



OPEN Thermal desorption GC/MS on human dental calculus detected minute millet consumption in medieval Ukraine

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This study presents a novel biomolecular approach for detecting broomcorn millet (*Panicum miliaceum*) consumption by analysing the plant biomarker miliacin (olean-18-en-3 β -ol methyl ether) in human dental calculus. Conventional methods, such as stable isotope analysis of bone collagen, often fail to identify low-level C₄ plant consumption due to the need for substantial dietary intake. To address this limitation, thermal desorption–gas chromatography–mass spectrometry (TD-GC/MS) was applied to dental calculus samples from individuals buried at the medieval Ostriv site in Ukraine. Miliacin, a pentacyclic triterpene methyl ether uniquely abundant in broomcorn millet, was identified in eight of the analysed samples. These findings provide the first molecular evidence for millet consumption at trace levels in human remains and demonstrate the viability of using dental calculus to recover dietary plant biomarkers. Stable isotope analysis of dentin collagen revealed depleted $\delta^{13}\text{C}$ values in several individuals with detected miliacin, confirming that millet consumption would have been overlooked by conventional isotopic approach. This methodological advance significantly improves the resolution of dietary reconstructions and opens new possibilities for studying underrepresented plant foods in archaeological contexts.

Keywords Miliacin, Ukraine, Plant biomarkers, Plant-derived molecules, Human diet, Gas chromatography–mass spectrometry

Despite its importance in the evolution of the human diet, direct plant consumption by humans is underrepresented in the archaeological record because plants often have much less protein and lipids than animals that were consumed by humans^{1,2}. Developing innovative proxies can improve the accuracy and resolution of archaeological reconstructions, reveal previously overlooked aspects of plant use, and expand our knowledge of cultural and environmental changes over time. Ultimately, these advances contribute to a more comprehensive picture of how humans interacted with and depended on plant resources throughout history.

The use of plants in human diets has been primarily studied through archaeobotanical methods that are very limited to plant processing and taphonomy, hindering the preservation of plants^{3,4}. For example, the intensity of plants exposed to fire and oxygen levels during charring strongly influence plant preservation, recovery and species identification^{5,6}. In most past human activities, however, the economically important plants perish after processing or consumption, and their use is simply not recognised. Recent application of molecular methods opened the possibility to recover and identify ancient plant proteins that were consumed in the past via paleo proteomics analysis^{7,8}. However, authors in the past had struggled to establish the presence of taxonomically specific plant proteins or other plant biomarkers.

The importance of the use of broomcorn millet (*Panicum miliaceum*) as a crop has received immense attention due to the impact it has had on past communities and its potential importance as one of the future superfoods⁹. Broomcorn millet utilises the C₄ photosynthetic pathway and has more enriched $\delta^{13}\text{C}$ values, as it discriminates less against the carbon isotope¹³C than C₃ plants during carbon capture from the atmosphere; this leads to a

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substantial difference between the ratios of heavy and light carbon isotopes in C_3 and C_4 plants^{10,11}. A relative proportion of isotopes from the plants eaten is absorbed into animal tissues and can later be directly measured using isotope ratio mass spectrometry. This allows for detecting millet consumers down to the individual level. However, for millet to be detected isotopically in human tissue, millet needs to make up more than 20% of the overall dietary protein consumed¹². Therefore, many millet consumers can be overlooked. Thus, we lack the proxies that will allow the identification of individuals consuming low levels of millet, which would otherwise not be detectable by stable carbon isotope analysis of skeletal remains.

Fortunately, broomcorn millet possesses another unique chemical property that can be measured and tracked through time. Broomcorn millet contains pentacyclic triterpene methyl ether (PTME) called miliacin. It is the principal (c. 99%) PTME in broomcorn millet and is absent in other commonly cultivated species¹³. The pentacyclic triterpene miliacin is an anti-microbial compound and is resistant to decomposition by bacteria^{13–15}. The durability of miliacin results in its survival in high concentrations in archaeological contexts. This specific biomarker has been identified in ancient agricultural fields, sediment cores^{16–19} and ceramic matrices of archaeological pottery vessels in Europe, as well as East and Central Asia^{20–24}. Yet, the identification of the miliacin biomarker in human dental calculus has never been previously attempted, as the miliacin extraction method required too much sample material (2–5 g used in previous studies); the average total dental calculus weight per adult human is 70.2 mg²⁵. Over the past years, methodological advancements in analytical technique have significantly reduced the sample size required for detection by mass spectrometry (e.g. less than 1.0 mg of sample material is usually sufficient), indicating that miliacin archived in human dental calculus could be successfully detected and analysed.

Miliacin is highly abundant in millet seeds, and human dental calculus has a high potential to trap these biomolecules during consumption. Therefore, the analysis of miliacin from human dental calculus will provide a molecular source to trace a wide range of millet consumption intensities and will be linked together with the obtained stable isotope values and archaeological data. Biomarkers recovered from human dental calculus hold immense potential for studying past human diet and physical health^{26,27}. This research presents a novel methodology for identifying the miliacin in human dental calculus through a case study on the medieval Ostriv cemetery in Ukraine, as the Ostriv population possessed extensive quantities of human dental calculus on their teeth.

To overcome the sample size issue, we adopt the thermal desorption–gas chromatography–mass spectrometry (TD-GC/MS) as the analytical method. This method has been used not only for archaeological ceramics²⁸ and mummies²⁹, but also for dental calculus from the Mesolithic population³⁰ and further Neanderthals³¹. Critically, this method requires much less sample mass than conventional methods with lipid extraction and a much shorter time for the preparation of analysis. Moreover, miliacin was detected using this method from an archaeological pottery sherd²⁸, opening the possibility for the detection of this compound in different materials such as dental calculus.

Simultaneously, stable carbon and nitrogen isotope analysis of dentin samples from the same individuals was conducted. By doing this, we aimed to confirm the novelty of TD-GC/MS method to detect minute millet consumption compared to the conventional isotopic methods. Additionally, while dental calculus is formed during adulthood, dentin is formed during childhood. This provides a good opportunity to compare the detection of millet biomarker in the former, and their tendency of dietary intake between C_3/C_4 in the latter at different stages of life.

Results

Thermal desorption – gas chromatography – mass spectrometry

Miliacin was detected in the eight dental calculus samples (MIL1_49, 52, 54, 57, 59, 65, 66 and 71), confirming that these humans directly consumed broomcorn millet (Table 1). Miliacin was not detected in the simultaneous blank control that was processed and analysed with dental calculus samples in the same setting. In addition to miliacin, free saturated fatty acid methyl esters (FAMES) such as those of palmitic and stearic acids, cholesterol and its derivatives, and Dimethyl phthalate, which is probably from plasticiser of containers used during the process of excavation, curation and storage³² were detected (Fig. 1). As FAMES and cholesterol are ubiquitous in a broad range of foods, it is difficult to determine the origins of these fatty acids and cholesterol derivatives. Because of the relatively lower concentration of miliacin, it was not possible to quantify this compound's abundance in the samples. In addition, an archaeological ceramic powder sample containing miliacin (MJR10, Heron et al. 2016) and the authentic miliacin reference substance (PhytoLab Certificate Report No. 74485154-99 002) were analysed in the same conditions, confirming the retention time and mass spectrum of miliacin (Fig. 1).

Stable isotope analysis in dentin

Stable carbon and nitrogen isotope analysis of the M2 dentin sample was conducted on 31 individuals, as they had larger quantities of dental calculus. As the dental calculus forms in adulthood, but dentin represents a childhood diet, stable isotope analysis aimed to understand dietary habits during the early stages of an individual's life and potentially identify C_4 plant consumers and those for whom the C_4 diet is not visible. The $\delta^{13}C$ in all analysed humans ranges from -15.0 to -20.8 ‰, with a mean value of -18.5 ‰. In $\delta^{15}N$ from 10 to 8.5 ‰, the mean value is 9.4 ‰ (Table 2). If the $\delta^{13}C$ cut-off value between predominantly C_3 and mixed C_3 and C_4 consumers -18 ‰^{33,34}, two dietary groups of individuals that consumed C_4 plants during childhood and the ones that had no or limited intake of C_4 plants could be classified.

Sample name	Laboratory ID	Sample mass (mg)	Presence of Miliacin	Other major compound detected
MIL1_40	MILWAYS012	1.3		FAC _{14:0-18:0} , FAC _{18:1} , DP
MIL1_41	MILWAYS013	11.4		FAC _{14:0-24:0} , FAC _{18:1}
MIL1_42	MILWAYS014	3.4		FAC _{14:0-24:0} , FAC _{18:1}
MIL1_43a	MILWAYS015	11.8		FAC _{16:0-18:0}
MIL1_45	MILWAYS016	3.0		FAC _{9:0-24:0} , FAC _{18:1}
MIL1_46	MILWAYS017	2.3		FAC _{16:0-24:0} , FAC _{18:1}
MIL1_47	MILWAYS018	5.1		FAC _{16:0-18:0}
MIL1_48	MILWAYS019	1.0		FAC _{14:0-18:0} , FAC _{18:1}
MIL1_49	MILWAYS020	7.4	X	FAC _{14:0-24:0} , FAC _{18:1}
MIL1_50	MILWAYS021	-		FAC _{8:0-24:0} , FAC _{18:1} , DP
MIL1_51	MILWAYS022	3.0		FAC _{14:0-24:0} , FAC _{18:1}
MIL1_52	MILWAYS023	9.8	X	FAC _{8:0-24:0}
MIL1_53	MILWAYS024	1.0		FAC _{14:0-18:0} , FAC _{18:1}
MIL1_54	MILWAYS025	14.7	X	FAC _{16:0-18:0} , Chol
MIL1_55	MILWAYS026	1.8		FAC _{16:0-18:0} , DP
MIL1_57	MILWAYS027	6.6	X	FAC _{16:0-24:0} , FAC _{18:1}
MIL1_58	MILWAYS028	10.2		FAC _{16:0-18:0}
MIL1_59	MILWAYS029	6.9	X	FAC _{14:0-24:0} , FAC _{18:1}
MIL1_60	MILWAYS030	6.6		FAC _{8:0-26:0} , FAC _{18:1}
MIL1_61	MILWAYS031	11.0		n/a
MIL1_62	MILWAYS032	5.2		FAC _{9:0-18:0} , FAC _{18:1}
MIL1_63	MILWAYS033	7.3		FAC _{16:0-18:0}
MIL1_65	MILWAYS034	10.8	X	FAC _{6:0-22:0} , FAC _{18:1} , Chol
MIL1_66	MILWAYS035	13.7	X	FAC _{14:0-18:0} , Chol
MIL1_67	MILWAYS036	5.4		FAC _{14:0-24:0} , FAC _{18:1}
MIL1_68	MILWAYS037	7.3		FAC _{14:0-24:0} , FAC _{18:1}
MIL1_69	MILWAYS038	3.2		FAC _{14:0-22:0}
MIL1_70	MILWAYS039	10.1		FAC _{9:0-24:0} , FAC _{18:1}
MIL1_71	MILWAYS040	7.7	X	FAC _{6:0-22:0} , FAC _{18:1}
MIL1_72	MILWAYS041	1.8		FAC _{9:0-22:0} , FAC _{18:1}
MIL1_73	MILWAYS042	1.1		FAC _{6:0-18:0} , FAC _{18:1}

Table 1. Summary of the results of the dental calculus samples analysed in this study. FACx: y: fatty acids with carbon length x and number of unsaturation y, chol: cholesterol and its derivatives, DP: dimethyl phthalate.

Discussion and conclusions

Applying the TD-GC/MS method to human dental calculus confirms millet consumption by the medieval populations in Ukraine, supported by the detected miliacin from eight samples out of 31 individuals. Indeed, the Ostriv population had dense layers of dental calculus covering their teeth, some of which caused severe periodontal conditions (Fig. 2).

Out of eight individuals with miliacin, six of them were female—one died at the age of 20–25, one at 20–30, one at 25–30, one at 25–35, two at 30–40, and one at 40–50. Male individuals were identified as aged 20–30 and 30–40 (Table 2). Interestingly, more depleted carbon isotope values less than -18‰ were observed in the adults (20–30, 30–40, and 40–50, Table 2). This might indicate that some children with less C_4 intake became millet consumers later on in their adulthood, although currently it is difficult to evaluate the amount or intensity of millet consumption solely based on the miliacin detection in dental calculus.

Some examples of intensive C_4 intake in childhood have previously been revealed in the Karatuma site in Kyrgyzstan³⁵ and the Taksai site in Kazakhstan³⁶ based on the bone collagen isotope analysis. The results from our study are beginning to reveal a different pattern whereby millet is more intensely consumed in adulthood. However, further evidence is needed to further confirm this pattern. Ostriv burials constitute two distinct populations of local origin and of potentially Baltic origin. According to multiple lines of evidence that include aDNA and artefact typology, the Baltic migrants moved to the vicinity of Ostriv and were buried there between 10 and 12 c CE³⁷. As millet was less common in northern Europe than in Southeastern Europe at the time, it is likely that the isotopic data of $\delta^{13}C$ in dentine, which represent childhood diet, were less positive in Baltic migrants. Millet became a part of the staple food of these individuals only upon their arrival in the present territory of Ukraine.

On the other hand, the absence of miliacin in dental calculus does not necessarily mean that the individual did not consume millet due to potential preservation biases. Therefore, the frequency of millet consumption could be underrepresented, as more research is needed to understand the underlying circumstances and taphonomy of miliacin preservation in human dental calculus. Nevertheless, miliacin was detected in both males and females,

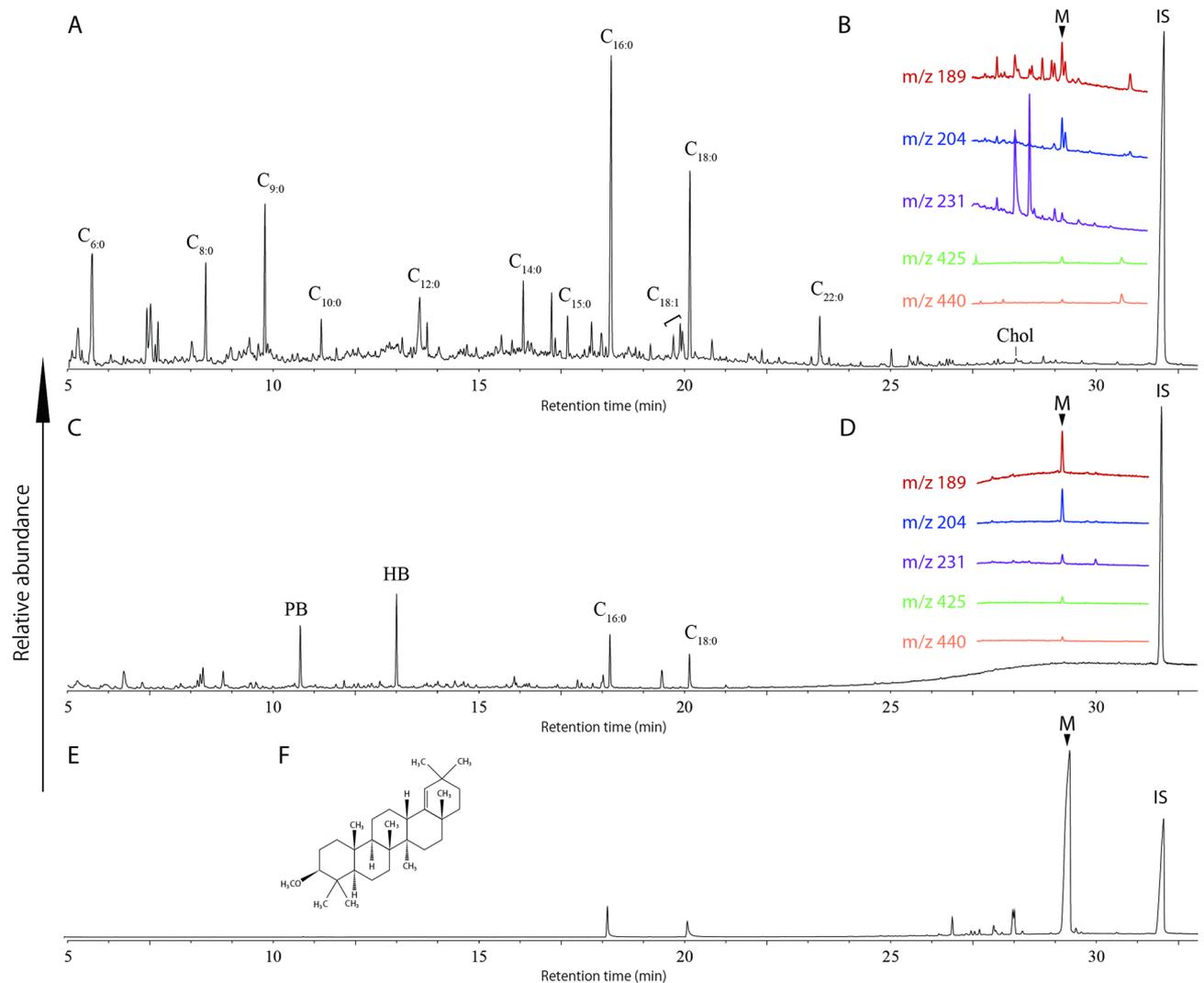


Fig. 1. (A) Typical partial TD-GC/MS chromatogram of dental calculus sample (MIL1_65) obtained by scan mode showing FAMES and cholesterol derivatives, (B) by SIM mode showing miliacin. (C) partial TD-GC/MS chromatogram of archaeological ceramic sample with miliacin (MJR10: Heron *et al.* 2016) obtained by scan mode, PB: Pentamethylbenzene, HB: Hexamethylbenzene, and (D) by SIM mode. (E) Partial TD-GC/MS chromatogram of an authentic sample of miliacin (PhytoLab Certificate Report-No. 74485154- 99 002) obtained by scan mode. (F) Chemical structure of miliacin.

as well as different age groups, without showing a correlation with a specific age group (Fig. 3), indicating that millet consumption itself was quite popular among these populations, although there should be differences in the intensity of consumption.

It is also tempting to compare the pathological features of these bones with the presence/absence of miliacin, but due to the sample size, it is difficult to find any statistically meaningful relationship among them. Therefore, reconstructing the foodways of millet consumption in these populations requires further research. Nonetheless, most importantly, this study successfully detected the millet-specific biomarker miliacin in human dental calculus for the first time.

The presence of miliacin in eight individuals provides direct molecular evidence for broomcorn millet consumption in medieval Ukraine. The detection of miliacin in individuals with more depleted $\delta^{13}\text{C}$ values, typically less than -18‰ in $\delta^{13}\text{C}$ in dentin, demonstrates that small quantities of millet, undetectable through stable isotope analysis, can now be identified. This shows that conventional isotope analysis which considers the value -18‰ as a threshold of millet consumption, underrepresents millet consumers, particularly those who consumed it intermittently or in minor amounts. The successful extraction of miliacin from microgram-scale dental calculus samples demonstrates the high potential of this matrix for recovering plant biomarkers. This opens new methodological avenues for studying low-level or occasional plant consumption in past human diets across different chronologies. Moreover, this potentially provides a way to compare the diet between childhood and adulthood, given the different formation period between dentins and dental calculus during their lives.

Sample ID	Lab ID	Miliacin	$\delta^{13}\text{C}$, ‰ _{dentin}	$\delta^{15}\text{N}$, ‰ _{dentin}	C/N _{atom}	Age	Sex
MIL1_40	MILWAYS012		-19.9	8.5	3.2	25–30	Unknown
MIL1_41	MILWAYS013		-20.3	9.7	3.1	30–40	M
MIL1_42	MILWAYS014		-20.0	8.9	3.3	18–25	M
MIL1_43 (a)	MILWAYS015		-20.7	9.6	3.2	45–55	M
MIL1_45	MILWAYS016		-20.1	9.9	3.2	20–25	M
MIL1_46	MILWAYS017		-19.0	9.6	3.2	35–45	F
MIL1_47	MILWAYS018		-17.4	9.6	3.2	25–30	F
MIL1_48	MILWAYS019		-19.0	9.1	3.2	45–50	M
MIL1_49	MILWAYS020	X	-18.0	8.9	3.2	40–50	F
MIL1_50	MILWAYS021		-18.6	9.7	3.2	20–30	M
MIL1_51	MILWAYS022		-16.6	9.6	3.1	16–18	M
MIL1_52	MILWAYS023	X	-16.5	10.3	3.1	25–30	F
MIL1_53	MILWAYS024		-19.7	8.9	3.2	16–18	F
MIL1_54	MILWAYS025	X	-19.5	9.4	3.1	20–30	F
MIL1_55	MILWAYS026		-19.3	9.2	3.1	25–35	Unknown
MIL1_57	MILWAYS027	X	-20.2	8.6	3.2	40–50	F
MIL1_58	MILWAYS028		-15.8	9.1	3.1	13–15	-
MIL1_59	MILWAYS029	X	-15.0	9.9	3.1	30–40 (45)	M
MIL1_60	MILWAYS030		-19.9	8.9	3.2	25–30	M
MIL1_61	MILWAYS031		-19.8	9.9	3.2	50–60	M
MIL1_62	MILWAYS032		-16.3	10.3	3.2	20–25	F
MIL1_63	MILWAYS033		-20.0	8.9	3.2	18–25	Unknown
MIL1_65	MILWAYS034	X	-16.9	10.5	3.2	25–35	F
MIL1_66	MILWAYS035	X	-19.5	9.3	3.2	30–40	M
MIL1_67	MILWAYS036		-16.4	9.6	3.2	55–65	F
MIL1_68	MILWAYS037		-18.8	9.2	3.1	30–40	M
MIL1_69	MILWAYS038		-17.0	9.6	3.1	20–25	M
MIL1_70	MILWAYS039		-20.8	9.9	3.2	40–50	Unknown
MIL1_71	MILWAYS040	X	-17.2	9.5	3.1	20–25	F
MIL1_72	MILWAYS041		-17.3	9.2	3.1	25–30	M
MIL1_73	MILWAYS042		-19.1	9.5	3.2	30–40	F

Table 2. The isotopic values of dentin, age, and sex of the analysed individuals from the Ostriv cemetery.

The use of thermal desorption GC/MS (TD-GC/MS) in this context demonstrates a micro-destructive, efficient, and scalable method for detecting specific plant compounds in archaeological dental calculus. This method can be extended to other chronologies, crops and contexts, contributing to more inclusive reconstructions of ancient plant parts in diets. This methodological breakthrough will aid future research in tracking the spread, intensity, and social context of millet and other underrepresented crops in human history.

Materials and methods

Archaeological site Ostriv and material selection

The medieval Ostriv cemetery is located c. 100 km south of Kiev in Ukraine. The burial ground of Ostriv is currently being investigated in the framework of a research grant by the German Science Foundation (DFG) at the Leibniz-Zentrum für Archäologie (LEIZA Schleswig) in cooperation with the Archaeological Institute of the National Academy of Science in Kyiv. It consists of Kievan Rus' inhumation graves, which are dated from the 10th to the 12th century, with a maximum in the 11th century AD. This burial site became the focus of this study due to a few reasons: a) previous research by Shiroukhov and colleagues³⁷ showed the possible consumption of C₄ plants as reflected in isotope values of bone collagen from a few graves at Ostriv site; b) millet is known to be used extensively in Ukraine during this period that provided higher chance in finding miliacin biomolecules and developing methodology; c) the population there contained huge quantity of dental calculus on their teeth that was an ideal material for further biomarker miliacin analysis; d) as this research aimed at developing methodology of miliacin extraction from dental calculus, we targeted more recent population for higher chances in finding miliacin.

The population in Ostriv burial was a part of the Kievan Rus' state that existed from the late 9th to the mid-13th century and encompassed a variety of polities and peoples³⁸. It constituted a crucial link between Scandinavia, Baltic and Byzantium via the Varangian trade routes, influencing the development of later East Slavic identities. The archaeological complex of Ostriv, discovered in 2017, presents a unique site characterised by burial practices and artefacts common not only to the local Slavic population. An analysis of the grave goods, such as flat ladder brooches, alongside burial orientations, suggests connections with Baltic tribes, including the



Fig. 2. The severe periodontal disease in grave 68 of the Ostriv cemetery site, caused by depositions of dental calculus, where a trace signal of miliacin biomarker was identified (photo by A. Kozak).

Prussians, Curonians, Scalvians, and Yotvingians. The multiproxy analysis of the population buried at this site suggests a multi-ethnic site with Baltic migrants and local Slavs³⁷, indicating human mobility during the Kievan Rus' period.

Ostriv cemetery probably belonged to the settlement, situated on the opposite bank of the river Ros'. The settlement was included to the south fortification line, which separated Rus' from the nomadic Steppe. The primary economic activities in the southern regions of Rus encompassed hunting, cattle breeding, and, to a lesser extent, agriculture. Paleobotanical studies conducted on materials excavated from several sites in the Porosia regions have demonstrated a predominance of crops such as rye, several types of wheat^{39,40}, peas, lentils, and millet, which was an important and widespread crop in this region⁴¹. In the city of Yuriev, which was also part of the Porosian defence line, archaeobotanists identified the presence of wheat and millet among the crops^{40,42,43}.

Thermal desorption – gas chromatography – mass spectrometry

By modifying the established method for archaeological ceramic powder²⁸, 31 dental calculus samples (Table 2) together with a blank control sample were analysed using thermal desorption–gas chromatography–mass spectrometry (TD-GC/MS). Powdered calculus samples (ca. 1–15 mg depending on the availability) were embedded in a sample cup to which 10 μ l of methyl derivatisation reagent (Tetramethylammonium hydroxide (TMAH) 25 wt% in methanol) and 10 μ l of the internal standard *n*-hexatriacontane dissolved in *n*-hexane (1 mg ml⁻¹), internal standard (*n*-hexatriacontane) was added. The sample was analysed by Thermally assisted hydrolysis and methylation thermal desorption (THM TD-GC/MS), using a multi-shot pyrolyser (EGA/PY-3030D, Frontier Laboratories Ltd, Koriyama, Japan) connected with GC/MS (GCMS-QP2010Ultra, Shimadzu Corporation, Kyoto, Japan). The sample cup was released in the pyrolyser, which was set to 550 °C for 0.20 min, then introduced to the GC at 300 °C by splitless mode. The Ultra ALLOY⁺-5 (30 m – 0.25 mm – 0.25

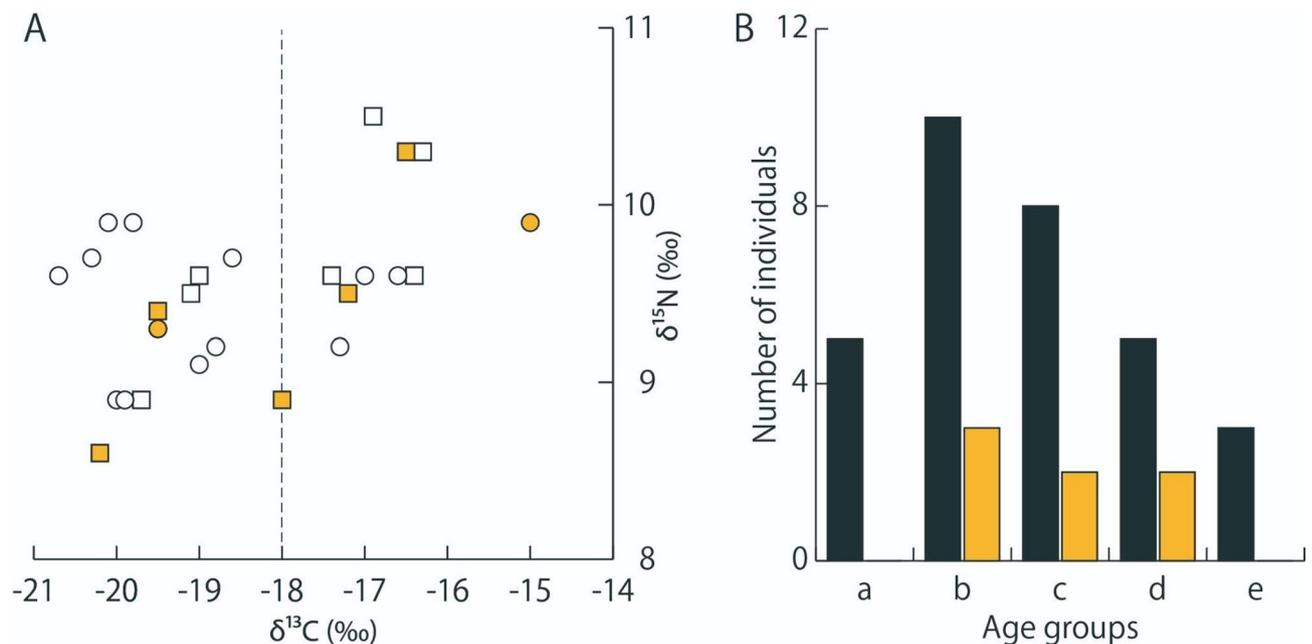


Fig. 3. (A) Scatter plots demonstrate the distribution of millet consumers against the dentin's stable carbon and nitrogen isotope values of the Ostriv humans. The cut-off value of -18% in $\delta^{13}\text{C}$ (dotted line) separates predominantly C_3 and mixed C_3 and C_4 consumers^{33,34}. Square symbols stand for females, while circle symbols represent males. Yellow: sample with miliacin. (B) Histogram of miliacin detection rates by age group. Group a: anthropologically identified as 13–15 years old, 16–18, and 18–25. b: 20–25, 20–30, and 25–30. c: 25–35, and 30–40. d: 35–45, 40–50, and 45–50. e: 45–55, 55–60, and 55–65. Black: total number of individuals, yellow: number of individuals with miliacin.

μm , Frontier Laboratories Ltd) column was used at a flow rate of 3.0 mL/min of helium gas and analysed at a split ratio of 1/20. FFAST (Fast Automated Scan/SIM) mode was adopted to detect miliacin efficiently while obtaining other major compound profiles with limited sample mass. For scanning, the temperature was set at $50\text{ }^\circ\text{C}$, then raised by $10\text{ }^\circ\text{C}/\text{min}$ until it reached $325\text{ }^\circ\text{C}$, where it was held for 5 min. The spectra were obtained between m/z 50 and 600. For SIM (Selected Ion Monitoring), m/z 189, 204, 231, 425, and 440 for the detection of miliacin were monitored between 27.0 and 31.3 min. The total acquisition time was 32.5 min. The Electron Ionisation (EI) energy of the MS was 70 eV. The temperature at the interface was kept at $300\text{ }^\circ\text{C}$, while the ion source was kept at $230\text{ }^\circ\text{C}$. Details of the Py-GC/MS instrumental settings in each section are shown in Figure S1.

Stable isotope analysis of human dentin collagen

The dentin in 31 individuals that also contained dental calculus subjected to thermal desorption were analysed for stable isotopes. The analysis was done on M2 human teeth. The second molar begins to calcify at 2.5–3 years and mineralisation of the crown is complete by 7–8 years of age⁴⁴. Collagen extraction took place in the Bioarchaeological Research Centre at the Department of Archaeology, Faculty of History, Vilnius University, following protocol as described in the previous study⁴⁵. Hydrochloric acid of 0.5 M was added to the samples until the inorganic component of the bone dissolved, and any debris was eliminated by multiple rinses with ultrapure water. Collagen was gelatinised in a pH 3 solution at $70\text{ }^\circ\text{C}$ for 48 h, frozen overnight in a $-30\text{ }^\circ\text{C}$ freezer, and then freeze-dried for at least 48 h. The collagen carbon and nitrogen stable isotope ratios were measured using an elemental analyser (Thermo Fisher Scientific, Flash EA 1112), which was connected to Isotope ratio mass spectrometer, EA-IRMS (Thermo Fisher Scientific Finningan Delta Advantage, Bremen, Germany) at the Centre for Physical Sciences and Technology in Vilnius, Lithuania (FTMC). Graphite USGS24, caffeine IAEA-600, ammonium sulfate IAEA-N1 and IAEA-N2 were used as calibration standards. High purity caffeine (99.7%, Alfa Aesar) was used as an internal laboratory standard.

Stable isotope data are presented as delta values and expressed in ‰, relative to the international standards V-PDB (Vienna Pee Dee Belemnite) and atmospheric air for carbon isotope values and nitrogen isotope values, respectively. To eliminate the possibility of contamination of diagenetic processes and ensure that the isotopic values represented the dietary signals, collagen quality indicators, such as collagen yields, the C/N atomic ratio, carbon weight%, and nitrogen weight%, were assessed. Precision ($u(Rw)$) was determined to be $\pm 0.16\%$ for $\delta^{13}\text{C}$ and $\pm 0.13\%$ $\delta^{15}\text{N}$ on the basis of repeated measurements of calibration standards. The total analytical uncertainty was estimated to be $\pm 0.20\%$ for $\delta^{13}\text{C}$ and ± 0.17 for $\delta^{15}\text{N}$.

Data availability

All the raw GC/MS data are available (mzML format) on the Zenodo repository (<https://zenodo.org/records/16892420>).

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

This study analyzed human skeletal remains recovered as archaeological heritage from the Ostriv site in Ukraine, dating to approximately 11th century AD (approximately 900 years ago). As research involving ancient human remains does not include living individuals or any personally identifiable information, it is exempt from ethics review under the policies of the authors’ affiliated institutions. We obtained permission to study these human skeletal materials, including the studied human bones, from the Institute of Archaeology of Ukrainian Academy of Science (Kyiv, Ukraine), which is responsible for the excavation and curation of the remains from the Ostriv site.

Additional information

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