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3D digital microscopy and taphonomy: two examples from Palaeolithic sites (Grotta dei Santi – Grosseto and Grotta Paglicci - Foggia)

La microscopia digitale 3D applicata alle indagini tafonomiche: l'esempio di due siti paleolitici (Grotta dei Santi – Grosseto e Grotta Paglicci - Foggia)

Summary - The Research Unit of Prehistoric Ecology of the University of Siena is testing the potentiality of a digital microscope that captures 3D images of the bone surface. The aim of this research is to develop new methods for understanding the origin of different bone modifications (e.g. scores, punctures, cut marks, chemical corrosion) using morphometry. It allows to find diagnostic criteria that can be processed through statistics, avoiding the exclusive use of subjective observations. In this paper different bone modifications have been analysed: cut marks produced during butchery experiments, modern tooth marks and striae located on macromammal remains coming from two Palaeolithic sites: Grotta dei Santi (Grosseto) and Grotta Paglicci (Foggia). The aim is to compare bone modifications of different origin and to test the method on archaeological samples, in order to understand how this can be employed to better interpret the taphonomic evidences in future works.

Riassunto - L'Unità di Ricerca di Ecologia Preistorica dell'Università di Siena sta portando avanti un ampio studio volto alla realizzazione di nuove metodologie di analisi tafonomiche, condotte attraverso l'utilizzo della microscopia digitale 3D. Le modificazioni sulle superfici ossee (scores, punctures, cut marks, alterazioni chimiche) possono essere infatti analizzate da un punto di vista morfometrico permettendo l'individuazione di caratteri diagnostici che possono essere elaborati statisticamente, evitando l'esclusivo utilizzo di osservazioni soggettive. In questo contributo vengono presentati alcuni dati riguardanti strie di macellazione ottenute in prove sperimentali, strie provocate da carnivori moderni e tracce rilevate su resti di macromammiferi provenienti da due siti paleolitici: la Grotta dei Santi (Grosseto) e Grotta Paglicci (Foggia). Lo scopo è quello di mettere a confronto tracce lasciate da diversi agenti e capire, testando il metodo su campioni archeologici, verso quale direzione possa essere sviluppato questo tipo di ricerca in modo da favorire in futuro una migliore interpretazione di alcune evidenze tafonomiche.

Keywords: 3D Digital Microscopy, Taphonomy, Grotta dei Santi, Grotta Paglicci, Statistics

Parole chiave: Microscopia Digitale 3D, Tafonomia, Grotta dei Santi, Grotta Paglicci, Statistica

Introduction

Methods for identifying the origin of marks on bone surfaces have been developed in several recent studies (e.g. Olsen, Shipman 1988; Selvaggio 1994; Blumenschine *et al.* 1996; Greenfield 1999, 2006; Choi, Driwantoro 2007; West, Louys 2007; Bello, Soligo 2008; Bello *et al.* 2009; de Juana *et al.* 2010; Yravedra *et al.* 2010). Some authors analysed the micromorphology of *striae* attempting to differentiate between trampling, carnivore and human modifications on the bone samples (Blumenschine 1995; Giacobini 1995; Domínguez-Rodrigo *et al.* 2009). Olsen and Shipman (1988) were the first to use a scanning electron microscope (SEM) in the investigation of marks in prehistoric contexts. As summarised by Bello and Soligo (2008), the scanning process of SEM produces a 2D image of a 3D surface, thus preventing the analysis of the cross sections of cut marks. These authors introduced a method for the analysis of the striae, using a 3D virtual reconstruction of bone surfaces captured with an Alicona 3D Infinite- Focus® imaging microscope. In this way they obtained reproducible, quantitative data to describe the micromorphology of each mark (e.g. slope angles, opening angles, bisector angle, shoulder heights, floor radius, depth of cut). Following their work, over the last three years we have included the use of an Hirox Digital Microscope KH-7700 in taphonomic analyses. The aim was to create a digital reference collection of qualitative and quantitative data of both natural and anthropogenic modifications on the bone surfaces, allowing the determination of diagnostic morphometrical parameters for a better identification of archaeological evidence.

Materials and methods

Butchery experiments were carried out on two fresh cattle autopodia (metapodials and phalanges) using a flint flake and a retouched flint tool. A diverse range of cut marks was produced. It resulted from the different force applied by the operators, hand position and action (skinning, disarticulation, cut of tendons). Bones were boiled in water and then buried for approximately 1 month for degreasing. A total amount of 27 cut marks on diaphyseal portions have been examined in this paper. The 58 analysed tooth marks are located on bones stored in the osteological comparative collection of the University of Siena. They were inflicted by various small wild carnivore species and by medium to large sized dogs. Bones were both recovered on the field or collected during experiments carried out with dogs.

The archaeological specimens analysed in this paper are coming from two Italian caves. Grotta Paglicci is an important Palaeolithic site located in the south-western edge of the Gargano Promontory (Apulia, Southeastern Italy). Excavations throughout the Upper Palaeolithic sequence yielded artefacts and both human and animal remains from the period ranging from the Aurignacian to the Final Epigravettian (Palma Di Cesnola 1993). The samples analysed for this article include 53 cut marks from layer 22F and 71 from layer 23C (both attributed to the Early Gravettian).

Grotta dei Santi opens onto a buhrstone slope in the South-Eastern side of Monte Argentario (Grosseto). The investigations of the deposit, still in progress under the direction of A. Moroni and M. Freguglia (University of Siena), suggest several alternate occupations of the cave by Neanderthals and large carnivores (Moroni *et al.* 2010). A lithic industry and a large amount of faunal remains were recovered in several layers. Deposit chronology, currently defined through stratigraphic criteria, is referable to a time period comprised between the Last Interglacial (Isotope stage 5e) and 40.000 years B.P. Layers 105 to 111 yielded a great amount of coprolites of large carnivores, whilst skeletal elements of *Panthera pardus* and *Crocuta crocuta spelaea* come from layer 110. A total amount of 26 *striae* on small to large-sized ungulate bones from layers 110 to 112 were analysed. Despite their provenance from different layers, they have been considered as a unique sample due to their small amount. The aim of the analysis of this set was to obtain preliminary data on the modifications of the bone assemblage caused by carnivores and humans.

The images were captured using a Hirox KH-7700 digital microscope with an MXG-10C body, OL-140II lens and an AD-10S Directional Lighting Adapter. Cut marks and tooth marks were examined under various levels of power (from 140 up to 700 magnification), depending on their dimensions. The Auto Multi Focus tool enabled the creation of a series of 100 images from different planes and, through the overlapping of focus levels, the construction of a 3D composite image. The entire cross section of each mark can be viewed, its morphology can be observed from several angles and the measurements of profiles can be taken. Three-dimensional images were observed using the software KH-7700 3DViewer Ver.1.2.00 (© HIROX CO., Ltd 2006), whereas the software tpsDig Version 2.15 (© 2010, F. James Rholf, Ecology & Evolution, SUNY at Stony Brook) was used to collect measurements. For this paper, one profile from the median part of each cut mark has been analysed. The selected measurements (Fig. 1) are the depth of the cut mark (DC), its breadth at the floor (BF) and at the top (BT), as defined by Bello and Soligo (2008) and by Boschin and Crezzini (2012). In addiction to these parameters also the distances from the middle of the floor to the edges of the cross section were measured (Fig. 1). To better describe the shape of the cross section three indexes were calculated: the ratio between the breadth at the top and the breadth at the floor (RTF = BT/BF), the ratio between the breadth at the top and the depth (RTD = BT/DC) and the ratio between the greatest and the smallest distances from the middle of the floor to the edges of the *striae* (SYM = GD/SD). Considering their shape, the profiles of the cross-sections were grouped into seven morphological categories, as defined by Boschin and Crezzini (2012): 1. profiles with a wide flat floor; 2. narrow V-shaped regular profiles; 3. narrow U-shaped regular profiles; 4. broad V-shaped profiles; 5. irregular V- or U-shaped profiles characterised by the presence of one ancillary groove or edge

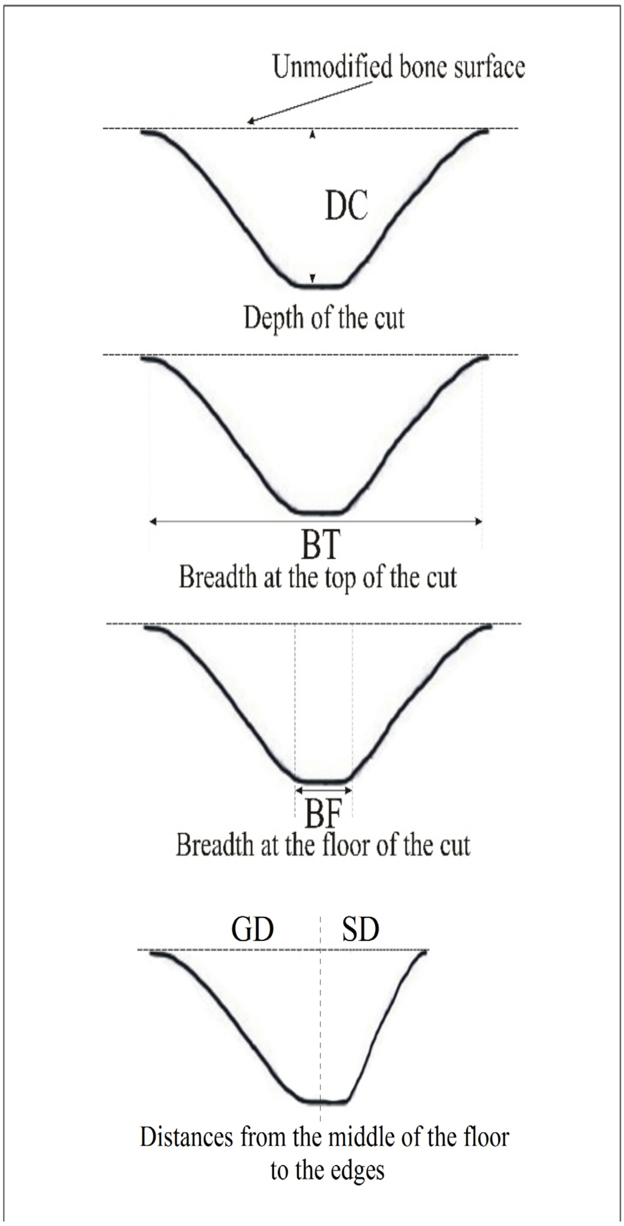

Figure 1. Measurements taken on the profiles.

on one side; 6. irregular V- or U-shaped profiles characterised by the presence of several ancillary parallel *striae*, on one or both sides, lateral to the apex of the cut and of uneven length and thickness; 7. profiles with two apexes occurring on the floor of the groove. Chi-square tests were performed to statistically verify the occurrence of morphological categories according to marks of different origin. Wilcoxon two-sample test and principal component analysis (PCA) were performed to evaluate the metrical parameters used to differentiate between cut marks and tooth marks. Statistics were performed using the R software version 2.12.0 (© 2010 The R Foundation for Statistical Computing) and the Past software (Hammer *et al.* 2001).

Results

The occurrence of morphological categories according to marks of different samples is shown in figure 2. The profiles of modern cut marks produced with the flint flake and the retouched stone tool are mainly grouped in categories 5, 6 and 7, which describe irregular profiles with one or two apexes occurring on the floor of the groove. The floor of the tooth marks instead is flat, wide or characterised by the occurrence of two apexes (categories 1, 3 and 7). The different distribution of the morphological categories according to the origin of marks was statistically proofed (χ^2 test = 49.85, df = 6, p<0.001). The profiles of cut marks from layers 22F and 23C of Grotta Paglicci are mainly grouped in categories 3 e 5. The distribution of the profiles according to the categories is not significantly different between the two samples (χ^2 test = 11.98, df = 6, p=0.062). On the contrary the results confirmed that the shape of tooth marks profiles is dissimilar (22F *vs* Modern tooth marks: χ^2 test = 68.58 df = 6, p<0.001; 23C *vs* Modern tooth marks: χ^2 test = 47.73 df = 6, p<0.001). The analysis carried out on the *striae* coming from Grotta dei Santi reveals several interesting characteristics: the morphologies of the cross-sections are statistically different from those of modern tooth marks (χ^2 test = 42.53 df = 6, p<0.001). Their distribution according to the morphological categories differs from that of modern cut marks (χ^2 test = 19.5 df = 6, p<0.01), but not from cut marks observed on the Paglicci specimens (χ^2 test = 5.8 df = 6, p = 0.44).
A PCA was performed on the measurements. The resulting 2D outcome shown in figure 3 explains approximately 97% of sample variability. The PC1, which accounted for 85.7% of variability, could differentiate between tooth marks and the other samples (Tab.1). This confirms that tooth marks are generally characterised by shallower and more U-shaped profiles. Modern cut marks are not different from the sample of Grotta dei Santi whilst the latter can be distinguished from that of Paglicci (Tab.1).
Also the PC2 (11% of the variance) could be used to separate between tooth marks and the other samples (Tab.1). On the contrary it is not useful to separate between modern cut marks and archaeological *striae*, whilst can distinguish

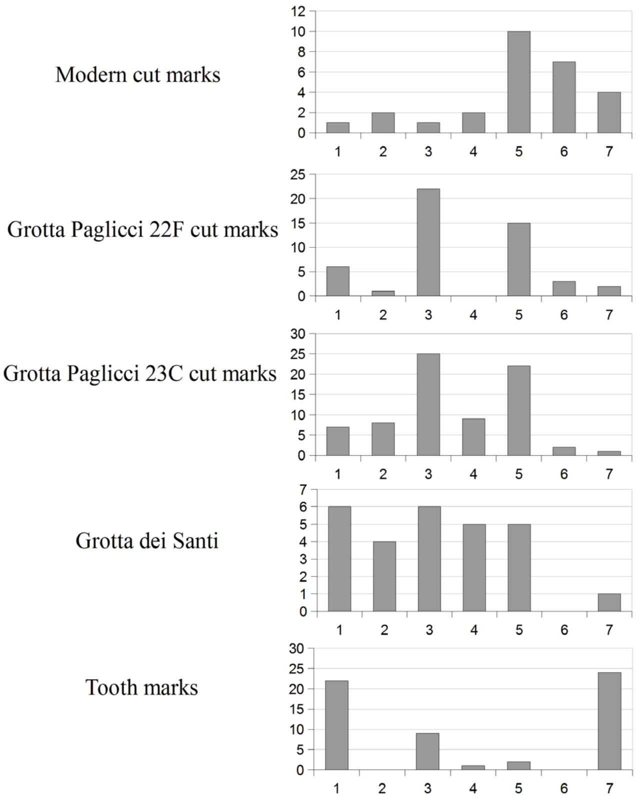

Figure 2. Frequency of each morphological category according to samples.

between Paglicci and Grotta dei Santi (Tab. 1). Furthermore it has to be pointed out that there is no statistical difference between PC1 and PC2 values of the cut marks from Paglicci 22F and Paglicci 23C (PC1: W=1967, $p = 0.3$; PC2: W=1452, $p = 0.06$). The scatter plot of the PCA can be interpreted as follows: the greater PC1 and PC2 scores are, the shallower and more U-shaped are the profiles; the smaller the scores of the PC1 are and the smaller are those of the PC2, the deeper and narrower and more V-shaped are the profiles.

RTF and RTD can be used to characterize the samples considered in this paper; the results are statistically proofed using a Wilcoxon two-sample test (Tab. 1). The two sub-samples from Paglicci (22F and 23C) show similar characteristics (RTF: W=2094, $p = 0.09$; RTD: W=1478, $p = 0.1$). Considering RTF, tooth marks are different from the other samples and Grotta dei Santi differs from both modern and Paglicci cut marks, significantly similar. Wilcoxon test carried out on RTD shows an analogue situation, with the exception of a similarity between Grotta

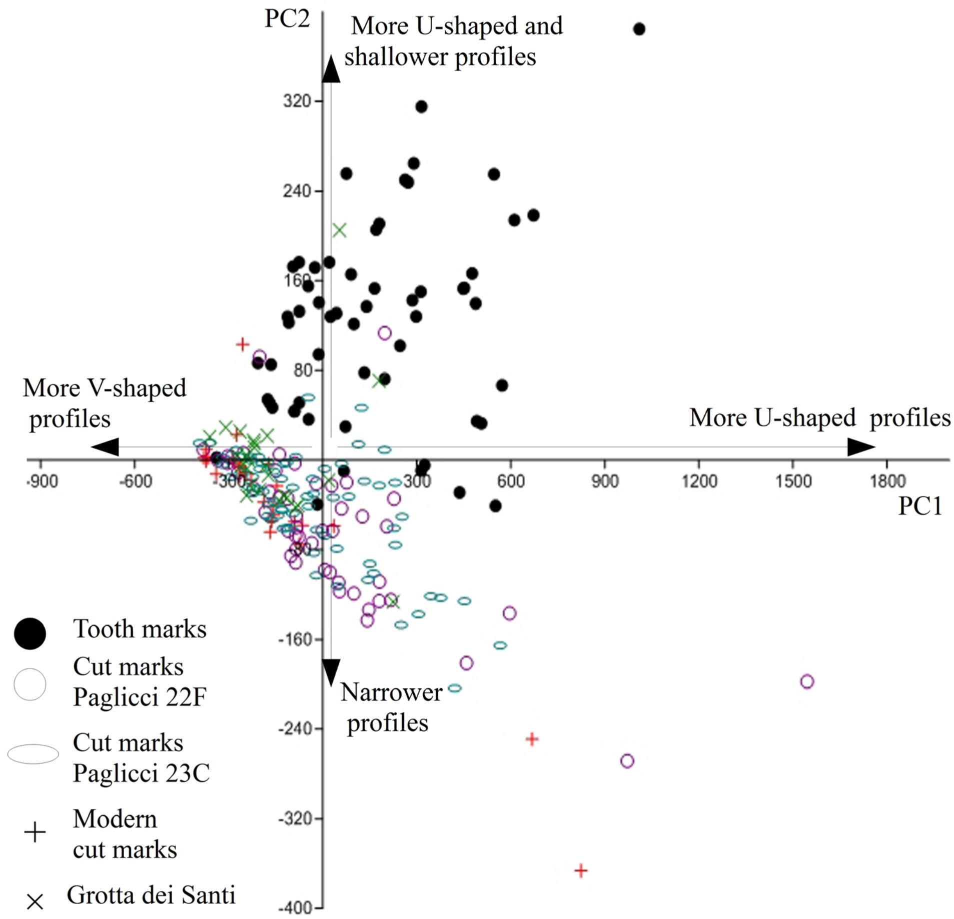

Figure 3. Principal component analysis performed of the measurements taken on the striae.

PC1	Paglicci	Grotta dei Santi	Tooth marks
Modern cuts	W=2427 p<0.001	W=433 p=0.2	W=231 p<0.001
Paglicci		W=2227 p<0.001	W=2134 p<0.001
Grotta dei Santi			W=224 p<0.001
PC2	Paglicci	Grotta dei Santi	Tooth marks
Modern cuts	W= 2013 p=0.08	W=263 p=0.08	W=474 p<0.01
Paglicci		W=894 p<0.001	W=1920 p<0.001
Grotta dei Santi			W=528 p<0.05
RTF	Paglicci	Grotta dei Santi	Tooth marks
Modern cuts	W=1952 p=0.15	W=518 p<0.01	W=1487 p<0.001
Paglicci		W=2060 p<0.05	W=319 p<0.001
Grotta dei Santi			W=153 p<0.001
RTD	Paglicci	Grotta dei Santi	Tooth marks
Modern cuts	W=1824 p=0.46	W=429 p=0.16	W=204 p<0.001
Paglicci		W=1029 p<0.01	W=6618 p<0.001
Grotta dei Santi			W=1359 p<0.001
SYM	Paglicci	Grotta dei Santi	Tooth marks
Modern cuts	W=1935 p=0.15	W=484 p<0.05	W=1143 p<0.001
Paglicci		W=2019 p<0.05	W=2330 p<0.001
Grotta dei Santi			W=707 p=0.6

Table 1. Results of the Wilcoxon two sample tests.

dei Santi and the modern sample. It has to be pointed out that the cross-sections of both tooth marks and the *striae* from Grotta dei Santi are more symmetric than those of the modern and Paglicci cut marks (Tab. 1, Figg. 4-6).

Discussion and conclusions

Our results confirm that the 3D digital microscope is a useful tool to separate between cut marks and tooth marks, through the analysis of both morphological and morphometric data. The method can be applied on large samples, recording a wide amount of measures in relatively brief time. Moreover the possibility to take reproducible measurements on the bone surfaces allows statistical data processing. The divergence coming out when comparing morphological categorisation with metrical measurements highlights the need to improve the subjective observations with more objective approach. Despite being promising, the application on a carnivore-modified archaeological assemblage is needed to test the validity of the proposed method. It is interesting to note that the comparison between the considered samples reveals some significant difference. In particular the cross-sections of the marks of Grotta dei Santi specimens are shallower, more symmetric and slightly less V-shaped than the analysed cut marks (Figg. 4-6). In spite of several archaeological evidences (coprolites, large carnivore skeletal elements) (Moroni *et al.* 2010) most of them do not show the shallow and broad U-section characterising modern tooth marks. If the set is considered as a whole, its characteristics do not seem compatible with those of a carnivore-modified sample (Fig. 3). Due to the shape of their cross-sections, the origin of these grooves could be related to butchery. Nevertheless trampling activities (by hominins and/or carnivores) on a sediment at Grotta dei Santi characterised by the presence of volcanic minerals can not be excluded. Olsen and Shipman (1988) pointed out that the abrasion of some types of crystals can leave on the bone surfaces V-shaped *striae* resembling anthropogenic marks.

The cut marks from Paglicci can be grouped according to bone tissue (rib cortical bone, diaphysis, epiphysis). In spite of the sample size, it seems that there are some differences between the sub-samples. For instance cut marks located on the diaphyses are shallower than the ones on the ribs or on spongy elements and epiphyses (Fig. 4). This depends from the force applied by the operator or from the different degree of penetration of cutting tools according to the hardness of the bone surfaces. Moreover cut marks on the diaphysis are characterised by a more evident asymmetry (Fig. 6), which can be related to a different action when these anatomical regions were treated (for instance a different hand position during skinning, disarticulation and defleshing). Although it is remarkable to observe the difference in the symmetry between modern cuts and those from Paglicci, this is not true if only the *striae* on the diaphyses are considered.

Moreover it is very interesting to point out that there is not similarity in the symmetry between the two archaeological samples, even if we considered only striation on the diaphyses. This suggests that the differences observed between Paglicci and Grotta dei Santi could be due both to a different agent (butchery *vs*. trampling) and to the size of the carcasses or to the different treatment of their parts (including the use of

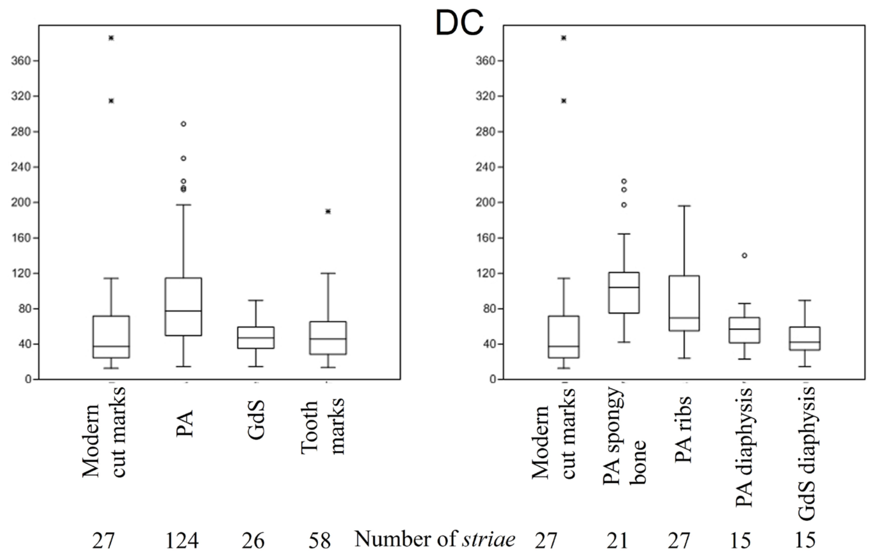

Figure 4. Differences in DC among the samples. Abbreviations: PA: Grotta Paglicci (22F and 23C together); GdS: Grotta dei Santi.

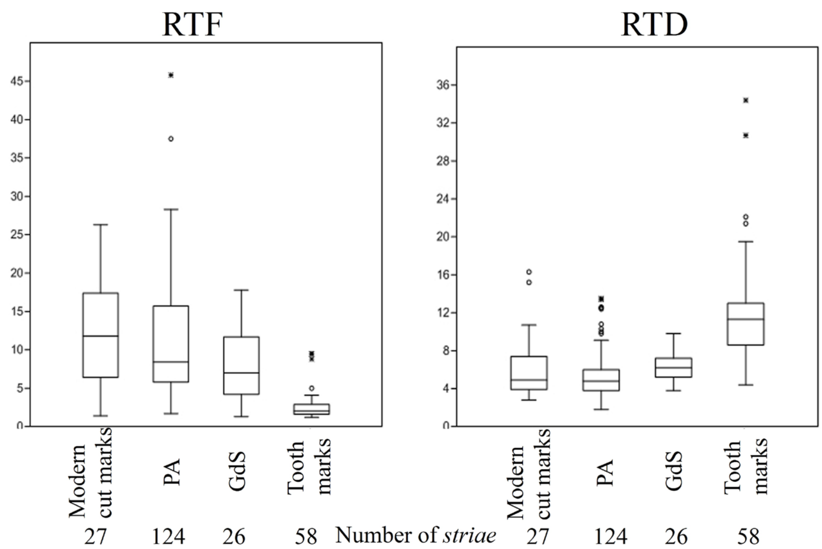

Figure 5. Differences in RTF and RTD among the samples. Abbreviations: PA: Grotta Paglicci (22F and 23C together); GdS: Grotta dei Santi.

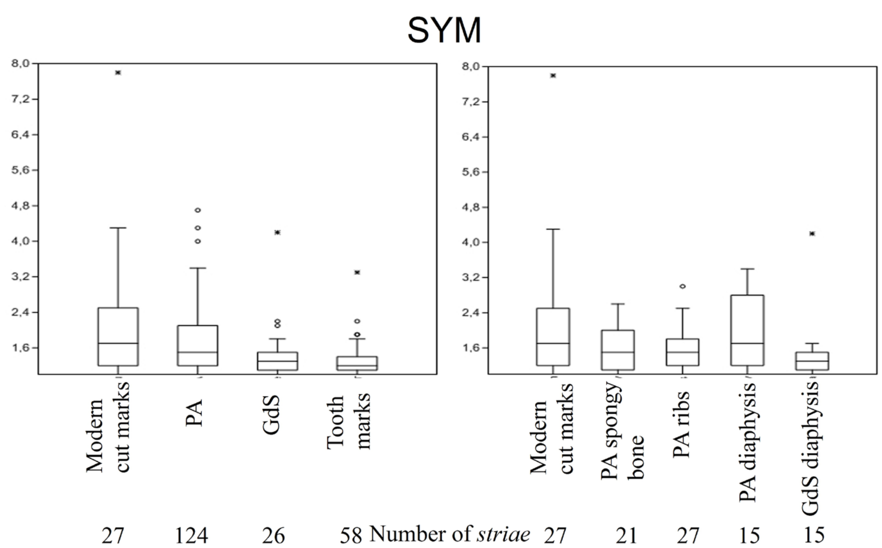

Figure 6. Differences in SYMamong the samples. Abbreviations: PA: Grotta Paglicci (22F and 23C together); GdS: Grotta dei Santi.

specific tools).

Although this kind of research is still at an initial stage, the obtained results confirm the potential of morphometrical data to explore the nature of the *striae* on bone surface. In facts this preliminary application of 3D microscopy in taphonomy shows some intriguing differences and similarities between analysed samples and, in a same sample (Grotta Paglicci), an intriguing variability of cut marks on different anatomical elements. It indicates that a morphometric study of striations at a microscopic level could help us to shed more light on the behaviour of past human groups (the use of different tools for butchering, different actions during their use) or on accumulation of bone assemblages (cut marks *vs*. trampling and tooth marks). Nevertheless these innovative data suggest that 3D studies have to be improved considering a dialogue between taphonomy, lithic technology, use-wear analysis, geology and experimental archaeology as a standard protocol of research.

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