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## Introducing 'trident': a graphical interface for discriminating groups using dental microwear texture analysis

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1 **Introducing ‘trident’: a graphical interface**  
2 **for discriminating groups using dental**  
3 **microwear texture analysis**  
4

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18

19 **Abstract**

20

21 This manuscript introduces trident, an R package for performing dental microwear texture  
22 analysis and subsequently classifying variables based on their ability to separate discrete  
23 categories. Dental microwear textures reflect the physical properties of the food, the feeding  
24 ecology of a given species, and niche partitioning when considering multi-specific communities.  
25 The trident package comes with independent functions and a user interface, trident, enabling  
26 easy and fast proficiency. It can import .SUR files, then remove aberrant peaks and possibly  
27 polynomial surfaces. Next, it can measure up to 24 texture parameters and their statistics of  
28 heterogeneity, generating 384 variables. It also ranks any number of variables using five  
29 different methods, displays the results in multivariate analyses, and exports the results into R,  
30 providing access to its large asset of libraries.

31 We then present these features in three case studies, showing how trident helps answer  
32 questions commonly investigated by paleontologists and archaeologists. In the first case study,  
33 we separate four groups of domestic pigs based on their dietary composition. In the second  
34 case study, we identify microwear texture patterns in a large database of 15 primate species  
35 and relate these patterns to biomechanical and ecological factors. The third case study  
36 investigates the dental microwear textures of four extant ruminants to infer the diet of an extinct  
37 antelope from the Pleistocene of Greece. These case studies show how trident can leverage  
38 dental microwear texture analysis results.

39

40 Keywords: Diet inference; DMTA; Multivariate analysis

## 41 **Introduction**

### 42 **Fifty shades of dental microwear**

43 Dental microwear analysis has been a prominent method for investigating the diet of extant and  
44 extinct species during the last 40 years (e.g., Walker et al., 1978; Kay, 1981; Teaford, 1985,  
45 1988; Ungar, 1996; Teaford et al., 1996; Solounias & Semprebon, 2002; Merceron et al., 2005;  
46 Merceron, Blondel, et al., 2005; Scott et al., 2005; Ungar et al., 2008; Rivals et al. 2011). It is  
47 based on the observation that food leaves microscopic wear marks on dental wear facets, and  
48 that those marks fade as the tooth wears, only to be replaced by new microwear marks (Walker  
49 et al., 1978; Gordon, 1982; Teaford & Oyen, 1989; Winkler et al., 2020). The nature (scratches  
50 or pits), size (small to large), and frequency of microwear depend on the food's physical  
51 properties, mostly hardness, and abrasiveness, while their spatial distribution and their  
52 anisotropy are related to chewing motions and food toughness (Teaford, 1988; Scott et al., 2006;  
53 Teaford et al., 2020; Kubo & Fujita, 2021). This allows us to infer an animal's diet during the last  
54 few weeks before its latest meal (Teaford & Oyen, 1989; Winkler et al., 2020). Apart from diet,  
55 the role of exogenous soil mineral particles and environmental conditions in dental microwear  
56 formation should not be minimized (Schulz-Kornas et al., 2019; Schulz-Kornas et al., 2020).  
57 Grit is indeed reportedly harder than enamel tissue and more abrasive than food particles  
58 (Sanson et al., 2007; Lucas et al., 2013). Still, controlled feeding experiments on sheep have  
59 shown that differences in dental microwear texture better reflect diet than the amount of  
60 exogenous particles (Merceron et al., 2016). Their digestive anatomy explains this as ruminants  
61 remove the largest external grit from food while the finest particles mimic organic amorphous  
62 silica (Clauss et al., 2023).

63

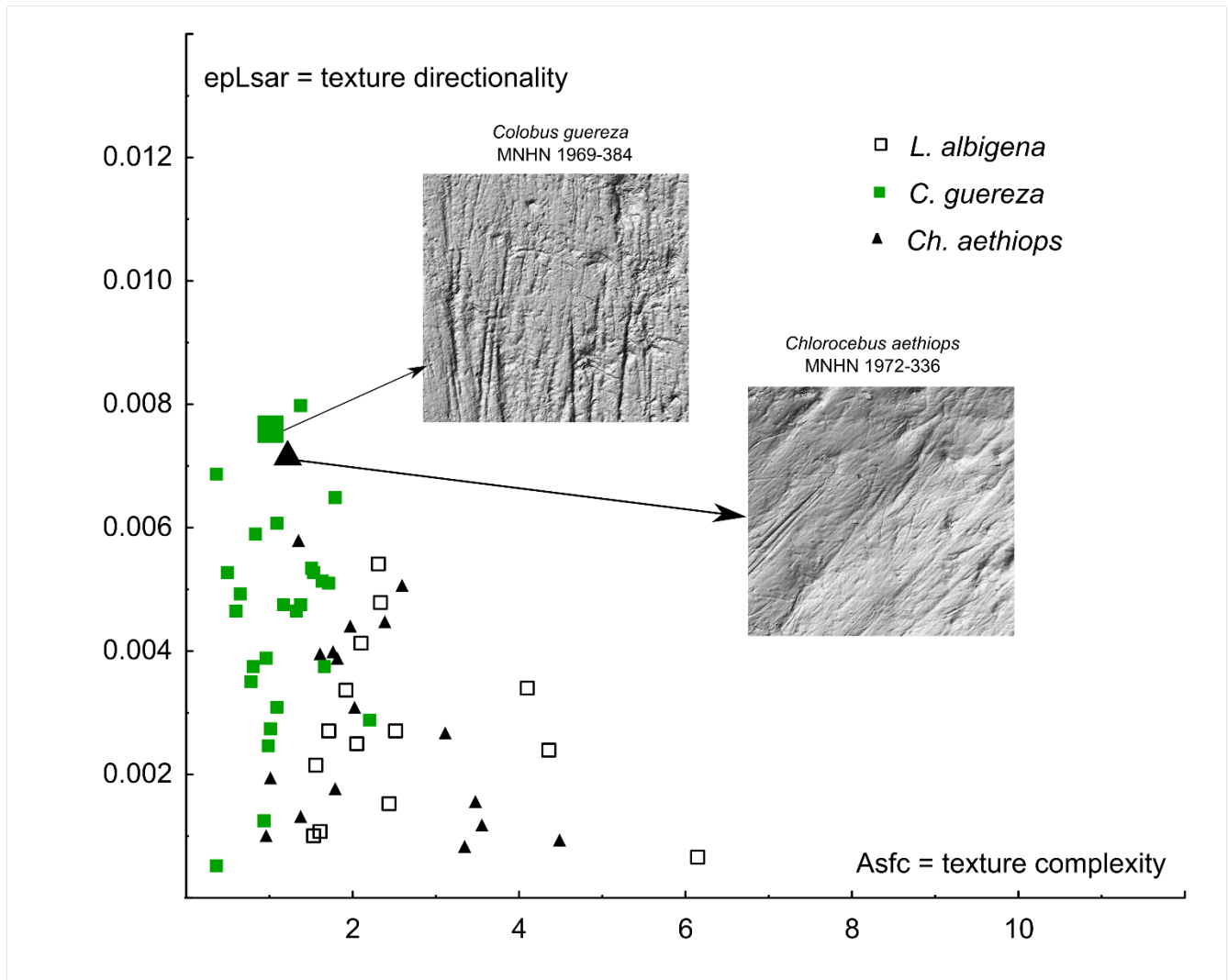
64 Initially, microwear analysis used scanning electronic or light microscopes to count pits and  
65 scratches left by the food on the enamel surface. But despite a proven ability to separate

66 surfaces according to diet, this method is reliant on the tuning of light orientation, the counting  
67 method, the observer experience, etc. (Grine et al., 2002; Galbany et al., 2005; Muhlbachler et  
68 al., 2012). With the advent of high-resolution digitization techniques, new methods for  
69 quantifying the three-dimensional surface texture have emerged. Scale Sensitive Fractal  
70 Analysis (SSFA) uses surface parameters strongly correlated to food material properties, such  
71 as surface scale roughness estimated from area-scale fractal complexity (Asfc) that correlates  
72 to food hardness, or anisotropy (epLsar) that correlates to food toughness (Ungar et al., 2003;  
73 Scott et al., 2005; Hua et al., 2020). A second approach, Surface Texture Analysis (STA)  
74 consists of computing ISO-25178 area parameters (Schulz et al., 2010; Kaiser et al., 2015).  
75 Both SSFA and STA methods belong to the larger field of dental microwear texture analysis  
76 (DMTA). They have been used to investigate the diet of animals from a broad range of species,  
77 including insectivorous mammals (Purnell et al., 2013) but also selachians (Weber et al., 2021),  
78 lepidosaurians (Winkler et al., 2019) or dinosaurs (Sakaki et al., 2022; Winkler et al. 2022).

### 79 **New methods, new challenges**

80 Despite their reliability, DMTA approaches are facing several methodological challenges. First,  
81 they sometimes fail to separate specimens that an expert eye could visually tell apart using the  
82 number of specific microwear marks (Fig. 1). This can be mitigated by using filters to enhance  
83 the microwear versus the raw shape of the tooth surface, for instance by removing the 2<sup>nd</sup> or  
84 8<sup>th</sup> order polynomial of the surface using least square approximation (Francisco et al., 2018a,  
85 2018b). Failure to tell surfaces apart can also come from distinct structures concealed in the  
86 average signal. One of the SSFA parameters can solve this issue by estimating heterogeneity  
87 of complexity (HAsfc, Scott et al., 2005, 2006). An alternative approach is to consider a grid of  
88 standard number and size of cells and to measure every parameter for each cell: the presence

89 of distinct structures would increase the dispersion of values, and could be detected using  
90 percentiles, minimal or maximal values, etc. (Francisco et al., 2018a).



91

92 Figure 1. Biplot showing variations of dental microwear texture anisotropy (epLsar) and complexity (Asfc) on  
93 crushing molar facets of wild-caught specimens belonging to three species of extant monkeys: the leaf-eating  
94 colobine *Colobus guereza*, the fruit/seed-eating mangabey *Lophocebus albigena*, and the opportunistic vervets  
95 *Chlorocebus aethiops*. There are quite notable overlaps between specific ranges. This is due to several specimens  
96 for which although an expert eye can visually differentiate surfaces (200 × 200 μm), dental microwear texture  
97 analysis cannot. For example, experts will count more wide scratches on the leaf-eating colobine (MNHN 1969-  
98 384) than on the opportunistic vervets (MNHN 1972-336). In contrast, DMTA fails to discriminate using both  
99 anisotropy (epLsar) and complexity (Asfc). Using trident and specifically the subsampling method to explore  
100 heterogeneity for all variables solves the issue.

101

102 Computing heterogeneity of DMTA variables helps track the most elusive structures, but it tends  
103 to generate a “jungle of parameters” (Francisco et al., 2018b), that is, too many variables to  
104 keep track of a given phenomenon. One way to bypass this issue is to compare the variables’

105 ability to discriminate groups, for instance using analyses of variance (ANOVAs) to find which  
106 groups can be separated using post-hoc analysis such as Tukey's HSD or Fisher's LSD. This  
107 analysis pipeline is very efficient and could separate animals according to their diet in several  
108 studies (Francisco et al., 2018a, 2018b; Louail et al., 2021; Merceron et al., 2021b).

109 Yet two more limitations can be identified. The first one concerns the repeatability of measures,  
110 as the code was not accessible in previous works. The second limitation is the ease of use  
111 since the pipeline made use of a combination of Fortran, Python, and R to collect the data.  
112 Consequently, fine-tuning the nature of data collection and analysis was only possible for  
113 people familiar with those languages. An open-source graphical user interface would solve both  
114 issues and make measuring, analyzing, and untangling DMTA data easier, faster, and more  
115 intuitive for beginners.

116 Here, we introduce trident, a graphical user interface, and its associated R source package  
117 trident for measuring dental microwear textures and analyzing the discriminant ability of DMTA  
118 variables. It can load .SUR files, remove abnormal peaks and measure 16 variables from 24  
119 DMTA parameters (for a total of 384 variables) on batches. The computed DMTA variables  
120 (along with variables possibly added by the user on the source .txt file) can be classified  
121 according to their ability to discriminate discrete categories such as species, diet, and any other  
122 categorical variable. trident also comes with tools to perform univariate, bivariate, and  
123 multivariate analysis. All the functions can be performed from the user interface. It is worth  
124 noting that trident is free of access and the code is available to the scientific community.

125 We then showcase its functionalities using three case studies, representative of research  
126 questions that could be answered using dental microwear analysis:

127 Case study A. Diet-related differences in dental microwear: The first case study is based  
128 on a controlled feeding experiment involving a single omnivorous species (*Sus*  
129 *domesticus*). Similarly to Louail et al. (2021), animals participated in trials only differing

130 in the dietary composition of their daily ration. The influence of diet on DMTA was then  
131 quantified using trident.

132 Case study B. Meta-analysis of a large multi-species sample: The second case study is  
133 based on a large sample grouping 260 specimens from 15 species of cercopithecoid  
134 primates of Asia and Africa. We used trident to detect patterns related to species, their  
135 tribe (Cercopithecini, Colobini, Papionini, and Presbytini), or their general diet according  
136 to the literature.

137 Case study C. Comparison with extant species to infer the diet of extinct species: The  
138 third case study is based on four sympatric species of ruminants from the Bauges Natural  
139 Regional Park, French Alps. In a previous study, SSFA variables could find differences  
140 between species, reflecting differences in dietary behavior and spatial use (Merceron et  
141 al., 2021a). We used trident to explore the dental microwear textures of this community  
142 and then used the most discriminating variables to make inferences on the diet of  
143 *Gazellospira torticornis*, an extinct antelope from Greece (Hermier et al., 2020).

## 144 **Material and Methods**

### 145 **Material**

#### 146 ***Case study A: Diet-related differences in dental microwear***

147 The first case study is based on three of the feeding trials with domestic pigs (*Sus domesticus*)  
148 detailed in Louail et al. (2021), to which was added another trial. The control group (N = 5) was  
149 fed exclusively with a base diet composed of ground cereal and soy seeds. The three other  
150 groups were also fed this base diet, with a supplement depending on their group:

- 151 • The *barley group* (N = 5) was fed 30 % of barley seeds.
- 152 • The *corn kernel* group (N = 5) was 20 % of corn (*Zea mays*) flour, supplemented with  
153 20 % (as dry matter weight) of corn kernels.



154 • The *corn silage* group (N = 5) was fed 100 % of the base diet but had access to corn  
155 silage at will.

156 We analyzed the deciduous upper fourth premolars of pigs aged between 6.5 and 9.5 months.

157 See Louail et al. (2021) for more details on the experiment.

### 158 ***Case study B: Meta-analysis of a large multi-species sample***

159 The second case study is based on skulls and jaws of extant cercopithecids from osteological  
160 collections of Europe, Asia, and Africa (for a detailed listing of institutions, see Supplementary  
161 Materials 1). A total of 260 casts of upper and lower second molars from 15 extant species were  
162 obtained as detailed in previous studies (Merceron et al., 2021b; Thiery et al., 2021). Each tribe  
163 of extant cercopithecids (Cercopithecini, Colobini, Papionini, and Presbytini) is represented by  
164 at least 2 species (Supplementary Materials 1). Overall, the selected taxa encompass a broad  
165 range of diets, from a large geographic range (see Rowe et al., 1996 and citations therein).

### 166 ***Case study C Comparison with extant species to infer the diet of extinct species***

167 The Bauges Natural Regional Park is a typical subalpine massif located in the French Alps. In  
168 the third case study, four extant ruminants from the Bauges have been investigated: *Cervus*  
169 *elaphus*, a mixed-feeding species; *Capreolus capreolus*, a selective browser; *Ovis gmelini*  
170 *musimon*, and *Rupicapra rupicapra*, two bovid species known to be mixed feeders. Mandibles  
171 were collected at the same locality, during a short period (for more details, see Merceron et al.,  
172 2021a), representing a hypothetical fossil assemblage composed of different species occupying  
173 different small-scale habitats (open alpine grassland, bushland, shrubland, deciduous, mixed,  
174 coniferous forests) in a common geographical range.

175 These four extant species were then compared to the extinct antelope *Gazellospira torticornis*  
176 (Bovidae), from the Early Pleistocene of Greece. Specimens come from the site of Dafnero and  
177 have been described by Hermier et al. (2020).

## 178 **Surface acquisition**

179 Each tooth surface was cleaned and molded as described in previous works (Louail et al., 2021;  
180 Merceron et al., 2021a; Merceron et al., 2021b) and on the TRIDENT website ([http://anr-  
181 trident.prd.fr/v/](http://anr-trident.prd.fr/v/)). For case study A, we investigated both the shearing (phase I) and crushing  
182 (phase II) dental facet of the very same tooth, whereas we focused on crushing facets for case  
183 study B (primates) and on shearing facets for case study C (ruminants). Each facet was  
184 scanned separately using a white-light confocal profilometer Leica DCM8, named “TRIDENT”,  
185 with a 100× objective housed at the PALEVOPRIM lab, CNRS and University of Poitiers, France  
186 (Leica Microsystems). All surfaces analyzed in the current study were pre-processed with  
187 LeicaMap (v. 8.2; Leica Microsystems) following Merceron et al. (2016). The procedure resulted  
188 in the obtention of .SUR files (saved as SUR version 7.2 or older), which were then imported  
189 into trident. It is worth noting that alternative free-of-access software, such as Gwyddion  
190 (<http://gwyddion.net/>) could be used to generate similar pre-treatments.

## 191 **DMTA with *trident***

### 192 ***Presentation of trident***

193 Here we introduce the R package trident, which is devoted to measuring microwear textures  
194 and classifying variables according to their discriminant power. It was implemented on two  
195 levels:

196 (1) Functions that can be launched from the R console, for which detailed instructions can  
197 be found in the metadata and the help files of the package.

198 (2) A shiny app named trident, which is launched from the console using the line  
199 `trident.app()`. The app is a wrapper for the package functions, connecting them to  
200 other packages for statistical analyses, multivariate analyses, or graphical rendering.

201 Below are summarized the functionalities of the interface used in the three case studies. The  
202 reader can find a more detailed description of the interface in the user manual, provided as  
203 supplementary materials (Supplementary Materials 2).

204 Beside R (R Core Team, 2021), trident was built using the following dependencies: car  
205 (Weisberg & Fox, 2011), DescTools (Signorell et al., 2024), doSNOW (Daniel et al., 2022a),  
206 dplyr (Wickham et al., 2023a), DT (Xie et al., 2024), factoextra (Kassambara & Mundt, 2020),  
207 FactoMineR (Lê et al., 2008), foreach (Daniel et al., 2022b), ggpubr (Kassambara, 2023),  
208 ggplot2 (Wickham et al., 2023b), MASS (Venables & Ripley, 2002), nortest (Gross and Ligges,  
209 2015), plyr (Wickham, 2011), shiny (Chang et al., 2024), shinyjs (Attali, 2021), shinyFiles  
210 (Pedersen et al., 2022), snow (Tierney et al., 2021) and stringr (Wickham, 2023)

### 211 ***Dental microwear texture analysis (DMTA)***

212 Surfaces were first enhanced using the polynomial removal procedure; all procedures  
213 mentioned below are detailed in Francisco, Brunetière et al. (2018). The primary surface S1  
214 was first numerically and automatically cleaned of any abnormal peaks. Then, considering the  
215 large-scale tooth surface geometry as an 8th-order polynomial (PS8), the latter was subtracted  
216 via a least square approximation. This procedure was performed on surfaces of the same size,  
217 as subtracting PS8 from smaller surfaces would remove larger amounts of relief. The software  
218 also allows to remove of the 2<sup>nd</sup> order polynomial (PS2).

219 Afterward, DMTA variables were computed. The program can currently compute four families  
220 of parameters (Table 1). The first one is complexity i.e., an estimation of the density of  
221 microwear textures across scales. The second family is height, or parameters describing the  
222 average height, its dispersion, and its variation over the surface whatever the location. The third  
223 family is spatial parameters, which describe the distribution and nature of the textures. The last  
224 family is topology, a combination of height and spatial parameters, measuring the proportion of

225 the surface above or below determined heights. A comparison of the height parameter values  
226 found for trident and the DigitalSurf Mountains software (here the Mountains-derived software  
227 LeicaMAP is used) is provided in the supplementary information  
228 (supplementaryMaterial\_Software comparisons.txt).

229

230 Table 1. DMTA parameters measured in trident

Parameter	Family	Significance	Description
Asfc2	Complexity	Area scale fractal complexity (#)	Asfc2 estimates roughness through scale-sensitive fractal analysis. Asfc2 would be low for smooth dental surfaces of leaf-eating colobine monkeys and high for rough surfaces frequent in hard seed-eating cercopithecine monkeys.
Sdar	Complexity	Relative area (developed area/projected area -1)	Sdar is higher for rough surfaces frequent in hard seed-eating cercopithecine monkeys than for smooth dental surfaces (of leaf-eating colobine monkeys; Sdar = 0 for a perfect horizontal plane surface)
Sa	Height	Arithmetic mean of the absolute of the heights (*)	Sa assesses surface roughness with low values for smooth dental surfaces of leaf-eating colobine monkeys and higher ones for rough surfaces frequent in hard seed-eating cercopithecine monkeys.
Sp	Height	Absolute of the largest height (*)	Sp is the height of the highest peaks, which is expected to be higher for rough surfaces found in species eating hard and brittle food items.
Sq	Height	Height standard deviation (*)	Sq is also expected to be higher for rough surfaces than smooth ones, such as the ones found for leaf-eating colobine monkeys.
Sv	Height	Absolute of the smallest height (*)	Sv is the height of the deepest pit, which is still expected to be higher for rough surfaces frequent in species eating hard and brittle food items.
Ssk	Height	Height skewness (*)	Ssk tends to be more negative for a surface with few deep pits as expected on dental surfaces of soft foliage and closer to 0 when a rough surface has many peaks and pits found in species eating hard and brittle food items.
Sku	Height	Height kurtosis (*)	Sku is expected to be higher (>>3) when the surface is smooth with few deep pits or/and high peaks, and close to 3 when peaks and pits have a wider range of height values around the mean
Sm	Height	Mean height (0 for the whole surface, but non-zero for its samples)	Useful when associated with statistics. It tells how the subsurface heights are distributed. Differences are expected with stepped surfaces
Smd	Height	Median height	Useful when associated with statistics. It tells how the subsurface heights are distributed.
Rmax	Spatial	Semi-major axis of the fACF ellipsis (**)	Rmax is higher for surfaces with long and parallel scratches usually found on molar facets of mammals avoiding hard and brittle foods that would require orthogonal crushing motion
Sal	Spatial	Semi-minor axis of the fACF ellipsis (**)	Sal is higher for wide and parallel scratches
Stri(*) = Str <sup>-1</sup>	Spatial	Rmax/Sal ratio (**)	Stri is high when there are numerous and long scratches.
b.sl	Spatial	Highest slope of fACF (**) at the distance rs from the origin <sup>β</sup>	Another way of quantifying the anisotropy. Highly correlated to Sal.
r.sl	Spatial	b.sl/s.sl ratio (**)	Another way of quantifying the anisotropy. Highly correlated to Stri.
s.sl	Spatial	Smallest slope of fACF (**) at the distance rs <sup>β</sup> from the origin	Another way of quantifying the anisotropy. Highly correlated to Rmax.
Std <sup>EX</sup>	Spatial	Texture direction	Std provides main orientation direction. Useful when combined with statistics, it shows how the direction of texture changes from one part of the surface to another.
Sk1, Sk2	Topology	Relative area of the surface above h1 <sup>ββ</sup> and h2 <sup>ββ</sup> respectively	Sk1 and Sk2 are higher for surfaces with many high heights.
Smc1, Smc2	Topology	Median relative area of the cells with heights exceeding h1 <sup>ββ</sup> and h2 <sup>ββ</sup> respectively	Smc1, Smc2 are higher for surfaces with many high heights arranged in large peaks.
Snb1, Snb2	Topology	Number of cells with heights exceeding h1 <sup>ββ</sup> and h2 <sup>ββ</sup> respectively	Snb1, Snb2 are higher for surfaces with many high heights arranged in thin peaks.
Sh	Topology	Percentage of quasi-horizontal faces (normal within a 4° cone)	Sh is low for rough surfaces and high for flat surfaces

231 <sup>EX</sup>, parameters available in trident, but excluded from the three case studies; \* ISO 25178; \*\* autocorrelation  
 232 function at z=0.5 (Francisco et al., 2018a; 2018b); <sup>β</sup> maximum slope radius; <sup>ββ</sup> h1 = 85% of total height (Sv+Sp)  
 233 and h2 = 95% of total height (Sv+Sp); #, Area Scale Fractal Complexity is labeled Asfc2 because its calculation  
 234 mode slightly differs from the Asfc computed in Scott et al., 2006).

235 Lastly, we estimated the heterogeneity of complexity, height, spatial, and topology variables.  
236 The heterogeneity of a (dental) surface is related to the spatial distribution of its features: for  
237 instance, a single pit in the enamel implies more heterogeneity than several pits uniformly  
238 distributed through the enamel surface (Scott et al., 2006). Following Francisco, Brunetière et  
239 al. (2018), trident uses a fast and intuitive approach for estimating heterogeneity: the surface is  
240 divided into  $n$  grid cells, and DMTA parameters are computed for each grid cell. Then, the  
241 distribution of DMTA parameters across grid cells is used to compute heterogeneity variables  
242 e.g., mean  $Asfc_2$ , maximal  $Asfc_2$ , or 25<sup>th</sup> percentile of  $Asfc_2$  (Table 2). Note that this way of  
243 assessing heterogeneity differs from SSFA parameters such as  $HAsfc$  (Box 1). In the end, a  
244 total of 384 variables can be computed, giving a highly detailed description of dental microwear  
245 textures. Out of these, 24 variables correspond to the 24 parameters from Table 1 measured  
246 on the whole surface, but the remaining 360 are estimates of surface heterogeneity (Table 2).

247 trident does not allow to compute SSFA variables. Still, the user can find equivalents  
248 among the 384 variables available in trident. For instance,  $Asfc$  can be approximated from  $Asfc_2$ ,  
249 which also strongly correlates with  $Sdar$ .  $EpLsar$ , which is an SSFA estimate of anisotropy, is  
250 related to spatial variables in trident. It is especially correlated with  $Rmax$ ,  $Sal$ , and  $Stri$  ( $Stri-1$ ),  
251 which are calculated with  $s = 0.5$ ; value adapted for enamel wear surface (Francisco et al.  
252 2018a, 2018b). Note that trident can open and manage any other variables (e.g., SSFA, furrows,  
253  $\delta^{13}C$  ...) that a user might want to add to the source .txt file. This way, it is easy to compare  
254 trident's variables with other parameters.

255 In each of the three case studies, all variables except minimal and maximal values were  
256 calculated on 23 parameters ( $Std$  was excluded because it is scanning orientation-dependent),  
257 for a total of 322 variables: this avoids measuring the effect of a single feature. To assess the  
258 heterogeneity, the resampling statistics were calculated for a grid of 256 cells ( $16 \times 16$ )

259 measuring 33 × 33 μm each (256 × 256 pixels) from the original surface measuring 200 × 200  
260 μm (1551 × 1551 pixels).

261

262 Table 2. Heterogeneity variables.

---

Statistics	Description
min <sup>EX</sup>	Minimal value
max <sup>EX</sup>	Maximal value
sd	Standard deviation
mean	Arithmetic mean
med	Median
fst.05	5 <sup>th</sup> percentile
lst.05	95 <sup>th</sup> percentile
min.05	Mean of values under the 5 <sup>th</sup> percentile
max.05	Mean of values above the 95 <sup>th</sup> percentile
fst.25	1 <sup>st</sup> quartile
lst.25	3 <sup>rd</sup> quartile
min.25	Mean of values under the 1 <sup>st</sup> quartile
max.25	Mean of values above the 3 <sup>rd</sup> quartile
skw	Skewness of the histogram of distribution
kurt	Kurtosis of the histogram of the distribution

---

263 <sup>EX</sup>, variables excluded from the analysis.

#### 264 **Multichecks**

265 The second most important part of trident relates to the classification of variables according to  
266 their ability to separate informed categories, such as diet, species, etc. In case study A, the

267 factor is diet whereas in case study B and C, the factor is species. The software proposes  
268 functions for adding a factor variable by combining variables from different datasets, by entering  
269 it manually or automatically (see Supplementary Materials 2).

270 Afterward, the discriminant ability of variables is calculated using a pipeline of analysis initially  
271 designed by Francisco and colleagues (Francisco, Blondel, et al., 2018; Francisco, Brunetière,  
272 et al., 2018):

273 (a) Normality: the normality of the data is tested using the Shapiro-Wilk test. If unsuccessful,  
274 we compute the skewness ratio, computed as the skewness divided by its confidence  
275 interval. If the skewness ratio is inferior to 2, the distribution is considered nearly normal.

276 (b) Homoscedasticity: for normally distributed data, the homogeneity of variances is tested  
277 using the Bartlett test. If the test fails, the groups are still nearly homoscedastic if the  
278 variance ratio, computed as the maximum variance divided by the minimum variance for  
279 each group, is lower than 3. For nearly normal data, Levene's test is performed to check  
280 the group variance homogeneity.

281 (c) Ability to separate categories: If both normality and homoscedasticity assumptions are  
282 respected, or if no more than one condition is nearly respected, then the discriminant  
283 ability of variables is tested using an ANOVA. Otherwise, the discriminant ability of  
284 variables is tested using a non-parametric test, the Kruskal-Wallis test.

285 All these tests are implemented with a default alpha of 0.05. They have been grouped in a 'multi  
286 check' function (Supplementary Materials 2). If data are not normally distributed, they can also  
287 be transformed using either a base 10 logarithm function or a Box-Cox transformation  
288 (Supplementary Materials 2).

### 289 ***Classification of variables***

290 We implemented five different classification methods, which can be used depending on the  
291 situation:



- 292 (1) Rank based on ANOVA: Performs an ANOVA and arranges variables by ascending p-  
293 value. The purpose here is to separate discriminant from non-discriminant variables. To  
294 make things easier for users, variables are ranked by the p values of the ANOVA. One  
295 could be interested to have a first glance at the discriminant variables and most  
296 discriminating ones whatever the number of groups involved in the study.
- 297 (2) Rank based on Kruskal-Wallis: Performs a Kruskal-Wallis rank-sum test and arranges  
298 variables by ascending p-value; in case the sample is far from normality or in case of  
299 small samples. This way of ranking is equivalent to the first mode but adapted for  
300 variables whose distribution does not respect conditions required for parametric tests,  
301 meaning that variables are ranked by the p values of the Kruskal Wallis analysis.
- 302 (3) Rank based on post-hoc (average): Performs an ANOVA and arranges by decreasing  
303 the number of significant p-values per pair and then ascending mean p-value from post-  
304 hoc tests. The mean p-value can be either arithmetic or geometric, and the user can  
305 choose to use only significant p-values to calculate it (this is the default option). This  
306 mode allows the users to go further in classifying discriminant variables. The aim is to  
307 target the variables that discriminate the highest number of groups. Then, among  
308 variables discriminating a same number of groups, we ordered them by the increasing  
309 values of the mean of the significant p-values of the post hoc tests (here we have chosen  
310 the Tukey test). For instance, with 4 discriminant groups among 7, the mean of the 6 p  
311 values of the 6 pairs of significantly different samples is computed for each discriminant  
312 variable. They are then ordered. One could then run a PCA on trident or export the  
313 dataset with only variables showing differences on the six or five pairs.
- 314 (4) Rank based on post-hoc (pairwise): Performs an ANOVA and then for a given pair,  
315 arranges variables by ascending pairwise post-hoc p-value. This mode of classification  
316 is similar to the former one. However, it differs in targeting variables that discriminate

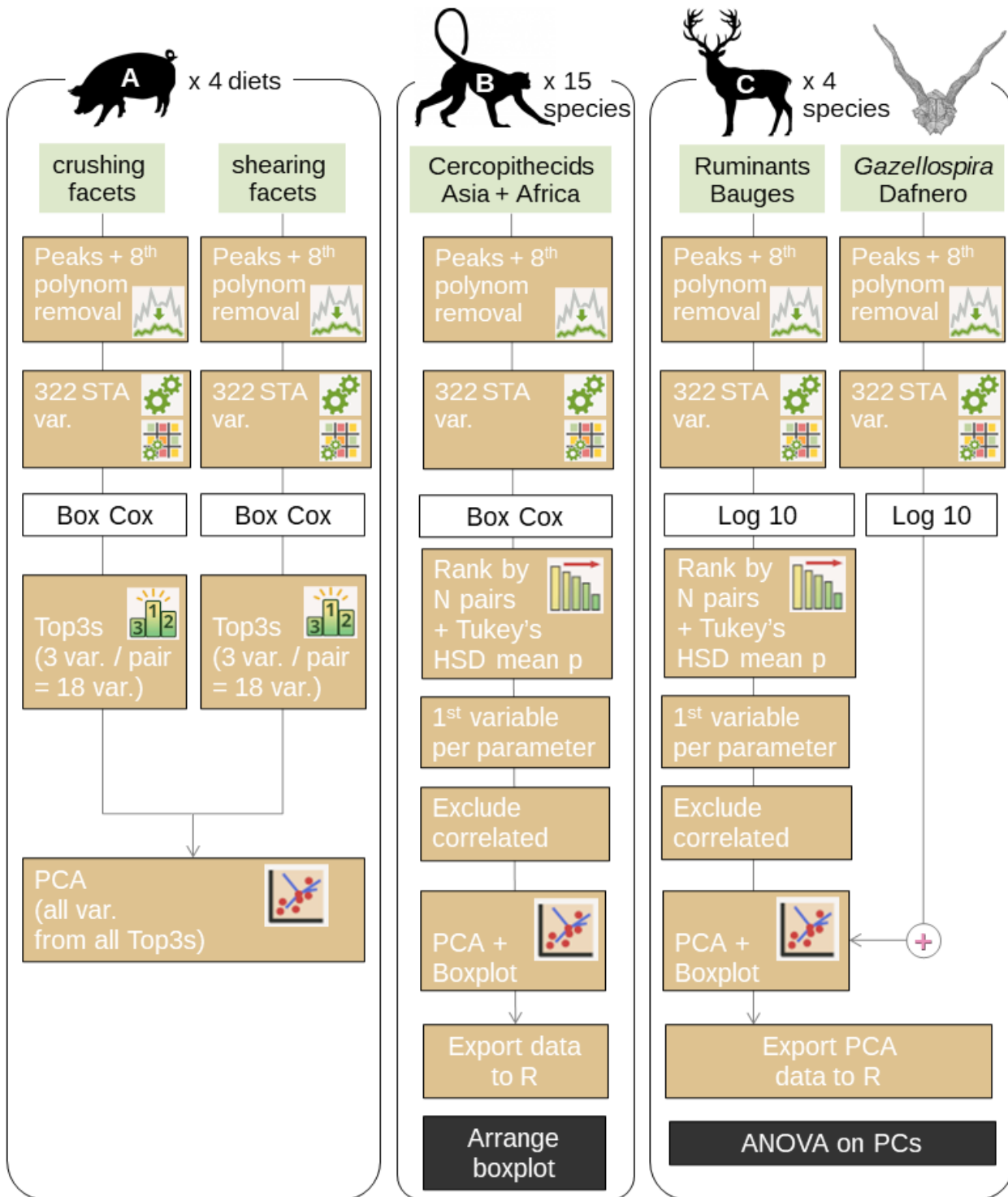
317 groups of interest. For instance, it can be used for a study including several species for  
318 which two of them overlap when considering traditional dental microwear textures  
319 although differences were expected because these two species have different feeding  
320 preferences. In such a case, trident can select the variables that discriminate the two  
321 species at priority.

322 (5) Top 3: For each pair of categories, arranged by the number of significant pairwise p-  
323 values from Tukey's HSD, then by the mean of significant p-values. The function returns  
324 the 3 best-classified variables. Because this function makes a new classification for each  
325 pair of groups, computation time and length of results increase exponentially as the  
326 number of categories goes up. Although it remains possible to use this function on any  
327 number of categories, we do not recommend using this approach for more than 5  
328 categories (10 pairs). In most cases when comparing a few groups, three parameters  
329 appear to be the easiest and fastest way to find out which and how variables discriminate  
330 the groups.

331

332 Note that all these tests are implemented with a default alpha of 0.05. Regardless of the chosen  
333 workflow, it is possible to visualize variables using boxplots and violin plots. It is also possible  
334 to perform a principal component analysis (PCA) on a selection of variables. It provides a  
335 histogram of the percentage of variance explained by each principal component (scree plot),  
336 as well as buttons for saving and exporting the PCA results, a bivariate diagram of the selected  
337 principal components, and circles of correlations. Graphics can be saved as images.

338 **Case-specific analysis**



339

340 Figure 2. Flowchart depicting the analysis for the three case studies; var. = variables; black boxes refer to step  
 341 done on R without the use of the graphic interface.

342 ***Case study A: Diet-related differences in dental microwear between controlled-fed pigs.***

343 For each wear facet (crushing and shearing), data were analyzed separately. They were Box-  
344 Cox transformed, then checked for normality, homoscedasticity, and their ability to discriminate  
345 categories (Fig. 2A). Variables that passed the multi check were classified using the mean of  
346 the significant p-values from Tukey's HSD post-hoc analysis of an ANOVA with diet as factor.  
347 They were ranked according to (1) the number of groups discriminated and (2) the arithmetic  
348 mean of Tukey's HSD discriminant p-values (the p-value of non-discriminated groups were  
349 ignored for calculating this arithmetic mean). Among these variables, we retained only the 3  
350 best-ranked variables (Top3). Afterward, all the retained variables for both crushing and  
351 shearing facets were combined. We performed a Principal Component Analysis (PCA) to  
352 explore their influence on data distribution. All analyses were done exclusively in trident.

353 ***Case study B: Meta-analysis of a large multi-species sample of cercopithecids.***

354 Data were Box-Cox transformed, then checked for normality, homoscedasticity, and their ability  
355 to discriminate categories (Fig. 2B). Variables that passed the checking step were classified  
356 using the results of a post-hoc analysis of an ANOVA with species as a factor. They were ranked  
357 according to (1) the number of groups discriminated and (2) the geometric mean of Tukey's  
358 HSD discriminant p-values (the p-value of non-discriminated groups were ignored for  
359 calculating this geometric mean). Then, for each parameter (e.g., Asfc2), the best-ranked  
360 variable out of 14 (central + heterogeneity statistics, see Table 2) was selected. Afterward, we  
361 selected parameters with a correlation of Pearson below 0.70: variables correlated to more than  
362 70 % with a better-ranked variable were systematically removed (calculated independently with  
363 R). The remaining variables were used for a PCA. All analyses were done in trident, but the  
364 boxplots of the first two principal components were modified for the purposes of this article in  
365 R using the ggplot2 package (Wickham et al., 2021).

366 **Case study C: Comparison with extant species to infer the diet of extinct ruminants**

367 First, the Bauges data were log-transformed (base 10), then checked for normality,  
368 homoscedasticity and their ability to discriminate species (Fig. 2C). Variables which passed the  
369 checking step were classified using (1) the number of groups discriminated by the post-hoc  
370 analysis of an ANOVA and (2) the geometric mean of Tukey's HSD discriminant p-values (the  
371 p-value of non-discriminated groups were ignored for calculating this geometric mean). Just  
372 like case study B, for each parameter (e.g., Asfc2, Sa), the best-ranked variable out of 14  
373 (central + heterogeneity statistics) was selected, and variables correlated to more than 70 %  
374 with a better-ranked variable were systematically removed (calculated independently with R).  
375 The remaining variables were used for a PCA. At this point, the surfaces of *Gazellospira*  
376 *torticornis* were added as supplementary individuals to the PCA. All analyses were done in  
377 trident, but the boxplots of the first two principal components were modified in R using the  
378 ggplot2 package. Afterward, the principal components were exported to R and used for an  
379 analysis of variance (ANOVA). The post-hoc analysis was performed using Tukey's HSD test.

## 380 **Results**

### 381 **Case study A: Diet-related differences in dental microwear between controlled-fed** 382 **pigs.**

383 The first analysis, a top-3 classification performed on crushing facets after Box-Cox  
384 transformation (Table 3), revealed that the most discriminant variables are central height  
385 skewness (Ssk), central topology variables (Sk1, Sk2, Smc1, Snb1) and heterogeneity  
386 variables for complexity (Asfc2), height (Sq, Sv, Smd) and topology parameters (Sh). In contrast,  
387 the same analysis performed on shearing facets (Table 4) revealed that the most discriminating  
388 variables are central height kurtosis (Sku), the standard deviation of central height skewness  
389 (Ssk.sd), mean and median of Smd, as well as skewness and kurtosis of spatial variables (Sal,  
390 r.sl). There are no common variables between the top 3 of crushing and shearing facets.

391 When combining the most discriminating variables from both crushing and shearing facets in a  
392 principal component analysis (Fig. 3), the first and second principal components (PCs) explain  
393 38.2 % and 21.1 % of the variances, respectively. Along PC1 and PC2, the control category  
394 overlaps with corn kernels and corn silage categories, but other groups are distinctly separated  
395 (Fig. 3A). In fact, PC1 separates barley-fed pigs from other categories, whereas PC2 separates  
396 seed-fed pigs (barley and corn) from silage-fed pigs. Other PCs failed to separate categories  
397 and were not pictured, but are available as supplementary materials (Supplementary Materials  
398 1).

399 Table 3. Case study A: Diet-related differences in dental microwear on crushing molars facets between controlled-  
 400 fed pigs, pairwise top 3 variables for each pair of compared groups ranked by the post hoc  $p$  values. All variables  
 401 were box-Cox transformed. Ba, barley; Co, control; CK, corn kernel; CS, corn silage (see Figure 2 and method  
 402 section).

pair $i$	TOP3 variables for the pair $i$	rank	$F$	$p$ value ANOVA	Post hoc $p$ values
Ba-Co	Snb1	1	9.02	<0.01	<b>0.09</b>
	Asfc2.fst.25	2	5.58	0.02	<b>0.09</b>
	Sh.min.05	3	3.69	0.06	<b>0.10</b>
Ba-CK	Sv.lst.25	1	4.47	0.03	<b>0.03</b>
	Sv.max.25	2	4.83	0.03	<b>0.04</b>
	Smc1	3	6.88	0.01	<b>0.04</b>
Ba-CS	Snb1	1	9.02	<0.01	<b>0.02</b>
	Sk1	2	8.33	0.01	<b>0.07</b>
	Asfc2.min.25	3	4.69	0.03	<b>0.07</b>
Co-CK	Smd.kurt	1	4.82	0.03	<b>0.26</b>
	Sk2	2	4.58	0.04	<b>0.39</b>
	Ssk	3	5.45	0.02	<b>0.57</b>
Co-CS	Ssk	1	5.45	0.02	<b>0.15</b>
	Sq.kurt	2	5.21	0.03	<b>0.45</b>
	Smd.kurt	3	4.82	0.03	<b>0.54</b>
CK-CS	Smd.kurt	1	4.82	0.03	<b>0.02</b>
	Sq.kurt	2	5.21	0.03	<b>0.07</b>
	Sk2	3	4.58	0.04	<b>0.35</b>

403 Table 4. Case study A: Diet-related differences in dental microwear between controlled-fed pigs, pairwise top 3  
 404 variables for the shearing facets. All variables Box-Cox transformed. Ba, barley; Co, control; CK, corn kernel; CS,  
 405 corn silage (see Figure 2 and method section).

pair <i>i</i>	TOP3 variables for the pair <i>i</i>	rank	<i>F</i>	<i>p</i> value ANOVA	Post hoc <i>p</i> values
Ba-Co	Sal.kurt	1	3.68	0.06	<b>0.01</b>
	r.sl.skew	2	5.49	0.02	<b>0.28</b>
	Smd.mean	3	5.18	0.03	<b>0.28</b>
Ba-CK	Smd.mean	1	5.18	0.03	<b>0.05</b>
	Sal.kurt	2	3.68	0.06	<b>0.06</b>
	Smd.median	3	5.76	0.02	<b>0.07</b>
Ba-CS	r.sl.kurt	1	5.68	0.02	<b>0.11</b>
	Sku	2	3.85	0.05	<b>0.13</b>
	r.sl.skew	3	5.49	0.02	<b>0.17</b>
Co-CK	r.sl.skew	1	5.49	0.02	<b>0.12</b>
	r.sl.kurt	2	5.68	0.02	<b>0.13</b>
	Ssk.sd	3	6.01	0.02	<b>0.62</b>
Co-CS	Sku	1	3.85	0.05	<b>0.03</b>
	Sal.kurt	2	3.68	0.06	<b>0.24</b>
	Ssk.sd	3	6.01	0.02	<b>0.36</b>
CK-CS	Sku	1	3.85	0.05	<b>0.02</b>
	r.sl.kurt	2	5.68	0.02	<b>0.03</b>
	Ssk.sd	3	6.01	0.02	<b>0.04</b>

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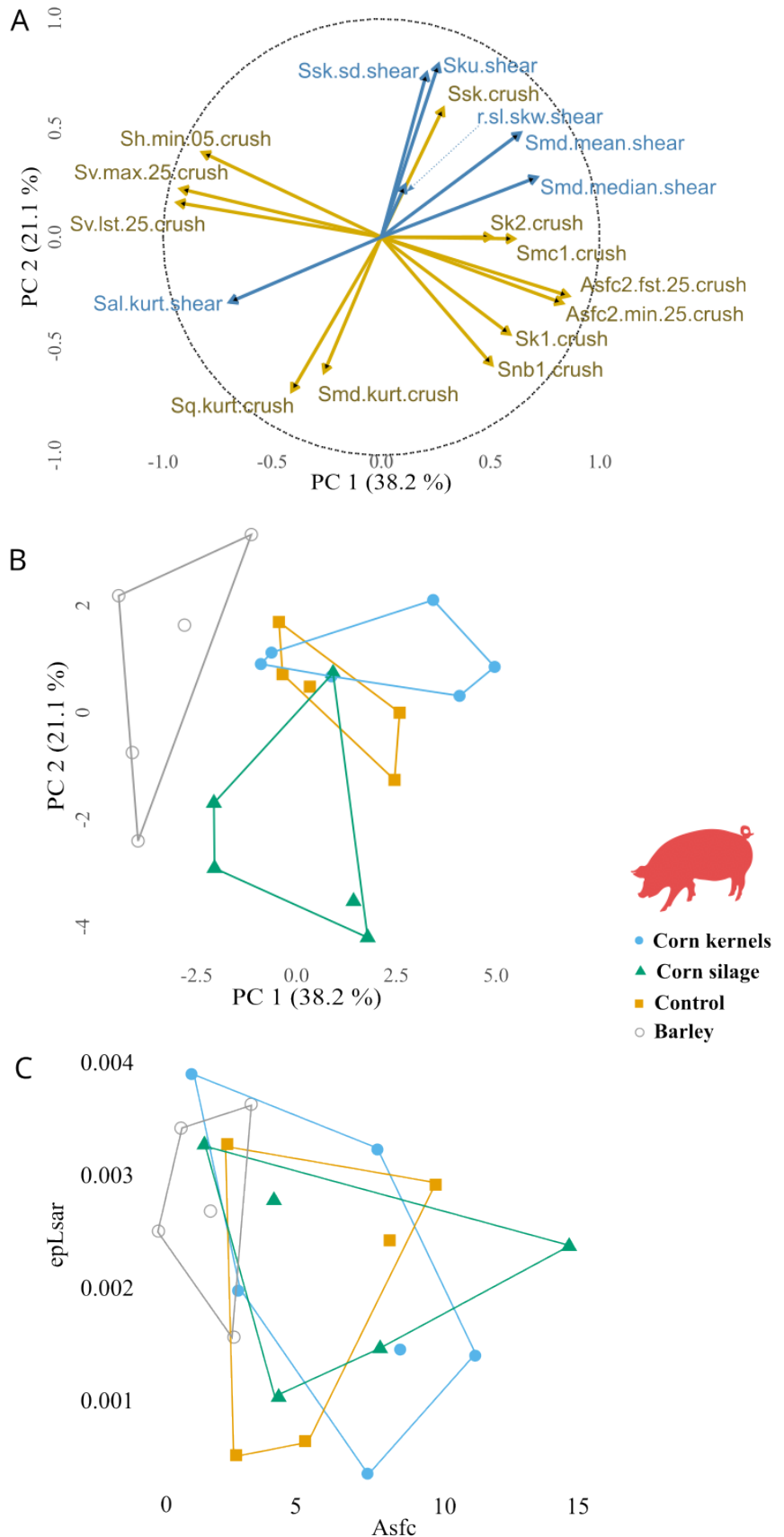
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Figure 3. Case study A: Diet-related differences in dental microwear between controlled-fed pigs, from crushing and shearing facets of upper deciduous fourth premolar. Principal component analysis from the top 3 variables for each pair of dietary categories (A & B) compared with the classical SSFA biplots. A, correlation circle, PC1 versus PC2; B, bivariate graph of individuals along PC1 versus PC2; C, bivariate graph of individuals with SSFA parameters Asfc and epLsar.



425 **Case study B: Meta-analysis of a large multi-species sample of cercopithecids.**

426 The analysis, a rank by post-hoc (mean) classification performed on crushing facets after Box-  
427 Cox transformation (Table 5), revealed that the most discriminant variables were a mix of central  
428 and heterogeneity variables. After removing variables correlated with the best ranked variables,  
429 the majority of variables are heterogeneity variables related to the highest percentiles among  
430 subsampled tiles (Sh.lst.05, Asfc2.max.05, Smc2.lst.25, s.sl.lst.05, b.sl.max.05 and Sku.lst.25).  
431 The major influence of the highest percentile variables is confirmed by the PCA (Fig. 4). Indeed,  
432 these variables contribute significantly to the first and second components, which explain 42.8 %  
433 and 22.6 % of the variance, respectively (Fig. 4B). This is also consistent with the difference in  
434 absolute surface height, which is clear on the maps: along the first component, the highest  
435 value has a height amplitude of 10.95  $\mu\text{m}$  (Fig. 4C, 4D) while the lowest value has a height  
436 amplitude of 0.71  $\mu\text{m}$  (Fig. 4C, 4E).

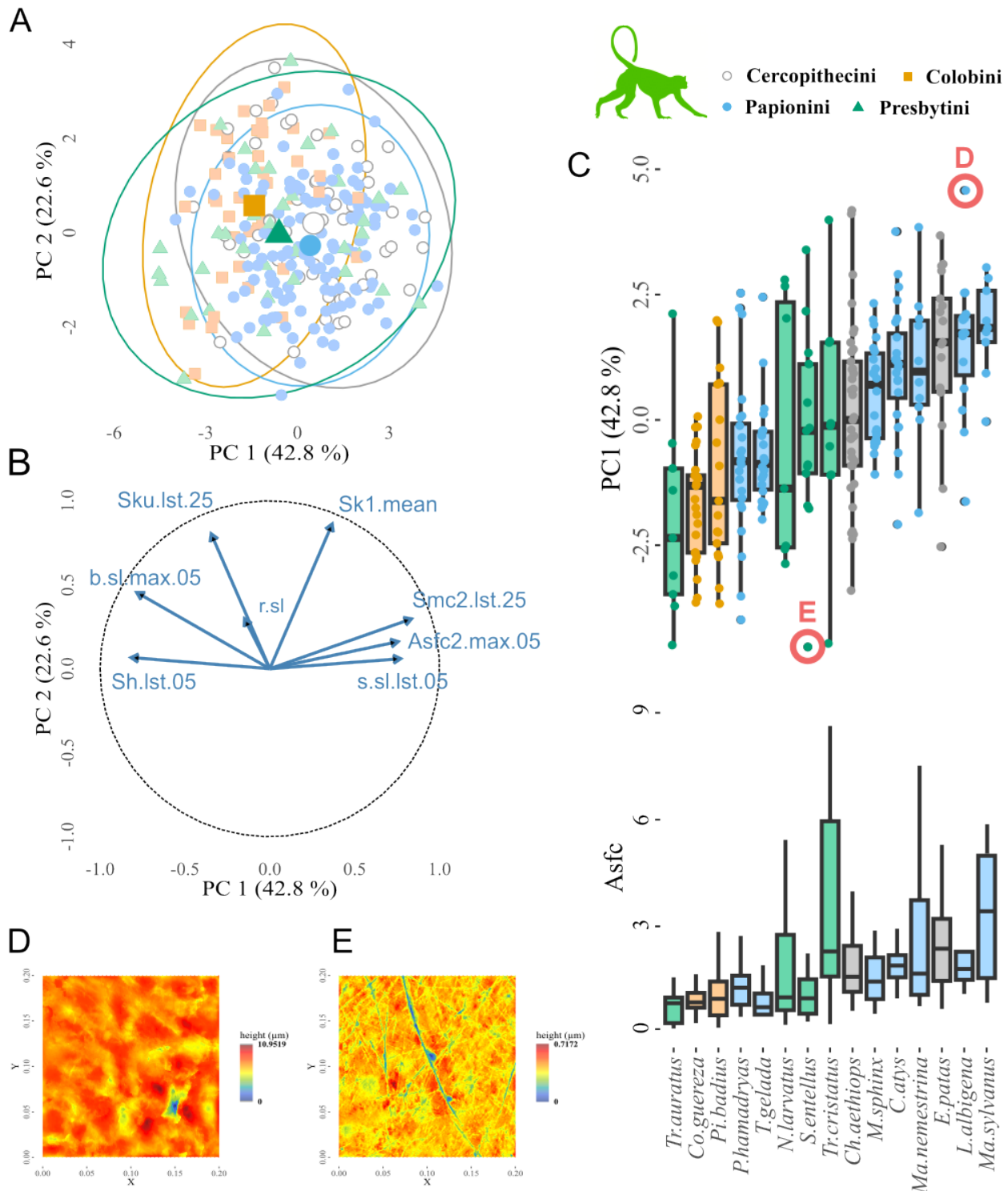
437 The bivariate graph of individuals for components 1 and 2, as well as the boxplot of component  
438 1, show that there is a large overlap between categories, both at the species and the tribe level  
439 (Fig. 4A, 4C). This is due to the broad dispersion of values. When comparing the means  
440 between species (Fig. 4C), the most folivorous species (*Trachypithecus auratus*, *Colobus*  
441 *guereza* and *Ptilocolobus badius*) have the lowest PC1 values. They are followed by terrestrial  
442 graminivorous papionines *Papio hamadryas* and *Theropithecus gelada*, then *Nasalis larvatus*,  
443 *Semnopithecus entellus* and *Trachypithecus cristatus*. The latter three are also folivorous but  
444 present higher Asfc2 values in our sample, indicating the opportunistic consumption of seeds  
445 (Thiery et al., 2021). This is supported by the surprisingly large breadth of PC1 value dispersion  
446 for these three species, especially *T. cristatus*. Then, opportunistic terrestrial cercopithecines  
447 and papionines show higher PC1 values, with the highest values found in the hard seed  
448 predator *Lophocebus albigena* (Lambert et al., 2004) and *Macaca sylvanus*, one of the most  
449 granivorous macaque (Kato et al., 2014).

450 Table 5. Case study B; Meta-analysis of a large multi-species sample of cercopithecids, the most discriminant  
 451 variables for each family, classified by the number of pair (N) that shows significant differences with the Tukey's  
 452 HSD p-value.

Variable	Position	ANOVA			Tukey's HSD	
		F	p value	mean p-value	geometric mean p-value	N (among 105 pairs)
Sh.lst.05	1	11.29	<0.01	0.01	<0.01	37
Asfc2.max.05	5	7.62	<0.01	0.01	<0.01	32
Sp.max.25	12	9.48	<0.01	0.01	0.01	24
Sa	13	9.39	<0.01	0.01	<0.01	23
Sq	15	9.03	<0.01	0.01	0.01	23
Sv.fst.05	16	6.11	<0.01	0.01	0.01	23
Sdar.max.05	18	6.37	<0.01	0.01	<0.01	22
Smd.max.25	25	5.84	<0.01	0.01	<0.01	17
Smc2.lst.25	29	6.58	<0.01	0.01	<0.01	15
Smc1.min.25	30	5.73	<0.01	0.02	<0.01	15
Sk2.mean	33	4.51	<0.01	0.02	0.01	15
Sm.min.25	36	6.41	<0.01	0.02	0.01	14
s.sl.lst.05	45	4.71	<0.01	0.02	0.02	13
b.sl.max.05	49	6.37	<0.01	0.01	<0.01	12
Snb2.mean	60	3.53	<0.01	0.02	0.01	12
Sal.min.05	73	5.85	<0.01	0.01	<0.01	10
Sku.lst.25	83	3.42	<0.01	0.01	<0.01	9
Sk1.mean	92	4.72	<0.01	0.03	0.02	9
r.sl	99	4.88	<0.01	0.01	<0.01	7
Stri	101	4.89	<0.01	0.01	<0.01	7
Ssk.fst.05	103	4.08	<0.01	0.02	0.01	7
Snb1.sd	149	3.30	<0.01	0.02	0.01	4

453 All variables Box-Cox transformed. For each parameter (e.g., Sh), only the variable with the best positioning was  
 454 selected (e.g., Sh.lst.05). Highlighted in grey are the best-positioned variables which are little correlated to each  
 455 other (threshold: 0.7).

456



457  
 458 Figure 4. Case study B: Meta-analysis of a large multi-species sample of cercopithecids. Analysis of dental  
 459 microwear textures from the crushing facets of upper and lower molars of cercopithecids from Asia and Africa.  
 460 Principal component analysis was performed using the best-ranked non-correlated variables. A, bivariate graph of  
 461 individuals along PC1 versus PC2 with ellipses depicting the confidence interval at 95 %; B, correlation circle, PC1  
 462 versus PC2; C, Boxplot of PC1 values, ordered by ascending mean, with species as a factor compared with  
 463 boxplots of Asfc values (individuals are not shown to make the figure easier to read.; D, height map of the surface  
 464 from the individual with the highest PC1 value (*L. albigena*\_NHMB-LP-2908); E, height map of the surface  
 465 from the individual with the lowest PC1 value (*S. entellus*\_BM30-11-1-4).

466 **Case study C: Comparison with extant species to infer the diet of extinct ruminants**

467 The first part of the analysis, a rank by post-hoc (mean) classification performed, after a base-  
468 10 log transformation, on the shearing dental facets of molars of the wild-caught ruminants from  
469 the Bauges Natural Regional Park (Table 6), revealed once again that most discriminant  
470 variables were a mix of central and heterogeneity variables. After removing variables correlated  
471 with the best-ranked variables, what remains are variables based on spatial parameters  
472 (Rmax.min.25, s.sl.mean, r.sl), the standard deviation of topology parameters (Sk1.sd, Smc1.sd)  
473 and Sm.fst.25, which is the first quartile of the lowest parts of the surface's height.

474 The first two components of the PCA encompass 43.9 % and 27.6 % of the variance,  
475 respectively. On the first component, we found that *G. torticornis* significantly differed from both  
476 *C. capreolus* and *C. elaphus* (Table 7). In contrast, there was no significant difference between  
477 *G. torticornis*, *R. rupicapra* and *O. gmelini*, which is visible on the bivariate graph and on the  
478 boxplot (Fig. 5C, D). On the second component as well on the third and fourth components, *G.*  
479 *torticornis* was significantly different from all extant species whereas no difference could be  
480 detected between extant species. Overall, *G. torticornis* had on average lower values than  
481 extant species for both the first and second components (Fig. 5D, E).

482

483

484 Table 6. Case study C: Comparison with extant species to infer the diet of extinct ruminants, the most discriminant  
 485 variables for each parameter, classified by the number of pair (N) that shows significant differences with the  
 486 Tukey's HSD p-value.  
 487

Variable	rank	F	p value			N (among 6 pairs)
			ANOVA	HSD <sub>arithmetic mean</sub>	HSD <sub>geometric mean</sub>	
Rmax.min.25	1	7.93	<0.01	0.02	0.01	4
Snb1.sd	2	9.19	<0.01	0.01	<0.01	3
Sku.lst.25	12	6.38	<0.01	0.01	<0.01	2
Sk1.sd	13	4.38	0.01	0.01	0.01	2
Ssk.max.05	14	5.08	<0.01	0.02	0.01	2
b.sl.min.25	21	5.10	<0.01	0.02	0.01	2
Sm.fst.25	27	3.65	0.02	0.03	0.02	2
s.sl.mean	30	5.49	<0.01	0.03	0.03	2
Smd.min.05	32	3.36	0.03	0.03	0.03	2
Smc1.sd	36	4.27	0.01	0.03	0.03	2
Snb2.sd	49	5.22	<0.01	<0.01	<0.01	1
Sal	55	4.26	0.01	0.02	0.02	1
r.sl	63	3.00	0.04	0.03	0.03	1
Sa.lst.25	70	3.45	0.02	0.04	0.04	1
Stri	72	2.27	0.09	0.04	0.04	1

488 All data log-transformed (base 10). For each parameter (e.g., Rmax), only the variable with the best positioning  
 489 was selected (e.g., Rmax.min.25). Highlighted in grey are the best positioned variables which are little correlated  
 490 to each others (threshold: 0.7).  
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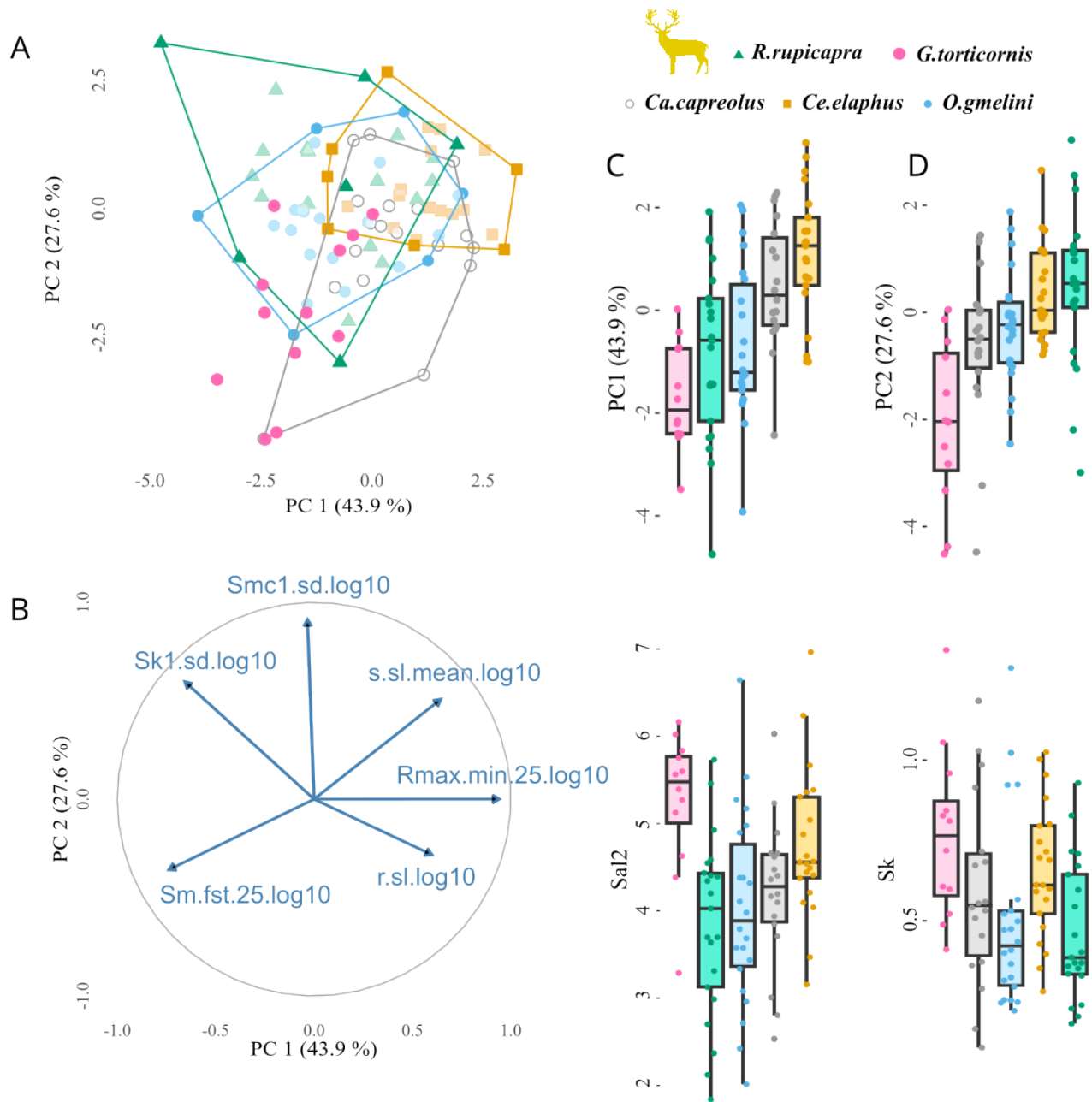
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495 Table 7. Case study C: Comparison between extant and extinct species to infer the diet of the extinct ruminants  
 496 thanks to ANOVA on the first four principal components and followed by pairwise comparison of the means using  
 497 Tukey's HSD.

Pair	PC1 (43.9 %)		PC2 (27.6 %)		PC3 (14.6 %)		PC4 (7.9 %)	
	Difference	p adjusted	Difference	p adjusted	Difference	p adjusted	Difference	p adjusted
CE-CC	0.61	0.66	1.02	0.10	-0.01	1.00	0.15	0.97
OG-CC	-1.14	0.09	0.39	0.87	-0.40	0.64	0.06	1.00
RR-CC	-1.31	0.04	1.12	0.06	0.28	0.87	0.15	0.96
OG-CE	-1.75	<0.01	-0.63	0.50	-0.39	0.63	-0.09	0.99
RR-CE	-1.92	<0.01	0.10	1.00	0.29	0.84	0.00	1.00
RR-OG	-0.17	0.99	0.73	0.34	0.68	0.11	0.09	0.99
GT-CC	-2.17	<0.01	-1.39	0.03	-1.54	<0.01	-1.97	<0.01
GT-CE	-2.78	<0.01	-2.41	<0.01	-1.53	<0.01	-2.12	<0.01
GT-OG	1.03	0.25	1.79	<0.01	1.14	0.01	2.03	<0.01
GT-RR	0.86	0.44	2.52	<0.01	1.82	<0.01	2.12	<0.01

498 Highlighted in grey are the differences supported by a significant adjusted p value. CE, *Cervus elaphus*; CC,  
 499 *Capreolus capreolus*; GT, *Gazellospira torticornis*; OG, *Ovis gmelini*; RR, *Rupicapra rupicapra*.

500



501  
 502 Figure 5. Case study C: Comparison with extant species to infer the diet of extinct species of ruminants, PCA on  
 503 selected variables with ruminants from the Bauges as individuals and fossil specimens of *Gazellospira torticornis*  
 504 as supplementary individuals. A, Bivariate graph of individuals along PC1 versus PC2, with the extinct species  
 505 from Dafnero *Gazellospira torticornis* as supplementary individuals (pink); B, Correlation circle, PC1 versus PC2;  
 506 C, Boxplot of PC1 values, ordered by ascending mean, with species as a factor in comparison with texture spatial  
 507 parameters (*Sal2*; *Sal* calculated with  $s = 0.5$ ) ; D, Boxplot of PC2 values, ordered by ascending mean, with species  
 508 as a factor in comparison with the *Sk* parameter related to vertical material distribution. All data log-transformed  
 509 (base 10).



## 510 **Discussion**

### 511 **Case study A: Diet-related differences in dental microwear between controlled-fed** 512 **pigs**

513 The broad spectrum of analytic tools and exploratory methods offered by trident maximizes the  
514 potential for detecting diet-related differences in dental microwear. In case study A, the four  
515 groups could be separated, which is consistent with Louail et al. (2021), but the difference  
516 between categories was enhanced. Dental microwear sometimes shows large within-species  
517 differences, including in wild animals (Calandra & Merceron, 2016, Percher et al., 2018) and  
518 extinct species (Scott et al., 2005; Thiery et al., 2021). In our case study however, even the  
519 subtlest variations in diet, for instance between the corn silage and the control groups, could  
520 be detected. These results are promising for paleontological and archaeological studies  
521 interested in diet variation across time and space.

522 In addition, trident is also compatible with other workflows, as it can easily combine multiple  
523 datasets, for instance microwear measured using different methods (SSFA, light microscopy...),  
524 from different teeth, or different parts of a tooth – as in case study A. In this case study, we  
525 found that shearing and crushing facets not only differ in microwear textures but also in the  
526 best-ranked variables diet-wise. Crushing different kinds of food influenced the skewness and  
527 heterogeneity of microwear height, whereas shearing different kinds of foods had a more visible  
528 influence on height kurtosis, on standard deviation of height skewness, on median height, as  
529 well as skewness and kurtosis of spatial parameters. For crushing, the presence of large and  
530 deep pits resulting from the processing of hard, seed-like foods is indeed expected to affect  
531 height and its heterogeneity. For shearing on the other hand, spatial parameters, and especially  
532 anisotropy, are expected to be more affected by the long shearing motions of tough, high-  
533 energy release rate foods such as leaves, grass etc. This is consistent with our results, and  
534 demonstrates that trident can not only integrate multiple methods, but also leverage their input.

## 535 **Case study B: Meta-analysis of a large multi-species sample of cercopithecids**

536 Sometimes the objective is not to separate groups of individuals but to identify patterns of  
537 variation imputable to dietary trends. This is exactly what trident enabled in case study B:  
538 despite PC1 values overlapping between cercopithecoid species, we can distinguish a  
539 continuum from strict leaf consumption to staple seed predation (Fig. 4). The most folivorous  
540 species (*Trachypithecus auratus*, *Colobus guereza* and *Ptilocolobus badius*) have the lowest  
541 PC1 values, whereas opportunistic terrestrial cercopithecines and papionines show higher PC1  
542 values, with the highest values found in *Lophocebus albigena* and *Macaca sylvanus*, two  
543 notable seed eaters (Lambert et al., 2004; Kato et al., 2014). Detecting this pattern required  
544 trident for ranking variables by mean p-value of Tukey's HSD and for performing multivariate  
545 analysis on the best non-correlated variables, but also the R environment for ordering species-  
546 related boxplots by ascending mean (Fig. 4C). It shows the interest of nesting trident within the  
547 R environment: accessing a broad range of libraries for complementing and leveraging  
548 functions from the R package trident.

549 The trident package in R (but not the graphic interface) also allows to inspect surfaces using  
550 2D and 3D maps – although these functions are not implemented into the interface, they can  
551 be launched from R (see Supplementary Materials 2). Here, the highest PC1 values are  
552 characterized by high maximal complexity, but also deeply worn surfaces (Fig. 4D). In contrast,  
553 the lowest PC1 values are characterized by a low complexity and shallow wear marks (Fig. 4E).  
554 Both a higher complexity (Ramdarshan et al., 2016) and larger, deeper pits (Teaford, 1985,  
555 1988) have been associated to the ingestion of large amounts of seed kernels, which is  
556 consistent with the pattern observed on Fig. 4C. It is also consistent with Asfc2 successfully  
557 separating seed-eating cercopithecids in previous studies (e.g., Thiery et al., 2021). In short,  
558 trident helps detect patterns in dental microwear textures, but it also and foremost helps  
559 interpret them in biomechanical or ecological terms.

560 **Case study C: Comparison with extant species to infer the diet of extinct ruminants**

561 The last key use of trident is the inference of diet, either in extant or in extinct species. In case  
562 study C, we could infer the diet of *Gazellospira torticornis*, an extinct antelope from the Early  
563 Pleistocene of Greece (Hermier *et al.*, 2020). To do so, we used trident to perform a PCA on  
564 the best ranked, non-correlated variables regarding their ability to separate four ruminants from  
565 the Bauges Natural Regional Park with known differences in diet, ranging from selective  
566 browsing to grass-dominated mixed feeding habits. We then added *G. torticornis* specimens as  
567 supplementary individuals to the PCA. This analysis showed that *G. torticornis* had low values  
568 of anisotropy, especially the 1<sup>st</sup> quartile (Rmax.min.25, Fig. 5), which is similar to *Ovis gmelini*  
569 and *Rupicapra rupicapra*. Both are mixed-feeding species: *O. gmelini musimon* eats grasses in  
570 complement with dicots foliages, shrubs and herbaceous dicots (Redjadj *et al.* 2014; see also  
571 Marchand *et al.*, 2013), while *R. rupicapra* alternates between grass and foliage depending on  
572 seasons (Redjadj *et al.* 2014; see also Pérez-Barberia *et al.*, 1997). *G. torticornis* likely was a  
573 mixed feeding species, incorporating both grasses and lignified tissues in its diet.

574 Once again, nesting trident in the R environment gives access to a broad range of methods for  
575 complementary analysis. To better understand the dietary behavior of *G. torticornis*, we  
576 performed an ANOVA on principal components to search for differences between extinct and  
577 extant taxa. For PC1, *G. torticornis* significantly differed from *Cervus elaphus* and *Capreolus*  
578 *capreolus*. *C. elaphus* is also a mixed-feeding species (Gebert & Verheyden-Tixier, 2001), but  
579 in the Bauges Natural Regional Park, its diet comprises a large proportion of grasses (Merceron  
580 *et al.*, 2021a). This likely increased its dental microwear anisotropy, which explains why it differs  
581 from other mixed-feeders that include more lignified tissues than the red deer. *C. capreolus* on  
582 the other hand is a selective browser (Redjadj *et al.*, 2014).

583 Lastly, variables that contribute to PC2 (Smc1.sd and to some extent, Sk1.sd and s.sl.mean)  
584 were significantly lower in *G. torticornis* compared to all four extant species. This point illustrates

585 how dental microwear textures can present original patterns in the fossil record, sometimes  
586 completely different to what is known for extant species – perhaps reflecting how different the  
587 environmental conditions were at the time.

## 588 **Conclusion**

589 trident, an R package for performing dental microwear texture analysis is proposed here and  
590 illustrated with three case studies, showing how trident helps answer questions concerning  
591 trophic ecology commonly investigated by paleontologists and archaeologists. In the first case  
592 study, we separate four groups of domestic pigs based on their dietary composition. In the  
593 second case study, we identify microwear texture patterns in a large database of 15 primate  
594 species and relate these patterns to biomechanical and ecological factors. The third case study  
595 investigates the dental microwear textures of four extant ruminants to infer the diet of an extinct  
596 antelope from the Pleistocene of Greece. These case studies show how trident can leverage  
597 dental microwear texture analysis results.

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## 607 **Authors' contributions**

608 AF, NB, and GM conceived the ideas and designed the methodology; GM, EB, CB, ML, and  
609 AW collected the data; AF, GM, ML, and GT developed the software; AF and GT coded the  
610 software; ML, GM and GT analyzed the data; GM acquired funding and managed the project  
611 administration. All authors contributed critically to the drafts and gave final approval for  
612 publication.

## 613 **Data availability**

614 The source package is available on Github at <https://github.com/nialsiG/trident> and on Zenodo  
615 [\(Thiery et al., 2023\)](#).

616 Dental Microwear Textures are available in the repository InDores. All raw scans of surfaces  
617 (plu ou plux files), the template used to pre-treat raw data (mnt files), as well as the surfaces  
618 (.sur files) used with trident are provided here:

619 Case A: Pig experiment:

620 <https://data.indores.fr:443/privateurl.xhtml?token=2a85b83e-2cf7-42aa-80ec-baee7c905784>

621 Case B: Extant species of Cercopithecids

622 <https://data.indores.fr:443/privateurl.xhtml?token=41e643b6-f389-4d70-b0ee-9cecd9c70ae5>

623 Case C: Extant species of ruminants from the Bauges, Alps, France

624 <https://data.indores.fr:443/privateurl.xhtml?token=fdf408d1-9997-48e6-89f4-5ed46e55256a>

625 Case C: Fossil specimens of *Gazellospira torticornis*

626 <https://data.indores.fr:443/privateurl.xhtml?token=d300097a-f0ba-4850-b096-561019fade2a>

## 627 **Conflict of interest disclosure**

628 The authors of this preprint declare that they have no financial conflict of interest with the  
629 content of this article.

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631 **References**

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