



Praca oryginalna  
Original paper

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## Verification of insertion-deletion markers (InDels) and microsatellites (STRs) as subsidiary tools for inferring Slavic population ancestry

## Weryfikacja markerów insercyjno-deleccyjnych (InDels) i mikrosatelitarnych (STR) jako narzędzi pomocniczych do wnioskowania o pochodzeniu populacji słowiańskiej

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### Abstract

Genetic markers for the prediction of biogeographical ancestry have proved to be effective tools for law enforcement agencies for many years now. In this study, we attempted to assess the potential of insertion-deletion markers (InDel) and microsatellites (STRs) as subsidiary polymorphisms for inference of Slavic population ancestry. For that purpose, we genotyped Slavic-speaking populations samples from Belarus, the Czech Republic, Poland, Serbia, Ukraine and Russia in 46 InDels and 15 STRs by PCR and capillary electrophoresis and analyzed for between-population differentiation with the use of distance-based methods ( $F_{ST}$ , principal component analysis and multidimensional scaling). Additionally, we studied a sample from a Polish individual of well-documented genealogy whose biogeographic ancestry had previously been inferred by commercial genomic services using autosomal single nucleotide polymorphisms (SNPs), mitochondrial DNA and Y-SNP markers. For comparative purposes, we used genotype data collected in the "forInDel" browser and allele frequencies from previously published papers. The results obtained for InDels and STRs show that the Slavic populations constitute a genetically homogeneous group, with the exception of the Czechs differing clearly from the other tested populations. The analysis of the known Polish sample in the Snipper application proves the usefulness of the InDel markers on the continental level only. Conversely, microsatellites not only improve prediction, but are also informative if considered as an independent set of ancestry markers.

**Key words:** insertion-deletion polymorphisms, microsatellites, the Slavs

## Streszczenie

Markery genetyczne do przewidywania pochodzenia biogeograficznego od wielu lat okazują się skutecznymi narzędziami dla organów ścigania. W tym badaniu podjęliśmy próbę oceny potencjału markerów insercyjno-delecyjnych (InDel) i mikrosatelitarnych (STR) jako pomocniczych polimorfizmów do wnioskowania o pochodzeniu populacji słowiańskiej. W tym celu genotypowaliśmy próbki populacji słowiańskojęzycznych z Białorusi, Czech, Polski, Serbii, Ukrainy i Rosji w w zakresie 46 markerów InDel oraz 15 loci STR za pomocą PCR i elektroforezy kapilarnej oraz analizowaliśmy pod kątem różnicowania między populacjami za pomocą metod bazujących na dystansach genetycznych ( $F_{ST}$ , analiza głównych składowych i skalowanie wielowymiarowe). Dodatkowo zbadaliśmy próbkę męzczyzny z populacji polskiej o dobrze udokumentowanej genealogii, którego pochodzenie biogeograficzne zostało wcześniej ustalone przez komercyjne usługi genomiczne przy użyciu autosomalnych polimorfizmów pojedynczych nukleotydów (SNP), mitochondrialnego DNA i markerów Y-SNP. Do celów porównawczych wykorzystaliśmy dane genotypowe zebrane w przeglądarce „forInDel” i częstości alleli z wcześniej opublikowanych artykułów. Uzyskane wyniki dla InDels i STR wskazują, że populacje słowiańskie stanowią grupę genetycznie jednorodną, z wyjątkiem Czechów wyraźnie różniących się od pozostałych badanych populacji. Analiza znanej polskiej próbki w aplikacji Snipper dowodzi przydatności markerów InDel jedynie na poziomie kontynentalnym. Z kolei, mikrosatelity nie tylko poprawiają wyniki predykcji, ale są informatywne jako niezależny zestaw markerów pochodzenia biogeograficznego.

**Słowa kluczowe:** polimorfizm insercyjno-delecyjny, mikrosatelity, Słowianie

## Introduction

Ancestry Informative Markers (AIMs) are genetic polymorphisms characterized by large differences in allele frequencies between populations [1] and employed in both population and forensic genetics. In the latter, they are most frequently applied for genetic prediction of ancestry of the donors of biological samples or in the identification of human remains when a reference material is unavailable [2]. There are well-established methods of determining biogeographical origin based on the analysis of the mitochondrial DNA molecule and the non-recombining fragment of the Y chromosome. However, these markers, due to their haploid nature, reflect the genealogy of small portions of the human genome, which can be misleading in reliable predicting the ancestry of the person with an admixed background. There are also several panels useful for predicting ancestry with continental accuracy, most of them being based on autosomal SNPs [3-6]. One of the panels, developed by Pereira et al. [7] contains 46 autosomal insertion-deletion markers (InDel), typed by multiplex PCR and capillary electrophoresis. Additionally, the “forInDel” database was developed (available at <http://spsmart.cesga.es/forindel.php?dataSet=forindel46>) to facilitate the handling of population based InDel data [7]. The obtained genotypes can also be analyzed using

the Snipper application (available at <http://math-gene.usc.es/index.php>) based on the Bayesian statistics, which allows a studied sample to be assigned to the population with the best match [8]. The Snipper also allows for the analysis of microsatellite markers (STR), used predominantly for human identification, but also being capable of improving the prediction of biogeographic ancestry [9].

In this study, we aimed at verifying two sets of genetic markers, i.e. InDels described by Pereira et al. [7] and STRs from the commercially available AmpFLSTR™ Identifiler™ Plus kit as potential subsidiary tools for prediction of ancestry among the Slavic-speaking populations of Europe. Previous studies of 46 InDel have not included Slavic-speaking populations, and their predictive power has been proved for the continental populations of Africans, Europeans, East Asians, and Native Americans. Microsatellite markers were included in the analysis due to their potential ability to improve the resolution of prediction. For this purpose, we genotyped different population samples representing Slavic populations and verified the degree of their differentiation using the distance-based methods. Additionally, we predicted the ancestry of one actual sample from a Polish individual of the well-documented genealogy using the Snipper application.

## Material and methods

### Population samples

The research material consisted of 409 DNA extracts from unrelated Slavic-speaking individuals including 61 samples from Belarus, 43 from the Czech Republic, 151 from Poland, 60 from Serbia, 57 from Ukraine and 37 samples obtained from persons of European descent (mostly Russian) from Magadan in Russia. Both maternal and paternal ancestry was confirmed for all sample donors for at least two generations. The study was approved by the Bioethics Committee of the Collegium Medicum, NCU (statement no. KB 423/2017). The part of the study concerning Serbian samples was approved by the Ethics Committee of the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade (decision no. O-EO-005/2018/1).

### Laboratory analyses

Concentrations of DNA isolates were measured using a Qubit® fluorimeter (Thermo Fisher Life Technologies). The amplification reaction for the 46 InDels was performed with 1 x Qiagen multiplex PCR master Mix and up to 5 ng of template DNA in a final reaction volume of 10 µl. PCR reactions were performed in GeneAmp® PCR System 9700 thermal cyclers. Amplification conditions were 95°C for 15 min, 28 cycles: 94°C for 30 s, 60°C for 90 s, 72°C for 45 s and 72°C for 60 minutes [10]. Concentrations of the PCR primers described by Pereira et al. [7] were experimentally optimized and presented in Table I. Microsatellite markers were tested with the commercially available multiplex amplification kit AmpFLSTR™ Identifiler™ Plus according to the manufacturer's recommendations (AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit USER GUIDE, number 4440211, revision H). Spectral calibration of the ABI PRISM® 3130 xl Genetic Analyzer (Applied Biosystems) capillary analyzer was carried out using PCR products obtained as a result of the InDel loci amplification (for the purposes of the analysis of insertion-deletion markers). Capillary electrophoresis was performed with 1 µl of product of PCR, 9 µl of Hi-Di Formamide (Applied Biosystems) and 0.6 µl of GeneScan™ 600 LIZ™ Size Standard. The GeneMapper® ID v.3.2 software was used to visualize the results (Applied Biosystems).

### Statistical and bioinformatic analyses

Allele frequencies of individual genetic markers,  $F_{ST}$  pairwise genetic distances between populations, deviations from the Hardy-Weinberg equilibrium and linkage disequilibrium (LD) were estimated at the statistical significance level of 0.05 with the Bonferroni correction [11] using the Arlequin 3.5.2.2 program [12]. Negative  $F_{ST}$  values were changed to 0 [13]. Multidimensional scaling (MDS) and a principal component analysis (PCA) were performed using allele frequencies of the tested markers (NIPALS algorithm, 5-fold cross-validation) in the Statistica 13 (StatSoft) program. For comparative purposes, genotype InDel data were retrieved using the “forInDel” browser. As far as microsatellite markers are concerned, allele frequencies from the published papers were used [14-40]. Three analyses were conducted: a binary classification at the continental level, i.e., into one of the groups: Europe, East Asia, Africa, America, Oceania; a classification using our own genotype results and the data retrieved from the “forInDel” browser using a set of 46 InDels; and a classification using frequencies of STR data (our own published data).

### The analysis of a sample with a well-documented genealogy

Additionally, the InDel and STR genotyping of a sample from a Polish male was performed, followed by the Snipper classification. The sample had previously been analyzed by the commercial genomic companies MyHeritage and 23andMe, using the Infinium® Global Screening Array-24 v1.0 (Illumina, 642 824 autosomal markers) and the Illumina OmniExpress platform (730 295 autosomal SNPs), respectively. In both cases, the sample had been reported as of mostly Eastern European ancestry (43% and 97.7%, respectively). The individual's self-inferred family genealogy also pointed to the Eastern European origins, with the most probable population ancestry of the present-day Ukraine.

**Table I.** Sequences and concentrations of primers ( $\mu\text{M}$ ) used for amplification of a multiplex set of 46 insertion-deletion polymorphisms.

| Marker | Product length (del/ins) | Labeled primer (5'→3')     | Unlabeled primer (5'→3')   | Concentration |
|--------|--------------------------|----------------------------|----------------------------|---------------|
| 1470   | 65/70                    | 6FAM-GAGTCTGACCCTTCATAAGC  | GCCATGGTGATATTACGTCCC      | 0.0475        |
| 777    | 75/78                    | 6FAM-TGGAAGACACGTCCTAAGAG  | GTATTCCTCCAGGCTCTTTGC      | 0.055         |
| 196    | 83/86                    | 6FAM-CCAAGTTCTAGCCATATGGA  | GTTTCTTGACTATCTTCTGACCATC  | 0.145         |
| 881    | 92/96                    | 6FAM-TTGGCTCCCTATGATAATCC  | GTTTCTTGTTCCCAAAGTTCTCC    | 0.1525        |
| 3122   | 104/108                  | 6FAM-TCACAAGTCCGGAATACCAG  | GAGTTATGGGATGGGAAGGAG      | 0.05          |
| 548    | 111/113                  | 6FAM-AGTCAGGACTGAAGAAACCC  | GTTTCAGTAAAACAAGAGCCCGTG   | 0.04          |
| 659    | 120/122                  | 6FAM-CACTGCATCAGACTGACTTC  | GTTTCTTGCTGCTTTGCTTTGAATTG | 0.06          |
| 2011   | 129/134                  | 6FAM-TGAGAACTAGGAGCTCTGG   | GTTTCCTAAGAGCCACTGCAT      | 0.075         |
| 2929   | 150/152                  | 6FAM-TGTGATGTGGATAGGCAAGG  | GAGGCTCCATTTGTTAAGAGG      | 0.05          |
| 593    | 159/161                  | 6FAM-TGCTCACTTTAGTGGAGACC  | GTTTGCCTTAGGTCCTTCTG       | 0.05          |
| 798    | 169/175                  | 6FAM-ACGACAGTGTTCACAAAGAG  | GCTGTTGTCTGACCTGTGAAG      | 0.055         |
| 1193   | 182/184                  | 6FAM-GCTGGGTAGTTTTTCTCC    | GTTCCACCATCTACCTTCTATG     | 0.095         |
| 1871   | 190/192                  | 6FAM-TTGTAGTCAGAGAGTGTGCC  | GAGCCTTTCCCTAACGTAC        | 0.0375        |
| 17     | 200/204                  | 6FAM-AGAACTGCAACCCTCCAAG   | GATCCCAGACACTGAAGATG       | 0.0725        |
| 2538   | 212/216                  | 6FAM-CTCGCAAAGTAGGCAAGTTC  | GACACCAACAATCTTGGCACC      | 0.05          |
| 1644   | 223/225                  | 6FAM-ACACCACTGAAGATCTGACC  | GGTCTAAAGTCAGTGCACAG       | 0.055         |
| 3854   | 58/62                    | HEX-TCACCCTTATTCAGGGTTGC   | GCCAGGGATTTAGTGTAGAG       | 0.13          |
| 2275   | 72/79                    | HEX-CTACCTGACTACCACCTATG   | GACCCAGCCTATCTGACTTTG      | 0.075         |
| 94     | 92/95                    | HEX-TGGTGGCTCATGCACTTTTG   | GTTTACAGGGTCTCGCTATGATGC   | 0.0475        |
| 3072   | 110/117                  | HEX-AGCTTTTTCCGGCAACTCTC   | GTTTGGATGTGTCTGAGCTCAAC    | 0.0475        |
| 772    | 120/123                  | HEX-GTCTCRTTTTCTGCAGTAG    | GTTTCTTATCCTTCTGCTACTCTACC | 0.12          |
| 2313   | 130/139                  | HEX-GCACACATGCAGAAATGCAG   | GTTGTAACATCTGTGAGGTC       | 0.085         |
| 397    | 163/167                  | HEX-TGGGCTTCTTCTGGGAAAAC   | GCCACATTCAGGCGTTTTGTC      | 0.0725        |
| 1636   | 174/176                  | HEX-TTAGGAAGAGGTGCTATGGG   | GCCTCCTTGAAGACACACAG       | 0.0675        |
| 51     | 183/188                  | HEX-AAGATTGGAGGGAAAAGTGC   | GCGTCTCCACCTTCTTTTTC       | 0.08          |
| 2431   | 209/213                  | HEX-AGGAGGAGCTGATAGACTTC   | GCAGTGTGCAACTGATACG        | 0.0775        |
| 2264   | 224/228                  | HEX-CTTTGGCTATCCTGTCTCAC   | GTAGGAGACCACTCACATTC       | 0.095         |
| 2256   | 61/64                    | TAMRA-ATCGAACCCTCCTAAGGAC  | GCAAGAAAAGGAATCCAGGC       | 0.05          |
| 128    | 70/73                    | TAMRA-ATCAGGAGACAATCCAGCAG | GTCCAGCCATTCAGACAAAGG      | 0.045         |
| 15     | 81/91                    | TAMRA-GGGTTATTTGCCTCATCTCC | GTTTCTAGGTATTCTCTGTTCCACG  | 0.115         |
| 2241   | 110/118                  | TAMRA-ACATACACGTGGAAGACTGC | GTTACTGTCGACTGATCCAATAG    | 0.0575        |
| 419    | 124/131                  | TAMRA-CAGGAAAGTATGGCCATTC  | GTCCATGTTTTCTTTGAGCATC     | 0.065         |
| 943    | 156/160                  | TAMRA-TCTTCCTACCCCTGTTAGTG | GACAAGATCACTAGCTTGAC       | 0.135         |
| 159    | 168/173                  | TAMRA-ACCAGAGCACTACAGCCTTT | GCAAGGYAGTAAATGAGGGG       | 0.1           |
| 2005   | 185/191                  | TAMRA-TGTAGCGGCAATATAGGACG | GAAAGTTGTGGCTTAACTGG       | 0.2775        |
| 250    | 202/204                  | TAMRA-ATGGAGCAGTAAAGCAGCAC | GTCACCTTGGTTTTTGCAGG       | 0.21          |
| 1802   | 213/216                  | TAMRA-ACGGTCAACTTTGTAGCTCC | GCCAGTTGAGAATCACTGCAC      | 0.1375        |
| 1607   | 221/223                  | TAMRA-TGTTGCAGAAGAACTCAACC | GATAAGCACCTAACTCCCAG       | 0.275         |
| 1734   | 56/60                    | ROX-TTCGTGTTCTCACACTGTCC   | GTGCATCCCATACTGAC          | 0.1           |
| 406    | 63/65                    | ROX-TGGCTGCTGTAGATTGTAGG   | GACAAATGGACAACGGCCAAG      | 0.1075        |

| Marker | Product length (del/ins) | Labeled primer (5'→3')   | Unlabeled primer (5'→3') | Concentration |
|--------|--------------------------|--------------------------|--------------------------|---------------|
| 1386   | 72/93                    | ROX-AGAGGATCATGGAGACCAAC | GTTTATGTTCCAAGTCAGCAGCAC | 0.15          |
| 1726   | 106/119                  | ROX-GGTCCAAATGCACCACAATC | GCTCTGCTATTTTGGTTTGC     | 0.225         |
| 3626   | 142/157                  | ROX-TGTTGGTTCTCTCCTTTTCC | GGTGACCCCTTCTTTATCTC     | 0.405         |
| 360    | 167/169                  | ROX-AGATCAACTGCCAATCTGGG | GCTCAAGTGACCAACCCACCT    | 0.1275        |
| 1603   | 214/218                  | ROX-TTACAATTTCAAGCCTCCGC | GGAGCTGTTAGTCTGAGTAG     | 0.275         |
| 2719   | 225/229                  | ROX-GTCAGGAGTCTAGAAACTTC | GGGTGATGAAATGTTCCGAA     | 0.425         |

**Table II.** Allele frequencies of insertion-deletion polymorphisms.

| Marker | Allele    | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|--------|-----------|-------|-------|-------|-------|-------|-------|
| 15     | deletion  | 0.385 | 0.36  | 0.354 | 0.311 | 0.4   | 0.333 |
|        | insertion | 0.615 | 0.64  | 0.646 | 0.689 | 0.6   | 0.667 |
| 17     | deletion  | 0.32  | 0.384 | 0.341 | 0.405 | 0.317 | 0.351 |
|        | insertion | 0.68  | 0.616 | 0.659 | 0.595 | 0.683 | 0.649 |
| 51     | deletion  | 0.68  | 0.628 | 0.728 | 0.716 | 0.642 | 0.693 |
|        | insertion | 0.32  | 0.372 | 0.272 | 0.284 | 0.358 | 0.307 |
| 94     | deletion  | 0.164 | 0.186 | 0.146 | 0.203 | 0.25  | 0.193 |
|        | insertion | 0.836 | 0.814 | 0.854 | 0.797 | 0.75  | 0.807 |
| 128    | deletion  | 0.41  | 0.535 | 0.477 | 0.446 | 0.525 | 0.526 |
|        | insertion | 0.59  | 0.465 | 0.523 | 0.554 | 0.475 | 0.474 |
| 159    | deletion  | 0.484 | 0.453 | 0.553 | 0.486 | 0.533 | 0.509 |
|        | insertion | 0.516 | 0.547 | 0.447 | 0.514 | 0.467 | 0.491 |
| 196    | deletion  | 0.434 | 0.585 | 0.52  | 0.459 | 0.5   | 0.561 |
|        | insertion | 0.566 | 0.415 | 0.48  | 0.541 | 0.5   | 0.439 |
| 250    | deletion  | 0.73  | 0.721 | 0.722 | 0.662 | 0.667 | 0.789 |
|        | insertion | 0.27  | 0.279 | 0.278 | 0.338 | 0.333 | 0.211 |
| 360    | deletion  | 0.803 | 0.767 | 0.851 | 0.892 | 0.842 | 0.746 |
|        | insertion | 0.197 | 0.233 | 0.149 | 0.108 | 0.158 | 0.254 |
| 397    | deletion  | 0.852 | 0.779 | 0.834 | 0.716 | 0.8   | 0.728 |
|        | insertion | 0.148 | 0.221 | 0.166 | 0.284 | 0.2   | 0.272 |
| 406    | deletion  | 0.885 | 0.791 | 0.858 | 0.878 | 0.775 | 0.781 |
|        | insertion | 0.115 | 0.209 | 0.142 | 0.122 | 0.225 | 0.219 |
| 419    | deletion  | 0.803 | 0.814 | 0.825 | 0.797 | 0.8   | 0.807 |
|        | insertion | 0.197 | 0.186 | 0.176 | 0.203 | 0.2   | 0.193 |
| 548    | deletion  | 0.23  | 0.209 | 0.212 | 0.257 | 0.25  | 0.237 |
|        | insertion | 0.77  | 0.791 | 0.788 | 0.743 | 0.75  | 0.763 |
| 593    | deletion  | 0     | 0     | 0.003 | 0     | 0     | 0.009 |
|        | insertion | 1     | 1     | 0.997 | 1     | 1     | 0.991 |
| 659    | deletion  | 0.098 | 0.093 | 0.086 | 0.108 | 0.142 | 0.123 |
|        | insertion | 0.902 | 0.907 | 0.914 | 0.892 | 0.858 | 0.877 |
| 772    | deletion  | 0.967 | 0.93  | 0.987 | 0.946 | 0.958 | 0.974 |
|        | insertion | 0.033 | 0.07  | 0.013 | 0.054 | 0.042 | 0.026 |
| 777    | deletion  | 0.402 | 0.36  | 0.377 | 0.351 | 0.267 | 0.307 |

| Marker | Allele     | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|--------|------------|-------|-------|-------|-------|-------|-------|
|        | insertion  | 0.598 | 0.64  | 0.623 | 0.649 | 0.733 | 0.693 |
| 798    | deletion   | 0.639 | 0.628 | 0.659 | 0.649 | 0.65  | 0.675 |
|        | insertion  | 0.361 | 0.372 | 0.341 | 0.351 | 0.35  | 0.325 |
| 881    | deletion   | 0.885 | 0.919 | 0.911 | 0.932 | 0.875 | 0.939 |
|        | insertion  | 0.115 | 0.081 | 0.089 | 0.068 | 0.125 | 0.061 |
| 943    | deletion   | 0.721 | 0.791 | 0.781 | 0.757 | 0.825 | 0.754 |
|        | insertion  | 0.279 | 0.209 | 0.219 | 0.243 | 0.175 | 0.246 |
| 1193   | deletion   | 0.23  | 0.163 | 0.156 | 0.203 | 0.175 | 0.123 |
|        | insertion  | 0.77  | 0.837 | 0.844 | 0.797 | 0.825 | 0.877 |
| 1386   | deletion   | 0.27  | 0.314 | 0.189 | 0.203 | 0.183 | 0.281 |
|        | insertion  | 0.73  | 0.686 | 0.811 | 0.797 | 0.817 | 0.719 |
| 1470   | deletion   | 0.631 | 0.477 | 0.682 | 0.595 | 0.653 | 0.623 |
|        | insertion  | 0.369 | 0.523 | 0.318 | 0.405 | 0.347 | 0.377 |
| 1603   | deletion   | 0.311 | 0.314 | 0.325 | 0.365 | 0.325 | 0.36  |
|        | insertion  | 0.689 | 0.686 | 0.676 | 0.635 | 0.675 | 0.64  |
| 1607   | deletion   | 0.23  | 0.163 | 0.281 | 0.284 | 0.233 | 0.272 |
|        | insertion  | 0.77  | 0.837 | 0.719 | 0.716 | 0.767 | 0.728 |
| 1636   | deletion   | 0.73  | 0.733 | 0.735 | 0.743 | 0.842 | 0.711 |
|        | insertion  | 0.27  | 0.267 | 0.265 | 0.257 | 0.158 | 0.289 |
| 1644   | deletion   | 0.967 | 0.942 | 0.94  | 0.946 | 0.975 | 0.982 |
|        | insertion  | 0.033 | 0.058 | 0.06  | 0.054 | 0.025 | 0.018 |
| 1726   | deletion   | 0.762 | 0.721 | 0.692 | 0.649 | 0.708 | 0.702 |
|        | insertion  | 0.238 | 0.279 | 0.308 | 0.351 | 0.292 | 0.298 |
| 1734   | deletion   | 0.861 | 0.791 | 0.894 | 0.892 | 0.9   | 0.842 |
|        | insertion  | 0.139 | 0.209 | 0.106 | 0.108 | 0.1   | 0.158 |
| 1802   | deletion   | 0.008 | 0     | 0.003 | 0.014 | 0.008 | 0.009 |
|        | insertion  | 0.992 | 1     | 0.997 | 0.986 | 0.992 | 0.991 |
| 1871   | deletion   | 0.41  | 0.326 | 0.404 | 0.419 | 0.442 | 0.465 |
|        | insertion  | 0.59  | 0.674 | 0.596 | 0.581 | 0.558 | 0.535 |
| 2005   | deletion   | 0.672 | 0.663 | 0.606 | 0.608 | 0.717 | 0.763 |
|        | insertion  | 0.328 | 0.337 | 0.394 | 0.392 | 0.283 | 0.237 |
| 2011   | deletion   | 0.762 | 0.721 | 0.719 | 0.703 | 0.692 | 0.737 |
|        | insertion  | 0.238 | 0.279 | 0.281 | 0.297 | 0.308 | 0.263 |
| 2241   | deletion   | 0.23  | 0.372 | 0.315 | 0.284 | 0.225 | 0.246 |
|        | insertion  | 0.77  | 0.628 | 0.685 | 0.716 | 0.775 | 0.754 |
| 2256   | deletion   | 0.213 | 0.174 | 0.149 | 0.162 | 0.167 | 0.246 |
|        | insertion  | 0.787 | 0.826 | 0.851 | 0.838 | 0.833 | 0.754 |
| 2264   | deletion   | 0.484 | 0.477 | 0.47  | 0.459 | 0.525 | 0.465 |
|        | insertion* | 0.066 | 0.035 | 0.043 | 0.027 | 0.058 | 0.044 |
|        | insertion  | 0.451 | 0.488 | 0.487 | 0.514 | 0.417 | 0.491 |
| 2275   | deletion   | 0.123 | 0.058 | 0.103 | 0.081 | 0.117 | 0.132 |
|        | insertion  | 0.877 | 0.942 | 0.897 | 0.919 | 0.883 | 0.868 |
| 2313   | deletion   | 0.246 | 0.291 | 0.268 | 0.324 | 0.35  | 0.237 |
|        | insertion  | 0.754 | 0.709 | 0.732 | 0.676 | 0.65  | 0.763 |
| 2431   | deletion   | 0.098 | 0.058 | 0.126 | 0.122 | 0.05  | 0.088 |

| Marker | Allele           | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|--------|------------------|-------|-------|-------|-------|-------|-------|
|        | <b>insertion</b> | 0.902 | 0.942 | 0.874 | 0.878 | 0.95  | 0.912 |
| 2538   | <b>deletion</b>  | 0.5   | 0.453 | 0.454 | 0.432 | 0.483 | 0.491 |
|        | <b>insertion</b> | 0.5   | 0.547 | 0.546 | 0.568 | 0.517 | 0.509 |
| 2719   | <b>deletion</b>  | 0.393 | 0.314 | 0.444 | 0.486 | 0.483 | 0.43  |
|        | <b>insertion</b> | 0.607 | 0.686 | 0.556 | 0.514 | 0.517 | 0.57  |
| 2929   | <b>deletion</b>  | 0.762 | 0.756 | 0.725 | 0.851 | 0.767 | 0.781 |
|        | <b>insertion</b> | 0.238 | 0.244 | 0.275 | 0.149 | 0.233 | 0.219 |
| 3072   | <b>deletion</b>  | 0.943 | 0.942 | 0.944 | 0.946 | 0.933 | 0.939 |
|        | <b>insertion</b> | 0.057 | 0.058 | 0.056 | 0.054 | 0.067 | 0.061 |
| 3122   | <b>deletion</b>  | 0.992 | 0.977 | 0.987 | 1     | 0.958 | 0.965 |
|        | <b>insertion</b> | 0.008 | 0.023 | 0.013 | 0     | 0.042 | 0.035 |
| 3626   | <b>deletion</b>  | 0.615 | 0.674 | 0.629 | 0.662 | 0.625 | 0.675 |
|        | <b>insertion</b> | 0.385 | 0.326 | 0.371 | 0.338 | 0.375 | 0.325 |
| 3854   | <b>deletion</b>  | 0.025 | 0.035 | 0.01  | 0.014 | 0.008 | 0.018 |
|        | <b>insertion</b> | 0.975 | 0.965 | 0.99  | 0.986 | 0.992 | 0.982 |

**Table III.** Allele frequencies of microsatellite polymorphisms.

| Marker  | Allele      | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|---------|-------------|-------|-------|-------|-------|-------|-------|
| D8S1179 | <b>8</b>    | 0.016 | 0.023 | 0.013 | 0     | 0.025 | 0     |
|         | <b>9</b>    | 0.008 | 0     | 0.003 | 0     | 0.008 | 0.018 |
|         | <b>10</b>   | 0.066 | 0.081 | 0.066 | 0.081 | 0.075 | 0.079 |
|         | <b>11</b>   | 0.033 | 0.07  | 0.06  | 0.122 | 0.058 | 0.07  |
|         | <b>12</b>   | 0.164 | 0.233 | 0.192 | 0.108 | 0.142 | 0.211 |
|         | <b>13</b>   | 0.328 | 0.233 | 0.341 | 0.392 | 0.35  | 0.316 |
|         | <b>14</b>   | 0.246 | 0.151 | 0.212 | 0.189 | 0.217 | 0.184 |
|         | <b>15</b>   | 0.115 | 0.186 | 0.083 | 0.095 | 0.108 | 0.088 |
|         | <b>16</b>   | 0.025 | 0.023 | 0.026 | 0     | 0.017 | 0.035 |
|         | <b>17</b>   | 0     | 0     | 0.003 | 0.014 | 0     | 0     |
| D21S11  | <b>26</b>   | 0.008 | 0.012 | 0     | 0     | 0     | 0     |
|         | <b>27</b>   | 0.008 | 0.07  | 0.017 | 0.014 | 0.042 | 0.035 |
|         | <b>28</b>   | 0.205 | 0.128 | 0.195 | 0.176 | 0.133 | 0.123 |
|         | <b>29</b>   | 0.246 | 0.174 | 0.219 | 0.243 | 0.217 | 0.228 |
|         | <b>29.2</b> | 0     | 0     | 0.007 | 0     | 0.008 | 0     |
|         | <b>30</b>   | 0.23  | 0.244 | 0.185 | 0.176 | 0.192 | 0.254 |
|         | <b>30.2</b> | 0.066 | 0.07  | 0.05  | 0.068 | 0.033 | 0.044 |
|         | <b>31</b>   | 0.033 | 0.047 | 0.05  | 0.014 | 0.05  | 0.053 |
|         | <b>31.2</b> | 0.074 | 0.07  | 0.089 | 0.122 | 0.125 | 0.105 |
|         | <b>32</b>   | 0.008 | 0.035 | 0.013 | 0.027 | 0.008 | 0     |
|         | <b>32.2</b> | 0.107 | 0.093 | 0.129 | 0.162 | 0.142 | 0.096 |
|         | <b>33.2</b> | 0.016 | 0.047 | 0.04  | 0     | 0.042 | 0.061 |
|         | <b>34.2</b> | 0     | 0.012 | 0.007 | 0     | 0.008 | 0     |
| D7S820  | <b>7</b>    | 0.008 | 0.012 | 0.01  | 0.041 | 0.025 | 0.018 |
|         | <b>8</b>    | 0.123 | 0.093 | 0.176 | 0.135 | 0.133 | 0.167 |
|         | <b>9</b>    | 0.115 | 0.256 | 0.162 | 0.135 | 0.117 | 0.167 |

| Marker  | Allele      | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|---------|-------------|-------|-------|-------|-------|-------|-------|
|         | <b>10</b>   | 0.311 | 0.221 | 0.275 | 0.243 | 0.308 | 0.263 |
|         | <b>11</b>   | 0.303 | 0.174 | 0.202 | 0.297 | 0.258 | 0.193 |
|         | <b>12</b>   | 0.107 | 0.198 | 0.142 | 0.122 | 0.142 | 0.158 |
|         | <b>13</b>   | 0.033 | 0.035 | 0.026 | 0.014 | 0.017 | 0.035 |
|         | <b>14</b>   | 0     | 0.012 | 0.007 | 0.014 | 0     | 0     |
| CSF1PO  | <b>8</b>    | 0     | 0     | 0.003 | 0     | 0     | 0     |
|         | <b>9</b>    | 0.049 | 0.047 | 0.046 | 0.081 | 0.017 | 0.053 |
|         | <b>10</b>   | 0.279 | 0.326 | 0.272 | 0.27  | 0.225 | 0.333 |
|         | <b>11</b>   | 0.295 | 0.244 | 0.301 | 0.176 | 0.367 | 0.228 |
|         | <b>12</b>   | 0.328 | 0.326 | 0.311 | 0.365 | 0.35  | 0.325 |
|         | <b>13</b>   | 0.049 | 0.047 | 0.053 | 0.081 | 0.025 | 0.061 |
|         | <b>14</b>   | 0     | 0.012 | 0.01  | 0     | 0.017 | 0     |
|         | <b>15</b>   | 0     | 0     | 0     | 0.027 | 0     | 0     |
|         | <b>16</b>   | 0     | 0     | 0.003 | 0     | 0     | 0     |
| D3S1358 | <b>13</b>   | 0     | 0.012 | 0     | 0     | 0     | 0     |
|         | <b>14</b>   | 0.115 | 0.07  | 0.199 | 0.162 | 0.125 | 0.184 |
|         | <b>15</b>   | 0.254 | 0.221 | 0.209 | 0.23  | 0.275 | 0.237 |
|         | <b>15.2</b> | 0     | 0     | 0.003 | 0     | 0     | 0     |
|         | <b>16</b>   | 0.246 | 0.279 | 0.272 | 0.23  | 0.242 | 0.211 |
|         | <b>16.2</b> | 0     | 0     | 0.003 | 0     | 0     | 0     |
|         | <b>17</b>   | 0.254 | 0.244 | 0.179 | 0.23  | 0.258 | 0.184 |
|         | <b>18</b>   | 0.131 | 0.163 | 0.123 | 0.149 | 0.092 | 0.167 |
|         | <b>19</b>   | 0     | 0.012 | 0.013 | 0     | 0.008 | 0.018 |
| TH01    | <b>5</b>    | 0     | 0     | 0.003 | 0     | 0     | 0     |
|         | <b>6</b>    | 0.18  | 0.279 | 0.245 | 0.27  | 0.317 | 0.202 |
|         | <b>7</b>    | 0.148 | 0.163 | 0.109 | 0.081 | 0.092 | 0.14  |
|         | <b>8</b>    | 0.082 | 0.07  | 0.07  | 0.162 | 0.158 | 0.114 |
|         | <b>8.3</b>  | 0     | 0.023 | 0     | 0     | 0     | 0     |
|         | <b>9</b>    | 0.213 | 0.105 | 0.202 | 0.149 | 0.183 | 0.219 |
|         | <b>9.3</b>  | 0.369 | 0.36  | 0.358 | 0.311 | 0.233 | 0.325 |
|         | <b>10</b>   | 0     | 0     | 0.01  | 0.027 | 0.017 | 0     |
|         | <b>10.3</b> | 0.008 | 0     | 0     | 0     | 0     | 0     |
|         | <b>11</b>   | 0     | 0     | 0.003 | 0     | 0     | 0     |
| D13S317 | <b>8</b>    | 0.139 | 0.07  | 0.172 | 0.23  | 0.158 | 0.175 |
|         | <b>9</b>    | 0.09  | 0.07  | 0.063 | 0.054 | 0.117 | 0.07  |
|         | <b>10</b>   | 0.041 | 0.07  | 0.02  | 0.027 | 0.067 | 0.018 |
|         | <b>11</b>   | 0.402 | 0.337 | 0.391 | 0.432 | 0.308 | 0.368 |
|         | <b>12</b>   | 0.197 | 0.314 | 0.228 | 0.122 | 0.233 | 0.219 |
|         | <b>13</b>   | 0.09  | 0.047 | 0.076 | 0.054 | 0.075 | 0.105 |
|         | <b>14</b>   | 0.041 | 0.093 | 0.05  | 0.068 | 0.042 | 0.044 |
|         | <b>15</b>   | 0     | 0     | 0     | 0.014 | 0     | 0     |
| D16S539 | <b>8</b>    | 0.008 | 0     | 0.007 | 0     | 0.017 | 0.018 |
|         | <b>9</b>    | 0.115 | 0.174 | 0.06  | 0.068 | 0.092 | 0.088 |
|         | <b>10</b>   | 0.008 | 0.058 | 0.033 | 0.054 | 0.058 | 0.061 |
|         | <b>11</b>   | 0.23  | 0.244 | 0.285 | 0.203 | 0.275 | 0.228 |
|         | <b>12</b>   | 0.352 | 0.302 | 0.384 | 0.351 | 0.283 | 0.377 |



| Marker  | Allele      | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|---------|-------------|-------|-------|-------|-------|-------|-------|
|         | <b>12.2</b> | 0     | 0     | 0     | 0.014 | 0     | 0     |
|         | <b>13</b>   | 0.246 | 0.198 | 0.182 | 0.257 | 0.225 | 0.211 |
|         | <b>14</b>   | 0.041 | 0.023 | 0.05  | 0.041 | 0.05  | 0.018 |
|         | <b>15</b>   | 0     | 0     | 0     | 0.014 | 0     | 0     |
| D2S1338 | <b>15</b>   | 0     | 0     | 0     | 0     | 0.008 | 0     |
|         | <b>16</b>   | 0.025 | 0.07  | 0.073 | 0.068 | 0.058 | 0.026 |
|         | <b>17</b>   | 0.254 | 0.174 | 0.205 | 0.257 | 0.258 | 0.193 |
|         | <b>18</b>   | 0.074 | 0.081 | 0.076 | 0.081 | 0.083 | 0.088 |
|         | <b>19</b>   | 0.139 | 0.128 | 0.116 | 0.041 | 0.083 | 0.123 |
|         | <b>20</b>   | 0.139 | 0.14  | 0.132 | 0.216 | 0.175 | 0.167 |
|         | <b>21</b>   | 0.057 | 0.035 | 0.02  | 0.014 | 0.033 | 0.044 |
|         | <b>22</b>   | 0.008 | 0.047 | 0.017 | 0.014 | 0.042 | 0.035 |
|         | <b>23</b>   | 0.09  | 0.14  | 0.096 | 0.068 | 0.1   | 0.114 |
|         | <b>24</b>   | 0.066 | 0.116 | 0.116 | 0.135 | 0.058 | 0.096 |
|         | <b>25</b>   | 0.115 | 0.07  | 0.126 | 0.081 | 0.083 | 0.105 |
|         | <b>26</b>   | 0.033 | 0     | 0.02  | 0     | 0.008 | 0.009 |
|         | <b>27</b>   | 0     | 0     | 0.003 | 0.027 | 0.008 | 0     |
| D19S433 | <b>10.2</b> | 0     | 0     | 0.003 | 0     | 0     | 0     |
|         | <b>11</b>   | 0     | 0     | 0.007 | 0     | 0.017 | 0     |
|         | <b>12</b>   | 0.057 | 0.058 | 0.096 | 0.122 | 0.067 | 0.132 |
|         | <b>12.2</b> | 0     | 0     | 0.003 | 0     | 0     | 0     |
|         | <b>13</b>   | 0.189 | 0.291 | 0.212 | 0.243 | 0.233 | 0.289 |
|         | <b>13.2</b> | 0.049 | 0.012 | 0.007 | 0.041 | 0.008 | 0.018 |
|         | <b>14</b>   | 0.418 | 0.349 | 0.361 | 0.324 | 0.317 | 0.281 |
|         | <b>14.2</b> | 0.041 | 0     | 0.017 | 0.027 | 0.008 | 0.009 |
|         | <b>15</b>   | 0.156 | 0.163 | 0.169 | 0.189 | 0.167 | 0.149 |
|         | <b>15.2</b> | 0.016 | 0.023 | 0.026 | 0.014 | 0.067 | 0.035 |
|         | <b>16</b>   | 0.041 | 0.081 | 0.046 | 0     | 0.092 | 0.026 |
|         | <b>16.2</b> | 0.016 | 0.012 | 0.046 | 0.027 | 0.017 | 0.035 |
|         | <b>17</b>   | 0.008 | 0     | 0     | 0.014 | 0.008 | 0     |
|         | <b>17.2</b> | 0.008 | 0     | 0.003 | 0     | 0     | 0.018 |
|         | <b>18.2</b> | 0     | 0.012 | 0.003 | 0     | 0     | 0.009 |
| vWA     | <b>13</b>   | 0     | 0     | 0.003 | 0     | 0.017 | 0     |
|         | <b>14</b>   | 0.139 | 0.07  | 0.076 | 0.081 | 0.125 | 0.079 |
|         | <b>15</b>   | 0.139 | 0.093 | 0.086 | 0.189 | 0.108 | 0.123 |
|         | <b>16</b>   | 0.197 | 0.163 | 0.219 | 0.176 | 0.158 | 0.237 |
|         | <b>17</b>   | 0.213 | 0.326 | 0.278 | 0.311 | 0.308 | 0.325 |
|         | <b>18</b>   | 0.254 | 0.267 | 0.222 | 0.189 | 0.183 | 0.175 |
|         | <b>19</b>   | 0.049 | 0.07  | 0.096 | 0.054 | 0.083 | 0.044 |
|         | <b>20</b>   | 0.008 | 0.012 | 0.017 | 0     | 0.017 | 0.018 |
|         | <b>21</b>   | 0     | 0     | 0.003 | 0     | 0     | 0     |
| TPOX    | <b>7</b>    | 0.008 | 0     | 0     | 0     | 0     | 0     |
|         | <b>8</b>    | 0.615 | 0.43  | 0.563 | 0.608 | 0.592 | 0.535 |
|         | <b>9</b>    | 0.066 | 0.14  | 0.116 | 0.068 | 0.083 | 0.088 |
|         | <b>10</b>   | 0.033 | 0.081 | 0.056 | 0.041 | 0.058 | 0.088 |
|         | <b>11</b>   | 0.254 | 0.291 | 0.222 | 0.23  | 0.233 | 0.272 |

| Marker | Allele      | BLR   | CZE   | POL   | RUS   | SRB   | UKR   |
|--------|-------------|-------|-------|-------|-------|-------|-------|
|        | <b>12</b>   | 0.025 | 0.058 | 0.04  | 0.054 | 0.033 | 0.018 |
|        | <b>13</b>   | 0     | 0     | 0.003 | 0     | 0     | 0     |
| D18S51 | <b>10</b>   | 0.008 | 0.012 | 0.01  | 0     | 0.025 | 0     |
|        | <b>11</b>   | 0.016 | 0     | 0.026 | 0.027 | 0.025 | 0.009 |
|        | <b>12</b>   | 0.09  | 0.128 | 0.076 | 0.054 | 0.108 | 0.061 |
|        | <b>13</b>   | 0.123 | 0.128 | 0.099 | 0.081 | 0.167 | 0.14  |
|        | <b>14</b>   | 0.131 | 0.174 | 0.152 | 0.122 | 0.133 | 0.175 |
|        | <b>15</b>   | 0.197 | 0.14  | 0.142 | 0.149 | 0.15  | 0.193 |
|        | <b>16</b>   | 0.197 | 0.163 | 0.152 | 0.176 | 0.2   | 0.158 |
|        | <b>17</b>   | 0.082 | 0.081 | 0.156 | 0.189 | 0.067 | 0.053 |
|        | <b>18</b>   | 0.057 | 0.093 | 0.086 | 0.081 | 0.083 | 0.061 |
|        | <b>19</b>   | 0.049 | 0.023 | 0.04  | 0.054 | 0     | 0.105 |
|        | <b>20</b>   | 0.033 | 0.023 | 0.043 | 0.041 | 0.025 | 0.035 |
|        | <b>21</b>   | 0.008 | 0.035 | 0.013 | 0.014 | 0.008 | 0.009 |
|        | <b>22</b>   | 0.008 | 0     | 0.003 | 0.014 | 0.008 | 0     |
| D5S818 | <b>7</b>    | 0.025 | 0.012 | 0.003 | 0     | 0.008 | 0     |
|        | <b>9</b>    | 0.074 | 0.047 | 0.04  | 0.041 | 0.025 | 0.044 |
|        | <b>10</b>   | 0.09  | 0.116 | 0.06  | 0.122 | 0.058 | 0.079 |
|        | <b>11</b>   | 0.303 | 0.291 | 0.344 | 0.365 | 0.292 | 0.351 |
|        | <b>12</b>   | 0.344 | 0.326 | 0.401 | 0.324 | 0.367 | 0.368 |
|        | <b>13</b>   | 0.156 | 0.198 | 0.149 | 0.135 | 0.25  | 0.132 |
|        | <b>14</b>   | 0     | 0.012 | 0.003 | 0.014 | 0     | 0.026 |
|        | <b>16</b>   | 0.008 | 0     | 0     | 0     | 0     | 0     |
| FGA    | <b>16</b>   | 0     | 0     | 0     | 0     | 0.008 | 0     |
|        | <b>17</b>   | 0     | 0     | 0.003 | 0     | 0     | 0.009 |
|        | <b>18</b>   | 0     | 0     | 0.023 | 0     | 0.008 | 0.018 |
|        | <b>19</b>   | 0.066 | 0.093 | 0.086 | 0.122 | 0.058 | 0.079 |
|        | <b>20</b>   | 0.213 | 0.093 | 0.113 | 0.095 | 0.133 | 0.14  |
|        | <b>21</b>   | 0.123 | 0.233 | 0.185 | 0.122 | 0.158 | 0.211 |
|        | <b>21.2</b> | 0.016 | 0     | 0.007 | 0.027 | 0     | 0     |
|        | <b>22</b>   | 0.139 | 0.244 | 0.179 | 0.27  | 0.133 | 0.167 |
|        | <b>22.2</b> | 0.025 | 0     | 0.007 | 0     | 0     | 0.009 |
|        | <b>23</b>   | 0.107 | 0.116 | 0.126 | 0.122 | 0.192 | 0.123 |
|        | <b>23.2</b> | 0     | 0.012 | 0.007 | 0     | 0.008 | 0.009 |
|        | <b>24</b>   | 0.18  | 0.081 | 0.132 | 0.149 | 0.167 | 0.114 |
|        | <b>24.2</b> | 0     | 0     | 0     | 0.014 | 0     | 0     |
|        | <b>25</b>   | 0.123 | 0.105 | 0.093 | 0.054 | 0.092 | 0.123 |
|        | <b>26</b>   | 0.008 | 0.023 | 0.036 | 0.027 | 0.042 | 0     |
|        | <b>27</b>   | 0     | 0     | 0.003 | 0     | 0     | 0     |

## Results and discussion

### Population data analyzed with the use of distance-based methods

The allele frequencies of the 46 InDel markers and 15 STRs are presented in Tables II and III, respectively. No significant results were observed in the linkage disequilibrium and Hardy-Weinberg tests after applying the Bonferroni correction. Matrices of pairwise genetic distances between populations are depicted in Table IV. Graphs of multidimensional scaling are shown in Figures I, II and III. The “stress” values in all MDS analyses indicate a very good fit between the distance matrices of the reconstructed distances and the distance matrices observed. Scatter plot of self-loads is presented in Figure IV. Snipper classification results for a set of 46 InDel markers are presented in Tables V, VI and Figure V, while Table VII shows the classification based on STRs.

The obtained results of genetic distances (greater than zero) reflect low genetic differentiation, which means high homogeneity of the studied Slavic-speaking populations (Table IV), the condition being proved previously for European gene pool in general with the use of different genetic markers. However, both MDS and PC analyses indicate that the population of the Czech Republic is the most genetically distant from the other studied Slavic populations (absence of genetic differentiation was observed relative to the population of Ukraine only, Table IV). Comparing both sets of markers while setting aside the subtle Czech outlier, it can be seen that 46 InDels gave similar results to the set of STR markers commonly used for human identification in forensics. The exceptions constitute positive (but not significant) InDel genetic distances observed between Poland and Ukraine, Serbia and Belarus, which were not noted using microsatellites. Conversely, positive distances between Poland and Belarus, Serbia and Russia, Serbia and Ukraine were not obtained in the study of InDels but were observed in STRs (Table IV).

### The case of individual prediction

According to the Snipper classification based on 46 InDel markers at the continental level, the studied sample of a Polish individual was assigned to the European population (probabilities are presented on a negative logarithmic scale, so a lower value indicates a better match). The profile of the tested person 25 893 434 times more likely belongs to the population of EUROPE than OCEANIA (Table V, Figure V). While at the continental level the classification result is correct, after further subdivision of Europe into subpopulations, the Polish sample was assigned with the highest probability to the population of France (Table VI). It should be noted here that the differences between the probabilities for successive matches are not large – the profile of the studied Pole only 2 times more likely belongs to the French population than for the Adygei and 355 times more likely belongs to the French population than for the Polish population. Considering the above, the set of 46 insertion-deletion markers is characterized by continental rather than population resolution. However, the Snipper microsatellite classification (Table VII) gave a much more plausible result in terms of the probability of assigning the sample to a particular European population. In this case, the sample was most probably assigned to the Belarusian population; the result being consistent with both previous high-resolution SNPs’ inference and the individuals’ self-inferred family genealogy. Simultaneously, small differences between the assignment probabilities indicate very small genetic differentiation between the populations on the STR level.

In conclusion, STRs but not InDels appear useful supplementary markers for inferring biogeographic ancestry within the Slavic-speaking population of Europe.

**Table IV.** Matrices of  $F_{ST}$  pairwise genetic distances between populations for insertion-deletion markers (InDel), microsatellite markers (IF) and both sets together (InDel + IF). Statistically significant distances are written in bold.

|            | BLR            | CZE            | POL            | RUS     | SRB     | UKR |
|------------|----------------|----------------|----------------|---------|---------|-----|
| InDel      |                |                |                |         |         |     |
| BLR        | 0              |                |                |         |         |     |
| CZE        | 0.00089        | 0              |                |         |         |     |
| POL        | 0              | <b>0.00413</b> | 0              |         |         |     |
| RUS        | 0              | 0.00025        | 0              | 0       |         |     |
| SRB        | 0.00122        | 0.00376        | 0.00206        | 0       | 0       |     |
| UKR        | 0              | 0              | 0.00231        | 0       | 0       | 0   |
| IF         |                |                |                |         |         |     |
| BLR        | 0              |                |                |         |         |     |
| CZE        | <b>0.00459</b> | 0              |                |         |         |     |
| POL        | 0.00051        | <b>0.00292</b> | 0              |         |         |     |
| RUS        | 0              | <b>0.00499</b> | 0              | 0       |         |     |
| SRB        | 0              | <b>0.00306</b> | 0.00162        | 0.00047 | 0       |     |
| UKR        | 0              | 0              | 0              | 0       | 0.00087 | 0   |
| InDel + IF |                |                |                |         |         |     |
| BLR        | 0              |                |                |         |         |     |
| CZE        | 0.00251        | 0              |                |         |         |     |
| POL        | 0.00007        | <b>0.00360</b> | 0              |         |         |     |
| RUS        | 0              | 0.00233        | 0              | 0       |         |     |
| SRB        | 0.00066        | <b>0.00346</b> | <b>0.00187</b> | 0       | 0       |     |
| UKR        | 0              | 0              | 0.00068        | 0       | 0.00036 | 0   |

**Table V.** Bayesian classification of the studied Polish sample using the Snipper application for a set of 46 insertion-deletion markers at the continental level.

| Population | -ln(likelihood) |
|------------|-----------------|
| EUROPE     | 36.4906         |
| OCEANIA    | 53.5601         |
| EAST ASIA  | 64.3729         |
| AMERICA    | 78.5671         |
| AFRICA     | 87.5719         |

**Table VI.** Bayesian classification of the studied Polish sample using the Snipper application for a set of 46 insertion-deletions. Both our own genotypic data and those downloaded from the "forInDel" browser were used in the analysis\*.

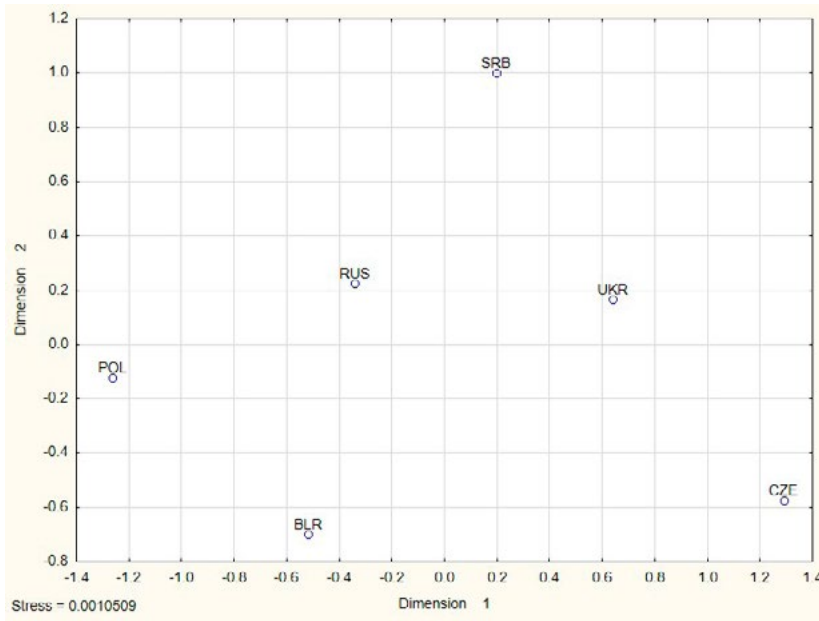
| Population     | -ln(likelihood) |
|----------------|-----------------|
| France         | 35.9454         |
| Adygei, Russia | 36.6048         |

|                    |         |
|--------------------|---------|
| Italy              | 36.9797 |
| Russia             | 38.0448 |
| Basque, France     | 38.9981 |
| Belarus            | 39.8187 |
| Middle East        | 39.8794 |
| Central-south Asia | 40.3405 |
| Serbia             | 40.4148 |
| Ukraine            | 40.557  |
| Poland             | 41.8174 |
| Magadan, Russia    | 41.893  |
| Orkney Islands     | 41.9066 |
| Czech              | 42.215  |
| Oceania            | 53.7207 |
| East Asia          | 64.3847 |
| America            | 78.5061 |
| Africa             | 87.5988 |

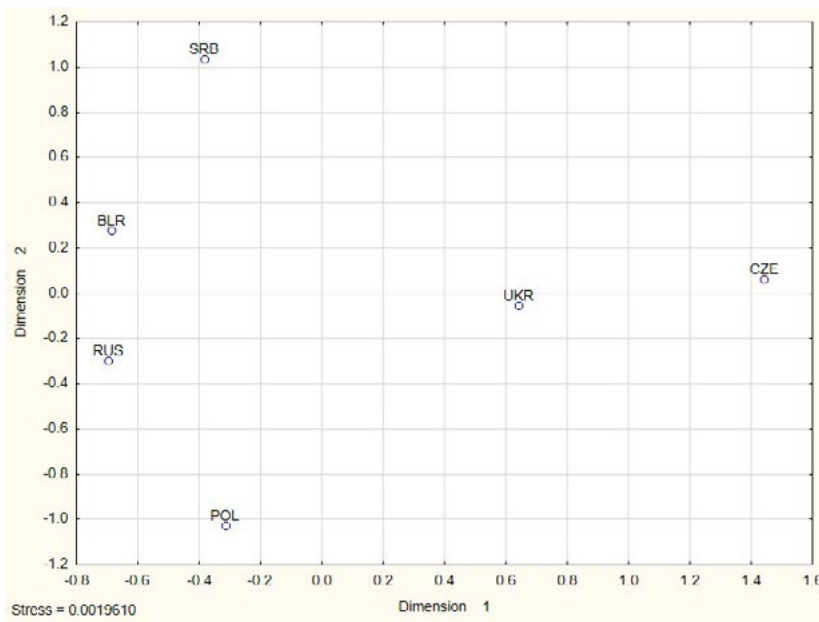
**Table VII.** Bayesian classification in the Snipper application for a set of microsatellite markers using our own data (population names in capital letters) and allele frequencies from published papers.

| Population                           | Likelihood  | -ln(likelihood) |
|--------------------------------------|-------------|-----------------|
| BELARUS                              | 3.55431E-24 | 53.9939         |
| Italy [14]                           | 2.51676E-24 | 54.3391         |
| Spain [15]                           | 1.1528E-24  | 55.1198         |
| Spain [16]                           | 8.65509E-25 | 55.4065         |
| Basque Country [17]                  | 8.44144E-25 | 55.4315         |
| Sweden [18]                          | 6.9279E-25  | 55.6291         |
| Greece [19]                          | 5.00861E-25 | 55.9535         |
| Croatia [20]                         | 4.07136E-25 | 56.1607         |
| Romania [21]                         | 3.87226E-25 | 56.2108         |
| Galicia [22]                         | 2.48123E-25 | 56.6559         |
| Ukraine [23]                         | 2.16913E-25 | 56.7903         |
| Poland- Pomorze Zachodnie [24]       | 1.404E-25   | 57.2253         |
| Denmark [25]                         | 9.73306E-26 | 57.5917         |
| Sweden [26]                          | 9.57944E-26 | 57.6076         |
| Serbia and Montenegro-Vojvodina [27] | 9.57835E-26 | 57.6077         |
| Serbia- Kosovo- Albanians [28]       | 8.48903E-26 | 57.7284         |
| Serbia [29]                          | 7.88233E-26 | 57.8026         |
| Czech [30]                           | 6.46332E-26 | 58.0011         |
| Poland [31]                          | 6.21621E-26 | 58.0401         |
| Poland- Łódź [32]                    | 5.77245E-26 | 58.1141         |
| Hungary [33]                         | 5.09134E-26 | 58.2397         |
| Croatia [34]                         | 4.24412E-26 | 58.4217         |
| The United Kingdom [35]              | 3.9333E-26  | 58.4977         |
| SERBIA                               | 3.77698E-26 | 58.5383         |
| POLAND                               | 2.82265E-26 | 58.8295         |
| Russia- Saratov [36]                 | 2.12107E-26 | 59.1153         |
| The Netherlands [37]                 | 2.02562E-26 | 59.1613         |
| UKRAINE                              | 1.06236E-26 | 59.8067         |
| CZECH                                | 3.93937E-27 | 60.7988         |
| Russia- Min-Vody [36]                | 3.59655E-27 | 60.8898         |
| Estonia [38]                         | 3.12041E-27 | 61.0318         |
| Russia- Orel [36]                    | 2.62156E-27 | 61.206          |
| Eastern Slovakia- Spis [39]          | 2.03594E-28 | 63.7614         |
| RUSSIA                               | 1.4934E-28  | 64.0713         |
| Eastern Slovakia- Abov-Gemer [39]    | 1.30146E-28 | 64.2089         |
| Eastern Slovakia- Saris [39]         | 3.66531E-29 | 65.4761         |
| Italy-Tuscany [40]                   | 1.54905E-29 | 66.3373         |

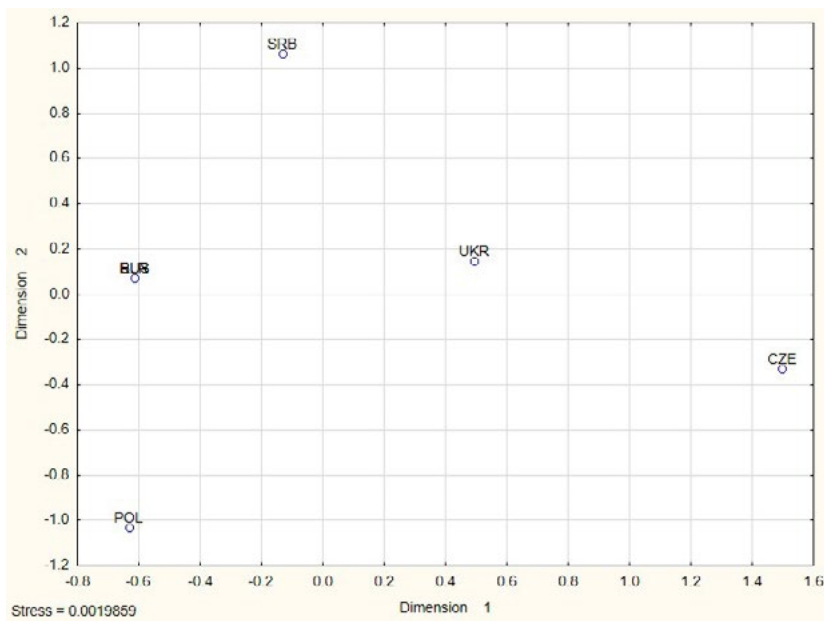




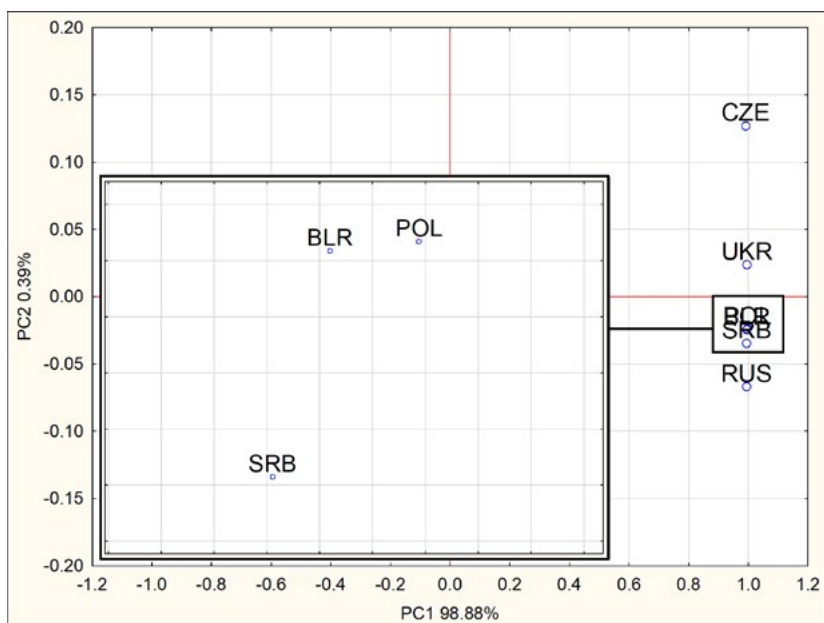
**Figure I.** Multidimensional scaling graph of studied populations regarding InDel markers. Population abbreviations: BLR – Belarus, CZE – Czech Republic, POL – Poland, RUS – Russia, SRB – Serbia, UKR – Ukraine.



**Figure II.** Multidimensional scaling graph of studied populations regarding STR markers. Population abbreviations: BLR – Belarus, CZE – Czech Republic, POL – Poland, RUS – Russia, SRB – Serbia, UKR – Ukraine.



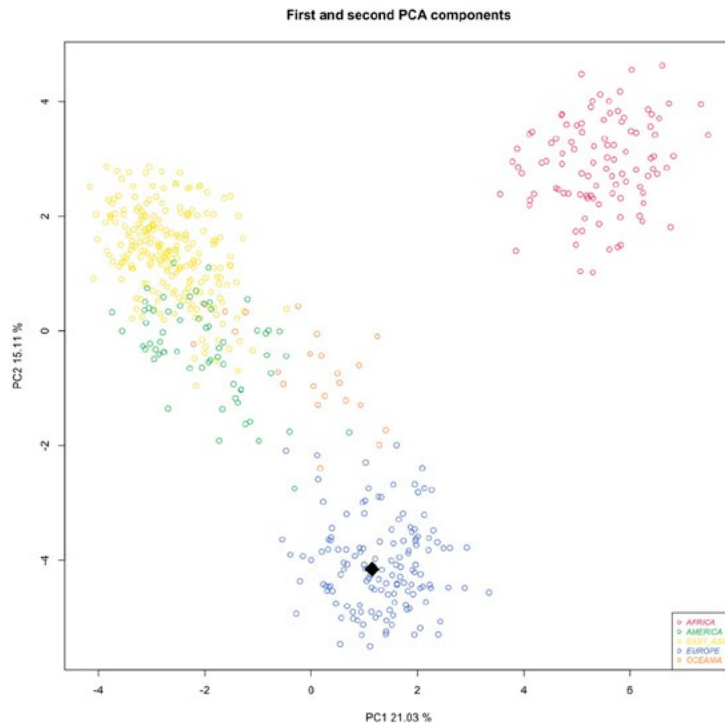
**Figure III.** Multidimensional scaling graph of studied populations regarding both InDel and STR markers. Population abbreviations: BLR – Belarus, CZE – Czech Republic, POL – Poland, RUS – Russia, SRB – Serbia, UKR – Ukraine.



**Figure IV.** Scatter plot of self-loads of studied populations regarding all studied markers. Population abbreviations: BLR – Belarus, CZE – Czech Republic, POL – Poland, RUS – Russia, SRB – Serbia, UKR – Ukraine.

PC1 – the first principal component that explains 98.88% of the observed genetic variation;

PC2 – the second principal component that explains 0.39% of the observed genetic variation.



**Figure V.** Scatter plot of self-loads generated in the Snipper application in continental level analysis for a set of 46 InDel markers. The black square on the graph represents a sample from the studied Polish individual.

PC1 – the first principal component;  
PC2 – the second principal component.

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Supplementary data in the electronic version: The component table for PCA analysis (Suppl. Table I).

## References

1. Shriver MD, Parra EJ, Dios S, Bonilla C, Norton H, Jovel C, Pfaff C, Jones C, Massac A, Cameron N, Baron A, Jackson T, Argypoulos G, Jin L, Hoggart CJ, McKeigue PM, Kittles RA. Skin pigmentation, biogeographical ancestry and admixture mapping. *Hum Genet* 2003; 112(4): 387-99.
2. Phillips C, Prieto L, Fondevila M, Salas A, Gómez-Tato A, Alvarez-Dios J, Alonso A, Blanco-Verea A, Brión M, Montesino M, Carracedo A, Lareu MV. Ancestry analysis in the 11-M Madrid bomb attack investigation. *PLoS One* 2009; 4(8): e6583.
3. Kidd KK, Speed WC, Pakstis AJ, Furtado MR, Fang R, Madbouly A, Maiers M, Middha M, Friedlaender FR, Kidd JR. Progress toward an efficient panel of SNPs for ancestry inference. *Forensic Sci Int Genet* 2014; 10: 23-32.
4. Kosoy R, Nassir R, Tian C, White PA, Butler LM, Silva G, Kittles R, Alarcon-Riquelme ME, Gregersen PK, Belmont JW, De La Vega FM, Seldin MF. Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. *Hum Mutat* 2009; 30(1): 69-78.
5. Phillips C, Parson W, Lundsberg B, Santos C, Freire-Aradas A, Torres M, Eduardoff M, Børsting C, Johansen P, Fondevila M, Morling N, Schneider P; EUROFORGEN-NoE Consortium, Carracedo A, Lareu MV. Building a forensic ancestry panel from the ground up: The EUROFORGEN Global AIM-SNP set. *Forensic Sci Int Genet* 2014; 11: 13-25.
6. Pereira V, Freire-Aradas A, Ballard D, Børsting C, Diez V, Pruszkowska-Przybylska P, Ribeiro J, Achakzai NM, Aliferi A, Bulbul O, Carceles MDP, Triki-Fendri S, Rebai A, Court DS, Morling N, Lareu MV, Carracedo A; EUROFORGEN-NoE Consortium,



- Phillips C. Development and validation of the EUROFORGEN NAME (North African and Middle Eastern) ancestry panel. *Forensic Sci Int Genet* 2019; 42: 260-267.
7. Pereira R, Phillips C, Pinto N, Santos C, dos Santos SE, Amorim A, Carracedo Á, Gusmão L. Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing. *PLoS One* 2012; 7(1): e29684.
  8. Phillips C, Salas A, Sánchez JJ, Fondevila M, Gómez-Tato A, Alvarez-Dios J, Calaza M, de Cal MC, Ballard D, Lareu MV, Carracedo A; SNPforID Consortium. Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. *Forensic Sci Int Genet* 2007; 1: 273-80.
  9. Phillips C, Fernandez-Formoso L, Garcia-Magariños M, Porras L, Tvedebrink T, Amigo J, Fondevila M, Gomez-Tato A, Alvarez-Dios J, Freire-Aradas A, Gomez-Carballa A, Mosquera-Miguel A, Carracedo A, Lareu MV. Analysis of global variability in 15 established and 5 new European Standard Set (ESS) STRs using the CEPH human genome diversity panel. *Forensic Sci Int Genet* 2011; 5(3): 155-69.
  10. Santos C, Phillips C, Oldoni F, Amigo J, Fondevila M, Pereira R, Carracedo Á, Lareu MV. Completion of a worldwide reference panel of samples for an ancestry informative Indel assay. *Forensic Sci Int Genet* 2015; 17: 75-80.
  11. Rice WR. Analyzing tables of statistical tests. *Evolution* 1989; 1: 223-225.
  12. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 2010; 10(3): 564-7.
  13. Nei M. *Molecular evolutionary genetics*. New York. Columbia University Press; 1987.
  14. Barbaro A, Phillips C, Formoso LF, Lareu MV, Carracedo Á. Distribution of allele frequencies of 20 STRs loci in a population sample from Calabria, Southern Italy. *Forensic Sci Int Genet* 2012; 6(5): e137-8.
  15. Hill CR, Duerwer DL, Kline MC, Coble MD, Butler JM. U.S. population data for 29 autosomal STR loci. *Forensic Sci Int Genet* 2013; 7(3): e82-3. Erratum in: *Forensic Sci Int Genet* 2017; Nov; 31: e36-e40.
  16. García O, Alonso J, Cano JA, García R, Luque GM, Martín P, de Yuso IM, Maulini S, Parra D, Yurrebaso I. Population genetic data and concordance study for the kits Identifiler, NGM, PowerPlex ESX 17 System and Investigator ESSplex in Spain. *Forensic Sci Int Genet* 2012; Mar; 6(2): e78-9. Erratum in: *Forensic Sci Int Genet* 2014; 9: 192.
  17. Yurrebaso I, Ajuariagera JA, Alday A, Lezama I, Pérez JA, Romón E, Uriarte I, García O. Allele frequencies and concordance study between the Identifiler and the PowerPlex ESX17 systems in the Basque Country population. *Forensic Sci Int Genet* 2011; 5(3): e79-80.
  18. Montelius K, Karlsson AO, Holmlund G. STR data for the AmpFISTR Identifiler loci from Swedish population in comparison to European, as well as with non-European population. *Forensic Sci Int Genet* 2008; 2(3): e49-52.
  19. Sánchez-Diz P, Menounos PG, Carracedo A, Skitsa I. 16 STR data of a Greek population. *Forensic Sci Int Genet* 2008; 2(4): e71-2.
  20. Barbarić L, Ozretić P, Horjan I, Korolija M, Mršić G. Forensic evaluation of the 20 STR loci in the population of Croatia. *Forensic Sci Int Genet* 2017; 28: e49-e50.
  21. Stanciu F, Popescu OR, Stoian IM. Allele frequencies of 15 STR loci in Moldavia region (NE Romania). *Forensic Sci Int Genet* 2009; 4(1): e39-40.
  22. Fernandez-Formoso L, Phillips C, Rodriguez A, Calvo R, Barbaro A, Lareu MV, Carracedo Á. Allele frequencies of 20 STRs from Northwest Spain (Galicia). *Forensic Sci Int Genet* 2012; 6(5): e149-50.
  23. Serga SV, Dombrovskiy IV, Maistrenko OM, Ostapchenko LL, Demydov SV, Krivda RG, Kozeretka IA. Allele frequencies for 15 STR loci in the Ukrainian population. *Forensic Sci Int Genet* 2017; 29: e40-e41.
  24. Piątek J, Jacewicz R, Ossowski A, Parafiniuk M, Berent J. Population genetics of 15 autosomal STR loci in the population of Pomorze Zachodnie (NW Poland). *Forensic Sci Int Genet* 2008; 2(3): e41-3.
  25. Hussing C, Bytci R, Huber C, Morling N, Børsting C. The Danish STR sequence database: duplicate typing of 363 Danes with the ForenSeq™ DNA Signature Prep Kit. *Int J Legal Med* 2019; 133(2): 325-334.
  26. Staadig A, Tillmar A. An overall limited effect on the weight-of-evidence when taking STR DNA sequence polymorphism into account in kinship analysis. *Forensic Sci Int Genet* 2019; 39: 44-49.
  27. Veselinović I, Kubat M, Furac I, Skavić J, Martinović Klarić I, Tasić M. Allele frequencies of the 15 AmpF ISTR Identifiler loci in the population of Vojvodina Province, Serbia and Montenegro. *Int J Legal Med* 2004; 118(3): 184-6.
  28. Kubat M, Skavić J, Behluli I, Nuraj B, Bekteshi T, Behluli M, Klarić IM, Perić M. Population genetics of the 15 AmpF ISTR Identifiler loci in Kosovo Albanians. *Int J Legal Med* 2004; 118(2): 115-8.
  29. Novković T, Panić B, Banjac A, Dekić TK, Tomisić-Kosić I, Vucetić-Dragović A, Stamenković G, Blagojević J, Marjanović D, Pojskić N. Genetic polymorphisms of 15 AmpFISTR Identifiler loci in a Serbian population. *Forensic Sci Int Genet* 2010; 4(5): e149-50.
  30. Simková H, Faltus V, Marvan R, Pexa T, Stenzl V, Broucek J, Horínek A, Mazura I, Zvárová J. Allele frequency data for 17 short tandem repeats in a Czech population sample. *Forensic Sci Int Genet* 2009; 4(1): e15-7.
  31. Ossowski A, Diepenbroek M, Szargut M, Zielińska G, Jędrzejczyk M, Berent J, Jacewicz R. Population analysis and forensic evaluation of 21 autosomal loci included in GlobalFiler™ PCR Kit in Poland. *Forensic Sci Int Genet* 2017; 29: e38-e39.
  32. Jacewicz R, Jędrzejczyk M, Ludwikowska M, Berent J. Population database on 15 autosomal STR loci in 1000 unrelated individuals from the Lodz region of Poland. *Forensic Sci Int Genet* 2008; 2(1): e1-3.
  33. Petrić G, Drašković D, Zgonjanin-Bosić D, Budakov B, Veselinović I. Genetic variation at 15 autosomal STR loci in the Hungarian population of Vojvodina Province, Republic of Serbia. *Forensic Sci Int Genet* 2012; 6(6): e163-5.



34. Haliti N, Carapina M, Masić M, Strinović D, Klarić IM, Kubat M. Evaluation of population variation at 17 autosomal STR and 16 Y-STR haplotype loci in Croatians. *Forensic Sci Int Genet* 2009; 3(4): e137-8.
35. Devesse L, Ballard D, Davenport L, Riethorst I, Mason-Buck G, Syndercombe Court D. Concordance of the ForenSeq™ system and characterisation of sequence-specific autosomal STR alleles across two major population groups. *Forensic Sci Int Genet* 2018; 34: 57-61.
36. Zhivotovsky LA, Malyarchuk BA, Derenko MV, Wozniak M, Grzybowski T. Developing STR databases on structured populations: the native South Siberian population versus the Russian population. *Forensic Sci Int Genet* 2009; 3(4): e111-6.
37. Westen AA, Kraaijenbrink T, Robles de Medina EA, Harteveld J, Willemse P, Zuniga SB, van der Gaag KJ, Weiler NEC, Warnar J, Kayser M, Sijen T, de Knijff P. Comparing six commercial autosomal STR kits in a large Dutch population sample. *Forensic Sci Int Genet* 2014; 10: 55-63.
38. Sadam M, Tasa G, Tiidla A, Lang A, Axelsson EP, Pajnič IZ. Population data for 22 autosomal STR loci from Estonia. *Int J Legal Med* 2015; 129(6): 1219-20.
39. Soták M, Petrejčíková E, Bůžiková A, Bernasovská J, Bernasovský I, Sovičová A, Boroňová I, Svičková P, Gabriková D, Mačeko-ová S, Carnogurská J, Rębała K, Vlček D. Population database of 17 autosomal STR loci from the four predominant Eastern Slovakia regions. *Forensic Sci Int Genet* 2011; 5(3): 262-3.
40. Carboni I, Nutini AL, Porfirio B, Genuardi M, Ricci U. Genetic STRs variation in a large population from Tuscany (Italy). *Forensic Sci Int Genet* 2007; 1(3-4): e10-1.

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