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ASSESSMENT OF GENETIC DIVERSITY OF BUŠA CATTLE FROM BOSNIA AND HERZEGOVINA USING MICROSATELLITE DNA MARKERS

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Abstract - The variability of 21 microsatellite loci was analyzed in order to estimate the genetic diversity of the Buša cattle breed. A total of 50 animals involved in the study were divided into two groups: Buša from eastern Herzegovina and Buša from western Herzegovina. The mean number of alleles per locus was 6.6. The average expected heterozygosities were 0.6885 and 0.6212 in the eastern and western populations, respectively. The observed heterozygosity values were 0.6579 and 0.6336, in eastern and western population, respectively. The degree of population differentiation (F_{ST}) ranged from 0.008 (for ILSTS006 locus) to 0.242 (for BM1818 locus), with a mean of 0.112. Cluster analysis showed that the two populations were clearly clustered into two distinct clades. This report represents the first genetic characterization of pure Buša cattle from Bosnia and Herzegovina. The obtained results are important for the future development of conservation and management strategies for this cattle breed.

Key words: Buša, cattle, microsatellites, genetic diversity, Bosnia and Herzegovina

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INTRODUCTION

Buša is an autochthonous cattle breed, characteristic for the area of the former Yugoslav republics. It inhabits areas with a poor economy and extensive agriculture, and it is perfectly adapted to the local climate, food supply and other local environmental factors. The loss of interest in autochthonous cattle breeds has almost caused the disappearance of Buša.

The biodiversity of autochthonous livestock breeds is widely recognized, since thorough information on the genetic diversity and population structures in cattle breeds is urgently needed to serve as a rational basis for the conservation, and possible use, of indigenous cattle breeds as a genetic resource to meet potential future demands (European Cattle Diversity Consortium 2006, Tapio et al., 2006, Li et al., 2007, Taberlet et al., 2008).

The protection of regional populations, autochthonous breeds and within-breed variation includes insight into the variability at several levels, from phenotypic to molecular and genome-wide. Microsatellite DNA markers are widely used in the population genetic studies of cattle breeds. They are especially valuable for their high variability and abundant distribution throughout the genome. In addition, their applications have been proven successful in assessing intrapopulation diversity, genetic differentiation and relationships between different cattle populations (Medugorac et al., 2009, Li et al. 2007, MacNeil et al., 2007, Brenneman et al., 2007).

Buša cattle have been previously studied with respect to microsatellite variability (Simčič et al., 2008, Ramljak et al., 2008, Li et al., 2007). However, the pure Buša populations from Bosnia and Herzegovina have never been studied before. Therefore, the aim of our study was to analyze the genetic variability and population structuring in the pure Buša cattle breed from Bosnia and Herzegovina

MATERIALS AND METHODS

Samples and DNA extraction

Blood samples were collected from 50 animals divided into two groups. The first group comprised 26 individuals from the area of the eastern Herzegovina (BEH), and the second group included 24 individuals from western Herzegovina (BWH). Genomic DNA from total blood was extracted using "DNeasy® Blood & Tissue Kit" (Qiagen, Valencia, CA) and kept frozen at -18°C until further processing.

Microsatellite analyses

In the present study we investigated 21 microsatellite loci recommended by the International Society for Animal Genetics (ISAG): BM1818, BM1824, BM2113, ETH3, ETH10, ETH152, ETH225, HEL1, HEL9, ILSTS005, ILSTS006, INRA05, INRA23, INRA32, INRA35, INRA63, TGLA053, TGLA122, TGLA126, TGLA227 and SPS115. Loci BM1818, BM1824, BM2113, ETH 3, ETH 10, ETH225, INRA 23, TGLA053, TGLA122, TGLA126, TGLA227 and SPS115 were amplified using "Bovine Genotypes Panel 1.2, F-904" kit (Finnzymes Oy, Keilaranta 16 A, 02150 Espoo, Finland) according to the supplier's protocol. Loci ETH152, HEL 1, HEL 9, ILSTS 005, ILSTS 006, INRA 05, INRA 32, INRA 35 and INRA 63 were amplified individually in a total volume of 10 µl containing 10 ng of DNA template, 1×PCR buffer with 5% DMSO, 1.5mM $MgCl_2$, 1 unit of Taq DNA polymerase, 0.2mM of each dNTP and 2 pmol of each primer. One primer of each pair was labeled with fluorescent dye. PCR conditions were as follows: the reactions were heated for 1 min at 98°C, followed by 30 cycles of denaturation (98°C, 20 s), annealing (60°C, 75 s), and extension (72°C, 30 s). A final extension was carried out at 72°C for 5 min. Allele sizes were scored according to the TAMRA 500 size standard on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

Microsatellite allele frequencies, observed (Ho) and expected (He) heterozygosity, were calculated using GENEPOP version 3.4 (Raymond & Rousset, 1995). Deviations from the Hardy-Weinberg equilibrium (HWE) and deviations from the pairwise linkage equilibrium (LE) between loci were determined by means of Fisher's exact test implemented in the GENEPOP, version 3.4 (Raymond & Rousset, 1995). Statistical significance was estimated using the Markov chain method with 10,000 dememorization steps, 500 batches and 5,000 iterations per batch. For each population, a strict Bonferroni test was used to correct the probabilities when many tests are carried out simultaneously.

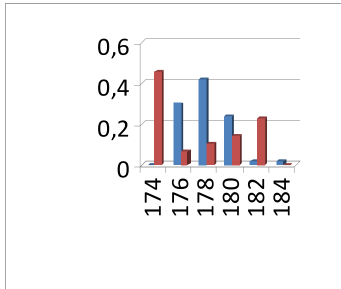
Population structure in our study was evaluated by Wright's F-statistics using Weir & Cockerham's method (1984) implemented in the FSTAT computer program (version 2.9.3.2) (Goudet, 2002). The null hypothesis (Ho) that the estimates were not significantly different from zero was tested by means of permutations as proposed by Goudet (2002). F-statistics have proven to be a very useful tool in elucidating the pattern and extent of genetic variation residing within and among natural populations of different species (Aguirre-Planteretal et al., 2000; Aranguren-Mendez et al., 2002; Jelic et al., 2009). Genetic similarities between the analyzed individuals were evaluated using the Dice coefficient of similarity. Clustering analysis was done based on the overall similarity between pairs of analyzed individuals. The UPGMA dendrogram was constructed from a matrix of pairwise distances by NTSYS-PC software package, Version 1.80.

RESULTS

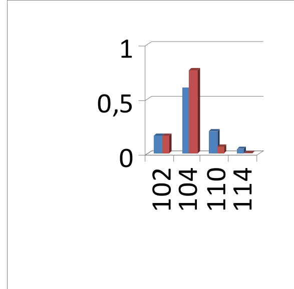
The highest number of alleles per locus was found at the TGLA227 and TGLA122 loci (11), while the lowest number of alleles (3) was observed at loci INRA05 and ILSTS005. The average value for all loci was 6.6 (Tab. 1). All loci, except INRA05, harbored from one to eight alleles specific to either one of the two populations. (Fig. 1).

In the BEH population, the number of alleles per locus ranged from 3 (INRA05 and ILSTS005) to 8 (BM2113, TGLA053, TGLA122 and TGLA227), with a mean of 5.4. Observed heterozygosity (Ho) ranged from 0.4231 (BM1818) to 0.9231 (TGLA227), with an average value of 0.6579. Expected heterozygosity (He) ranged from 0.4661 (ETH3) to 0.8379 (INRA23), with an average value of 0.6885.

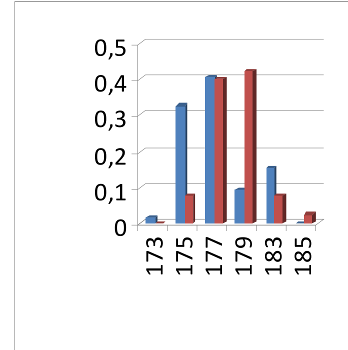
INRA32



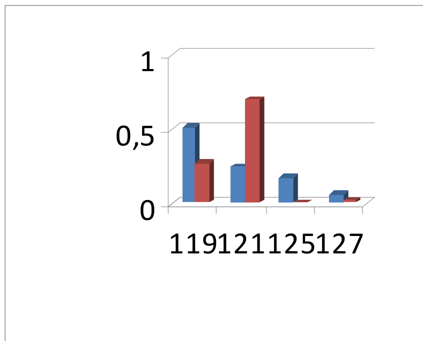
INRA32



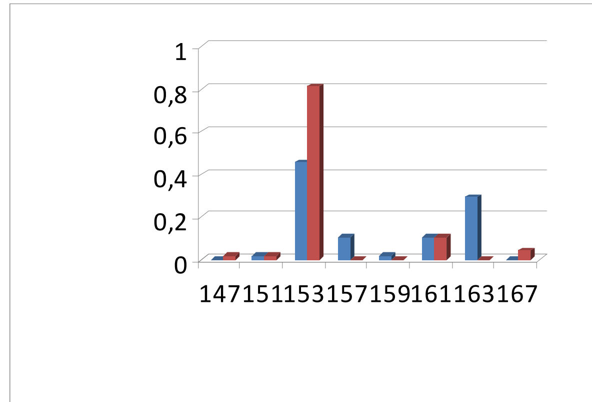
INRA32



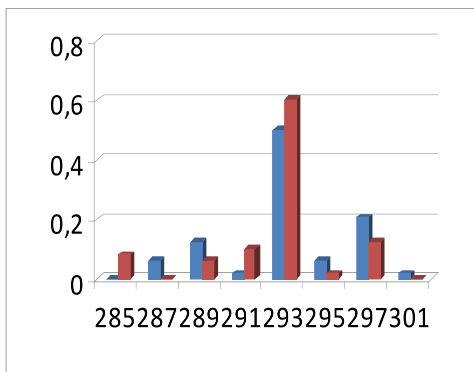
TGLA126



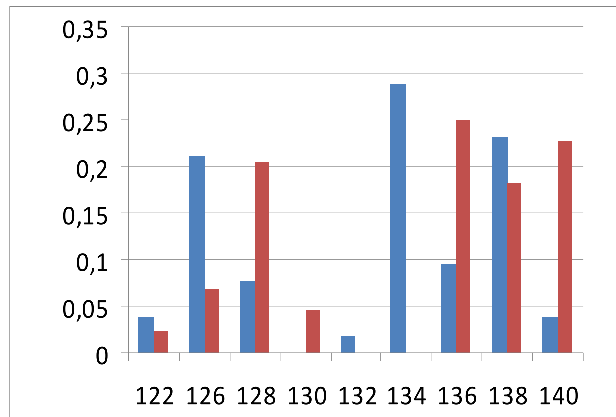
HEL9



ILSTS006



BM2113



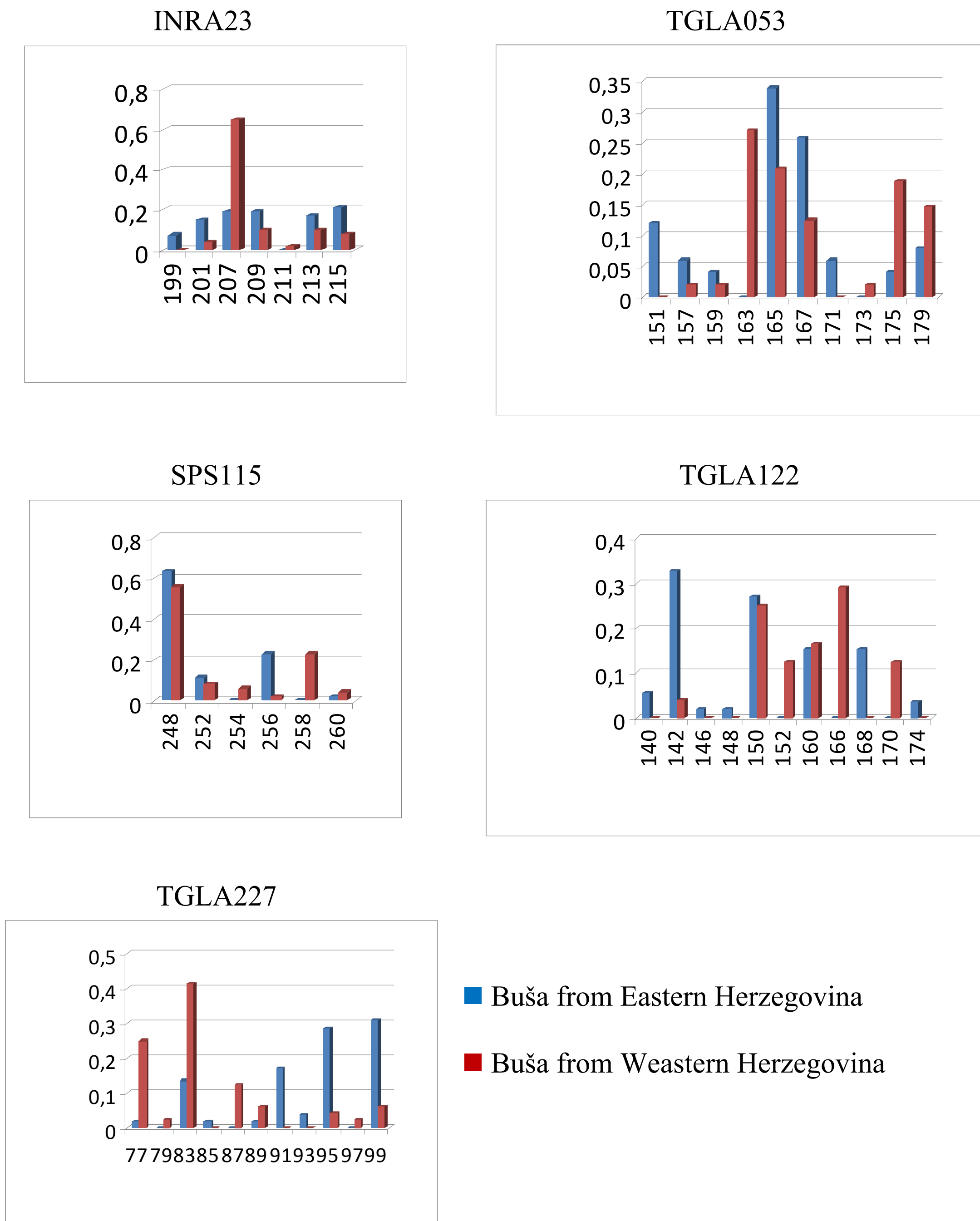


Fig. 1. Distribution of alleles for Buša from Eeastern and Western Herzegovina

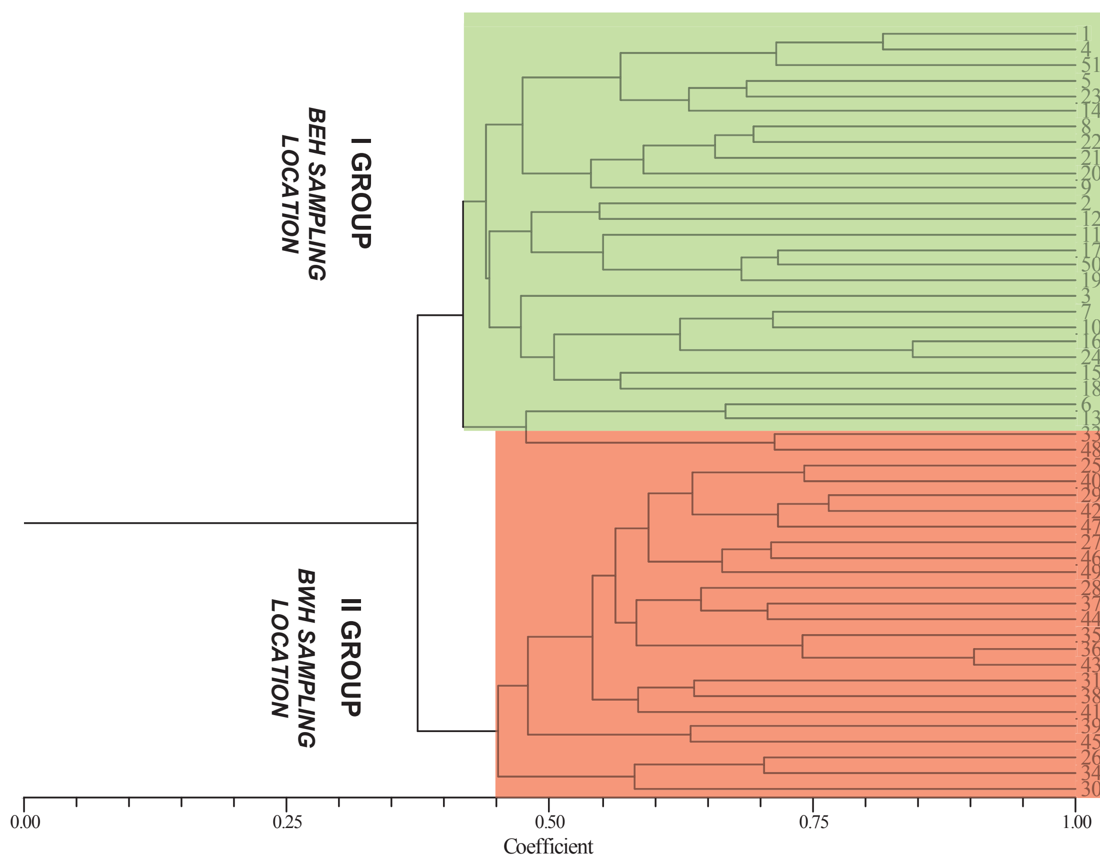


Fig. 2. UPGMA dendrogram showing overall genetic similarities between analyzed individuals

In the BWH population, the number of alleles per locus ranged from 2 (ILSTS005) to 8 (TGLA053 and TGLA227), with a mean of 5.1. Observed heterozygosity (Ho) ranged from 0.1818 (INRA35) to 0.9167 (TGLA053), with an average value of 0.6336. Expected heterozygosity (He) ranged from 0.2837 (ILSTS005) to 0.8271 (TGLA053), with an average value of 0.6212.

An exact test for LE between all pairs of loci was performed for both populations. Out of 420 locus pair/population combinations, the tests resulted in 4 significant ($p<0.05$) outcomes, which were sporadically distributed among the populations, i.e. the loci that showed significant deviation from LE were different ones in the two populations. The proportion of significant pairs (n = 4, 0,95%) was similar, as expected by chance. Significant departure from HWE was not detected ($p<0.05$) for either of the loci in both populations. (Tab. 1).

The values of genetic differentiation estimator (F_{ST}) are shown in Table 1. The level of differentiation (F_{ST}) ranged from 0.008 (ILSTS006) to 0,242 (BM1818), with a mean of 0.112. F_{ST} estimates were significantly different from zero for all loci, except for ETH152, ILSTS005, ILSTS006 and INRA35.

In order to visualize the overall similarity between the analyzed individuals, a dendrogram was constructed (Fig. 2). The UPGMA analysis clustered two separate populations in two distinct clades. Overall genetic similarity between pairs of individuals varied from 0.348 to 0.903.

Table 1. Parameters of genetic information content of 21 microsatellite loci: Nt-total number of alleles per locus, F_{ST} - fixation index, He - expected heterozygosity, Ho - observed heterozygosity, *p<0,05, **p<0,01

Locus	Nt	F_{ST}	He		Ho		P	
			BEH	BWH	BEH	BWH	BEH	BWH
BM1818	6	0,242**	0,5739	0,6786	0,4231	0,7727	0,1518	0,2900
BM1824	5	0,017*	0,7572	0,6995	0,6538	0,7083	0,2904	0,2822
BM2113	9	0,070**	0,8150	0,8224	0,5385	0,8182	0,0197	0,4049
ETH3	6	0,08**	0,4661	0,7333	0,5385	0,6522	0,7791	0,0093
ETH10	5	0,190**	0,7579	0,5168	0,7308	0,5417	0,9434	0,7754
ETH152	6	0,011	0,7690	0,6516	0,6800	0,6190	0,3906	0,8332
ETH225	7	0,075**	0,7813	0,7225	0,7308	0,8333	0,4948	0,4265
HEL1	6	0,144**	0,7053	0,7712	0,7391	0,8571	0,0935	0,2460
HEL9	8	0,162**	0,6968	0,3333	0,5417	0,3750	0,0786	1,0000
ILSTS005	3	0,057	0,5619	0,2837	0,5652	0,2500	0,7210	0,5011
ILSTS006	8	0,008	0,6968	0,6099	0,5000	0,6250	0,0721	0,5098
INRA05	3	0,078*	0,5340	0,4435	0,4783	0,5217	0,1301	0,6226
INRA23	7	0,138**	0,8379	0,5638	0,8461	0,6250	0,9531	0,7625
INRA32	6	0,216**	0,6890	0,7163	0,7200	0,5417	0,9469	0,1297
INRA35	4	0,022	0,5845	0,3816	0,5200	0,1818	0,0816	0,0133
INRA63	6	0,098**	0,7104	0,6641	0,6923	0,8000	0,9503	0,1707
TGLA053	10	0,072**	0,8016	0,8271	0,8800	0,9167	0,4024	0,9798
TGLA122	11	0,103**	0,7934	0,8085	0,7308	0,8333	0,2852	0,6195
TGLA126	4	0,206**	0,6471	0,4335	0,6923	0,5833	0,1377	0,1655
TGLA227	11	0,151**	0,7866	0,7535	0,9231	0,6250	0,8632	0,4263
SPS115	7	0,069**	0,5407	0,6312	0,6923	0,6250	0,5478	0,1240
Mean	6,6	0,112**	0,6885	0,6212	0,6579	0,6336		

DISCUSSION

The number of alleles identified in our study varied from three to 11, with a mean of 6.6. In the analysis of the genetic variability of Croatian Buša cattle, Simčič et al. (2008) detected an average number of alleles that is lower compared to our study (5). A similar number of alleles (6.7) was found in Serbian Buša cattle in the study of Li et al. (2007). In their study the lowest number of alleles was found for locus ILSTS005, which is the same as in our research. The greatest number of alleles in our study was observed for locus TGLA122, as was the case in the study of Medugorac et al. (2009) and Li et al. (2007). The average observed heterozygosity (Ho) values for the BEH and BWH populations were, 0,6579 and 0.6336, respectively, as was the case in the population from Serbia (0,6522) (Li et al., 2007). The Croatian Buša cattle (Simčič et al., 2008) show a higher values of Ho (0.7775). According to Ho, the level of genetic variability in the analyzed Buša cattle population is slightly smaller compared to other neighboring countries. The different number of loci analyzed by Simčič et al. (2008) and Li et al. (2007) may be the reason for these different results. Locus BM2113 exhibits the highest level of heterozygosity and was the most informative locus for the BEW and BEH populations, as was the case in Simčič et al. (2008).

The average F_{ST} value was 0.112, which indicates that nearly 11% of the genetic variation among these populations was due to breed differentiation. Estimation of a genetic subdivision using classical drift-based models showed that the average proportion of genetic differentiation among the two populations of Buša cattle was equivalent to other diversity studies using microsatellites, e.g. 7-11% for European cattle (Kantanen et al., 2000, Mateus et al., 2004, Ramljak et al. 2008, Medugorac et al., 2009).

Cluster analysis based on overall similarities between pairs of individuals showed two major groups of genotypes. These two clades almost perfectly correspond to the BEH and BWH populations. Overall genetic similarity differentiated the samples with regard to their geographical origin. Twenty six individuals clustered to the first group belong to the same location of sampling (BEH), while only two individuals belong to the BWH sampling location (individual 33 and 48, Fig. 2.). All individuals (22) clustered to the second group belong to the BWH location of sampling. The observed results are in accordance with the obtained FST values between the BEH and BWH populations.

In conclusion, the obtained values of the parameters of genetic heterogeneity and the fact that all the examined loci are in Hardy-Weinberg equilibrium (HWE) indicate a high and preserved genetic variability of the Buša cattle. According to the number of private alleles in each population from our study and the genetic differentiation among them, we can conclude that all markers, except ETH152, ILSTS005, ILSTS006 and INRA35, are very powerful in assessing genetic differentiation between cattle populations. According the F_{ST} value and cluster analysis, two major groups of genotypes can be noticed, which indicates that the genetic differentiation between the two populations of Buša cattle is significant. The significance of our study is the genetic analysis of the pure Buša cattle bred in the region of Bosnia and Herzegovina, where this breed has not been analyzed before. The results are important since they show that Buša populations inhabiting different geographical regions harbor some genetic uniqueness which may prove to be important in conservation and management strategies for this breed.

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REFERENCES

Aguirre-Planter, E., Furnier, G. R., and *L. E. Equiarte* (2000). Low levels of genetic variation within and high levels of genetic differentiation among populations of species of *Abies* from southern Mexico and Guatemala. *Am. J. Bot.* **87 (3),** 362-371.

Aranguren-Mendez, J., Gomez, M., and *J. Jordana* (2002). Hierarchical analysis of genetic structure in Spanish donkey breeds using microsatellite markers. *Heredity* **89,** 207-211.

Brenneman, R.A., Chase Jr, C.C., Olson, T.A., Riley, D.G., and *S.W. Coleman* (2007). Genetic diversity among Angus, American Brahman, Senepol and Romosinuano cattle breeds. *Anim. Genet.* **38**, 50-53.

European Cattle Diversity Consortium (2006). Marker-assisted conservation of European cattle breeds: an evaluation. *Anim. Genet..* **37**, 475-481.

Goudet, J (2002). FSTAT, a program to estimate and test gene diversities and fixation indices (version2.9.3.2) Institut d'Ecologie, Batiment de Biologie, Universite de Lausanne, Dorigny.

Jelic, M., Kenig, B., Kurbalija, Z., Stamenkovic-Radak, M., and *M. Anđelković* (2009). Intra-species differentiation among *Drosophila suobscura* from different habitats in Serbia. *Arch. Biol. Sci. (Belgrade)* **61 (3),** 513-521.

Li, M.H., Tapio, I., Vilkki, J., Ivanova, Z., Kiselyova, T., Marzanov, N., Cinkulov, M., Stojanovic, S., Ammosov, I., Popov, R., and *J. Kantanen* (2007). The genetic structure of cattle populations (*Bos taurus)* in northern Eurasia and the neighboring Near Eastern regions: implications for breeding strategies and conservation. *Mol. Ecol.* **16**, 3839-3853.

Kantanen, J., Olsaker, I., Holm, L.E., Lien, S., Vilkki, J., Brusgaard, K., Eythorsdottir, E., Danell, B., and *S. Adalsteinsson* (2000). Genetic diversity and population structure of 20 North European cattle breeds. *J. Hered.* **91**, 446-457.

MacNeil, M.D., Cronin, M.A., Blackburn, H.D., Richards, C.M., Lockwood, D.R., and *L.J. Alexander* (2007). Genetic relationships between feral cattle from Chirikof Island, Alaska and other breeds. *Anim. Genet.* **38**, 193-197.

Mateus, J.C., Penedo, M.C.T., Alves, V.C., Ramos, M., and *T. Rangel-Figueiredo* (2004). Genetic diversity and differentiation in Portuguese cattle breeds using microsatellites. *Anim. Genet*. **35**, 106-113.

Medugorac, I., Medugorac, A., Russ, I., Veit-Kensch, C.E., Taberlet, P., Lunty, B., Mix, H.M., and *M. Forster* (2009). Genetic diversity of European cattle breeds highlights the conservation value of traditional unselected breeds with high effective population size. *Mol. Ecol*. **18**, 3394-3410.

Ramljak, J., Ivanković, A., Međugorac, I., Caput, P., and *M. Konjačić* (2008). Microsatellite genotyping of Busha in Croatia, 42nd Croatian & 2nd International Symposium on Agriculture, 201.

Raymond, M., and *F. Rousett* (1995). Population genetics software for exact tests and ecumenicism. *J Hered.* **86**, 248-9.

Simčić, M., Čepon, M., Horvat, S., Jovanovac, S., Ganter, V., Dovč, P., and *D .Kompan* (2008). Genetic characterization of autochthonous cattle breeds, Cika and Busha, using microsatellites. *Acta. Agr. Slov.* **2**, 71-77.

Taberlet, P., Valentini, A., Rezaei, H.R., Naderi, S., Pompanon, F., Negrini, R., and *P. Ajmone-Marsan* (2008). Are cattle, sheep, and goats endangered species? *Mol. Ecol.* **17**, 275-284.

Tapio, I., Värv, S., Bennewitz, J., Maleviciute, J., Fimland, E., Grislis, Z., Meuwissen, T.H., Miceikiene, I., Olsaker, I., Viinalass, H., Vilkki, J., and *J. Kantanen* (2006). Prioritization for conservation of northern European cattle breeds based on analysis of microsatellite data. *Conserv. Biol.* **20**, 1768-1779.

Weir, C.A., and *C.C. Cockerham* (1984). Estimating F-statistics for the analysis of population structure. *Evolution,* **38**, 1358-1370.