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Dalmatian Bušak horse

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Abstract

One of the Croatian autochthonous breeds in the Mediterranean region that has been classified as uncategorized or unknown is the Dalmatian Bušak horse. It has been noted that in Dalmatia a small number of individuals are still bred and body measurement (as part of the exterior description) and genotyping research were carried out. Nine measurements were taken from 25 horses. Hair samples were taken from 23 horses for preliminary genotyping at 17 microsatellite loci. Lydtin stick was used to measure the height at withers (129.88 ± 3.94 cm), height at croup (129.62 ± 4.33 cm), body length (136.11 ± 7.04 cm), and chest depth (58.14 ± 4.48 cm); ribbon to measure the chest (156.81 ± 10.6 cm) and cannon bone circumference (17.57 ± 1.29 cm); and caliper measure the chest width (28.57 ± 2.62 cm), croup width (41 ± 2.76 cm), and croup length (43.98 ± 2.65 cm). Microsatellite analysis showed that each locus had between 4 and 12 different alleles, the average observed heterozygosity was 0.74 and expected 0.76, and, consecutively, the inbreeding coefficient (F_{IS}) was 0.02. The polymorphic information content (PIC) revealed that 16 out of 17 markers were highly informative. The results indicated that the Dalmatian Bušak horses are a relatively homogeneous, but not inbred, population and that the next steps leading to the official recognition of this autochthonous breed in Croatia should be taken.

Keywords: Dalmatian Bušak horse, autochthonous breed, exterior, microsatellite

Introduction

Small horses known as the Dalmatian Bušak horse have been observed in the Mediterranean part of Croatia over the past ten years. Researching various historical sources, it was established that they have been found in that area for more than two centuries. Despite the breed currently being classified as not evaluated by the Food and Agriculture Organization (FAO) and as insufficiently known by the categorization of the International Union for Conservation of Nature (IUCN) (BARAĆ et al., 2011), there is still a small population of horses in Dalmatia whose conformation corresponds to the Dalmatian Bušak breed.

The first mention of small horses in Dalmatia can be found in the report of the Split district from 1822, compiled by district chief Enrico Reha. The horses were described as miserable,

poorly groomed, and shod, and they bred in herds on pasture without shelter. They were used exclusively as pack horses, sometimes for riding, and were not suitable for military purposes (REHA, 2016). The next written document was from the 1830s, where governor Lilienberg, in his description of the Habsburg province of Dalmatia, also mentioned very small horses that were often poorly kept, fed, and shod (TROGRLIĆ and CLEWING, 2015). As in the previous report, the author noted that horses of this breed were not used for any kind of pulling but as pack and/or riding animals.

The name Dalmatian Bušak horse was mentioned for the first time in the “Report of the National Economic Administration of the Kingdom of Croatia and Slavonia for the period 1896-1905”, where Prof. dr. Oton Frangeš, in the chapter on horse breeding, wrote about small horses, stating that certain regions (Dalmatia) do not have good conditions for horse breeding (BARAĆ et al., 2011). Beautiful horses like the Posavina horse did not develop in these areas, but horses of a smaller build, with heavy heads, short necks, long backs, large abdomen, steep hindquarters, thin legs with dry muscles, and solid hooves, whose height at withers ranged from 110 to 140 cm. He also mentioned that they could safely carry heavy loads or riders through steep and rocky roads in all kinds of weather (storm and rain).

Along with the historical records, quite a large number of postcards and photos of the Dalmatian Bušak horse were found (Figures 1 and 2). Although the horses in the photos are in different positions and, in some of them, they are not even fully visible, it is visible that they have quite a similar exterior.



Figure 1. Postcard from webpage Forgotten Dalmatia (www.zaboravljenadalmacija.hr), Split surroundings, 1899.

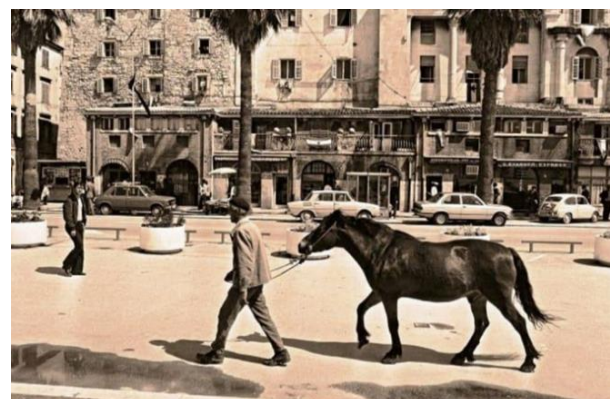


Figure 2. Photo from 1980s from “Slobodna Dalmacija” by Feđa Klarić: "A man and a horse on Split riva".

The next important record was Giacomo Gospodnetich Martinovich’s booklet “Libretto delle cavalle” from 1935 (given by his great-grandson). It is a notebook in which he entered data about bought mares: name, origin, age, time of arrival at his property in Dol on the island of Brač, height at withers (average height was 126.71 cm), time of foaling, whether the foal was a mule or a horse, and the picture of the mare. Another interesting record is the documentary film “Autumn on the island of Brač” (Croatia film, 1957), which talks about life, people, and customs on the island of Brač, and it is also possible to see horses in the Dalmatian Bušak type. In this study, we presented the exterior body measurements of the Dalmatian Bušak horse and the preliminary genotyping data (research is still ongoing).

Materials and Methods

A total of 38 horses (11 males and 27 females; Figure 3) aged 6 months to 16 years, situated on 10 farms in the Split-Dalmatia and Dubrovnik-Neretva counties, were included in this study. Nine body measurements were taken from 25 horses, as most of them were not used to any kind of human manipulation. Lydtin/measuring stick was used to measure the height at withers, height at croup, body length, and chest depth; ribbon was used to measure the chest and cannon bone circumference; and caliper was used to measure chest with, croup width, and croup length. Hair samples, 10-50 hairs from the main region, were taken from 23 horses. DNA was isolated using NucleoSpin Tissue (Macherey-Nagel, Germany) following the supplier's manual. Seventeen (17) horse-specific, ISAG recommended microsatellite (MS) loci were amplified using StockMarks™ for Horses 17-Plex Genotyping Kit (Thermo Fisher Scientific, Finland) according to supplier's protocol instructions: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1, and CA425. MS PCR products were analysed using genescan™ 500 LIZ® Size Standard (Applied Biosystems) on ABI Prism™ 3130 Genetic Analyzer. Determination of the size of the amplified fragments was performed using GeneMapper 4.0 (Applied Biosystems).

Descriptive statistics of the acquired measurements were calculated with Statistica v.14 (TIBCO SOFTWARE INC., 2020). Allele frequency, polymorphic information content (PIC), observed (H_o), and expected heterozygosity (H_e) were analysed with Cervus v.3.0.7 (MARSHALL et al., 1998; KALINOWSKI et al., 2007) while the fixation index (F_{IS}) and testing for the deviation from the Hardy-Weinberg equilibrium with R software (R CORE TEAM, 2021) adegenet package (JOMBART and AHMED, 2011). All loci were analysed together except LEX3, located on the X chromosome, which was analysed separately only in mares ($N = 16$).



Figure 3. Dalmatian Bušak stallion, about 3-year-old

Results and Discussion

The results of the assessed body measurements are given in Table 1. The obtained values indicate the relative homogeneity of the population. The most variable measurement was chest circumference, due to it being the most affected by horse weight variations (measured horses varied from underfed to overweight). Determined heights at withers and croup of 130 cm, body length of 136 cm, and cannon bone circumference of 17.6 cm indicate that the population belongs to a group of smaller horses and that their body conformation is rectangular (a little bit longer than taller).

Table 1 Mean values of the taken body measurements in cm (N = 25). Results are presented as arithmetic mean (\bar{X}), standard deviation (SD), minimal (min), and maximal (max) values.

Measurement	\bar{X}	SD	min	max
Height at withers	129.88	3.94	122	136
Height at croup	129.62	4.33	123	137
Body length	136.11	7.04	124.50	148
Chest circumference	156.81	10.60	140	180
Cannon bone circumference	17.57	1.29	15	20
Chest width	28.57	2.62	23	34.50
Croup width	41	2.76	34	44
Croup length	43.98	2.65	38	49
Chest depth	58.14	4.48	50	65

Considering the limited availability of literature data on the Dalmatian Bušak horse, the measurements from this research could have been compared only with the measurements from "Libretto delle cavalle" and IVANKOVIĆ et al. (2012). When comparing the height at withers of the mares in this study to the mean height at withers value from "Libretto delle cavalle", it was found that it was higher for 3.82 cm (the average height for mares is 130.5 cm). In relation to IVANKOVIĆ et al. (2012), the horses measured in this research were 2.9 cm higher at withers, 1.72 cm at croup, and had a 1.1 cm longer body, 4.8 cm larger chest, and 1.6 cm cannon bone circumference. Slightly lower values were recorded for chest width (3.23 cm), croup width (4.4 cm), croup length (1.82 cm) and chest depth (0.66 cm). The conclusion is that these are small deviations, which are partly possible due to differences in the way of taking measurements from person to person (personal communication with other researchers), and that the horses are very similar in appearance.

In Table 2. are statistics of MS loci (N = 23) analysed presented: locus name, number of alleles found, range allele sizes in base pairs (bp), observed (H_o) heterozygosity, expected heterozygosity (H_E), fixation index (F_{IS}), polymorphic information content (PIC), Hardy-Weinberg (H-W) equilibrium (P-value), chromosome on which the locus is found.

Table 2. Statistics of microsatellites investigated

Locus	No of alleles	Range allele sizes (bp)	H_o	H_E	F_{IS}	PIC	H-W (P)	Chromosome
VHL20	9	83-102	0.83	0.84	0.01	0.80	0.93	30
HTG4	5	116-137	0.52	0.44	-0.19	0.40	0.98	9
AHT4	8	140-166	0.83	0.83	0.00	0.79	0.79	24
HMS7	7	167-187	0.74	0.79	0.06	0.74	0.28	1
HTG6	6	74-103	0.39	0.68	0.43	0.62	<0.01	15
AHT5	6	126-147	0.74	0.74	0.00	0.69	0.19	8
HMS6	6	154-170	0.74	0.77	0.04	0.71	0.34	4
ASB23	7	176-212	0.61	0.83	0.27	0.79	<0.01	3
ASB2	9	237-268	0.83	0.85	0.03	0.81	0.04	15
HTG10	10	83-110	0.96	0.83	-0.15	0.79	0.32	21
HTG7	4	114-128	0.83	0.63	-0.23	0.61	0.11	4
HMS3	7	146-170	0.65	0.74	0.13	0.70	0.46	9
HMS2	8	215-236	0.83	0.83	0.00	0.78	0.23	10
ASB17	12	104-106	0.87	0.86	-0.01	0.82	0.06	2
HMS1	6	166-178	0.61	0.72	0.16	0.66	0.16	15
CA425	6	224-247	0.96	0.82	-0.17	0.77	0.91	17
$\bar{X} \pm SD$	7.25 ± 2.02		0.74 ± 0.15	0.76 ± 0.11	0.02 ± 0.17	0.72 ± 0.11		

The Dalmatian Bušak horse measurements differ greatly from the measurements of all other recognized Croatian autochthonous horse breeds (Croatian Coldblood, Croatian Posavina horse, and Međimurje horse; LISTEŠ et al., 2019), which is not surprising given that they belong to the group of cold-blooded animals, and the Dalmatian Bušak belongs to a group of smaller horses.

The preliminary genotyping data (research still ongoing) showed that all investigated MS loci were polymorphic and at least 4 different alleles were determined per locus. The total number of alleles found on autosomal loci was 116 (Table 2), and an additional 9 for the LEX3 locus (Table 3). The presence of a large number of alleles in different microsatellites is a good indicator of the genetic variability of the population.

Table 3. Statistics of locus LEX3 (N = 16)

Locus	No of alleles	Range allele sizes (bp)	H _O	H _E	F _{IS}	PIC	H-W (P)	Chromosome
LEX3	9	141-161	0.75	0.88	0.15	0.84	0.33	X

The average H_O was 0.74 and H_E 0.76. Consequently, F_{IS} was slightly positive (0.02), meaning a slight deficit in heterozygosity. The relatively high H_E indicates that the population has retained the presence of several alleles. The observed heterozygosity being not significantly different from the expected (0.74 and 0.76, respectively) is in accordance with the fact that the population has a random-mating structure as there is no organized selection scheme systematically applied. Five out of 17 MS loci had negative F_{IS}, nine had positive F_{IS} and three had equal H_O and H_E with F_{IS} being 0 (Table 2). Even from the nine positive F_{IS}, four had F_{IS} lower than 0.06. Looking at the overall F_{IS}, and the ones separately for each locus, it indicates a low level of inbreeding. These results confirm the relatively high heterozygosity test and indicate that there are variations between the individuals within the population. Of 17 investigated loci, 16 showed highly informative PIC (BOTSTEIN et al., 1980), which could be attributed to a large number of alleles or heterozygosity. Despite a small population, almost all observed loci were in genetic equilibrium according to the Hardy–Weinberg law.

Conclusions

According to the results of this study, the Dalmatian Bušak horse population is quite homogeneous with a low level of inbreeding. The relatively high expected heterozygosity and PIC, along with a quite low inbreeding coefficient, imply a higher amount of genetic variability. The aforementioned can be exploited and used in planning breeding strategies, particularly in populations of small size. The next steps for the Dalmatian Bušak horse are focused on increasing the number of individuals, popularization, and receiving formal recognition in Croatia.

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