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REGULAR ARTICLES

Zavot cattle genetic characterization using microsatellites

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Abstract

This study aimed to reveal the genetic diversity and phylogenetic relationship between intra- and inter-breeds of Zavot cattle raised locally in and around Kars province, Türkiye. A total of 209 [Zavot (ZAV) $n=49$, Eastern Anatolian Red (EAR) $n=40$, Simmental (SIM) $n=40$, Brown-Swiss (BS) $n=40$, and Holstein (HOLS) $n=40$] non-related cattle without any clinical health problems were evaluated. Using the standard phenol–chloroform method, deoxyribonucleic acid (DNA) was isolated from blood samples and amplified by multiplex polymerase chain reaction (PCR) using 19 bovine-specific microsatellite markers. A capillary electrophoresis process was applied to the denatured PCR products. A total of 274 different alleles were identified, with an average of 10.29 and an average of effective alleles of 5.38. According to the genetic distance matrix between populations, the largest genetic distance was found between ZAV-HOLS (0.358) populations, while ZAV-EAR populations were located at the same roots. The largest F_{ST} value (0.072) was found among ZAV-HOLS populations. According to the factorial correspondence analysis (FCA) graph, each population was located separately but also showed a mixture, especially the ZAV, EAR, and BS populations. The average polymorphism information content (PIC) values were the lowest (0.44) for the BM2113 marker and the highest (0.92) for the TGLA53 marker. In conclusion, ZAV cattle bred in the Kars region were found to be completely separate from the BS and SIM breeds which were claimed to have contributed to the formation of the ZAV breed. Since currently the native breeds, which are symbolic of the region, inbreeding cannot be prevented, an increase in studies devoted to the protection of these breeds and the establishment of pure herds will be useful for the future of native cattle in Türkiye.

Keywords Genetic diversity · Molecular method · Native breed · Polymorphism · Zavot

Introduction

Restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), and microsatellites are used in DNA polymorphism studies (Fatima 2004; Özşensoy and Kurar 2012). The size of microsatellite PCR products vary according to each locus and are generally between 75 and 300 base pairs in length. These molecular markers are used in various species and are highly preferred since they have codominant inheritance patterns, are specific to the locus, have a uniform and wide distribution in the genome, and can be determined via PCR-based techniques (Kurar 2001; Özşensoy 2011; Özşensoy and Kurar 2012). Microsatellite markers, as suggested by the International Society for Animal Genetics-Food and Agriculture Organization (ISAG-FAO) Advisory Group, should exhibit high polymorphism and heterozygosity, be located on different chromosome regions, and have four or more alleles (Korkmaz Ağaoğlu and Ertuğrul 2010; Özşensoy and Kurar 2012).

The FAO has reported that breeds that are becoming extinct should be preserved and thereby protected for the sake of genetic diversity (FAO 2018). The Turkish National Strategy and Action Plan on Animal Genetic Resources emphasized the protection and improvement of indigenous breeds (Zavot (ZAV), Eastern Anatolian Red (EAR), etc.), as well as ensuring their continuity. The characterization of genetic resources should be performed since some of the indigenous cattle breeds of Türkiye were faced with the threat of extinction.

The present study aimed to use microsatellite markers to genetically characterize ZAV with EAR, Simmental (SIM), and Brown-Swiss (BS) breeds, which purportedly

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contributed to the formation of this breed, and with the Holstein (HOLS) breed, which was bred in the same region and said to be interrelated with the ZAV breed. The data obtained can provide information related to genetic variations within and between the breeds. This is the first genetic characterization study to evaluate ZAV cattle together with SIM, BS, and HOLS breeds using microsatellite markers.

Material and methods

In the study, a total of 209 cattle, consisting of 49 ZAV (15 male and 34 female), 40 EAR (19 male and 21 female), 40 SIM (20 male and 20 female), 40 BS (20 male and 20 female), and 40 HOLS (15 male and 25 female) were evaluated. The cattle were clinically healthy, between 1 and 5 years of age, and unrelated. Sampling was carried out at 156 farms, including 30 for ZAV, 23 for EAR, 39 for SIM, 38 for BS, and 26 for HOLS, with a maximum of 2–3 cattle originating from the same farm.

Blood samples were taken from the *vena jugularis* of each animal using 4-mL K_3-EDTA tubes which were transported to the laboratory under cold conditions and stored at − 20 °C until analyzed.

DNA was extracted from the collected blood samples by the standard phenol–chloroform method used by Özşensoy (2011) (Sambrook et al. 1989).

The DNA samples were uploaded to a 0.6% agarose gel electrophoresis system and measured using a 260/280 nm UV spectrophotometer. Quantity and quality controls were performed for the DNA samples that had been stored at − 20 °C until used.

Microsatellite markers and polymerase chain reaction

The DNA samples were tested using 19 cattle-specific microsatellite markers (Supplementary Table 1) which had high informatics value, represented different chromosomes, were suitable for multiplex studies, and recommended by ISAG and FAO measurement of domestic animal diversity (MoDAD) (Hoffmann et al. 2004). The multiplex PCR method was used according to the study by Özşensoy (2011). For reference studies, three different multiplex PCRs were created, consisting of six, six, and seven microsatellite markers. The tests were carried out using the profile and protocol of the Touchdown PCR method (Don et al. 1991) evaluated and proven suitable by Özşensoy et al. (2010).

Capillary electrophoresis

A mixture was prepared with 20 μL of Hi-Di™ formamide (Applied Biosystems), 0.5 μL of GeneScan™ 600 LIZ® Size Standard, and 1 μL from the group of PCR products that were oxidized using fluorescence-labeled primers. The samples were denatured at 95 °C for 3–5 min and then in ice for 2 min. The prepared mixture was loaded on an ABI 310 Genetic Analysis System for capillary electrophoresis, and the PCR products were separated using the fragment protocol (5-s injection at 15 kW, 30-min run at 15 kW and 60 °C). Genotypes were identified for each microsatellite marker using the GeneMapper 5.0 (Rinehart 2004) fragment analysis program.

Statistical analysis

During statistical analysis of the genotypes, different genetic parameters were calculated using different software packages. Among these genetic parameters, the total number of alleles, allele frequencies, private alleles and frequencies, expected and observed heterozygosity values, assignment values of each individual to the population (assignment test), and the inter-population genetic identification and genetic distance matrix values (Nei 1972) were calculated using GenAlEx6 (Peakall and Smouse 2012). F statistic values of F_{IT}, $F_{ST,}$ and F_{IS}, cross-population F_{ST}, and F_{IS} of the genetic differences between the populations were calculated using the FSTAT (Goudet 1995) software package. In addition, to determine whether the populations were in danger of extinction, the results of the Bottleneck 1.2.02 (Piry et al. 1999) and Factorial Correspondence Analysis (FCA) graphs were calculated using Genetix 4.05 (Belkhir et al. 2004). Results of the analysis of molecular variance (AMOVA) and the Mantel test were calculated using Arlequin 3.1 (Excoffier and Lischer 2010). The polymorphism information content (PIC) values were calculated using Cervus 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007). Results of the structure test were calculated using Structure 2.3.4 (Pritchard et al. 2009) software. Additionally, the radial tree and phylogenetic relationship graphs of the populations were drawn using Population 1.2.32 (Langella 2011) software and visualized using TreeView (Page 1996) software.

Results

Alleles in populations

The quantity and range of alleles are presented in Table 1. The average allele number determined from 274 different

Table 1 Active alleles, total number, ranges, and expected and observed heterozygosity per cattle population and marker

Marker	ZAV				EAR				SIM				HOLS				BS				Total number of alleles	Allelic size range (bp)	Mean		
	Na	Ne	He	Ho	Na	Ne	He	Ho	Na	Ne	He	Ho	Na	Ne	He	Ho	Na	Ne	He	Ho			He	Ho	H_T
CSRM60	11	3.73	0.732	0.653	12	4.73	0.789	0.775	11	4.80	0.792	0.875	7	4.19	0.761	0.600	12	5.35	0.813	0.825	16	75–107	0.777	0.746	0.797
CSSM66	13	6.18	0.838	0.673	14	6.07	0.835	0.700	14	5.10	0.804	0.650	12	5.47	0.817	0.625	13	7.22	0.862	0.700	15	174–202	0.831	0.670	0.849
SPS115	10	3.83	0.739	0.449	10	5.27	0.810	0.475	9	4.28	0.767	0.500	8	3.85	0.740	0.475	9	4.81	0.792	0.550	13	231–259	0.770	0.490	0.791
ILSTS006	9	6.23	0.839	0.531	9	5.83	0.828	0.500	10	6.39	0.843	0.625	9	5.05	0.802	0.650	10	5.95	0.832	0.725	11	280–302	0.829	0.606	0.852
HEL9	12	5.56	0.820	0.735	14	7.71	0.870	0.775	13	5.11	0.804	0.775	11	7.58	0.868	0.925	12	6.41	0.844	0.775	17	138–174	0.841	0.797	0.864
ETH03	8	3.50	0.714	0.755	10	3.01	0.668	0.800	7	2.85	0.649	0.725	5	3.52	0.716	0.800	7	3.61	0.723	0.625	12	100–128	0.694	0.741	0.713
BM2113	11	5.25	0.809	0.571	6	1.30	0.231	0.100	5	1.23	0.188	0.050	3	1.23	0.184	0.000	5	1.17	0.143	0.025	11	116–138	0.311	0.149	0.417
ETH10	3	1.31	0.239	0.143	9	3.97	0.748	0.350	6	2.88	0.653	0.475	8	3.20	0.687	0.325	8	3.44	0.710	0.350	10	203–221	0.607	0.329	0.678
TGLA53	13	9.15	0.891	0.592	20	14.61	0.932	0.625	15	11.03	0.909	0.800	14	8.02	0.875	0.600	17	10.09	0.901	0.750	22	141–193	0.902	0.673	0.925
ETH185	11	4.95	0.798	0.255	14	7.15	0.860	0.184	13	5.57	0.821	0.436	12	5.24	0.809	0.450	12	6.58	0.848	0.500	18	210–246	0.827	0.365	0.843
ETH225	12	5.63	0.822	0.653	12	4.57	0.781	0.650	10	3.57	0.720	0.750	10	4.93	0.797	0.625	11	6.54	0.847	0.875	13	133–157	0.794	0.711	0.820
BM1818	9	4.64	0.785	0.531	9	5.28	0.811	0.625	7	3.51	0.715	0.625	9	4.03	0.752	0.600	12	6.25	0.840	0.700	15	255–277	0.780	0.616	0.799
TGLA227	12	8.07	0.876	0.878	13	9.25	0.892	0.925	11	6.88	0.855	0.875	11	7.57	0.868	0.775	14	8.40	0.881	0.925	17	68–102	0.874	0.876	0.896
INRA005	9	6.13	0.837	0.229	8	6.43	0.844	0.225	8	6.87	0.854	0.350	8	5.49	0.818	0.400	11	6.49	0.846	0.425	12	134–154	0.840	0.326	0.874
HEL13	11	5.88	0.830	0.592	11	5.88	0.830	0.564	9	5.55	0.820	0.550	9	3.88	0.743	0.600	10	5.66	0.823	0.675	13	173–201	0.809	0.596	0.827
TGLA126	7	2.99	0.665	0.449	6	2.76	0.638	0.425	6	3.19	0.687	0.600	5	2.67	0.626	0.500	7	3.16	0.683	0.525	9	111–127	0.660	0.500	0.678
TGLA122	17	8.83	0.887	0.673	17	9.61	0.896	0.800	13	6.50	0.846	0.625	17	8.94	0.888	0.625	18	8.72	0.885	0.750	24	132–180	0.880	0.695	0.900
HAUT27	9	3.98	0.749	0.510	9	4.82	0.793	0.525	11	3.41	0.707	0.675	10	5.01	0.800	0.850	8	5.34	0.813	0.675	14	127–157	0.772	0.647	0.808
BM1824	9	4.99	0.800	0.694	9	4.77	0.790	0.625	8	4.36	0.771	0.650	10	4.13	0.758	0.675	9	4.48	0.777	0.750	12	169–195	0.779	0.679	0.808
Mean	10.32	5.31	0.772	0.556	11.16	5.95	0.781	0.560	9.79	4.90	0.748	0.611	9.37	4.95	0.753	0.584	10.79	5.77	0.782	0.638	-	-	-	-	-

ZAV Zavot, *EAR* Eastern Anatolian Red, *SIM* Simmental, *HOLS* Holstein, *BS* Brown-Swiss, *Na* allele numbers, *Ne* effective allele numbers, *bp* base pairs, *He* expected heterozygosity, *Ho* observed heterozygosity, H_T total heterozygosity

alleles was 10.29. The quantity of alleles was generally between 3 and 20, with the highest in EAR (TGLA53) and the least in ZAV (ETH10) and HOLS (BM2113) populations. The mean quantity of alleles (Na) was the lowest in HOLS (9.37) and the highest in EAR (11.26) populations. The mean quantity of effective alleles (Ne) was the lowest in SIM (4.90) and the highest in EAR (5.95) populations. The allele ranges of the 19 markers were between 68 and 302.

Marker allele frequency

Among the markers used in the study, the most frequent alleles in the five populations were 87 and 97 in the CSRM60 marker; 180 in the CSSM66 marker; 239 and 241 in the SPS115 marker; 150, 158, and 160 in the HEL9 marker; 116 in the ETH03 marker; 115 and 117 in the TGLA126 marker; 138 in the TGLA122 marker; 147 in the HAUT27 marker; and 230 in the ETH185 marker.

Private allele quantities observed in populations and their frequencies

A total of 51 private alleles were identified. The highest quantity (15) of private alleles in the populations was in the EAR breed, and the lowest quantity (6) of private alleles was in the HOLS breed.

Expected and observed heterozygosity in populations

The expected and observed heterozygosity values in the populations are presented in Table 1. When the markers used in the study were examined, the mean total heterozygosity (H_T) value was between 0.417 and 0.925, the mean expected heterozygosity (He) value was between 0.311 and 0.902, and the mean observed heterozygosity (Ho) value was between 0.149 and 0.876. When the populations were evaluated, the mean He values varied between 0.748 and 0.782, and the mean Ho value varied between 0.556 and 0.638. The highest mean He value (0.782) was in the BS population, and the lowest mean He value (0.748) was in the SIM population. The highest mean Ho value (0.638) was in the BS population, and the lowest mean Ho value (0.556) was in the ZAV population.

Phylogenetic trees

Genetic distance matrices between populations are presented in Table 2. The highest genetic distance (0.358) was found between ZAV and HOLS populations, while the lowest genetic distance (0.081) was found between EAR and SIM populations. While the closest genetic structure was determined as 0.922 between EAR and SIM breeds, the most distant genetic structure was determined as 0.699 between ZAV and HOLS breeds. Radial tree and phylogenetic relationship graphics of the populations are presented in Fig. 1 A and B. When the radial trees of the populations were evaluated, the HOLS and BS populations were observed to be completely separated from the others and genetically distant from each other despite sharing the same roots. The SIM population was observed to root from a completely different point from the other populations. On the other hand, ZAV and EAR populations were observed to have rooted from the same point; however, they separated over time.

Individual population assignment tests

Among the 209 individual cattle analyzed in the study, 65 were found to have been assigned to different populations (8 of 49 ZAV, 22 of 40 EAR, 16 of 40 SIM, 6 of 40 HOLS, and 13 of 40 BS).

F statistics

When the F_{ST} values used for the binary comparison of the populations (Table 2) were examined, all populations were different from each other ($P < 0.05$). The highest F_{ST} value (0.072) was observed among the ZAV-HOLS populations, and the lowest F_{ST} value (0.009) was observed among the EAR-SIM populations. The F_{IT}, F_{ST}, and F_{IS}

Table 2 Inter-population F_{ST} values and genetic distance matrix

Populations	ZAV	EAR	SIM	HOLS	BS
ZAV	–	0.032**	0.037**	0.072**	0.039**
EAR	0.183	–	0.009*	0.029**	0.010**
SIM	0.185	0.081	–	0.039**	0.014**
HOLS	0.358	0.164	0.188	–	0.030**
BS	0.216	0.097	0.098	0.167	–

*$P < 0.05$; **$P < 0.01$, F_{ST} values (above diagonal) and Nei's genetic distance (below diagonal). *ZAV* Zavot, *EAR* Eastern Anatolian Red, *SIM* Simmental, *HOLS* Holstein, *BS* Brown-Swiss

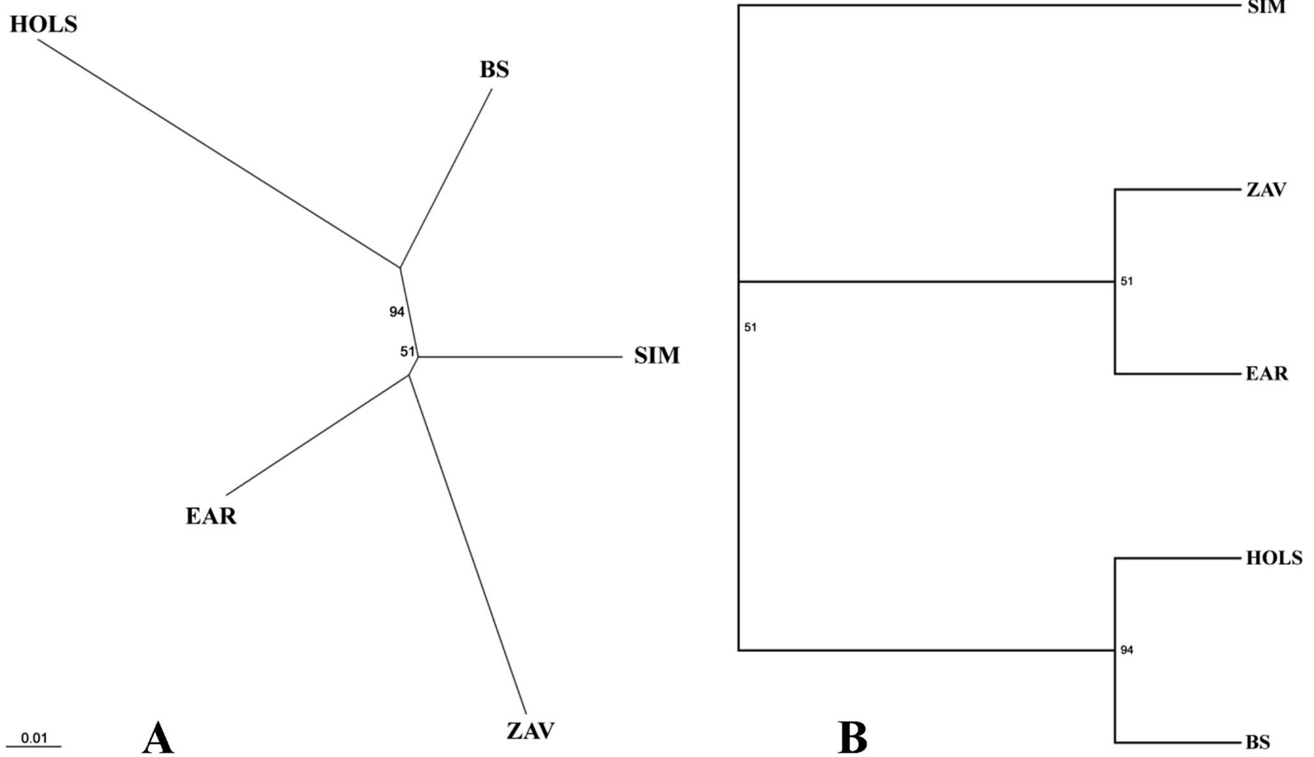

Fig. 1 **A** Radial tree drawn using genetic distance for populations; **B** phylogenetic relationship plotted using genetic distance for populations. BS and HOLS with a frequency of 94% and ZAV and EAR with a frequency of 51% were clustered together. SIM was completely separate from other breeds. ZAV, Zavot; EAR, Eastern Anatolian Red; SIM, Simmental; HOLS, Holstein; BS, Brown-Swiss

Table 3 Average F_{IT}, F_{ST}, and F_{IS} values of all markers

Marker	F_{IT}	F_{ST}	F_{IS}
CSRM60	0.073	0.018	0.056
CSSM66	0.216	0.013	0.206
SPS115	0.386	0.017	0.376
ILSTS006	0.297	0.017	0.286
HEL9	0.087	0.021	0.068
ETH03	-0.032	0.021	-0.054
BM2113	0.592	0.468	0.395
ETH10	0.535	0.124	0.469
TGLA53	0.281	0.017	0.269
ETH185	0.572	0.005	0.570
ETH225	0.146	0.027	0.123
BM1818	0.238	0.013	0.229
TGLA227	0.029	0.019	0.011
INRA005	0.636	0.030	0.625
HEL13	0.283	0.011	0.276
TGLA126	0.270	0.017	0.258
TGLA122	0.235	0.014	0.224
HAUT27	0.213	0.040	0.182
BM1824	0.166	0.029	0.141
Mean	0.275	0.048	0.248

ZAV Zavot, *EAR* Eastern Anatolian Red, *SIM* Simmental, *HOLS* Holstein, *BS* Brown-Swiss

values calculated for all markers are presented in Table 3. The mean F_{IT}, F_{ST}, and F_{IS} values of all markers were 0.275, 0.048, and 0.248, respectively. The F_{IS} values of the populations are presented in Supplementary Table 2. A negative F_{IS} value was determined for one marker in the ZAV population (ETH03), two markers in the EAR population (ETH03, TGLA227), four markers in the SIM population (CSRM60, ETH03, ETH225, TGLA227), three markers in the HOLS population (HEL9, ETH03, HAUT27), and three markers in the BS population (CSRM60, ETH225, TGLA227). The mean F_{IS} values of the populations were observed to range between 0.210 (SIM) and 0.301 (EAR). When analyzed using the Jackknife technique, the mean F_{IS} values were 0.244 ± 0.042, as 0.167 at a 95% confidence interval, and 0.145 at a 99% confidence interval. The overall F_{IS} value was 0.253. The F_{IS} values in all populations were found to be statistically significant ($P < 0.001$, Supplementary Table 2).

The bottleneck test

According to the Wilcoxon results, the population values (ZAV 0.445, EAR 0.414, SIM 0.767, HOLS 0.325, BS 0.067) were $P > 0.05$, with a normal L distribution; therefore, it was determined that the populations were not in danger of extinction (Fig. 2). According to the two-phase model

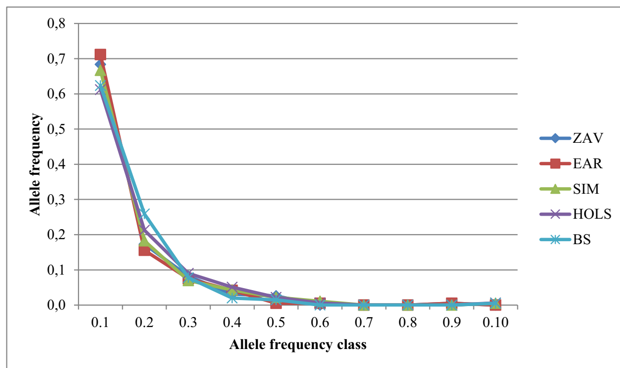

Fig. 2 Allele frequency distribution of all populations. ZAV, Zavot; EAR, Eastern Anatolian Red; SIM, Simmental; HOLS, Holstein; BS, Brown-Swiss

results for the bottleneck test of the populations, the Heq values varied between 0.390 and 0.938 in the ZAV population, between 0.662 and 0.920 in the EAR population, between 0.604 and 0.880 in the SIM population, between 0.404 and 0.900 in the HOLS population, and between 0.609 and 0.908 in the BS population. While no significance was determined in ZAV and HOLS populations in terms of all markers, four markers in the EAR population (ETH03, BM2113, TGLA53, INRA005), four markers in the SIM population (BM2113, TGLA53, INRA005, HAUT27), and one marker in the BS population (BM2113) were found to be significant.

Factorial correspondence analysis (FCA)

For FCA, the breeds were placed on a three-dimensional plane. The genetic relationship between the populations and between individuals belonging to the populations is presented in Fig. 3 A–B. When the FCA graph was examined, the EAR, SIM, and BS populations were located together on a single plane. The EAR and SIM populations were located closer, while the HOLS and ZAV populations were on a different plane compared to the other populations (Fig. 3B).

Analysis of molecular variance

When all populations were evaluated as a single group, 96.45% of the total genetic variation was within the populations, and 3.55% was among the populations ($P<0.001$, Table 4). Three groups (group 1, ZAV and DAK; group 2, SIM; group 3, HOLS and ESM) were formed according to FCA and neighbor-joining method results. Accordingly, the total genetic variation was 96.42% among the populations, 3.42% among the populations in the groups, and 0.16% between the groups (Table 4, $P<0.001$).

Mantel test analysis

The Mantel test was conducted in a single group of populations. The results obtained showed a strong positive correlation and were statistically significant ($r=0.999909$, $P=0.004$).

Polymorphism information content (PIC)

The overall mean PIC value of the populations was 0.74. Moreover, when the populations were examined, the mean PIC values were between 0.72 (SIM) and 0.76 (EAR and BS). When the markers were examined, the mean PIC values were the lowest (0.44) for the BM2113 marker and the highest (0.92) for the TGLA53 marker. The mean PIC values were greater than 0.50 in all markers except for the BM2113 marker; therefore, it was observed that they were highly informative.

Genetic structure test

As a result of the experiments performed at different K values between one and seven, it was determined that the best K value that differentiated the populations was three.

Discussion

This is the first genetic characterization study in which SIM, BS, and HOLS cattle of ZAV were evaluated together using microsatellites in Türkiye. ZAV and EAR cattle are raised locally in the Kars and Ardahan provinces of Türkiye and have adapted to the region, but their number has decreased significantly. EAR, SIM, and BS cattle contribute to the formation of the ZAV breed (Aksoy et al. 2006; Yilmaz et al. 2012). The information in the literature (Decker et al. 2014) that there is a genetic relationship between the ZAV and HOLS breed was investigated for the first time in Türkiye.

In Türkiye, 39 (14.66%) private alleles were determined in the local cattle breeds. The highest quantity of private alleles was found in the TGLA122 and TGLA53 markers (Özşensoy 2011). Similarly, in our study, the marker with the highest quantity of private alleles was TGLA53. The TGLA122 marker had been identified with the highest quantity of 31 (Ngono Ema et al. 2014) or 34 private alleles (Amigues et al. 2011; Gororo et al. 2018). In our study, the TGLA122 marker had the third highest quantity of alleles, consistent with other studies. Previous studies have also reported the lowest number of alleles at the TGLA126 marker (Amigues et al. 2011; Filho et al. 2014; Gamarra et al. 2017; Radhika et al. 2018) and the highest number of alleles at the TGLA122 marker (Ilie et al. 2015; Keros et al. 2015; Vargas et al. 2016; Radhika et al.

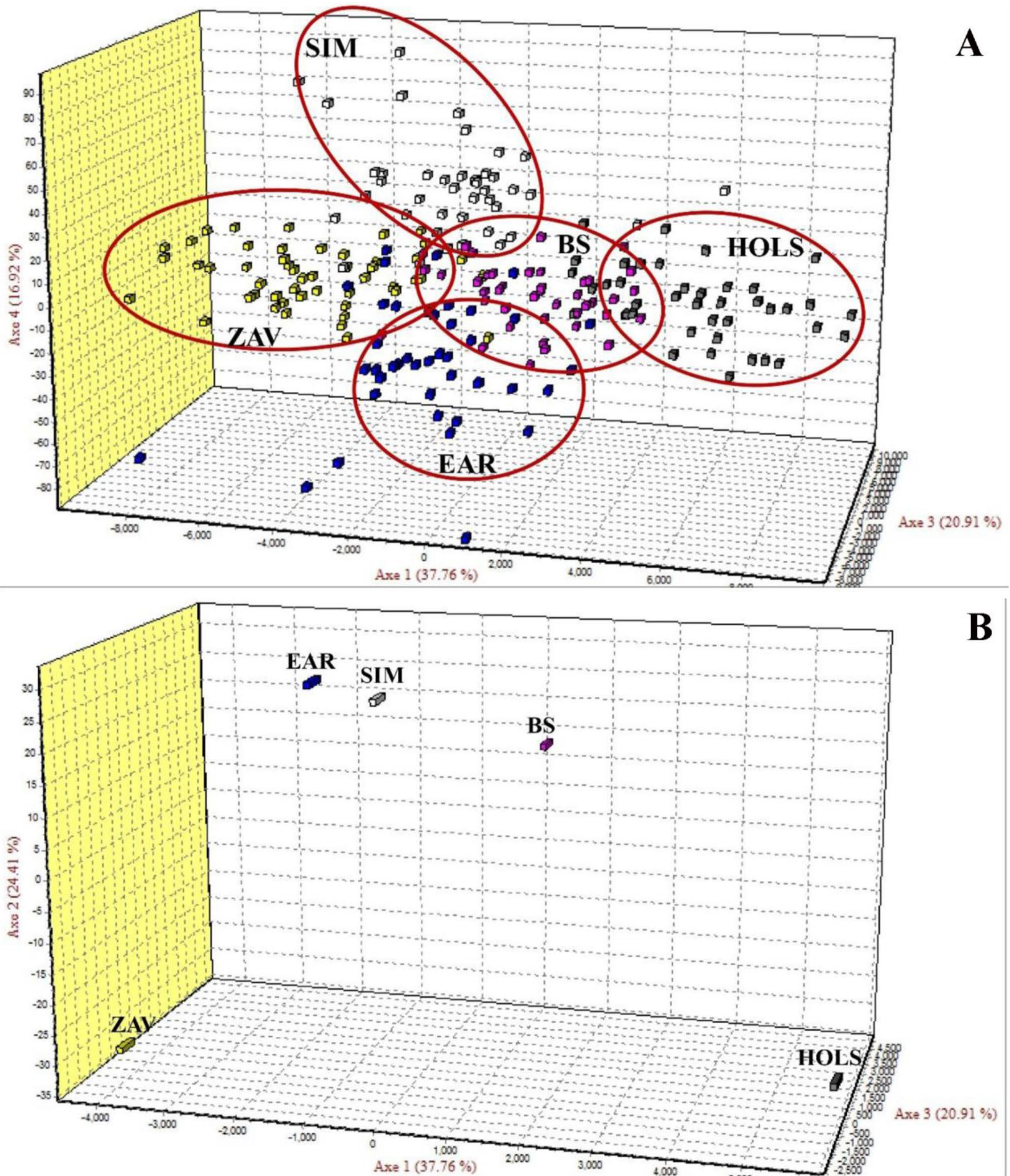

Fig. 3 **A** Factorial correspondence analysis between individuals belonging to breeds. **B** Factorial correspondence analysis between breeds. The percent value in each axis indicates the contribution to the total genetic variation. ZAV, Zavot; EAR, Eastern Anatolian Red; SIM, Simmental; HOLS, Holstein; BS, Brown-Swiss

Table 4 Analysis of molecular variance in populations evaluated as a single group and three groups

Group	Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	P
Single group	Inter-population	4	119.640	0.27034 Va	3.55	0.000
	Intra-population	413	3036.281	7.35177 Vb	96.45	0.000
	General	417	3155.921	7.62211	-	
Three groups*	Between groups	2	61.090	0.01192 Va	0.16	0.447
	Between populations in groups	2	58.550	0.26085 Vb	3.42	0.000
	Within populations	413	3036.281	7.35177 Vc	96.42	0.000
	General	417	3155.921	7.62454	-	

*Group 1, ZAV and DAK; group 2, SIM; group 3, HOLS and ESM

2018; Swathi et al. 2018). In this study, when the number of alleles in the markers was compared, the lowest number (9) of alleles was in the TGLA126 marker and the highest number (24) in the TGLA122 marker.

Studies in some cattle breeds have identified alleles ranging from 71 to 259 using 11–22 microsatellite markers (Amigues et al. 2011; Delgado et al. 2012; Gororo et al. 2018; Kramarenko et al. 2018). In our study, 274 different alleles were detected using 19 microsatellite markers. The fact that the number of alleles we obtained was higher than that in the literature may be due to the number of animals and microsatellite markers.

In the SIM cross-breed cattle in West Sumatra, a total of 317 alleles were determined with 12 microsatellite markers (Agung et al. 2016). In Indian cattle, a total of 359 alleles have been identified with 21 microsatellite markers (Sharma et al. 2015). In Ethiopian cattle, a total of 292 alleles have been identified with 30 microsatellite markers (Dadi et al. 2008). Factors such as the number of animals and differences in the number of breeds in the region may affect the number of alleles obtained in studies.

In a study performed in Türkiye on native cattle breeds, the He value was between 0.669 and 0.877, and the Ho value was between 0.619 and 0.852 (Özşensoy et al. 2014). While the lowest He value was determined in the ZAV breed, the highest He value was determined in the Southern Anatolian Yellow (SAY) breed. The lowest Ho value was determined in the Anatolian Grey (AG) breed, and the highest Ho value was determined in the SAY breed (Özşensoy 2011). In our study, the He value was determined to be between 0.748 and 0.782 and the Ho value between 0.556 and 0.638. In addition, the mean He value was determined to be between 0.311 and 0.902, and the mean Ho value was determined to be between 0.149 and 0.876. The lowest He value was in the SIM breed, and the highest He value was in the BS breed, while the lowest Ho value was in the ZAV and the highest Ho value was in the BS breed. Differences in the results from the literature may be due to breed diversity. The low Ho value in ZAV may be due to the breeding of ZAV only in the Kars region, Türkiye.

In this study, the overall mean F_{IS} value was determined to range between 0.210 and 0.301 among the populations. Particularly, the F_{IS} values were found to be at the highest level in ZAV (0.292) and EAR (0.301) populations. Positive F_{IS} values in our study indicate a heterozygous deficiency in populations that may have resulted from inbreeding and small herd size. In our study, the F_{ST} value ranged from 0.011 (HEL13) to 0.468 (BM2113). The mean F_{ST} value was 0.048. The highest F_{ST} value (0.072) was observed between the ZAV-HOLS populations ($P<0.01$), and the lowest F_{ST} value (0.009) was observed between the EAR-SIM populations ($P<0.05$). There was a low level of genetic similarity between ZAV and HOLS in terms of F_{ST} value; however, genetic similarity between the EAR and SIM populations was high. According to pairwise comparisons between populations, differences in F_{ST} values were found ($P<0.05$). Based on the finding of the small degree of differentiation, Türkiye's indigenous gene resources should be protected.

According to the radial tree findings drawn using the genetic distance, the genetically distant position of the EAR population from the HOLS and BS populations was consistent with the findings obtained by Özkan (2005). Similarly, Altınalan (2005) found that the EAR population was genetically distant from the HOLS population. Özşensoy (2011) stated that according to the clustering of the populations, the EAR was in a distant position compared to the ZAV and other populations. Unlike Özşensoy (2011), we found in this study that the ZAV and EAR populations originated from the same location compared to other populations; however, they were genetically distant. This result may be due to the limited breeding of EAR and ZAV in similar regions, particularly in recent years.

Decker et al. (2014) argued that the ZAV had a different history with a large ancestral structure similar to the HOLS. They also reported that the EAR clustered in a separate position compared to the other breeds. In Türkiye, indigenous breeds are close to each other, and ZAV, EAR,

SIM, and BS share the same roots. Interestingly, Decker et al. (2014) reported that ZAV has a genetic structure very distant from EAR, SIM, and BS. In our study, the furthest genetic structure and the highest genetic distance were found in ZAV and HOLS. The findings were rather inconsistent with the findings of the study conducted by Decker et al. (2014). In the relevant literature, the samplings of ZAV and EAR were very low ($n=5$ and $n=8$, respectively), which may have led to the differences in results compared to our findings. In our study, ZAV and HOLS population samples were taken from farms located in the same region; however, the ZAV and HOLS populations were determined to have completely different origins, contrary to the findings of the study by Decker et al. (2014).

In a study of Türkiye's native cattle breeds, 213 (87%) of 245 cattle were assigned to their own population and 32 (13%) to different populations. Assignment to a different population in the ZAV was not determined, while assignment to a different population was determined in the EAR with a smaller ratio compared to the other breeds (Özşensoy 2011). In our study, the percentage of assignment to different populations was low in the ZAV, while the EAR was the breed with the highest percentage of assignment to different populations. According to the data obtained, it can be argued that ZAV, which is one of the native breeds, has a more specific genotype compared to the others. The EAR is a low yielding native breed, and breeders want to increase their yield by crossbreeding bulls of high yielding breeds (BS and SIM), but they cannot prevent inbreeding due to the low number of breeding bulls. As a result, pure breeding of EAR has declined in recent years. In this study, EAR may have been assigned to different populations at the highest rate compared to other breeds because of these factors.

A study found that Anatolian Black (AB), Anatolian Grey (AG), South Anatolian Red (SAR), and SAY populations having a $P > 0.05$ and normal L distribution were not in danger of extinction; however, despite having a $P < 0.05$, ZAV and EAR populations were also not in danger of extinction due to normal L distribution (Özşensoy 2011). In this study, ZAV and other breeds had normal L distribution and a $P > 0.05$ and were not in danger of extinction. The number of cattle evaluated may have affected the difference in P values in the ZAV and EAR populations in our study and Özşensoy's (2011).

In a study conducted on Türkiye's native cattle breeds, ZAV and EAR clustered in separate locations from other breeds (AG, SAY, AB, and SAR) and from each other in FCA (Özşensoy 2011). EAR was not completely far from other native breeds (AB, AG, and SAR) (Özkan 2005). In our study, ZAV and EAR were mostly clustered separately. In another study, EAR clustered distantly from the HOLS population (Altınalan 2005). Similarly, in the present study, the HOLS population clustered distantly from ZAV and EAR.

A study conducted in different regions of Italy with 13 different breeds using 30 microsatellites found that the highest PIC value used for measuring polymorphism in the populations was in the Podolian population. The PIC value obtained in the Podolian breed was followed by BS, HOLS, and SIM populations (D'Andrea et al. 2011). In our study, the PIC value (0.76) was found to be the highest in EAR and BS. Similar to the literature (D'Andrea et al. 2011), the lowest PIC value was determined in the SIM population.

As a result, the highest genetic distance was between ZAV and HOLS populations. The ZAV, EAR, and SIM populations clustered distantly from the HOLS population. The ZAV and EAR populations originated from the same point and diverged over time. The ZAV cattle raised in the Kars region are separated from the BS and SIM, which contributed to the formation of the ZAV. The populations analyzed have not been in danger of extinction in the recent past, but the numbers of ZAV and EAR are decreasing due to SIM and BS hybrids. Thus, it is believed that the ZAV and EAR in the region need to be protected, and pure herds should be formed.

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Author contribution This work was carried out in collaboration between all authors. BBK, YÖ, and TK: designed the experimental procedures. BBK and YÖ: laboratory analyses and contributed to the statistical and genetic analyses of data and writing of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The present study was conducted with approval from Kafkas University Ethical Committee for Animal Experiments, Kars, Türkiye (KAU-HAYDEK/2015–036).

Statement of animal rights All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of interest The authors declare no competing interests.

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