

## Integrated multidisciplinary ecological analysis from the Uluzzian settlement at the Uluzzo C Rock Shelter, south-eastern Italy

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**ABSTRACT:** The Middle to Upper Palaeolithic transition, between 50 000 and 40 000 years ago, is a period of important ecological and cultural changes. In this framework, the Rock Shelter of Uluzzo C (Apulia, southern Italy) represents an important site due to Late Mousterian and Uluzzian evidence preserved in its stratigraphic sequence. Here, we present the results of a multidisciplinary analysis performed on the materials collected between 2016 and 2018 from the Uluzzian stratigraphic units (SUs) 3, 15 and 17. The analysis involved lithic technology, use-wear, zooarchaeology, ancient DNA of sediments and palaeoproteomics, completed by quartz single-grain optically stimulated luminescence dating of the cave sediments. The lithic assemblage is characterized by a volumetric production and a debitage with no or little management of the convexities (by using the bipolar technique), with the objective to produce bladelets and flakelets. The zooarchaeological study found evidence of butchery activity and of the possible exploitation of marine resources, while drawing a picture of a patchy landscape, composed of open forests and dry open environments surrounding the shelter. Ancient mitochondrial DNA from two mammalian taxa were recovered from the sediments. Preliminary zooarchaeology by mass spectrometry results are consistent with ancient DNA and zooarchaeological taxonomic information, while further palaeoproteomics investigations are ongoing. Our new data from the re-discovery of the Uluzzo C Rock Shelter represent an important contribution to better understand the meaning of the Uluzzian in the context of the Middle/Upper Palaeolithic transition in south-eastern Italy. © 2021 The Authors. *Journal of Quaternary Science* Published by John Wiley & Sons Ltd.

**KEYWORDS:** ancient DNA of sediment; lithic technology; Uluzzian; zooarchaeology; ZooMS

### Introduction

The Middle to Upper Palaeolithic (MP/UP) transition corresponds to the period between around 50 000 and 40 000 years ago and is a key period of change in the prehistory of the Old World. This timespan covers the last millennia of Neanderthal presence in the fossil record, together with the appearance of Modern Human (MH) populations in Europe (Benazzi *et al.*, 2011a; Douka *et al.*, 2014; Higham *et al.*, 2014).

Mediterranean Europe, and in particular Italy, plays an essential role in the study of this period due to: (i) its geographical position and ecological variability; (ii) the presence of key archaeological sites dating back to the MP/UP transition; and (iii) the evidence of different techno-complexes – such as Late Mousterian, Uluzzian and Protoaurignacian – associated with human fossil remains. In the Palaeolithic context of Mediterranean Europe, Neanderthals are associated with Mousterian assemblages, while, according to Benazzi *et al.* (2011b, 2015) MHs are associated with Uluzzian and Protoaurignacian assemblages.

The Uluzzian is one of the first lithic assemblages related to the arrival of MHs in Europe. It is characterized by its own

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originality and coherence, observable in lithic technology (Riel-Salvatore, 2009, 2010; De Stefani *et al.*, 2012; Moroni *et al.*, 2013, 2018; Ronchitelli *et al.*, 2018; Villa *et al.*, 2018; Peresani *et al.*, 2019; Arrighi *et al.*, 2020a; Collina *et al.*, 2020; Marciani *et al.*, 2020a), manufacturing of formal bone tools (d'Errico *et al.*, 2012; Peresani *et al.*, 2016; Villa *et al.*, 2018; Peresani *et al.*, 2019; Arrighi *et al.*, 2020a) and ornaments (Arrighi *et al.*, 2020b, 2020c), which point towards the sharing of common behaviours among its social groups and group members.

From a technological point of view, the Uluzzian is in fact characterized by the following. (i) A specific conceptualization of production that consists in the application of a straightforward debitage method, which implies the collection of raw blocks presenting convexities, angles and guide ribs appropriate for knapping; the striking platform being either opened by a single or few removals, or not opened at all by using a natural or cortical plan; the debitage surfaces are roughly managed, and the production of blanks follows unidirectional, bidirectional or orthogonal directions. (ii) The deliberate selection of the bipolar technique (aiming at the production of small blades/bladelets and small flakes/flakelets). (iii) The idea of a 'simple' production for complex tools (the use of composite tools) (Riel-Salvatore, 2009, 2010; Moroni *et al.*, 2013, 2018; Sano *et al.*, 2019; Collina *et al.*, 2020; Marciani *et al.*, 2020a).

Among retouched tools we note the production of lunates (tools characterized by a curved backed side opposite to a rectilinear cutting edge), which at Grotta del Cavallo were used as armatures (Sano *et al.*, 2019), and the systematic production of end-scrapers (Palma di Cesnola, 1964, 1993, 2004; Gambassini, 1997).

Zooarchaeological data suggest that human adaptive strategies changed over time to cope with variations in the local topographic and ecological conditions, as well as to deal with the uncertainty of resource availability (Romandini *et al.*, 2020). However, while in southern Italy more substantial data on the distribution of ungulate limb elements might point to a marked change in ungulate exploitation between the Late Mousterian and the Early Upper Palaeolithic (Boscato and Crezzini, 2012; Romandini *et al.*, 2020), in northern sites a higher variety of processing techniques were already present at the beginning of the Late Mousterian (Romandini *et al.*, 2020). Accordingly, our understanding of the Uluzzian is far from exhaustive, and additional evidence is required to empirically test inferences regarding Late Mousterian, Uluzzian and Protoaurignacian contexts.

The Uluzzo C Rock Shelter provides another piece of evidence towards a better understanding of the role of the Uluzzian within the MP/UP transition in south-eastern Italy. The site is located in the Bay of Uluzzo, where numerous Uluzzian sites have already been reported (Moroni *et al.*, 2013, 2018; Marciani *et al.*, 2020a) – including Grotta del Cavallo – and where the Uluzzian was first discovered and described (Palma di Cesnola, 1964). Moreover, the site of Uluzzo C includes a long stratigraphic sequence composed of several levels, including Romanellian, Uluzzian and a long and rich Mousterian sequence. Here we report the results from our multidisciplinary investigation on the sediments from the cave entrance and the archaeological materials from Uluzzo C Rock Shelter, which were collected during previous and new excavations (i.e. in the 1960s and between 2016 and 2018). Our approach includes the analysis of lithic technology and use-wear, zooarchaeological remains, sedimentary DNA and palaeoproteomics to provide further insights into the lithic behaviour (as far as reduction sequences, goals and knapping techniques are concerned) and the hunting strategies of the human groups that inhabited Uluzzo C during the MP/UP transition. Moreover, we present palaeoecological evidence on the composition of the landscape surrounding the rock shelter during this period.

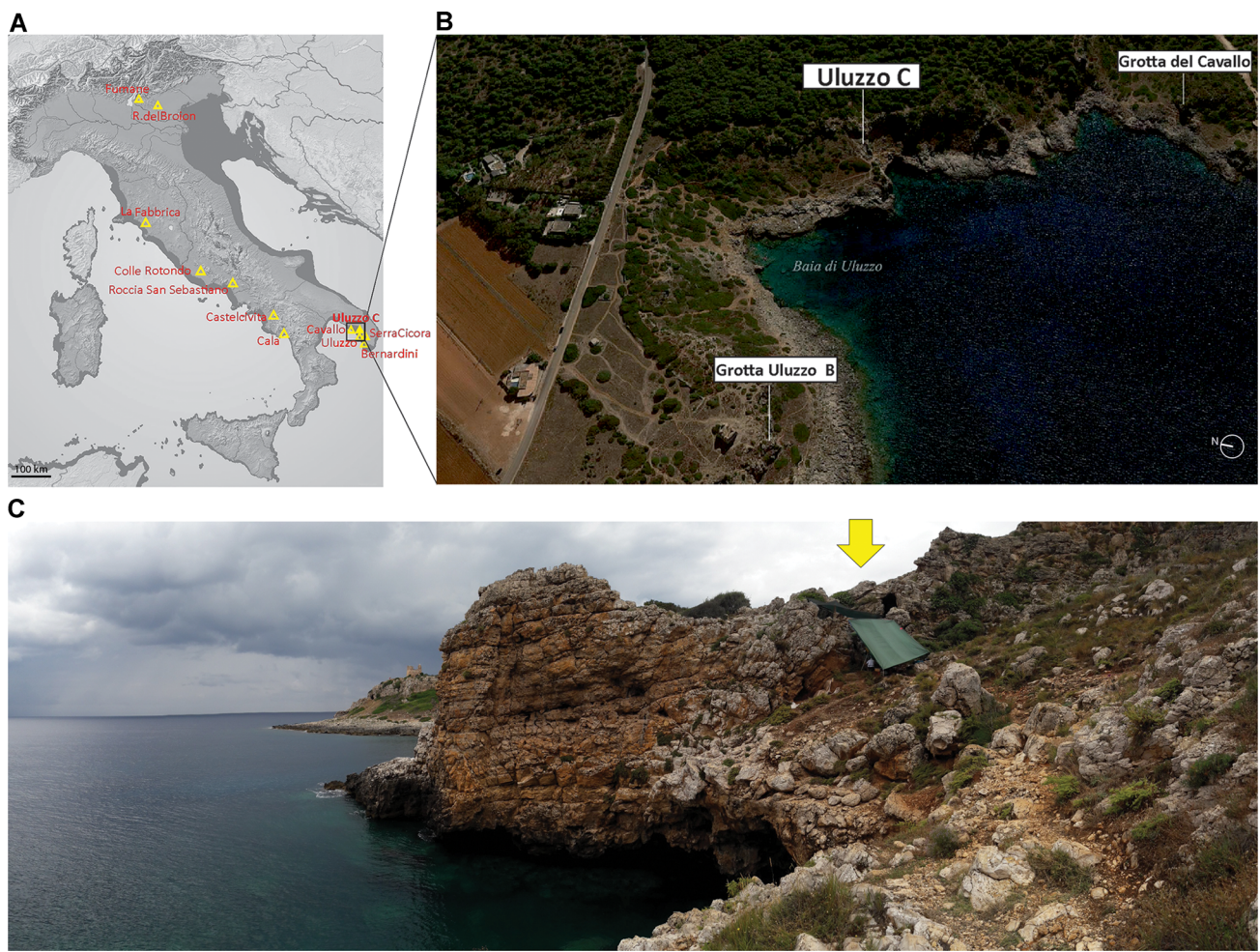
## Archaeological and chronological context

The Uluzzo C Rock Shelter is located in the Parco Naturale di Porto Selvaggio (Nardò, Lecce, southern Italy), on the western side of the Apulian Coast facing the Ionian Sea (40°9'27.84"N, 17°57'35.34"E) (Fig. 1). The Uluzzo C site was discovered during archaeological investigations carried out in the area in the 1960s by the Italian Institute of Prehistory and Protohistory and led by Borzatti von Löwenstern (Borzatti von Löwenstern, 1965; Borzatti von Löwenstern and Magaldi, 1966). During this first exploration, the stratified deposit of the cave yielded significant lithic assemblages that spanned from the Mousterian to the Bronze Age, including the Uluzzian techno-complex.

Today, the site is formed by a central hall with a smaller cavity on its right, whereas the stratigraphic sequence consists of 10 different layers (Borzatti von Löwenstern, 1965). From a cultural point of view, the stratigraphic sequence of Uluzzo C includes: a Romanellian layer (A); a sterile layer (B); two Uluzzian layers (C, D); a layer composed of a mixture of Upper Palaeolithic and Mousterian deposits (E); and a large sequence of Mousterian layers (F–L) (Borzatti von Löwenstern, 1965; Borzatti von Löwenstern and Magaldi, 1966; Spinapolice, 2018) (Fig. 2A). This sequence was confirmed by recent excavations, as well as by sedimentological and micromorphological analyses (for a detailed description see Spinapolice *et al.* in this special issue), which further detailed the sequence by identifying 22 stratigraphic units (SUs) within the layers originally identified by Borzatti.

In the new excavation, the site of Uluzzo C was divided into three sectors: A, B and C. Sectors A and B are located inside the rock shelter and correspond to the surface of the actual deposit (sector A) and to the bottom of Borzatti's trench (sector B) (Fig. 2B), whereas sector C is outside the rock shelter and has been explored to identify possible archaeological deposits on the terrace (Fiorini *et al.*, 2018, 2019). The recent investigations (2016–2018) focused on the area inside the rock shelter (sector A) and involved the stratigraphic excavation of the Uluzzian occupation (Borzatti's layer C), which in the new excavations corresponds to SUs 3, 15 and 17. Samples for sedimentological analysis and optically stimulated luminescence (OSL) dating, however, were not taken only from this layer, but from the entire stratigraphic succession accessible in sector A (i.e. Borzatti's layers B, C–L) (Fig. 2).

We performed specific investigations on sediments. The technical details of the sedimentological and micromorphological analyses and of the OSL dating campaign, as well as the individual OSL ages, are reported in Spinapolice *et al.* (this issue). Based on this single-grain OSL chronology, the grand weighted mean age for the Uluzzian occupations – layers C, D and E (OSL samples ULOC 3, 4 and 5) is  $40.6 \pm 1.4$  ka (Fig. 2A). These OSL time constraints generated for the Uluzzian layers at Uluzzo C match the chronology of the main Uluzzian sequences of the region (e.g. Grotta del Cavallo). The Uluzzian occupation of Grotta del Cavallo (which probably corresponds to layers D and E at Uluzzo C) is constrained to between  $45.5 \pm 1.0$  ka (Layer Fa/Y-6) and  $39.85 \pm 0.14$  ka (CI) by Zanchetta *et al.* (2018). At Uluzzo C the Mousterian layer that underlies the Uluzzian complex (Layer G) has been OSL dated as well (i.e.  $46 \pm 4.0$  ka, Layer G – OSL sample ULOC 1; Fig. 2A) (Spinapolice *et al.* in this special issue). Hence, despite the stratigraphic diversity and difficulties in matching the Uluzzian layers between Grotta del Cavallo and Uluzzo C, the chronological constraints for the end of the Mousterian appear to be congruent in the two caves:  $45.5 \pm 1.0$  ka at Grotta del Cavallo (Zanchetta



**Figure 1.** A. Uluzzian sites in Italy. B. Location of Uluzzo C in the bay of Uluzzo (Apulia, southern Italy) from Google Earth. C. General view of the site. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)].

*et al.*, 2018) and OSL dated at  $46 \pm 4.0$  ka at Uluzzo C (Layer G – ULOC 1) (Spinapolice *et al.* in this special issue).

## Materials and methods

This paper considers the archaeological finds (i.e. lithics and faunal remains) and sediment samples from the SUs 3, 15 and 17 of the new excavations (2016–2018), which correspond to Borzatti's layer C. In addition, it includes the lithics from layers C, D and E from the excavations carried out by Borzatti during the 1960s (Tables S1–S2). SUs 3, 15 and 17 are three components of the same level of occupation (Layer C) (Fig. 3).

The whole lithic assemblage was analysed by using a technological approach. Furthermore, traceological analysis, with both low- and high-power approaches, was performed on the six retouched items.

Taxonomic and taphonomic evaluations were performed on the macromammal, micromammal, avifaunal and malacofaunal remains. Moreover, DNA analysis of sediments and palaeoproteomics were used to corroborate the zooarchaeological studies.

### Lithic technological analysis

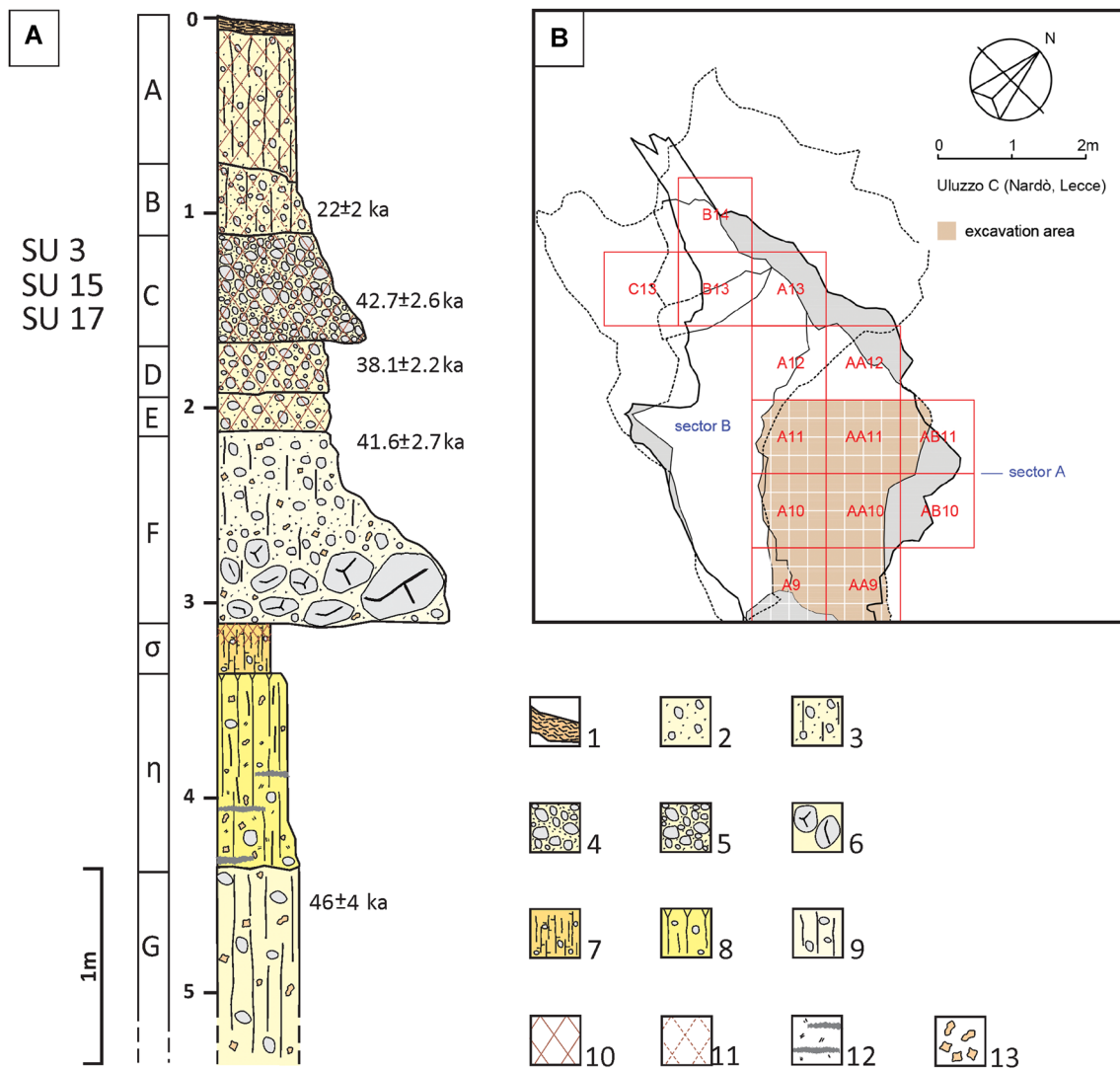
The technological analysis was carried out on the lithic materials coming from layers C, D and E of Borzatti's excavations and on the materials coming from SUs 3, 15, 17 of the new excavations.

We decided to include all the layers excavated by Borzatti because of the limited number of the items (i.e. layer C, corresponding to the SUs 3, 15 and 17 included only five lithic items).

Borzatti von Lowerstern and Magaldi (1966) report retrieving 145 lithic items from layers C, D and E. Currently, only 61 of these pieces are stored at the Museo della Preistoria di Nardò (MPN) (Table S1). A total of 339 pieces from SUs 3, 15 and 17 of the new excavations were here analysed (Table S2).

This lithic assemblage was analysed by using a technological approach: Geneste (1991) was used as a fundamental conceptual text on how to approach the reduction sequences; Inizan *et al.* (1999) was used for the definition of technological categories, whereas Boëda (2013) was used to approach lithic technology and, more precisely, regarding the description of the cores. All the archaeological material was sorted according to the lithology and texture of the raw material (chert, jasper, siliceous limestone, limestone and quartz sandstone), the colour of the cortex, the colour of the inner portion of each item, and the presence and type of post-depositional alteration (chemical, mechanical or thermal). Subsequently, items were divided into five dimensional classes (DC) (DC1: 0–50 mm<sup>2</sup>, DC2: 50–100 mm<sup>2</sup>, DC3: 100–150 mm<sup>2</sup>, DC4: 150–200 mm<sup>2</sup>, DC5: >200 mm<sup>2</sup>) on the basis of the area covered by each specimen (Marciani *et al.*, 2020b; Spagnolo *et al.*, 2020). Complete items that were larger than the first DC were additionally measured according to both their technological and morphological axes.





**Figure 2.** A. Log of the stratigraphic sequence of the deposit of the Uluzzo C Rock Shelter. For a full interpretation of the log the reader is referred to Spinapolice *et al.* (in this special issue). OSL dating results are indicated. Key: (1) flowstone; (2) sandy unit with rock fragments and bioturbation; (3) sandy unit with rock fragments and bioturbation; (4) clast-supported breccia displaying weak oblique lamination (sandy matrix); (5) matrix-supported breccia (sandy matrix); (6) large blocks due to roof collapse; (7) clay-rich deposit with scarce rock fragments; (8) slightly weathered silty deposit with scarce rock fragments; (9) silty deposit with scarce rock fragments; (10) CaCO<sub>3</sub>-cemented deposit; (11) weakly CaCO<sub>3</sub>-cemented deposit; (12) charcoal fragments and ash-rich lenses; (13) CaCO<sub>3</sub> nodules and/or concretions. B. New excavation in sector A. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

As the material recorded at Uluzzo C is few in number, and the Uluzzian techno-complex is characterized by an absence of standardization of the categories, in particular regarding production (i.e. Collina *et al.*, 2020), we decided to define the categories of blank based on metric attributes.

Thus, we considered the following technological categories: flakes and blades based on the ratio between length and width, i.e. flakes (ratio length/width ratio < 2) and blades (length/width ratio ≥ 2) (Laplace, 1966). This definition of flakes and blades was used for the Uluzzian layer EIII of Grotta del Cavallo (Moroni *et al.*, 2018). Moreover, flakes whose length is <2.5 cm are named flakelets (<2.5 cm), whereas blades whose length is <2.5 cm are named bladelets (<2.5 cm). The other technological classes are cores, pebbles, debris (items smaller than DC1) and indeterminates (fragmented pieces bigger than DC3, altered pieces, non-orientatable pieces).

For complete flakes, flakelets, blades and bladelets, we registered the localization and extension of the cortex. We identified the concept and methods of debitage and considered: volumetric aspects (morphology, symmetry, profile and section shape); the number and orientation of dorsal scars; the type of butt and bulb; and the position of the impact point (if

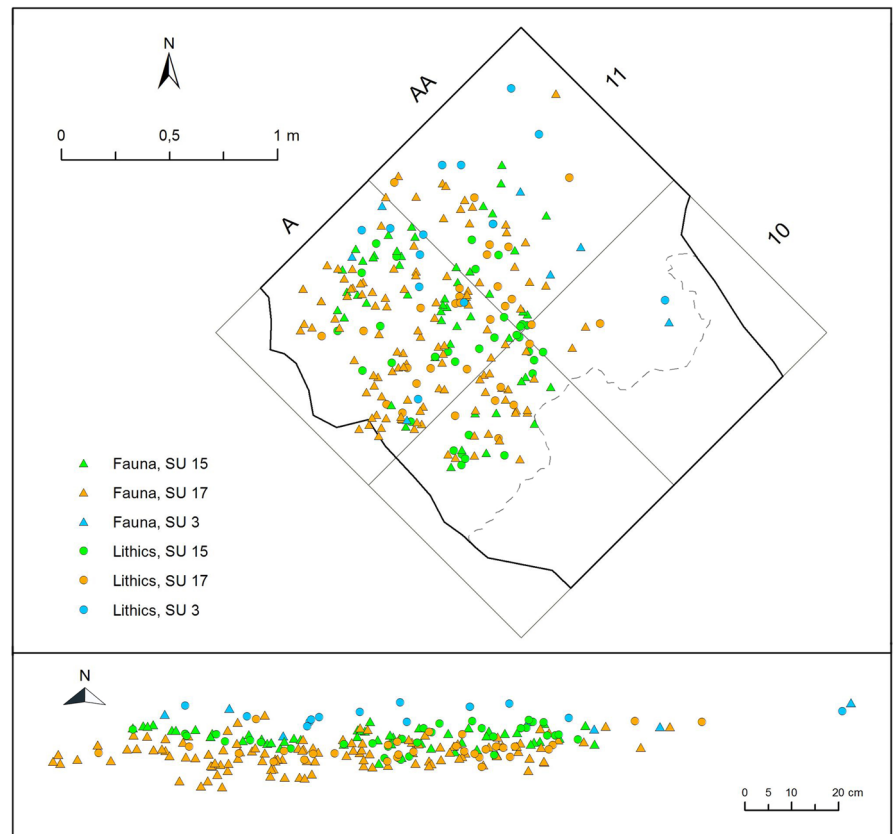
present). Lastly, the occurrence, type and localization of the retouch or use-wear were also noted.

For each core we observed: the nature and morphology of the raw block; the volumetric conception of the exploitation; the hierarchy of surfaces; the type, location and preparation of the striking platform; the number and direction of the negatives on the surface of the debitage; the level of exhaustion and the possible reason for its abandonment.

Use of the bipolar technique on an anvil was evaluated on the basis of specific traits that characterized this kind of technique: rectilinear longitudinal profile of the ventral face, similar ventral and dorsal faces, pronounced ripple marks, shattered point-form or linear butts, diffused impact points, sheared bulbs of percussion, and the presence of a parasite scar (e.g. Guyodo and Marchand, 2005; Bietti *et al.*, 2010; Soriano *et al.*, 2010; Duke and Pargeter, 2015; de la Peña, 2015; Collina *et al.*, 2020).

### Lithic use wear analysis

To evaluate the functional potential of the lithic assemblage from Uluzzo C, a preliminary use-wear analysis was carried



**Figure 3.** Vertical and horizontal distribution of lithic and faunal remains of SUs 3, 15 and 17 from Uluzzo C Rock Shelter. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

out on the retouched items recovered during the 2016–2018 excavations (six specimens). Traceological analysis was undertaken following both the low-power approach (LPA) (Tringham *et al.*, 1974; Odell and Odell-Vereecken, 1980; Odell, 1981) and the high-power approach (HPA) (Keeley, 1980; Plisson, 1985; Van Gijn, 2010). Traces were observed by means of a Hirox KH-7700 3D digital microscope using an MX-G 5040Z body equipped with an AD-5040 Lows and an AD-5040HS lens working at low magnification (20–50 $\times$ ) to observe the macro-traces (fractures, edge damage, diagnostic impact fractures), and an MXG-10C body and an OL-140II lens (140–480 $\times$ ) used to analyse micro use-wear (polishes, abrasions and striations). Before examination with the microscope, the artefacts were washed in fresh water and subsequently cleaned with pure acetone to remove traces of soil and finger grease.

### Macromammal remains and zooarchaeological analysis

Identifications of both the skeletal element and the taxa were based on the reference collection stored at the Laboratory of Osteoarchaeology and Paleoanthropology (BONES Lab) of the Department of Cultural Heritage of the University of Bologna (Ravenna, Italy). Microscopic analysis of the bone surfaces was carried out using a Leica stereomicroscope. To identify the nature of the surface alterations on the bones, and to discriminate human from animal traces, trampling abrasion and modern mechanical modifications produced by excavation tools, a well-established taphonomic literature was used as a reference (Binford, 1981; Potts and Shipman, 1981; Shipman, 1981; Brain, 1983; Shipman and Rose, 1984; Blumenschine and Selvaggio, 1988; Capaldo and Blumenschine, 1994; Lyman, 1994; Blumenschine, 1995; Fisher, 1995). The degree of combustion was evaluated according to Stiner *et al.* (1995). Sex and age at death were estimated to reconstruct the exploitation strategies of the different species

(Aitken, 1974; Mariezcurrena and Altuna, 1983; Vigal and Machordom, 1985; D'Errico and Vanhaeren, 2002; Fiore and Tagliacozzo, 2006). To evaluate species abundance, the number of identified specimens (NISP) was considered (Grayson, 1984).

### Micromammal sorting and palaeontological and taphonomic study

The few recovered micromammal fossil remains analysed in this study were disarticulated mandible fragments and isolated teeth (which were collected by water screening the sediments from the excavation of 2018 with a 0.5-mm mesh screen). The fragments were identified following the general criteria given by Galán-García (2019) for bats and rodents (Berto, 2013). The specific attribution of this material is based principally on the most diagnostic elements: mandible for the genus *Myotis*; and mandible and isolated teeth for the subfamily Arvicolinae and genus *Apodemus*. Moreover, the identified remains were grouped by using the minimum number of individuals (MNI) method, by which we determined the sample by counting the most represented diagnostic elements. Finally, a preliminary taphonomic study was performed to investigate the alterations caused by digestion, which were present in the first lower molars of the arvicoline rodent species (according to Andrews, 1990; Fernández-Jalvo *et al.*, 2016a), ultimately attesting to the action of predation.

### Avifaunal remains

The fossil avifauna from Uluzzo C analysed in this contribution consists of 12 bone remains. The bird remains were recovered after dry/wet sieving of the sediment, numbered with the acronym UC and subjected to taxonomic and taphonomic analyses. Bone identification was possible thanks to the comparison with modern skeletal specimens from two comparative collections: the Marco Pavia Osteological

Collection at the Department of Earth Sciences of the University of Torino and the collection at the Department of Humanities of the University of Ferrara. For identification of the remains, we also used osteology handbooks on particular bird families and orders (Janossy, 1983; Tomek and Bochenki, 2000). Taphonomic analysis was carried out with a 30× lens and a Leica S6D Verde Ough 0.75–70× stereomicroscope, available at the Laboratory of Archaeozoology and Taphonomy of the Prehistoric and Anthropological Sciences Section of the University of Ferrara.

### Malacofaunal remains

The classification and nomenclature used for taxonomic analysis of the malacofaunal remains were based on the updated datasets available online on the World Register of Marine Species (WoRMS). The NISP was used to define the number of specimens in the assemblage. The taphonomic study focused on three main groups of alterations (Claassen, 1998): pre-depositional alterations (e.g. marine abrasion, predation by other molluscs and bioerosion); intentional/unintentional anthropogenic alterations (e.g. thermic alterations, anthropic damage caused by consumption); and post-depositional transformations (e.g. fragmentation, abrasions, root marks, decalcification and excavation damage).

### ZooMS

We selected 12 unidentified bone samples (>1 cm in size) for zooarchaeology by mass spectrometry (ZooMS) analysis. We tested the protocol designed by Van Doorn *et al.* (2011) and used a warm (65 °C) ammonium bicarbonate buffer (50 mM) to leach bone collagen without acid digestion. Then, trypsin digestion was carried out for 18 h at 37 °C using 0.5 µL of sequencing-grade trypsin (Sigma). Enzymatic digestion was ended using 5 µL of 5% formic acid (FA), then the tryptic digests were purified and concentrated using C18 SpinTips (Thermo Scientific). Peptide elution was performed with 15 µL of 50% acetonitrile (ACN)/0.1% FA (v/v). Samples were dried overnight under a class 100 laminar flow hood. After re-suspension, each sample (1 µL) was spotted on a target steel plate, and mixed with 1 µL α-cyano-4-hydroxycinnamic acid (CHCA; Sigma) as matrix. The samples were then analysed in duplicate with MALDI-ToF (Bruker) over a mass-to-charge range of 700–3500 *m/z*. Spectra were manually inspected and averaged using mMass (Strohalm *et al.*, 2010), after setting a signal-to-noise ratio equal to 4. Taxonomic identification was performed comparing identified peptides with a database of peptide markers for all European, Pleistocene medium to large size mammals (Welker *et al.*, 2016).

### Ancient DNA analysis

A total of 14 sediment samples, which were collected from the site on two different occasions, were tested for the preservation of ancient faunal or hominin DNA. The first set consisted of 11 samples from squares A11 and AA11 in SU 15. The second set was composed of three samples collected from square A11 in SU 17 (Table S3). Subsamples of each sample, ranging between 42 and 100 mg, were used as input for DNA extraction using a silica-based protocol (Dabney *et al.*, 2013), which was performed either manually or using a liquid handling platform (Bravo NGS workstation, Agilent Technologies) as described by Rohland *et al.* (2018).

All subsequent laboratory procedures were performed on the liquid handling platform as described by Slon *et al.* (2017). A single-stranded DNA library was prepared from each DNA

extract using the procedure described by Gansauge *et al.* (2017, 2020), the efficiency of which was assessed by quantitative PCR. The number of library molecules generated from sediment samples was higher than those prepared from associated negative controls (Table S3), which demonstrates that the library preparation procedures were successful (Gansauge and Meyer, 2013).

The number of molecules generated from a control oligonucleotide was similar between the sediment libraries and the controls, thus indicating that potential inhibitory substances that may have been co-extracted with the sediment were not interfering with the library preparation procedure to an appreciable extent (Glocke and Meyer, 2017).

Each library was then amplified to PCR plateau and tagged with two indices (Kircher *et al.*, 2012). Libraries originating from sample set 1 were enriched by hybridization capture for mammalian mitochondrial DNA (mtDNA) (Slon *et al.*, 2016), as well as specifically for human mtDNA (Maricic *et al.*, 2010; Fu *et al.*, 2013), while libraries from set 2 were only enriched for human mtDNA. Paired-end sequencing was performed on an Illumina MiSeq platform using 76 cycles. We note that all sample libraries were sequenced to an adequate depth, as indicated by the duplication rate observed (Table S3). Negative controls for the DNA extraction and library preparation procedures were carried along with the samples.

The processing of sequencing data was performed as described in detail by Slon *et al.* (2017). After removing PCR duplicates and fragments shorter than 35 bases, taxonomic identification at the family level was carried out for each sequenced DNA fragment by comparing it to a database of reference mammalian mtDNA genomes (Altschul *et al.*, 1990; Huson *et al.*, 2007). At least 10 fragments and at least 1% of identified fragments were required to be assigned to a family for it to be deemed present in a sample. For each identified family, we then tested whether the DNA fragments assigned to it displayed elevated frequencies (i.e. significantly higher than 10%, tested using an exact binomial test) of terminal cytosine (C) to thymine (T) nucleotide substitutions compared to a reference genome. These substitutions are a typical feature of ancient DNA and allow us to determine whether ancient DNA fragments are present in a sample (Briggs *et al.*, 2007). None of the negative controls tested positive for the preservation of ancient DNA (Table S3).

## Results

### Lithic technology

#### Technological analysis: Borzatti's excavation

The 61 lithic artefacts from Borzatti's excavation in 1964 (five items from layer C, six items from layer D, 50 items from layer E), which are stored at the Museo della Preistoria di Nardò (Lecce, Apulia), all have fresh margins, except for nine items with blunted edges. Moreover, 11 pieces show traces of a yellow or white patina, and traces of combustion are visible on four items. Generally, their state of preservation is coherent with the lithics coming from the current excavations. In all the layers there is a predominance of fine-grained chert, followed by siliceous limestone. Occasional limestone and jasper are attested to in layer E (Table 1). Most of the pieces, corresponding to 57.4% of the collection, pertain to DC 5 (corresponding to pieces >200 mm<sup>2</sup>) (Tables 2 and 3). No lithics pertain to DC 1 (0–50 mm<sup>2</sup>) and only seven pertain to DC 2 (50–100 mm<sup>2</sup>) (Table 2). After comparison with the lithics from the more recent excavation (see below), it is evident that a selection bias must have affected the retrieval of

**Table 1.** Raw material (*Excavation Borzatti*)

Raw material	Layer C	Layer D	Layer E	Total	%
Chert	3	4	38	45	73.8
Siliceous limestone	2	2	7	11	18.0
Limestone	0	0	4	4	6.6
Jasper	0	0	1	1	1.6
Total	5	6	50	61	100

**Table 2.** Dimensional classes (*Excavation Borzatti*)

Dimensional classes	Layer C	Layer D	Layer E	Total	%
DC 2	0	0	7	7	11.5
DC 3	0	1	7	8	13.1
DC 4	1	0	10	11	18.0
DC 5	4	5	26	35	57.4
Total	5	6	50	61	100

the lithic artefacts in the 1960s, probably because researchers were more interested in large diagnostic items, such as retouched pieces. Given the biased composition of the collection and the high degree of the retouch, it was not possible to determine from which reduction sequence these items had come from.

Layer C is represented by only five items, and noteworthy is the presence of two cores that were reduced by the bipolar technique, one of which was then retouched (Fig. 4C). Layer D presents six items, among which there are two flakes and one bladelet. Layer E is the one with the most items (50), including one core, 16 flakelets, eight flakes and five blades. Complete cortex coverage is present in only two flakes, whereas three flakes present between 50 and 75%; all the other items present between 25% and 0 cortex coverage. The bipolar technique is attested to on 18 items in almost all the categories (Table 3).

The assemblage includes 20 retouched pieces made mainly on flakes, flake fragments and indeterminate fragments. Most of the retouched tools occurred on pieces with a length >3 cm. Typologically, there is a prevalence of scrapers ( $n=11$ ). Noteworthy is the presence of one end scraper and two lunates (Fig. 4A <B). The latter is the most representative retouched tool of the Uluzzian (Table 4).

From a diachronic point of view, chert is the prevailing raw material in all technological classes in the three layers. A greater variety of raw material and greater quantity of pieces is found in layer E. Layers C and D present very few entire pieces,

in both cases the majority being retouched tools. Layer E displays a set of large retouched tools and a smaller component of flakelets (Fig. 5).

#### Technological analysis: 2016–2018 excavation

The industry is not particularly affected by post-depositional alterations and there are no pieces presenting double patinas. All lithics have fresh margins, except for two pieces that present blunt edges, and only six pieces show traces of combustion.

Regarding the raw materials, we observe a selection of fine-grained raw materials of different nature: nine pieces were extracted from pebbles, four pieces from slabs of local siliceous limestone and 11 from local chert lists. Even without evident traces of cortex, it was still possible to associate the siliceous limestone with the local outcrops, used in the nearby sites throughout the Mousterian and the Uluzzian (Spinapolice, 2012, 2018; Moroni *et al.*, 2013; Spinapolice *et al.*, 2018). The chert, by contrast, is partly local (white and blue chert), coming from the same outcrops where the siliceous limestone was found, and partly (beige or very glassy grey chert) similar to the materials of the Apennines or of the Gargano area. We also noted the sporadic presence of jasper and quartzite (Table 5), probably coming from the Bradano River basin (Basilicata, southern Italy).

Most of the lithics, corresponding to 80.7% of the collection, are included in the first (71.4%) and second (11.5%) dimensional classes (Table 6). Most of them (250 items) are debris, which is the waste of the debitage and indicates that flaking activities were performed *in situ*. For these fragments, it is not possible to detect the concept of debitage. Among these artefacts, 26.2% (corresponding to 89 items) are the more diagnostic pieces, which are useful to infer information on the reduction sequences performed at the site. The initialization of the reduction sequence is attested to by only two semi-cortical flakes, and hence the first stage of debitage is not documented at the site (or at least in the portion of the site under investigation).

The phases of full debitage are mainly attested to by the production of flakelets (Fig. 6), blades and bladelets (Fig. 7) (Table 7). Also, a significant number of fragmented flakes and fragmented blade-bladelets are present (Table 7). The blanks show predominantly unidirectional scar patterns ( $n=43$ ). Some blanks show convergent ( $n=8$ ), orthogonal ( $n=8$ ) or bidirectional ( $n=5$ ) scar patterns. Most pieces exhibit a rectilinear profile ( $n=58$ ), but there are some that present a convex ( $n=7$ ), wavy ( $n=4$ ) or concave ( $n=2$ ) profile. The butts are mainly flat ( $n=15$ ), point-form ( $n=15$ ) or linear

**Table 3.** Technological categories: R indicates the retouched pieces, B indicates the pieces produced by the bipolar technique (*Excavation Borzatti*)

Technological category	Layer C			Layer D			Layer E			Total		
	Total	R	B	Total	R	B	Total	R	B	Total	R	B
Cortical flakes							2			2		
Flakes	1	1R		2	1R		8	3R	1B	11	5R	1B
Flakelets <2.5 cm							16	1R	6B	16	1R	6B
Flakes fragmented				2	2R		7	2R	2B	9	4R	2B
Blades							5	3R	4B	5	3R	4B
Bladelets <2.5 cm	1			1	1R	1B				2	1R	1B
Cores	2	1R	2B				1	1R		3	2R	2B
Indeterminate fragment >DC 3	1			1	1R	1B	7	3R	1B	9	4R	2B
Debris DC 1–2							4			4		
Total	5	2R	2B	6	5R	2B	50	13R	14B	61	20R	18B