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DIETS IN THE LATE NEOLITHIC OF THE CENTRAL PORTUGAL – PRELIMINARY RESULTS OF DENTAL MICROWEAR ANALYSIS IN GRUTA DOS OSSOS

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Abstract

In the present work, we study dental microwear signature on sample of teeth from Late Neolithic cave collective burial Gruta dos Ossos. The material dates to the second half of 4th – mid 3rd millennia cal BC. It belongs to the cultural assemblage of Nabão valley in North Ribatejo.

The main objective of the study was to collect more information about buccal microwear striation patterns of Neolithic populations and to collocate these data in context of other dietary reconstructions, socio-economic processes in Portuguese Late Prehistory, and geographical and taphonomical background.

31 teeth were molded, their casts were analyzed using optical and scanning electron microscopy. Two of them belonged to sub-adults.

The most important information received from the sample characterizes taphonomic alterations of the material and diagenetic history of the site. It shows important role of trampling in altering tooth enamel surface and adding new yet recognizable micro-striations. Extensive sedimentation in the karstic context is reflected in the sediment spots formation on both macro- and micro-level of observation.

Although significantly altered by taphonomical processes, material was used in a trial of dietary reconstruction. The results of striation counting have shown low frequency of micro-features, suggesting either low abrasiveness of the diet or erasing microstriations by taphonomic factors.

Buccal microwear presence on a deciduous tooth suggests that age of weaning was just under three years.

Key-words: buccal microwear, taphonomy, North Ribatejo, Late Neolithic, collective burials, diet reconstruction.

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Introduction

General view of Neolithization and Neolithic in the Central Portugal

Neolithic economies seem to arrive to Central and Southern Portugal as quite complete "Neolithic package" (Zilhão, 1993; Rowley-Conwy, 2011). First domesticates appear in Portugal around 5600-5400 cal BC, more or less simultaneously with the rest of the South-Western Europe (Martins, 2015; García-Puchol, 2017). It allowed Zilhão (1993, 2001) to suggest sea faring as the route by which the first Neolithic farmers arrived to Portugal, since normal velocity of terrestrial spread is calculated to be much lower (Vierra & Carvalho, 2019).

These first Neolithic cultures are marked by emergence of Cardial ceramics (Zilhão, 1993). The presence of parallel or later non-Cardial traditions is described, such as Furninha (Oosterbeek, 1994; Zilhão, 1993). The profile of domestic animals here was likely dominated by goats and sheep with cattle playing a minor role (Vierra & Carvalho, 2019). Although archaeobotanical data are almost absent in the archaeological record of Portugal, we can conclude the agricultural plants used in the region from the general situation in Iberia. In this regard, although wheats and barleys were apparently widely grown, pulses like lentils, beans, pea and vetch made much more important part of the agricultural complex than in the rest of the Europe (Zapata, 2004). Fragmented nature of the archaeological record often creates picture of transitional economies or Neolithic populations relying only on part of the typical Neolithic package, for example, on husbandry. But it was shown to be a bias deriving from the predominance of caves as sources of archaeological material (Vierra & Carvalho, 2019).

Importantly, Late Mesolithic populations and way of life survived until around 4000 BC or even later, at least south of the Tejo (Zilhão, 1997). Those had essentially estuarine settlements concentrated in valleys of big rivers: Tejo, Sado and Mira. Their subsistence strategy was balancing between use of terrestrial and aquatic resources (Lubell, 1994). The important role of aquatic resources is witnessed by the great amount of shells from which the "shell-middens" are named. Thick cultural layers, big mounds (several meters high and about 100m in diameter) and up to 300 individual burials evidence way of life with more or less lengthy stays on the same place if not sedentary (Soares, 2013).

Mesolithic cultures and settlements constitute an important aspect to bear in mind characterizing the Neolithization because some of them could coexist with the Neolithic farmers for some 1500 years after the first contact (Zilhão, 1997). This fact means that hunter-gatherers on one side and farmers on another shared the same environment, had to contact and influence each other (Carvalho, 2007). In this regard it is important to view their geographical distribution. Mesolithic archaeological sites, including famous shell-middens of Muge and Sado, are concentrated in three big groups in Central and Southern Portugal. The territories have no Mesolithic archaeological site, creating situation of hiatuses without typical Mesolithic cultural signature before the emergence of Neolithic groups, for example, in Portuguese Estremadura (Vierra & Carvalho, 2019). Although they could be still exploited or controlled by the groups of Mesolithic hunter-gatherers they clearly was not permanently populated like the river banks. Such territories could present a suitable first environment for newly arrived farmers in the sixth millennium.

Nevertheless, soon Neolithic farmer groups made new enclaves in valleys of big rivers and their affluents (Zilhão, 1997). In case of Muge valley, ceramic sherds, characteristic of transition to Neolithic economy, appear in the upper layers of shell-midden. Findings like this sparkled a lot of speculations in the past about local autonomous transition from Mesolithic to Neolithic (Oosterbeek, 1994).

Although there are some open-air Early Neolithic sites (Silva and Soares 1981; 1987) most of the evidence from the period comes from caves and gives little idea about the habitation sites. Other archaeological data like sites´ distribution indicate a pattern of estuary or riverine groups of round or oval huts, without natural or artificial defenses, corresponding to a still mobile settlement (Oosterbeek, 1994).

In the 4th millennium, the characteristic trait of the Portuguese Middle Neolithic appears: collective burials (Oosterbeek, 1994). In inland Alentejo and in Algarve megalithic burials appear at this period, first of them being individual. Then, in the second half of the millennium, megalithic interments typically also serve as burial place for a group of persons. They can be viewed as evidence of demographic growth.

All the types of collective and megalithic burials become even more developed and widespread in the first half of 3rd millennium. In the second half of this millennium another important change occurs: emergence of Mediterranean hillforts and bell beaker culture. The hillforts became indicative of the following archaeological period – Chalcolithic. They strongly suggest growing social inequality, emergence of closed dominating family groups and hierarchization. These latter hypotheses are further supported by other evidences of warfare: rock-art depictions of archers' combats, increasing amount weapons, skeletons pierced by arrows (Oosterbeek, 1994).

North Ribatejo and Nabão valley

North Ribatejo region is special by the fact that it represents the contact area of three different great geomorphological units – the Ancient Massif to the East, the Estremadura Limestone Massif to the West and the Tagus tertiary basin to the South. These geological features influenced the landscape use by Neolithic people. Namely, in the karstic area of the Western part, communities associated with Cardial tradition extensively used natural caves for collective burials. Contrary, the Tagus and Zêzere valley are dominated by megalithic tombs (fig.1, Tomé & Oosterbeek, 2011).

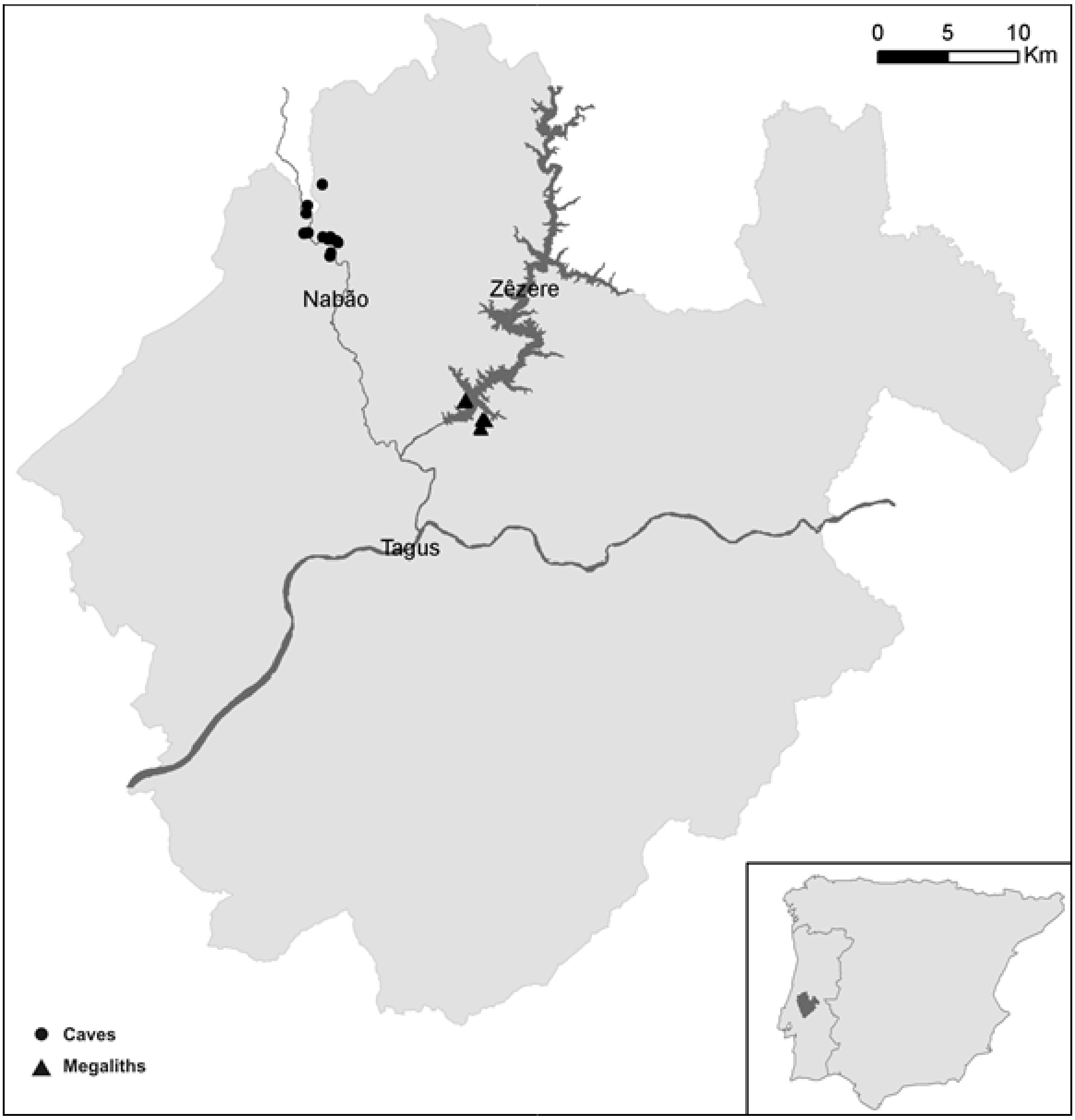

Figure 1. North Ribatejo region on the map of Iberia with indicated types of archaeological sites. Figure adapted from Tomé & Oosterbeek, 2011

Different geological conditions in different parts of the region lead to various issues of material preservation. While the main problem of the Western part is calcium carbonate concretions typical for karstic caves, osteological material in megalithic burials of East and South suffers from high acidity of the Portuguese soil (Oosterbeek, 1994; Gonçalves, 1999).

The cave necropolises of the limestone area are disproportionally represented by the collective cave burials of Nabão valley, first of all of Canteirões do Nabão area. Here, such caves as Gruta do Cadaval, Gruta doa Ossos, Gruta de Nossa Senhora das Lapas, Gruta do Morgado Superior and Gruta Sobreirinho are presented. All of them are small cavities situated close to the river stream. Another important fact is that they are directly visible from each other (Tomé & Silva, 2013).

Regarding the chronology, cave burials of Nabão valley are distributed between the end of 6th and the second half of the 3rd millennia. The most ancient site of Nabão valley Gruta de Nossa Senhora das Lapas – the first period of this cqve use is second half of 6th – first half of 5th millennia. The most recent of them is Gruta dos Ossos, having its most recent chronology boundary in 2200 BC and overlapping in its late chronology with the first Chalcolithic sites of Portugal. Megalithic sites like Murta and Souto appear in the region only in the 3rd millennium (Tomé & Silva, 2013; Oosterbeek, 1994).

Dietary and paleopathological studies in the Portuguese Neolithic

Mesolithic economy was typically based on wild resources, focusing significantly on aquatic and marine prey, but including terrestrial resources as well. This applies as well to the Portuguese Mesolithic. Most human remains of the Mesolithic period in Portugal are found in so-called shell middens. Archaeozoological context there, as well as the very their names tell about the predominance of marine food resources in the diet of Mesolithic hunter-gatherers (Soares, 2013; Jackes & Meiklejohn, 2004).

Those conclusions are approved by the stable isotopes studies. Average $\delta^{13}C$ and $\delta^{15}N$ values in shell middens of Muge and Sado valleys, together with those of isolated individuals found in Alentejo and Algarve regions, indicate dietary focus on the exploitation of wild resources (Guiry et al, 2016; Jackes, 1997). To be precise, they reveal both more pronounced carnivorousness and higher percentage marine and estuarine foods. Moreover, marine resources consumption seems to be specialized on a single kind of prey or a mixture of marine foods in well-defined proportions (because of very close $\delta^{15}N$ values with low variability). All the isotopic data are considerably more variable in Mesolithic and rather uniform in Neolithic despite a greater number of human remains in the latter (Lubell et al. 1994).

There is a major turn toward the terrestrial food resources observed in the European Neolithic. Marine foods were not completely discarded – even when domestic ungulates made up a good portion of the diet, marine bivalves were still eaten, including inland sites. Still, proportion of marine foods strikingly falls in Neolithic communities comparing to Mesolithic hunter-gatherers, uniformly in inland and coastal sites and even in islands (Lubell et al., 1994; Waterman, 2016; 2014).

Archaezoological and archaeobotanical evidence in Portugal between Mesolithic and Late Neolithic is sparse (Davis & Moreno-García, 2007; Zapata, 2004) because most known Neolithic sites are graves and little remains of settlements are known (Carvalho & Petchey, 2013). From that material which is found it is possible to say that as late as in Chalcolithic some people still relied significantly on hunting, leaving bones of wild and domestic mammals in nearly equal quantities (Davis & Mataloto, 2012). However, most of the contemporaneous sites present 75% to 90% abundance of domestic mammals relative to the wild (Cardoso & Detry, 2002).

Portuguese Neolithic has been extensively studied using stable isotope analyses, further extended by numerous similar studies throughout all the Iberia. Although the isotope data are sparse in the Early Neolithic (Guiry et al., 2016) some limited sample from this time supports evidence received by other methods about the shift away from the emphasized marine resources use (Carvalho & Petchey, 2013). This trend towards terrestrial diets remains visible throughout all the Neolithic. Average $\delta^{13}C$ values from both humans and fauna are consistent with C_3-based terrestrial staple, and comparison of considerable samples grouped by Early/Early Middle, Middle, Middle Late and Late Neolithic, show no significant changes over the Neolithic period. Moreover, a uniform decrease in human bone $\delta^{13}C$ values from The Neolithic sites, regardless of geographic region or absolute chronology provides firm evidence for a pervasive and fundamental dietary shift from varied Mesolithic subsistence practices to a more homogeneous Neolithic diet (Guiry, 2016).

$\delta^{15}N$ values from at least Middle Neolithic shows that people then obtained protein predominantly from herbivores, supported by archaeozoological remains of terrestrial animals, it may be a proof of both husbandry development and terrestrial-centered hunting. Despite the general homogeneity of the Neolithic isotope signature, in some cases it fits better marine-based diets. It usually happens to sites on seashore or near estuaries, while most of such sites show little or no evidence of marine-based diets (Carvalho & Petchey, 2013).

Although all the isotope studies agree in defining Neolithic diets as C_3-based, some data suggest presence of minor C_4-resources (or CAM plants) in Late Neolithic-Chalcolithic sites near Zambujal, either as domesticates or wild-growing plants (Waterman, 2014; 2016). It needs further clarification, since the first evidence of C_4-plants presence in Iberia is indicated in the Bronze Age (Rovira Buendía, 2007) with no reliable earlier finds.

Dental evidence somewhat complicates the picture. After the milestone volume of the American anthropologists Cohen & Armelagos (1984), it is commonly believed that caries rate strikingly increases after the adoption of agriculture. In Portugal, there are two lines of evidence regarding this pathological condition. Lubell (1994) and Jackes (1997) argue that caries rates do not increase significantly from Mesolithic into Early Neolithic or even decrease. They emphasize on a very high caries rate in the Mesolithic shell middens of Muge valley and on significant variability of this feature in the Portuguese Neolithic. This, in turn, points to either less importance of agriculture at this period in Portugal, or lower cariogeneity of the local domestic plants. On the other hand, more recent large-scale paleopathological study (Cunha et al., 2007) of Meso/Neolithic and later Chalcolithic period shows the opposite tendency, more in raw with the initial hypothesis. Here, caries rates in Muge and Sado are estimated as 4.1 and 3.9 percent respectively. No statistically significant difference was found between the Neolithic samples and their mean value is equal to 8.0 percent. This statement agrees also with some particular cases in Neolithic such Feteira II and Paimogo I, with 7.9 to 7.0 and 6.7 percent respectively (Waterman, 2014).

Although one can expect increase in the abrasiveness of diet with the transition to agriculture, rates of attrition are higher in Mesolithic samples than in Neolithic. An extremely intensive attrition is indicated in Moita do Sebastião, where over 80 per cent of first lower molars are heavily worn with dentine broadly exposed by the eruption of the third molar. This percentage gradually falls with time, reaching lower value in the late Mesolithic (Lubell 1994). Large-scale comparisons have not found any clear pattern of flat and cupped wear distribution among the Mesolithic foragers and Neolithic farmers (Cunha et al., 2007).

Microwear studies on the Neolithic material from Portugal are still rare. One study on Late Neolithic/Chalcolithic sites Feteira II and Bolores (Horwath, 2014) examines occlusal microwear. It suggests soft but abrasive diet thus being consistent with feeding mostly on cereals milled using fragile abrasive stones. Still, even in this case it is impossible to identify by 100% if the major part of the abrasives comes from agricultural plant sources or from grit taken with sea-foods. Undoubtedly, more microwear studies of the Portuguese Neolithic material are needed in order to make up comparable samples of this data and to parallel it to the other lines of evidence like stable isotopes and oral pathology.

Scientific problems and objectives of the study

Continuous coexistence of Neolithic and Mesolithic cultures in the same territory raises questions about population mixing, transitional or mixed forms of economy and about social structure in hunter-gatherer and farmer communities. These questions are even more salient taking in account aDNA evidence of genetic mixing between Meso- and Neolithic populations (Lazaridis, 2014). Also, such facts as ceramics finding in Muge shell midden can evidence adoption of some technologies and elements of culture by local Mesolithic hunter-gatherers from Neolithic farmers. In turn, concentration of early farming communities in river valleys can potentially mean inclusion of the "local" subsistence strategy of aquatic resources consumption into every-day life of farmers. This makes dietary studies very important for further social, technological and household characterization of the Neolithization process in Portugal.

Another important aspect of the studies of Neolithic is tracking development of hierarchies and inequalities in agriculturalist societies. The growing number of material manifestation of inequalities appears only in Chalcolithic. But although previous Neolithic cultures in the region do not show any undisputable evidence of status inheritance and inequality within groups, subtle social changes, not visible in the material culture, could already start in Neolithic. For such slight changes in social status, dietary studies and dietary variability within group can provide a suitable diagnostic data. Connected with studies of kinship, occupational health and stress status they can tell more about the social-political dynamics in the region in late Prehistory.

The rich portuguese osteological collections of Mesolithic and Neolithic periods were already studied with both problems of diets and of inequality in mind. Archaeozoological evidence in Mesolithic clearly shows the prevalence of marine foods with a significant proportion of terrestrial game. Archaeozoological and archaeobotanical evidence of Neolithic is sparser, but allows talking about a sustained use of plant and animal domesticates. The latter are still diversified by the presence of wild game which, together with semi-permanent settlements, make the "Neolithic package" look somewhat incomplete. However, the cultural difference between the Portuguese Mesolithic and Neolithic is quite pronounced without evident intermediate forms. The same applies for the diets of those different communities. Even though local Mesolithic and Neolithic cultures clearly overlap in time, stable isotope and paleopathological evidence shows their distinctiveness and discontinuity. Mean $\delta^{13}C$ and $\delta^{15}N$ values in Mesolithic follow the archaeozoological data and suggest quite uniform marine-based diet throughout the territory of Portugal, the same is found even in the late specimens contemporaneous with the Early Neolithic. Contrary, a pronounced shift away from marine-oriented diets is shown on the stable isotope values of Neolithic people. They still consumed fish if lived near a sea-coast, but even in this case, seldom. C_3-based plant food and meat of herbivorous mammals should have made up the remaining greater part of the diet. Despite a bigger sample of Neolithic people, their isotope signature seems to be much more standardized. Dental evidence mostly follows the aforementioned array of data. Most likely, caries rates corroborate the typical diet of agriculturalists, rising on the Meso/Neolithic transition. One interesting observation in this case is that dental attrition decreased from Mesolithic into Neolithic.

Although some dental microwear studies were started on the Neolithic material, they are still few to be correlated with other proxies such as isotopes or pathologies. Therefore the objectives of our study became:

(1) start assessing odontological material of Nabão valley with buccal microwear approach;

(2) look for possible intra-group variability of the dietary data in the studied sample;

(3) compare results of dental microwear dietary analysis with other previously known dietary-related data such oral pathology, stable isotope values, cultural context of the findings;

(4) check how the situation of material in certain geological context influenced its conservation, how taphonomical processes depended on the environment and interfere with results of microwear analyses.

Site description and the osteological material

Gruta dos Ossos archaeologiocal site

Gruta dos Ossos (GRO) is a natural horizontal karst cave on the left bank of Nabão River, Portugal (fig.2-3). Administratively it belongs to Santarém district, municipality of Tomar, Além de Ribeira parish, Canteirões zone, location Casais Novos. Its absolute altitude equal 70m. The cave consists of a single chamber with interior area of 22 m^2. A stalagmite formation nearly 3 m from the entrance constitutes a "bottle-neck" of the chamber (Oosterbeek, 1993; Tomé, 2006).

Figure 2. Location of the site on the Iberian peninsula with its coordinates. Screenshot from https://earth.google.com

It was first visited in 1984 by Luiz Oosterbeek and Ana Rosa Cruz. They found there two human crania on the surface of the sediment. The first archaeological campaign in Gruta dos Ossos was initiated by them in 1986 and defined the secondary nature of the archaeological context. In 1987, the first inhumation was found on the cave's floor. The excavation of the site was entirely completed in 1989 and the protective structures were constructed (Oosterbeek, 1993).

During the excavation, 14 sedimentary moments and 5 Layers were identified. The first moment (sedimentary moment n+1) is a thick stalagmite cover; the second (n+2), a reduction cone, is based on the latter and enclosed in layer 5 (n+3). Layer 5 consists of sand clayey brown-reddish deposit with abundant blocks and mean thickness of 20 cm. Distribution of sediments divides the cave into two halves: western and eastern. The western part, the one which is closer to the entrance, does not have layers 4 and 5 (fig.3). In turn, layer 1 is thinner in the eastern part and distributed mostly in the western one (read below). Layer 4 (n+6) is a margalitic-sand-silt whitish deposit with 20 cm mean thickness. It contains the second reduction cone (n+5). Layer 4 is very disturbed throughout all its extent.

Layers 3 (n+7), 2 (n+8 and n+9) and 1 (n+11 and n+12) do not differ significantly by texture and color: they all are margalitic-sand-silt brown-reddish deposits. Layer 3 has a mean thickness of 30 cm and encloses the base level of the bone assemblage. Level 2's mean thickness is 20 cm. The layer contains remains of another reduction cone (n+10) and intermediate level of the bone complex. Layer 1 (n+11) contains the

Figure 3. A – Map showing Gruta dos Ossos (indicated here as "Lapa dos Ossos") and other nearby cave burials. Gruta de Nossa Senhora das Lapas is situated in the North-East part, to the North from Gruta da Areia,; in the western part – Gruta do Morgado; in the southern – Gruta do Cadaval. Picture taken from an open source website https://www.openstreetmap.org/
B – Aerial picture of the Nabão valley with location of Gruta dos Ossos indicated

uppermost bones. It is believed to appear later than geological alterations of layers 5, 4 and partially 2 (reduction cone n+10) occurred. The area of this layer is generally limited to the western part of the cave. There, near the entrance, its thickness reaches 50 cm, while mean thickness of the layer is 20 cm.

There are two moments related to human burials in the cave. The first of them was found in layer 4 and indicates individual burials of early use of the cave. One certain individual burial was located in the eastern part of the cave, near the wall in the basis of the chamber (quadrate 10 IX, fig. 4). It has the only case of bones found in anatomical connection: tibia, fibula and metatarsals. Also, it is the only area of layer 4 which has no displacements. Radiocarbon dating of bones from this burial suggests a chronology around the mid-III millennium cal BC (fig. 5).

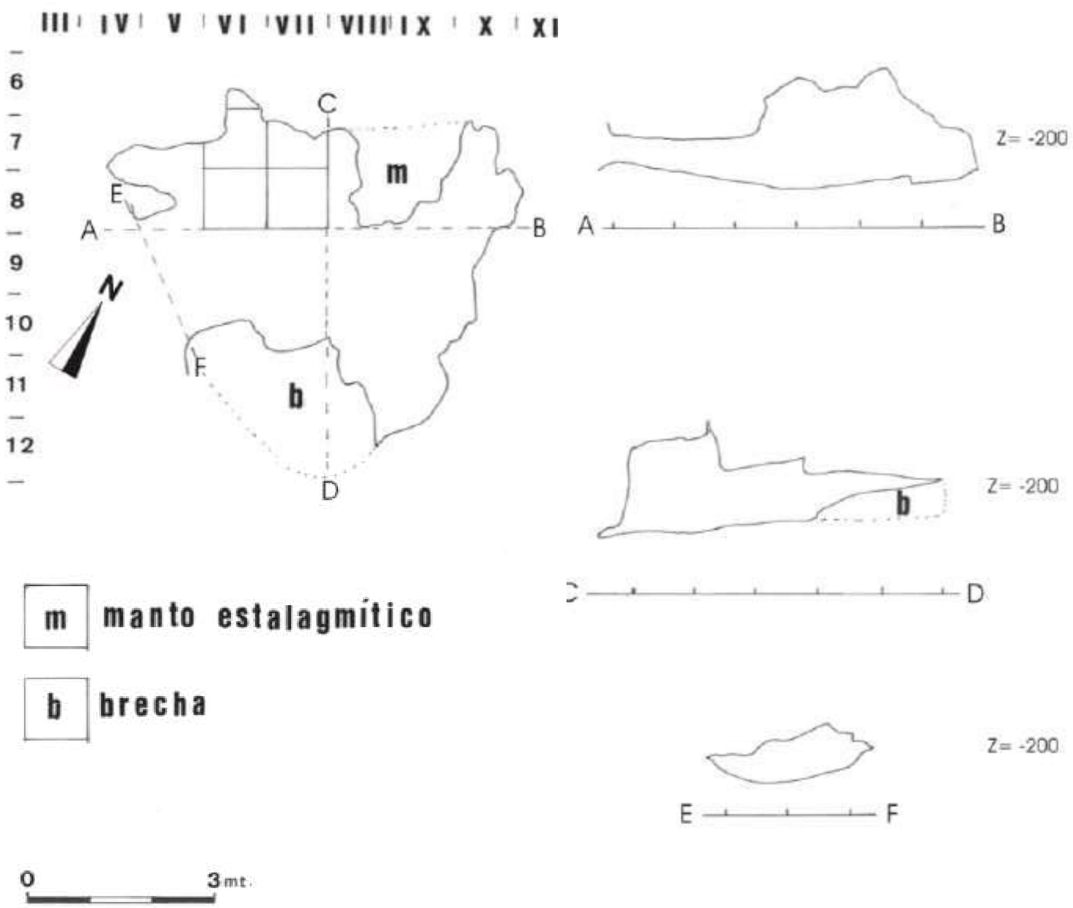

Figure 4. Left-above: Map of the cave "Gruta dos Ossos". Right: topographic sections of the cave.

remains of an ossuary. It occupies three layers of cave stratigraphy: layers 1, 2 and 3. Layer 1 integrates crania and mandibles. Contrary to all other layers, bones in layer 1 were not covered with sediment. Instead, the crania frequently bear limestone of stalagmite nature on their surfaces. They were lying in an arch (or crescent), arrow of which was directed to South-East. An alignment of limestone blocks extended from the western side of the cave in direction WSW-ENE on this level. This "wall" delimited the area of ossuary.

Layer 2 has two levels of bones: lower, with tibias, fibulas and femurs, and upper, with humeri, radii and ulna. All of these long bones have horizontal position and follow a spatial distribution clearly based on the anatomical recognition of different types of bones. In this layer, the core part of the ossuary is well-visible: it has area of approximately 4 m^2, where the layers of bones are perfectly differentiated.

Layer 3 contains pelvic bones: sacra and ilia. It is the lowest part of the ossuary and it rests on a plain platform of big slabs taken from a reduction cone present in the same stratigraphic layer. Smaller bones, like metapodia, ribs, kneecaps and vertebrae are more abundant in layer 2, but are distributed throughout all the ossuary. Teeth sampled in the study, are found either in the ossuary (layers 1-3) or in the individual burial (layer 4) (Oosterbeek, 1993).

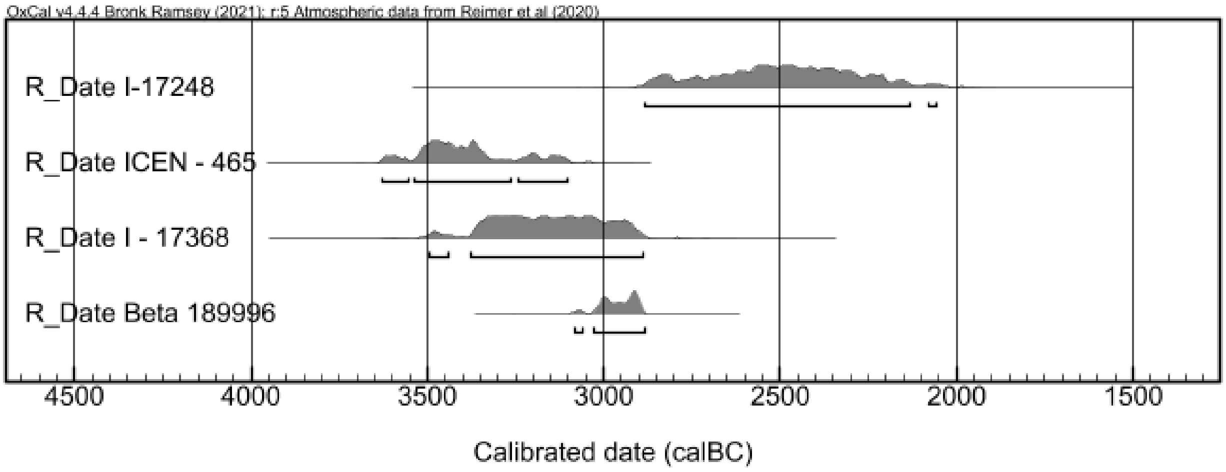

Figure 5. Radiocarbon dating of the Gruta dos Ossos site. R_Date I-17248 – individual burial (layer 4); dates (R_Date ICEN – 465, R_Date I-17368, R_Date Beta 189996)

Importantly, there are no bones in anatomical connection among those making up the ossuary. Also, the sediment around shows no signs of hole digging or other spatial perturbations. Probably, the ossuary was artificially constructed on a prepared place with deliberately constructed platform in the center of the chamber. The absence of natural layers 4 and 5 in the western half of the cave also could be explained by intentional removal in order to prepare place for ossuary. Bones for the construction were collected from already decomposed bodies and could include those extracted from the individual burials in the eastern part of the cave. Alternatively, taking in account a later dating of the individual burials, we can suppose that they were dug aside the ossuary later and thus represent completely another tradition.

Total minimal number of individuals is 26 including 19 adults and 7 sub-adults. Adults include at least 6 men and 8 women.

Typically for an ossuary, the bones were re-arranged regarding the type of bone that is visible between and within the layers (Oosterbeek, 1993; Tomé, 2006).

There is no evidence that all those individuals died at one moment (of a disease or inter-group violence), so most likely, the decomposed, defleshed osteological remains were gradually accumulated before the construction of the ossuary.

The fact of ossuary construction and certain other features of the site suggest ritual use of the cave. Size of the cave and situation of the ossuary places the latter in a kind of "lobby" with the access restricted by limestone blocks. The entrance to cave is one meter high therefore it requires any "visitor" to squat or kneel in order to enter, thus imposing a “worship posture” over them. In the same time, ossuary’s position in the western part of cave, near the entrance, should have made it visible from outside. Finally, the overwhelming part of archaeological artifacts is found near the entrance with significant part possibly washed out of the cave and lost (Oosterbeek, 1994).

The artefacts found in the cave are very scarce and fragmented. Ceramic assemblage presents brittle sherds with minor firing and smooth surfaces. The typical shape is a spherical low cup. Only one vessel was decorated: a “glass” with light horizontal fluting more or less parallel to the rim. The lithic industry includes hummerstones, geometric (triangle, trapezoidal) and non-geometric microliths, flint and quartzite bladelets and a greywacke adze. Bone industry is represented by several punches, a possible flute of bone and a perforated conch of *Mytilus edulis*. Deer bones attest large game hunting. Generally, the artifact assemblage was culturally dated to Late Neolithic/Early Chalcolithic (Oosterbeek, 1993).

Cultural chronology is supplemented by sedimentology and radiocarbon dating. Moments n+1 – n+3 in Gruta dos Ossos correlate with analogous thick stalagmite cover of nearby Gruta do Cadaval. In both caves they are attributed to Pleistocene-Holocene transition and are archaeologically sterile. The second reduction cone (n+5) embedded in layer 4 in GRO was equally observed in Gruta do Cadaval where it has two radiocarbon dates of late IV/early III millennium common with layer D (Neolithic). Radiocarbon analyses of GRO completely support this dating and also place use of the cave to late IV/early III millennia (ICEN-465: 3300 cal B.C. [3628-3100]; more recent accelerator mass spectrometry, Beta 189996: 3020-2890 cal B.C.) (Tomé, 2006).

To sum up, Gruta dos Ossos is a natural cave in Nabão valley with a Late Neolithic collective burial. Evidence of human presence here is dated to relatively narrow range of time in late IV – mid III millennia and are represented by an ossuary and rests of two inhumations. The most important part of its use by people is the construction of an ossuary which apparently played a ritual role.

Description of the osteological material

The entire osteological assemblage of the site was described and analyzed from point of view of physical anthropology by Tiago Tomé (2006, 2011).

Most remains have code given to them upon their recovery in field. It consists of code of the (GRO) followed by square of origin (Arabic and Roman number, e.g. 8-VI) and then by a sequential number (e.g. GRO 8VI 202). We use these codes as the main names for the analyzed teeth.

In total, osteological assemblage of Gruta dos Ossos includes 2987 bone and dental fragments of which 2583 are bones and 404 are teeth.

More than half of bones (51.91%) have depositions of calcium carbonate on their surface. In some cases the deposition was so abundant that its weight overwhelmed one of a bone itself. Several pieces of bone suggest to be gnawed by rodents.

Minimal number of individuals is identified as 20 for adults after the number of left humeri found at the site. Talking about long bones in general, this number is followed by the 19 right humeri, other long bones, such as clavicles, femurs, tibias, ulnae and radii are fewer but still numerous, being 15 to 18 for every side. Together with under-representation of small bones it suggests an intentional selection of long bones for the construction of ossuary.

Sex of 12 adult individuals was diagnosed from calcaneus bones: 8 males and 4 females. Other bones gave smaller numbers, leaving calcaneus bone limiting for the MNI of adults of both sexes.

Minimal number of sub-adults is identified as 11 individuals using dental eruption, long bones measures and epiphyses fusion. Of them, age at death of 3 individuals is 0-4 years, of 5 – 5-9 years, of 2 – 10-14 years and only one individual has age at death 15 to 19 years. One of the youngest children from the first category died at the age between 3 and 12 months. Thus, MNI of sub-adults being 11 constitutes 36% of the total MNI of the sample. Age at death of adult individuals concluded from crania attests the presence of at least one young adult (32±12 years), one middle-age adult (42±15 years) and four older individuals (50+ years). Pelvic bones show presence of at least two young (25-35 years) and two individuals between 35 and 50 years. Results obtained from clavicle bone suggest presence of four individuals over 30 years. Unfortunately, because the bones were rearranged to the artificial structure, it is difficult to associate a lot of them to a particular individual. It potentially could reduce the minimal number of individuals, both general and of different categories.

Such age and sex profile of human remains allowed the previous researcher to assume the ossuary to represent a natural population. The selection of individuals here does not seem to be based on any demographic criterion. Although identified male remains are more than female ones, relatively small size of the sample does not allow excluding random statistical nature of this disequilibrium. Children under a year and 1 to 4 years are underrepresented not only in Gruta dos Ossos, but also in other coetaneous late Neolithic cave burials of this and neighboring regions.

Mean height estimated using talus is 169.37 cm for males (using 7 specimens) and 157.22 cm for females (using 3 specimens). Neither its values nor intra-group variability do not differ noticeably from those of other similar Late Neolithic burials in Portugal and Spain (Gruta de Nossa Senhora das Lapas, Gruta do Cadaval, Gruta do Morgado Superior, Cueva de los Postes, Cerro de las Baterías).

Another important parameter of the osteological complex of Gruta dos Ossos is oral pathologies registered on its teeth. Among them the most important are caries and dental calculus since they allow to make some conclusions about diets of population. Caries prevalence in Gruta dos Ossos sample is 11.63%. Comparison provided in the study of Tomé shows that this is the highest values among other Late Neolithic caves mentioned above, where this value varies between 2.04% (Gruta do Morgado Superior) and 9.26% (Gruta de Nossa Senhora das Lapas). Expectedly, caries prevalence on post-canine teeth overwhelms that of the frontal teeth, suggesting its dietary origin.

Dental calculus occurrence on the teeth is 35.14% in case of Gruta dos Ossos – a bit elevated respecting values of other similar caves (21.92% to 32.62%) if we exclude somehow extreme value of Gruta de Nossa Senhora das Lapas (62.96%). However, the dental calculus data should be taken with certain degree of carefulness because this trait is highly subjected to the taphonomical alterations, to be precise the calculus itself can be likely removed post-mortem.

Sample of the teeth used for analysis

A part of osteological material of Gruta dos Ossos was accessed and processed in the Instituto Terra e Memória in Mação, Santarém district, municipality of Tomar. It was transferred there from the Archaeology laboratory in the Instituto Politécnico de Tomar in agreement with Ana Rosa Cruz, co-director of excavation at Gruta dos Ossos.

Unfortunately, at the moment of interment to Mação, all the osteological material from Gruta dos Ossos was not accessible due to technical works in the Politechnical Institute of Tomar. Only the isolated teeth and one piece of mandible were transferred to the museum in Mação for the microwear analysis. Of this material, 31 molars were chosen for further study. This is about half of the total number of pluriradicular loose teeth in the osteological complex (66 including upper first premolars) and 17.8% of total pluriradicular teeth (31/174).

As well as distinction between loose second and third molars is often complicated task, those which were difficult to determine during the work with osteological material were checked later, after making epoxy resin casts. It resulted in 7 casts being discarded from the dietary analysis: GRO 9-VII n3 SC104; GRO 7-VIII SC8; GRO 9-VII n2 SC20; GRO 8-V 15; GRO 11-VIII 3; GRO 9-VI n5 SC158; GRO 9-VI n5SC143.

Two of the sampled teeth are deciduous: one deciduous lower right first molar (GRO 8-VI 62) and another - deciduous lower right second molar (GRO 8-VI 81). Another arrow of samples clearly attributed to children consists of 4 permanent teeth with roots not closed yet. Three of them are first upper molars (GRO 7-VI SC5; GRO 8-VI 240; GRO 9-VI n5 SC157) and one – lower first molar (GRO 8-VI SC19). All these teeth were excluded from general dietary analysis and used to characterize children of the paleopopulation.

Among the adult teeth upper first molars were much more common than lower first molars: 6 vs. 1 (respectively: GRO 8-VII SC 85; GRO 9-VII n2 SC9A; GRO 10-VI n1 SC22; GRO 10-IX 1; GRO 8-VI 153; GRO 10-VIII n1 SC59 and GRO 7-VII 1). Importantly, all the upper first molars are of the same laterality – left. It means that all of them belonged to different individuals and caused their inclusion into the dietary analysis.

Sampled second molars are twice as many as first: 14 against 7. Eight of them are lower: GRO 11-IX 2A; GRO 11-IX 11B; GRO 11-IX 11C; GRO 9-VII SC50; GRO 8-VI 24; GRO 9-VI n5 SC144; GRO 9-VI n5 SC145; GRO 9-VII n3 73. Only in one case a molar was molded directly in the mandible (GRO 11-IX 2A). Among upper second molars, one had a particular type of macro-wear suggestive of occupational use (as a tool). Its surface of wear is not parallel to the occlusal surface, instead it is inclined and is oriented meso-cervical to disto-occlusal. This tooth was excluded from the dietary analysis.

Methodology

Sampling of the tooth surface

Silicon molds were taken in the museum of Pre-Historic art, Mação, Portugal. Following the commonly accepted protocol the molars chosen for analyses were cleaned first. For this, we used cotton swabs soaked in ethanol (Rose, 1983). When nothing more could be removed with cotton swabs, the cleaning was over, even though in our case a lot of sediment stayed firmly attached to some teeth. The silicone molds were taken right after the cleaning. For this, we used high precision dentist silicone of polyvinylsiloxane “President microSystemTM” (regular body, Coltène®), following widely accepted methodology (Galbany et al., 2005). Mechanical mixing pistol dispenser was used to directly apply the silicon onto a tooth surface. After 10 minutes, when the polymerization process was complete, the molds were carefully removed from the teeth, so they remained intact. In most cases the first mold was used as the final one. Only on several teeth, which stayed covered with sediment after initial cleaning, the first mold removed some sediment and opened enamel beneath it. On such teeth the second mold was taken and then, we used it as the final one. Since most sampled teeth are isolated, the molds were taken from the entire enamel surface. In case of the mandible GRO 11-IX 2A, where two molars are present in a row, the mold of both of them together was taken.

Each mold was put in an individual plastic bag to avoid any dust contamination. Every mold received its own number identical to the number of tooth, this number was written on the bag.

Then, all the silicon molds were brought to Tarragona, Spain. Here, in the molecular laboratory of IPHES they were used to make epoxy resin casts. For that all the molds were fixed in vertical position in fume hood, upside-down. Additional silicon was used on some of them to reinforce walls where they were thin, and putty were used in order to fix their position.

Epoxy resin and hardener EPO-TEK 301 were mixed in proportion 1:1. Using a plastic pipette we filled the molds with the prepared epoxy resin. For this we wetted the internal surface of mold first in order to avoid air bubbles formation on the epoxy resin/silicone interface. Then, the epoxy resin was slowly spilled into the mold with the tip being immersed into the liquid.

After 24 hours of solidification, hard casts were carefully removed from the mold-preforms. They received the same numbers as the corresponding teeth and molds and were placed into individual plastic bags.

Light microscopy investigation

All epoxy resin casts were examined using an optical microscope. The equipment we used is ZEISS Axio Scope A1 reflected light microscope which is situated in the laboratory of lithic technology in IPHES. It is equipped with a differential interference contrast system (DIC) and a Nomarski prism and thus suited to examine transparent objects like epoxy resin casts. The objective we used is 10x/0.20 with Epiplan® lenses (Ollé et al., 2016). Magnification was uniform for all the casts and equal 100x (10x objective and 10x oculars). The camera used with the OM is a 6.3 MP Color Blackfly S USB 3.0 Camera, 1/1.8″, FLIR, and the software used to control image acquisition is Kivy Capture MIC Z. Helicon Focus 5.3 software was used to automatically create one deep-field-focused image of a series of photos with different focus distance.

Environmental Scanning electron microscope investigation

Only after the optical microscopy examination sample of tooth casts was created for scanning electron microscopy. These casts were glued to aluminum stabs to fix them in the SEM chamber and to increase conductivity of the samples. Then, all of them were sputter coated with gold for 3 min using current of 20 kV and 30mA. It resulted in layer of gold about 20 nm thick.

High vacuum SEM imaging was accomplished using an Environmental Scanning Electron Microscope (ESEM) Fei Quanta 600, located in the Scientific and Technical Resources Service of the Rovira i Virgili University (Tarragona, Spain). This microscope has two regimes: ESEM and SEM. We used only SEM regime with secondary electron detector (Everhart Thornley Detector – ETD) for all SEM analyses. Secondary electron detector is recommended for use with dental microwear because contrary to the Backscatter electron detector it does not lose striations arranged parallel to the detector alignment (Galbany et al., 2005).

Acceleration voltage used in the SEM examination is 20 kV following the standard methodology (Pérez-Pérez, 1999). Following optical microscopy examinations, here we used magnification 100× as well. The micrographs were taken with horizontal field of view 2070 μm. Working distance we used was between 15 and 18 mm. Each micrograph was centered on the middle third of the buccal surface (Pérez-Pérez et al., 1994) and we sought to find the same areas which already presented microwear during optical microscope investigation.

Image processing

The obtained photos, first of all from optical microscope, were then treated in Adobe Photoshop 2013. We adjusted exposition, contrast, and color saturation of the photos. It was made to (1) decrease shining of polished enamel surfaces; (2) highlight shadowed areas; (3) make small striations clearer and distinguishable one of another; (4) make taphonomical alterations more contrast and visible.

Some photos were rotated before striation counting in order to correspond anatomical orientation of the studied teeth. In all cases sides were marked on the photos with letters: occlusal (O), cervical (C), mesial (M), and distal (D).

All the photos, then were cropped, also in Adobe Photoshop 2013. After cropping they represented square of 0.56 mm^2 with equal sides.

Counting the striations and statistical treatment

Striations were counted on the cropped images using open-source software ImageJ. Scale bars made during microphotograph capture were used for calibration on every photo. Only those striations were counted which start and finish in the cropped area. Three parameters were recorded: striation length, angle and number of striations per analyzed field of view. Since in most cases 100 x magnification is not enough to identify the beginning and the end of scratch we needed only positive angles for the analysis. For that reason, all the striations were measured from the lowest point to the highest point, because in the opposite case ImageJ gives negative value. All the striations were divided into four 45° groups due to their orientation: horizontal (H) – 0° to 22.5° and 157.5° to 180°; vertical (V) – 67.5° to 112.5°; mesio-occlusal to disto-cervical (MD) - 112.5° to 157.5° for the lower left and upper right teeth, but 22.5° to 67.5° for the lower right and upper left teeth; disto-occlusal to mesio-cervical (DM) - 22.5° to 67.5° for the lower left and upper right teeth, but 112.5° to 157.5° for the lower right and upper left teeth (Pérez-Pérez et al., 1994). Scratches were classified into these groups automatically using a logical function in Microsoft Office Excel 2013.

Statistical treatment of the data was made in software STATISTICA 7. Shapiro-Wilk W test was used to check the normality of values distribution in samples. Since in all cases it showed non-normal distribution, the samples were further characterized using values typical for non-normally distributed samples: median, upper and lower quartiles.

Results I. Taphonomical alterations

At least 14 out of 31 molded teeth (45%) present some features indicative or suggestive of trampling. One of their main typical traits of trampling is presence of parallel striations, either straight or curved, while dietary striations are characterized by fine straight scratches of various direction (King, 1999; Martìnez & Pérez-Pérez, 2004). Another important tip which can point to the possible trampling is the striation outline (Domìnguez-Rodrigo, 2009).

One particular variation of parallel striations can be met as a bundle with multiple (more than 3) short straight fine scratches (fig. 6, A-B). Alternatively, straight scratches produced by trampling can be organized into smaller bundles of two or three – another option found in our sample (fig.6, C-D). In our case it happened to longer striations. Apparently, long straight striations can be characteristic of post-mortem alterations as well (Weber 2021).

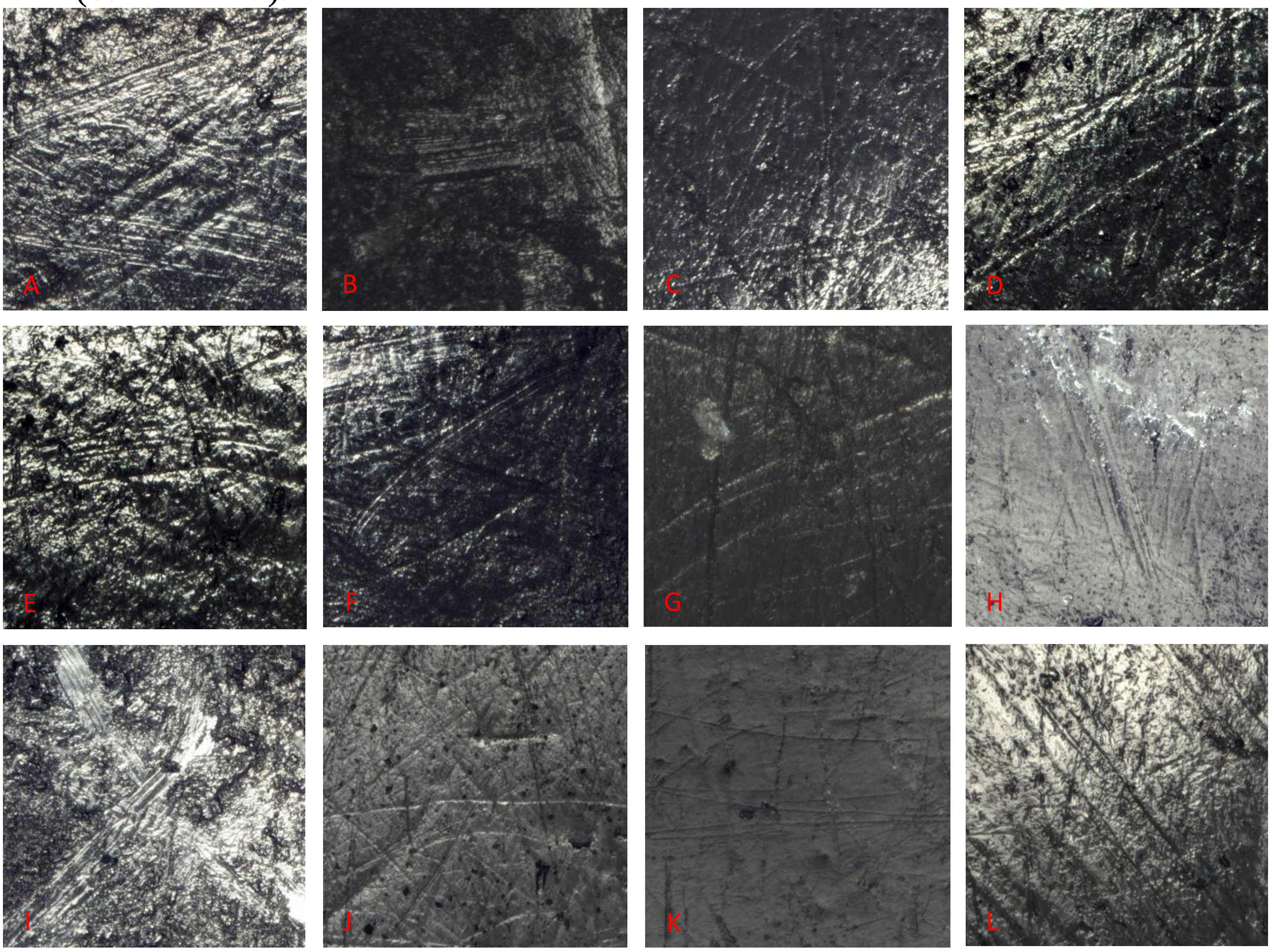

Figure 6. Optical microphotographs (350 µm × 350 µm) representing various evidences of trampling

On the other hand, curved striations can be results of trampling (Domìnguez-Rodrigo, 2009) and they were also found in the studied sample (fig. 6, E-G). Their attribution to the trampling marks instead of dietary signature is additionally proved by the fact that being curved they are still united into parallel bundles.

Another important typology of marks produced by trampling and found in our study is short or medium but wide furrows with a flat bottom presenting micro-grooves (fig. 6, H). In one particular case (fig. 6, I) such marks with micro-grooves were two, they were curved and crossed. The general appearance of those features, therefore was similar to traces of a hard tool (Teaford, 1988).

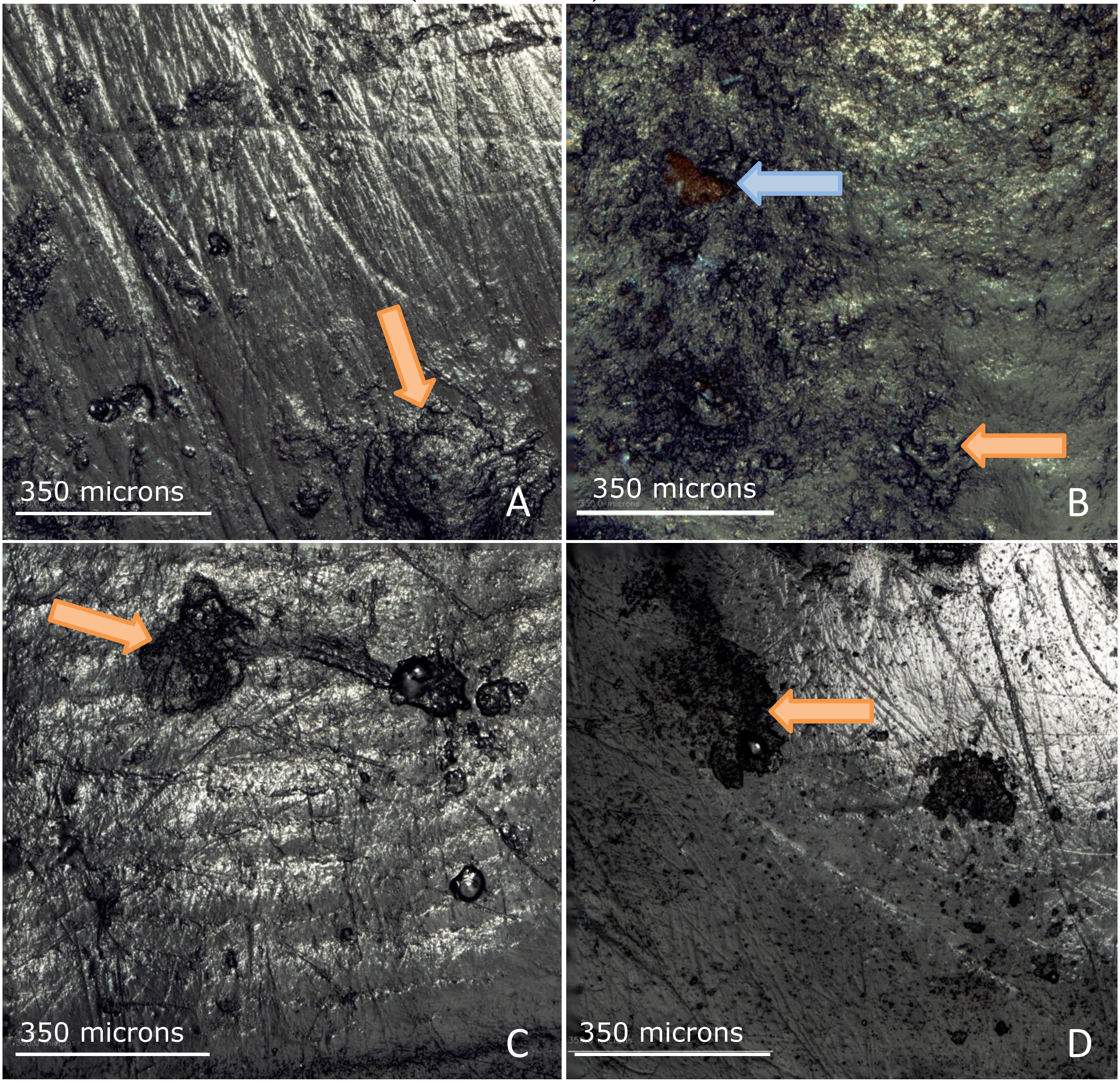

Figure 7. Enamel with spots of sediment. Light microscopy, magnification 100x. Blue arrow points to the piece of sediment stack to the cast; Orange arrows point to the imprints of sediment spots

The third typical morphology described for trampling marks on bone material is sinusoid striation (Domìnguez-Rodrigo, 2009). In our case no strictly sinusoid marks were found, but some striations approached this morphology. Namely, very fine, shallow striae with a bit fluctuating direction (fig. 6, J) or bent in several points (fig. 6, K) can be considered as examples of this group of defects.

One tooth have surface widely covered with parallel straight striations (fig. 6, L). Following one more time analogy found in studies with bone material (Gaudzinski-Windheuser 2010), we can supposedly attribute this alteration to trampling, too.

The next most common post-mortem alteration is spots of dirt or a sediment. In 11 out of 35 molded teeth (31%) it was impossible to avoid them capturing photo with micro-striation (fig.7, 12 and 17). However, lots of other teeth had traces of sediment reflected on the casts which nevertheless could be avoided during taking micro-photographs. Undoubtedly, these reflections of sediment on the casts represent the sediment which was hard enough to resist cleaning and then was not removed by the molding process. Still in some cases (fig.7 B) pieces of sediment were stack to the cast. Such cases give possibility to take a close look to this sediment. In case of pieces stack to the casts it is likely that the sediment is the same as described by the excavators: margalitic-sand-silt brown-reddish (Oosterbeek, 1993) and seen on the teeth themselves during their examination (e.g. fig. 8).

Figure 8. Tooth **GRO 9-VII n2 SC 9A** in its buccal (A) and distal (B) aspect

Another possible content of the sediments found on the examined teeth is calcium carbonate. It is expected to find in karstic caves like Gruta dos Ossos especially taking in account that skulls on the top of the ossuary were covered with a layer of calcium carbonate. This type of sediment expectedly can be even harder than the silt or clay.

Coming back to the striations we should also mention ordinary long curved striae (fig.8). They are more rarely described in taphonomical studies, but still can be potentially characteristic of trampling, since they are absent in tumbling experiments (Uzunidis, 2021) and require more complex movement for their production than can be expected during sediment transportation. On the other hand, it is possible to imagine that masticatory movements of human can produce curved striations because of their flexible nature in several dimensions.

In this regard it is important to pay attention to the margin and cross-section of such striations. Those of them which are shallow and with smooth margins can derive from dietary particles and masticatory movements (fig.9 B, D). But in any case we avoided to count such striations because it is impossible to determine their direction and length correctly using conventional methodology. Long curved striations which

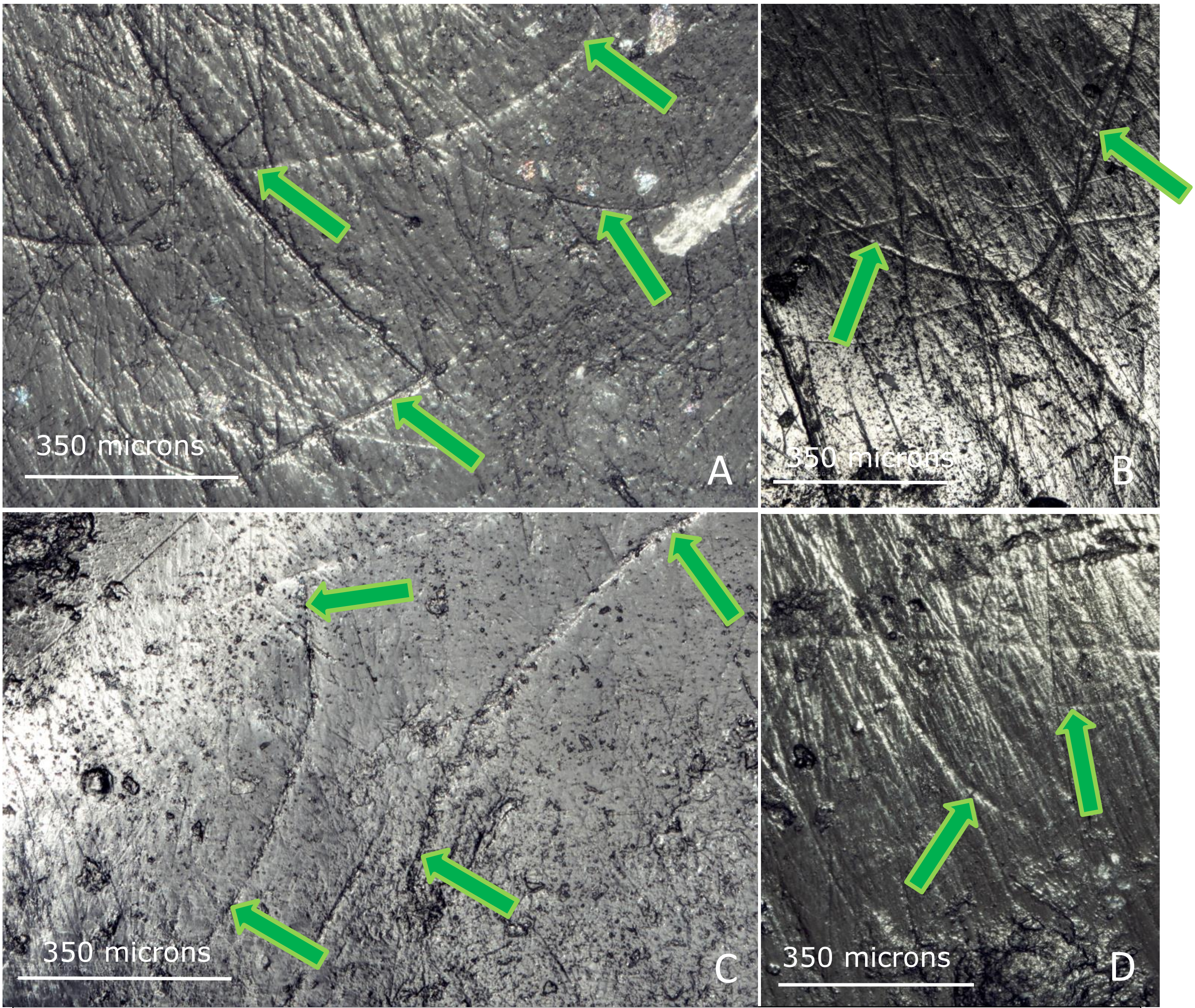

Figure 9. Traces of trampling on counted (A, B) and discarded (C, D) teeth: A, B, C – long wide curved scratches (pointed by green arrows); D – multi-parallel scratches (pointed by red arrows). Light microscopy, magnification 100x

are deep, with sharp margins and flat bottom are more likely represent one more type of trampling marks (fig.9 A). Other can have rough, uneven, flaked margin (fig.9 C) similar to scars found on bones after trampling (Domìnguez-Rodrigo, 2009) although enamel should behave a harder material than the bone.

Four of the molded teeth presented another type of surface often found in buccal microwear studies – perikymata. In all cases this feature was associated to the teeth with roots not shaped or not closed yet at the moment of the death. Therefore perikymata seems to be the initial morphology of enamel surface before the tooth comes to occlusion and gets buccal wear. It is corroborated by the fact that three of these teeth have no striations at all (fig.10 B, D). Only in one case perikymata was found together with dietary striation – on an upper third molar **GRO 9-VII n2 SC20** (fig.10 A). Here, the elevations of perikymata seem erased in the area with striations.

Two teeth did not present perikymata but had clearly visible enamel prisms. Although King (1999) suggested that such surface can be indicative of acidic influence in our case it seems to correspond to early stages of the tooth use, too. One of the teeth presenting enamel prisms is a deciduous molar with scares striation, it will be discussed in details below. Another tooth is permanent upper second molar (fig.10 C), but it is only slightly worn on the occlusal surface (Smith, stage 1).

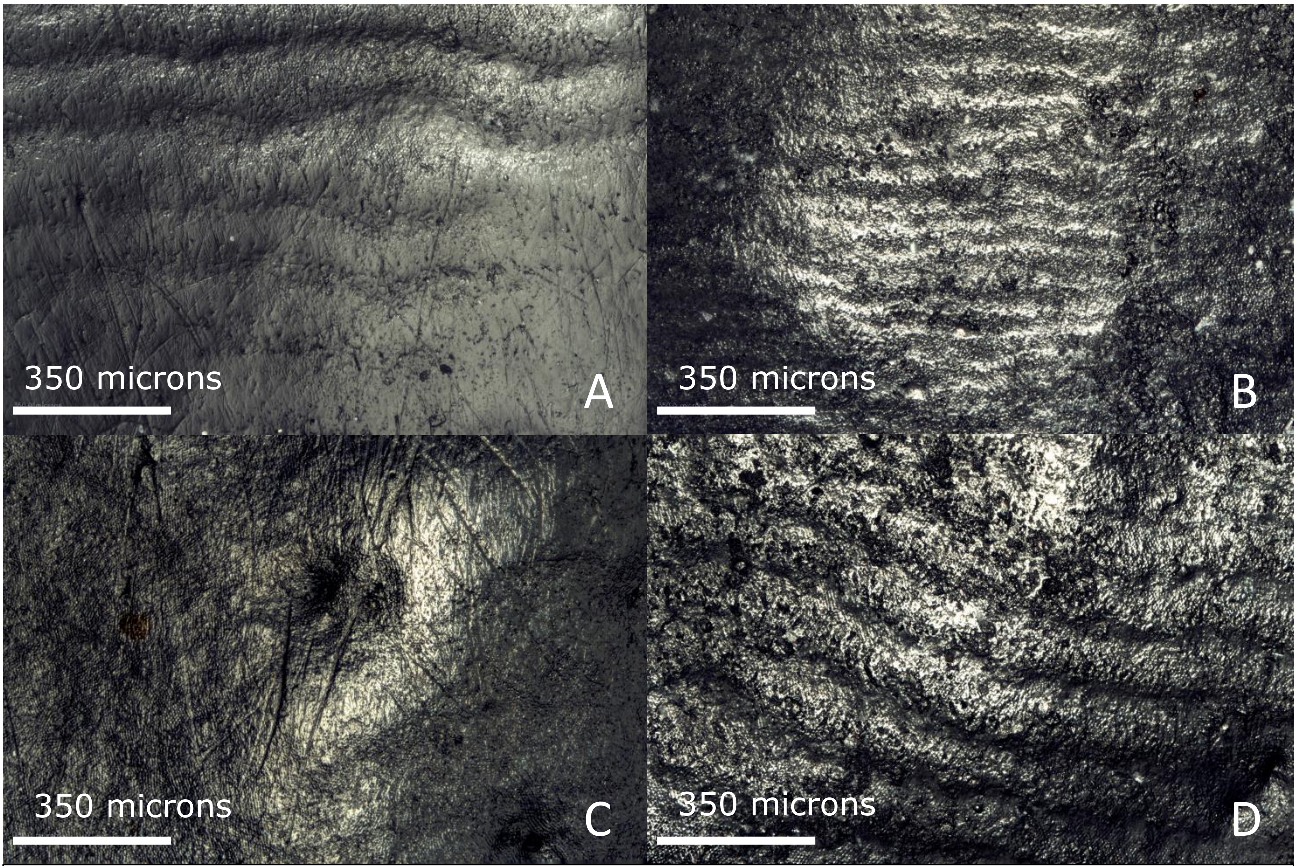

Figure 10. Buccal surface of teeth with perikymata and enamel prisms at 100x magnification

In summary taphonomic profile of the odontological material from the Gruta dos Ossos site suggests that trampling was an important process altering isolated teeth. It is manifested in plenty of different patterns of striation. Covering with sediment accompanied trampling as another most common post mortem alteration capable to reduce sample or obscure analysis results. Here, in Gruta dos Ossos it can be caused by both silt and carbonate sediments sticking to the enamel surface.

The taphonomical examination of tooth surface is important not only to evaluate the limitation which appear before us in the dietary reconstruction, but first of all to understand better the methodology in general, to learn how to find unaltered surfaces with dietary striations and how to discern them from various post-mortem marks. Also, taphonomic description of the material allows describing its diagenetic history.

Results II. Measurements and descriptive statistics of light microscopy results

Although the sample of teeth proved to be significantly altered by taphonomic processes, we decided to try creating a sample for dietary reconstruction of the least altered molars. Eight permanent molars were selected for. Four of them are upper left first molars thus representing four different individuals. Four others are lower molars one of which is the first and three others are second, including two left and one right.

Table 2. Number and length of striations in analyzed teeth

Tooth	Type of molar	Total number of scratches (**NT**)	Median length, μm	**NV**	**NH**	**NMD**	**NDM**
GRO 9-VII SC9A	left M^1	44	118.97	19	4	7	5
GRO 10-IX 1	left M^1	58	138.60	11	4	14	11
GRO 8-VI 159	left M^1	41	232.69	18	8	3	35
GRO 8-VII-SC85	left M^1	30	165.31	12	9	6	11
GRO 11-IX 2A	left M_2	38	149.05	14	5	3	20
GRO 7-VII-1	left M_1	41	175.32	25	3	13	17
GRO 9-VII SC50	right M_2	64	124.49	22	3	4	12
GRO 11-IX-11B	left M_2	39	150.71	8	2	1	17
Total		346	146.67	129	38	51	128

* - NT – total number of striations; NV – number of vertical striations; NH – number of horizontal striations; NMD – number of mesio-occlusal to disto-cervical striations; NDM – number of disto-occlusal to mesio-cervical striations

The raw data of total number of scratches, their length and angle are presented in table 2. Assessment of frequency distribution of the lengths of scratches with Shapiro-Wilk W-test shows that all the analyzed teeth show non-normal distribution (Table 3). The latter is an important aspect to underline taking in account that in other studies of buccal microwear length of striations is distributed normally (Salazar-García et al., 2016; Pérez-Pérez et al., 2003; Lalueza-Fox et al., 1996). The total sample of striations length of all the teeth has non-normal distribution as well (table 3). For that reason, we used median instead of mean value to assess and compare the striation length.

Table 3. Distribution of length of striation in the studied samples

Tooth	Valid N	Shapiro-Wilk p-value	Normal distribution
GRO 9-VII SC9A	44	0.000	no
GRO 10-IX 1	58	0.006	no
GRO 8-VI 159	41	0.011	no
GRO 8-VII-SC85	30	0.003	no
GRO 4-IX 2A	38	0.004	no
GRO 7-VII-1	41	0.000	no
GRO 9-VII SC50	64	0.000	no
GRO 11-IX-11B	39	0.002	no
Total	350	0.000	no

Striation length. Median, quartiles minimal and maximal values of these samples are shown on the box-whiskers plots of figure 5. It can be seen (table 2, fig.11) that most median values of scratch length lie between 100 and 200 µm, only in one case

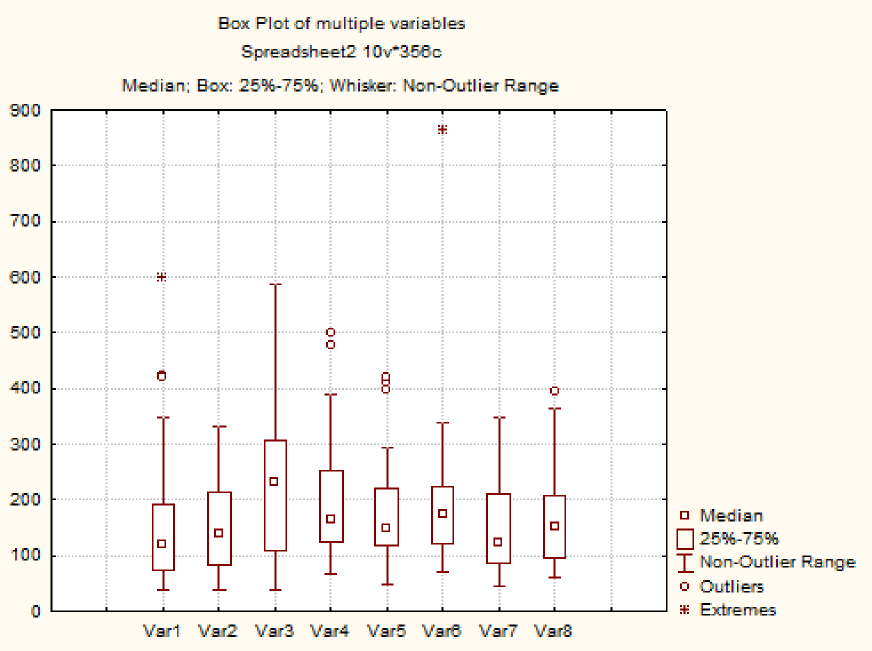

Figure 11. Characterization of scratches lengths on the analyzed teeth as non-normally distributed data. The teeth analyzed are as follows: **Var1** - GRO 9-VII SC9A; **Var2** - GRO 10-IX 1; **Var3** - GRO 8-VI 159; **Var4** - GRO 8-VII-SC85; **Var5** - GRO 4-IX 2A; **Var6** - GRO 7-VII-1; **Var7** - GRO 9-VII SC50; **Var8** - GRO 11-IX-11B

being more than 200 (232.7 µm). Median scratch length of the total sample is 146.67 µm. The minimal value of this variable is 118.97 µm. Thus, in all cases this figure is greater than 100 µm. Length of striations is distributed non-normally. When the total sample of all analyzed teeth is analyzed for normality, log-normal distribution is evident, that was found as well in other studies (Pérez-Pérez, 2003).

Table 4. Total number of striations and macro-wear rate of analyzed teeth

Tooth	NT	Macro-wear rate, Smith
GRO 9-VII SC9A	44	3
GRO 10-IX 1	58	2
GRO 8-VI 159	41	4
GRO 8-VII-SC85	30	8
GRO 4-IX 2A	38	1
GRO 7-VII-1	41	6 to 7
GRO 9-VII SC50	64	4
GRO 11-IX-11B	39	2
GRO 8-VI 81	36	1
GRO 8-VI 62	none	1

Striation frequency. Striations frequency fluctuates between 30 and 64 (tables 2 and 3), thus always being below 100 scratches per view field. In the table 4 total numbers of striations on all teeth including the deciduous one are correlated with their macro-wear stage of Smith.

Relative frequency of striations. Number of vertical, horizontal, mesio-occlusal to distally-radical and distally-occlusal to mesio-radical striations are shown table 5. NH/NT and NV/NT show significant prevalence of vertical striations over horizontal ones. Its interpretation and correlation with the results of other authors is developed in the discussion section.

Table 5. General and relative number of striations on the studied teeth

Tooth	**NT***	**NV**	**NH**	**NMD**	**NDM**	**Relations**	
						NH/NT	**NV/NT**
Lower molars							
GRO 4-IX 2A	35	19	4	7	5	***0.11***	***0.54***
GRO 7-VII-1	40	11	4	14	11	***0.10***	***0.28***
GRO 9-VII SC50	64	18	8	3	35	***0.13***	***0.28***
GRO 11-IX-11B	38	12	9	6	11	***0.24***	***0.31***
Upper molars							
GRO 9-VII SC9A	42	14	5	3	20	***0.12***	***0.33***
GRO 10-IX 1	58	25	3	13	17	***0.05***	***0.43***
GRO 8-VI 159	41	22	3	4	12	***0.07***	***0.54***
GRO 8-VII-SC85	28	8	2	1	17	***0.07***	***0.29***
Total	346	129	38	51	128	***0.11***	***0.37***

* - NT – total number of striations; NV – number of vertical striations; NH – number of horizontal striations; NMD – number of mesio-occlusal to disto-cervical striations; NDM – number of disto-occlusal to mesio-cervical striations

Results III. SEM and light microscopy results comparison

Environmental scanning electron microscopy was also used to analyze tooth enamel surface. Of 7 teeth studied using this methodology some countable striations were found only on two of them. One of them, deciduous lower right second molar **GRO 8-VI 81** will be discussed in the next chapter.

The data received from the electron microscope expertize were not enough for an independent microwear study, but gives a possibility to compare two microscope techniques on the same tooth improving the methodological value of this work.

Scanning electron microphotographs of the tooth **GRO 10-IX 1** (fig.12) generally have shown significantly less striations than were on the same tooth under light microscope (Table 6). Less number of striations found on scanning electron

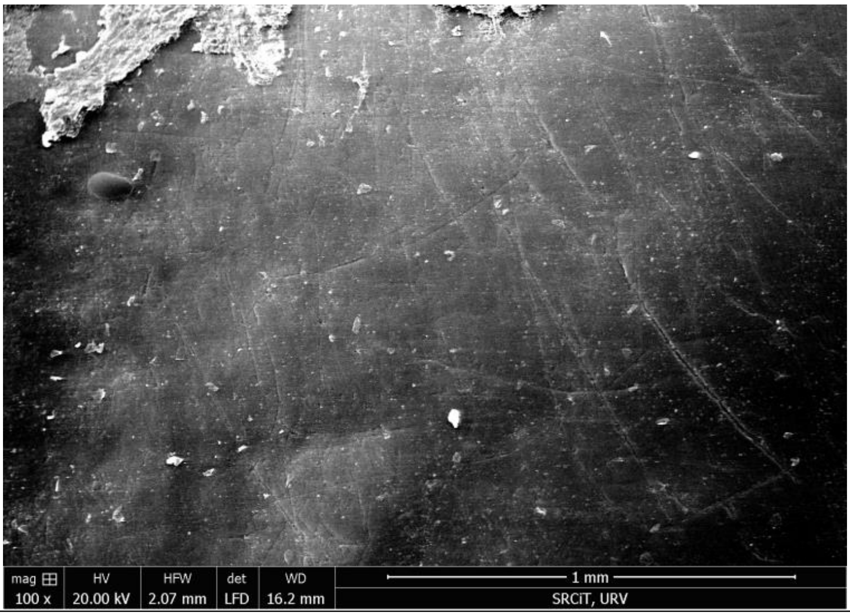

Figure 12. Scanning electron microphotographs of the **GRO 10-IX 1** at 100x

microphotographs comparing to the light microscopy is generally speaking an expected outcome taking in account studies of Hernando et al. (2020). Still, in this article authors have found light microscopy to be capable of distinguishing in average 17.5% more striations than SEM is.

Table 6. Comparison of Optical and electron microscopy results on one tooth

GRO 10-IX 1	NT	NV	NH	NMD	NDM	NV/ NT	NH/ NT	Length Median, µm
Light microscopy	58	25	3	13	17	0.43	0.05	138.60
SEM	26	13	5	5	3	0.5	0.19	181.66

Another important fact we can see from this comparison is significantly greater median length of striations in electron microscopy.

Results IV. Deciduous teeth and child nutrition

Deciduous teeth description and age-at-death identification

GRO 8-VI 81 is a deciduous lower right second molar (fig.13). It has only slight macrowear (table 4). Roots are damaged on the tips but seem to be closed by the time of death. It allows us to determine the age of the child as at least 3 years old, when the roots of the second deciduous molars close. However, it does not allow us to draw a

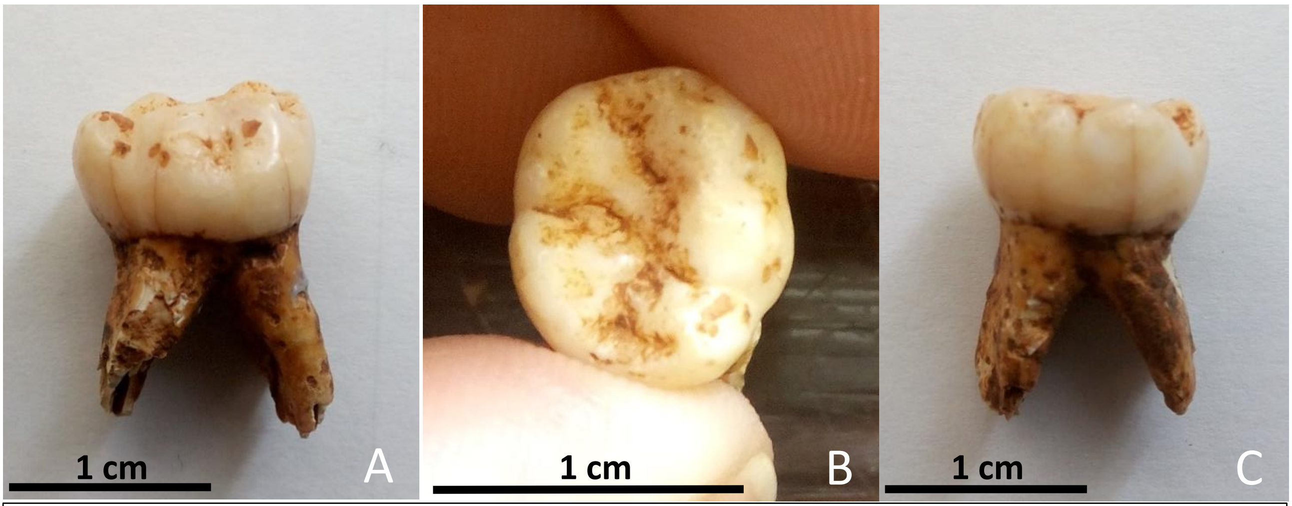

Figure 13. Deciduous lower right second molar GRO 8-VI 81: A – buccal surface; B – lingual surface

clear upper boundary of the age of the child, because root resorption of these teeth starts only after 7 years with the development of permanent premolars under them (Schour, 1941; Ubelaker, 1999). Thus, basing on stage of tooth development, the age-at-death of the child is between 3 and 7 years. But taking in account initial stage of macro-wear, the age-at-death could be closer to 3 years.

GRO 8-VI 62 is a deciduous lower right first molar (fig.14). Roots are closed, the mesial one is wide and branched at the apex. The distal facet is present, although not

Figure 14. Deciduous lower right first molar GRO 8-VI 62: A – buccal surface; B – occlusal surface; C – lingual surface

visible on the photos. Macro-wear is either absent or hardly-identifiable because of weathering. In case of the first deciduous molar roots can be closed already at 2 years, however here we see also the distal facet which attests for the second deciduous molar also being in occlusion. Since resorption of the roots starts in the first and the second deciduous molar at roughly the same time (Schour, 1941; Ubelaker, 1999), we can suppose here the same age range as in the previous case: 3-7 years. However, since the teeth are loose and since there were rests of several sub-adult individuals in the osteological material, it is difficult to say if the teeth belonged to the same individual.

Microwear signature on the deciduous teeth

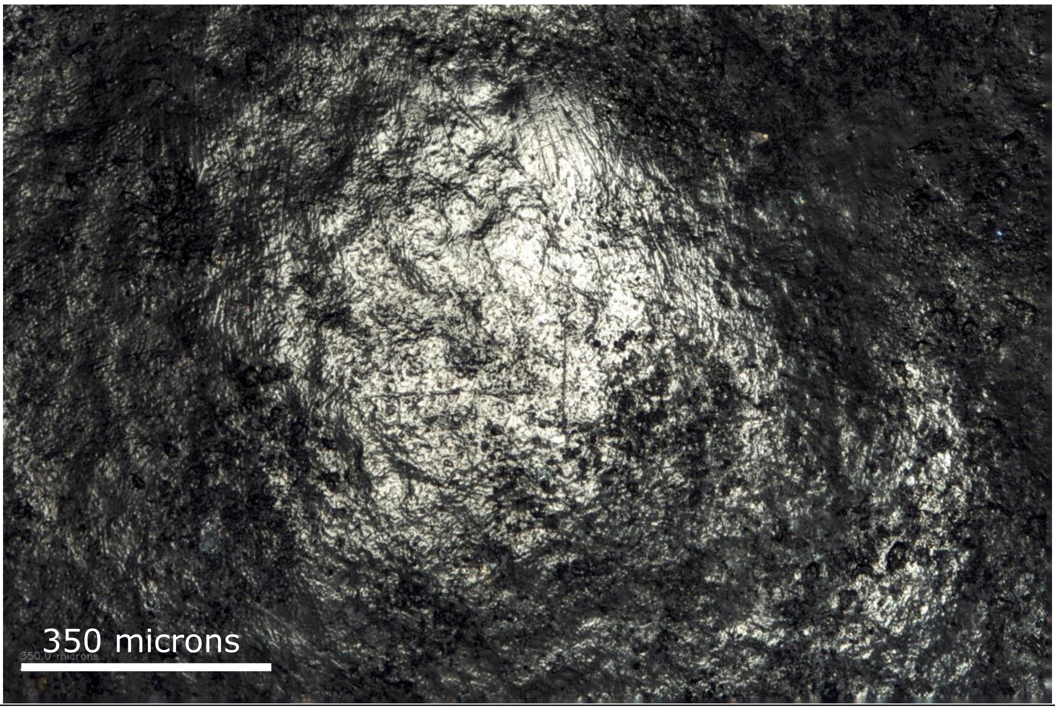

Figure 15. Buccal surface microphotograph of the **GRO 8-VI 81** tooth. Optical microscopy, magnification 100x

Tooth **GRO 8-VI 81** presented some countable striations, which were counted following the standard methodology. However, as a possibly just erupted tooth, it presented enamel prisms on its surface (fig.15).

Table 4. Microwear of the **GRO 8-VI 81** tooth

	NT	Number of striations by categories				Relative frequencies		Striation length, Median, µm
		NV	NH	NMD	NDM	NV/NT	NH/NT	
OM*	36	20	3	5	8	0.56	0.08	120.99
ESEM	14	11	1	1	1	-	-	181.66

OM – Optical microscopy; ESEM – Environmental scanning electron microscopy

Results of the striation counting on this tooth are shown in the table 4. Number of striations, however, should be taken with carefulness again. Figure 11 clearly shows that lots of striations could be easily overshadowed on the enamel curvature or hidden by the enamel prisms. Just as in the rest of the teeth analyzed in this study, here the distribution of the length of striation is not normal (Shapiro-Wilk W=0.87389; p=0.00071).

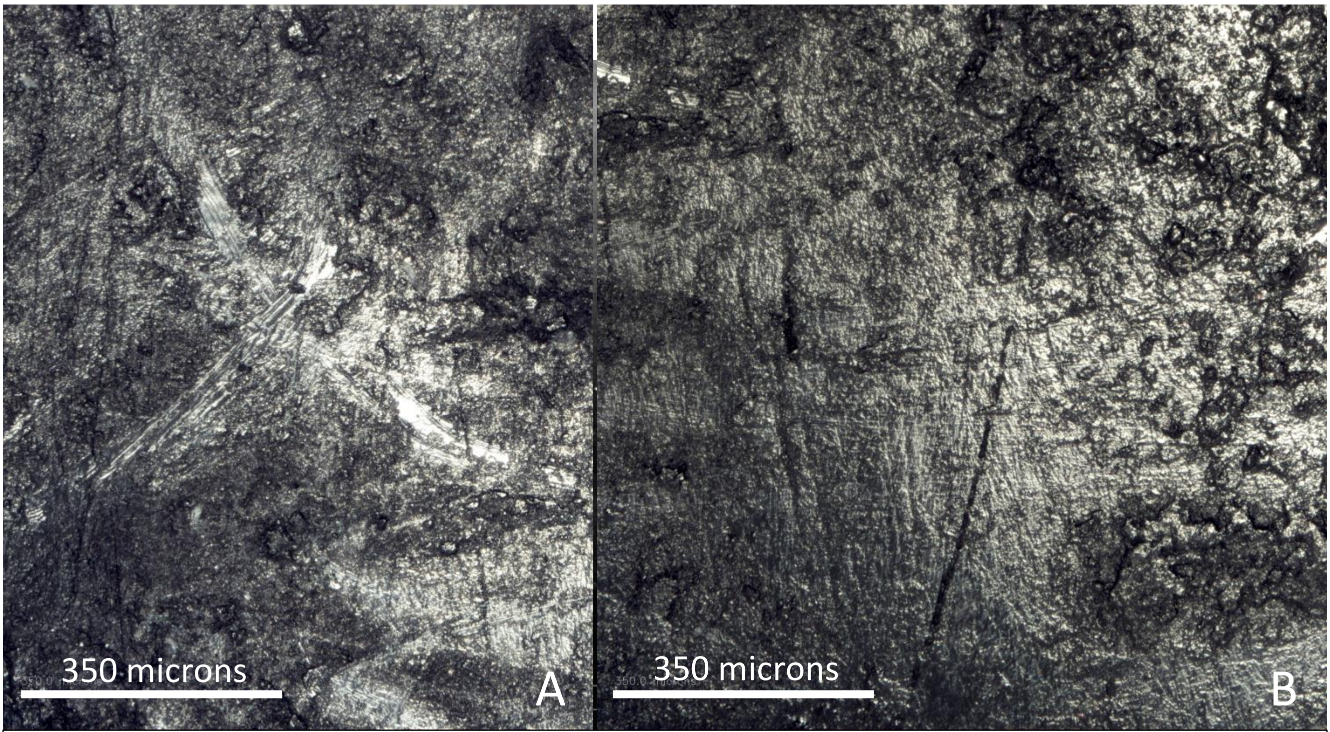

Figure 16. Buccal surface microphotograph of the **GRO 8-VI 62** tooth

microscopy, magnification 100x

Just as in other teeth, in **GRO 8-VI 81** SEM has shown significantly less striations than the optical microscopy (table 4). Once more we can see pronounced sediment deposition exactly near the area studied for the microwear (fig.17).

Another deciduous tooth **GRO 8-VI 62** having significant taphonomical issues (fig.16) does not show any countable microwear striations. Its rate of macro-wear is the same as in the previous – 1. Although there are some tiny straight scratches (Fig.16 B), they are surrounded by abundant sediment depositions. In the same time enamel surface of the tooth presents clear traits of post-mortem alterations such as short multi-parallel striations (fig.16 A). Therefore, no diet is identifiable apart from this tooth, instead the main question is “are there any dietary striations on this tooth at all?”

Discussion

Taphonomy and diagenesis of the site

The isolated teeth examined in our study presented all the spectrum of microscopic alterations typical for trampling. It suggests that human activities or frequent visits of animals took place in the cave after the construction of the ossuary and distribution of isolated teeth around it. Such evidences of frequent activities inside the cave are in agreement with the main premise about the function of this cave. We can suggest that after the construction of the ossuary its ritual use was more or less long and intensive and involved entering the cave. In that case trampling marks we can see on the teeth could be produced by the younger generations of the same community from which the human rests derive. But of course, possible trampling by animals should not be disregarded as well.

Trampling of teeth can be responsible for disappearance of dietary striation from the enamel surface, therefore results of dietary reconstructions should be taken with carefulness because they potentially can be underestimated. However, as Domìnguez-Rodrigo et al. (2009) point out on bone material, trampling marks such as furrows with micro-grooves inside are often accompanied by randomly oriented fine striations created by the trampling as well. Thus, it is important to understand if such fine singular randomly oriented striations can appear as well during trampling of dental enamel. It seems that such a morphology can be mistakenly recognized as dietary striations much more likely than other trampling marks described here.

Expectedly for a closed cave environment no signs of fluvial sediment transport were found on the teeth. It narrows the scope of taphonomic alterations from which the dietary striations should be differed.

Covering with sediment is another important taphonomical alteration typical for both bones and teeth in Gruta dos Ossos. Deposition of calcium carbonate in the cave resulted not only in formation of stalagmite floor but also covered bones with thick layer of sediment or even impregnated them inside. Calcium carbonate was frequently found also on the tooth enamel, both on macro and micro level. The sediment making up stratigraphy around the ossuary – sand-silt mix also was often stack to the enamel. Apparently, in some cases it was solidified by carbonate and therefore stack stronger to the teeth.

Another aspect of taphonomy which was not directly evidenced in our study but still can affect microwear dietary studies is soil acidity. High acidity of soil in Portugal is well-known cause of poor preservation of osteological material (Gonçalves 1999). Although bones in Gruta dos Ossos are not deposited in the soil, its excavators told about the human origin of the sediment of layers where the bones were found. It means that likely a lot of the substrate could be carried into the cave from outside. Soil acidity is thus another factor which could possibly affect the number of striations on the enamel surface of analyzed teeth.

Interaction of different taphonomic factors between them is also important to mention. Sand-silt sediment, similar to sand-clay mixture could increase effect of trampling. It was shown that the finest the sediment the more microscopical striations it produces either during trampling or during sediment transport (references).

Dietary reconstruction trial

Striations frequency found on both permanent teeth and deciduous tooth is among the lowest values usually found in microwear studies. It can have several explanations. One of the analyzed teeth, the same one that had the lowest number of recorded striations (30) is heavily worn – it has macro-wear attrition score “eight” after Smith. In this exact case, it could be caused by the need to take mold near the cervical line where striation frequency is typically lower.

The rest of analyzed teeth have various macro-wear score. In the same time we know from Tomé’s studies (2011) that the odontological material from Gruta dos Ossos has generally quite high mean value of dental attrition – 3.64, the highest among other similar Neolithic burials in the region. From this point view, it is difficult to interpret low frequency of striations observed on the teeth as a sign of low abrasiveness of diet.

This makes us to return to the taphonomic characterization of the site. It is widely believed that most taphonomic alterations are rather likely to erase existing dietary striation on the teeth than to create new ones. We saw serious taphonomic alterations on a great number of teeth. Mainly they are trampling marks – traces of a process quite destructive for the surface. This fact together with potential effect of acidic soils typical for Portugal with high probability can explain results we received.

Contrary to total number of striations, relative frequencies should be less subjected to bias related to taphonomic erasing of the striations. We can suppose this notion if we accept premise that taphonomic processes erase dietary striations in a random manner, independently of their direction. Distribution of the NH/NT and NV/NT indexes in permanent teeth is shown on the Figure 5 in comparison with these indexes in modern pre-industrial societies studied by Lalueza-Fox et al. (1996, fig.18 & 19). The indexes suggest that the diet of studied palaeopopulation was most close to the carnivorous or arid hunter-gatherers. Low NH/NT indexes appear where the horizontal striations are few. On the other hand, high NV/NT values are result of significant vertical striation prevalence. Such a distribution seems to correspond to a diet with high quantity of meat. The latter is a tough, but not abrasive food, which requires vertical chewing instead of horizontal shearing.

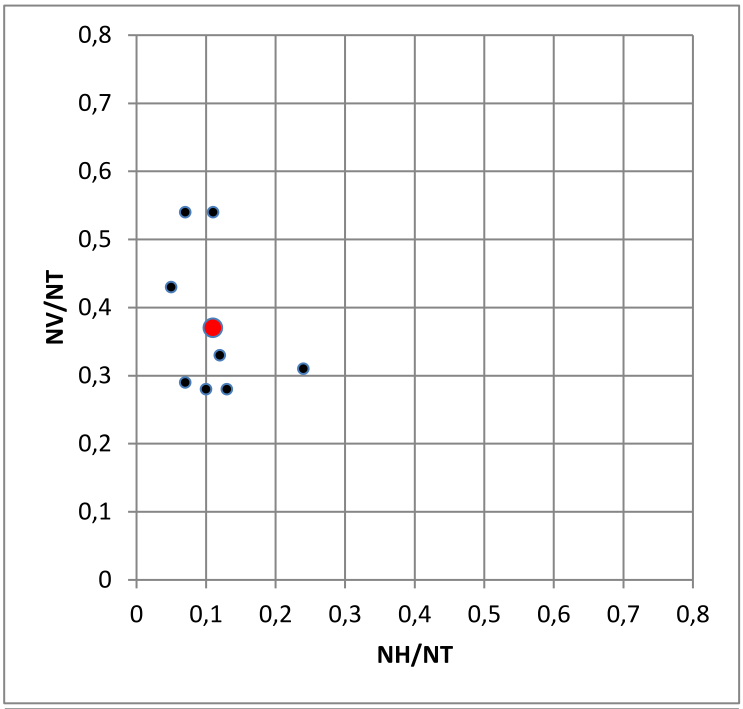

Figure 18. Plot of NH/NT index vs. NV/NT. Black circles represent individual values of teeth, red one – general index of overall data

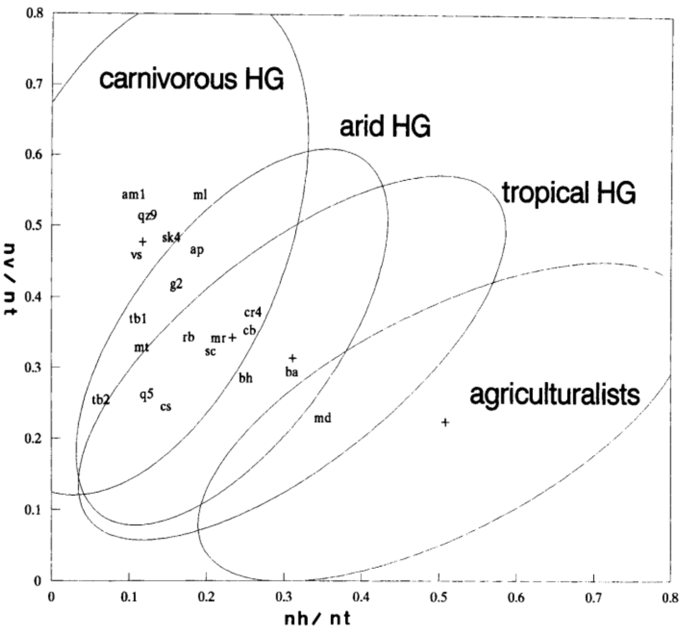

Figure 19. NV/NT and NH/NT indexes in modern hunter-gatherers. Picture adapted from Lalueza et al. (1996).

Suggestions about high meat proportion in the diet, however, should be correlated with the previous stable isotope analyses conducted on the site and in the region. Studies of Guiry et al. (2016) show that $\delta^{15}N$ values in Gruta dos Ossos are in the range typical for other Neolithic sites of the region and quite different from such values of Mesolithic populations with their fish and game based ration. Gruta dos Ossos is not characterized by an extremely high outlier. Unfortunately, even this isotope study does not allow for a precise reconstruction regarding meat consumption because $\delta^{15}N$ base level of the soils in the region is too variable and seems to explain most of variability of the value in Neolithic.

The last data we can employ for a comparison is dental pathology – another important proxy for diet reconstruction. Dental caries is believed to increase its prevalence in societies that ingest large quantity of carbohydrates and to be low in populations that are more carnivorous. General carious prevalence in Gruta dos Ossos assemblage is quite high – 11.63% (Tomé, 2011). As Cunha (2007) underlines, caries prevalence grows after Mesolithic and fluctuates around 7% in Neolithic. In case of Nabão valley this value gets both lower (Gruda do Cadaval, 5.6%) and higher values (Gruta de Nossa Senhora das Lapas, 9.3%) (Tomé, 2011). Thus, Gruta dos Ossos presents not just high caries prevalence but the higher one among other similar sites of Central Portugal and nearby Spain. Although carious potentially can depend also on other factors (such as generalized physiological stress) such a level of this pathological condition generally suggests rather plant-based diet of this palaeopopulation. It further questions applicability of the microwear data we obtained.

In addition, it is possible to imagine the situation in which mostly plant food would produce microwear signature typical for low abrasive diet and for one requiring little horizontal shearing. It can happen in case of significantly processed starchy products, for example producing semi-liquid porridges and soups of cereals carefully washed before. In that case not only "soft" microwear pattern can be explained, but also elevated carious prevalence and tartar frequency observed in Gruta dos Ossos – these pathological conditions are indicators of sticky starchy food.

The last microwear striation variable we analyzed is striation length. Median length of striations on analyzed teeth is generally between 100 and 200 μm, in one case being even more than 200 μm. This is quite a great median value which can be characteristic of high proportion of a dense food which requires chewing. Mean striation length values of farmers are typically around 100 μm because of abundant short scratches, characterizing diet made up of highly abrasive crumbly plant food (Salazar-García, 2016; Galbany, 2008). Contrary, less but longer striations seem to be typical for diet of hunters who rely more on meat. However, in line with the previous data here we should be very careful before making any firm conclusions about diets.

Given (1) the limited sample studied in present work, (2) particular type of material (isolated teeth), and (3) significant burden of taphonomic alterations found on the enamel surface, our buccal microwear study conducted at Gruta dos Ossos gives little new dietary information. The main points for the future microwear studies at this site and in similar contexts are: (1) to pay more attention to *in situ* teeth; (2) to take bigger samples; and (3) to further take in account carefully all the taphonomic alterations, geological and cultural context of the site.

Sub-adult teeth and methodolical comparisons

Loose deciduous teeth although outnumbered manage to bring an additional information. Only one of them, a lower right deciduous second molar, presents microwear traces proving consumption of hard "adult" food at age between 3 to 7 years. Microwear present on its buccal surface is generally similar to the microwear on the permanent teeth studied in this work. Initial stage of macro-wear suggests that the individual rather died around 3 years old. Therefore, this deciduous tooth is humble evidence suggesting that (1) weaning in the Gruta dos Ossos community took place before 3 years and (2) diet of children was not significantly different from that of adults. However, unfortunately, one deciduous tooth is obviously not enough for confident conclusions – and the future studies should look into the rest of collection as well.

Since we used both scanning electron microscopy and optical microscopy in the study, in some cases we had a possibility to compare results of these two techniques. Only two scanning electron microphotographs had some countable striations, one of which is of deciduous tooth. In both cases SEM microphotographs had less striations than Optical ones from the same tooth. This result generally agrees with the conclusions of Hernando et al. (2020), that optical microscopy tends to find more striations than scanning electron one. Another interesting fact when we compare the samples of striations from the two techniques is that on both teeth median value of striation length is greater for SEM microphotograph than for the optical microphotograph. It suggests that the number of visible striations is reduced in scanning electron microscope for the expense of short striations. However, here again the sample is too small for any serious conclusions, thus it presents an interesting topic for future studies on bigger samples.

Conclusions

Study of a sample of isolated teeth from Late Neolithic collective cave burial Gruta dos Ossos was undertaken. Taphonomic alterations of the teeth, visible on the micro-level, show abundant evidence of trampling in accordance with suggested recurrent ritual use of the site. Sediment depositions on the enamel are typical for the given type of the context: calcium carbonate resulted from the stalagmite sedimentation process; softer sand-silt sediment either solidified with carbonate or alone often covered the surface as well. No signs of fluvial sediment movement were found suggesting that most of the taphonomic history of the osteological material passed in the same closed environment of the cave.

A limited comparison between optical and scanning electron microscopy results shows one more time higher capability of the modern optical microscope equipment to find more striations on the enamel surface.

The alterations likely affected the number of micro-striations found on the buccal enamel. This, in turn, potentially undermines the results of the dietary reconstruction trial. Likelihood of taphonomic alteration of the results is even higher given the low frequency of striation we registered in this study. Alternatively, it can evidence low-abrasion diet, but clearly new studies on bigger samples are of need in this context. Analysis of two deciduous teeth has shown that children could get weaned and pass to a hard “adult” diet at the age of approximately 3 years old. This latter conclusion, however, needs further studies of the osteological assemblage as well.

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