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A Multi-Dimensional Approach to Investigate Use-Related Biogenic Residues on Palaeolithic Ground Stone Tools

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ABSTRACT

Recent advances in the role played by dietary carbohydrates in human food webs during the Palaeolithic highlight that starchy foods were part of the diet well before crop domestication. Although certain plants can be eaten raw, intentional processing such as mechanical reduction using stone tools and thermal treatment readily increases the assimilation of nutrients for metabolic functions and for storing. We present a multi-techniques approach designed to combine micro to nanoscale analyses applied to percussive stones to identify their function using micro-wear traces and use-related biogenic residues. The starch grains extracted from functionally active areas of the ground stone tools were scanned using optical microscopy (OM) down to the nanoscale (SEM) and by applying different spectroscopic and spectrometric techniques like FTIR, ToF-SIMS, and IRMS. The combined analyses carried out at different resolutions - morpho-structural and molecular levels - contribute to an unprecedented methodological refinement regarding the intentional processing of starch-rich plants as early as 40,000 years ago at the boreal latitudes. Our preliminary data on pestles and grinding stones from Early Upper Palaeolithic sites of the Pontic steppe (Moldova and Russia) show the suitability of the analytical techniques involved and also the difficulties encountered in detailing authentication procedures of ancient starch candidates.

KEYWORDS
Ground stones; wear-traces; starch; OM; SEM; spectroscopy

Introduction

Stone tools are the evidence of human intentional activity, and they can be analyzed at different scales and with different resolutions. Within this broad and long-lasting evidence, two main groups can be observed: the flaked tools (obtained from a wide range of different rocks which can be reduced into flakes and blades by means of a skilled operational sequence) and the non-flaked assemblage (generally used as they are for percussive activities and collected as pebbles, slabs, blocks, etc.). Our integrated analysis includes river pebbles, coarse gravels, and cobble-sized stones brought to the site by humans living in Early Upper Palaeolithic (EUP) sites across the Pontic steppe. These stones, which were not further transformed prior to their use, were collected along creeks, in ravines or in glacial deposits. Nonetheless, the selection of these materials shows consistent preference criteria regarding the raw materials (sandstones, quartzite, dolomitized limestones, and volcanic rocks such as diabase, etc.), their overall shape (size, roundness, flatness, weight), and, finally, their coarseness. Pebbles were used for combined percussive activities spanning pestling, grinding, threshing, tenderizing, knapping, mineral transforming, bone breaking, etc. The ground stone tools (hereafter GSTs) can be stationary, serving as the lower passive tool (known as the grinding stone), and also mobile, usually consisting of a smaller hand-held active tool that serves as a pestle (Aranguren et al. 2015).
Given the variety of uses and features of GSTs, the focus of this paper is to present the methodological strategy we developed using both wear-traces (WT) and use-related biogenic residues (U-RBR) to investigate the processing of plant starch-rich storage organs (PSRSO) by actions like pestling, grinding, and threshing carried out with non-modified pebbles, slabs and rocks from EUP sites (Longo et al. 2018; Skakun et al. 2019; Skakun et al. 2020). Among all the possible U-RBR, starch grains were given special attention. We detail the techniques employed to analyze the residues and propose our reasoned evaluation on the significance of the unprecedented methodological refinement we attempted in order to address intentional starchy plant processing in EUP records.
Facing the challenge of detecting the presence of starch grains despite their dimensions (typically between 1-50 urn, however, they can reach up to 100 μm)) and their genuine origin. we developed a multi-dimensional 'tailored' approach devoted to the extraction procedure, sample preparation. analysis. and characterization of starch grains still adhering to functional areas of the ground stones. This is an attempt to go beyond the presence *vs* absence assessment of starch grains belonging to economically relevant plants which allows us to interpret these remains as the result of genuine intentional processing of dietary carbohydrates since the EUP period. Furthermore, when it comes to dealing with plants available during Marine Isotope Stage 3 (hereafter MIS 3) at the boreal latitudes - putatively no longer in use - there are issues regarding the simple side by side comparisons of ancient and modern reference starch grains. Although one may consider our results modest compared to the effort, we maintain that the compound analytical chain, never applied before and presented here for the first time, is worth it in order to go beyond the unsatisfactory and questionable results obtained up to now based on the sole optical microscope analysis and the morphological comparison with present-day plants.
Our multi-dimensional approach aims at collating analytical information to:

(i) identify WT on GSTs by means of both optical (OM) and digital (DM) microscopy;
(ii) streamline a procedure to extract U-RBR (chiefly starch grains) from Palaeolithic GSTs;
(iii) detect the presence of U-RBR (mainly starch grains) by micro- and nanoscale analyses;
(iv) observe the structural/morphological characteristics of starch grains at different

resolutions (and their modifications);

(v) identify ancient starch candidates (ASC, Birarda et al. 2020) among all the possible URBR adhering to the GSTs;

(vi) address the chemo-profiling of starch grains (amylose and amylopectin) and of other minor components such as parenchyma (cell-wall fragments), raphides, etc., attempting to narrow down their taxonomic attribution;

(vii) control putative causes of contamination along the pipeline: excavation. conservation and extraction/analytical procedures.

Based on these observations, we made a significant attempt to characterize starches from EUP sites across the European Pontic steppe. We critically present the application of this multi-dimensional analytical approach - from the micro- down to the nanoscale - to a key selection of ground stones (N = 9) from four sites in Moldova, Crimea and the Russian plain (Table 1).

Table 1. Terms and abbreviations used in our research referring to plant involvement.

Acronym	Definition	Type	Description
U-RBR	Use-related biogenic residues	Any kind of biological remain connected with utilized areas	Feathers,raphides, hair, parenchyma, phytoliths, starch grains, tar, etc.
PSRSO	Plant starch-rich storage organs	Those parts of the plant where starch is stored or accumulated	
ASO	Above surface storage organs	Rich in starch	Fruits, seeds, grains, legumes, cambium, shoots, and sprouts.
USO	Under surface storage organs	It includes all the organs that store nutrients for the plant. **Roots** absorb water and nutrients from the soil through the root hairs, and anchor the plant. **Tubers** are storage organs that do not multiply, while new plants grow from roots and rhizomes. **Bulbs** are the layered fleshy underground storage structure. **Rhizomes** are swollen stems growing horizontally. **Corms** are the underground swollen part of the plant.	Roots, tubers, bulbs, rhizomes, corms
Geophytes (Eoin, 2016)	It can be used as a synonym for an USO (Deacon, 1993)	Prolific, energy-rich staples	As above
ASC	Ancient starch candidate	Putative grains related to functional active areas of GSTs	Grains with shape, size, structure and chemical composition compatible with starch

The Working Hypothesis

The present working hypothesis, that percussive stone tools were used by *Homo sapiens* to transform starch-rich organs into highly energetic food since its early occurrence at the northern latitudes. was launched by the first author (LL.) in the project 'Unfolding the complexity of nutrition at the dawn of modern humans in Eurasia' (2015-2018: Longo 2016; Longo, et al. 2018). The preliminary results were encouraging but also called for the development of an integrated multi-dimensional contextual approach to investigate putative GSTs and their associated U-RBR by involving several research teams. highly experienced in the application of micro- and nanoscale analytical techniques. Our investigation demonstrates how the involvement of different analytical techniques necessitates reflection and to eventual redirection of the research while sampling archaeological stone tools and also during the extraction and preparation procedures for U-RBR (namely starch) to allow further structural and chemical-physical analyses.

Our attempts successfully reveal the existence of different-sized GSTs at sites dating back to the Early Upper Palaeolithic/Initial Upper Palaeolithic and show that their utilized areas are still suitable for the micro-sampling of biogenic residues. The first phase of the project (2015-2017), devoted to the survey of pebbles and putative percussive tools, ended with a standardized procedure to select GSTs in museum collections and to extract suitable samples for micro- and nanoscale qualitative analysis of biogenic residues.

Evidence for Starchy Plant Consumption Before Crop Domestication

Evidence for the intentional processing of starchy plants in Palaeolithic sites across the western Eurasian boreal latitudes is scant. At Hohle Fels, an Aurignacian site in southern Germany, remains referred to as Asteraceae, Poaceae and berries have been reported (Riehl et al. 2015). Starch residues associated with raphides were identified on flakes from Crimean Early Upper Palaeolithic sites (Hardy et al. 2001). Direct isotopic data obtained from a burial discovered in an early Gravettian layer from Buran Kaya III (Crimea) supports the hypothesis that plant consumption was significantly higher for *Homo sapiens* compared with Neandertals living in the same area (Drucker et al. 2017). Use-wear traces and starch grains from Gravettian grinding stones in the Italian Peninsula, the Czech Republic and along the Don River have been interpreted as the intentional transformation of a broad variety of starchy plants such as cattail *(Typha* sp.), burdock *(Aretium lappa),* tuberous lettuce *(Laetuea tuberosa* and *L. vulgaris),* a fern known as the common moonwort *(Botryehium* sp.), cheat/rye brome *(Bromus seealinus)* and acorns *(Quereus* sp. a shelled fruit) around 30,000 yr cal BP (Revedin et al. 2010; Aranguren et al. 2015). These latter findings are considered to date the oldest known evidence of grinding and pounding stones used to process starchy plants during the Late MIS 3 (40-25 kyr BP). We have already reported preliminary results of the study of a large grinding stone retrieved in the bottom layer of the Upper Palaeolithic site of Kamennaya Balks II on the lower Don River (19,030-18,820 yr cal BP), where USOs belonging to reeds (i.e. *Arundo donax, Phragmites* sp.), cattail, water lily *(Nelumbo* sp.), ferns (Polypodiales) and ASOs from the Triticeae tribe and the Fabaceae family were mechanically processed to obtain calorific foods (Skakun et al. 2019, 2020, 2021). At Central European Epipalaeolithic sites. the record of plants remains became more consistent during the climatic amelioration of the Late Glacial Interstadial (LGI 14,670 - 12,890 yr BP), which created the conditions for the colonization of certain warm-weather flora (and fauna). Starch retrieved from stone tools is reported for the Mesolithic sites of Pod Zubem and Pod křídlem in Bohemia (Czech Republic, Hardy 1999; Divisova and Sida 2015; Hardy and Kubiak-Martens 2016). In China the processing of roots and seeds is documented from around 20,000 years ago, along the banks of the middle Yellow River. revealing starchy plant transformation even at the eastern Eurasian boreal latitudes (Liu et al. 2013).

Further south in the Levant, around 20,000 years ago, at Ohalo II, an Epipalaeolithic dwelling on the shore of the Galilee Lake (Israel), a broad range of plants are reported. and some were processed with grinding stones (Piperno et al. 2004; Dubreuil and Nadel 2015; Snir et al. 2015).

By contrast, outside of Eurasia, in totally different climatic and ecological conditions compared to those under investigation here. endocarp and mesocarp. vegetative parenchyma and stem tissue of non-wood plants have been identified through flotation and interpreted as being part of the plant-based diet 65,000 years ago at Madjedbebe in northern Australia (Florin et al. 2020). In Africa, charred remains of rhizomes have been reported in ashy layers from South African sites dated to 170,000 years ago (Blombos Cave, Wadley et al. 2020) and at the Klasies River main site, parenchyma of charred starchy plants have been identified by Larbey and colleagues (Larbey et al. 2019). In Mozambique, Mercader (2009) reported starch grains attributed to Poaceae family seeds *(Sorghum* spp.) from the Middle Stone Age layer of Ngalaue cave and interpreted them as evidence for plant consumption. Interestingly, in both Australian and South African contexts the authors report that the same plants are available even today around the sites. These observations offer some clues to the different environmental settings of the Eurasian Pontic steppe around 36,000-32,000 years ago.

Landscape Resources

In light of the above, it seems important to consider what kind of starchy plants were available in the investigation area - the Eurasian Pontic steppe - during MIS 3, and moreover, among those plants, which were actually mechanically transformed for consumption in the biomes colonized by early waves of *Homo sapiens.* MIS 3 was characterized by fluctuating, but generally cold temperatures and snow coverage, both limiting factors for the development of plant coenosis (Gerasimenko 2007; Dolukhanov and Arslanov 2009; Staubwasser et al. 2018). The cold and well-aerated soils of the mammoth steppe and the anaerobic permafrost *(yedoma,* in Russian) covered an extended area at the boreal latitudes during MIS 3, resulting in shallow and immature soils (less than 2-3 meters deep), not adapted to support most of the plants characterizing the post Late Glacial Maximum (LGM, 21,000 yr BP) coenosis (Zimov and Zimov 2014; Murton et al. 2015). Microbotanical evidence retrieved in the intestinal remains of frozen mammoths and woolly rhinoceroses in Yakutia (Russia) (Blinnikov et al. 2011) indicates the presence, until 1l,000 yr BP, of cold-tolerant, water-loving plants such as cattail *(Typha* sp.), whose rhizomes are rich in starch. These environmental conditions also affected both ruminants (bison and reindeer) and non-ruminants (mammoth and horses) and the plants they were feeding on. Such fauna was the usual food during the Upper Pleistocene for Boreal Hemisphere autochthonous huntergathers (Neandertals), considered hypercarnivores based on isotopic data (Drucker et al. 2017; Jaouen et al. 2019), and *Homo sapiens* who, on the other hand, was favored by a broader dietary plasticity and versatile behavior (Pratt 2011, p. e20834; Vicedomini et al. 2021). The decline of such fauna affected traditional animal food supplies and in turn put humans at risk as they turned to hunting lean animals, a dietary strategy that can have disadvantages and lead to 'rabbit starvation' (Speth 1987; Noli and Avery 1988; Cordain et al. 2000). These conditions were also attested in the Don and the Prut River valleys, and in the Crimea Peninsula when steppe covered the whole area and very cold conditions were experienced by modern human colonizers (Hoffecker et al. 2017; Allsworth-Iones et al. 2018).

Only after the LGM, when the climate began slightly but significantly to warm during several brief interstadials, periglacial loess-steppe environments prevailed across the East European Plain (Staubwasser et al. 2018). This warming was reflected in marked zonation of biomes (Hoffecker 2011) and the emergence of proto weeds in the Levant, on the southern edge of the Mediterranean (Snir et al. 2015). Finally, the glacial retreat started, and the improved climatic conditions of the Holocene (around 11,700 yr BP) allowed for the development of coenosis where graminoids and legumes could flourish. The Atlantic climatic optimum (8,000-5,000 yr BP) triggered the transformation of immature soils into fertile ones which were finally available for proto-weed expansion, so that they became an attractive resource for humans who began to increase long-standing seasonal mobility (Richerson et al. 2001). Therefore, taxa like oats or barley are unlikely to be part of the foraged plants prior to warming and drying trends observed in the Holocene. Thus, the conditions for the permanent flourishing of cereals and grains matured much later, after the Late Glacial Maximum was over, and allowed crops to be finally cultivated by a biologically and behaviorally fully adapted modern human population ready to exploit their new niches: domestication and then agriculture (Snir et al. 2015). However, analyses carried out in some Upper Pleistocene sites reported the presence of starch from grasses: 73 starch grains were entrapped in the dental calculus of Neandertals from Shanidar cave (Iraq) and attributed to barley (Triticeae tribe), and also to date palm (Henry et al. 2011). Moreover, according to Mariotti Lippi et al. 2015, starch grains from the Poaceae family were widely collected and processed with a pestle retrieved in a Gravettian layer in the Paglicci cave (Italian Peninsula) and identified as pertaining to oats, millet, and oak. These very limited remains attributed to the Triticeae tribe have already led some scholars to claim that their presence might be due to contamination (Coli ins and Copeland 2011), which may result from biases in sampling, preparation, or inadequate conservation practices (Crowther et al. 2014; Mercader et al. 2018), whereas starch grains from the transformation of USOs retrieved on other Gravettian ground stones seem to be more consistent with the environmental conditions occurring during MIS 3 (Revedin et al. 2010; Aranguren et al. 2015; Skakun et al. 2019) and less likely to be part of contemporary plant processing.

Nonetheless, macrobotanical and palynological data recovered from the frozen stomach contents of herbivores (mammoths, rhinoceroses, horses, bison, lions) (Arslanov et al. 1980) clearly indicates the predominance of wild Triticeae grasses along with sedges (Cyperaceae) and other families (i.e. Amaranthaceae, Typhaceae) and a range of other woody plants (birch, willow), typical of the periglacial forest-tundra/steppe for the period of the Bryansk interstadial within MIS 3 (Kupriyanova and Alyoshina 1972; Siniakova and Puzachenko 2005; Ukraintseva 2002, 24). Given the very limited yet undisputable presence of starch grains attributed to ASOs - keeping in mind that proto-weeds become available in later periods - we would argue that during MIS 3 it may be safer, for the time being, to include some plants (i.e. the Triticeae) into a broader group - graminoids - which encompasses many edible geophytes growing in wetlands and grasslands which have not yet been considered for comparison but which we are continuously being added to our reference collection. It is clear that additional work is required to resolve the issue of plants actually entering the food web of *Homo sapiens* when it ventured into Eurasian northern latitudes. Hence, we propose a multi-dimensional approach aimed at characterizing starch grains not only on the basis of their morphology but also by their chemical profile to achieve crucial information for their taxonomic attribution (chernotaxonomy, Jardine et al. 2019; Singh 2016).

Materials and Methods

Selected Archaeological Materials

The research includes items from some of the oldest EUP sites attesting the presence of *Homo sapiens* in the European steppe environment, who exploited the broad range of available food webs (vegetal and faunal resources). Having investigated dozens of putative percussive tools surveyed from 21 sites across the Eurasian steppe, we here report on the combined techniques we approached and applied in detail in the case of nine ground stones (Table 2) selected from four sites located in the central and south-western Russian plain, namely Kostenki 14 - Markina Gora (KI4), Kostenki 16 - Uglianka (KI6), Surein 1 (Crimea), and Brînzeni I cave (Moldova) (Figure 1).

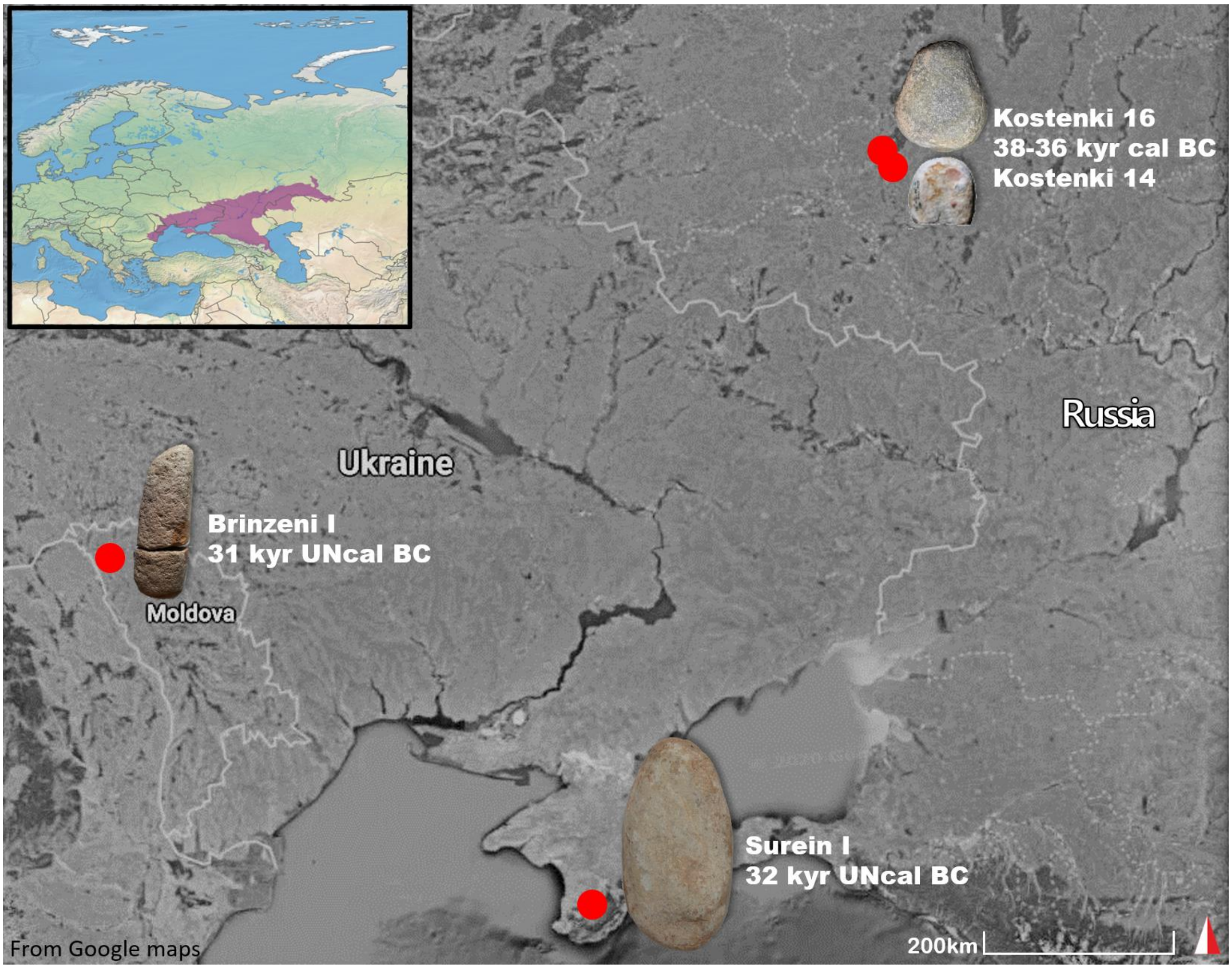


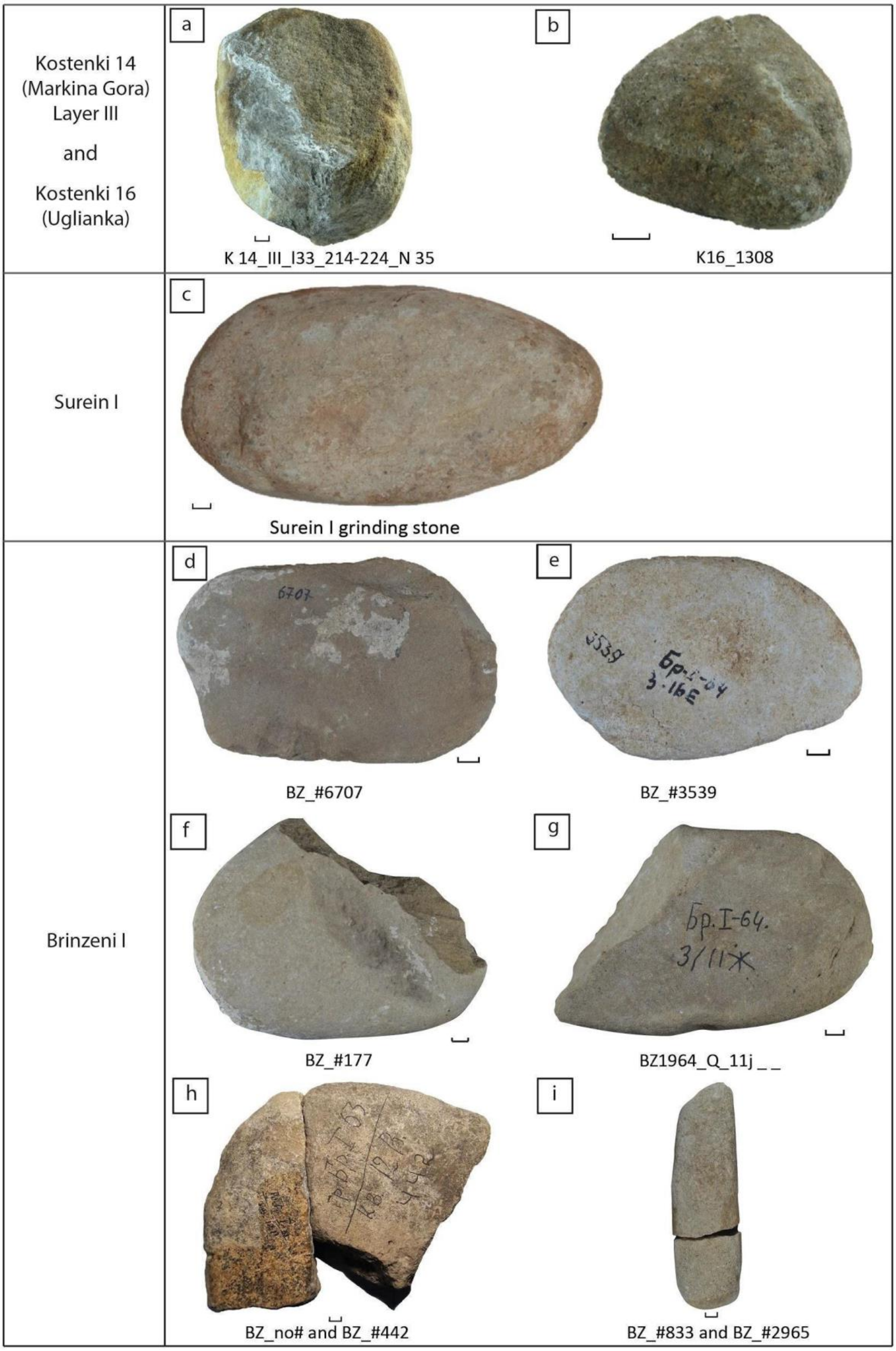
Figure 1. The Pontic Steppe Zone: location of the investigated sites presented in this paper.

From a biogeographical point of view this area corresponds to the Pontic steppe where rivers such as the Prut and the Dniester served as dispersal corridors for the migrations of both Neanderthals and early *Homo sapiens,* as supported by the high number of sites along their valleys. Intriguingly, these lithic assemblages consist of some 'tools' also present in Crimea and in the Caucasus, supporting possible connections with south-western Asian complexes already observed by Vekilova (1957) in her detailed study of the site of Surein 1. K 14 and K16, along the Don River, were targeted as they hold several ground stones, already reported by Russian Scholars (Rogachev 1973) as involved in plant processing during the Palaeolithic. Among a total of 14 ground stones from layer III and IV of K 14, the layers yielding the Campanian Ignimbrite tephra dating back to 38-36 kyr cal BP (Dinnis et al. 2019), one small grinding stone was tested for the present study (Tab 2: a). The material considered from K 16 is a fragmented pestle (Tab 2: b), which was selected among a total of eight objects, and pairs with the pestle previously studied by L.L. and N.S. (Revedin et al. 2010).
Regarding the other sites under study, they are located in south-eastern Europe and are characterized by a broader environmental diversity. Surein 1 is a rockshelter in the easternmost Mediterranean peninsula of Crimea. and Brînzeni I is a cave in the Prut River valley (Moldova). According to Hoffecker (2011), they are listed among the oldest eastern European Aurignacian

sites and were occupied during the early presence of *Homo sapiens,* as attested by one rare tooth found in Layer 3 at Surein 1, attributed to the Aurignacian. This makes Surein 1 a key site for the eo-occurrence of both late Neanderthals and early *Homo sapiens* in Crimea (Pratt et al. 2011; Demidenko 2014). In 1926, within Layer 3, a very large (236 x 122 x 68 mm) flat and oval-shaped, biogenic limestone (Tab 2: c) was unearthed and interpreted as being associated with a complex structure - with several remains of a horse still anatomically connected during the Bonch-Osmolovsky excavations (1926- 29). Moreover, the perfect preservation conditions ensured by the MAE RAS Museum in St Petersburg (Longo et al. 2021a; Longo et al. 2021b; Birarda et al. 2020) represent a further reason to apply biogenic residue analysis even to materials excavated almost a century ago.

In Brînzeni I cave, cultural layer 3 is the oldest cultural level showing the most relevant industry and has been shown to be the richest site in GSTs among the 21 sites surveyed at present. More than 100 percussive stones were identified and detailed during the excavations that began in 1963 and continued for a number of years, by N. Chetraru and I. Borziak (1987). They carefully numbered and plotted their locations, curated the GSTs in wooden boxes and stored them in the National Museum collections, where they remained untouched until this present study (Allsorth-Jones et al. 2018, 62). Among the large record of river pebbles from Brînzeni I cultural layer 3, 36 were recognized as tools by Moldovan archaeologists. Here we report the detailed study of eight fragments (Tab 2: d-i). After our analysis, four fragments were refitted: a grinding stone and a pestle (Tab 2: h, i). This is another fact supporting the infrequent handling of the material from Brinzeni I, as already mentioned by AlIworth-Jones (ibid.), which strengthens the suitability of the assemblage for analysis.

Table 2. The GSTs from Kostenki 14 (K14-III-11-k33-214-224 #35, grinding stone), and from Kostenki 16 (# 1308 pestle, fragment); from Surein 1, a large grinding stone; from Brînzeni 1, 6 percussive tools, among which a long pestle broken in 2 pieces (#2965, small fragment, and #833, large fragment) and one large slab broken in several parts of which we identified two that refitted (#442, large fragment, and # NN, small fragment). The scale is 1 cm



An Integrated Multi-dimensional Contextual Approach

From a methodological point of view, our attempt to investigate percussive stones from the micro- all the way down to the nanoscale is unprecedented. not only because of the involved techniques but also because it was designed so as not to reciprocally influence the other types of analyses and their interpretations. The results were only collectively discussed once all the analyses were performed. Therefore, it was conceived as a sort of blind-test on both the suitability of the techniques to investigate U-RBR (i.e., starch grains) and of the feasibility of results being obtained independently by the research teams involved. The *ArchaeOrganics* Conference, held in Rome in 2019, allowed for the first presentation on the overall pivotal results obtained through this integrated study.

Special care was devoted to collecting information to reconstruct each stone tool's biography, from the excavation process to the conservation strategies. This crucial information has very rarely been reported on the ground stones analyses published to date (Longo et al. 202Ia). We are aware of the concern expressed by the archaeological community regarding items with a long-life history, namely 'legacy' objects retrieved up to nearly one hundred years ago (i.e. Surein 1; Koh and Birney 2020). Nonetheless, the scrupulous and convenient methodologies applied by the Russian archaeological tradition, the precise information accompanying the stone tools, and the naturally cold storage conditions of Russian museums (below 0°C for most of the year) are unmatched in the vast majority of museums in Western countries and at lower latitudes. As such, we are confident in the preservation of the biogenic residues (Longo et al. 2021a). However, as washing and handling the stones without wearing powder- free gloves is 'the' common practice even in current-day excavations all around the world, the presence of finger- grease and keratin cannot be ruled out in almost any of the study cases published to date. Hence, we devoted attention to this issue.

WT analysis of the ground stones was carried out by N.S. and V.T. on the spot by means of a metallographic microscope Olympus BHMJ with magnifications from 50x to 500x, while digital microscopy and the SEM analysis were carried out by L.L. and G.S. on the molds or on araldite positives (see below) and results are detailed elsewhere (Longo et al. 2018; Longo and Skakun 2017; Longo et al. 2021b; Skakun et al. 2019; Skakun et al. 2020).

Within the study of U - RBR, we focused our attention on the recovery of putative starch grains. To characterize these residues, we developed the integrated multi-dimensional contextual approach including both micro- and nanoscale heuristics. Very significant information was gained by using (i) several microscopies (including visual light - VLM and LED - and electron source - SEM, scanning electron microscopy) to locate and identify the starch grains; (ii) Fourier Transform Infrared (FTIR) imaging and microscopy with infrared Synchrotron Radiation (SR) and other conventional sources; (iii) Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), and (iv) isotopes analyses (Isotopic Ratio Mass Spectrometry, IRMS) conducted on the same biogenic samples. The selection of these characterization methods (FTIR, ToF-SIMS and IRMS) was driven by an attempt to move beyond the morphological identification of the starch grains with respect to VLM, and also aimed at characterizing some of their peculiar components such as fatty acids and other chemical compounds, thus providing qualitative data for a more consistent attribution of their botanical origin (chemotaxonomy, Jardine, Goslin, and Lomax 2019). Based on different physical principles, these spectroscopic analytical techniques provide compositional information, namely molecular, elemental, and isotopic information. The preliminary results obtained are reported below, in turn providing evidence of their potential for reliable starch grain analysis.

FTIR (Fourier Transform Infra-Red) spectroscopy is based on the interaction of infrared radiation with matter. The spectra obtained represent the energies absorbed by covalent chemical bonds between atoms that form different molecules, depending on their nature. The atoms are in constant motion and this activity provides each of them with a stretching and bending vibrational energy. When hit by an infrared photon from an external source, these vibrational states change, causing an absorption which can be measured. As each bond (e.g. C-O, C-H, C = O, etc.) is characterized by a specific energy and this energy depends also on the chemical neighbours, the bonds can be identified and thus the whole molecular architecture. A FTIR spectrum is the sequence of absorbance peaks and it is like a fingerprint of a substance which can be compared with the reference libraries.

FTIR spectroscopy is considered a non-destructive and versatile technique, which can be applied to analyze micro- to nanoscale remains (Buleon et al. 1997; Perez and Bertoft 2010). The vibrational spectrum of the various U-RBR makes it possible to distinguish the particular signals of the different compounds from both inorganic origin (namely, minerals) and biological origin (such as carbohydrates, fatty acids, proteins and other biogenic molecules). In Table 3 we present the main signals for carbohydrates, which can be used to distinguish ASCs from the minerals or other contaminants, like bacteria or plastic particles (Longo et al. 2021a). Since references are unavailable for ancient plant compounds, we built an ad hoc reference collection to perform a chemical cross-check with the material recovered from the EUP grinding stones. This reference collection is continuously being updated.

Table 3. Infrared signals for carbohydrates and carbonates (Socrates G. 2004; E. Wiercigroch et al. 2017; R. Kizil, et al. 2002).

Infrared band	*Infrared band assignment*
3520-3100 cm^{-1}	O–H stretching
~2900 cm^{-1}	C–H stretching
1775-1735 cm^{-1}	C=O stretching
~1642 cm^{-1}	O–H bending
1530-1500 cm^{-1}	Aromatic ring deformation vibration
1460-1200 cm^{-1}	C–H and O–H deformation (multiple bands)
1420-1415 cm^{-1}	$C–H_2$ bending , C–O–O stretching
1350-1345 cm^{-1}	C−O−H bending, CH2 twisting
~1340 cm^{-1}	O−H deformation vibration
~1240 cm^{-1}	CH_2OH (side chain) related mode
1160-1000 cm^{-1}	C−O and C−C stretching, C−O−H bending
960-730 cm^{-1}	C−H deformation vibrations, ring vibrations

ToF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry) can be listed as a non-destructive, highly surface-sensitive analytical technique that provides elemental and molecular information about surfaces of solid matter, allowing the detection of low molecular weight fragments from biomolecules such as lipids, amino acids, and metabolites. This is achieved by scanning a micro-focused ion beam across the surface of the starch grain. Elemental ions and charged fragments of the molecules obtained as a result of the impact are recognized by the time required to arrive at the end of the line in a particle accelerator, which depends on the mass/charge ratio. The information is acquired with both high-mass and high-spatial resolution. The surface is analysed within a depth of approximately 1 nm and with a typical lateral resolution less than 0.1 μm. Moreover, by combining the primary ion beam with a sputter ion beam, the depth distribution of elements and molecules can be determined.

IRMS (Isotopic Ratio Mass Spectrometry) is based on the separation of ions of different masses, even when referring to the same chemical element, measuring their relative abundance in several kinds of matters. The principle is that physical and chemical processes can induce *isotopic fractionation* thus discriminating between the isotopes of an element. The transformation or translocation of element compounds from one environmental compartment to another leads to an enrichment of or decrease in the heavier isotope in a specific compound of interest, compared to the lighter one, and allows the tracing of the pathway of a given substance in the environment (Mook 2005). The differences in isotope abundance are typically very small in nature, so the isotopic ratio of a sample to a standard is conventionally measured in units per thousand (delta notion 6), where the ratio R is that of the heavier isotope with respect to the lighter one. Therefore, the delta notion is δ (‰) = $(R_s/R_{std} - 1) \times 1000$, where R_s is the isotopic ratio of the sample and R_{std} that of the reference standard. Plants with different photosynthetic pathways (i.e. C_3, C_4, and CAM) can be differentiated based on their natural ^{13}C isotope content.

The contextual integrated analyses briefly mentioned above (i) allows for the establishment of novel procedures to analyze the U-RBR (i.e. starch from grinding stones), (ii) highlights the fact that hard sciences can provide unprecedented data on invisible traces, indicative of past human practices (i.e. plant processing for consumption purposes), and (iii) informs archaeologists and museum curators that a rich and delicate 'palimpsest' of data calls for a new understanding of ground stones as rich bioarchives. Hence the need to put in place standardized procedures that span the moment they are excavated to the moment they are stored in museums, and to keep a detailed record of their individual biographies.

Criticalities Encountered to Document U-RBR on GSTs

As described above, we are aware of the risk of potential contamination given that the objects were excavated a long time ago and curated in museums since then; therefore, reconstructing the tool's biography was key to our selection criteria. One object - the Surein 1 grinding stone - was excavated up to 100 years ago and stored in the MAE RAS Museum 'The Kunstkamera' in St Petersburg. To our knowledge, even for ongoing excavations, it is very difficult to guarantee an uncontaminated life history to an artefact from the moment it is excavated (i.e., the almost unmatchable 'clean' conditions) until the moment in which the implement is analyzed in a laboratory and during the different analytical steps (Cuthrell and Murch 2016). As noted by other scholars, current archaeological practices, including the studies of lithic tools as well as museum curation and exhibition protocols, are unsuitable for preventing both on- and off-site contamination (Crowther et al. 2014; Louderback et al. 2015; Mercader et al. 2018). Moreover, the single-tool/object biography and its conservation history are only seldom reported in publications (Longo et al. 2021a). However, challenging as it might be, scholars have been investigating the

possible causes/conditions that might produce ambiguous remains or even reduce data (i.e., lack of retrieval, heavy washing and brushing, handling with bare hands, labelling, drawing, refitting, etc.). These conditions are more constraining when micro- and nanoscale investigations are to be carried out, and while we are aware that the pre-treatment of our ground stones might raise some red flags with regards to the authenticity of the residues we retrieved, hence this concern can be applied to almost all other records (Haslam 2004; Hart 2011; Pearsall 2015; Croft et al. 2016; Ma et al. 2017). On the other hand, in this study some of these concerns were resolved by the specific features of the analytical techniques chosen, such as in the case of human finger-grease. The presence of finger-grease can be identified by suitable techniques such as mass-spectrometry for the peculiar qualitative molecular composition. in which ceramides, squalene. cholesterol and sapienic acid are present (Pappas 2009). Squalene and ceramides are also produced in vegetal tissues. but their occurrence is very different in terms of concentration from that of human skin in which it is significantly higher (Pappas 2009; Lozano-Grande 2018). Skin ceramides are also qualitatively different from vegetal ones in chain length, hydroxylation pattern, number and position of unsaturation (Tessema et al. 2017). The previous observations may also hold for cholesterol, as high amounts of this compound are typically present only in animals while plant production is several hundreds to thousands of times smaller (Sonawane et al. 2016). Although sapienic acid is scarcely found in plants and is produced by human glands, it can be distinguished from other molecules common in nature (such as palmitoleic acid, with the same molecular formula $C_{16}H_{30}O_2$) only from the position of double bonds (Pappas 2009). The simultaneous presence of these substances can thus reveal handling contamination. In this view, in addition to grease, keratin could also be an indication of recent human residues (Barry 1992; Vasconcelos 2008). Given the high characteristic limits of detection of FTIR technique for mixtures, the detection of grease (Crane et al. 2007) - although unlikely to be present in the residues after in-water extraction from archaeological materials - would represent a warning of sure (high) contaminant concentration.
Regarding starch contamination, concerns were raised on the presence of starch granules in the surrounding environment of labs (Crowther et al. 2014). Starting with the control of the sampling conditions, our approach aims at overcoming the drawbacks related to the sole morphological comparison between 'ancient' starch grains and their putative modern analogues, since many of the plants possibly in use during MIS 3 are neither acknowledged nowadays nor present in the available reference collections. Our experience led us to another concern: the weathering and ageing processes that occurred in the sediments during the last 40,000 years definitely affect the appearance of the grains, making them unlikely to be identifiable using side by side comparison with native (modern) starches alone. These concerns led us to spend more time characterizing the U-RBR with different advanced molecular techniques to avoid drawing conclusions that might be the result of contamination, lack or limitations of a reference collection, and issues related to ageing/taphonomic processes. Experiments on aged starch grains in the climatic chamber will apply the same analytical protocol (ongoing experiment).

Being fully aware of these caveats, we designed the sampling and the following analytical procedures in order to avoid as much as possible further contamination, while at the same time searching for evidence of previous contamination in order to subtract those insidious data from the ancient data (false positives). In the cases where samples came from old excavations or museum collections, we focused during our analyses on building a detailed object biography and highlighting possible contamination and other sources that could obscure actual U-RBRs. Many of the difficulties we encountered are connected to uncontrolled retrieval and/or management strategies before samples entered the museum storerooms (Longo et al. 2021a; Longo et al. 2021b).

We here present our first attempts at studying starch grains from EUP ground stones, which we consider to be positively ancient, for consideration by the scientific community. Far from resolving this issue, since almost all the ground stones retrieved in Palaeolithic records follow the same excavation trajectory, we would also like to raise awareness among scholars so that they may in turn make an informed decision concerning the published results.

Ground Stones Sampling Pipeline (Obtaining Molds and ASC Extraction)

Although sampling procedures were carried out using powder-free gloves and on clean surfaces (cleaned using bleach), it is fair to say that we cannot exclude the likelihood that bare hands handled artefacts during previous management and curation, along with the techno-typological measurement, as it is common practice for all lithic studies. As a matter of fact, FTIR analyses evidenced the presence of keratin chips on some of the observed samples (K 14 and K 16), but they were easily detectable and distinguished from the target particles, as it is demonstrated that any skin cells, or any oily contamination derived from bare-hand handling of the stones are characterized by a different infrared spectrum (Ali et al. 2013).

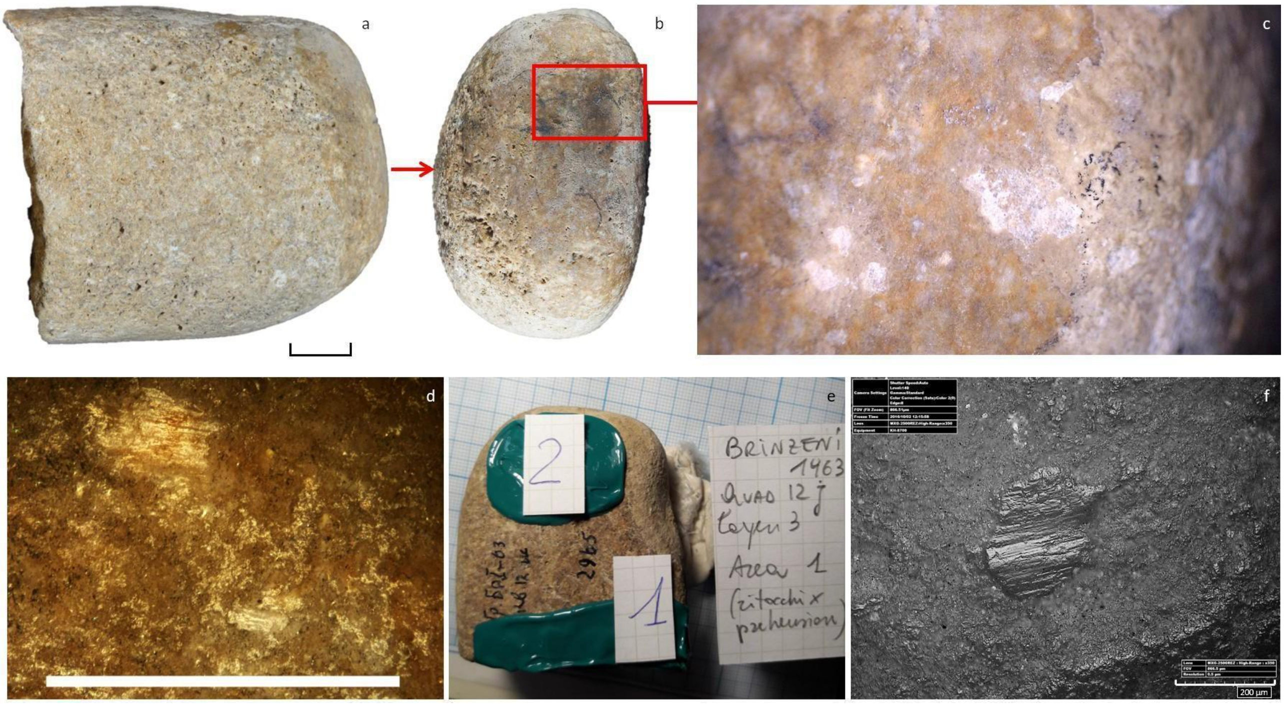


Figure 2. Example of the procedure for use-wear documentation: Brînzeni 1, # 2965 (pestle, small frag.) Layer 3, q 12 j: a: general view of the side of the pestle fragment; b: zenithal view of the used surface; c: detail (macrophoto); d: OM, metallographic micrograph taken on the original stone: the flattened and polished areas covered with micro-striations (scale bar: 1 mm); e: position of the molded areas; f: DM micrographs (1000x) taken on the molds, flattened areas covered with striations are visible in localized areas (same area as d).

Phase A: In museum: inspection of the pebbles via the naked eye, photos are taken (Figure 2 a-c); (1) soft brushing or hair dryer used to blow off of the dusty surfaces; (2) observation using a binocular stereoscope (Low Power Approach); (2a) when available on site, observations were made with a metallographic microscope (High Power Approach, up to 500 x) (Figure 2 (d)); (3) sampling the dust adhering to the box and shelf where the ground stones were curated.

Phase B: Photographic documentation with macros (Figure 2(c)) and multiple shots for photogrammetric post- processing; (1) 3D scanning to obtain a 3D model for off-site future analysis and evaluation. Several scanners were used to streamline the best cost-benefit procedure but also depending on the availability of the instrument (Longo, Skakun, and Sorrentino 2018).

Phase C: Water extraction of the U-RBR. (1) localized extraction of putative U - RBR in used and NON-used areas (for cross-reference) using water; (2) the procedure may differ according to the conditions (presence of ultrasonic tank, etc.) (Figure 3(a)): (2a) immersion of the selected area in dd-H_2O using a new, sealed container each time; (2b) sonication with ultrasonic tank for 10 min (this operation can be repeated); (3) liquid poured into new 50 ml Falcon tubes; (4) centrifugation for 3-5 min at 1500-3000 rpm (depending on the available device on site); (5) pouring the centrifugation liquid into another tube, saving the pellet in a small vial (3-5 mL) and pipetting (a few drops) of ethanol (EtOH) - to improve starch preservation in water-based solutions and to avoid bioactivity - before storage in a refrigerator.

Another concern is raised by the water-insoluble remains, which might provide information on other biomolecules (i.e., fatty acids and proteins), also deriving from 'invisible processing' of food sources. This constitutes the next methodological refinement we will address when carrying out new sampling campaigns.

Phase D: Molding is carried out using Provil® novo light (polyvinyl siloxane, manufactured by Heraeus Kulzer GmbH), a two-component addition-reaction silicone elastomer (Figure 3(b)). When mixed, the two components produce a flexible mold characterized by long-lasting dimensional stability with excellent thixotropic properties. Both this technique and this material have been widely used by L.L. since 1990 (Padergnana et al. 2016). Thus, a large collection derived from past studies can be compared with the present molds. The steps are as follows: (1) molding the putatively used areas; (2) molding other areas (i.e., non-functionally active surfaces) for reference; (3) the molding of the same area is repeated to obtain up to three molds for each potentially used area; (4) each mold is individually placed into a zip lock bag and labelled; (5) all the steps are documented, and photos/videos are taken. Surprisingly, this procedure has revealed itself to be rewarding in identifying starch grains still entrapped in crevices even after the sonication of the stone (Figure 3). The potential entrapment of starches deep in the pores of the rocks constitutes a further investigation that we have already planned to address in the future. The last mold (usually the third) represents the very clean copy of the surface, to be used for the sole purpose of wear-traces analysis (Figure 2(e-f)) (Longo et al. 2021b).

Phase E: Curation of the samples and of the molds in the Laboratory (VCH Lab, NTU Singapore/DAIS, Ca' Foscari, Venice, IT): (1) the sonication of the molds in water enables the extraction of additional starches that were adhering to the polyvinyl siloxane surface (Figure 3(c)). The vials with the starches extracted from the stone surfaces and from the molds were kept in the refrigerator until delivered to the involved labs (LP. Vladivostok and c.c. Nanterre); (3) direct observation of the molds under DM (Figure 2(f)) for use-wear inspection and at SEM for residue analysis (Figure 3(h)).

Phase F: Making positives by pouring araldite (1:5 bicomponent, LY 554 (base) and HY 956 (hardener)) to obtain a high-resolution positive copy of the surface to be investigated. This operation is carried out under the fume hood. Usually, the positive is created from the third mold, considered the cleanest, and after hardening (24-36 h), is bagged separately. Both molds and araldite positives can be observed under the microscope. In order to fit inside the SEM chamber, molds can be cut into smaller pieces and positives can be sized down to the required dimensions (Figure 3(f)).

Phase G: Analysis of the sonication containing U-RBR with SEM, FTIR, and OM (detailed below, Figure 3(d-j)).

Wear- Traces Analysis

During the 2015-2017 campaigns, we surveyed several dozens of pebbles, reviewed field notes and excavation reports, and also checked the storage conditions which might have changed over time. The tool biography reconstruction was compelling preliminary work which drove the selection of the putative GSTs.

Further selection was made on the basis of a first on-site analysis with the Low Power Approach, using a stereomicroscope with magnification up to 80x. This first step, carried out jointly with N. S. and V.T. of the IHMC and MAE of the RAS in St Petersburg (Longo et al. 201S; Longo et al. 2021b; Skakun et al. 2019; Skakun et al. 2020) allowed the initial identification of candidate functional areas. The functional active areas and the test non-used areas (identified by cross-checking) were then molded to undergo a refined subsequent examination.

Finally, the High-Power Approach was used to study the wear-traces on a selected sample using a metallographic microscope with magnification up to 500x (Figure 2(d)), while molds and positives were investigated with different microscopes with a broad range of magnifications and resolutions, i.e. Digital Microscopy (DM, Figure 2(1) and SEM (Longo, Skakun, and Sorrentino 2018; Longo et al. 2021b; 2021). The integrated results of the three approaches (Low Power Approach, High Power Approach and Ultra High-Power Approach) permitted us to identify the functionally active portions of the percussive tools, contributing to elucidating their potential polysemic utilization on different materials as already proposed by Rogachev (Rogachev 1973; Stepanova 2020). However, on the nine GSTs we identified several features that could be attributed to the grinding and pounding of plants, further supported by the still adhering starch grains indicative of starchy organ (PSRSO) processing. The detailing of the wear-traces analysis is beyond the scope of this paper, which is devoted to sharing the multi-dimensional contextual approach; the former can be found in several publications (Longo et al. 201S; Longo et al. 2021b, 2021c; Skakun et al. 2019; Skakun et al. 2020, 2021).

A methodological refinement developed during this study is the systematic use of microscopes with different resolutions which corresponds to higher magnification (Ultra High-Power Approach) using both visual light *(VLM/OM)* and scanning electron microscopy (SEM). Digital Microscopy covers a magnification range from 35 to 2500x (times) and generates a 3D model of the surface enabling the efficient observation of an area from various angles, reaching a magnification of 2000X and was carried out independently in two laboratories: the Cyprus Institute (Hirox KH-S700) and VCH Lab at the Nanyang Technological University (NTU) in Singapore (Keyence VCH- 7000, Longo et al. 2021c). The new generation FEG-SEM (field emission gun) working with low-energy electron beams provides extremely focused imaging with a spatial resolution of < 2nm, yielding a resolution three to six times better than conventional SEM and at least ten times better than the visual light microscopy (optical microscopy), allowing direct investigation without coating at very low potentials (maximum 5 kV). This setting also permits the observation of the starch grains adhering to the molds, which could have later been extracted by sonication and analyzed with FTIR spectroscopy and imaging for their further chemo-profiling (Birarda et al. 2020; Longo et al. 2021b). Digital and SEM microscopy offered an increasingly higher resolution intended as the separating power of two adjacent points according to Abbe's formula which is about 0.2 mm for the optical microscopy. These technologies were applied mainly to the molds, to the experimental stone tools, and DM was applied to a selection of the original implements from the four sites here considered (an example from Brînzeni I is presented in Figure 2). Images were acquired at various magnifications deriving from scanning between 35x and 2000x, averaging between 500x and 1500x, which enabled the identification of features frequently observed using both 3DMs and SEM. Starch grains were also imaged with a SEM.

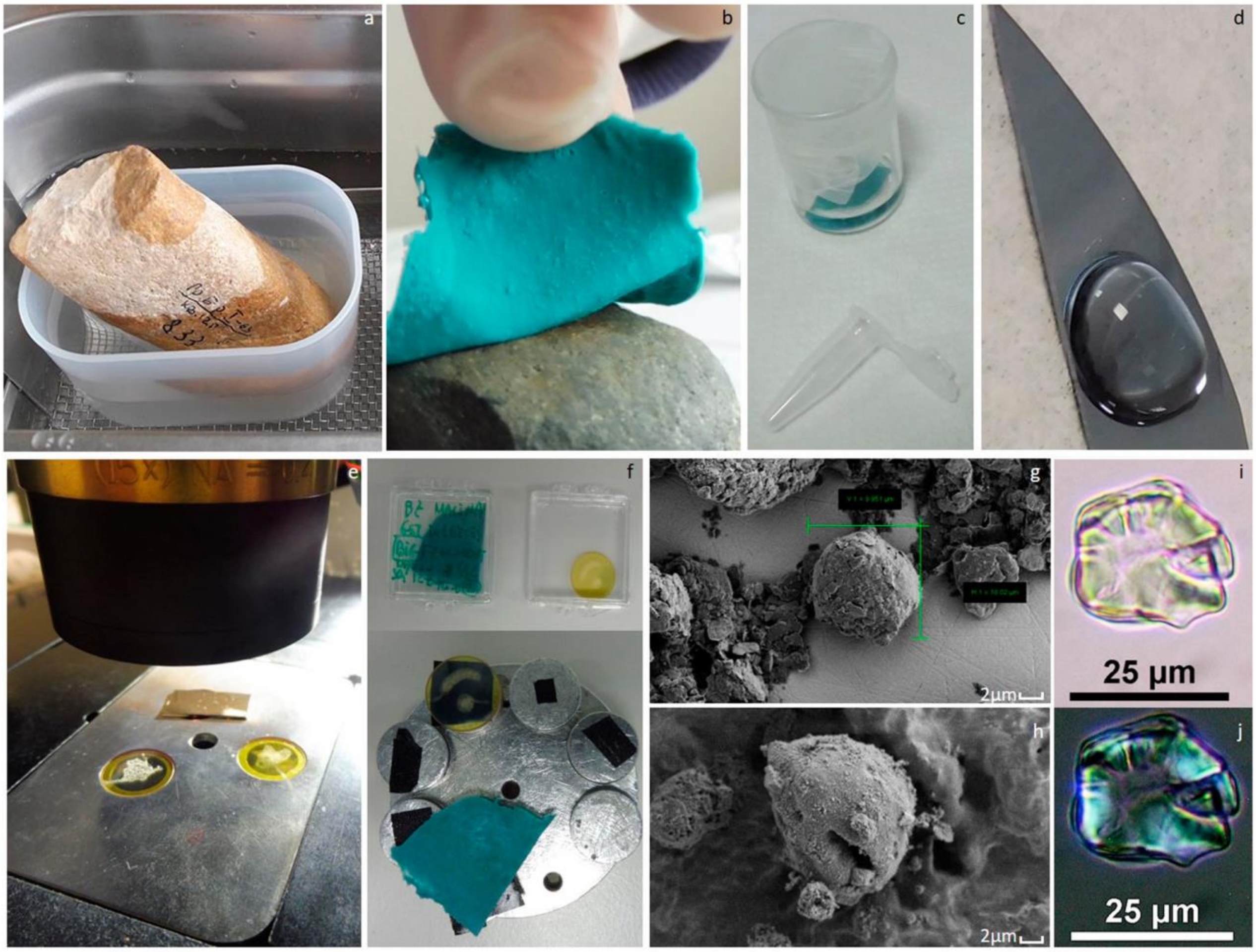


Figure 3. Starch grains extraction and analysis procedure from Brînzeni I grinding stones (EUP). a: sonication of percussive tool (#833, pestle); b: molding of a selected functional area; c: sonication of the impression (mold) to extract adhering starch grains; d–e: drops of sonicated solution deposited on different holders for FTIR spectroscopy: d, silica holder, e, ZnSe windows; f: SEM holder with sectioned mold and droplets of sonication solutions on ZnSe window; g: SEM image of a starch grain extracted from sonication; h: SEM image of a starch grain still adhering to the mold; i–j: optical microscope at 600 X magnification of a starch grain extracted from sonication (viewed in plane polarized and cross polarized light).

Starch Grain Sample Preparation and Analytical Pipeline

The starch sample preparation consists in isolating starch candidates from the other materials resulting from Phase C of the sampling procedure ('sediment', which is composed of both inorganic soil remains and other biological material such as parenchymal tissue; it can also contain modern starch grains, fungi, bacteria, etc.). This operation was undertaken in two separate laboratories and on separate occasions. The 2016 sampling addressed the sites of Kostenki 14, Kostenki 16 and Surein 1 and a first group of (N=6) ground stones from Brînzeni I; in 2017 the stone tools (N=36 out of more than 100) from the Moldovan cave were sampled. The Russian-related sets were prepared by Irina Pantyukhina at the Institute of History, Archaeology and Ethnology, Far East Branch-RAS, (IHAE FEB RAS) Vladivostok (Russia). The second set of samples (2017 campaign in Moldova) was also processed by Clarissa Cagnato at the Maison des Sciences Humaines (MSH) Mondes in Nanterre (France). The involvement of two separate laboratories was intended to avoid the same conceivable laboratory contamination sources; if contamination occurred, differences should be noted among the starch grains involved (Crowther et al. 2014; Dozier 2016; Ma et al. 2017). The laboratory methods followed those outlined by scholars (Pearsall, Chandler-Ezell, and Zeidler 2004; Therin and Lentifer 2006; Torrence and Barton 2006; Yang et al. 2012) and used by the authors in other studies (Cagnato and Ponce 2017; Skakun et al. 2019; Skakun et al. 2020). Prior to the analysis, all the laboratory equipment such as beakers and stirrers. for example. were washed using Alconox or bleach. The two laboratories used different heavy liquids, namely Caesium chloride (CsCl) in Vladivostok and Sodium polytungstate (SPT) in France. These salts behave slightly differently and call for multiple rinsing episodes to be washed out of the solution. The slightly different procedures followed in the two laboratories for the dispersion, pretreatment, and heavy-liquid flotation are presented next. Detailed reporting on single site results is to be published elsewhere.

The sediment from sonicated artefacts was transferred into a 50 ml plastic test tube and concentrated by centrifuging. To dissolve limescale in the soil, 10 ml of 0.1% ethylenediaminetetraacetic acid (EDTA) was added to the test tube, and then rinsed with distilled water. This was followed by adding hydrogen peroxide (5.75%) to ensure that non-starch organics would be destroyed and to aid in breaking up the soil. Once again. rinsing of the samples was necessary. Heavy-liquid flotation was performed using CsCl or SPT, which allowed the separation of the starch from the rest of the sediment since 'starches with a specific gravity of mostly 1.5 and below, will float to the top of the tube' (Piperno 2006, 96). Using a specific gravity of this high-density heavy liquid ensures that the starch floats or remains suspended (Coil, Korstanje, and Archer 2003). Fresh starch typically has a density of 1.5 g/rnl., and therefore the specific gravity of the CsCl and SPT solutions was set at 1.8 and 1.7 gl ml respectively. The test tube was filled with distilled water and centrifuged, at this point, the specific gravity of the solution is lowered, and therefore the starch will sink to the bottom of the test tube. The supernatant was decanted once again, the test tube was filled using distilled water and centrifuged. Once the solution was clear it was transferred to a clean, labelled slide, and a coverslip was placed on top. A water-glycerin solution (1:1) is typically used to mount the samples for standard optical microscopy observation. With regards to the optical microscopy starch grains were observed under a cross-polarized microscope (Nikon Eclipse E600 Poll and photographs and measurements were taken using the software NIS-Elements (located at the Archeoscopie Platform at the MSH Mondes). The optical microscope used in Vladivostok is a Zeiss AXIO Scope Al with magnification up to 800x, while in France magnification reached 600x. Photographs were taken of every starch grain at 600x and 800x in both cross- polarized and transmitted light. Starch grains were identified by the presence of the distinct extinction cross, quantitative data such as the size of the grains, but also qualitative data such as the angularity of the facets, and whether lamellae, fissures, or the hilum were visible among other features (Figure 3(i-j)).

For this research, which integrates both visual light/optical microscopy (VLM/OM) and SEM observation and further chemo-profiling of the starch grains (i.e. FTIR and ToF-SIMS), the extracted starches were mounted in two different ways: in a water: glycerin solution, and simply in water (thus omitting glycerin). A select set of samples was mounted on ZnSe windows to avoid adding additional elements to the FTIR spectrum, to be analysed at the nanoscale with a SEM and further studied by chemo-profiling the starch grains. It is worth noting that SEM observations highlight that sediment dissolution proved to be a critical issue when nanoscale investigation was involved, therefore new samples are under preparation for a future round of analyses.

Regarding the modern reference collection, a 'side-by-side comparison' is generally used as proof in the identification of a specific taxon recovered in the archaeological record. We want to stress the following as regards the reference collection: (i) it should be composed of taxon actually comparable with those present at the time of the archaeological dwelling, (ii) it should include the most common present day taxon that can be a potential source of contamination, and (iii) the appearance and the features of fresh starches are to be considered simply as an example since we have doubts about the feasibility of comparing fresh starches to those that are 40,000 years old. In view of this, we are in the process of running ageing experiments on modern starch using the controlled parameters of the climatic chamber inspired by the very few similar experiments carried out for later assemblages from Staff Car (Croft et al. 2016; High et al. 2016), which we adjusted to Palaeolithic climatic conditions. It will be interesting to observe those 'weathered' starches with our integrated approach and then attempt to consider them 'side by side' for meaningful comparison.

Overall, on the basis of (i) the small amount of starch grains (if modern contamination was at play, we could expect a far higher number of granules - Crowther et al. (2014) reported hundreds of contamination starches) and (ii) their variety, we can assume that our conclusions about the ancient anthropogenic origin of starch granules on the Palaeolithic ground stones are likely to be correct. Furthermore, considering the long history of the artefacts, modern starch would not be damaged at all and would not acquire the dilapidated and 'shabby' look that we observed. Also, we stress that no starch grains comparable with the archaeological ones have been identified in the very few starches (less than ten) recognized in the control samples collected in the box and on the shelf where the cages with the stone tools were stored.

Nonetheless, in order to overcome the limited diagnostic efficacy of morphological observations, we considered it worthwhile to carry out a series of molecular analyses described below.

FTIR spectroscopy is a routine analytical technique applied to starches in academic and industrial research (Buleon et al. 1997; Perez and Bertoft 2010). Nevertheless, this is the very first time that this technique has been applied in ASC research, allowing the measuring of isolated starch grains at the Chemical and Life Sciences branch of SISSI beamline at Elettra Sincrotrone Trieste (Basovizza, Italy).

In order to prove the effectiveness of FTIR on archaeological use-related starches, a first experiment was performed by dropping 1 ml of sonicated liquid on two different types of substrates: silicon and ZnSe (Elettra beamtime N. 20170057).

Figure 4 shows a powder deposit of minerals and organic particles. By integrating the signals that belong to the carbohydrate materials, like the C- H stretching (Figure 4(b)) and the O-H stretching (Figure 4), it is possible to generate false colour maps, like those in the figures, and identify the particles of interest mixed among all the others, as the areas where 'hotspots' co-localize. Then, SEM analysis provided additional information about the fine structure of the particles identified by FTIR (Figure 4). Following the inspection of all the samples it was possible to develop a protocol to identify ASCs inside the powder deposits:

- the particles have to be 'roundish', around 10-30 microns;
- a hotspot has to be present in at least two of the three heatmaps: 0-H stretching, C-H stretching and C-O-C stretching (see Tab. 3);
- if inspected under polarized light, they should present the 'Maltese cross'.

During the same beamtime, the direct survey of the molds using a SEM revealed starch grains still entrapped in the crevices of the used areas, which could be parsimoniously interpreted as processed starch. However, polyvinyl siloxane was unsuitable for further analyses with either FTIR or ToF-SIMS.

Finally, with the isolation of purified archaeological starch (according to established starch extraction protocols, in Vladivostok and Nanterre), during a new beamtime, it was possible (i) to confirm the FTIR data acquired and (ii) authenticate the presence of starch in the dried droplet (Figure 4). Thus, the established procedure provided evidence of the morphological (VLM/OM and SEM) and chemical features characterizing ancient starch and was demonstrated to be a valuable tool for discriminating against modern contaminants.

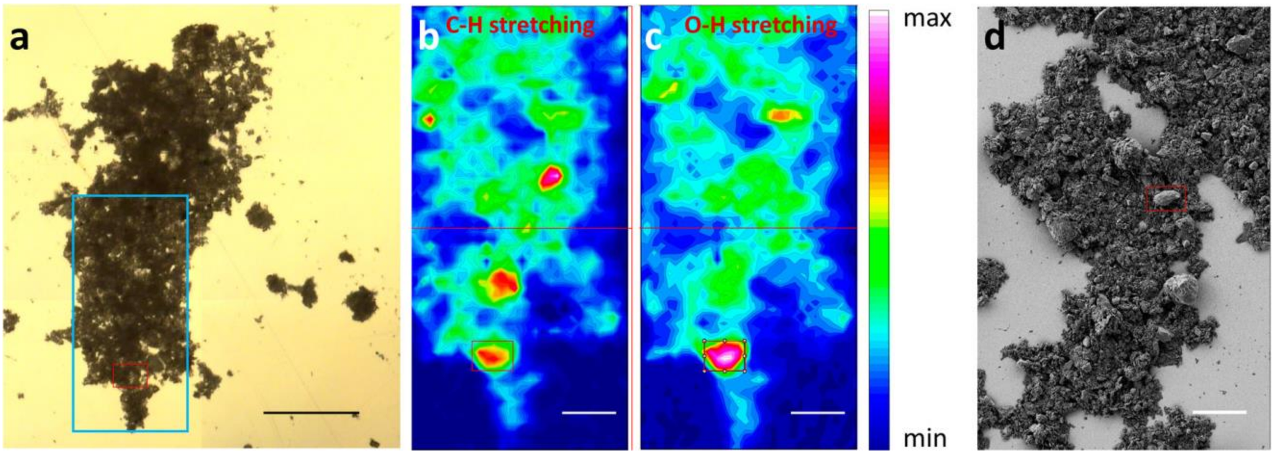


Figure 4. FTIR imaging of the sonicated dispersion (residues on mold) deposited on the ZnSe window, the blue rectangle indicates the sampled area, scale bar 150 microns; b–c: False-colour image of the chemical distribution of the C−H stretching signal and the O−H stretching signal, respectively; the presence of both signals confirms the ASC nature of the sample (red square), scale bars 50 microns; d: SEM micrograph of the sonicated dispersion, scale bar 50 microns.

ToF-SIMS is a unique system in Italy made available at the INFN and Roma Tre University Surface Lab. It has been applied to investigate dyes, pigments, metal leaves, corrosion and degradation products among inorganic compounds (Biocca et al. 2016; Ingo et al. 2019; Iorio et al. 2019, 2021; Sodo et al. 2019). The capability to detect small biomolecules without the aid of chemical reagents has opened new perspectives in the discovery of biomarkers and their spatial localization even for archaeological remains. Finally, ToF-SIMS is a very suitable technique for the investigation of archaeological starch granule chemistry in that the high depth resolution enhances the detection of specific plant-related minor components and elements (lipids, proteins, hexose, pentosan, phosphorus, silicon) which are mostly concentrated on the surface of the granules, as demonstrated by Baldwin et al. (1997) and Baldwin (2001) using this particular technique. For experiments on Paleolithic residues, a customized ToF-SIMS 5 instrument with a 30 keV bismuth liquid metal ion gun, 0.25-11 keV Caesium sputtering ion-gun, and ToF detector was used. The system was also equipped with a large load lock chamber to allow archaeological samples to be studied. Among the preliminary experiments on ASC, ToF-SIMS spectra from five different contemporary starch samples were recorded, thus allowing the identification of ions arising from a starch grain's surface: carbohydrates, proteins, and lipids were detected, while several peaks remained unassigned due to a lack of reference standards. However, others have been tentatively assigned as arising from the proteins on the starch's surface. Therefore, a relevant outcome of the work to date is the creation of a reference database devoted to the study of starch grains.

The **IRMS** methodology permits the differentiation of plants with different photosynthetic pathways (i.e. C_3, C_4, and CAM), based on their natural ^{13}C isotope content. The $\delta^{13}C$ analysis was widely used as a criterion for C_3 and C_4 classification (Li et al. 2006; Marchese et al. 2006), on the basis of the photosynthetic cycles and so of the adaptation to their typical environment. In fact, this results in a different carbon isotopic signal: C_4 plants have $\delta^{13}C$ tissue values about 17‰ higher than the corresponding components of C_3 plants (Farquhar, Ehleringer, and Hubick 1989).

In addition, the ^{15}N of plants can indicate the principal nitrogen source used, i.e. nitrogen fixing species (such as legumes) have values near to zero due to the direct use of atmospheric N_2 (which have ^{15}N=0 ‰). So, nitrogen is more variable than carbon, and is site-specific to the location where the plant grew (Dawson et al. 2002). The isotopic technique to detect the use of plants by ancient populations has been widely applied, especially in the case of organic residues inside vessels or on artefacts (Evershed 2008; Shoda et al. 2018). This methodology was applied to detect the possible

presence of vegetal materials on a selection of grinding stone residues from Brînzeni I, and to classify the plants according to their photosynthetic pathways as a means to reconstruct the taxa available in the Pontic steppe around 32 kyr BP. The carbon and nitrogen isotopic ratios were measured at the iCONa lab (isotopic Carbon, Oxygen and Nitrogen analysis) of the University of Campania 'L. Vanvitelli'.
Additional information can be obtained by compound-specific isotope analysis, which allows the measurement of isotopic ratios in specific molecules of a compound, chromatographically separated upstream. In this case, such analysis can be useful to identify the carbon isotopic signal of an individual fatty acid (i.e. palmitic and stearic acid) on stone materials, indicative of the biological origin of the source, and will be part of the future development of the research.

Results

Use-wear Traces and Functional Analysis of the GSTs

As stated above, the aim of this paper is to share the multi-dimensional contextual approach designed for this comprehensive study and the difficulties we faced when sampling was carried out according to conventional and in use procedures. The wear-trace analysis results are reported in recent publications (Longo, Skakun, and Sorrentino 2018; Skakun et al. 2019; Skakun et al. 2020; Longo et al. 2021b, 2021c; Sorrentino et al. in preparation). The analysis was applied to non-flaked, raw pebbles and slabs collected by the ancient settlers in the rivers nearby the archaeological sites (Tab 2). Non-destructive (or limited by nature), the application of optics in the study of stone tools began with the methodology called 'Traceology', developed in the early 1930s by S.A. Semenov (Longo and Skakun 2005). Microscopy is clearly difficult to apply outdoors (during fieldwork) and even in indoor situations (i.e. in museums). and is therefore infrequently used on site. SEM (coupled with EDAX/EDS*)* is a large instrument, and it implies the use of sometimes invasive sampling and. in general. it is limited by the dimensions of the sample to be analyzed.
The traceological study was performed in two phases. A survey of museum collections was necessary. since most of the Upper Palaeolithic non-flaked stone tools and macro-lithic assemblages are not usually reported in publications (except for Rogachev's seminal work (1973), recently reviewed, summarized, and enriched with more recent findings by Stepanova 2020). Here, we report on the methodological refinements applied to the pivotal functional study of nine ground stones and we include the experimental reference collection designed for this purpose, which is available at the traceological laboratory established by S. A. Semenov at the Institute for the History of Material Culture in S. Petersburg, Russia (Skakun et al. 2020; Sorrentino et al. in preparation).

In the strategy developed for this study, principles of tribology, metrology, and experimental archaeology have been applied to the GSTs. Use-wear analysis embraced the systematic application of photogrammetry and 3D scanning of the stones (point cloud models are useful for laboratory activities and ultimately for museum outreach) and the use of different microscopes (with light. scanner. and electronic beams) with increasing magnification and resolution power to perform the Surface Texture Analysis (STA, Longo et al. 2018; Caricola et al. 2018). 3D digital microscopy observation was supported by software for metrological application. The fatigue that occurs during the interaction of surfaces in relative motion with different worked material in between (in the present case plant processing) is what tribology studies: adhesive wear (deposited residues), fatigue wear (induced fractures, crystal warning and/or removals), abrasive wear (creating striations, levelling, or rounding the salient surfaces), and tribo-chemical wear (i.e. producing polishes) (Adams

2014a). This approach allows the reconstruction of the utilization of the percussive stones, which might not show a highly developed degree of wear.

The first macro- and microscopic observation by means of stereo and metallographic microscopy drove the selection of the areas to subsequently be molded (Figure 5), after the sonication of the putative used area to dislodge and extract the U - RBR. The molding techniques (imprints) revealed themselves to be very useful in the case of ground stones, since their large dimensions did not allow for easy scanning under microscopes, and molds could be further investigated with different resolution and magnification. The molds were photographed, mapped, and sealed in separated plastic bags to avoid further contamination. Both the imprints (negative) and the positive - obtained with araldite - were observed under high-power magnification, 3D digital microscope (Hirox and Keyence), and the SEM (without coating). Molding proved to be very helpful to control the experimental steps, allowing the inspection of the surfaces during the development of the wear-traces (Sorrentino et al. 2021).

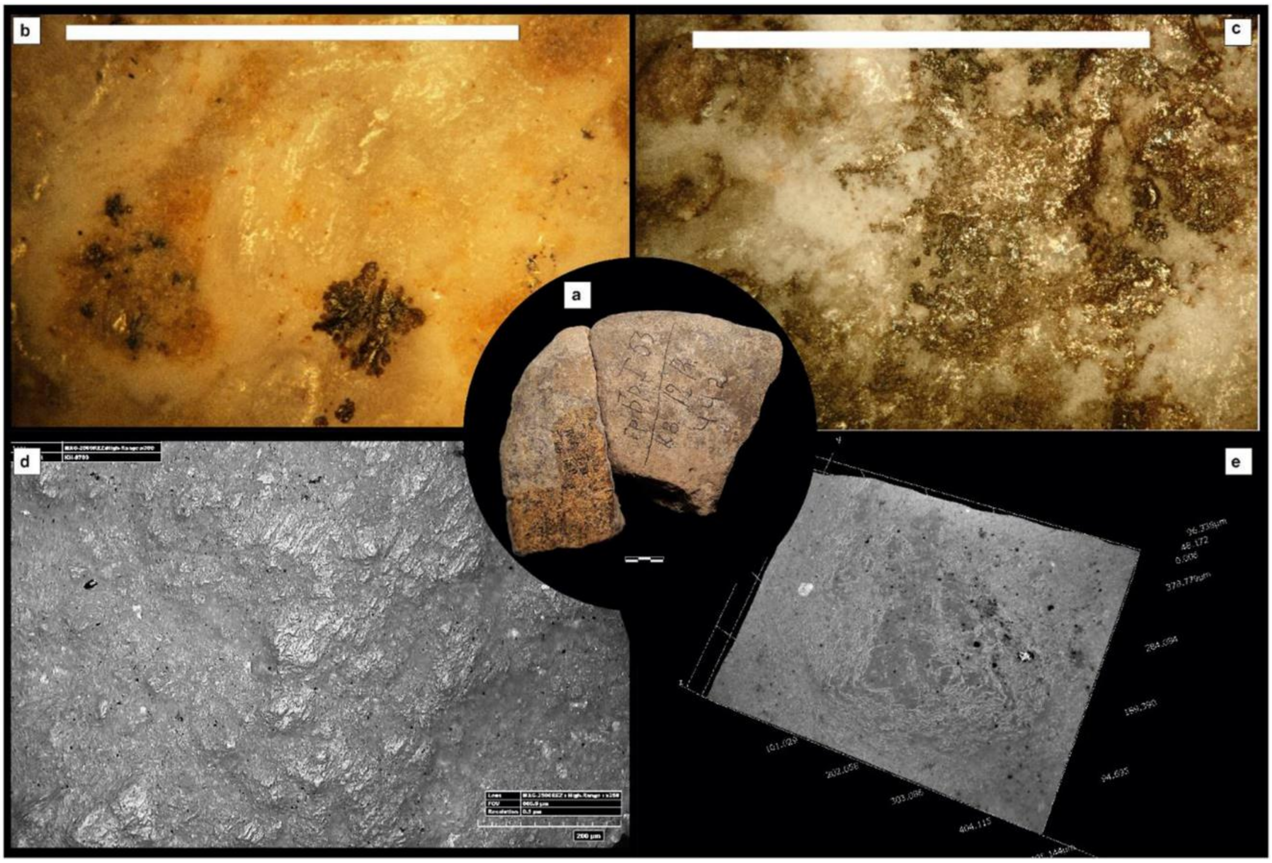


Figure 5. Examples of wear-traces under different microscopes. a: Brînzeni I grinding stone (incomplete refitting of #442, large fragment, and a small fragment without number). b–c: alignments of polish and spot of polish observed on the original surface by using VLM (Olympus metallographic microscope, 200 X); d–e: the same areas as observed with 3D DM (Hirox), scanning of the mold (copied area of small fragment: centre, toward the fracture); d: the uneven microtopography of the used surface is affected by alignments of short striations with the same orientation; e: polish as seen under the digital microscope, appearing as matt, different scale of grey and flattened areas that follow the salient relief of the microtopography (as observed with the 3D function) with the same orientation as the striae, informing on the direction of the gesture.

Starch Residue Analysis (Tables 4-5)

The samples were processed in two different labs and starch grains extracted were identified according to the analysts' individual criteria (Pearsall et al. 2004; Liu et al. 2013; Mariotti Lippi et al. 2015), used by the authors in other studies (Cagnato and Ponce 2017; Skakun et al. 2019; Skakun et al. 2020). Starch grains were analysed for the morphological (VLM/OM and SEM) and physico-chemical characterization. However, not all the samples underwent the same analysis. FTIR was applied to samples from all the sites, while pivotal ToF-SIMS was essayed on Surein 1 samples, and IRMS was attempted on those from Brînzeni I.

Brînzeni I. The large grinding stone is broken, and we were able to refit two fragments (others are still missing): both yielded starch grains. More specifically, the large fragment (#442, sampled area 2x6 cm) had nine grains (N=9) along with a starchy mass, while the small fragment (no accession number) contained three elements; two grains along with a starch mass (in the sampled area of 2x6 cm).

Moreover, one pestle was refitted from two broken fragments (#2965 and #833). The pestle (small fragment, #2965) yielded a larger number of starch grains, N=29, in addition to a starchy mass (Figure 6(a–b)). Five of these grains have been identified as belonging to the Triticeae tribe in the Poaceae family (Figure 6(c–d)). The remaining starch grains could not be identified further by the sole use of optical microscopy, while others were extremely damaged (broken edges, deformed, extinction cross no longer visible for two of these). From sample #833 (pestle, large fragment), nine grains (N=9) were recovered, including a very damaged one.

From the flat limestone pebble used as a grinding stone (#3539, from a 2x3 cm sampled area), a total of six starch grains (N=6) were recovered. The smallest of these grains measures 17 × 13 µm and is polyhedral. Four of the grains are rounded, and measure between 15 and 20 µm in width. The last one is slightly larger (25 × 23 µm) and is polyhedral in shape (similar to what Mariotti Lippi et al. 2015 identified as morphotype I). Five of the grains exhibit damage (circular and uneven depressions), for the most part affecting the central portion where the hilum is located (Figure 6(e–f)). This central damage has also been observed on numerous starches recovered from the other tools from Brînzeni I. Finally, two starchy masses were recovered from this sample (Figure 6(g—h)). The large slab used as a grinding stone (#6707, from a sampled area of 2x3 cm) was also rich in starch grains (N = 21). For the most part, these grains were damaged, with broken or missing edges, crushed, and sometimes with faint extinction crosses, which might suggest different types of mechanical or processing activities, but could alternatively be the result of taphonomic processes. On average, they measure 22 µm in length. Based on the extensive damage, it is not possible to identify the majority of these further. One of these grains, measuring 33 × 24 µm, may belong to a member of the Triticeae tribe (Figure 6 (i–j)). One very large grain (60 × 55 µm) with damaged edges probably belongs to a USO (Figure 6(k–l)), but it could belong to a very swollen grain (the extinction cross is almost absent). Two grains in this same sample have a hilum that projects outwards, creating a darkened centre (Figure 6(m–n)): this type of damage is typical of roasting (Babot 2006) and popping (at least in certain taxa; CC personal observations, and see Mariotti Lippi et al. 2015). Finally, the broken large pebble (#177), probably used as a pestle, yielded 15 grains, while nine were identified on the other pebble from square Q 11 j (GST with no #). Some of the grains recall those retrieved in calculi from Mesolithic burials in the central Balkans (Cristiani 2016).

Kostenki. K 14 (#35 from layer III) is a small grinding stone which yielded (sampled area 3x4 cm) four starch grains, attributable to both USOs and ASOs (Figure 6(o-p)). The fragmented pestle from K16 (#1308) yielded a larger sample (N=15) suggesting its involvement in the transformation of possible Triticeae (type 1) and tubers or rhizomes (type 2 and type 4, Figure 6(q–r)). Regardless of their shape and dimensions, the starch grains are broken and show cracks of different intensity in the center. Lamellae are always visible, while the Maltese cross is in some cases partially lost. Although there were a limited number of grains observed with VLM/OM, other starch grains were spotted on the molds when observed under the SEM at varying magnifications (Figure 6(s–u)). These grains clearly present lamellae and hila (Longo et al. 2020c, 2021c).

Surein 1. The large grinding stone yielded seven starch grains which can be divided into two groups. The first includes four polyhedral starch grains that range between 15 and 23 µm in size. The extinction

cross varies from (X) to (+) type and the arms are straight or curved. Their hilum is located in the center, rounded. Some granules exhibit radial cracks. Two starch granules have surface damage in the form of craters, similar to the result of an enzymatic attack. The other three grains, about 18-19 µm in size, have oval-like forms, but could not be rotated to determine their precise shape. The extinction cross varies from (X) to (+) type and the arms are straight and curved. Their hilum is located in the center, with a rounded
outline. Two of them have lamellae and one show a transverse crack. The starch grains from Surein I appear evidently crushed or broken. Other plant remains, such as raphides, parenchyma, and phytoliths, were also recovered from this object and are further detailed in Birarda et al. (2020). Starch grain analysis is conducive to interpreting starch grains as pertaining to three to four different geophytes (Longo et al. 2021c).

Table 4. Samples analyzed for starch presence and type. In total, we report on nine percussive tools. The results reported here were obtained according to conventional VLM/OM observation. It should be noted that SEM inspection allows for the detection of more starch grains.

Brînzeni I In total 8 samples belonging to 6 percussive tools * pestle in two fragments ** large GST composed of two fragments (other fragment is still missing)										
Stone tools	**#833***	**#2965***		**#442****		**no #****	**Q 11 j # --**	**#177**	**#3539**	**#6707**
Laboratories Vladivostok (A) Paris (B)	A	A	B	A	B	A	A	A	B	B
N. Analysed Starch	9	26	3	8	Few	3	9	15	6	21
Recurring Type	Prevalent Types 3-4	Types 1-5		Types 1-3		Types 4, 6, NI	Prevalent Types 6-8, NI	Types 3, 6-7, NI		
Condition			Starch mass		Damaged				Starch masses	Damaged, Crushed
Total Starch N	9	29		8+		3	9	15	6	21

Table 5. Samples analyzed for starch presence and type. In total, we report on nine percussive tools. The results reported here were obtained according to conventional VLM observation. It should be noted that SEM inspection allows for the detection of more starch grains.

Site	Kostenki 14 (Markina Gora) K14-III-11-k33- 214-224 #35 (grinding stone)	Kostenki 16 (Uglianka) #1308 (pestle)	Surein 1 Large grinder
Laboratories Vladivostok (A) Paris (B)	A	A	A
Total starch N	4	15	7
Recurring Type (A)	T1, 3, 6	T3, 4, 6, 7	T3, 4
Damaged		2	-
NI		1	1
Plant tissue	+	+	+
Plant fibre	+	+	-
Hair/Feather	-	-	-

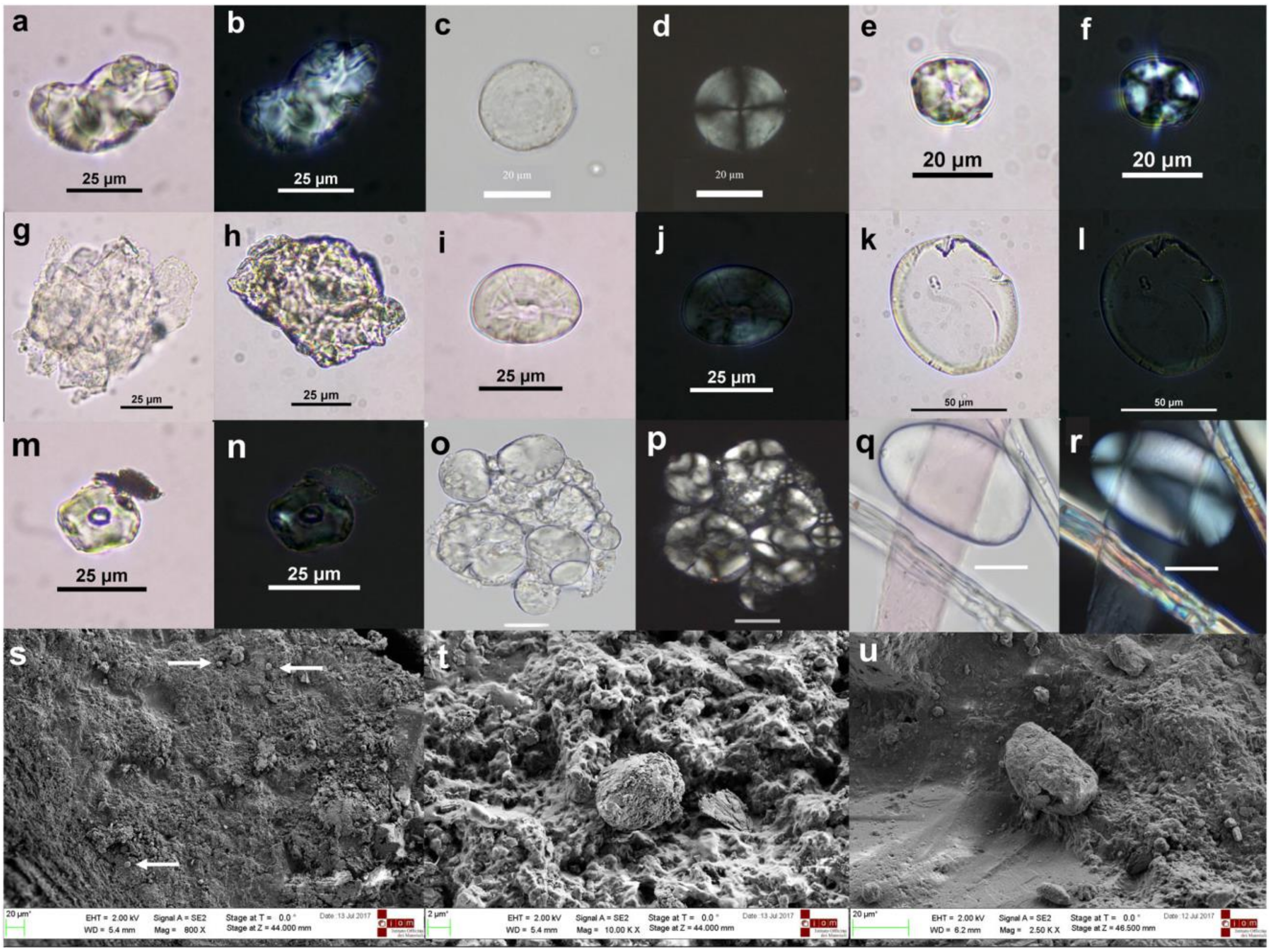


Figure 6. Selection of starch grains recovered on GSTs from Brînzeni 1 and Kostenski, K 14 and K 16. Brînzeni 1: a–b: #2965, starchy mass and c–d: Triticeae tribe starch grain; e–f: #3539, damaged starch grain and g-h: starchy masses; i–j: #6707, Triticeae tribe starch grain, k–l: possible starch grain belonging to a damaged USO, and m–n: damaged starch grain. Kostenski 14 #35 (layer III): o–p: Triticeae tribe type cluster (scale 20 um); q–r: starch grain likely belonging to a USO, a fibre can also be seen (scale 20 um). s–u: SEM images of starch grains from K 14 adhering to the molds observed at different scales s: 800x, arrows show location of some starch grains; t: 10000x u: 25000x. (OM photos I. P. and C.C.; SEM by L.L.).

In 2017, pivotal ToF-SIMS was applied for the very first time to samples from Surein 1 and Brînzeni I with the aim of studying the surface chemistry of ancient starch grains. In these experiments, fragments of polyvinyl siloxane molds with evidence of material adhering to the inner surface of the mold were selected, the working hypothesis being that they might have peeled off the stone surface a mixture of ancient starch grains, inorganic soil remains, and other biological material such as parenchymal tissue, fungal hyphae, and bacteria. The presence of starch grains was preliminarily verified by SEM inspection. To promote the solubilization of the structural fatty acids in starch grains (Baldwin et al. 1997), the collected material was placed in a 3 mL vial with reagent-grade EtOH and sonicated for 15 min. The supernatant was then drop-casted onto a silicon wafer and finally subjected to ToF-SIMS analysis. The sample obtained by this procedure entails further dilution of the finger-grease or handling residuals which could have eventually been captured by the mold as a result of the previous stages of pebble handling.

In Figure 7 the negative mass spectrum shows the signals of fatty acids - deprotonated palmitic and stearic acids - at high mass values, originating from the sample holder surface covered by the dried extracted residuals. The natural source of these signals is not fully assessable as the extraction in ethanol was carried out on the entire residual material, including any possible contaminants as well as parenchyma. However, the intensity of the signals allows us to exclude finger-grease contamination which would not have been so intense. The presence of other unassigned peaks is also reported. In Figure 8 the distribution of both fatty acids and unknown substances on the sample holder surface can be appreciated.

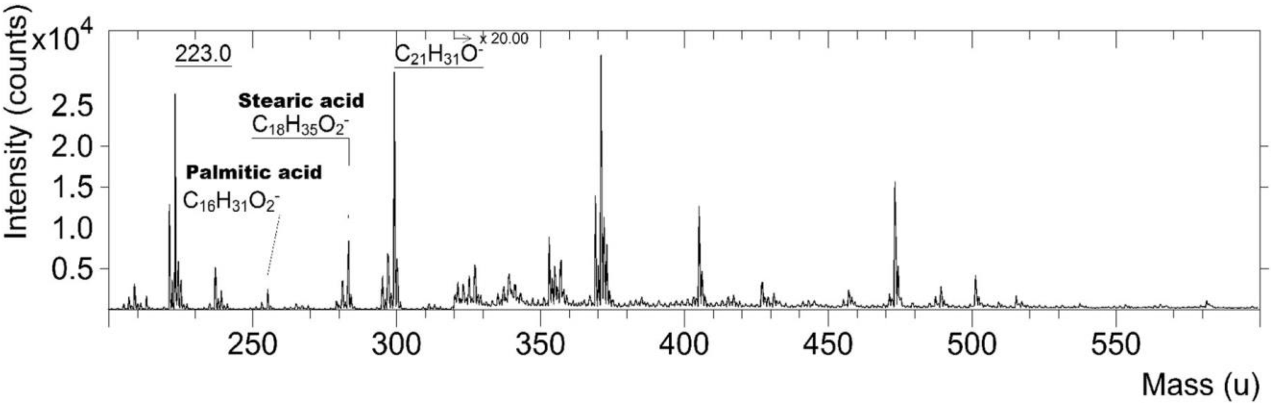


Figure 7. Portion of ToF-SIMS mass spectrum of ethanol soluble components coming from the extraction procedure.

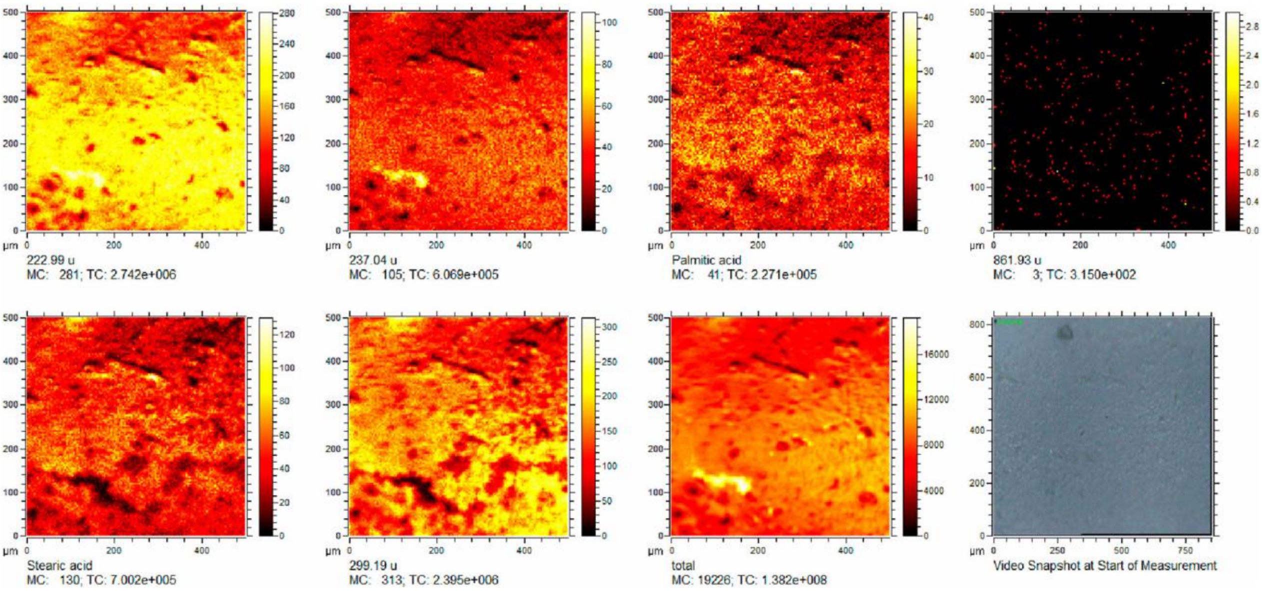


Figure 8. ToF-SIMS images of fatty acid ions obtained from the extraction procedure.

Discussion

Dietary carbohydrates - mainly starch - are contained in the amyloplasts of a plant's storage organs. This principal energetic reservoir is concentrated in underground storage organs (USOs), namely tubers and rhizomes' fruits and seeds (ASOs). Mechanical transformation, by means of pounding and grinding tools, greatly enhanced the tenderization of these starch-rich storage organs and helped to obtain a sort of flour that could be mixed with water and eventually further thermally treated. This complex processing increases starch bioavailability to salivary amylase breakdown during the oral phase of digestion (Babbitt et al. 2011; Butterworth 2011). Starch grains have only recently emerged as relevant sources of energetic food well before crops were domesticated, hence their intentional processing is key to the understanding of plant consumption and even for other uses (Hardy 2010; Revedin et al. 2010). However, demonstrating the intentional processing of plants during the early phase of modern humans' colonization of the Pontic steppe is not straightforward and calls for complex diagnostic steps. First, the putative economic exploitation of plants has been assumed mostly on the basis of pollen and phytoliths, which represent the vegetation surrounding the sites, and are not necessarily indicative of the intentional use/consumption of vegetal resources. Also, plant remains are perishable and tend to be recovered in special circumstances (permafrost, waterlogged, desert-like conditions, carbonized, etc.). Finally, when it comes to intentional processing, most of the evidence regarding ground stones dates to post-LGM and Holocene contexts (Hamon 2006; Adams 2014b), whereas scant data on starch proces-sing are available for ground stones dating to the Palaeolithic (Revedin et al. 2010; Aranguren et al. 2015; Mariotti Lippi et al. 2015; Barton et al. 2018; Birarda et al. 2020) and Epipalaeolithic periods (Piperno et al. 2004; Cristiani and Zupancich 2021; Dubreuil and Nadel 2015; Liu et al. 2018; Skakun et al. 2020). Besides the difficulties mentioned above concerning the reconstruction of plants used by humans in their diet, we faced an additional hurdle as the great majority of the reference collections cover the range of plants typically assumed to be in use from the time crops were first domesticated (Zeder 2012; Liu et al. 2013; Zeder 2015). Therefore, one of the goals - and at the same time an outcome - of our research is to increase the starch grain reference collection by adding plants available in the Pontic steppe during MIS 3 and, more generally, at the northern latitudes during the colonization of the Pontic steppe by modern humans (Hardy 20 10; Blinnikov et al. 2011). Our results suggest that early waves of modern humans during their colonization of the boreal latitudes were already capable of transforming starchy plants into energy-rich foods.

Methodological Refinements and Technical Enhancements

Since plants are liable to decompose, a first challenge posed by the present investigative approach is achieving sufficient methodological and instrumental sensitivity and resolution for starch identification and characterization. In documenting starch grains, the identification of which, to date, relies mainly on morphological analysis (OM/VLM, Piperno et al. 2004; Cristiani 2016), but the use of light microscopy limits the resolution and proved to be problematic. Starch characterization is generally based on the shape and size of the grains: the grouping of the grains into types is done according to the discretion of the specialist based on criteria referring to grain morphology, size, shape of the extinction cross, position of the hilum, lamellae, folds, etc. according to ICSN (ICSN 2011; Liu et al. 2013; Mariotti Lippi et al. 2015; Skakun et al. 2019). This methodology, including sampling techniques, has already raised issues regarding the reliability of the results, since the presence of contaminants of any kind (as a result of fieldwork, collection management, and laboratory procedures) cannot be completely ruled out (Crowther et al. 2014; Dozier 2016; Ma et al. 2017; Mercader et al. 2018). Considering the age (MIS 3), the conditions of retrieval and the curation of the ground stones under investigation, light microscopy alone is not a failsafe method for identifying starches and their putative transformations across time or to distinguish ancient from modern contaminating starch grains (Crowther et al. 2014; Mercader et al. 2018). In order to overcome this limitation, our multi-dimensional integrated approach conveys refined procedures established from the beginning of the research: the selection and sampling of the ground stones and the starch extraction, the involvement of different laboratories for the processing of the samples, the systematic use of SEM to visualize features of the starch grain, to which other molecular analysis could then be further applied, and the coherent inclusion in the reference collection of taxa which could have been collected and processed in cold and northern latitudes.

A second challenge is represented by the age of the investigated sites. The mainstream narrative for Palaeolithic economies does not consider that percussive tools were used for processing plants, and as a result many candidate grinding stones and pestles were not collected during the excavations (Gravel - Miguel et al. 2017); hence it is safe to say that the entire record of EUP sites is biased and lacking in these types of tools (De Baune 1997). During our survey we were positively surprised by the quantity of percussive tools - available in the assemblages from Russian EUP sites located across the Eurasian Pontic Steppe - bearing clear traces of their intentional use. We argue that Late Pleistocene dwellers used ground stones to transform ASOs and USOs, the latter more likely to have been available in MIS 3 climatic conditions at boreal latitudes.

The high number of retrieved ground stones, their conservation conditions (in dark places and store-rooms, below 0°C for most of the year), the study conditions, and the informative object biography reconstructed by accessing the field notes and the museum documents revealed that even these 'legacy' objects are suitable for investigation with our multidimensional approach. Therefore, another outcome of our study is that stone tool biographies have become a relevant piece of information to be taken into consideration along with the laboratory preparation/conditions for compelling starch grain analysis. Acknowledging the relevance of the immense archaeological record preserved in museums, we devoted a paper to the role played by stone tool biographies, which drove the selection process and the validation to apply suitable STEM techniques to analyze ancient starch grains (Longo et al. 2021b). We are confident that the synergistic results of wear-traces analysis on the selected ground stones and the morpho-chemical characterization of the associated starch grains can be considered as actual evidence for starchy plant processing during the EUP.

Critical Evaluation of the Collected Data

U-RBRs extracted from the nine ground stones were investigated through conventional approaches, i.e. visual light microscopy with polarization for their morphological identification *(OM/VLM,* Torrence and Barton 2006; Revedin et al. 2015), and characterized with other techniques ranging from SEM to spectroscopy (Birarda et al. 2020 and this paper). The results obtained by the two laboratories that prepared and observed the starches (Vladivostok and Nanterre) were very consistent, and in the case of Moldova, greatly overlap. Considering the observed morpho-types, it is likely that several different species of starchy plants were mechanically processed with the studied percussive tools. Data from Brînzeni I show the highest diversity compared to that obtained from Surein and Kostenki, whose grains are far less numerous. However, this might be due to the number of tools investigated because the larger selection corresponds to a higher number of percussive tools retrieved in the Moldovan site. Although skepticism might remain in the community over the idea that even if two separate labs were processing the same 'contaminated' samples the results may turn out equally biased, we stress the limited number of grains retrieved and their damaged features (i.e. granule splitting), with uncharacteristic cracks (for modern starch), and the loss of the extinction cross (Maltese cross). Additional evidence for the presence of genuine starch grains is their association in clumps and with parenchyma fragments (vascular and epithelial tissues), and also with raphides and plant fibers, an ensemble of features which are not typical of modern commercial! industrially processed starch. Hence, visually, the state of the grains supports their antiquity. The contextual presence of different plant-related remains and other use-related biogenic residues has already been observed by other scholars (Hardy et al. 2001; Pearsall 2015), and the observed features are here interpreted as resulting from intentional pounding and grinding of the plant organs and unlikely to be the result of contamination. The pollen analysis from the Kostenki-Borshevo suite (Velichko et al. 2009) identified the presence of Triticeae (ASOs) and USOs such as cattail and anemone, and also ferns, which supports ancient processing of such taxa. Previous analysis on another pestle from K 16 attributed the presence of several starch grains to *Lactuca tuberosa,* cattail, and burdock *(Arctium lappa)* (Aranguren et al. 2015). Interestingly, this taxon is very rich in insulin, supplying a sweet taste even for the spoiled modern receptors, used to sugar - as experienced by IP - and might have played a role in feeding infants high-calorie foods, before they could consume more nutritional foods. It would thus appear that grasses, sedges, and ruderal species were collected together with species from marshy areas surrounding the open-air settlements, brought back to the campsites and finally tenderized by means of ground stones, demonstrating the exploitation of a complex food web starting around 36-32 kyr BP. However, not all starch-rich organs necessitate processing and many berries or fruits available in the Pontic steppe may have been eaten raw and therefore remain outside the resolution of this analysis.

We observed that SEM resolution increased the number of visible starch grains (mostly for those <30 μm),), but also evidenced other U-RBRs adhering to the molds (i.e. fibers and raphides, Hardy et al. 2020), which had a deep peel-off effect and extracted the more deeply entrapped plant microremains. Besides, this effect was observed on the molds obtained from all the used areas of the ground stones but not on the molds or the sonication from non-used areas. Given the small number of starch grains usually recognized by OMIVLM, the SEM increased the record of identified grains, even when ranging <30 μm), and also their variety. We argue that these are further elements to identify starches less likely to be the result of contamination.

The FTIR imaging provided the chemo-profiling of the different starch samples prepared in Vladivostok and in Nanterre, data that can also be recovered with the ToF-SIMS. Moreover, infrared spectra can be used to determine modification or aging processes undergone by the ASCs, like oxidation or mineralization. The coupling of morphological characterization techniques, namely OM and SEM associated with the chemical information of FTIR, allowed for a complete and positive identification of the inspected starches by using the typical vibrational signals of saccharides, which, to our knowledge, has never been attempted before on archaeological starches. This technique proved to be useful in obtaining spectra that allows for discrimination between different carbohydrates and provided elements to distinguish true archaeological starch from those derived from putative contemporary contamination. On the other hand, ToF-SIMS can detect fatty acids composing archaeological starch grains and therefore has the potential to be highly informative on the taxa supplying short and long fatty acids if supported by a dedicated reference collection.

Plants can be sources of vital nutrients such as dietary carbohydrates and fatty acids. Preliminary ToF-SIMS results obtained on the starch grains from the Surein 1 sample show the presence of fatty acids at high mass values (deprotonated palmitic and stearic acids), providing one more line of evidence for the transformation of plant foods by means of ground stones. It will be of utmost importance in our future research to be able to distinguish between the source of the fatty acids supplied through the diet (either plant or animal based) of *Homo sapiens,* as the duplication of the genes involved in their efficient metabolism in ancient genomes suggests the potential for archaic humans (i.e. Denisovans and Neandertals) to transform short chain fatty acids (SC-FA) into long chain fatty acids (LC-FA). This duplication might have been crucial for the development and the functioning of the human brain and to maintain homeostasis. Today, Inuit populations present the same duplication, and they mostly feed on animal fats, a similar feeding behavior to that of Neandertals (Ameur et al. 2012; Fumagalli et al. 2015). The rest of the modern human population can source polyunsaturated fatty acids by efficiently metabolizing ARA (arachidonic acid n-6) from plant food supplements - seeds, nuts, mosses, ferns, *Chenopodium* and *Artemisia* - or EPA (eicosapentaenoic acid n-3) of animal origin. Buckley et al. (2017) proposed that plant-derived fatty acids may be interpreted as an adaptation resulting from the spread of agriculture. We argue that the capacity to efficiently metabolize plants was developed well before crop domestication and may have played a crucial role in the success of *Homo sapiens.* Although our analyses are still at a preliminary stage, it will be highly informative to cross-check the genetic pattern encoding for starch and fatty acid metabolism and the other food sources available to both archaic humans and *Homo sapiens* in our targeted area (Vicedomini et al. 2021).

In addition, the IRMS technique has given its first indications of the type of plant material present in the residues (C_3 or C_4). At the same time, it highlighted the problems associated with sampling - the resulting amount of sample after the extraction was below the limit of quantification for the instrument. We have, therefore, to develop a more suitable sampling strategy devoted to the isotopic study, as this methodology promises to disclose plants with photosynthetic pathways coherent with conditions typical of cold northern latitudes.

The isotopic analysis of the biogenic residues, combined with the other types of investigation, can be useful to reconstruct the types of plants used for human nutrition, in relation to the availability of the species in the context of the Eurasian steppe during the investigated period. The information obtained can be relevant in terms of understanding the nutrient content of a plant (carbohydrates, fatty acids, and proteins) as well as their digestibility. Additionally, the residues collected from the grinding stones (preliminary analysis herein presented refers to Brînzeni I cave in Moldova) also allow us to create a reference database by measuring the $\delta^{13}C$ and the $\delta^{13}N$ of some vegetal species that can be expected to have been present at the site during the EUP.

The very preliminary carbon isotopic ratios measured on the starch grains from sample #6707 showed a prevalence of C_4 plants. On the other hand, the $\delta^{13}C$ in other samples suggests the absence of plant material or possible contamination, a bias resulting from excavation and conservation procedures (stones were washed), as well as to the sampling strategy (carbonate material from stones could have been mixed with biogenic residues). These conditions might be the case for the samples less rich in starch grains. The samples used for IRMS analyses were collected by simply scraping some sediment still adhering to the stone. The quality and the efficiency of the sampling is key, thus future measurements will be carried out on samples obtained through sonication. The limited amount of starch would explain the $\delta^{13}C$ of carbonates with values near to zero per mil, which could affect the isotopic signal of the organic material. In this view, for samples showing carbon isotopic signals near to zero, we might exclude the presence of vegetal material. Given the unprecedented design of our study, sampling and preparation procedures proved to be problematic, being limited by the lack of soil from the same stratigraphic units for comparison. In addition, the starches collected from the stones were not sufficient to measure nitrogen isotopic ratios, because vegetal samples have a very high carbon content with respect to nitrogen, which is more difficult to detect, especially in very old and degraded material and according to the instrument's sensitivity. Despite all the difficulties, the preliminary $\delta^{13}C$ results obtained from the pestles and from the GST #6707 suggest the prevalence of possible C_4 plants with respect to C_3 plants. Regarding the *compound-specific isotope* analysis, the methodology is in development and even in this case the limiting factor is the low quantity of starch-candidate materials extracted from the grinding stones. The typical procedure involves the extraction of the lipid component from the original samples, using specific solvents, and subsequent chemical preparation (derivatization) of the compounds of interest (Stott et al. 2003). Therefore, low masses of a residue sample risk rendering the chemical phases inefficient. Again, this methodology will be applied to both the scraped and sonicated samples from Brînzeni I.

Finally, the starch grains were compared with a modern reference dataset composed of over 230 taxa comprising both USOs and ASOs, which, we are aware, may not fully represent those starchy plants most likely in use during Late MIS 3 at boreal latitudes. To narrow this gap, we are building a reasoned reference collection of native modern plants reported at Late Pleistocene sites along boreal latitudes. The plants are targeted according to the pollen lists, the macrobotanical remains found in the guts of fauna (Arslanov et al. 1980; Kosintsev et al. 2019; Ukraintseva 2002), and from starch grain lists (Hardy 2010; Magyari et al. 2014; Kovarnik and Benes 2018). Hence, compiling a coherent reference collection of plants available at the northern latitudes during MIS 3, building an image database of starch grains viewed under MO and SEM associated with their related spectra obtained during physico-chemical characterization, is an urgent matter. Our integrated methodology will allow us to build a reasonable list of taxa based not only on morphological comparison but also on the criteria proposed by chemotaxonomy (Jardine et al. 2019). The reference collection will be continuously updated with new taxa, but also with plants collected at different maturation stages, during different seasons, from different parts of the same plant, and from different geographical areas. Starches from industrial foods or flours will also be included for contamination comparison.

While all primates living in intertropical regions rely on plants in their diet, they differ according to their natural environment. To make better sense of the evolutionary importance of a starch-based diet and the environmental history of dietary carbohydrates available to humans, it is important to stress that *Homo sapiens* is a tropical species that evolved in Africa around 300,000 years BP (Scerri et al. 2018). During the Middle Pleistocene, Africa underwent climatic changes as suggested by isotopes studies, facing an overall shift towards C_4 and CAM (Crassulacean Acid Metabolism) plants. African C_4 plants include tropical savanna grasses, sedges (Cyperaceae), and other monocotyledons, while CAM plants include succulents that are more typical of deserts (Katzenberg 2008; Sponheimer et al. 2013). These climatic fluctuations continued during the Middle to Late Pleistocene, meaning that hominins moved back and forth from woodland tree and shrub environments towards more open and drier nutriotopes (nutritional environments), where they possibly increased their intake of savanna grassland resources (i.e. USOs). As a result, out of this patchwork of environmental pressures and hominins mingling and mating, *Homo sapiens* emerged in Africa with a genetic make-up and technological/behavioral skills predisposed to resources diversity and with dietary flexibility, possibly consuming a wide range of plant foods from both C_4 and C_3 sources. When *Homo sapiens* finally reached the Pontic steppe in the Boreal Hemisphere during MIS 3, the region was already colonized by archaic humans, Neandertals and Denisovans (Fu 2016), who had evolved in the northern latitudes of Eurasia, in a much colder environment. Therefore, it is crucial to our investigation to identify the stable isotopic signature of the plants available in the Eurasian steppe during the period known as Out of Africa 2, in order to identify those plants putatively tenderized by means of ground stones found in EUP sites.

Final Remarks and Future Perspectives

We cannot stress enough our awareness regarding the risk of contamination, which can affect the results, and which cannot be completely excluded in any archaeological study. The integrated multi-scale approach presented here was applied for the first time to the study of ground stones from EUP sites across the Pontic steppe, with the aim of extracting and maximizing the types of information that can be acquired from coupling wear-traces and starch grain analyses. Our research question - was starchy food processed by means of ground stones during the EUP? - drove the selection of the methodologies and techniques that were applied. Our approach was demonstrated to be worthwhile in identifying ground stones among a selection of pebbles, and characterizing archaeological starch still adhering to their functionally active areas.

For the starch grain characterization, both optical and SEM microscopy revealed to be crucial to determine morphology, while the latter was key when nano-techniques were called into play, as in the case of FTIR spectroscopy. The systematic application of this set of procedures improved the assessing the authenticity of the starch grains to a certain extent.

As a general consideration, sampling turned out to be a crucial issue that should be carefully planned and duly detailed in the report, a compulsory procedure worth to address the different requirements set by each analytical technique involving the nanoscale. The state of preservation of the items - with the assessment of the tool's biography - and the steps devoted to U-RBR extraction require appropriate sampling procedures to avoid contamination and to obtain concentrated amounts of starch grains suitable for nano-scale analysis. For all these reasons, this pivotal study proved challenging but provided important clues to develop a new procedure for the extraction of biogenic residues from ground stones, specifically for the molecular analysis to be successfully applied even to 'legacy' objects from museum collections.

Our multi-dimensional integrated contextual approach can provide information about the elusive vegetal resources entering the dietary strategies of early *Homo sapiens* in Eurasia. Future studies will provide the building blocks to understand how the efficient exploitation of a new nutriotope - the starchy food web - led to modern humans becoming the main player. Our approach represents an important step in trying to bridge the gap between the humanities and the hard sciences by applying sophisticated heuristics in order to unfold the complexity of dietary habits at the dawn of modern humans colonizing Eurasia.

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No potential conflict of interest was reported by the author (s).

Notes on contributors

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