**Supplementary Text 1. Description of the sample and methods of the analyses of mtDNA and NRY.**

**The sample for mtDNA and NRY analyses**

Blood samples were collected from 175 unrelated and healthy adult individuals of both sexes, residing in Bosnia and Herzegovina (n=77), Croatia (mainland, Zagreb region, n=19), Serbia (Belgrade area, n=21), Monte Negro (Podgorica, n=18), Kosovo (Pristina and Prizren, n=18) and Macedonians from the F.Y. Republic of Macedonia (Skopje, n=22) (Figure 1). The sample of 77 individuals from Bosnia and Herzegovina consisted of 17 Bosniacs (Sarajevo and Zavidovici), 41 Bosnian Croats (20 from Central Bosnia - Zepce and Maglaj - and 21 from South Bosnia and Herzegovina - Mostar, Grude, Livno and Capljina) and 19 Bosnian Serbs (Doboj and Banjaluka region).Out of the 175 Western Balkan samples, all were genotyped for mtDNA variation and 85 were genotyped for NRY variation. 7 mtDNA samples of our sample set were sequenced completely (Figures S19-21), completely sequenced mitochondrial genomes are available in the National Center for Biotechnology Information (NCBI), in Genbank (accession numbers KM103653 - KM103659). 70 individuals were randomly chosen for autosomal genotyping of 660 000 genome-wide markers in this study, 20 Croatian samples of this sample set has been characterized for autosomal variation in Behar et al. [1].

We have used the published data of 3202 samples of mtDNA from the European and Middle Eastern populations to put the mtDNA variation of 175 Western Balkan samples of this study (Table S5) into a wider context. The total mtDNA sample used for principal component analysis (PCA, Figure S13A and C) consisted of 273 Austrians [2], 267 Belarusians [3], 179 Czechs [4], 374 Slovaks [5], 211 Hungarians [6], 319 North Greeks [7], 341 South Italians [8], 94 Romanians [9], 996 Bulgarians [9]and 148 Iranians [10].

The Western Balkan NRY sample consisted of 9 Bosniacs, 14 Bosnian Serbs, 29 Bosnian Croats (8 from Central Bosnia, 21 from Herzegovina), 3 Croatians, 11 Serbians, 7 Montenegrins, 6 Macedonians of the Republic of Macedonia and 6 Kosovars. Due to the small individual sample sizes, the obtained NRY data were analyzed jointly with previously published NRY data of 84 Bosniacs, 90 Bosnian Croats, 81 Bosnian Serbs, 118 Croatians, 64 Macedonian Albanians (FYROM Albanians) and 55 Albanians from Battaglia et al. [11], and 113 Serbians from Pericic [12] for PCA (Table S6). The NRY sample of a wider context consisted of 2424 samples of 258 Austrians[13],565 Belarusians [3], 149 Greeks (57 from the former Yugoslav Republic of Macedonia, and 92 from Athens, Greece), 67 northeast Italians, 75 Czechs and 53 Hungarians from Battaglia et al.[11], 150 Slovaks [14], 149 Romanians and 808 Bulgarians from Karachanak et al. [15]and 150 Iranians from Regueiro et al. [16] (Figure S13B, D).

**Materials and methods**

***MtDNA and NRY genotyping***

Observed maternal lineages were determined into the hgs by the sequencing of the first and the second hypervariable segment (HVS1 and HVS2) of mtDNA control region and by the use of the high-resolution genotyping. The sequences of mtDNA HVS1 and HVS2 regions between nucleotide positions (nps) 16020 and 16519 and 29 and 510, respectively, as well as relevant coding region sequences of mtDNA and sequences of NRY were amplified by Biometra T1 Thermocycler 96 (Biometra GmbH, Goettingen, Germany)  and sequenced by ABIPrism 3130xl Genetic Analyser (Applied Biosystem, Foster City, CA, USA). The mtDNA samples were first analyzed for diagnostic nps of control region and thereafter hierarchically genotyped for putative hg-specific coding region mutations by RFLP or by direct sequencing, following the most recent established classification and nomenclature available at [www.phylotree.org](http://www.phylotree.org)[17]. The protocols of amplification and sequencing of mtDNA HVS1, HVS2 and coding region sequences were performed as described in [18–21]. All sequences were aligned and analysed by the use of ChromasPro software (Technelysium Pty Ltd). The complete sequencing of mtDNA genomes was done according to the procedures and conditions described in Rieder et al. [22]. The frequencies of mtDNA haplogroups and the list of the mtDNA HVS1 and HVS2 haplotypes observed in the sample of three ethnic groups of Bosnia and Herzegovina and in other studied Western Balkan populations are presented in Table S5.

The phylogenetic analysis of mtDNA haplotypes (Figures S14-18) was carried out by the use of the software Network 4.5.0.2 and Network Publisher (<http://www.fluxus-engineering.com>). The principle of maximum parsimony was applied using a reduced median algorithm (r = 2) [23], followed by a median joining algorithm (ε = 0) [24] and corrected by hand, if needed. The weights of mutations were assigned according to their observed rate of evolution [25–27].

The NRY variation (Table S6) was genotyped with the set of biallelic markers either by determining the restriction fragment length polymorphisms (RFLP) or sequencing, according to the current NRY phylogeny by Battaglia et al.[28] and Karafet et al. [29].

PCA based on the frequencies of mtDNA and NRY hgs was performed by the use of the software POPSTR (http://harpending.humanevo.utah.edu/popstr/). The results are given in Figure S13.

***Statistical analysis***

MtDNA HVS1 sequences were used to calculate the number of haplotypes and polymorphic sites, haplotype diversity, mean number of pairwise differences and nucleotide diversity of the populations (AMOVA) by the use of software Arlequin, v3.5 for all studied populations (Table S7). For testing the genetic structure of the Western Balkan populations, AMOVA was performed by the use of mtDNA HVS1 haplotypes (Tables S5 and S7).Genetic distances were estimated using the haplogroup (for Mantel test) or HVS-I haplotype frequency (for AMOVA) based linearized FST-s [30]. Mantel test with 10000 permutation steps was used to test the correlation between the genetic and geographic distances. In order to generate geographical matrix, we used program Geographic Distance Matrix Generator v1.2.3. [31]*.* For AMOVA (Table S9) and Mantel test (Table 1) the populations were grouped first according to their geographic and thereafter to their linguistic and religious affiliations – the last grouping was done as follows: Kosovars were grouped with Bosniacs to the Islamic (Muslim); Croats, both from Croatia and from Bosnia and Herzegovina into Catholic; Macedonians of former Yugoslav Republic of Macedonia, Montenegrins, Bosnian Serbs and Serbians into Orthodox group.

Bayesian 95% credible regions (CRs) for haplogroup frequencies were calculated with the computer program SAMPLING, provided by Dr. Vincent Macaulay.The coalescence time estimates and their standard deviations of mtDNA haplotypes were calculated according to Forster et al. [32] and Saillard et al. [33]. Estimated coalescense ages for the largest hgs found in studied Western Balkan populations are presented in Table S10.

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