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The Complete Mitochondrial Genome of a Neglected Breed, the Peruvian Creole Cattle (*Bos taurus*), and Its Phylogenetic Analysis

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Abstract: Cattle spread throughout the American continent during the colonization years, originating creole breeds that adapted to a wide range of climate conditions. The population of creole cattle in Peru is decreasing mainly due to the introduction of more productive breeds in recent years. During the last 15 years, there has been significant progress in cattle genomics. However, little is known about the genetics of the Peruvian creole cattle (PCC) despite its importance to (i) improving productivity in the Andean region, (ii) agricultural labor, and (iii) cultural traditions. In addition, the origin and phylogenetic relationship of the PCC are still unclear. In order to promote the conservation of the PCC, we sequenced the mitochondrial genome of a creole bull, which also possessed exceptional fighting skills and was employed for agricultural tasks, from the highlands of Arequipa for the first time. The total mitochondrial genome sequence is 16,339 bp in length with the base composition of 31.43% A, 28.64% T, 26.81% C, and 13.12% G. It contains 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, and a control region. Among the 37 genes, 28 were positioned on the H-strand and 9 were positioned on the L-strand. The most frequently used codons were CUA (leucine), AUA (isoleucine), AUU (isoleucine), AUC (isoleucine), and ACA (threonine). Maximum likelihood reconstruction using complete mitochondrial genome sequences showed that the PCC is related to native African breeds. The annotated mitochondrial genome of PCC will serve as an important genetic data set for further breeding work and conservation strategies.

Keywords: zoogenetic resources; organelle; genomics; NGS; cattle; *Bos taurus*

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1. Introduction

Cattle are recognized as among the most important species for livestock, economic, and cultural influence in the world [1]. The global population of cattle (*Bos taurus* and *B. indicus*) is around 1.5 billion [2], making them one of the most common types of livestock. The American creole cattle (*B. taurus*) presumably descend from animals that were introduced from the Iberian Peninsula in the 15th century [3,4]. The origin of cattle in Peru dates back to the history of the Spanish conquest. In the year 1521, the arrival of bovines to America began, after which a process of establishment and evolution generated cattle adapted to the new environment [5]. The current Peruvian creole cattle (PCC) could be descendants of Retinta, Berrenda, Cacereña, and Andaluza Negra breeds [6]. In addition, a level of crossbreeding among original breeds in PCC can exist. The cattle population in Peru is about 5.5 million head, of which 63% are considered creole cattle [7]. However, that percentage does not consider the level of crossbreeding PCC possesses due to the introduction and use of specialized breeds in Peru in recent years. On the other hand, M. Rosenberg (UC del Sur, pers. comm.) estimates that only 5% of the cattle population in

Peru corresponds to creole. PCC, in contrast to other exotic breeds, has developed the ability to survive in environments with food limitations and under climatic factors that heavily affect its performance. Therefore, PCC is the basis for milk and beef production in the Peruvian highlands. In some Peruvian regions, PCC is also used for agricultural purposes and cultural events. For example, cattle from Arequipa city have been specialized for bullfighting events in local celebrations. Their breeding has been committed to the cultural manifestation of Arequipa citizens [8].

Studies on PCC are mostly limited to zoometric characterization. Espinoza and Urviola [9] determined body measurements of 140 creole cattle from Puno and concluded that the PCC can be described as a small animal size with a shallow chest and short rump of little amplitude that corresponds to the type of elipometric cattle. In addition, PCC presents an oblique rump and narrow pin bones. Similarly, Dipas Vargas [10] analyzed 254 creole individuals from Huamanga, Ayacucho, and indicated that the zoometric indices scored distinguished them as dolichocephalic (cephalic index = 45.96 $\pm$ 2.98), mesomorphic (body index = 87.79 $\pm$ 4.01), brachiothoracic (thorax index = 51.72 $\pm$ 4.95), brachypelvic (pelvic index = 90.68 $\pm$ 6.53), and dolichomorphic (depth index relative to the thorax = 50.81 $\pm$ 2.26). Moreover, Montoya [11] evaluated the phaneroptic and morphometric traits of 421 PCC individuals in Ayacucho, Cajamarca, and Puno and showed that the creoles from Puno showed a milk orientation and had more body development than the creoles from Ayacucho, which do not correspond to a dairy or beef orientation. He concluded that the individuals showed differences in their phaneroptic and morphometric traits according to their geographic origin, which may be used in breeding programs. In a more recent study, Encina Ruiz et al. [12] characterized the zoometry of creole cattle from the Southern Amazonas region of Peru and distinguished three biotypes of creole cattle with characteristics for meat and milk production. Molecular analyses are urgently needed to reinforce all these zoometric characterizations.

The current genomic evaluation methods have created favorable conditions to employ mitochondrial DNA as a tool for phylogenetic and biodiversity research [5]. Methods used for phylogeny verification have changed over time, from morphometric studies to molecular genetics methods. Genomic studies with the use of single nucleotide polymorphisms (SNPs) have been performed for the last 15 years. For instance, Hiendleder et al. [13] analyzed *B. taurus* and *B. indicus* mitochondrial genome sequences to investigate their sequence divergences and to study their taxonomic status by molecular methods. Estimated divergence times indicated that the two cattle lineages separated 1.7–2.0 million years ago. This molecular method provides new insights into intra-species taxonomy. Moreover, mitochondrial DNA studies have been carried out in American creole cattle in order to elucidate their maternal genomic origin. Lirón et al. [14] analyzed published D-loop mtDNA sequences from Creole, Iberian, and African cattle breeds and identified two subclades within the African T1 haplogroup. These subclades were clearly separated between creole cattle from Brazil and the cattle restricted in the American region colonized by the Spaniards. The authors hypothesized that there were two independent sources for the origin of American creole cattle from Africa. Similarly, Bonfiglio et al. [15] agreed on an African influence on the American creole cattle. In an assessment of mtDNA from European, African, and American breeds, they found six distinct sub-haplogroups. They hypothesized that seven to eight independent female lineages, belonging to haplogroup T1, were domesticated in the Near East and scattered throughout America by human migration activities. Recently, Ginja et al. [5] used mitochondrial DNA sequence data, Y-chromosome haplotype, and autosomal microsatellite marker information and showed that there was a differentiated contribution from African cattle to American creole genetic composition. Their results showed that there was a mixed ancestry of American creole cattle and each group was genetically differentiated from the others. They also recommended that a deep study of each creole bred would be beneficial for their conservation and use.

These molecular tools are greatly necessary in order to conduct studies on genetic distance and differentiation among cattle breeds in Peru. However, genomic tools and

mitochondrial DNA studies are still in their infancy in Peru. Research is mainly focused on determining the allelic and genotypic frequency of the kappa casein gene (*κ-CN*). Veli et al. [16] determined the allelic component of ß-lactoglobulin (*BLG*) in 267 creole cattle of rural communities of Ancash, Ayacucho, and Puno and indicated that the genotypic frequency of BLG^{AA} was lower than the BLG^{AB} and BLG^{BB} genotypic frequencies. In another work, Almeyda et al. [17] screened 48 creole cattle from nine villages of Bambamarca, Cajamarca, and demonstrated that those individuals possessed low genotypic frequencies that favor cheese production. In a recent study, Yalta-Macedo et al. [4] identified the genetic diversity and paternal origin of Peruvian creole cattle by using seven Y-chromosome-specific markers in 229 individuals from six regions of the Peruvian highlands. They showed that PCC possessed low genetic diversity and a lack of population structure. In addition, their results revealed unique characteristics of PCC and suggested that PCC derived from the Iberian Peninsula cattle; however, the authors mentioned that African cattle also had an influence on PCC.

To the best of our knowledge, this is the first time a complete mitochondrial genome of the Peruvian creole cattle was sequenced employing next-generation sequencing (NGS). We here determined the genomic features of the mitochondrial genome of the PCC and provided insights into its phylogenetic relationship within the family Bovidae.

2. Results

2.1. Mitochondrial Genome Organization

The complete assembled mitochondrial genome of the Peruvian creole cattle (*Bos taurus*) was 16,339 bp in length and consisted of 22 tRNA genes, 13 protein-coding genes, 2 rRNA genes, and a control region (Table 1, Figure 1). Most of the genes (28) were encoded within the heavy (H) strand, and nine genes were positioned on the L-strand. The base composition of this genome was 31.43% A, 28.64% T, 26.81% C, and 13.12% G. The D-loop region is located between the $tRNA^{\mathrm{Pro}}$ and $tRNA^{Phe}$ genes. In the range from 1 to 40 bp, there were a total of 78 overlapping nucleotides in seven different regions. The largest overlapping region (40 bp) was emplaced between the *Atp8* and *Atp6* genes. Furthermore, the intergenic spacer (IGS) comprised 14 regions across the mitochondrial genome ranging from 1 to 7 bp, which summed up to a total length of 30 bp. The largest intergenic spacer (7 bp) was located between $tRNA^{Ser2}$ and $tRNA^{Asp}$ genes. The entire mitochondrial genome sequence was submitted to the GenBank database with accession number OK135155. The associated Bioproject, Biosample, and SRA numbers are PRJNA763011, SAMN21419641, and SRR15883111, respectively. The assembly coverage was 6073.6X.

Table 1. Gene organization of the mitochondrial genome of Peruvian creole cattle. The (+) and (−) values correspond to intergenic nucleotides and overlapping regions between the genes, respectively.

Gene	Nucleotide Positions	Size (bp)	Strand [1]	Codon	Intergenic Spacer (bp)
$tRNA^{Phe}$	364–430	67	H	TTC	
12S rRNA	431–1386	956	H		
$tRNA^{Val}$	1387–1453	67	H	GTA	
16S rRNA	1454–3023	1570	H		
$tRNA^{Leu2}$	3025–3099	75	H	TTA	1
Nd1	3102–4057	956	H		2
$tRNA^{Ile}$	4058–4126	69	H	ATC	
$tRNA^{Gln}$	4124–4195	72	L	CAA	−3
$tRNA^{Met}$	4198–4266	69	H	ATG	2
Nd2	4267–5309	1043	H		
$tRNA^{Trp}$	5309–5375	67	H	TGA	−1
$tRNA^{Ala}$	5377–5445	69	L	GCA	1
$tRNA^{Asn}$	5447–5519	73	L	AAC	1
Rep_origin	5522–5552	31	H		2
$tRNA^{Cys}$	5552–5618	67	L	TGC	−1
$tRNA^{Tyr}$	5619–5686	68	L	TAC	

Table 1. *Cont.*

Gene	Nucleotide Positions	Size (bp)	Strand [1]	Codon	Intergenic Spacer (bp)
Cox1	5688–7232	1545	H		1
tRNA^{Ser2}	7230–7298	69	L	TCA	−3
tRNA^{Asp}	7306–7373	68	H	GAC	7
Cox2	7375–8058	684	H		1
tRNA^{Lys}	8062–8128	67	H	AAA	3
Atp8	8130–8330	201	H		1
Atp6	8291–8971	681	H		−40
Cox3	8971–9755	785	H		−1
tRNA^{Gly}	9755–9823	69	H	GGA	−1
Nd3	9821–10170	350	H		−3
tRNA^{Arg}	10171–10239	69	H	CGA	
Nd4L	10240–10536	297	H		
Nd4	10530–11907	1378	H		−7
tRNA^{His}	11908–11977	70	H	CAC	
tRNA^{Ser}	11978–12037	60	H	AGC	
tRNA^{Leu}	12039–12109	71	H	CTA	1
Nd5	12110–13930	1821	H		
Nd6	13914–14441	528	L		−17
tRNA^{Glu}	14442–14510	69	L	GAA	
CytB	14515–15654	1140	H		4
tRNA^{Thr}	15658–15727	70	H		3
tRNA^{Pro}	15727–15792	66	L	CCA	−1
D-loop	15793–16339, 1–363	910	H		

[1] Strand: H (Heavy), L (Light).

2.2. Protein Coding Genes (PCGs) and Codon Usage

A total of 13 PCGs were encoded in the PCC mitogenome and were 11,409 bp in length, representing 69.83% of this genome. This mitochondrial genome encodes 3792 amino acids. These PCGs were represented by (i) seven NADH dehydrogenase subunits, (ii) two ATPase subunits, and (iii) a cytochrome *b* gene. These PCGs are AT-biased as AT content ranges from 55.9% for *Cox3* to 68.2% for the *Atp8* gene. Moreover, the length of PCGs varied greatly from *atp8* (201 bp) to *Nad5* (1821 bp) (Table 2). The most abundant start and stop codons were ATG and TAA, respectively. On the other hand, genes *Nd1*, *Nd2*, *Cox3*, *Nd3*, and *Nd4* presented incomplete stop codons (TA- or T–). The length of the 13 genes varied from 201 bp (*Atp8*) to 1821 bp (*Nd5*). Similarly, protein length (aa) ranged from 66 to 606 for these two genes. The five codons with the highest relative synonymous codon usage (RSCU) values in the PCGs were: CUA (2.86), CGA (2.67), UCA (2.15), ACA (1.99), and GUA (1.83) (Figure 2A). CUA (leucine), AUA (isoleucine), AUU (isoleucine), AUC (isoleucine), and ACA (threonine) were the most frequently used codons (Figure 2B).

Table 2. Characteristics of protein-coding genes identified in the mitochondrial genome of Peruvian creole cattle.

Gene	Gene Length (bp)	A + T Content (%)	Start/Stop Codon	Protein Length (aa)
Nd1	956	59.4	ATG/TA-	318
Nd2	1043	64.6	ATA/TA-	347
Cox1	1545	58.3	ATG/TAA	514
Cox2	684	61.7	ATG/TAA	227
Atp8	201	68.2	ATG/TAA	66
Atp6	681	61.5	ATG/TAA	226
Cox3	785	55.9	ATG/TA-	261
Nd3	350	58.0	ATA/TA-	116
Nd4L	297	63.9	ATG/TAA	98

Table 2. *Cont.*

Gene	Gene Length (bp)	A + T Content (%)	Start/Stop Codon	Protein Length (aa)
Nd4	1378	60.9	ATG/T–	459
Nd5	1821	60.3	ATA/TAA	606
Nd6	528	63.1	ATG/TAA	175
CytB	1140	56.4	ATG/AGA	379
Total	11,409			3792

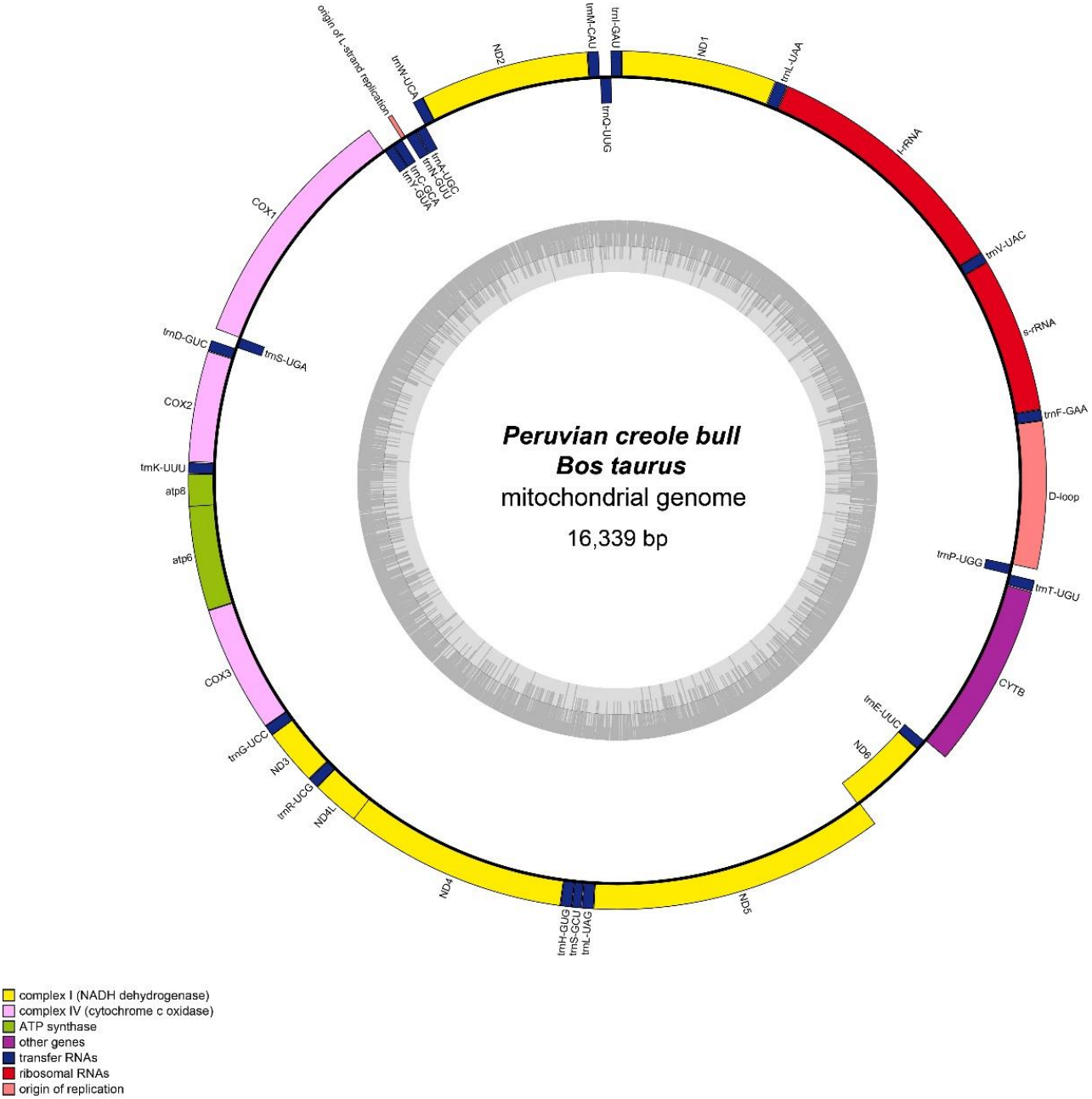

Figure 1. The mitochondrial genome map of the Peruvian creole cattle. Genes encoded by the heavy strand are shown outside the circle, while those encoded by the light strand are shown inside.

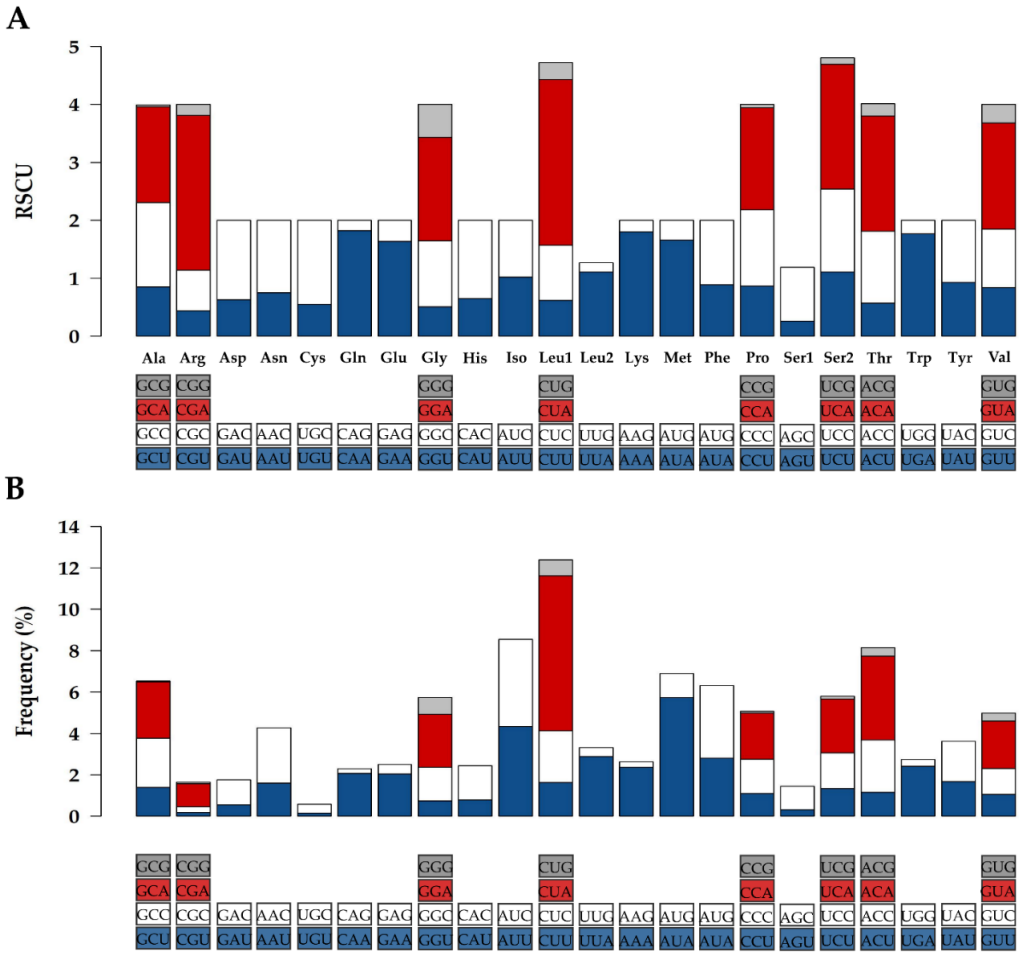

Figure 2. Codon usage of the mitochondrial genome protein-coding genes of the Peruvian creole cattle. (**A**) Relative synonymous codon usage (RSCU). (**B**) Codon usage frequency. Codon families are plotted on the X-axis and represented by different colors.

2.3. Ribosomal RNA, Transfer RNA, and Non-Coding Regions

The total size of the two rRNA genes (*12S* and *16S*) was 2526 bp, and they were flanked by $tRNA^{Phe}$ and $tRNA^{Leu2}$ (Table 1, Figure 1). We identified 22 *tRNA* genes with a total size of 1511 bp, which varied in length from 60 ($tRNA^{Ser}$) to 75 ($tRNA^{Leu2}$) bp (Table 1). The H-strand contained 14 *tRNA* genes, and eight *tRNA* genes were encoded by the L-strand. All *tRNA* genes exhibited the cloverleaf secondary structure except two of them, $tRNA^{Lys}$ and $tRNA^{Ser}$ (Figure 3). Non-coding regions comprised the origin of replication, the intergenic spacers, and the control region. The origin of replication possessed 31 bp and was located between $tRNA^{Asn}$ and $tRNA^{Cys}$. In addition, with a total length of 910 bp, the control region (D-loop) was flanked by $tRNA^{Pro}$ and $tRNA^{Phe}$ genes (Table 1, Figure 1).

2.4. Phylogenetic Inference

Our maximum likelihood (ML) phylogenetic tree topology showed three well-supported clades. Species *B. taurus* is placed within a monophyletic clade and is sister to a clade formed by *B. primigenius*; sister to them is a clade formed by *B. indicus*. Members of *B. gaurus* and *B. frontalis* were placed within clade 2. In addition, *B. grunniens* and *B. mutus* form clade 3, which is sister to clades 1 and 2 (Figure 4). ML analysis revealed that within clade 1, members of *B. taurus* are placed in two subclades, 1 and 2. Peruvian creole cattle was placed in subclade 1 together with other creole cattle from Paraguay and Mexico, and with individuals from Africa mainly. On the other hand, subclade 2 comprised mainly

cattle from Europe that are mostly specialized for beef and milk production (Figure 4, Supplementary Table S1).

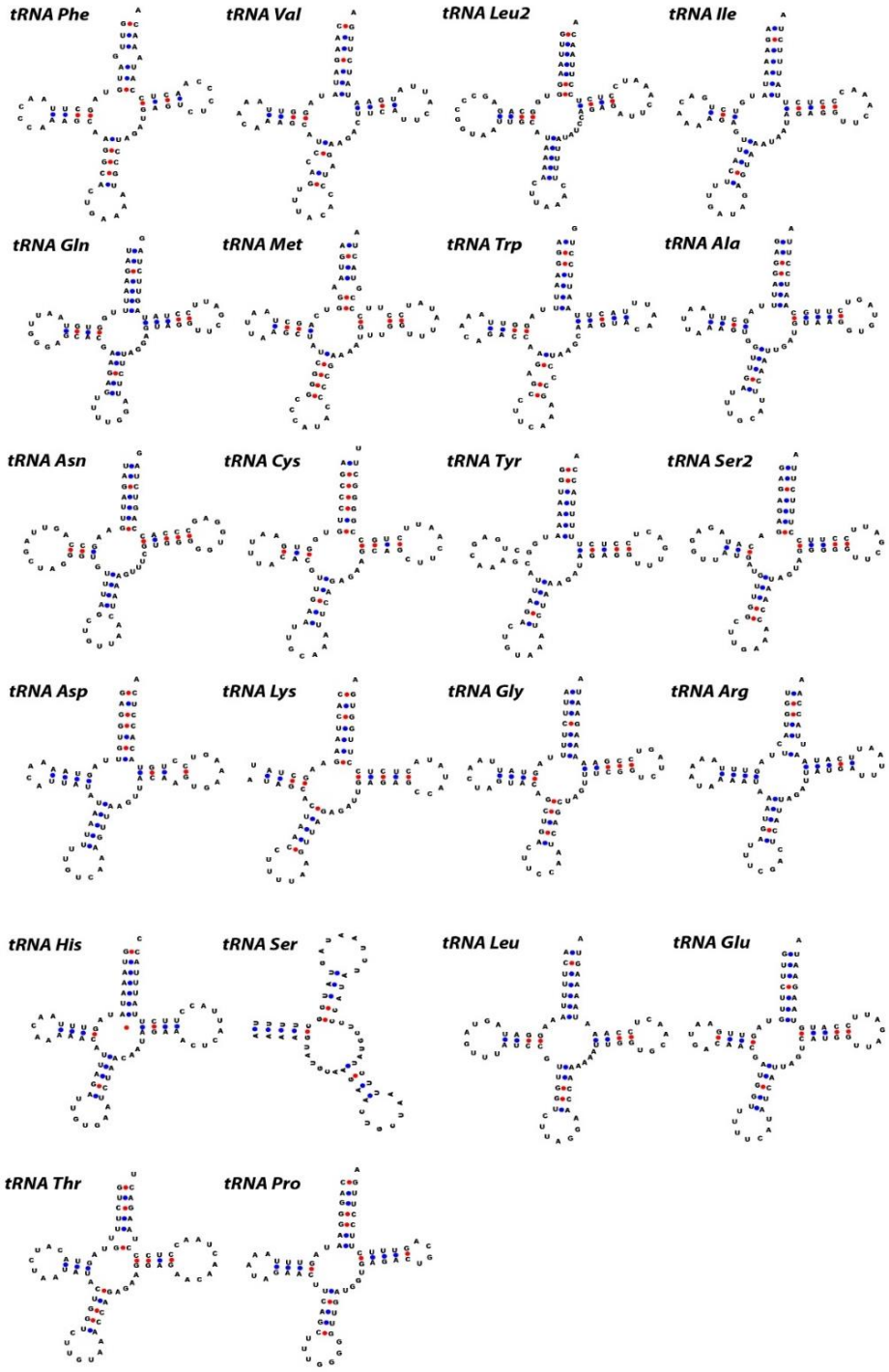

Figure 3. The predicted secondary structures of 22 transfer RNA genes of Peruvian creole cattle.

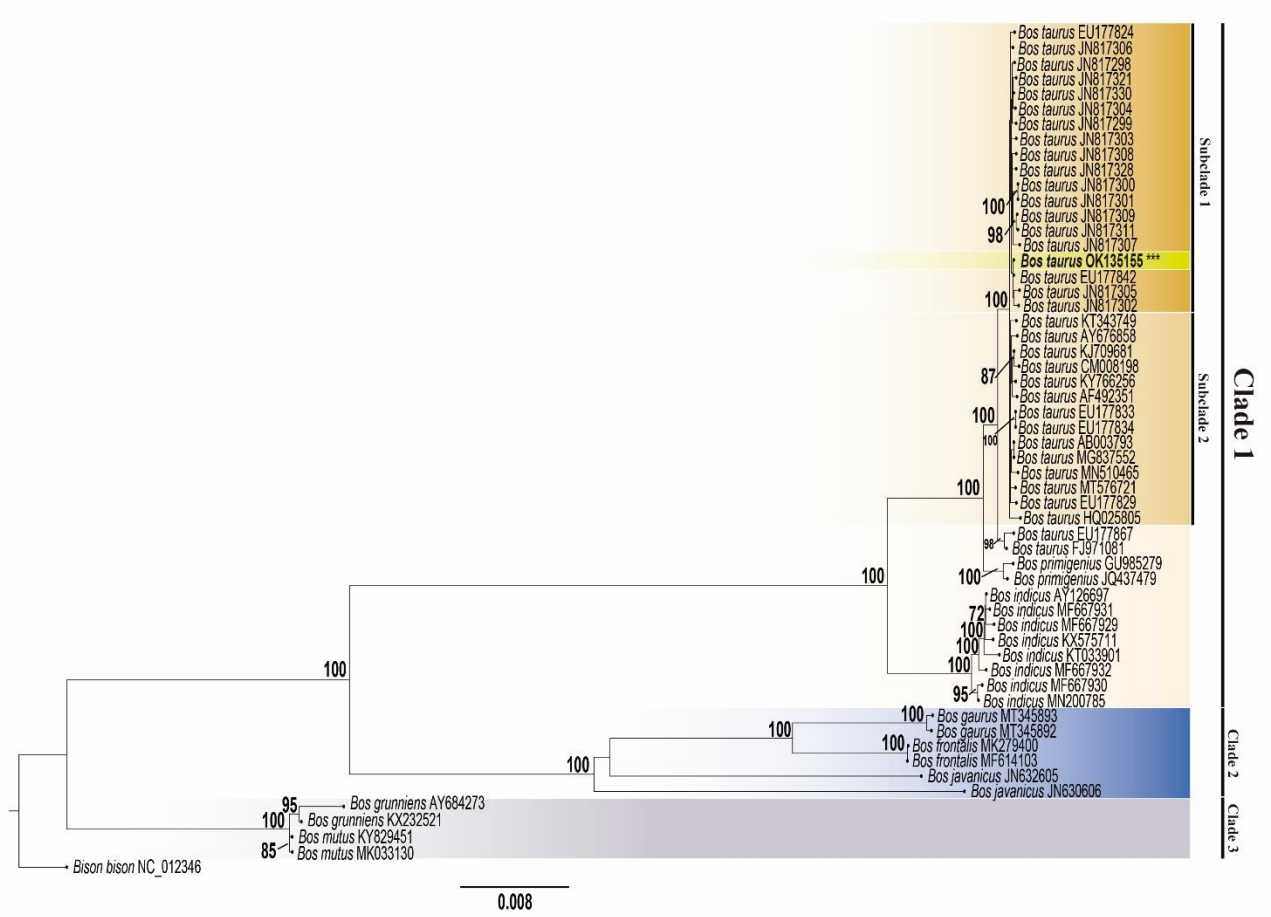

Figure 4. Maximum likelihood phylogenetic tree of *Bos* species inferred from mitochondrial genomic sequences. Bootstrap support is shown only for branches with >70% support. *Bison bison* was set as the outgroup. The PCC is designated by three asterisks.

3. Discussion

During the last five centuries, creole cattle went through multiple random crosses, conferring not only zoometric characteristic diversity, but also adaptive traits that allowed them to survive in different environmental conditions [18]. Due to the introduction of specialized breeds, now the Peruvian creole cattle (PCC) breed is mainly distributed in rural communities in southern Peru (Ayacucho, Arequipa, Huancavelica, and Apurimac) (M. Rosenberg, UC del Sur, pers. comm.), and it represents a significant protein source and agricultural labor force and is part of cultural traditions. Due to the reduction in sequencing costs, livestock geneticists have sequenced over 2500 cattle genomes [19]. Nevertheless, PCC is still a neglected breed as it has been poorly studied.

The PCC is a unique local breed adapted to the Peruvian conditions and was developed by the introduction of the European or African cattle in Peru, according to the historical records and traditional knowledge [3,4]. The PCC breeds mainly in the highlands, where the geography naturally isolates the lands. Due to the interest in the use of exotic breeds in Peru, there are some crossbreed specimens, which are commonly called "creole improved cattle". However, these individuals are not fully resistant to the tough conditions of the Andean environment. PCC is typically morphologically distinguished from other exotic breeds by the body structure [9,10,12]. Its body is unbalanced with the topline being higher on the front and becoming smaller toward the rear. The hooks to pin are lower-level hipped when compared to other breeds of cattle, dairy or beef. The length of the body is shorter, as is the topline. Colors of hair have multiple variations. The diversity of colors ranges from a total color cover to mixed ones and spotting ones.

Thus, in the present work, we sequenced and characterized the PPC mitochondrial genome, which was 16,339 bp in length, for the first time. This size is in agreement with mitogenomes of other individuals of *Bos taurus* (16,338 bp), *B. indicus* (16,339 bp), *B. frontalis* (16,346 bp), *B. grunniens* (16,324 bp), and *B. gaurus* (16,345 bp) [13,20–22]. Our result confirmed that mitogenomes in the Bovinae subfamily are very similar in size. In addition, gene order and structure were similar to previously reported *B. taurus* mitogenomes [23,24]. The AT and GC contents were 60.07% and 39.93%, respectively, revealing a nucleotide composition bias of the PCC mitogenome towards adenine and thymine, which is commonly observed in other mammals [20,25,26].

Indices of codon usage bias show differences in the occurrence of codon usage, indicating genomic evolutionary patterns [27]. Start (ATG) and stop (TAA) codons were the most abundant in the PCC mitogenome, which is in agreement with the mitochondrial genome of other mammals [26,28,29]. Incomplete stop codon presence is commonly found in mitogenomes of other vertebrates [30–32], which might be further completed by the posttranscriptional poly-adenylation of the 3′ end of the mRNA [33,34]. Codon usage bias occurs in a wide variety of species. We here report the codon usage bias for the first time in PCC, providing useful information about the abundance of tRNA, GC composition gene expression level, and mutation frequency. Codon usage is determined based on the relative synonymous codon usage (RSCU) index. According to Sharp et al. [35], this index is calculated as the ratio of the observed frequency of a codon to the expected frequency of that codon, assuming uniform codon usage. An RSCU of 1 indicates that there is no codon usage bias for that amino acid and the codons are chosen randomly or equally. RSCU values above and below 1 correspond to positive and negative codon usage bias, respectively [35,36].

Non-coding regions are known to possess controlling elements for transcription and replication [37] and include intergenic spacers, L-strand origin of replication, and control region [38]. The organization of the origin of replication of the PCC mitogenome is similar to that of other mammal species [26,39,40]. Similar to previous work on other *Bos* mitogenomes [20,21], the length of the control region of the PCC mitochondrial genomes was 910 bp.

In order to understand the phylogenetic relationship of the Peruvian creole cattle, an ML phylogenetic tree with other cattle breeds and *Bos* species was constructed. Our entire mitochondrial genome analysis provided a well-supported topology of the *Bos* genus, as reported by Kamalakkannan et al. [21]. The ML phylogenetic tree showed three clades, and clade 1 was further divided into two subclades. The PCC is distributed in subclade 1 together with cattle from Paraguay, Mexico, and Africa mainly. Interestingly, the PCC and an Italian breed (Cinisara) were sisters to a clade formed by two African indicine breeds, Boran and Arsi. Porter et al. [41] indicated that the Boran breed underwent selection and improvement with European taurine cattle in the early 20th century. In addition, Ginja et al. [5] used autosomal microsatellites to demonstrate that American creole cattle occupied an intermediate position between European and African breeds. However, they did not use individuals from Peru. Therefore, it is very likely that African cattle played a role in the development of PCC, as showed by Yalta-Macedo [4] with Y-chromosome-specific markers. This may be explained by the role that African cattle had in the development of Iberian breeds or imported cattle directly from Africa to Peru.

Mitogenomes possess limitations such as neutrality and effective population size, introgression, accelerated rates of substitution, and maternal inheritance [42], as well as variation in rates of gene loss accompanied by functional transfer to the nucleus and rates of genome rearrangement [43]. Therefore, other nuclear markers should be employed to validate our phylogenetic results found for the PCC in this work. Our next step is to continue developing molecular tools for the PCC. Further investigation is needed to identify putative genes of the PCC that confer resilience in adverse climate conditions, among other traits. These molecular tools may shed light on developing a modern cattle breeding program in Peru by exploiting the alleles that this neglected breed possesses.

Moreover, sustainable local management and implementation of conservation programs of the PCC are urgently needed.

4. Materials and Methods

4.1. Sample Collection, DNA Extraction, and Sequencing

A hair sample from the tail was collected from a single male specimen from the Andagua district, Castilla province, in Arequipa (3574 masl; −15.499548°, −72.359927°). This individual was considered an "Arequipa fighting bull" as it possessed exceptional fighting skills and was part of the traditional bullfight activity of Arequipa (Figure S1). We selected this individual as it possessed most of the classical characteristics of a PCC. In addition, the owners indicated its parents were also creole. It was also employed for agricultural labor.

We extracted genomic DNA with the Wizard Genomic DNA Purification Kit (Fitchburg, WI, USA) following the manufacturer's instructions. The quality and quantity of genomic DNA were assessed using agarose gel electrophoresis and a Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA), respectively. An Illumina paired-end (2×150 bp) genomic library was constructed by following the standard protocol (Illumina, San Diego, CA, USA) and sequenced using an Illumina HiSeq 2500 platform by GENEWIZ (South Plainfield, NJ, USA). Briefly, NEBNext Ultra DNA Library Prep Kit for Illumina, clustering, and sequencing reagents was used throughout the process following the manufacturer's recommendations. The genomic DNA was fragmented by acoustic shearing with a Covaris S220 instrument. Fragmented DNA was cleaned up and end repaired. Adapters were ligated after adenylation of the 3′ ends followed by enrichment by limited cycle PCR. The DNA library was validated using a High Sensitivity D1000 ScreenTape on an Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA) and was quantified using a Qubit 2.0 Fluorometer. The DNA library was also quantified by real-time PCR (Applied Biosystems, Carlsbad, CA, USA). The sequencing library was clustered onto a flowcell. After clustering, the flowcell was loaded onto the Illumina instrument according to the manufacturer's instructions. Image analysis and base calling were conducted using the Illumina Control Software. Raw sequence data (.bcl files) generated from the Illumina instrument were converted into fastq files using Illumina bcl2fastq 2.17 software. One mismatch was allowed for index sequence identification.

4.2. Assembly, Annotation, and Sequence Analysis

Adapters and low-quality reads were removed using Trim Galore [44]. We used clean data and *Bos taurus* (MN510465) as a reference to assemble the mitochondrial genome using GetOrganelle v1.7.2 pipeline [45], in which SPAdes v3.11.1 [46], bowtie2 v2.4.2 [47], and BLAST + v2.11 [48] were employed. The annotations of the protein-coding genes (PCGs), transfer RNAs (tRNAs), and rRNA genes from the mitogenome were performed using the automatic annotators of mitochondrial genes online, Geseq in CHLOROBOX web service [49] and MITOS 2 [50], and curated manually. The tRNA secondary structure was analyzed with tRNAs-can-SE 2.0 [51]. The codon usage bias was analyzed by using MEGA v11 [52]. We obtained the circularized drawing of the mitochondrial genome with OGDRAW v1.3.1 [53].

4.3. Codon Usage and tRNA Analysis

Codon usage analysis was performed in MEGA v11. For this purpose, each coding gene sequence was verified by alignment with its orthologs in the reference *B. Taurus* mitochondrial genome (MN510465) using CLUSTALW implemented in MEGA v11. The stop codon was removed. Then, 13 nucleotide sequences were concatenated using the Concatenate Sequence Alignment option, and we evaluated the codon usage using the RSCU option in MEGA v11. To predict the secondary structure of each tRNA, we used the tRNAscan-SE website server (http://lowelab.ucsc.edu/tRNAscan-SE/ accessed on 12 April 2022). We

analyzed the sequences in FASTA format and kept the default parameters except for the sequence source, which corresponded to mammalian mitochondrial.

4.4. Phylogenetic Analysis

We employed 55 mitochondrial genomes of other *Bos* species available in GenBank to determine the genetic relationship of PCC (Table S1). As an outgroup, we used a species of the genus Bison (*Bison bison*) from the same subfamily Bovinae. Each genome was aligned using MAFFT v7.475 [54], and with a GTR + GAMMA model of evolution, we obtained the best-scoring maximum likelihood (ML) tree; then, 1000 nonparametric bootstrap inferences were performed with RAxML v8.2.11 [55]. The resulting trees were viewed in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/ accessed on 18 April 2022).

5. Conclusions

Here, we first reported the complete mitochondrial genome of a neglected breed, the Peruvian creole cattle. Our results showed that the organization and characteristics of this mitogenome are in agreement with the mitochondrial genomes of other mammals, possessing a length of 16,339 bp, 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, and a control region. Moreover, 28 genes were positioned on the H-strand and 9 genes were positioned on the L-strand. Phylogenetic analysis showed that PCC is potentially related to native African breeds. However, more work employing nuclear markers and additional individuals of creole cattle from Peru is needed. We hope this work will help pave the way toward establishing conservation policies and conducting more modern research on the PCC.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/data7060076/s1, Table S1: Species, breed, origin, accession code, and clade assignment of the 56 individuals employed in this study. Figure S1: Bull from Andagua, Arequipa, whose mitochondrial genome was sequenced in the present work. This individual possessed exceptional fighting skills and was part of the traditional bullfight activity of Arequipa in Peru, and it was employed for agricultural labor. It was considered a PCC by its owners (Family Ojeda). Source: Pasion y Coraje (https://pasionycoraje.com/ accessed on 27 May 2022).

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Informed Consent Statement: Written informed consent was obtained from the owner of the bull studied here.

Data Availability Statement: All data generated during this study are included in this published article.

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