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Paternal genetic history of the Vlax Roma

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ABSTRACT

Romanies constitute the largest minority group belonging to different subgroups in Hungary. Vlax Romanies are one of these Romani subgroups. The Gypsies came to Hungary from the Balkans in two large migrations. The Carpathian Romanies arrived in the 15th century and the Vlax Romanies came in the 19th century. The Carpathian Gypsies speak Hungarian and the Vlax Romanies speak Hungarian and Romani languages.

Only a limited number of genetic studies of Y-chromosomal haplotypes/haplogroups have been done before, moreover most studies did not contain information regarding the investigated Roma populations which subgroups belong to.

In the present study, we analyzed a wide set of Y-chromosomal markers to do comparable studies of the Vlax Roma in eastern Hungarian regions. The results can be compared in the context of previously published data on other Romani groups, Indian and Hungarian reference populations.

Haplogroups H1a-M82 and J2a2-M67 were most common in the investigated population groups. A median-joining network of haplogroup H1a-M82 has demonstrated the sharing of identical Indian specific Y-chromosomal lineages between all Romani populations including Malaysian Indians as well as the Vlax Romanies. This common lineage of haplogroup H1a-M82 represents a common descent from a single ancestor provides a strong genetic link to the ancestral geographical origin of the proto-Gypsies.

The detected haplogroups in the Vlax Romani population groups can be classified into two different Y-chromosomal lineages based on their putative origin. These lineages include ancestral Indian (H1a-M82), present-day Eurasian (J2a2-M67, J2*-M172, E1b1b1a-M78, I1-M253, R1a1-M198 and R1b1-P25) Y-chromosome lineages. Presence of these lineages in the paternal gene pool of the Roma people is illustrative of the Gypsy migration route from India through the Balkan to the Carpathian Basin.

1. Introduction

The Gypsies arrived in Europe 900–1100 years ago, when they first appeared in the Balkans. The present-day Gypsy population groups in Europe are the compound product of the early migrations from the Balkans into Europe [1].The Gypsies came to Hungary from the Balkans in two large migrations. The Carpathian Romanies arrived in the 15th century and the Vlax Romanies came in the 19th century. The Carpathian Gypsies speak Hungarian and the Vlax Romanies speak Hungarian and Romani languages.

Vlax Romani is a dialect group of the Romani language. Vlax Romani varieties are spoken mainly in Southeastern Europe by Romani people [2]. Most Vlax Romani speakers live in Bosnia-Herzegovina followed by Romania, Albania and Hungary [3].

Romanies constitute the largest minority group in Hungary, which means approximately 6–700 thousand people [4]. They live dispersed in all territories of the country. Their presence is very high in the three northeastern counties and in the southern county of Hungary. About thirty percent of the Romanies, especially, Vlax Romanies, live in northeastern counties.

Only a limited number of genetic studies based on mtDNA and Y-chromosomal haplotypes/haplogroups has been done on European and Hungarian Roma groups before [1,5–11], moreover, most studies did not contain information regarding the investigated Roma populations which subgroups belong to. The only paper published by Gresham et al. [1] provided a study on Roma males and determined both Y-STRs and Y-SNPs of Vlax Roma and other groups. Füredi et al. [7] investigated two Hungarian Roma groups previously based on only Y-STRs. Gusmão et al. [6] studied Y-STRs and Y-SNPs for Iberian Gypsies who probably are not belonging to the Vlax Romanies.

This study is an attempt to address the issue of relatedness between Vlax Roma subgroups and to outline the origin of paternal lineages.

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In the present study, we have analyzed a wide set of Y-chromosomal markers to do comparable studies of the Vlax Roma in eastern Hungarian regions. The results were compared in the context of previously published data on other Romani groups, Indian and Hungarian reference populations [1,5–7,9–13], thus expanding the information base regarding the genetic structure of the Hungarian Vlax Romanies and their demographic history.

2. Materials and methods

Two Vlachian Romani groups originating from Tiszavasvári ($N = 29$) and Tokaj ($N = 39$) in Eastern Hungary were studied. Twelve Y-STRs (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS 439) were tested with the Powerplex Y PCR kit (Promega). Fifty-one Y-SNPs were tested with Taqman Assays (Fig. 1). The haplogroups tested and the markers used in the study originated from YCC [14]. The nomenclature of haplogroups follows Jobling and Tyler-Smith [15] and Karafet et al. [14]. According to the journals new policy concerning the publication of forensic population genetic data [16] the new samples published herein were sent to the YHRD for external evaluation prior to publication and received the following YHRD accession numbers: Tokaj, Hungary [Romani] YA003660, Tiszavasvari, Hungary [Romani] YA003658 and Taktaköz, Hungary [Romani] YA003659. The new populations as well as the previously published populations Eastern Slovakia [Romani] with accession number YA003186, Hungary [Romani] YA003188, Hungary [Hungarian] YA003187 and Malaysia [Indian] YA003277 used for comparisons can be searched at www.yhrd.org [17] by population name, contributor or accession number. Each person gave their informed consent prior to their inclusion in the study.

Y-STR and Y-SNP data of the Hungarians and Malaysian Indians for the comparison were used from the previous papers published and submitted [12,13].

Haplotype and haplogroup frequencies and their diversity values were calculated as before [18]. Population pairwise genetic distances (Rst) were calculated from haplotype frequencies with Arlequin 2.0 [19]. A multidimensional scaling (MDS) plot was generated withVista 7.2.4. Networks were constructed using the Network 4.5.1.0 program [20], the Y-STR loci were weighted according to the average of their variability in corresponding haplogroups.

3. Results

3.1. Y-SNP results

The haplogroup frequencies and diversity values of the Tiszavasvári and Tokaj Vlax Romanies are presented in Table 1.

Tiszavasvari Vlax Romani group presented the lower haplogroup and haplotype diversity values compared to the corresponding values for the Tokaj Vlax Romani group.

The investigated and compared population data are included in the Supplementary Table 1.

3.2. Phylogenetic analysis

A median-joining (MJ) network of haplogroup H1a-M82 of the investigated and compared populations [1,5,6,12,13,21] based on minimum haplotype (7 Y-STRs) is shown in Fig. 2A.The network includes 327 H1a-M82 chromosomes. The common H1a-M82 haplotype cluster was shared by 215 individuals. The circle size is proportional to the frequency of the haplotype.

Fig. 2B depicts a network construction of 104 individuals on 7 Y-STR loci within H1a-M82 haplogroup of Tiszavasvári, Tokaj and different Bulgarian Vlax Romani groups from the published source

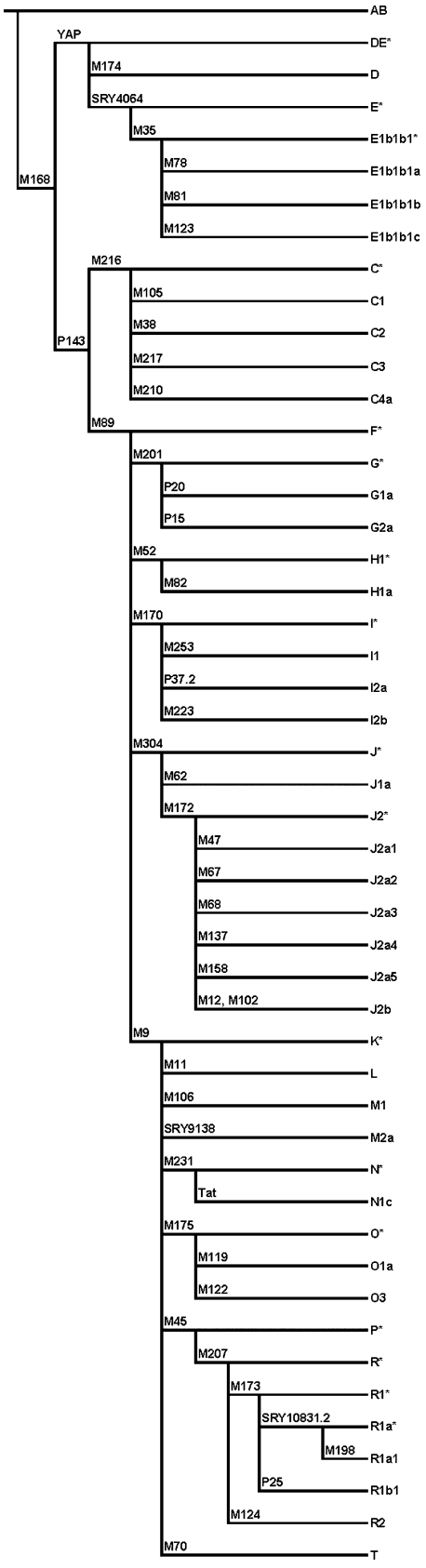

Fig. 1. A phylogenetic tree of the investigated 51 Y-SNP loci.

[1]. It shows a star-like network as well, as in Fig. 2A where a high number of individuals (77 males) belong to a modal haplotype shared by all Romani population groups. The remaining haplotypes differ from the common haplotype by one or two molecular steps.

Table 1
Y-chromosomal haplogroup distributions and diversities in the two investigated Hungarian Vlax Romani populations.

Haplogroups	Mutation	Tiszavasvári Vlax Roma (*N* = 29)		Tokaj Vlax Roma (*N* = 39)	
		N	%	*N*	%
E1b1b1a	M78	2	6.90	6	15.38
G2a	P15	1	3.45	1	2.56
H1a	M82	17	58.62	8	20.51
I1	M253	3	10.34	1	2.56
I2b	M223	0	0.00	1	2.56
J2*	M172	2	6.90	1	2.56
J2a2	M67	3	10.34	9	23.08
R1a1	M198	0	0.00	8	20.51
R1b1	P25	1	3.45	3	7.69
R2	M124	0	0.00	1	2.56
Haplogroup diversity		0.62813		0.85223	
No. of STR haplotypes		11		30	
Haplotype diversity		0.78876		0.91498	

The same MJ network constructed within haplogroup J2a2-M67 of the Romani groups is shown in Fig. 2C. The network included 104 J2a2-M67 chromosomes and out of those, 52 individuals belonged to a core haplotype [1,5,6,12,13]. The core haplotype was shared by all Romani population groups, except for Tiszavasvári and Taktaközi Roma groups. The modal haplotype represents 50% of all J2a2-M67 chromosomes (104 individuals) compared.

3.3. Genetic structure

Based on Rst values of the haplotypes of the Vlax Romani population groups, Hungarian and Malaysian Indian populations investigated [12,13,22], including some other Romani population data available in publications [1,5,12,13,23], pairwise genetic affinities were estimated (Supplementary Table 2) and an MDS plot was constructed as shown in Fig. 3.

4. Discussion

To examine the genetic variation in the Romani groups, we used evolutionary stable Y-SNP markers.

Haplogroups H1a-M82 and J2a2-M67 were most common in the investigated population groups (Table 1). Haplogroup R1a1-M198 appeared with relatively high frequency in the Tokaj Vlax Romanies and it was absent in Tiszavasvari Vlax Romanies. The haplogroup was detected at higher frequency in the Hungarians as

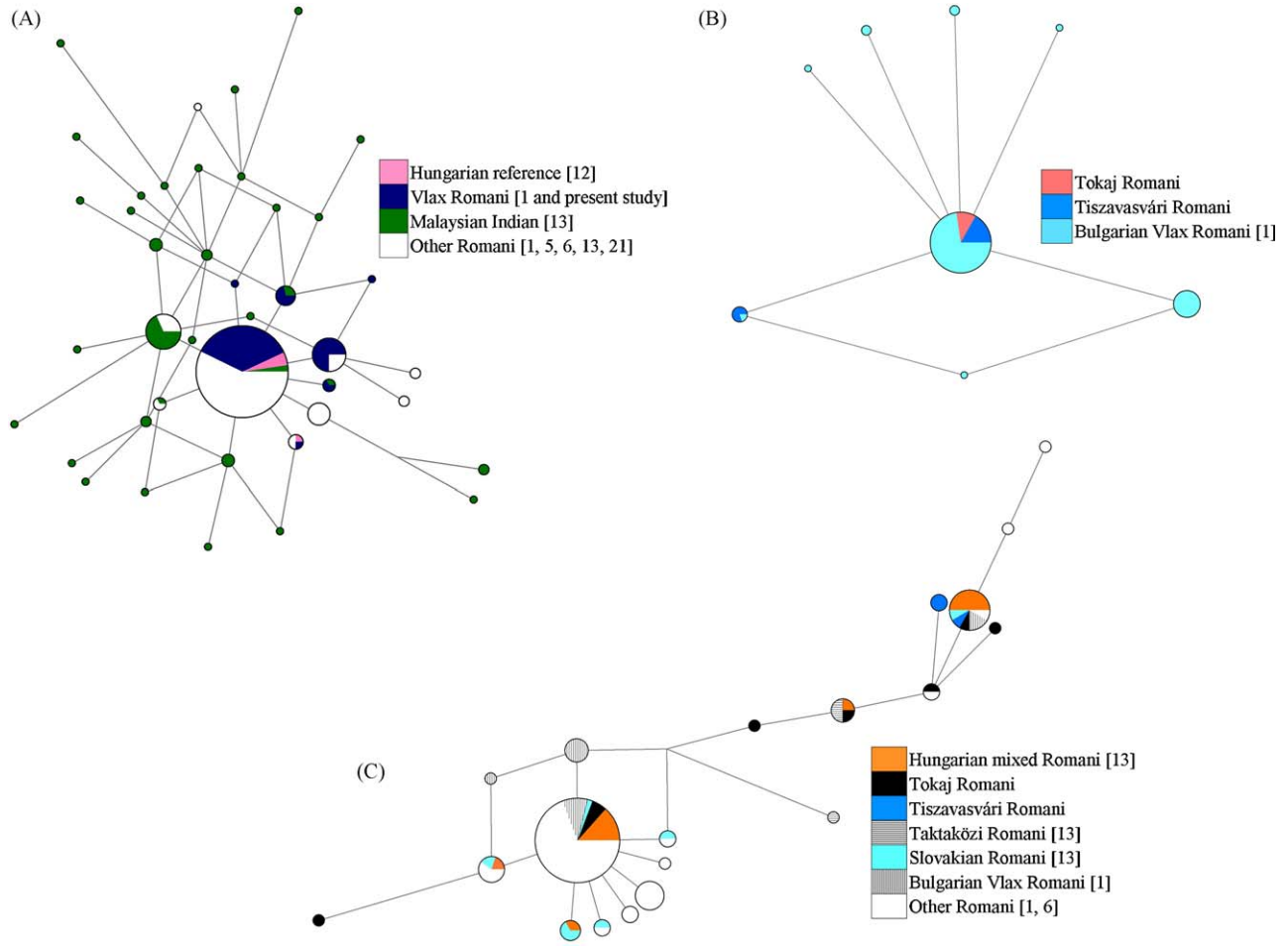

Fig. 2. Median-joining networks for the Romani population groups compared. (A) Median-joining network of Y-STRs within H1a haplogroup for the Romani population groups and Malaysian Indians compared. Vlax Romani includes Tiszavasvári Vlax Romani and Tokaj Vlax Romani from present study and Bulgarian Vlax Romani groups (Lingurari South, Intreni, Montreni, Lom, Kalaidjii South, Kalderash) [1]; other Romani includes Hungarian mixed Romani [12], Taktaközi Romani [13], Slovakian Romani [5], Iberian Gypsy [6], Bulgarian non-Vlax Romani (Turgovzi, Feredjelli, Kalaidjii North, Koshnichari South Central, Koshnichari Southwest) [1], Spanish Roma [1], Lithuanian Roma [1] and Albanian Romani [21]. (B) Median-joining network of Y-STRs within H1a haplogroup for the Tiszavasvári, Tokaj and Bulgarian Vlax (Lingurari South, Intreni, Montreni, Lom, Kalaidjii South, Kalderash) Romani [1] population groups. (C) Median-joining network of Y-STRs within J2a2 haplogroup for the Romani population groups compared. Bulgarian Vlax Romani [1] includes Lingurari South, Intreni, Montreni, Lom, Kalaidjii South and Kalderash; Other Romani includes Iberian Gypsy [6] and Bulgarian non-Vlax Romani groups (Turgovzi, Feredjelli, Kalaidjii North, Koshnichari South Central, Koshnichari Southwest) [1]. The circle sizes are proportional to the haplotype frequencies. The smallest area is equivalent to one individual.

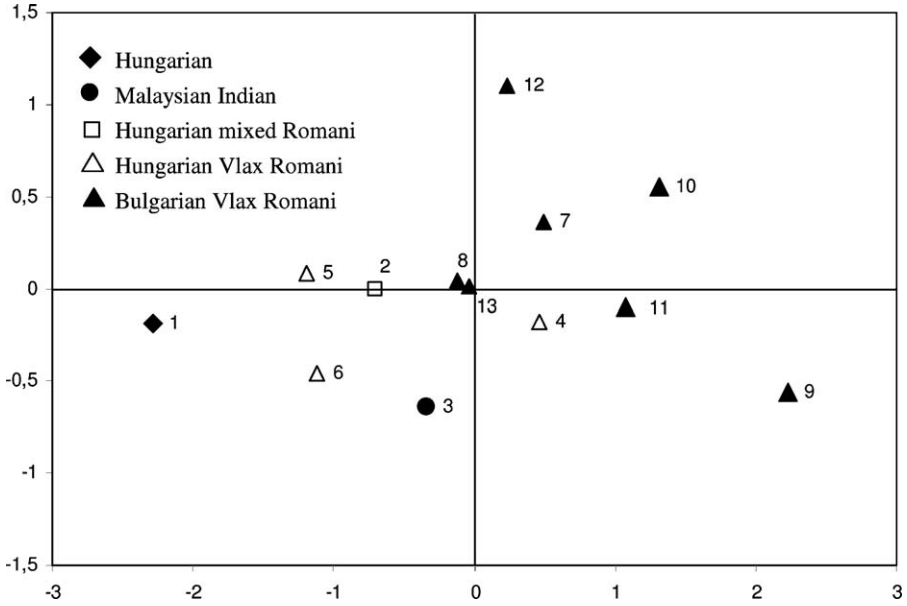

Fig. 3. An MDS plot of 13 population groups based on the Rst genetic distances. 1: Hungarian Caucasian [12], 2: Hungarian mixed Romani [13], 3: Malaysian Indian [22], 4: Tiszavasvári Vlax Romani, 5: Tokaj Vlax Romani, 6: Debrecen Vlax Romani [23], 7: Lingurari North Vlax Romani [1], 8: Lingurari South Vlax Romani [1], 9: Intreni Vlax Romani [1], 10: Montreni Vlax Romani [1], 11: Lom Vlax Romani [1], 12: Kalaidjii South Vlax Romani [1], 13: Kalderash Vlax Romani [1].

compared to in the Malaysian Indians. Haplogroups I1-M253 and E1b1b1a-M78 were detected in all the investigated population groups, except for the Malaysian Indians. All other haplogroups were observed at low frequencies or absent in the investigated population groups (Table 1).

The detected haplogroups in the Vlax Romani population groups can be classified into two different Y-chromosomal lineages based on their putative origin. These lineages include ancestral Indian (H1a-M82), present-day Eurasian (J2a2-M67, J2*-M172, E1b1b1a-M78, I1-M253, R1a1-M198 and R1b1-P25) Y-lineages. Presence of these lineages in the paternal gene pool of the Roma people may be illustrative of the Gypsy migration route from India through the Balkan to the Carpathian Basin.

A median-joining (MJ) network of haplogroup H1a-M82 has demonstrated the sharing of identical Indian specific Y-chromosomal lineages between all Romani populations including Malaysian Indians as well as the Vlax Romanies (Fig. 2A and B). This common lineage of haplogroup H1a-M82 represents a common descent from a single ancestor providing a strong genetic link to the ancestral geographical origin of the proto-Gypsies [1]. According to Sengupta et al. [24] the age of microsatellite variation within haplogroup H1 in Indian populations is more than 9.7 ± 4.4 ky. This time was estimated to be 992 years (95%CI 425–3472) in the Romani populations investigated by Gresham et al. [1] suggesting the Indian H1 haplogroup is the ancestral one. Low presence of this haplogroup in the Hungarian population [12] is due to an unselected sampling method in which the population data applied was primarily for forensic genetic purposes.

Relatively high frequencies of the haplogroup J2a2-M67 in all Romani population groups, its lower frequency in the reference Hungarian population [12,13], and an absence in Indians [24], may indicate a genetic admixture with a population during their migration to the Carpathian Basin. This observation is also supported by the J2a2-M67 network constructed (Fig. 2C) where each haplotype can be traced back to the modal haplotype indicating the common origin of the Romani groups. The spatial distribution of the haplogroup in Eurasia makes it likely that indeed the proto-Roma already carried J2a2-M67 when they first arrived in Europe [6]. Gusmão et al. detected a higher frequency of J2a2-M67 chromosomes (~20%) in the Iberian Gypsies and according to the authors the Iberian Gypsies are likely to be a branch of the group that crossed the Pyrenees in the first quarter of the 15th century and reached to the Iberian Peninsula. Taking into account this observation, the J2a2-M67 marker might have been introduced in the Romani gene pool before of their fragmentation from the Balkans.

The presence of haplogroups E1b1b1a-M78 and I1-M253 in all Romani groups investigated in Europe and absence for Malaysian Indians [1,6,11,12,13,21,22,25] may refer to these chromosomes, at least in part, might have been incorporated into the founder Romani gene pool probably in the Balkans before their fragmentation and migration started from the Balkans to the Carpathian Basin [26]. According to Semino et al. [27] the spatial distribution and network analysis of the E1b1b1a-M78 chromosomes were in agreement with the hypothesis of their ancient presence in the Middle East and their subsequent expansion into the southern Balkans. Based on the previously published paper, haplogroup I-M170 is widespread over Europe, but virtually absent elsewhere, including Near East, suggesting that it arose in Europe [28]. Gusmão et al. [6] noted that the I1-M253 chromosomes occurred at inverted proportions in the Romani groups investigated compared to their host populations if it is considered to have been incorporated into Romani gene pool in the Balkans, in spite of their frequency being high in Scandinavia. This finding can easily be explained by genetic drift, but these events may lead to misinterpretation when addressing the linage origin.

The presence of haplogroup R1b1-P25 at lower frequency in Tokaj and Tiszavasvári Romanies is due to the local population admixture, because there were no shared haplotypes among any Romani groups (data not shown).

The occurrence of R1a1-M198 lineage in the Tokaj Vlax and Lithuanian Romani [1] groups and absence in the Tiszavasvári, Bulgarian and Iberian Gypsies [1,6], may reflect it might have been incorporated into the Romani gene pool later on after fragmentation of the Gypsy groups from the Balkan region by the host population admixture. Besides, possible alternative explanation for absence of the haplogroup in some Roma groups could be caused by genetic drift. Haplogroup R1a1-M198 is particularly common in a large region extending from South Asia to Central Europe and Scandinavia [29].

An MDS plot (Fig. 3) compares ten Vlax Roma population groups with Hungarian, mixed Hungarian Romani and Malaysian Indian populations, through use of 7 Y-STR haplotypes. The Y-chromosome MDS appears less structured, but it seems to mirror the historical and geographical background of the Vlax Romani population groups compared (Fig. 3). The Hungarian Roma population groups (2, 5 and 6 in Fig. 3) were loosely associated with the Hungarian reference population, while other Vlax Romani groups from Bulgaria constitute another cluster indicating different admixtures and the STR substructural patterns of Y-chromosome. The Tiszavasvari Romanies (4 in Fig. 3) interspersed with other Bulgarian Vlax Romani groups, which is due to small population size and the high frequency of H1a-M82 haplogroup detected as well as in the Bulgarian Roma groups. The Malaysian Indians appeared to be a link between Hungarian and other Bulgarian Vlax Romani groups.

The reduced haplotype and haplogroup diversities in Tiszavasvari Romani group due to genetic drifts acting in their pool of Y-chromosomal lineages and these effects can be explained by small population size, together with social organization, which is characteristic for the Romanies [1,30].

Two lineages of the Romani paternal gene pool were identified: ancestral Indian and contemporary Eurasian. Additional admixture, in the presence of the low and moderate frequencies of typical Eurasian haplogroups E1b1b1a-M78, I1-M253, J2*-M172, J2a2-M67, R1b1-P25 and R1a1-M198 took place primarily during their migration route or early settlement in the Balkans and their subsequent influx in the Carpathian Basin.

Conflict of interest statement

The authors state that they have no interests which might be perceived as posing a conflict or bias.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2010.08.017.

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