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)¹, Aderbal Cavalcante-Neto (Cavalcante-Neto, A)³, Carlos Fonseca (Fonseca, C)⁴ and Maria N. Ribeiro (Ribeiro, MN)²

¹Federal University of Paraíba, Animal Science Dept., Areia, Paraíba State, Brazil. ²Federal Rural University of Pernambuco, Animal Science Dept., Recife, Pernambuco State, Brazil. ³Federal University of Pará, Animal Science Dept., Guamá, Belém, Pará State, Brazil. ⁴Aveiro 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 were 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 Piauí 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: JKG. Critical review of the manuscript regarding intellectual content: ECPF, CAN, CF, and MNR. Supervision of the work and coordination of the research Project: MNR.

Citation: Silva, NMV; Pimenta-Filho, EC; Arandas, JKG; Medeiros, RBN; Cavalcante-Neto, A; Fonseca, C; Ribeiro, MN (2019). Genetic relationships between local Brazilian goat breeds based on mtDNA D-loop region similarity. Spanish Journal of Agricultural Research, Volume 17, Issue 4, e0407.

Received: 10 Feb 2019. Accepted: 07 Jan 2020.

Copyright © 2019 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Funding agencies/Institutions

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Conselho Nacional de Desenvolvimento Científico e Tecnológico

Competing interests: The authors have declared that no competing interests exist.

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 semi-arid 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, Guruguêa, 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 reflecting 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) n° 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 Piauí 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’-CGCTCGCTACACACAAATA-3’ and the reverse primer 5’-GAAGAGTGGGCCGATTAGG-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 breeds, the genetic diversity was calculated using the number of polymorphic sites (π) and diversity (h). Tajima’s D and Fu’s FS were also calculated.
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, Fst (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 Fs 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 0.8842 (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, p, Tajima’s D and Fu’s Fs 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
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.

### 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 \( p<0.05 \).

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

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.

References

Amills M, Ramírez O, Tomás A, Badaoui B, Marmi J, Acosta J, Sánchez A, Capote J, 2009. Mitochondrial DNA diversity and origins of South and Central American goats. Anim Genet 40: 315-322. https://doi.org/10.1111/j.1365-2052.2008.01837.x

Câmara TS, Nunes JF, Diniz FM, Silva GR, de Araujo AM, 2017. Genetic diversity and relatedness between Canindé and British Alpine goat breeds in Northeastern Brazil accessed by microsatellite markers. Gen Mol Res 16 (1): gmr16019569. https://doi.org/10.4238/gmr16019569

Carvalho GMC, Paiva SR, Araújo AM, Mariante A, Blackburn HD, 2015. Genetic diversity and relatedness between Canindé and British Alpine goat breeds from Brazil and the United States: Implications for conservation and breeding programs. J Anim Sci 93: 4629-4636. https://doi.org/10.2527/jas.2015-8974

Coelho EGA, Oliveira DAA, Teixeira C, Sampaio IBM, Rodrigues S.G, Alves C, 2004. Comparação entre métodos de estocagem de DNA extraído de amostras de sangue, sêmen e pelos e entre técnicas de extração. Arq Bras Med Veterinária e Zootec 56: 111-115. https://doi.org/10.1590/S0102-09352004000100017
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*. 
Colli L, Milanesi M, Talenti A, Bertolini F, Chen M, Crisâ A., Daly KG, Del Corvo M., Gulbrandsen, B., Lenstra, J.A., et al., 2018. Genome-wide SNP profiling of worldwide goat populations reveals strong partitioning of diversity and highlights post-domestication migration routes. Genet Sel Evol 1: 50-58. https://doi.org/10.1186/s12711-018-0422-x

Deng J, Feng J, Li L, Zhong T, Wang L., Guo J, Ba G, 2018. Polymorphisms, differentiation and phylogeny of 10 Tibetan goat populations inferred from mitochondrial D-loop sequences. Mitochondrial DNA A DNA Mapp Seq Anal 29 (3): 439-445. https://doi.org/10.1080/24701394.2017.1303491

Excoffier L., Lischer HEL., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564-567. https://doi.org/10.1111/j.1755-2099.2010.02847.x

Ginja C, Gama LT, Martinez A., Sevane N., Martin-Burriel I, Lanari MR., Revidatti MA, Aranguren-Mendéz JA, Bedotti DO, Ribeiro MN, Sponenberg P, Aguirre EL., et al. 2017. Genetic diversity and patterns of population structure in Creole goats from the Americas. Anim Genet 48: 315-329. https://doi.org/10.1111/age.12529

Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B, Pilling D, Negrini R, Finlay EK, Jianlin H, Groeneveld E, Weigend S, 2010. Genetic diversity in farm animals - A review. Anim Genet 41: 6-31. https://doi.org/10.1111/j.1755-260X.2010.00208.x

Hassanin A, Ropiquet A, Couloux A, Cruaud C, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Lima P, Souza D, Pereira G, Torreao J, Moura J, Gomes J, Lopez Acosta J, Rey S, Ribeiro M, Pimenta Filho E, 2007. Gestao genetica de raças caprinas nativas no Estado da Paraiba. Arch Zootec 56: 623-626.

Lopes DO, Fernández GP, Poli M, Moreira GRP, Gonçalves GL, 2016. Ancestry analysis of locally adapted Creusa goats from southernmost Brazil. Genet Mol Res 15: 1-16. https://doi.org/10.4238/gmr.15028324

Luikart G, Gielly L, Excoffier L., Vigne JD, Bouvet J, Taberlet P, 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proc Natl Acad Sci 98: 5927-5932. https://doi.org/10.1073/pnas.091591198

Naderi S, Rezaei HR, Taberlet P, Zundel S, Rafat SA, Naghash HR, El-Barody, Mohamed AA, Ertugrul O, Pompanon F, Abo-Shehada M, et al., 2007. Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. PLoS One 2(10): e1012. https://doi.org/10.1371/journal.pone.0001012

Othman OE, Mahfouz ER, 2016. Genetic biodiversity, affinities and phylogeny of four goat breeds in Egypt. J Biol Sci 16: 86-92. https://doi.org/10.2592/jbs.2016.86.92

Pakpahan S, Artama WT, Widayanti R, Gede Suparta I, 2016. Genetic characteristics and relationship in different goat populations of Indonesia based on cytochrome B gene sequences. Asian J Anim Sci 10: 29-38. https://doi.org/10.4393/ajas.2016.29.38

Pereira L, Van Asch B, Amorim A, 2004. Standardization of nomenclature for dog mtDNA D-loop: a prerequisite for launching a Canis familiaris database. Forensic Sci Int 141: 99-108. https://doi.org/10.1016/j.forsciint.2003.12.014

Pereira ML, Lui JF, De Oliveira JV, 2005. Citogenética de jumentos da raça Marchadora Brasileira. Pesqui Agropec Bras 40: 179-182. https://doi.org/10.1590/S0100-204X2005000200012

Parma P, Feligini M, Greppi GF, Giuseppe E, 2003. Short communication: The complete nucleotide sequence of goat (Capra hircus) mitochondrial genome. DNA Seq 14 (3): 199-203. https://doi.org/10.1080/1042517031000089487

Ribeiro MN, Bruno-de-Sousa C, Martinez-Martinez A, Ginja C, Menezes MPC, Pimenta-Filho EC, Delgado JV, Gama LT, 2012. Drift across the Atlantic: Genetic differentiation and population structure in Brazilian and Portuguese native goat breeds. J Anim Breed Genet 129: 79-87. https://doi.org/10.1111/j.1439-0388.2011.00942.x

Sardina MT, Ballester M, Marmi J, Finocchiaro R, Van Kaam JBCHM, Portolano B, Folch JM, 2006. Phylogenetic analysis of Sicilian goats reveals a new mtDNA lineage. Anim Genet 37: 376-378. https://doi.org/10.1111/j.1365-2052.2006.01451.x

Sevane N, Cortés O, Gama LT, Martinez A, Zaragoza P, Amills M., Bedotti DO, Bruno de Sousa C, Cañon J, Dunner S et al., 2018. Dissection of ancestral genetic contributions to Creole goat populations. Animal 12 (10): 10 pp. https://doi.org/10.1017/S175171117003627

Simonsen RC, 1937. História econômica do Brasil: 1500-1820. Companhia Editora Nacional.

Sultana S, Mannen H, Tsuji S, 2003. Mitochondrial DNA diversity of Pakistani goats. Anim Genet 34: 417-421. https://doi.org/10.1071/0003-582X.017.1303491

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: Molecular evolutionary genetics analysis, vers. 6.0. Mol Biol Evol 30: 2725-2729. https://doi.org/10.1093/molbev/ms3197

Wang GZ, Pi XS, Ji ZB, Qin ZJ, Hou L, Chao TL, Wang JM, 2015. Investigation of the diversity and origins of Chinese dairy goats via the mitochondrial DNA D-loop. J Anim Sci 93: 949-955. https://doi.org/10.2527/jas.2014-8420