ORIGINAL RESEARCH

Revised: 30 April 2018

MicroWeaR: A new R package for dental microwear analysis

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Funding information

Sapienza Università di Roma, Grant/Award Number: C26A153RNH; AEI/FEDER EU, Grant/Award Number: CGL2016-76431-P

1 | INTRODUCTION

Dental microwear analysis studies microscopic wear patterns produced on the occlusal enamel surfaces of teeth during mastication.

Abstract

Mastication of dietary items with different mechanical properties leaves distinctive microscopic marks on the surface of tooth enamel. The inspection of such marks (dental microwear analysis) is informative about the dietary habitus in fossil as well as in modern species. Dental microwear analysis relies on the morphology, abundance, direction, and distribution of these microscopic marks. We present a new freely available software implementation, MicroWeaR, that, compared to traditional dental microwear tools, allows more rapid, observer error free, and inexpensive quantification and classification of all the microscopic marks (also including for the first time different subtypes of scars). Classification parameters and graphical rendering of the output are fully settable by the user. MicroWeaR includes functions to (a) sample the marks, (b) classify features into categories as pits or scratches and then into their respective subcategories (large pits, coarse scratches, etc.), (c) generate an output table with summary information, and (d) obtain a visual surface-map where marks are highlighted. We provide a tutorial to reproduce the steps required to perform microwear analysis and to test tool functionalities. Then, we present two case studies to illustrate how MicroWeaR works. The first regards a Miocene great ape obtained from through environmental scanning electron microscope, and other a Pleistocene cervid acquired by a stereomicroscope.

KEYWORDS

diet reconstruction, open-source software, paleoecology, R package, tooth microwear

It is one of the most valuable methods to assess dietary preferences in vertebrate taxa. Since the 1970s (see, among others, Gingerich, 1972; Grine, 1977; Puech, 1979; Walker, Hoeck, & Perez, 1978), microwear analysis has been successfully applied by anthropologists

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and paleontologists to gain insights into the diet of several extinct groups, such as primates, including humans and hominins (DeSantis, 2016; Scott et al., 2005; Teaford & Walker, 1984), ungulates (DeMiguel, Fortelius, Azanza, & Morales, 2008; Kaiser & Brinkmann, 2006; Mihlbachler, Campbell, Ayoub, Chen, & Ghani, 2016; Semprebon & Rivals, 2007: Solounias & Havek, 1993: Solounias & Semprebon, 2002), and carnivores (Schubert, Ungar, & DeSantis, 2010: Van Valkenburgh, Teaford, & Walker, 1990). Dental microwear analysis relies on the microscopic marks on the occlusal surfaces of tooth enamel (and/or dentin), left by the food chewed by an individual up to a few hours, days, or weeks before its death-a phenomenon referred to as the "Last Supper effect"-, depending on the rate of turnover in dental microwear of a particular consumer and food (Grine, 1986). The abundance, morphology, size, distribution, and orientation of marks are a consequence of the mechanic abrasion produced by mastication and are distinctive between different diets, depending on the fracture properties of the food items. In ungulates, a higher number of scratches over pits indicate tough-food (e.g., grasses) consumption. In contrast, a high number of pits indicate consumption of brittle, soft material such as leaves, fruits, and seeds (Solounias & Semprebon, 2002). In primates, a high occurrence of pits and coarse scratches is typical of hard-object feeders (which primarily feed on nuts and roots, and unripe fruits). Conversely, diet rich in leaves and soft fruits, which is typical of folivorous and frugivorous primates, is characterized by a low percentage of pits and narrower scratches (King, Aiello, & Andrews, 1999; Teaford, 1988).

The most common way to observe and study enamel marks is using high definition, two-dimensional pictures of a selected tooth crown region under either low or high magnification. The former, well-established approach, known as Low magnification microwear (LMM), employs high-precision casts of enamel surfaces observed by a standard stereomicroscope at 35× or 100× (for small mammals) magnification. Because it is fast and relatively low-cost, LMM is probably the most common dental microwear method today (Bastl, Semprebon, & Nagel, 2012; Rivals & Athanassiou, 2008; Rodrigues, Merceron, & Viriot, 2009; Semprebon, Taob, Hasjanova, & Solounias, 2016; Solounias & Semprebon, 2002). High magnification microwear (HMM) relies instead on pictures obtained through scanning electron microscope (SEM; DeMiguel et al., 2008; Galbany, Martínez, & Pérez-Pérez, 2004; King et al., 1999; Solounias, McGraw, Hayek, & Werdelin, 2000; Solounias & Moelleken, 1994), typically at 500× magnification. With environmental SEM (ESEM) devices, teeth can be observed directly without any damage, avoiding the risk of losing fine details during cast preparation. The downside of HMM is that it is more expensive and slower than LMM. Under both methods, enamel marks are classified, counted, and measured on a standard square area, whose size depends on the specific magnification adopted.

The recently introduced Dental microwear texture analysis (DMTA) (Merceron et al., 2009; Scott, Teaford, & Ungar, 2012; Scott et al., 2005; Ungar, Krueger, Blumenschine, Njau, & Scott, 2012) provides an alternative to both LMM and HMM. DMTA works with 3D surfaces and scale-sensitive fractal data. Unlike the traditional

methods, DMTA does not require the identification of any individual feature, and the analysis is automated, thus being faster and less affected by observer error than more traditional methods (Scott et al., 2005). However, DMTA is an expensive method, as it requires the use of white-light scanning confocal microscopes (rather than simple 2D micrographs), and uses specific commercial software (Surfract[®], ©2007; http://www.surfract.com/) and additional plugins (e.g., ToothFrax and SFrax) that increase the economic burden of the approach. Moreover, whereas traditional approaches record individual wear features to better understand individual morphologies and their orientations, DMTA focuses only on the overall pattern.

Both traditional (LMM and HMM) methods and DMTA require a software application to count and score enamel marks. Such software, except for *Microware* (Ungar, 1995), has never been specifically designed for microwear analysis and usually requires a costly license. In the case of *Microware*, one disadvantage is that it cannot discern between different subtypes of microscopic marks (e.g., large pits, coarse scratches). We therefore feel it is time to develop a freely available tool, specifically designed for microwear analysis, which allows for a more in-depth and complete investigation of the tooth occlusal features.

Here, we introduce MicroWeaR, a new free, open-access tool stored as an R package (Profico, Strani, Raia, & DeMiguel, 2018) that examines and scores microwear marks in a semiautomatic way. The method is designed to optimize sampling and classification of microscopic marks on high-resolution pictures of tooth surfaces, under different magnification levels. Using a picture of a dental surface (provided with a metric reference for the definition of the scale factor) as the input, the operator defines the size and position of a working area first, and then tracks the microwear features. Each mark is automatically classified into one of the two main categories, either "scratch" or "pit." It is important that, for each of these two categories, the tool recognizes two subcategories "small" and "large" pits, and "fine" and "coarse" scratches, and provides the user with summary statistics for each category and subcategory (count, mean, and standard deviation). We also provide MicroWeaR R code (R Development Core Team, 2009) along with the description of the application procedure. To illustrate the effectiveness of MicroWeaR, we further examined two case studies belonging to different taxonomic groups and different methodological procedures to obtain microwear information: a molar of the Miocene great ape Anoiapithecus brevirostris (see DeMiguel, Alba, & Moyà-Solà, 2014) and a molar of the Pleistocene cervid Cervus elaphus eostephanoceros (Strani et al., 2018).

2 | DESCRIPTION: MICROWEAR AS A TOOL FOR ESTIMATING MAMMAL DIETS

MicroWeaR has been developed to sample and semiautomatically classify multiple features from a picture at once. The tool functions (Table 1) support a variety of image file formats (i.e., "bmp," "png," "jpg," and "tif") and convert the input image into an .lco object. The

TABLE 1 List and descriptions of the functions embedded in the *MicroWeaR* R package

Function	Description
class.lco	Convert an image into an object of class Ico. At present, the formats "jpeg," "png," and "tiff" are supported. Limited to grayscale images
plot_lco	Plot an image of class Ico. Setting the matrix that contains the coordinates of the microwear marks as set, the function returns to the image
scale_lco	Scale an image of Ico class by an interactive plot selecting two points on the metric reference and defining the length of the latter
Warea.lco	Select a working area of an image of class Ico through an interactive plot. The operator has to select the center of the working area and its dimensions
samp.traces	Record detectable microwear marks through the interactive plot. <i>samp. traces</i> has an option to zoom in or out of the image of class Ico
autom_class	Classify the microwear marks in different subcategories as recorded by <i>samp.traces</i> (object type). The output also provides a matrix (object Matrix), where the length and the width in micron are reported for each mark. In addition, the image with recorded marks is produced
cross.parallel	Detect pairs of scratches, which are "parallel" or "crisscross"
output.lco	Print a summary statistics table reporting the number of pits and scratches (and the size of any subcategory)
mw.check	Check (via interactive multi-plot) the classification provided by the autom_class function. Before running <i>output.lco</i> using the a posteriori classification, the user must run again <i>cross.parallel</i> using the updated microwear classification

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R code provides the user with an interactive plot to scale the .lco object to its original size using a metric reference that should be embedded in the picture. For each microscopic feature sampling is achieved by recording two distances using the left-click: the first one records the mark length, and the second its width. During the sampling procedure, the user may use the undo command to revert to a previous step and to zoom the picture in or out.

At the end of the sampling session, the function *autom_class* provides an automatic classification of the marks as either pits or scratches. In turn, each pit is categorized as either "large" or "small" and each scratch is classified as either "fine" or "coarse." Automatic classification parameters can also be set manually to customize the sampling procedure. The tool provides an additional function of direction to detect pairs of "parallel" and "crisscross" scratches. The *autom_class* function outputs a summary statistics table that can be exported in different format files (.txt, .sav for SPSS Statistics software, .csv for Excel spreadsheet), which includes the number of features of each type, the standard deviation and mean diameter of the pit, fine and coarse scratch lengths, and coarse scratch widths. Using the function *autom_class*, the user is able to save the original picture overlaid by a transparent layer of the identified microscopic marks highlighted with a distinctive, user-defined color. The graphical rendering of the final output is itself fully customizable.

3 | APPLICATION OF THE MICROWEAR PROCEDURE USING REAL CASE STUDIES

We provide two case studies as examples of the step-by-step application of *MicroWeaR*. These are the enamel occlusal surfaces of a lower left second molar (m2) ("Phase II" crushing/grinding facet 9) of the Miocene great ape A. brevirostris (see DeMiguel et al., 2014) and an upper right first molar (M1) (antero-lingual enamel band of the paracone) of the Middle Pleistocene cervid C. e. eostephanoceros (see Strani et al., 2018). The photomicrograph of the former was acquired through ESEM (at ×500 magnification) on the original specimen (Figure 1a), whereas the image of the latter was obtained using a stereomicroscope (×35 magnification) from a cast (Figure 1b). The mold and the cast of the molar tooth crown of C. e. eostephanoceros were prepared following standard procedures (Semprebon, Godfrey, Solounias, Sutherland, & Jungers, 2004; Solounias & Semprebon, 2002). The impression was made using high-resolution Elite HD+ polysiloxane for the mold, and Araldite epoxy polymer for the cast. According to that, we provide microwear examples obtained from both high (×500) and low (×35) magnification and using either tooth originals or replicas. More comprehensive information on the taxa and the full description of the cleaning, molding/casting and examination procedures are available in DeMiguel et al. (2014) and Strani et al. (2018).

The MicroWeaR package supports the file formats "bmp," "jpg," "tif," and "png." As the first step, the MicroWeaR library is loaded into the R workspace. All the dependencies will be automatically installed or loaded as well. To begin the session, the user specifies the arguments *path* and *image.type* to import the image specifying where the file is located and its file format respectively.

require(devtools)
install _ github("MicroWeaR/MicroWeaR",local=FALSE)
library(MicroWeaR)

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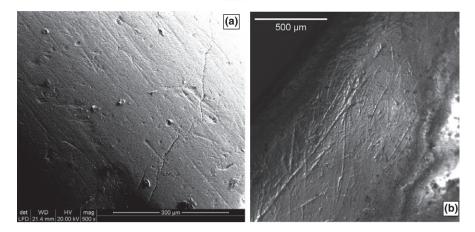


FIGURE 1 Enamel surface of the molars of *Anoiapithecus brevirostris* (a) and *Cervus elaphus eostephanoceros* (b)

library(zoom)
#load picture of C. e. eostephanoceros
data(C_el_pic)
#or load your picture typing:
#class.Ico(path, image.type = c("jpg", "png", "tiff"))

The function *scale_lco* scales the picture to the real size in micron (μ m). The scaling procedure requires the selection of two points on the image. In a successive way, the operator will specify the scale length on the console.

#load scaled picture of C. e. eostephanoceros
data(C_el_sca)
#or scale your picture typing:
#scale Ico(image.ico)

After loading and scaling the image, the operator defines a working area (e.g., $200 \times 200 \ \mu$ m) and a magnification factor to be applied. The argument *sizes* of the function *area.param* allows setting the default square working area size to be displayed in the interactive 2D plot during the sampling session. By default, either $200 \times 200 \ \mu$ m, $400 \times 400 \ \mu$ m or $600 \times 600 \ \mu$ m working areas are selected, yet the user can define a custom area by choosing the "select" option and typing the desired size (side length) on the console (Figure 2a).

```
#load the selected working area
data(C _ el _ war)
#or select the working area typing:
#Warea.Ico(image.ico)
```

Once the working area is defined, the sampling session begins (Figure 2b). The operator defines four points for each mark: the first two record the mark length, and the last two its width (Figure 2c).

```
#load the sampling session
data(C_el_sam)
#or start the sampling session typing:
#samp.traces(image.ico)
```

The arguments *cexp* and *lwdp* define the size and width of the points and lines of the marks, respectively. Considering that the

image is scaled in micron, we suggest setting these parameters in respect to the dimension of the scaled picture, or inserting any other reasonable number (e.g., cexp = 50; lwdp = 1). In any case, if the *cexp* and the *lwdp* parameters are set as *NULL* the *samp.traces* function will adjust the values of these parameters automatically.

After the manual sampling, the tool automatically classifies each mark within one of the two categories of features: "scratch" and "pit" (Figure 2d). The classification is based on the length/width ratio; by default, this is set to 4 μ m (≤4 for Pit and >4 for Scratch as proposed by Ungar, 1995). For each of these two categories, the tool recognizes different subcategories based on the diameter (for pits) and width (for scratches): "small" and "large" for pits (by default diameter ≤8 and >8 μ m, respectively), and "fine" and "coarse" for scratches (by default the width ≤3 and <3 μ m, respectively). All default discriminating values can be changed by the user in the *autom_class* function by editing the *Pit_Scr, Sm.Lg_pit* and *Fi.Co_Scr* arguments.

```
# run type classification
class<-autom_class(C_el_sam,C_el_war$image)
#or run the automatic classification typing:
#autom_class(big_matrix, image.ico, Pit_Scr = 4,
Sm.Lg Pit = 8, Fi.Co Scr = 3)</pre>
```

The function *cross.parallel* calculates all the combinations of scratches and finds crossed and parallel scratch pairs. In detail, this function calculates the linear equation of the line passing through the two points that define the length of each mark. *MicroWeaR* uses the regression model parameters (intercepts and slopes) to classify scratch pairs as parallel (if the distance between the two scratches and their intersection point is greater than two-times the square of the working area), or crisscross (if otherwise). In the latter case, the angle between intersecting scratches is calculated and produced in the output.

scratches.ana<-cross.parallel(big_matrix= C_el_ sam,image.ico= C el war\$image,Type=class\$Type)

In addition, *MicroWeaR* provides a summary statistics report for each category and subcategory (including count, mean, and standard deviation) and the input picture with the sampled marks that can be

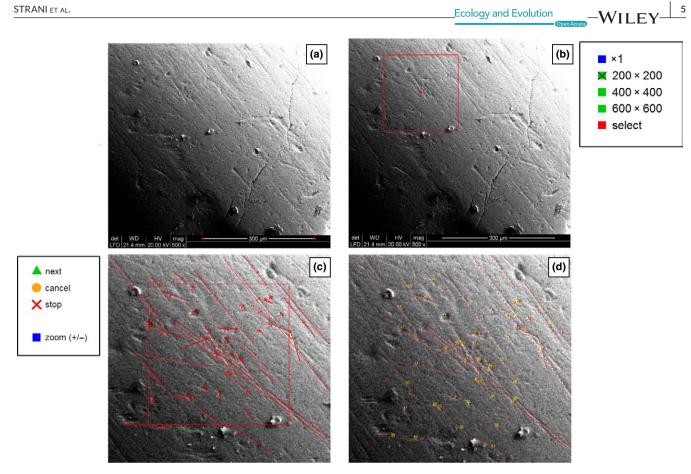


FIGURE 2 Step-by-step summary of semiautomatic enamel mark recognition performed using MicroWeaR. (a) Selection of two points on the reference metric scale to scale the image (top left). (b) Selection of the working area and size ("x1": the size of the working area corresponds to the size of the input image; "select": by selecting this option, the user can customize the size of the working area). (c) Sampling session (the "next" command allows to sample a new feature, the "cancel" command undoes the last sampling step, the "stop" command stops the sampling session, the "zoom" command allows to zoom in and out). (d) Sampled features displayed on the output image

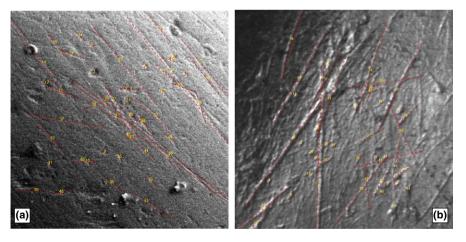


FIGURE 3 Final output images of Anoiapithecus brevirostris (a) and Cervus elaphus eostephanoceros (b). Microwear features were sampled on a 200 and a $400 \,\mu\text{m}^2$ area, respectively

exported in different file formats. Automatic classification parameters can also be manually edited and set allowing customizing each sampling session.

At last, using the function output. Ico and specifying the matrix with the coordinates of the microwear marks, an image with the displayed marks is loaded as a plot (Figure 3).

output.Ico(C _ el _ sam, class\$Type, scratches. ana,C_el_war)

We provide a video tutorial as Supporting Information (Video S1) for the application of the tool in R environment.

3.1 | Case studies interpretation

Regarding the occurrence of pits (N = 17), A. brevirostris resembles extant frugivores/mixed feeders such as Cebus nigrivittatus. It further displays somewhat wide scratches (Mean_width = $2.77 \mu m$), in the range of *Pan troglodytes* (Mean_width = 2.6 μ m) and *Pongo pygmaeus* (Mean_width = 2.8 μ m), which suggests a certain degree of sclerocarpy. The results obtained by DeMiguel et al. (2014) show that, on average, *A. brevirostris* diet is somewhat intermediate in between *P. pygmaeus* and extant frugivores/mixed feeders such as *P. troglodytes* in terms of pitting incidence (*N* = 22), whereas it is similar to extant frugivores/ mixed feeders in scratch width (Mean_width = 1.98 μ m). These results confirm a soft-fruit diet (albeit with some sclerocarpic components) and are fully consistent with those obtained using *MicroWeaR* (Table 2).

The dental microwear pattern of the Pleistocene deer *C. e. eostephanoceros* has a similar amount of pits (N = 21) and scratches (N = 25) according to the *MicroWeaR* semiautomatic classification (Table 3). Most scratches are short and finely textured with a few long coarse scratches (Mean_length = 415.92 µm). Cross scratches are also detected (N = 15). Small pits are more abundant than larger ones (N = 13and N = 8, respectively). A high number of pits and scratches with a prevalence of finely textured features indicates that *C. e. eostephanoceros* fed on a variety of plant types (both soft and abrasive), as commonly observed in modern mixed feeders (Solounias & Semprebon, 2002). The findings obtained using *MicroWeaR* are thus consistent with those obtained by Strani et al. (2018) where a larger, more indicative sample of *C. e. eostephanoceros* studied using both LMM and dental mesowear analysis, indicated a mixed feeder diet for this species.

4 | SIGNIFICANCE OF THE TOOL

Using traditional LMM and HMM methods, one key factor affects the validity of the results, that is how different operators count

and discriminate among microscopic marks (DeSantis et al., 2013; Mihlbachler, Beatty, Caldera-Siu, Chan, & Lee, 2012). The use of a semiautomatic approach minimizes the intraobserver error because the only manual step in the whole procedure is the definition of the initial and the end point of each enamel mark. The automatic differentiation between subcategories also helps to reduce interobserver error rates when it comes to detailed interpretation of microwear features, which are usually high with traditional semiautomatic approaches (Galbany et al., 2005; Grine, Ungar, & Teaford, 2002; Mihlbachler et al., 2012). Given that MicroWeaR can be used for the analysis of any 2D image containing scars, it is also useful for recording lineal striations (i.e., number, length and breadth of scratches) in micrographs taken on nonocclusal tooth surfaces and, therefore, extensible to buccal enamel microwear quantification (Galbany & Pérez-Pérez, 2004; Pérez-Pérez, Lalueza, & Turbón, 1994; Puech, 1981) as well.

Since the creation of the R platform, libraries addressing natural science applications have rapidly increased (R Core Team, 2000). The open-access nature of the R platform allows tools to be rapidly improved, by introducing new functionalities that are under immediate diffusion and testing through the R community. According to that, we designed *MicroWeaR* in order to work under different operating systems (i.e., Windows, OSX, Linux).

MicroWeaR allows the automatic classification of the marks left on the enamel surface by the last foods (Grine, 1986) processed. Such automaticity helps keeping inter- and intraobserver error low (categories automatically assigned to each mark can be nonetheless manually edited using the *mw.check* function; Figure 4) and makes

	N.pits	N.sp	N.lp	%р	Р	N.scratches	N.fs	N.cs	S	N.Ps	N.Xs	%Ps	%Xs
Count	17	9	8	33.3	425	34	20	14	850	62	9	85.3	26.5
Mean_length	7.64	5.29	9.73	/	/	20.94	22.38	18.87	/	/	/	/	/
Sd_length	3.75	1.06	4.08	/	/	19.24	23.14	12.21	/	/	/	/	/
Mean_width	2.86	2.54	3.14	/	/	2.77	1.13	5.12	/	/	/	/	/
Sd_width	1.95	1.54	2.31	/	/	2.41	1.41	1.35	/	/	/	/	/

TABLE 2 Results of the microwear analysis applied to a tooth of Anoiapithecus brevirostris

Note. N.pits: number of pits; N.sp: number of small pits; N.lp: number of large pits; %p: percentage of pits; P: pits/mm²; N. scratches: number of scratches; N.fs: number of fine scratches; N.cs: number of coarse scratches; S: scratches/mm²; N.Ps: number of pairs of parallel scratches; N.Xs: number of scratches that cross each-other; %Ps: percentage of parallel scratches; %Xs: percentage of scratches that cross each-other.

TABLE 3 Results of the microwear analysis applied to a tooth of Cervus elaphus eostephanoceros

	N.pits	N.sp	N.lp	%р	Р	N.scratches	N.fs	N.cs	S	N.Ps	N.Xs	%Ps	%Xs
Count	21	13	8	45.7	131	25	17	8	156	4	15	20.0	36.0
Mean_length	20.38	11.96	34.06	/	/	240.52	157.98	415.92	/	/	/	/	/
Sd_length	14.52	5.79	14.11	/	/	178.58	108	176	/	/	/	/	/
Mean_width	4.52	2.73	7.43	/	/	1.66	0.73	3.62	/	/	/	/	/
Sd_width	4.73	2.24	6.3	/	/	2.36	0.93	3.25	/	/	/	/	/

Note. N.pits: number of pits; N.sp: number of small pits; N.lp: number of large pits; %p: percentage of pits; P: pits/mm²; N. scratches: number of scratches; N.fs: number of fine scratches; N.cs: number of coarse scratches; S: scratches/mm²; N.Ps: number of pairs of parallel scratches; N.Xs: number of scratches that crosses each-other. %Ps: percentage of parallel scratches; %Xs: percentage of scratches that cross each-other.

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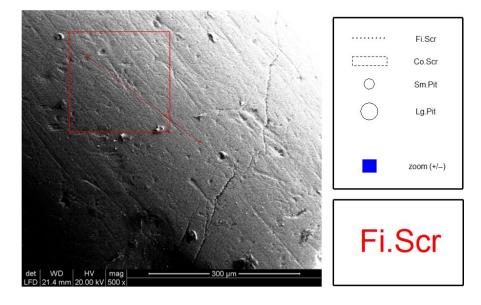


FIGURE 4 Additional *MicroWeaR* functionality: classification editing. The automatic classification of each mark can be manually edited at the end of the procedure using a multiplots interactive interface. Co.Scr: coarse scratch; Fi.Scr: fine scratch; Lg.Pit: large pit; Sm.Pit: small pit

the dental microwear analysis faster, more robust, and cheaper than with any other comparable application.

CONFLICT OF INTEREST

None declared.

5 | CONCLUSIONS

A new software implementation for dental microwear analysis, *MicroWeaR*, offers a semiautomatic open-access tool for quantification and classification of the microscopic enamel marks, stored as an R package. *MicroWeaR* is less time-consuming and less prone to observer errors in comparison with the conventional microwear analysis with twodimensional imaging methods (LMM, HMM), as it is inexpensive compared to a new three-dimensional method (DMTA). It works for any 2D image containing microwear scars. Thus, it is useful for the quantification of marks as observed under either high or low magnification, on both occlusal and nonocclusal (e.g., buccal) tooth surfaces (dentin or enamel), and from either tooth originals or replicas. *MicroWeaR* is designed to work in different operating systems (e.g., Windows, OSX, Linux) and due to its intrinsic characteristics, it is unique to be developed further.

ACKNOWLEDGMENTS

This work has been possible thanks to Scienze della Terra—Sapienza, Università di Roma, to Ricerche Universitarie, Sapienza 2015 (ref. C26A153RNH to Raffaele Sardella) and to Grandi Scavi 2016 (ref. SA116154CD9592F3 to Raffaele Sardella). We also thank support from the AEI/FEDER EU (CGL2016-76431-P). We thank Mikael Fortelius for the helpful comments and advices. We acknowledge Fabio Parenti and the Italian Institute of Human Palaeontology for preserving the collection and allowing the study of the *C. e. eostephanoceros* specimen and Laura Sadori (Dipartimento di Biologia Ambientale, Sapienza—Università di Roma) for providing access to the laboratory facilities where the dental cast has been analyzed. We are grateful to three anonymous reviewers for providing insightful comments on the earlier version of the manuscript.

AUTHORS' CONTRIBUTIONS

F.S., A.P., P.R., and D.DM. conceived the ideas and designed methodology; D.DM. and F.S. collected the data; F.S. and A.P. wrote the R code with the contribution of P.R. and D.DM.; F.S., A.P., P.R., and D.DM. led the writing of the manuscript and contributed to the implementation of example analyses. D.P., R.S., and G.M. contributed helpful comments and provided inputs for the manuscript. All authors revised the manuscript and gave final approval.

AVAILABILITY AND DATA ACCESSIBILITY

MicroWeaR can be downloaded from https://github.com/MicroWeaR (https://doi.org/10.5281/zenodo.1233505). We encourage authors to cite Strani et al. (this paper) if you use *MicroWeaR* for research, education, and outreach. As an application designed to be part of R, *MicroWeaR* is available as a package to run on different operating systems (Windows, Mac OS or Linux).

The results reported in this paper were obtained using *MicroWeaR* R package. The code and real examples of use are available in the *MicroWeaR* R package.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Strani F, Profico A, Manzi G, et al. MicroWeaR: A new R package for dental microwear analysis. *Ecol Evol*. 2018;00:1–9. https://doi.org/10.1002/ece3.4222