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High-resolution genomic assessment of diversity and conservation risks in Croatian indigenous cattle

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Introduction

Cattle domestication and subsequent breed dispersal are closely linked to human migrations and the spread of agriculture (Zeder, 2011). Over tens of thousands of years, modern cattle diverged into two major lineages, *Bos taurus* and *Bos indicus*, originating from multiple domestication centers, including the Fertile Crescent, the Indus Valley, and possibly North Africa (Decker et al., 2014). Following Neolithic expansion, taurine cattle colonized Europe, Asia, and Africa, shaping the present geographic distribution of cattle breeds (Zeder, 2011). Genomic studies have revealed complex patterns of admixture, including introgression from African taurine and indicine cattle into several southern European breeds (Decker et al., 2014). The Balkan Peninsula represents a historical crossroads of human movements and cultural exchange, repeatedly influenced by Roman, Ottoman, and other empires. These migrations involved the introduction of foreign livestock, leading to admixture with local cattle populations (Decker et al., 2014; Olalde et al., 2023). However, indigenous breeds have undergone local adaptation over a much longer timeframe, whereas modern breeding practices based on intensive selection and repeated crossbreeding are relatively recent and may threaten their genetic integrity, eroding locally adapted variation and increasing conservation risk. (Ouédraogo et al., 2021). High-density single nucleotide polymorphism (SNP) arrays now provide powerful tools for assessing genome-wide diversity, inbreeding, and introgression. Despite their cultural and agricultural importance, Croatian indigenous cattle breeds remain poorly characterized at the genomic level. Buša (CCB, N_c = 5000 (VU)), Istrian cattle (CCIG, N_c = 1500 (EN)), and the Slavonian–Syrmian Podolian (CCSSP, N_c = 400 (EN)) represent valuable genetic resources adapted to local environments and traditional production systems. The objective of this study was to perform a high-resolution genomic assessment of diversity, inbreeding, and introgression in these three Croatian indigenous cattle breeds. Using genome-wide SNP data and comparative datasets from European, African, and indicine breeds, we estimated population genetic parameters and evaluated conservation risks to support evidence-based management and preservation strategies.

Materials & Methods

Dataset. Genotypes for the three Croatian indigenous cattle breeds were obtained from 112 blood samples using the Illumina BovineHD (777K) SNP array. Comparative genotype data for breeds of taurine and indicine background were downloaded from publicly available databases and standardized to ensure compatibility. All datasets were merged based on shared marker positions, resulting in the removal of non-overlapping SNPs. Quality control was performed in PLINK v1.9 (Purcell et al., 2007) by excluding individuals and markers with more than 10% missing data (--mind 0.1; --geno 0.1) and variants with minor allele frequency below 0.10 (--maf 0.1). After filtering, the final dataset included 439,608 SNPs and 674 individuals representing 25 cattle breeds.

Genetic Diversity and Demographic History. Population genetic diversity parameters, including observed (H_o) and expected (H_e) heterozygosity and the F_{IS}, were estimated for each population using PLINK v1.9. Allelic richness was calculated using Allelic Diversity AnalyZEr (ADZE), which employs a rarefaction approach to account for differences in sample size (Szpiech et al., 2008). To further assess inbreeding, coefficients based on runs of homozygosity (F_{ROH}) were characterized using the detectRUNS package (Biscarini et al., 2019) in R. Finally, contemporary and historical effective population sizes (N_e) were estimated using the GONE software (Santiago et al., 2020).

Relatedness, Population Structure, and Admixture. Pairwise genetic differentiation (F_{ST}) among populations was estimated using PLINK v1.9. To minimize bias associated with small sample sizes, F_{ST} was calculated only for populations represented by more than 20 individuals. Population structure was further explored using principal component analysis (PCA) implemented in R to characterize linear patterns of genetic variation among breeds. In addition, nonlinear relationships were investigated using a variational autoencoder (VAE) approach with POPVAE (Battey et al., 2021). Phylogenetic relationships among breeds were inferred using a Neighbor-Net network based on Nei's genetic distances (Nei, 1972). Pairwise distances were calculated in the R package StAMPP (Pembleton et al., 2013), and the resulting network was visualized with SplitsTree5 (Huson and Bryant, 2006). Population structuring was examined using NeuralADMIXTURE (K = 1 – 27). The analysis of gene flow among the 25 analyzed breeds was estimated using TreeMix (Pickrell and Pritchard, 2012).

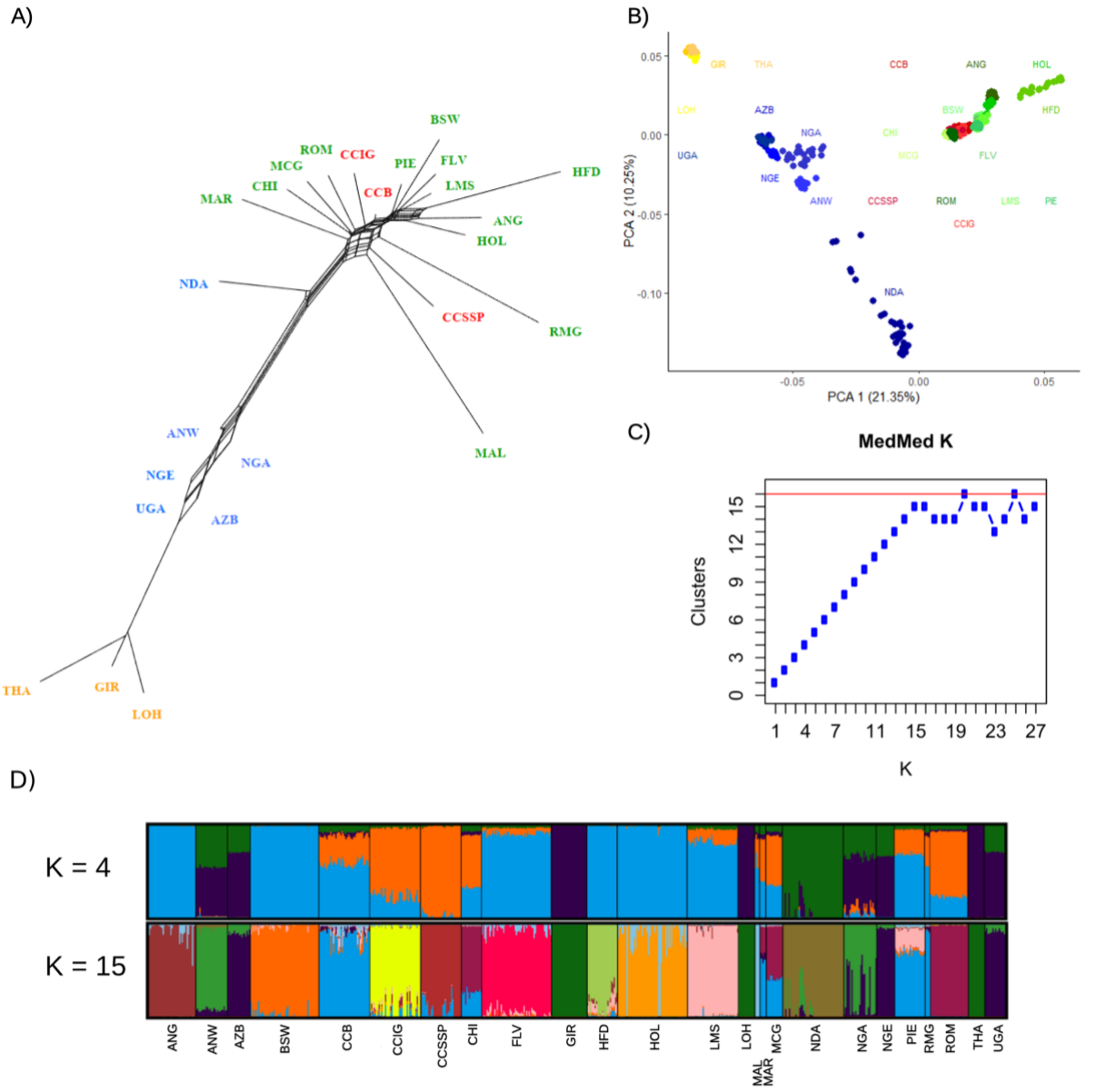
Results & Discussion

Genetic Diversity and Effective Population Size. Observed (H_o) and expected (H_e) heterozygosity ranged from 0.18 (ROM) to 0.33 (CCIG) and from 0.16 (ROM) to 0.32 (CCIG), respectively (see Table 1). Among Croatian breeds, CCB showed the lowest heterozygosity (H_o = 0.28; H_e = 0.27). CCSSP exhibited heterozygosity like RMG, consistent with their shared Podolian subtype (see Table 1). F_{IS} did not differ significantly from zero across all breeds. ROH-based inbreeding coefficients were high in CCIG ($F_{ROH>1Mb}$ = 0.23; $F_{ROH>4Mb}$ = 0.10) and CCSSP ($F_{ROH>1Mb}$ = 0.23; $F_{ROH>4Mb}$ = 0.11), exceeding those observed in Holstein cattle. In contrast, CCB exhibited considerably lower remote inbreeding ($F_{ROH>1Mb}$ = 0.13; $F_{ROH>4Mb}$ = 0.05). (see Table 1). Contemporary N_e estimates revealed small effective population sizes in Croatian breeds (CCB = 138; CCIG = 60; CCSSP = 43), with historical N_e indicating severe long-term declines (see Table 1).

Table 1. Genomic observed (H_o) and expected (H_e) heterozygosity, Wright's inbreeding coefficient (F_{IS}), genomic inbreeding from rule-based consecutive ROH ($F_{ROH>1Mb}$, $F_{ROH>4Mb}$), effective population size (N_e), and historical effective population size 20 generations ago (N_{e20}).

Breed	N	H_o	H_e	F_{IS}	$F_{ROH>1Mb}$	$F_{ROH>4Mb}$	N_e	N_{e20}
ANG	37	0.29±0.19	0.29±0.18	0.005±0.02	0.271±0.015	0.087±0.015	166	152
CCIG	40	0.33±0.17	0.32±0.16	0.015±0.03	0.230±0.021	0.104±0.023	60	5312
CCSSP	32	0.29±0.18	0.29±0.17	-0.043±0.03	0.236±0.025	0.114±0.026	43	205
CCB	40	0.28±0.20	0.27±0.18	-0.005±0.02	0.129±0.015	0.057±0.021	138	18768
HFD	28	0.21±0.19	0.21±0.18	0.053±0.05	0.248±0.012	0.143±0.045	164	90
HOL	24	0.29±0.18	0.31±0.17	-0.019±0.02	0.321±0.040	0.065±0.008	68	2378
RMG	4	0.30±0.19	0.29±0.17	-0.345±0.19	0.156±0.110	0.006±0.005	51	50
ROM	30	0.18±0.23	0.16±0.19	-0.028±0.01	0.205±0.008	0.060±0.009	103	2255

ANG-Angus, CCIG-Istrian Cattle, CCSSP-Slavonian–Syrmian Podolian, CCB-Buša, HFD-Hereford, HOL-Holstein, RMG-Romanian Grey, ROM-Romagnola



ANG-Angus, ANW-Ankole-Watussi, AZB-African Zebu, BSW-Brown Swiss, CCB-Buša, CCIG-Istrian Cattle, CCSSP-Slavonian–Syrmian Podolian, CHI-Chianina, FLV-Flechviech, GIR-Gir, HFD-Hereford, HOL-Holstein, LMS-Limousin, LOH-Lohari, MAL-Maltese, MAR-Maremmana, MCG-Marchigiana, NDA-N'dama, NGA-Nganda, NGE-Sokoto Gualdi, PIE-Piedmontese, RMG-Romanian Grey, ROM-Romagnola, THA-Tharparkar, UGA-Karamojong Zebu

Figure 1. Population structure of 25 analyzed breeds: (A) Neighbor-Net network of all breeds. (B) PCA analysis for breeds with sample size N > 10. (C) Most suitable K determined by StructureSelector MedMed K. (D) Graphical representation of Bayesian unsupervised clustering (K = 4, 15) visualized with the software CLUMPAK.

Genetic Relatedness and Population Structure. Contrary to expectations based on phenotype, CCB and CCIG showed low pairwise differentiation (F_{ST}=0.05), while both CCB versus CCSSP and CCIG versus CCSSP were more strongly differentiated (F_{ST}=0.10). PCA analysis demonstrated close genetic clustering of Croatian breeds with Italian (CHI, MCG, ROM) and European beef breeds (ANG, PIE, LMS, FLV) (see Figure 1B). Neighbor-Net analysis grouped CCSSP with Maltese and Romanian Grey cattle, consistent with their Podolian background, although the absence of Hungarian Grey and Serbian Podolian data limits

phylogenetic resolution (see Figure 1A). POPVAE dimensionality reduction achieved a clear separation of all Croatian breeds. Population-structure analysis was used to characterize genetic partitioning and admixture across breeds. Based on the most suitable K values, K = 4, 15 were selected. At K = 4, the initial genetic differentiation of CCSSP and CCIG becomes apparent, while CCB exhibits noticeable admixture. K = 15 was identified as the optimal clustering level for the dataset, corresponding to the first plateau of the MedMed K curve (see Figure 1C). K = 15 shows that even at high resolution, CCSSP and CCIG maintain their genetic integrity with only minor signs of admixture. In contrast, CCB displays more pronounced population mixing, consistent with historical breeding practices (see Figure 1D). Migration analyses did not detect recent gene flow among the Croatian breeds or between them and neighboring local populations.

Conclusions

Our findings demonstrate that CCB, CCIG, and CCSSP are genetically vulnerable and require urgent, diversity-oriented breeding strategies. CCIG and CCSSP are particularly at risk due to small population sizes (N_e, N_c), which may lead to the accumulation of genetic load. Conversely, while CCB maintains a larger effective population size (N_e), its high admixture suggests a lack of breed homogenization that requires improved management to preserve its genetic identity. Ultimately, this study confirms the distinct genetic signature and rich history of all three breeds, justifying immediate conservation efforts.

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