



# Microremains from El Mirón Cave human dental calculus suggest a mixed plant–animal subsistence economy during the Magdalenian in Northern Iberia



Robert C. Power<sup>a, \*</sup>, Domingo C. Salazar-García<sup>b, c, d, e</sup>, Lawrence G. Straus<sup>f, g</sup>, Manuel R. González Morales<sup>g</sup>, Amanda G. Henry<sup>a</sup>

<sup>a</sup> Research Group on Plant Foods in Hominin Dietary Ecology, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

<sup>b</sup> Department of Archaeology, University of Cape Town, Cape Town, South Africa

<sup>c</sup> Departament de Prehistòria i Arqueologia, Universitat de València, València, Spain

<sup>d</sup> Aix Marseille Université, CNRS, Ministère de la culture et de la communication, LAMPEA UMR 7269, 13090 Aix-en-Provence, France

<sup>e</sup> Department of Human Evolution, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany

<sup>f</sup> Department of Anthropology, University of New Mexico, Albuquerque, NM, USA

<sup>g</sup> Instituto Internacional de Investigaciones Prehistóricas de Cantabria, Universidad de Cantabria, Santander, Spain

## ARTICLE INFO

### Article history:

Available online 13 April 2015

### Keywords:

Upper Palaeolithic  
Archaeobotany  
Palaeolithic diet  
Starch grains  
Phytoliths  
Fungus

## ABSTRACT

Despite more than a century of detailed investigation of the Magdalenian period in Northern Iberia, our understanding of the diets during this period is limited. Methodologies for the reconstruction of Late Glacial subsistence strategies have overwhelmingly targeted animal exploitation, thus revealing only a portion of the dietary spectrum. Retrieving food debris from calculus offers a means to provide missing information on other components of diet. We undertook analysis of human dental calculus samples from Magdalenian individuals (including the “Red Lady”) at El Mirón Cave (Cantabria, Spain), as well as several control samples, to better understand the less visible dietary components. Dental calculus yielded a diverse assemblage of microremains from plant, fungal, animal and mineral sources that may provide data on diet and environment. The types of microremains show that the individuals at El Mirón consumed a variety of plants, including seeds and underground storage organs, as well as other foods, including possibly bolete mushrooms. These findings suggest that plant and plant-like foods were parts of her diet, supplementing staples derived from animal foods. As faunal evidence suggests that the Magdalenian Cantabrian diet included a large proportion of animal foods, we argue here for a mixed subsistence pattern.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

Although the Magdalenian in France, Belgium and Germany is commonly characterised as the period of the ‘reindeer hunters’, this was not the dominant dietary pattern in other European regions. The subsistence of this period in general focused heavily on the hunting of large game, supplemented by fishing and fowling (Álvarez-Fernández, 2011). In the coastal environments along the Atlantic Coast of Northern Iberia, faunal profiles are dominated by ibex (*Capra pyrenaica*), and red deer (*Cervus elaphus*), with smaller

proportions of horse (*Equus* sp.), bison (*Bison* sp.), chamois (*Rupicapra rupicapra*), salmon (*Salmo salar*) and shellfish (e.g. Marín-Arroyo, 2009; Straus, 1977; Straus and González Morales, 2012). Red deer and ibex were the most commonly hunted species, perhaps due to their abundance, large size and gregarious behaviour.

However, there is little information on the use of vegetal resources in Northern Iberia. Plant remains including seeds from oak (*Quercus* sp.), hazelnut (*Corylus* sp.), raspberry (*Rubus* sp.), soft-grass (*Holcus* sp.), and chenopods (*Chenopodium* sp.) were recovered during flotation of Lower Magdalenian sediments from El Juyo Cave. Yet they are only tenuously associated to the occupation of the site, and may instead have been introduced by natural processes (Freeman et al., 1988). The only direct evidence for plant consumption in the region comes from a dental microwear study

\* Corresponding author. Tel.: +49 (0)341 3550 789, +49 (0)15237044289.

E-mail addresses: [robert\\_power@eva.mpg.de](mailto:robert_power@eva.mpg.de), [r.c.power@umail.ucc.ie](mailto:r.c.power@umail.ucc.ie) (R.C. Power).

carried out on two human teeth found in the Magdalenian levels of Rascaño Cave, roughly 20 km from El Mirón (Guerrero and Lorenzo, 1981). Microwear indicated that these individuals had mixed diets, and possibly even tending toward a degree of reliance on plant foods. This scarcity of evidence occurs despite there being a strong theoretical basis for a reliance on plant foods in some Upper Palaeolithic Iberian diets (e.g. Jones, 2009; Haws, 2004; Owen, 2002; Zaatari and Hublin, 2014), although it is likely that major variation was present within Iberia's complex topography and ecosystem. Even where plant biomass is low, plant consumption may have allowed circumvention of the energy ceiling created from relying on high protein ungulates (Speth, 2010). For this reason, it is important to expand our knowledge to the full range of diet.

El Mirón Cave provides a rare opportunity to look for direct evidence of plant use in Cantabrian Iberia, due in part to the wealth of information about the local and regional environments at the time of the Magdalenian burial, which is the subject of our study. The immediate environment around the site during the Lower Magdalenian was an open landscape with a low biomass of vegetal food. It was a hilly area dominated by heath, grasses and dwarf shrubs, with only some scattered pine (*Pinus* sp.), and rare juniper (*Juniperus* sp.) and birch (*Betula* sp.) (Straus et al., 2013). Pollen analysis of a sediment sample from the burial layer itself reveals a very cold open and only moderately humid landscape at the time of the burial, with some pines but a high percentage of chenopods (Iriarte-Chiapusso et al., 2015). Yet with the large ranging behaviour seen in this time period (Langley and Street, 2013), a Magdalenian hunter-gatherer likely had access to a much wider range of resources than what was found in the near vicinity. Available trees that may have been targeted as a source of nutritional and non-nutritional plant resources include Scots pine (*Pinus sylvestris* L.), birch (*Betula* sp.) and rarer temperate trees species such as oaks (*Quercus* sp.), hazel (*Corylus avellana* L.), linden (*Tilia* sp.) and elder (*Sambucus* sp.). These temperate species would have been found during periods of climatic amelioration (Carrión et al., 2010), but would have been confined to the narrow lowland coastal belt. The limited isolated areas where trees and damp meadows persisted could have supported several calorie-rich plant foods (e.g. mast of oak, hazelnut) and a wealth of other forms of vegetal foods such as fleshy, starch-rich rhizomes (e.g. *Bistorta vivipara* L. Delarbre, *Argentina anserina* L.). These plants resources (oak mast, hazelnut, *Bistorta officinalis*, *Argentina anserina*) are all foods whose consumption is well documented by recent Eurasian and North American northern populations (e.g. Cuenca-Bescós et al., 2009; Kuhnlein and Turner, 1991).

To investigate plant and other, less-understood foods consumed by the Magdalenian population in Northern Iberia, we analysed dental calculus from the human burial at El Mirón. Analysis of dental calculus is an emerging archaeobotanical method that can provide evidence on specific plant taxa and organs consumed during life (e.g. Armitage, 1975; Boyadjian et al., 2007; Henry et al., 2014; Horrocks et al., 2014; Salazar-García et al., 2013; Mickleburgh and Pagan-Jimenez, 2012), as well as information on other foods that produce morphologically distinct microremains, such as fungal spores and diatoms. Dental calculus forms due to precipitation of salivary calcium phosphate on the bacterial biofilm that covers the teeth, and therefore acts as a mineralised reservoir of the oral environment, including food fragments (Warinner et al., 2014; Power et al., 2014; Kucera et al., 2011; Boyadjian et al., 2007; Dobney and Brothwell, 1986). Studies of calculus in living populations demonstrate that the preserved food remains can reflect the known diet, though its coverage may be highly stochastic and it is constrained to foods where microremains are present. The dental calculus record may also reflect oral processing of non-dietary plants as well as plants introduced to the mouth through the

consumption of prey animal stomach contents (Buck and Stringer, 2013), but is expected to overwhelmingly preserve staple foods. The results from calculus analysis have been used to complement those from other methods of dietary reconstruction such as microwear (e.g. Estalrich et al., 2011) and stable isotopic analysis (e.g. Salazar-García et al., 2014a).

## 2. Methods

We examined the dental calculus from the Lower Magdalenian human burial in Level 504, also known as the “Red Lady”, to better understand the diet of this individual. The burial is from an adult female, who was deliberately interred (Straus et al., 2013). We also included in the study an isolated human molar from younger deposits dated to the late Lower or early Middle Magdalenian deposits (level 105 in Square W7). We first used scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) to study the elemental composition of a dental calculus sample, and to look for *in-situ* microremain inclusions on the surface of the calculus. Then, we performed optical microscopy on separate human dental calculus samples, along with control samples from faunal calculus, sediment and bone, looking for preserved microremains.

### 2.1. Calculus sampling

We collected dental calculus from four human teeth in the Laboratorio de Evolución Humana, Universidad de Burgos, Spain (Table 1). Three of the samples were from the “Red Lady” (samples eM1-3), and only one sample was from the isolated tooth of the second Magdalenian individual (sample eM5). The sampled teeth had a thick pristine band of hard and darkly stained supragingival calculus situated on the enamel surface. Each calculus band that was to be sampled was carefully photographed. The sampling surface was gently brushed clean with a sterile dry toothbrush. Then, a dental scaler was used to remove small areas of calculus (~4 mm<sup>2</sup>) onto aluminium foil (Table 1). Each sample was transferred a microcentrifuge tube and weighed with a microbalance (Mettler Toledo MX5). Finally, the teeth with the remaining *in situ* calculus were photographed. We also took a comprehensive variety of control samples to ensure replicability (Weyrich et al., 2015); one sample of plastic storage foam, eight of animal calculus from isolated fauna teeth, two of trabeculae exposed from aDNA sampling of the centre of a human phalange, and five of sediment from four localities near to where the human remains were recovered.

### 2.2. Electron microscopy analysis

We conducted SEM-EDX analysis at University College Dublin's Nano-Imaging and Materials Analysis Centre (NIMAC) in Dublin, Ireland. One sample (eM2) was examined to check if the sample chemically matched dental calculus, as well as to visualise microremains that might have been trapped on the sample's exterior. The calculus sample was mounted on a stub using double-sided carbon tape, and sputter coated with gold for 20 s using an Emitech K575X Sputter Coating Unit to prevent surface charging by the electron beam. The calculus was mounted so that the exterior surface was visible. We then examined the sample using a FEI Quanta 3D FEG DualBeam (FEI Ltd, Hillsboro, USA) SEM attached to an EDAX ED APOLLO XV Silicon Drift Detector with a 5–10 kV accelerating voltage. The EDX created chemical maps of all elements.

### 2.3. Optical microscopy analysis

The remainder of the human calculus from the two individuals, fauna calculus and sediment samples were examined using optical

**Table 1**  
Calculus samples from El Mirón analysed using electron microscopy and optical microscopy.

Sample	Type	Stratum	Find no.	Individual	Tooth	Tooth surface
Electron microscopy only						
eM2	Human calculus	504	–	“Red Lady”	LR M1	Distal, buccal
Optical microscopy only						
eM1	Human calculus	504	–	“Red Lady”	LR M1	Buccal
eM3	Human calculus	504	–	“Red Lady”	LR PM2	Lingual
eM5	Human calculus	105	180	Individual 2	M	–
eM6	Capra calculus	504	696	–	–	–
eM7	Capra calculus	17	5285	–	–	–
eM8	Capra calculus	17	5286	–	–	–
eM9	Capra calculus	–	5289	–	–	–
eM10	Capra calculus	17	6101	–	–	–
eM11	Site sediment	504	–	–	–	–
eM12	Site sediment	504	–	–	–	–
eM12b	Site sediment	504	–	–	–	–
eM13	Site sediment	105	–	–	–	–
eM15	Grey plastic storage foam	–	–	–	–	–
eM16	Human phalanx	506	1884	–	–	–
eM17	Human phalanx	506	1884	–	–	–

microscopy (OM) at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany. Unlike many other studies of archaeological calculus, we did not use hydrochloric acid (HCl) as a means to break down the calculus. In a clean laminar flow cabinet set to positive pressure, the archaeological and control samples were wet ground with a micro pestle in a 1.5 ml micro-centrifuge tube containing ~25 µl of a 25% glycerine (C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>) solution to reduce sample loss due to static electricity. The samples were then centrifuged at 2000 × g (Carl Roth Rotilabo®-mini-centrifuge) for 10 min, and then mounted on glass slides and examined under brightfield and variable cross-polarized light on a A1 Zeiss Axioscope microscope at 400 × magnification. When larger mounted samples were found to be too voluminous for analysis, these were subdivided and remounted onto several slides and were later recounted.

The sediment samples were treated differently from the other control samples. In order to reduce the risk of microremains being trapped in clays and not visible, in half of the sediment samples (eM12b and eM14) we deflocculated the sediments using 6 ml of a 5% sodium hexametaphosphate (Na<sub>6</sub>P<sub>6</sub>O<sub>18</sub>) solution for 30 min. We then used centrifugation to create a pellet and to remove excess supernatant. The samples were then mounted with ~30 µl of remaining supernatant. The other half of sediment samples was treated identically to the other samples.

Microremains were photographed and described using international protocols (ICSN, 2011; Madella et al., 2005). Images of all microremains are deposited on the Archaeological Microremain Database of the Plant Foods and Hominin Dietary Ecology Research Group in Leipzig. Where possible, the microremain types were identified to plant part and lowest level of taxonomic origin based on comparisons to published sources (Seidemann, 1966; Reichert 1913) or to our reference collection of Eurasian plants curated at the MPI-EVA, or from consultation with a mycologist.

Some studies have highlighted the risks of laboratory contamination from modern plant microremains (Crowther et al., 2014; Wesolowski et al., 2010). To address the possibility of contamination we conducted a regime of weekly laboratory cleaning at the MPI-EVA to remove contamination. All work surfaces were wiped with hot water, washed with starch-free soap, and wiped with 5% Sodium hydroxide (NaOH). We additionally performed wipe tests before and after cleaning to quantify contamination and assess type. Wipe tests retrieved all settled particles of the surface area (74 × 43 cm<sup>2</sup>) of the laboratory positive-pressure laminar flow hood used for mounting. Wiped dust was transferred into a 50 ml

centrifuge tube, centrifuged, decanted and the resulting pellet mounted and analysed.

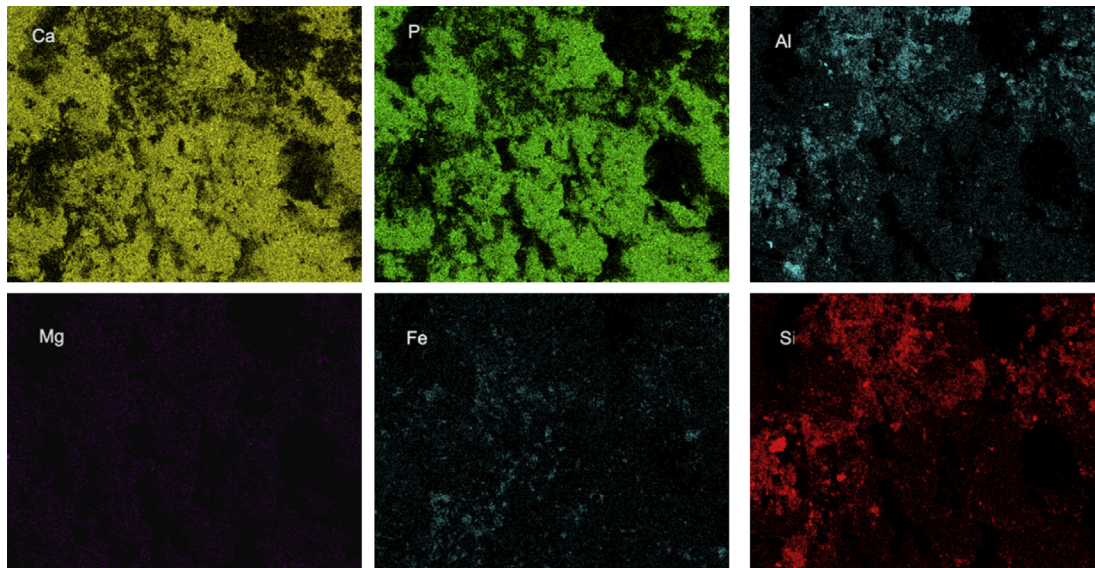
### 3. Results

The EDX spectra reveal that the composition of the matrix of sample eM2 is mostly calcium and phosphorus, with lesser amounts of iron, oxygen, magnesium, silicon, fluorine, nitrogen, aluminium and manganese (Figs. 1 and 2; SI Table 1). Dental calculus is predominantly oral calcium phosphate in different mineral arrangements, with many organic and other inclusions (Jin and Yip, 2002). The spectra from eM2 match what is expected for calculus (Power et al., 2014; Salazar-García et al., 2014b). SEM-EDX failed to locate plant or animal microremains, but did identify siliceous mineral particles and lumps of iron trapped in the matrix's surface (Figs. 1 and 2). Iron particles may be iron oxides that have deposited onto the calculus surface from exogenous haematite, which is abundant in the sediments of this cave layer.

During the optical microscopy analysis of the human calculus from the “Red Lady”, we found phytoliths such as long-cell psilates, cylindroid psilates, hairs and a mesophyll form (Table 2; SI Tables 4 and 2; Fig. 3). We additionally recovered a variety of starches such as sub-polyhedral, eccentric and elongated ellipsoid types. These reflect energy storage organs, most likely including starchy endosperm (seeds) and possibly underground storage organs (tubers) of a variety of plants, but unfortunately none of the starches were diagnostic to any specific plant taxon. We also observed an unusually broad range of other microremains, including several that are rarely reported in other human calculus studies, such as fungal spores, intact and unsilicified plant cells, calcium oxalate crystals and pollen. Some of the fungal spores could be identified as coming from *Alternaria* sp., which is an Ascomycete mould fungus genus that includes both ubiquitous agents of plant decay and pathological agents of specific plants. This fungus is ubiquitous in nature (air, plants, soil) (Rotem, 1994). Other spores could only be identified to particular fungal components, with spores from bolete mushrooms (with sponge caps, often found in the Boletales order) and others from agaric mushrooms (with gilled caps, common to several different fungal orders). Several types of pollen were also recovered (e.g. *Pinus* sp., *Betula* sp.), with pine pollen being particularly abundant (SI Tables 2 and 3).

Two of the three samples from the “Red Lady” (eM1 and eM3) had very similar microremain profiles, with abundant phytoliths and fungal remains. The third sample's surface was analysed with





**Fig. 1.** Element maps of the calculus surface from sample eM2 (disto-buccal surface of the LR M1) of the “Red Lady” from El Mirón, showing the distribution of calcium (Ca), phosphorus (P), aluminium (Al), magnesium (Mg), iron (Fe) and silicon (Si).

scanning electron microscopy (eM2) but yielded few microremains, despite coming from the same tooth as eM1. This may be due to the inherent differences in the ability of SEM and OM to visualize microremains (Power et al., 2014), or it may be that food remains are preferentially trapped in certain areas on the calculus far from the surface and removed elsewhere. This could occur possibly due to bacterial activity, intentional oral cleaning or diagenesis.

The sample from the isolated tooth (eM5) also had relatively poor preservation, with few starches and phytoliths. Despite this low preservation, this sample also preserved several other microremains, including a number of calcium oxalate crystals, two starches, and a sponge spicule. We were not able to identify the source of any of these microremains, and no fungal remains were recovered.

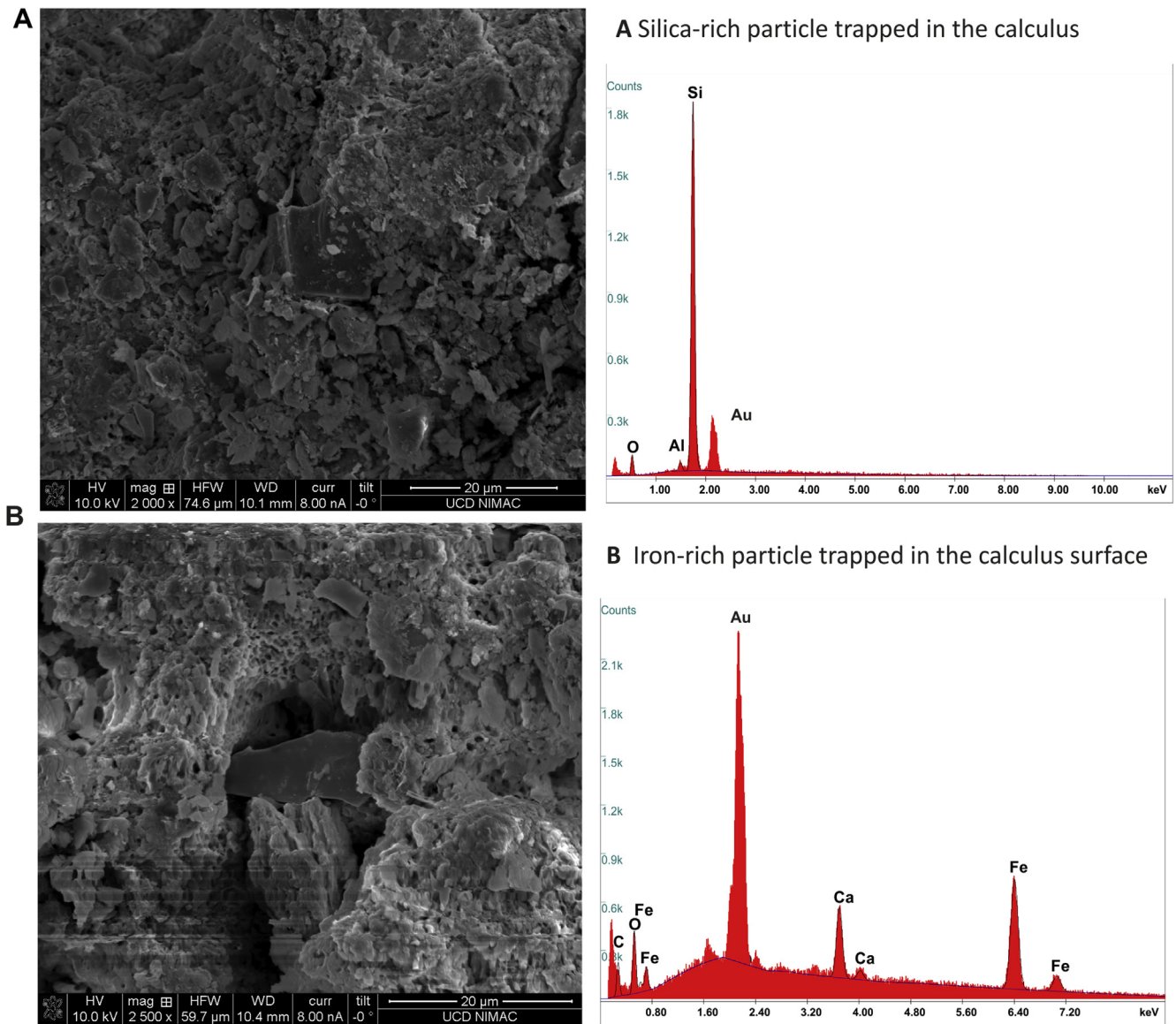
The various controls and contamination tests suggest that the human calculus results are valid. The wipe tests carried out in the MPI-EVA examined for any airborne microremains entering the slide mounting area. Larger microremains like unsilicified epidermal plant remains were not found in our contamination tests. Only modest quantities of starch were found and comprised mainly of wheat or barley (*Triticeae*) and potato starches (SI Table 2). One starch from eM3 potentially matched those from potatoes, but none of the other archaeological starches resembled the modern contaminants, suggesting a low likelihood of contamination. Control tests from the site (animal calculus, bone, sediment) were also carried out. We retrieved microremains from *Capra* calculus only in low numbers, which were mostly grass and some dicot phytoliths. These results show the survival of grass phytoliths and calcium oxalate, which match the expected herbivorous diet. In addition, a single weathered Protozoa skeleton was also recovered in *Capra* calculus. This Protozoa must have originally derived from a marine or brackish environment, but could have entered the *Capra* calculus from the ingestion of reworked soils too. In contrast, sediment found adjacent to the human remains yielded fewer microremains (moderate quantities of grass phytoliths, calcium oxalate and pollen), suggesting low post-depositional contamination. Human bone samples revealed no microremains at all, indicating no contamination in the Burgos lab (SI Tables 2 and 3).

#### 4. Discussion

Analysis recovered a diverse assemblage of microremains from the human calculus. Samples from the herbivores contemporary to the human remains helped to validate and contextualise the human microremain assemblages by providing an expected herbivore pattern. Contamination tests, sediment and bone samples allowed us to assess if microremains found within calculus may be exogenous particles contaminating the dental calculus or not. Microremains from human calculus were distinct from that of bone (absent), sediment (patterns of phytoliths, calcium oxalate and pollen assemblages distinct in types and abundances) and *Capra* calculus (compatible with a herbivore diet -folivore, lignivore). This confirms human results as reflecting micro-remain in-vivo entrapment rather than exogenous post-depositional, excavation or lab contamination. The variation in microremain preservation among the samples from the Lower Magdalenian adult female suggests that different areas in the mouth preserve different information about diet, and indicates that researchers could take multiple samples from the same individual to better approximate diet.

Considering only the samples from the Lower Magdalenian female, the morphology of several starches indicates consumption of at least several varieties of plants, some with seeds containing hard, starchy endosperm, and possibly others that produce starchy rhizomes (SI Tables 2 and 3). Unfortunately, these starches were not diagnostic of any particular taxon, thus limiting our interpretation of these plant remains. Likewise, the sample from the more recent isolated tooth had very few starches. This paucity of starches in the human calculus samples may indicate that: i) starchy plant staples were eaten but the starches have degraded, ii) plants which might have been consumed did not produce abundant starches or iii) there was no major reliance on starchy plants as dietary staples. We are currently unable to differentiate among these possibilities.

Monocot phytoliths recovered from human and *Capra* calculus are suggestive of grass or sedges. Due to the high production of phytoliths in grasses and many graminoids, phytoliths from this group of plants are more likely to be overrepresented in archaeological contexts. Their presence in human calculus may result from



**Fig. 2.** SEM-EDS results of two inclusions in the calculus surface from sample eM2 (disto-buccal surface of the LR M1) of the “Red Lady” from El Mirón. Left side is the SEM image, right side is the elemental spectra from the EDS. A) A silica-rich particle, B) An iron-rich particle.

a variety of non-dietary behaviours. These include the production of containers, the intentional or inadvertent use of grasses as fuel (thus aerosolizing the phytoliths or possibly coating cooked foods), the consumption of herbivore stomach contents (Buck and Stringer, 2013), the use of grass to clean the teeth or even from grass bedding or matting (Ryan, 2011).

The survival of unsilicified plant tissue in human calculus is notable as it is seldom encountered. Among the unsilicified tissues, the fungal remains are of particular interest, because they have scant attention in dental calculus literature, despite having been found in calculus in at least one previous study (Afonso-Vargas et al., 2015). It is possible that our finding of fungal spores relates to our use of a less harsh calculus preparation method (i.e. without using HCl) compared to other studies. Alternatively, the discrepancy may relate to a failure by past researchers to recognise these forms.

Because fungi are known to be a functional part of the human oral microbiome (e.g. Ghannoum et al., 2010), including native taxa like *Candida* and diverse transient environmental species associated with plants, soil and air, it is possible that fungus in calculus

could represent this non-dietary signal. Yet the spores we identified are not consistent with fungus endemic to the mouth. The identification of spores from mushroom-forming fungi (boletes and agarics) suggests the intentional consumption of fungi, particularly since both of these groups include many edible and medicinal mushrooms (e.g. Kalač and Svoboda, 2000). This finding could possibly imply the earliest documented human mushroom use or consumption, which until this point has been unidentified in the Palaeolithic. Recent hunter-gatherers have commonly used fungi as food, flavouring and medicine (Kuhnlein and Turner, 1991; Marles et al., 2008). Mushroom use has firmly been identified from as early as the European Chalcolithic. The Chalcolithic Tyrolean Iceman “Ötzi” carried several types of fungi (Peintner and Pöder, 2000). However, fungi consumption is still almost always invisible in the archaeological record, giving particular importance to finds like the one here reported.

The recovery of pollen from human dental calculus is harder to interpret, as for example in sample eM1, in which pine pollen was particularly common. Scots pine does produce edible nuts but

**Table 2**  
Microremain counts from all calculus and sediment samples from El Mirón.

Lab no.	Material	Wt (mg)	Starch	Phytolith	Unsilicified plants	Calcium oxalate	Cystolith	Pollen	Fungal remain	Protozoa skeleton	Spicule	Sediment mineral	Other microremain	Microremains/mg
<b>Human calculus</b>														
eM1	LR M1	1.40	4	10	4	2	–	17	38	–	2	–	5	55
eM2	LR M1	2.52	–	1	–	–	–	–	–	–	–	1	3	0.79
eM3	LR PM2	3.95	5	29	6	14	1	6	22	–	3	–	4	21.8
eM5	M	0.78	2	1	–	11	–	–	–	–	1	–	–	19.2
<b>Human bone</b>														
eM16	Phalanx	0.11	–	–	–	–	–	–	–	–	–	–	–	–
eM17	Phalanx	0.53	–	–	–	–	–	–	–	–	–	–	4	–
<b>Capra calculus</b>														
eM6a	696	2.72	–	–	–	–	–	–	–	–	–	–	–	–
eM6.b	696	0.64	1	3	–	2	–	–	–	–	–	–	–	9.32
eM7	5285	2.5	1	3	–	2	–	–	–	–	–	–	–	2.4
eM8	5286	4.29	–	2	–	–	–	–	–	1	–	–	–	0.7
eM9	5289	0.64	2	3	–	–	–	–	–	–	–	–	–	7.76
eM9b	5289	0.04	–	2	–	–	–	–	–	–	–	–	–	57.1
eM10a	6101	–	–	–	–	–	–	–	–	–	–	–	–	–
eM10b	6101	0.16	–	–	–	–	–	–	–	–	–	–	–	–
<b>Sediment</b>														
eM11	Sediment	7	–	–	–	–	–	–	–	–	–	–	–	–
eM12b	Sediment	4.29	–	6	2	3	–	3	–	–	–	–	–	3.26
eM13	Sediment	1.57	–	2	–	1	–	–	–	–	–	–	–	1.91
eM14	Sediment	–	–	–	–	–	–	–	–	–	–	–	–	–
<b>Other controls</b>														
eM15	Packing foam	–	–	–	–	–	–	–	–	–	–	–	–	–

unlike some other pines, this is a marginal resource because of its high foraging costs. However, it produces pollen that may be gathered as a significant dietary addition. Therefore, pine stands in the vicinity may have been targeted for pollen collection or for other edible pine foods or non-dietary products such as edible young buds or the inner bark – notably, bark is a pollen trap add, in between name and ear (Groenman-van Waateringe 1998). Yet as pine is particularly effective at massive airborne dispersal of its pollen (Pessi and Pulkkinen, 1994), regular exposure and inhalation of pollen is expected. Therefore, the origin of these microremains is ambiguous and it is inappropriate to interpret these as exclusively dietary or behavioural markers; this pollen may have been inhaled in life or else may be a contaminant.

The results from the isolated tooth (eM5) are difficult to interpret because the sample is small and the preservation of microremains is low. The environment around El Mirón in the late Lower and early Middle Magdalenian period was more temperate and possibly slightly more wooded than during the period in which the “Red Lady” lived, so we might have expected to see an increase in plant consumption for this individual. However, there is no strong evidence in this sample to support that view.

Overall, the diverse plant and other microremain assemblage may show that the “Red Lady” exploited a range of vegetal resources, likely distinct from wild herbivores of the area. Vegetal resources were part of a diet highly rich in ungulate meat and some fish (García-Gonzalez et al., 2015). Absence of specific plant remains may not necessarily indicate non-consumption. However, there is no evidence of caries from the “Red Lady” or the second individual, potentially suggesting diet was not dominated by carbohydrates such as would be the case through high plant use. There are almost no other teeth of Magdalenian age recovered from the region with which to compare this, and the few that exist have had no reports of caries.

Even taking this in mind, and although the methods for recovering information about diet are very different, our results broadly match those from the two nearby contemporaneous sites (El Rascáño, c.20 km west in the mountains, and El Juyo, c.35 km west-northwest on the coastal plain) in that they indicate at least a moderate amount of plant use. There may be some differences in intensity

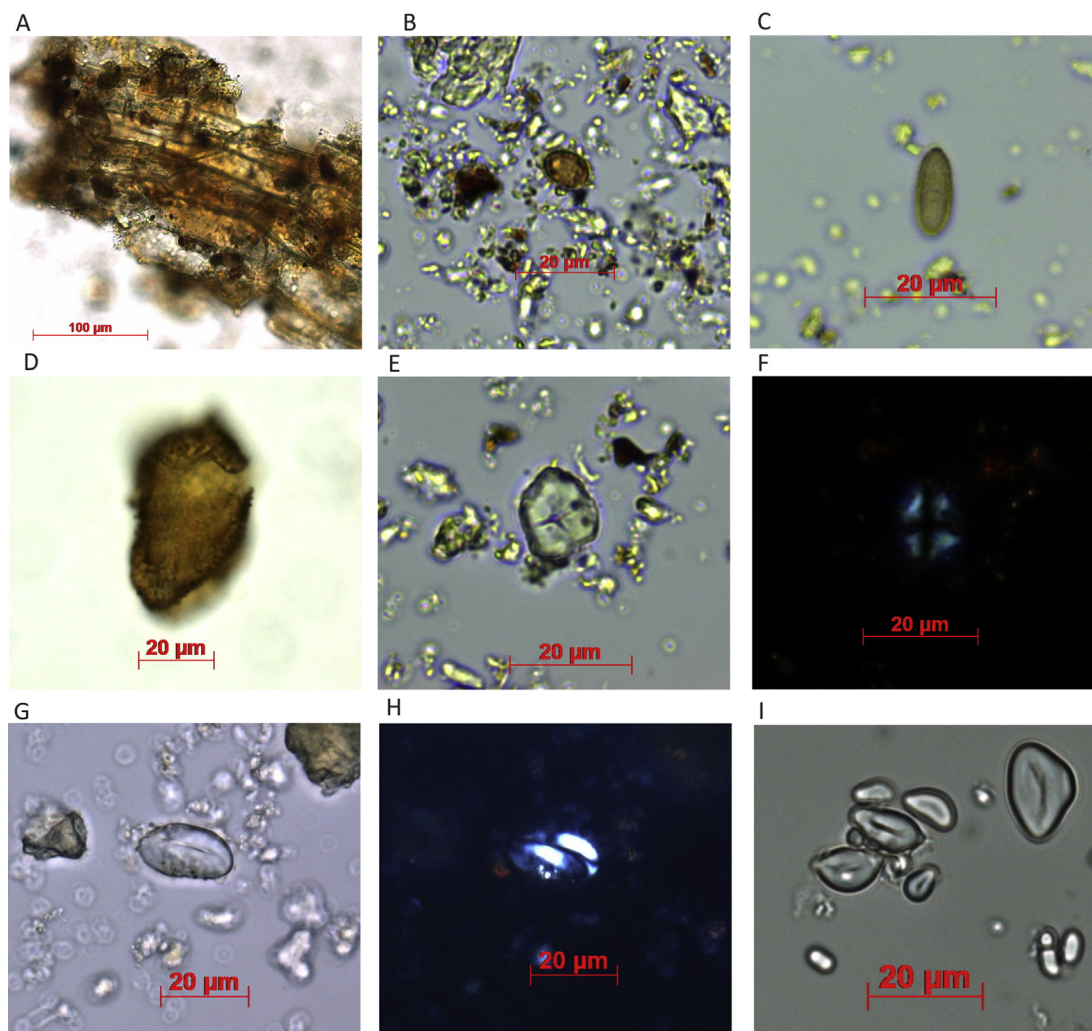
of plant consumption among these sites, with the seed assemblages from El Juyo potentially suggesting a trend toward sophisticated vegetal resource strategies (Zapata et al., 2002; Freeman et al., 1988). This variation may be due to differences in site location (i.e. montane slopes vs. coastal plain), but too few sites from the Magdalenian in this region have been so far sampled to draw meaningful conclusions about habitat-specific foraging strategies.

## 5. Conclusions

The diversity of microremains recovered from human dental calculus at El Mirón Cave exceeds what is usually reported in past studies, which are typically limited to starch grains and phytoliths. At El Mirón Cave, the stability of the calculus microenvironment has preserved other plant tissues, such as unsilicified structures and robust plant remains that are unlikely to survive in sediments. Our results verify that particles such as unsilicified plant wall tissue and fungal spores that inform us on ancient behaviour and subsistence can be identified within ancient human calculus. These particles may be underrepresented in past studies due to attrition during the extraction of microremains from calculus or analyst unfamiliarity. And, although past calculus studies are strikingly deficient in microremains other than phytoliths and starches, these alternative microremains may be useful for reconstructing diet and behaviour in certain contexts.

At El Mirón Cave, microremains recovered from calculus indicate human consumption of plants during the Magdalenian in Northern Iberia, although many microremains cannot be tied to particular types of plants. Agaric and bolete spores may suggest the use of several species of mushrooms as part of their diet or for other purposes, but further research is needed to understand the presence of airborne particles such as spores and pollen in calculus. Microremains from human calculus at El Mirón indicate consumption of plants, but do not specifically suggest that starchy foods were dominant in their diet. Calculus, combined with other lines of evidence such as the study of macrobotanical remains and of dental microwear, may suggest a growing reliance on vegetal foods as they became more available with incipient climate amelioration in the Late Glacial period.





**Fig. 3.** Images of recovered microremains from human dental calculus from El Mirón. A) Unsilicified fragment of plant cells from sample eM3. B) Spore of gilled fungi (agaric) group from sample eM3. C) Spore of fungus with pores (boletes) from sample eM3. D) Indeterminate spore. E) Endosperm starch from sample eM3 in brightfield. F) Same endosperm starch as E) in cross-polarized light. G) Elongated ellipsoid starch from sample eM1 in brightfield, likely derived from a starchy rhizome. H) Same elongated ellipsoid starch as G) in cross-polarized light. I) Modern reference starch from the rhizome of *Argentina anserina* (L.) Rydb.

## Acknowledgements

Special thanks are due to Pat O'Reilly of First Nature.com who assisted with the fungal identifications. The authors also wish to thank Peter Otto, Ian Reid (NIMAC UCD), José Miguel Carretero Díaz, Antje Hutschenreuther, Joerg Watzke, Simone Schmidt, Thomas Büdel, and Arlene M Rosen. We also wish to acknowledge funding from the Max Planck Society. DCSG acknowledges support from the Generalitat Valenciana (VALi+d APOSTD/2014/123), the BBVA Foundation (1 Ayudas a investigadores, innovadores y creadores culturales) and the European Union (FP7/2007–2013 - MSCA-COFUND, n°245743 via a Braudel-IFER-FMSH).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jas.2015.04.003>.

## References

Álvarez-Fernández, E., 2011. Humans and marine resource interaction reappraised: archaeofauna remains during the Late Pleistocene and Holocene in Cantabrian Spain. *J. Anthropol. Archaeol.* 30 (3), 327–343.

- Afonso-Vargas, J., La Serna-Ramos, I., Arnay-de-la-Rosa, M., 2015. Fungal spores located in 18th century human dental calculi in the church “La Concepción” (Tenerife, Canary Islands). *J. Archaeol. Sci. Rep.* 2, 106–113.
- Armitage, P.L., 1975. The extraction and identification of opal phytoliths from the teeth of ungulates. *J. Archaeol. Sci.* 2 (3), 187–197.
- Boyadjian, C.H.C., Eggers, S., Reinhard, K., 2007. Dental wash: a problematic method for extracting microfossils from teeth. *J. Archaeol. Sci.* 34 (10), 1622–1628.
- Buck, L.T., Stringer, C.B., 2013. Having the stomach for it: a contribution to Neanderthal diets? *Quat. Sci. Rev.* 16, 161–167.
- Carrión, J.S., Fernández, S., González-Sampériz, P., Gil-Romera, G., Badal, E., Carrión-Marco, Y., López-Merino, L., López-Sáez, J.A., Fierro, E., Burjachs, F., 2010. Expected trends and surprises in the Lateglacial and Holocene vegetation history of the Iberian Peninsula and Balearic Islands. *Rev. Palaeobot. Palynol.* 162 (3), 458–475.
- Crowther, A., Haslam, M., Oakden, N., Walde, D., Mercader, J., 2014. Documenting contamination in ancient starch laboratories. *J. Archaeol. Sci.* 49, 90–104.
- ICSN, 2011. The International Code for Starch Nomenclature. <http://fossilfarm.org/ICSN/Code.html> (accessed 04.03.13.).
- Cuenca-Bescós, G., Straus, L.G., González Morales, M.R., García Pimienta, J.C., 2009. The reconstruction of past environments through small mammals: from the Mousterian to the Bronze Age in El Mirón Cave (Cantabria, Spain). *J. Archaeol. Sci.* 36 (4), 947–955.
- Dobney, K., Brothwell, D., 1986. Dental calculus: its relevance to ancient diet and oral ecology. *Teeth and anthropology*. In: Cruwys, E., Foley, R.A. (Eds.), *Teeth and Anthropology*, BAR International Series, vol. 291, pp. 55–81.
- Estalrich, A., Rosas, A., García Vargas, S., García Taberner, A., Santamaría, D., de la Rasilla, M., 2011. Brief communication: subvertical grooves on interproximal wear facets from the El Sidrón (Asturias, Spain) Neanderthal dental sample. *Am. J. Phys. Anthropol.* 144 (1), 154–161.

- Freeman, L.G., Echegaray, J.G., Klein, R.G., Crowe, W.T., 1988. Dimensions of research at El Juyo. In: Dibble, H.L., Montet-White, A. (Eds.), *Upper Pleistocene Prehistory of Western Eurasia*. University Museum, pp. 3–39.
- García-González, R., Carretero, J.M., Rodríguez, L., Richards, M.P., Quam, R., 2015. Dietary inferences through dental microwear and isotope analyses of the Lower Magdalenian individual from El Mirón Cave (Cantabria, Spain). *J. Archaeol. Sci.* 60, 28–38. <http://dx.doi.org/10.1016/j.jas.2015.03.020>.
- Ghannoum, M.A., Jurevic, R.J., Mukherjee, P.K., Cui, F., Sikaroodi, M., Naqvi, A., Gillevet, P.M., 2010. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathog.* 6 (1), e1000713.
- Groenman-van Waateringe, W., 1998. Bark as a natural pollen trap. *Rev. Palaeobot. Palynol.* 103 (3), 289–294.
- Guerrero, L., Lorenzo, J., 1981. Antropología física en Rascaño. In: González Echegaray, J., Barandiarán, I. (Eds.), *El Paleolítico Superior de la Cueva del Rascaño*. Santander, vol. 3. Monografías del Centro de Investigación y Museo de Altamira, pp. 277–321.
- Haws, J.A., 2004. An Iberian perspective on Upper Paleolithic plant consumption. *Promontoria* 2, 49–106.
- Henry, A.G., Brooks, A.S., Piperno, D.R., 2014. Plant foods and the dietary ecology of Neanderthals and early modern humans. *J. Hum. Evol.* 69, 44–54.
- Horrocks, M., Nieuwoudt, M.K., Kinaston, R., Buckley, H., Bedford, S., 2014. Microfossil and Fourier Transform InfraRed analyses of Lapita and post-Lapita human dental calculus from Vanuatu, Southwest Pacific. *J. R. Soc. N. Z.* 44 (1), 17–33.
- Iriarte-Chiapusso, M.J., Arrizabalaga, A., Cuenca-Bescós, G., 2015. The vegetational and climatic contexts of the Lower Magdalenian human burial in El Mirón Cave (Cantabria, Spain): implications related to human behavior. *J. Archaeol. Sci.* 60, 66–74. <http://dx.doi.org/10.1016/j.jas.2015.02.008>.
- Jin, Y., Yip, H., 2002. Supragingival calculus: formation and control. *Crit. Rev. Oral Biol. Med.* 13 (5), 426–441.
- Jones, M., 2009. Moving north: archaeobotanical evidence for plant diet in Middle and Upper Paleolithic Europe. In: Hublin, J.J., Richards, M.P. (Eds.), *The Evolution of Hominin Diets: Integrating Approaches to the Study of Palaeolithic Subsistence*. Springer, pp. 171–180.
- Kalač, P., Svoboda, L., 2000. A review of trace element concentrations in edible mushrooms. *Food Chem.* 69 (3), 273–281.
- Kucera, M., Pany-Kucera, D., Boyadjian, C.H., Reinhard, K., Eggers, S., 2011. Efficient but destructive: a test of the dental wash technique using secondary electron microscopy. *J. Archaeol. Sci.* 38 (1), 129–135.
- Kuhnlein, H.V., Turner, N.J., 1991. *Traditional Plant Foods of Canadian Indigenous Peoples: Nutrition, Botany, and Use*. Taylor & Francis.
- Langley, M., Street, M., 2013. Long range inland–coastal networks during the Late Magdalenian: evidence for individual acquisition of marine resources at Andernach-Martinsberg, German Central Rhineland. *J. Hum. Evol.* 64 (5), 457–465.
- Madella, M., Alexandre, A., Ball, T., 2005. International code of phytolith nomenclature 1.0. *Ann. Bot.* 96, 253–260.
- Marín-Arroyo, A.B., 2009. Exploitation of the Montane zone of Cantabrian Spain during the Late Glacial: faunal evidence from El Mirón Cave. *J. Anthropol. Res.* 65 (1), 69–102.
- Marles, R.J., Clavelle, C., Monteleone, L., Tays, N., Burns, D., 2008. *Aboriginal Plant Use in Canada's Northwest Boreal Forest*. UBC Press (University of British Columbia).
- Mickleburgh, H.L., Pagán-Jiménez, J.R., 2012. New insights into the consumption of maize and other food plants in the pre-Columbian Caribbean from starch grains trapped in human dental calculus. *J. Archaeol. Sci.* 39 (7), 2468–2478.
- Owen, L., 2002. Reed tents and straw baskets? Plant resources during the Magdalenian of Southwestern Germany. In: Mason, S.L.R., Hather, J.G. (Eds.), *Hunter-Gatherer Archaeobotany: Perspectives from the Northern Temperate Zone*. Left Coast Press, pp. 156–173.
- Peintner, U., Pöder, R., 2000. Ethnomycological remarks on the Iceman's fungi. In: Bortenschlager, S., Oeggl, K. (Eds.), *The Iceman and His Natural Environment, The Man in the Ice*, vol. 4. Springer, pp. 143–150.
- Pessi, A.M., Pulkkinen, P., 1994. Temporal and spatial variation of airborne Scots pine (*Pinus sylvestris*) pollen. *Grana* 33 (3), 151–157.
- Power, R.C., Salazar-García, D.C., Wittig, R.M., Henry, A.G., 2014. Assessing use and suitability of scanning electron microscopy in the analysis of microremains in dental calculus. *J. Archaeol. Sci.* 49, 160–169.
- Reichert, E.T., 1913. *The Differentiation and Specificity of Starches in Relation to Genera, Species, Etc.* The Carnegie Institution of Washington, Washington, DC.
- Rotem, J., 1994. *The Genus Alternaria: Biology, Epidemiology, and Pathogenicity*. APS Press, St Paul.
- Ryan, P., 2011. Plants as material culture in the near Eastern Neolithic: perspectives from the silica skeleton artifactual remains at Çatalhöyük. *J. Anthropol. Archaeol.* 30 (3), 292–305.
- Salazar-García, D.C., Power, R.C., Serra, A.S., Villaverde, V., Walker, M.J., Henry, A.G., 2013. Neanderthal diets in central and southeastern Mediterranean Iberia. *Quat. Int.* 318, 3–18.
- Salazar-García, D.C., Aura, E., Olària, C., Talamo, S., Morales, J.V., Richards, M.P., 2014a. Isotope evidence for the use of marine resources in the Eastern Iberian Mesolithic. *J. Archaeol. Sci.* 42, 231–240.
- Salazar-García, D.C., Richards, M.P., Nehlich, O., Henry, A.G., 2014b. Dental calculus is not equivalent to bone collagen for isotope analysis: a comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the Medieval site of El Raval (Alicante, Spain). *J. Archaeol. Sci.* 47, 70–77.
- Seidemann, J., 1966. *Stärke-atlas*. Paul Parey.
- Speth, J.D., 2010. Big-game hunting: protein, fat, or politics. In: Speth, J.D. (Ed.), *The Paleoanthropology and Archaeology of Big-game Hunting*. Springer, New York, pp. 149–161.
- Straus, L.G., 1977. Of deerslayers and mountain men: Paleolithic faunal exploitation in Cantabrian Spain. In: Binford, L.R. (Ed.), *For Theory Building in Archaeology: Modeling Essays on Faunal Remains, Aquatic Resources, Spatial Analysis, and Systemic Modeling*. Academic Press, pp. 41–76.
- Straus, L.G., González Morales, M., Marín Arroyo, A.B., Iriarte Chiapusso, M.J., 2013. The human occupations of El Mirón Cave (Ramales de La Victoria, cantabria, Spain) during the Last Glacial Maximum/Solutrean period. *Espac. Tiempo Forma. Ser. Prehist. Arqueol.* 5.
- Straus, L.G., González Morales, M.R., 2012. The Magdalenian settlement of the cantabrian region (Northern Spain): the view from El Mirón Cave. *Quat. Int.* 272, 111–124.
- Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R.Y., Fiddyment, S., Speller, C., Hendy, J., Charlton, S., Luder, H.U., Salazar-García, D.C., Eppler, E., Seiler, R., Hansen, L.H., Castruita, J.A.S., Barkow-Oesterreicher, S., Teoh, K.Y., Kelstrup, C.D., Olsen, J.V., Nanni, P., Kawai, T., Willerslev, E., von Mering, C., Lewis Jr., C.M., Collins, M.J., Gilbert, M.T.P., Ruhli, F., Cappellini, E., 2014. Pathogens and host immunity in the ancient human oral cavity. *Nat. Genet.* 46 (4), 336–344.
- Wesolowski, V., de Souza, S.M.F.M., Reinhard, K.J., Ceccantini, G., 2010. Evaluating microfossil content of dental calculus from Brazilian sambaquis. *J. Archaeol. Sci.* 37 (6), 1326–1338.
- Weyrich, L.S., Dobney, K., Cooper, A., 2015. Ancient DNA analysis of dental calculus. *J. Hum. Evol.* 79, 119–124.
- Zaatar, S., Hublin, J.J., 2014. Diet of Upper Paleolithic modern humans: evidence from microwear texture analysis. *Am. J. Phys. Anthropol.* 153, 570–581.
- Zapata, L., Cava, A., Iriarte, M.J., Baraybar, J.P., De la Rúa, C., 2002. Mesolithic plant use in the western Pyrenees: implications for vegetation change, use of food and human diet. In: Mason, S.L.R., Hather, J.G. (Eds.), *Hunter-Gatherer Archaeobotany: Perspectives from the Northern Temperate Zone*, pp. 96–107.