The ancient DNA of the Malomirovo graves 5, 14 and 19: A brief overview

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FROM THE STEPPES TO THE BALKANS

YAMNA CULTURE IN UPPER THRACE

Edited by

Stefan Alexandrov and Piotr Włodarczak

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THE YAMNAYA IMPACT ON PREHISTORIC EUROPE

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ERZSÉBET JEREM and WOLFGANG MEID

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Yamna Culture in Upper Thrace

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Anthropomorphic stele from Grave 17 in Malomirovo. Photo by Michał Podsiadło

Back Cover
Excavations of Barrows 4 and 5 in Mogila. Photo by Magdalena Mazurkiewicz

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The ancient DNA of the individuals buried in Malomirovo Graves 5, 14 and 19: A brief overview

YOAN DIEKMANN – JENS BLÖCHER – VOLKER HEYD – JOACHIM BURGER

Abstract

Four samples from the Malomirovo kurgan were analysed for ancient DNA. Grave 19 had good DNA preservation (60%) and was full-genome sequenced, while Graves 5 and 14 only underwent target-enrichment sequencing due to low DNA content. Libraries were sequenced on the Illumina NovaSeq platform, resulting in, a genome-wide average depth of >60,000 SNPs for Graves 5 and 14, sufficient for genomic analyses. Grave 19 contained a female with U4 mitochondrial haplogroup, dated to the late 4th millennium BC, showing 100% steppe ancestry. Graves 5 and 14, from the 28th/27th centuries BC, turned out to be third-degree relatives. Both males have H2 mitochondrial and R1b Y-chromosomal haplogroups, indicative of steppe ancestry and relation via maternal descent. PCA and ancestry modelling showed Graves 5 and 14 aligned with Yamna, Corded Ware, and Fatyanovo cultures, while the individual from Grave also exhibited a small Mesolithic hunter-gatherer component. The findings suggest a steppe population origin dominance for both, with minor local admixture in the case of Grave 5.

Keywords: *Ancient DNA, steppe ancestry, R1b-Z2103, kinship, maternal descend*

Four samples from the Malomirovo, Pamukli Bair kurgan (Alexandrov *et al.* 2024) were screened for ancient DNA (results summarised in *Table 1*). One individual from Grave 19 showed very good ancient DNA preservation of nearly 60% and was full-genome shotgun sequenced. The other three had an endogenous DNA content below 1%, rendering full-genome sequencing economically non-viable. Therefore, two individuals (from Graves 5 and 14) were sequenced using a target-enrichment approach. The sample from Grave 17 was not processed any further.

Table 1. Malomirovo samples information

lab ID	archaeological information	endogenous DNA content [in screening]	capture?	avg. read depth	nb. of 1240k SNPs covered	sex	MT	YHG	
Malo1	Malomirovo, Grave 5, Individual 107, 7-7-21	0.31	X	0.003	60 093	XY	H2a2a1	R1b1a1b2a2b1	R-Z29769
Malo2	Malomirovo, Grave 14, Individual 110, 7-7-21	0.45	X	0.004	65 050	XY	H2a2a1	R1b1a1b1b	R-S20902
Malo3	Malomirovo, Grave 17, Individual 113, 7-7-21	0.36							
Malo5	Malomirovo, Grave 19, Individual 112, 7-7-21	59.98		1.841	1 009 959	XX	U4a2	*NA*	*NA*

The three samples were sequenced on an Illumina HiSeq3000 or an Illumina NovaSeq6000 (single end, 100 cycles) at the Next Generation Sequencing Platform at the University of Bern, Switzerland. After removing residual adapters and reads shorter than 30 base pairs, reads were aligned against the hg19 reference assembly. Reads with mapping quality below 30 were removed, along with duplicates, before realigning around known InDels. Pseudo-haploid majority-allele calls were produced in a post-mortem damage-aware manner. This approach yielded a genome-wide average depth of ~1.8x for Grave 19 and >60,000 SNPs from the 1240k capture array for Graves 5 and 14, i.e., enough for all basic genomic analyses, including ancestry.

The individual buried in Grave 19 belongs to the end of the 4th millennium BC and is thus labelled 'Pre-Yamna.' This first individual buried in the Malomirovo mound was a female with U4 mitochondrial haplogroup. Contrary to her, Graves 5 and 14—being a few centuries younger and likely dated to the 28th/27th century BC—contained males with H2 mitochondrial haplogroups. The latter two are inferred to have Y-chromosomal haplogroups R1b, already suggesting that at least their male ancestry line is rooted in the steppe. Even more so, Grave 14's exact Y-chromosome of R1b1a1b1b (R-S20902) belongs to the Z2103 family, being the typical Y-chromosome of almost all Yamna individuals tested so far (see also Lazaridis *et al. in press*). Grave 5's Y-chromosome R1b1a1b2a2b1 (R-Z29769) also belongs to the wider network including a large number of Yamna men. Both individuals are roughly contemporary based on their radiocarbon dates. However, Grave 5 is the younger one, as this grave was established above Grave 14. Results of the tool/method KIN (Popli – Peyrégne – Peter 2023) suggest third-degree biological relatedness between the two individuals. Given the distinct Y-chromosomal markers, a connection through the maternal line is likely.

To investigate further the genetic ancestry of the individuals, we projected them onto a principal component (PCA) reference space spanned by modern Eurasians, together with reference ancient genomes from Meso-, Neolithic, and Early Bronze Age Eurasia (*Fig. 1*). All three Malomirovo individuals project outside the space covered by modern Eurasian reference populations, close to ancient reference

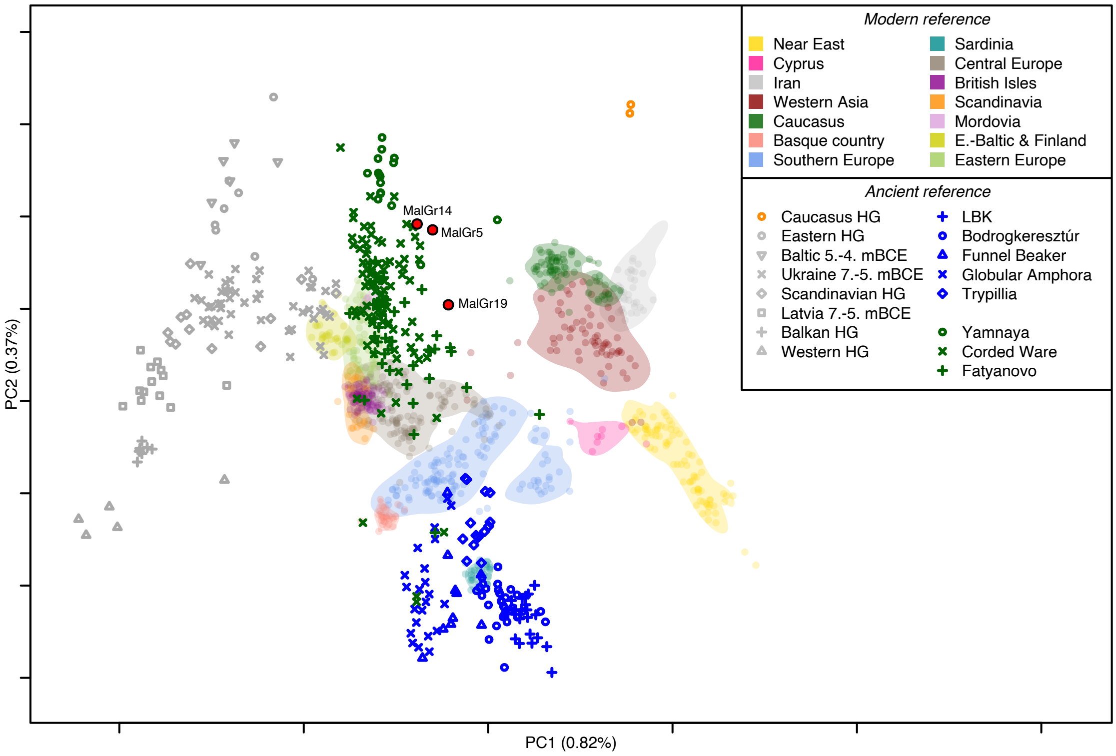

Fig. 1. Principal component (PCA) reference space spanned by modern Eurasians, together with reference ancient genomes from Mesolithic, Neolithic, and Early Bronze Age Eurasia. The three Malomirovo individuals are highlighted

individuals annotated to belong to the Yamna, Corded Ware, and Fatyanovo cultures. Yamna from the Samara region of Russia served as the original exemplar genomes based on which steppe ancestry was defined, and the Corded Ware and Fatyanovo individuals plotting close to Yamna in PCA space have previously been shown to have a dominant steppe ancestry component as well (Malmström *et al.* 2019; Saag *et al.* 2021).

Lastly, we directly quantify the genomic ancestry profile by modelling the three genomes as potential mixtures of the three main European ancestry components, representing three ultimate source populations: Mesolithic hunter-gatherers from the Iron Gates, Neolithic Aegean-Anatolian farmers, and pastoralists from the Pontic-Caspian steppe. Consistent with their location in PCA, we infer the Malomirovo individuals from Graves 14 and 19 to be best described by 100% steppe ancestry. At the same time, the individual interred in Grave 5 may have an additional small Mesolithic hunter-gatherer component of < 20% (*Fig. 2*). None of our individuals has any detectable Neolithic Aegean-Anatolian farmer ancestry despite living in a region where there is ample evidence for the contemporary settling of their descendants, particularly in the form of tell and occasional flat settlements of the Early Bronze Age Ezero A culture (Valchev 2023; Valchev – Sobotkova 2023).

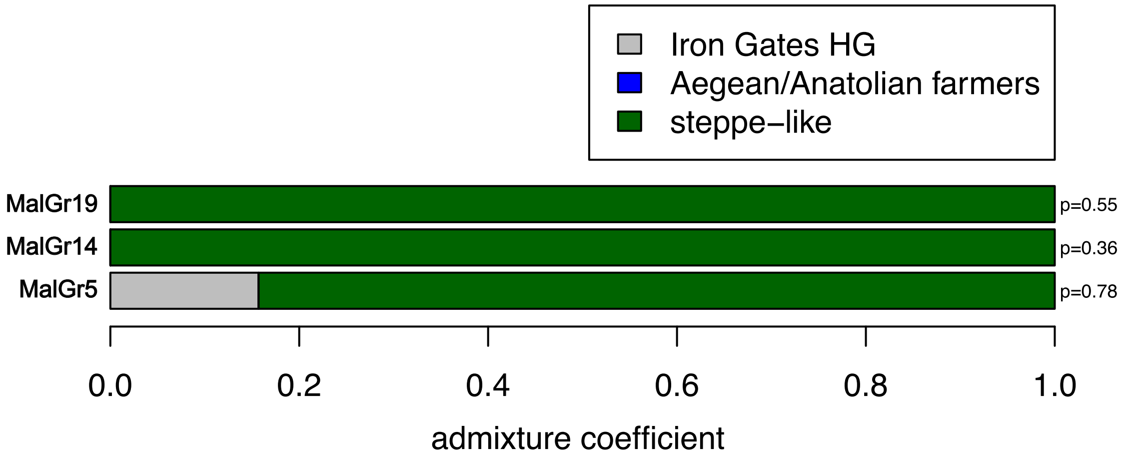

Fig. 2. Model of the three Malomirovo genomes as admixtures of the three main European ancestry components, representing three ultimate source populations: Mesolithic hunter-gatherers from the Iron Gates, Neolithic Aegean-Anatolian farmers, and pastoralists from the Pontic-Caspian steppe

We conclude that most of the ancestors of all three individuals belonged to steppe populations, which geographically extended across the Pontic-Caspian steppe ecozone, including the westernmost region beyond the Danube estuary, reaching deep into the historical region of Thrace. While the relatively small amount of genomic data obtained for Grave 5 precludes secure inference, the presence of Mesolithic hunter-gatherer ancestry tied to local populations suggests some admixture between steppe and unspecified neighbouring populations based on the ancestry profile of this man. Both—related—individuals from Grave 5 and Grave 14 plot close to other Yamna and Corded Ware individuals in PCA (*Fig. 1*). Yamna is also the right keyword regarding their Y-chromosome profiles. However, the woman from Grave 19 is different, and the distance in PCA space between 5&14 and 19 is striking. She also displays a non-admixed, 100% steppe ancestry. However, her position in our PCA is slightly shifted towards Neolithic farmer ancestries, similar to the Cernavodă I and Usatove peoples described in Penske *et al.* 2023, which is also more congruent with her dating into the last centuries of the 4th millennium BC. However, archaeologically, and taking her 'right-sided, crouched hocker'/'semi-supine to the right' burial position with the head towards the northeast, in a rectangular pit without any ochre, she can best be described as linked to the likewise Pre-Yamna Nizhna-Mikhailivka culture of the northern Pontic Region.

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Barrows in Upper Thrace from the 4th and 3rd millennia BC were the subject of field research conducted in 2018–2019 in cooperation with the Institute of Archaeology and Ethnology of the Polish Academy of Sciences and the Regional Historical Museum in Yambol. This research was part of the project 'From the Steppes to the Balkans: Yamna Culture in Thrace', financed by the National Science Centre (Kraków, Poland). Five barrows were excavated near Yambol and Elhovo in the Middle Tundzha Region. The most significant results emerged from the investigation of the barrow on Pamukli Bair Hill in Malomirovo (Elhovo municipality, Yambol Province). Numerous Early Bronze Age graves (starting from the Pre-Yamna phase) and burials from the Middle Bronze Age and Late Antiquity were discovered in this barrow. This book presents the research results on the barrow in Malomirovo, incorporating a series of analyses of the find material. The volume also includes research results from two other barrows in Mogila, Malkia Kayryak, and Mogila, Golemia Kayryak, which were recently excavated by international expeditions, along with the chronological evaluation of the barrows at Gabrova Mogila and Shekerdzha Mogila in Kamen. Together, these findings greatly enhance our understanding of funerary practices in Upper Thrace, the southernmost zone of the Eurasian steppe, where expanding kurgan communities settled in the 4th and 3rd millennia BC.

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