

The Genetic Landscape of Serbian Populations through Mitochondrial DNA Sequencing and Non-Recombining Region of the Y Chromosome Microsatellites

Gabriele Scorrano¹, Andrea Finocchio¹, Flavio De Angelis¹, Cristina Martínez-Labarga¹, Jelena Šarac³, Irene Contini¹, Giuseppina Scano¹, Domenico Frezza², Natalija Novokmet³, Olga Rickards¹

¹ Center of Molecular Anthropology for Ancient DNA Studies, Department of Biology, University of Rome Tor Vergata, via della Ricerca Scientifica 1, 00133 Rome, Italy.

² Department of Biology, University of Rome Tor Vergata, via della Ricerca Scientifica 1, 00133 Rome, Italy.

³ Institute for Anthropological Research, Zagreb, Croatia

ABSTRACT

The Balkan Peninsula is known to represent a complex cultural mosaic and it is a strategic area because it represents a gateway into Europe from the Near East. This research seeks to evaluate the variability of both uniparental markers (mtDNA and non-recombining region of the Y chromosome) to dissect the genetic makeup of Serbians. The whole sample pertains to 257 Serbians (87 from the central region and 170 from the southern area) who have been analyzed for both uniparental genetic markers. The results showed that the extant inhabitants of the Balkan Peninsula have a homogeneous genetic background, despite their linguistic and cultural differences. The obtained data were compared with those of neighboring populations to detect possible relationships among groups. On the whole, the genetic variability of the Balkan populations seems to be due to an admixture process of European and Asian lineages in different proportions whose contributions constitute the current maternal and paternal genetic landscape.

Key words: Balkan peninsula, genetic variability, mtDNA, NRY, Serbian population

Introduction

Several studies have suggested that the high complexity of the Balkan Peninsula seems to be due to its geographical position, which has influenced the genetic history of its inhabitants. In fact, since the pioneering occupation by modern humans in the Paleolithic period, the Balkan Peninsula has been the location of multiple migration processes increasing their settlements and density in the Mesolithic Age^{1,2}. During the Neolithic period, populations from the Near East moved westward to Europe, probably using the Balkan Peninsula as a corridor³. During the Bronze Age, the peninsula was interested in the migration of the Urnfield people, who eventually travelled southward to colonize the entire area—from the Baltic to the Alps to the Adriatic Sea and the Apennines⁴. Since 1,000 BCE the Balkan area was inhabited by the Illyrians in the west and by the Thracians in the south-east. The dispersion of Slavic languages («Slavicization»)

is one of most important events of the peopling of Europe. It started with pre-Proto-Slavs (or Balto-Slavs) which splitted from other Indo-European languages around 2,600–5,050 BCE^{5–9} and whose origin is mapped to Central Europe⁹. Kushniarevich et al.¹⁰ pointed out the key role played by the Balkan Peninsula in the expansion of the Balto-Slavic speaking populations in Europe in particular the South Slavs populations (Bosnians, Bulgarians, Croatians, Macedonians, Montenegrins, Serbian and Slovenians) which were separated from other Balto-Slavic speaking populations¹⁰. Various Slavonic tribes began to move toward the area around the 5th or 6th centuries CE¹¹. Although the genetic history of the Slavonic populations is still unclear¹² it seems that tribes settled in the Balkan peninsula in the first half of the 7th century CE, and that, together with the Illyrians, they became the

ancestors of modern Slovenians, Croatians, and Bosnians. Meanwhile, the Slavs, along with the Thracians, are reported to have given rise to the Serbians¹³.

It is undisputed that one of the most important events affecting the cultural, ethnic, and religious structure of the Balkans was the expansion of the Ottoman Empire during the 14th century CE^{13–15}. In this period, several waves of Serbian migrations occurred as the result of numerous wars. The most important migrations occurred during the 15th century (to the Kingdom of Hungary), and the 17th and 18th centuries (the so-called Great Serbian Migrations to Austria)¹⁴.

These processes of diachronic admixture of various human groups shaped the complex cultural landscape of the Balkan Peninsula and are mirrored by the region linguistic and religious patchwork. Several genetic surveys have been performed on the Balkan populations, but most of them focused on the impact of different human expansions across Europe, comparing the Paleolithic and Neolithic genetic contributions^{16–20}. Moreover, only few works aimed to find evidence of genetic stratification related to several cultures by typing both mitochondrial DNA (mtDNA) and the non-recombining region of the Y chromosome (NRY) of the Serbian population.

This paper aims to evaluate the genetic variability of mtDNA and NRY in the Serbian population in order to

identify putative genetic relationships between Serbians and neighboring populations and to determine whether historical processes could have contributed in shaping their extant genetic compositions.

Materials and Methods

Population samples

A total of 257 mouth swab samples were collected from 4 villages in Serbia: 56 from the Studenica district and 31 from the Sumadija district, both in the central region, and 30 from Brestovac and 140 from Nish, both in the southern region (Figure 1). All the samples were taken from unrelated and apparently healthy people. Appropriate informed consent to anonymously use their data was obtained from all the donors. Ethical approval for this study was further provided by the Ethical committee of the University of Rome Tor Vergata (Protocollo di Studio Area Mediterranea, Registro sperimentazioni 92/11 date 14/06/2011). DNA was extracted in accordance with the standard protocols, as described by Budowle *et al.*²¹.

mtDNA amplification

mtDNA hypervariable segments I and II (HVS I, HVS II) of the D-loop control region were amplified using prim-

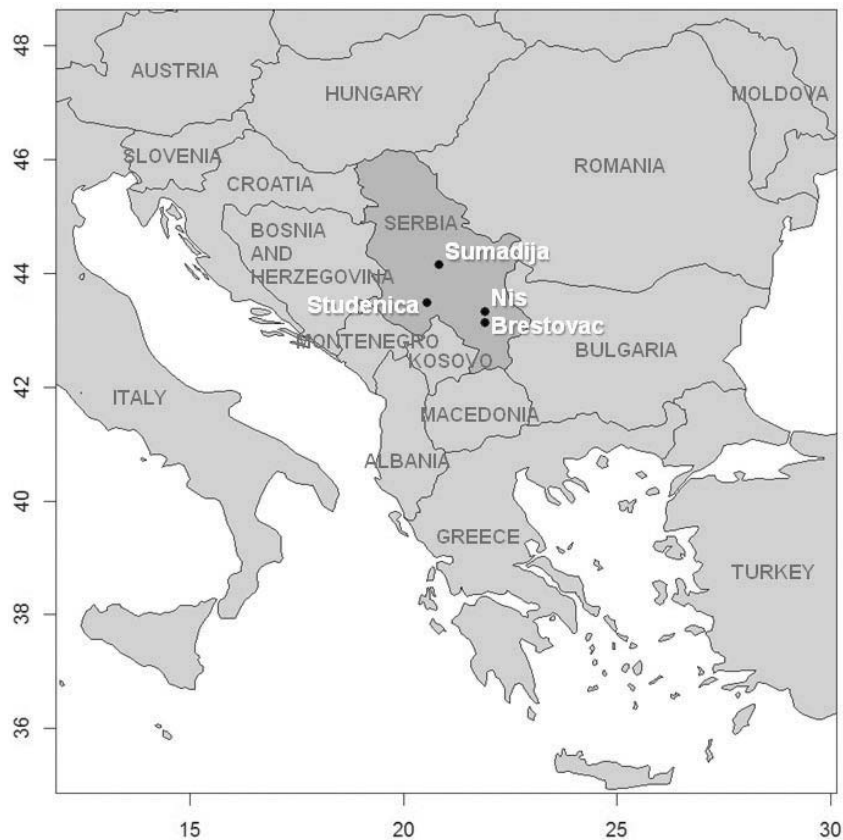


Fig. 1: Geographical location of the Serbian samples.

ers L15996 and H16401, as well as L29 and H408²² respectively. The amplification cycle conditions have previously been reported in Messina *et al.*²³. The sequencing reaction was performed for each strand, using primers L15996/H16401 and L29/H408, with the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems by Life Technologies, Foster City, CA, USA) in accordance with the supplier's recommendations and was run through the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems by Life Technologies, Foster City, CA, USA).

The sequences were then compared with the revised Cambridge Reference Sequence (rCRS)^{24,25}. Each mtDNA haplotype was phylogenetically assigned to a haplogroup following the classification proposed by van Oven and Kayser (2009) along with several additional (properly selected) diagnostic coding region SNPs for mtDNA phylogeny typing. Table S1 reports upon the informative coding region SNPs analyzed with the primers and the PCR conditions used. Published mtDNA data from several western Eurasian populations were used for comparison (Table S2).

NR1 Genotyping

Male individuals ($n=67$) were genotyped for 17 Y-STR loci (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and YGATAH4) using the AmpFISTRy filer kit (Applied, Foster City, CA, USA by Life Technologies) following the manufacturer's recommendations²⁶. STR amplification products were analyzed on ABI Prism 3130 Avant (Applied Biosystems by Life Technologies, Foster City, CA, USA). Allele analysis was carried out using GeneMapper v. 4.1 (Applied Biosystems by Life Technologies, Foster City, CA, USA) and Y-STR haplogroups were inferred using Whit Athey's Haplogroup Predictor²⁷. In order to confirm haplogroup assignments, 10 NR1 mutations catalogued by Karafet *et al.*²⁸ (M12, M70, M215, M253, M267, M343, M410, P15, P37.2, SRY10831.1) (Table S3) were genotyped by TaqMan assays using Real-Time PCR System (Real Time 7500 Fast Applied Biosystems by Life Technologies, Foster City, CA, USA) as manufacturing description. The nomenclature used in this study is adopted from the YCC2008 tree. Since the literature data on paternal lineages have different phylogenetic resolution, such as 17 STR, 9 STR and 7 STR, the inferred genetic structures were homogenized in order to allow population comparisons. Therefore, data on 9 Y-specific microsatellites (DYS19, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS385a and b) were compared with several published results (see Table S2).

Statistical Analyses

The genetic diversity of the four Serbian groups: (from the central, southern, and western regions) were assessed by evaluating the 95% credible regions for the haplogroup frequencies with the Sampling software (kindly provided by V. Macaulay, Department of Statistics, University of Glasgow).

The genetic diversity in the Balkan populations was evaluated through genetic distance analysis considering the sampled populations and neighboring human groups. FST²⁹ and RST³⁰ distance matrices were calculated respectively for mtDNA sequences and NR1-chromosome profiles using Arlequin v.3.5³¹. The relationships among these populations were graphically displayed by non metric Multi-Dimensional Scaling (nmMDS) (Kruskal, 1964)³² using Past software v.2.08b³³.

Results

The mtDNA haplotypes found in the different Serbian samples are shown in Table S4.

In 257 analyzed individuals, 178 different mtDNA haplotypes for HVS-I and HVS-II were identified. Of these, 138 mtDNA types were unique, 19 haplotypes were found twice, 13 haplotypes were found three times, and 8 haplotypes were shared by more than three individuals. The Serbians' mtDNA haplogroup frequencies are shown in Table 1. As reported in other Europeans^{20,34–39}, the most common haplogroup detected in the Serbian population is haplogroup H, with a frequency of 43.59%. To identify sub-haplogroups, diagnostic SNPs were analyzed. The most frequent is H* (11.67%), which comprises haplotypes that could not be assigned to any of the sub-groups studied. Upon grouping all the sub-haplogroups, the H1 (5.85%) and H2 (4.66%) appeared to be the most frequent sub-clades in the Serbian population. The H1 appears considerably more frequently (in around 30% of haplogroups H) in Western Europeans and in Slavic-speaking Eastern Europeans^{35,36,40}. The two most common sub-clades of H1 in Europe⁴¹ are H1a and H1b, with the latter reaching a frequency of only 2.34% in Serbian sample. The U cluster has a frequency of 14%, and only some of the sub-haplogroups are represented in Serbians. The most common U sub-haplogroups is U5, with a weak frequency of 3.12%; it appears with relative frequency in Central and Eastern Europe⁴² but is unrepresented in Serbians. Haplogroup HV is present with a frequency of 8.17%, which is consistent with the rate reported for Europe. Haplogroups T1 (5.04%), J (8.95%), and U3 (1.95%) suggest the presence of Neolithic lineages^{34,43} in Serbia. MtDNA sub-haplogroups descending from the macro-haplogroup N—such as N1a, N1b, W, and X2—are found in few individuals, while the R and V clades are present in two and three individuals respectively. The frequency distribution of haplogroup I was in accordance with a typical European mtDNA pool³⁴ and with the published data for Serbia^{44,45}. Non-European haplogroups L2a1, C, and D4* are also found only twice, once, and three times respectively.

To deepen the differentiation among the four sub-samples analyzed and among the neighboring populations, a nmMDS was generated considering the five Serbian regions (Central, Southern, the Vojvodina province, Western, and Eastern areas) (Figure 2a).

Studenica and Sumadija mtDNA haplotypes were pooled to other data from the Central regions^{20,46}, and the

TABLE 1
 MtDNA HAPLOGROUPS FREQUENCIES IN THE SERBIAN POPULATION ANALYZED.

Haplogroups	Sub-haplogroups	Frequencies	Haplogroups	Sub-haplogroups	Frequencies
H		43.59	HV		8.17
	H*	11.67		HV*	4.65
	H1	0.78		HV2	0.39
	H1ah2	0.39		HV4a	0.78
	H1aj1	0.39		HV0	0.39
	H1b	0.39		HV0a	0.78
	H1b1	1.17		HV10	1.17
	H1b1b	0.78	T		7.39
	H1c	1.17		T1a	0.78
	H1e	0.39		T1a1	2.70
	H1m1	0.39		T1a1'3	1.56
	H2a1	1.55		T2	0.78
	H2a2a	3.11		T2b	1.17
	H3p	0.39		T2c1d	0.39
	H5	3.11	J		8.95
	H5a4	0.39		J*	2.33
	H5a5	0.39		J1b1a1	1.17
	H5q	0.39		J1c	1.55
	H6	3.89		J1c1c	0.39
	H66a	0.39		J1c2	0.39
	H7a1	0.39		J1c2a2	0.39
	H7c1	0.39		J1c2e	0.78
	H7c4	0.39		J1c2e1	0.39
	H8	0.39		J1c3c	0.39
	H11	0.78		J2a1a1	0.78
	H11a	1.55		J2b1c1	0.39
	H11a1	1.17	K		5.45
	H11a2	0.39		K*	2.33
	H13a1b	2.72		K1a	0.39
	H13a1c	0.39		K1b1c	1.55
	H13b1	1.17		K1b2	0.39
	H20	1.17		K2	0.39
	H24	0.78		K2a5	0.39
	H28	0.39			1.56
	H74	0.39	N1		0.39
U		14.00		N1a1a	0.39
	U*	0.39		N1b1	0.39
	U2e	0.39		N1b1a	0.39
	U2e1'2'3	1.17		N1b1b	0.39
	U2e1b	0.78	I1		2.33
	U3	0.39		I1	1.55
	U3a	0.78		I1a	0.39
	U3b2b	0.78		I1d	0.39
	U4	2.70	V		1.17
	U4a2	1.94		V	0.39
	U4a2a	0.39		V6	0.39
	U4b3	0.39		V19	0.39
	U5a	0.78	W		2.33
	U5a1	0.39		W*	1.17
	U5a2e	0.39		W1*	0.78
	U5b1b1	0.78		W1e1a	0.39
	U5b2a5	0.39			1.94
	U5b3	0.39	X		1.17
	U8b1	0.39		X2	1.17
	U8b1a	0.39		X2m'n	0.39
				X2n	0.39
			R		0.78
				R*	0.39
				R31	0.39
			C		0.39
			D4*		1.17
			L2a1		0.78
			Total		100.00

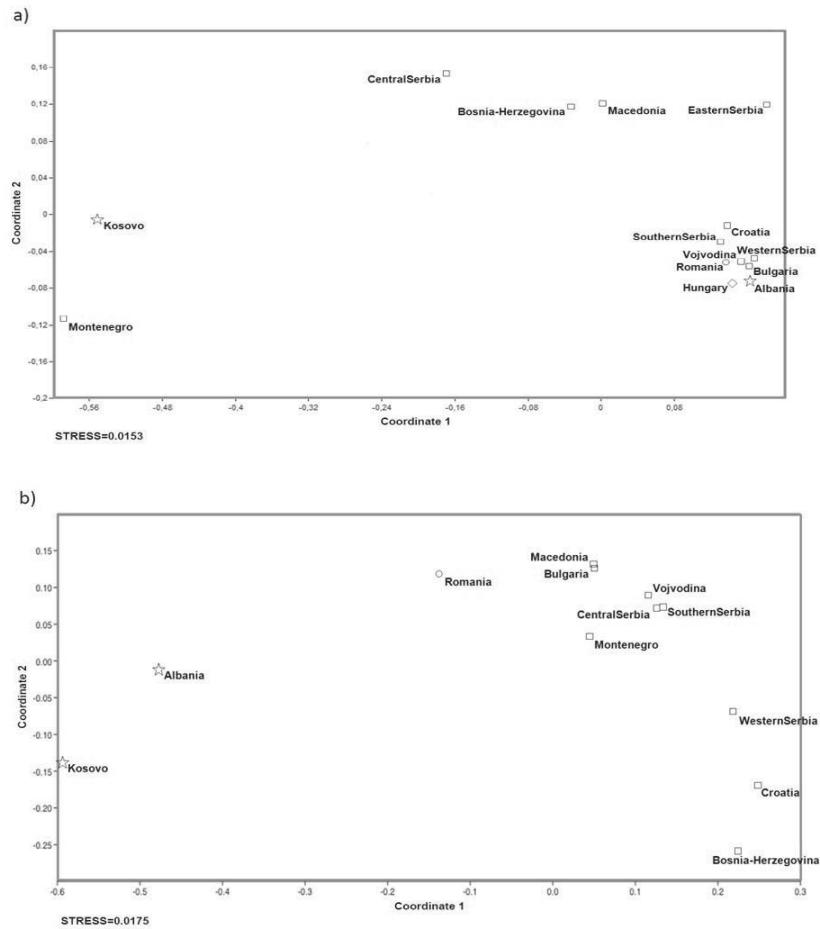


Fig. 2 Non Metric Multidimensional Scaling (nmMDS) analysis from distance matrices among haplotypes of the different Serbian regions and neighboring populations: a) *FST* genetic distances for mtDNA; b) *Rst* genetic distance for the Y-chromosome.

Brestovac and Nish results are added to the southern Serbian regions⁴⁶ because no significant differences were found among them ($p > 0.05$ for each pair of groups). For Serbians from the West, East⁴⁶ and the Vojvodina province^{45,46} published data were used for comparison. The low stress value³² which measures the goodness of fit of the obtained distances in the configuration space, suggests that the degree of distortion of the pairwise distances in the two-dimensional space was acceptable. The nmMDS (Figure 2a) highlights a scenario where the populations are not clustered according to languages or geographic locations. Additionally, no cluster is highlighted when the different religions present in the Balkan Peninsula are taken into account. The majority of the populations cluster together in a corner of the plot (Figure 2a), suggesting a degree of genetic affinity among them, but the Central and Eastern Serbians are placed in an external position that could be attributed to genetic diversity among the different regions. However, this result could be due to the scar-

city of the sample size. Moreover, among the Balkan populations, only groups in Kosovo and Montenegro fall into external positions, suggesting a heterogeneous mitochondrial composition of the populations.

The list of different STR haplotypes and Y chromosome haplogroups found in the Serbian samples are shown in the Table S5. The haplogroup frequencies for Serbian populations are shown in Table 2. Out of the 67 males collected, 55 different Y-chromosome haplotypes, belonging to 11 haplogroups, were identified: 46 were unique, 6 were found twice, and 3 were found three times. The most frequent haplogroup is I (43.5%), particularly the subclade I2a (42%), that shows the same frequencies previously reported for Serbians⁴⁷. The haplogroups E1b (16.5%)—especially E1b1b (12%)—and J2 and G2 show higher frequencies in the Balkan Peninsula populations than in other European populations,⁴⁸. The E1b1, J1, and T were observed only in three individuals (4.5%).

TABLE 1 (S1)
 PRIMERS (5' TO 3') AND PCR CONDITIONS USED FOR THE CODING REGION MTDNA SNPS ANALYSIS.

Position	Nucleotide change	Forward Primer	Reverse Primer	T _a annealing °C	Total size (bp)
426	A to T	LF393 CoAAATTTTATCTTTT	H605 ATTGCTTTGAGGAGGTAAGC	54	212
461	C to T	L393 CAAATTTTATCTTTT	H605 ATTGCTTTGAGGAGGTAAGC	54	212
482	T to C	L393 CAAATT TTATCTTTT	H605 ATTGCTTTGAGGAGGTAAGC	54	212
629	T to C	L580 ATGTAGCTTPACCTCCTCAA	H690 ACGGGGATGCTTGCATGTGT	56	110
1118	A to G	L920 TCAATAGAAGCCGGCGTAAAG	H1451 TCAGGGCCCTGTTCAACTAA	60	531
1189	T to C	L920 TCAATAGAAGCCGGCGTAAAG	H1451 TCAGGGCCCTGTTCAACTAA	60	531
1391	T to C	L1361 GGCTACATTTTCTACCCCA G	H1451 TCAGGGCCCTGTTCAACTAA	60	531
1438	A to G	L1361 GGCTACATTTTCTACCCCA G	H1451 TCAGGGCCCTGTTCAACTAA	60	531
1811	A to G	RL1654 TAACTTGACCGCTCTGAGAT	H1874 TTTGGCTCTCCTTGCAAAGT	59	220
2755	A to G	L2281 ACCCTATAGAAGAACTAATG	H2791 CGAAATTTTTAATGCAGGTT	50	510
3010	G to A	L2978 GTCCATATCAACAATAGGG	H3221 GCCATCTTAACAACCCTGT	54	243
3027	T to C	L2978 GTCCATATCAACAATAGGG	H3221 GCCATCTTAACAACCCTGT	54	243
3336	T to C	L3108 TTCAAATTCCTCCCTGTACG	H3361 CGTTCGGTAAGCATTAGGAA	55	253
3348	A to G	L3108 TTCAAATTCCTCCCTGTACG	H3361 CGTTCGGTAAGCATTAGGAA	55	253
3392	G to C	L3108 TTCAAATTCCTCCCTGTACG	H3630 AACGGCTAGGCTAGAG	51	522
3394	T to C	L3108 TTCAAATTCCTCCCTGTACG	H3630 AACGGCTAGGCTAGAG	51	522
3480	A to G	L3108 TTCAAATTCCTCCCTGTACG	H3630 AACGGCTAGGCTAGAG	51	522
3992	C to T	L3816 CACCTCTGATTACTCCTGC	H4102 GAAGTAGGGTCTTGGTGAC	57	286
4024	A to C	L3816 CACCTCTGATTACTCCTGC	H4102 GAAGTAGGGTCTTGGTGAC	57	286
4188	A to G	L4091 AGACCCTACTTCTAACCTCC	H4281 TACTCTATCAAAGTAACTCT	53	190
4216	T to C	L4180 AACTTCTACCCTCACC	H4281 TACTCTATCAAAGTAACTCT	51	101
4336	T to C	L4180AACTTCTACCCTCACC	H4621 TGGCAGCTTCTGTGGAAC	55	481
4491	G to A	L4180 AACTTCTACCCTCACC	H4621 TGGCAGCTTCTGTGGAAC	55	481
4580	G to A	L4519 CACTC ATCACAGCGCTAAGC	H4621 TGGCAGCTTCTGTGGAAC	57	142
4646	T to C	L4590 TTCCAGTTPCTAACCAAAAAA	H4661 GAAGGATTATGATGCGGTT	52	71
4715	A to G	L4590 TTCCAGTTPCTAACCAAAAAA	H4861 GGCTAGTTTTTGTGTCATGTGA	56	217
4745	A to G	L4590 TTCCAGTTPCTAACCAAAAAA	H4861 GGCTAGTTTTTGTGTCATGTGA	56	217
4793	A to G	L4770 CTATAGCAATAAAACTAGG A	H4861 GGCTAGTTTTTGTGTCATGTGA	51	91
5178	C to A	L5147 AAACCTCCAGCACCACG	H5193 GGTGTTAGTCATGTTAGCTT	52	46
5471	G to A	L5221TAGGAGGCCTGCCCCGCTA	H5611 TGATTTGCGTTCAGTTGGATG	59	300
5656	A to G	L5604 CACTCTGCATCAACTGAACG	H6e263 GGCCTCCACTATAGCAG	58	659
5913	G to A	L5604 CACTCTGCATCAACTGAACG	H6263 GGCCTCCACTATAGCAG	58	659
5999	T to C	L5604 CACTCTGCATCAACTGAACG	H6263 GGCCTCCACTATAGCAG	58	659
6221	T to C	L6149 AGTTCCCCTAATAATCGGTG	H6263 GGCCTCCACTATAGCAG	55	114
6776	T to C	L6740 TGGTCTGAGCTATGATATCA	H7041 GGCAAATACAGCTCCTATTGATAGGAC	58	301
7028	C to T	L6948 ACCGTAGGTGGCCTGACTGG	H7041 GGCAAATACAGCTCCTATTGATAGGAC	63	93
7094	T to C	L6948 ACCGTAGGTGGCCTGACTGG	H7115 CGTAGGTTTGGTCTAGG	58	167
7705	T to C	L7458 GAATCGAACCCCCAAAGCTG-GTTTCAAGC	H7817 GGGCGATGAGGACTAGGTTAGTTAGTTT TG	68	359

TABLE 1 (S1)
Continued

Position	Nucleotide change	Forward Primer	Reverse Primer	T _a annealing °C	Total size (bp)
8818	C to T	L8571 AGGCCTACCCGCCGAGTAC	H9014 TAGGTGGCCTGCAGGTAATGT	61	443
8994	G to A	L8908 TTCTTACCACAAGGCACACC	H9014 TAGGTGGCCTGCAGGTAATGT	57	106
9000	A to G	L8908 TTCTTACCACAAGGCACACC	H9014 TAGGTGGCCTGCAGGTAATGT	57	106
9055	G to A	L9003 CCTAA CCGCTAACATTACP	H9105 TGTA AACGACGGCCAGTGAAGATGATAAGT GTAGA	60	102
9335	C to T	L9200 CACATAATGACCCACCAATC	H9580 GGAGTGGGACTTCTAGGGGA	58	380
9698	T to C	L9500 TGAGCCTTTTACCCTCCAG	H10088 GTAGTAAGGCTAGGAGGGTG	56	588
9899	T to C	L9500 TGAGCCTTTTACCCTCCA G	H10088 GTAGTAAGGCTAGGAGGGTG	56	588
10034	T to C	L9976 TTGATGAGGGTCTTACTC	H10088 GTAGTAAGGCTAGGAGGGTG	53	112
10238	T to C	L10163 ACGGCTACATAGAAAAATCCR	H10466 ATTTATGTAATGAGGGGCATTTGG	303	49
10398	A to G	L10359 GTCTGGCCTATGAGTGACTAC	H10466 ATTTATGTAATGAGGGGCATTTGG	59	107
10400	C to T	L10359 GTCTGGCCTATGAGTGACTAC	H10466 ATTTATGTAATGAGGGGCATTTGG	59	107
10550	A to G	L10359 GTCTGGCCTATGAGTGACTAC	H10840 ATAATTAGGCTGTGGGTGGT	58	481
10664	C to T	L10359 GTCTGGCCTATGAGTGACTAC	H10840 ATAATTAGGCTGTGGGTGGT	58	481
10873	T to C	L10830 AATCAACACAACCACCACA	H10900 GGGGAACAGCTAAATAGGTT	55	70
10907	T to C	L10830 AATCAACACAACCACCACA	H11141 GATGATAGCCAAGGTGGG	56	311
11251	A to G	L11200 TACTTCCTATTCTACACCCT	H11271 TAGAATGTTTAGTGAGCCTA	52	71
12308	A to G	L12216 CACAAGAAGTGTAACTCATGC	H12595 TGGAGAAGTAGTCTAGTTTGAA	57	379
12414	T to C	L12216 CACAAGAAGTGTAACTCATGC	H12595 TGGAGAAGTAGTCTAGTTTGAA	57	379
12636	C to T	L12580 TAGACTACTTCTCCATAATA	H12661 TTAATGTTTGGGTCTGAGTT	50	81
12705	C to T	FL12641 GAATTCTCACTGTGATATATA	H12761 TCTCAGCCGATGAACAGTTG	54	120
12753	A to G	L12641 GAAT TCTCACTGTGATATATA	H12761 TCTCAGCCGATGAACAGTTG	54	120
12771	G to A	L12641 GAATTCTCACTGTGATATATA	H13121 TTCTGCTAGGGGTGGAAGC	56	480
12950	A to C	L12641 GAATTCTCACTGTGATATATA	H13121 TTCTGCTAGGGGTGGAAGC	56	480
13449	C to T	L13041 AGCCATAGAAGCCCCACCC	H13741 GCGGGGAAATGTTGTTAGT	60	700
14470	T to C	L14398 AACACTCACCAAGACCTCAACC	H14509 TTTTTTAATTTATTTAGGGGGGGT	57	111
14569	G to C	L14398 H14832 AACACTCACCAAGACCTCAACCRAAGTGAGCC- GAAGTTTCATCATG	59	434	
14766	C to T	L14729 TCAACTACAAGAACACCAATGACC	H14832 AGTGAGCCGAAGTTTCATCATG	60	103
14798	T to C	L14729 TCAACTACAAGAACACCAATGACC	H14832 AGTGAGCCGAAGTTTCATCATG	60	103
14872	C to T	L14821 CAACATCTCCGCATGATGAA	H14900 GTCTGGTGAGTAGTGCATGG	57	79
15218	A to G	L14821 CAACATCTCCGCATGATGAA	L15682 GGCGAAATATTATGCTTTTGT	53	861
15904	C to T	L15861 TTGAAAACAAAATACTCAAAA	H 15971 AGACTTTTCTCTGATTTGT	47	110
16526	G to A	L16453 CCGGGCCATAACACTTGGGG	H16543 CGTGTGGGCTATTTAGGC	61	90

TABLE 2 (S2)

POPULATIONS, REFERENCES AND NUMBER OF SAMPLES OF MTDNA AND THE Y-CHROMOSOMES USED FOR THE COMPARISONS

Population	mtDNA		Y-chromosome	
	N	References	N	References
Albanians	83	Belledi and colleagues ¹ , Bosch and colleagues ²	151	Bosch and colleagues ² , Pericic and colleagues ³
Bosnians	587	Malyarchuk and colleagues ⁴ , Sarac and colleagues ⁵ , Kovacevic and colleagues ⁶	292	Klaric and colleagues ⁷ , Kovacevic and colleagues ⁸
Bulgarians	140	Richards and colleagues ⁹ , Calafell and colleagues ¹⁰	373	Zaharova and colleagues ¹¹ , Karachanak and colleagues ¹²
Croats	603	Sarac and colleagues ⁵ , Kovacevic and colleagues ⁶ , Babalini and colleagues ¹³	684	Barac and colleagues ¹⁴ , Lovrecic and colleagues ¹⁵ , Sarac and colleagues ¹⁶
Hungarians	211	Irwin and colleagues ¹⁷	116	Furedi and colleagues ¹⁸
Kosovo	26	Kovacevic and colleagues ⁶ , Davidovic and colleagues ¹⁹	117	Pericic and colleagues ³
Macedonian	241	Bosch and colleagues ² , Kovacevic and colleagues ⁶ , Zimmermann and colleagues ²⁰	135	Bosch and colleagues ² , Pericic and colleagues ²¹
Montenegro	26	Kovacevic and colleagues ⁶ , Davidovic and colleagues ¹⁹	404	Mirabal and colleagues ²²
Romanians	193	Bosch and colleagues ² , Richards and colleagues ⁹	171	Bosch and colleagues ² , Barbarii and colleagues ²³
Serbian	481	Present research; Kovacevic and colleagues ⁶ , Davidovic and colleagues ¹⁹ , Zgonjanin and colleagues ²⁴	876	Present research; Mirabal and colleagues ²² , Lauc and colleagues ²⁵ , Stevanovic and colleagues ²⁶ , Veselinovic and colleagues ²⁷ , Regueiro and colleagues ²⁸

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TABLE 3 (S3)
SNPS IN THE NON-RECOMBINING REGION OF THE Y CHROMOSOME GENOTYPED AND PRIMERS (5' TO 3')
USED IN AMPLIFICATION.

Marker-	Nucleotide change	Position (bp)	Forward Primer	Reverse Primer	Total size (bp)
M215	A to G	163	GTA AAAACTCAGATATATACATCCCATG	AAAAAAAAGAATCACTATCTTAACG	386
P15	C to T	138	AGAGAGTTTTCTAACAGGGCG	TGGGAATCACTTTTGCAACT	191
M253	C to T	283	GCAACAATGAGGGTTTTTTG	CAGCTCCACTCTATGCAGTTT	400
P37.2	T to C	135	CGTCTATGGCCTTGAAGA	TCCGAAAATGCAGACTTT	447
M267	T to G	148	TTATCCTGAGCCGTTGTCCCTG	TGTAGAGACACGGTTGTACCCT	287
M410	A to G	115	CAATCATTGACCTTAAGTCTGAGTCCC	ACTGGATACCTTTCTAGGAAGAATTG	395
M12	G to T	286	ACTAAAACACCATTAGAAACAAAGG	CTGAGCAACATAGTGACCCC	309
SRY_10831.2	G to A	135	CCACAACCTCTTTTCATC	AATAAAAATCCCCTAAAATA	536
M343	C to A	402	TTTAACCTCCTCCAGCTCTGCA	ACCCCCACATATCTCCAGG	424
M70	A to C	45	GGTTATCATAGCCCCTACTACTTTG	ATCTTTATCCCTTTGTCTTGCT	257

TABLE 2
Y-CHROMOSOME HAPLOGROUPS FREQUENCIES IN THE
SERBIAN POPULATION ANALYSED.

Haplogroups	Sub-haplogroups	Frequencies
E1b		16.5
	E1b1b	12.0
	E1b1	4.5
I		43.5
	I1	1.5
	I2a	42.0
J		13.5
	J1	4.5
	J2a	4.5
J2b		4.5
	J2b	4.5
	R1	
R1a		15.0
R1b		6.0
T		4.5
G2a		1.5
Total		100.0

The two most frequent sub-clades of R1 in Europe are R1a, found in the North-East, and R1b, found in the West. This geographic distribution could reflect episodic population expansions during the post-glacial period, including those associated with the spread of agricultural/pastoral culture.^{49–53} The frequency of R1b in Serbians (6%) is lower than what is present in other southeastern European populations but it is consistent with the frequency reported for another Serbian sample⁴⁷. Even for the Y chromosome nmMDS was generated considering the STR haplotypes of four split Serbian regions and neighboring populations. Studenica and Sumadija mtDNA haplotypes were pooled to other data from the Central regions^{47,54,55} because no significant differences were found among them ($p > 0.05$ for each pair of groups). To what concerns Southern Serbian region, Brestovac and Nish results are used for the analysis. For Serbians from the West⁵⁶ and the Vojvodina province⁵⁷ published data were used for comparison while for the East it was not possible to use published data. The Y-chromosome nmMDS plot was first generated considering the same populations used for mtDNA analysis (Figure S1). In this case, the Hungarian population appeared apart while all the other populations clustered together. At a later step, the RST matrix and nmMDS were reconstructed, excluding this population from the analysis.

TABLE 6 (S4)
 mtDNA HAPLOTYPES, NUMBER OF INDIVIDUALS (N); HAPLOGROUPS; HVS-I, HVS-II, AND MTDNA CODING REGION SNPS OF THE DIFFERENT SERBIAN SAMPLES.

Haplotype	N	Haplogroup	Polymorphisms								
Brestovac 1	2	K1b1c	16224C	16311C	73G	94A	263G	315.1C	1189C	5913A	
Brestovac 2	3	T1a1'3	16126C	16163G	16186T	16189C	16294T	73G	152C	195C	263G
Brestovac 3	2	K*	16224C	16311C	73G	263G	315.1C	3480G	9055A		
Brestovac 4	1	U3a	16311C	16343G	16390A	73G	150T	263G	309.1C	315.1C	1811G
Brestovac 5	1	U2e1'2'3	16051G	16129C	16183C	16193.1C	16209C	16362C	73G	217C	228A
Brestovac 6	6	HV	16311C	263G	315.1C	3010G	5471G	7028T	7094T	8994G	9000A
Brestovac 7	1	H20	16218T	16256T	263G	315.1C	3010G	6776T			
Brestovac 8	1	H7a1	16048A	16203G	16252T	16261T	200G	263G	309.1C	315.1C	1438G
Brestovac 9	1	J*	16069T	16126C	73G	188G	263G	295T	309.1C	315.1C	4216C
Brestovac 10	2	J1c2e	16069T	16126C	16366T	73G	185A	188G	228A	263G	295T
Brestovac 11	2	W*	16223T	16292T	73G	189G	194T	195C	204C	207A	263G
Brestovac 12	4	H11a	16293G	16311C	152C	195C	263G	309.1C	315.1C	1438G	3010G
Brestovac 13	1	H11	16293G	16311C	263G	315.1C	1438G	14872C			
Brestovac 14	2	H13b1+200	16261T	200G	263G	309.1C	315.1C	1438G	3010G	7028C	14872T
Brestovac 15	1	H*	16189C	16193.1C	16356C	16362C	16376T	195C	204C	263G	309.1C
Nish 1	1	H13b1	16048A	16261T	200G	263G	309.1C	315.1C	3010G	7028C	14872T
Nish 2	1	U5a2e	16189C	16192T	16234T	16256T	16270T	16311C	16362C	73G	151T
Nish 3	1	H*	16287T	195C	263G	315.1C	1438G	3010G	4024C	7028C	14766C
Nish 4	1	W1e1a	16223T	16292T	16295T	16324C	73G	189G	195C	204C	207A
Nish 5	1	U5b2a5	16189C	16270T	16311C	73G	150T	263G	309.1C	315.1C	12308G
Nish 6	1	T2b	16126C	16294T	16296T	16304C	73G	263G	315.1C	4216C	12705C
Nish 7	1	U5a	16192T	16256T	16270T	73G	263G	315.1C	12308G		
Nish 8	1	N1a1a	16172C	16223T	16248T	16355T	73G	199C	204C	263G	315.1C
Nish 9	1	N1b1b	16145A	16176A	16223T	16390A	73G	195C	263G	272G	315.1C
Nish 10	1	T2c1d	16126C	16290T	16292T	16294T	73G	146C	263G	279C	309.1C
Nish 11	1	V6	16162G	16298C	72C	263G	294C	309.1C	315.1C	14766C	15904T
Nish 12	1	H1	16261T	16319A	263G	315.1C	1438G	3010A			
Nish 13	1	H*	16169T	263G	309.1C	315.1C	7028C	14766C			
Nish 14	1	H11	16311C	195C	263G	309.1C	315.1C	1438G	3010G	7028C	
Nish 15	1	K2	16224C	16311C	73G	146C	263G	309.1C	315.1C	1811G	12308G
Nish 16	1	X2	16183C	16189C	16223T	16264T	16278T	16376T	73G	153G	225A
Nish 17	1	U3	16189C	16343G	73G	150T	263G	309.1C	315.1C	1811G	12308G
Nish 18	1	R31	16362C	73G	239G	263G	309.1C	315.1C	12705C		
Nish 19	1	J2a1a1	16069T	16126C	16145A	16231C	16261T	73G	150T	152C	195C
Nish 20	1	U8b1	16172C	16183C	16189C	16234T	16311C	73G	150T	195C	263G
Nish 21	1	U4a2	16356C	73G	195C	263G	310C	629T	1811G	8818T	10907T
Nish 22	1	H6	16362C	239C	263G	315.1C	3010G	7028C			
Nish 23	1	L2a1	16051G	16223T	16278T	16294T	16309G	16390A	73G	143A	146C
Nish 24	1	K*	16224C	16311C	73G	150T	152C	263G	309.1C	315.1C	1811G
Nish 25	1	H1e	16129A	263G	315.1C	1438G	3010A	7028C			
Nish 26	1	H13a1c	16244A	16399G	146C	207A	263G	309.1C	315.1C	3010G	7028C
Nish 27	1	X2	16183C	16189C	16278T	16376T	73G	153G	195C	263G	309.1C
Nish 28	1	H2a2a	263G	315.1C	1438A						
Nish 29	1	D4*	16223T	16362C	73G	195C	263G	309.1C	315.1C	14668T	
Nish 30	1	V	16162G	16298C	263G	294C	309.1C	315.1C	4580A	7028T	14766C
Nish 31	1	H*	143A	263G	309.1C	315.1C	1438G	3010G	7028C		
Nish 32	1	H*	16059G	16155G	16318G	263G	309.1C	315.1C	1438G	3010G	7028C
Nish 33	1	N1b1	16145A	16176G	16223T	16390A	73G	152C	185A	263G	315.1C
Nish 34	1	HV0	16298C	72C	263G	315.1C	7028T	14766C			
Nish 35	1	U3a	16343G	16390A	73G	150T	263G	315.1C	1811G	12308G	
Nish 36	1	U4	16270T	16356C	73G	195C	263G	315.1C	340T	389A	1811G
Nish 37	1	J1c2e1	16069T	16126C	16324C	16366T	16390A	73G	185A	188G	228A
Nish 38	1	U2e	16051G	16129C	16169T	16183C	16189C	16193.1C	16362C	16376T	73G
Nish 39	1	J2b1c1	16069T	16126C	16193T	16319A	73G	150T	152C	263G	295T
Nish 40	1	HV0a	16217C	16243C	16261T	72C	73G	152C	195C	263G	309.1C
Nish 41	1	J1c3c	16069T	16126C	16160G	16222T	16342C	73G	185A	228A	263G
Nish 42	1	R0	16092C	16189C	16294T	16376T	73G	195C	263G	315.1C	1391T
Nish 43	1	W1*	16193T	16223T	16292T	73G	119C	152C	189G	195C	204C
Nish 44	1	U2e1b	16051G	16129C	16189C	16256T	16274A	16362C	73G	152C	217C
Nish 45	2	K1b1c	16224C	16311C	73G	94A	263G	315.1C	1189C	5913A	
Nish 46	1	J1b1a1	16069T	16126C	16145A	16172C	16222T	16261T	73G	242T	263G
Nish 47	2	H11a1	16278T	16293G	16311C	195C	263G	309.1C	315.1C	3010G	7028C
Nish 48	2	U5b1b1	16093C	16189C	16270T	16376T	73G	150T	263G	315.1C	5656G
Nish 49	1	U5a1	16075C	16256T	16270T	16399G	152C	214G	263G	309.1C	315.1C

315.1C	4216C					
12308G						
263G	309.1C	315.1C	1811G	12308G		
13449C	14766C					
4793G	7028C					
12705C						
309.1C	315.1C	4216C	12705C			
315.1C	10398A	12414C				
7028C						
315.1C	3010G	6776T	7028C			
152C	263G	309.1C	315.1C	12308G		
263G	315.1C	10398A	12414C			
10398A						
3027T	5178C	10238C	10398A	12308A		
315.1C	4216C	12705C				
263G	309.1C	315.1C	10398A			
215G	263G	295T	311T	319C	4216C	12705C
309.1C	315.1C	1811G	3480G	9698C	12308G	
12308G						
152C	195C	263G	315.1C	10664C		
12308G						
14872T						
315.1C	10398A					
360G	10398A					
5999C	12308G					
263G	295T	309.1C	315.1C	4216C	12705C	
152C	263G	315.1C	1811G	12308G		
309.1C	315.1C	4216C	12705C			
315.1C	4580G	7028T	12308A	14766C	15904C	
295T	309.1C	315.1C	12705C	14798C		
1438G	2755A	3010G	7028T	12705C		
207A	263G	315.1C	10398A			
263G	315.1C	340T	1811G	12308G		
295T	315.1C	4216C	12705C			
12308G						
12308G	15218G					

TABLE 4 (S4)
Continued

Haplotype	N	Haplogroup	Polymorphisms								
Nish 50	2	H24	16293G	262T	263G	315.1C	1438G	7028C	8448T		
Nish 51	1	U4	16356C	73G	195C	263G	315.1C	629T	1811G	7705T	8818C
Nish 52	1	H8	16288C	263G	315.1C	7028C					
Nish 53	1	C	16223T	16298C	16327T	73G	249.d	263G	309.1C	315.1C	4715G
Nish 54	1	H5	16304C	16362C	263G	315.1C	1438G	7028C			
Nish 55	2	H5	16304C	263G	315.1C	426T	1438G	3010G	7028C	14766C	
Nish 56	1	H7c4	16265C	263G	309.1C	315.1C	4793G	7028C			
Nish 57	1	U3b2b	16062G	16074G	16172C	16189C	16343G	16362C	73G	150T	263G
Nish 58	1	X2m'n	16183C	16189C	16278T	16376T	73G	185A	188G	195C	225A
Nish 59	1	H5q	16192T	16304C	16335G	263G	315.1C	7028C	8975C		
Nish 60	1	T1a1	16126C	16163G	16186T	16189C	16294T	73G	195C	263G	309.1C
Nish 61	1	H*	16129A	16189C	195C	263G	3010G	7028C			
Nish 62	1	U2e1b	16051G	16129C	16183C	16189C	16256T	16258C	16362C	152C	183G
Nish 63	4	J*	16069T	16126C	16261T	73G	263G	295T	309.1C	315.1C	4216C
Nish 64	1	T2b	16126C	16221T	16294T	16296T	16304C	73G	263G	309.1C	315.1C
Nish 65	1	U*	16189C	16301T	16319A	73G	150T	263G	315.1C	1811A	12308G
Nish 66	1	H5a5	16304C	146C	152C	263G	309.1C	315.1C	1438G	4336C	
Nish 67	1	H1	16162G	263G	309.1C	315.1C	3010A	7028C			
Nish 68	1	H5	16183C	16189C	16304C	16376T	263G	315.1C	1438G	7028C	
Nish 69	1	J2a1a1	16069T	16126C	16145A	16231C	16261T	16362C	73G	150T	152C
Nish 70	1	HV2	16217C	16243C	16261T	73G	124C	152C	195C	263G	309.1C
Nish 71	1	W*	16223T	16292T	16311C	73G	143A	189G	195C	204C	207A
Nish 72	1	H1m1	16311C	146C	263G	315.1C	1438G	3010A	7028C		
Nish 73	1	H*	182T	263G	315.1C	1438G	3010G	6776T	7028C		
Nish 74	1	H11a1	16278T	16293G	16311C	195C	263G	315.1C	1438G		
Nish 75	1	U4b3	16356C	73G	195C	215G	263G	309.1C	315.1C	1811G	12308G
Nish 76	1	H66a	16172C	16233C	103C	263G	309.1C	315.1C	1438G	3010G	7028C
Nish 77	1	I1	16129A	16223T	16311C	16390A	16391A	73G	146C	199C	204C
Nish 78	1	K1b2	16224C	16311C	73G	146C	195C	263G	315.1C	1811G	3480G
Nish 79	1	L2a1+143	16189C	16209C	16223T	16278T	16294T	16309G	73G	143A	146C
Nish 80	1	K*	16224C	16304C	16311C	73G	150T	263G	315.1C	1811G	10550G
Nish 81	1	T2	16126C	16294T	16296T	16344T	73G	263G	315.1C	4216C	12705C
Nish 82	1	H1aj1	16192T	150T	263G	309.1C	315.1C	1438G	3010A	7028C	
Nish 83	1	J1c2	16069T	16126C	73G	146C	185C	188G	228A	263G	295T
Nish 84	2	T1a	16126C	16163G	16186T	16189C	16294T	73G	263G	315.1C	4216C
Nish 85	1	U5b3	16192T	16270T	16304C	73G	150T	152C	228A	263G	315.1C
Nish 86	1	H*	16184T	263G	315.1C	1438G	3010G	7028C	14766C		
Nish 87	1	T1a1'3	16126C	16163G	16186T	16189C	16294T	73G	152C	195C	263G
Nish 88	1	U8b1a	16172C	16183C	16189C	16311C	16376T	73G	150T	195C	263G
Nish 89	1	H1b	16189C	16356C	16376T	263G	315.1C	3010A			
Nish 90	1	H*	16142T	16325C	146C	263G	309.1C	315.1C	1438G	3010G	7028C
Nish 91	1	H5a4	16093C	16294T	16304C	16390A	257G	263G	309.1C	315.1C	1438G
Nish 92	1	H*	16042A	152C	263G	315.1C	1438G	3010G	5178C	6776T	7028C
Nish 93	1	J1c1c	16069T	16126C	16145A	16183C	16189C	16231C	16261T	16376T	73G
Nish 94	1	T1a1	16126C	16163G	16186T	16187T	16189C	16193.1C	16294T	73G	151T
Nish 95	2	U4a2	16356C	73G	183G	195C	263G	310C	1811G	12308G	
Nish 96	1	H11a2	16092C	16293G	16311C	146C	195C	263G	315.1C	1438G	
Nish 97	1	H1ah2	16212G	16222T	263G	315.1C	7028C	14766C	3010A		
Nish 98	1	H*	16163G	16287T	195C	263G	315.1C	1438G	3010G	3992C	4024A
Nish 99	1	J1c2a2	16069T	16126C	16256T	73G	185A	188G	228A	263G	295T
Nish 100	1	I1d	16129A	16148T	16223T	16311C	73G	199C	204C	250C	263G
Nish 101	1	K*	16124C	16224C	16311C	73G	150T	263G	315.1C	1811G	10550G
Nish 102	2	H2a2a	263G	309.1C	315.1C	1438A					
Nish 103	1	H6	16362C	239C	263G	315.1C	1438G				
Nish 104	1	X2n	16092C	16183C	16189C	16193.1C	16223T	16266T	16278T	16376T	64T
Nish 105	1	H6	16171G	16362C	239C	263G	309.1C	315.1C	7028C	14766C	
Nish 106	2	HV4a	16221T	152C	263G	315.1C	1438G	7028T	14766C		
Nish 107	1	H2a1	16354T	263G	309.1C	315.1C	1438A				
Nish 108	1	U4	16356C	73G	143A	195C	252C	263G	1811G	5999C	12308G
Nish 109	1	H*	16311C	249d	263G	310C	315.1C	1438G	3010G	6776T	7028C
Nish 110	3	H1c	152C	263G	315.1C	1438G	3010A	6776T			
Nish 111	1	H*	16278T	263G	309T	310C	311T	315.1C	3010G	7028C	14872C
Nish 112	2	H6	16183C	16189C	16362C	16376T	146C	152C	239C	263G	309T
Nish 113	1	U3b2b	16093C	16343G	16362C	73G	150T	195C	263G	315.1C	1811G
Nish 114	1	K2a5	16224C	16311C	73G	146C	152C	263G	315.1C	324T	1811G
Nish 115	4	HV	16311C	263G	315.1C	3010G	5471G	7028T	7094T	8994G	9000A

10907T	12308G											
309.1C 226C	315.1C 263G	1118G 309.1C	12308G 315.1C	6221C	10398A							
315.1C	4216C	12705C										
217C 12705C 4216C 15218A	263G 12705C	340T	1811G	12308G								
195C 315.1C 263G	215G 7028T 309.1C	263G 3010G 315.1C	295T 14872C 10398A	311T	319C	4216C	12705C					
250C 12308G 152C 12308G	263G 195C	315.1C 199C	10398A 263G	309.1C	315.1C	10664C						
315.1C 12705C 12308G	11251G	12705C	14798C									
315.1C 309.1C	4216C 315.1C	12705C 1811G	2755A	3348A	3480G	12308G	12705C	12753A	12771A	12950A	15218A	
14766C 3010G 12308A 150T 195C	14872C 7028C 152C 263G	195C 309.1C	198T 315.1C	263G 4216C	295T 12705C	315.1C	4216C	12705C				
7028C 315.1C 309.1C 12308G	4216C 315.1C	12705C 10398A										
73G	153G	159C	195C	225A	226C	6221C	10398A					
14766C												
310C 4188G 3480G 13449C	7028C 12308G 12308G 14766C	14766C										

TABLE 4 (S4)

Continued

Haplotype	N	Haplogroup	Polymorphisms								
Nish 116	1	W1*	16292T	73G	119C	189G	195C	204C	207A	263G	315.1C
Nish 117	1	J*	16069T	16126C	73G	185A	263G	295T	315.1C	4216C	12705C
Nish 118	1	K*	16224C	16311C	73G	263G	315.1C	3480G	9055A		
Nish 119	1	J1c	16069T	16183C	16189C	16376T	73G	185A	228A	263G	295T
Nish 120	1	H74	16218T	16256T	72G	263G	315.1C	3010G	6776T	7028C	12753G
Nish 121	1	V19	16298C	16362C	72C	263G	309.1C	315.1C	328G	14766C	15904T
Nish 122	1	H28	186A	263G	309.1C	315.1C	1438G				
Studenica 1	1	H*	16142T	16325C	146C	263G	309.1C	315.1C	1438G	3010G	7028C
Studenica 2	1	H7c1	16093C	16265G	263G	309.1C	315.1C	1438G	3010G	4793G	6776T
Studenica 3	6	H*	16051G	16312G	195C	263G	309.1C	315.1C	1438G	3010G	3392C
Studenica 4	2	T1a1	16126C	16163G	16186T	16189C	16294T	73G	152C	195C	232G
Studenica 5	3	T1a1	16126C	16163G	16186T	16189C	16292T	16294T	73G	195C	263G
Studenica 6	1	HV*	16311C	263G	309.1C	315.1C	330T	343T	4577C	5471G	7028T
Studenica 7	3	H*	16311C	249d	263G	310C	315.1C	1438G	3010G	6776T	7028C
Studenica 8	2	H1b1b	16189C	16193.1C	16355T	16356C	16362C	199C	263G	315.1C	3010A
Studenica 9	1	H5	16304C	263G	315.1C	426T	1438G	3010G	7028C	14766C	
Studenica 10	1	U4a2a	16183G	16254G	73G	153G	195C	263G	310C	1811G	4646C
Studenica 11	1	D4*	16174T	16362C	16384A	73G	183G	263G	309.1C	315.1C	461C
Studenica 12	3	H5	16051G	16304C	263G	315.1C	456T	1438G	3010G	7028C	14766C
Studenica 13	1	U2e1'2'3	16051G	16129C	16182C	16183C	16189C	73G	152C	200G	217C
Studenica 14	1	U2e1'2'3	16051G	16129C	16182C	16183C	16189C	73G	152C	200G	217C
Studenica 15	3	H13a1b	16189C	16193.1C	146C	153G	204C	263G	309.1C	315.1C	1438G
Studenica 16	1	H*	195C	263G	309.1C	315.1C	3010G	7028T	12308A	14766C	
Studenica 17	1	H*	16189C	16193.1C	263G	315.1C	3392C	4793A	6776T	7028C	12308A
Studenica 18	1	T2b	16126C	16294T	16296T	16304C	73G	263G	309.1C	315.1C	4216C
Studenica 19	2	J1b1a1	16069T	16126C	16145A	16172C	16261T	73G	242T	263G	295T
Studenica 20	1	N1b1a	16145A	16176G	16223T	16244A	16390A	73G	146C	263G	315.1C
Studenica 21	1	HV0a	16217C	16243C	16261T	72C	73G	152C	195C	263G	309.1C
Studenica 22	3	HV10	16311C	263G	309.1C	315.1C	5471G	7028T	8994G	13449T	14766C
Studenica 23	1	H13a1b	16189C	16193.1C	153G	204C	263G	315.1C	1438G	3010G	3392C
Studenica 24	1	HV	16311C	263G	315.1C	3010G	5471G	7028T	7094T	8994G	9000A
Studenica 25	1	H*	16183C	16189C	16193.1C	146C	153G	204C	263G	315.1C	1438G
Studenica 26	1	D4*	16174T	16362C	73G	263G	315.1C	461C	3336T	4491G	10400T
Studenica 27	1	U5a	16192T	16256T	16270T	73G	175G	263G	309.1C	315.1C	12308G
Studenica 28	1	X2	16182C	16183C	16189C	16223T	16248T	16278T	73G	153G	195C
Studenica 29	1	H3p	16222T	73G	152C	263G	309.1C	315.1C	1438G	6776C	12308A
Studenica 30	4	U4	16356C	73G	195C	263G	315.1C	629T	1811G	7705T	8818C
Studenica 31	1	I1a	16129A	16172C	16223T	16311C	73G	199C	204C	250C	263G
Studenica 32	1	K1a	16093C	16224C	16311C	73G	146C	195C	263G	309.1C	315.1C
Studenica 33	2	U4a2	16356C	73G	195C	263G	310C	629T	1811G	8818T	10907T
Studenica 34	1	J1c	16069T	16126C	73G	185A	263G	295T	309.1C	315.1C	482T
Sumadija 1	1	H2a2a	263G	309.1C	315.1C	1438A					
Sumadija 2	2	H20	16218T	16256T	263G	315.1C	3010G	6776T			
Sumadija 3	1	H2a2a	263G	309.1C	315.1C	1438A					
Sumadija 4	3	I1	16129A	16223T	16311C	16390A	16391A	73G	199C	204C	250C
Sumadija 5	2	H1b1	16189C	16193.1C	16356C	16362C	16380T	263G	315.1C	1438G	3010A
Sumadija 6	1	H2a1	16354T	146C	263G	315.1C	1438A				
Sumadija 7	1	H13a1b	16189C	16193.1C	16376T	146C	153G	263G	315.1C	7028C	14872T
Sumadija 8	3	H2a2a	263G	315.1C	1438A						
Sumadija 9	2	H13a1b	16189C	16193.1C	146C	153G	263G	315.1C	7028C	14872T	
Sumadija 10	1	H1b1	16189C	16193.1C	16356C	16362C	16376T	263G	315.1C	1438G	3010A
Sumadija 11	1	H*	16189C	16193.1C	16380T	146C	152C	153G	263G	315.1C	7028C
Sumadija 12	1	H*	16189C	146C	152C	153G	263G	315.1C	7028C	14872C	
Sumadija 13	1	H*	16093C	16291T	195C	263G	315.1C	7028C	1438G	3010G	
Sumadija 14	2	H2a1	16354T	263G	315.1C	1438A					
Sumadija 15	1	H6	16362C	152C	239C	263G	315.1C	1438G			
Sumadija 16	3	H6	16362C	152C	239C	263G	309.1C	315.1C	1438G		
Sumadija 17	1	T2	16126C	16294T	16296T	73G	263G	315.1C	4216C	12705C	
Sumadija 18	1	H6	16362C	152C	183G	239C	263G	309.1C	315.1C	7028C	
Sumadija 19	2	J1c	16069T	16126C	73G	185A	228A	263G	295T	315.1C	4216C
Sumadija 20	1	H*	16192T	16261T	16289G	118A	152C	183G	187A	263G	315.1C

10398A

309.1C 315.1C 4216C 12705C
12950A 14766C

14766C 14872C
7028C 14766C
4793A 6776T 7028C 14470T 14766C 14872C
263G 315.1C 4216C 9899C 12705C
309.1C 315.1C 4216C 9899C 12705C
13449C 14766C
14766C
7028C 14766C

8818T 12308G
3336T 4491G 10400T 10873C 12308A 12636T 14569C 14668T

263G 315.1C 1811G 12308G
263G 309.1C 315.1C 1811G 12308G
3010G 3392C 4793A 6776T 7028C 12308A 14470T 14766C 14872T

14470T 14766C
12705C
309.1C 315.1C 345T 4216C 12705C
9335T 10238C 10398A
315.1C 4580G 7028T 12308A 14766C 15904C

4745G 4793A 6776T 7028C 12308A 14470T 14766C 14872T
13449C 14766C
3010G 6776T 7028C 14766C
10873C 12308A 12636T 14569C 14668T
15218A 16526A
263G 315.1C 10398A 10400C 10873T 12308A 12705T
12705C
10907T 12308G
315.1C 4216C 10034C 10398A
9055A 12308G
12308G
3010A 3394T 4216C 12705C

263G 315.1C 10398A

14872C

12705C
3010G 7028C 14872C

TABLE 5 (S6)
 Y-STR HAPLOTYPES, NUMBER OF INDIVIDUALS (N), SNP, AND HAPLOGROUPS OF SERBIAN MALES ANALYZED.

Haplotypes	N	DYS 393	DYS 390	DYS 19	DYS 391	DYS 385a	DYS 385b	DYS 439	DYS 389 1	DYS 392	DYS 389 2	DYS 458	DYS 437	DYS 448	H4	DYS 456	DYS 438	DYS 635	SNP	Haplogroups
Brestovac 1	3	13	24	16	11	13	14	14	13	11	30	18	15	19	11	14	10	23	P37.2_C	I2a
Brestovac 2	2	13	24	16	11	14	15	12	13	11	30	17	15	19	11	15	10	23	P37.2_C	I2a
Brestovac 3	1	12	23	14	11	14	18	11	14	11	30	16	15	20	11	15	9	23	M410_G	J2a
Brestovac 4	3	13	24	15	11	14	15	13	13	11	31	18	15	19	11	15	10	23	P37.2_C	I2a
Brestovac 5	1	13	24	16	12	14	16	12	13	11	31	17	15	19	11	15	10	22	P37.2_C	I2a
Brestovac 6	1	13	22	14	10	13	17	10	13	11	30	15	14	20	11	15	10	23	M215_G	E1b1b
Brestovac 7	2	13	22	14	10	13	17	10	13	11	30	15	14	20	11	16	10	23	M215_G	E1b1b
Brestovac 8	1	13	24	15	12	14	16	12	13	11	31	17	15	19	11	15	10	22	P37.2_C	I2a
Brestovac 9	1	13	25	15	10	11	14	10	13	11	30	15	14	20	12	15	11	23	SRY_10831.2_A	R1a
Brestovac 10	2	13	24	16	10	14	15	12	13	11	31	17	15	20	11	16	10	22	P37.2_C	I2a
Brestovac 11	3	13	23	17	10	14	16	14	13	12	32	17	15	20	11	15	10	24	P37.2_C	I2a
Nish 1	1	13	24	13	10	16	17	11	13	11	30	16	14	20	12	16	10	22	M215_G	E1b1b
Nish 2	1	13	25	17	10	11	14	11	13	11	29	17	14	20	12	18	11	23	SRY_10831.2_A	R1a
Nish 3	1	13	24	14	11	14	15	13	13	11	31	17	14	19	12	15	10	24	P37.2_C	I2a
Nish 4	1	13	25	13	10	16	17	12	13	11	31	17	14	20	11	16	10	22	M215_G	E1b1b
Nish 5	1	13	24	13	10	16	17	12	13	11	31	17	14	20	12	16	10	22	M215_G	E1b1b
Nish 6	1	12	24	14	11	12	15	12	13	13	29	16	15	20	12	17	12	23	M343_A	R1b
Nish 7	1	13	24	14	11	15	16	11	13	13	30	18	14	18	11	15	9	22	M70_C	T
Nish 8	1	13	24	13	10	17	18	12	13	11	31	16	14	20	12	16	10	22	M215_G	E1b1b
Nish 9	1	13	23	14	10	13	14	11	12	11	28	15	16	20	12	14	10	22	M253_T	I1
Nish 10	1	13	25	16	10	11	14	11	13	11	29	16	14	20	12	17	11	23	SRY_10831.2_A	R1a
Nish 11	1	13	25	17	10	11	14	12	13	11	29	16	14	20	12	17	11	24	SRY_10831.2_A	R1a
Nish 12	1	13	24	16	11	14	15	13	13	11	30	17	15	19	11	15	10	22	P37.2_C	I2a
Nish 13	1	13	24	16	11	14	15	12	13	11	31	17	15	20	11	15	10	22	P37.2_C	I2a
Nish 14	1	12	23	14	10	12	18	11	12	11	29	18	14	19	12	15	10	20	M267_G	J1
Nish 15	1	13	24	16	11	14	15	12	13	11	29	17	15	20	11	15	10	24	P37.2_C	I2a
Nish 16	1	13	24	15	12	11	15	11	13	11	31	15	14	20	12	16	11	23	SRY_10831.2_A	R1a
Nish 17	1	12	23	15	10	15	18	12	12	12	28	16	14	19	12	15	9	22	M12_T	J2b
Nish 18	1	12	24	14	10	11	14	12	13	14	28	19	15	20	12	17	12	23	M343_A	R1b
Nish 19	1	13	24	14	11	10	11	12	13	13	29	15	15	19	12	15	12	24	M343_A	R1b

Haplotypes	N	DYS 393	DYS 390	DYS 19	DYS 391	DYS 385a	DYS 385b	DYS 439	DYS 389 1	DYS 392	DYS 458	DYS 437	DYS 448	DYS H4	DYS 456	DYS 438	DYS 635	SNP	Haplogroups
Nish 20	1	13	22	15	10	14	15	12	12	11	29	16	16	21	10	15	20	P15_T	G2a
Nish 21	1	13	22	17	10	10	15	11	14	11	31	17	14	20	12	16	23	SRY_10831.2_A	R1a
Nish 22	1	12	23	15	10	14	17	7	12	11	28	17	16	19	12	15	21	M12_T	J2b
Nish 23	1	13	25	13	10	17	18	12	13	11	31	16	14	20	11	17	23	M215_G	E1b1b
Nish 24	1	12	22	16	10	14	15	6	13	11	31	17	15	21	11	15	24	M410_G	J2a
Nish 25	1	12	23	15	10	15	19	12	12	12	28	16	14	19	12	14	21	M12_T	J2b
Nish 26	1	13	24	15	11	14	15	13	14	11	32	17	15	20	11	16	24	P37.2_C	I2a
Nish 27	1	13	24	17	11	14	15	11	13	11	31	16	14	19	11	15	22	P37.2_C	I2a
Nish 28	1	11	24	14	10	13	17	11	13	11	29	18	14	20	11	14	21	M267_G	J1
Nish 29	1	13	25	15	11	11	17	11	12	11	29	15	14	20	12	15	24	SRY_10831.2_A	R1a
Nish 30	2	13	24	16	11	14	15	13	13	11	31	17	15	19	11	15	23	P37.2_C	I2a
Nish 31	1	12	23	14	10	13	19	5	13	11	30	18	14	20	11	15	20	M267_G	J1
Nish 32	1	13	24	17	10	14	15	13	13	11	30	17	15	20	11	15	23	P37.2_C	I2a
Nish 33	1	13	24	16	10	13	14	13	13	11	31	17	15	19	11	15	23	P37.2_C	I2a
Nish 34	1	13	24	16	11	14	15	14	13	11	31	17	14	20	11	15	23	P37.2_C	I2a
Nish 35	1	13	25	15	10	11	14	10	14	11	31	15	14	20	12	16	23	SRY_10831.2_A	R1a
Nish 36	1	12	24	14	11	11	12	12	13	13	29	17	15	21	12	15	23	M343_A	R1b
Nish 37	1	12	23	16	10	14	16	12	13	11	29	15	14	21	12	16	23	M410_G	J2a
Studentica 1	1	12	23	13	10	16	18	11	13	14	28	16	14	20	11	15	20	M70_C	T
Studentica 2	1	13	23	16	11	15	16	13	13	11	32	18	15	19	12	15	23	P37.2_C	I2a
Studentica 3	1	13	24	16	11	14	15	13	13	11	33	16	15	20	11	15	21	P37.2_C	I2a
Sumadija 1	2	13	24	13	10	15	18	9	14	11	31	17	14	19	12	16	21	M215_G	E1b1b
Sumadija 2	1	13	22	13	10	13	15	12	13	13	29	17	15	19	12	16	21	M70_C	T
Sumadija 3	1	13	24	13	10	16	17	12	14	11	32	15	15	19	11	16	21	M215_G	E1b1b
Sumadija 4	2	13	25	15	10	11	14	11	13	11	28	14	14	20	13	15	23	SRY_10831.2_A	R1a

In the new representation (Figure 2b), the populations cluster in different areas according to their languages. For the mtDNA data, no clear clusters are detected when taking different religions into account (Figure 2b). In fact, subjects from Kosovo and Albania speak Albanian and are located in a corner of the plot, while Romanians who speak romance languages appear in the center, and people speaking Slavic languages appear to the left. Moreover, the four Serbian regions group each other along the first coordinate, suggesting their genetic similarity at the Y chromosome level, with a certain tendency religion-wise plotting. In fact, the central of the pictures is characterized by predominantly Orthodox populations, while mostly Catholic populations are placed in the right side.

Discussion

Previous genetic data supported the idea, proposed on the basis of archeological evidence, that the Balkan Peninsula was one of the most important migration corridors for the initial peopling of Europe, a crucial stage in the human genetic history of the continent⁴³. The present findings contribute to the understanding of the various phenomena that led to the extant genetic landscape of the Balkan Peninsula. The presence in the sample of haplogroups involved in the Neolithization process, such as U4a2⁵⁸, seems to suggest that the Balkans might have been used as a bridge by ancient populations to colonize Europe⁵⁹. On the other hand, the identification in the Serbians of the Pre-Neolithic U5 lineage, which originated before the Last Glacial Maximum (LGM) around 27 ky ago⁶⁰, shows that the mitochondrial genetic landscape of the Balkan Peninsula seems to be a mixture of Pre-Neolithic and Neolithic lineages, as highlighted in other European population⁶¹. The occurrence, at a very low frequency, of African haplogroups indicates movement from Africa to Europe of mtDNA lineages^{58,62}, although it is not possible to date them.

Serbiens' Y-chromosomes show six macro-haplogroups, shared throughout southeastern Europe and Asia^{60,63–66}. The frequency of the R1 haplogroup is 21%, very similar to the value previously found by Regueiro *et al.*⁵⁵, and is probably associated with early settlements during the Southern dispersal of South-Western Asian populations⁶⁷.

Specifically for the R1a sub-haplogroup, three major events of gene flow have been described to explain its presence in the Balkans area: (i) the expansion of the R1 haplogroup from a Ukrainian refuge (early post-LGM, ~20–12 KYA)^{68,69}; Yamnaya culture migrations from the steppe region of the Caspian Sea territory in Late Neolithic, 4.5–3 KYA^{39,70–73}; and, more recently, (iii) the Balto-Slavs and the massive Slavic migration (5th–7th centuries)^{10,65,74}. Concerning the sub-haplogroup R1b, our data seem to suggest that R1b haplogroup expansion into the Balkan Peninsula might be associated with a dispersal from the Iberian refuge after LGM, as well as with the origin in West Asia and the migration into Europe before the glaciations^{64,74,75}. Recent studies indicate that both R1a and R1b are present in the steppe much earlier than in

other European regions, making this region a likely source for these male-specific region of the Y chromosome (MSY) expansion lineages⁷⁶. Therefore, the overlapping of the aforementioned migrations involving R1a individuals that penetrated Europe, place the R1a sub-haplogroup more recent in time, rather than represent a deeper Palaeolithic signal in Europe^{68,74}.

The high frequency of I haplogroup is consistent with the values present in literature^{77,78}. Haplogroup I is thought to have played a central role in the process of human recolonization of Europe from isolated glacial refugia after the LGM. Furthermore, the expansion of the sub-haplogroup I2a has occurred later following the post-Younger Dryas recovery, as suggested by recent studies^{39,73}. The sub-haplogroup I2a seems to have come out of the Neolithic period for reasons that are not yet clear. In fact, the subhaplogroup I2a is by far the largest branch of I2 and the most strongly linked to Neolithic cultures in South-East, South-West and North-Western Europe.

Finally, the high frequency of E1b1b, probably descending from the first farmers⁶⁰, suggests a Neolithic male contribution to the Serbian populations.

The whole data indicate admixture processes among Pre Neolithic substrata, Neolithic genetic lineages and probably Slavic genetic component. The role of the Slavs expansions, as previously suggested by Kushniarevich *et al.*¹⁰, could have played a key role in the peopling of the Balkan Peninsula but in lesser amounts than the genetic introgression from the Near East during the diffusion of agriculture⁴⁷. Furthermore, the Serbian population represents a connection not only between the easternmost (Bulgarians and Macedonians) and (Slovenians, Croatians, Bosnians, and Herzegovinians) Slavic populations, as suggested by Davidovic *et al.* (2015)⁴⁶, but also between northern Balkan populations from Romania and Hungary and southern Balkan populations from Greece, Macedonia and Albania.

The patrilineal component differences between Turks and Balkans, as Regueiro *et al.* (2012) 5s suggested, seem to indicate limited gene flow between the populations despite their long-term shared history throughout the Roman, Byzantine, and Ottoman Empires. The genetic similarity with northern Europe could be due to the population expansions from the Ukrainian refuge after the LGM^{79–81} suggesting a preferential demic route southwards to the Balkan Peninsula. Thus, concerning the Y chromosome, these results indicate a high level of Palaeolithic NRY lineages, suggesting an acculturation of indigenous peoples during the Neolithic Period. The comparison between the Serbians and their neighboring populations highlights an overall genetic similarity within the Balkan Peninsula, even though it is spotted only by Aromus and Bosnian samples. Bosch *et al.*³⁷ suggested a genetic drift in the Aromus population, probably due to its small effective population size. However, Marjanovic *et al.*⁷⁸ suggested a prolonged gene flow from several glacial refuges of Europe, the Middle East, and Africa in shaping the Bosnian modern gene pool and differentiating it from the Serbian population, where the NRY lineages are represented mostly by Palaeolithic components.

Conclusion

The analysis of the mtDNA and Y-chromosome in Serbians allowed us to support hypothesis related to the genetic make up of the Balkan region. The Y-chromosome and mtDNA haplogroups found in the Balkans support a genetic admixture between European and Near Eastern groups—although some differences regarding the matrilineal and patrilinear side of the history could not be ruled out. In fact, the mtDNA suggests an overall homogeneity with the neighboring human groups, with evidence of contributions from Neolithic people. Meanwhile, the Y chromosome suggests a specific genetic background for Serbians that could be the result of a western Asian re-peopling of the area from Ukrainian refuge. Thus, the data seem to be consistent with the hypothesis that the Balkan Peninsula acted as a bridge—not only from the Middle East but also from northern Europe—to reach Western Europe during prehistoric and historic times.

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Conflict of Interest Statement

The authors state there is no conflict of interest. The authors alone are responsible for the content and writing of this article.

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G. Scorrano

Center of Molecular Anthropology for Ancient DNA Studies, Department of Biology, University of Rome Tor Vergata, via della Ricerca Scientifica 1, 00133 Rome, Italy.

E-mail addresses: gabrielescor@gmail.com

GENETSKI KRAJOLIK SRPSKE POPULACIJE DOBIVEN SEKVENCIRANJEM MITOHONDRIJSKOG DNA I REGIJSKE NE-REKOMBINACIJE Y KROMOSOMSKIH MIKROSATELITA

SAŽETAK

Poznato je da Balkanski poluotok predstavlja složeni kulturni mozaik i to je strateško područje jer predstavlja put prema Europi s Bliskog Istoka. Ovo istraživanje nastoji procijeniti varijabilnost oba uniparentalna markera (mtDNA i ne-rekombiniranog područja Y kromosoma) kako bi se analizirala genetska struktura Srba. Cijeli uzorak odnosi se na 257 Srba (87 iz središnje regije i 170 iz južnog područja) koji su analizirani i za uniparentalne genetske markere. Rezultati su pokazali da postojeći stanovnici Balkanskog poluotoka imaju homogenu genetsku podlogu, unatoč njihovim jezičnim i kulturnim razlikama. Dobiveni podaci uspoređeni su s onima susjednih populacija kako bi se otkrili mogući odnosi među skupinama. Čini se da je u cjelini genetska varijabilnost balkanskih populacija posljedica procesa miješanja europskih i azijskih loza u različitim omjerima čiji doprinosi tvore trenutni rodni i očinski genetički krajolik.