DNA ANALYSIS OF TWO SARMATIAN FUNERARY CONTEXTS FROM EASTERN ROMANIA

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Cuvinte cheie: *analize ADN, haplogrup, sarmați, estul României.*
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Rezumat: *Acest studiu își propune să analizeze ADN-ul și să identifice haplogrupurile corespunzătoare pe baza unor fragmente osteologice selectate din două contexte funerare aparținând comunităților sarmatice din estul României. Probele pentru analizele ADN au fost selectate din necropola sarmatică de la Isaiia (județul Iași) amplasată pe o terasă a Prutului și din situl de la Vorniceni (județul Botoșani) situat în bazinul Jijiei. Cele două descoperiri funerare au fost atribuite sarmaților, îndeosebi, pe baza inventarului funerar. Analizele ADN realizate pe fragmente osteologice atribuite populației sarmatice contribuie la înțelegerea migrațiilor care au avut loc în estul României.*

Abstract: *The aim of this study was to analyse the ancient DNA and their correspondent haplogroups, presenting also the first genetic results on bone samples selected from two funerary contexts assigned to the Sarmatian communities identified in Eastern Romania. The archaeological sites are located on the terraces integrated in the Jijia and Prut river basins, in the Vorniceni village (Vorniceni commune, Botoșani*

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county) and Isaiia village (Răducăneni commune, Iași county) and were largely attributed based, mainly, on the grave goods, to the Sarmatian populations. The ancient DNA analysis performed on bone samples assigned to the Sarmatian population offered us the possibility to infer the ancient human population admixtures from Eastern Romania using amtDNA isolated from bone remains.

Introduction

The nomadic tribes of the Sarmatians have dominated for more than six centuries the Eurasian Steppe and have played an important role in the emergence, evolution and disappearance of different political powers in the region. The raids of the Sarmatians on the area west of the Don River started as early as the 3rd century BC, while their domination followed only in the 2nd century BC. During the 1st century BC, the Sarmatians already controlled the entire area between the Don and the Dnieper. They continued to integrate new territories until they reached the Northwest Pontic area, which they occupied by the end of the 1st century BC and the first half of the 1st century AD. Throughout numerous raids, the Sarmatian tribes have extended their dominance, in the 1st century BC, in the area situated west of the Dnieper. The presence of the Sarmatians in the North Pontic area, during the 2nd century BC, corresponds to both archaeological discoveries and classical authors' accounts[1].

The systematic analysis and interpretation of the literary, epigraphic and archaeological sources related to the Sarmatians expansion in the region between the Don and the Danube has allowed understanding their appearance in the region and the complex network of contacts they developed with the surrounding world[2]. Most of our knowledge regarding their material culture consists within the metal artefacts found in hoards and the inventory of the funerary discoveries. Moreover, the systematic analysis of the grave goods facilitated a detailed analysis of the different degrees of interaction they had with the Roman Empire, the North-Pontic Greek cities and the "barbarian" neighbours[3].

During the 2nd century BC – 4th century AD, the Sarmatian tribes were one of the most fiercely enemy of the Roman Empire and of the Greek cities in the North and North-Western Black Sea region. They developed very complex relationships with the North-Pontic Greek cities and the surrounding "barbarian" world. Throughout their evolution in the region, they influenced the material culture of the Zarubineck communities, of the Late Scythian tribes from the Lower Nipper and Crimea, and of the Greek cities[4].

Although the Sarmatian tribes from the North Pontic and Eastern Carpathian regions have been extensively analysed in recent archaeological studies[5], their genetic identity remains uncertain. The archaeogenetics is a powerful tool able to track the communities dynamic and population admixture, which can represent a step forward to decode the cultural evolution of the Sarmatian tribes in relation to

[1] BÂRCĂ 2014, p. 167.
[2] BICHIR 1971; BÂRCĂ & SYMONENKO 2009.
[3] BÂRCĂ 2006a.
[4] BÂRCĂ 2006b.
[5] BÂRCĂ 2006a; 2006b.

neighbouring cultures. Most of our current knowledge about the genetic origin of the Sarmatians resides on few samples included in the extended studies dedicated to the so-called *Scythian cultural horizon*[6].

Despite the high informativeness, the genetic characteristics and haplogroup composition of the praehistoric and early historic populations from Eastern Romania is highly unknown. To infer the ancient population from Eastern Romania admixture degree with neighbouring population associated with Sarmatians, we combined the genetic analysis of the mitochondrial hypervariable region 2 (Hv2) and the funerary context. This paper presents the first genetic results on bone samples selected from two funerary contexts assigned to the Sarmatian communities identified in Eastern Romania aiming to identify the membership to a human haplogroup.

Material and methods

Archaeological context

The archaeological human bones samples presented in this study are part of larger database collected from a number of sites analysed for DNA analysis throughout the project *Genetic Evolution: New Evidences for the Study of Interconnected Structures. A Biomolecular Journey around the Carpathians from Ancient to Medieval Times* (GENESIS).

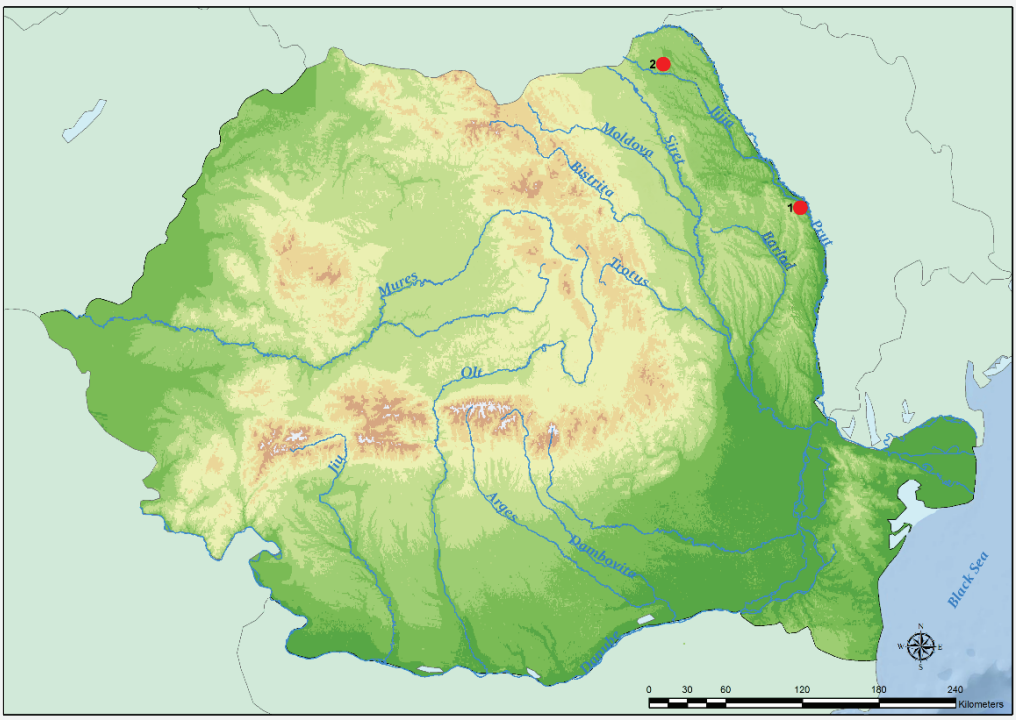

Fig. 1: The location of the archaeological sites from where the human remains were selected (1- Isaiia, Iași County, 2-Vorniceni, Botoșani County)

[6] SARKISSIAN 2011; UNTERLÄNDER *et alii* 2017.

Osteological material for the present study comes from two different burial contexts situated in Eastern Romania (**Fig. 1**), which belongs, based on the grave goods, to the Sarmatian populations[7]. The archaeological site located in the Isaiia village (Răducăneni commune, Iași County) has a complex stratigraphy identified throughout more than six archaeological research campaigns initiated in 1996, mainly for investigating the Precucuteni settlement[8]. The stratigraphic layers identified at Isaiia-*Balta Popii* covers a large timespan, starting with the Neolithic (Linear Pottery culture), Chalcolithic (Precucuteni culture), early Hallstatt (Corlăteni culture), the 2nd – 3rd centuries AD and the beginning of the 2nd millennium AD (Răducăneni culture). From the 16 graves identified in the Isaiia necropolis, 11 belongs to the Sarmatian tribes based on their inventory while other four graves have no funerary goods[9] which makes their cultural and chronological identification difficult in the absence of ^{14}C dates.

The other archaeological site from where we have selected bone samples for this study is in the Vorniceni village (Vorniceni commune, Botoșani County) and consists in an isolated grave identified due to a rescue excavation conducted in the proximity of the Cucuteni A-B settlement located in the Vorniceni village[10]. The osteological remains were assigned to the Sarmatian population based on the associated grave goods.

Samples M8, M13, M15 and M18 were taken from the Sarmatian necropolis identified in the Isaiia village (Răducăneni commune, Iași county) (**Fig. 1**) located on the lower terraces of the Prut river[11]. The osteological remains identified in M8 belongs to an adult woman (age 30) with intentional deformed cranial features. The funerary context contains glass beads, distributed around the legs[12]. The human bones from M13 belong to a young woman (age 25-30) which is relatively well preserved. As grave goods, glass beads and a bone pendant are present[13]. M15 represents the richest grave containing the badly preserved remains of an old woman (age 60-65). The rich funerary inventory consists in glass beads, amber and lapis lazuli beads, a spindle whorl, an iron fibula and a bronze mirror[14]. M18 contains the remains of an adult woman (age 40-45) buried with no grave goods.

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[7] We express our gratitude to Professor PhD Nicolae Ursulescu and to Researcher PhD Felix Adrian Tencariu who provided the samples and the context information for the human remains from Isaiia (Iași County). The archaeologist Maria Diaconescu, from the Botoșani County Museum, offered the human remains from Vorniceni (Botoșani County) and we are very grateful to her.

[8] URSULESCU & KOGĂLNICEANU 2002-2004, p. 27-59.

[9] URSULESCU & KOGĂLNICEANU 2002-2004, p. 36.

[10] The funerary remains identified in the proximity of the Cucuteni A-B settlement represents unpublished material from the collection of the Botoșani County Museum. The samples and the context informations were provided by the archaeologist Maria Diaconescu to which we express our gratitude.

[11] URSULESCU & KOGĂLNICEANU 2002-2004, p. 27-59.

http://cronica.cimec.ro/detaliu.asp?k=3148&d=Isaiia-Raducaneni-Iasi-Balta-Popii-2004, accessed on 22nd June 2017.

[12] URSULESCU & KOGĂLNICEANU 2002-2004, p. 30-31.

[13] URSULESCU & KOGĂLNICEANU 2002-2004, p. 33.

[14] URSULESCU & KOGĂLNICEANU 2002-2004, p. 34-35.

The human remains buried in M18 are in a very poor state of preservation[15]. For M8, M13, M15 we have for DNA analysis the *humerus*, while for M18 we have selected samples from the femur.

Sample M1S consists in a *humerus* fragment selected from the human remains identified at Vorniceni (Botoșani County). The human osteological remains were in a good state of preservation without any additional anthropological analysis.

Genetic analysis

The extraction of aDNA from bone remains followed a two steps protocol, as following: samples preparation and aDNA extraction protocols. To avoid contamination, beside the extraction strict condition (total isolation, UV decontamination overnight, facemask, gloves and disposable lab coat), the bones remains were cleaned of potential debris with a dry brush, washed with sodium hypochlorite (NaOCl) and exposed to UV light (**Fig. 2**). For a higher confidence degree, we have used two extraction protocols: phenol-chlorophorm-isoamyl alcohol (PCI) and DNA IQ (Promega, USA). For both extraction protocols, blank control samples were used to assess the potential reagents or lab contamination. In order to identify any possible contamination that might have occurred in the different stages of the samples preparation and mainly in the aDNA isolation, at least two extraction blank controls and multiple PCR non-template controls were included in each amplification reaction. The PCR was carried out in a 25 μL reaction volume using GoTaq® Hot Start Polymerase (Promega, USA) to amplify the mitochondrial hyper variable region 2 (Hv2). Specific pairs of primers were used: MPS3A_f – MPS3A_r, MPS3B_f – MPS3B_r, MPS4A_f – MPS4A_r and MPS4B_f – MPS4B_r [16]. The amplicons were purified using the Agencourt AMPure XP (Beckman Coulter, USA) and direct sequenced using the Genome Lab DTCS Quick Start Kit (Beckman Coulter, USA) in the CEQ 8000 Genetic Analysis System (Beckman Coulter).

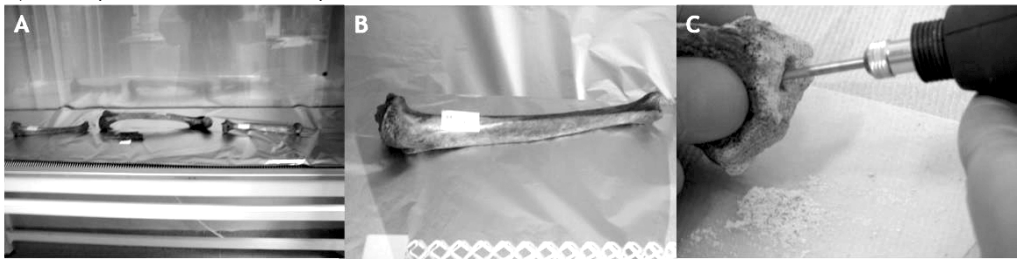

Fig. 2: Samples preparation steps: (A) drying after sodium hypochlorite wash, (B) UV exposure, and (C) bone powder sampling

Results and discussion

The aDNA was successfully isolated free of contaminants from all the human bones samples and used to amplify through PCR method the mitochondrial hypervariable region 2 (Hv2). DNA sequence of approximately 315 bp of was successfully obtain overlapping 4 Hv2 partial sequences. We have checked for

[15]http://cronica.cimec.ro/detaliu.asp?k=3148&d=Isaiia-Raducaneni-Iasi-Balta-Popii-2004 accessed on 22nd June 2017.

[16] GABRIEL *et alii* 2001, p. 247- 253.

similarity the obtained sequences by using BLAST module from the National Center for Biotechnology Information (NCBI).

Table 1: Sample assignation to human haplogroup variants using MITOMASTER[17]

Sequence	Predicted Haplogroup	Total Variants	Variants
M8	B4a(B4a1c3a)	12	C151T, A197AT, T223TA, A243AT, G251GC, A263G, T310C, C311CTCC, T318C, T319TTCT, C332CC, T344TT
M15	B4a(B4a1c3a)	2	T310C, T310TTC
M18	H2a(H2a2a)	4	T223TA, A243T, G251GC, A263G
M13	H2a(H2a2a)	1	A263G
M1S	U5a(U5a2a1b1)	14	T196A, A197T, T223TA, G229d, A243T, A249T, T252C, C253T, T254C, A263G, T310C, T310TTC, C317CC, C332CC

To ensure the haplogroup assignation for the obtained sequences, a double check was made using MITOMASTER[18], a database of human mitochondrial DNA (mtDNA) able to identify nucleotide variants relative to the rCRS and to determine the haplogroup. Both comparison analysis revealed a high similarity score with three human haplogroups: B4a, H2a and U5a (**Table 1, Fig. 4**).

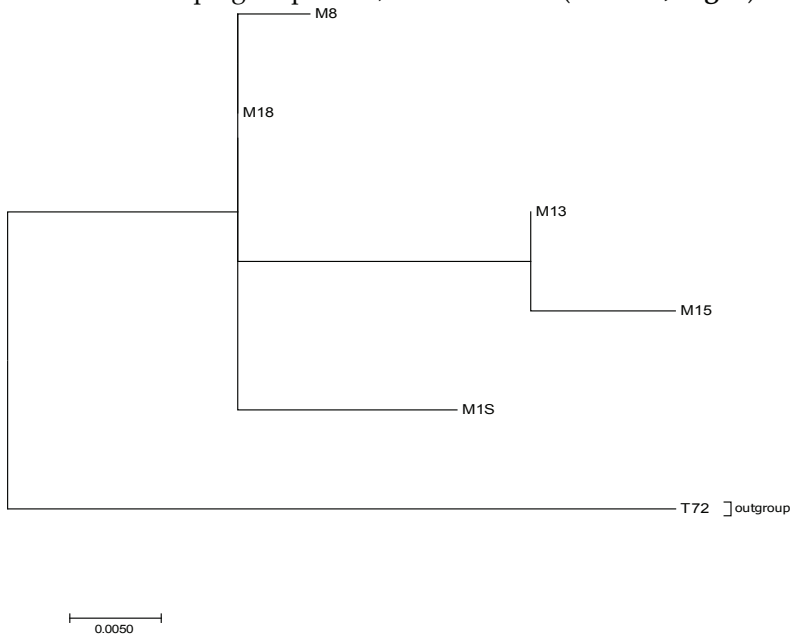

Fig. 3: Molecular Phylogenetic analysis by Maximum Likelihood method

For inferring the evolutionary history of the analysed human bone samples, we have used the Maximum Likelihood method based on the Tamura-Nei

[17] MITOMAP, http://www.mitomap.org, accessed on 10 July 2017.
[18] LOTT *et alii* 2013, 1.23.1-26s.

model[19]. The tree with the highest log likelihood (-467.2804) is shown in **Fig. 3**. Initial tree(s) for the heuristic search obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances and estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The obtained tree drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved six nucleotide sequences. We have eliminated all positions containing gaps and missing data. There were 254 positions in the final dataset. Then, for the evolutionary analyses, we have used MEGA7 software[20].

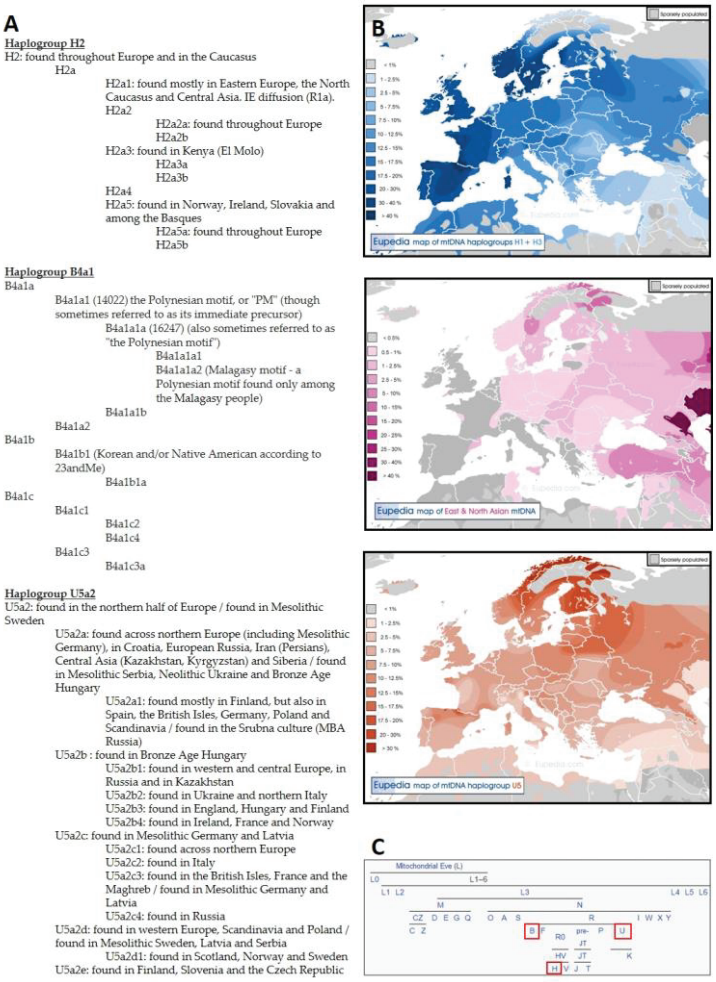

Fig. 4: Subclades and geographic occurrence of the identified haplogroups according to Eupedia[21]. A – Haplogroups subclades and their geographic occurrence; B – Geographic distribution across Europe of the identified haplogroups; C – European mtDNA haplogroups chart.

[19] TAMURA & NEI 1993.
[20] KUMAR *et alii* 2016.
[21] http://www.eupedia.com/europe/, accessed on 7 July 2017.

The H haplogroup includes more than 40% of the total mtDNA variation in most of Europe, originating in the Near and Middle East[22]. More likely, the subclades of the haplogroup H that have a high frequency today, some of which correlating with the post-Late Glacial Maximum reoccupation, were already common before the glacial period and were not highly affected by the large-scale climatic fluctuations. Most importantly, it occurs that after the initial migrations of the populations belonging to haplogroup into Europe, probably before or during the Gravettian period, there almost no admixture between the West Asian and European H haplogroup lineages[23]. The known history for H2 haplogroup is very general; this haplogroup was determined to be quite successful in Europe during the Last Ice Age. While H2 is common throughout all of Europe (**Fig.** 4), recent studies has shown that H2a is concentrated near the Caspian Sea[24].

Unlike H2 haplogroup found mostly in Europe, the B haplogroup has relatively high frequencies in mainland South-Eastern Asia (20.6%), in the islands of South-Eastern Asia (15.5%), Oceania (10.2%), Eastern Asia (10.5%) and America (24%), but occurs as rarely as 0.1–1% in the Volga-Ural region, the Caucasus, Western and Southern Asia[25]. In Europe, it shows very low frequency in some populations (**Fig. 4**). The spread of the Eastern Asian mtDNA haplogroups was identified in the Neolithic skeletons from archaeological sites located in the Carpathian Basin[26]. The presence of B4a haplotype in Sarmatian population was quite expectable regarding the Iranian origin of the Sarmatian culture, suggesting an admixture with Altaic populations.

In our days, the U5 haplogroup is relatively rare in Europe (**Fig. 4**). Despite this, an ancient DNA study of Stone Age hunter-gatherers from central and Eastern Europe has shown that most of the samples analysed shared mtDNA haplotypes belonging to this specific haplogroup[27]. Most of the ancient hunter-gatherers (82%) carried U clade (particularly the U5 subclades) which is relatively rare in the present-day population from Central Europe. The relatively high diversity of the U haplogroup observed for the hunter-gatherers European populations together with their uninterrupted presence for 11 millennia and the scattered presence outside Europe, increases the possibility of having this high frequency due to the post Late Glacial Maximum repopulation of the Central European region[28]. In modern populations, closest relatives are among eastern Europeans (in Latvians, Russians, Tatars and Mordvins)[29]. Therefore, the presence of this haplogroup in our sample is justified, being, previously, reported in Scythian culture in the Altai Region[30] and across Europe in different timelines[31].

[22] ROOSTALU *et alii* 2007, p. 436-437.
[23] ROOSTALU *et alii* 2007, p. 445.
[24] van OVEN & KAYSER 2009, p. E386-E394.
[25] DERENKO *et alii* 2012, p. e32179.
[26] GUBA *et alii* 2011, p. 793.
[27] BRAMANTI *et alii* 2009, p. 137–140.
[28] BRAMANTI *et alii* 2009, p. 137–138.
[29] MALYARCHUK *et alii* 2010, p. e10285.
[30] GONZÁLEZ-RUIZ *et alii* 2012, p. e48904.
[31] MALYARCHUK *et alii* 2010, p. e10285.

aDNA study of the Iron Age burials attributed to the Scythian Pazyryk culture from the Altai Mountains showed that the population displayed mitochondrial lineages already present in the region before the Iron Age. This investigation offers an accurate perspective into the regional dynamics of the gene pool and provides support for a demographic expansion of the local people of Altai region instead of westward or eastward migratory scenarios[32]. Previous genetic investigations of the Scythian Pazyryk culture have reported the presence of U5a1 and HV2 haplogroups. The major centres of haplogroup U5a1 within modern populations resides considerably west and northwest from the Altai Mountains. The origin and the subsequent diversification of the U5a1 haplogroup are in close connection with the Northeastern European population[33].

Recent aDNA extended study of the Iron Age groups from the North Pontic region revealed an east Eurasian ancestry common to varying degrees between the western and eastern steppe groups. Although, they do not share a common origin and have enormous geographic distances between them, the genetic analysis inferred ongoing and substantial gene flow between eastern and western groups, which offers a reliable demographic mechanism to explain the high degree of material culture similarities[34].

Previously aDNA analysis of Sarmatian populations from the Cis-Asov region included 16 skeletons dated to the 1st century BC – 2nd century AD and reported haplogroups distributed mostly in Europe and Central and Western Asia[35]. Besides that, two human bone samples attributed to Pazyryk culture and one to Sarmatian population belongs to U4a1 haplogroup which correlates very well with the complex anthropological composition of the population both in the Scythian and the successive Sarmatian periods[36]. The genetic study of the Scythian and Sarmatian nomadic groups revealed strong evidence for Caucasus hunter-gatherers and Easter European hunter-gatherers ancestry. The results are in good agreement with previous studies, which advanced European hunter-gatherers and Caucasian elements in the Yamnaya communities formed in the European Steppe region and, then, spread into Central Asia and Siberia[37].

Conclusions

To our knowledge, this is the first genetic study on bone samples selected from funerary contexts assigned to the Sarmatian tribes identified in Eastern Romania. Our analysis reveals a high similarity score with three human haplogroups (B4a1, H2a and U5a2) based on both, comparison analysis (BLAST and MITOMASTER) and phylogenetic reconstruction.

M8 and M15 human bone samples selected from the Isaiia Sarmatian necropolis (Iași County) belongs to B4a1 haplogroup. This represents the first identification of a B4 haplogroup subclade, which is characteristic for Eastern

[32] GONZÁLEZ-RUIZ *et alii* 2012, p. e48904.
[33] PILIPENKO *et alii* 2010, p. 233.
[34] UNTERLÄNDER *et alii* 2017.
[35] MOROZOVA *et alii* 2013, p. 969-970.
[36] GUBINA *et alii* 2016, p. 101-102.
[37] HAAK *et alii* 2015, p. 210.

Asia, in a Sarmatian funerary context. If we combine the aDNA results with the archaeological data which reports a typical Sarmatian inventory for M15 and the anthropological data, which describes an intentional cranial deformation typical to Sarmatian populations.

The other two samples selected from the Isaiia necropolis (M13 and M18) belongs to H2a haplogroup. In a recent study, H2a1 haplogroup was identified in an Early Scythian context (9th-7th centuries BC) from East Kazakhstan, in a Classic Scythian context (6th-2nd centuries BC) from the North Pontic region and in an Early Sarmatian context (5th – 2nd centuries BC) from the Southern Ural region[38]. H2 haplogroup was attested at Sultana-*Malu Roșu*, in a Neolithic context from Southern Romania[39].

The human remains identified in Vorniceni (Botoșani County) which can be associated based on the grave goods to the Sarmatian populations have revealed the U5a2 haplogroup. This haplogroup was previously reported in Early Scythian contexts from Tuva region (7th-6th centuries BC), Khakassia region (5th century BC), Classical Scythian contexts from the Russian Altai region (4th-3rd centuries BC), North-Western Mongolia (4th-3rd centuries BC), North Pontic region (6th -2nd centuries BC) and in the Early Sarmatian contexts from Southern Ural (5th -2nd centuries BC)[40]. Previous aDNA analysis performed on Neolithic, Chalcolithic and Late Bronze Age human remains selected from Romania have revealed the presence of the U5 haplogroup in samples selected from Curățești and Sultana-*Valea Orbului* (Southern Romania) and Florești-*Polus* (central Romania)[41].

The presence of haplogroups previously identified in European and in Eastern Asian ancient and modern population in the analysed Sarmatian samples is not so surprising, but needs to be confirmed by more extended studies. This will offer a broader understanding of the gene flow occurrence from east to west Eurasia and vice versa, which will allow us to understand the underlying population dynamics that may have driven the cultural dynamics so relatively understood.[42]

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[38] UNTERLÄNDER *et alii* 2017.

[39] HERVELLA *et alii* 2015.

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