doi:10.14720/aas.2024.120.2.19117 Original research article / izvirni znanstveni članek

Mitochondrial DNA analysis of the the Yugoslavian Shepherd Dog – Sharplanina and its phylogenetic relationship within and between breeds

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Received April 19, 2022; accepted June 03, 2024.
Delo je prispelo 19. aprila 2022, sprejeto 03. junija 2024

Mitochondrial DNA analysis of the the Yugoslavian Shepherd Dog – Sharplanina and its phylogenetic relationship within and between breeds

Abstract: The Yugoslavian Shepherd Dog – Sharplanina belongs to the group of Molosser type dog breeds and is an autochthonous dog breed in southwestern Kosovo and northwestern North Macedonia. This breed is characterised by its genetic diversity in the mitochondrial DNA. In our research we found nine haplotypes grouped into three main clades A, B and C, with distribution rates of 43 %, 43 % and 14 %, respectively. Our analyses show that the "Sharplanina dog" exhibits a remarkable genetic heterogeneity, which makes it very difficult to determine its origin and to correlate the haplotypes with the geographical location of the collected samples. The geographical proximity of the breed's origin to the habitat of the extinct ancient Molossian hound and the similarities of its haplotypes with certain dog breeds in Europe and East Asia make it a very interesting breed for further research.

Key words: livestock guardian dogs, breeds, Sharplanina shepherd dog, genetics, phylogenetics, haplotype, mitochondrial DNA

Analiza mitohondrijske DNA pri psih pasme šarplaninec in njena uporaba za ugotavljanje filogenetskih povezav med pasmami in znotraj pasme

Izvleček: Šarplaninec spada v skupino moloških psov in je avtohtona pasma na področju jugozahoda Kosova in v severozahodni Severni Makedoniji. Za to pasmo psov je značilna genetska pestrost mitohondrijske DNA. V naši raziskavi smo odkrili devet različnih haplotipov, razvrščenih v tri glavne veje, A, B in C, z deleži 43 %, 43 % in 14 %. Naše analize kažejo, da je šarplaninec genetsko izjemno heterogen, zaradi česar je zelo težko določiti njegov izvor in povezati haplotipe z geografsko lego izvora zbranih vzorcev. Zaradi geografske bližine področja razširjenosti pasme področju habitata izumrlih starodavnih moloških psov in podobnosti haplotipov šarplanincev z določenimi pasmami psov v Evropi in vzhodni Aziji, je pasma zelo zanimiva za nadaljnje raziskave.

Ključne besede: pastirski psi, pasme, šarplaninec, genetika, filogenetika, haplotip, mitohondrijska DNA

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1 INTRODUCTION

The "Sharplanina dog" is an autochthonous breed of the Sharri Mountains, a region located southeast of Kosovo and northwest of North Macedonia. For the first time, the breed was registered with the Federation Cynologique Internationale (FCI) in 1931 under the name "Sharplanina dog". In 1957 the FCI accepted the name change into "Yugoslavian shepherd dog - Sarplaninac". It is one of the representatives of Molossoid dog breeds that are grouped into section 2 of group 2 according to the FCI breed nomenclature, as a Yugoslavian shepherd dog - Sharplanina (http://www.fci.be) In Kosovo it is known as the "Deltari Ilir" and it represents one of the most popular dogs in the country. The "Sharplanina dog" was traditionally used for herding sheep and guarding, especially against wolves, as it has natural guarding abilities and an independent mind. Today, it is also used as a pet by people living in the urban areas and therefore spread all over the world. The phylogeny of molossoid dog breeds is highly controversial. It is debated that they were either domesticated in Mesopotamia or that they originally came from Tibet. Some researches go further and postulate that the Tibetan mastiff originated from the Molosser dog and not vice-versa (Savolainen et al., 2002). So far, no molecular genetic studies have been conducted to determine the phylogeny or to evaluate the existing diversity within the breed and/or between the "Sharplanina dog" and other breeds. In Western Balkan it is hard and complex to follow the genetic lineage of dog breeds (Ceh et al. 2014). Here we provide and insight in the genetic diversity of the "Sharplanina dog" by evaluation of its D-loop region on the mtDNA. To determine the diversity within the species and the genetic relationships between species, dogs belonging to the Molossoid breeds were taken for the comparison: the Portugal Serra da Estrela mountain dog, the Caucasian Shepherd Dog and the Anatolian Shepherd Dog. Although recognized as separate breeds by Turkey and several western kennel clubs, the FCI has combined Kangal and Akbash into one breed called the Anatolian Shepherd Dog with the breed number 331. Consequently, this breed reveals, in fact, a broad range of different characteristics and a high genetic variety (Vila et al; 1997).

Sequencing and analysis of the canine mitochondrial DNA has been used to determine origin of domestic dogs (Savolainen & Lundeberg, 1999; Angleby & Savolainen, 2005) and in forensic analyses (Angleby & Savolainen, 2005; Pereira et al., 2004). The phylogenetic relationship is determined by comparing the mitochondrial D-loop region and more specifically the hypervariable control region I, which are 262 and 582 base pairs (bp) long, respectively (Savolainen & Lundeberg, 1999; Angleby & Savolainen, 2005).

Six haplogroups or clades (A-F) have been reported so far (Vila et al; 1997; Angleby & Savolainen, 2005; Wayne & Ostrander, 1999). More than 71 % of all DNA samples analysed to date had haplotypes assigned to clade A (Angleby & Savolainen, 2005). More than 95 % of all haplotypes belong to the three main phylogenetic clades A, B, and C, which suggests that almost all dog populations worldwide originate from a common gene pool (Savolainen & Lundeberg, 1999). The genetic diversity of dog breeds is explained by the fact that they have a common ancestor that originated from a diverse and well-mixed gene pool. Most of them originated from east Asia and then spread througout the world (Savolainen et al., 2002; Wayne & Ostrander, 1999; Leonard et al., 2002). The high number of different mitochondrial haplotypes suggests that females were more involved in the development of a given breed than males (Sundqvist et al., 2006). Moreover, genetically diverse founders from occasional crossbreeding between different breeds and between dogs and wolves (Vila et al; 1999, Vila et al; 1997), contributed significantly to the increase of genetic heterogeneity. Most of the dog breeds show remarkable heterogeneity, which sometimes is higher between the individuals of the same breed than it is for individuals of different dog breeds (Angleby & Savolainen, 2005). Similarly, the Molosser group of dogs has a high number of haplotypes per breed.

2 MATERIALS AND METHODS

2.1 RESEARCH POPULATION

Swabs of 72 "Sharplanina dogs" (40 male and 32 female) were collected in the Sharri Mountain region in Kosovo for sequence analyses of the mtDNA. All animals belonged to private owners and were selected based on breed-specific morphological characteristics and with respect to the pedigree information (in cooperation with the Kennel Kosova Federation). The swabs were deep-frozen immediately after collection until DNA extraction. Mitochondrial DNA (mtDNA) was isolated with the Qiagen Cell Kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). We amplified the fragment of 721bp. For comparing the mtDNA data within samples we analysed only 582 bp in the first hypervariable segment of the mtDNA control region (HV1) of D-loop. Primers were designed according to the sequence deposited in GenBank (Acc. Nr. U96639). Primers were designated as H15404 (5′-CTCTTGCTCCACCATCAGC-3′) and L16125 (5′-AAACTATAT-

GTCCTGAAACC-´3). PCR and sequencing reactions were performed on a Biometra PCR thermocycler (Biometra, Goettingen). PCR was performed using 20 ng DNA, 0.2 µM of each primer, 5 µl Q-solution, 200 mM of each dNTP and 0.2 µl Taq polymerase (1U) in 1x PCR buffer as recommended by the manufacturer (Qiagen, Hilden, Germany) in a final volume of 25 µl. Reactions were performed for 30 cycles (denaturation at 93 °C for 30s, annealing at 58 °C for 30 s, extension at 72 °C for 30s) following pre-denaturation at 95 °C for 2 min and ended with a final extension at 72 °C for 2 min. Sequencing was performed bi-directionally using the Big Dye Terminator (v 3.1) cycle sequencing kit (ABI, Weiterstadt, Germany). All sequencing reactions were performed on an ABI PRISM® 3100 DNA analyzer (ABI, Weiterstadt, Germany). DNA sequencing was performed using 10 µM of the respective oligonucleotide, 3 µl Big Dye premix and 20 ng of purified PCR product as a template in a total volume of 10 µl. he sequencing conditions were 95 °C for 10 s, followed by 29 cycles of 95 °C for 10 s, annealing at 58 °C for 5 s and an extension at 60 °C for 4 min. After sequencing, a BLAST comparison was performed (http://www.ncbi.nlm.nih.gov). Analyses of the sequenced raw data were performed using Sequencing Analysis Software 3.7 (ABI, Weiterstadt, Germany). The processed data was assembled into a contig using the DNAstar SeqMan software (DNASTAR Inc. Madison, USA).

2.2 PHYLOGENETIC ANALYSES

The sequences obtained were compared by alignment with all available D-loop sequences from Molosser breed dogs (ftp://ftp.ebi.ac.uk/pub/software/clustalw2) and haplotypes were generated using Collapse1.2 (http://darwin.uvigo.es/). Medium–spanning networks were calculated using TCS software (Clement et al., 2000) and the median-joining network algorithm (Bandelt et al., 1999) using Network version 4.1 at http://www.fluxus-engineering.com. Analysis of molecular variance (AMOVA), diversity measures and FST distances were determined using Arlequin 2.0 software (Schneider et al., 2000). For Kangal and Akbash dogs, samples were collected from NCBI GenBank with accession numbers from EF660078 to EF660191. For the Caucasian Shepherd Dog, GeneBank accession numbers AF531664 and AF531731) were used. The haplotype data for the Serra da Estrela mountain dog was taken from Van Asch et al. (2005). The sequence with accession number U96639 was used as a reference for the mitochondrial DNA of the dog.

3 RESULTS AND DISCUSSION

After mitochondrial DNA analysis, we found a total of 10 haplotypes, of which one was newly described and 9 previously reported, in the 72 DNA samples analysed, as shown in Table 1.

Table 1: Total of 10 haplotypes founded on "Sharplanina dog" based on mtDNA analyses

Base pair position	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	16	16
	55	55	55	56	56	56	56	56	56	56	56	56	56	56	58	58	58	59	59	59	60	60	
	08	26	95	12	12	20	27	26	36	93	43	05	25	50	04	15	52	18	35	52	03	25	
Haplotype																							
U96639	C	C	C	T	T	T	A	C	T	T	A	T	G	T	T	C	T	C	G	C	A	T	
A11	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	C	
A17	.	.	.	.	.	C	G	.	.	A	.	.	.	.	.	T	.	.	.	T	.	.	
A20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	
A24	.	.	.	.	.	.	G	.	.	G	.	-	-	-	-	T	.	.	.	T	.	.	
A71	.	.	.	.	.	C	G	.	.	A	.	.	.	C	.	T	.	.	.	.	.	C	
B1	.	T	T	.	C	.	-	T	.	G	G	.	A	.	C	T	C	T	.	T	G	.	
B10	.	T	,	.	C	.	.	T	.	G	G	.	A	.	C	T	C	T	.	T	G	.	
nB1	.	T	T	.	C	.	.	T	C	G	G	.	A	.	C	T	C	T	.	T	G	.	
C5	T	T	.	C	.	.	.	.	.	A	.	C	.	.	C	T	.	T	_	T	G	.	

The new haplotype is very similar to the haplotype B1and differs only in one nucleotide base pair (T15636C). For understanding and standardization purposes the names of clades and haplotypes were kept the same as in the previous nomenclature used in several studies (Pereira et al., 2004). We found that the nucleotide frequencies for the entire D-loop region of the mtDNA in the "Sharplanina dog" were A = 0.268, C = 0.2730, T = 0.299, and G = 0.159. Almost the same frequencies were found in the Kangal and Akbash dog breeds, which are common in the mtDNA of all vertebrates (Tamura & Nei, 1993). Our analyses revealed the presence of 21 polymorphic sites, 20 transitions, one transversion and one insertion-deletion (indel).

The haplotypes belong to three main clades A, B and C with a frequency of 43 %, 43 % and 14 % respectively. B1 is the main haplotype found in 29.3 % of the samples, followed by A17 with a frequency of 26.3 %, while the new haplotype was found in 4.1 % of the samples analysed (Table 2).

The median joining network algorithm was used with the sequencing data generated from the samples, and the results were confirmed by the medium spanning network performing calculations using a statistical parsimony algorithm with the TCS software, as shown in the following figure.

Both methods revealed that there are 17 different single nucleotide polymorphisms (SNPs) between the haplotypes. The samples analysed were collected in 5 different regions of Kosovo and we observed that they did not always have the same haplotypes, both within breed and within samples collected in the same region. Although Kosovo is a relatively small region, we did anticipate some level of heterogeneity. However, the extent of genetic diversity revealed by the mtDNA analysis of the "Sarplanina dog" exceeded our expectations. According to Ceh (2014), a total of 15 haplotypes of this breed have been identified thus far. The pair-wise genetic distance is significant for some of the inter-region correlation, indicating no geographical correlation (data not shown). This could be explained by the high number of haplotypes within the breed and the random sampling.

Table 2: Haplotype distribution - frequencies of haplotypes for the total number of "Sharplanina dog"samples, showing nine haplotypes. The haplotype nomenclature is similar as in Angelby and Savolainen (2005). Designation nB1 represents the newly discovered haplotype.

Haplotype ID	No of samples	Frequency (%)
A11	1	1.4
A17	19	26.3
A20	3	4.2
A24	2	2.7
A71	6	8.3
B1	21	29.2
B10	7	9.7
C5	10	14.0
nB1	3	4.2
Total	72	100

3.1 COMPARISON ANALYSIS WITH THE OTHER BREEDS

Four breeds were chosen for comparative sequence analyses of the D-loop region with "Sharplanina dog". The chosen breeds were Kangal (113 dogs), Akbash (20 dogs), for which the data was collected from the NCBI repository and the literature (Savolainen et al., 2002), Caucasian Shepherd Dog (3 dogs) (Savolainen et al., 2002), and Cao de Serra da Estrela (34 dogs) (Van Asch et al., 2005). We decided to make a comparative analysis with these breeds because of their desired specifics, belonging to the same group and geography. There were

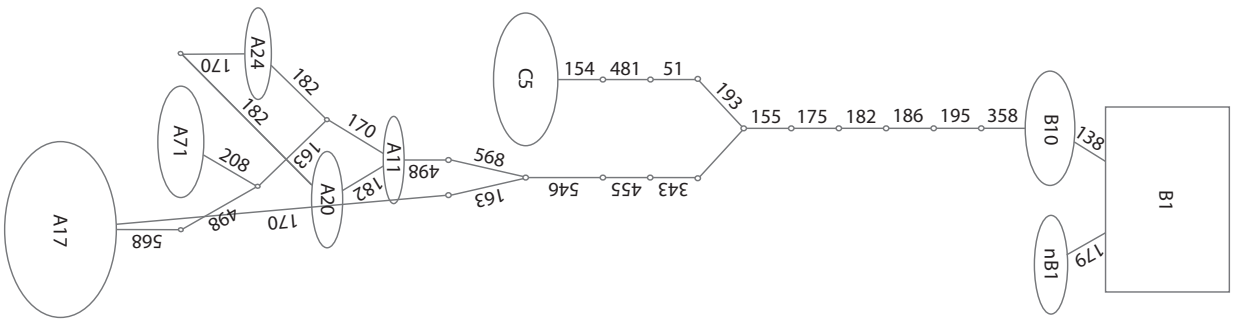

Figure 1: Sequencing data generated from the "Sharplanina dog" samples and the results were confirmed by the Medium-spanning network that performs calculations by using statistical parsimony algorithm with TCS software

6 haplotypes shared between the "Sharplanina dog" and Kangal breed, 2 haplotypes with Akbash dog, 2 haplotypes with Caucasian Ovcharka that are the two known haplotypes for this breed so far, and three haplotypes with Serra da Estrela dog, as shown in the (Fig. 2).

Only the haplotype A17 and the new haplotype B1 are not found within the breeds used for comparison. Based on the total number of dogs reported so far, the common haplotypes in percentages are as follows: 50 % of the Kangal breed dogs share the same haplotypes with the "Sharplanina dog", 52 % of the Serra da Estrela, 40 % of the Akbash and 100 % of the Caucasian Ovcharka breed populations share the same haplotypes with the Sharplanina Dog. It is noteworthy that only four haplotypes have been reported so far for the Caucasian Shepherd Dog, but the number of samples analyzed was very small (Ceh et al., 2014). The frequency of haplotype A17 was high in the "Sharplanina dog" (approx. 26 %). The haplotype is mainly found in dogs living in the northern part of Europe and in East Asia, but not in Southern Europe, except for the Azorean Mountain Dog in the Azores. This observation suggests that this breed may be maternally descended from the northern parts of Europe rather than the Portuguese mainland dog, which is consistent with the historical report (Van Asch et al., 2005). Among the ten haplotypes, haplotype A11 is the least frequent. This haplotype together with B1 and A17 represent the most dispersed haplotypes in the world, including in the mountain/molosser dogs, such as Kangal, Akbash and Caucasian Shepherd breeds. The frequency of haplotype A20 was found to be 4 % in the "Sharplanina dog". This haplotype is also rare in the Kangal and Akbash dogs. A20 haplotype is characteristic of northern Europe, and not found in Asia. The haplotype A24 was found in only two samples of the "Sharplanina dog" and was also found in Pyrenean Mountain dog, Serra da Estrela Mountain dog, and in one sample of Kangal dog. A24 haplotype seems to be characteristic for the south of Europe, except for the Irish wolfhound which is located in the British Isles in the northern part of Europe. A71 haplotype, represented in Sharplanina breed with a frequency of 6 %, was previously found in Kisha dog and reported only in Japan (east Asia) (Savolainen et al., 2002), but latter it has been found at a very high frequency in the Serra da Estrela Mountain Dog (around 26 %) (Van Asch et al., 2005). B1 haplotype is one of the haplotypes found in all regions of Europe and Asia, and we found that it is the most prevalent in the "Sharplanina dog" (29 %). A frequency is also high in the Kangal (44 %) and Serra da Estrela (17 %) breeds. B10 is one of the two haplotypes found in Caucasian Ovcharka (Savolainen et al., 2002), and this haplotype is represented with a frequency of approximatelly 10 % in our samples. Also, it was found in one sample of the Kangal breed and based on the published data it was not found in other breeds selected for our comparative analysis. C5 haplotype, represented in 14 % of the studied population has been reported in one China dog of the unknown breed (Savolainen et al., 2002), one Mongolian dog (Tsuda et al.,

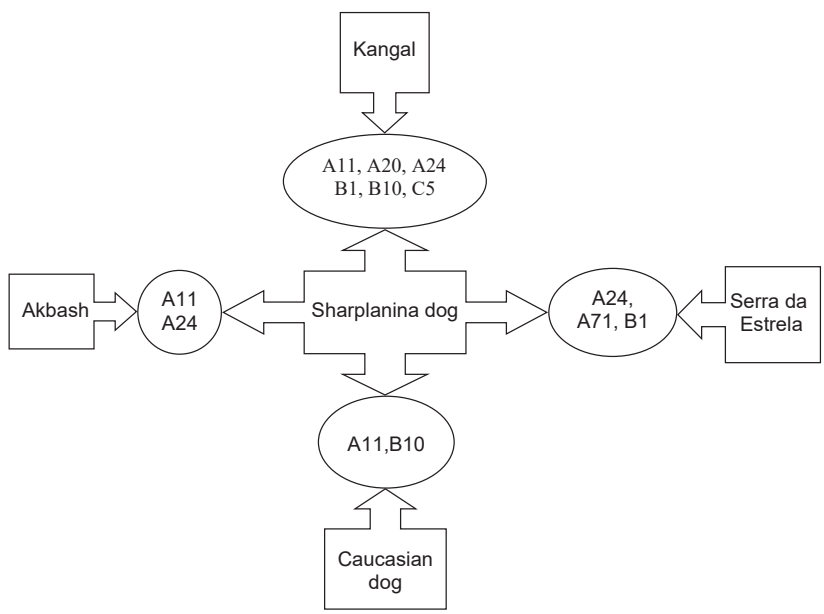

Figure 2: Schematic connection of sharing the haplotype, between the "Sharplanina dog" and the other breeds. In the square are the names of breed and in the circle the shared haplotype.

Table 3: Diversity statistics of the mtDNA D-loop region across selected dog breeds

Breed	N	Number of Haplotypes	Haplogroups	Haplotype diversity	Mean n° of pairwise differences	Nucleotide diversity
Sharplanina dog	72	9	3	0.816 ± 0.024	8.212 ± 3.852	0.014 ± 0.007
Kangal	104	21	4	0.796 ± 0.036	8.369 ± 3.906	0.014 ± 0.007
Akbash	20	10	3	0.836 ± 0.064	4.241 ± 2.195	0.007 ± 0.004
Serra da Estrela	24	8	4	0.852 ± 0.030	9.802 ± 4.601	0.017 ± 0.009
Tibetan Masttif	5	2	1	0.600 ± 0.175	3.673 ± 2.228	0.006 ± 0.004
Kaukazskaia Outcharka	3	2	2	0.666 ± 0.314	8.000 ± 5.126	0.014 ± 0.011

1997), in Anatolian Shepherd Dog (Gundry et al., 2007), and in three dogs of Kangal breed. Also, this haplotype has been reported in three samples of the "Sharplanina dog" from two different sources (Gundry et al., 2007), one originating from North Macedonia and two other samples of unknown origin. A very common clade for breeds of Europe and Asia is clade A with frequencies of about 71 %, and is also common for the Molossian dogs (Savolainen et al., 2002). In the "Sharplanina dog" clade A, was represented in 43 % of the studied dogs and is equally represented with clade B (43 %), the latter not being present in other European breeds. Also, clade A is present in 35 % of the population of Kangal breed with 12 different haplotypes. Clade A is represented in 55.9 % of the Serra da Estrela breed population, and in 90 % of the Akbash breed. In Caucasian shepherd dog a fixation index (F_{ST}) was used as a measure of genetic distance between the dog breeds and showed no geographical correlation among breeds, which might be explained by the high genetic heterogeneity of the mtDNA (Savolainen et al., 2002) . The "Sharplanina dog" has different haplotypes and this is typical of other breeds as well. Also the Tibetian Mastif showed similar distribution of clades as "Sharplanina dog", with 51.4 % of haplotypes belonging to clade A, 45.9 % to clade B and only 1 % on clade C (Li et al., 2017). It represents a breed that has a large number of haplotypes, compared with for example Serra de Estrela Mountain dog (Van Asch et al., 2005) that has 8 haplotypes classified in 4 clades or German Shepherd dog that has 7 haplotypes classified in 2 clades (Volkel, personal communication) or Shiba, the Japanese dog that has 8 haplotypes in three clades (Okumura et al., 1996). A large number of haplotypes was also observed in the Kangal breed (21 haplotypes in four clades) and in the Akbash breed (10 haplotypes in two clades), as shown in Table 3.

The reason for such a high number of haplotypes found in the Kangal dogs is explained (Altunok et al., 2005). The diversity observed in Kangal dogs may be due to repeated mating between Kangal and wild dogs, a crossbreeding known to produce offspring that resemble the Kangal type (Altunok et al., 2005). In general, the haplotype diversity found in the "Sharplanina dog" is most likely due to the origin of their ancient ancestors, that may have come from a very diverse gene pool. To this argument, we would add the fact that for thousands of generations, before the advent of modern breeding methods, dogs around the world were mating randomly (Savolainen et al., 2002). The "Sharplanina dog" itself may not originate directly from the wolf lines, but most likely descended from the oldest dogs in Europe (Molosser dogs) and was subsequently mixed with other dogs in the region.

Our results are not in accordance with the theory that the Molosser dogs are descendants of the Tibetan mastiff. We did not find any sample containing the A44 or A45 haplotype (Savolainen et al., 2002), which are typical of the mastiff breed. Not only the breeds we studied but also all other Molosser type breeds studied previously share no haplotypes with the new Tibetan mastiff which is the descendant of the old Tibetan mastiff. (Savolainenet al., 2002). However, because deep molecular analyses were not performed yet for the mastiff breeds and the Tibetan mastiff specifically, we cannot claim the above with certainty.

The second theory is that the old ancestors of the "Sharplanina dog" were from the Caucasus. Our haplotype analyses suggest that the "Sharplanina dog" might have common ancestor with the Caucasian shepherd breed. The B10 and A11 haplotypes are represented in 11 % of all our samples, the frequency that is very similar to the Caucasian Shepherd Dog. However, it cannot be considered as a fact since the haplotypes are represented with a moderate frequency value (11 %), and second because it is well-known that breeds from different regions can also have more similar haplotypes than breeds that co-exist in the same habitat (van Asch et al., 2005). The breed exists many years in the region, and is considered autochthonous, and unique for Europe. However, sampling strategy is very important for evolutionary studies (Webb et. al, 2010). The "Sharplanina dog" has three haplotypes, C5, A17, and nB1, with a cu-

mulative frequency of 37 %, which is not characteristic for any other breed in Europe. What makes this breed very specific is that clades A and B are represented in an equal frequency of 43 % and 43 %, respectively. This could be expected, as was suggested that clade A (the oldest clade) has its origin from east Asia and clade B from Europe and South Asia (Savolainen et al., 2002). We suggest that the dog ancestors of this breed came from old breed of south Asia and new breed of Europe.

Similar to other breeds, the "Sharplanina dog" has a large number of haplotypes in three main clades. Based on this discovery, it is assumed that at least ten female lines from the breed's population probably played a role in the development of the breed. Nevertheless, Ceh and Dovc (2014) identified 15 haplotype lines, but this discrepancy could be due to the sampling distribution, suggesting that the lower number of haplotypes could be a result of the sampling procedure, which was only conducted in the Kosovo region. Genetic diversity within breeds of the Molossian dog group is often high, reflecting the origin of a genetically diverse founder population, followed by occasional interbreeding between breeds and between dogs and wolves (Vila et al., 1999). Given that the Molossian group is heterogeneous, we should consider both possibilities. First, the grey wolf is very heterogeneous, and as a descendant, so is the dog.

Secondly, in the past, owners neglected to maintain the purity of the dog breed because they were not interested in genetics. Females were often copulated with males of other breeds and could also randomly copulate with wolfs (Sundqvist et al., 2006). Anyway, the breeds of the Molloser group survived through history, because of the practical utility - guarding the flock and house against predators or foreigners. The "Sharplanina dog" has high genetic diversity and very unique distribution of haplotypes, representing a mix between breeds of Europe and Asia and between northern and southern Europe. Three haplotypes are very specific for this breed especially the haplotype C5, found only in this breed in Europe, haplotype A71 only in this breed and in Serra da Estrela (Portugal), and a newly discovered haplotype, nB1 which we suggest to name haplotype B20, if we consider the haplotype designations from other studies (Angleby & Savolainen, 2005), there is no haplotype with the initials B20.

4 CONCLUSION

Looking at the origin of this breed and the breeds studied, it appears that they are descended from the ancient dog, which originated in Mesopotamia, for example, or, as some assume, in Tibet. The archaeological data, the historical data and the phylogenetic data make it seem more likely that the Molosser group breed originated somewhere more nearby. The fact that the breed's ancestors were well known at the time of Aristotle and Alexander the Great also confirms that the breed could have originated in this region. This may be explained with better genetic characterisation of other breeds in the region and the other breeds in Asia, and also wolfs in this region. The data does not give a perfect view of the phylogenetic relationship between this breed and the similar breeds, also taking into consideration that the phylogenetic correlation between breeds is/was done with different methods, for example using the chromosome Y or SNP analyses or combining maternal and paternal DNA analyses. Our study characterises the breed in a way of maternal factors and represents a step forward in phylogenetic studies of "Sharplanina Dog", which could also be used as tools for forensic purposes, especially in Kosovo and North Macedonia, representing regions with a high number of the "Sharplanina dog".

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