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The site of 'Cova de Les Gralles', a burial cave (Catalunya - ES): a paleoenvironmental reconstruction based on palynological analysis and a diet reconstruction of the individuals buried within the cave by the investigation of the micro - remains contained inside the dental calculi.

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*“mutat enim mundi naturam totius aetas,
ex alioque alias status excipere omnia debet,
nec manet ulla sui simili res: omnia migrant,
omnia commutat natura et vertere cogit”*

(Titus Lucretius Carus – De rerum natura, V, 828-834)

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Abstract

The following work is focused on the paleoenvironmental reconstruction of the area surrounding the site of 'Cova de Les Gralles', on the mountains of Prades (Catalunya - ES) using the palynological analysis of some sediment samples collected in situ. Furthermore, this type of investigation will be complemented by a diet study of certain individuals buried within the cave, through the analysis of the dental calculi from twelve osteological samples. In this paper has been developed a methodology compatible with its sampling, its treatment with chemical agents and the consequent observation and analysis of the fossil micro-remains trapped in it, at the time of the individuals' death: principally pollen, phytoliths and NPPs, non-pollen palynomorphs.

Key words: ancient diet; dental calculus; burial cave; micro-remains; archaeopalynology.

Objectives

The main objective of this study was to carry out a reconstruction of the natural environment that dominated the surrounding area of the site of Cova de Les Gralles, in the Prades Mountains in Catalonia (ES), between the Final Chalcolithic and the Early Bronze Age.

In addition to this type of work, it was decided to perform a complementary investigation by analysing the dental calculus of fossil human remains belonging to sub-adult

individuals buried in the cave in question, with the aim of reconstructing the ancient diet and verifying if a correspondence was present, in particular between the plant species ingested and those that grew in the mountain environment in question.

This work attempts to insert the apparently 'isolated' case of Cova de Les Gralles into a broader context characterised by mountain settlements chronologically placed in this time range (as mentioned earlier between the Final Chalcolithic and the Early Bronze Age), taking as its focus the reconstruction of the ancient environment and the subsistence economy of these social groups.

Introduction

Catalunya has a very varied geography with very different landscapes as a result of the confluence of the various mountain ranges and the Mediterranean coast. This lack of uniformity characteristic of the Catalan territory has conditioned the climatic features: an alpine and subalpine climate in the Pyrenees, a continental climate in the central depression and a Mediterranean climate along the coast and the surrounding areas. Cova de Les Gralles is located in the Prades Mountains, which are quite close to the coast and for this reason displays typical Mediterranean vegetation.

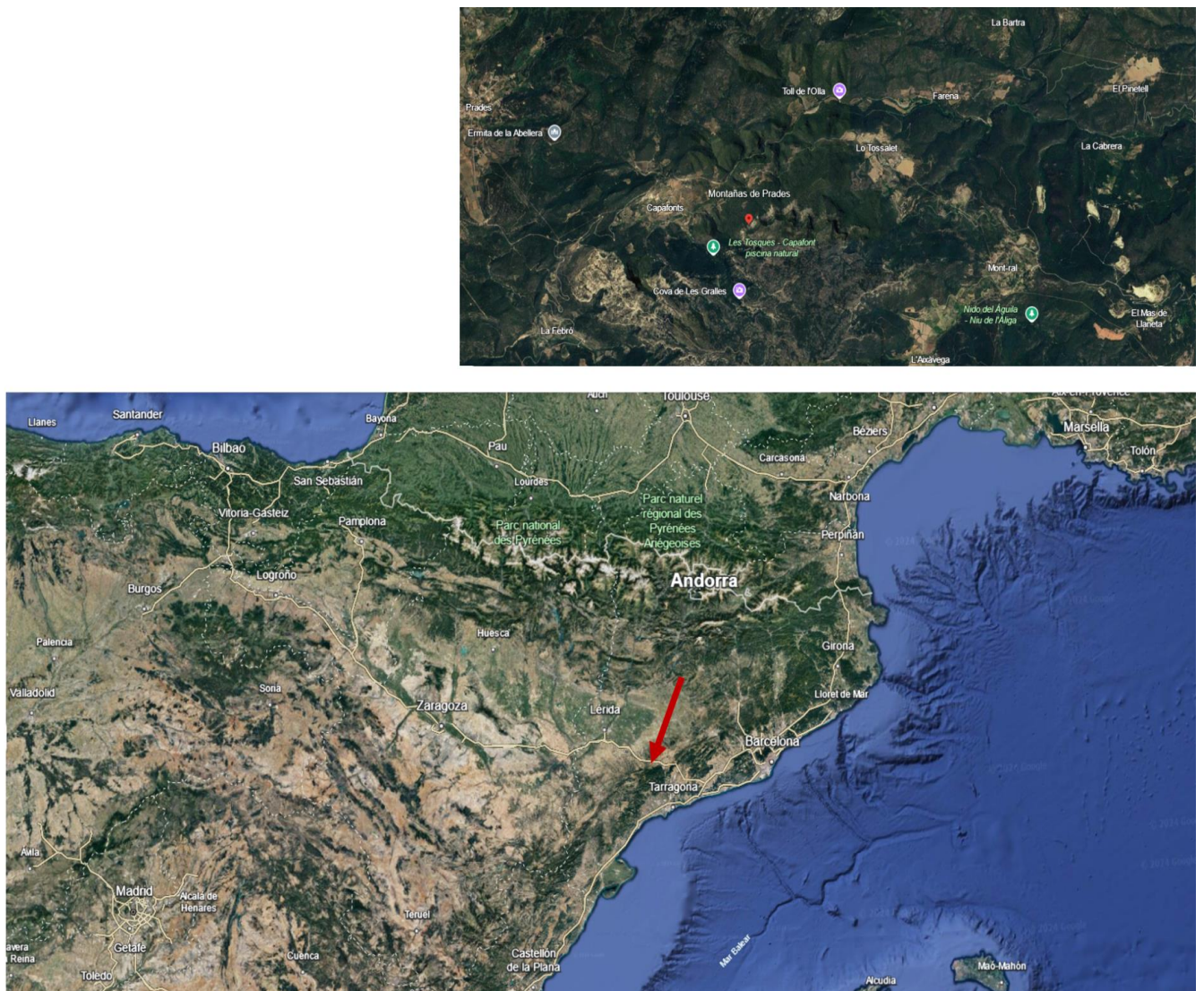
Not much is known about the burial practices implemented by the first farming and/or breeding communities in the second half of the 6th millennium BC. Even though the scarcity of burial remains prevents us from characterising such practices, it seems that the few burials found so far are single or double interments, accompanied by few grave goods. However, this paucity seems significant in that it may be due to certain symbolic concepts linked to a very different funerary treatment from the one that began to take place in the first half of the 5th millennium and developed between the second half of the 5th and the first half of the 4th millennium.

At the beginning of the 5th millennium, the first burials in caves and shelters were observed: they were characterised by few grave goods and the body were interred as inhumations, sometimes in pits and covered with stones; the use of these places (caves and rock shelters) for funerary purposes is sometimes complemented by other functions related to their habitability or to various subsistence-related tasks (Gibaja, 2004).

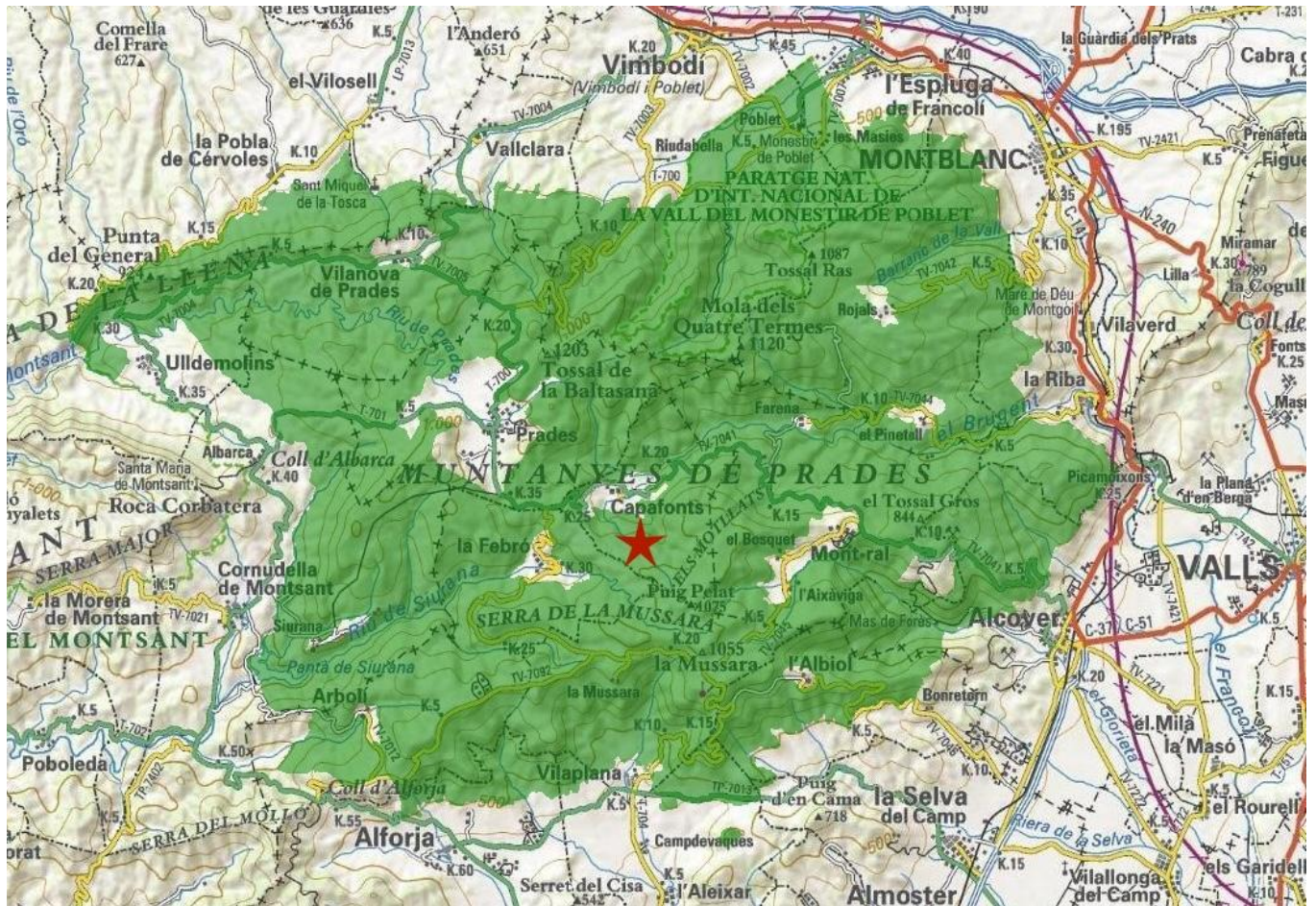
The known cases are thus not many and in-depth studies on this subject equally scarce; often, as in the case of Cova de Les Gralles, it is not even possible to interpret the stratigraphy, to reconstruct the disposition of the bodies and the burial pattern as these sites have been manipulated, by people not skilled in the profession, since the early 20th century and only partially studied a posteriori.

A work like the following is potentially useful for a reconstruction of the cultural context, especially in terms of the ancient diet and subsistence politics, to add an extra piece to this patchwork that is still too incomplete. This type of investigation can also serve as an impetus to broaden studies on various aspects of the population of the Iberian Peninsula (in this case the ones who lived in the territory of Catalonia) between Chalcolithic and Bronze Age.

1. The site and its surroundings



Pic. A Position of Prades mountains and the site of Cova de Les Gralles in the Iberian Peninsula



Pic. B The mountains of Prades and the approximate location of Cova de Les Gralles

1.1. Territory and vegetation of Prades mountains

The Prades mountains are a natural area located in the south-center of Catalunya (ES), which is characterised by a high degree of environmental diversity and it forms part of the Catalan Mediterranean system. The entire mountainous area constitutes a perfectly identifiable geographical unit between the counties of Alt and Baix Camp, Conca de Barberà and Priorat, with mountains culminating in highlands and peaks exceeding 1,000 m, all defined by great orographic and tectonic complexity. The mountainous compound incorporates special protected natural spaces within it, such as the Titlar and Trinitat partial nature reserves.

About the geology of the territory, while in the northernmost part we find Paleozoic sediments (slates and sandstones) and granodiorites, Triassic (sandstones, clays and limestones) and Jurassic (dolomites and limestones) sediments predominate in a large part of the territory.

As far as the soil is concerned, it shows a great edaphic diversity: in the limestone soils, the soils are quite stable, on the contrary, in the area characterised mainly by granite, the soils are much more fragile.

Furthermore, the entire territory has a complex hydrographic network composed mainly of the following watercourses: Brugent, Montsant, Siurana, Glorieta and Francolí; the powerful erosive action of these has been the main factor of the formation of these mountains.

In terms of vegetation, the Mediterranean complex of the Prades mountains coexist with others from Central Europe, and even boreal and this, from a biogeographical point of view, implies different changes in the physical landscape as the variation of the slopes, the altitudinal gradient, the different substrates, the anthropic action, etc. The entire area is also interested in the existence of some paleontological sites of great scientific value that, as in this case study, are caves that have been affected by human presence.

The Mediterranean vegetation of the Prades Mountains is mainly characterised by oak groves, a type of forest consisting of evergreen species, which in some places can be quite dense and very stratified: up to 900 - 1,000 metres, holm oaks (*Quercus rotundifolia*) predominate and, above this altitude, dry oaks (*Quercus pubescens* - and *Quercus petraeae*) and in sub-humid wood, the red pine (*Deschampsio-Pinion*). There are also Mediterranean and sub-Mediterranean secondary pine forests of white pine (*Pinus halepensis*), red pine (*Pinus sylvestris*), Pinassa (*Pinus nigra subsp. salzmannii*) and maritime pine (*Pinus pinaster*).

In Prades it is possible to find some Iberian, continental Iberian or Euro-Siberian plant species that have their limit of dispersion in these lands. The flora of this territory is home for a good number of remarkable species, many of them strictly protected by the PEIN (Pla d'Espais d'Interès Natural de Catalunya), which stand out for their rarity and high biogeographical interest.

In this territory there are the only forests of Pyrenees oak (*Quercus pyrenaica*), the cliff willow community (*Hieracio-Salicetum tarraconensis*), *Lavandula pedunculata*, *Cistus populifolius*, the *Evax pygmaea* (also known as *Filago pygmaea*), the *Jasione Crispa*, the Lemon Coral (*Sedum forsteranum*), the plantain-leaved leopard's-bane (*Doronicum plantagineum*), the *Galium rotundifolium*, etc., many of which are endemic to the southern Catalan mountains.

Lastly, the presence of cryptogamic plants, especially bryophytes, is worth mentioning, as they are excellent representatives of Mediterranean biogeographical elements. In the specific case of the Prades mountains, one example is the lichen flora due to its diversity of epiphytic and stony lichens and the presence of very rare species (e.g. *Lethariella soleirolii*).

Finally, it should be pointed out that the landscape of the Prades Mountains, over the years, has been altered by agricultural activities that developed on the main plateaus, from traditional forestry exploitation, ranging from coal mining to grass sod harvesting, and the existence of modern population cores.

Deforestation processes took place since the demolition of the Poblet Monastery in 1835. However, between the 1970s and 1980s, lost forested areas were recovered especially through the repopulation of various plant species, mainly pine trees.

A second characteristic of the current population inhabiting the Prades Mountains is the depopulation of the early decades of the 20th century. This phenomenon has favored the

revival of an extensive natural landscape with little humanization, despite its relevant historical past, from prehistory to the 20th century. (Guidi Sánchez 2013).



C1



C2



C3



C4



C5



C6



C7



C8



C9



C10



C11

Pic. C (1-11) Some of the plant species found in the natural landscape overlooking the mountains of Prades (C1. *Cistus laurifolius*; C2. *Cistus salviifolius*; C3. *Erica arborea*; C4. *Juniperus oxycedrus*; C5. *Pinus sylvestris* a; C6. *Pinus sylvestris* b; C7 *Pinus sylvestris* c; C8 *Pinus sylvestris* d; C9 *Quercus rotundifolia*; C10 *Rubus ulmifolius*; C11 *Ruscus aculeatus*) - Photos by Isabel Expósito Barea

1.2. The cave

The site of Cova de Les Gralles, from which the fossil of human remains examined in this paper come, is located in the heart of the Prades Mountains, in the surroundings of the municipal district of Capafons, Tarragona; it is one of the many caves that characterize the karst system of the area concerned.

After leaving the paved road, the place of interest is easily accessible and, after about twenty minutes of dirt trails, it is possible to reach a hiking level route, walkable in approximately fifteen minutes. Upon reaching the cave, we are presented with a majestic entrance followed by several natural corridors that intersect each other before entering into a large hall, at the foot of which is the very narrow access to the site proper.

Several humans remains and ceramic fragments, listed below, were found here in the 1970s (source: original document pertaining to the material found and classified under identification number 4360) and are now preserved in the Museu d'Arqueologia Salvador Vilaseca in Reus:

- 22 fragments of “campaniforme” pottery
- 213 fragments of smooth pottery
- 1 fragment of “llengüetes” pottery
- 6 fragments of “mugrons” pottery
- 2 fragments of ribbed pottery
- 4 fragments of stringed pottery
- 1 fragment of smooth-stringed pottery
- 1 axe fragment

- 1 punch made of bone
- 1 stalactite
- 1 flint
- 1 polished bone
- 2 bones possibly polished
- 2 bone fragments
- 1 ceramic rim
- 29 flints (from all over the site)
- 19 cranium fragments
- 35 mandible fragments
- 13 teeth
- 7 faunal bones
- 1 fragment of ovoid vase with hole on the rim

Unfortunately, in the excavation memoirs, there are no data inherent in the reconstruction of the stratigraphy, nor details about the finds, nor the relationships that there were between them and the sediment, all this because unfortunately in those years archaeology still lacked a proper methodology, consequently the "excavations" were more than earthworks, carried out only for the purpose of recovering the material found. Cova de Les Gralles is located in a weathered area, over 1000 m high. It is a burial cave, by this expression we mean a natural cavity of karst origin that was exploited in the past as a place for the burial of individuals belonging to communities of the past. More broadly, caves, but also rockshelters, found all over Europe, have frequently been occupied by human groups, from prehistory (Clottes, 2012) right up to the present days. Some of them appear to have only traces of short occupations, while others contain deep cultural deposits, indicating longer and multiple occupations. Above all, there is great variability in their use, including the 'sacred', as in our case (Robin, Bergsvik 2012). In the

case of the site of interest for this paper, its occupation has been placed chronologically at the final stages of the Chalcolithic (ca. around 4200 B.P.).



D1



D2



D3



D4

Pic. D (1-3) D1. Entrance to the Cova de Les Gralles; D2. The narrow tunnels inside the cave before reaching the site location; D3. The large chamber before descending into the site cave; D4. Inside the burial cave, the arrow in red indicates the pit that originally contained the human remains - Photos by Arianna Giordano and Josep Maria Vergès

A brief parenthesis should be opened here: initially there were two sites examined, Cova de Les Gralles and Cova del Buldò, this second cave is also located in the Prades mountains and is also a burial cave in which human remains were found.

The finds were observed and selected in the same way as those from Cova de Les Gralles. To be precise, they consist of four specimens from which dental tartar was taken: one skull and three mandibles, the data of which will be reported in a table below (*Table 1.*) this paragraph. In this case, the chronology is slightly later and lies between the end of the Chalcolithic and the beginning of the Early Bronze Age.

These data will not be treated in the detail here as for the first site, as it was not possible to carry out a complete study such as for the samples from Cova de Les Gralles due to the impossibility of accessing the Cova del Buldò during the period in which the surveys were carried out, however it is considered appropriate to introduce the data collected here as a starting point for a future study.

1	Cranium 5136-11	Big accumulation in the molar (right).
2	Mandible 5136-06	Deposit on the side of the molar (right).
3	Mandible 5136-04	Small deposit between the two incisors.
4	Mandible 5136-01	Small ridge deposits.

Table 1. Sample classification and location of dental calculi (Cova del Buldò)



E1



E2

Pic. E (1-2) E1. Collection of some of the dental calculi inside a silver paper sheet; E2. Removal of large tartar accumulation on the specimen 5136-11 (Cova del Buldò)

1.3. The environment outside the cave

During the site survey, it was possible to collect some samples of plants that inhabit the environment at the entrance of the cave and along the path to reach it.

In the diagram below (*Table 3.*), it is possible to observe the presence of the various species marked by a frequency index according to the following legend (*Table 2.*):

minimum presence	-
poor presence	+
medium presence	++
moderate presence	+++
high presence	++++

Table 2. Legend of the frequency index of the plants in the modern environment

species	family	presence	location	notes
<i>Asparagus</i> sp.	Asparagaceae	++	Entrance	
<i>Asplenium trichomanes</i>	Aspleniaceae	+++		
<i>Bupleurum fruticosens</i>	Apiaceae	++		-
<i>Campanula</i> sp.	Campanulaceae	++		
<i>Cistus albidus</i>	Cistaceae	++		
<i>Euphorbia characias</i>	Euphorbiaceae	+		
<i>Galium</i> sp.	Rubiaceae	+		(<i>tricornotum</i> or <i>lucidum</i> ?)
<i>Geranium rotundifolium</i>	Geraniaceae	+		
<i>Hedera hélix</i>	Arialiaceae	+++		
<i>Onnonis minutissima</i>	Fabaceae	+		
<i>Polypodium cambricum</i>	Polypodiaceae	++		
<i>Quercus ilex</i>	Fagaceae	++++		
<i>Rubia peregrina</i>	Rubiaceae	++		
<i>Rubus ulmifolius</i>	Rosaceae	+		
<i>Ruscus aculeatus</i>	Asparagaceae	++		
<i>Salvia</i> sp.	Lamiaceae	+		
<i>Salvia rosmarinus</i>	Lamiaceae	+++		
<i>Sedum álbium</i>	Crassulaceae	+		
<i>Smilax aspera</i>	Smilacaceae	++		
<i>Teucrium</i> sp.	Lamiaceae	+		
<i>Acer granatense</i>	Sapindaceae	+	Road	
<i>Bupleurum fruticosum</i>	Apiaceae	+		
<i>Genista scorpius</i>	Fabaceae	++		
<i>Juniperus oxycedrus</i>	Cupressaceae	+++		
<i>Juniperus phoenicea</i>	Cupressaceae	+++		
<i>Lonicera caerulea</i>	Caprifoliaceae	++		
<i>Pinus halepensis</i>	Pinaceae	+		
<i>Quercus pubescens</i>	Fagaceae	-	Not visible, just leaves	

Table 3. Outline on the species observed and sampled during the survey in the environment around the cave

As can be observed from the diagram, the holm oak predominates in the modern plant landscape followed by ivy, asplenium (a perennial fern), rosemary, red juniper, and Phoenician juniper, all species that are featured of the environment in which they are located, which is a mountain habitat but still very close to the coast and hence characterised by Mediterranean connotations.

These data will then be useful in the next stages of this work for the purpose of comparison between the modern and ancient landscape.



Pic. F (1-6) Some of the plant species found outside Cova de Les Gralles and along the path (F1. *Bupleurum fruticosens*; F2. *Juniperus phoenicea*; F3. *Lonicera caerulea*; F4. *Quercus pubescens*; F5. *Salvia rosmarinus*; F6. *Sedum album*; F7 *Asparagus sp.*; F8. *Campanula sp.*; F9. *Polypodium – Quercus ilex*; F10. *Smilax aspera*) - Photos by Isabel Expósito Barea

2. The history of the disciplines

Before going on and entering in the details of the work that has been conducted, it is good to open a few brief parentheses on the disciplines on which this study has been relied: palynology and the study of dental tartar. These areas are worthy of mention in terms of both the history of the studies, the techniques they rely on and the methodology they follow.

2.1. Palynology (discipline, background, objectives, methodology)

Palynology is the discipline that studies pollen, spores and non-pollen palynomorphs (NPP's). This type of investigation is particularly based on the observation of the morphology (diagnostic characters such as wall, size, shape, ornamentation etc. are taken into account) of the pollen grains and spores of the different plant species and their geographical distribution. Since they are persistent for individual species and similar for related plants, they are used for classification and phylogeny.

Palynology is particularly connected with palaeobotany (pollen analysis of Quaternary and Pre-Quaternary deposits) and taxonomy and is also often associated with archaeology, this is because pollen grains and spores are characterised by a high level of preservation since their walls (sporoderm) have a very high resistance to chemicals, and are therefore easily recognisable, even in the fossil state. Palynology within palaeobotany and archaeobotany deals precisely with the study of micro-remains belonging to this group (pollen grains, spores and NPP's), which abound in deposits dating back millions of years, especially in wetland contexts such as marshes and lakes. Thanks to this discipline, it has been possible to reconstruct the climatic history of specific chronological periods and the ancient vegetation of numerous prehistoric and historical contexts.

The research conducted on pollen is also used in other fields, such as the analysis of the origin of honeys, the investigation of parasitic fungi that are injurious to certain cultivated plant species, and finally even in the field of allergic diseases.

The word “palynology” was coined by Hyde and Williams (1945) as a substitute for “the science of pollen grains and spores”. It comes from the Greek verb *paluno* (παλύνω, ‘to strew’ or ‘to sprinkle’), the Greek noun *pale* (παλη, in the sense of “dust” and very close to the Latin word pollen, meaning “fine flour, dust”), in recognition of the fact that many pollen grains and spores are easily carried by the wind and subjected to dispersion (Erdtman, 1963).

When was palynology born?

The invention of the first microscopes and especially the compound microscope in the late sixteenth century represents the starting point of the observation and study of the micro remains. This was an important contribution to the study of pollen morphology. Marcello Malpighi in his “*Anatomia Plantarum*” was the first to describe pollen grains as having germination furrows while Nehemiah Grew noted in his famous work “*The Anatomy of Plants*” (Pic. G) the constancy of pollen characters within the same species. They are both considered the founders of pollen morphology studies.

Later, Rudolf Jakob Camerarius described several pollination experiments (Camerarius, 1694). He stated that male “seed dust” is necessary for seed development.

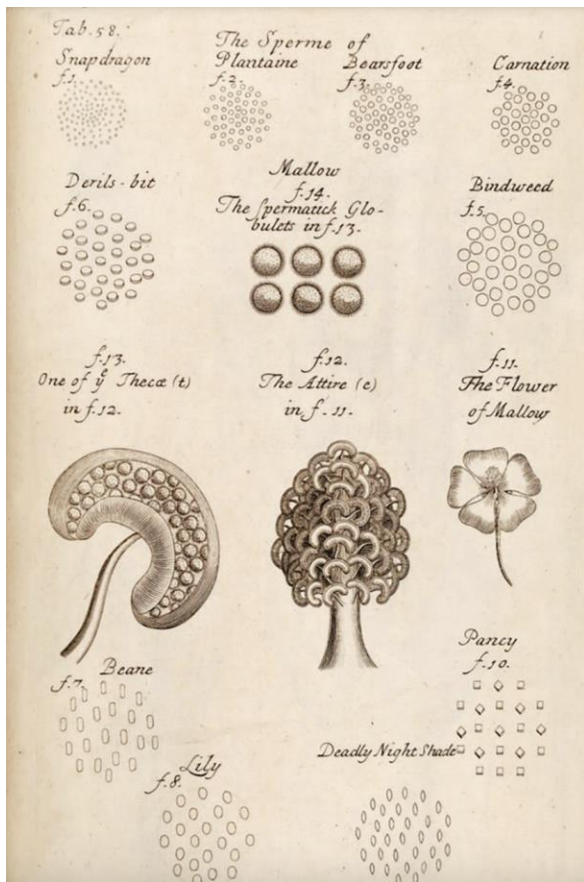
Carl Von Linné (also known before his ennoblement as Carl Nilsson Linnæus) first used the term pollen (in 1750). In the 18th and the early nineteenth centuries, there was considerable progress in pollen research and the understanding of pollination.

Later, in the first decade of the 1800s, Joseph Gottlieb Kölreuter discovered that the pollen wall consists of two distinct layers and made the first attempt to classify pollen based on their morphology.

During 1834 H. von Mohl wrote his fundamental work entitled “Über den Bau und die Formen von Pollenkörner/On the structure and diversity of pollen grains”, which was a major contribution to the knowledge of pollen structure and descriptive classification.

Paleopalynology was established at the end of the nineteenth century, when P. Reinsch published the first photomicrographs of fossil pollen and spores from Russian coals (Reinsch, 1884). Moreover, the rise of stratigraphic palynology started shortly before 1950 and played a prominent role in petroleum explorations during the second half of the twentieth century.

The first and especially the second half of the twentieth century saw palynology at its peak, combining light microscopy with electron microscopy techniques: the introduction of SEM (Scanning Electron Microscope) in palynology in the mid 1960s was a key innovation in the study of the fine relief (sculpture) of pollen and spore surfaces. The advantages of SEM include the relatively simple and rapid preparation methods and the supreme depth of focus (Halbritter, 2018).



Pic. G First drawings of pollen. Grew (1682) "The anatomy of plants"

2.2. Dental calculus (discipline, background, objectives, methodology)

In order to find out what the diet of ancient populations was, especially those whose culture was not yet characterised by written and iconographic sources, it is possible to resort to various types of analysis, including the study of the vegetal macro remains inside the 'food containers', the study of the traces of wear on the teeth (microwear) and finally the study of the vegetal micro remains trapped inside dental calculi (deposits consisting mainly of concretions formed by insoluble calcium salts, phosphates and desquamated epithelial cells from the oral mucosa). The latter type of study is the one that was used for this work.

Archaeological dental calculus is a rich source of ancient DNA and proteins, providing insights into past oral microbial communities and ancient diets. Dental plaque accumulates on the tooth surface during life and, in the presence of calcium and phosphate ions in saliva and gingival crevicular fluid, mineralizes to form dental calculus (tartar). By doing this, dental calculus entombs and preserves biomolecules associated with the oral microbiota, the inhaled and/or ingested micro debris, including environmental or occupational debris and food particles such micro remains as starches grains and phytoliths, pollen grains and NPP's (Hendy, et al., 2018).

The word calculus is derived from Latin word 'Calcis' (limestone), the first sources referring to this composition being deposited and calcified (precisely) on teeth date back to Hippocrates (460–377 BC) which was the foremost person whose writings showed a relation between dental deposits and oral diseases. He noticed the harmful effects of calculus on dental gums and teeth. Thereafter Albucasis (936–1013 AD) evidently illustrated the association between calculus and disease and advocated thorough removal of deposits. Later Paracelsus (1493-1541), a Swiss/German physician and alchemist introduced the term tartar as a description for a variety of stony concretions that form in humans (Aghanashini, et al., 2018).

In the past 25 years calculus has been viewed just as a fossilized remnant of minor significance, and not as in the past as the cause of major teething diseases, but in despite of this, the ancient deposits of dental tartar are so precious from an academic point of view because both ancient DNA and micro remains belonging to the 'categories' mentioned above can be extracted from it.

In the 1970's, the first studies of ancient dental calculus took place on archaeological samples of cattle, sheep, and horse teeth; and only later on human teeth (Weyrich, et al., 2015). Over the last decade and a half, starch granules, phytoliths, and pollen obtained from dental calculus have been used as a basis for exploring both the diet and paleoenvironment of ancient humans, Neanderthals, and even *Australopithecus sediba* (Henry, Piperno, 2008; Henry, et al., 2010; 2012). In the past 30 years, the use of dental calculus in anthropological and archaeological research has advanced significantly.

No matter considering to study genetics or ancient diet, the protocol for extracting a dental calculus follows the same steps; in general, a calculus deposit remains separate from the enamel surface of the tooth, making it possible to simply pry or apply pressure to dislodge the calculus deposit (Henry, Piperno, 2008).

The calculus sample should be collected from the specimen (isolated tooth, skull, or jaw) over clean aluminum foil, to catch fragments of the calculus as it is removed from the teeth. Once the calculus is removed from the tooth, the sample on the aluminum foil should be collected and tipped into a small labeled, sterile plastic bag or screw cap tube. One of the biggest challenges is to avoid any possible contamination of the samples (soil contamination or bacterial contamination) in any possible way, moreover the rugose, pitted, and porous nature of dental calculus makes dental calculus one of the most challenging ancient DNA samples to examine. Rigorous decontamination procedures must be followed to remove or irreparably damage contaminating DNA on the outer surface of the calculus sample: sample decontamination should involve UV irradiation;

the sample can be manipulated during this process with sterile tweezers that are ideally pre-treated with 4% bleach and UV irradiated. Following UV irradiation of the calculus sample, bleach treatment is strongly recommended to decontaminate beyond the exterior surface (Weyrich, et al., 2015).

Most ancient DNA extraction protocols recommend decalcification as the first step; this process can be actuated by placing the sample in a mixture of EDTA, sodium dodecyl sulfate (SDS), and proteinase K and incubating for 24 h at 55 C°. Once the sample is decalcified there are several DNA extraction methods available, for example commercial DNA extraction kits may be used in the case of the sample is well preserved and not ancient in origin, whereas poorly preserved or ancient samples generally require more rigorous extraction methods. In-house silica-based methodologies are common in the ancient DNA field and appear to be several orders more effective at retrieving DNA from dental calculus samples than the use of the kits (Hoss, Pääbo, 1993; Weyrich, et al., 2015). Regarding protocols related to the treatment of dental tartar, in terms of extracting micro remains to investigate the ancient diet, please refer to paragraph 3.2 (*Cleaning and sampling of the dental calculi*) of this work. It is good at this point to make a small observation on a limit of this type of investigation, i.e. that although satisfactory results may or may not be achieved in terms of the amount of micro-remains extracted from dental calculus, the diet picture may also remain partial and therefore this type of study could be combined with an analysis concerning the examination of micro wear on dental enamel.

3. Materials and method (dental calculi)

3.1. Fossil observation and selection

As a first step for this study, the human osteological remains found in Cova de Les Gralles, preserved in the Salvador Vilaseca archaeological Museum of Reus, were picked

for being observed and reviewed in order to see which ones had dental calculi or remnants of them. These are fossil human remains belonging to several individuals, mainly juveniles and sub-adults; all lived in a chronological period between late Chalcolithic and early Bronze Age (ca. 4200 B. P.), (Vilaseca, Iglésies, 1932; Vilaseca 1973). The count of observed remains is as follows: total number of mandibles → 27; total number of maxillae → 6; total number of single teeth → 13.

A total of 14 pieces were selected from all these finds, including 10 mandibles, 2 maxillae and 2 single teeth. As will be shown in the diagram below (*Table 4.*), many of the fossils have been numbered in their time (N° of reference), others have no numbers at all.

1	Tooth	Premolar, line of tartar following the dental gum.
2	Tooth	Premolar, micro line of tartar following the dental gum.
3	Maxilla	Very big deposit on two molars.
4	Maxilla	Small deposit on the first molar (right) and on the first premolar (left). Three cut-marks.
5	Mandible	N° of reference = 1; small deposits on the carious molar and the one preceding it.
6	Mandible	Small deposits on the premolar.
7	Mandible	Small deposit in both the teeth.
8	Mandible	N° of reference = 9; micro deposit on the canine.
9	Mandible	Small ridge deposits on the three molars.
10	Mandible	N° of reference = 10; small ridge deposits on both the teeth.
11	Mandible	N° of reference = 12; deposits in the internal part between the molars. Several cut-marks.

12	Mandible	N° of reference = 2; ridge deposits on two molars, in addition, small deposits on the penultimate molar (right) and incisor (right). Several cut-marks.
13	Mandible	N° of reference = 3; deposits on the molars, small crests especially between the teeth.
14	Mandible	N° of reference = 4; small deposits especially between the incisors and behind the first molar (right).

Table 4. Sample classification and location of dental calculi (Cova de Les Gralles)

N.B. Following this stage of fossil selection, it was observed that premolar number 1 belongs to mandible 12, while premolar number 2 belongs to mandible 8.



H1



H2



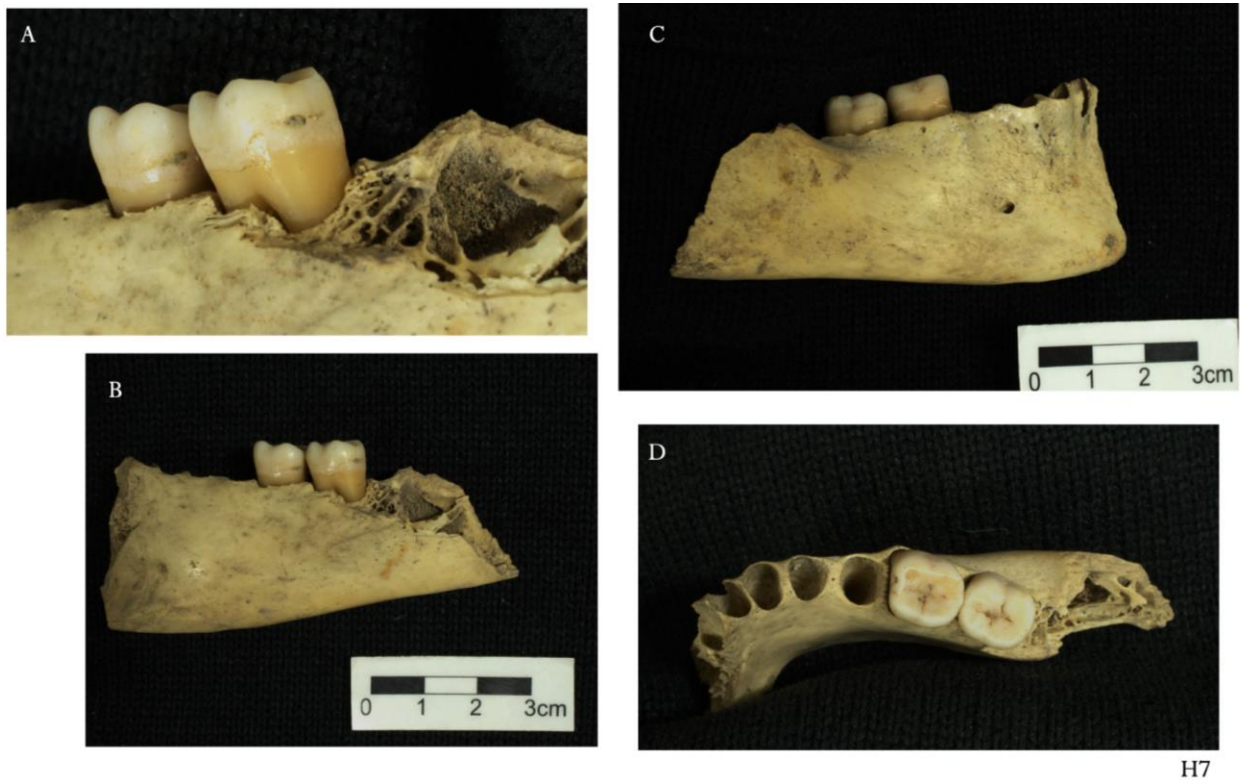
H3



H4



H5





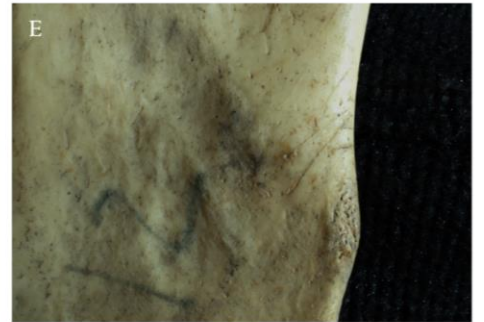
H8



H9



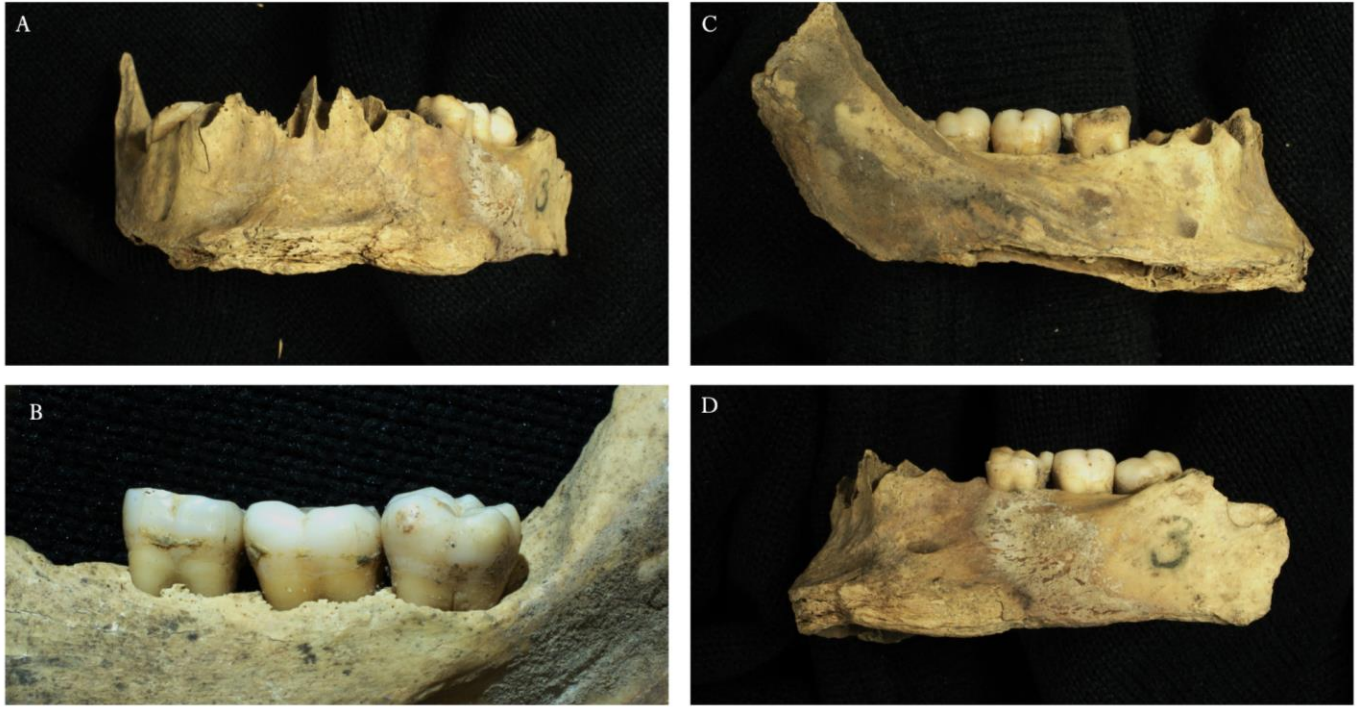
H10



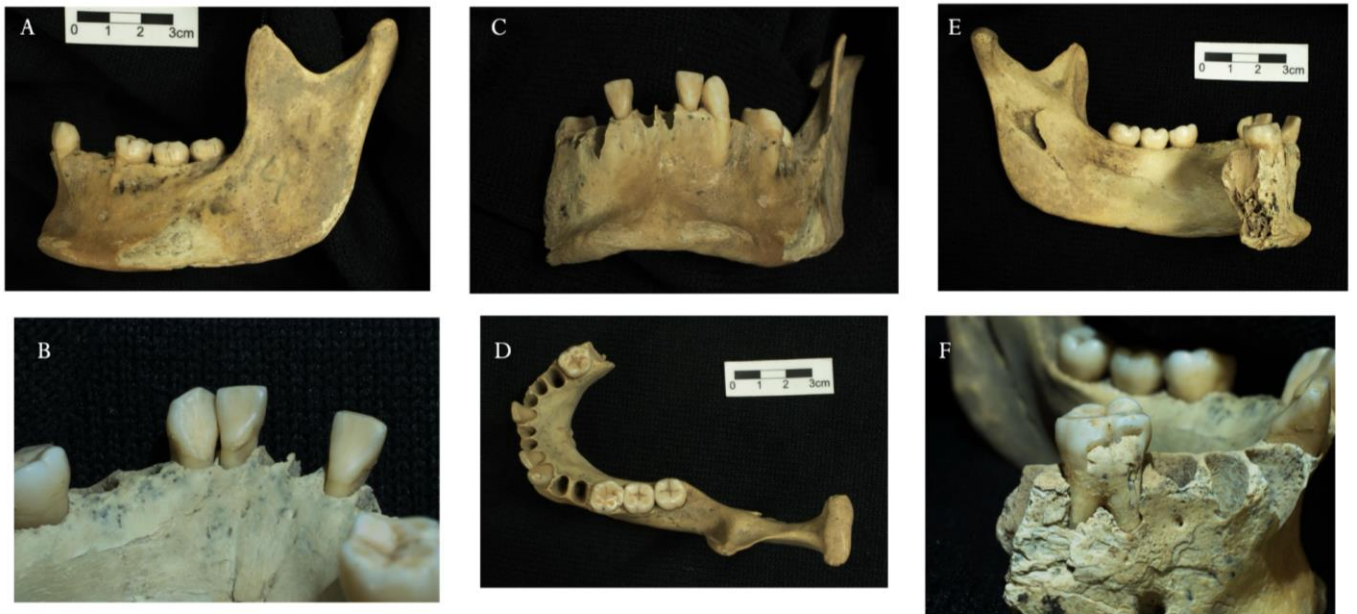
H11



H12



H13



H14

Pic. H (1-14) The human remains belonging to 12 subadult individuals found in Cova de Les Gralles and some details of the dental calculi collected.

N.B. Tooth 1 (Pic. H1.) matches mandible 12 (Pic. H12.), while tooth 2 (Pic. H2.) matches mandible 8 (Pic. H8.).

H1. (A/C) Tooth; H2. (A-B-C) Tooth; H3. (A-B) Maxilla; H4. (A/D) Maxilla; H5. (A-B-C-D) Mandible; H6. (A/D) Mandible; H7. (A/D) Mandible; H8. (A/D) Mandible; H9. (A/D) Mandible; H10. (A/D) Mandible; H11. (A/F) Mandible, E and F details of the striations due to post-mortem manipulation of the individual's bones; H12. (A/G) Mandible, from E to G details of the striations due to post-mortem manipulation of the individual's bones; H13. (A/D) Mandible; H14. (A/F) Mandible. Photos by Josep Maria Vergès and Arianna Giordano

3.2. Cleaning and sampling of the dental calculi

The dentition of the individuals, most of them subadults, from Cova de Les Gralles appears very clean, just with few remnants of dental calculus (mostly small accumulations and ridge-like striae following the course of the dental gums), consequently, the sampling process required extreme care and precision.

Before the collection of dental calculi, it is good to keep in mind that it is possible that these could be contaminated by modern micro remains, to avoid this, therefore, it is necessary to take some precautions during the export phase of a dental calculus. For this purpose, a very precise protocol was followed, which premised on limiting the “pollution” of the evidence to a minimum.

The sampling procedure was then carried out as follows:

- first of all, the cover of the work surface with silver paper;
- subsequently squares of silver paper were cut;
- then the working instruments (scalpels and specils) were disinfected with ethanol;
- after that, the samples were taken by gently scraping the dental calculus without affecting the enamel and dropping the powder inside the silver paper squares. In this way, the ‘heart’ of dental tartar has been exported; in the other hand, the surface is more susceptible to contamination by modern micro remains;
- between one fossil and the other, the work tools have been disinfected with a gauze soaked in ethanol;
- finally, they were placed inside labeled small bags.

N.B. As anticipated above, in this work the micro remains analysed are focused on the extraction of fossilised pollen, phytoliths and NPP's (non-Pollen Palynomorphs). An analysis of starch grains, which in archaeology contexts are identified on the basis of the size and the shape, was not possible because the laboratory where the analysis was conducted (in the IPHES) does not have the appropriate instruments for this type of test, so, it would have been impossible to perform analyses without contamination occurring by modern starch grains that look exactly morphologically identical to fossil grains (Hardy et al. 2009).

It is necessary here to open a note-worthy parenthesis in so far as direct observation of the fossils revealed some elements, not related to the main topic of this thesis work, that are worthy of mention, especially if in the future someone wants to investigate other aspects related to the human remains of Cova de Les Gralles.

First of all, some fossil specimens, three in particular (maxilla 4 and mandibles 11 and 12) under study, present several striations on the surface that suggest postmortem manipulations. However, there is not enough proof to know something more about these marks just from an initial observation, this requires more in-depth specific analyses.

Most of the specimens show the absence and/or fracture of the dental enamel capsule. These are probably post-depositional fractures, as there is a sharp clear cut of the fractures.

It is still unclear what this detachment of the enamel capsule is due to since there are no diseases in life that cause such a situation *post - mortem*. It must also be ensured that there are no deficiencies in life that could cause a similar situation after the death of the individuals.

The only plausible hypothesis could be one related to the composition of the soil in which the human remains were immersed, since the acids produced by some organic constituents in the soil can, in some cases, drastically weaken the structure of the teeth, decalcifying them, a factor that consequently also causes a loss of dental enamel. As the pH changes, the equilibrium of the dissociation/salt formation reaction changes, and above certain values the direction of the reaction shifts, and this is also the principle behind caries formation (Manoilescu et al. 2015).

3. Treatment of dental calculus samples

Once the dental tartar samples had been collected, they were transferred to the Archaeobotanical laboratory of the IPHES (Institut Català de Paleoecologia Humana i Evolució Social) research center, Tarragona. Here, the treatment of the samples began with the purpose of removing the calcium carbonate and other residues that would have denied the observation of the sample, until the material was obtained to mount the glass slides for observation under an optical microscope.

The protocol for the chemical treatment to be achieved on dental tartar was adapted to the needs dictated by the state of the samples (cf. the second point of the webography; Gancz et al., 2024).

As previously mentioned, the part that is potentially most contaminated with modern micro remains is obviously the outer part of the calculus. However, most of these samples are pulverized (so the inner part of the dental tartar was taken), therefore, the larger samples were cleaned with ethanol before being picked up (Dobney K., Brothwell 1987). In consideration of this, as a first step, the silver paper sheets in which the samples were stored, were opened and the powder were firstly weighed dry with a precision balance; then a second weighing was carried out as a final step after the stove passage, but we will deal with this phase later. (*Table 5.*)

Once the samples were weighed, they were introduced into the Eppendorf's tubes, following which the second step consisted of introducing hydrochloric acid (HCL) into them in order to dissolve possible traces of calcium carbonate.

Firstly, HCL 1 N was used, however the reaction did not take place probably due to the absence of a high concentration of calcium carbonate within the samples; anyway, has been observed them partially disintegrating; for this reason, for the following treatments, it has been decided to use a higher concentration of acid: HCL 3 N, as the calcium carbonate resulted more consistent.

N.B. → No samples coming from Cova de Les Gralles showed a strong reaction to HCL 3 N except for number 13 which had a slight reaction when in contact with the acid.

During the next stage, distilled water (DH₂O) was added up to the cap of the tube, followed by a passage through the agitator and then 3 minutes into the centrifuge.

Finally, the last step consisted in the decantation (pipetting) of the liquid solution by means of a pipette until the sediment was almost completely free of water.

Before the samples were finally introduced into the oven, the same step just described was repeated for the last time but with the addition of ethanol (C₂H₆O) in view of facilitating evaporation and to 'wash away' the last remaining particles that were not useful for the analysis of the micro-rests contained within the dental calculi.

During the last passage, as anticipated, the Eppendorf containing the samples were placed inside the stove and left to dry for about 24 hours at 50 degrees celsius. After this, a second weighing of the samples was performed with the aim of obtaining the weight of the residue removed from the difference between the initial and final weights of the samples.

Once the samples were ready, they were placed on the labeled glass slides, drops of phenol glycerine were added on them and then sealed with the sandwich slide by means of an adhesive resin. At this point, the samples were ready to be examined under the microscope.

	Starting weight	Residual weight	Final weight
Gralles (1+12)	0,0119 g.	0,0089 g	0,0030 g.
Gralles (2+8)	0,0012 g.	0,0005 g.	0,0007 g.
Gralles 3	0,0062 g.	0,0057 g.	0,0005 g.
Gralles 4	0,0014 g.	0,0011 g.	0,0003 g.
Gralles 5	0,0084 g.	0,0076 g.	0,0008 g.
Gralles 6	0,0010 g.	0,0002 g.	0,0008 g.
Gralles 7	0,0012 g.	0,0009 g.	0,0003 g.
Gralles 9	0,0008 g.	0,0006 g.	0,0002 g.
Gralles 10	0,0007 g.	0,0006 g.	0,0001 g.
Gralles 11	0,0018 g.	0,0013 g.	0,0005 g.
Gralles 13	0,0038 g.	0,0028 g.	0,0010 g.
Gralles 14	0,0034 g.	0,0031 g.	0,0003 g.

Table 5. Initial, residual and final weight of dental calculus samples (Cova de les Gralles)

3.4. Counting and data processing

Before the direct observation of the dental calculus samples, a study of the reference collection stored in the archaeobotanical laboratory of the IPHES had to be carried out regarding the various pollen types. As far as phytoliths and NPP's are concerned, the study was achieved by means of the observation of samples containing micro - remains coming from previous excavations.

Prior to moving on, it is good to give a brief excursus on why these fossil remains are so resistant as they remain trapped within the dental calculus.

First of all, we know that inside the pollen there is the biological fluid matrix, the cytoplasm, what makes the pollen granule 'alive', this is obviously not preserved when we speak about fossil pollen, what we see is only the external structure, the sporoderm (which has various decorations depending on the pollen's family of origin).

This does not deteriorate in the course of time, especially the outermost part of the sporoderm, the exine, because it is made up of a complex of carotenoid polymers, called 'sporopollenins', which are among the most resistant substances in the organic world; The only 'enemy' of sporopollenins is oxygen, which causes their immediate corrosion and destruction (Shaw, 1971).

The case is different for phytoliths, since they do not belong to the category of palynomorphs. Their formation consists of the following process: plants absorb silicon in the form of monosilicic acid $[\text{Si}(\text{OH})_4]$ through their roots. Then silicon is deposited into the aerial parts of the plants in the form of opal or hydrated silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$), in those cells related to transpiration (Currie, Perry, 2007). It's true that phytolith production is regulated by plant genetics, however environmental factors (soil type, evapotranspiration and the amount of water) can affect the accumulation of these inorganic structures.

During the plant's necrolysis, phytoliths are deposited in the soil; they can survive millions of years because of their inorganic structure, they are also resistant to fire, acidic pH environments and to the digestive tract of the animals. They can also accumulate in the dental calculi of animals and humans (Henry et al. 2010, 2012; Henry, Piperno 2008; Power et al. 2015, 2018) and based on this we can study the ancient diet of them (more specifically the proportion of plants in diet); the most well-known phytoliths are those relating to herbaceous species, mainly in the Poaceae and Cyperaceae families (Lalauze et al., 1996; Cummings et al., 2018; Ge, et al., 2022), while the ones produced by woody plants are less well investigated. According to recent studies, they are mostly produced by conifers, some families of deciduous trees such as the genus *Acer* and *Quercus* (An, Xie, 2022).

In the view of this, while for pollens it is possible to define the family and, in some cases, even the genus, this does not apply to phytoliths which, as it has just been explained, are siliceous structures that "replicate" the specialised cells of certain types of plants, identifying even just the family requires a greater effort due to the complexity often associated with the state of preservation and the variety of these micro remains.

The same goes for NPP's, a group which includes micro remains belonging to the plant, animal and in some cases also the inorganic (mineral) kingdom; it is such a broad and 'new-born' field of investigation that it is very frequent being not able to recognise the type to which the observed non-pollen palynomorph belongs.

4. Pollen and NPP's analysis (sediment samples)

On 19 February 2024, an inspection of the Cova de Les Gralles was conducted in order to collect some sediment samples from the inside of the cave and observe the surrounding modern vegetation, both on the path and just outside the site (see 1.2 *The cave*).

4.1 Sampling

The section that constitutes the actual site within the cave consists of a larger room that ends in a blind wall, while below, on the left-hand side, there is a cavity about two meters deep, which becomes narrower the more forward one goes, and constitutes the very site where the human remains were found.

Inside this chamber there was a pit (*Pic. D4.*), approximately 20/25 cm deep, which most probably originally contained human remains (due to the presence of some remains still in situ).

Two of the sediment samples examined were collected at this very spot, one in the center of the pit (sample number 1), and the second (sample number 2) just outside it.

The remaining two samples had been taken on a wall of stratified sediment, just outside the cavity that forms the site of the bone discovery (sample 3 lower and sample 4 higher).

The sediment picked up was then packed in sterile bags, labeled and taken to the IPHES laboratory for treatment with chemical agents.

The following scheme (*Table 6.*) describes the sediment matrix collected as samples:

1. Inner sediment (Gralles interior, cavitat)	Dove-brown colour, very fine and compact matrix. Weight at the beginning of the treatment = 14,55 g.
2. Inner sediment (Gralles interior, zona boca)	Dove-brown colour, more granular and coarser matrix. It contains carbon. Weight at the beginning of the treatment = 12,22 g.

3. Outer sediment (Gralles columna, exit n° 1)	Darker brown sediment than the previous one. Fine matrix. Weight at the beginning of the treatment = 13,97 g.
4. Outer sediment (Gralles columna, exit n° 2)	Darker brown sediment than the previous one. Slightly grainier matrix than before. Weight at the beginning of the treatment = 13,47 g.

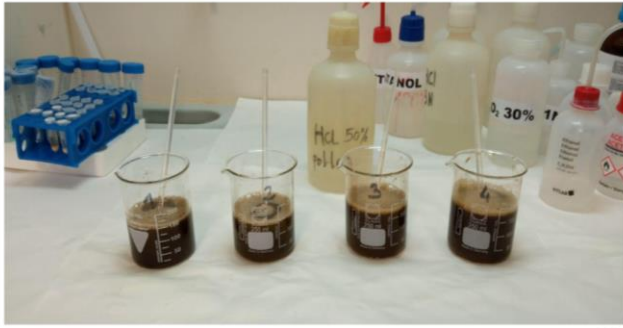
Table 6. Matrix description of the four sediment samples

N.B. → In this first weighing, all sediment samples were taken in quantities between 12 and 15 grams.

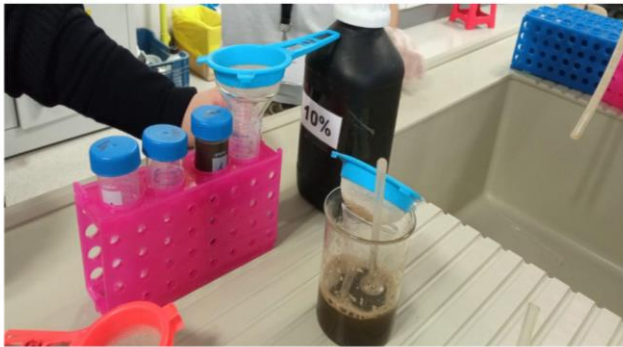
4.2 Treatment

First of all, these quantities of sediment were introduced into four sterile glass bechers and 50 per cent hydrochloric acid was added. All the samples had a strong reaction to acid addition (*Pic. I1.*).

Once the reaction was complete, the contents were imported into the test tubes, after which the entire mixture was filtered (*Pic. I2.*). Next, distilled water was added to the contents so that all weights were balanced to introduce the tubes into the centrifuge (program 1, 3 min, 2500 rpm).



I1



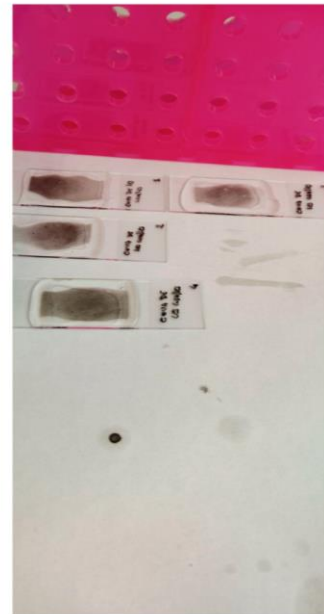
I2



I3



I4



I5

Pic. I (1-5) Some of the treatment passages (I1. The HCL reaction inside the glass bechers; I2. Filtration of the sediment samples; I3. Filtration in the ampoule after the addition of the 'Thoulet's solution'; I4. One of the samples, inside the Eppendorf, ready to be mounted on a glass slide; I5. The four samples assembled on the glass slides and ready to be examined under an optical microscope).

After several washes with hydrogen peroxide, the last ones (normally three) were with soda (NaOH) and introduced in a 40° water bath (soda at 10%- and 10-minutes bain-marie).

N.B. → At the third wash with soda, and at each further wash with distilled water, three drops of 50% HCL were always added to balance the pH.

At this point, after several hours, the mixture inside the test tubes dried and only approx. 20 ml were taken (for each sample) to add the 'Thoulet's solution', a cadmium iodide and potassium iodide solution with a density equal to 2 g/cm³, used in laboratory to separate palynological remains from a sediment.

In the next phase the contents of the test tubes were equilibrated on the weighing scales, always using liquor to balance, in order to centrifuge them again.

After these steps, filtration took place by using an ampoule and glass fiber filters → the contents of the test tubes were poured into the ampoule, after which the filter was removed (*Pic. I3.*).

The penultimate passage is the addition of hydrofluoric acid at 48% (HF): the tubes were placed inside glass jars (to guarantee greater safety and stability), and special gloves were used to manipulate this type of acid; after that the whole mixture was left to react for at least one hour minimum.

Finally, the last step consists in the addition of hydrochloric acid (HCL) and distilled water up to the cap of the tubes, the decantation into the acid jug and then the last washings with the distiller water and the final centrifugation.

While all these passages were carried out, the residual sediment was dried inside the strainers and weighed, the following is the weight and the matrix description of the four samples:

1. 0,38 g. → Brown and gray charcoal stones. Is the one with the most quantity.
2. 0,28 g. → Brown and gray charcoal stones.

3. 0,06 g. → Just a little charcoal.
4. 0,02 g. → Just a little charcoal. Is the one with less quantity.

Lastly, the samples are assembled on the glass slides with the aid of glycerine phenol (*Pic. 14. and 15.*). The values (in ml) of the montage are shown in the following table (*Table 7.*):

	Glycerine	Collected	Mounted
1	1000	1272	20
2	980	1200	20
3	1010	1372	20
4	990	1221	20

Table 7. Montage values

4.3. Counting and data processing

The glass slides containing the sediment samples were subjected to careful observation at 400 x magnification, ocular x15, with the aid of the Euromex iScope optical microscope; for each specimen, the micro - remains contained within five lines (six for specimen number 3) were counted (minimum 150 pollen grains counted for each line).

The counted micro remains were annotated and, in some cases, drawn on four appropriate sheets, after that they were entered on the Tilia Neotoma database (Grimm, 1991-2011) in view of recording all taxonomic groups and affiliations present. After this was completed, a diagram was created to check which palynomorphs are represented, in which samples and their concentration.

5. Data presentation and interpretation

The two paragraphs below (5.1. and 5.2.) are focused on the data obtained from this study and the assumptions concerning their interpretation.

5.1. Dental calculus

The twelve samples of dental calculus were observed under an optical microscope, and what resulted from this careful analysis can be deduced from the table below (*Table 8.*):

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Dental calculus samples (Gralles) (1+12)	(2+8)		3	4	5	6	7	9	10	11	13	14
2	Vegetal tissue	22	15	18	7	18	5	15	19	34	17	48	9
3	Phytoliths	2	1	2	1		3	2	1		2	4	1
4	Protists	2											
5	Poaceae			1									
6	Undeterminate pollen								1			2	
7	Microcharcoal	7	3	7	1	25	62	6	1	2	3	65	14
8	Type HdV S2L			1								1	1
9	Hiphae	1	1	1				1		1		1	
10	Fungi undet.	2											
11	Sordaria		1	2				2					
12	Polyadosporites	2											
13	Polyporisporites											1	
14	Type HdV 303							1				1	
15	Glomus								1				
16													
17													
18													
19													
20	Total all the evidences	38	21	32	9	43	70	27	23	37	22	123	25

Table 8. Counting of micro-rests observed within dental calculus samples (Cova de Les Gralles)

The first thing that stands out is the fact that the presence of micro-remains is sporadic and rare with a prevalence of undefined plant tissues, microcharcoal (only fragments with a size between 5 and 20 μm were included) followed by the presence of some elements classifiable as NPP's and finally the almost total absence of pollen grains.

Inside these dental calculi a total of 19 phytoliths (in samples 1+12; 2+8; 3; 4; 6; 7; 9; 11; 13; 14) were observed. Unfortunately, it was not always possible to identify their exact

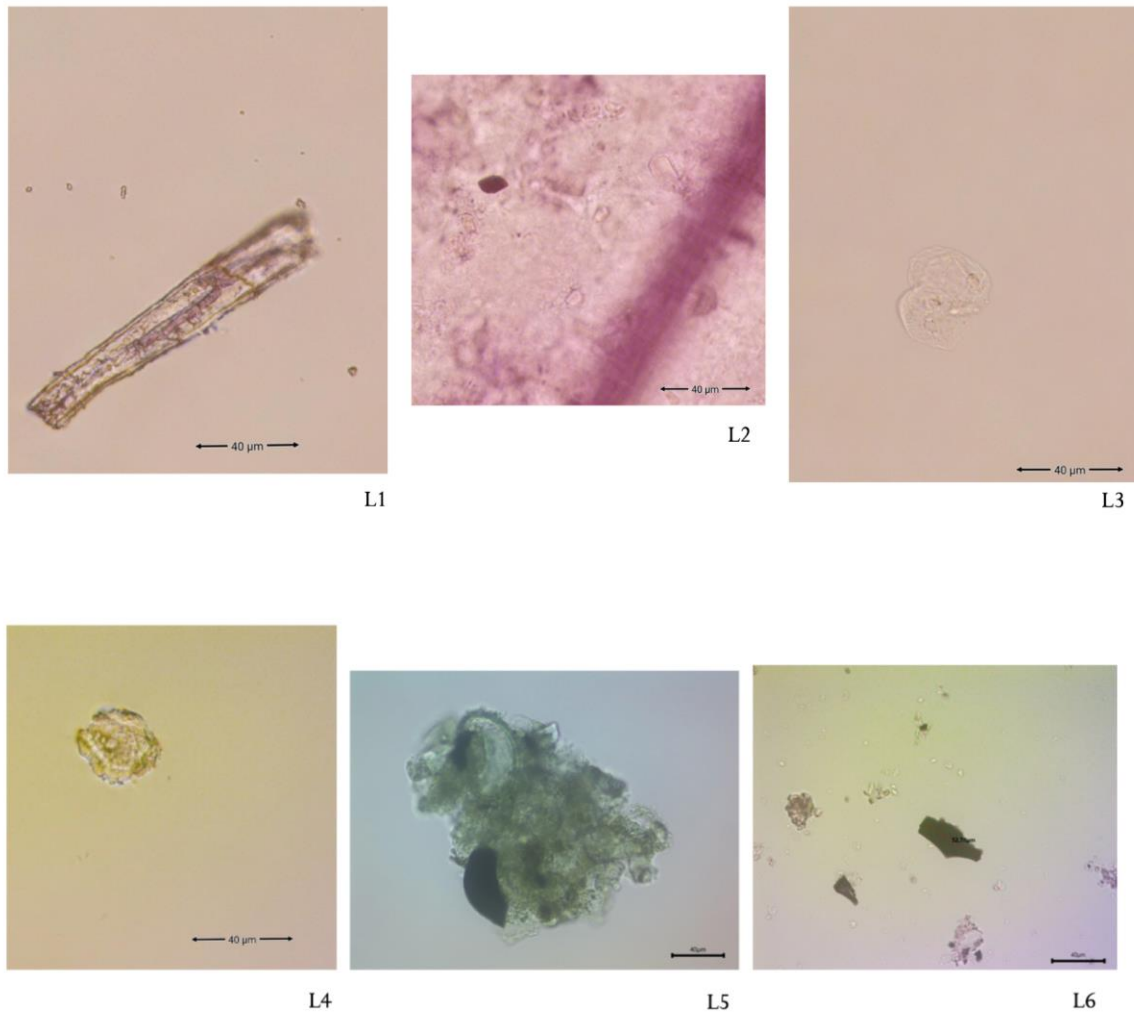
morphology, except for a few cases for which the following forms were determined: scutiform, bulliform and cylindrical (ICPN 1.0, 2005); elongate multicell, acicular (ICPN 2.0, 2019); blocky irregular (Gao et al. 2018); wavy top rondels (Pagán-Jiménez, et al., 2020). As far as the pollen is concerned, the only recognised case is within sample number 3, a pollen belonging to the Poaceae family, whereas the three pollen grains contained in samples number 9 and 13 could not be classified due to their poor state of conservation. More frequent than the rest of the micro remains, but in any case, still scarce, is the presence of non-pollen palynomorphs (NPP's), of which various classes are represented: protists in sample (1+12); type HdV S2L in samples 3, 13 and 14; hiphae in samples (1+12), (2+8), 3, 7, 10 and 13; sordaria in sample (2+8), 3 and 7; polyadosporites in sample (1+12); polyporisporites in sample 13; type HdV 303 in samples 7 and 13; glomus in sample 9 and indeterminate fungi in sample (1+12).

A more detailed description of the nature of the NPP's encountered follows.

Within the sample (1+12), a small lump formed by two protists recognised by the eyespot (first primitive form of 'sight') was observed, followed by a variety of non-pollen palynomorphs belonging to the fungi kingdom (especially fungal spores and other diagnostic parts of these organisms) and algal forms:

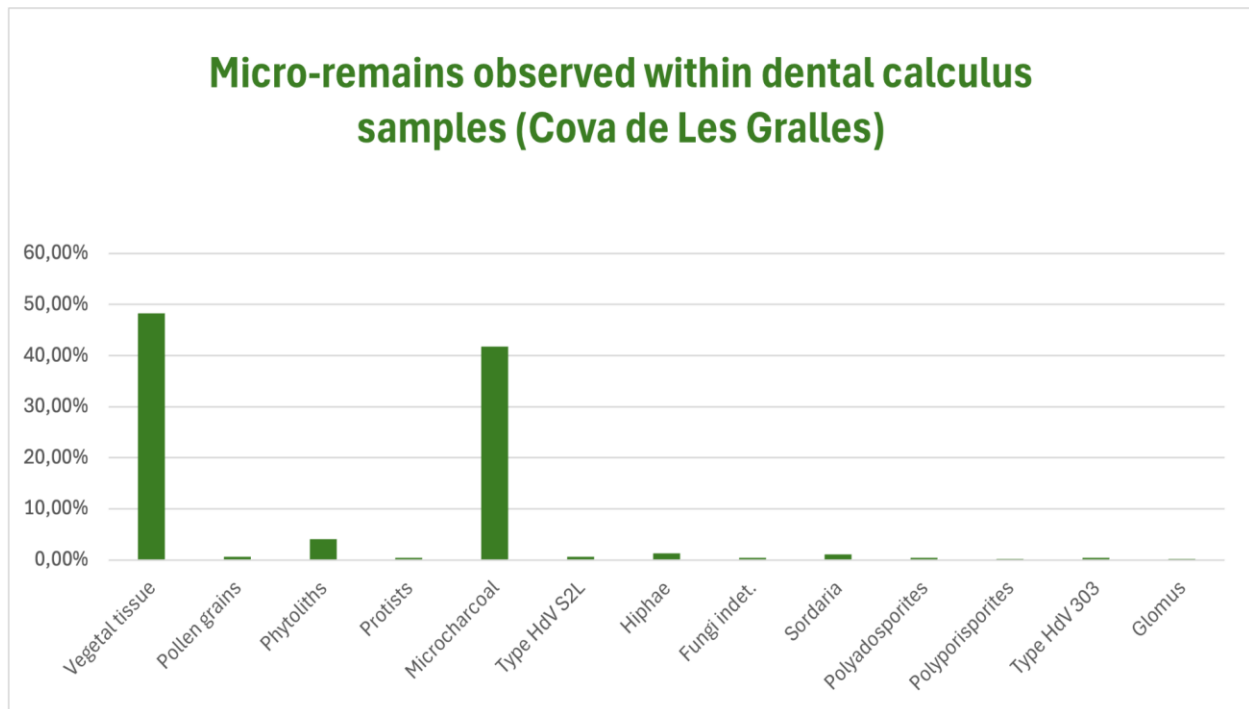
type HdV S2L in samples number 3, 13 and 14; hyphae, the basic cellular unit of filamentous fungal structures formed by cells which may be uninucleate or multinucleate (Zabel, Morrel, 2020) in samples number (1+12), (2+8), 3, 7, 10 and 13; undetermined fungi in sample (1+12); sordaria, a fungal spore commonly found in the faeces of herbivores as these types of fungi exploit the digestion mechanism of animals to propagate (Cugny, et al., 2012; van Geel, 1976; 1978; van Geel et al., 2003; Gelorini, et al., 2011) in samples number (2+8), 3 and 7; polyadosporites, septate fungal spores, (Jarzen, Elsik, 1986; van Geel, et al., 1989) in sample number (1+12); polyporisporites, septate fungal spores, (López Sáez, López García, 2003) in sample number 13; type HdV 303, a type of

transparent unicellular algae, (Llimona, et al., 1985; Miola, et al., 2006, 2012; van Geel, et al. 1981) in sample number 7 and 13 and finally glomus, a genus of arbuscular mycorrhizal fungi, all species form a symbiotic relationship with plant roots, (van Geel, et al., 1989; Gelorini, et al., 2011) in sample number 9.

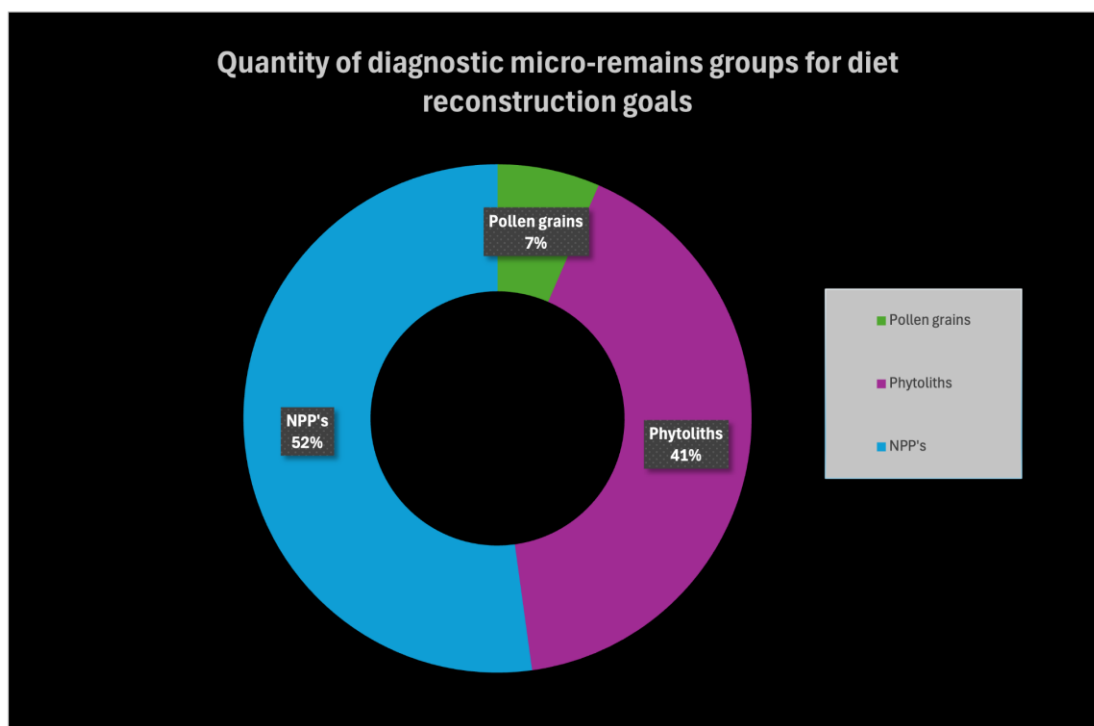


Pic. L (1-6) Some of the micro remains found inside the dental calculi - Cova de Les Gralles. (L1. Elongate phytolith multicell, sample 1+12; L2. Sordaria inside a fragment of vegetal tissue, sample 3; L3. A lump formed by two protists, sample 3; L4., L5. Indefinite pollen, samples 9 and 13; L6. Micro coal, sample 13). Euromex iScope Microscope.

Based on the processed data just discussed, it was possible to elaborate the following two graphs. In the first one (*Graph 1.*) we can observe the total quantity and all types of micro-remains that were observed within the Cova de les Gralles dental calculus samples, while in the second (*Graph 2.*) only the micro-remains that were diagnostic and useful for the reconstruction of the ancient diet were considered: pollen grains, phytoliths and NPP's. In the first graph (*Graph 1.*) we can see that the quantity of micro-rests present in the samples is very limited: as far as the vegetal world is concerned, non-diagnostic remains such as plant tissue parts and microcharcoal predominate; pollen grains are almost totally absent, while there is a scarce presence of phytoliths. The same situation also occurs with the NPP's whose class in this case is predominantly made up of taxa belonging to the fungi kingdom, followed by algal forms and protists of an indefinite kind.



Graph 1. Micro-remains observed within dental calculus samples (Cova de Les Gralles)



Graph 2. Quantity of diagnostic micro-remains groups for diet reconstruction goals (Cova de Les Gralles)

5.1.1. Interpretation

In view of the data examined, it came out by observing the human remains from Cova de les Gralles, that the great majority of the individuals show little presence or almost complete absence of dental tartar, furthermore, many of the teeth are affected by postmortem breakage of the dental enamel capsule. As anticipated, it is not possible to establish the cause of this phenomenon with surety, but it is hopeful that the issue will be investigated more deeply in future studies.

From the evidence emerging in the content analysis of dental calculi we can also observe that there is a very low presence of plant micro remains, this could be interpreted as a trend toward a diet based on a greater consumption of animal rather than plant-based foods.

It is also not possible to comment fully on the cause of this as the site has not returned any other evidence, of various kinds, that can be linked to this fact.

The only observation that can be formulated about this is that probably living high up in the mountains, this society did not devote much time to the cultivation of vegetable species and lived more on animal proteins, however, regarding this theory too, there is not enough evidence to establish that this statement is correct.

5.2. Pollen and NPP's analysis

Regarding the pollens, in the four samples studied, there is a prevalence of those from herbaceous plants (indicated with 'group B' in the database) compared to the ones from tree plants ('group A').

The taxa represented inside the samples are the following:

Arboreal pollen	Herbaceous and arbustive pollen
<i>Pinus</i> sp (6,1%-27,7%) <i>Quercus</i> deciduous (3,1%-7,9%) <i>Quercus ilex-coccifera</i> (4%-9,2%) <i>Alnus</i> (2%-23%) <i>Salix</i> (1%-3,1%) <i>Castanea</i> -type (0%-0,5%) <i>Corylus</i> (0%-2%) <i>Olea-Phillyrea</i> (0%-1,1%)	Poaceae (17,8%-26,5%) Asteraceae liguliflorae (6,7%-15,2%) Apiaceae (3,1%-22,4%) Cyperaceae (2%-9%) <i>Erica</i> sp (1,6%-6,1%) <i>Ephedra distachya</i> - type (0%-5,1%) Asteraceae tubuliflorae (0%-2%) <i>Artemisia</i> (0%-1,1%) Amaranthaceae (0%-1,1%) <i>Plantago</i> sp (0%-1%) Lamiaceae (0%-1%) Thymelaeaceae (0%-1%) <i>Scabiosa</i> (0%-1%) <i>Typha-Sparganium</i> (0%-1%) Caryophyllaceae (0%-0,5%)

Table 9. The main pollen taxa observed in the four sediment samples. In brackets, the percentages of all taxa with their minimum and maximum values represented.

Pteridophytes ('group D') with predominance of monolete spores and *Polypodium* are also present.

There are different types of algae ('group E') and fungi ('group F') too, many of them are unfortunately impossible to recognise mainly due to poor conservation status.

The same applies to zoostats ('group G'), some recognisable as the eggs of certain parasites and the body parts of some microorganisms such as mites, and others not very clear. Finally, there is the last group ('group H'), that refers to the unknown taxonomy but clearly recognisable as non-pollen palynomorph.

Below are reported the main taxa encountered:

Pteridophyta	Algae	Fungi	Zooests	Unknown
<i>Polypodium</i> (7,7%-15,9%) Monolete spores (2,8%-17,6%) Trilete spores (0%-0,4%)	<i>Spirogyra</i> (4,5%-10,9%) <i>Botryococcus</i> (1,4%-9,2%) <i>Debarya</i> (0,4%-1,7%) <i>Rivularia</i> (0,3%-1,8%) <i>Anabaena</i> (0%-1,1%) HdV 721 (0%-0,9%) HdV 128 (0%-0,7%) <i>Mougeotia</i> (0%-0,4%) <i>Zygnema</i> (0%-0,3%)	<i>hyphae</i> (14,2%-19%) <i>Glomus sp</i> (0,4%-11,3%) UG 1180 (0%-3,5) <i>Polyporisorites</i> (0,3%-2,6%) UKA 33 (0%-1,8%) HdV 1013 (0%-1,8%) <i>Coprinus sp</i> (0%-1,8%) <i>Cladosporium</i> (UKA 1a) (0%-1,4%) UKA 30 (0%-1,4%) BM 5 (0%-1,1%) <i>Polyadosporites</i> (0%-1%) HdV 1032 (0%-0,7%) UG 1079 (0%-0,7%) HdV 55b (0%-0,7%) <i>Thecaphora sp.</i> (HdV 364) (0%-0,4%) UG 1172 (0%-0,4%) UG 1282 (0%-0,4%) <i>Spegazzinia Tessarthra</i> (TI 039) (0%-0,3%)	HdV 52 (8%-13,2%) HdV 36 (1,1%-1,7%) HdV 36c (0,7%-1,3%) HdV 106 (0%-0,7%) <i>Trichuris</i> (0%-0,7%) <i>Scolecodonte</i> HdV 88 (0%-0,7%) HdV 37 (0%-0,4%) <i>Assulina sp</i> (0%-0,4%)	HdV 303 (8,8%-15,8%) protists (1,2%-4,9%) BFA 30 (0%-1,5%) UKA 38 (0%-0,7%) UY 134b (0%-0,6%) UG 1319 (0%-0,6%) UG 1312 (0%-0,6%) HdV 115 (0%-1%) <i>Pseudoschizaea</i> (NN61) (0%-0,3%) UY 134a (0%-0,3%) BM 1 (0%-0,3%)

Table 10. The main NPP's taxa observed in the four sediment samples. In brackets, the percentages of all taxa with their minimum and maximum values represented.

Once all the data had been uploaded on Tilia database (Grimm, E.C. 1991-2022: TILIA and TILIA Software [computer Program], 3.0.3 Version), the calculation of the sum base and total sum of each group of micro-rests examined was performed, with the aim of creating percentages that served as the basis for the construction of the graph presented below (Graph. 3).

Inside this graph NPP's and pollen values were expressed in concentrations (particles-grains/g of dry sediment) calculated using the volumetric method (particles/g of dry sediment) (Loublier, 1978; Burjachs, et al., 2003).

5.2.1. Interpretation

From the first reading of the graph, it results in a variegated ancient plants landscape. In order to reconstruct the paleoenvironment type, it is necessary to consider arboreal, arbustive and herbaceous plant species.

Regarding the first typology, there are pollens belonging to the following taxa represented: *Pinus* sp, *Quercus ilex-coccifera*, *Quercus* deciduous, *Corylus*, *Alnus*, *Salix*, *Castanea*-type and *Olea-Phillyrea*. Most of these plants live in temperate places but with a very wet climate, preferring damp grasslands and riparian forests, apart from *Pinus* sp., *Quercus ilex-coccifera* and *Olea-Phillyrea* that favor a more Mediterranean and sunnier climate.

The arbustive species found within the four specimens, namely *Erica* sp. and *Ephedra distachya*, live mainly in open and very sunny rocky and/or sandy locations, in any case they do not tolerate shady areas very well.

Finally, a lot of herbaceous taxa are represented: Poaceae, Asteraceae liguliflorae, Asteraceae tubuliflorae, *Artemisia*, *Plantago* sp, Amaranthaceae, Apiaceae, Caryophyllaceae, Lamiaceae, Thymelaeaceae, *Scabiosa*, Cyperaceae, *Typha-Sparganium*.

Most of these taxa generally prefer open environments such as grasslands, woodland edges, but also man-made rural landscapes such as paths, seasonally grazed meadows (especially Asteraceae, *Plantago* and *Artemisia*), and plough areas (especially *Plantago*) (López-García, López-Sáez, 2000; López-Sáez, et al., 1998; 2000).

There are not many correspondences with the vegetation that dominates the modern landscape, because the only modern taxa also found in ancient pollen analysis are Lamiaceae, Pinaceae and Apiaceae.

The current mountain landscape in the Prades territory is characterised mostly by forests dominated by pine and oak trees, there are also a large number of taxa typical of rural but anthropised areas, such as the Euphorbiaceae, Rubiaceae, Fabaceae, Rosaceae,

Campanulaceae, Cistaceae (the latter are also pyrophilous and therefore highly resistant in the event of fires, as we know, unfortunately very frequent in the mountains frequented by grazing land, especially during the summer period).

As regards non-pollen palynomorphs, we should remember that their dispersion is not comparable to that of pollen, which can take advantage of wind (anemophilous), animals (zoophilous) and water (hydrophilous) to reach even considerable distances. In contrast, NPP's only disperse into the surrounding environment, depending on their nature.

Diverse groups of taxa were observed, including those belonging to the mushroom kingdom, those related to microalgae, those related to the animal kingdom and many 'unknowns' whose origin and affiliation is still obscure to us.

It is also worth of mention the presence of Pteridophyta spores, especially monolete and *Polypodium* spores, which disperse not very far from the mother plant and are characteristic of very wet underbrush environments and spread, as in our case, just outside the caves.

With respect to the fungi kingdom, the following taxa were identified: *hyphae*, *Polyadosporites*, *Polyporisporites*, *Glomus* sp, *Cladosporium*, (UKA 1a), *Thecaphora* sp., (HdV 364), *Coprinus* sp, UG 1172, *Spegazzinia Tessarthra* (TI 039), HdV 55b, BM 5, UG 1180, UKA 30, UKA 33, UG 1282, HdV 1032, UG 1079, HdV 1013.

Several of these micro-rests, mostly fungal spores, are also, like pollen, diagnostic for understanding the ancient environment; first, these are taxa that once again prefer a humid environment (as do most species belonging to the kingdom of fungi), these include some saprophiles (UG 1180), some parasites of plants since they meet their roots. Then they penetrate the epidermal cells and grow between the cortical cells of the roots (Gehlot, Singh 2015). Most interestingly, is that a large majority of the taxa observed in the sediment samples from Cova de Les Gralles are coprophilous, i.e. they exploit the intestine, and consequently the excrement, of herbivores to reproduce themselves

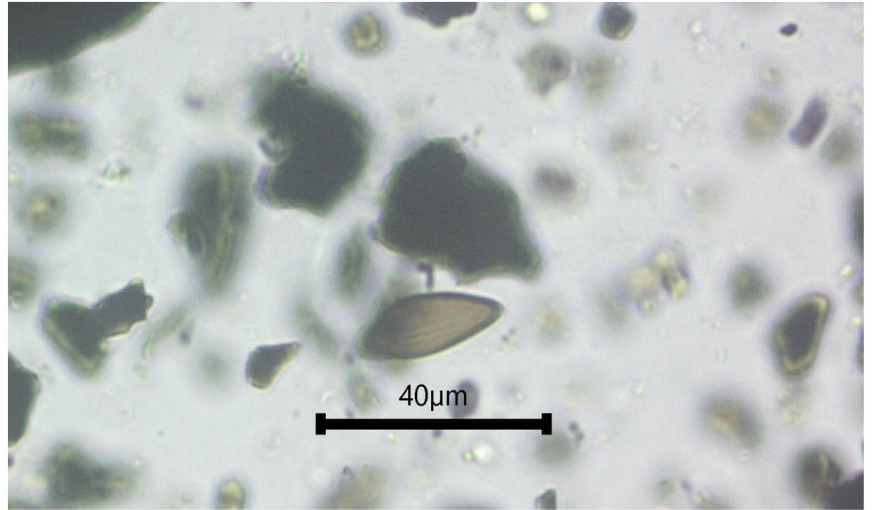
(*Polyadosporites*, *Polyporisporites*, HdV 55b; UG 1180 and HdV 1013). This is another indicator of the presence of possible upland grazing meadows.

The microalgae encountered include the following: *Spirogyra*, *Debarya*, *Botryococcus*, *Rivularia*, HdV 721, HdV 128, *Mougeotia*, *Zygnema* and *Anabaena*. There are also some noteworthy taxa with regard to environmental recognition such as, for example, HdV 128 which is eutrophic, *Spirogyra*, *Debarya*, *Botryococcus* and *Zygnema* meso eutrophic, and finally some oligotrophic species such as *Rivularia* and *Anabaena*. So, once again we are in the presence of very humid areas.

Finally, but not least, diagnostic micro remains of animal origin include *Assulina* sp and *Trichuris*, eggs of coprophilic parasites (Chmura et al., 2006; Komarek et al., 2003; van Geel, 1976; 1978; Livingstone, Jaworski, 1980; van Geel et al., 1983; 1986; 1996; Llimona, et al, 1985; van Geel, et al. 1981; van Geel, et al., 1989; Cugny, et al., 2012; van Geel et al., 2003; Gelorini, et al., 2011; Jarzen, Elsik, 1986; van Geel, et al., 1989, López Sáez, López García, 2003, Llimona, et al., 1985; Miola, et al., 2006, 2012; Gelorini, et al., 2011).



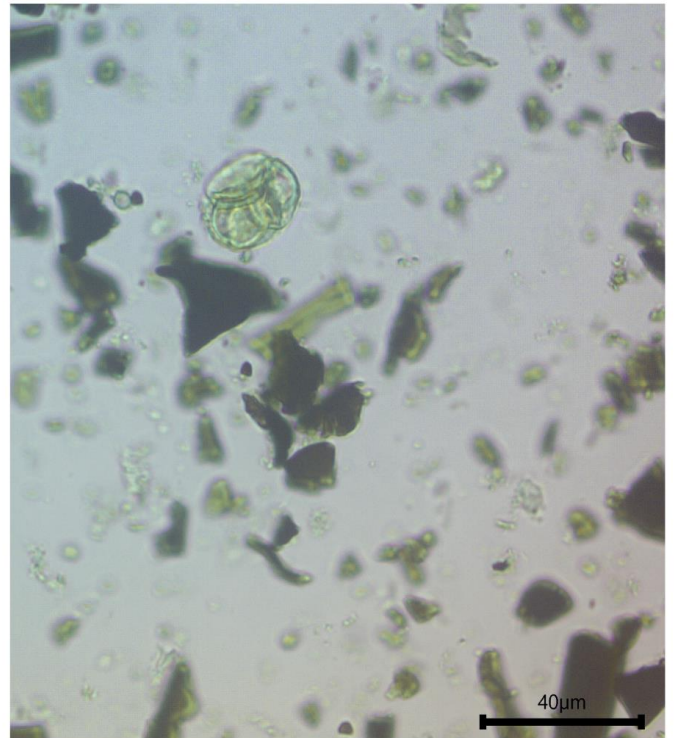
M1



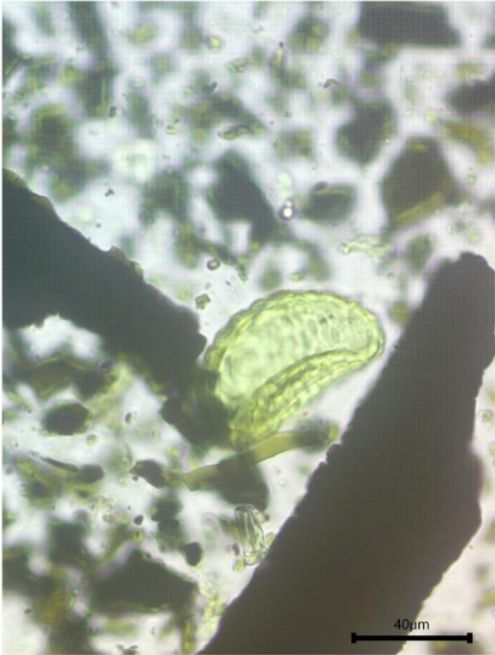
M2



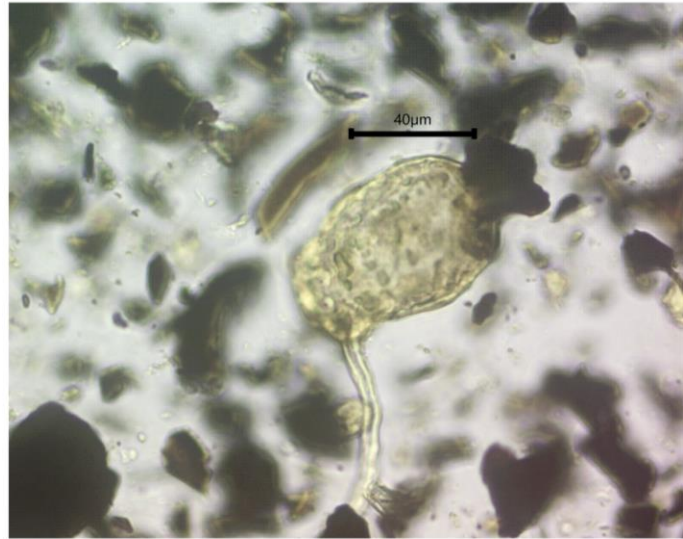
M3



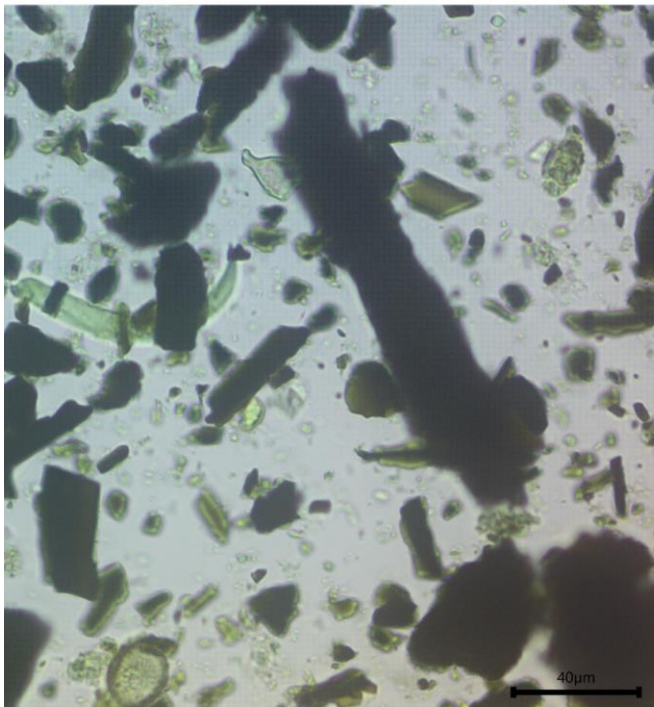
M4



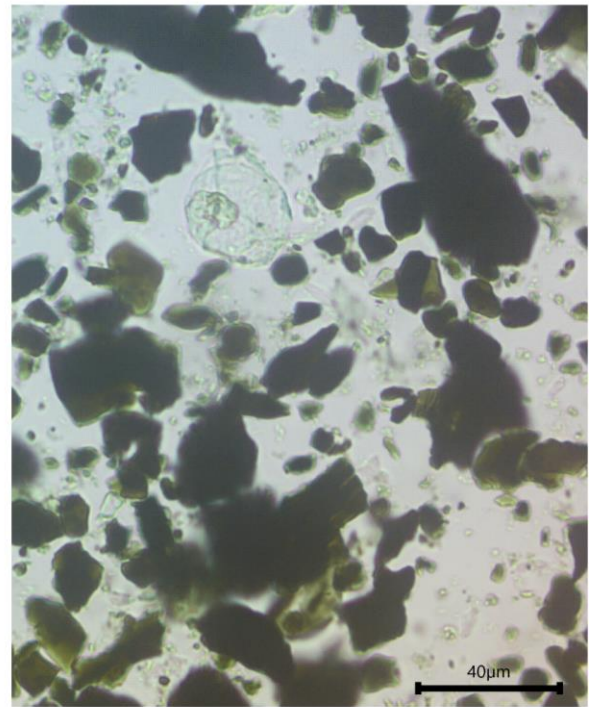
M5



M6



M7



M8

Pic. M (1-8) Some examples of the micro - remains observed inside the sediment samples. (M1. *Trichuris sp.* egg; M2. *Sordaria*; M3. Pollen of *Pinus sp.*; M4. Pollen of *Erica*; M5. *Polypodium*; M6. *Glomus*; M7. Some zoorests, an HdV-36 and an indefinite one; M8. Indefinite protist.

Conclusions

The territory of the Prades mountains in Catalunya (ES) is studded with archaeological sites chronologically placed from prehistory to the Middle Ages. With specific regard to the period between the Late Chalcolithic and the Early Bronze Age, numerous caves in the area's karstic system are interested in deposits, especially those belonging to the funerary sphere peculiar to the social groups that inhabited these heights.

The environment surrounding these cave sites is varied and heterogeneous as the vegetation is mostly composed of arboreal, arbustive and herbaceous species typical of the Mediterranean landscape but also characteristic of the humid continental climate.

The analysis of the micro-remains present within the four samples showed that the paleoenvironment in ancient times was characterised by different types of arboreal plants typical of a temperate but at the same time humid environment, characterised by riparian forests as we are reminded that the area is affected by the presence of several watercourses (Brugent, Montsant, Siurana, Glorieta and Francolí. See par. 1.1).

The two arbustive taxa (*Erica* and *Ephedra*), on the other hand, are typical of mountainous elevations as they prefer open spaces and therefore greater heights than the forests just mentioned.

Finally, the herbaceous plants are excellent indicators of man-made rural landscapes such as paths and pasture lands.

Correspondences with modern taxa are rather limited, as current vegetation is composed by plant species that prefer a drier climate than in the past.

The site of Cova de Les Gralles fits right into this environmental and chronological landscape (ca. 4200 B.P.), and the remains of some of the individuals buried inside it were chosen with the objective of collecting their dental calculus to initiate research on what their diet was, and whether the plant remains trapped in their teeth corresponded more or less to those of modern vegetation.

With a lot of surprise, it was observed that the amount of dental tartar in these individuals was quite poor. By analysing these small quantities for each sample, it emerged not only that the micro-rests were represented quite reductively in all categories, but that those specific to food-related plants (pollen and phytoliths) were almost absent: one pollen of Poaceae and three indeterminate ones, which could not be classified because of their poor state of preservation. The nineteen phytoliths observed within ten of the twelve samples examined are also not directly attributable to specific taxa.

As a result of all this, it has not been possible to determine what the diet of these social groups was; presumably, due to this scarcity, it can be supposed that the climate and the territory were not entirely suitable for cultivar areas, neither that there were frequent exchanges related to the subsistence economy with other groups, perhaps because of the territory that was not exactly suitable for these activities.

This work aimed to open an overview of two topics that have not yet been fully investigated: the reconstruction of the paleoenvironment and of the ancient diet of certain individuals coming from the Prades mountains.

This is intended to be a starting point for other possible studies that may also be undertaken to complement this one, for example, about the second macro-topic dealt with in this work, the investigation of diet through the study of starches and microwear on dental enamel.

Other possibilities can be represented by studies conducted in parallel, for example the analysis of teeth whose enamel capsule has been completely damaged and separated from the tooth.

In short, the possibilities are many and hopefully this is only the beginning for this type of investigation in this region of the Iberian Peninsula.

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