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BoLA-DRB3 genetic diversity in Highland Creole cattle from Bolivia

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The genetic diversity of the *BoLA-DRB3* gene has been reported in different cattle breeds owing to its central role in the immune response. However, it is still unknown in hundreds of cattle breeds, especially native populations. Here, we studied *BoLA-DRB3* genetic diversity in Highland Creole cattle (CrAl) from Western Bolivia, raised at altitudes between 3800 and 4200 m. DNAs from 48 CrAl cattle were genotyped for *BoLA-DRB3* exon 2 alleles using polymerase chain reaction-sequence-based typing (PCR-SBT). The results were compared with 1341 previously reported data from Tropical Creole cattle and other breeds raised in the region. Twenty-three *BoLA-DRB3* alleles were identified in CrAl, including the *BoLA-DRB3*029:02* variant previously detected in other Creole cattle. Observed and expected heterozygosity were 0.87 and 0.93, respectively. Nucleotide diversity and the number of pairwise difference values were 0.078 and 19.46, respectively. The average number of nonsynonymous and synonymous substitutions were 0.037 and 0.097 for the entire *BoLA-DRB3* exon 2, and 0.129 and 0.388 for the antigen-binding site, respectively. Venn analysis and the review of the IPD-MHC database and the literature showed that 2 of 64 alleles were only detected in CrAl, including *BoLA-DRB3*029:01* previously reported in African cattle and **048:01* detected in Philippine cattle. Two additional alleles, *BoLA-DRB3*007:02* and **029:02*, were only present in CrAl and Lowland Creole cattle. Principal Component Analysis (PCA) showed that Bolivian Creole cattle breeds were closely located but they were distant from the Colombian Hartón del Valle Creole. F_{ST} analysis showed a low degree of genetic differentiation between Highland and Lowland Bolivian Creole cattle ($F_{ST} = 0.015$). The present results contribute to increasing our knowledge of *BoLA-DRB3* genetic diversity in cattle breeds.

KEYWORDS

BoLA-DRB3, Creole cattle, genetic diversity, principal component analysis, sequence-based genotyping

1 | INTRODUCTION

Genes belonging to the major histocompatibility complex, named bovine major histocompatibility complex (BoLA) in cattle, play a central role in the immune response and have been associated with resistance/susceptibility to different infectious diseases in human beings, experimental animals and livestock species.[1] In cattle, class II *BoLA-DRB3* polymorphism is mainly located in exon 2, which codes for the antigen binding site (ABS),[1,2] and exhibits an outstanding level of polymorphism within and among breeds/populations. Previous studies showed that certain *BoLA-DRB3* alleles were associated with resistance/susceptibility to infectious disease (ie, bovine leukemia virus-induced lymphocytosis, mastitis and dermatophilosis), different immunological and production traits (ie, milk yield) and different vaccine responses (eg, to foot-and-mouth disease and *Theileria parva*).[1] Despite the main role of the *BoLA-DRB3* gene in the immune response, its extensive polymorphism has only been studied in a few highly selected cattle breeds by polymerase chain reaction-sequence-based typing (PCR-SBT) and targeted next generation sequencing.[3-12] By contrast, native or local breeds have been poorly studied to date, even though previous work has shown that these populations are sources of additional genetic diversity (new alleles) in the *BoLA-DRB3* gene.[5,10,11]

Creole cattle are the direct descendants of bovines introduced by the Spanish and Portuguese into the American continent during the first 50 years of colonization.[13] After more than 500 years, these cattle have adapted to a wide range of environments, such as tropical and subtropical forests, seasonal flood plains, dry forests, temperate valleys and highland plains. Although local Creole cattle breeds remain in most American countries, from the United States to Argentina (http://www.ansi.okstate.edu/breeds/cattle/), the *BoLA-DRB3* polymorphism has been characterized by PCR-SBT in only two Creole cattle breeds adapted to subtropical environments: Hartón del Valle (Colombia) and Yacumeño (Boliva).[11] The aim of this work was to study *BoLA-DRB3* genetic diversity in Highland Creole cattle raised in Western Bolivia (Departments of Oruro, Potosí and La Paz) between 3800 and 4200 m of altitude in a cool and dry environment (SENAMHI, 2008), and compare the results with data from Creole cattle with the same historical origin but adapted to a humid tropical environment. We hypothesized that *BoLA-DRB3* genetic diversity would be driven through natural selection in Creole cattle populations sharing the same ancestral origin but adapted to different environments (highland plain vs tropical savanna); consequently, intra-population *BoLA-DRB3* genetic diversity could have diverged during the last centuries. Furthermore, these Creole populations could have been exposed to different processes of gene introgression with Zebu breeds in the tropical savanna and Holstein in the highland plain.

2 | METHODS

Genomic DNA from 48 hair samples belonging to Bolivian Highland Creole cattle (CrAl) was purified using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany). Samples were collected in two communities (Colpa Collana and Huacanapi) located around 4000 m above sea level in the San Pedro de Totora Province (latitude 17°49′ and 18°7′S; longitude 67°54′ and 68°20′W), Oruro Department, Bolivia (Figure 1). All animal procedures were reviewed and approved by the Institutional Committee on Care and Use of Experimental Animals from the School of Veterinary Sciences of the National University of La Plata (Buenos Aires, Argentina).

The obtained DNAs were genotyped for *BoLA-DRB3* exon 2 alleles using the PCR-SBT assay implemented by Takeshima et al.[14] Briefly, *BoLA-DRB3* exon 2 was amplified by PCR using the DRB3FRW and DRB3REV primers designed by Baxter et al.[15] The 280 bp PCR fragments were purified using an ExoSAP-IT PCR Product Purification Kit (USB Corp., Cleveland, OH) and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA).

The raw DNA electropherograms were analyzed using Assign 400ATF v. 1.0.2.41 software (Conexio Genomics, Fremantle, Australia). This software uses a database of all reported *BoLA-DRB3* alleles (https://www.ebi.ac.uk/ipd/mhc/) to semi-automatically resolve the ambiguities among ambiguous polymorphic sites in heterozygous individuals and determine the genotypes. Additionally, previously reported data from 1341 cattle belonging to 2 Creole, 2 Holstein and 3 Zebu breeds were included for comparison (Table 1).[7,11,12] Genetic diversity at allele and molecular level, allele distribution, Hardy-Weinberg equilibrium (HWE), Ewens-Watterson-Slatkin exact test for neutrality, genetic structure and genetic differentiation among breeds were estimated from raw data using ARLEQUIN 3.5,[16] GENEPOP 4.0,[17] PAST v. 2.28 software,[18] MEGA 5,[19] and VennDiagram of the R package (http://cran.r-project.org/). To condense the genetic variation at the *BoLA-DRB3* locus, allele frequencies were used to perform a Principal Component Analysis (PCA) according to Cavalli-Sforza,[20] implemented in the Past software.

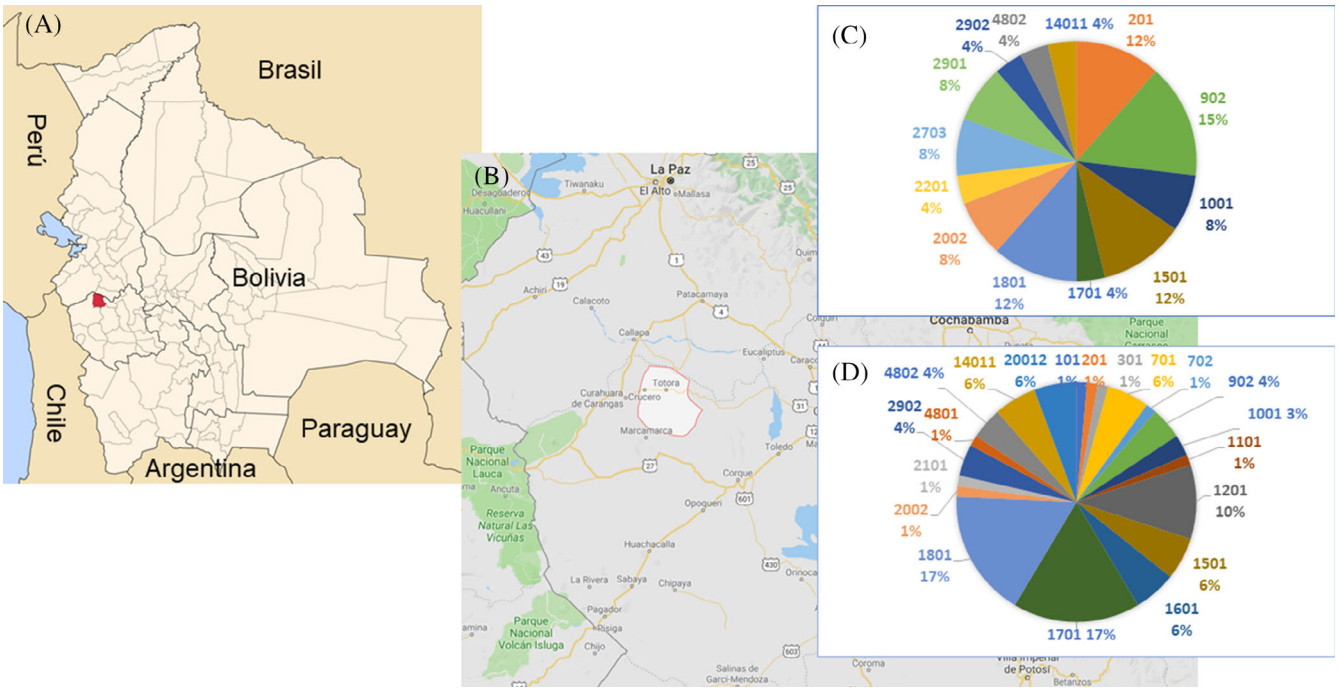

FIGURE 1 Geographic distribution of the sampled farms in the Department of Oruro (Bolivia). A, Map of Bolivia. B, Map of San Pedro de Totora Province (Oruro Department, Bolivia; 17° 48′ 00” S, 68° 10′ 00” W). C, *BoLA-DRB3* gene frequencies estimated in Colpa Collana. D, *BoLA-DRB3* gene frequencies estimated in Huacanapi

TABLE 1 Characteristics of the analyzed populations

Acronym	Sample size	Breed	Type	Origin country	Sampling country	Reference
CrAl	48	Highland Creole	Taurine	Bolivia	Bolivia	Present work
CrHV	66	Hartón del Valle Creole	Taurine	Colombia	Colombia	Giovambattista et al[11]
CrYa	112	Yacumeño Creole	Taurine	Bolivia	Bolivia	Giovambattista et al[11]
NeBo	116	Nellore	Zebuine	Brazil	Bolivia	Takeshima et al[12]
GirBo	110	Gir	Zebuine	India	Bolivia	Takeshima et al[12]
NexBrPe	195	Nellore × Brahman	Zebuine mixed	—	Perú	Takeshima et al[6,7]
HoAr	424	Argentine Holstein	Taurine	Netherlands	Argentina	Takeshima et al[6,7]
HoBo	318	Bolivian Holstein	Taurine	Netherlands	Bolivia	Takeshima et al[6,7]

Abbreviations: CrAl, Highland Creole; CrYa, Yacumeño Creole; CrHV, Hartón del Valle Creole; NeBo, Bolivian Nellore; GirBo, Bolivian Gir; NexBrPe, Peruvian Nellore-Brahman crossbreed; HoAr, Argentine Holstein; HoBo, Bolivian Holstein.

3 | RESULTS AND DISCUSSION

Genotyping by PCR-SBT allowed us to identify 23 *BoLA-DRB3* alleles in CrAl, including the *BoLA-DRB3*029:02* variant previously detected only in other Creole cattle breeds. No new alleles were detected in the analyzed population. The presence of this allele in the CrAl confirms that part of the genetic diversity of the BoLA-DRB3 gene would be found in local breeds or in group of related breeds, evidencing the importance of the study and conservation of these important bovine genetic resources.

Gene frequencies are described in Table 2. Allele frequency within the CrAl population was >5% for six alleles (*BoLA-DRB3*018:01*, **017:01*, **009:02*, **012:01*, **015:01* and **014:01:01*), accounting for 55.21% of the cumulative allele frequency (Figure 2). Some of these common alleles were also frequent in previously

TABLE 2 Allele frequencies in Latin American Creole cattle breeds

	CrAl (N = 48)	CrYa (N = 113)	CrHV (N = 66)
BoLA-DRB3 allele	Allele no. 94	Allele no. 226	Allele no. 132
*BoLA-DRB3*001:01*	1.04	2.23	0.76
*BoLA-DRB3*002:01*	4.17	**6.70**	0.00
*BoLA-DRB3*003:01*	1.04	0.00	0.00
*BoLA-DRB3*005:01*	0.00	4.02	0.76
*BoLA-DRB3*006:01*	0.00	3.13	0.00
*BoLA-DRB3*007:01*	4.17	**10.71**	0.00
*BoLA-DRB3*007:02*	1.04	1.34	0.00
*BoLA-DRB3*009:01*	0.00	4.46	0.00
*BoLA-DRB3*009:02*	**7.29**	**8.48**	0.00
*BoLA-DRB3*010:01*	4.17	3.13	3.79
*BoLA-DRB3*010:02*	0.00	0.00	**6.82**
*BoLA-DRB3*011:01*	1.04	3.57	**12.12**
*BoLA-DRB3*011:04*	0.00	4.91	0.76
*BoLA-DRB3*012:01*	**7.29**	1.79	0.00
*BoLA-DRB3*013:01*	0.00	1.34	0.00
*BoLA-DRB3*014:01:01*	**5.21**	**6.70**	0.76
*BoLA-DRB3*015:01*	**7.29**	4.46	**7.58**
*BoLA-DRB3*016:01*	4.17	4.46	**6.06**
*BoLA-DRB3*017:01*	**13.54**	1.79	0.00
*BoLA-DRB3*018:01*	**15.63**	**8.48**	3.79
*BoLA-DRB3*020:01:02*	4.17	0.45	1.52
*BoLA-DRB3*020:02*	3.13	0.00	0.00
*BoLA-DRB3*020:06*	0.00	0.00	**9.85**
*BoLA-DRB3*021:01*	1.04	0.45	0.00
*BoLA-DRB3*022:01*	1.04	1.79	**5.30**
*BoLA-DRB3*025:01:01*	0.00	0.00	2.27
*BoLA-DRB3*025:02*	0.00	0.89	0.00
*BoLA-DRB3*027:03*	2.08	1.79	**9.85**
*BoLA-DRB3*027:10*	0.00	1.79	0.00
*BoLA-DRB3*028:01*	0.00	1.34	**7.58**
*BoLA-DRB3*028:02*	0.00	0.89	1.52
*BoLA-DRB3*029:01*	2.08	0.00	0.00
*BoLA-DRB3*029:02*	4.17	0.89	1.52
*BoLA-DRB3*030:01*	0.00	0.89	**6.06**
*BoLA-DRB3*032:02*	0.00	0.45	0.00
*BoLA-DRB3*033:01*	0.00	2.23	0.00
*BoLA-DRB3*035:01*	0.00	0.89	0.76
*BoLA-DRB3*036:01*	0.00	0.89	**7.58**
*BoLA-DRB3*037:01*	0.00	0.89	0.00
*BoLA-DRB3*039:01*	0.00	0.45	0.76
*BoLA-DRB3*044:01*	0.00	0.00	1.52
*BoLA-DRB3*048:01*	1.04	0.00	0.00

(Continues)

TABLE 2 (Continued)

	CrAl (N = 48)	CrYa (N = 113)	CrHV (N = 66)
*BoLA-DRB3*048:02*	4.17	1.34	0.76

Note: Frequent alleles in each breed are given in bold (>5%).
Abbreviations: CrAL, Highland Creole; CrHV, Hartón del Valle Creole; CrYa, Yacumeño Creole; N, number of animals analyzed.

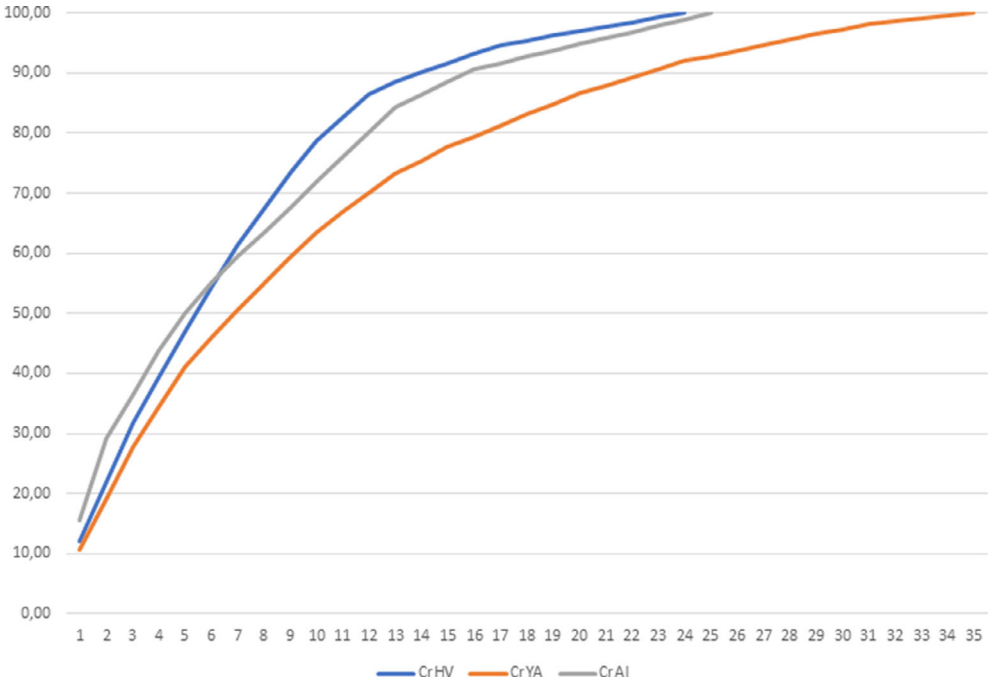

FIGURE 2 Cumulative gene frequency plot of *BoLA-DRB3* alleles in Highland (CrAl), Yacumeño (CrYa), and Hartón del Valle (CrHV) Creole cattle breeds

TABLE 3 Number of alleles (n_a), observed (h_o) and expected (h_e) heterozygosity, Hardy-Weinberg equilibrium (HWE) measured with F_{IS}, and Slatkin's exact test calculated in the cattle breeds studied. N = sample size. F_{IS} *P* values are indicated between parenthesis. Significant values ($P < .05$) are highlighted in bold

Breed	N	n_a	h_o	h_e	HWE F_{IS} (*P* value)	Slatkin's *P* value
Highland Creole[a]	48	23	0.87	0.93	**0.0633 (.0198)**	.077
Yacumeño Creole[b]	112	35	0.92	0.95	0.0344 (.5318)	**.005**
Hartón del Valle Creole[b]	66	24	0.97	0.94	**−0.0360 (<.0001)**	.121
Bolivian Nellore[c]	116	26	0.78	0.87	0.0990 (.6038)	.310
Bolivian Gir[c]	110	19	0.88	0.92	0.0406 (.1331)	**.012**
Peruvian Nellore × Brahman[c]	195	27	0.89	0.85	**0.1131 (<.0001)**	.458
Argentine Holstein[d]	424	33	0.84	0.91	0.079 (.0040)	.398
Bolivian Holstein[d]	318	23	0.93	0.90	−0.035 (.0285)	.210

[a]Present work.
[b]Giovambattista et al.[11]
[c]Takeshima et al.[6,7]
[d]Takeshima et al.[12]

analyzed Creole cattle breeds (*BoLA-DRB3*018:01*, **017:01*, **009:02*, **015:01* and **014:01:01*; Table 2). Additionally, 10 alleles with frequencies between 2% and 5% were detected (*BoLA-DRB3*002:01*, **007:01*, **010:01*, **016:01*, **020:01:02*, **029:02*, **048:02*, **020:02*, **027:03* and **029:01*). In the remaining seven variants (*BoLA-*

*DRB3*001:01*, **003:01*, **007:02*, **11:01*, **021:01*, **022:01* and **048:01*), gene frequency was <2%.

The number of alleles (n_a) and gene frequency distribution resulted in an observed (h_o) and expected (h_e) heterozygosity of 0.87 and 0.93, respectively. DNA genetic diversity showed nucleotide diversity (π) and number of pairwise difference (NPD) values of 0.078 and 19.46, respectively. The average numbers of nonsynonymous (d_n) and synonymous (d_s) substitutions in CrAl were 0.037 and 0.097 for the entire *BoLA-DRB3* exon 2, and 0.129 and 0.388 for the antigen-binding site (ABS), respectively. As expected, the d_n/d_s ratio was higher when only ABS was analyzed. The degree of diversity observed at allele, DNA, and amino acid levels within CrAl was similar to that estimated in other Creole cattle breeds analyzed by PCR-SBT ([11]; Tables 3 and 4).

Regarding HWE, the analysis showed a significant deviation from the theoretical values due to a marked excess of homozygote animals ($F_{IS} = 0.0633$; $P = .0198$). The *BoLA-DRB3* locus was also in HWE disequilibrium in other cattle breeds analyzed (Table 3). In the case of CrAl, the observed excess homozygosity could be the consequence of population structure, considering that the cattle analyzed belonged to different farmers with only a few animals from the Colpa Collana and Huacanapi districts (San Pedro de las Totoras Province). As shown in Figure 1, the cattle population of the Colpa Collana and Huacanapi districts varied in allele composition and gene frequency. Slatkin's exact test showed that gene frequency distributions in CrAl were compatible with neutral selection because it is not as homogeneous as expected in a population under balancing selection (Table 3). However, this test did not allow detection of the presence of evolutionary forces within the CrAl population, as had been reported in other cattle breeds (eg, Yacumeño Creole cattle).

Venn analysis of the genetic relationship between the *BoLA-DRB3* gene from CrAl and other cattle breeds raised in Bolivia showed that 2 of the 64 alleles identified in the 4 cattle groups (Highland and Lowland Creole, Zebu and Holstein) were only detected in CrAl (Figure 3): alleles *BoLA-DRB3*029:01* previously reported in African native cattle,[21] and *BoLA-DRB3*048:01* detected in Philippine cattle.[5] On the other hand, variants *BoLA-DRB3*007:02* and **029:02* were only present in Creole cattle breeds.[11] *BoLA-DRB3*021:01* and *BoLA-DRB3*048:02* were detected in Creole and Zebu groups. These variants had been previously reported in Asian, African, and Creole breeds,[5,11,21-23] and their presence in both Highland and Lowland population could be a

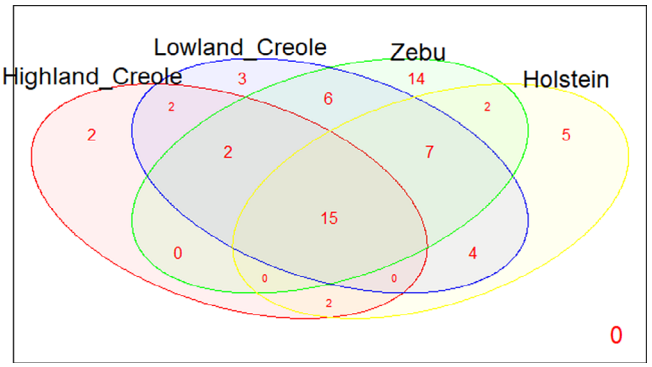

FIGURE 3 Venn diagram showing *BoLA-DRB3* allele distribution among cattle breeds grouped according to geographic origin: Highland Creole, Lowland Creole (Yacumeño and Hartón del Valle), Holstein from Argentina and Bolivia, and Zebu (Nellore, Gir, and Brahman)

TABLE 4 Values of nucleotide diversity (π), mean number of pairwise differences (NPD), and mean number of non-synonymous (dn) and synonymous (ds) nucleotide substitutions per site

			Total			ABS		
Breed	**Π**	**NPD**	**ds**	**dn**	**dn/ds**	**ds**	**dn**	**dn/ds**
Highlander Creole[a]	0.078	19.46	0.037	0.097	2.62	0.114	0.293	2.57
Yacumeño[b]	0.078	19.59	0.036	0.099	2.75	0.128	0.391	3.05
Hartón del Valle[b]	0.076	19.00	0.029	0.096	3.31	0.109	0.386	3.54
Bolivian Nellore[c]	0.070	17.54	0.035	0.097	2.77	0.117	0.388	3.32
Bolivian Gir[c]	0.078	19.45	0.038	0.096	2.53	0.133	0.385	2.89
Nellore × Brahman[c]	0.068	16.95	0.039	0.097	2.48	0.128	0.376	2.94
Argentine Holstein[d]	0.093	23.24	0.036	0.098	2.72	0.122	0.396	3.25
Bolivian Holstein[d]	0.080	20.07	0.042	0.104	2.48	0.146	0.409	2.80

Abbreviation: ABS, antigen binding site.
[a]Present work.
[b]Giovambattista et al.[11]
[c]Takeshima et al.[6,7]
[d]Takeshima et al.[12]

consequence of the African influence on Latin American native cattle rather than Zebuine gene introgression because the latter are not raised in the Bolivian highlands.[24,25] Two other variants were shared by CrAl and Holstein (*BoLA-DRB3*003:01* and *BoLA-DRB3*020:02*), but they were absent in Yacumeño and Hartón del Valle Creole cattle, supporting the hypothesis of Holstein gene introgression in Highland population in order to increase milk yield. Most of the remaining alleles were present in all bovine groups because of their wide geographical distribution.

The PCA results based on allele frequencies are shown in Figure 4. The first two components together accounted for 59.77% of data variability. The first component accounted for 43.67% of the total variance and, as shown in a previous work,[12] it clearly exhibited a differentiation between Zebu (negative values) and Taurine (positive values) breeds. The first PC was primarily determined by differences in the frequency of the same alleles, such as *BoLA-DRB3*022:01*, **028:01*, **027:01*, **030:01*, **031:01*, **035:01*, **036:01*, and **057:02* with the higher negative axis 1 values, while, the alleles *BoLA-DRB3*001:01*, **002:01*, **006:01*, **007:01*, **009:02*, **010:01*, **011:01*, **012:01*, **015:01 *016:01*, **018:01*, *and *027:01* had the higher positive values for this axis. The second component explained 16.10% of the total variation and showed a gradient among Taurine and Zebuine breeds. This second PC was primarily determined by differences in the frequency of the same alleles, *such as BoLA-DRB3*002:01*, **006:01*, **007:01*, **009:02*, **012:01*, **017:01*, **018:01*, **022:01*, **027:10*, and **057:02* with the higher negative axis 1 values, while, *the alleles BoLA-DRB3*010:02*, **011:01*, **014:01:01*, **015:01 *016:02*, **021:01*, **020:06*, **028:01*, **028:02*, **030:01*, **031:01*, **036:01*, **041:01*, and **043:01* had the higher positive values for this axis. PC 2 showed that Bolivian Creole cattle breeds were closely located but they were distant from the Colombian Hartón del Valle Creole breed. In

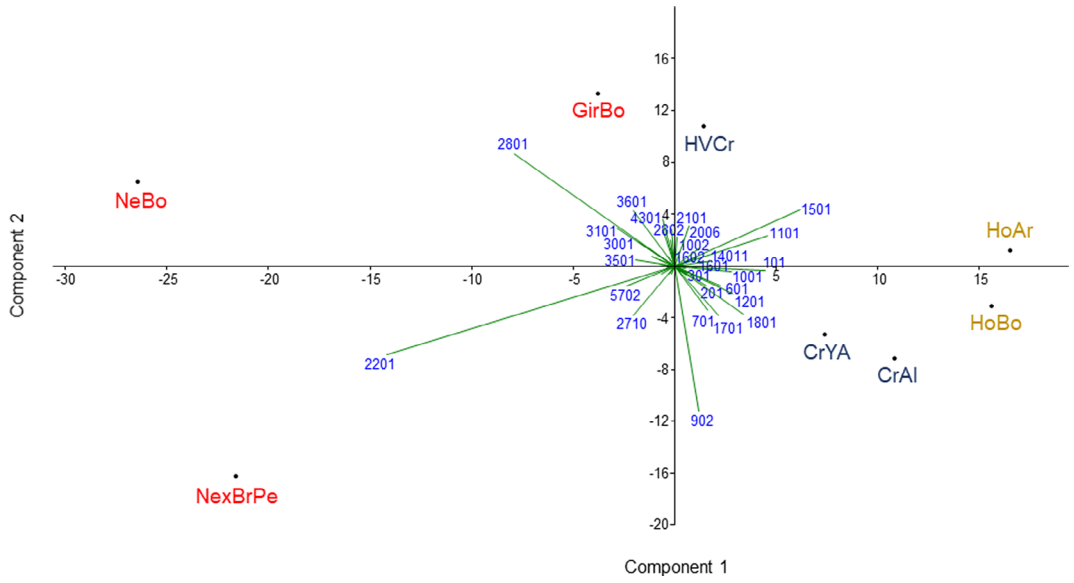

FIGURE 4 Principal components analysis of allele frequencies from the *BoLA-DRB3* gene in Highland Creole (CrAl), Yacumeño Creole (CrYa) and Hartón del Valle Creole (CrHV), Argentinean Holstein (HoAr), Bolivian Holstein (HoBo), Bolivian Nellore (NeBo), Bolivian Gir (GirBo), and Peruvian Nellore × Brahman (NexBrPe) cattle breeds/crossbreeds

	CrAl	CrYa	CrHV	HoAr	HoBo	GirBo	NeBo	NexBrPe
CrAl	0.0000	0.0149	0.0446	0.0406	0.0414	0.0548	0.0930	0.0876
CrYa		0.0000	0.0348	0.0348	0.0325	0.0438	0.0765	0.0706
CrHV			0.0000	0.0380	0.0419	0.0419	0.0602	0.0765
HoAr				0.0000	0.0193	0.0590	0.1049	0.0979
HoBo					0.0000	0.0578	0.1078	0.0900
GirBo						0.0000	0.0627	0.0743
NeBo							0.0000	0.0511
NexBrPe								0.0000

TABLE 5 Genetic distance between pair of populations estimated through F_{ST} (above) between Highland Creole (CrAl), Yacumeño Creole (CrYa) and Hartón del Valle Creole (CrHV), Argentinean Holstein (HoAr), Bolivian Holstein (HoBo), Bolivian Gir (GirBo), Bolivian Nellore (NeBo) and Peruvian Nellore × Brahman (NexBrPe) cattle breeds/crossbreeds

agreement with PCA, the F_{ST} analysis showed a low degree of genetic differentiation between Highland and Lowland Creole cattle from Bolivian native breeds (F_{ST} = 0.01485). On the one hand, this genetic structure value was similar to that estimated in Argentine and Bolivian Holstein populations (F_{ST} = 0.01928).[7] On the other hand, F_{ST} values between CrAl and Holstein varied from 0.0325 to 0.0414 (Table 5). However, Colombian Hartón del Valle Creole breeds exhibited a high degree of genetic divergence with respect to Bolivian populations, probably owing to a higher Zebu gene introgression.[25] Furthermore, the observed relationships among Creole Breeds would also reflect historical rather than environmental factors.

In conclusion, this work is the first report of *BoLA-DRB3* genetic diversity in a Bolivian Highland Creole cattle population using PCR-SBT. This analysis confirmed the presence of the *BoLA-DRB3*029:02* variant in this American Creole cattle breed. Furthermore, PCA showed that Bolivian Highland and Lowland Creole cattle were closely related. Venn analysis provided evidence of the influence of African and Zebuine genes in the Creole cattle germplasm. This study increases our knowledge about the worldwide variability of the *BoLA-DRB3* gene, an important locus for immune response and protection against pathogens.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Guillermo Giovambattista, **Juan A. Pereira Rico**, **Yoko Aida**: Conceived and designed the experiments. **Guillermo Giovambattista**, **Kyaw Kyaw Moe**, and **Ariel Loza Vega**: Performed the experiments. **Juan A. Pereira Rico**, **Ariel Loza Vega**, **Orlando N. Arce Cabrera**: Sample collection. **Guillermo Giovambattista**, **Kyaw Kyaw Moe**, **Meripet Polat**, and **Shin-Nosuke Takeshima**: Analyzed the data. **Yoko Aida**: Contributed reagents/materials/analysis tools. **Guillermo Giovambattista** and **Yoko Aida**: Wrote the manuscript. **Guillermo Giovambattista**, **Shin-Nosuke Takeshima**, **Kyaw Kyaw Moe**, **Juan A. Pereira Rico**, **Meripet Polat**, **Ariel Loza Vega**, **Orlando N. Arce Cabrera** and **Yoko Aida**: Final approval of the version to be published.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Takeshima SN, Aida Y. Structure, function and disease susceptibility of the bovine major histocompatibility complex. *Anim Sci J*. 2006;77:138-150.
2. Brown JH, Jardetzky TS, Gorga JC, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*. 1993;364:33-39.
3. Takeshima S, Nakai Y, Ohta M, Aida Y. Short communication: characterization of DRB3 alleles in the MHC of Japanese shorthorn cattle by polymerase chain reaction-sequence-based typing. *J Dairy Sci*. 2002;85(6):1630-1632.
4. Takeshima S, Saitou N, Morita M, Inoko H, Aida Y. The diversity of bovine MHC class II DRB3 genes in Japanese black, Japanese shorthorn, Jersey and Holstein cattle in Japan. *Gene*. 2003;316:111-118.
5. Takeshima SN, Miyasaka T, Polat M, et al. The great diversity of major histocompatibility complex class II genes in Philippine native cattle. *Meta Gene*. 2014;2(0):176-190.
6. Takeshima SN, Miyasaka T, Matsumoto Y, et al. Assessment of biodiversity in Chilean cattle using the distribution of major histocompatibility complex class II BoLA-DRB3 allele. *Tissue Antigens*. 2015a;85(1):35-44.
7. Takeshima SN, Giovambattista G, Okimoto N, et al. Characterization of bovine MHC class II DRB3 diversity in South American Holstein cattle populations. *Tissue Antigens*. 2015b;86(6):419-430.
8. Miyasaka T, Takeshima SN, Matsumoto Y, et al. The diversity of bovine MHC class II DRB3 and DQA1 alleles in different herds of Japanese black and Holstein cattle in Japan. *Gene*. 2011;472(1–2):42-49.
9. Miyasaka T, Takeshima SN, Sentsui H, Aida Y. Identification and diversity of bovine major histocompatibility complex class II haplotypes in Japanese black and Holstein cattle in Japan. *J Dairy Sci*. 2012;95(1):420-431.
10. Lee BY, Hur TY, Jung YH, Kim H. Identification of BoLA-DRB3.2 alleles in Korean native cattle (Hanwoo) and Holstein populations using a next generation sequencer. *Anim Genet*. 2012;43(4):438-441.
11. Giovambattista G, Takeshima SN, Ripoli MV, et al. Characterization of bovine MHC DRB3 diversity in Latin American creole cattle breeds. *Gene*. 2013;519(1):150-158.
12. Takeshima SN, Corbi-Botto C, Giovambattista G, Aida Y. Genetic diversity of BoLA-DRB3 in South American Zebu cattle populations. *BMC Genet*. 2018;22;19(1):33.
13. Primo AT. El Ganado bovino ibérico en las Américas 500 años después. (the IBERIC cattle in the Americas: 500 years later). *Arch Zootec*. 1992;41:421-432.

14. Takeshima SN, Matsumoto Y, Miyasaka T, et al. A new method for typing bovine major histocompatibility complex class II DRB3 alleles by combining two established PCR sequence-based techniques. *Tissue Antigens*. 2011;78(3):208-213.
15. Baxter R, Craigmile SC, Haley C, Douglas AJ, Williams JL, Glass EJ. BoLA-DR peptide binding pockets are fundamental for foot-and-mouth disease virus vaccine design in cattle. *Vaccine*. 2009;28:28-37.
16. Schneider S, Roessli D, Excoffier L. *Arlequin Version 2.000: A Software for Population Genetics Data Analysis. In., 2.000 Edn: Genetics and Biometry Laboratory*. Geneva, Switzerland: University of Geneva; 2000.
17. Rousset F. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour*. 2008;8(1):103-106.
18. Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electron*. 2001;4(1):9.
19. Kumar S, Nei M, Dudley J, Tamura K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform*. 2008;9(4):299-306.
20. Cavalli-Sforza LL, Menozzi P, Piazza A. *The History and Geography of Human Genes*. Vol 35. Princeton, NJ: Princeton University Press; 1994.
21. Gelhaus A, Schnittger L, Mehlitz D, Horstmann RD, Meyer CG. Sequence and PCR-RFLP analysis of 14 novel BoLA-DRB3 alleles. *Anim Genet*. 1995;26(3):147-153.
22. Mikko S, Andersson L. Extensive MHC class II DRB3 diversity in African and European cattle. *Immunogenetics*. 1995;42:408-413.
23. Russell GC, Marello KL, Gallagher A, McKeever DJ, Spooner RL. Amplification and sequencing of expressed DRB second exons from *Bos indicus*. *Immunogenetics*. 1994;39:432-436.
24. Lirón JP, Peral-García P, Giovambattista G. Genetic characterization of Argentine and Bolivian Creole cattle breeds assessed through microsatellites. *J Hered*. 2006;97(4):331-339.
25. Ginja C, Gama LT, Cortés O, et al. The genetic ancestry of American Creole cattle inferred from uniparental and autosomal genetic markers. *Sci Rep*. 2019;9(1):11486.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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