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

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Abstract

This manuscript introduces trident, an R package for performing dental microwear texture analysis and subsequently classifying variables based on their ability to separate discrete categories. The trident package comes with independent functions and a user interface, trident, enabling easy and fast proficiency. It can import .SUR files, then remove aberrant peaks and possibly polynomial surfaces in the 2^{nd} or 8^{th} order. Next, it can measure up to 384 variables corresponding to 24 parameters and their heterogeneity. It can also rank any number of variables using five different methods, display the results in multivariate analyses, and export the results in R, providing access to its large asset of libraries.

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

Keywords: Diet inference; DMTA; Multivariate analysis

Introduction

Fifty shades of dental microwear

Dental microwear analysis has been a prominent method for investigating the diet of extant and extinct species during the last 40 years (e.g., Walker et al., 1978; Kay, 1981; Teaford, 1985, 1988; Ungar, 1996; Teaford et al., 1996; Solounias &Semprebon, 2002; Merceron et al., 2005; Merceron, Blondel, et al., 2005; Scott et al., 2005; Ungar et al., 2008; Rivals et al. 2011). It is based on the observation that food leaves microscopic wear marks on dental wear facets, and that those marks fade as the tooth wears, only to be replaced by new microwear marks (Walker et al., 1978; Gordon, 1982; Teaford & Oyen, 1989; Winkler et al., 2020). The nature (scratches or pits), size (small to large), and frequency of microwear depend on the food's physical properties, mostly hardness, and abrasiveness, while their spatial distribution and their anisotropy are related to chewing motions and food toughness (Teaford, 1988; Scott et al., 2006; Teaford et al., 2020). This allows us to infer an animal's diet during the last few weeks before its latest meal (Teaford & Oyen, 1989; Winkler et al., 2020). Apart from diet, the role of exogenous soil mineral particles and environmental conditions in dental microwear formation should not be minimized (Schulz-Kornas et al., 2019; Schulz-Kornas et al., 2020). Grit is indeed reportedly harder than enamel tissue and more abrasive than food particles (Sanson et al., 2007; Lucas et al., 2013). Still, controlled feeding experiments have shown that differences in dental microwear texture better reflect diet than the amount of exogenous particles (Merceron et al., 2016).

Initially, microwear analysis used light microscopes to count pits and scratches left by the food on the enamel surface. But despite a proven ability to separate surfaces according to diet, this method is reliant on the tuning of light orientation, the counting method, the observer experience, etc. (Grine et al., 2002; Galbany et al., 2005; Mihlbachler et al., 2012). With the advent of high-resolution digitization techniques, new methods for quantifying the three-

dimensional surface texture have emerged. Scale Sensitive Fractal Analysis (SSFA) uses surface parameters strongly correlated to food material properties, such as surface scale roughness estimated from area-scale fractal complexity (Asfc) that correlates to food hardness, or anisotropy (epLsar) that correlates to food toughness (Ungar et al., 2003; Scott et al., 2005; Hua et al., 2020). A second approach, Surface Texture Analysis (STA) consists of computing ISO-25178 area parameters (Schulz et al., 2010; Kaiser et al., 2015). Both SSFA and STA methods belong to the larger field of dental microwear texture analysis (DMTA). They have been used to investigate the diet of animals from a broad range of species, including insectivorous mammals (Purnell et al., 2013) but also selacians (Weber et al., 2021), lepidosaurians (Winkler et al., 2019) or dinosaurs (Williams et al., 2009).

New methods, new challenges

Despite their reliability, DMTA approaches are facing several methodological challenges. First, they sometimes fail to separate specimens that an expert eye could visually tell apart using the number of specific microwear marks (Fig. 1). This can be mitigated by using filters to enhance the microwear versus the raw shape of the tooth surface, for instance by removing the 2^{nd} or 8^{th} order polynomial of the surface using least square approximation (Francisco, Brunetière, et al., 2018). Failure to tell surfaces apart can also come from distinct structures concealed in the average signal. One of the SSFA parameters can solve this issue by estimating heterogeneity of complexity (HAsfc, Scott et al., 2005, 2006). An alternative approach is to consider a grid of standard number and size of cells and to measure every parameter for each cell: the presence of distinct structures would increase the dispersion of values, and could be detected using percentiles, minimal or maximal values, etc. (Francisco, Blondel, et al., 2018).

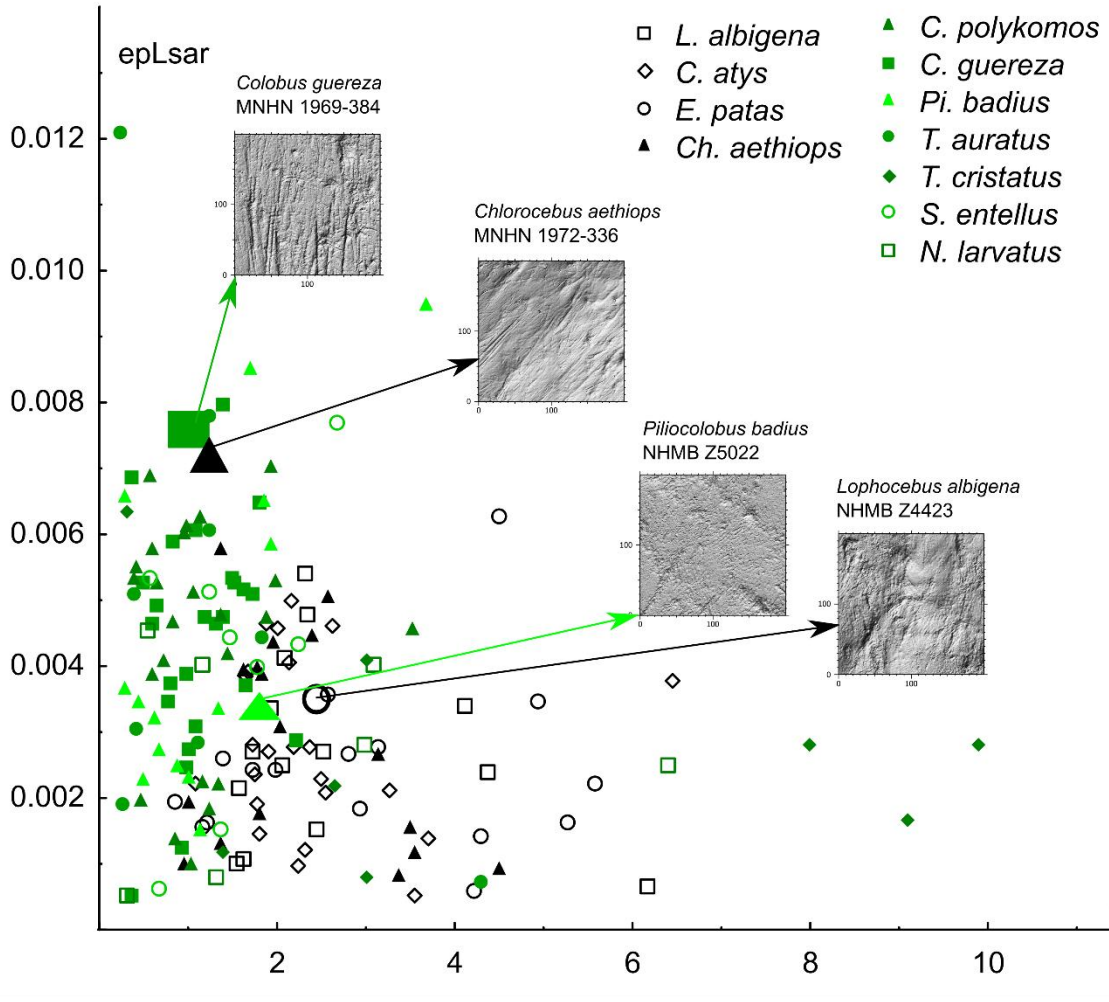

Figure 1. Cases where an expert eye can visually differentiate two surfaces, but dental microwear texture analysis cannot. For example, experts will count more wide scratches on *Colobus guereza* (MNHN 1969-384) compared to *Chlorocebus aethiops* (MNHN 1972-336) whereas DMTA fails to discriminate the two using both anisotropy and complexity. Similarly, experts will count more large pits on *Lophocebus albigena* (NHMB Z4423) compared to *Piliocolobus badius* (NHMB Z5022) whereas DMTA fails to discriminate the two. In the latter example, we expect heterogeneity to solve the issue.

Computing heterogeneity of DMTA variables helps track the most elusive structures, but it tends to generate a "jungle of parameters" (Francisco, Brunetière, et al., 2018), that is, too many variables to keep track of a given phenomenon. One way to bypass this issue is to compare the variables' ability to discriminate groups, for instance using analyses of variance (ANOVAs) to find which groups can be separated using post-hoc analysis such as Tukey's HSD or Fisher's LSD. This analysis pipeline is very efficient and could separate animals

according to their diet in several studies (Francisco, Blondel, et al., 2018; Francisco, Brunetière, et al., 2018; Louail et al., 2021; Merceron, Kallend, et al., 2021).

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

Here, we introduce trident, a user interface, and its associated R source package trident for measuring dental microwear textures and analyzing the discriminant ability of DMTA variables. It can load .SUR files, remove abnormal peaks, and measure 16 variables from 24 DMTA parameters (for a total of 384 variables) on batches. The computed DMTA variables (along with variables possibly added by the user on the source .txt file) can be classified according to their ability to discriminate discrete categories such as species, diet, etc trident also comes with tools to perform univariate, bivariate, and multivariate analysis. All the functions can be performed from the user interface.

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

Case study A. Diet-related differences in dental microwear: The first case study is based on a controlled feeding experiment involving a single omnivorous species (*Sus domesticus*). Similar to Louail et al. (2021), animals participated in trials only differing in the dietary composition of their daily ration. The influence of diet on DMTA was then quantified using trident.

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

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

Material and Methods

Material

Case study A: Diet-related differences in dental microwear

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

- The *barley group* (N = 5) was fed 30 % of barley seeds.
- The *corn kernel* group (N = 5) was 20 % of corn (*Zea mays*) flour, supplemented with 20 % (as dry matter weight) of corn kernels.
- The *corn silage* group (N = 5) was fed 100 % of the base diet but had access to corn silage at will.

We analyzed the deciduous upper fourth premolars of pigs aged between 6.5 and 9.5 months. For more details on the experiment, see Louail et al. (2021).

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

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

Case study 3: Comparison with extant species to infer the diet of extinct species

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

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

Surface acquisition

Each tooth surface was cleaned and molded as described in previous works (Louail et al., 2021; Merceron, Berlioz, et al., 2021; Merceron, Kallend, et al., 2021) and on the TRIDENT website (http://anr-trident.prd.fr/v/). For case study A, we investigated both the shearing (phase I) and crushing (phase II) dental facet of the very same tooth, whereas we focused on crushing facets for case study B (primates) and on shearing facets for case study C (ruminants). Each facet was scanned separately using a white-light confocal profilometer Leica DCM8, named “TRIDENT”, with a 100× objective housed at the PALEVOPRIM lab, CNRS and University of Poitiers, France (Leica Microsystems). All surfaces were pre-processed following Merceron et al. (2016). The procedure resulted in the obtention of .SUR files (saved as SUR version 7.2 or older), which were then imported into trident.

DMTA with *trident*

Presentation of trident

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

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

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

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

Dental microwear texture analysis (DMTA)

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

Afterward, DMTA variables were computed. The program can currently compute four families of parameters (Table 1). The first one is complexity i.e., an estimation of the density of microwear textures. The second family is height, or parameters describing the average height, its dispersion, and its variation over the surface. The third family is spatial parameters, which

describe the distribution and nature of the textures. The last family is topology, a combination of height and spatial parameters, measuring the proportion of the surface above or below determined heights.

Table 1. DMTA parameters measured in trident

Parameter	Family	Significance
Asfc2	Complexity	Area scale fractal complexity($^{\#}$)
Sa	Height	Arithmetic mean of the absolute of the heights (*)
Sp	Height	Absolute of the largest height (*)
Sq	Height	Height standard deviation (*)
Sv	Height	Absolute of the smallest height (*)
Ssk	Height	Height skewness (*)
Sku	Height	Height kurtosis (*)
Sm	Height	Mean height (0 for the whole surface, but non-zero for its samples)
Smd	Height	Median height
Rmax	Spatial	Semi-major axis of the fACF ellipsis(**)
Sal	Spatial	Semi-minor axis of the fACF ellipsis (**)
Std ^{EX}	Spatial	Texture direction (**)
Stri(*) = Str^{-1}	Spatial	Rmax/Sal ratio
b.sl	Spatial	Highest slope of fACF (**) at the distance rs from the origin
r.sl	Spatial	b.sl/s.sl ratio
s.sl	Spatial	Smallest slope of fACF (**) at the distance rs^{β} from the origin
Sdar	Complexity	Relative area (developed area/projected area)
Sk1, Sk2	Topology	Relative area of the surface above $h1^{\beta\beta}$ and $h2^{\beta\beta}$ respectively
Smc1, Smc2	Topology	Median relative area of the cells with heights exceeding $h1^{\beta\beta}$ and $h2^{\beta\beta}$ respectively
Snb1, Snb2	Topology	Number of cells with heights exceeding $h1^{\beta\beta}$ and $h2^{\beta\beta}$ respectively
Sh	Topology	Percentage of quasi-horizontal faces (normal within a 4° cone)

^{EX}, parameters excluded from the analysis; * ISO 25178 ; ** autocorrelation function at z=0.5 (Francisco, Blondel, et al., 2018; Francisco, Brunetière, et al., 2018) ; $^{\beta}$ maximum slope radius ; $^{\beta\beta}$ h1 = 85 % of total height (Sv+Sp) and h2 = 95 % of total height (Sv+Sp)$^{\#}$; Area Scale Fractal Complexity is labeled Asfc2 because its calculation mode slightly differs from the Asfc computed in Scott et al., 2006).

Lastly, we estimated the heterogeneity for complexity, height, spatial, and topology variables. The heterogeneity of a (dental) surface is related to the spatial distribution of its features: for instance, a single pit in the enamel implies more heterogeneity than several pits uniformly distributed through the enamel surface (Scott et al., 2006). Following Francisco, Brunetière et

al. (2018), trident uses a fast and intuitive approach for estimating heterogeneity: the surface is divided into *n* grid cells, and DMTA parameters are computed for each grid cell. Then, the distribution of DMTA parameters across grid cells is used to compute heterogeneity variables e.g., mean Asfc2, maximal Asfc2, or 25th percentile of Asfc2 (Table 2). Note that this way of assessing heterogeneity differs from SSFA parameters such as HAsfc (Box 1). In the end, a total of 384 variables can be computed, giving a highly detailed description of dental microwear textures. Out of these, 24 variables correspond to the 24 parameters from Table 1 measured on the whole surface, but the remaining 360 are estimates of surface heterogeneity.

Box 1. trident and the traditional SSFA variables

trident does not allow to compute of SSFA variables. Still, the user can find equivalents among the 384 variables available in trident. For instance, Asfc can be approximated from Asfc2, which also strongly correlates with Sdar. EpLsar, which is an SSFA estimate of anisotropy, is related to spatial variables in trident. It is especially correlated with Rmax, Sal, and Stri (Str^{-1}). Note that trident can open and manage any other variables (e.g.. SSFA, furrows, δ^{13}C …) that a user might want to add to the source .txt file. This way, it is easy to compare trident's variables with other parameters.

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

Table 2. Heterogeneity variables.

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

[EX], variables excluded from the analysis.

Multichecks

The second most important part of trident relates to the classification of variables according to their ability to separate informed categories, such as diet, species, etc. In case study A, the factor is diet whereas in case study B and C, the factor is species. The software proposes functions for adding a factor variable by combining variables from different datasets, by entering it manually or automatically (see Supplementary Materials 2).

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

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

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

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

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

Classification of variables

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

(1) Rank based on ANOVA: Performs an ANOVA and arranges variables by ascending p-value.

(2) Rank based on Kruskal-Wallis: Performs a Kruskal-Wallis rank-sum test and arranges variables by ascending p-value; in case the sample is far from normality.

(3) Rank based on post-hoc (average): Performs an ANOVA and arranges by decreasing the number of significant p-values per pair and then ascending mean p-value from post-hoc tests. The mean p-value can be either arithmetic or geometric, and the user can choose to use only significant p-values to calculate it (this is the default option).

(4) Rank based on post-hoc (pairwise): Performs an ANOVA and then for a given pair, arranges variables by ascending pairwise post-hoc p-value.

(5) Top 3: For each pair of categories, arrange by the number of significant pairwise p-values from Tukey's HSD, then by the mean of significant p-values. The function returns the 3 best-classified variables. Because this function makes a new classification for each pair of groups, computation time and length of results increase exponentially as the number of categories goes up. Although it remains possible to use this function on any number of categories, we do not recommend using this approach for more than 5 categories (10 pairs).

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

Case-specific analysis

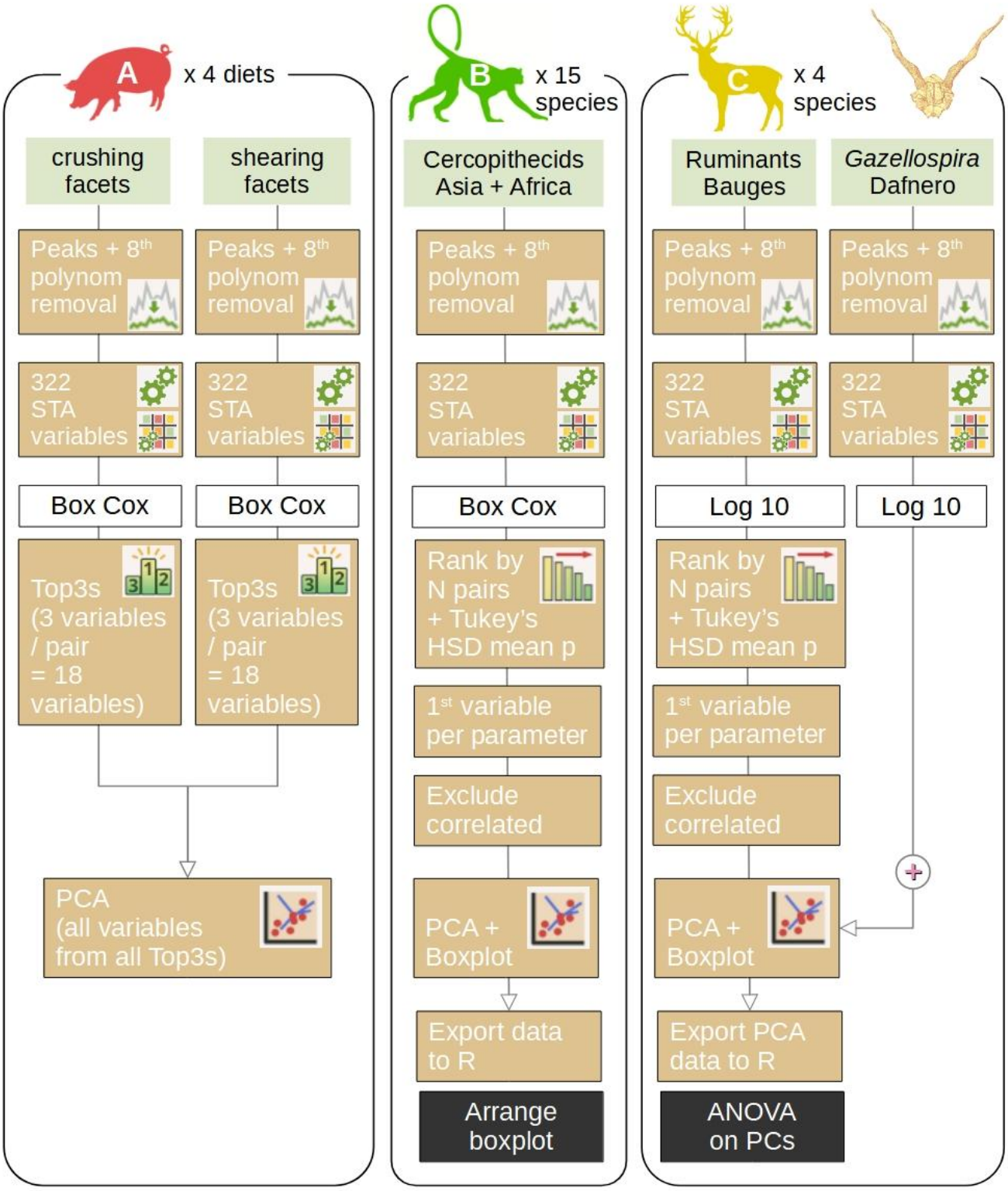

Figure 2. Flowchart depicting the analysis for the three case studies.

Case study A: Diet-related differences in dental microwear

For each wear facet (crushing and shearing), data were analyzed separately. They were Box-Cox transformed, then checked for normality, homoscedasticity, and their ability to

discriminate categories (Fig. 2A). Variables that passed the multi check were classified using the mean of the significant p-values from Tukey's HSD post-hoc analysis of an ANOVA with diet as factor. They were ranked according to (1) the number of groups discriminated and (2) the arithmetic mean of Tukey's HSD discriminant p-values (the p-value of non-discriminated groups were ignored for calculating this arithmetic mean). Among these variables, we retained only the 3 best-ranked variables (Top3). Afterward, all the retained variables for both crushing and shearing facets were combined. We performed a Principal Component Analysis (PCA) to explore their influence on data distribution. All analyses were done exclusively in trident.

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

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

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

First, the Bauges data were log-transformed (base 10), then checked for normality, homoscedasticity and their ability to discriminate species (Fig. 2C). Variables which passed

the checking step were classified using (1) the number of groups discriminated by the post-hoc analysis of an ANOVA and (2) the geometric mean of Tukey's HSD discriminant p-values (the p-value of non-discriminated groups were ignored for calculating this geometric mean). Just like case study B, for each parameter (e.g., Asfc2), the best ranked variable out of 14 (central + heterogeneity statistics) was selected, and variables correlated to more than 70 % with a better ranked variable were systematically removed (calculated independently with R). The remaining variables were used for a PCA. At this point, the surfaces of *Gazellospira torticornis* were added as supplementary individuals to the PCA. All analyses were done in trident, but the boxplots of the first two principal components were modified in R using the ggplot2 package. Afterwards, the principal components were exported to R and used for an analysis of variance (ANOVA). The post-hoc analysis was performed using Tukey's HSD test.

Results

Case study A: Diet-related differences in dental microwear

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

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

Table 3. Case study A: Diet-related differences in dental microwear, pairwise top 3 variables for the crushing facets. All variables Box-Cox transformed. Ba, barley; Co, control; CK, corn kernel; CS, corn silage.

pair *i*	TOP3 variables for the pair *i*	rank	*F*	*p* value ANOVA	Post hoc *p* values					
					Ba-Co	Ba-CK	Ba-CS	Co-CK	Co-CS	CK-CS
Ba-Co	Snb1	1	9.02	<0.01	**0.09**	0.10	0.02	1.00	0.89	0.80
	Asfc2.fst.25	2	5.58	0.02	**0.09**	0.06	0.09	1.00	1.00	1.00
	Sh.min.05	3	3.69	0.06	**0.10**	0.06	0.07	1.00	1.00	1.00
Ba-CK	Sv.lst.25	1	4.47	0.03	0.15	**0.03**	0.20	0.84	1.00	0.77
	Sv.max.25	2	4.83	0.03	0.15	**0.04**	0.18	0.93	1.00	0.89
	Smc1	3	6.88	0.01	0.42	**0.04**	0.23	0.58	0.97	0.83
Ba-CS	Snb1	1	9.02	<0.01	0.09	0.10	**0.02**	1.00	0.89	0.80
	Sk1	2	8.33	0.01	0.20	0.08	**0.07**	0.97	0.93	1.00
	Asfc2.min.25	3	4.69	0.03	0.13	0.07	**0.07**	0.99	0.99	1.00
Co-CK	Smd.kurt	1	4.82	0.03	1.00	0.18	0.66	**0.26**	0.54	0.02
	Sk2	2	4.58	0.04	0.80	0.08	0.84	**0.39**	1.00	0.35
	Ssk	3	5.45	0.02	0.51	1.00	0.84	**0.57**	0.15	0.74
Co-CS	Ssk	1	5.45	0.02	0.51	1.00	0.84	0.57	**0.15**	0.74
	Sq.kurt	2	5.21	0.03	0.92	0.33	0.81	0.70	**0.45**	0.07
	Smd.kurt	3	4.82	0.03	1.00	0.18	0.66	0.26	**0.54**	0.02
CK-CS	Smd.kurt	1	4.82	0.03	1.00	0.18	0.66	0.26	0.54	**0.02**
	Sq.kurt	2	5.21	0.03	0.92	0.33	0.81	0.70	0.45	**0.07**
	Sk2	3	4.58	0.04	0.80	0.08	0.84	0.39	1.00	**0.35**

362 Table 4. Case study A: Diet-related differences in dental microwear, pairwise top 3 variables for the shearing
363 facets. All variables Box-Cox transformed. Ba, barley; Co, control; CK, corn kernel; CS, corn silage.

pair *i*	TOP3 variables for the pair *i*	rank	*F*	*p* value ANOVA	Post hoc *p* values					
					Ba-Co	Ba-CK	Ba-CS	Co-CK	Co-CS	CK-CS
Ba-Co	Sal.kurt	1	3.68	0.06	**0.01**	0.06	0.33	0.70	0.24	0.78
	r.sl.skw	2	5.49	0.02	**0.28**	0.98	0.17	0.12	0.99	0.07
	Smd.mean	3	5.18	0.03	**0.28**	0.05	0.98	0.81	0.46	0.10
Ba-CK	Smd.mean	1	5.18	0.03	0.28	**0.05**	0.98	0.81	0.46	0.10
	Sal.kurt	2	3.68	0.06	0.01	**0.06**	0.33	0.70	0.24	0.78
	Smd.median	3	5.76	0.02	0.35	**0.07**	0.59	0.79	0.97	0.53
Ba-CS	r.sl.kurt	1	5.68	0.02	0.33	0.95	**0.11**	0.13	0.90	0.03
	Sku	2	3.85	0.05	0.86	0.85	**0.13**	1.00	0.03	0.02
	r.sl.skw	3	5.49	0.02	0.28	0.98	**0.17**	0.12	0.99	0.07
Co-CK	r.sl.skw	1	5.49	0.02	0.28	0.98	0.17	**0.12**	0.99	0.07
	r.sl.kurt	2	5.68	0.02	0.33	0.95	0.11	**0.13**	0.90	0.03
	Ssk.sd	3	6.01	0.02	1.00	0.69	0.30	**0.62**	0.36	0.04
Co-CS	Sku	1	3.85	0.05	0.86	0.85	0.13	1.00	**0.03**	0.02
	Sal.kurt	2	3.68	0.06	0.01	0.06	0.33	0.70	**0.24**	0.78
	Ssk.sd	3	6.01	0.02	1.00	0.69	0.30	0.62	**0.36**	0.04
CK-CS	Sku	1	3.85	0.05	0.86	0.85	0.13	1.00	0.03	**0.02**
	r.sl.kurt	2	5.68	0.02	0.33	0.95	0.11	0.13	0.90	**0.03**
	Ssk.sd	3	6.01	0.02	1.00	0.69	0.30	0.62	0.36	**0.04**

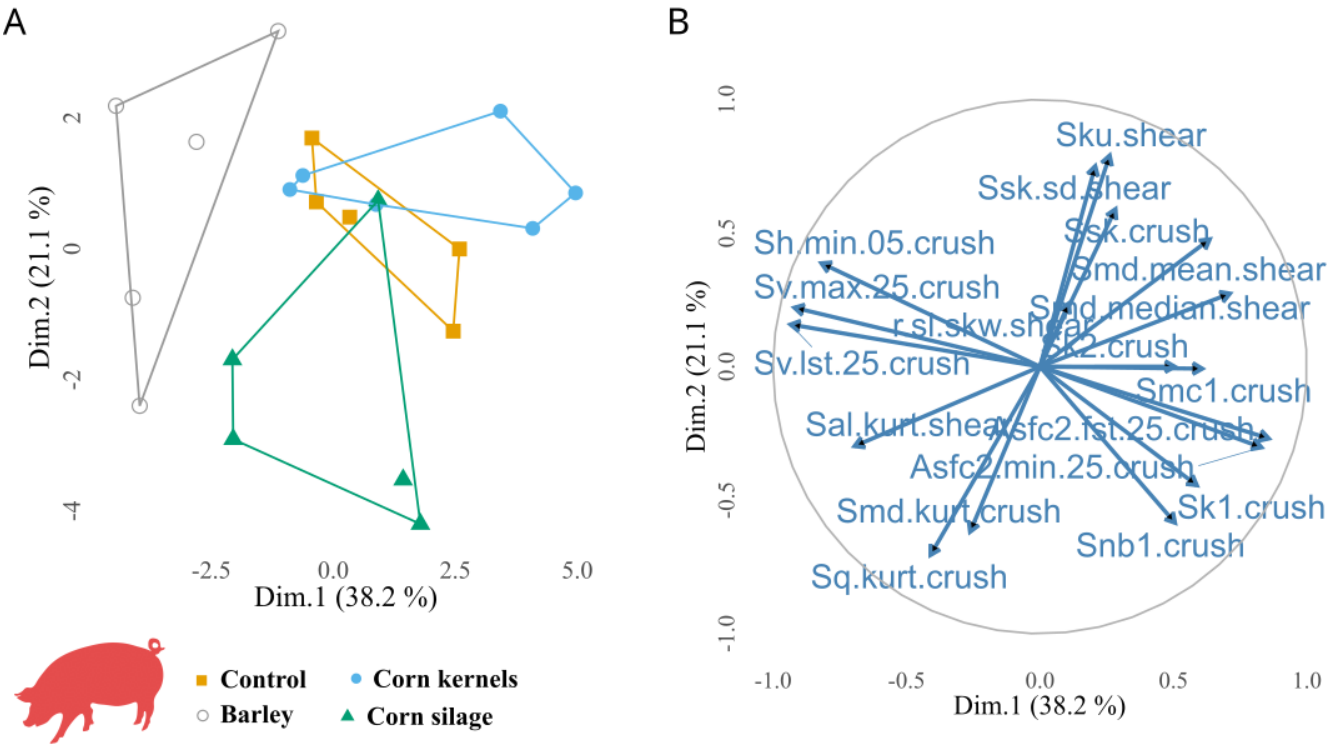

Figure 3. Case study A: Diet-related differences in dental microwear, from crushing and shearing facets of upper deciduous fourth premolar of domestic pigs. Principal component analysis from the top 3 variables for each pair of dietary categories. Before the PCA, data of crushing and shearing facets have been Box-Cox transformed separately. A, bivariate graph of individuals along PC1 versus PC2; B, correlation circle, PC1 versus PC2.

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

The analysis, a rank by post-hoc (mean) classification performed on crushing facets after Box-Cox transformation (Table 5), revealed that the most discriminant variables were a mix of central and heterogeneity variables. After removing variables correlated with the best ranked variables, the majority of variables are heterogeneity variables related to the highest percentiles among subsampled tiles (Sh.lst.05, Asfc2.max.05, Smc2.lst.25, s.sl.lst.05, b.sl.max.05 and Sku.lst.25).

The major influence of the highest percentile variables is confirmed by the PCA (Fig. 4). Indeed, these variables contribute significantly to the first and second components, which explain 42.8 % and 22.6 % of the variance, respectively (Fig. 4B). This is also consistent with the difference in absolute surface height, which is clear on the maps: along the first

component, the highest value has a height amplitude of 10.95 µm (Fig. 4C, 4D) while the lowest value has a height amplitude of 0.71 µm (Fig. 4C, 4E).

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

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

Variable	Position	ANOVA		Tukey's HSD		
		F	p value	mean p-value	geometric mean p-value	N
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

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

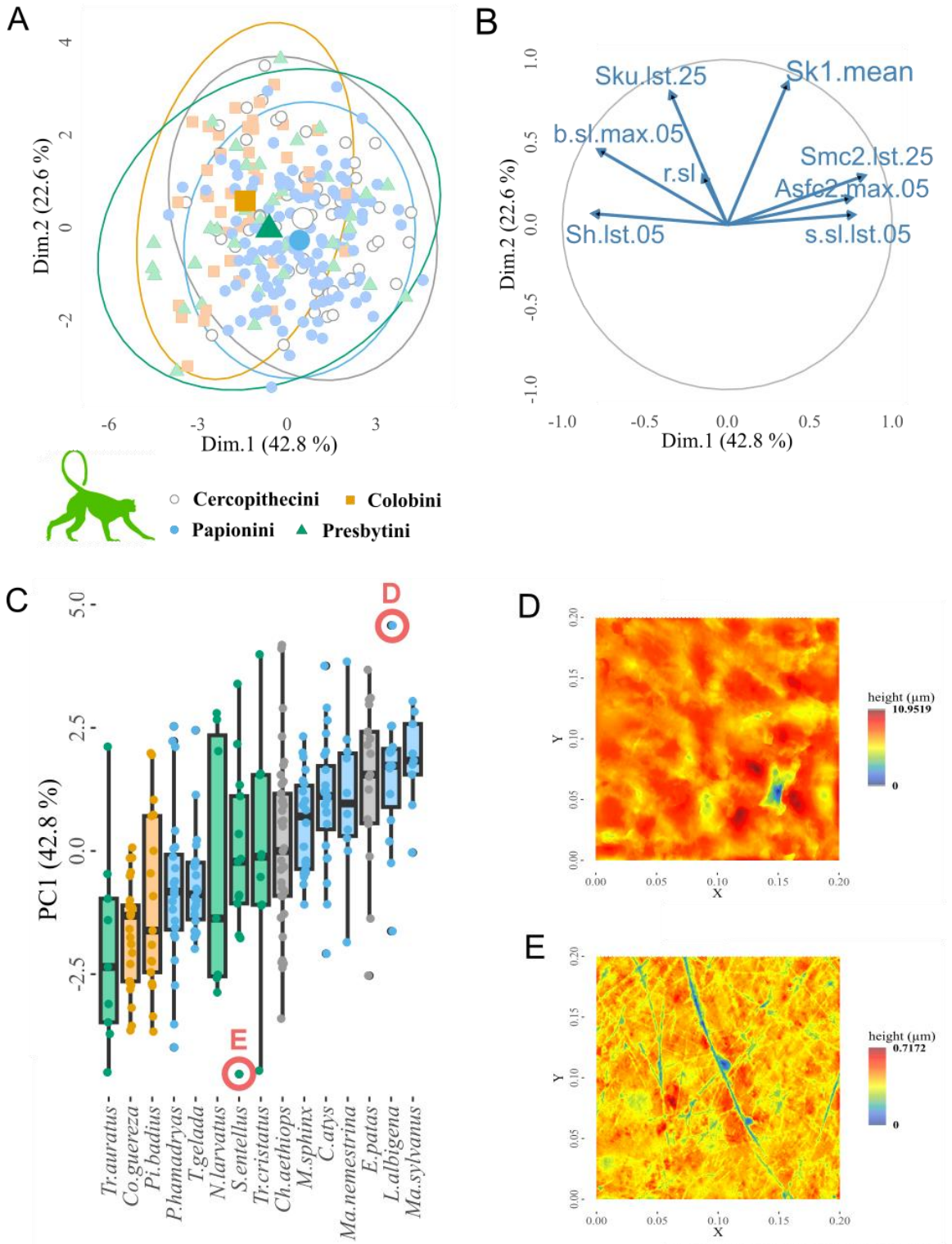

Figure 4. Case study B: Meta-analysis of a large multi-species sample. Analysis of dental microwear textures from the crushing facets of upper and lower molars of cercopithecids from Asia and Africa. Principal component analysis was performed using the best-ranked non-correlated variables. Before PCA, data were Box-Cox transformed. A, bivariate graph of individuals along PC1 versus PC2; B, correlation circle, PC1 versus PC2; C, Boxplot of PC1 values, ordered by ascending mean, with species as factor. Colors indicate the tribe (blue, papionines; green, presbytines; grey, cercopithecines; orange, colobines); D, height map of the surface from the individual with the highest PC1 value (*L.albigena*_NHMB-LP-2908); E, height map of the surface from the individual with the lowest PC1 value (*S.entellus*_BM30-11-1-4). Ellipses depict the confidence interval at 95 %.

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

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

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

Table 6. Case study C: Comparison with extant species to infer the diet of extinct species, the most discriminant variables for each parameter, classified by the number of groups significantly discriminated using Tukey's HSD p-value.

			p value			
Variable	rank	F	ANOVA	HSD arithmetic mean	HSD geometric mean	Number of significant differences
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

All data log-transformed (base 10). For each parameter (e.g., Rmax), only the variable with the best positioning was selected (e.g., Rmax.min.25). Highlighted in grey are the best positioned variables which are little correlated to each others (threshold: 0.7).

Table 7. Case study C: Comparison between extant and extinct species to infer the diet of the extinct species thanks to ANOVA on the first four principal components and followed by pairwise comparison of the means using Tukey's HSD.

	PC1 (43.9 %)		PC2 (27.6 %)		PC3 (14.6 %)		PC4 (7.9 %)	
Pair	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

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

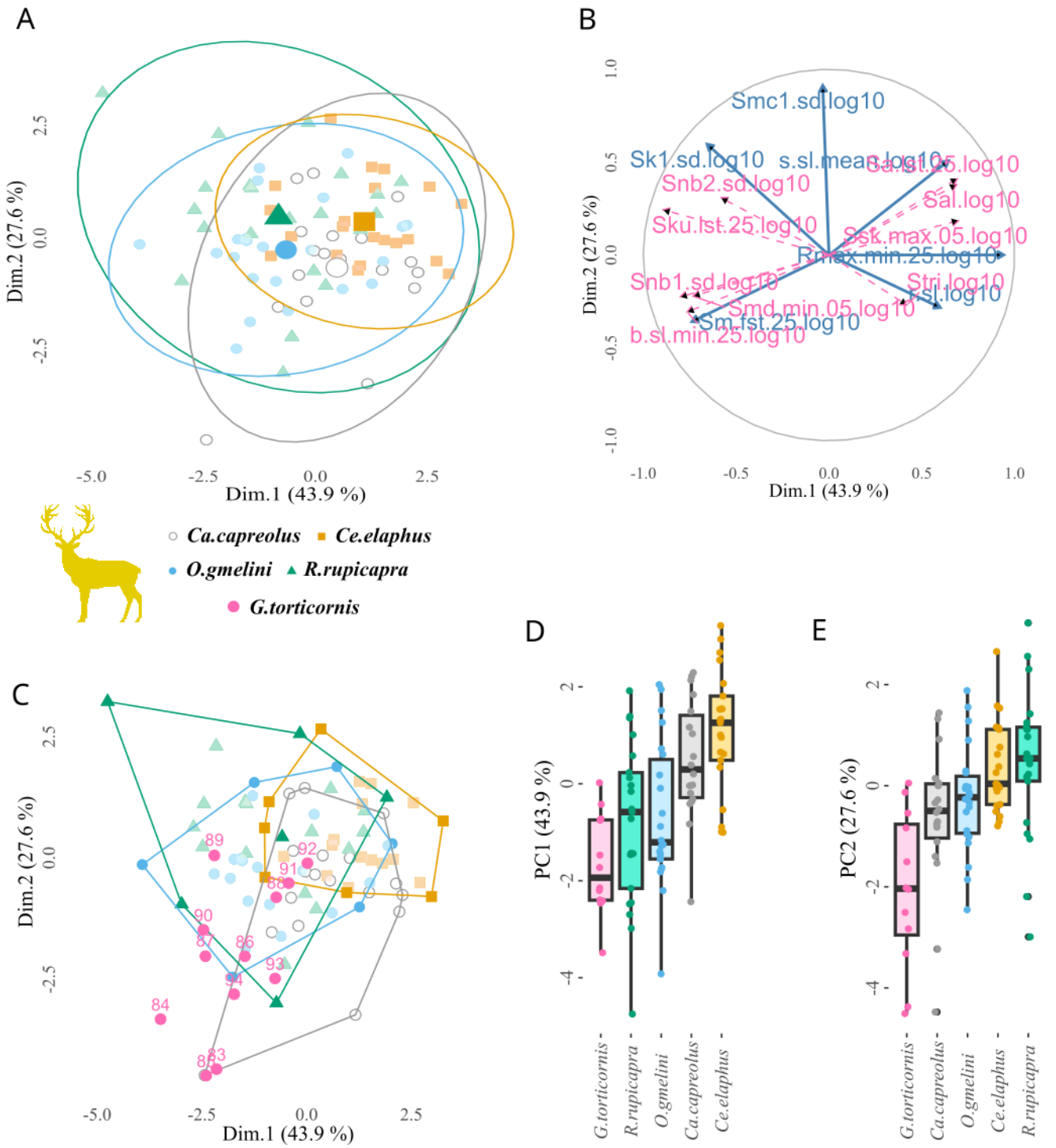

Figure 5. Case study C: Comparison with extant species to infer the diet of extinct species, PCA on selected variables with ruminants from the Bauges as individuals and fossil specimens of *Gazellospira torticornis* as supplementary individuals. A, Bivariate graph on extant specimens, PC1 versus PC2; B, Correlation circle with non-selected variables as supplementary variables (pink dashed lines), PC1 versus PC2; C, Bivariate graph of individuals along PC1 versus PC2, with the extinct species from Dafnero *Gazellospira torticornis* as supplementary individuals (pink); D, Boxplot of PC1 values, ordered by ascending mean, with species as factor; E, Boxplot of PC2 values, ordered by ascending mean, with species as factor. All data log-transformed (base 10). Ellipses depict the confidence interval at 95 %.

Discussion

Case study A: Diet-related differences in dental microwear

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

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

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

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

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

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

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

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

Lastly, variables that contribute to PC2 (Smc1.sd and to some extent, Sk1.sd and s.sl.mean) were significantly lower in *G. torticornis* compared to all four extant species. This point illustrates how dental microwear textures can present original patterns in the fossil record, sometimes completely different to what is known for extant species – perhaps reflecting how different the environmental conditions were at the time.

Conclusion

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

Acknowledgments

This study was funded by the French National Agency for Research (ANR-13-JSV7-0008-01 Trident, PI: Gildas Merceron; ANR-17-CE27-0002-02 DIET-Scratches, PIs: Gildas Merceron, Stéphane Ferchaud) and the Region of Nouvelle Aquitaine (ALIHOM #210389; PI: Gildas Merceron). We thank all the people who helped improve trident during its development, and especially Anusha Ramdarshan, Antoine Souron and Franck Guy. Our gratitude also goes towards the colleagues who allowed or participated in data collection for the three case studies, notably Jérôme Surault (PALEVOPRIM).

Authors' contributions

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

Data availability

The source package is available on Github at https://github.com/nialsiG/trident.

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