Genetics and Molecular Research

Ancestry analysis of locally adapted Crespa goats from southernmost Brazil

D.D. Lopes[1], G.P. Fernández[2], M. Poli[3], G.R.P. Moreira[4], G.L. Gonçalves[1,5] and T.R.O. Freitas[1]

[1]Departamento de Genética,
Programa de Pós-Graduação em Genética e Biologia Molecular,
Universidade Federal do Rio Grande do Sul,
Porto Alegre, RS, Brasil
[2]Centro de Bioinvestigaciones, CITNOBA,
Universidad Nacional del Noroeste de la Provincia de Buenos Aires,
Buenos Aires, Argentina
[3]Instituto de Genética Ewald Favret, CICVyA-INTA CC 25, Castelar, Argentina
[4]Departamento de Zoologia, Programa de Pós-Graduação em Biologia Animal,
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[5]Instituto de Alta Investigación, Universidad de Tarapacá, Arica, Chile

Corresponding author: G.L. Gonçalves
E-mail: lopes.goncalves@ufrgs.br

Genet. Mol. Res. 15 (2): gmr.15028324
Received December 22, 2015
Accepted February 11, 2016
Published June 24, 2016
DOI http://dx.doi.org/10.4238/gmr.15028324

ABSTRACT. Crespa goats are phenotypically similar to the Angora breed, and are traditionally reared in small, low-tech farms in southernmost Brazil. Whether they represent degenerated remnants of pure Angora goats or result from foreign breeds introduced during colonial times and recently mixed with commercial breeds is unknown. Since the degree of relatedness of Crespa in relation to other goats is completely unknown, we performed a comparative assessment of the genetic similarity between Crespa and foreign commercial breeds reared in the region (Angora, Alpine, Anglo-Nubian, Boer, and Saanen), particularly the Angora. We used 11 microsatellites to score alleles

in 148 individuals and performed a Bayesian assignment test, which revealed six clusters (K = 6; Ln likelihood = -5047.6). In addition, a segment of the mitochondrial DNA (mtDNA) control region was sequenced to investigate the relatedness of Crespa goats to Portuguese autochthonous breeds (Algarvia, Bravia, Charnequeira, Serpentina, and Serrana). The origin of the Crespa breed could not be ascertained from the mtDNA, but it does not only descend from the Angora. It is probably related to other introduced and autochthonous Portuguese breeds, in particular the Algarvia. Therefore, our results indicate that this distinctive source of genetic diversity is partly a remnant of animals that were introduced during the colonial period. By recognizing it as genetically distinct, we provide further support for the protection of this particular gene pool.

Key words: Autochthonous ecotype; Naturalized livestock breed; Control region; Genetic variation; Microsatellite

INTRODUCTION

South American goatherds are mainly formed from breeds of the Iberian Peninsula brought during the colonial period (Amills et al., 2009; Ribeiro et al., 2012). Over the centuries, many regional ecotypes have emerged, i.e., phenotypically unique populations with no formal taxonomic recognition that are adapted to harsh environmental conditions. However, the absence of an effective conservation genetic strategy and uncontrolled introgression with foreign breeds seriously threatens the future of these particular genetic resources (Pariacote, 2006).

In the beginning of the 20th century, the Angora goat was traditionally bred in the Pampean Province (Morrone, 2006) of Rio Grande do Sul (RS), the southernmost State of Brazil. This province can be roughly divided into two physiognomic types. The grass-dominated matrix that predominates in the Northeast is more or less interwoven with fragments of semideciduous forests and herbaceous and shrub vegetation, which form a mosaic in the East. This area is also known as the Southeastern Highlands, because it reaches higher elevations than other Pampean areas (Figure 1A). The steppe savanna is interspersed with a mixture of shrub vegetation, boulders, and gallery forests (Figure 1B and C). Farming has employed traditional land-management practices since the region was colonized, primarily by the Portuguese, in the late 18th century (Rambo, 1942).

In addition to the Angora, which originated in Turkey and is the only breed that produces mohair (Shelton, 1993) (a fiber only slightly coarser than cashmere), other European breeds were introduced into the region to increase either milk production (e.g., Alpine and Saanen) or both milk and meat (e.g., Anglo-Nubian) (Pires et al., 2012). The South African Boer was introduced later to improve meat production. The rearing of Angoras in southernmost Brazil mainly occurred in the first half of the 20th century, when it was the main goat breed in RS (Hervé, 1922) and was reared primarily for the production of mohair. As far as we are aware, the last recorded importation of purebred Angora bucks and does by Brazilian authorities was from the USA in 1950. Subsequent to the decline of this economic activity due to the increasing use of synthetic fibers by the end of the last century, the demand for mohair by the Brazilian textile industry decreased substantially, and animals were no longer sheared on a

regular basis. Consequently, most Angora herds were exterminated or crossed with other breeds.

A particular ecotype that resembles Angora, commonly named Crespa (curly), has been recognized in the region (Figure 1C and D), and might represent degenerated remnants of pure Angora goats (Oliveira et al., 2012). Alternatively, the Crespa could have resulted from the introgression of breeds introduced by the Portuguese during colonial times with other improved commercial breeds also introduced into the region. Interbreeding in combination with minimum management and microevolutionary processes could have produced the phenotypic differences found in the Crespa ecotype. However, the degree of relatedness of Crespa in relation to other goats currently being bred in southernmost Brazil is unknown. Therefore, genetic characterization is required in order to recognize Crespa as a pure breed.

In this study, we used molecular markers to assess the extent of genetic similarity between remnant herds of Angora-style goats in southernmost Brazil and foreign commercial breeds reared in the same region (Angora, Alpine, Anglo-Nubian, Boer, and Saanen). We used 11 microsatellite loci to score alleles and perform a Bayesian assignment test. In addition, a segment of the mitochondrial DNA (mtDNA) control region was sequenced to investigate the relatedness of Crespa to Portuguese autochthonous breeds (Algarvia, Bravia, Charnequeira, Serpentina, and Serrana) and the six main global haplogroups (A, B1/B2, C, D, F, and G; Naderi et al., 2007).

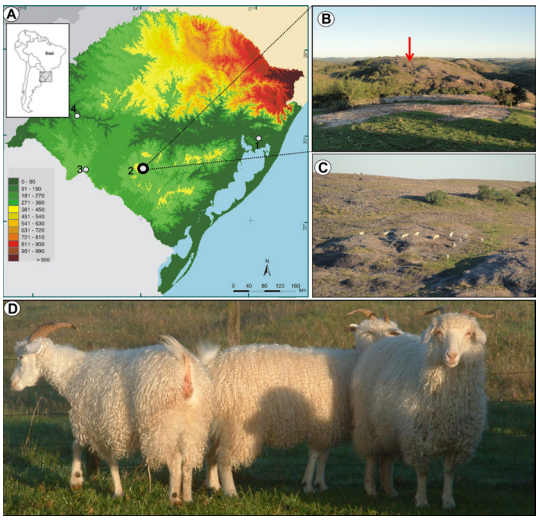

Figure 1. Crespa herds. **A.** sampled farms in Rio Grande do Sul State, southernmost Brazil: 1, CBR; 2, CBA; 3, CSL + CCA; 4, CAL (see **S1 Table** for a further description of each herd). **B.** Detail of the Palmas region and a distant view of a small herd (arrowed) arriving near sunset to overnight on a hilltop. **C.** Closer view of the same herd (zoom lens). **D.** One-year-old fleeces from CSL farm. Photographs (B, C, and D): G.R.P. Moreira.

MATERIAL AND METHODS

Goats and samples

DNA samples were obtained from 148 individuals of five goat breeds and the relict Crespa (**S1 Table**). Four remnant herds (N = 51) of Crespa reared in the Pampean region of RS

were investigated (Figure 1 and **S1 Table**). In addition, purebred [i.e., animals registered by the South Brazilian Goat Breeders' Association (CAPRISUL)] Alpine (N = 10), Anglo-Nubian (N = 21), Boer (N = 19), and Saanen (N = 20) goats reared in the same region were included. Pure Angora goats (N = 28) were obtained from a herd officially recognized by the National Agricultural Technology Institute (INTA) of Bariloche, Argentina (**S1 Table**).

Crespa goats are small (morphometric data are shown in **S1 Figure** and **S2 Table**), with a typical white, silky, and curly fleece, which distinguishes them from all other goat breeds in the region [for a comparison with the body sizes of Saanen, Alpine, Anglo-Nubian, and Boer breeds, see Agraz-Garcia (1976), Pesmen and Yardimci (2008), and Pires et al. (2012)]. They are phenotypically uniform and very similar in size (e.g., in wither height, chest depth, and body length) to the Angora, in particular, when compared to data from Angora herds found in the mid-20th century at Lalahan, near Ankara, Turkey (Bilgemre, 1953). They differ from the Angora, however, in having a much shorter fleece, with the head, distal half of the legs, and belly not covered with mohair, and smaller ears. The mohair is coarse, with fibers varying widely in length and diameter, with abundant kemp. Farmers do not usually shear these goats, because the fleece is shed during the summer.

Genetic data collection

Total genomic DNA was extracted from tissue or blood samples using a cetyltrimethylammonium bromide protocol. Eleven microsatellite markers (SRCRSP5, SRCRSP8, SRCRSP9, OarFCB48, MAF65, MAF209, ILSTS005, ILSTS011, ILSTS029, ETH10, and TGLA53) were chosen based on Luikart and England (1999) (**S3 Table**). All of the forward primers carried an 18-bp universal sequence (5'-TGTAAAACGACGGCCAGT-3') that was fluorescent-labeled with hexachloro-6-carboxy-fluorescine (Perkin-Elmer/Applied Biosystems, USA). These markers were polymerase chain reaction (PCR)-amplified under the protocol and conditions described by Schuelke (2000). Then, 1 µL of the PCR product was added to 22 µL formamide and 0.5 µL ROX standard (Perkin-Elmer) and run on an ABI3730XL automatic sequencer (Applied Biosystems) at Macrogen (Seoul, Republic of Korea). The results were directly analyzed in the Peak Scanner™ 1.0 (Applied Biosystems) software to score allele sizes. In addition to nuclear markers, a fragment of the mtDNA control region (hypervariable region, HVR) was PCR-amplified using the universal primer pair HVR1 forward (5'-CGCTCGCCTACACACAAATA-3') and HVR1 reverse (5'-AATGCCCATGCCTACCATTA-3') (Amills et al., 2004). The PCR contained 1 U *Taq* polymerase (Invitrogen) and 1.2 mM $MgCl_2$ at a 50°C annealing temperature in a 20-µL reaction volume. The thermocycling conditions were as follows: 94°C for 3 min, 31 cycles at 94°C of denaturation for 1 min, 64°C of annealing for 1 min, 72°C of extension for 1 min, and a final extension at 72°C for 10 min. The PCR products were cleaned by the ExoSAP (GE Healthcare) enzymatic method, sequenced with BigDye®, and analyzed on an ABI3730XL (Applied Biosystems) at Macrogen. Chromatograms were edited in Chromas 2.4 (Technelysium Pty Ltd., South Brisbane, Australia), and the sequences were automatically aligned using Clustal X in the MEGA 6 software (Tamura et al., 2013). HVR haplotypes found in each breed were deposited in GenBank under the accession numbers KM260501-KM260545 (**S4 Table**).

Analyses of genetic variation

Allele frequency distribution, presence of private alleles, and mean values of observed (H_O) and expected heterozygosities (H_E) were evaluated specifically for the Angora and Crespa goats using Arlequin 3.5 (Excoffier and Lischer, 2010) and FSTAT 2.9.1 (Goudet, 1995). Differences in heterozygosity were tested using the Wilcoxon signed rank test. In addition, we used Genepop (http://genepop.curtin.edu.au/) to test pairwise linkage disequilibrium (LD) at all loci over all breeds, and to compute pairwise estimates of genetic differentiation (F_{ST}) (Weir and Cockerham, 1984). Values of F_{IS} (inbreeding coefficient), which estimates the deficit of heterozygotes within populations (F_{IS} significantly >0), were computed using the method of Weir and Cockerham (1984). Departures from the Hardy-Weinberg equilibrium (HWE) were tested for all locus-population combinations, and overall. Significance levels were adjusted using the sequential Bonferroni method for multiple tests on the same dataset. Analysis of molecular variance (AMOVA) in Arlequin 3.5 (Michalakis and Excoffier, 1996) using F_{ST} and R_{ST} (a measure of genetic divergence analogous to F_{ST}; Slatkin 1995), also assessed the differentiation between Angora and Crespa. Measures of mtDNA diversity, such as definitions of haplotypes (H), haplotype diversity (H_d), and nucleotide diversity (π) were calculated using DnaSP v5 (Librado and Rozas, 2009).

Ordination plotting and clustering of individual genotypes

Inter-individual genetic distances (Nei's D_a) were obtained using the Populations v1.2.30 program (http://bioinformatics.org/~tryphon/populations) with samples from Angora, Crespa, and the four commercial breeds (Alpine, Anglo Nubian, Boer, and Saanen). Distance matrices were then used to construct neighbor-joining trees with the NEIGHBOR program in PHYLIP 3.5 (Felsenstein, 1995) and edited in FigTree v3.1 (http://tree.bio.ed.ac.uk/software/figtree). The robustness of the tree was assessed by bootstrap re-sampling procedures using 1000 replicates. Specifically for Angora and Crespa, each individual, for all allelic classes at all loci, was scored as 0.0 (the allele was not observed), 0.5 (one copy of the allele was observed in heterozygotes), or 1.0 (two copies were observed in homozygotes). The matrix was then ordinated in a multidimensional space by principal coordinate analysis (PCoA) using pCAGEN (http://www2.unil.ch/popgen/softwares/pcagen.htm). We used this analysis to depict the degree of relatedness between genotypes at a fine scale.

Bayesian admixture analysis

A Bayesian clustering method was used to detect the presence of cryptic population structure and to perform assignment testing in Structure 2.1 (Pritchard et al., 2000). We performed this analysis in order to define the number of ancestral populations underlying the breeds surveyed, and the proportion of mixed ancestry of these breeds, in order to determine whether Crespa would be assigned to a unique cluster. The number of inferred ancestral populations (K) was evaluated with 10 independent runs from K = 1-7 using 2,000,000 Markov chain Monte Carlo iterations and a burn-in period of 1,500,000 steps, assuming an admixture model, correlated allele frequencies, and no prior information for each sample (option USEPOPINFO = 0). The most probable number of clusters was based on the second-order rate of change in the log probability of the data (ΔK; Evanno et al., 2005). We performed

50 additional runs for the most probable *K*, using the same parameters as described above. We present the results for the run that retrieved the highest mean of the estimated logarithm of probability of the data [Ln Pr (*X*/*K*)]. An arbitrary threshold of membership probability of $q \geq 0.90$ was assumed, in order to ensure that specimens with more than 90% of their genomic content represented in one cluster would be assigned to it.

Phylogenetic HVR haplotype analysis

Capra hircus (domestic goat) was grouped into six major haplogroups: A, B1/B2, C, D, F, and G by Naderi et al. (2007). A phylogenetic tree based on the HVR segment (696 bp) was generated by selecting haplotypes of each breed surveyed in this study (in addition to Crespa) and incorporating GenBank sequences from each global haplogroup (Naderi et al., 2007), in order to investigate the origin and relatedness of Crespa in a broad evolutionary scenario. We also incorporated autochthonous Portuguese breeds into the dataset, including Algarvia (AY961894, AY961895, AY961910, and AY961913), Bravia (AY961898, AY961899, AY961901, and AY961902), Charnequeira (AY961887, AY961896, AY961897, and AY961891), Serpentina (AY961854, AY961911, AY961915, and AY961916), and Serrana (AY961905, AY961906, AY961908, and AY961909), as most of the goats introduced into Brazil came from Portugal (Ribeiro et al., 2012). Recently, sequences of Angora from Turkey (the native location of the breed) became available on GenBank and were added to the analysis (KC574285-KC574380).

Phylogenetic trees were constructed using the neighbor-joining distance method in MEGA 6 (Tamura et al., 2013), as the shallow history of these populations does not require the weight that most character-based methods, such as Bayesian and maximum likelihood, give to mutation as a factor of differentiation. Relationships between the mtDNA haplotypes were estimated using a median-joining approach (Bandelt et al., 1999) in the program Network 4.6.1 (www.fluxus-engineering.com).

RESULTS

Genetic variability and HVR1 haplogroups

All of the microsatellites were polymorphic, with 3-14 different alleles per locus (mean ± standard error: 7.2 ± 1.9) and values of H_E ranging from 0.53 to 0.68 (Table 1). All of the markers were in HWE (P = 0.05), considering the entire sample. Pairwise allelic combinations were in LD at all loci ($P < 0.05$, Bonferroni corrected for all comparisons). Genetic variability across the four herds of Crespa is summarized in Table 1. Specifically for Crespa and Angora, the allele frequency distributions varied across loci and showed marked differences between them (Figure 2). At a threshold frequency of 5% that was chosen to reduce the effects of sampling error, there were 14 private alleles: eight in Angora and six in Crespa (Table 2). Genetic diversity was similar in Crespa, which showed a higher allele number and heterozygosity than Angora (Table 2). H_O and H_E were not significantly different between Crespa and Angora (Z-value = -1.172, P = 0.252) (Table 2). The mean number of pairwise allelic differences between these breeds was negligible. Crespa and Angora showed significant deficits of heterozygotes in 9 and 2 of the 11 loci, respectively ($P < 0.05$, Bonferroni corrected). The mean F_{IS} was significantly positive in Crespa ($F_{IS} = 0.214$, $P < 0.01$) and Angora (F_{IS} =

0.146, P < 0.01) (Table 2). Microsatellite variability was significantly partitioned between Crespa and Angora (F_{ST} = 0.13 and R_{ST} = 0.12, P < 0.001). Estimates of F_{ST} and R_{ST} were similar, suggesting that Crespa and Angora goats have comparable patterns in the distribution of allele frequency and allele size (Slatkin, 1995). F_{ST} values were calculated between Crespa and the commercial breeds reared in the same region, and are shown in Table 3.

Table 1. Genetic variability per breed and population (Pop.) of Crespa goats based on 11 microsatellite loci and 696-bp sequences of HVR1.

			Microsatellites				HVR1 (mtDNA)		
Breed	Pop.	N	A	H_O	H_E	F_{IS}	nh	H_d	π
Alpine	-	10	3.20	0.43	0.53	0.194	3	0.73 ± 0.07	0.010 ± 0.001
Anglo-Nubian	-	21	4.00	0.41	0.56	0.278	6	0.71 ± 0.07	0.012 ± 0.001
Boer	-	19	4.36	0.57	0.67	0.153	6	0.76 ± 0.07	0.013 ± 0.001
Saanen	-	20	5.09	0.57	0.63	0.100	9	0.91 ± 0.03	0.012 ± 0.000
Angora	-	28	6.36	0.57	0.67	0.146	10	0.86 ± 0.04	0.021 ± 0.006
Crespa		64	7.18	0.53	0.68	0.213	12	0.74 ± 0.03	0.007 ± 0.000
	CAL	10	4.09	0.54	0.53	-0.019	2	0.38 ± 0.13	0.001 ± 0.000
	CBA	19	5.27	0.59	0.63	0.063	4	0.76 ± 0.06	0.010 ± 0.001
	CSL + CCA	18	5.81	0.54	0.60	0.089	4	0.74 ± 0.02	0.008 ± 0.001
	CBR	4	4.27	0.55	0.63	0.197	2	0.64 ± 0.12	0.002 ± 0.000

nh = number of haplotypes.

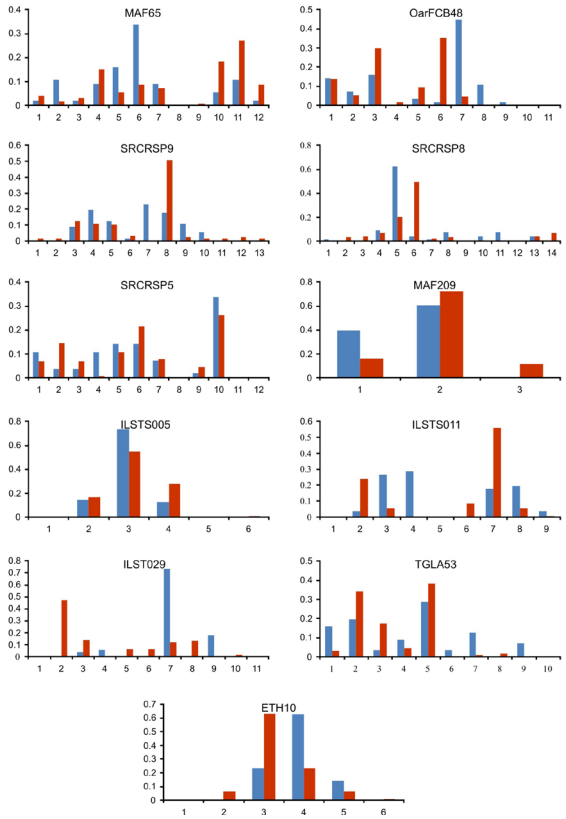

Figure 2. Histograms depicting the frequency distributions of microsatellite lengths (x-axis, the number of alleles; y-axis, their frequency) in Angora (blue bars) and Crespa (red bars) goats for the 11 loci studied.

Table 2. Summary of genetic diversity indices based on 11 microsatellite loci for Crespa and Angora breeds.

Statistic	Crespa	Angora
Mean No. of alleles per locus	7.2 (2.7)	6.3 (2.8)
No. of private alleles (P > 0.05)	6	8
No. of pairwise differences	0.667 (0.348)	0.671 (0.353)
Observed heterozygosity (H_O)	0.536 (0.137)	0.574 (0.196)
Expected heterozygosity (H_E)	0.682 (0.123)	0.671 (0.172)
Hardy-Weinberg equilibrium (F_{IS})	0.214*	0.146*

Standard deviations across loci are in parentheses. *P < 0.05.

Table 3. Pairwise estimates of genetic differentiation (F_{ST}) between goat breeds based on 11 microsatellite loci.

	Alpine	Anglo-Nubian	Boer	Saanen	Angora
Alpine	-				
Anglo-Nubian	0.265*	-			
Boer	0.300*	0.237*	-		
Saanen	0.188*	0.254*	0.217*	-	
Angora	0.236*	0.205*	0.161*	0.205*	-
Crespa	0.198*	0.177*	0.169*	0.133*	0.140*

In relation to mtDNA, 61 polymorphic sites were found in the 696 bp of HVR1 sequences across the whole dataset, corresponding to 36 haplotypes (Tables 1 and **S4 Table**). Specifically for populations of Crespa, 24 variable sites were detected, resulting in 12 haplotypes. Haplotype diversity was moderate (0.74 ± 0.03) and nucleotide diversity was lower (0.007 ± 0.000) than in Angora (0.86 ± 0.04 and 0.021 ± 0.006, respectively) and the other breeds (Table 1). The tree topology generated using samples of different haplogroups showed that Crespa is closely related to Angora from Argentina and Turkey, as well as to the Algarvia breed from Portugal (Figure 3). In addition, as with Angora and all four commercial breeds, it belongs to haplogroup A. Sequences representative of haplogroups C and F were placed as basal in the tree, followed by a clade formed by two subgroups of haplogroup B (B1 and B2). Haplogroup G was placed as a sister clade of haplogroups A and D. Haplogroup D was more closely related to all other sequences, including those representative of haplogroup A (Figure 3A).

The two high-frequency haplotypes found in Crespa were shared with foreign breeds reared in southern Brazil (Anglo-Nubian, Saanen, Boer, and Angora) (Figure 3B). Crespa and Angora shared two haplotypes. The ecotype also had one haplotype that was closely related to Saanen, Angora, and to the Algarvia Portuguese autochthonous breed.

Clustering and ordination plot of individuals

The neighbor-joining tree that clustered inter-individual microsatellite distances is presented in Figure 4. Each breed was split into distinct clusters. The results of the PCoA of the individual genotypes, focusing specifically on Angora and Crespa, are given in Figure 5. Individual scores were plotted on the two principal axes (PC-I and PC-II), which cumulatively explained 31.2% of the total genetic diversity. This showed a marked separation of the individual scores into two different groups on PC-I; most Angora and Crespa goats were placed on the right and left sides of the plot, respectively, with a few individuals placed as admixture forms.

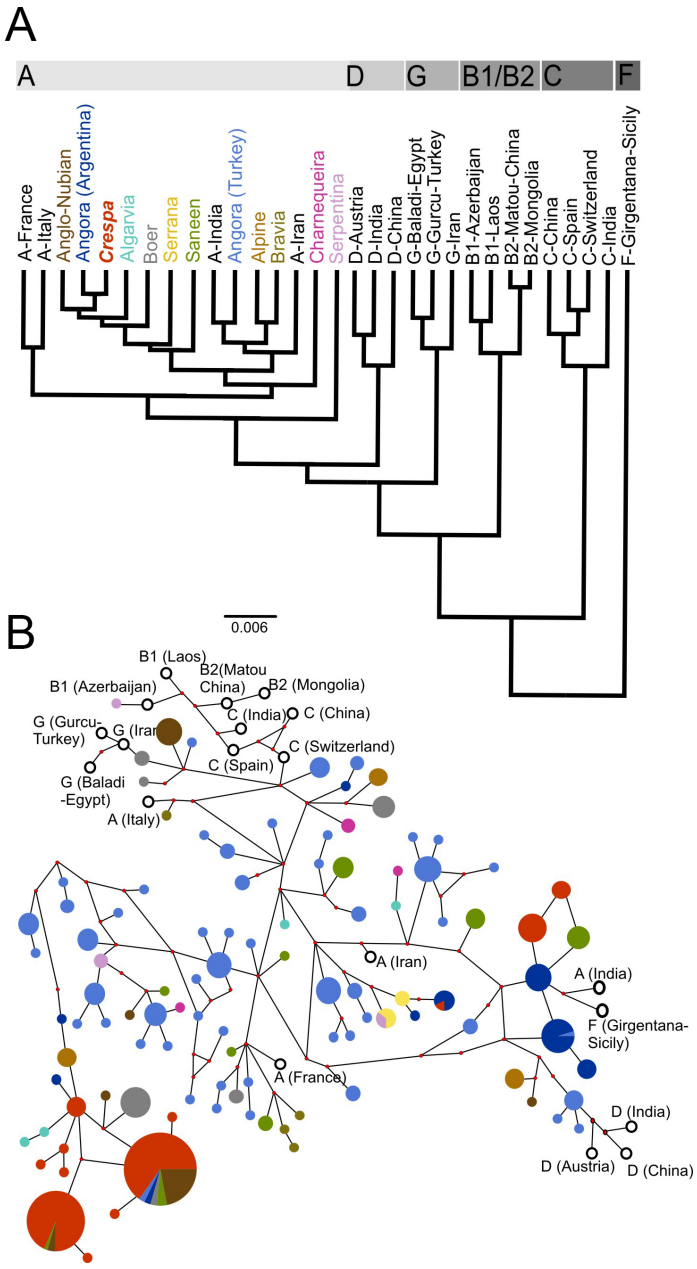

Figure 3. Evolutionary relationships of the Crespa. **A.** Maximum-likelihood phylogenetic tree based on a 696-bp section of the HVR1 region of mtDNA from Crespa and representative haplotypes of breeds reared in southernmost Brazil (Alpine, Anglo-Nubian, Boer, and Saanen), purebred Angora from Argentina and Turkey, representative haplotypes from autochthonous Portuguese breeds (Algarvia, Bravia, Charnequeira, Serpentina, and Serrana), and from the six major global haplogroups (see Material and Methods for details). **B.** Median-joining haplotype network reconstructed based on HVR1 haplotypes, with the same terminals (and colors) used in the phylogenetic tree. Circle size indicates haplotype frequency; small red circles indicate median vectors.

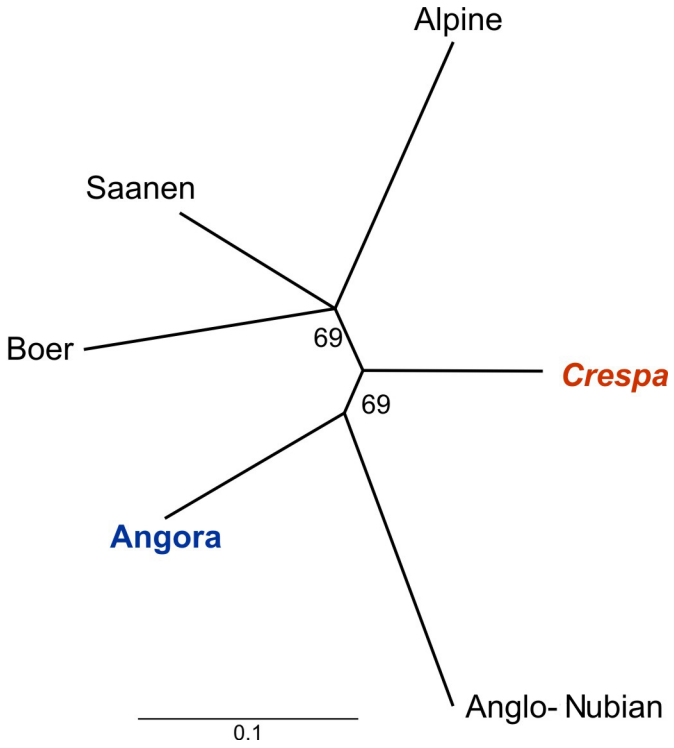

Figure 4. Individual cluster analysis of Crespa based on microsatellite variation. Neighbor-joining tree showing the genetic relationships among Crespa, Angora, and commercial breeds (Alpine, Anglo-Nubian, Boer, and Saanen) obtained from Nei's D_a genetic distances based on 11 loci. Numbers at the nodes indicate bootstrap values (%).

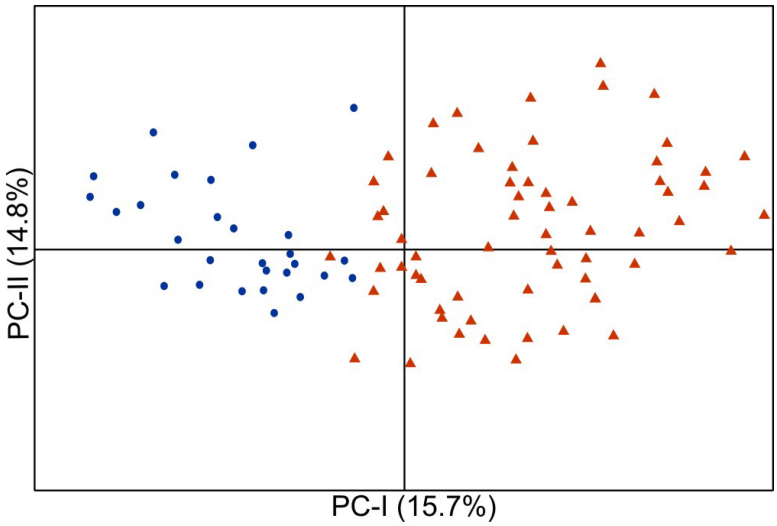

Figure 5. Scores of individual Crespa and Angora genotypes plotted on the first two axes (PC-I and PC-II) of a principal coordinate analysis performed using pCAGEN.

Genetic admixture analysis of Crespa

The probability of the number of populations (goat breeds) of the data was estimated by comparing the Ln likelihood (LnL) (Figure 6). The Ln probability of the data was minimum with $K = 1$ (LnL = -6056.9) and maximum with $K = 7$ populations (LnL = -5180.6) and decreased from six, suggesting that the pooled "population" of goats including Crespa, Angora, and the commercial breeds was heterogeneous, and may have contained six genetically distinct groups. We also estimated the proportion of membership (q) of each group in six clusters representing "cryptic" genetic populations (Table 4). Crespa was assigned to the sixth cluster with $q = 0.92$ (Figure 7 and Table 4), indicating a distinct identity. Only one sample showed a small fraction of membership assigned to a commercial breed, the Saanen. Angora and the other breeds exhibited a proportion of membership of over 93% assigned to one of the clusters (Table 4).

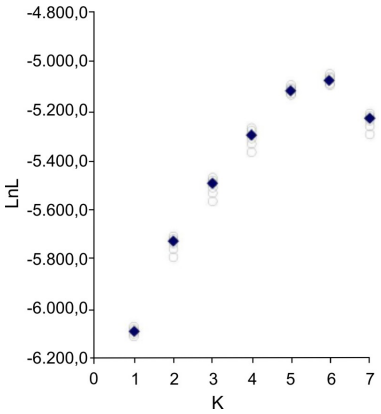

Figure 6. Bayesian assignment test. Estimated posterior probability of the data for different numbers of inferred clusters (K = 1-6). Likelihood obtained for individual runs (circles) and the mean (lozenges) of six runs in each K.

Table 4. Bayesian clustering analysis of the goats studied (148 individuals, 11 loci).

	Genetic cluster					
Breed	I	II	III	IV	V	VI
Alpine	0.970	0.008	0.004	0.009	0.005	0.005
Anglo-Nubian	0.007	0.972	0.005	0.004	0.006	0.007
Boer	0.009	0.010	0.957	0.007	0.007	0.010
Saanen	0.020	0.014	0.009	0.935	0.009	0.012
Angora	0.018	0.007	0.007	0.009	0.937	0.022
Crespa	0.012	0.013	0.014	0.027	0.013	0.921

The identity of each breed is presented for each of the six clusters (K = 6) inferred by the Bayesian algorithm.

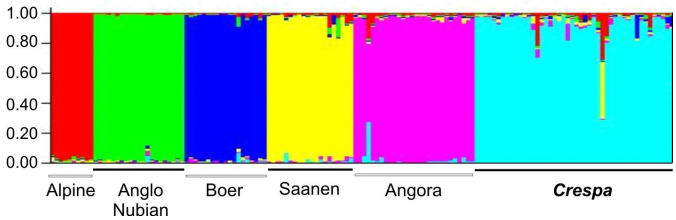

Figure 7. Bayesian assignment test. Graphical representation of the estimated membership fractions for 148 individuals of five goat breeds and Crespa analyzed for K = 6.

DISCUSSION

Crespa as a distinct genetic cluster

The microsatellite and mtDNA data support the hypothesis that Crespa herds represent a distinct breed from southernmost Brazil that did not descend from Angora. Crespa is related to other introduced commercial breeds that are still being raised in the region (particularly Saanen) and to autochthonous Portuguese breeds, particularly the Algarvia and Serrana. Therefore, our results suggest that Crespa goats are in part a remnant of animals that were introduced during the colonial period, and have become adapted over hundreds of years.

Over the last few years, considerable effort has been devoted to characterizing the genetic diversity of African, Asian, and European goats (e.g., Azor et al., 2005; Pereira et al., 2005; Cañón et al., 2006; Naderi et al., 2007; Çinar Kul and Ertuğrul, 2011), but locally adapted Latin American breeds have received much less attention. In Brazil, a few studies have recently addressed the existence of local breeds using either microsatellites or mtDNA, and all have focused on those from the northern region of the country, for example the Canindé, Graúna, Marota, Moxotó, Repartida, and Serrana Azul (Araújo et al., 2006; Menezes et al., 2006; Oliveira et al., 2007, 2010). Recently, a next-generation sequencing approach was employed to investigate genetic variation in Brazilian naturalized breeds (Moura et al., 2015). Although out of the scope of the present study, a genetic comparison of Crespa with such breeds should also be conducted in the near future.

Interestingly, the influence of environmental characteristics and management practices in shaping locally adapted domestic breeds has also often been neglected, particularly in developing countries (Egito et al., 2002; Gonçalves et al., 2010). Only in the late 19th century were foreign goat breeds recognized as economically important, and therefore managed in Brazil (Oliveira et al., 2007). In RS, however, improvements in goat breeding, including phenotypic selection to increase meat and milk production, started only in the 1980s with the foundation of CAPRISUL (Ponciano V, personal communication). Furthermore, in the Palmas region in particular, there is no indication that the present low-tech management practices have changed significantly since colonial times. Therefore, these conditions may have contributed substantially to molding the phenotypic attributes of Crespa goats, particularly those related to their hardiness, a prerequisite to succeed in this harsh environment and that is not found in highly productive commercial breeds.

mtDNA haplotype and nucleotide diversity are important indices with which to assess polymorphisms and population genetic differentiation. Pereira et al. (2005) analyzed 481 bp of the mtDNA control region in 288 individuals from several native Portuguese breeds, and obtained 118 variable sites. The 696 bp obtained in our study covered the same mitochondrial marker, which we analyzed for 50 individuals of Crespa and found 24 variable sites. This difference, i.e., the low variability, could be explained by founder effects, low effective population size, and/or the short evolutionary time elapsed compared to native Portuguese herds. In addition, Pereira et al. (2005) found two events of insertion and two deletions, in contrast to our results, where no indels were observed. The Crespa nucleotide diversity (π = 0.0075) was high compared to that of the other breeds reared in the same region, and Crespa had the highest number of shared haplotypes, with Anglo-Nubian, Boer, and Saanen in the same haplotype (Figure 3).

Genetic relatedness and ancestry

In the Bayesian admixture analysis, the probable number of ancestral populations assumed was $K = 6$. Over 90% of Crespa individuals had a unique membership, as was the case in Angora and the commercial breeds surveyed (Alpine, Anglo-Nubian, Boer, and Saanen). The lowest level of gene flow was found between Angora and Crespa, which have become differentiated through years of local management, uncontrolled crossings with other breeds, and the lack of new imports of Angora goats into Brazil in the last 60 years. Regarding microsatellite variation, Crespa and Saanen showed genetic similarity, which might indicate some level of crossing for increasing production, possibly due to their phenotypic resemblance in color. This aspect raises a fundamental conservation genetics issue, the recurring end of non-recognized ecotypes: outcrossing with phenotypically similar commercial breeds that are considered more productive.

Analysis of the mtDNA haplogroups indicated that Crespa belongs to haplogroup A, which corresponds to almost 90% of breeds worldwide (Naderi et al., 2007). This is probably due to the high prevalence of this group of haplotypes in southern Europe, the major source of the breeds that are currently reared in southern Brazil. Although genetic relationships between Brazilian (specifically from the northern region of the country) and Portuguese breeds have already been addressed (Oliveira et al., 2010; Ribeiro et al., 2012), the degree of relatedness among foreign breeds reared in southernmost Brazil, particularly the locally adapted Crespa, has never been studied. We therefore inserted these breeds into the global scenario using worldwide haplogroups, native Portuguese breeds, and native Turkish Angora. A pattern of divergence (no haplotypes shared) between the breeds surveyed in our study and from Portugal was observed, similar to that observed by Ribeiro et al. (2012). However, in the case of Crespa, a haplotype was closely related to two autochthonous breeds, Serrana and Algarvia from the northern and southern regions of Portugal, respectively. Due to the lack of goat phylogeographic structure, particularly using the HVR region, it is difficult to determine the origin of Crespa, but the haplotype inferences were able to rule out the possibility that they are only related to Angora, and indicated a probable source in the Portuguese breeds.

Conservation efforts

Our findings elucidate the genetic status of the first locally adapted goat lineage from southernmost Brazil. The population of this ecotype is about 500 individuals at most; therefore, a management plan is urgently needed so that this breed can be conserved and economically sustained. The main concern regarding these animals is not directly related to genetic variability, which had moderate to high values for mtDNA and nuclear markers, but to the lack of proper management, which allows intercrossing with other breeds. This mischaracterizes the animals, and it will certainly affect the homogeneity that is currently present in the remnant herds. The historical importance and unique genetic characteristics of this newly identified ecotype may not be sufficient to encourage its conservation, making it necessary to implement economic incentives. The maintenance of any domestic population is the direct responsibility of the owners, and it is important to promote it accordingly, in cooperation with official authorities (Mariante et al., 2009). The most important point is that these animals should be considered part of the history of the Palmas region, i.e., a cultural heritage, which might increase their commercial value. In addition, Crespa goats should become a better source of income for

farmers, e.g., their mohair, until now disregarded, could be promoted for use in handicrafts. Finally, improving the morphological characterization of Crespa goats for the purpose of establishing a breed standard, as well as determining their effective population size, are essential in order to petition for their official recognition and conservation.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Thanks are due to Vera Ponciano, the head of CAPRISUL, for her kindness in facilitating contact with the purebred goat breeders Ronald M. Rauter (Saanen), Zuleika B. Torrealba (Alpine), and Jaqueline A.Vecchi/Vitor Severo (Boer), and for providing her own Anglo-Nubian animals to be sampled. We are also grateful to several Crespa breeders for allowing us to take samples from their animals: Antonio C. Costa, the late Carlos J.F. Rodrigues, Luiz Carlos Gasperin on behalf of Dinarte Ribeiro, Júlio César Barbosa, and Régis Collares. Thanks to Janet W. Reid for editing the text. G.L. Gonçalves and G.R.P. Moreira were supported by grants from CNPq (#156153/2011-4 and #309676/2011-8, respectively).

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SUPPLEMENTARY MATERIAL

S1 Figure. Schematic representation of body parameters measured in Crespa herds, modified from Agraz-Garcia (1976). Red and green lines represent linear and circumferential measurements, respectively.
S1 Table. Description of goat specimens used for molecular analysis by breed, including sample size (N), corresponding identification in tissue collection (sample ID), collection site (location, as plotted in Figure 1), and owner.
S2. Table. Mean, standard error (SE), maximum (Max), and minimum (Min) values of linear body measurements of selected Crespa does and bucks from CBA (N = 9♀/1♂), CCA (N = 6♀/1♂), and CSL (N = 4♀/1♂) farms. Measurements were performed following the parameters and apparatus described by Agraz-Garcia (1976); see S1 Figure for a schematic representation of the measurements and geographical locations of the farms.
S3 Table. Microsatellite markers used in this study.
S4 Table. Haplotypes based on hypervariable region (HVR) sequences found in all goat breeds surveyed in this study.

