Similar results have also been found in previous studies (Pereira *et al*., 2005; Amills *et al*., 2009).

All Brazilian goat breeds were included in maternal lineage A and separated from the remaining groups, which clustered together (Fig. 3). The haplogroup A is the most frequent group and has been identified in all goat breeds reported so far (Luikart *et al*., 2001; Wang *et al*., 2015; Deng *et al*., 2018). The diversity among members of haplogroup A results from the beginning of domestication in the fertile crescent region and its global expansion (Luikart *et al*., 2001). The majority of the goat breeds in the world belong to this haplogroup, which agrees with the theory that the place of domestication of goats is the fertile crescent region (Othman & Mahfouz, 2016). After domestication, the goats that already carried differentiated gene pools spread to Europe, Africa and Asia (Colli *et al*., 2018).

The highest bootstrap value for external and internal branches was 98%, sequences referring to *C. pyrenaica* as well, and haplogroups (Fig. 3). We did not detect the mitochondrial lineages B, C, D, F, and G since they are rarely found in most of the European continent (Naderi *et al*., 2007; Amills *et al*., 2009). More than 90% of the goats studied with the D-loop region worldwide are from haplogroup A. On the other hand, the B lineage

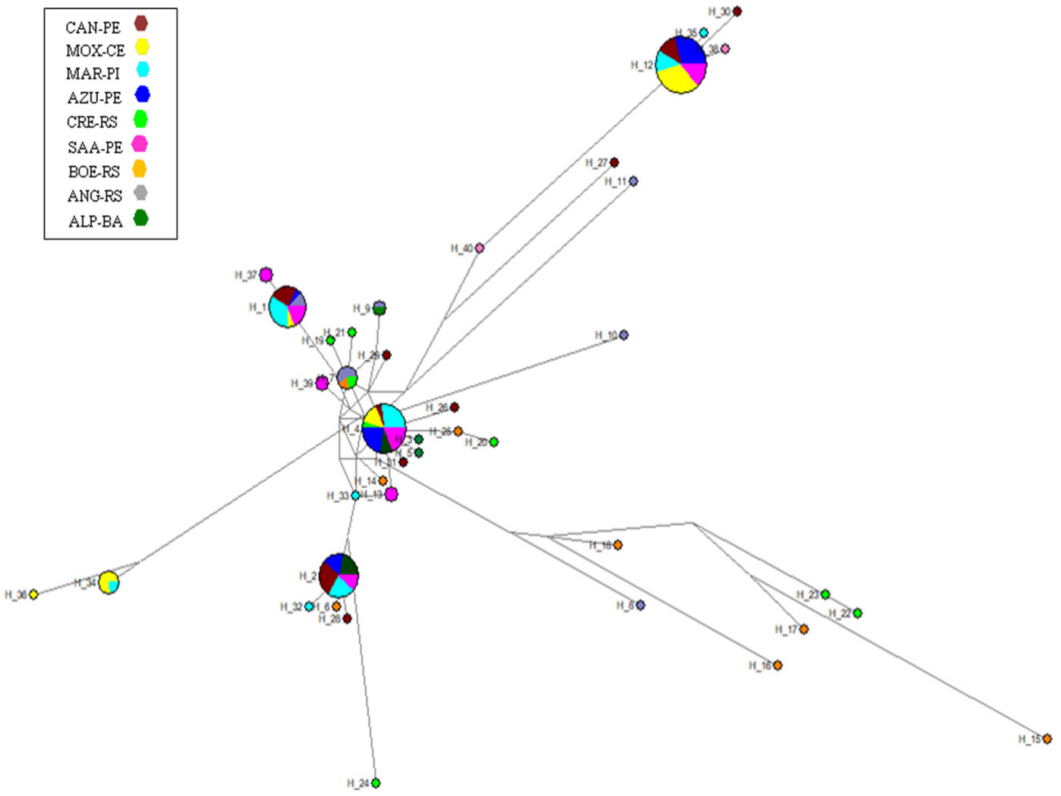

Figure 2. Relationship between the haplotypes (H) of five local Brazilian goat breeds [Canindé (CAN-PE), Moxotó (MOX-CE), Marota (MAR-PI), Azul (AZU-PE), and Crespa (CRE-RS)] and four exotic breeds [Saanen (SAA-PE), Boer (BOE-RS), Anglo-Nubian (ANG-RS) and British Alpine (ALP-BA)]. In the comparative pie charts the areas are proportional to the haplotype frequencies.

has been found only in Asia and South Africa, while the C lineage is found in southern Europe, the D lineage in Asia, the F lineage in Italy and the G lineage in southwest Asia and North Africa (Groeneveld *et al*., 2010; Pakpahan *et al*., 2016).

Recent studies using other molecular markers have found divergences regarding the ancestry of Brazilian goats. Colli *et al*. (2018), using SNP markers, found that Canindé (CAN-RN) and Moxotó (MOX-PE) breeds strongly exhibit ancestry with West African animals, while Ginja *et al*. (2017), with microsatellite markers, showed a European origin of these breeds. The present study revealed European goats as the dominant stock for Brazilian goats, but there are some haplotypes within haplogroup A, clustering with African and Asian goat breeds.

The large variety of local goats represent an important genetic reservoir. Even in areas far from domestication centers, there is a significant genetic diversity that can benefit conservation programs. These results could be suitable for creating a strategic conservation program, potentially benefitting future breeding programs.

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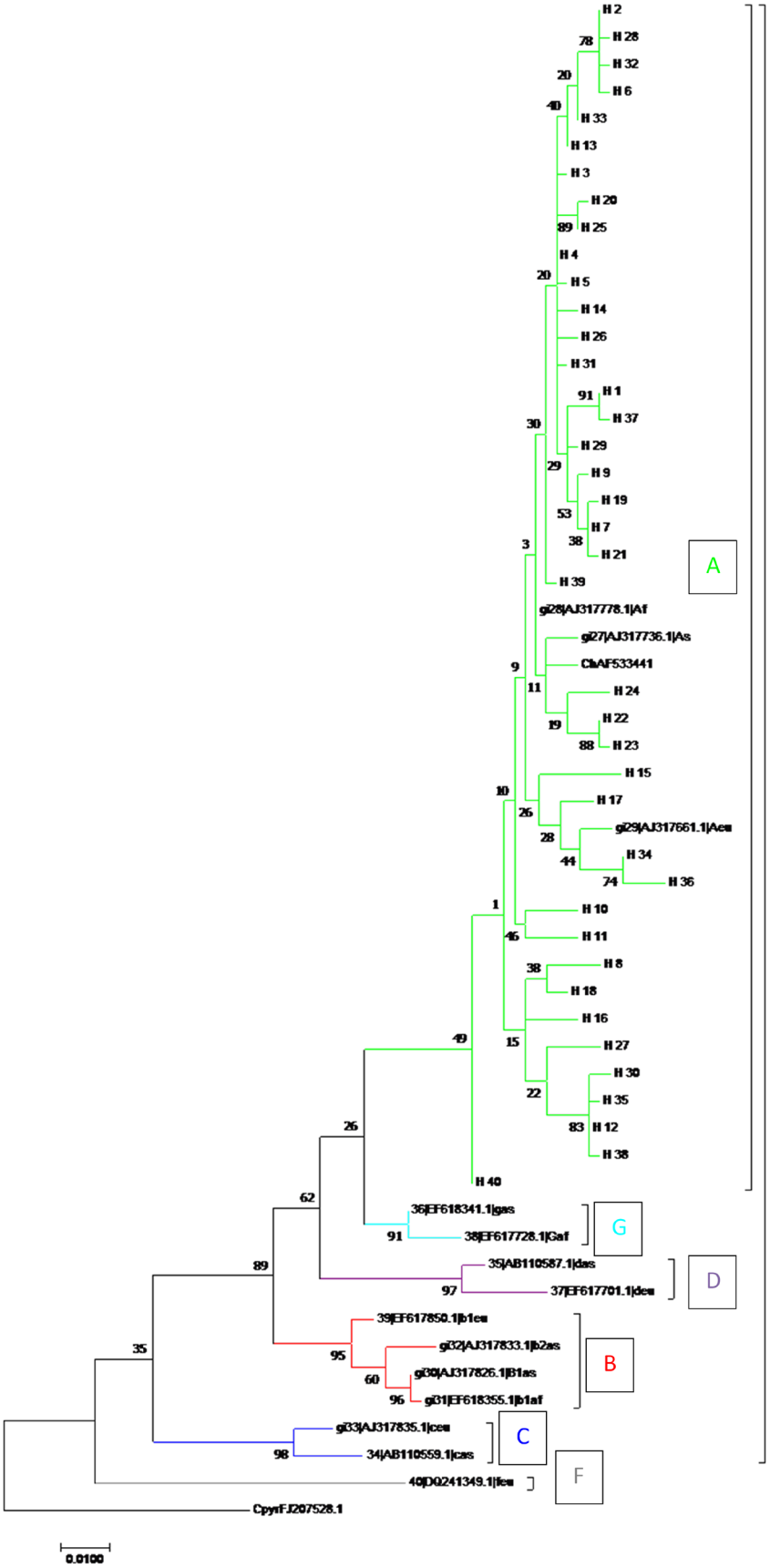

Figure 3. Phylogram is showing the molecular phylogenetic analysis using the maximum likelihood method: the trees of local Brazilian goat are based on 40 mtDNA haplotypes (H); fifteen sequences as references for domestic goat haplogroups A, B, C, D, F, G; outgroup, *Capra pyrenaica*.

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