

RESEARCH ARTICLE

Folded: A toolkit to describe mammalian herbivore dentition from 2D images

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Handling Editor: Nicolas Lecomte**Abstract**

1. Dental morphology is a major aspect of ecological and evolutionary studies of both extant and fossil mammalian species. Mammalian dentitions are diverse feeding systems that can be defined through continuous numerical descriptors of the enamel pattern.
2. We developed a comprehensive toolkit to quantify complex occlusal enamel patterns from two-dimensional images of herbivore mammals, widespread in the scientific literature, in form of three novel enamel complexity descriptors: two-dimensional orientation patch count (2D OPC), enamel folding (EF), and enamel thickness (ET). Previously proposed parameters such as occlusal enamel index or indentation index are implemented as well.
3. The current method is devised for extracting continuous variables of enamel complexity from macro and microherbivore mammalian species with conspicuous wear facets. A general case study is proposed using two clades within the Family Rhinocerotidae containing species regarded as hypsodonts. The results show that antagonist dental adaptations were achieved through disparate evolutionary strategies in both groups. To test the robustness of this tool under different practical scenarios, other mammalian groups have been evaluated as well. Additional sensitivity analyses include the impact of image size, rotation, or differences in dental wear.
4. Our approach differs from previous 2D techniques in its affordability, versatility, and control over individual regions within each tooth while delivering continuous numerical data. Additionally, the 2D reference images required as input are widespread in the literature and easier to process in comparison to 3D data alternatives.

KEYWORDS

dental complexity, dental topology, dentition, enamel orientation, enamel thickness, macroecology, palaeontology, ungulates

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1 | INTRODUCTION

The enamel pattern of herbivore teeth and tooth crown height are regarded as phenotypic features with sound ecological effects and provide important information on the ability of individual species to cope with habitat conditions (Archer & Sanson, 2002; Damuth & Janis, 2011; Evans et al., 2007; Famoso et al., 2013; Fortelius, 1985; Fortelius & Solounias, 2004; Heywood, 2009; Kaiser et al., 2009). Large-scale analyses of dental morphological data have received increasing attention over the past decades, providing insights into current and past environmental settings (Kaya et al., 2018; Žliobaitė et al., 2016) and their impact on deep-time diversity patterns (Cantalapiedra et al., 2021; Žliobaitė et al., 2017). Additionally, teeth are relevant due to the eminent role of dental morphology in taxonomy, as systematics studies strongly rely on dental diagnostic characteristics. Finally, teeth are common elements in museum collections as a result of their better preservation, being available for most living and fossil species.

Occlusal features of molars capture several aspects of the mechanics and durability of dental pieces during mastication (Famoso & Davis, 2014; Kaiser et al., 2009; von Koenigswald et al., 2014). Thus, these occlusal traits are good candidates to serve as proxies for the diet and the environment of extinct species, having attracted a lot of interest in evolutionary and paleoecological studies. One of the dental features that have received more attention is occlusal 'dental complexity'. Dental complexity, complexity from here on, has been defined as any parameter measuring the number of features, 'tools' or 'breakage sites' on a tooth (Evans et al., 2007; p. 78). The degree of enamel complexity is correlated with both body size and diet in different ungulate lineages: highly convoluted enamel on cheek teeth is linked with diets focused on higher abrasiveness and/or tough items like monocotyledonous grasses and/or grit. (Evans & Pineda-Munoz, 2018; Famoso et al., 2013).

A series of 'topometric' descriptors (sensu Gailer et al., 2016) have been proposed for parametrizing tooth complexity from two-dimensional (2D) images. These can be broadly divided into three groups depending on the variables used to estimate them. The first one makes use of the length of the enamel bands through their midline (OEL) and the projection of the tooth's 2D occlusal total area (OTA) in occlusal view. In some cases, OEL and OTA help quantify the ratio of enamel length per area: a higher enamel length provides an increased resistive cutting area for food processing during mastication. Two examples are the enamel index (EI; $EI = OEL/OTA$; Becerra et al., 2012) or the Occlusal Enamel Index (OEI; $OEI = OEL/\sqrt{OTA}$; Famoso et al., 2013). In others, like dental structural density, OEL and OTA are used to estimate the deviation of the occlusal enamel morphology from circularity, as exemplified by the indentation index (D; $D = OEL^2/(4 \cdot \pi \cdot OTA)$; Schmidt-Kittler, 1984, 2002). A second group uses the discrepancies between the outer and the inner enamel ridges using both area and length. An example of this approach is the relative inner enamel width (EW). EW is the quotient of the inner enamel area-length ratio by the area-length ratio of the total enamel, or, in other words, the width of the internal

enamel structures relative to the overall width of occlusal enamel (Gailer et al., 2016). A third alternative to estimate 2D complexity is fractal dimensionality (FD), which captures how enamel edges fill the available image area in form of a ratio between image scale and increasing enamel detail (Mandelbrot, 1982). FD is typically calculated by breaking down the image into boxes of gradually decreasing dimensions and quantifying the detail within. Depending on the degree of complexity, FD ranges from 1, a single straight line traversing a certain box, to 2, complex sinuous patterns filling nearly all the available space. The main advantage of FT relies on its ability to quantify enamel complexity independently from body size (Famoso & Davis, 2016).

The use of three-dimensional (3D) data opened the study of additional topographic descriptors of dental complexity that aim to quantify diet-based dental adaptations. For example, 3D orientation patch count (3D OPC) measures the number of separate surface patches facing eight compass directions (Evans, 2013; Evans et al., 2007; Ledogar et al., 2013; Santana et al., 2011; Wilson et al., 2012; Winchester et al., 2014). The number of patches is a robust measure of a broad diet. A higher patch count reflects more intricate molar surfaces and higher resistance to dietary abrasion (Boyer et al., 2010; Evans & Pineda-Munoz, 2018; Wilson et al., 2012). Other studies mapped enamel thickness (ET) variations in 3D at a local scale (e.g. Martín-Francés et al., 2020). Despite the increasing number of techniques using 3D data and the initiatives to make surface and volumetric models accessible and freely available, 2D images are still prevalent in the literature, as their availability vastly surpasses their 3D counterparts. Additionally, 3D scanned data often require computationally expensive pre-processing and the use of specialized and costly software to improve raw mesh/voxel information, further biasing its potential users (Das et al., 2017).

Tooth walls tend to be thicker on cheek teeth, as they constitute mechanical protection against the maximum bite forces going through them (Dumont, 1995; Schwartz et al., 2020 among others). Variations in ET within a single dental piece can be quite remarkable: labio-lingually oriented outer walls of Perissodactyla and Artiodactyla show a considerable enamel thickening while the internal, mesio-distally oriented ones are thinner and more intricate. Thus, ET is a valuable source of information for the masticatory performance of the tooth (Gailer et al., 2016). Previous attempts to use ET with 2D metrics include linear measurements at homologous spots through different dental cross sections (Winkler & Kaiser, 2015a, 2015b) or measuring EW of the internal enamel structures relative to the overall width of occlusal enamel (relative inner EW; Gailer et al., 2016). More recently, 3D techniques allowed for mapping and analysing 3D ET and to gauge the ratio between enamel volume and the tooth's total volumes (e.g. Buti et al., 2017; Schwartz et al., 2020). All these studies stress the importance of enamel wall thickness as a key descriptor of dental performance, being, therefore, included in the present workflow as an additional numerical variable.

'Folded', the new toolkit described in this work, provides the instruments necessary to perform a comprehensive numerical description of the 2D occlusal patterns of herbivore mammal enamel

by means of three parameters: 2D orientation patch count (2D OPC), enamel folding (EF), and local ET. In the case of 2D OPC, adjacent linear segments with similar orientations are grouped into patches. Frequent changes in enamel orientation reflect complex tooth's configurations. Alternatively, orientation is quantified by means of EF, defined as the inverse of 'local coherency' as described in Püspöki et al. (2016). Coherency analysis has been demonstrated to be a reliable method of quantifying organic tissues (Clemons et al., 2018; Rezakhanliha et al., 2010), but has never been applied to dental enamel data. A folding index of zero would represent a completely straight, filamentary structure (such as a single, unbend enamel band) and tends to be one for pixels with no dominant orientation like in the case of sharp curves. Besides these newly proposed complexity parameters (2D OPC, EF, and ET), previously described ones such as OEI, EI, D, and FD are estimated for further evaluation. The method's pipeline is automatic, requires traced 2D images as input (Figure 1) and can process large numbers of specimens in seconds. The input can be obtained from images of worn molars in occlusal view (including the immense number of photographs/diagrams published in the literature), thus providing the opportunity for analysing vast amounts of data at virtually zero cost. Our approach will be of particular interest to the many institutions with extensive neontological or paleontological collections lacking access to 3D scanning devices or cutting-edge processing software, as well as research teams with limited budgets to visit museum collections.

2 | MATERIALS AND METHODS

2.1 | Step-by-step user guide

This first section details a step-by-step guide on how to integrate the script 'folded' into the user's workflow. All these steps were run on an Intel Core i9-9900K CPU @ 3.60GHz computer with 32GB of usable RAM. A more detailed version of this guide can be found in Supplementary Data 1 and at <https://github.com/iarganda/folded>.

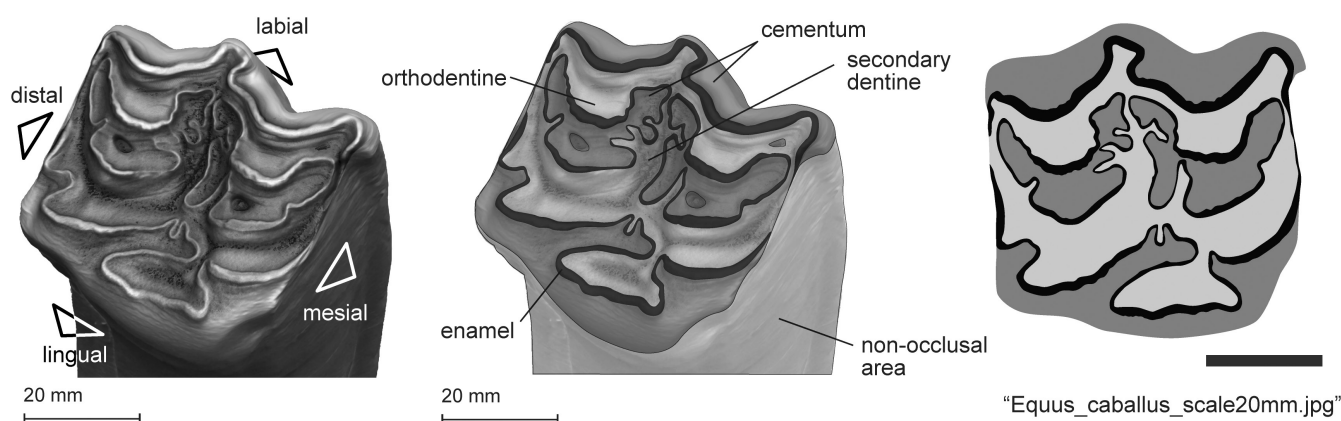


FIGURE 1 Example of tooth (M2 of *Equus ferus caballus*) showing the three main tissues (enamel, dentine, and remaining tooth) segmented as an input for the present analysis.

2.2 | Image preparation

1. **Select the reference images used for the analysis:** All specimens should show at least some wear. Exposed enamel and dentine should be visible at the occlusal surface.
2. **Digitize the sample:** This step could be done with any graphics software. We recommend using vector graphics due to its ability to generate clean boundaries between colour patches. In our case, the open-source vector graphics editor Inkscape v.1.2 (Inkscape development team, 2022) was used to trace the boundaries of enamel, dentine, and the rest of the tooth for each reference image and colour them according to the following hexadecimal colour coding: #000000, occlusal enamel of both attritional and abrasional facets; #CACACA, occlusal exposed dentine (orthodentine + secondary dentine); #5D5D5D, tooth projection of non-occlusal areas including cementum (the latter according to the criteria described in Famoso & Davis, 2014, p. 5) and #1E1E1E for scale (Figures 1 and 2a,b). Variations in ET were traced when clearly visible, otherwise assumed constant. We exported the vector traced files in RGB colour mode and JPEG format using the antialiasing export option to prevent interpolated pixel values between the mentioned colour coding (PNG and TIFF formats are also supported by our script but generate larger file sizes). 'folded' allows the user to define the size of the processed images. Image size effect is negligible above 500 pixels for the studied dataset (as demonstrated in Supplementary Data 2; Figure 2). However, we recommend using 1000 pixels along the largest image dimension to optimize computational performance on resolution-dependent steps. All traced images should be included in the same folder. Additional information on how to obtain ready-to-use images can be found in Supplementary Data 1.

2.3 | Image data analysis

3. **Install ImageJ/Fiji software:** Our script makes use of open-source image processing toolkit Fiji (with ImageJ v. 1.53q).

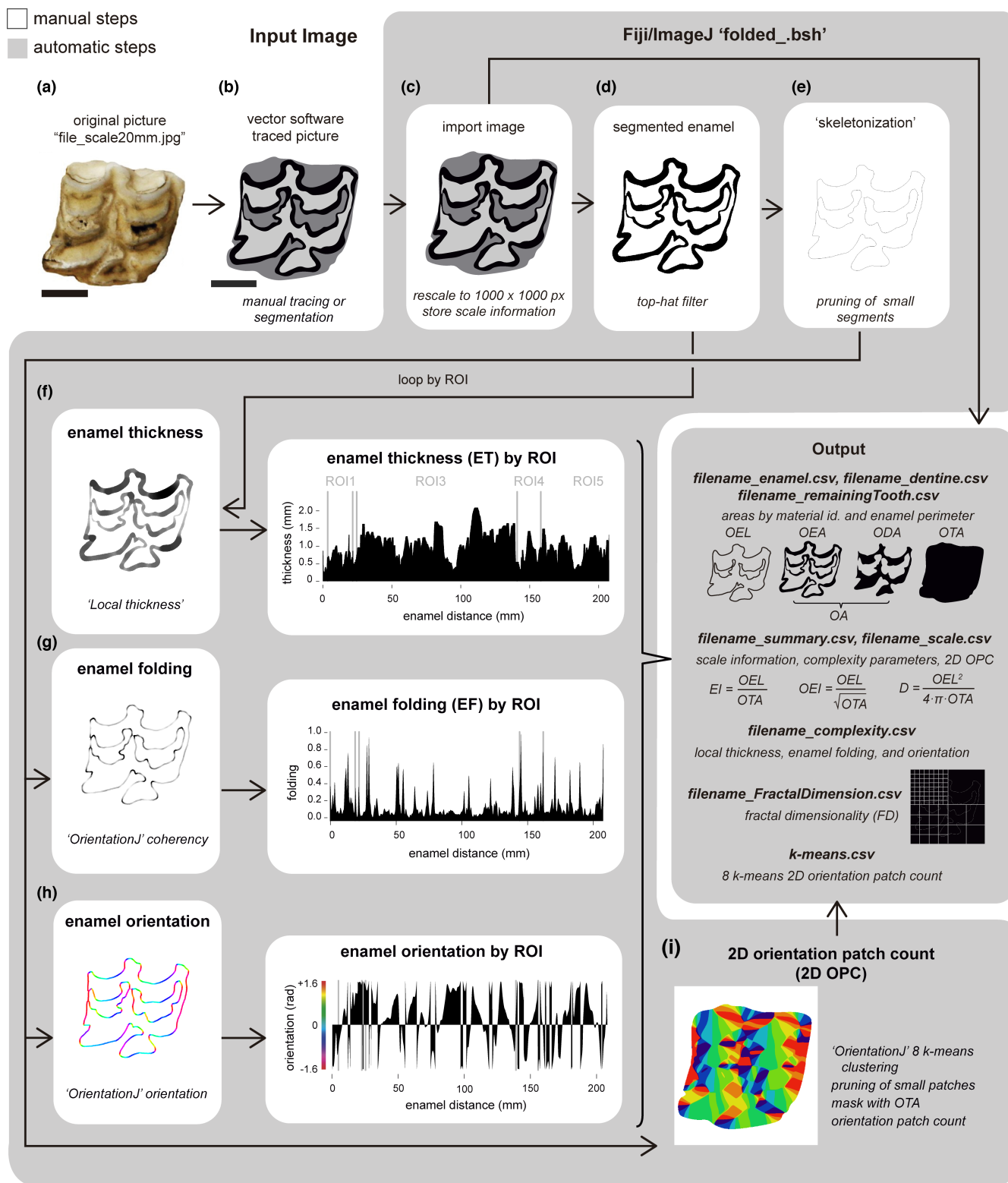


FIGURE 2 Scheme of the workflow followed in the present study using an upper third premolar of *Equus ferus caballus* MVZ 154358 originally figured in Famoso and Davis (2016) as an input. The resulting parameters are encoded by 'folded' as coherency, thickness, and orientation per region of interest (ROI). Additionally, the script performs a 2D orientation patch count analysis together with the estimation of additional, previously proposed complexity descriptors. See material and methods for a comprehensive description of each step (a-i). The inset figures included for enamel folding have been enhanced for visualization purposes. Similarly, the background colour of the generated images for 2D OPC, enamel folding, enamel thickness, and orientation images have been inverted. Scale bar equals 10 mm.

This software is freely available at <https://fiji.sc/> (Schindelin et al., 2012).

4. **Install required ImageJ/Fiji plugins:** Enamel complexity is described in terms of three different topological parameters: EF, 2D OPC and ET. 'folded' calculates them by means of the ImageJ plugins 'OrientationJ' (Fonck et al., 2009; Püspöki et al., 2016; Rezakhaniha et al., 2010), 'LocalThickness' (Dougherty & Kunzelmann, 2007), 'AnalyzeSkeleton' (Arganda-Carreras et al., 2010) and 'MorphoLibJ' (Legland et al., 2016). These plugins are required to use 'folded' and can be installed in the [Help > Update] menu, by selecting 'Manage update sites' and, from the list within, picking 'BIG-EPFL' and 'IJPB-plugins'. Finally, select the 'Close' and 'Apply changes' buttons.
5. **Install the 'folded' script, and download the test data:** both the 'folded_bsh' script and the image examples included in this work can be downloaded via the following Github repository: <https://github.com/iarganda/folded>. Next, copy the file to the directory <Fiji root>/plugins/Scripts/Plugins/Analyze/ (you may need to create it) and restart Fiji. The script then appears as a new command under the menu [Plugins/Analyze] (in the last position of the menu).
6. **Run 'folded'.** Once installed, folded can be accessed in the Plugins menu [Plugins > Analyze > folded].

Practical example: the evolution of dental complexity in hypsodont rhinoceroses.

In this second part, we illustrate the process by exploring the evolutionary fingerprint of dental complexity in two clades of rhinoceros species, the Subfamily Elasmotheriinae and the Subtribe Rhinocerotina within the Subfamily Rhinocerotinae. The dataset includes 23 Elasmotheriinae and 24 Rhinocerotina species, 85% of which are extinct (as detailed in Supplementary Data Table S1). Hypsodonty, or high crowned teeth, is the most frequently observed evolutionary solution to prevent tooth loss due to wear in mammals and the consequence of a delayed onset of root formation. Hypsodont and the more specialized euhyposodont dentitions (i.e. continuously growing molar teeth; see von Koenigswald, 2011) are part of the same continuum of dental adaptations for grazing and/or abrasive diets and have been reported in horses, ruminants, rodents or rhinoceros species (von Koenigswald, 2011). The practical case presented in this work aims to describe dental variations that led to highly complex dentitions in hypsodont rhinoceroses and to outline their underlying evolutionary processes.

2.4 | Image preparation

We selected a total of 47 images of the occlusal surface of the second upper molar of different rhinoceros species for this analysis (see Table S1 for a comprehensive description of the data sample). Molars were chosen if their wear stage fell within the individual dental age stage 3 (i.e. adult dentition with fully functional molar teeth where inner occlusal features like dental valleys are still visible; Anders

et al., 2011), to make them comparable. Image sources include direct photographs of the specimens and photographs and illustrations obtained from the literature (as detailed in Table S1).

2.5 | Image data analysis

Once 'folded' is executed, the following steps are processed automatically. First, each dental tissue is segmented (binarized) according to the previously described colour coding. Subsequently, the script stores scale information from the maximum dimensions of the corresponding binarized region of interest (ROI). and the scale information included in the input image file name (e.g. 'Equus_cabalus_scale20mm.jpg'). In the case of the enamel, the binarized areas (Figure 2d) are transformed by means of a 'top hat' filter with a radius of 3 and then transformed into linear ROIs by means of a skeletonize process (Figure 2e). In addition, a pruning process has been performed to remove short spurious segments generated by the process on thick and sharp enamel turns or bridges in closely located areas. The minimum pixel number of pruned branches can be established in the initial options of the workflow (being set here at 50 pixels).

As mentioned, 'folded' calculates ET (Figure 2f) using the plugin 'LocalThickness', which uses the algorithms described in Hildebrand and Rüegsegger (1996) for stacked sequential images. 'folded' divides each binarized enamel area into a series of equidistant points with a threshold of 1 and quantifies the largest diameter that fits inside the enamel area while containing the point through the path. Thickness is then exported as continuous values through all points of each ROI's and as an absolute total (stored in 'filename_complexity.csv' and 'filename_summary.csv' respectively).

Our script obtains orientation data by means of the plugin 'OrientationJ' 19.11.2012 (Sage, 2012). This plugin computes how structures are oriented in a 2D space from a structure of tensors for each pixel in the image. The local tensor, or gradient structure tensor, is a second-moment matrix that summarizes the predominant directions of the gradient at a specific area around a point by sliding the Gaussian analysis window over the entire image. In this case, we use a gaussian window size $\sigma = 3$ pixels. The orientation output indicates the angle to which the local features are oriented (Figure 2h). As orientation could be an informative feature at both local and global scales, it has been stored as a continuous variable in radians along the ROIs.

Structural coherency (C), another parameter computed by 'OrientationJ', can be defined as the ratio between the difference and the sum of the eigenvalues (λ):

$$C = (\lambda_{\max} - \lambda_{\min}) / (\lambda_{\max} + \lambda_{\min})$$

Coherency is low if there are changes in the orientation of the structures included in the ROI and high when these show a consistent, prevailing orientation. For a more direct interpretation of the results, we consider EF as the inverse of the enamel coherency.

$$EF = 1 - C$$

EF values are provided by the script for each linear unit through the ROIs (giving a value of the sharpness of the individual turns of the enamel walls; Figure 2g). As thickness variations through the enamel ridge can alter local orientation values, ET has been considered constant through all ROI's when estimating coherency with a width of 3 pixels.

Additionally, 'folded' performs a 2D OPC analysis on the binarized image using the ImageJ plugin 'OrientationJ Clustering'. This analysis clusters orientation data into patches of 8 orientation classes based on their neighbouring orientation values using a K-means algorithm, making it less sensitive to changes in the tooth's image orientation (Figure 2i and Figure S2). Patches with areas smaller than 10 pixels have been discarded to prevent the inclusion of stretched and small and isolated patches.

The output data are exported as data tables in comma-separated value (CSV) format by the script. Scale information is stored in 'filename_scale.csv', whereas areas and perimeters by tooth material are stored independently ('filename_enamel.csv', 'filename_dentine.csv' and 'filename_remainingTooth.csv'). Finally, EF and local thickness data are included in 'filename_complexity.csv', whereas 2D OPC is stored as 'k-means.csv'. In addition to the parameters generated by our model, previously published ones are replicated. These include indentation index (D; Gailer & Kaiser, 2014), OEI (Famoso et al., 2013), Enamel Index (EI; Becerra et al., 2012), and FD (following Mandelbrot, 1982). These previously published complexity parameters are stored in 'filename_summary' and 'filename_FractalDimension.csv'. More information about the files generated by 'folded' can be found in Supplementary Data 1.

2.6 | Output processing

The open-source software R 2.15.2 (R Core Team, 2020) was used to merge the CSV files and execute statistical and evolutionary analyses. To summarize the results per specimen, all local thickness and EF values through the ROIs have been averaged. 'folded.R', the R script necessary to perform all the analyses detailed in this section, can be found in the Github repository <https://github.com/iarganda/folded>.

2.7 | Phylogenetic framework

To analyse topological enamel descriptors from an evolutionary perspective, we built an informal supertree that incorporates the current understanding of evolutionary relationships within the studied rhinocerotid clades in Mesquite software (Maddison & Maddison, 2019) using established relationships from the literature. Early cladistic hypotheses of Elasmotheriinae and Rhinocerotini coded their specialized skulls and dentitions similarly, leading to the species' interspersing of both clades (e.g. Cerdeño, 1995). Posterior phylogenetic studies revealed that

these two groups are not only unrelated but nested in two different subfamilies deeply rooted in the rhinoceros life history. Their converging craniodental traits are the result of similar feeding habits and the intrinsic simplification of the character coding process. We have used the phylogenetic framework originally proposed by Antoine (2003), a standard for phylogenetic hypotheses within the subfamily Elasmotheriinae. Except for minor discrepancies, Elasmotheriinae clade topology has proven to be stable through recent phylogenetic iterations (Antoine, 2003; Deng, 2008; Geraads & Zouhri, 2021; Sanisidro et al., 2012). Contrariwise, phylogenetic relationships within Rhinocerotini have shown some topological ambiguity across studies. In this case, we followed the topologies proposed by Pandolfi et al. (2020); Pandolfi, Antoine, et al. (2021); Pandolfi, Bartolini-Lucenti, et al. (2021). The tree was time-scaled using published first occurrence date ranges detailed in Supplementary Data Table S1 with the 'timePaleoPhy' function using the 'equal' method and a 'randObs' date treatment as implemented in the PALEOTREE (v.3.30) R package (Bapst, 2012). To compare the obtained variables with previously published hypsodonty data, an hypsodonty index has been coded according to the combination of characters 62 (Cheek teeth: crown: 0, low; 1, high) and 63 (Cheek teeth: crown: 0, high; 1, partial hypsodonty; 2, hypsodonty; 3, euhypsodonty) based on the last character coding detailed in Antoine et al. (2022). These characters have been recoded from 1 to 5 and can be broadly described as follows: 1, brachydont; 2, mesodont; 3–4 hypsodont; 5, euhypsodont. Coding of the included species is provided in Supplementary Data Table S2.

We used the R package SURFACE (Ingram & Mahler, 2013) to identify macroevolutionary adaptive landscapes for the studied rhinoceros species, allowing the assessment of phenotypic convergence evolution without an a priori designation of the number of selective regimes. This method generates a sequential fitting of the observed data with multiple Ornstein-Uhlenbeck (OU) selective regimes and identifies cases where different lineages explore the same adaptive zone (convergence). Once the list of possible competing macroevolutionary models of increasing complexity (i.e. increase in the number of regimes shifts) is identified, a step-wise Akaike's Information Criterion (AICc) balances improvements in the log-likelihood against increases in model complexity finally selecting the optimal one (see Ingram & Mahler, 2013 and references included therein). In this case, we set a minimum improvement of 6 AICc units to accept a more complex candidate model ('aic-threshold' argument in the 'runSurface' function). At the same time, the 'phenogram' function of the R package PHYTOOLS was used to visualize the phenotypic trajectories of the studied species (Revell, 2012). Finally, to test the correlation between teeth features (EF, 2D OPC, and ET), hypsodonty, and occlusal area, we performed ordinary least square (OLS) and phylogenetic least square regressions (PGLS), using the packages NMLE to test for an evolutionary correlation among the different variables (Table S3; Supplementary Data 3).

This work makes use of photographs of living and fossil specimens previously published in the literature. Ethics approval was not required.

3 | RESULTS

3.1 | Performance and limitations of the current methodology

Three novel enamel complexity descriptors with potential evolutionary signals: 2D OPC, mean EF, and ET, are provided by the present workflow. First, we explored the potential range of applications and limitations of 'folded'. In terms of image size, the studied sample delivers consistent outputs beyond 500×500 pixels (Supplementary Figure S2). In our practical example, image resampling performed by 'folded' has been set to a safer dimension of $1000 \times 1,000$ pixels to prevent the potential consequences of more intricate and/or thinner enamel samples (as shown in Figure 2c). In addition, our method is insensitive to image rotation (see Supplementary Figure S2 and related Supplementary Data): images at different angles deliver similar EF and 2D OPC values. Noteworthy, our method outperforms the 3D orientation patch analysis (3D OPC; see Figure S6) due to the susceptibility of the latter to variations in specimen's orientation (although this problem has been accounted for in latter versions; Wilson et al., 2012). Furthermore, 2D OPC clearly discriminates through samples with distinct degrees of complexity, as in the case of equid genera (Figure S6). The generated parameters could be dependent on body size (measured here as the total molar area; OTA), as in the example of the rhinoceroses presented here (Table S3). In this case, we controlled for the occlusal molar area (OTA; see below) prior to any phylogenetic modelling, but the decision of controlling for molar size will ultimately depend on the hypothesis to test. Yet, size dependency is not pervasive in the generated metrics and should be tested on a case-by-case basis (see Supplementary Data 2).

Enamel architecture changes through the tooth's lifespan hand in hand with dental wear, transforming its grinding and shearing performance. These changes in enamel could potentially drive the output produced by our method. This is not a limitation of the method itself. In fact, one of the toolkit's potential applications is to track the evolution of dental features across different wear stages (Figure S3). However, the current methodology does not allow to discriminate completely unworn teeth (as tooth shearing structures should be functional) and works best when the dentine in between the enamel walls is clearly exposed, being, therefore, restricted to the analysis of occlusal surfaces with conspicuous wear facets in form of lophs

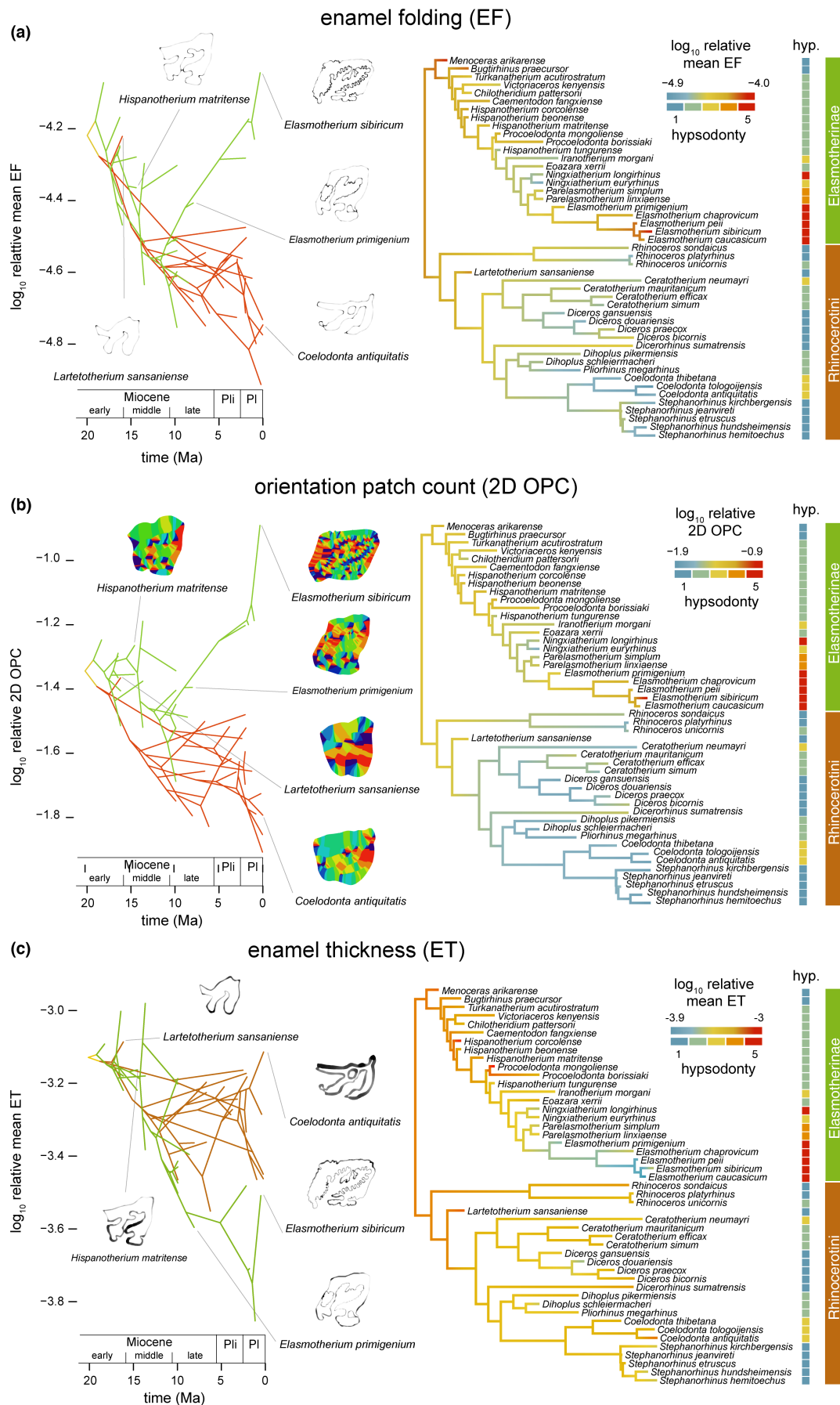
or cusps. Tooth wall components located more gingivally such as peripheral cingula are thus excluded from the input traced image at early wear stages, although they might play an important masticatory role through the latter functional stages of the tooth. If the sample is limited, avoiding controlling for wear is in most cases impossible. Overall, we recommend that, when a larger sample is available, inter-specific studies should be restricted to comparable wear stages. It is advised to evaluate the descriptors of exposed enamel at multiple age stages to gain a broader scope on how the analysed parameters vary, allowing for ontogenetic inferences.

Finally, multiple samples of macro and micromammals teeth of the orders Perissodactyla, Artiodactyla, Proboscidea and Lagomorpha have been analysed (Figures S4 and S5). Their analysis shows that this workflow can be used in multiple scenarios, including partially bunodont morphologies (see additional information included in Supplementary Data 4 for further details).

3.2 | The evolutionary signal of complexity in fossil rhinoceroses

We showcase our framework by assessing the evolutionary dynamics of molar occlusal complexity for hypsodont rhinoceros' species, as detailed in the descriptive statistics summarized in Supplementary Data Table S3. The two complexity parameters, 2D OPC and mean EF, are strongly correlated in the studied dataset. Complexity parameters show a very distinct pattern between the two groups included in this study (Figure S6). However, these trends are likely reflecting a certain degree of allometric scaling. The two metrics (2D OPC and EF) correlate with occlusal area in the OLS models and phylogenetic regressions (PGLS; Table S3). Thus, complexity is affected by size beyond expected just from phylogenetic inertia. In the case of ET, the correlation seems to just reflect some phenotypic differentiation basally rooted in the tree: elasmotheres have thinner enamel and rhinocerotines are generally smaller and have thicker enamel. To evaluate dental complexity with the independence of molar occlusal area, size-corrected alternatives have been used (named here as relative EF, 2D OPC and ET) for further analyses. After accounting for size, enamel complexity on phenograms describe a clear pattern: relative EF and 2D OPC show a steady decline from the early to middle Miocene (~23–11 Ma) in Elasmotheriinae and Rhinocerotini species (Figure 3). This is the result of molar size increasing at a higher pace than enamel complexity. During this early phase, elasmotheres and rhinocerotine EF and local thickness share a common adaptive peak that rendered similar values in both lineages (Figures 3 and 4a,b,e). By contrast, early elasmotheres evolved in a singular adaptive zone regarding 2D OPC, showing

FIGURE 3 Left column: phenogram depicting divergence times plotted against the complexity variables obtained with the method present in this study. Right: plot of the same variables mapped on the studied tree of selected Elasmotheriinae and Rhinocerotini species. All three variables have been corrected for size using the total area of the tooth (OTA). (a), $\log_{10}$ relative enamel folding (EF); (b), $\log_{10}$ relative 2D orientation patch count (2D OPC); (c), relative mean enamel thickness (ET). Additionally, a colour scale showing differences in hypsodonty has been included by combining characters numbered as 62 and 63 in Antoine et al. (2022). A hypsodonty level of 5 (red colour) stands for euhypsodont dentitions. Hyp: Hypsodonty; Pli: Pliocene; pl: Pleistocene. The inset figures included for EF have been enhanced and the background colour of the output images for 2D OPC, EF and ET have been inverted for visualization purposes.



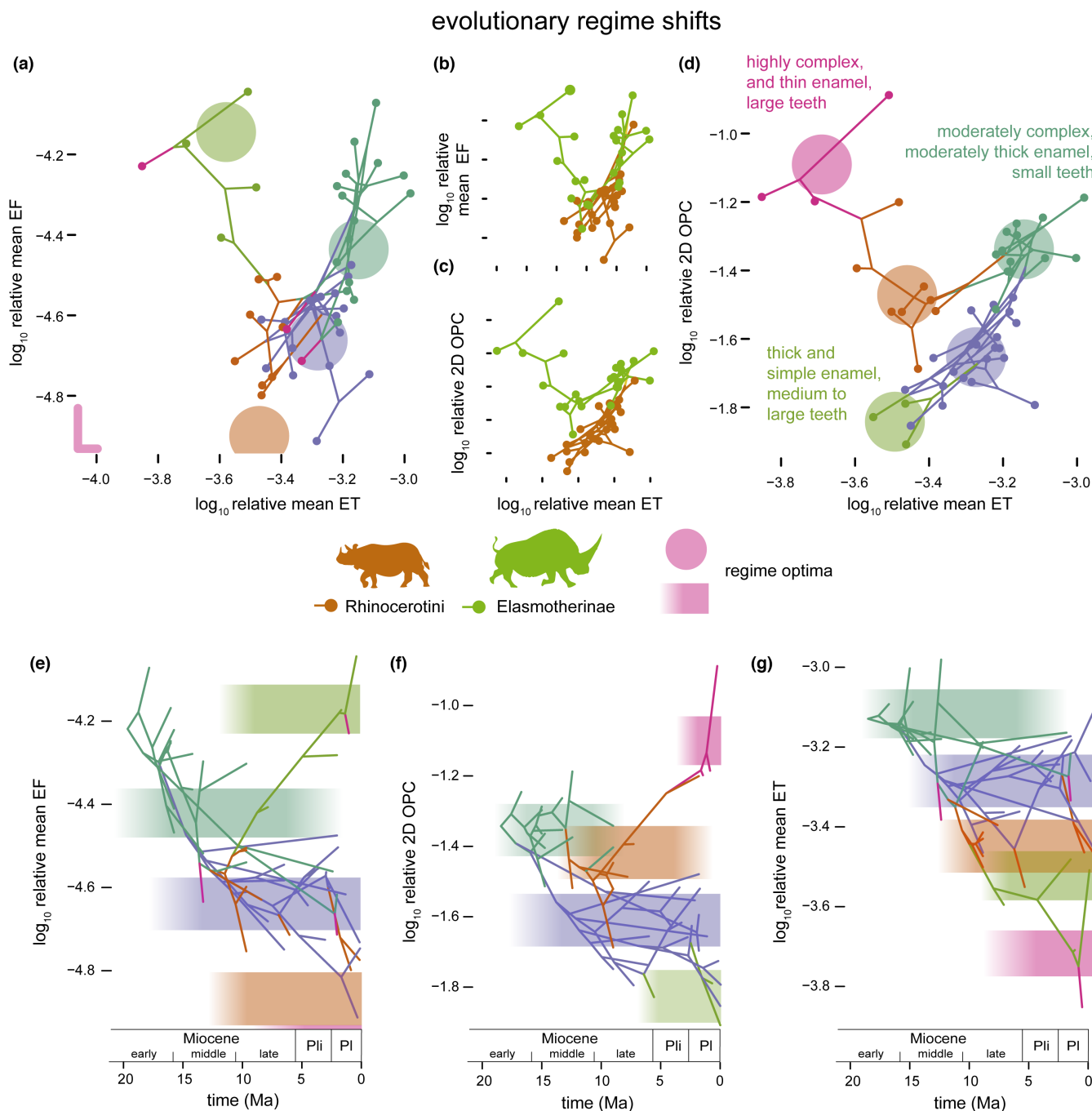


FIGURE 4 (a, d), phylomorphospaces showing the phyletic relations of the studied sample using mean enamel folding (EF) and 2D orientation patch count (2D OPC) respectively versus mean local thickness, all corrected for size (OTA), as input for the 'surface' evolutionary regime modelling. Colours represent unique regimes and are not equivalent across variables. Two additional phylomorphospaces (b, c) with the same topological configuration have been coloured according to the clade correspondence for comparative purposes. (e–g), phenogram representing mean enamel folding and 2D OPC coloured according to the different adaptive regimes. Shaded circles (a, b) and squares (e–g) correspond to projected optima. Unidimensional regime optima recovered by 'surface' in (e–g) slightly differ from those using local thickness as a secondary variable.

from the beginning a higher number of orientation patches than rhinocerotines, which were selected towards lower 2D OPC values from early in their history (Figures 3b and 4c,d,f). After the middle to late Miocene transition at around 11.6 Ma, relative complexity values remained decreasing in most Elasmotheriinae, but the lineage leading to the genus *Elasmotherium* decouples from the remaining rhino species,

as complexity is gained at higher rates than size (Figure 3b). Our phylogenetic model captures the signal of this new selective regime operating in this branch of the elasmother tree (Figure 4f). In addition to the increasingly complex enamel patterns, late Miocene elasmother genera show a gradual delay of root formation that ultimately led to the acquisition of true euhypsodonty, as reported in *Elasmotherium*

and, possibly, in *Ningxiatherium* (Antoine, 2003). Both euhypsodonty and the intricate enamel pattern present in *Elasmotherium* are only two aspects of the uniqueness of the last surviving representative of the Elasmotheriinae in terms of craniodental adaptations. Stable isotopic data demonstrated that *Elasmotherium* inhabited dry steppe and/or cold desert environments, very specific habitats restricted to central Asia (Kosintsev et al., 2019). Environmental changes during the last glacial/interglacial periods reduced these environments and would have predisposed the last *Elasmotherium* species to extinction as part of the so-called 'Late Quaternary extinction event' of mega-fauna at around 40–38 ka BP.

With some exceptions, relative ET shows a general diminishing trajectory through the entire life history of both Elasmotheriinae and Rhinocerotini (Figure 3c). But from the middle Miocene onwards (~15 Ma), the two groups show disparate evolutionary pathways driven by the unfolding of distinctive adaptive regimes (Figure 4g). From this moment, Rhinocerotini retained relatively thick enamel despite their increases in size (Figures 3c and 4g). Lineages representing these configurations include species of the genera *Ceratotherium*, *Dihoplus*, *Pliorhinus*, *Stephanorhinus* and, more specifically, *Coelodonta*. In contrast, elasmotheres kept evolving towards configurations where enamel thinned as complexity increased (Figures 3c and 4g), with genera like *Eoazara* or *Ningxiatherium* (Figure S6), reducing its values thereafter and reaching thickness minima by the Pleistocene with *Elasmotherium*.

Our multifaceted approach reveals a complex relationship between relative enamel complexity and thickness, molar size (occlusal area) and hypsodonty. The modelled macroevolutionary optima show that enamel with high relative complexity and low thickness were selected for in elasmotheres, whereas the opposite is true for rhinocerotines. However, this procedure does not consider potential correlations between variables (Ingram & Mahler, 2013). A set of additional regression tests illuminates this issue. A set of regression tests illuminates this issue. Our PGLS analyses reveal that relative complexity correlates positively with relative thickness, although no correlation is found in the OLS models. This suggests that the pattern is only evident among closed related taxa and is likely determined by the underlying changes in OTA, which is the denominator in the ratio. However, when the correlation between complexity and thickness is tested prior to controlling for OTA, the significance of the PGLS models is lower (see Table S3), reflecting that the broad differentiation 'thinner and convoluted versus thicker and simple' took place at the basal radiation event that separated elasmotheres and rhinocerotines but is not to be regarded as a universal correlation across the tree. A mixed pattern is found when correlations between occlusal features and hypsodonty are explored. When using OLS, complexity and thickness correlate with hypsodonty, but no correlations govern as we zoom in on closer phylogenetic relationships (PGLS). Relative ET only correlates with hypsodonty among closely related taxa (in PGLS), but this significance weakens when absolute mean ET is used instead, also capturing some positive relation between hypsodonty and OTA.

A general pattern emerges when all these findings are taken together: most of the correlations observed in the dental features

between elasmotheres and rhinocerotines have an origin in major phenotypic and developmental divergences taking place at the basal split of the two lineages and are only marginally explained by piecemeal adaptation and coevolution later in their evolutionary history. Trends in individual features are apparent along rhinos' history, but their conspicuous evolutionary correlations among these traits were set deep in their past. The underlying factors triggering more complex (Elasmotheriinae) or thicker (Rhinocerotini) dentitions around the middle to late Miocene transition in Eurasia are still to be explored in detail. The intensification of the dry conditions during the late Miocene (at or prior to 9 Ma) in Asia, a geographic hotspot for elasmothere and rhinocerotine diversity, and the subsequent onset of more arid conditions, would have increased inter-specific competitive pressures among hypsodont herbivores and driven the evolution of morphologies allowing for greater niche differentiation within the large herbivore guild, in a similar way to that observed in other groups such as bovids and proboscideans (Bibi, 2007; Cantalapiedra et al., 2021). Unfortunately, the scarce middle and late Miocene elasmothere fossil record may have dampened the discovery of transitional forms that would fill this phenotypic gap and help to refine its evolutionary tempo and mode at a finer scale, a gap that has only recently started to fill with the description of species such as *Elasmotherium primigenium* (Sun et al., 2021).

4 | CONCLUSION

Enamel ridge architecture is keystone to understanding deep-time evolutionary and ecological interactions in herbivore communities. However, potential ecological factors modulating enamel wall complexity are still to be explored in 2D with larger samples and modern deep-time quantitative techniques. In this note, we developed a new procedure to quantify enamel complexity based on 2D data using an ImageJ/Fiji script. The methodology proposed here will allow for the assessment of ecological and macroevolutionary hypotheses, as exemplified with the case of hypsodont rhinoceros species. A step-by-step description of the methodology is included while demonstrating its main features. An evolutionary study resulting from its outcome is provided. The two distinct strategies to increase masticatory durability in hypsodont rhinos has their origin in two differing phenotypic and developmental pathways that diverged early in their evolutionary history: members of the Elasmotheriinae increased their enamel folding thus generating a higher number of orientation patches, while some Rhinocerotini thickened their enamel walls. Our method has been tested for other practical scenarios, demonstrating that variations in image size or rotation do not affect the output.

The complexity descriptors used in the present workflow are a novel approach to objectively quantify enamel morphology, outlining the potential of this technique for the reconstruction of dietary variability, mastication behaviour or evolutionary adaptation in fossil and modern species using 2D images as an input.

AUTHOR CONTRIBUTIONS

Oscar Sanisidro conceived and designed the study methodology, prepared sample images, and led the writing of the manuscript. Ignacio Arganda Carreras and Oscar Sanisidro created the Fiji script. Juan L. Cantalapiedra and Oscar Sanisidro contributed with the phylogenetic modelling. Juan L. Cantalapiedra and Ignacio Arganda Carreras contributed to the manuscript and approved its final version.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials included in the Github repository <https://github.com/iarganda/folded>.

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