Proceedings of the 12th World Congress on Genetics Applied to Livestock Production (WCGALP)

Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges

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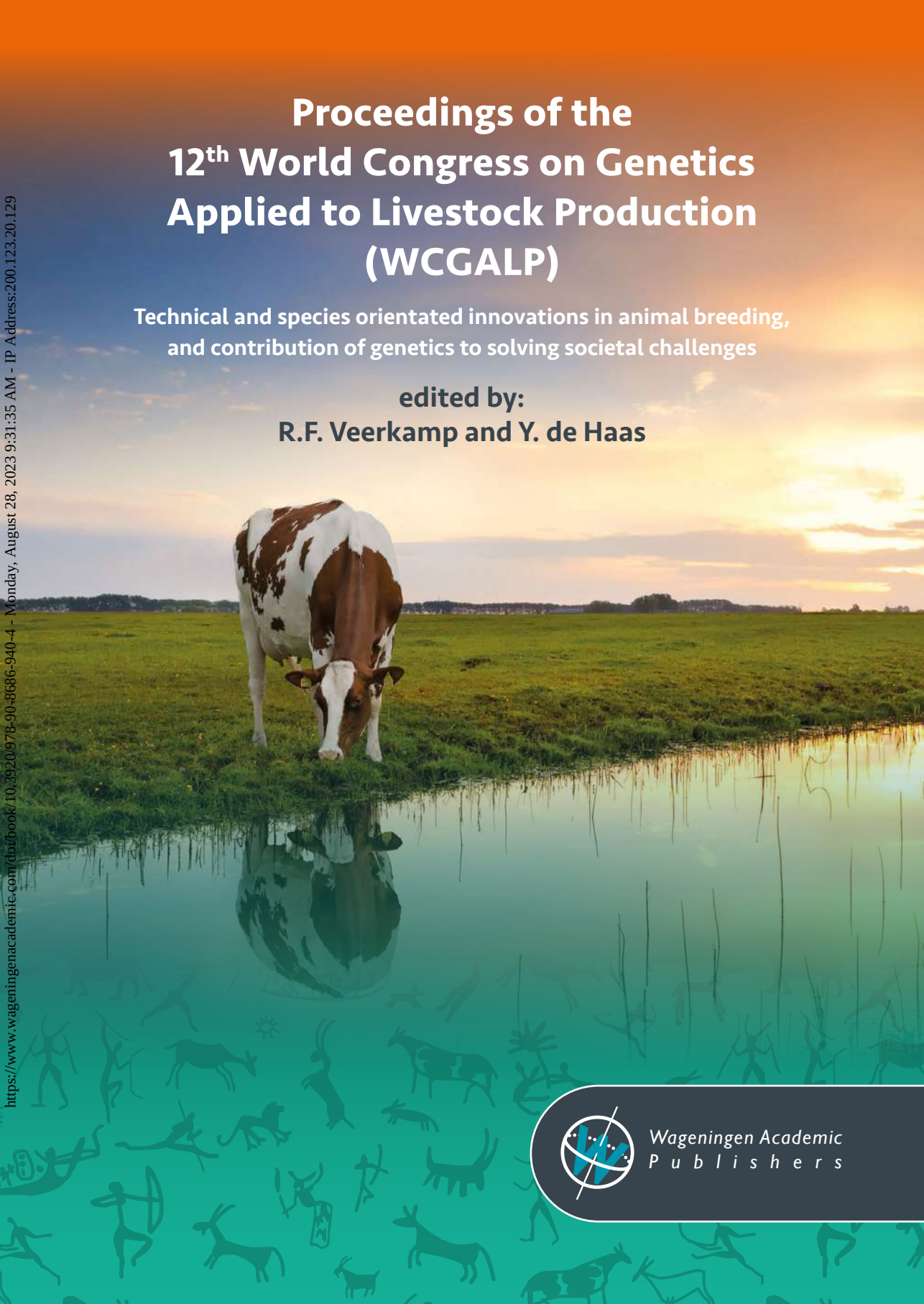

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264. The complete mitochondrial genome of a Peruvian creole cattle (*Bos taurus*) and its phylogenetic analysis

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Abstract

The population of Peruvian creole cattle (PCC) is decreasing mainly due to the introduction of more productive breeds in recent years. We report the complete mitochondrial genome sequence of a PCC bull for the first time. This genome was 16,339 bp in length with the base composition 31.43% A, 28.64% T, 26.81% C, and 13.12% G. It contained 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 analysis clearly demonstrated that PCC are strongly related to a native African breed, giving insights into the maternal ancestry of PCC. The annotated mitochondrial genome of PCC would serve as an important genetic data set for further breeding work and conservation strategies.

Introduction

The global population of cattle (*Bos taurus* and *Bos indicus*) is around 1.5 billion (McLeod, 2011), making it one of the most common livestock species. The cattle population in Peru is about 5.5 million head (MIDAGRI, 2019) of which 5% are creole cattle (M. Rosenberg, UC del Sur, pers. Comm.). PCC, in difference to other exotic breeds, have developed the ability to survive in environments with food limitations and under climatic factors that heavily affect their performance. Therefore, PCC are the basis for milk and beef production in the Peruvian highlands. In some Peruvian regions, PCC are also used for agricultural purposes and cultural events. In this work, for the first time, we sequenced the complete mitochondrial genome of a Creole bull from Peru.

Materials & methods

Sample collection and DNA extraction. A tail hair sample was collected from a single male specimen from the Andagua district, Castilla province in Arequipa (3,574 masl; -15.499548°, -72.359927°). This individual named as 'Tremendo Cachorro' was considered an 'Arequipa fighting bull' as it possessed exceptional fighting skills and was part of the traditional bullfight activity of Arequipa. We extracted genomic DNA with the Wizard® Genomic DNA Purification Kit (Wisconsin, USA) following the manufacturer's instructions. The quality and quantity of genomic DNA were assessed by agarose gel electrophoresis and NanoDrop One (Thermo Fisher Scientific, USA), respectively. Library preparation and high-throughput sequencing with Illumina HiSeq Sequencing System (Illumina, San Diego, CA) were conducted by Genewiz (New Jersey, USA).

Assembly, annotation and sequence analysis. Pair-end clean reads were obtained by PE 150 library and the Illumina HiSeq 2500 platform. Adapters and low-quality reads were removed using Trim Galore (Martin 2011). We used clean data to assemble the mitochondrial genome using GetOrganelle v1.7.2 pipeline (Jin *et al.* 2020). The annotations of the protein-coding genes (PCGs), transfer RNAs (tRNAs) and rRNA genes from mitogenome were performed using the automatic annotators of mitochondrial genes

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online: Geseq (Tillich *et al.* 2017) and MITOS 2 (Donath *et al.* 2019), and curated manually. The tRNA secondary structure was analyzed with tRNAscan-SE 2.0 (Chan *et al.* 2021). The codon usage bias was analyzed using MEGA X (Kumar *et al.* 2018). The circularized drawing of the mitochondrial genome was performed with OGDRAW 1.3.1. (Greiner *et al.* 2019).

Codon usage and tRNA analysis. Codon usage analysis was performed in Molecular Evolutionary Genetics Analysis 11 (MEGA 11) software (https://www.megasoftware.net/). For this purpose, each nucleotide sequence was cured manually, removing the STOP codon and only keeping the codons that synthesize amino acids. Then, 13 nucleotide sequences were concatenated using the Concatenate Sequence Alignment option and we evaluated the Codon Usage using RCSU option in MEGA 11. To predict the secondary structure of each tRNA, we used the tRNAscan-SE website server (http://lowelab.ucsc.edu/tRNAscan-SE/). We analysed the sequences in FASTA format and kept the parameters by default except the Sequence Source that corresponds to Mammalian mitochondrial.

Phylogenetic analysis. To ascertain the genetic relationship of PCC with other *Bos* species, we also included 32 complete mitochondrial genomes from *Bos* species available in GenBank. As outgroup, we used a species of the genus Bison (*Bison bison*) from the same subfamily Bovinae. We used GTR+GAMMA model of evolution to obtain the best-scoring ML tree, and then 1000 nonparametric bootstrap inferences were performed with RAxML v8.2.11 (Stamatakis 2014).

Results

The complete mitochondrial genome of PCC was 16.339 bp in length, which included 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes and a control region (Figure 1). Most of the genes (28) were encoded within the heavy (H) strand, and 9 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 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 relative synonymous codon usage (RSCU) analysis showed the highest utilization of CUA, AUA, AUU, AUC and ACA codons among PCGs. Our phylogenetic analysis showed that PCC clustered as expected in a well-supported clade that included *B. taurus* individuals (Figure 2). Interestingly, this PCC was within subclade 1 that contains most African breeds.

Discussion

We sequenced for the first time the complete mitochondrial genome of a Peruvian creole bull using next generation sequencing. The genome size and order was in agreement to previous work on native cattle (Liu *et al.*, 2020). The nucleotide composition of the PCC mitochondrial genome was biased towards adenine and thymine and was in agreement with other studies in *Bos* (Kamalakkannan *et al.*, 2020). This feature is a common characteristic for the bovine mitochondrial genome (Zhou *et al.*, 2019). Similar to other studies in the *Bos* genus, amino acids leucine, threonine, and isoleucine were the most abundant among the protein coding genes (Kamalakkannan *et al.*, 2020). It was possible to recover the three clades that were reported for *Bos* (Kamalakkannan *et al.*, 2020). In addition, our study provided further evidence about the influence of African and European founders. However, more samples from a wider geographic area in Peru, especially form the Andean region, needs to be analysed using both mitochondrial and nuclear DNA to confirm PCC origin. This newly generated sequence would serve for phylogenetic, evolutionary and conservation work for this orphan breed.

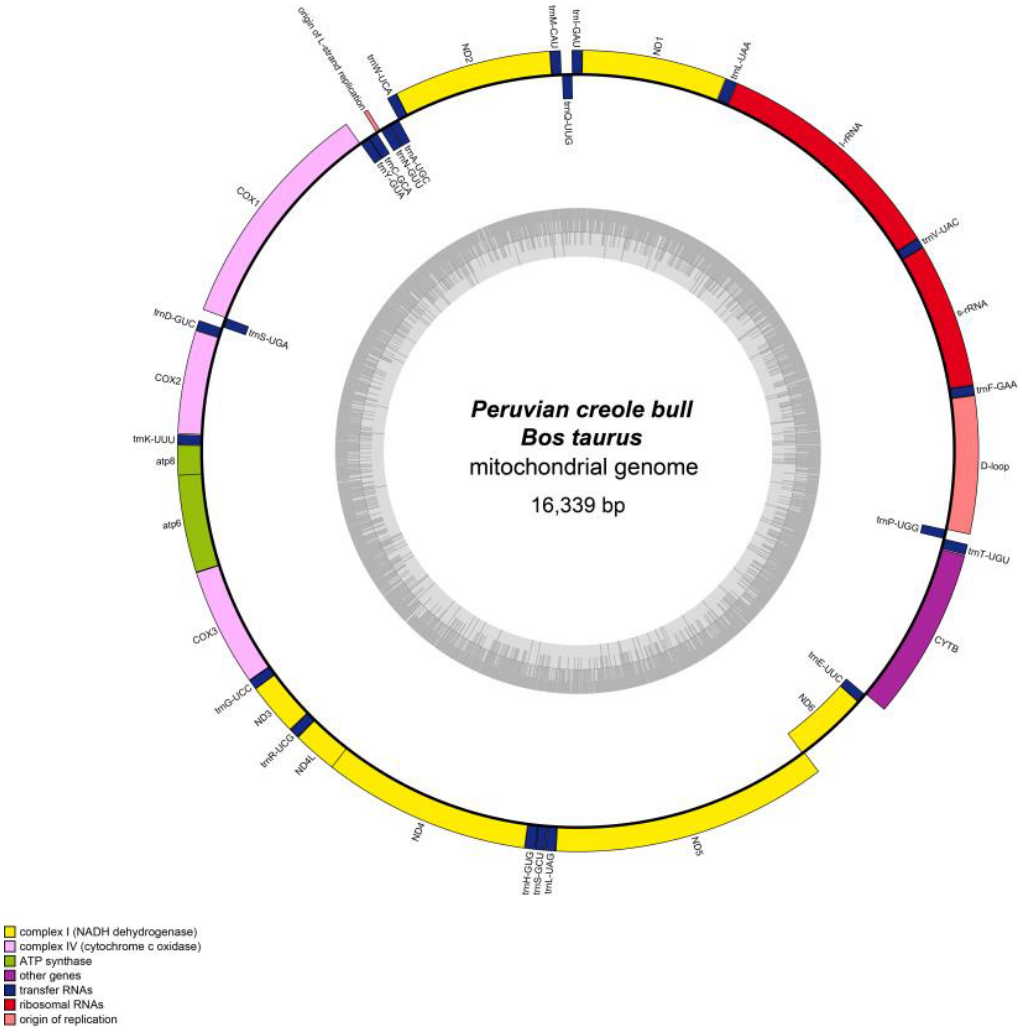

Figure 1. Representative map of the mitochondrial genome of the Peruvian creole cattle. Genes encoded by the heavy strand are shown outside the circle, while those encoded by the light strand are shown inside.

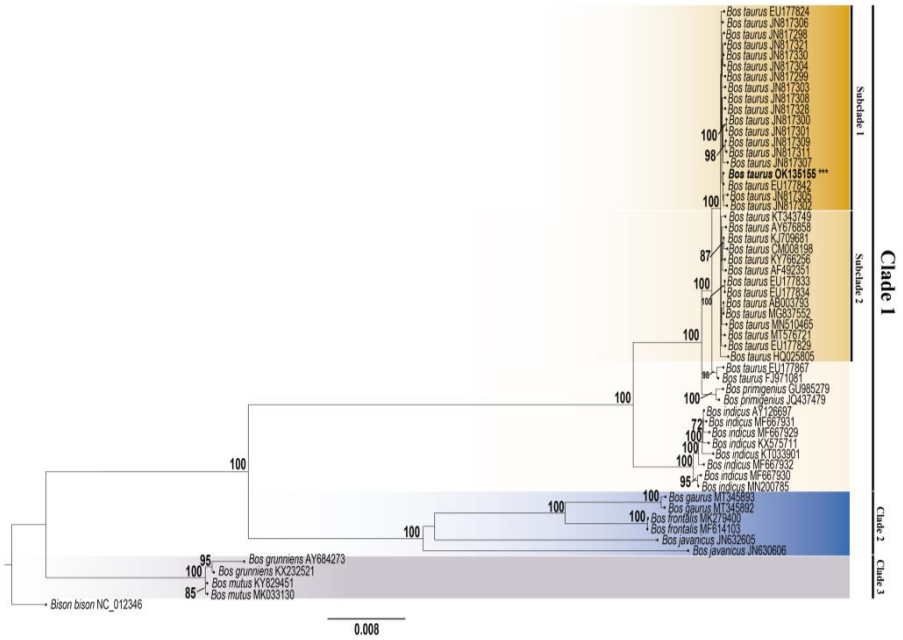

Figure 2. Phylogenetic relationship of *Bos* species inferred from mitochondrial genomic sequences. Maximum likelihood bootstrap support is shown only for branches with <72% support. The *Bison bison* mitochondrial genome sequence was used as an out-group.

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