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Preliminary Report

# Frequencies of mtDNA Haplogroups in Southeastern Europe

## – Croatians, Bosnians and Herzegovinians, Serbians, Macedonians and Macedonian Romani

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### ABSTRACT

*Mitochondrial DNA polymorphisms were analyzed in of 1,610 randomly chosen adult men from 11 different regions from southeastern Europe (Croatians, Bosnians and Herzegovinians, Serbians, Macedonians and Macedonian Romani). MtDNA HVSI region together with RFLP sites diagnostic for main Euroasian and African mtDNA haplogroups were typed to determine haplogroup frequency distribution. The most frequent haplogroup in studied populations was H with the exception of Macedonian Romani among whom the most frequent were South Asian (Indian) specific variants of haplogroup M. The multidimensional scaling plot showed two clusters of populations and two outliers (Macedonian Romani and the most distant from mainland Croatian island of Korčula). The first cluster was formed by populations from three Croatian islands (Hvar, Krk and Brač) and the second cluster was formed by Macedonians, Serbians, Croatians from mainland and coast, Herzegovinians, Bosnians, Slovenians, Poles and Russians. The present analysis does not address a precise evaluation of phylogenetic relations of studied populations although some conclusions about historical migrations could be noticed. More extended conclusions will be possible after deeper phylogenetic and statistical analyses.*

**Key words:** mtDNA, haplogroups, southeastern Europe

## Introduction

Major advantages of mitochondrial genome are high mutation rate, strictly maternal inheritance and the absence of recombination. These features make mtDNA an invaluable tool for studying human origins and human genetic diversity, e.g.<sup>1–5</sup>. Southeastern Europe has been viewed as one of the key areas in major Upper Paleolithic, Neolithic and later migratory episodes and understanding of there present maternal and paternal (Y-chromosome) lineages is essential in the reconstruction of the genetic history of European populations and in understanding of its present-day diversity. The aim of this preliminary report is to present mtDNA haplogroup frequencies.

## Sample and Methods

### Sample

All blood samples were collected after obtaining informed consent. Details of sampling design and molecular analysis have been described previously. Total sample comprised of 1610 individuals from 11 different regions from southeastern Europe (Figure 1) as followed: 277 from Croatian Mainland, 133 from the island of Krk, 105 from the island of Brač, 108 from the island of Hvar and 98 from the island of Korčula (our previous investigations<sup>6,7</sup>); 96 from Croatian Coast<sup>8</sup>; and 247 from Bosnia, 130 from Herzegovina, 117 from Serbia, 146 from Macedonia and 153 Macedonian Romani (this preliminary report).

### MtDNA analysis

The hypervariable segment I (HVS-I) of the control region of mtDNA was PCR-amplified, purified using shrimp alkaline phosphatase and exonuclease treatment, followed by sequencing of the segment between nps 16024 and 16402 on MegaBace 1000 Sequencer (Molecular Dynamics/

Amersham Life Sciences) using the DYE-namic2 ET terminator cycle sequencing premix kit (Amersham). To confirm the exact haplogroup affiliation of mtDNA HVS-I lineages, a set of RLFP sites, diagnostic for main Eurasian haplogroups<sup>1,2,9,10</sup> were typed in a hierarchical order: 73 Alw44I, 1715 DdeI, 4577 NlaIII, 7025 AluI, 8249 AvaII, 9052 HaeII, 10028 AluI, 10394 DdeI, 10397 AluI, 12308 HinfI, 12406 HincII, 13366 BamHI, 13704 BstOI, 14465 AccI, 14766 MseI, 15606 AluI, 15904 MseI, following the previously established phylogenetic structure of Eurasian mtDNA lineages and haplogroups<sup>2,3,9–11</sup>.

## Results

The frequency distribution of mtDNA haplogroups in southeastern Europe (present study and our previous data<sup>6–8</sup>), Slovenia, Bosnia (1), Poland and Russia<sup>12</sup> is shown in Table 1. The most frequent haplogroup in all studied Slavonic speaking populations was H with the exception of Macedonian Romani in which the most frequent was Asian-specific haplogroup



Fig. 1. Map of the investigated region.

M, represented by sub-variants typical for South Asians<sup>13</sup>. Predominant haplogroups in Croatian Mainland were H (45.10%), J (11.90%) and U5 (11.60%) and Croatian Coast H (45.80%) and U5 (10.40%). Tolk et al.<sup>6</sup> found H the most frequent haplogroup in Croatian islands although each island showed excess frequencies of certain lineages such as I and W on Krk, HV on Brač, U5 on Hvar and H on Korčula. The most characteristic haplogroups in Bosnians were H (47.00% in this study and 47.92% in Malyarchuk et al. 2003<sup>12</sup>), U5 (8.5% in this study and 6.94% in Malyarchuk et al. 2003<sup>12</sup>) and J (7.30% in this study and 6.94% in Malyarchuk et al. 2003<sup>12</sup>), and in Herzegovinians H (43.10%), K (9.20%) and J (8.50%). Serbians had the highest frequencies of H (41%), followed by U5 (9.40%), J and U4 (6.80%). Most frequent lineages in Macedonians were H (41.10%), U5 (8.90%), J (7.50%), T2 and X (6.20%). Macedonian Romani had highest fre-

quencies of M (39.90%), followed by H (19.60%), X (11.10%) and J (8.50%). Slovenians, Poles and Russians also showed the highest frequencies of H followed by either J, T or U5.

Figure 2 shows the multidimensional scaling derived from the data in Table 1. The stress coefficient of 0.06263 implies accurate presentation of Euclidian inter-population distances, whereas RSQ coefficient of 0.99227 confirms that the 2D configuration represents a high percentage of total variance among investigated populations. The plot shows two clusters of populations and two outliers. The first cluster was formed by populations from three Croatian islands (Hvar, Krk and Brač) and the second cluster was formed by Macedonians, Serbians, Croats from mainland and coast, Herzegovinians, Bosnians, Slovenians, Poles and Russians. The Macedonian Romani are clearly separated from all considered populations due to the prevalence of the Asian-spe-

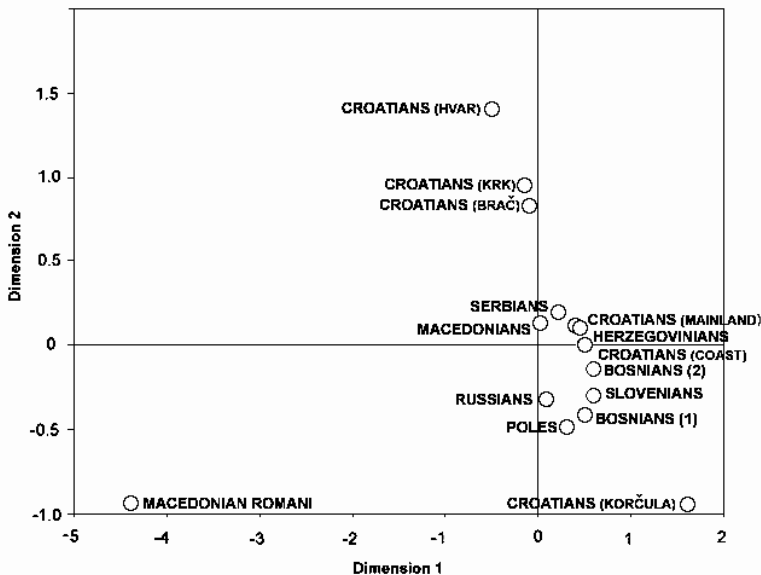


Fig. 2. Multidimensional scaling of data in Table 1. Legend: Bosnians (1) from Malyarchuk et al. 2003<sup>12</sup>; Bosnians (2) from this study.

TABLE 1  
HAPLOGROUP FREQUENCIES

Population	N	A	D4	F	H	HV	I	J	K	L2a3	L3d	M	M3c	N	N1a	N1b	pre-HV
Croatian Mainland	277	.00	.00	.40	45.10	.40	1.40	11.90	3.60	.40	.00	.40	.00	.00	.70	.40	.70
Croatian Coast*	96	.00	.00	.00	45.80	2.10	3.10	3.10	6.30	.00	.00	2.10	.00	.00	.00	.00	.00
Krk	133	.80	.00	.00	33.80	4.50	11.30	9.00	6.80	.00	.00	.00	.00	.00	.00	.00	.00
Brač	105	.00	.00	.00	35.20	10.50	1.00	8.60	9.50	.00	.00	.00	.00	.00	1.90	2.90	.00
Hvar	108	.00	.00	.80	27.80	4.60	1.90	9.30	3.70	.00	.00	.00	.00	.00	.00	.00	.00
Korčula	98	.00	.00	.00	60.20	4.10	1.00	6.10	4.10	1.00	.00	.00	.00	.00	.00	.00	1.00
Herzegovinians	130	.00	1.50	.00	43.10	5.40	0.80	8.50	9.20	.00	.00	.00	.00	.00	1.50	.00	.80
Bosnians 2	247	.00	.00	.00	47.00	5.70	2.40	7.30	5.30	.00	.00	.00	.00	.00	.00	.40	1.60
Serbians	117	.90	.00	.00	41.00	0.90	3.40	6.80	4.30	.00	.00	.00	.00	1.70	.00	1.70	.90
Macedonians	146	.00	.00	.70	41.10	2.70	1.40	7.50	3.40	.00	.00	.70	.70	.00	.00	.70	.70
Macedonian Romani	153	.00	.00	.00	19.60	0.70	4.60	8.50	2.00	.00	1.30	39.90	.00	.00	.00	1.30	.00
Slovenians**	104	.00	.00	.00	47.12	0.00	1.92	9.62	3.85	.00	.00	.00	.00	.00	.00	.00	.00
Bosnians 1**	144	.00	.00	.00	47.92	0.69	2.78	6.94	4.17	.00	.00	1.39	.00	.00	.00	.69	1.39
Poles**	436	.00	.00	.00	45.18	0.92	1.83	7.80	3.44	.00	.00	1.83	.00	.00	.00	.23	.00
Russians**	201	.00	.00	.00	42.29	1.99	2.49	7.96	2.99	.00	.00	1.49	.00	.00	.00	.00	.50

Population	N	pre-V	R	T	T1	T2	U	U1	U2	U3	U4	U5	U6	U7	U8	V	W	X
Croatian Mainland	277	0.00	0.00	0.70	2.20	5.80	0.70	0.40	1.40	0.70	2.20	11.60	.40	.00	.40	4.00	2.20	2.20
Croatian Coast*	96	.00	1.00	1.00	2.10	3.10	2.10	1.00	2.10	2.10	2.10	10.40	.00	1.00	.00	5.20	4.20	.00
Krk	133	.00	.00	0.80	0.80	3.80	0.00	0.00	1.50	.00	3.00	11.30	.00	.00	.00	5.30	7.50	.00
Brač	105	1.90	.00	1.00	1.90	2.90	1.90	.00	.00	2.90	1.00	8.60	.00	.00	.00	5.70	2.90	.00
Hvar	108	1.90	.00	.00	1.90	12.00	.00	.00	4.60	.00	0.90	14.80	.00	.00	.00	4.60	1.90	1.90
Korčula	98	.00	.00	.00	5.10	.00	2.00	1.00	2.00	.00	1.00	2.00	.00	.00	.00	8.20	3.10	.00
Herzegovinians	130	3.10	.00	0.80	2.30	3.10	.00	1.50	2.30	.00	4.60	4.60	.00	.00	.00	3.80	3.30	.80
Bosnians 2	247	2.00	0.80	2.40	0.40	.00	3.20	.00	0.80	.00	2.40	8.50	.00	1.60	.00	2.40	2.60	2.00
Serbians	117	1.70	2.60	.00	3.40	1.70	1.70	.00	1.70	0.90	6.80	9.40	.00	1.70	.00	3.40	3.40	.00
Macedonians	146	2.10	.00	.00	4.80	6.20	0.70	0.70	1.40	2.10	3.40	8.90	.00	.00	.00	1.40	2.70	6.20
Macedonian Romani	153	.00	.00	.00	2.60	0.70	.00	0.70	1.30	3.90	.00	2.00	.00	.00	.00	.00	11.10	.00
Slovenians**	104	6.73	.00	4.81	0.96	.00	.00	.00	0.96	1.92	5.77	7.69	.00	.00	.00	.00	4.81	.96
Bosnians 1**	144	6.25	.00	3.47	1.39	.00	.00	1.39	.00	0.69	5.56	6.94	.00	.00	.00	.00	1.39	1.39
Poles**	436	4.82	0.46	9.40	2.06	.00	0.23	.00	0.92	0.46	5.05	5.28	.00	.23	.46	.00	3.67	1.83
Russians**	201	5.47	0.50	8.96	1.99	.00	.00	1.00	1.49	1.00	3.48	7.46	.00	.50	.00	.00	1.99	3.48

\* from Rickards et al.<sup>8</sup>, \*\* from Malyarchuk et al.<sup>13</sup>

cific haplogroup M. The island of Korčula is distinguished from other Croatian islands mostly due to exceptionally high frequency of haplogroup H (60.20%).

Based on the presented preliminary results it may be concluded that: 1) Macedonian Romani are the most distant population group due to high frequency of haplogroup M as a presumed consequence of the origin of their mtDNA genomes and the level of their socio-cultural and reproductive isolation; 2) the effect of genetic drift and multiple founder effect followed by bottleneck in the island populations<sup>14</sup> could have been the main determinants of population history and settlement of the Croatian islands out of which the island of Korčula appears to be most

strongly affected; 3) the structuring of the remaining population cluster (consisting of Macedonians, Serbians, Croats from mainland and coast, Herzegovinians, Bosnians, Slovenians, Poles and Russians) indicates that the observed frequency distribution of maternal lineages should be applied cautiously to discussions of genetic history, different intensity of mobility and migration directions of various populations of southeastern Europe.

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## UČESTALOST HAPLOGRUPA mtDNA U JUGOISTOČNOJ EUROPI

### SAŽETAK

Polimorfizmi mitohondrijske DNA analizirani su na uzorku od 1613 slučajno odabranih odraslih muškaraca iz jedanaest regija Jugoistočne Europe. MtDNA HVSI zajedno s RFLP biljezima karakterističnim za glavne euroazijske i afričke mtDNA haplogrupe određene su kako bi se utvrdila razdioba haplogrupa. Najučestalija haplogrupa u izučavanim populacijama bila je haplogrupa H s izuzetkom populacije makedonskih Roma kod kojih je najučestalija haplogrupa M specifična za azijske populacije. Multidimenzionalnim skaliranjem dobivena su dva klastera i dvije izdvojene populacije (makedonski Romi i otok Korčula). Prvi klaster sačinjavaju populacije tri jadranske otoka (Hvar, Krk i Brač), a drugi populacije Makedonije, Srbije, kontinentalne i obalne Hrvatske te Hercegovci, Bošnjaci, Slovenci, Poljaci i Rusi. Razina rezolucije prikazanih preliminarnih rezultata nije dovoljna za precizno procjenjivanje filogenetičkih odnosa izučavanih populacija te će konačni zaključci biti mogući nakon daljnjih i neophodnih dubljih filogenetičkih i statističkih analiza.