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Complete Mitogenome, Phylogenetic and SNP-data Analysis of Jakumaru (*Bos taurus*), a Prime Bull from a Peruvian Breeding Core Herd

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ABSTRACT

This study provides a comprehensive mitochondrial genome sequence and a phylogenetic analysis of Jakumaru (*Bos taurus*), a prominent bull from a Peruvian breeding program. The mitochondrial genome, spanning 16,340bp, comprises 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, with a GC content of 40%. The gene arrangement and structural organization are highly conserved, closely resembling those of related Bovine species, indicating a stable genomic architecture essential for mitochondrial function. Phylogenetic analysis, incorporating 35 complete bovine mitochondrial genomes, positions Jakumaru within Subclade I, revealing a close relationship with European Simmental cattle. The study emphasizes the biological diversity and the evolution of the Simmental breed, influenced by natural selection, breeding practices, and genetic drift. These findings enhance our understanding of the breed's genetic variation and its evolution, particularly in the context of its importance in Peru.

Keywords: *Bos taurus,* Simmental breed, mitochondrial genome, phylogenetic analysis, SNP-data, Peruvian Genetic Nucleus

INTRODUCTION

The mitochondrial genome genetic features provide crucial understanding in molecular biology, given its straightforward structure, ease of analysis, and exclusive maternal inheritance, making it particularly useful for investigating genetic evolution and phylogenetic relationships (Di Lorenzo et al. 2016). The Simmental breed (SB) has predominantly been selected in North America for its meat production capabilities, whereas in Europe, the focus has been on enhancing its milk production performance (ASA 2018). While the Austrian Simmental breed initially exhibited significant genetic diversity, recent research suggests a decline in genetic variability, which has adversely affected reproductive performance (Sölkner et al. 1998; Maximini et al. 2011). In Peru, the extensive adoption of artificial insemination for the Simmental breed commenced in 2002, leading to the nationwide distribution of offspring derived from bulls of diverse genetic origins (Montoya Barrera 2018). During 2020, 10 genetic centers were set up across Peru by INIA-MINAGRI, resulting in the production of 710,000 premium semen straws designed to boost milk and meat output in breeds like Simmental (INIA 2020), which is vital in regions such as Amazonas because of its adaptability and significant economic impact (Maicelo and Bardales 2017). The objectives of this study were to assemble and validate the mitochondrial genome, compare it with other bovine reference genomes, perform phylogenomic analysis, and analyze SNP-data for genomic characterization.

MATERIALS AND METHODS

Blood sample from Jakumaru (*Bos taurus*) was collected from the National Institute of Agrarian Innovation’s Central Genetic Hub, situated at the Donoso Agricultural Research Facility in Huaral, Lima. Genomic DNA was isolated with the Wizard Genomic DNA Purification Kit (Fitchburg, WI, USA) and stored at −20°C for subsequent analysis. The complete genome of Jakumaru was sequenced on the Illumina HiSeq 2500 platform, with raw sequencing data processed using

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Illumina Control Software and converted to fastq format via bcl2fastq 2.17. Low-quality reads and adapter sequences were filtered out using TrimGalore v0.6.7 and Trimmomatic v0.36 (Bolger et al. 2014). The mitochondrial genome assembly was performed using the GetOrganelle pipeline (Jin et al. 2020), incorporating tools such as SPAdes v3.11.1 (Bankevich et al. 2012), bowtie2 v2.4.2 (Langmead and Salzberg 2012), and BLAST+ v2.11 (Camacho et al. 2009). Gene annotations were conducted using Geseq in the CHLOROBOX web service (Tillich et al. 2017). To determine Jakumaru phylogenetic placement, a tree was constructed using 34 complete bovine mitochondrial genomes, with *Bison bison* serving as the outgroup. Genome alignment was carried out using MAFFT v7.475 (Katoh and Standley 2013), followed by phylogenetic analysis using the GTR + GAMMA model and 1000 bootstrap replicates with RAxML v8.2.11 (Stamatakis 2014). The resulting phylogenetic trees were visualized using iTOL (Letunic and Bork 2021). The accurate annotated mitogenome sequence of Jakumaru was submitted to GenBank with the accession number PQ152950. In addition, Jakumaru's DNA sample was genotyped using the Illumina GGP Bovine 100K BeadChip, provided through a commercial genotyping service (Neogen, Geneseek, NL, United States), with the 100K chip containing 95,256 SNPs. The sample collection from the cattle specimen was conducted in accordance with the Peruvian National Law No. 30407: “Animal Protection and Welfare”.

RESULTS AND DISCUSSION

The mitogenome is 16,340 bp in length (Fig. 1), comprising 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, with a GC content of 40% (Table 1). The gene order and structural organization highly conserved, closely matching those reported in previously sequenced mitochondrial genomes of related species, which highlights the evolutionary stability within this group (Hiendleder et al. 2008; Wu et al. 2016; Guo et al. 2019). The length of this mitogenome is comparable to those observed in other species within the Bovinae subfamily (Chu et al. 2016; Kamalakkannan et al. 2020). This consistency in mitogenome size across different species within the subfamily suggests a conserved genomic architecture, which is critical for maintaining key mitochondrial functions (Rahman and Rahman 2018).

The phylogenetic analysis of the mitochondrial genome confirmed the similarity among genera within the *Bos* family (Fig. 2), highlighting the close evolutionary relationships between these species (Ren et al. 2018; Arbizu et al. 2022). The phylogenetic tree, constructed from 35 mitogenomes, identified two major clades. The first clade further splits into two subclades: Subclade I, where Jakumaru is found, shows a close relationship with Pumpo (PP780079.1), which belongs to the genetic nucleus of Huaral (Estrada et al. 2024). This subclade includes species from Italy, Mexico, France, Germany, Spain, China, Uruguay, Egypt, and Malta. Subclade II groups species from Italy, Egypt, and Peru. In contrast, Clade II consists exclusively of species from India.

Table 1: Organization of the mitochondrial genome of Jakumaru cattle

Gene	Nucleotide Positions	Size (bp)
tRNA^{Phe}	1 - 67	67
12S rRNA	68 - 1023	956
tRNA^{Val}	1024 - 1090	67
16S rRNA	1091 - 2661	1571
tRNA^{Leu}	2662 - 2736	75
ND1	2739 - 3694	956
tRNA^{Ile}	3695 - 3763	69
tRNA^{Gln}	3761 - 3832	72
tRNA^{Met}	3835 - 3903	71
ND2	3904 - 4945	1042
tRNA^{Trp}	4946 - 5012	67
tRNA^{Ala}	5014 - 5082	69
tRNA^{Asn}	5084 - 5156	73
tRNA^{Cys}	5189 - 5255	67
tRNA^{Tyr}	5256 - 5323	68
COX1	5325 - 6869	1545
tRNA^{Ser}	6867 - 6935	69
tRNA^{Asp}	6943 - 7010	68
COX2	7012 - 7695	684
tRNA^{Lys}	7699 - 7765	67
ATP8	7767 - 7967	201
ATP6	7928 - 8607	670
COX3	8608 - 9391	784
tRNA^{Gly}	9392 - 9460	69
ND3	9461 - 9807	347
tRNA^{Arg}	9808 - 9876	69
ND4L	9877 - 10173	297
ND4	10167 - 11544	1378
tRNA^{His}	11545 - 11614	70
tRNA^{Ser}	11615 - 11674	60
tRNA^{Leu2}	11676 - 11746	71
ND5	11795 - 13567	1773
ND6	13551 - 14078	528
tRNA^{Glu}	14079 - 14147	69
Cytb	14152 - 15291	1140
tRNA^{Thr}	15295 - 15364	70
tRNA^{Pro}	15364 - 15429	66

The Simmental breed, prominently represented in Subclade I, has been extensively studied due to its economic and agricultural significance. Detailed analyses of its genetic variability have revealed intricate phylogenetic patterns, indicative of the complex interplay between natural selection, breeding practices, and genetic drift that have shaped the breed's development over time (Amaya et al. 2020).

SNP-Data analysis

SNP markers genotypes related to reproductive and productive traits were analysed (Table 2).

Table 2: Genotype for principal SNP markers.

SNP marker	Genotype
COQ9_rs109301586	AG
PKP2-988	ID
Lactoferrin (LTF)	AA
Beta-Lactoglobulin	BB
Kappa-Casein	AB
Beta-Casein	A2A2

Reproductive traits

Jakumaru possesses an AG genotype for COQ9-rs109301586 marker, and A allele has been linked to an increased genetic potential for daughter fertility rate and cow conception rate, also it has been associated with

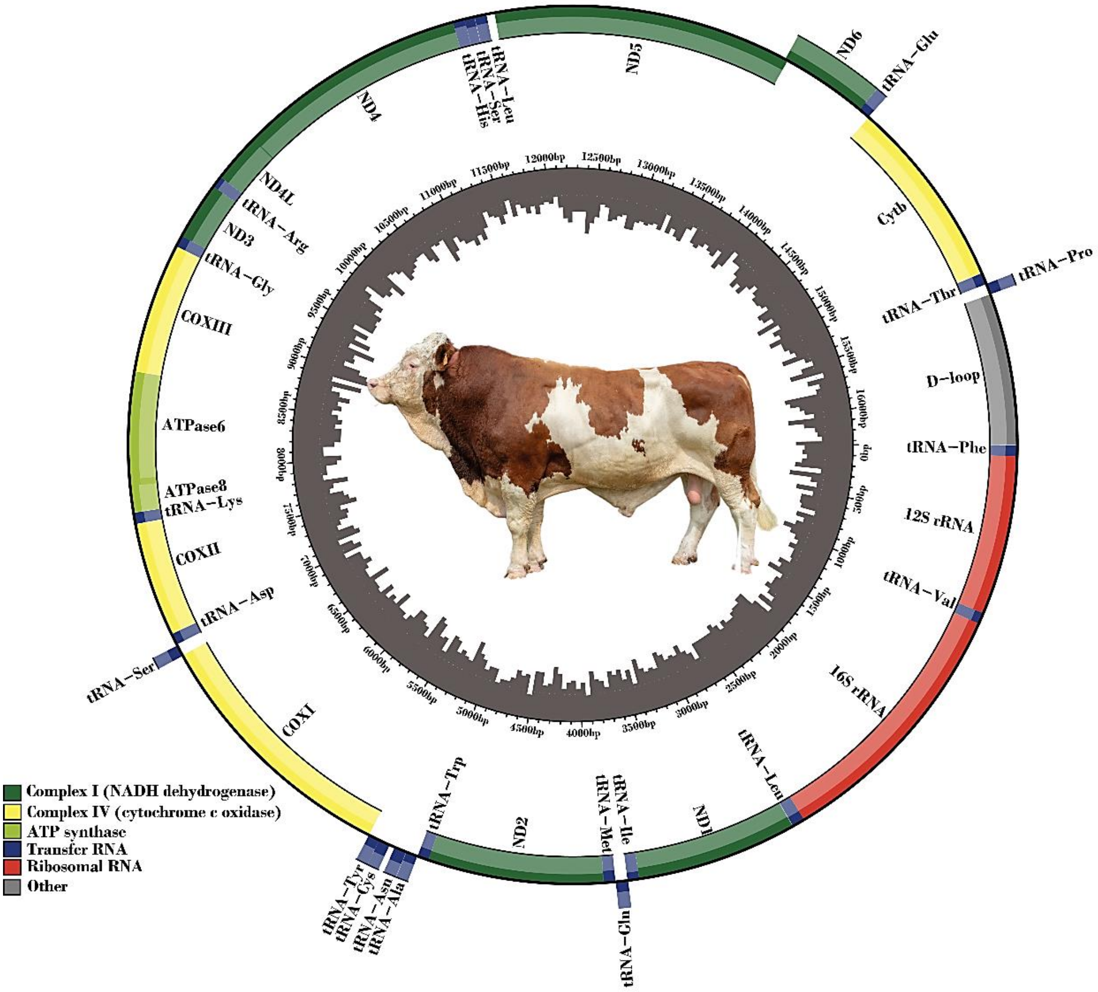

Fig. 1: Mitochondrial genome map of Jakumaru.

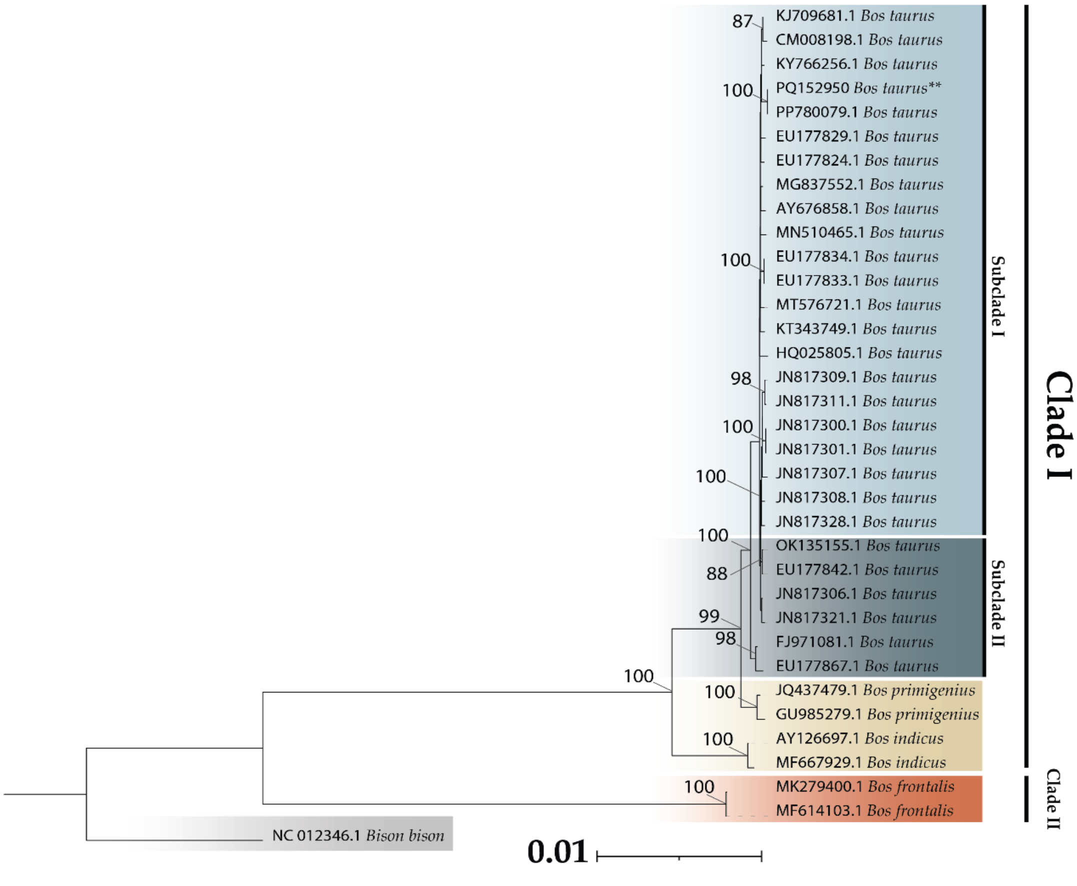

Fig. 2: A phylogenetic tree of complete mitochondrial genomes from *Bos* species, with *Bison bison* as the outgroup, was inferred using the Maximum Likelihood method with 1000 bootstrap replicates.

lower oxygen consumption on mitochondrial respiration and high electron transport capacity (Ortega et al. 2017). For PKP2-988 marker it has an Ins/Del genotype and according to Sugimoto et al. (2013), the Del/Del genotype has been associated with higher conception rate as it enhances gap junction communication and embryo implantation, even though Jakumaru doesn't have that genotype it is heterozygous for the mentioned marked, carrying an Del allele.

Productive traits

Simmental–Fleckvieh breed is dual-purpose cattle; its milk is especially respected for cheese specialties, and for beef purposes, its high final weights are remarkable (Averdunk 2002). Lactoferrin (LTF) has been linked to somatic cell count (SCC) in milk; where the genotype AA has the lowest value while the AG is linked to an elevated SCC (Wojdak-Maksymiec et al. 2006). Jakumaru has an AA genotype for this marker, suggesting its genetics can improve the physiological health, milk quality and safety of his daughters as dairy cows.

Jakumaru has a BB genotype for the Beta-lactoglobulin marker, which has produced a substantial increase in both the actual and dried weight of the cheese yield (Meza-Nieto et al. 2013). For Kappa-casein marker, Jakumaru had AB genotype and Lavon et al. (2023) observed that cows with the AA or AE genotype exhibited lower curd firmness compared to those with the BB, AB, or BE genotype. Several studies have indicated that the β-Casein A2 allele may be more easily tolerated by humans, as it reduces gastrointestinal inflammation in individuals with the A2A2 genotype (Jianqin et al. 2016; He et al. 2017).

Conclusion

The comprehensive analysis of Jakumaru's complete mitochondrial genome (*Bos taurus*) from a Peruvian Genetic Nucleus has provided valuable insights into the genetic architecture and evolutionary relationships within the *Bos* genus. Jakumaru mitogenome, consistent with other Bovine species, highlights the conserved genomic features critical for mitochondrial function. The phylogenetic analysis indicated a strong genetic similarity between Jakumaru and European Simmental cattle, particularly Pumpo from the Huaral genetic nucleus, emphasizing the breed's significant genetic diversity and evolutionary stability. These results enhance our knowledge of the genetic diversity and evolutionary background of the Simmental breed, reflecting the influence of natural selection, breeding practices, and genetic drift over time.

Authors' Contribution: Carlos I. Arbizu, Carlos Quilcate, Jorge L. Maicelo and David Casanova designed the concept of this study and secured funding. Richard Estrada, Yolanda Romero, and Deyanira Figueroa conducted the field and laboratory work. Silvia Leon collected and analyzed the data. Richard Estrada, Yolanda Romero, and Deyanira Figueroa drafted the manuscript. All authors have read and approved the final manuscript.

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