

## HOW HOMOGENEOUS ARE MICROWEAR PATTERNS ON A FOSSIL HORSE TOOTH? PRELIMINARY TEST ON A PREMOLAR OF *EQUUS ALTIDENS* FROM BARRANCO LEON 5 (SPAIN)

Andrea Maria Francesco Valli<sup>1,2</sup>, Maria Rita Palombo<sup>3,2</sup> & Maria Teresa Alberdi<sup>4</sup>

<sup>1</sup> 78 Rue du Pont Ginguet, 03000 Moulins sur Allier (France)

<sup>2</sup> Istituto Italiano di Paleontologia Umana, Rome (Italy)

<sup>3</sup> Dipartimento di Scienze della Terra, Università "Sapienza" and CNR, Istituto di Geologia Ambientale e Geoingegneria, Rome (Italy)

<sup>4</sup> Departamento de Paleobiología, Museo Nacional de Ciencias Naturales, CSIC, Madrid (Spain)

Corresponding author: A.M.F. Valli <[andrea\\_vallifr@yahoo.fr](mailto:andrea_vallifr@yahoo.fr)>

**ABSTRACT:** Microwear patterns of twenty seven points on the enamel surface of a fossil horse tooth (upper P3-4; *Equus altidens granatensis*, late Early Pleistocene, Barranco Leon 5, Guadix-Baza basin, Spain) were analysed by a correspondence analysis test with the dual aim of testing the suitability of this premolar in analysing microwear of horses, and of analysing how consistent the microwear patterns are across different micro-areas on a single cheek tooth. The results obtained stress that remarkable differences in dental scars are restricted to few zones. The microwear patterns of the lingual enamel surface of the paracone, the point classically analysed on upper M2 for studying microwear in ruminants, and of the pli caballin are the most consistent with those of the other analysed micro-areas. The pattern of dental microwear shown at these points by the upper P3-4 of *Equus altidens granatensis* from Barranco Leon 5 is closer to those shown by seasonal mixed feeders than of those by strict grazers.

**Keywords:** Equidae, Guadix-Baza basin, Late Early Pleistocene, Microwear analysis, Spain.

### 1. INTRODUCTION

Analysis of dental wear, particularly the microscopic marks produced during chewing on occlusal enamel surfaces, both by tooth/food (abrasion) and tooth/tooth (attrition) interaction, is among the most significant tools applied by palaeontologists to infer dietary behaviour and the environmental context of extinct herbivore mammals.

During the last forty years studies on this subject studies have more and more enhanced and new methodological approaches have continuously been developing (e.g. Gordon, 1984; Teaford, 1986; Young & Marty, 1986; Hayek et al., 1991; Solounias & Moelleken, 1992; Franz-Odenaal & Solounias, 2004; Semprebon et al., 2004; Merceron & Ungar, 2005; Merceron et al., 2005, 2006, 2007; Palombo et al., 2005; Charles et al., 2007; Schubert et al., 2006; Scott et al., 2006; DeMiguel et al., 2008; Ungar et al., 2008, in press; Valli & Palombo, 2008; Merceron et al., 2010; Schulz et al., 2010, and references therein).

Although the use of microwear analysis undoubtedly is an useful tool in palaeoecological studies, thus far, few or no studies have been performed trying to test differences (if any) in microwear patterns shown by wear facets formed during the different masticator phases (but see for example Gordon, 1982; Schulz et al., 2010).

Butler (1972) first described the mastication process of herbivore mammals, and mapped the position of wear facets and the direction of the striae formed during the two phases of the power stroke of the masticatory cycle: Phase I, when vertical movements prevail up to

centric occlusion and Phase II, when the initial vertical movements become horizontal and finally downward out of the occlusal contact. Following Butler's (1972) idea, other authors (see e.g. Hiiemae, 1978; Janis, 1979, 1990; Rensberger et al., 1984; Joomun et al., 2008) investigated the progression of the cheek tooth pattern. They recognized that in phase I, shearing is predominant, while in phase II, grinding prevails. However, in hypsodont grazing ungulates, shearing becomes predominant and phase II tends to be lost (Janis, 1979).

Pleistocene *Equus* representatives have generally been regarded as typical grazers, because the microwear pattern of the lingual side of the paracone of the second upper molar (M2), the canonical point to perform analysis (see below), consists of fine, nearly parallel scratches, few pits, and no or very few gouges, and the number of scratches definitively outnumbers to the number of pits. This has been regarded as an important factor in distinguishing grazers from browsers (e.g. Solounias & Semprebon, 2002). Therefore, *Equus* cheek teeth seem to be particularly appropriate to test the consistency of microwear patterns across the whole occlusal enamel surface, because most of its surface is affected by shearing only (e.g. Janis, 1979), avoiding disturbances related to the presence of enamel scars produced by differently oriented masticator forces.

Examination of microwear in equids, as well as in ruminants, has generally been carried out on the enamel surface of the paracone of M2 by means of a either a scanner microscope or stereomicroscope (Solounias & Moelleken, 1993; Mainland, 1998; Solounias & Semprebon, 2002; Rivals et al., 2008; Muhlbachler et al., 2011

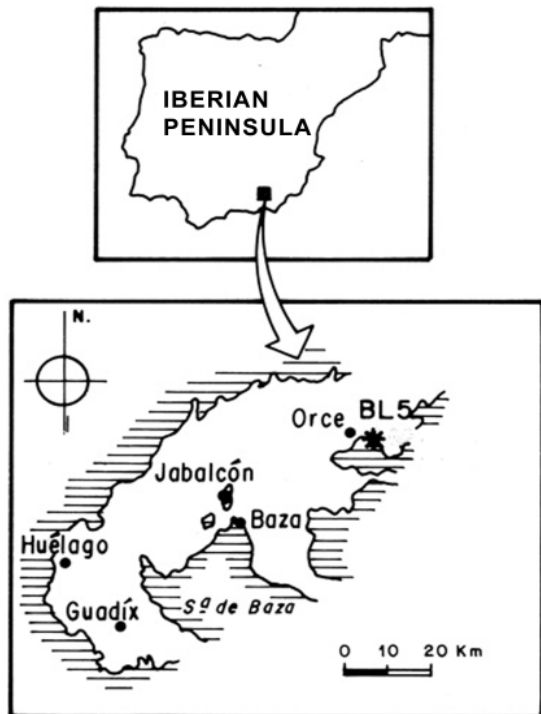


Fig. 1 - Position of the fossil deposit of Barranco Leon 5 (BL5; Guadix-Baza Basin, Spain; modified from Alberdi, 2010). The white area represents the Guadix-Baza Basin.

and references in those papers). However, in equids, the attrition/abrasion forces differentially affect the enamel surface of the cheek teeth because chewing pressures may vary along the tooth row. The anterior and posterior edges of the tooth row participate less in food processing, therefore very anterior and posterior teeth are affected by a more pronounced attrition (tooth to tooth contact), with higher pressures on molars than on premolars. Taking into account the peculiar development of anterior/posterior tilts characterising cheek teeth in horses, analysing the occlusal surface of P4 and M1 could be particularly appropriate in inferring dietary behaviour because, in these teeth, the main stress is almost perpendicular to the tooth mesio-distal axis, thus the enamel prisms are not much affected by this tooth tilt.

This is the reason why we have decided to analyze the microwear patterns on several points of the enamel of an upper premolar in the twofold aim of investigating on the suitability of the P3-4 in inferring dietary behaviour of fossil horse, and testing the consistency of the microwear patterns on the whole enamel occlusal surface of a grazer. We have chosen to carry out the test on an extraordinarily well preserved upper premolar (either P3 or 4) of a stenonoid horse, *Equus altidens granatensis*, from Barranco Leon 5 (late Early Pleistocene, Guadix-Baza Basin, Spain, Alberdi, 2010) (Fig. 1). This species has been regarded as a grazer (Alberdi, 2010; Alberdi & Palombo, 2012 and references therein) therefore the arrangement of enamel scars on the whole enamel of the tooth occlusal surface should be roughly constant. Although we are aware that, due the intrinsic characteristic of the microwear methods, reliable results can be obtained only by analysing a statistically consistent sample, we consider to preliminarily test the

soundness of the method as a first fundamental step before developing and improving the study by means of an adequate number of specimens.

## 2. MATERIALS AND METHODS

Analysis was performed on a left upper premolar (either P3 or P4 = P3-4; catalogue number: BL02 31 1692) from the latest Early Pleistocene fossiliferous deposit of Barranco Leon 5 (Guadix-Basin, Spain, Blain et al., 2011; Duval et al., 2009; Toro-Moyano et al., 2009). The specimen, stored at the Museum of Orce (Granada, Spain), has been chosen because of its good preservation status, allowing the whole surface of the enamel to be analysed. Observations were done directly on the tooth at 500x magnification, using the QUANTA200 FEI SEM at 25 kV (Museo Nacional de Ciencias Naturales, Madrid). Quantitative analysis was performed using the Microwear 4.02 software program (Ungar, 2001) on a surface of 500x450  $\mu\text{m}^2$ . Scars are categorised as pits if their width to length ratio is  $> 1/4$  and scratches if their width to length ratio is  $< 1/4$  (Solounias et al., 1988; Teaford, 1988; Solounias & Hayek, 1993). Pits are considered to be "large" or "small" according to their maximum diameter (whether it exceeds 15  $\mu\text{m}$  or not; Merceron et al., 2004). Scratches are described as "coarse" (= "wide") or "fine" according to their width (whether it exceeds 15  $\mu\text{m}$  or not; Merceron et al., 2004). They are also categorised as "long" or "short" depending on their length (whether it is equal to or longer than 250  $\mu\text{m}$ , or less than this value; the scars which could extend out of the field of the picture have not been considered; Valli & Palombo, 2008). The number of "gouges" (scars deeper and several times larger than large pits with irregular outlines; Solounias & Semprebon, 2002), and "cross scratches" (defined as the number of crosses among the scratches, independent of their orientation) were counted.

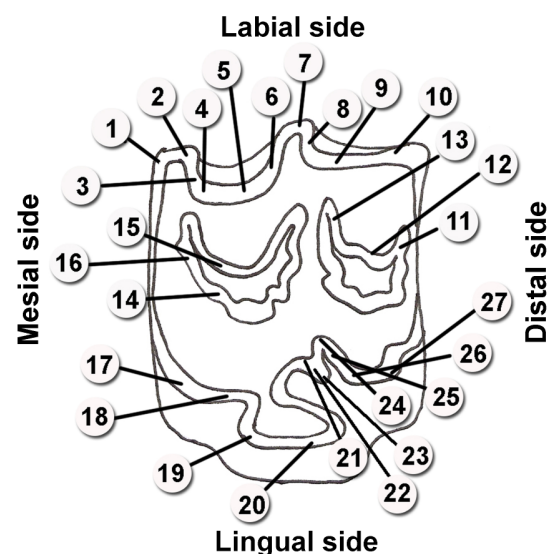


Fig. 2 - Left upper P3-4 (BL02 31 1692) of *Equus altidens granatensis* from Barranco Leon 5, Spain. The numbers indicate the points (loci) where the microwear analyses have been carried out.

	Pits								Scratches								Gouges		
	Large	Small	Tot	length	SD	width	SD	%	Short Fine	Long Fine	Long Coarse	Tot	length	SD	width	SD	%	Cross	
Point 1	2	7	9	4.14	2.48	1.87	0.93	13.85	49	6	1	56	31.35	27.71	1.07	0.60	86.15	38	1
Point 2	0	41	41	2.78	1.27	1.33	0.35	26.11	116	3	0	116	16.70	19.32	0.82	0.33	73.89	51	0
Point 3	0	53	53	2.06	0.80	1.03	0.31	35.33	97	0	0	97	16.65	13.27	0.89	0.41	64.67	46	0
Point 4	0	34	34	2.37	1.73	1.12	0.60	15.38	181	6	0	187	16.99	18.99	0.81	0.41	84.62	85	0
Point 5	0	31	31	1.98	1.37	1.06	0.43	24.80	91	3	0	94	19.27	17.67	0.94	0.45	75.20	38	0
Point 6	0	13	13	1.93	0.78	0.99	0.41	13.00	81	5	1	87	22.18	24.47	0.89	0.56	87.00	28	0
Point 7	0	22	22	2.82	1.43	1.34	0.53	17.32	97	8	0	105	22.13	23.26	0.99	0.46	82.68	44	0
Point 8	1	5	6	5.00	2.49	2.41	2.19	7.89	64	6	0	70	28.51	27.40	1.03	0.51	92.11	32	0
Point 9	3	12	15	3.83	4.34	1.74	1.60	15.00	80	4	1	85	22.35	18.71	0.90	0.71	85.00	26	0
Point 10	1	23	24	2.06	0.96	1.05	0.43	27.27	57	4	0	64	26.25	22.69	0.85	0.43	72.73	17	0
Point 11	1	43	44	2.54	2.33	1.29	0.76	30.77	95	3	1	99	16.70	18.58	0.86	0.60	69.23	19	0
Point 12	1	11	12	1.96	0.94	1.32	1.04	21.43	37	7	0	44	34.28	31.51	0.82	0.33	78.57	29	0
Point 13	4	13	17	4.45	2.65	2.79	2.21	27.42	42	1	2	45	24.42	24.70	1.15	1.34	72.58	8	2
Point 14	0	10	10	2.47	1.65	1.11	0.45	17.54	47	0	0	47	17.15	11.14	0.92	0.40	82.46	11	0
Point 15	2	15	17	3.24	2.42	1.67	1.29	20.99	60	4	0	64	22.81	25.56	0.86	0.33	79.01	24	0
Point 16	2	17	19	2.91	3.79	1.53	1.59	22.09	65	2	0	67	15.95	22.32	0.74	0.38	77.91	14	0
Point 17	1	23	24	2.99	1.92	1.50	1.12	21.82	81	5	0	86	20.31	20.92	0.99	0.52	78.18	33	1
Point 18	0	11	11	2.04	0.74	1.10	0.36	16.18	55	2	0	57	18.98	18.44	0.94	0.52	83.82	15	1
Point 19	2	27	29	3.81	3.12	1.70	1.14	33.33	50	7	1	58	27.41	29.17	1.14	1.14	66.67	20	0
Point 20	1	22	23	2.46	1.35	1.39	0.59	26.74	56	6	1	63	29.00	28.26	1.00	0.77	73.26	58	0
Point 21	0	16	16	2.32	1.54	1.50	1.43	21.33	53	6	0	59	28.10	29.57	0.71	0.45	78.67	35	0
Point 22	0	14	14	2.15	1.22	1.31	1.05	17.07	61	6	1	68	29.42	25.33	0.88	0.78	82.93	46	0
Point 23	0	25	25	1.77	0.72	0.95	0.37	25.25	69	5	0	74	24.58	24.95	0.74	0.48	74.75	35	0
Point 24	1	18	19	3.25	3.46	1.32	1.09	22.89	62	1	1	64	19.38	15.16	0.86	0.66	77.11	18	1
Point 25	1	11	12	2.60	3.63	1.48	1.89	16.00	60	3	0	63	24.67	17.23	0.86	0.53	84.00	25	0
Point 26	0	35	35	2.03	1.17	1.14	0.55	25.55	97	5	0	102	21.20	21.23	0.74	0.42	74.45	35	0
Point 27	0	20	20	2.23	24.31	1.07	0.37	32.26	40	2	0	42	26.93	24.31	0.89	0.59	67.74	19	0
M1-2 36-1520	3	34	37	2.60	1.75	1.41	0.69	39.36	55	1	1	57	13.45	7.98	1.09	0.67	60.64	13	2
M1-2 I54 13	4	51	56	2.47	1.25	1.51	0.99	36.36	95	3	0	98	11.81	12.47	0.74	0.29	63.64	19	0

Tab. 1 - Results of the microwear analysis of three teeth of *Equus altidens granatensis* from Barranco Leon 5. Analyses carried out on 27 enamel points of the left upper P3-4 (BL02 31 1692) and on the lingual side of the paracone of M1-2 (specimens BL02 36-1520, right, and BL03 I54 13, left).

Photomicrographs of the dental occlusal surface were taken at the following points (loci; Fig. 2): 1-2 on the parastyle, 3 to 6 on the labial side of the paracone, 7-8 on the metastyle, 9-10 on the labial side of the metacone, 11-13 on the postfossette, 14 to 16 on the prefossette, 17-18 on the lingual side of paraboloph, 19-20 on the lingual side of protocone, 21 to 23 on the pli caballin, 24 to 27 on the lingual side of the hypocone. The results of the analyses are shown in Table 1.

Results obtained have been compared with those resulting from the analysis of microwear patterns, of two other specimens of *E. altidens granatensis* from the same locality, a right (BL02 36-1520) and a left (BL03 I54 13) upper molars (both either M1 or M2 = M1-2). In the present study (because of the preservation of the molar surface), only the locus n° 15 has been analysed. The results are shown in Table 1.

In order to evaluate the consistency of microwear patterns at different points of the enamel on the occlusal surface, as well as to test the significance of differences in pattern found among the examined micro-areas, six independent variables (number of small and large pits; number of short, long coarse and long fine scratches; and number of gouges) were analysed by means of a multivariate analysis test (Sokal & Rohlf, 2001). The analysis was performed using the correspondence analysis (CA) test of the Past software program (Hammer et al., 2001), which is especially appropriate for count data, such as ours.

### 3. MICROWEAR PATTERNS

On the lingual side of the paracone (locus 15), the microwear pattern shows parallel fine scratches, located mainly at the centre of the analysed micro-area (Fig. 3). The inclination with respect to the lingual-labial axis ranges from about 0° to 15°. Small pits and more inclined scratches can be detected on the lateral side of the photomicrograph, but scratches are more abundant than pits on the whole of the examined surface (Tab. 1). Short or very short scratches (scratches with width to length ratio near to 1/4), sometimes not much different from pits, prevail. A few long scratches ( $\geq 250 \mu\text{m}$ ) are present. Some look like they consist of several shorter marks arranged along the same line. Cross scratches are fairly abundant, comprising more than one third of the total number of scratches (24 against 64; Tab. 1).

The microwear patterns of other analysed micro-areas are roughly comparable, but some differences are detectable especially with regards to the scratch (always the most numerous) to pit ratio, which varies from 92.11% on the posterior side of the mesostyle (locus 8), to 64.67% on the labial side of the paracone (locus 3) (Tab. 1). Short (sometimes very short) scratches and small pits predominate. Long scratches are not very abundant. Long coarse scratches are scanty, clearly detectable only on about one third of the examined micro-areas particularly on the postfossette and on some loci of the labial and lingual sides of the tooth (Tab. 1). Gouges, definitely larger than large pits, are rare, with

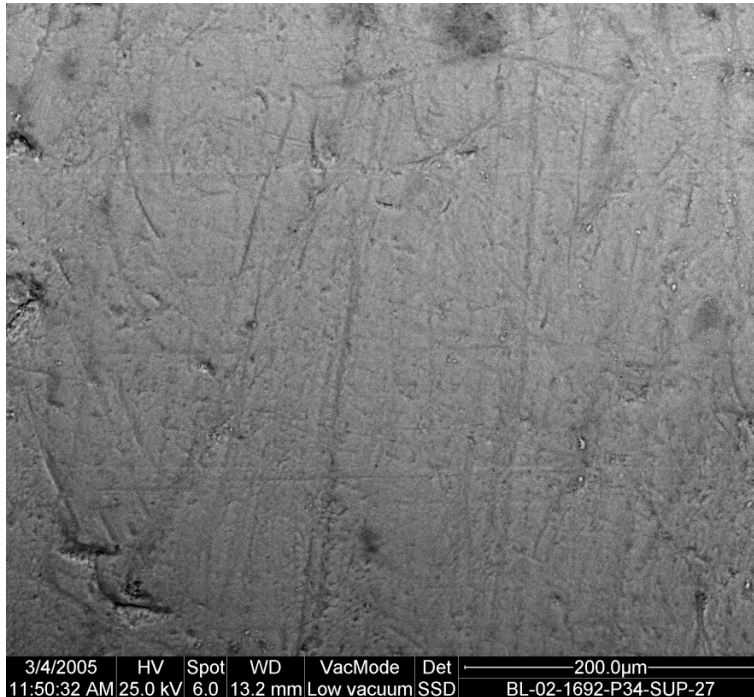


Fig. 3 - Photomicrograph (x500) the point n° 15 (see Fig. 2) of the enamel surface of *Equus altidens granatensis* left upper P3-4 (BL02 31 1692).

only six being found on five points, whilst cross scratches can be locally abundant: their frequency ranges from 52% to 85% (Tab. 1).

#### 4. STATISTICAL ANALYSIS

Results of statistical analysis are shown in Fig. 4 and Fig. 5, where the data are plotted into the new coordinates. The eigenvalues and the variance explained for each eigenvector are given in Table 2.A. The first three eigenvectors explain 89.06% of the variance.

Considering new axes 1 and 2 (Fig. 4), the position of the loci along axis 1 is directly proportionate to the number of small pits and inversely proportionate to the number of gouges, coarse scratches, large pits, fine and short scratches. The position of the loci on axis 2 is directly proportionate to the number of gouges, coarse scratches, large and small pits and inversely proportionate to the number of short and fine scratches. The more gouges, coarse scratches and large pits are counted in one locus the more it is inclined towards the negative values on axis 1 and the positive values on axis 2 (as for the loci 13, 1 and, in lesser proportion, 24). The more fine scratches and fewer small pits are counted (in the absence of gouges and coarse scratches) the more the locus plots along the negative value of the axis 2 (like locus 8). The central area of the 95% confidence ellipse contains two loci on the prefossette (15 and 16), two on the lingual side of paraloph (17 and 18) and one on the lingual side of protocone (20). Very near are positioned loci on the pli caballin (21, 22 and 23), on the lingual side of the hypocone (25), on the prefossette (14, just under locus 15), on the labial side of the metacone (10), and on the lingual side of the hypocone (26). The most peripheral loci from the centre

(but still inside the ellipse) are the locus n° 1 (on the parastyle), the n° 3 (on the labial side of the paracone), the n° 8 (on the metastyle), the n° 9 (on the labial side of the metacone), the n° 11 (on the postfossette) and the n° 27 (on the lingual side of the hypocone). These loci, except n° 1 and n° 3, fall in the posterior half of the tooth. The locus n° 13, on the postfossette, is the only one falling outside the 95% confidence ellipse.

The position of the loci along axis 3 (Fig. 5) is directly proportionate to the number of fine scratches, large pits, coarse scratches and small pits and is inversely proportionate to the number of short scratches. The higher the ratio of "fine scratches/short scratches" the more the locus will fall far within the positive values, like loci 12 (which scores "0.19"), 19 ("0.14"), 20("0.11"), and 21 ("0.11"). The central area of the 95% confidence ellipse contains loci on the labial side of the paracone (5), on the prefossette (15 and 16), on the lingual side of paraloph (17), on the pli caballin (23), on the lingual side of the hypocone (26), and on the parastyle (2). Very near are positioned loci on the parastyle (1), on the metastyle (7), on the labial side of the metacone (9 and 10), on

the pli caballin (22) and on the lingual side of the hypocone (25). The most peripheral loci are n°12 (on the labial side of the metacone), n°19 (on the lingual side of protocone), and n° 8 (on the metastyle). Again, the locus n° 13 is the only one falling outside the 95% confidence ellipse. As in Fig. 4, loci 15, 16 and 17 fall within the central area of the ellipse. The loci on the pli caballin (21-23) even if they do not fall within the centre, lie in proximity to the locus n°15, the classical point chosen in the micro-wear analysis, in both Fig. 4 and Fig. 5. Again, some of the most peripheral points are situated on the posterior half of the tooth (8, 11, 13, 24, but not 9, 10).

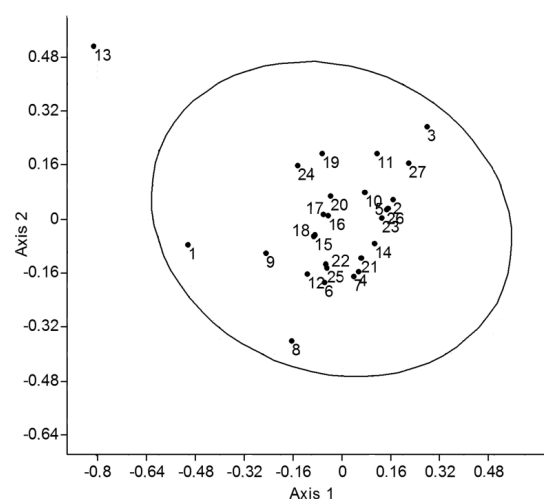


Fig. 4 - Correspondence analysis among 27 points (loci) on a upper P3-4 of *Equus altidens granatensis*. First and second eigenvectors. The 95% confidence ellipse is indicated.

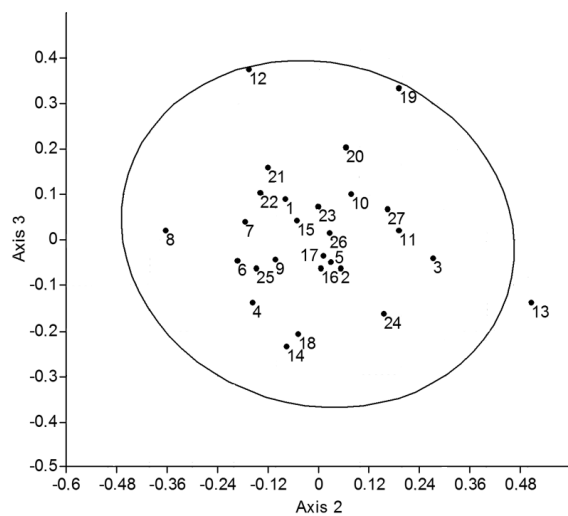


Fig. 5 - Correspondence analysis among 27 points (loci) on a upper P3-4 of *Equus altidens granatensis*. Second and third eigenvectors. The 95% confidence ellipse is indicated.

Adding to the multivariate analysis data from specimens BL02 36-1520 and BL03 I54 13, the eigenvectors change slightly (Tab. 2.B) but without any substantial change in the general pattern of the setting of analysed loci (Fig. 6). In the axis 2 versus axis 3 graphic, the M1-2 loci fall well inside the 95% confidence ellipse, while in the axis 1 versus axis 2 upper M1-2 (36-1520), it is closer to the border of the 95% confidence ellipse (because of the two gouges found on it).

### 5. DISCUSSIONS

Whether the differences in microwear patterns of nearly contiguous enamel areas are related to the intrinsic properties of the food, to the presence of exogenous

grit (see *inter alios* Rivals & Semprebon, 2006; Semprebon & Rivals, 2007, 2010), or to stochastic factors connected to the local resistance to tensile and pressure stress during masticatory processes, is a still unsolved question. As *Equus* from Barranco Leon is concerned, results obtained indicate that the main differences in macrowear patterns (in terms of dental scars among the various areas of enamel on the same tooth) are limited to a few zones of the enamel surface. Nonetheless, considering the quite homogeneous masticatory stress across the tooth, its flat occlusal surface, and the processing of a roughly similar food, it is not easy to understand what is producing the inconsistencies among microwear patterns shown by loci close to each other. Differences depend mainly on the pits/scratches ratio, but also on presence/absence of coarse scratches, large pits and gouges. The latter two scars are believed to be related to feeding habits that include seeds, hard fruits or even roots (Solounias & Semprebon, 2002; Semprebon et al., 2011) but in the present study, a few gouges and/or large pits might be caused by exogenous grit, swallowed together with food, which locally damaged the enamel surface. Indeed, scars herein regarded as “large pits” mainly show a more irregular outline than the outline of the large pits detectable on the teeth of animals whose diet includes seed and hard fruits.

On the other hand, the microwear patterns shown by the lingual enamel of the paracone (locus n°15), the point classically chosen for analysing microwear in ruminants, falls very near the central area of the 95% confidence ellipse. As a result, the lingual enamel band of the paracone, can be considered the best point for analysing microwear in *Equus* representatives in M2 as well as in P3-4, according to the results obtained. In addition, on the latter also the pli caballin could be confidently analysed to infer the dietary behaviour of horses. According to our result, if in a sample of horse teeth to be analysed, the number of available M2 is scanty, it is possible to increase the sample size, adding upper P3-4 (see Fig. 6).

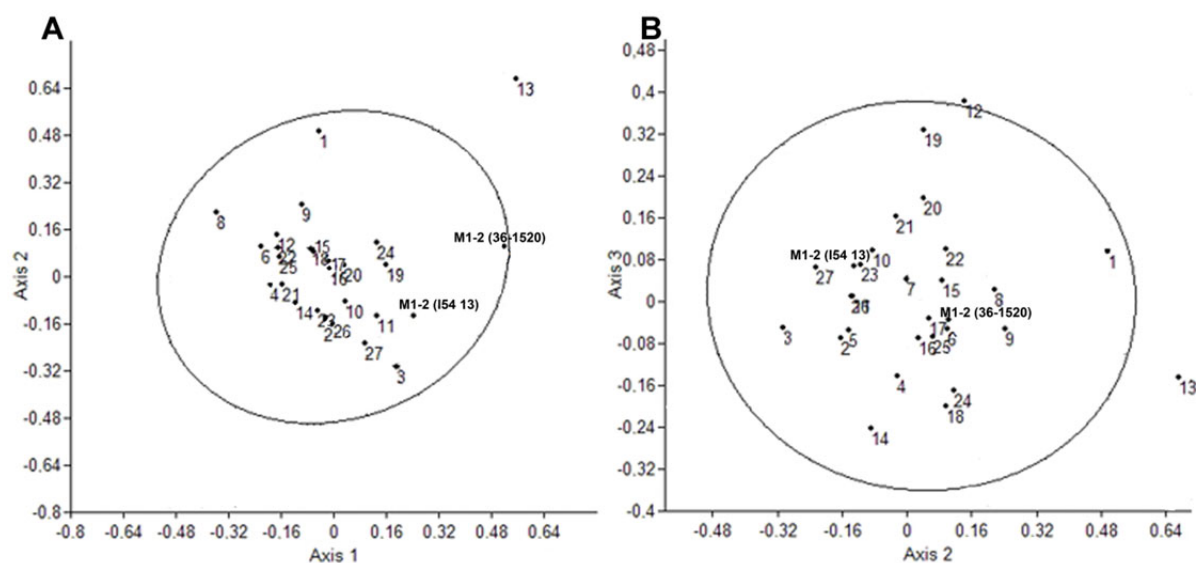


Fig. 6 - Correspondence analysis among 27 points (loci) on a upper P3-4 and 2 upper M1-2 of *Equus altidens granatensis*. A) axis 1 versus axis 2. B) axis 2 versus axis 3. The 95% confidence ellipses are indicated.

A) Eigenvector	Eigenvalue	% Variance	B) Eigenvector	Eigenvalue	% Variance
1	0.03885520	42.144	1	0.03639830	38.289
2	0.02715360	29.452	2	0.03309950	34.819
3	0.01610000	17.463	3	0.01521050	16.000
4	0.00609858	6.615	4	0.00625209	6.577
5	0.00398883	4.326	5	0.00410246	4.315

Tab. 2 - Eigenvectors of the correspondence analysis test (CA), with respective eigenvalues and % variance, for **A)** 27 loci on a upper P3-4 of *Equus altidens granatensis*, **B)** 27 loci on a upper P3-4, and 2 upper M1-2 of *Equus altidens granatensis*.

Our results are also consistent with those obtained by Kaiser T.M. (2002), according to which in hypsodont perissodactyls, like *Equus*, the chewing direction, along the horizontal plane, tends to become exclusively transverse. Indeed, the loci which appear as anomalous with respect to the average pattern shown by the microwear of the *E. altidens granatensis* from Baranco Leon, are mostly located on the posterior part of the tooth (e.g. loci 11 and 13). Despite the reliability of most of the analysed loci with the masticatory stress and movements, some inconsistencies still remain unexplained. For instance, what about loci 24-26, which may possibly fall within the phase II area, albeit they show a pattern different from that of other loci of the same area? Why does locus 3 present a microwear pattern so different from locus 2, which is located on the same enamel band? Could these differences have explained by a different orientation relative to the chewing direction? Finally, how many stochastic factors, such as the ingestion of sediments particles, may affect microwear patterns?

Results obtained, albeit preliminary, on the one hand stress once more the individual variation in food assumption and consumption, as well as the need of large samples and the support of complementary analyses – such as mesowear (Kaiser et al., 2000; Kaiser & Fortelius, 2003) and stable isotopes (e.g., Cerling et al., 1997; Muhlbachler et al., 2011) – to validate inferences drawn by microwear analysis. On the other hand, they indicate the potential efficacy of the additional test herein proposed to better understand the reliability and limits of microwear analysis.

## 6. PALAEODIETARY INTERFERENCES

As far as the diet of the horse from Barranco Leon 5 is concerned, plotting the number of scratches of this horse and extant ruminants (data from Solounias et al., 2000) versus the number of pits, our data forms a main cloud lying between the grazing and the mixed feeding taxa (Fig. 7). A few loci are nested inside the grazers, a few inside the mixed feeders and some are scattered over both groups. Only locus 4 (on the labial edge of the paracone) falls far from the others, because of its large number of scratches (Tab.1). Loci on the prefossette (15 and 16), and on the pli caballin (22 and 23) fall between grazers and mixed feeders, with locus 22 nearest to

the second group. All in all, the pattern of dental microwear shown by *E. altidens granatensis* from Barranco Leon 5, for upper P3-4, is closer to those shown by seasonal mixed feeders than by strict grazers. Adding the two M1-2, one (BL02 36-1520) falls near a grazer taxon, the other (BL03 154 13) in the range of the mixed feeders. These data, in the first approximation, can be considered consistent with those analysed on the P3-4.

In any case, more data are necessary in order to support the hypothesis of any relationship between the increase in moisture between 1.3 and 1.0 My (Blain et al., 2011) pointed out for the localities of Barranco Leon and the dietary behaviour inferred by microwear analysis.

## 7. CONCLUSIONS

Our study is a preliminary work aimed at understanding the consistencies in microwear patterns on the occlusal surface of dental enamel and the use of single locus for palaeodietary analyses. Our data suggest that the differences, at least for horses, are limited to a few zones. In particular, the lingual side of the paracone, the classic locus chosen for microwear analysis in ruminants and equids, falling at the centre of the distribution obtained, considering all the loci on Fig. 2. On our sample, the loci on the pli caballin could also be confidently analysed to infer dietary behaviour. They are all judged consistent with the data obtained on M1-2 from the same locality. Results obtained also suggest that the use of P3-4 can be useful in inferring palaeodietary behaviour when the number of available M2 is scanty.

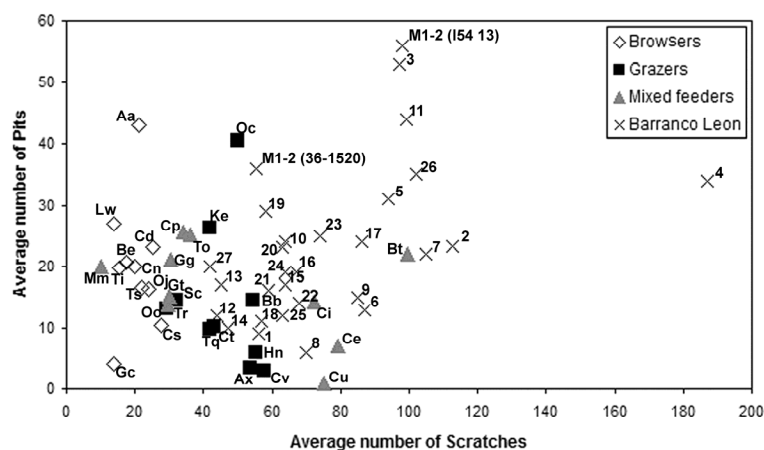


Fig. 7 - Bivariate plot of the average number of scratches versus average number of pits for extant ruminants, mixed feeders, grazers (data from Solounias et al., 2000; Aa *Alces alces*, Lw *Litocranius walleri*, Cd *Cephalophus dorsalis*, Be *Boocercus euryceus*, Ti *Tragelaphus imberbis*, Cn *Cephalophus niger*, Ts *Tragelaphus strepsiceros*, Oj *Okapia johnstoni*, Cs *Cephalophus sylvicultor*, Gc *Giraffa camelopardis*, Cp *Capriicornis sumatraensis*, To *Taurotragus oryx*, Gg *Gazella granti*, Tr *Tragelaphus scriptus*, Gt *Gazella thomsoni*, Mm *Muntiacus muntjak*, Bt *Boselaphus tragocamelus*, Ci *Capra ibex*, Ce *Cervus canadensis*, Cu *Cervus unicolor*, Oc *Ovis canadensis*, Ke *Kobus ellipsiprimnus*, Ct *Connochaetes taurinus*, Tq *Tetraceros quadricornis*, Bb *Bison bison*, Hn *Hippotragus niger*, Ax *Axis axis*, Cv *Cervus duvauceli*, Oo *Ourebia ourebi*, Sc *Syncerus caffer*), left upper P3-4 (BL02 31 1692; 27 loci), right M1-2 (BL02 36-1520) and left M1-2 (BL03 154 13) of *Equus altidens granatensis* from Barranco Leon. Numbers = P3-4 loci.

Thus, our analysis shows that microwear methodology could be extended to other loci and teeth in addition to the classic one considered. Of course, more data and more studies would be necessary in order to further improve the methodology and fully understand its limits.

#### Acknowledgements

We have to thank to Tormo L. (QUANTA200 FEI SEM; Museo Nacional de Ciencias Naturales, Madrid) for her help in analysing samples, Hammer Ø., for his helpful discussions and advices on statistical analysis, and Rivals F. and an anonymous referee for useful comments that contributed to improving the quality of the manuscript. Haire S. revised the English text.

Work supported by the BIODIBERIA project of the European Commission HUMAN POTENTIAL PROGRAMME (2004).

#### REFERENCES

- Alberdi M.T. (2010) - Estudio de los caballos de los yacimientos de Fuente Nueva-3 y Barranco León-5 (Granada). In: Toro I., Martínez-Navarro B. & Agustí J. (Eds.): Ocupaciones humanas en el Pleistoceno inferior y medio de la Cuenca de Guadix-Baza. Arqueología Monografías, Junta de Andalucía, Consejería de Cultura, 6, 291-30.
- Alberdi M.T., Palombo M.R. (2011) - The late Early to early Middle Pleistocene stenooid horses from Italy. *Quaternary International*, doi:10.1016/j.quaint.2011.12.005.
- Blain H.G., Bailon S., Agustí J., Martínez-Navarro B., Toro I. (2011) - Paleoenvironmental and paleoclimatic proxies to the Early Pleistocene hominids of Barranco León D and Fuente Nueva 3 (Granada, Spain) by means of their amphibian and reptile assemblages. *Quaternary International*, 243, 44-53.
- Butler P.M. (1972) - Some functional aspects of molar evolution. *Evolution*, 26, pp. 474-483.
- Cerling T.E., Harris J.M., MacFadden B.J., Leakey M.G., Quadek J., Eisenmann V., Ehleringer J.R. (1997) - Global vegetation change through the Miocene/Pliocene boundary. *Nature*, 389, 153-158.
- Charles C., Jaeger J.-J., Michaux J., Viriot L. (2007) - Dental microwear in relation to changes in the direction of mastication during the evolution of *Myodonta* (Rodentia, Mammalia). *Naturwissenschaften*, 94, 71-75.
- DeMiguel D., Fortelius M., Azanza B., Morales J. (2008) - Ancestral feeding state of ruminants reconsidered: earliest grazing adaptation claims a mixed condition for Cervidae - *BMC Evolutionary Biology*, 8, 1-13.
- Duval M., Falguères C., Bahain J.J., Voinchet P., Grün R., Aubert M., Agustí J., Martínez-Navarro B., Toro I. (2009) - ESR dating of the Lower Pleistocene sites of Orce (Guadix-Baza basin, Andalusia, Spain): Fuente Nueva III, Barranco León and Venta Micena. Abstracts and field trip Guide, 2009 annual meeting SEQS, The Quaternary of southern Spain: a bridge between Africa and the Alpine domain, September 28th-October 3rd, 2009. 24, Orce and Lucena.
- Franz-Odendaal T.A., Solounias N. (2004) - Comparative dietary evaluations of an extinct giraffid (*Siva-therium hendeyi*) (Mammalia, Giraffidae, Sivatheriinae) from Langebaanweg, South Africa (early Pliocene). *Geodiversitas*, 26, 675-685.
- Gordon K.D. (1982) - A Study of Microwear on Chimpanzee Molars: Implications for Dental Microwear Analysis. *American Journal of Physical Anthropology*, 59, 195-215.
- Gordon K.D. (1984) - Hominoid dental microwear: complications in the use of microwear analysis to detect diet. *Journal of Dental Researches*, 63, 1043-1046.
- Hammer Ø., Harper D.A.T., Rayan P.D. (2001) - Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4, 1-9.
- Hayek L.-A., Bernor R.L., Solounias N., Steigerwald P. (1991) - Preliminary studies of hipparionine horse diet as measured by tooth microwear. In: Forsten A. (Ed.): Björn Kurtén memorial Volume. *Annales Zoologici Fennici*, 28, 187-200.
- Hiiemae K.M. (1978) - Mammalian mastication: a review of the activity of the jaw muscles and the movements they produce in chewing. In: Butler P.M. & Josey K. (Eds.): Development, Function and Evolution of Teeth. Academic Press, 359-398.
- Janis C.M. (1979) - Mastication in the hyrax and its relevance to ungulate evolution. *Paleobiology*, 5, 50-59.
- Janis C.M. (1990) - The correlation between diet and dental wear in herbivorous mammals, and its relationship to the determination of the diets in extinct species. In: Boucot, A.J. (Ed.): *Evolutionary Paleobiology of Behavior and Coevolution*. Elsevier Science, 241-259.
- Joomun S.C., Hooker J.J., Collison M.E. (2008) - Dental wear variation and implications for diet: an example from Eocene perissodactyls (Mammalia). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 263, 92-106.
- Kaiser T.M. (2002) - Functional significance of ontogenetic gradients in the enamel ridge pattern of the upper cheek dentition of the Miocene hipparionine horse *Cormohipparion occidentale* (Equidae, Perissodactyla). *Senckenbergiana Lethaea*, 82, 167-180.
- Kaiser T.M., Fortelius M. (2003) - Differential Mesowear in Occluding Upper and Lower Molars: Opening Mesowear Analysis for Lower Molars and Premolars in Hipsodont Horses. *Journal of Morphology*, 258, 67-83.
- Kaiser T.M., Solounias N., Fortelius M., Bernor R.L., Schrenk F. (2000) - Tooth mesowear analysis on *Hippotherium primigenium* from the Vallesian Dinotherien-sande (Germany) - A blind test. *Carolinea*, 58, 104-114.
- Mainland I.L. (1998) - Dental microwear and diet in domestic sheep (*Ovis aries*) and goats (*Capra hircus*): distinguishing grazing and fodder-fed Ovicaprids using a quantitative analytical approach. *Journal of Archaeological Science*, 25, 1259-1271.
- Merceron G., Blondel C., de Bonis L., Koufos G.D., Viriot L. (2005) - A New Method of Dental Microwear Analysis: Application to Extant Primates and *Ouranopithecus macedoniensis* (Late Miocene of Greece). *Palaios*, 20, 551-561.

- Merceron G., Blondel C., Brunet M., Sen S., Solounias N., Viriot L., Heintz E. (2004) - The Late Miocene paleoenvironment of Afghanistan as inferred from dental microwear in artiodactyls. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 207, 143-163.
- Merceron G., Blondel C., Viriot L., Koufos G.D., de Bonis L. (2007) - Dental microwear analysis of bovids from the Vallesian (late Miocene) of Axios Valley in Greece: reconstruction of the habitat of *Ouranopithecus macedoniensis* (Primates, Hominoidea). *Geodiversitas*, 29, 421-433.
- Merceron G., Escarguel G., Angibault J.-M., Verheyden-Tixier H. (2010) - Can dental microwear textures record inter-individual dietary variations? *Plos one*, 5, 1-9.
- Merceron G., Taylor S., Scott R.S., Chaimanee Y., Jaeger J.-J. (2006) - Dietary characterisation of the hominoid *Kharatpithecus* (Miocene of Thailand): evidence from dental topographic and microwear texture analyses. *Naturwissenschaften*, 93, 329-333.
- Merceron G., Ungar P.S. (2005) - Dental microwear and palaeoecology of bovids from the Early Pliocene of Langebaanweg, Western Cape province, South Africa. *South Africa Journal of Science*, 101, 365-370.
- Mihlbachler M.C., Rivals F., Solounias N., Semprebon G.M. (2011) - Dietary Change and Evolution of Horses in North America. *Sciences*, 331, 1178-1181.
- Palombo M.R., Filippi M.L., Iacumin P., Longinelli A., Barbieri M., Maras A. (2005) - Coupling tooth microwear and stable isotope analyses for paleodiet reconstruction: the case study of Late Middle Pleistocene *Elephas* (*Palaeoloxodon*) antiquus teeth from Central Italy (Rome area). *Quaternary International*, 126-128, 153-170.
- Rensberger J.M., Forsten A., Fortelius M. (1984) - Functional evolution of the cheek tooth pattern and chewing direction in Tertiary horses. *Paleobiology*, 10, 439-452.
- Rivals F., Semprebon G.M. (2006) - A comparison of the dietary habits of a large sample of the Pleistocene pronghorn *Stockoceros onusrosagris* from the Papago Springs Cave in Arizona to the modern *Antilocapra americana*. *Journal of Vertebrate Paleontology*, 26, 495-500.
- Rivals F., Schulz E., Kaiser T.M. (2008) - Climate-related dietary diversity of the Ungulate fauna from the middle Pleistocene succession (OIS 14-12) at the Caune de l'Arago (France). *Paleobiology*, 34, 117-127.
- Schubert B.W., Ungar P., Sponheimer M., Reed K.E. (2006) - Microwear evidence for Plio-Pleistocene bovid diets from Makapansgat Limeworks Cave, South Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 241, 301-319.
- Scott R.S., Ungar P.S., Bergstrom T.S., Brown C.A., Childs B.E., Teaford M.F., Walker A. (2006) - Dental microwear texture analysis: technical considerations. *Journal of Human Evolution*, 51, 339-349.
- Schluz E., Calandra I., Kaiser T.M. (2010) - Applying tribology to teeth of hoofed mammals. *Scanning*, 32, 162-182.
- Semprebon G.M., Janis C.M., Solounias N. (2004) - The diets of the Dromomerycidae (Mammalia: Artiodactyla) and their response to Miocene vegetational change. *Journal of Vertebrate Paleontology*, 24, 427-444.
- Semprebon G.M., Rivals F. (2007) - Was grass more prevalent in the pronghorn past? An assessment of the dietary adaptations of Miocene to recent Antilocapridae (Mammalia: Artiodactyla). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 253, 332-347.
- Semprebon G.M., Rivals F. (2010) - Trends in the paleodietary habits of fossil camels from the Tertiary and Quaternary of North America. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 295, 131-145.
- Semprebon G.M., Sise P.J., Coombs M.C. (2011) - Potential bark and fruit browsing as revealed by stereomicroscopic analysis of the peculiar clawed herbivores known as chalicotheres (Perissodactyla, Chalicotherioidea). *Journal of Mammalian Evolution*, 18, 33-55.
- Sokal, R.R., Rohlf, F.J. (2001) - *Biometry*. W.H. Freeman and Company, 3rd ed., 1-887.
- Solounias N., Hayek L.-A. (1993) - New methods of tooth microwear analysis and application to dietary determination of two extinct antelopes. *Journal of Zoology*, 229, 421-445.
- Solounias N., McGraw M.S., Hayek L.-A., Werdelin L. (2000) - The paleodiet of Giraffidae. In: Vrba E.S. & Schaller G.B. (Eds.): *Antelopes, Deer, and Relatives. Fossil record, behavioral ecology, systematics, and conservation*. Yale University Press, 84-95.
- Solounias N., Moelleken S.M.C. (1992) - Tooth microwear analysis of *Eotragus sansaniensis* (Mammalia: Ruminantia) one of the oldest known bovid. *Journal of Vertebrate Paleontology*, 12, 113-121.
- Solounias N., Moelleken S.M.C. (1993) - Tooth microwear and premaxillary shape of an archaic antelope. *Lethaia*, 26, 261-268.
- Solounias N., Semprebon G. (2002) - Advances in the reconstruction of ungulate ecomorphology with application to early fossil Equids. *American Museum Novitates*, 3366, 1-49.
- Solounias N., Teaford M.F., Walker A. (1988) - Interpreting the diet of extinct ruminants; the case of a non-browsing giraffid. *Paleobiology*, 14, 443-457.
- Teaford M.F. (1986) - Dental microwear and diet in two species of *Colobus*. In: Else J., Lee P. (Eds.): *Proceedings of the tenth annual international primate ecological conference. Volume 2: Primate Ecology and Conservation*. Cambridge University Press, 63-66.
- Teaford M.F. (1988) - A review of dental microwear and diet in modern mammals. *Scanning Microscopy*, 2, 1149-1166.
- Toro-Moyano I., de Lumley H., Fajardo B., Barsky D., Cauche D., Celiberti V., Grégoire S., Martínez-Navarro B., Espigares M.P., Ros-Montoya S. (2009) - L'industrie lithique des gisements du Pléistocène inférieur de Barranco León et Fuente Nueva 3 à Orce, Grenade, Espagne. *L'Anthropologie*, 113, 111-124.
- Ungar P.S. (2001) - Microwear software, Version 4.02. A semi-automated image analysis system for the



- quantification of dental microwear. Available on web.
- Ungar P.S., Krueger K.L., Blumenschine R.J., Njau J., Scott R.S. (2011) - Dental microwear texture analysis of hominins recovered by the Olduvai landscape Paleonthology Project, 1995-2007. *Journal of Human Evolution*, doi:10.1016/j.jhevol.2011.04.006.
- Ungar P.S., Scott R.S., Scott J.R., Teaford M. (2008) - Dental microwear analysis: historical perspectives and new approaches. In: Irish J.D. & Nelson G.C. (Eds.): *Technique and Application in Dental Anthropology*. Cambridge University Press, 389-425.
- Valli A.M.F., Palombo M.R. (2008) - Feeding behaviour of middle-size deer from the Upper Pliocene site of Saint-Vallier (France) inferred by morphological and micro/mesowear analysis. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 257, 106-122.
- Young W.G., Marty T.M. (1986) - Wear and microwear on the teeth of a moose (*Alces alces*) population in Manitoba, Canada. *Canadian Journal of Zoology*, 64, 2467-2479.

Ms. received: January 31, 2012  
Final text received: February 21, 2012