GENETIC DIVERSITY AND STRUCTURE OF AUTOCHTHONOUS CATTLE BREEDS FROM BOSNIA AND HERZEGOVINA BASED ON MICROSATELLITES

Biljana ROGIĆ[1], Božo VAŽIĆ[1], Mihajla ĐAN[2], Marina STAMENKOVIĆ RADAK[3]

[1]University of Banja Luka, Faculty of Agriculture, Banja Luka, Republic of Srpska, B&H
[2]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia
[3]University of Belgrade, Faculty of Biology, Belgrade, Serbia

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Genotype data from 21 microsatellites were used to evaluate genetic diversity and relationships of Gatacko cattle and Busha breeds. A total of 100 animals included in the study were divided into three groups: Gatacko cattle (GC), Busha from eastern Herzegovina (BEH) and Busha from western Herzegovina (BWH). Hardy-Weinberg equilibrium was observed for all loci in all groups, except for single locus (INRA35) in group of Busha from western Herzegovina. A total number of 166 alleles were detected in a total sample. The average number of alleles per locus was 7.9. The highest observed mean heterozygosity (0.688) was found in the Gatacko cattle and the lowest (0.634) in Busha from western Herzegovina. A pairwise population matrix of F_{st} values among analyzed groups showed significantly high genetic differentiation among all three groups: Gatacko cattle and Busha from western Herzegovina, Gatacko cattle and Busha from eastern Herzegovina and Busha from western and Busha from eastern Herzegovina. This report represents the first genetic characterization of Gatacko cattle and Busha cattle, traditional cattle breed from Bosnia and Herzegovina. The obtained results are valuable for the future development of conservation and management strategies for these cattle breeds.
Keywords: genetic diversity and structure, microsatellites, Gatacko cattle, Busha

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Corresponding authors: Biljana Rogić, University of Banja Luka, Faculty of Agriculture, Bulevar vojvode Petra Bojivića 1a, 78000 Banja Luka, Republic of Srpska, B&H
biljana.rogic@agro.unibl.org

INTRODUCTION

The number of extinct cattle breeds throughout the world is 184, endangered 67 and endangered-maintained 54 (FAO 2015). Most of endangered breeds are specialized in a particular habit or production system and represent, in both developed and developing countries, a unique genetic resource to meet present and future breeding objectives (MEDUGORAC *et al*., 2009). Characterization of genetic resources of domestic animals includes all activities related to the identification of qualitative and quantitative traits, collecting information on races and populations, their habitats and production systems. Based on the genetic characterization of the autochthonous breeds (populations) can be defined and planned appropriate sustainable and conservation programs. Busha and Gatacko cattle are autochthonous cattle breeds from Bosnia and Herzegovina. Busha is a small size cattle with poor production characteristics, but with excellent adaptation to harsh climatic conditions and their resistance to diseases. Busha is an autochthonous cattle breed of the Balkans, which survives in small, highly endangered populations. Today, Busha from Bosnia and Herzegovina inhabits on two areas, foremost is the western Herzegovina where it lives in the Centre for the protection and preservation of the domestic breed, in the area of Široki Brijeg. Second, is located within the various individual households in the area of Berkovići (eastern Herzegovina) which is characterized by better conditions for breeding. Gatacko cattle is the mongrel of the Tyrolean Grey cattle and the autochthonous Busha. Historically, important role in cattle production in this area is attributed to the Austro-Hungarian empire. Upon invitation of the Bosnian government, a stock expert Leopold Adametz suggested a planned crossing of Busha with the purebred foreign breeds (Pinzauger, Montafon and Tyrolean Grey) in order to get a crossbred that would have better exterior and producing characteristics (ILANČIĆ, 1952). As the result of the planned breeding process in Herzegovina originated cattle named Gatacko cattle, which gave the best results in productivity and resilience. Gatacko cattle is widespread in eastern Herzegovina and it is the most frequently breed in the total cattle production of eastern Herzegovina. Today, Gatacko cattle have medium production characteristics with average milk production about 3700-3800 kg per lactation (ROGIĆ, 2012). The average values of the external measures among named breeds (withers height, body length, chest circumference and cannon circumference) is the highest in the Gatacko cattle, followed by the Busha from eastern Herzegovina and the lowest in the Busha from western Herzegovina. A pair-wise comparisons of the average values for all of the three groups showed that there is a significant difference in the external measurements (ROGIĆ *et al*., 2011a).

It is expected that the studies of genetic diversity and structure of the contemporary cattle populations using molecular markers provide implications for revealing breeding practices implemented in the past, and for the conservation and utilization of genetic resources planned in the future (LI *et al*. 2007). Microsatellites have been widely used to analyze the genetic diversity and population structure of many traditional cattle breeds, including European unselected breeds (MEDUGORAC *et al*., 2009), Croatian (KEROS *et al*., 2013), Slovenian (SIMČIĆ *et al*., 2008), Spanish (MARTIN-BURRIEL *et al*., 2007), Indonesian (SUTARNO *et al*., 2015), Latin-American (DELGADO *et al*., 2011), Indian (DEPIKA and SALAR, 2014) and Zebu cattle (HUSSEIN *et al*., 2015; CHAUDHARI *et al*.,2009). This approach has also been successfully used to analyze the genetic

diversity in other traditional livestock species, such sheep (ĆURKOVIĆ *et al.*, 2016), dogs (DIMITRIJEVIĆ *et al.*, 2013), horses (ACHMANN *et al.*, 2004). Some genetically characterization of Busha from Bosnia and Herzegovina have already reported (ROGIĆ *et al.*, 2011b). There is no data for Gatacko cattle. This work is actually a continuation of research in order to genetically characterize autochthonous breeds from Bosnia and Herzegovina, with modern software packages to determine genetic diversity and structure. The aim of this study was to analyze: (I) genetic variability and population structure, and (II) genetic differentiation and relationships between Gatacko cattle and Busha cattle from Bosnia and Herzegovina, using microsatellites.

MATERIAL AND METHODS

Blood samples were collected from 100 animals by jugular vein puncture, using Vacutainer blood collection tubes treated with 0.25% ethylene diamine tetra acetic (EDTA) as an anticoagulant. All animals were divided into three groups. The first group included 50 individuals of Gatacko cattle (GC) cows from the Agriculture complex in Gacko town area, the second group included 26 Busha cattle from the area of the eastern Herzegovina (BEH) located within the various individual households in the area of Berkovići, and the third group included 24 Busha cattle from western Herzegovina (BWH) living in the Centre for the protection and preservation of the domestic breed, in the area of Široki Brijeg. Genomic DNA from total blood was extracted using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions and kept frozen at -20°C until further processing.

Twenty-one microsatellites, recommended by the International Society for Animal Genetics (ISAG), were used for evaluation of genetic diversity. Amplified PCR products were analyzed with ABO 3100 sequencer (Applied Biosystems, Foster City, CA). The number of different alleles and their frequencies, observed and expected heterozygosity values, deviations from Hardy Weinberg equilibrium (HWE), deviations from linkage equilibrium (LE), genotype assignment test, analysis of molecular variance (AMOVA) and pairwise genetic distances among the groups (F_{ST}) were calculated using ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010). The significance level for multiple comparisons was modified using Bonferroni correction (Rice, 1989). The polymorphism information content (PIC) were calculated using GENPOPO 3.4 (RAYMOND and ROUSSET, 1995).

RESULTS

In the present study genetic diversity of autochthonous cattle breeds of Bosnia and Herzegovina and their pairwise relationships were assessed using microsatellite markers. The number of alleles per locus ranged from 2 (ILSTS005) to 12 (TGLA053), with an average value for a total sample of 7.9 (Table 1). All markers were found to be polymorphic in all three analyzed groups. Average values for all three populations indicated that locus TGLA053 was found to be the most informative marker for this population, because it showed the highest PIC value (0.857) an also the highest average number of alleles (9.33). The lowest PIC value (0.371) was detected at the ILSTS005 loci which is in accordance with the low average number of alleles (2.67).

Populations of Busha cattle showed the lower values of parameters number of alleles and heterozygosity than Gatacko cattle (Table 2). Gatacko cattle showed the highest number of average alleles (6.43), followed by BWH (5.47) and BEH with the lowest number (5.43).

Observed heterozygosity ranged from 0.634 (BWH) to 0.688 (GC) and confirmed the remarkable level of diversity in the studied populations (Table 1). In populations of Gatacko cattle expected heterozygosity ranged from 0.368 (SPS115) to 0.844 (TGLA053) with an average value of 0.659. Busha from eastern Herzegovina had the highest expected heterozygosity, ranged from 0.466 (ETH3) to 0.816 (BM2113), with an average value 0.689. Busha from western Herzegovina had the lowest expected heterozygosity, ranged from 0.274 (ILSTS006) to 0.838 (BM2113) with an average value 0.63.

Table 1. Information about the 21 analyzed microsatellite loci: microsatellite names, chromosome number, PIC value, size range (bp) and total number of alleles referred to the sample analyzed in the present study

Microsatellite	Chromosome number	Size range (bp)	PIC	Total number of alleles
BM1818	16	256-270	0.695	8
BM1824	21	179-197	0.698	8
BM2113	20	122-142	0.838	11
ETH3	18	109-131	0.608	9
ETH10	23	211-223	0.676	6
ETH152	1	191-203	0.662	6
ETH225	2	139-159	0.745	7
HEL1	19	103-113	0.707	6
HEL9	5	147-167	0.730	10
ILSTS005	5	182-194	0.371	4
ILSTS006	9	285-303	0.678	10
INRA05	12	139-157	0.500	4
INRA23	3	199-215	0.802	9
INRA32	11	174-186	0.723	7
INRA35	16	96-114	0.464	5
INRA63	18	173-185	0.586	6
TGLA053	15	151-181	0.857	13
TGLA122	10	138-194	0.780	14
TGLA126	7	119-127	0.559	4
TGLA227	15	77-101	0.839	12
SPS115	8	246-260	0.482	7
mean				7.90

Considering all cattle groups, the majority of the markers were in HWE, except locus BM 1824 and INRA35 in GC group, BM 2113 in BEH group, and ETH 3 and INRA 35 in BWH group which had deviation from HWE ($p<0.05$). Likelihood of individual genotypes in all populations showed that only one individual from GC group is not well assigned. Test for linkage disequilibrium between all loci pairs was conducted for each cattle group separately. Out of 441 locus pair/group combinations, the tests resulted in 148 in GC, 112 in BEH and 96 in BWH group significant deviation from the LE ($p<0.05$) outcomes.

Table 2. Genetic diversity parameters based on 21 microsatellite loci for three groups (PIC value, Ho-observed heterozygosity, He-expected heterozygosity)

Microsatellite	GC				BEH				BWH			
	Num. allele	H_o	H_e	HWE	Num. allele	H_o	H_e	HWE	Num. allele	H_o	H_e	HWE
BM1818	6	0.776	0.699	ns	4	0.423	0.574	Ns	7	0.783	0.687	ns
BM1824	8	0.729	0.757	*	4	0.654	0.757	Ns	5	0.68	0.704	ns
BM2113	9	0.837	0.812	ns	8	0.538	0.816	*	9	0.826	0.838	ns
ETH3	7	0.735	0.676	ns	4	0.538	0.466	Ns	5	0.625	0.721	*
ETH10	5	0.680	0.643	ns	5	0.731	0.758	Ns	4	0.52	0.504	ns
ETH152	5	0.814	0.708	ns	6	0.68	0.769	Ns	3	0.636	0.647	ns
ETH225	5	0.620	0.64	ns	5	0.731	0.781	Ns	7	0.84	0.727	ns
HEL1	6	0.576	0.623	ns	6	0.739	0.705	Ns	5	0.857	0.771	ns
HEL9	8	0.810	0.789	ns	6	0.542	0.697	Ns	7	0.4	0.386	ns
ILSTS005	3	0.5	0.496	ns	3	0.565	0.517	Ns	2	0.24	0.274	ns
ILSTS006	7	0.811	0.701	ns	7	0.5	0.697	Ns	6	0.625	0.61	ns
INRA05	4	0.325	0.375	ns	3	0.478	0.53	Ns	3	0.5	0.43	ns
INRA23	9	0.776	0.84	*	6	0.846	0.838	Ns	6	0.6	0.591	ns
INRA32	7	0.615	0.649	n.s	5	0.72	0.689	Ns	5	0.56	0.718	ns
INRA35	3	0.522	0.512	n.s	5	0.52	0.584	Ns	3	0.174	0.420	*
INRA63	4	0.636	0.511	ns	5	0.692	0.71	Ns	5	0.81	0.674	ns
TGLA053	12	0.896	0.844	ns	8	0.88	0.802	Ns	8	0.92	0.832	ns
TGLA122	9	0.84	0.726	ns	8	0.731	0.793	Ns	7	0.84	0.822	ns
TGLA126	4	0.68	0.631	ns	4	0.692	0.647	Ns	3	0.6	0.467	ns
TGLA227	10	0.939	0.842	ns	8	0.923	0.787	Ns	8	0.64	0.767	ns
SPS115	4	0.327	0.368	ns	4	0.692	0.541	Ns	7	0.64	0.638	ns
mean	6.43	0.688	0.659		5.43	0.659	0.689		5.47	0.634	0.630	

[ns] non significant (p>0.05), *significant (p<0.05)

Table 3. Pairwise population matrix of F_{st} values among analyzed groups

	GC	BEH	BWH
GC	0	+	+
BEH	0.057	0	+
BWH	0.124	0.113	0

The highest F_{st} was found between GC and BWH , subsequently between BEH and BWH, and the lowest F_{st} was found between GC and BEH (Table 2). All three values were statistically significant, indicating significant differentiation among all three groups. The AMOVA indicated higher intragroup (88.91%) than intergroup (11.09%) variability, with statistically significant intergroup percentage of variation (p<0.05).
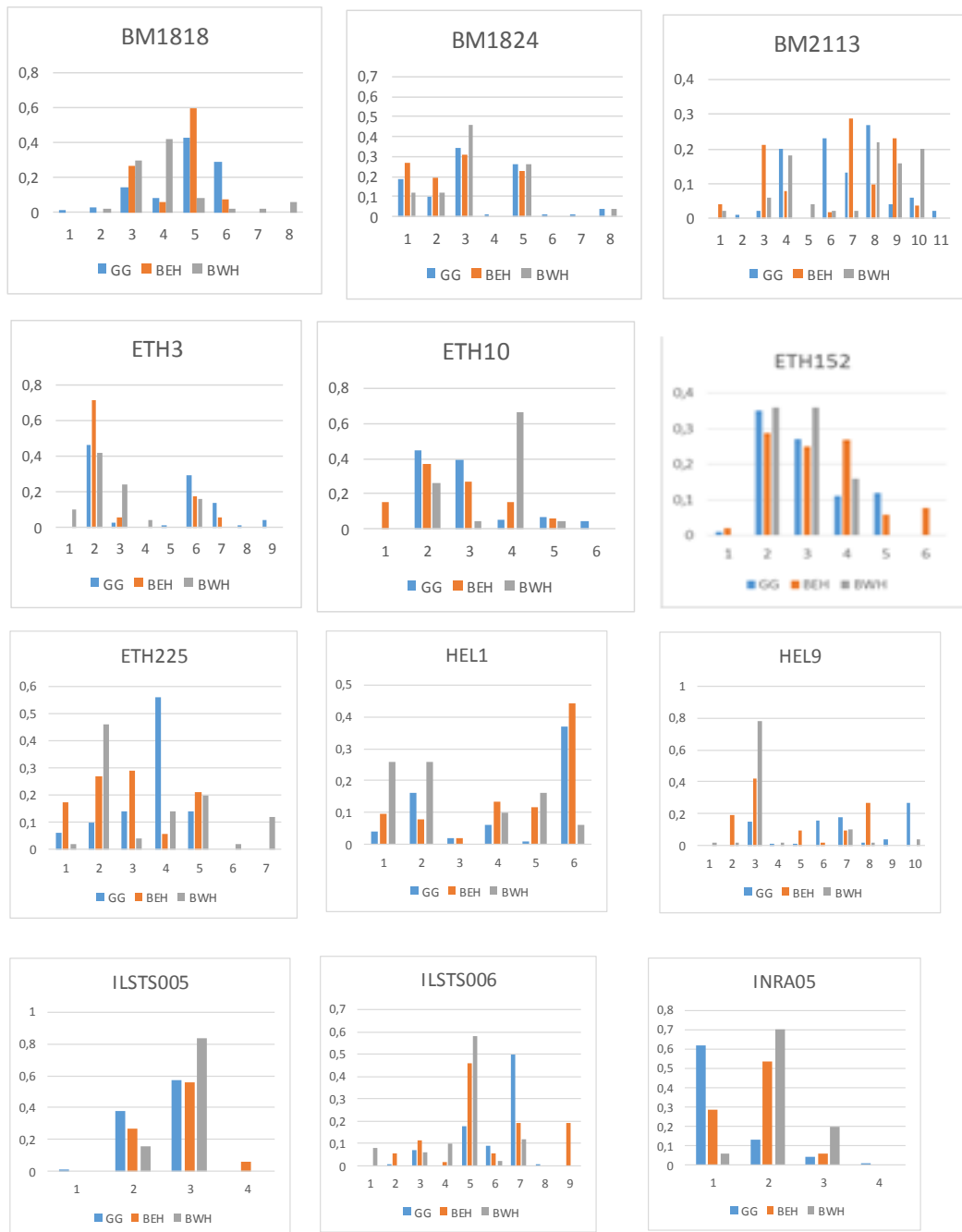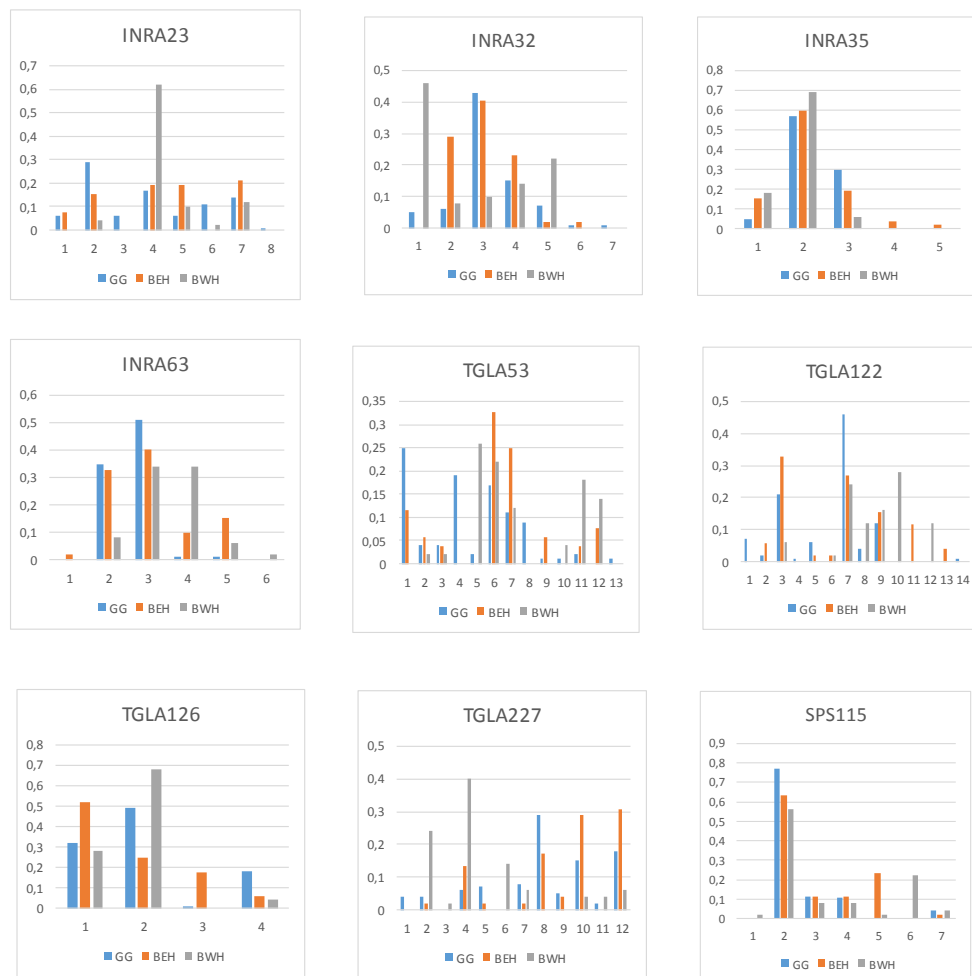
Figure 1. Allele frequency distribution for 21 microsatellite markers in the Gatacko cattle and Busha from Eastern and Western Herzegovina

DISCUSSION

From the long-term perspective, nowadays facing challenges e.g. climate change, emerging diseases, pressure on land and water, human nutritional needs, at the present time it is more important than ever to ensure animal genetic resources are conserved and used sustainably. Busha and Gatacko cattle are autochthonous cattle breeds and valuable animal genetic resources from Bosnia and Herzegovina. They characterized poor or medium production performance, but excellent adaptation to harsh climatic conditions and their resistance to diseases. Busha cattle is

considered to be endangered due to registered population decline, which is not a case with Gatacko cattle.

All microsatellite loci used in this research showed ample polymorphism for evaluating within breed genetic variability and exploring genetic differences between breeds. Locus TGLA122 showed the highest number of alleles (14) as was the case in the study of genetic diversity of European cattle, Eurasian and Near Eastern cattle (MEDUGORAC et al., 2009; LI et al., 2007; KEROS et al., 2013). According to LI et al. (2007) the lowest number of alleles was found for locus ILSTS005, which is in accordance with our results. The mean number of alleles across all the loci was 7.9 and was higher than in other traditional cattle from neighboring countries like Croatia, FYRO Macedonia, Albania, Serbia and Slovenia (MEDUGORAC et al., 2009; LI et al., 2007; SIMČIĆ et al., 2008). Higher allelic diversity than in our studied populations have been reported in Anatolian Black, Illyrian Mountain and Red Metohian Busha (MEDUGORAC et al., 2009), and Croatian Podolian cattle (KEROS et al., 2013). Estimates of observed heterozygosity ranged from 0.34 to 0.687 and were similar to values estimated for other traditional European cattle breeds. MEDUGORAC et al. (2009) analyzed genetic variability of six Busha populations from the Balkan region and showed that the average observed heterozygosity ranged from 0.650-0.708. Similar values were found in Busha (0.652) and Podolian cattle (0.613) from Serbia (LI et al., 2007).

This study revealed significant genetic differentiation among three cattle groups. Pairwise genetic differentiations quantified by Fst value showed statistically significant differences. These differences are in coordinance with the significant differences in the external measures reported by ROGIĆ et al. (2011). Significant genetic differentiation between Gatacko cattle and Busha is in coordinance with report of MEDUGORAC et al. (2009), who found that Gatacko cattle (or Gacko Grey Busha) are in intermediate position between Tyrolean Grey and Busha breeds from the Balkan neighbouring countries.

CONCLUSION

Our results represent the first report of genetic differentiations between Busha and Gatacko cattle from Bosnia and Herzegovina. The results of this study indicate that during the past century and breeding Gatacko cattle led to a significant genetic differentiation between Gatacko cattle and their founders (Busha and Tyrolean Grey cattle). This work contributes the characterization of genetic resources of autochthonous cattle breed from Bosnia and Herzegovina. The obtained values of the parameters of genetic heterogeneity indicate a high and preserved genetic variability of the Busha and Gatacko cattle. Furthermore, new modern methods which allow the application of genomic selection most successfully in cattle should be applied in future research. SNPs are currently used for massive genotyping and has proven to be useful to genetically characterize populations of domestic cattle and to assess their degree of divergence. However, genetic characterization of Busha and Gatacko cattle using microsatellites represent a valuable information and high genetic diversity in Busha and Gatacko cattle together with described qualitative traits should be considered in further conservation and breeding strategies.

The obtained results indicate a need for further study of populations of Gatacko cattle, and its genetic, productive and reproductive parameters, to define an appropriate breeding

program. Also, the obtained results are applicable in the field of conservation genetics, and protection of the genetic potential of autochthonous Busha breed, according to FAO recommendations.

ACKNOWLEDGMENTS

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PROCENA STRUKTURE I GENETIČKOG DIVERZITETE AUTOHTONIH RASA GOVEDA BOSNE I HERCEGOVINE NA OSNOVU MIKRSATELITA

Biljana ROGIĆ [1], Božo VAŽIĆ[1], Mihajla ĐAN[2], Marina STAMENKOVIĆ RADAK[3]

[1]Univerzitet Banja Luka, Poljoprivredni fakultet, Republika Srpska, B&H
[2]Univerzitet Novi Sad, Prirodno-matematički fakultet, Departmant za biologiju i ekologiju, Srbija,
[3]Univerzitet Beograd, Biološki fakultet, Srbija

Izvod

Genotipizacija 21 mikrosatelita je urađena da bi se procjenio genetički diverzitet i povezanost gatačkog govečeta i buše. Ukupno je genotipizirano 100 životinja podijeljenih u tri grupe: gatačko goveče (GG), buša istočne Hercegovin (BEH) i buša zapadne Hercegovine (BWH). Odstupanje od Hardi-Vajnebergovog ekvilibrijuma je zabilježeno samo za lokus INRA35 i to u grupi buše zapadne Hercegovine. Ukupno je otkriveno 166 alela a prosječan broj alela po lokusu je bio 7,9. Najviša dobijena heterozigotnost (0,688) je zabilježena kod gatačkog govečeta a najniža (0,634) kod buše zapadne Hercegovine. Dobijene vrijednost Fst su pokazale statistički visoko značajnu genetičku diferencijaciju između sve tri grupe goveda: gatačkog govečeta i buše istočne Hercegovine, gatačkog govečeta i buše zapadne Hercegovien, kao i između buše zapadne i buše istočne Hercegovine. U ovom radu je po prvi put urađena genetička karakterizacija gatačkog govečeta i buše. Dobijeni rezultati su od značaja za buduće programe očuvanja tradicionalnih rasa goveda Bosne i Hercegovine.