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The Mitochondrial landscape of the Konjuh and Majevisa mountains of northeastern Bosnia: The view in the context of genetic and demographic history

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Abstract

This study is the first report on the mtDNA profile of human settlements of the Konjuh and Majevisa mountains of northeastern Bosnia. The aims of this study were: a) determination of mitochondrial genetic structure of populations of the Konjuh and Majevisa mountains of northeastern Bosnia; b) detection of trace of ancient of mtDNA variations; and c) assessment of genetic relations with other Bosnian and Herzegovina populations and neighboring populations from the Balkan region. The genetic structure of populations of Konjuh and Majevisa is shaped by western Eurasian maternal signals, which may trace their ancestry to the Paleolithic, pre-Neolithic and Neolithic. Especially interesting is the feature of the Neolithic expansion in this area. This applies especially to the presence of the pre-Neolithic lineages HV* and N1a in northeastern Bosnia, which can indicate an early settlement of this region of Bosnia by pre-Neolithic populations from the Middle East. This region abounds with resources of salt sites, which might suggest in favor of the thesis that the early-Neolithic colonists needed a safe source of salts so as to settle in the Balkan area. The populations of mountains of northeastern Bosnia indicate elements of the local population history, but they do not show strict genetic closure in relation to the neighboring populations of the Balkans. This may be a consequence of the population size, degree of geographic isolation and events of migration.

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Introduction

Archaeological (Mihailović 2014; Dogandžić et al., 2014; Dogandžić and Đuričić, 2017; Whallom, 2017) and genetic studies (Balaesque et al., 2010; Pala et al., 2012; Brotherton et al., 2013; Mathieson et al., 2018) indicate that the Southeastern European region has had an important role in Late Glacial recolonization of the rest Europe and early influence of the Neolithic expansion from the Middle East. Bosnia and Herzegovina is one of the countries in the Southeastern European region with almost continuous complex colonization events from the prehistoric period to the present day. The archaeological data in central Bosnia (about 100,000 years old) and northern Bosnia (about 50,000 years old) point to the fact that Bosnia and Herzegovina was probably inhabited as early as the Paleolithic period (Imamović, 1998). Although some stone artifacts found in these parts of Bosnia date back to the Paleolithic era, the most famous prehistoric period of civilization dates back to the Neolithic, as evidenced by numerous archaeological investigations of Neolithic cultures. After the Neolithic period, Illyrian peoples, followed by the Romans, Goths, Slavs, Avars, Ottomans and many other conquerors left their historical mark on the soil of Bosnia and Herzegovina.

The influx of data on mtDNA variation in the field of biological anthropology indicates that they can be used very successfully in the assessment of the demographic and genetic history of human populations, migration routes and settlement of different geographical regions of the world. Traces of early human dispersal, late glacial and post-glacial re-expansion and Neolithic expansion can be traced back to early mitochondrial lineages, which are still present in recent populations (De Fanti et al., 2015). Numerous studies indicate that the genetic profile of geographically and/or culturally isolated populations, and populations with specific historical events, is very informative and interesting in a more detailed understanding of

the genetic structure of populations and the relationships of microevolutionary, migration and genetic variations that occurred in the recent demographic past (Tolk et al., 2000; Colonna et al., 2007; Jeran et al., 2009; Capocasa et al., 2013; Šarac et al., 2014). Previous studies of maternal ancestry and population history of northeastern Bosnia (general population) (Ahmić et al., 2013, 2014) indicate that genetic structure of populations of northeastern Bosnia was probably affected by waves of prehistoric and historic migrations, and later migrations with a slightly lower contribution. However, in order to get a more complete picture of the microgeographic structuring of the mtDNA variations of northeastern Bosnia, in the context of assessment of the demographic and genetic history, in this study we analyzed for the first time of the mtDNA diversity of the settlements of Konjuh and Majevisa. Mountainous regions of Konjuh and Majevisa testify to rich historical heritage in northeastern Bosnia. Due to its extremely great cultural-historical and natural value and potential, part of the Konjuh mountain area has been declared the protected landscape "Konjuh" (Official Gazette of Tuzla Canton, number 13, 09.10.2017). Traces of human settlement in the area of Konjuh date back to the prehistoric period, as evidenced by the drawings found in Djevojačka Pećina near the Brateljevići settlement (Basler, Mulaomerović, 1984). Researchers have revealed the remains of old road communications from the period of the Roman Empire (close to village Pauč), which were used in the Middle Ages, and later during the Ottoman rule, the remains of the caravan road, and many other historical artifacts (Imamović, 2021). Mountain Majevisa belongs to the region of Northeastern Bosnia, which is characterized by of the oldest settlements since the Middle Neolithic - bearers of the Starčevo culture (Perić, 2002), then the buildings of the Neolithic Vinča culture, the Bronze Age, and the medieval era (Archaeological Lexicon of Bosnia and Herzegovina, National Museum of Bosnia and Herzegovina, Sarajevo, 1988).

According to the above, objectives of our study were to: a) present for the first time the maternal ancestry of the local settlements of Konjuh and Majevisa mountains of northeastern Bosnia; b) evaluate whether these mountain communities contain traces of ancient genetic variations and of the local population history; and c) assess the genetic relations with other previously studied populations from Bosnia and Herzegovina and neighboring populations from the Balkan region (Croatia, Macedonia, Serbia, Slovenia and Montenegro).

Material and methods

Population samples and DNA extraction

Buccal swabs were collected from a total of 79 autochthonous and maternally unrelated individuals from two mountain regions: Konjuh and Majevisa (34 from Konjuh and 45 from Majevisa) of northeastern Bosnia. The analysis included individuals from following villages: Tuholj, Pauč, Goletići, Noćajevići, Gojsalići, Brateljčići, Kuljani, Kršići, Džebari (the mountain Konjuh) Stari Teočak, Tursunovo Brdo, Srednja Trnova, Janjari, Atmačići, Glinje, Sapna and Vitinica (mountain Majevisa). According to the population census from the period of 2013 ("Census of population, households and apartments in Bosnia and Herzegovina 2013") compared to other periods, there is an evident decrease in the total number of inhabitants in these mountain villages, which affected the number of autochthonous and maternally unrelated individuals. All individuals gave their consent to participate in this study and provided written consent for the collection of samples and subsequent analyses. Our study follows the principles of the Declaration of Helsinki. Total DNA was extracted using salting out method (Miller et al., 1998).

MtDNA analysis

The combined information of RFLPs (Restriction Fragment Length Polymorphisms) and HVSI

(Hypervariable Segment I) motifs were used for determination of the haplogroups. PCR reaction (Polymerase Chain Reaction) of the HVSI region sequence was performed with the set of primers F15971/R16411. GeneAmp® PCR System 9700 (Applied Biosystems) was used for amplification of the appropriate HVSI fragments. The success of the amplification reaction was checked by the process of separation of the obtained fragments on 1.5% agarose gel in 1xSB buffer. Sequencing service of the PCR products was performed in the company Macrogen Korea Inc.

Samples nucleotide sequences of the HVSI were matched and aligned with the revised Cambridge reference sequence (Anderson et al., 1981; rCRS, Andrews et al., 1999) using BioEdit software (Hall, 1999). The mtDNA haplogroups were determined by the Mitomap software (www.mitomap.com) and Haplogrep software (Kloos-Brandstätter et al., 2011; Weissensteiner et al., 2016) according to the PhyloTree build 17 (van Oven and Kayser, 2009). Additional polymorphisms of coding region of the mtDNA of the cytochrome b in populations of northeastern Bosnia (Ahmić et al., 2019) were applied for a more precise determination of the haplogroup R according PhyloTree build 17 (van Oven Kayser, 2009). The detection of relevant restriction site for mtDNA haplogroup affiliation was implemented according to the hierarchical mtDNA RFLP scheme (Santos et al., 2004). Specific primer pairs and amplification conditions for each polymorphic site were available from the literature (Torrioni et al., 1996; Santos et al., 2004).

The mtDNA data for four samples with new polymorphisms were submitted to GenBank under accession numbers MF969051, MF969048, MF969049, and MF969050.

Population-data analyses

Estimation of genetic profile of the mtDNA of the populations of Konjuh and Majevisa mountains of northeastern Bosnia and its comparison with the populations from different regions of the Balkans was analysed using methods implemented in the

Arlequin ver.3.11 software (Excofier et al., 2005). For the assessment of genetic differentiation between populations pairwise F_{ST} analysis (Weir and Cockerham, 1984) and exact-test (Reymondt and Rousset, 1995) based on haplogroup frequencies was used. Principal component (PC) analysis was performed on mtDNA haplogroup frequencies implemented by PAST software (Hammer et al., 2001).

The mtDNA data for comparative populations from the following countries of the Balkan area were included: Bosnia (region of mountain Bjelašnica) (Pojskić-Kapur et al., 2014); Bosnia 1 (general population) (Malyarchuk et al., 2003); Bosnia 2 (general population) (Cvjetan et al., 2003); Herzegovina (general population) (Cvjetan et al., 2003); Croatia (mainland) (Cvjetan et al., 2003); Croatia (island Krk) (Augustin et al., 2012); Croatia (island Cres) (Jeran et al., 2009); Croatia (Žumberak region) (Šarac et al., 2014); Serbia (general population) (Davidović et al., 2015); Slovenia (general population) (Šarac et al., 2014); Montenegro (general population) (Šarac et al., 2017).

Results and Discussion

MtDNA variations as indicators of maternal history of populations of Konjuh and Majevisa mountains of Northeastern Bosnia

Previous studies of variations of the mtDNA in populations of Bosnia and Herzegovina, including part of northeastern Bosnia, have shown that the majority of detected mtDNA haplogroups belong to the common West Eurasian gene pool with very minor portion of haplogroups which originate from East Eurasia and Africa (Malyarchuk et al., 2003; Cvjetan et al., 2004; Ahmić et al., 2013; 2014; Kovačević et al., 2014). Using the HVSI profile of the mtDNA in combination with the haplogroup-specific sites in the coding region, a total of 9 haplogroups of West Eurasian gene pool (HV*, H, J, T, K, U, X, W and N) with corresponding sub-lineages and representative Asian haplogroup R

were identified in genetic structure of settlements of Konjuh and Majevisa of northeastern Bosnia ([supplementary table 1](#)). The frequencies of the haplogroups along with corresponding sub-lineages are shown in Table 1.

Table 1. Frequencies of the haplogroups in populations of the mountains Konjuh and Majevisa

Haplogroup/population	Konjuh (N=34)	Majevisa (N=45)
H	2.94	0.00
H1	14.70	13.13
H2a	17.64	24.44
H5	2.94	2.22
H6	0.00	2.22
H11a	5.88	0.00
H13a1a1*	2.94	0.00
H24	0.00	4.44
H33c	0.00	2.22
H85	0.00	4.44
R	2.94	0.00
HVO	5.88	4.44
HV4	2.94	0.00
HV12b	2.94	0.00
N1b1b	2.94	0.00
J	0.00	2.22
J1	2.94	17.17
T2	0.00	4.44
U1a	2.94	0.00
U4c1	2.94	0.00
U5	0.00	2.22
U5a	5.88	4.44
U5b	5.88	0.00
U7	0.00	2.22
U8b1a1	2.94	0.00
K	0.00	4.44
K1a	5.88	0.00
X2e	5.88	0.00
W	0.00	2.22

Within the HV phylogeny, haplogroup H (with its corresponding sub-lineages) was found. In our study, 47.05% of the studied individuals from Konjuh mountain and 52.91% from Majevisa mountain belong to what is undoubtedly the most common haplogroup in Europe (H). The dominance of this lineage in settlements of the mountains of northeastern Bosnia is consistent with previous studies of haplogroup H distribution in populations from Bosnia (Malyarchuk et al., 2003; Cvjetan et al., 2004; Pojskić-Kapur et al., 2014) and populations from the Balkan region (Cvjetan et al., 2004; Jeran et al., 2009; Augustin et al., 2012; Davidović et al., 2015; Šarac et al., 2014, 2017). Subcluster diversification of the haplogroup H revealed nine sub-haplogroups (table 1). Haplogroups H1, H2 and H5 were shared by both populations. The most frequent haplogroups were H2a (17.64% in Konjuh and 24.24% in Majevisa), and H1 (14.70 in Konjuh; 13.13 in Majevisa). In our study, two lineages H1 and H13 have new polymorphisms according a recent global mtDNA phylogenetic tree (van Oven and Kayser, 2009), and are reported to GeneBank (accession numbers MF969049, MF969051). Previous studies indicate that common haplogroup H in Europe has arrived in Europe from the Near East prior to the Last Glacial Maximum (LGM) (22 thousand years ago, kya), and have been strongly involved in late-glacial expansions of population from ice-age refugia in Southwest Europe after the LGM (Torroni et al., 1998; Achilli et al., 2004; Pereira et al., 2005, Richards et al., 2000; Soares et al., 2010). In the study on the presence of Y-haplogroups (as equivalent to H of the mtDNA) in the Bosnian-Herzegovinian populations, Marjanovic et al. (2005) report that an increased frequency of Y- subhaplogroup I (P-37) is an indicator of re-expansion after the LMG in the Balkan populations. However, some research based on ancient human remains (Brotherton et al., 2013) indicates that Neolithic processes played a significant role in recent diversity and distribution

of haplogroup H in Europe. The composition of different sublineages within H (H1, H2a, H5, H6, H11a, H13, H24, H33, H85) including and new polymorphisms of the subhaplogroup H1a and H13 in mountain populations from northeastern Bosnia can help in the better understanding of recent diversity of haplogroup H in the Balkan region and Europe and its role during the expansion after the LGM, the Late Pleistocene, early Holocene and neolithic events. Namely, sub-haplogroup H1, one of the largest sub-branches of haplogroup H, which comprises about 13% of H haplogroup in the Balkans and 18% among Slovaks (Pereira et al., 2005; Loogväli et al., 2004), has coalescence age around 22000 YBP (years before present) (based on HVSI analysis) (Roostalu et al., 2007). The higher frequency of haplogroup H5 in southern European populations and coalescence age of about 16000 YBP coincides with the period of increased migration from southern to northern parts of Europe after the LGM (Mielnik-Sikorska et al., 2013). H6, one of the oldest haplogroup in the Near East and the Caucasus, shows in contrast, an expansion age of a mere 3400 YBP in Europe (Roostalu et al., 2007). The oldest H13 mitogenomes known are from the early Holocene of Georgia (Jones et al., 2015) and Mesolithic period in Romania and Serbia, close to 10000 YBP (Mathieson et al., 2018).

Within the HV phylogeny, we found sub-lineages very rare in European populations HV*(xH,V) (without H, V) resulting from at least two (post-Last Glacial Maximum and Neolithic) waves of migration (De Fanti et al., 2015; Shamooun-Pour et al., 2019). HV* is not particularly common in Europe, with frequencies ranging from 0% to 10% (De Fanti et al., 2015). HV* is mainly distributed in southern Europe (e.g., HVO in northern Italy and Spain; HV4 in southern Italy) and Asia (e.g., HV12 in Turkey, the Caucasus, and India) (De Fanti et al., 2015; Shamooun-Pour et al., 2019). In our study, the specificity of settlements of Konjuh mountain is reflected in the presence of rare

European lineages of the HV* clade (HVO, HV4, HV12b), that are very informative in the study of prehistoric movements after LGM populations from the Middle East to Europe and South Asia (Shamoon-Pour et al., 2019). A total of 11.76% of individuals from Konjuh mountain belong to the European sub-haplogroup HVO and haplogroups HV4a and HV12b which originate from the Near East and Caucasus (Torroni et al., 2006). Only 4.44% of individuals from Majevisa mountain belong to the HVO lineage. HV4, one of the most common HV*(xH,V) subclades in Europe, originated in Eastern Europe about 14 kya, and has been present in the Franco-Cantabrian region since 5 kya (Gómez-Carballea et al., 2012). Our sample which falls into a lineage HV4 is defined by transition 16221T, which is observed in Italian population (De Fanti et al., 2015). The pre-Neolithic haplogroup HV12 is absent in Europe (except in population of the Italian peninsula) and it is found primarily in the Near East, the Caucasus and South Asia (De Fanti et al., 2015). Although the lineages HV4, HV12b and HVO in our study are represented by a reduced frequency, it is interesting that they were also observed in the population from Herzegovina (our unpublished data), in one sample from Bosnia in the study by De Fanti et al. (2015) and in previous studies of Bosnian populations (Malyarchuk et al., 2003; Šarac et al., 2014). These data can indicate a possible early influx of pre-neolithic settlers from Middle East to these parts of the Balkans. Due to the lack of data on the phylogeny and expansion of this haplogroup in the Balkan region, our suggestion for the further research is to include individuals which belong to haplogroups HV* cluster from board population sampling of the entire Bosnian territory, as a country of the Balkan region which would enrich the HV* phylogeny database in the assessment of variations of the oldest non-European HV* (xHV) sublineages (De Fanti et al., 2015; Shamoon-Pour et al., 2019) and their role in settlement of the Balkan region.

The second most common haplogroup was U. In the gene pool of settlements in the mountains of northeastern Bosnia, two major sub-haplogroups U5a and U5b (Table 1) were detected, which appeared among the first European settlers in the early Upper Paleolithic (Richards et al., 2000; Malyarchuk et al., 2010). The haplogroup U5 participated in LGM post re-colonization from refugia zones located in the Balkans, in the coastal regions of the Mediterranean, and in the Franco-Cantabrian region (Malyarchuk et al., 2010). In the Konjuh population, in subcluster diversification of the haplogroup U5, we found ancestral type of sequence 16192-16256-16270 (U5a1), whose evolutionary time for the European population dates back to 15600-24300 YBP (according to Richards et al., 2000). The observed subclusters U5a1 indicate the possibility that they were involved in the settlement of the region of northeastern Bosnia, probably at the end of LGM. Although other characterizing haplogroups of the hunter-gather (U4, U1 and U8) (Bramanti et al., 2009) are less frequent, however, they may indicate influence of expansions from different periods of the Late Glacial and LGM post re-colonization, in the area of northeastern Bosnia, especially the Konjuh region. The Middle Upper Paleolithic haplogroups, U4 and U1 (Richards et al., 1998; 2000) were detected only in the Konjuh population with a low frequency (2.94%). The geographic distribution of haplogroup U4 in Europe ranges from 5.9% in populations of northeastern Europe (Finns, Karelians, and Estonians) to 10% in eastern Finnish populations of the Volga-Ural region and to peaks of 17% in Ugro-Samoyed populations of western Siberia (Malyarchuk, 2004). We identified only one sample of the haplogroup U4c1 in the population from Konjuh mountain. The subhaplogroup U1a with the characteristic nucleotide motif 16129-16189-16249 was only present in one sample from Konjuh. Also, one sample of the U7 sequence with HVSI motif 16309G-16318T was present in the

Majevisa population. The presence of U7 in the Balkans indicates genetic flow to Europe via Anatolia (Tambes, et al. 2003). The genetic structure of the population from Konjuh contains a rare haplogroup of European Paleolithic hunter-gatherers (U8, 2.94%), whose frequency in recent European populations is less than 1% (Cabrera, 2022).

Haplogroup J, determinant of the gene flow brought to Europe by the early Neolithic expansion from the Near East (~19 kya) whose main expansion in Europe occurred in the Late Glacial period (~16-12 kya) (Pala et al., 2012), occurs at a greater frequency in the Majevisa population (19.39%), compared to its frequency in Konjuh (2.94%) and populations of the Balkan region (Malyarchuk et al., 2003; Cvjetan et al., 2003; Jeran et al., 2009; Augustin et al., 2012; Pojskić-Kapur et al., 2014; Šarac et al., 2014; 2017; Davidović et al., 2015). The haplogroup J comprises 11% of the mtDNA variation in Europe, with the highest frequency of 13,7% in populations of Western Balkan (Kovačević et al., 2013). In addition to the present European sub-haplogroup J1b1a, dating to ~14000 ago (Pala et al., 2012), three samples with new polymorphisms designated as haplogroup J1c3f lineage (GenBank: MF969048.1) are part of the gene pool composition of the mountain region Majevisa. Namely, we found three different haplotypes of the haplogroup J1 in the Majevisa samples, of which haplotype 16063-16069-16126-16193-16348 ([supplementary table 1](#)) is designated as J1c3f lineage (GenBank: MF969048.1), and according to the mtDNA phylogenetic tree (mtDNA tree Build 15, van Oven & Kayser, 2009) none of the sublineages could be assigned to this haplotype. The subhaplogroup J1 is the most frequent and widespread in all Western Balkan populations (Kovačević et al., 2013). The cluster J1c dates to ~16000 ago is found primarily in Europe, especially central Europe, the Balkans, and Ukraine, where it encompasses almost 80% of

total J1 lineages (Pala et al., 2012). Mathieson et al. (2018) tested the DNA of Early Neolithic farmers from western Anatolia and from the Starčevo culture in neighbouring Serbia and Croatia, and found that J1c was present in this culture, along other typical European Neolithic lineages like H5, K1a, N1a, T2, W and X2 which are considered to be haplogroups of Early/Middle Neolithic (Haak et al., 2010). Other haplotypes of the Majevisa samples belong to haplogroup J1b. The increased frequency of haplogroup J1 in the Majevisa region could be a result of different effects of demographic history, but also a result of influence by different micro-evolutionary processes. In the Majevisa population, second typical Neolithic haplogroup T (with a frequency of 4.44%) is widely distributed in Europe with an average frequency of about 9% and predominantly concentrated in northwestern Italy (Babalini et al., 2005).

In addition to the standard determinants of the Neolithic expansion (J, T), the important genetic signature of the Early Neolithic populations was haplogroup N1 (Palanichamy et al., 2010) in Konjuh mountain (a frequency of 2.94%), defined by the HVS I motif 16145-16176-16223-16390 and +1081MnII site ([supplementary table 1](#)). In the neighboring Croatian population the frequency was found to be 0.83%, in Herzegovina 1.50% (Cvjetan et al., 2003), while on Cres island it was 9.24% (Jeran et al., 2009). Also, in our samples of Northeastern Bosnia other haplogroups of the mitochondrial 'Neolithic package' (K1, X and W) (Haak et al., 2010) were found. Only the subhaplogroup K is shared by both populations. Haplogroup K1, according to studies of ancient DNA, reached the European continent around 8 kya by migrations of Early Neolithic people from the Near East through continental and Mediterranean routes (Lacan et al., 2011). Interestingly, the autochthonous haplogroup of South Asia, the Indian-specific haplogroup R (Palanichamy et al., 2004; Thangaraj et al., 2009;

Sylvester et al., 2019), which is almost not present in previous studies on European populations, was found in the Konjuh sample.

Our study of variations of mitochondrial DNA of two mountain regions of northeastern Bosnia reveals mostly a West Eurasian composition of their gene pool. However, northeastern Bosnia appears to be microgeographically structured based on the distribution of some genetic signals of demographic history. This study shows that local populations of Konjuh and Majevisa are shaped by some ancient genetic signals of the Paleolithic and Neolithic period that can help in the understanding of migration and demographic events in this region. Mitochondrial profile of genetic structure of settlements of Konjuh mountain contains lineages which are related to different periods of the Late Glacial and post-glacial re-colonization of Europe (H, U5, U4, U1), the late Upper Paleolithic–Mesolithic (K) (Richards et al., 2000), pre-neolithic period (HV* clade), while typical Neolithic haplogroups (JT clade and N1) are present in a small percentage (table 1). Also, the genetic structure of the Majevisa population contains haplogroups of subsequent population expansion in the Upper Paleolithic (H and U5), of the haplogroup K which is spread by human expansion after the LGM (Richards et al., 2000) and the haplogroup J of Neolithic expansion from the Middle East with an increased frequency (table 1). The results of archaeological studies in the municipalities of northeastern Bosnia indicate traces of the Paleolithic, which date probably from 20000 to 30000 years ago. The most significant indicators of the early Neolithic period are archaeological remains of Starčevo culture (6640 to 5580 years ago) in northeastern Bosnia (Perić, 2002). The earliest archaeological evidence of the Neolithic Starčevo culture in northeastern Bosnia is associated with places of salt source where one of the expansion waves of the early Neolithic complex ended (Tasić, 2000; Perić, 2002). The

observed ancestral haplotypes (16147G) in the previous study (Ahmić et al., 2013) and ancient lineages HV* and N1a in this study, can indicate the early settlement of this region of Bosnia by pre-Neolithic populations from the Middle East. The region of Northeast Bosnia abounds resources of salt sites, might suggest in favor of the thesis that the early-Neolithic colonists needed a safe source of salts so as to settle in the Balkan area (Perić, 2002). Also, other haplogroups of the mitochondrial ‘Neolithic package’ (K1, X and W) (Haak et al., 2010) indicate a significant role of the Neolithic period in the region of northeastern Bosnia.

Genetic relations between populations of the Konjuh and Majevisa mountains of northeastern Bosnia and neighboring populations from the Balkan region

In addition, we compared inter-population differentiation based on frequency of mtDNA haplogroups in the analyzed populations and comparative populations in order to understand to what extent the discrete open and isolate populations correspond to the way in which their genetic mtDNA diversity is structured. The analysis of the exact-p value test (Raymond and Roasset, 1995) revealed a significant statistical ($p < 0.05$) difference in the frequency of mtDNA haplogroups between the populations from Konjuh and Majevisa. This result may also be a consequence of the small sample size. Pairwise F_{st} values for the distances between the mtDNA haplogroup frequencies in analyzed populations from Konjuh and Majevisa mountains of northeastern Bosnia and those from the Balkan region are illustrated in the [supplementary table 2](#). Interestingly, no significant pairwise F_{st} difference was found between the population from Konjuh and comparable populations from the Balkan region (except for the populations from Bjelašnica mountain and the island of Cres). Significant

values of genetic differentiation ($p < 0.05$) were found between Majeвица - Bjelašnica, Majeвица - Hercegovina, Majeвица- Krk, Majeвица - Žumberak and Majeвица - Cres. The summarized data on the distribution of mtDNA haplogroups of the analyzed population from northeastern Bosnia and their comparison with the European populations are presented in Figure 1.

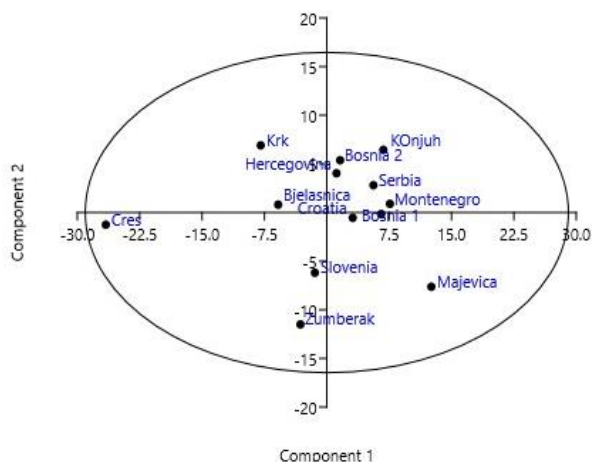


Figure 1. PC plot based on mtDNA haplogroup frequencies between comparative populations constructed from the following data: Bosnia (region of mountain Bjelašnica) (Pojskić et al., 20014); Bosnia 1 (general population) (Malyarchuk et al., 2003); Bosnia 2 (general population) (Cvjetan et al., 2003); Herzegovina (general population) (Cvjetan et al., 2003); Croatia (mainland) (Cvjetan et al., 2003); Croatia (island Krk) (Augustin et al., 2012); Croatia (island Cres) (Jeran et al., 2009); Croatia (Žumberak region) (Šarac et al., 2014); Serbia (general population) (Davidović et al., 2015); Slovenia (general population) (Šarac et al., 2014); Montenegro (general population) (Šarac et al., 2017).

The plot illustrates the distribution of settlements of Konjuh and Majeвица mountains in northeastern Bosnia in two quadrants. In the upper right quadrant, population of Konjuh is close to the populations of Serbia, Montenegro and Bosnia 2. In the lower right quadrant, on the other hand, there is only the population of Majeвица mountain. As previously suggested in studies by Pojskić-

Kapur et al. (2014), Šarac et al. (2014), Augustin et al. (2012) and Jeran et al. (2009), the isolation populations from Bjelašnica (Bosnia) and Žumberak (Croatia), then the populations of islands of Cres and Krk (Croatia) clearly show elements of the isolation populations in relation to neighboring populations of the Balkan region. Although each of the analyzed populations of the mountains of northeastern Bosnia contains the local demographic history, they do not indicate strict genetic closure, especially in the case of the population of Konjuh. This may be a consequence of the population size, degree of geographic isolation and discretionary migration events.

Conclusion

The analysis of mtDNA variation of mountain communities of northeastern Bosnia revealed new additional details on the mtDNA diversity of the Bosnian-Herzegovinian populations. Different mitochondrial lineages of Paleolithic, pre-Neolithic and Neolithic origin were found in two mountain regions of northeastern Bosnia, and can indicate input of different migrations flows in the region of northeastern Bosnia. These data can have an important role in accepting the hypothesis of LGM refugia zones located in the Balkans, as well as important pre-neolithic and neolithic impact in settlement not only in the territory of Bosnia and Herzegovina, but also the Balkans. In addition, results support previous studies that indicate that genetic structure of local populations with rich history value represents a useful model for testing hypotheses about the detection of traces of some ancient genetic variations that are still present in recent populations. The populations of mountains of northeastern Bosnia indicate elements of local population history, but they do not show strict genetic closure in relation to neighboring populations of the Balkans. This may be a consequence of the population size, degree of geographic isolation and events of migration.

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Conflict of interest

The authors report no conflicts of interest.

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