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# Aurignacian Grinding Stone from Surein I (Crimea): "trace-ing" the roots of starch-based diet

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## Abstract

This study is applied to the unique grinding stone from Surein I retrieved in the Aurignacian layers of the rockshelter located in the south of the Crimea Peninsula. Our research enables us (i) to make reliable inference on the agency establishing the functional modification on the surface of the Surein I grinding stone, (ii) to demonstrate this grinding stone served as steady surface (Face A) to mechanically process plant material including roots and tubers (under surface storage organs, USOs) and (iii) to set a chrono-cultural framework for starchy plant tenderization, also responding to key issues relating to the dietary breadth of early waves of *Homo sapiens* at the northern latitudes. We present a pilot research design which integrates data derived from macro and micro-scales techniques, by coupling use-wear traces analysis and use-related starch granules observation. The multi-scale approach allows distinctive resolutions for surface texture analysis thanks to the combined use of stereo, metallographic and digital microscopes; whereas transmitted and polarized light microscopes were used to observe use-related biogenic residues (U-RBR), namely starch granules, and SEM provided resolution down to the nano-scale. Our data suggest that *Homo sapiens* was exploiting the rich environment of the Pontic steppe-grassland since its earliest presence in south-eastern Europe by processing starchy plants to obtain calorific food. Moreover, this study brings fresh lines of evidence to the broadening of dietary strategies during late MIS 3 (40-25 ka calBP) by

analysing ground stones from the poorly investigated non-flaked industry, and opens new scenarios for the reasoning on *Homo sapiens* successful colonization of Eurasia.

**Keywords:** Wear-traces, Microscopy, Resolution, Methodological refinement, Use-Related Biogenic Residues (U-RBR), Starch

## Highlights

- Methodological refinement for functional analysis of Palaeolithic ground stones
- Optical light and scanning electron microscopy applied for surface texture and starch analysis
- Mapping of the functional related features
- Surein I (Crimea) is the oldest direct evidence of grinding stone used to process USOs
- *Homo sapiens* dietary flexibility and adaptation to Pontic steppe plant resources

## 1. Introduction

Within the broad assemblage of non-flaked industry, Palaeolithic ground stones represent an under investigated source of data on different materials' transformation among which plants, listed in the perishable edibles, are highly informative to reconstruct dietary strategies. The term ground stones (macrolithic tools) is comprehensive of a multitude of stone objects '*which were manufactured and/or used according to motions*' (Dubreuil et al. 2015 p. 106). In the case of Early Upper Palaeolithic items, we are referring to those '*not altered from their natural rock shape*' (Adams, 2014 p. 15) used in both passive and active motions (Ebeling and Rowan 2004, p. 108), such as lower steady grinding stone (i.e. Surein I presented here) and movable items as pestles as we already applied in our previous work on Gravettian: the terms grinding stone and pestles were used with reference to grinding and pounding activities before crop domestication (Longo et al., 2018; Revedin et al., 2010; 2015). The present research focuses on a task-specific tool used to mechanically transform plant starch-rich storage organs (PSRSO) during early Upper Palaeolithic, when seedy cereals - to which processing is referred most of the literature - were unlikely to be available. We evaluated that wear-traces and starch granules extracted from ground stones may provide reliable information about plant foraging and the mechanical processing aimed specifically at starch extraction from the under and above surface storage organs (USOs and ASOs, Longo, 2016; Longo et al., 2018; Revedin et al., 2010; 2015). We are fully aware that PSRSO processing cannot be considered only as a technological/cultural phenomenon as it is intimately connected with other biological and behavioural adaptations (Butterworth et al., 2016; Perry et al., 2015; Longo, 2016), determining interactions that require complex yet careful analysis: a challenge that goes beyond the scope of this work (Longo, 2016; Longo et al., 2020a). Here, our main objective is to present the methodological refinements implemented in order to

investigate a large slab from Surein I (Crimea), aiming to locate and directly relate the active surface of the ground stone with the adhering starch granules, by ensuring the provenience of the U-RBR from the intentionally used areas across the large steady stone. This was obtained by sampling the residues directly from the stone and also from the mold that revealed the deep peel-off effect (see 3.5, 3.6 Fig. 9), a procedure that, to the authors knowledge, represent an original contribution to both functional and residues analysis. An attempt to identify the functionally active areas was already performed by roughness analysis on the 3D model (Next Engine, Longo et al. 2018). Moreover, the mapping of the wear-traces provided insights for the spatial distribution of the utilized areas and the main direction of use.

The project "*Unfolding the complexity of nutrition at the dawn of modern humans in Eurasia*" started in 2016 (L.L. SUG, Singapore), already surveyed and sampled twenty sites dating back to Late Pleistocene - Initial/Early Upper Palaeolithic (EUP) and Upper Palaeolithic (UP), spanning western Eurasia to Central Asia (from Moldova to Crimea, to the Don river reaching the Altai Mountains, in Siberia). Our data disclosed the processing of PSRSO by means of ground stones since around 40 ka, possibly starting with the very early waves of *Homo sapiens* venturing out of Africa towards boreal western Eurasia.

The experience acquired during our previous studies on palaeolithic ground stones supports the development of a multistep analysis at macro and micro scale for the surface texture modifications of the limestone slab, and down to the nanoscale for the starch granules (1-100 µm). Although surface texture is difficult to define, among tribological studies there is a general agreement in considering it as the "*the features of surface relief*" (Myshkin and Grigoriev, 2013). Texture analysis of the grinding stone from Surein I displayed evidence of man-induced surface modification, allowing to interpret its possible uses, amongst which the very likely processing of PSRSO.

Our new procedures have proved to be useful to acquire relevant data supporting strong evidence of plant processing in the Pontic steppe since the Aurignacian. Here, we present the pilot study on the grinding stone from the Aurignacian layer 3, the lowermost according to 1926-29 excavation carried out by G. A. Bonch-Osmolovsky at Surein I rockshelter (Crimea) (Fig. 1a-b). The Crimea peninsula is recognized for the richness of its Late Middle/and Early Upper Palaeolithic record represented by both behavioural and biological remains referable to Neandertal man (Kiik Koba, Ak-Kaya, Zaskalnaya VI, Stepanchuk et al., 2017) and to *Homo sapiens* (Buran Kaya III burials, Pratt et al., 2011, and a molar tooth from Surein I unfortunately lost as reported in Chabai et al., 2004, p. 56). The two species lived in the same environment, therefore were exposed to the same range of available food sources, although isotopic data support a diversified access to food sources by the two species (Drucker et al., 2017).



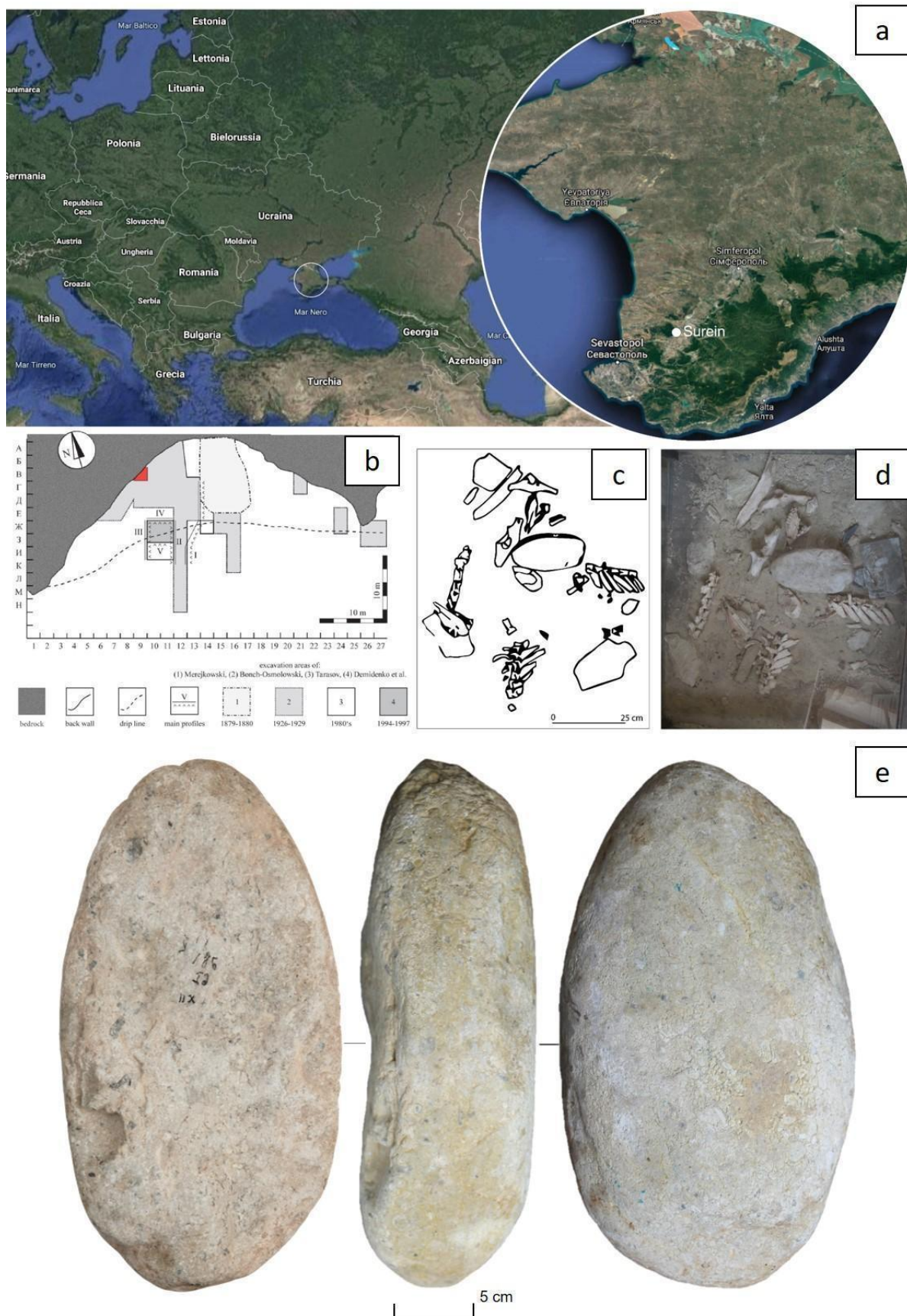


Fig. 1. a) The Surein I rockshelter is located on the southern rim of the Crimea Peninsula (Pontic Steppe); b) Map of Surein I rockshelter (modified from Bataille, 2016, Fig. 3: 53). In red, square 9B where the grinding stone was found; c) Drawing of the “structure” retrieved in square 9B, where the grinding stone is reported according to E.A.

Vekilova (1957) in Fig. 34: 307; d) the restaging at MAE RAS, St. Petersburg (photo L. Longo, June 1991). The exhibit is based on the information published by Vekilova. Surein I grinding stone is clearly visible with the water-weathered dorsal face exposed, surrounded by fauna remains still in anatomic connection. In plain black, flint artefacts; e) Surein I grinding stone: left, the unused surface (Face B); middle, the side; right, the coarse used surface (Face A).

Until recently, the study of the lithic assemblages mainly revolved around very detailed analysis of the flaked industry. Despite the numerous efforts based on this approach, the study of the material culture failed to clarify the conundrum of the so-called “transitional industry” and to pinpoint the actual dwellers of the EUP layers that have not, as yet, been identified unequivocally (Bataille et al., 2016, p. 53; Demidenko, 2014a).

The study of the Palaeolithic non-flaked industry might bring new and unexplored information regarding the dietary habits of the hominins roaming in the refugia of Crimean Peninsula around 40,000 years ago. The analysis of the grinding stone involved a methodological refinement which includes the use of different resolution microscopes such as stereomicroscope, reflected and transmitted light (IHMC, RAS), digital microscopy (hereon DM) and electron scanning microscope, SEM, covering wear-traces and U-RBR, namely starch grains. Bulky macro tools are difficult to examine under the standard structural designs of most conventional light microscopes. We decided to use digital microscopes (Hirox and Keyence) to scan large experimental stones, molds and araldite positives. Starch analysis was then carried out according to established procedures at IHAЕ FEBRAS (Vladivostok) using transmitted and polarized light and under SEM. We are confident that the methodological and technological refinements applied to the study of Surein I grinding stone have enabled optimal conditions for functional interpretation, which involves contextual different scales of resolution, impacting on our research outcomes. The investigation, aiming at identifying functional features supported by fine-grained data on the non-flaked stone tool from Surein I finally demonstrates this is a grinding stone that serves as a steady surface to mechanically process storage organs for modern human consumption.

## **2. The archaeological material**

The large rockshelter of Surein I in the Crimea Peninsula (alias Siuren or Suiren or Surein, according to different transliterations, Demidenko et al., 2012; Demidenko, 2014a; Bataille et al., 2018; Vekilova, 1957) is situated in the eastern slope of Bel'bek gorge, not far from Sebastopol, on the second ridge of the Crimean Mountains (Fig 1a). The rockshelter is 43 m long, 15 m deep and 9-10 m high (Fig 1b), and is located 110 m above the sea level and 15-17 m above a small creek flowing nearby. K.S. Merezhkovski (alias Merejkowski and Ruev, 2018) in 1879-1880 excavated a first trench in the central part of the rockshelter and retrieved 1150 flint artefacts, not thoroughly published, conserved in part at the Historical Geology Chair in St. Petersburg University, and in part at the Department of Archaeology of the Peter the

Great Museum of Anthropology and Ethnography (Kunstkamera) of the Russian Academy of Sciences, St. Petersburg, Russia (hereinafter MAE RAS).

In 1926-1929 G.A. Bonch-Osmolovsky, continued excavations at Surein I and identified three cultural layers by pioneering cutting-edge methodologies as he carefully collected all the materials related to human activities and applied complex statistical analysis for the interpretation of the finds (Vasil'ev, 2008, pp. 25-27), not yet in use by contemporary western archaeologists. The excavated surface was divided into a grid (2 x 2 m) and Surein I stratigraphy was presented during the Second (proto-INQUA) Congress held in 1932 (Leningrad, Soviet Union). Describing the flint inventory from Surein I rockshelter, the Russian scholar attributed the findings to the western European Aurignacian complexes - the only comparisons available at the time - and dated Surein I horizons to the second half of the last Würm glaciation (Bonch-Osmolovsky, 1926; 1932). During 1926-29 excavation about 1000 tools were discovered in the bottom level together with a single molar tooth attributed to *Homo sapiens* (Chabai et al., 2004, p. 56; unfortunately, the molar is lost, at present). The blade industry includes scrapers, Busquet burins, small size hand axes, retouched blades, Mousterian scrapers. 30 years later, E.A. Vekilova (Vekilova, 1957) re-addressed the study of Surein I, comparing the materials with Trans-Caucasian assemblages (the Upper Palaeolithic of Georgia) at that time finally available for comparisons to the Russian scholars. More recently (1994-1997), a Ukrainian-Belgian team excavated the rockshelter intercepting the 1926-1929 trench and layers (F-G-H) obtaining the oldest absolute radiocarbon dating around 30 ka BP which are considered "*too young* [...] *and* *not represent the true age*" (Demidenko, 2014a; 2014b, p. 6720; Demidenko et al., 2012). The directly dated human bones at Buran Kaya III recently retrieved in layer C demonstrate the presence of *Homo sapiens* in Crimea as early as 31,900±240/-222 BP (Pratt et al., 2011) and further consolidate evidence for modern humans as the actual users of the Surein I grinding stone.

Whatever demic or emic model can be called into the play, the attribution of the lower layers of Surein I to the Aurignacian is still undisputed as demonstrated by the new excavation and the radiocarbon dating obtained in 1994-1997, endorsing the previous stratigraphy, although detailing 8 successive dwelling episodes (Bataille, 2016, Fig 2, p. 52). The oldest phases are confirmed as the Krems/Dufour type and attributed to (proto) Aurignacian, and the faunal composition is also similar to that retrieved in Bonch-Osmolovsky trench (Demidenko, 2014b).

The present paper focused on the large, oval-shaped, biogenic limestone grinding stone from Surein I (236x122x68 mm; 3477 g; Fig. 1e) unearthed during the 1926–29 excavations in the lowermost layer 3 of the rockshelter (corresponding to layer G of 1994-97 excavation, Bataille, 2016, Fig. 2, p. 52), and dated back to >31 ka uncal BP. The large

grinding stone was retrieved in an intriguing close relationship with horse remains, still in anatomical connection (Fig. 1c-d) and was immediately noticed during the excavation, photographed, mapped and drawn (Fig. 1b-c, adapted from Vekilova, 1957; Bataille, 2016, Fig. 3). It is worth mentioning the cutting-edge methodology applied by Bonch-Osmolovsky, who paid careful attention to the documentation with notes, photographs and drawings devoted to this exceptional context, unusual at the time the excavation was carried out. On the basis of the forerunning scientific approach applied by Bonch-Osmolovsky, a real innovator in those times, the whole area (about 1 m<sup>2</sup>) was philologically reconstructed in the museum (MAE RAS, St. Petersburg), where the structure is restaged under a glass cage (Fig. 1d), which avoid dust and other putative contaminations, and it is available for study. Such detailed documentation of the artefact biography represents a strong case to clearly assess the contextual association of the grinding stone with the flaked industry and the horse remains still in anatomical connection, all referred to the layer 3 (the layer G of 1994-1997 excavation) and attributed to the Aurignacian. In 1957 E.A. Vekilova duly published such well documented material and also reported functional remarks derived from S.A. Semenov observations on the flint assemblages.

The stone shows two very different faces (Fig 1e): one strongly weathered by water when immersed in the river water (Face B, which faced upwards when retrieved during the excavation), whereas the opposite (Face A, leaning on the soil) is still rough and it is the one that was certainly used, after its collection from the river. The research design applied a fresh multi-scale approach to investigate both wear-traces and starch granules extracted from the functionally active areas of the large grinding stone. Shape, size and general morphology were acquired by means of 3D scanning and photogrammetry, while the comparative surface texture analysis of the used and unused areas of both faces, was integrated with the morphological characterization of ancient “starch granules candidate” still adhering to the active areas, and carried out by means of microscopes with different resolutions (Birarda et al., 2020; Longo et al., 2020a).

### 3. The multi-scale contextual approach

For this study several microscopes with different resolution and magnification were involved (Tab. 1): Optical microscope (OM), Digital microscope (DM), Scanning Electron Microscope (SEM).

Model	Microscope	Magnification	Location
MBC-10	OM, Stereomicroscope	8.4-98 x	IHMC, St. Petersburg (Russia)



OLYMPUS BHMJ	OM Metallographic (DIC lenses) Canon EOS 400D camera Helicon Focus software	50-500 x	IHMC, St. Petersburg (Russia)
AXIO Scope A1	OM Transmitted/Polarized light	200-800 x	IHAЕ FEBRAS, Vladivostok (Russia)
Hirox KH-8700	3D Digital Microscope (DM)	35-2500 x (2D and 3D modality)	STARC, The Cyprus Institute, Nicosia, Cyprus
Keyence VHX 7000	3D Digital Microscope (DM)	35-2000 x (2D and 3D modality)	VCH Lab, ADM School, NTU, Singapore
Jeol JSM-6700F	Field Emission Gun (FEG) SEM	200-2000 x acceleration voltage (5 kV)	NTU, Singapore
Zeiss EVO 40	SEM with EDS OXFORD INCA energy	200-2000 x acceleration voltage (5 kV)	UniFi, Florence, Italy
Zeiss Supra 40	Field Emission Gun (FEG) SEM with Gemini column	800-25000 x low acceleration voltage (2 kV)	IOM-CNR, Elettra Sincrotrone, Basovizza, Italy

Tab. 1: microscopes involved in the study. Surein I grinding stone was directly investigated in St. Petersburg by means of optical microscopes (OM), and sonicated to extract the starch. The molds (imprints) and the araldite positives were analysed by means of DM in Cyprus and Singapore. Both molds and starch grains were analysed with SEM.

The study for attributing the function of Surein I grinding stone was carried out at different scales of resolution and magnification and with various methodologies based on tribological principles (Longo et al., 2018; 2020a; Birarda et al., 2020). Among them, surface texture can be considered as “*the features of surface relief*” (Myshkin and Grigoriev, 2013), and texture analysis is intended as the study of the deviation of the surface from a plane. This can be applied to both macro and microscale as texture reflects the appearance of distinctive surface patterns that can be analysed according to two main approaches: comparative and parametric. Comparative wear-traces detection is based on qualitative analysis which reflects analyst's experience and it is buffered by the resolution power and magnification of the inspection technology. The comparative approach is applied in this analysis based on “*the expert visual evaluation of the similarities*” between the observed tool and the experimental reference (Myshkin and Grigoriev, 2013) by applying conventional Optical Microscopy (stereo and reflected/metallographic microscopes), enhanced by the increasing resolution and magnification lended by Digital Microscope and SEM. The resolution power based on visual light, led and electron sources is influenced by the wavelength of the source beams and it affects the discrimination between two adjacent points, hence the detailing capacity of the obtained image. Our strategy is to analyse the functionality of grinding stone involving different resolutions with the aim to increase the reliability of the comparative observation and to

understand the possible functions of the lower steady tool under scrutiny. The direct observation of the archaeological grinding stone was carried out at MAE RAS, St. Petersburg, by N.S. and V.T. using both low power and high-power approaches (optical microscopy). Replicative experiments were carried out by N.S. to detail the reproduction of wear-traces and worked materials transformation, and by L.L. and I.P. to build a suitable reference collection for plants residues calibrated on the Pontic steppe coenosis (Longo et al., 2020a; Skakun et al., 2019). In order to investigate the tool function, all “classical” approaches to wear-traces analysis were applied (stereomicroscope and metallographic microscope, pioneered by S.A. Semenov (1964) and L.H. Keeley (1980) and the following development, and the microscopes involved are reported in Tab. 1.

During the survey (2015) the stone was 3D scanned to obtain a digital model by means of Next Engine (Longo and Skakun, 2017; Longo et al., 2018). The putative used areas were molded (impressions taken) using high-definition polyvinyl siloxane (PVS, Provil® novo light, Heraeus Kulzer GmbH), selected after preliminary direct inspection by means of stereo and metallographic scopes. The surface texture nanoscale replication was ensured by polyvinyl siloxane impressions, a long-tested procedure for the authors (Longo, 2003) and the molds were mapped on the actual surface of the stones. Repeated molding proved to be a very useful practice during the experimental reproduction of the gestures and the kinematics of the pounding activities. Also, molding disclosed an unexpected positive outcome due to its peel-off effect, since the sequential peeling (up to three per used-area) was at first cleaning the surface (from dust and putative contaminants adhering the surface) and, in second instance, it dislodged unpredictable micro to nanoscale residues (i.e. starch, raphides and fibers adhering to the molds) out of the inner pits, the crevices and the unevenness of the surface. The following sonication (standard ultrasonic tank) of peeled/molded areas were still testifying the presence of starch grains and other microremains (see paragraph 3.5)

In laboratory conditions, the molds were thus photographed by G.S. Digital microscopy and SEM investigation was carried out on those obtained during 2015 and 2016 sampling campaigns by L.L. and G.S. who worked independently by using digital microscopes: Keyence VHX 7000 at VCH Lab at NTU (Singapore) and Hirox KH-8700 at Cyl-STARC (Cyprus). Starch granules were observed at both light microscope and SEM (I.P. and L.L.). Finally, the observations were cross-checked and the results discussed within the three groups of wear-traces analysts working in St. Petersburg, Cyprus and Singapore.

Use-Related biogenic residue analysis (i.e. starch granules) was carried out by I.P., using a Zeiss AXIO Scope A1 and L.L. using several SEM, at low vacuum and with no coating, to be furtherly chemoprofiled by means of FTIR spectrometry and ToF-Sims (Birarda et al., 2020; Longo et al., 2020a). By using low and high-powered microscopes we feel confident that our functional interpretation is supported by a solid methodological and technological

refinement which involves contextual different scales of resolution impacting on our research outcomes.

### ***3.1 Traceology with direct optical observation***

The traceological analysis of the stone was carried out at low and medium magnification (8.4–98x) with MBC-10 binocular microscope and oblique illumination. When studying at micro level (50–500x), an Olympus BHMJ metallographic microscope was used, equipped with a reflected light illuminator and differential-interference contrast (DIC) lenses. The DIC produces a bias retardation which enhances the perception of the micro topographical variations of the surface, and of the overall contrast, an effect that is obvious in direct observation (Plisson, 2015, p. 221) and reduces chromatic aberration, a phenomenon to which digital sensors are particularly sensitive. In fact, even when the sample texture or micro-relief does not require this kind of vertical resolution enhancement, the DIC still improves the quality of the photography (Plisson, 2015, p. 221). Micro-level shooting was carried out using a Canon EOS 400D camera mounted on the metallographic microscope. To obtain high-quality photographs of the stone surface by focusing over the entire area in one frame, the Helicon Focus software was used. The surface of the tool is usually embossed, but with the help of this focus stacking software, we are able to quickly and correctly combine several (5 to 20 or more) source images, at different focusing distances, into a fully sharp image (Fig. 2a-b). During this study, special attention was paid to the following diagnostic features: macro changes of the relief, linear traces, gloss and polishing.

When describing flint and bone inventory from the Surein I rockshelter, G.A. Bonch-Osmolovsky made remarks about the characteristics of putative working traces. However, this is the first time that the functional study of the grinding stone is reported. The large pebble belongs to the structure in the area 9B from the lowermost layer G as reported in E.A. Vekilova (1957). The grinding stone shows an irregular-oval shape with a flat-convex cross section (Fig. 1e: center). The large oval stone shows two very different surfaces: Face A (Fig. 1e: right) is quite coarse and still showing evidence of microfossils composing the biogenic original rock (Fig. 9a), while Face B (Fig. 1e: left) is highly smoothed by water weathering (typical of stone surface long exposed to water running in a river, which actually flows not far from the shelter). Face A was downwards and embedded in the sediment, as carefully reported during the excavation and in the philological restaging in the museum (Fig. 1c-d), and it is that on which we concentrate our analysis: use-wear traces and adhering starch granules. Macro and micro traces of utilization were concentrated in the central part of Face A, which is fairly flat with negligible relief elevations. Significant macroscopic features are recorded only in certain areas of the surface like polishing and flattening of the central area of the stone; no modern damage was observed. Microscopic examination reveals spotty micropolishing with blurred edges,

317 situated on the highest points of the relief; it looks as dense, smooth, with varying degrees of  
318 brightness, fading towards the periphery. In some areas we observed craters formed as a  
319 result of light blows. The boundaries of the smoothed areas, when compared with the natural  
320 surface of the stone, are slightly worn out and even, the protruding tops are polished. Linear  
321 traces in polished areas look like short and long shallow lines with blurred, softly outlined  
322 edges, less often as parallel scratches directed along the long axis of the object (Fig. 2 a-b).  
323 However, the kinematic shows a general trend of the motion along the main axis of the  
324 implement (Fig. 6). Well-defined wear traces are concentrated in the central part of the stone,  
325 where molds 3 and 5 were taken, and to a lesser extent on peripheral areas. The features of  
326 wear (striations, spotted polishes and the clear general abrasion of the central area which is  
327 slightly lowered) drove us to identify this tool as a lower steady grinding stone. The direction  
328 of linear traces, sub parallel to the long axis of the tool indicates that when working, the upper  
329 mobile stone moved back and forth along one main direction.

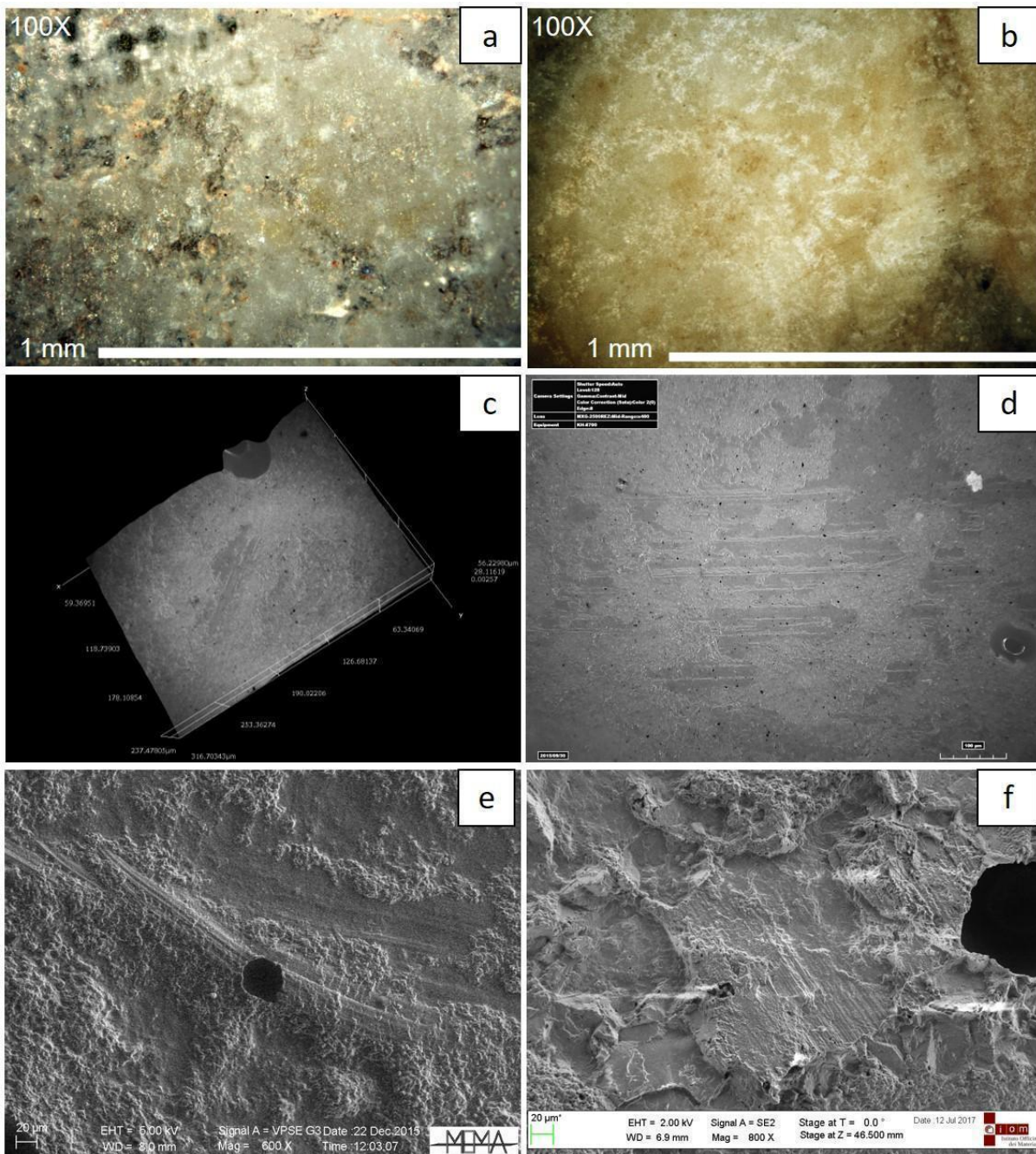


Fig. 2. Use-wear traces observed with different microscopy; a-b) optical microscope (OM) images of use-wear traces, flattened areas, groups of striations and rubbed areas are visible (b); c-d) DM images of the use-wear traces on the molds 3 and 5; c, 3D elaboration of the wear features at 1000x; d, bunches of striations visible at 400x) as observed with Hlrox KH-8700. The length of parallel striae ranges from 70 to 200  $\mu\text{m}$  and are visible on flattened areas; e-f) SEM images of striation and rubbed areas as observed on the mold (mold 3; e, 600x; f, 800x).

### 3.2 Replicative experiment to reproduce the use of the grinding stone

To verify the results of use-wear analysis and to obtain a sufficient number of standards, we undertook a series of experiments that further the work begun by S.A. Semenov (1974), G.F. Korobkova (1972, 1987, 1999) and other researchers (Hamon and Plisson 2008; Revedin et al. 2010; Skakun and Plisson 2014; Skakun et al. 2020). This was necessary since the Palaeolithic ground stones are unmodified pebbles and slabs contrary to those used for



processing plant materials from later periods (Neolithic) which are shaped to fit their utilization, hence their morphology became highly diagnostic. Firstly, they were used to process wild plants, and not cultivated cereals, which are qualitatively different in structure. Secondly, their working parts, as a rule, preserve the stone's natural surface and are not processed, like the Neolithic ones, with special picketage for better adhesion of the processed material (mostly hard seeds) to the working surface of the tool, and therefore show evident shaping and other signs of wear. Our experiments were carried out according to a specifically developed protocol, which included the selection of blanks for tools similar to archaeological samples in shape and raw materials, selection of plant materials, identification of macro and micro use-wear traces at different stages of work, detailed recording of the experiment. Large pebbles/slabs with a flat surface were selected for the lower, steady grinding stones, and small flat-convex in profile pebbles as the upper active tools (Fig. 3a). A total of six experiments were carried out. For the main experiment on grinding vegetation, *Typha* sp. (USO) roots were used as modified material (Fig 3b-c). This plant was chosen because recent studies of the lower cultural layer (Layer G) of Surein I (AMS OxA-5154  $28450 \pm 600$ ), from where the studied stone originates, show that the Crimean foothills of this time were characterized by a forest-steppe landscape (Demidenko et al., 2012; Chabai, 2000). The proximity to the water of the settlement is also confirmed by the remains of water vole and beaver (Vekilova 1971, p. 124, Table 3 and p. 126, Table 4), indirectly suggesting the likely presence of *Typha* sp. (USO) along the reservoir. When preparing the experiments, the roots of *Typha* sp. (USO) were preliminarily dried by a fire and peeled manually. Light crushing of the roots, kneading and grinding were carried out in two ways: in a circular and reciprocating motion, with increased pressure of the upper active tools on the processed material (Fig. 3b).



Fig. 3. The replicative experiment enacted to reproduce plant processing a) The raw material is consistent with the archaeological ones (limestone); b) N.N.S. during the replicative experiment; c) the stones are used for mechanical



processing of the cattail dried rhizomes (*USO*, *Typha* sp.) to produce a fine flour; d-e) optical microscopy images of wear traces created during the experimental work of the pebbles used as grinding stones and upper active stones.

The first macro-traces of use in the form of a slight sheen on the protruding parts of the surface, a slight deformation in the form of flattened asperities of the rock original surface arising from light impacts, were recorded after two hours of operation (Fig. 3d). Clearer wear-traces, similar to those found on archaeological objects: slight grinding of the protruding sections of the relief of the working part, spots of a rather bright shine with uneven boundaries, fading towards the periphery of the working part, weakly expressed micro-traces in the form of thin shallow lines on the lower stone and upper stone were produced after 5 hours of intensive work (Fig. 3e). As a result of rubbing the roots of *Typha* sp. (*USO*), a thin, light substance (Fig. 3d) was obtained, similar in appearance to the product acquired during the experimental work with replicas of instruments from the Palaeolithic site of Bilancino (Italy) and Pavlov VI (Revedin et al., 2010).

It should be noted that there were no traces of friction between the upper and lower grinding stones on both experimental and original tools. Apparently, this was hindered by the fibrous layer of the processed plant material.

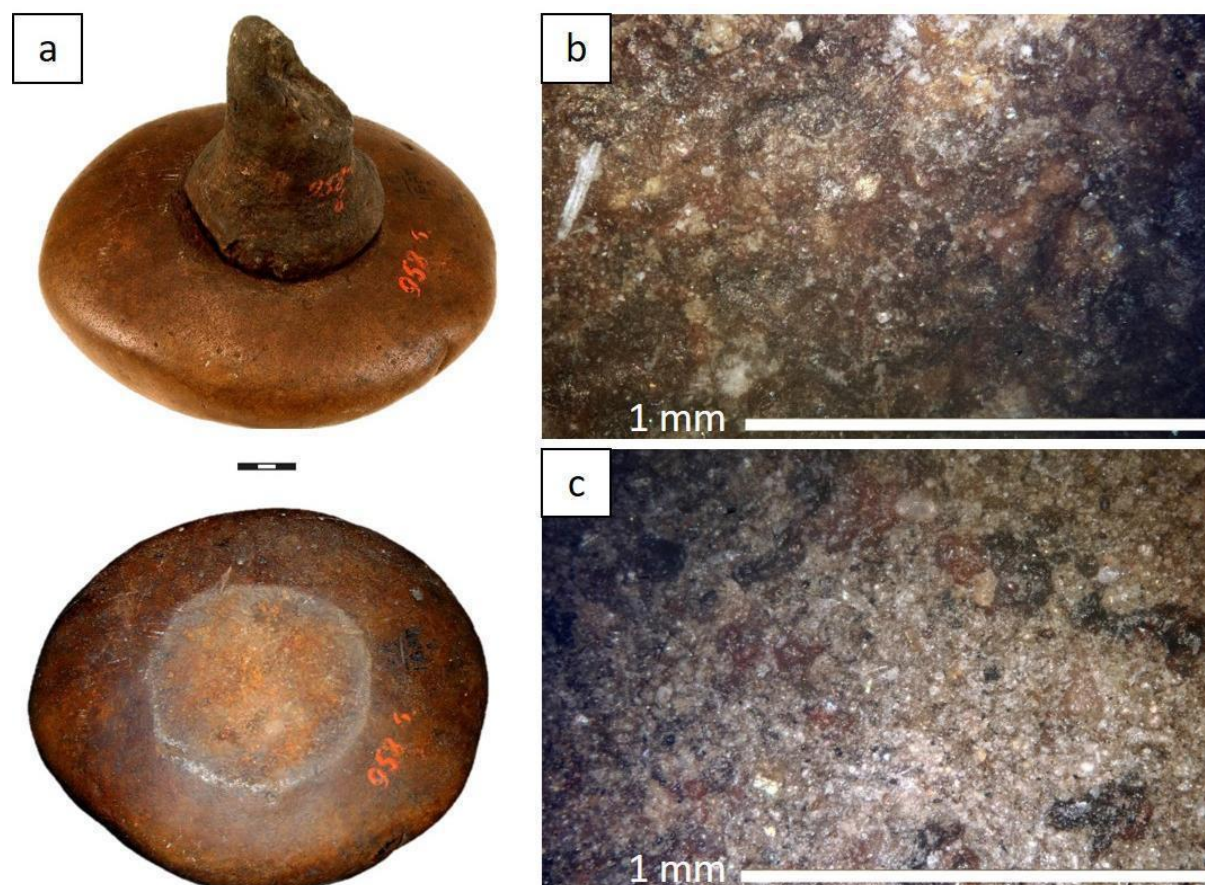


Fig. 4. a) Ethnographic tool (lower stone and mano) for grinding berries and grains (MAE RAS No. 958-6 / b-c)

Cheyenne, Wyoming, USA, Late 19th century; b-c) microphoto of the working surfaces of the lower grinding stone (b, 100x, scale bar = 1 mm) and mano (c, 100x, scale bar = 1 mm).

According to the palynological data obtained from the study of the cultural layer G of Surein I, it is possible to reconstruct that near the rockshelter grew mountain ash - *Sorbus aucuparia* L., buckthorn - *Rhamnus cathartica* L., and juniper - *Juniperus* sp. (Vekilova, 1957, p. 251). However, we could not report on starches referable to these plants. Nonetheless, we tested wear traces on a mortar used for grinding berries and grains belonging to the ethnographic collection of the MAE RAS (No. 958-6, Cheyenne, Wyoming, USA, Late 19th century). The microscopic features of utilization on the ethnographic mortar are close to the traces we described on the original grinding stone from Surein I and its experimental replica. The most significant differences are expressed by the absence of macro deformation of the working surface of the bottom of the mortar, that shows, instead, more even polishing covering the bottom and partly the walls of the mortar, together with randomly located linear microscopic features were found on its bottom (Fig. 4, modified after Skakun et al., 2020).

Further experimental work will be devoted to the study of use-wear features occurring on different types of rocks (sandstone, diabase, quartzite, granite) used for processing plant materials, aiming at identifying the dependence of the degree of wear development according to the duration of use of the tools, the type of processed plant materials and the tribological performance of the different rocks.

### **3.3 Digital microscopy and SEM**

Direct investigation of large and bulky tools like ground stones by means of conventional OMs is constrained by their structural design, as the stand and head are designed for thin samples (e.g. slides), making it difficult to accommodate bulky shaped tools. Similarly, the design of the SEM chamber is not suitable for oversized specimens. Therefore, the design of DM is a relevant aspect in the selection of the most suitable microscope for grinding stone wear-traces analysis. The DM stand is tall and adjustable, making it possible to orient the optical head with respect to the surface of the stone, hence facilitating the observation of large implements from multi-angle observation, as demonstrated during the study of experimental grinding stone (VCH lab, Singapore). Moreover, the manual mode allows for the tilting of the lens up to 90° and rotating the stage of 180°, facilitating observation of the stone from various angles without handling. During inspection, all adjustments were made without stone manipulation, using a small motorized console that greatly facilitates the stitching operation (mosaicking pictures), enhancing precision and saving time for precisely locating/mapping of the relevant spots on the tool surface.

Besides the issue raised by the dimension of the macrolithic tools, DM and SEM can also overcome the depth-of-field and the focal distance constraints when inspecting highly

uneven surface texture and facilitate the scanning at different resolutions and higher magnifications. Visual light microscopy is limited in the resolution by the classical Abbe diffraction limit ( $d = \lambda/2.8$ ) and the magnification of metallographic microscopy is generally capped at a spatial resolution of hundreds of nanometers due to the wavelength range of visual light. In the case of the ground stone's uneven surface, the overall capacity of DM revealed to be highly informative when observing raw materials with marked roughness, crevices, holes, contours and, in this case, microfossils. The DM technology can greatly influence image quality because of its combined lenses, video-camera and graphic software which are developed to optimize the relationship between depth-of-field, resolution and brightness, providing images that appear to have higher resolution than allowed by the diffraction-limited optics. The combination of the images - taken by the digital video camera at several focal planes (up to 120) - allows to visualize the fine topography of a very large area (highly improving the image processing obtained with Helicon Focus, already mentioned in 3.1) and to build a 3D imaging. The field emission gun characterizing FESEM allows for direct investigation at very low potentials (we used maximum 5 kV) without coating, nevertheless providing extremely focused high and low-energy electron beams with a spatial resolution < 2nm (that means 3 or 6 times better than conventional SEM) (Borrel et al., 2014). However, during scanning some samples may overcharge, ending with white striations on the images as happened with our starch granules (e.g. Fig. 8a' and Fig. 9d). The option "no coating" revealed crucial when carrying out further chemoprofiling of the U-RBR by applying FTIR spectroscopy and ToF-SIMS (Longo et al., 2020a). In the case of rough surfaces (like those of the ground stones, very different from the fine and smooth surface texture of rocks used in flaked industries) the wide depth of field available in DM and SEM reveals itself as crucial.

In order to overcome both structural and optical constraints, replicas of the surface (i.e. molding compound polyvinyl siloxane) were taken to allow the higher scale observation, being a familiar and long-tested procedure for the authors (Longo, 2003; Longo et al., 2020a; 2020b; Macdonald et al., 2018; Pedergrana et al., 2016). In the case of macrolithic tools the position of the impressions on the grinding stone was documented to map the observed traces (Fig. 6). Molds are suitable for direct investigation under any optical microscope and prove to be highly reliable even when scanned with DM (Fig. 2c-d) and SEM (Fig. 2e-f). The molds of the putative used areas were analysed with two different 3D digital microscopes: Hirox KH-8700 (at Cyl-STARC in Cyprus, by G.S. and L.L.) and Keyence VHX-7000 (at VCH Lab, NTU, Singapore, by L.L.) which cover a magnification range from 35 to 2500x (times). The DM higher resolution details the features already observed under lower resolution microscopy (Fig. 2 c-d: striations polish) and adds evidence of lesser-developed traces, decidedly increasing the ultimate functional attribution. In Fig. 5 we exemplify the key features observed with DM on the 4 molds (out of 5) taken out of Face A. Fig. 5a shows the mapping, on mold 3, of the

460 bunches of striae on the flattened area - surrounded by the unmodified original surface -  
461 outlined in red (5b and 5c). The following microphotos are imaging polished areas and  
462 striations on molds 2, 4 and 5. It is noticeable that molds 3 and 5 are covering the central area  
463 of the grinding stone, where the most intense and lasting pressure was exercised, therefore  
464 flattening and lowering the area causing a slight shadowing of the surface that can recall the  
465 look of tribochemical wear, observed as spotted polish with different degrees of brightness.  
466 Moreover, bunches of shorter and lighter striae testify a coupled motion such as pounding and  
467 dragging as the alignment of the striae is suggesting (Fig. 5a-c), and accordingly their  
468 orientation is both parallel and perpendicular to the main axis of the grinding stone. On the  
469 molds we observed U-RBR such as starch granules and fibres (Fig. 5e and h), that were then  
470 observed under the SEM (Fig. 10). Molds 2 and 4, taken in peripheral areas affected by a  
471 lesser prolonged activity, show more defined and sharp striae and polished areas (Fig. 5e and  
472 5f). The unprecedented results of our study demonstrate DMs as the most feasible equipment  
473 for the study of large implements with rough surfaces as ground stones enabling easy  
474 inspection at both high resolution and magnification.



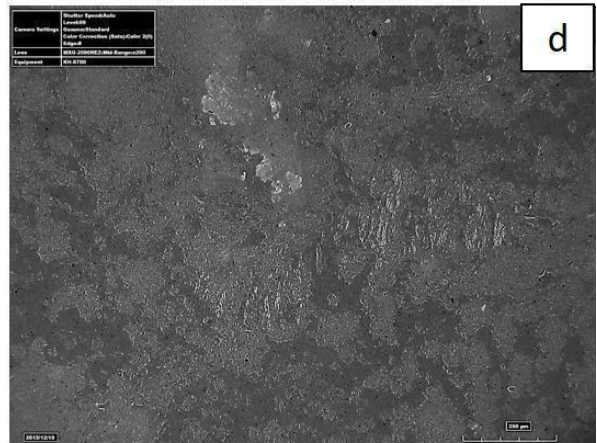
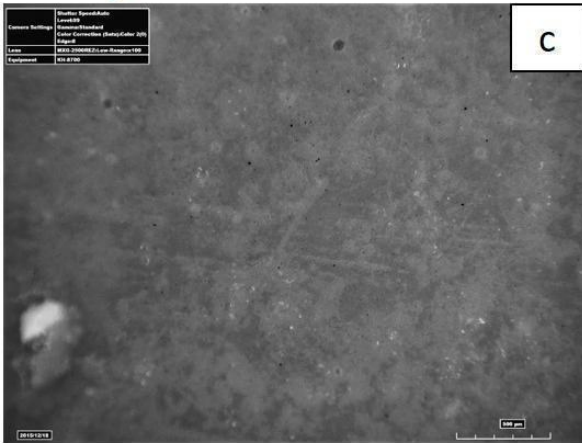
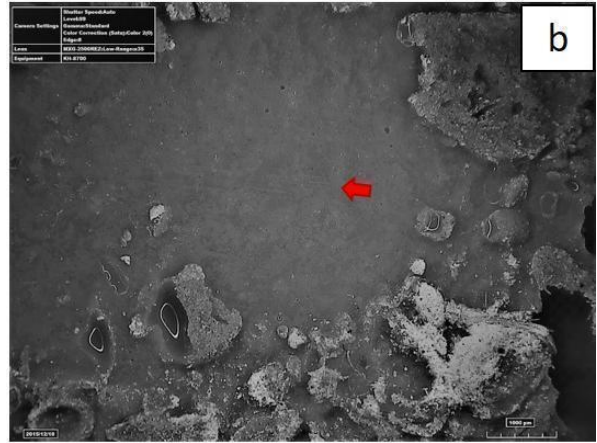
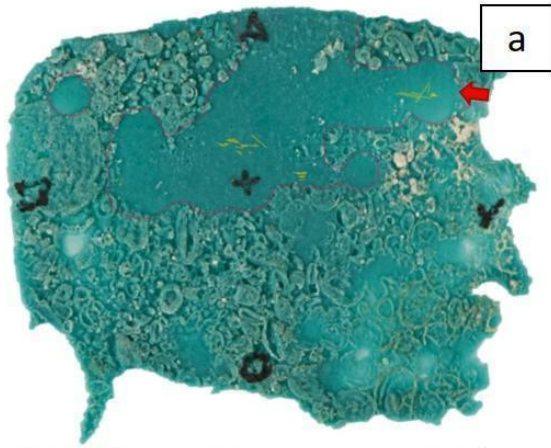


Fig. 5. a) Mold 3 with flattened areas highlighted in red and striation in yellow; b) smashed biogenic formations visible around a flattened area where bunches of parallel striations (indicated by the red arrow) are visible and they are crossed by a couple of longer striae parallel to the main axis of the stone (35x and c at 100x); d) striations on a rubbed area on mold 5 (200x); e) series of sharp striae visible already at low magnification (35x) on mold 4; f) bunches of parallel micro-striations on a polished area on mold 2 (600x); g) plant fiber on a rubbed area, next to a striation and to parallel striations on mold 5 (100x); h) the same residue at higher magnification (2500x), part of the striation is still clearly visible next to it.

### 3.4 Mapping of the wear-traces

The identified use-wear traces on the stone (shapeless rubbed-down traces, weak and light linear traces, spotted polished areas, other wear features), detailed by scanning the molds with the DMs, were photographed at increasing magnification (within a consistent standard: 200X, 500X, 1000X, 1500X, 2000X), and one or more details were identified as reference to map the same trait/s when imaged at higher magnification (Fig. 5a-c). Moreover, the contact side of the molds (impressions) were photographed and orthorectified.

In addition to molding, Surein I stone tool was 3D scanned (Next Engine, at maximum intensity) for digital reproduction and for off-site inspection (Longo et al., 2018; McCartney and Sorrentino, 2019, pp. 69-73). In order to elaborate the 3D model and the digital elevation model (DEM) (McCarty, 2014), the stone tool was also photographed and referred to a coordinate system. The orthophotos of the used surface (Face A), were matched into a final orthomosaic. This procedure allowed us to import in AutoCAD the orthorectified images of the molds of Face A, together with the images acquired with the different microscopes. The wear-traces microphotos were scaled (according to the magnification) and co-registered through the recognition of three common points. When entered in AutoCAD each magnification was treated as a different layer. The final goal was to relocate the microscopic features in each relative position on the molds, and the impressions were finally mirrored and aligned to the stone surface. This procedure aims to create a schematic map of the featured traces of use on the grinding stone's original surface, assigning the utilized areas and the main direction of use (e.g. see the direction of the striations and the areas of their higher concentration, Fig. 6).



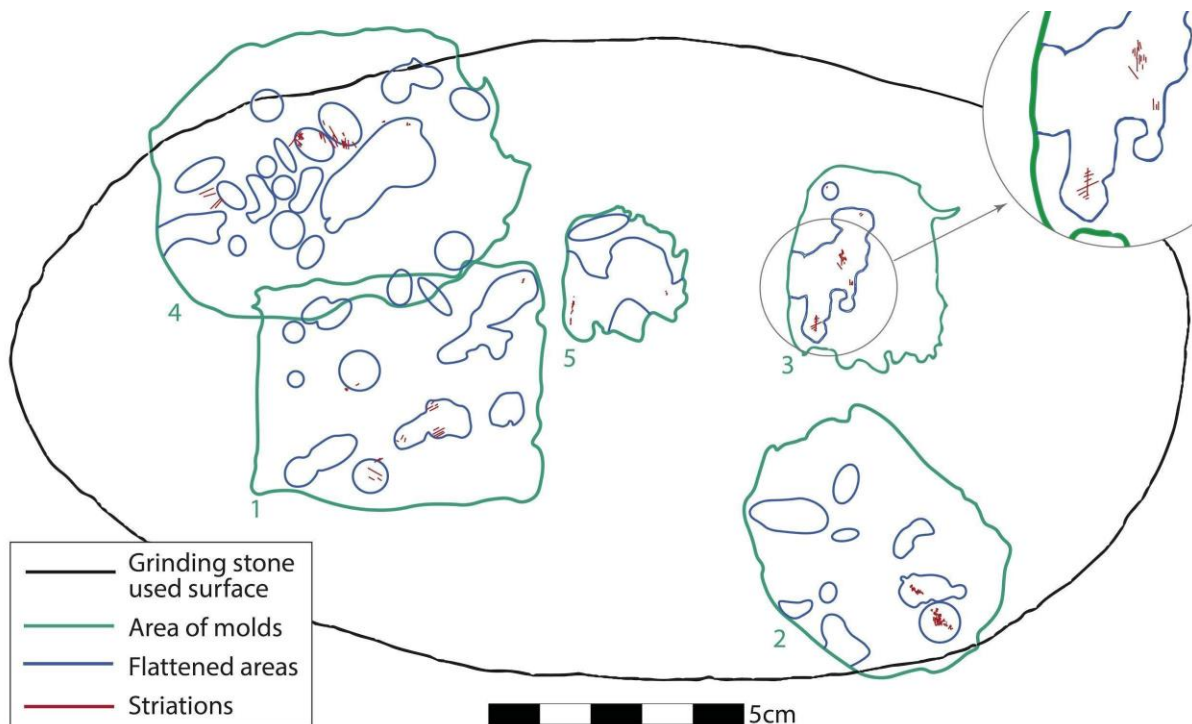


Fig.6. Schematic map of the molded areas: flattened areas (outlined in blue) and striations alignment (in red) are highlighted, revealing their actual location on the central part of Face A and the striations can be even oblique to the main axes of the stone (evidence for a widened motion).

### 3.5 The use-related starch granules

Use-related starch granules were extracted through standard ultrasonic tank cleaner at room temperature (double-frequency ultrasonic power 180 W, 28 kHz is used for overall clean, 40 kHz for precise clean) by soaking part of the tool. The operation occurred at the MAE RAS in St. Petersburg (2017), after both the preliminary inspection through stereomicroscope and metallographic microscope and the molding carried out as described in 3.1. Surein I liquid sample was then processed by I.P. at the Institute of History, Archaeology and Ethnology, Far East Branch, RAS, (IHAE FEB, Vladivostok, Russia). The preparation followed the methods in use by scholars (Torrence and Barton, 2006; Therin and Lentifer, 2006; Yang et al., 2012). Bleaching and careful cleaning of the lab surfaces and consumables is routine prior to starch granule extraction. Cesium chloride (CsCl) was the salt added to prepare the heavy liquid to segregate the starch granules from the accompanying sediment. The addition of this salt calls for multiple rinsing episodes in order to be carefully washed-off the final solution and to ensure the effectiveness of the further nanoscale analysis (Birarda et al., 2020; Longo et al. 2020a).

A Zeiss AXIO Scope A1 was used to scan the isolated residues. The solution (10% glycerol and 90% distilled water) with the isolated starch granules was mounted on a slide and observed at 200-800x under unpolarized (Fig. 7 a-e) and polarized transmitted light using DIC-contrast modalities (Fig. 7 a'-c'). Micrographs and measurements were taken using the microscope software. All starch granules showed the typical Maltese cross (extinction cross

under polarized light) and other diagnostic features (Gott et al., 2006) (Fig. 7 a'-c'). The starch granules are classified according to criteria proposed in previous studies (Piperno et al., 2004; Torrence and Barton, 2006): the shape of the granules in various projections, surface features, position and shape of the hilum, the presence and features of facets, the type of polarization-cross and characteristics of rays, the presence of lamellae and damage allowing for a confident identification of genuine starch granules.

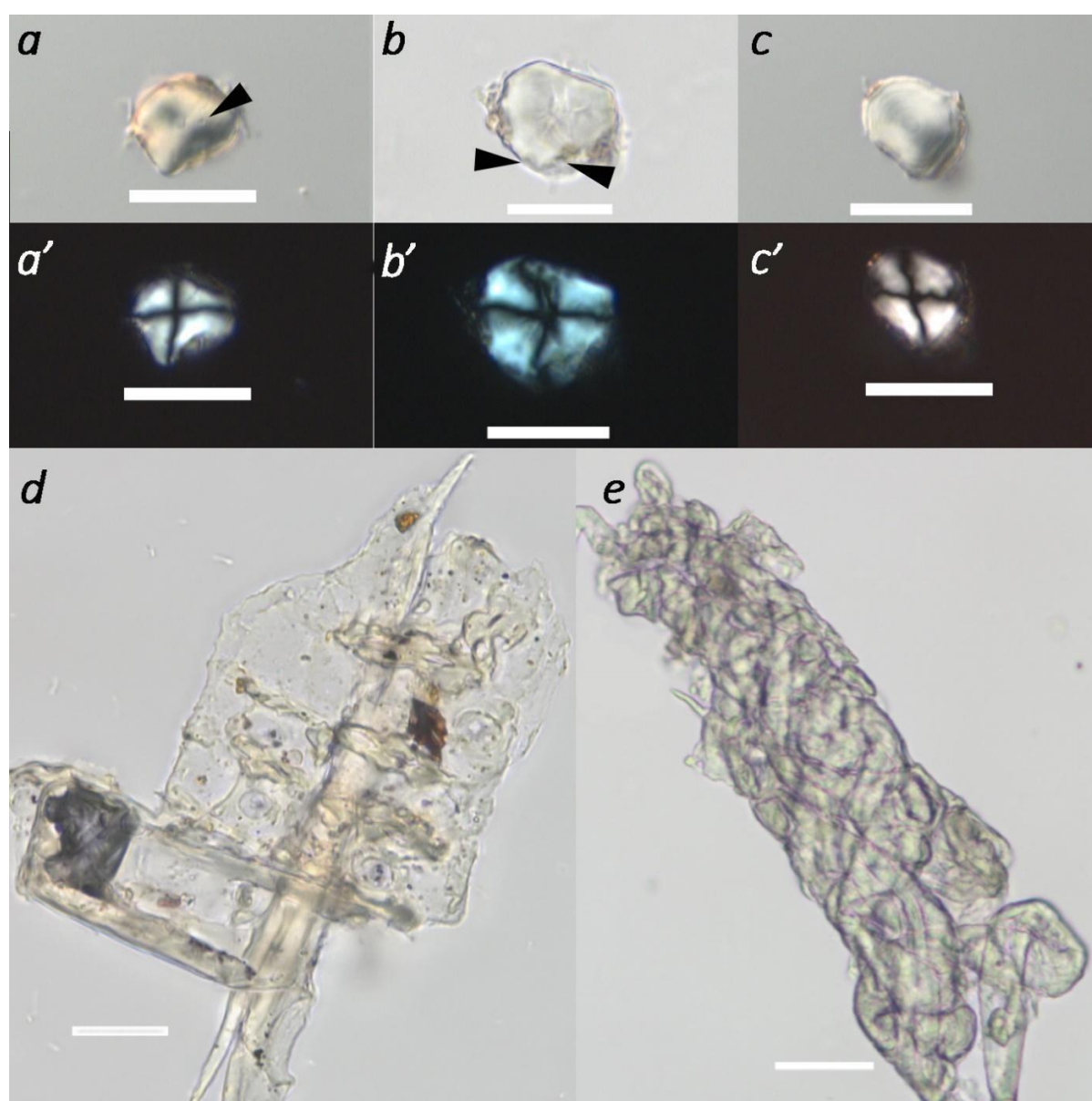


Fig. 7. Surein I: Starch granules and U-RBR isolated from the utilized areas on face A, bar 20  $\mu\text{m}$ , e) – 50  $\mu\text{m}$ ; a-b) Polyhedral granules, black arrows – surface craters after enzymatic attack; c) Ellipsoidal granule; a'-c') the same starches granules under polarised light. The extinction crosses are visible; d) Plant vascular tissue; e) Plant tissue or fiber.

Seven granules of starch and fragments of plant tissues were obtained out of the sonicated sample of Surein I. None of them has a complex of attributes reliably comparable with any sample of the reference collection in Vladivostok lab, which includes 120 species

from 24 families encompassing wild and cultivated cereals, pulses, plants with USO and ferns. This collection is mainly composed and constantly replenished with plants present in the Russian Far East that do not correspond to the studied region, either geographically and paleoecologically. At DAIS (Ca' Foscari, Venice, Italy) we are building a reference collection with plants consistent with the western and south-eastern coenosis of boreal Eurasia, selected among the list reported in recent reviews (Hardy, 2010; Kovárník and Beneš, 2018; Shipley and Kindscher, 2016). As well, SEM imaging and FTIR spectrometry are applied to characterize the starch granules in order to build a physical-chemical reference of starches from the PSRSO record coherent with the Pontic steppe coenosis during Late MIS 3.

The seven starch granules can be divided into two groups. The first includes 4 granules of a polyhedral shape. Size range is 15-23  $\mu\text{m}$ . The extinction cross varies from (X) to (+) type and the rays are straight or curved. 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 (Fig. 7 a-b).

The other 3 grains have oval-like forms. Another section-view was not available. Size range is 18-19  $\mu\text{m}$ . The polarization cross varies from (X) to (+) type and the rays are straight and curved. Hilum is located in the center, with a rounded outline. Two of them have lamellae and one show transverse crack.

In addition to starch granules, the sample contained other residues. There are fragments of plant tissues such as vascular and epithelial tissues and plant fibers (Fig. 7 d-e). Therefore, the contextual presence of different plants-related remains and other use-related biogenic residues (as already observed by Hardy et al., 2001; Pearsall, 2015) are supportive of the genuine origin of the starch granules observed on Surein I grinding stone.

The limited amount of starch granules in the liquid sample can be explained by the history of the artifact (surely washed and most probably brushed since the discovery), its curation and in the last instance by the starch sampling strategy. It is relevant to recall that polyvinyl siloxane imprints removed the main residues from the working surface and, due to the geometry and dimensions of the ground stone, only a small part of the tool original area was fitting in the ultrasonic tank, hence the sonication interested just a small peripheral area of the tool (Skakun et al., 2019). Because of this constraint, the sonicated area is not one referable to the most active areas. Such a small amount of starch granules extracted from the sonication, in comparison with the more consistent number extracted from the molds, is considered a good evidence of the absence of modern contamination and the localization of residues in areas with use-wear traces is conducive to their authenticity. Therefore, we can confidently say that the detected residues are mainly associated with the ground stone utilization, and not due to soil deposits or other biasing conditions.

When comparing images of the starch granules we evaluate that the set of starch types observed under OM and SEM did not differ significantly. There are distinguishable types and shapes in the SEM samples: flattened round-oval granules corresponding to the Triticeae tribe (Fig. 8), which is the most common group composing the Pontic steppe grassland. The first group of OM starch granules finds analogies with another type of starch granules in SEM. They have a multifaceted elongated shape. The presence, in the OM samples, of such characteristics as the shape of the hilum, lamellae, and cracks are the projection of the internal structure of the grain which can be detailed with the resolution of the SEM (Fig. 11). This is the very first report on the coupled observation (OM and SEM) of ancient starch granules (Fig. 8).

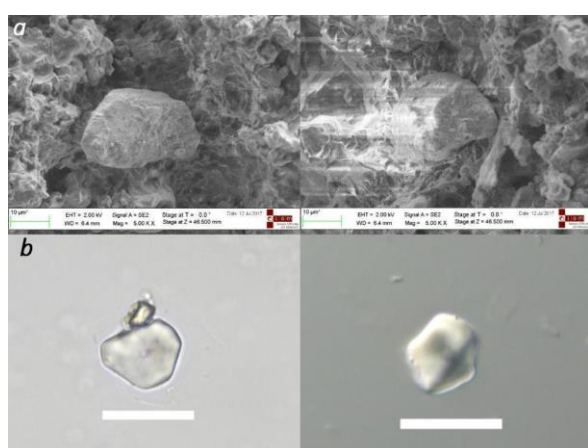


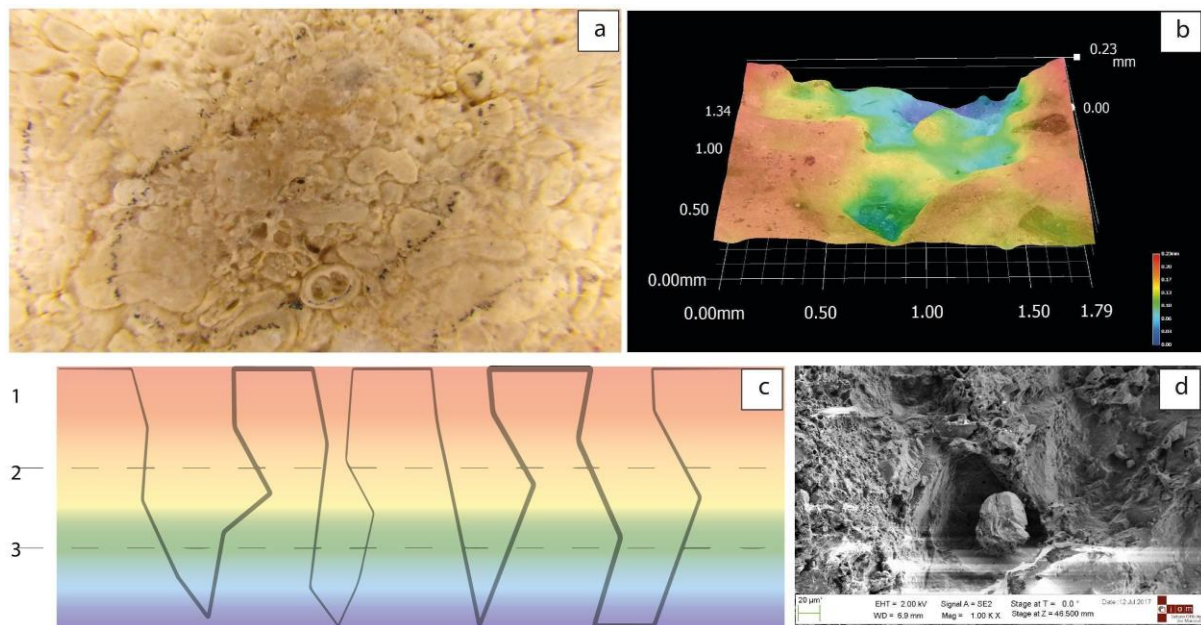
Fig. 8. Surein I grinding stone. Correlated starch granules form as observed with SEM and OM microscopy; a) Polyhedral shaped grains on the mold, bar 10 µm; b) Polyhedral shaped grains from the sonicated sample, bar 20 µm.

### 3.6 Starch granules under SEM

During the sampling, we developed a standardized procedure for the analysis of both wear-traces and U-RBR (i.e. starch). It included wearing powder-free gloves, careful dusting of the stone surface with a clean soft brush and running a first macroscopic inspection of the putative used areas, taking close-up macro photos (Longo et al., 2020b). Once the suitable areas are identified (see above 3.1), the further steps include deep cleaning of the areas by means of the molding peel-off effect exerted by the thixotropic property of the polyvinyl siloxane that enters in the unevenness, holes and crevices of the stone surface and extract actual ancient starch granules (Fig. 9). The sonication was performed at MAE RAS, St. Petersburg, to extract putative “genuine” use-related biogenic residues (Collins and Copeland, 2011; Copeland and Hardy, 2018). Molding obtained prior to sonication can peel-off putative contaminants (a case still under analysis, since contamination cannot be ruled out in any archaeological condition). However, the starch granules extracted do not look modern and both DM and SEM inspections proved that starch granules and other U-RBR, adhering to the



molds, are associated with wear-traces (Fig. 9d and Fig. 8a: SEM images of starch adhering to mold 3; Fig. 5g-h and Fig. 10: DM and SEM images of other U-RBR adhering to molds 1, 3 and 5). Molding generates peels from plant remains still entrapped into the crevices of the archaeological grinding stone: this demonstrates the direct relationship of the starch granules with the functionally active areas (as exemplified by the wear-traces on molds 5, pictured in Fig. 5g-h), a case never reported before, to our knowledge. This unexpected discovery proved to be fruitful for extracting starch granules out of the molds during the chemo-profiling of the granules by means of SR-FTIR spectroscopy. A selection of starch granules isolated in Vladivostok underwent the nanoscale analysis with FEG-SEM (Fig. 11; Longo et al., 2020a). To perform the very first systematic observation of Palaeolithic starch granules under scanning electron microscopy a Zeiss SUPRA 40 high resolution FEG-SEM, based on the 3rd generation GEMINI column (available at ION CNR-Elettra Sincrotrone, Basovizza, Italy) was used. The optimal structural characteristic of this FEG-SEM enables for direct observation of the starches (and other residues) with no coating, thus enabling further analysis (e.g. SR-FTIR and TOF-SIMS, Longo et al., 2020a; Birarda et al., 2020).



*Fig. 9. a) The uneven surface of the active side (Face A) of Surein I grinding stone. Limestone microfossils are detailed with the function “digital microscope mode” provided by the Ricoh WG-30 camera (7,5x); b) DM false colour 3D model of the mold microtopography: the deeper areas are in green and blue, while the higher are in yellow and red (Keyence VCH 7000); c) we propose an ideal model of the crevices according to b). This unevenness can serve to entrap both the contaminants and the U-RBR. In our hypothesis 1: the first mold extracts putative contaminants, together with sediment, dust, and other biases and can be used as cross-check reference; 2-3, second and third mold progressively clear the microtopography and increase the extraction of genuine ancient starches as demonstrated in d) where a starch granule is entrapped in the crevices of the 3rd mold (view under SEM).*

As mentioned above, the limits imposed by the resolution of conventional light microscopy to detect starch granules, mostly when dealing with those falling in the lowest size range ( $<50\ \mu\text{m}$ ) can be overcome by SEM: this allows a further level of analysis of the U-RBR

morphological features, and has proved useful in examining the small starches extracted from Surein I grinding stone. Hence, SEM did help to identify new features appreciable at higher magnification, and, compared to visible light microscopy, has enabled us to count more starch granules (ten in a single 0,5  $\mu$ L droplet) and to observe other plant remains (i.e. fibres and raphides, Fig. 10 and Fig. 11 d, f). SEM inspection renders the surface sculpturing and the morphological features highly evident, highlighting attributes never featured before for such ancient remains (Fig. 11).

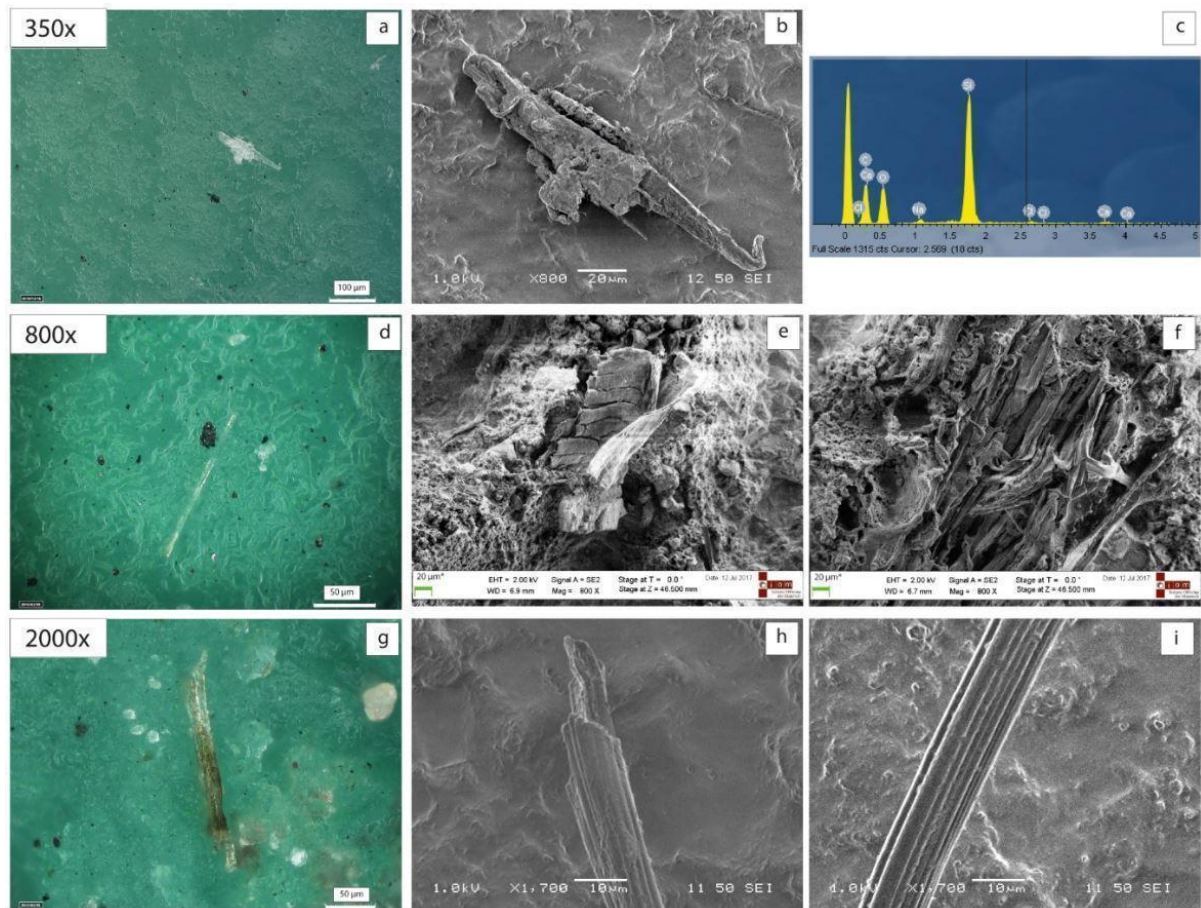


Fig. 10. Surein I, molds 1, 3 and 5: adhering plant remains. a, d, g) DM direct observation of the molds at different magnifications showing adhering plants remains; a, b, c) putative plant remains under DM and SEM with EDAX analysis evidencing Cl, C, Ca, O, Na, Si; d, e, f) plant tissues at DM and SEM; g, h, i) DM and SEM images of candidate phytolith.

In the case of Surein I, starch granules are clustering in two types with evident hilum, distal or central, and the shape looks as “roundish with a stretch” in the central portion of the grain and also lamellae were clearly visible (Fig. 11). During the scanning, several granules evidently crushed or broken were observed. Accompanying plants remains were also evidenced such as raphides, parenchyma, and phytoliths (Fig. 11 c-d and f: raphides, in light blue), that are thoroughly discussed elsewhere (Birarda et al., 2020; Longo et al., 2020a; 2020b). As mentioned above, the lack of tailored reference collection for plants used for economic purposes during the Late MIS 3, when *Homo sapiens* was living in the Pontic steppe,



653 makes it challenging to speculate on the taxonomic attribution of the identified starch granules  
654 (Hardy et al., 2001). Therefore, we are implementing the reference collection with plants  
655 available in the Pontic steppe today. The list includes those present during late MIS 3, i.e.  
656 *Betula* sp., *Rhamnus cathartica* L., *Sorbus aucuparia* L., *Juniperus* sp., willow *Salix* sp, *Populus*  
657 *tremula* as already reported by Vekilova (1957; 1971) and shrubs and grasses with storage  
658 organs (Hardy, 2010) suitable to be collected and processed for the extraction of dietary  
659 carbohydrates like *Stipa* sp. (among ASO) and *Typha* sp., *Arundo donax*, and *Phragmites* sp.  
660 (among USO), already reported in pollen lists of the area available in the literature  
661 (Hammerman, 1929; 1934; Gerasimenko, 2004; Demidenko et al., 2012). Modern plants are  
662 collected with the dual purpose (i) to extract starch, under lab-controlled conditions, for  
663 physical-chemical and morpho-structural characterization (including ageing under controlled  
664 parameters) and (ii) to be used in experiments of mechanical tenderization and traces  
665 development (G.S. PhD project, ongoing).

666 Another positive mark of SEM observation is the “3D like” effect which makes the  
667 observation of the U-RBR much detailed and allows for the identification of features at the  
668 nanoscale like lamellae and exfoliation, interpreted as due to mechanical processing as well  
669 as to other physical or chemical events occurring during the tenderization, hence man-made  
670 (Birarda et al., 2020, Fig. 4). However, the phenomenon might have occurred during  
671 diagenesis, and the on-going ageing experiments in a climatic chamber (at DAIS, Venice) will  
672 possibly shed light on the mechanism of the exfoliation and other features observed.

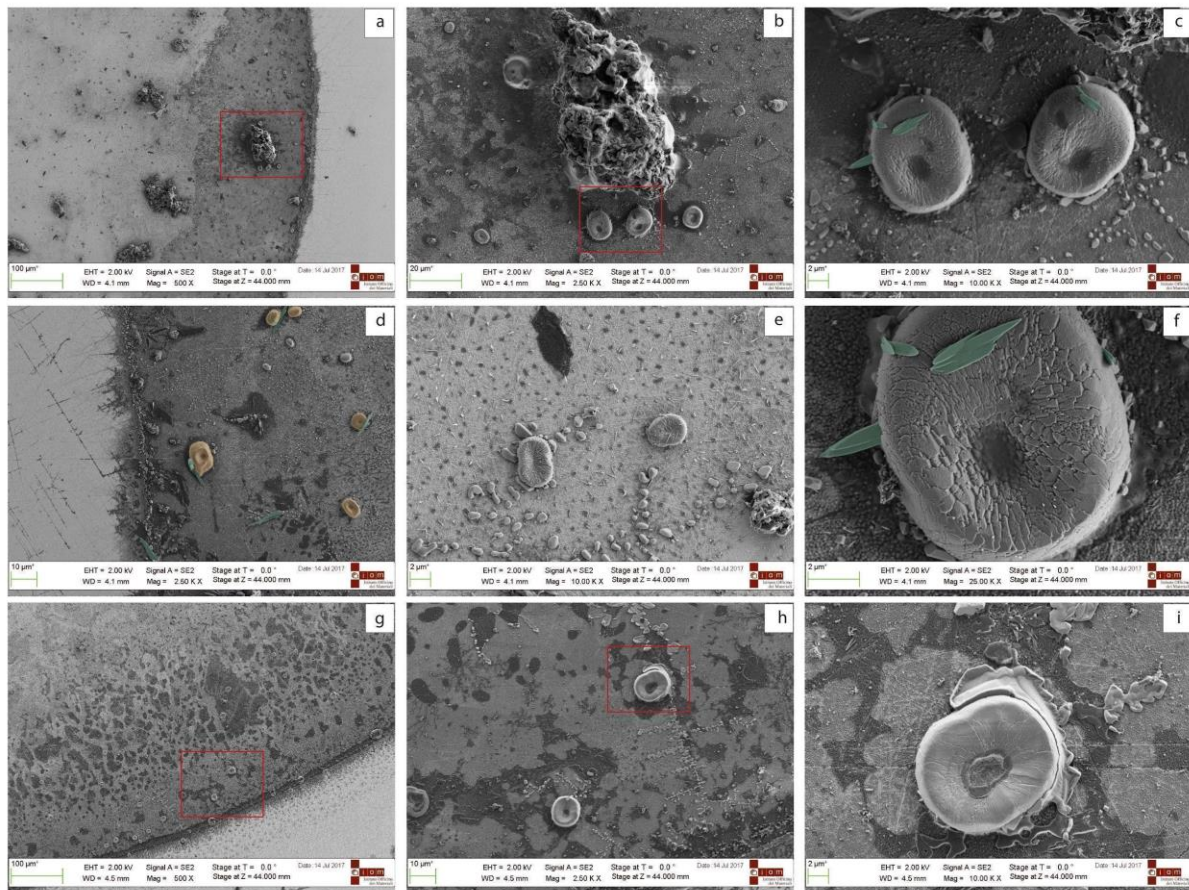


Fig. 11. SEM: Starch granules extracted from the sonication of molds 3 and 5, usually they are distributed along the outline of the droplet. Starch granules in a-d and g-i are imaged at different magnifications with the lower range resembling those obtained under OM. Starches (on purpose coloured in light orange in 11d) and raphides (calcium oxalate crystals) on purpose coloured in light blue 11c-f) are still adhering to different granules, testifying their consistency with USO attribution of the granules; e-f; h-i) Starch different morphology, surface structure and central hilum.

#### 4. Discussion

Our research aimed at: (i) collecting evidence supporting Surein I grinding stone was used to mechanically transform PSRSO into food, (ii) implementing the investigative procedures with *ad hoc* methodological refinements for both wear-traces and U-RBR analysis. Finally, the results obtained with this innovative research design are supporting fine-grained data to unfold the complexity of nutrition at the dawn of modern human colonization of the south-eastern Eurasian steppe during late MIS 3 (40-25 ka calBP). By that time, the boreal continent was already inhabited by Eurasian archaic humans, namely Neandertals and Denisovans further to the east, well adapted to their ancestral boreal environments, who were thriving on a highly carnivorous diet (Jaouen et al., 2019). Even for the ancient settlers of Crimea, prompted to be one of the south-eastern refugia for late Neandertals, the bulk collagen  $\delta^{15}\text{N}$  high values are suggesting they fed on a typical terrestrial-based diet (Drucker et al., 2017). The early occurrence of *Homo sapiens* in the peninsula is represented by the remains of Buran Kaya III, layer C, which direct dating supports for modern humans presence

694 before the harsh HS 4 conditions (Heinrich Stadial) (Pean et al., 2013). Together with the  
695 recent radiocarbon dating of Bacho Kiro cave (Bulgaria) it is possible to set back the early  
696 presence of anatomically modern humans in the Pontic Steppe around 40 ka calBP (Fewlass  
697 et al., 2020), extending the length for the overlapping of the newcomers with the late  
698 Neandertals in the area. Vekilova (1957) anticipated that Surein I oldest assemblages recall  
699 features comparable with the Transcaucasian Upper Palaeolithic (Zamiatnin, 1957, and lately  
700 Korkia, 1998), among which some instruments show similarities with the Levantine industries,  
701 and with the EUP assemblage of Mezmaiskaya cave (Golovanova et al., 2006). However, in  
702 this mixed peopling scenario, with late Neandertals' resilient presence and early *Homo*  
703 *sapiens* appearance, there is much debate over who actually may have developed the so  
704 called "transitional industries" reported for the Pontic Steppe sites. These industries include  
705 innovative elements - and are listed with different names i.e. Late Micoquian, Streletskayan,  
706 or eastern Szeletian, Proto-Aurignacian, Aurignacian, etc. (Demidenko, 2014b; Bataille et al.,  
707 2016, just to name a few within a vast literature). Therefore, it is crucial to explore other lines  
708 of evidence to disentangle the long chain of circumstances that allow us to identify who actually  
709 developed the transitional/Initial/EUP cultures: the resilient Neandertals or modern humans  
710 newcomers. Although flaked industries represent – along with bones - the most frequent  
711 physical remains left by hunter-gatherer activities, they cannot be considered as the only  
712 source of behavioural information and to reconstruct nutritional strategies.

713 Off the beaten track, we addressed the broad assemblage of non-flaked industry  
714 specifically focusing on ground stones retrieved in boreal latitude EUP sites associated with  
715 *Homo sapiens*, in order to elucidate their high potential as reliable source of information for  
716 dietary strategies (Birarda et al., 2020; Longo, 2016; Longo et al., 2020a). The investigation  
717 of Surein I task-specific tool - the large biogenic limestone slab retrieved in the lowermost  
718 Layer 3 of G.A. Bonch-Osmolovsky 1926-29 excavation (layer G, Bataille, 2016) referred to  
719 Aurignacian – provided data to demonstrate its use to transform PSRSO by mechanical  
720 tenderization such as grinding, pounding and threshing.

721 The methodological refinement applied to the study of Surein I grinding stone  
722 consistently integrates the research design with different stages: from the sampling strategy -  
723 to avoid further potential contamination - to the concerns arisen during the complex integration  
724 of the probes in use, and analysis from the macroscale down to the nanoscale. We developed  
725 a coupled macro to nanoscale investigation of the Surein I grinding stone, considering the  
726 contextual occurrence of wear-traces and U-RBR, namely starch granules. The application of  
727 different microscopes with increasing resolution and magnifying capability (Optical, Digital and  
728 Scanning Electron Microscopy) made it possible to couple the different methodologies and to  
729 overcome the limits due to the resolution power of the applied technologies. Our procedures

combine the acquisition of 3D geometry, the direct observation of wear-traces (in the museums) and the further detailing of the features on the stone tool molds (imprints). Mapping and relocating the functional features (the wear-traces, the flattened areas, and the orientation of the striations) from the molds to the original stone is a step forward in functional analysis of ground stones when investigated at high resolution. We are aware that due to methodological constraints, e.g. heuristic requirements, equipment availability, and time restraints, these techniques are applied at present to a limited number of samples and to address specific questions (i.e. wear-traces on different raw materials pebbles and starch granules, Longo et al., 2020a; Birarda et al., 2020).

Furthermore, we identify a best practice to extract U-RBR correctly from the used areas of the stones with reference to the internal and external constraints of the available facilities. The starch granules adhering to the grinding stone are tiny (in the case of Surein I  $< 50\ \mu\text{m}$  average size, within a range of 1-100 $\mu\text{m}$ ), thus, requires micro and nanoscale analysis to be appreciated. One of the main constraints of use-related residues analysis is that it can be affected by the unpredictable effects of contamination (Crowther et al., 2014; Dozier, 2016; Mercader et al., 2018 for review), which is an even greater concern when sampling from museum collections and old excavations. The topic is widely treated by several scholars (Pearsall, 2015; Ma et al., 2017) who also report on experiments carried out to verify the feasibility of starch migrating from the soil or from the surrounding weeds, to the surface of the stone tools. Hart (2011) has demonstrated that is not the case, and if contamination occurred it is limited to the very external surface of the tool, since he did not find any contaminants starch granules in the sonication of the control experiments. Hart's results can be taken as reference for the reliability of U-RBR entrapped at the bottom of crevices, holes, etc. of the coarse ground stone surface. We actually paid great attention, through all the analytical steps to control putative pollution and other biases that may hinder actual U-RBR and specifically those related to starchy plants intentional processing. The practice of taking three successive molds, progressively clearing possible biases, made the ultrasonic extraction suitable for the identification of ancient use-related biogenic residues, although it can limit the number of remains extracted as experienced in our study (only seven starch grains were extracted from the sonication). The contextual extraction of U-RBR signifies the presence of different plant-related remains (starch, raphides, parenchyma, phytoliths) on the areas bearing clear wear-traces and proved to be supportive of the genuine origin of the starches observed on Surein I grinding stone (Hardy et al., 2001; Pearsall, 2015). The standardized procedure we developed to extract starch granules has enabled us to correlate residues and wear-traces – intended as an evidence of PSRSO intentional processing - as well as to confirm the presence of genuine ancient starches. Considering the long history of the grinding stone examined, any trace of

modern starch on it would not display the dilapidated appearance that all our starch granules displayed.

Plant remains are perishable and difficult to track. For the southern hemisphere, J. Mercader was the first reporting on sorghum seeds from Ngalaue cave (Mozambique), a site dated to 105-55 ka (Mercader, 2009). Further to the south, parenchyma remains, retrieved in hearths and ash-rich layers, were attributed to charred roots and rhizomes at Border cave (177 ka), and at Klasies River cave since 65 ka (Larbey et al., 2019; Wadley et al., 2020). Although no ground stones have been retrieved in the mentioned sites, therefore no direct evidence for intentional processing is documented, the findings are consistent with the presence of *Homo sapiens*. Phytoliths and starch granules were recovered from two slabs retrieved in the Middle Sangoan occupations of Sai Island (Sudan) and interpreted as evidence of plants processing dating back to 200 ka ago (Van Peer et al., 2003). One can speculate that *Homo sapiens* in Africa was already including in his dietary breadth starch-rich foods, mostly rhizomes, the veritable plant storage organs. Therefore, it is possible that plant processing was a technological practice that *Homo sapiens* exported with its northward explorations.

At boreal latitudes, putative plant foods have been reported throughout the late Pleistocene (Hardy, 2010; Kovárník and Beneš, 2018; Shipley, 2016); however, the intentional plant processing by means of ground stones is not very frequent until the Holocene. Hence, ours is a challenging hypothesis for the considered time period (late MIS 3). In the Levant phytolith from grass husks were associated with Neandertal (70-55 ka) at Amud cave (Madella et al., 2002), whereas charred legumes and nuts (i.e. acorns and pistachio) were recognized at Kebara (48–59 ka; Lev et al., 2005). Pulses (i.e. lentil, chickpea, pea, vetchling), fruit and nuts are reported at Theopetra Cave in Greece (Mangafa, 2000). Starch granules are reported on 3 artefacts from the Swabia Aurignacian sites by Hardy B.L. et al. 2008, although they caution that it is unlikely that flaked tools were used in the starchy plants processing. Plant remains correlated with prevailing shrub tundra have been reported from the Aurignacian site of Hohle Fels (Swabia, Germany), where few seeds attributed to Asteraceae and to the broad group of Poaceae are referred to be used by humans around 44,2 ka calBP, together with bark fragments interpreted as bearberry (Riehl et al., 2015). In Crimea, the very first publication reporting intentional plant processing dates back to 2001, thanks to the pivotal and inspiring study on 50 flaked flint tools from the Late Middle Palaeolithic layers of Starosele and from Buran Kaya III layer C, attributed to the Early Upper Palaeolithic (Streletskaya) (Hardy et al., 2001). The coupled investigation of wear-traces and residues analysis highlighted the retrieval of soft plant parenchyma and wood tissues, the latter interpreted as hafting traces for spear heads and, more in general, woodwork undertaken in both the sites. What is particularly

relevant for the present study is the data derived from the analysis of the trapezoidal microlith from Buran Kaya III. The OM compelling analysis of the residues revealed the presence of starch granules and raphides (calcium oxalate crystals) and the wear-traces suggest the tool “*was hafted and used to plane or to scrape a starchy substance*” (Hardy et al., 2001, p. 10976). As said, for late Pleistocene starches, it is very difficult to “*identify the plant material to taxon*” (ibid.), however, the presence of raphides suggests they originated from starch-rich storage organs like rhizomes (USOs, Hardy et al., 2001). Intriguingly enough, our analysis of Surein I grinding stone revealed not only wear-traces compatible with plant processing, but OM and SEM inspection clarify the presence of both starches and raphides, that are conducive to interpret starch granules as pertaining to three to four different geophytes (Fig. 11). Our previous work on ground stones from Gravettian hunter-gatherer settlements clearly demonstrated the intentional tenderization of geophytes (USOs) such as *Typha* rhizomes, *Botrychium ternatum*, *Lactuca tuberosa* and *Arctium lappa* which were processed in the Italian peninsula and in Central Europe, therefore throughout the cold boreal territories (Longo et al., 2020a; Longo et al., 2018; Revedin et al., 2010; Skakun et al., 2019).

Ethnographic sources report starch-rich water-lilies rhizomes are foraged from spring to autumn across Eurasia northern latitudes (Gubanov et al., 2002). The use of baking or boiling USOs is well reported in the Far East to prepare a sort of porridge or soup, and kasha is still the traditional meal in Russia (Podmaskin, 2007). Also, it is acknowledged that mechanical processing, leaching and even roasting are necessary steps that make plant food bioavailable for the further metabolization occurring once plant foods are ingested. The practice of mechanical processing into flour and then drying the crumble, which is therefore losing most of the mass and the weight, reduces the bulkiness and concentrates the nutritional power and can readily be consumed or stored for a delayed utilization, making the staple starchy-food easy to store and to transport, i.e. a highly suitable reservoir for Palaeolithic mobile hunter-gatherers.

Plants are predictable and their perennating undersurface storage organs - meaning available all year round - are rich in highly nutritious carbohydrates and short-chain fatty acids, which enable plants to survive ecological and climatic downturns. This turns out to be vital even for *Homo sapiens* who was the hominin who could efficiently transform PSRSO into calorific food due to the ptialin, the salivary enzyme regulated by the AMY 1 gene that is duplicated in *Homo sapiens* but not in the archaic humans (Perry et al., 2015; Butterworth et al. 2016; Longo, forthcoming). It is our opinion that the step-change capacity of efficiently metabolizing highly calorific starch-rich food revealed a crucial adaptation to survive the adverse climatic conditions *Homo sapiens* faced throughout late MIS 3 “volcanic winters” (Golovanova et al., 2010), enabling *Homo sapiens* to access different nutrients in the course of climatic downturns occurring at the northern latitudes. The Surein I grinding stone was



possibly used to process geophytes (USOs) surviving in cold and more arid steppe, where mostly lean animals such as horses, deers and steppe bisons were supplying fats and meat. Direct isotopic evidence from the *Homo sapiens* burial retrieved Buran Kaya III supports that plant food consumption in EUP modern humans was significantly higher compared to the Neandertals diet, and the two species most probably co-existed in Crimea (Drucker et al., 2017), supporting they accessed different foods. Although still speculative, it is worth considering that starches might have supplied *Homo sapiens* with those nutrients that spared him from the constraints of a strictly carnivore diet (i.e. rabbit starvation; Speth and Spielmann, 1983; Speth, 2018).

## Final remarks

Altogether, our study enabled us to identify Surein I macrolithic tool as a grinding stone, used by *Homo sapiens* to mechanically process starch-rich storage organs into more easily bioaccessible chunks and gross-grained flour. Our approach represents a due methodological refinement, highly relevant towards establishing the ground stone's function within the elusive processing strategies of plant originated food since the very beginning of Early Upper Palaeolithic. We trust that our multi-scale contextual approach supports fine-grained data that Surein I task-specific macrolithic tool was used to process under surface starch-rich organs (USOs). Relying on the results of wear-traces and starch granules (including fibers and raphides) directly associated to the used areas, we speculate that *Homo sapiens*, while roaming across the Pontic Steppe, was foraging plants devoted to starch extraction from under surface storage organs (USO, such as cattail, cane, reed) by mechanically processing them into raw food. The chronology for modern humans' presence in south-eastern Europe, makes Surein I grinding stone the oldest direct evidence of intentional geophyte processing during Aurignacian settling, providing proof for a breakthrough in the dietary strategies of modern humanity.

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