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Morphological variation and covariation in the human postcanine dentition

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Ďakujem!

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Abstract

The understanding of dental morphology plays a key role in the advancement of human biology as well as anthropology, phylogeny and medicine. Since odontogenesis is driven by complex genetic signaling patterns, but still responds to various developmental and functional stimuli, it remains an open field of study with many valuable information still to be uncovered. The study of teeth has presented various obstacles in the past, due to the geometric complexity of their morphology that can be easily affected by extrinsic factors like wear. However, recent advancements in morphological analysis have moved beyond classical methods, exploring inner structures and shape variation in 3D using virtual, non-destructive techniques.

This thesis comprised three studies, investigating dental morphology using geometric morphometrics and 3D imaging techniques. Our work focuses on the determination of morphological variation of the postcanine dentition among and within modern human populations, as well as on the covariation between various tooth types within the postcanine dentition. Two studies were performed on separate samples of upper and lower deciduous second molars, and upper third and fourth premolars, focusing on determination of morphological variations and possible differences between populations. The final study considered a large sample of postcanine dentitions (third molars excluded) from individuals of various geographical origin, investigating covariation and its morphological patterns between tooth types.

The conducted results and outcomes of this thesis contribute significantly to the knowledge of 3D dental morphological variation in modern humans, and lay the ground work for the future study of dental covariation and possible phenotype-genotype associations. The used methods proved suitable for the investigation of dental morphology, however, we report on various limitation factors that should be addressed and considered in the future research.

Zusammenfassung

Das Verständnis der Zahnmorphologie spielt eine Schlüsselrolle in der Weiterentwicklung der Humanbiologie sowie der Anthropologie, Phylogenie und Medizin. Da die Odontogenese von komplexen genetischen Signalmustern gesteuert wird, aber dennoch auf verschiedene Entwicklungs- und funktionale Reize reagiert, bleibt sie ein offenes Forschungsfeld, in dem erst noch viele wertvolle Informationen entdeckt werden müssen. Die Untersuchung von Zähnen konfrontierte uns in der Vergangenheit mit verschiedenen Herausforderungen, insbesondere aufgrund der geometrischen Komplexität ihrer Morphologie, die durch äußere Einflüsse wie Abnutzung beeinträchtigt werden kann. Fortschritte in der morphologischen Analyse haben jedoch die bisher üblichen klassischen Methoden überwunden und erlauben die Untersuchung von inneren Strukturen sowie der Formvariationen in 3D mithilfe virtueller, nicht-destruktiver Techniken.

Diese Dissertation umfasst drei Studien, die die Zahnmorphologie mit Hilfe von geometrischer Morphometrie und 3D-Bildgebungstechniken untersuchen. Unsere Arbeit konzentriert sich auf die Bestimmung der morphologischen Variation im postcaninen Gebiss sowohl innerhalb als auch zwischen modernen menschlichen Populationen sowie auf die Kovariation zwischen verschiedenen Zahntypen des postcaninen Gebisses. Zwei Studien wurden an getrennten Proben von oberen und unteren zweiten Milchmolaren sowie oberen dritten und vierten Prämolaren durchgeführt, wobei der Fokus auf der Bestimmung der morphologischen Variation und möglichen Unterschieden zwischen Populationen lag. Die abschließende Studie untersuchte eine große Stichprobe von postcaninen Gebissen (dritte Molaren wurden ausgeschlossen) von Individuen unterschiedlicher geografischer Herkunft und analysierte die Kovariation und deren morphologische Muster zwischen den Zahntypen.

Die durchgeführten Ergebnisse und Erkenntnisse dieser Dissertation tragen signifikant zum Wissen über die 3D-Zahnmorphologie moderner Menschen bei und legen das Fundament für zukünftige Studien zur Zahnkovariation und möglichen Phänotyp-Genotyp-Assoziationen. Die angewandten Methoden erwiesen sich als geeignet für die Untersuchung der

Zahnmorphologie, jedoch berichten wir auch über verschiedene Einschränkungsfaktoren, die in zukünftigen Forschungen berücksichtigt werden sollten.

1. Introduction

Dental morphology, the study of the shape, size, and structure of teeth, has been a crucial area of investigation in fields of biological anthropology, dental medicine, and human evolution. It provides significant insights into evolutionary relationships among species, the dietary habits of ancient populations, and the genetic foundations of dental traits. Given the extensive history of research in this area, one might assume that most knowledge about modern human teeth has already been gathered. However, this is not the case.

Previously, studies relied primarily on qualitative descriptions of the outer enamel surface (OES), non-metric traits, and classical two-dimensional measurements, such as buccolingual/mesiodistal diameters or crown height, to characterize teeth (Hanihara, 2008; Hanihara & Ishida, 2005; Lovell & Haddow, 2007; Martinón-Torres et al., 2006; Scott, 1980; Scott & Irish, 2017). Despite their contributions, significant advancements in the study and description of the three-dimensional (3D) morphology of teeth have only emerged in the past two decades (Bailey et al., 2011; Benazzi et al., 2012, 2014; Fornai et al., 2014; Hershkovitz et al., 2018; Krenn et al., 2019; Macchiarelli et al., 2013; Morita et al., 2014; Ortiz et al., 2017; Pan et al., 2016; Sarig et al., 2021; Weber et al., 2016; Zanolli et al., 2018; Chapter 2.1, 2.2, 2.3). With the development of new methods, we now have the ability to explore the inner structures of teeth without causing damage to the material. This has opened up new possibilities for studying how the 3D shape and size of different tooth types vary among populations, identifying which regions of teeth exhibit greater shape variation versus those more constrained by functional demands, and also analyzing how different teeth covary with each other.

1.1 Dental Morphology

A tooth consists of four primary dental tissues: enamel, dentine, cementum, and pulp. The enamel, consisting of 97% inorganic material, is the hardest and most durable substance of the human body. Enamel covers and protects the underlying dentine that is more similar to the

bone in terms of organic and inorganic material composition (Scott & Pilloud, 2018). The development of most human teeth begins before birth and terminates at different times throughout the individual's life, according to the tooth type. Dentine formation (dentinogenesis) precedes enamel formation (amelogenesis), with odontoblasts and ameloblasts responsible for dentine and enamel production, respectively, being separated by the basal lamina. After the cell differentiation, the enamel-dentine junction (EDJ) is formed as the exact copy of the basal lamina (Hillson, 1996), being the primary developmental structure of the crown. EDJ thus serves as a shape-precursor of the enamel cap (Bailey et al., 2011; Guy et al., 2015; Monson et al., 2020). The formation of deciduous teeth differs from permanent teeth, with faster rates of development (Boyde, 1964; Liversidge & Molleson, 2004).

Surprisingly little is known about odontogenesis, considering how genetic and epigenetic factors influence the development of cusp patterns and the overall shape of the dental crown. Crown morphogenesis is a critical embryological phase during which the shape, size, and position of the dental cusps are established (Kenessey et al., 2024). The crown of the tooth is completely formed within its crypt in the jaw, before it erupts and comes into function, i.e. into occlusion. Unlike bony structures, the dental tissues are highly mineralized, and enamel does not remodel in response to biomechanical demands. Under normal conditions, changes to the dental crown after eruption occur only through erosion (chemical dissolution of hard tissues) or abrasion (physical removal of material, i.e. contact with food) and attrition (contact between teeth) (Lucas, 2004). As the enamel is the part of the tooth that is affected by chemical and mechanical destruction in the first place and thus serves as a protection of the inner structures of the tooth (i.e. dentine), its morphology is also affected the most.

Over 2,400 genes are implicated in odontogenesis (Landin et al., 2012; Sehic et al., 2017). This process is initiated by the formation of the primary enamel knot (PEK), and further formation of secondary enamel knots (SEKs; Jernvall & Thesleff, 2012; Thesleff, 2003). The termination of PEK cell proliferation, followed by its apoptosis, is driven by the MSX2, BMP2, and BMP4 genes, along with the cell cycle inhibitor P21 (Jernvall et al., 1998; Tucker & Sharpe, 1999), marking the transition to the bell stage. During this stage, activator signals that promote

epithelial cell differentiation and inhibitor signals that stimulate mesenchymal growth determine the positioning of SEKs, which in turn predict the future locations of the dental cusps (Jernvall et al., 1994; Sarkar & Sharpe, 1999). The interaction between activators and inhibitors regulates cell proliferation rates across different regions of the crown, leading to the lateral expansion of epithelial tissue (Jernvall et al., 1994; Salazar-Ciudad & Jernvall, 2002). In addition to spatial positioning, the number of cusps is also influenced by SEKs and the space available between them (Burroughs, 2019; Thesleff & Tummers, 2008), resulting in diversification of morphological features. The final morphology of the crown is shaped by genetic inheritance and developmental processes, with its shape and size also reflecting functional adaptations, defining the overall morphology of the various tooth types.

Humans are heterodont (Scott & Pilloud, 2018). Teeth are classified into different tooth classes and tooth types based on their shape and function, occupying specific positions within the dental arches. The deciduous teeth follow a 2-1-2 pattern (2 incisors, 1 canine, 2 molars), with the eruption beginning around six months after birth, typically completed with 2.5 years of age, following a sequence: incisors, canines, first molars, and second molars (Al-Jasser & Bello, 2003; Scott & Irish, 2017). Deciduous tooth eruption varies by population, sex, and even within the same individual (Al-Jasser & Bello, 2003), though tooth development remains a reliable indicator of developmental age (Liversidge & Molleson, 2004). The first permanent molar erupts around 5.5-6 years, starting the transition to the permanent dentition that follows a 2-1-2-3 formula (2 incisors, 1 canines, 2 premolars, and 3 molars; Cunningham et al., 2016; Hillson, 1996; Schaefer et al., 2009; Scott & Pilloud, 2018). The permanent human dentition thus consists of 32 teeth, while the deciduous dentition comprises only 20 teeth (Hillson, 1996; Liversidge & Molleson, 2004; Scott & Irish, 2017; Scott & Pilloud, 2018). In general, in modern human dentitions we distinguish between the anterior and the posterior dentition.

Postcanine dentition

In this thesis, we focus on the posterior dentition, also called the postcanine dentition, consisting of two tooth classes: premolars and molars (only molars in the deciduous dentition)

(Hillson, 1996; Scott & Irish, 2017). The postcanine teeth exhibit more articulated surfaces than anterior teeth, as their morphology is tightly linked to their function. The postcanine dentition serves mainly as the grinding masticatory tool, the third premolars (P3s) though, as the tooth type connecting the anterior teeth to the rest of the dentition, engage in tearing function as well (Kraus et al., 1969). Usually, premolars are bicuspids, each cusp continuing into a root cone that can either form two separate roots or fuse into one. Occasionally, three-rooted premolars can be observed. Upper molars normally consist of four cusps and three roots, while lower molars modal cusp number is five (second molars only exhibiting four) with two roots. These however, count as normative characterizations and the exceptions in them create a weighty part of dental morphology (Scott & Pilloud, 2018).

1.2 Virtual 3D imaging

In the past, examining the internal structures of teeth often required destructive methods to reveal them (Corruccini, 1987; Sakai et al., 1967). However, since the end of the 20th century, advancements in virtual imaging technologies have revolutionized dental anthropology and related fields (Weber, 2015; Weber & Bookstein, 2011). Various tools now allow the creation of 3D virtual models of dental surfaces, to be analyzed either together or separately. For example, CT scanning (computed tomography) enables non-destructive imaging of the internal structures of specimens. It gives us the ability to separate the substantial structures of the objects, according to the different X-ray absorption levels of the materials (Weber, 2015). For detailed analysis of small structures like teeth, high-resolution data can be obtained via micro-CT (µCT) technology (Figure 1A). Moreover, synchrotron facilities can generate ultra-high-resolution images, to capture even microscopic details (Tafforeau & Smith, 2008). Using these methods, virtual models can be reused, reanalyzed, shared, and serve as precise replicas of the original specimens once scanned (Weber, 2015).

If analyzing separated structures of the provided volumetric data, the various materials have to be segmented to be further reconstructed as separate surfaces. In this thesis, the enamel has been segmented and separated from the dentine, for us to be able to measure and analyze

the dentinal crowns of the teeth (Figure 1A, 1B). As mentioned in Chapter 1.1, the EDJ correlates highly with the outer enamel surface, but profits from physical protection of the enamel. This makes the study of the EDJ morphology advantageous over enamel cup's morphology. These methods are discussed in Chapter 2 in more detail.

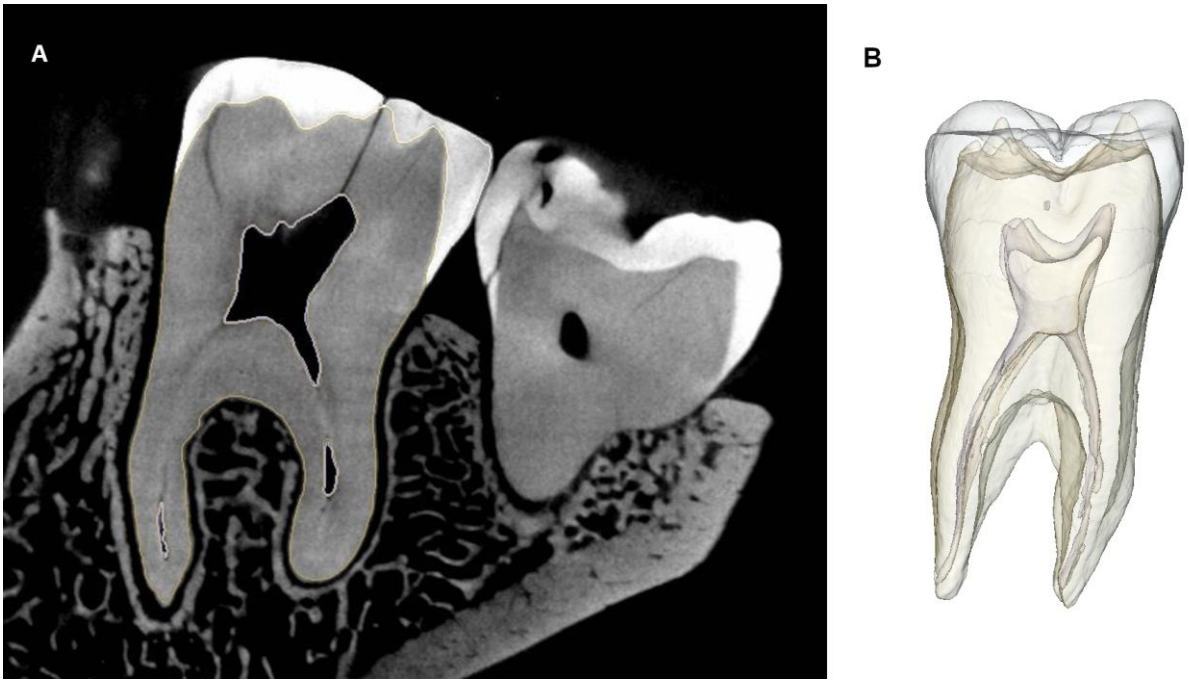


Fig. 1 – Lower permanent first molar, captured by micro-CT scan in 44µm resolution (**A**) and the resulting 3D virtual model of the tooth (**B**).

1.3 Geometric morphometrics (GM)

For more than a century, physical anthropology has relied on methods such as linear measurements, distance ratios, and angular measurements derived from anatomical landmarks to analyze shape variation in skeletal structures (Martin & Saller, 1957). However, the limitations of these methods, particularly in capturing complex morphological variations, led to the evolution of morphometrics as a field of study. Morphometrics, the study of shape and form variation, initially progressed with the introduction of multivariate statistical techniques in the early 20th century. These techniques enabled researchers to analyze multiple variables simultaneously, forming the basis of multivariate morphometrics (Mitteroecker &

Gunz, 2009). This early work paved the way for more sophisticated approaches to shape analysis.

In the latter part of the 20th century, the field of morphometrics underwent a significant transformation with the advent of coordinate-based methods and the computational implementation of deformation grids. These advances, combined with the development of statistical shape theory, culminated in what is now known as Geometric Morphometrics (GM) (Bookstein, 1986, 1991a; Rohlf & Slice, 1990). GM revolutionized the study of shape by allowing the preservation of geometric relationships in landmark data, providing a powerful tool for analyzing biological forms in three dimensions. This approach has since been widely applied across various biological disciplines, including dental anthropology, to study evolutionary and developmental patterns in human and fossil dentitions (Benazzi et al., 2011, 2012, 2014; Davies et al., 2019; Fornai et al., 2014, 2016; Gómez-Robles et al., 2007, 2008, 2011, 2012; Hershkovitz et al., 2018; Kato et al., 2011; Krenn et al., 2019; Weber et al., 2016; Chapter 2.1, 2.2, 2.3), as well as other mammalian teeth.

The core principle of GM is the use of landmarks, specific anatomical points that can be identified in all studied specimens throughout the sample. According to Bookstein (1991a), landmarks are defined as anatomical loci represented by Cartesian coordinates, which can be either two-dimensional or three-dimensional. These landmarks are critical for capturing the geometry of a structure, and they adhere to the principle of homology, which is essential for analyzing biological variation based on the spatial relationships of structures (Weber & Bookstein, 2011). Weber and Bookstein (2011) classified landmarks into six categories based on their geometric properties (Figure 2):

- Type I landmarks: Fixed landmarks located on discrete juxtapositions of tissues
- Type II landmarks: Fixed landmarks determined by extremes of curvature
- Type III landmarks: Landmarks characterized locally by information from multiple curves and by symmetry.
- Type IV landmarks: Semilandmarks placed along curves

- Type V landmarks: Semilandmarks located on surfaces
- Type VI landmarks: Geometrically constructed semilandmarks, also known as pseudolandmarks.

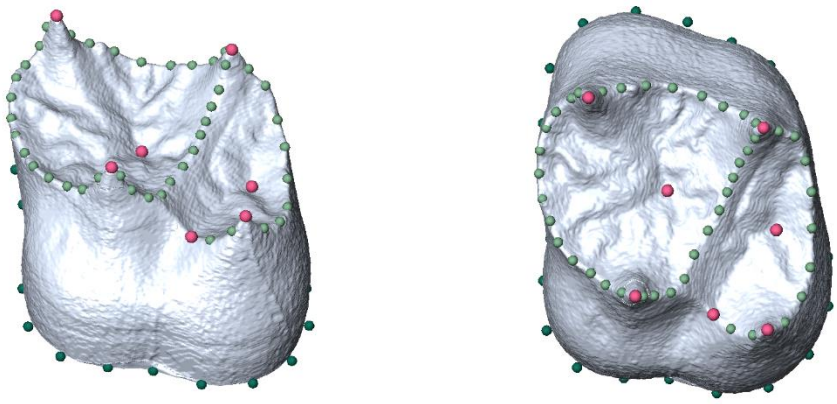


Fig. 2 – Landmark configuration capturing the morphology of the dentinal crown of an upper permanent first molar in lingually oblique view (**left**) and occlusal view (**right**). Fixed landmarks represented in **pink**, semilandmarks in **light green** (along the ridges of the occlusal surface), and pseudolandmarks (along the cervical line) in **dark green**.

Landmarks in our approach are essential for analyzing rigid structures such as bones and teeth, as well as flexible structures like soft tissues. Semilandmarks (sLMs), in particular, are used to quantify the morphology of surfaces and curves in areas where no fixed landmarks can be defined and significantly increase and improve information about the geometry of objects. These points are allowed to "slide" along the structure (a curve or a surface) in relationship to landmark configurations of the other specimens, which helps to achieve geometric homology within the sample, a key concept in the accurate comparison of shapes (Bookstein, 1997; Mitteroecker & Gunz, 2009). The sLMs can only be established on virtual objects, since a mathematical algorithm needs to be applied on the data to locate them.

One of the significant advances in GM is the possibility to separate the actual shape of an object from its size, its position in space, and its orientation. This is achieved using methods

like Generalized Procrustes Analysis (GPA; Gower, 1975). Shape is defined as the geometric properties of an object that are invariant to transformations such as translation, rotation, or scaling. For example, two objects are considered to have the same shape if they can be repositioned, rotated and resized to align perfectly (Mitteroecker et al., 2013). Form, on the other hand, retains size information and thus can be referred to as "size-and-shape" (Dryden & Mardia, 1998). GPA plays a central role in GM by applying Procrustes superimposition and aligning all landmark configurations. Procrustes superimposition is a widely used technique for aligning landmark configurations in morphometrics, involving three main steps: translating landmarks to a common centroid, scaling configurations to a uniform size (typically based on Centroid Size), and rotating configurations to minimize the sum of squared distances between corresponding points (Mitteroecker & Gunz, 2009). This process results in Procrustes shape coordinates that contain shape information only. The mean of these coordinates across specimens represents the consensus shape which can be visualized. Differences from this mean shape, known as Procrustes residuals, provide insights into shape variation within a sample (Gower, 1975; Rohlf & Slice, 1990). In addition to GPA, several other registration methods have been developed to eliminate irrelevant information in morphometric data (Bookstein, 1986, 1991a; Lele & Richtsmeier, 2001), with Procrustes superimposition becoming one of the most useful methods for comparing shapes, as it retains the complete geometric relationships within an object under study and does not distort variation. Shape coordinates can thus be analyzed using various multivariate statistical approaches, allowing researchers to explore patterns of shape variation and covariation.

Among these, Principal Component Analysis (PCA) is the most commonly used tool for estimating the parametric structure of a sample. PCA reduces the dimensionality of data by identifying orthogonal linear combinations of variables that explain the variability within the sample with the minimum of variables (Figure 3). PCA is especially useful for uncovering patterns of variation among shape variables, and thus the actual shape changes along the principal components, that can be closely observed using visualization techniques. Furthermore, covariation patterns between two different sets of variables can be examined

through Partial Least-Squares (PLS) analysis, a technique frequently used in morphometrics as well. PLS (Bookstein, 1991a; Rohlf & Corti, 2000) investigates and informs about the association between two sets of landmark configurations (e.g., between two tooth types), and enables the investigation of differences in morphological covariation between groups by utilizing the mean shape and visualizing the covariance of shape in every specimen.

While Procrustes superimposition is effective in removing size and positional differences, sLMs offer a way to extend landmark-based analyses to smooth curves and surfaces. The sLMs are adjusted using a Thin-Plate Spline algorithm to minimize bending energy and are particularly useful in studies where fixed landmarks are missing, for instance, on large areas of the neurocranium, the shaft of a tibia, or along the ridges of the EDJ between tooth cusps. By allowing the landmarks to slide along curves or surfaces, sLMs ensure that the geometric homology necessary for the investigation of the sample can be established, even for complex structures such as teeth or skulls (Gunz et al., 2005; Gunz & Mitteroecker, 2013). The preservation of spatial relationships between landmarks is one of the key advantages of GM, allowing researchers to virtually re-enter the Euclidean space and visualize shape differences on the screen as actual deformations of structures. This can be done through various graphical tools, such as vector diagrams and thin-plate spline deformations, which provide intuitive representations of how one shape transitions into another (Figure 4; Bookstein, 1989, 1991b).

The utility of GM extends beyond simple shape analysis. Researchers have also applied these techniques to explore the relationships between size and shape in evolutionary and developmental contexts. For instance, form space (or size-shape space) incorporates both Centroid Size and shape coordinates, making it possible to study how size influences shape and vice versa (Mitteroecker et al., 2013). This is particularly relevant in studies of allometry, which examines how biological structures change in size and shape during growth and development.

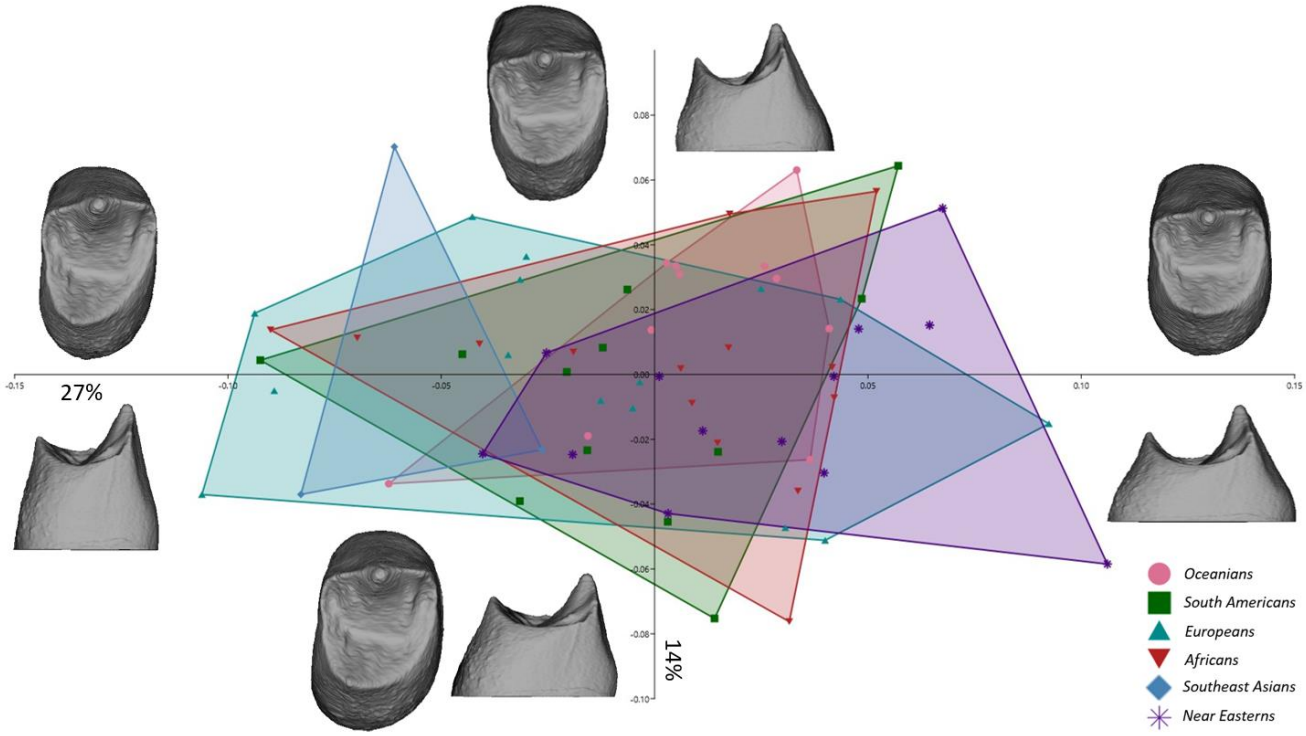


Fig. 3 – PCA plot representing PC1 and PC2 for upper third premolars' dentinal crowns. The warpings at the end of each axis show the real shape variation in occlusal and distal view (Chapter 2.2 - modified).

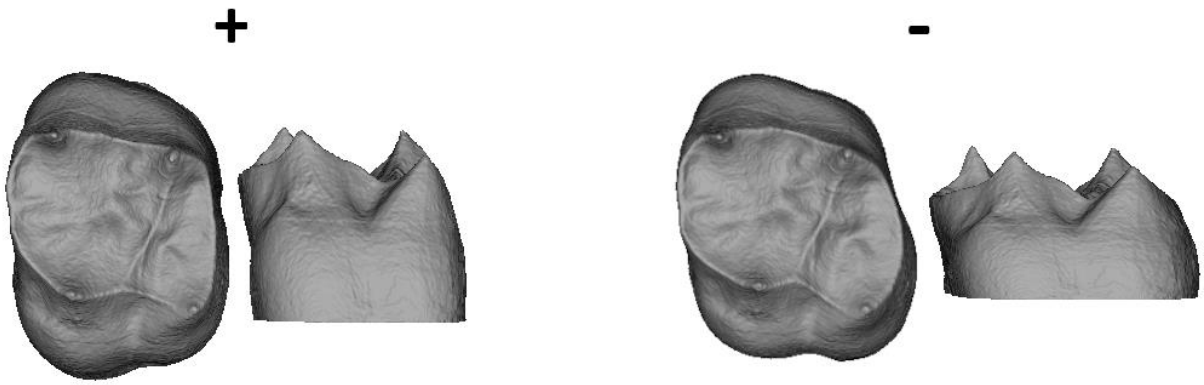


Fig. 4 – The shape change observed on the extremes of the Singular Warp 1 of a 2B-PLS analysis in the sample of upper first premolars.

GM remains an invaluable tool for studying biological shape and form. Its application in dental anthropology sheds light on the evolutionary and functional adaptations of dentition in humans as well as in other primates. It has transformed the study of biological shape by providing robust methods for capturing and analyzing complex morphological data which is also the main goal of this thesis.

1.4 Morphometric studies in this thesis

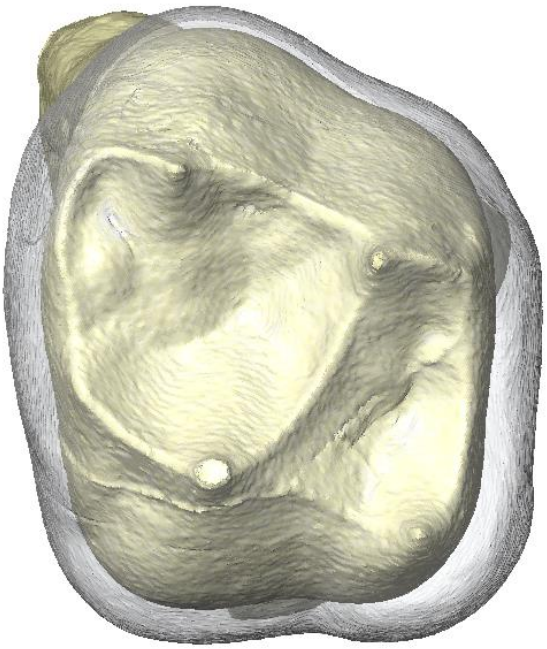
The backbone of this thesis is represented by three studies (Chapter 2) that are based on Geometric Morphometrics in combination with 3D virtual imaging. All of them focus on various dental types of the postcanine dentition. In two of these studies (Chapter 2.1, 2.2), we thoroughly investigated the morphological variation within and between various geographical modern human groups, namely on a sample of upper and lower deciduous second molars (Chapter 2.1), and a sample of upper third and fourth premolars (Chapter 2.2). This research, hence this thesis, culminates in a study building upon the first two chapters mentioned, as well as on a series of dental morphometric data from previous co-workers of the Virtual Anthropology group at University of Vienna. For the first time, a large sample of modern human postcanine teeth (only third molars excluded) was used to define the 3D shape covariation patterns between various tooth types of the postcanine dentition (Chapter 2.3). The dentinal crown and the morphology of the EDJ, thus the inner structures of the teeth, have been of main interest in all papers of this thesis. Using virtual 3D imaging enabled the visualization and further manipulation of these structures with no invasive methods needed throughout the whole work process. This thesis presents a detailed investigation of the morphological variation and covariation of the human postcanine dentition, determining the patterns of the actual shape changes of dentinal crowns in 3D.

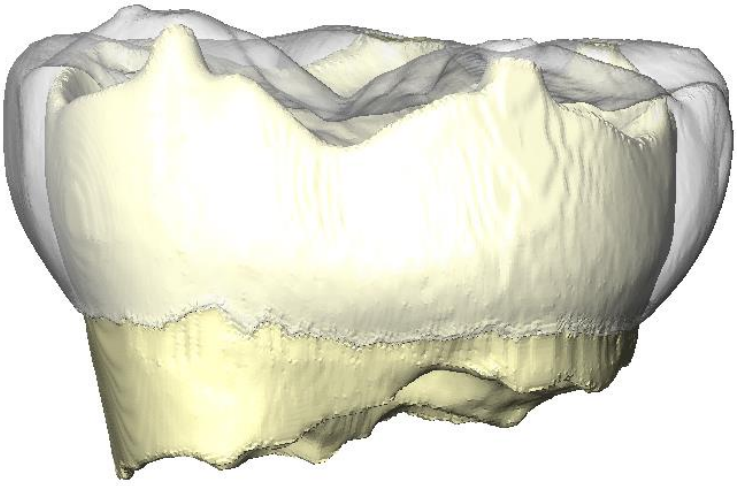
2. Research

2.1 Morphological variation of the deciduous second molars in the Baka Pygmies

Šimková, P. G., Weber, G. W., Ramirez Rozzi, F. V., Slimani, L., Sadoine, J., & Fornai, C. (2021). Morphological variation of the deciduous second molars in the Baka Pygmies. Scientific Reports, 11(1), 16480. https://doi.org/10.1038/s41598-021-95524-3

Contribution: This project was designed and supervised by Dr. Cinzia Fornai and Prof. Gerhard Weber, in collaboration with Dr. Fernando Ramirez Rozzi, who has conducted extensive longitudinal biometric studies with the Baka Pygmies of Cameroon. Over the years, Dr. Ramirez Rozzi collected naturally shed deciduous second molars from children of the village La Bosque, with the consent of both the children and their parents. He made µCT scans of this material available, giving us the opportunity to investigate aspects of dental development and growth in this unique population. I have collected additional specimens for the comparative sample, performed the image data acquisition and processing (e.g., segmentation, outline collection), landmark data collection, and I performed the analyses. Furthermore, I wrote the paper with contributions from Dr. Fornai, Dr. Ramirez Rozzi and Prof. Weber, and compiled all visual representation of the data and results (figures and tables).





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Morphological variation of the deciduous second molars in the Baka Pygmies

Petra G. Šimková[1]✉, Gerhard W. Weber[1,2], Fernando V. Ramirez Rozzi[3,4], Lotfi Slimani[5], Jérémy Sadoine[5] & Cinzia Fornai[1,6,7]✉

The Baka Pygmies are known for their short stature resulting from a reduced growth rate during infancy. They are peculiar also for their teeth erupt earlier than in any other African population, and their posterior dentition is larger than in non-Pygmy populations. However, the Baka's dental morphology, like several other aspects of their biology, is still understudied. Here, we explore the variation of the Baka's deciduous upper and lower second molars (dm2s) in comparison to a geographically heterogeneous human sample by means of 3D geometric morphometrics and analysis of dental traits. Our results show that the different populations largely overlap based on the shape of their dm2s, especially the lower ones. Their distal region and the height of the dentinal crown differ the most, with the Baka showing the most extreme range of variation. Upper and lower dm2s covary to a great extent (RV = 0.82). The Baka's and South Americans' dm2s were confirmed among the largest in our sample. Despite the Baka's unique growth pattern, long-lasting isolation, and extreme dental variation, it is not possible to distinguish them from other populations based on their dm2s' morphology only.

The Baka people are a Central African population with an average male height under 155 cm[1]. Like many other Central African human groups, the Baka have chosen to refer to themselves as Pygmies[2], accordingly, we will use the term Pygmy in this manuscript for the reasons detailed in Methods.

Until recently, most African Pygmies maintained semi-nomadic lifestyles based mainly on hunting and gathering[1,3]. From the mid-twentieth century, the missionary and sedentarization programs influenced the lifestyle of the Baka populations[4–9] which moved closer to village sites inhabited by Bantu-speaking agriculturists. Some of the Baka started practicing horticulture in particular seasons of the year and trade products with their neighbors[5,7,8]. Nowadays, the Baka's hunting camps in the rainforest last only for a few months per year during the major dry season[5,8], but they remain an essential source of wild resources the Baka are highly depending on[7].

Short average stature in Pygmy populations results from their particular growth patterns, but the actual underlying evolutionary mechanisms have been long debated[10,11]. Pygmies' small body size was mainly interpreted as an adaptation to environmental conditions, food shortage[11–13], and thermoregulation[14]. It is now established that the genetic basis of this specific phenotype is polygenic, including genes related to skeletal growth, allometric patterning, immunity, and metabolism, with effects on the growth hormone axis, in particular the expression of the growth hormone-insulin-like growth factor I[15–17].

The life history variables in the Baka Pygmies seem to correspond with those of other non-Pygmy populations, including puberty growth spurts and maturity reached at a very similar age[10]. Furthermore, their birth size and birth weight are within the standard limits of modern humans, differently from other Pygmy populations that are already born with smaller body size[6,10]. However, during the first two years of life, the Baka experience a significant deceleration of growth which causes a long-lasting growth delay throughout their whole development and results in a short adult stature (average women's height = 146.7 cm; average men's height = 153.5 cm)[10]. Although the timing of tooth eruption is commonly strongly correlated with other life history variables, in the Baka Pygmies the permanent dentition erupts earlier than in any other African groups, including other Pygmy

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populations (in females upper and lower first molars erupt respectively 0.75 and 0.71 years earlier, while in males 0.63 and 0.39 years earlier)[3]. The Baka's permanent dentition has been studied with respect to other non-Pygmy populations as well as in relation to their body size[3,7,18–20]. Their post-canine dentition was found to be larger than in their Bantu neighbors[18] and other non-Pygmy populations. Sexual dimorphism of the Baka's permanent molars has been identified, being male molars significantly larger than in females[18]. However, no consistent pattern of correlation was found between the Baka's dental size and body height or weight[19].

Several important aspects of the Baka's biology are still unknown, and the morphological variation of the Baka's teeth is yet unexplored. Given the peculiar set of phenotypical expressions shown by the Baka, we want to contribute to the understanding of the Baka's morphological variation by investigating their deciduous upper and lower second molars (dm2) compared to a geographically diverse dm2 sample (Table 1). For this purpose, we use two approaches: 3D landmark- and semilandmark-based methods and analysis of qualitative dental traits. Working with µCT-derived 3D surface models, the geometry of both the enamel-dentine junction (EDJ) and the outer enamel surface[21] can be safely captured, while the roots cannot be investigated since they are completely resorbed in our Baka sample. Similarly, the moderate degree of wear of the occlusal aspect of the crowns permits the investigation of a limited number of discrete dental traits. Based on their results, Sardi and Ramirez Rozzi[22] inferred that the genetic pathways regulating cranial growth and dental morphogenesis are most likely distinct. If this is the case, we can expect that a reduced growth rate during infancy, resulting in short adult stature in the Baka Pygmies, will not necessarily correspond to dental changes. Thus, we predict that the Baka's dental shape does not differ substantially from other human groups. Additionally, considering the outcomes of previous work[18], we expect to find sex-related size differences within the Baka's dm2s.

Results

The most salient finding originating from our geometric morphometric investigation of upper and lower dm2s can be summarized as follows. The populations showed an overlapping distribution (Fig. 1; Fig. S1, S2, S3, S4). The dm2s varied from low-crowned and broad to tall and narrow. This pattern of variation was concordant between the upper and lower dm2s. Additionally, a reversed pattern of variation was detected for the upper and lower dm2s: the Baka's lower dm2s (ldm2s) showed a wider range of variation in comparison to the upper dm2s (udm2s), while the opposite was found for the European upper and lower dm2s. Depending on the analysis, 58 to 71% of the total variance could be explained by the first three principal components (PCs; Table 2).

Lower dm2 shape variation. The results of the ldm2's dentinal crowns analysis (using landmarks resampling the EDJ marginal edge and the cervical outline; Fig. 1a), revealed a broad overlap of all populations with the Baka sample. Shape variation along PC1 (explaining 32% of the total variance) concerned the relative bucco-lingual position of the hypoconulid corresponding with the relative bucco-lingual distal expansion of the dentinal crown, as well as its height. Variation along PC2 (16%) was driven by the mesio-distal elongation of the dentinal crown. Both along PC1 and PC2, the Baka exceeded the full range of shape variation within this geographically diverse comparative sample for their particularly short and mesio-distally elongated dentinal crowns, and relatively bucco-lingually narrow distal aspect. We observed a 20% higher variance for the Baka sample (0.006) than for the European ldm2s (0.005) (Supplementary Table 1 online). Supplementary Figure S1 shows the sample distribution along PC3. The Baka male and female means did not differ significantly ($P = 0.758$) in terms of ldm2 dentinal crown shape. Sexual dimorphism could not be tested in the other populations since the individuals' sex was unknown. As expected, the analysis of the EDJ alone did not provide any additional information with respect to the analysis of the dentinal crown, therefore, we found reporting on this analysis redundant.

The ldm2 cervical outline (see Supplementary Fig. S2a online) varied mainly between round and hourglass-shaped, with the Baka encompassing almost the full range of variation of the considered sample, along PC1 (30%). Along PC2 (26%), the cervical outlines varied from elongated and narrow, to short and broad. The Baka and the South American ldm2s reached the most extreme configurations in terms of bucco-lingual constriction and mesio-distal elongation. Similar to the cervical outline, the crown outline (see Supplementary Fig. S2b online) varied from mesio-distally elongated and bucco-lingually constricted, to rounded along PC1 (32%), while along PC2 (21%), variation occurred in the buccal aspect, with differing relative expansion of the mesial angle with respect to the distal angle.

Upper dm2 shape variation. The results for the udm2s dentinal crowns (Fig. 1b) showed again an overlapping distribution of Europeans and Southeast Asians with the Baka. Along PC1 (29%), the udm2 dentinal crowns varied between short with squared occlusal aspects, and taller with lingually displaced distal cusps (i.e., metacone and hypocone). Along PC2 (20%), variation was driven by the relative expansion of the trigon (consisting of the three main cusps and forming the central fovea) with respect to the talon (consisting of the disto-lingual cusp and fossa). In other words, the dentinal crowns possessed variably expanded central fovea with respect to their base. The Baka tended to have low, square-shaped dentinal crowns, while Europeans are more variable and are characterized also by tall teeth with lingually shifted distal cusps and reduced metacone. A clear separation between the Baka and the Egyptians, Bedouins, and South Americans was found, although these groups were represented by only two to four specimens. The analysis of variance (see Supplementary Table 1 online) revealed a 40% higher variance in the Europeans' udm2s (0.007) with respect to the Baka (0.005). Shape variation along PC3 is shown in Supplementary Figure S3 online. The Baka male and female means did not differ significantly ($P = 0.456$) in terms of ldm2 dentinal crown shape. The other groups could not be tested because the information on the individuals' sex was missing. Similar to the ldm2s, the EDJ analysis for the udm2s was not a source of additional information with respect to the analysis of the dentinal crown.

Population	Nr	Sex	Wear[23]		Dental outline analyses		EDJ analysis		EDJ reconstruction	
			U	L	U	L	U	L	U	L
Baka [a]	01	f		3		+		+		+
	02	f	3	3	+	+	+	+	+	+
	03	f	3–4		+		+		+	
	04	f	3	3–4	+	+	+	+	+	+
	05	m	3		+		+		+	
	06	f	3–4	3	+	+	+	+	+	+
	08	f	3	3	+	+	+	+	+	+
	09	f		3		+		+		+
	10	f	3	3	+	+	+	+	+	+
	11	m	3		+		+		+	
	12	f	3		+		+		+	
	13	f	3	3	+	+	+	+	+	+
	14	f		3		+		+		+
	16	m	3–4		+		+		+	
	17	m	3–4	3–4	+	+	+	+	+	+
	18	m	3	4–5	+	-	+	+	+	-
	19	f		3		+		+		+
	20	f	3–4	3–4	+	+	+	+	+	+
	22	f		3		+		+		+
	23	m	3	3	+	+	+	+	+	+
	24	f		3–4		+		+		+
	25	m	3	3	+	+	+	+	+	+
	28	m	3		+		+		+	
	29	f		3		+		+		+
	30	m		3		+		+		+
	31	f		3–4		+		+		+
	32	f		3		+		+		+
	33	f		3		+		+		+
	34	m	3	3–4	+	+	+	+	+	+
	35	m	5	3	-	+	+	+	-	+
	36	m		3		+		+		+
	37	m		3		+		+		+
	38	m	3	3	+	+	+	+	+	+
	39	m	3	3	+	+	+	+	+	+
	40	m	3	2	+	+	+	+	+	+
	41	f		3		+		+		+
	43	m	2		+		+		+	
	44	m		5		-		+		-
	45	m		5		-		+		-
Europeans	44[b]	?		1		-		+		+
	52[b]	?		1		-		+		+
	57[b]	?		1		-		+		+
	75[b]	?		1		-		+		+
	93[b]	?		1		-		+		+
	96[b]	?		1		-		+		+
	105[b]	?		1		-		+		+
	113[b]	?		1		-		+		+
	115[b]	?		1		-		+		+
	116[b]	?		1		-		+		+
	319[b]	?	1		-		+		+	
	322[b]	?	1		-		+		+	
	429[b]	?		1		-		+		+
	513[b]	?	1		-		+		+	
	549[b]	?	1		-		+		+	
	Nr75[b]	?	1		-		+		+	
Continued										

Population	Nr	Sex	Wear[23]		Dental outline analyses		EDJ analysis		EDJ reconstruction	
			U	L	U	L	U	L	U	L
	Nr115[b]	?	1		-		+		+	
	Nr116[b]	?	1		-		+		+	
	Nr396[b]	?	1		-		+		+	
	Cs13[c]	?	2		-		+		+	
	Cs305[c]	?	2		-		+		+	
	Cs444[c]	?	1		-		+		+	
	EH-U21[d]	?	1		-		+		+	
	EH-U56[d]	?	1		-		+		+	
	EH-U57[d]	?	1		-		+		+	
	CA_T19[e]	?	1		-		+		+	
	Med1_Batch1[e]	?	1		-		+		+	
	Guid_T49[e]	?	1		-		+		+	
	PM[e]	?	1		-		+		+	
	Tb36[e]	?	1		-		+		+	
	Tb37[e]	?	1		-		+		+	
	Tb44R[e]	?	1		-		+		+	
	Tb49[e]	?	1		-		+		+	
Bedouins[f]	BLZ_004	?		3		+		+		+
	BLZ_273	?		3		+		+		+
	BLZ_279	?		1		-		+		+
	BLZ_294	?		1		-		+		+
	BLZ_441	?	1		-		+		+	
	RCEH036	?	2		-		+		+	
South Americans[g]	A5380	?	3	2	+	-	+	+	+	+
	A5381	?	3	3	+	+	+	+	+	+
Egyptians[g]	A113	?	4–5		-		+		-	
	C120	?	3	3	+	+	+	+	+	+
	C392	m	4	2–3	+	+	+	+	+	+
	CN101	?		2		-		+		+
	CN141	?		3		+		+		+
	CN17	?	3		+		+		+	
	CN233	?		2		-		+		+
	CN26	?	3	3	+	+	+	+	+	+
	CN61	?	3	3	+	+	+	+	+	+
Southeast Asians[g]	SI3256	?	1		-		+		+	
	FI3528	?	2	3	-	+	+	+	+	+
	I9664	?	1	1	-	-	+	+	+	+
	I9665	?	4–5		-		+		-	
	NZ_3108	?		3		+		+		+
	NZ_3125-11	?	3		-		+		+	

Table 1. List of deciduous second molars used in this study, with associated information such as repository, sex, degree of wear, and analyses performed. It is also reported whether the dentine horns were virtually reconstructed. U = upper; L = lower; f = female; m = male; + = yes; − = no; ? = unknown; EDJ = enamel-dentine junction. [a]Université de Paris, Plateforme Imageries du Vivant. [b]Anatomy Collection of the Medical University of Vienna. [c]University of Vienna, Department of Evolutionary Anthropology. [d]Université de Poitiers, Centre de Microtomographie. [e]University of Bologna, Department of the Cultural Heritage. [f]Tel Aviv University, Department of Anatomy and Anthropology, The Sackler Maculty of Medicine. [g]Natural History Museum, Vienna.

For the cervical outline (see Supplementary Fig. S4a online), PC1 (34%) reflected the relative expansion of the disto-buccal cusp (or metacone) with respect to the disto-lingual cusp (or hypocone), while, along PC2 (23%), the cervical outlines varied from mesio-distally constricted (hourglass-shaped) to mesio-distally expanded with reduced hypocone. The crown outlines changed from oval to rounded along PC1 (37%; see Supplementary Fig. S4b online). This shape change was associated with the relative expansion of the paracone. Variation along PC2 (18%) consisted in the relative expansion of the talon with respect to the trigon.

Covariation. The covariation between upper and lower dm2s could be assessed only for individuals represented by both tooth types (n = 20 of which 13 Baka). For the dentinal crown, the percentage of total squared covariance of udm2s and ldm2s for the Singular Warp scores 1 was 66% and the pairwise shape correlation between the antagonists was r1 = 0.82 (Table 3). Upper and lower dm2s clearly showed common trends of variation (Fig. 2), being either short-crowned with reduced central fossa (in udm2s) and distal fossa (in ldm2s), or taller with expanded opposing fossae. Moreover, lower dentinal crowns were associated with relatively higher horn tips than taller crowns. It was not possible to clearly separate the populations from each other based on the upper and lower dm2 dyads, although the Baka showed extreme expression towards low and elongated dentinal crowns. Within the same tooth type, the highest covariation coefficient was found between the ldm2s cervical and crown outlines (r1 = 0.81), while cervical and crown outlines in udm2s covaried the least (r1 = 0.74).

Size. The comparison of the Baka's natural logarithm of Centroid Size (lnCS) for the dentinal crown with respect to the rest of the sample, showed that the Baka's upper and lower dm2s were among the largest specimens within our sample following the South Americans (Fig. 3). With respect to the various populations, the Baka's upper and lower dm2s differed significantly from Europeans, which similarly to the Egyptians were among the smallest specimens. Comparable results were obtained by observing the crown outline. The cervical and crown outlines of the Baka's udm2s were significantly larger than the rest of the sample (Table 4). Interestingly, while the Baka possessed the largest udm2s cervical outline, the Egyptians showed the highest values for the ldm2s. However, the interpretation of the findings for the groups with the smallest sample size (i.e., Egyptians, South American, and Southeast Asian individuals) require caution.

The multivariate regression showed that only a low percentage of morphological variation of the dentinal crown could be explained by lnCS (ldm2: ~ 3%; udm2: ~ 9%). In ldm2s, we observed size-related shape variation mainly connected to the expansion and reduction of the distal and occlusal aspects, and the height of the dentinal crown. Larger ldm2s showed low dentinal crowns with expanded distal aspects and mesio-distally elongated occlusal aspects. This underlines the fact that the largest teeth (among which the Baka) are short, bucco-lingually narrow, and mesio-distally elongated, while the smallest (including Europeans) tended to be taller, bucco-lingually broader and mesio-distally shorter. Similarly, size-related shape changes in udm2s were associated to the height of the dentinal crown, the relative expansion of the metacone, and the bucco-lingual position of the hypocone. Larger udm2s (including the Baka) showed lower dentinal crowns with expanded metacone and buccally shifted hypocone, resulting in a squared crown as seen in occlusal view. Conversely, smaller udm2 specimens, including the Europeans, typically showed high crown, reduced metacone and lingually shifted hypocone.

The dm2 size differences between the Baka males and females were not statistically supported (see Supplementary Fig. S5 and Supplementary Table S2 online) based on the lnCS of the dentinal crowns or crown outlines. These analyses could not be carried out on the whole sample due to the lack of necessary meta-data.

Non-metric traits. The prevalence of the hypocone and Carabelli's cusp for the udm2s, and entoconulid and metaconulid for the ldm2s are presented in Table 5. The high degree of occlusal wear along with the resorption of the roots in the Baka specimens hampered the observation of further discrete traits. The hypocone was present in each of the udm2 specimens in our sample, and was the most variable trait although it was highly expressed in most of the individuals (grade 4 to 6)[24]. The Baka showed a significantly higher frequency of a massive hypocone manifestation (96%) in comparison to the rest of the sample ($Chi^2 = 11.7$; $P = 0.001$). Most specimens, however, showed a medium grade of expression (3–4). In our sample, we found no or slight expression of Carabelli's cusp, of the entoconulid, and the metaconulid, with no significant differences between populations.

Discussion

The Baka Pygmies from south-eastern Cameroon, western Central African Republic, and northern Congo[5–8,10,18] are characterized by their peculiar life history and growth pattern with respect to other Pygmy and non-Pygmy populations. They are also known for their large permanent molars and early eruption times of the permanent teeth. However, in terms of dental morphology, we found that their dm2s are indistinguishable from other non-Pygmy populations. In fact, we observed a great overlap between populations in our ldm2 sample, which hampered the possibility to distinguish the diverse geographical groups based only on their tooth shape. Similar findings were obtained for permanent upper and lower premolars[25,26], and first and second molars[27,28] from non-Pygmy populations.

Based on dental crown diameters, most of the variation occurs within rather than between populations[29,30]. The high degree of shape variation observed in the Baka's ldm2s is compatible with previous observations on Sub-Saharan populations, which have been proven highly variable in terms of both dental[29] and cranial dimensions[31,32]. Surprisingly, the extended shape distribution of the Baka's lower dm2s is not paralleled by the upper dm2s. Interestingly, the pattern of variation in European dm2s follows the opposite trend, with less variable lower dm2s and highly variable upper dm2s. This finding is consistent with our knowledge of the European permanent upper molars showing a high degree of hypocone and metacone reduction[28].

Given the morphological resemblances of the dm2s to first molars (M1s)[33–37], we draw comparisons of our results with those previously published on M1s[28,38,39]. Distal and lingual aspects appear to be the most variable regions in both upper and lower dm2s. This finding is in agreement with Halász's[28] research on M1s from various populations, and with Polychronis et al.[39], who studied M1s from Greek individuals. There seems to be a clear trend that points to an increased shape variation in the distal aspect of the crown. The pattern of shape covariation observed between upper and lower dm2s, from tall-crowned with expanded opposing fossae to short-crowned with reduced opposing fossae, was also observed in upper and lower M1s[28]. High pairwise correlation between

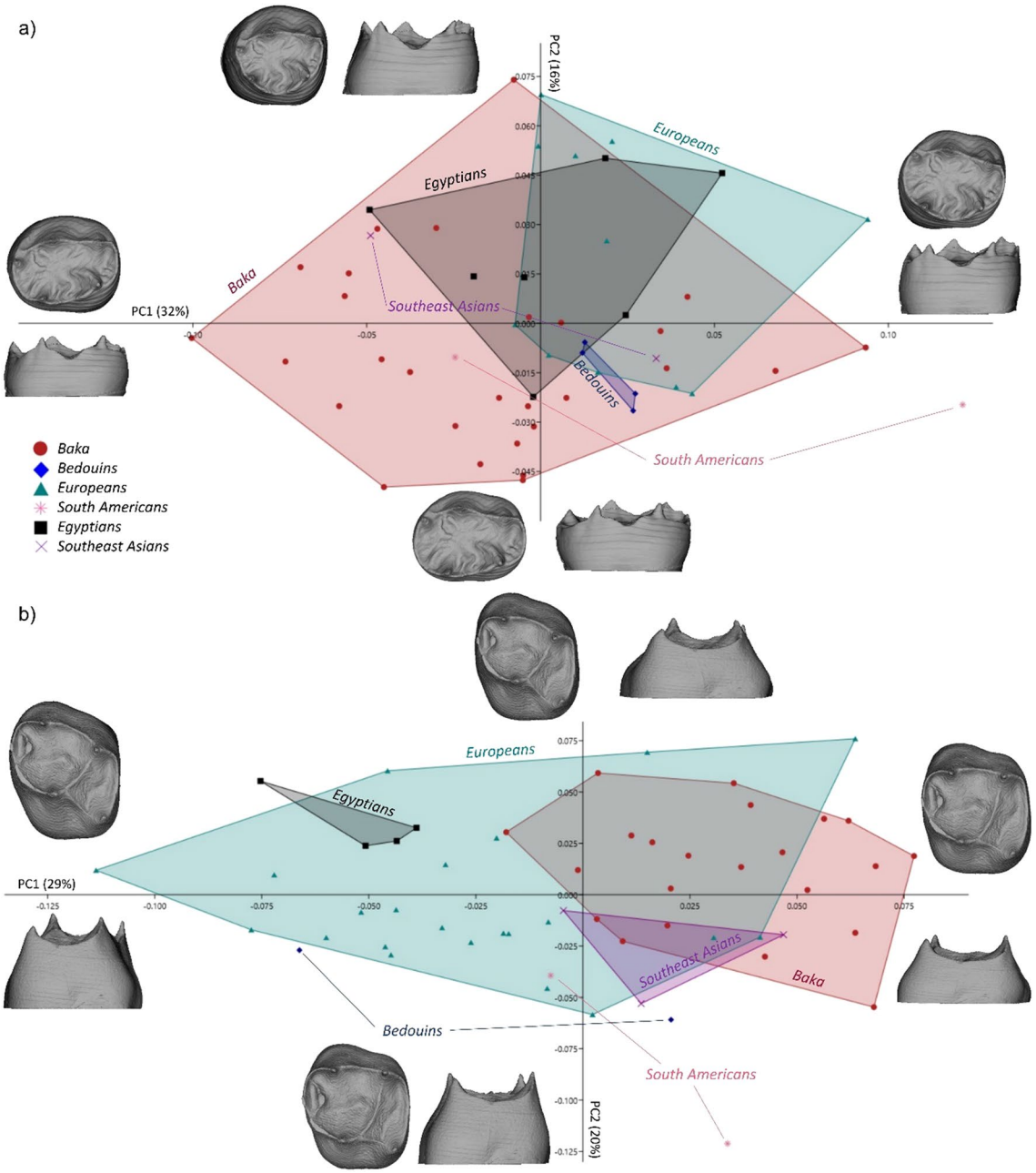

Figure 1. PCA plots for deciduous lower and upper second molar (ldm2 and udm2, respectively) dentinal crowns. (**a**) PC1—PC2 plot for ldm2s in shape space for the combined enamel-dentine junction (EDJ) and cervical outline (warpings at values ± 0.15/ ± 0.07). The Baka show a broader range of morphological variation than the Europeans; **b)** PC1—PC2 plot for udm2s in shape space for the combined EDJ and cervical outline (warpings at values ± 0.15). The Baka show a less variable morphology, while the Europeans exceed the range of variation.

opposing teeth developing in different jaws can be interpreted as a strong hint to tight genetic control over the dental development and is explained by the fact that antagonists have to occlude optimally to facilitate effective mastication. Likely, these functional constraints concern all posterior teeth, in fact similar results were achieved for dyads of other dental types[26,28].

In terms of non-metric traits, our results for the hypocone contrasted the findings of Edgar and Lease[33] who observed a high percentage of reduced hypocone expression in deciduous molars in general. Instead, we found expression of larger hypocones in the Baka than in Europeans, as Lease[40] found between African Americans and Europeans. Today it is known that the manifestation of Carabelli's cusp is equally common in all world populations[41], which was confirmed by our findings. However differently from Halász[28] who observed a prevalence of 100% in African and Near Eastern M1s, and in accordance to Scott[42] we found that most of the specimens lacked Carabelli's cusp. According to Harris[43] the grade of expression of Carabelli's cusp in M1s and

		PC1 (%)	PC2 (%)	PC3 (%)	Total (PC1-PC3) (%)
ldm2	Dentinal crown	32	16	10	58
	Cervical outline	30	26	15	71
	Crown outline	32	21	12	65
udm2	Dentinal crown	29	20	9	58
	Cervical outline	34	23	12	69
	Crown outline	37	18	12	67

Table 2. Percentage of variance explained by the first three PCs as resulting from the analysis of the deciduous upper and lower second molars' (udm2s and ldm2s, respectively) cervical outline, crown outline and combined enameldentine junction and cervical outline (dentinal crown).

	Pairwise correlation (r1)	% of total covariance
	udm2 dentinal crown	udm2 dentinal crown
ldm2 dentinal crown	0.82	66
	udm2 cervical outline	udm2 cervical outline
udm2 crown outline	0.74	61
	ldm2 cervical outline	ldm2 cervical outline
ldm2 crown outline	0.81	51

Table 3. Results of the 2B-PLS (single warp score 1) for the deciduous upper and lower second molars (udm2 and ldm2, respectively). The covariation between upper and lower dentinal crowns (combined enamel-dentine junction and cervical outline), and between cervical and crown outline in both udm2s and ldm2s was calculated.

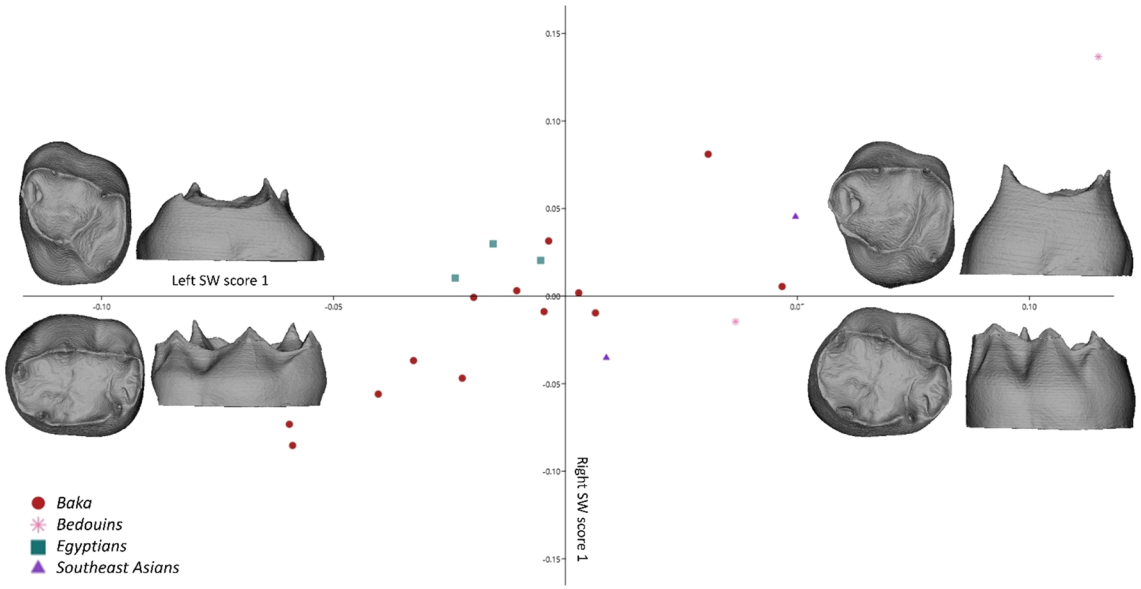

Figure 2. 2B-PLS plot capturing the covariation between dentinal crowns (combining enamel-dentine junction and cervical outlines) of deciduous upper and lower second molars (dm2s; warpings at values ± 0.15). The upper and lower dm2 dyads vary from low-crowned with expanded opposing fossae to high-crowned with reduced opposing fossae. The various populations can be hardly distinguished, although the subsample sizes are too small to allow definite statements. Within the Baka sample the most extreme expression towards the low-crowned variation is observed.

the tooth size are connected, with larger teeth showing this trait more often. We cannot support this result, since we did not find any significant differences in Carabelli's cusp manifestation between the populations in spite of the significant size differences. Likewise, the various populations used in our sample did not differ in terms of entoconulid and metaconulid frequency or degree of expression.

Variation in dental size of the Pygmies' permanent dentition in comparison to non-Pygmy populations was assessed previously for various samples testing different hypotheses[18–20,44]. The general findings include the

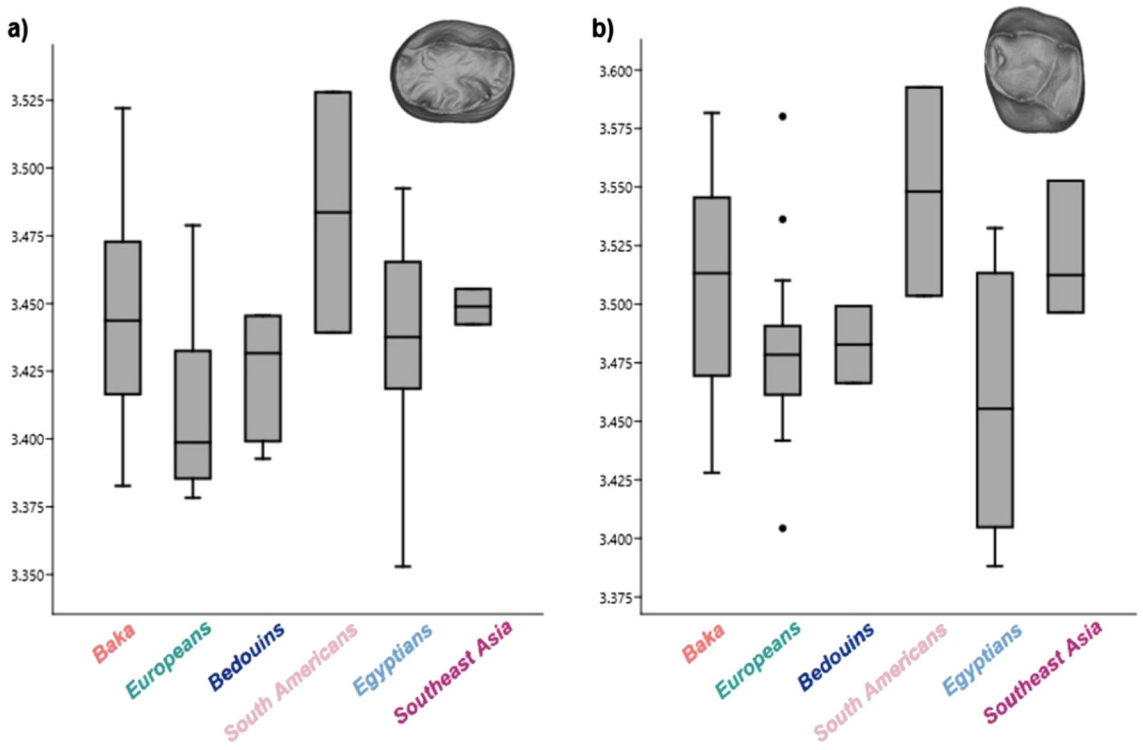

Figure 3. Boxplots of the natural logarithm of Centroid Sizes from the dentinal crown (combining enamel-dentine junction and cervical outline) in deciduous (**a**) lower and (**b**) upper second molars. South Americans and the Baka possess the largest deciduous second molars. The smallest specimens are within the Europeans and Egyptians.

		n (B/ n-B)	Z	*p*-value	n (B/E)	Z	*p*-value	Kruskal–Wallis test	*p*-value
ldm2	**Dentinal crown**	28/26	1.462	0.143	28/11	2.325	**0.020**	7.83	0.165
	Cervical outline	32/28	1.044	0.296	32/12	2.095	**0.036**	10.01	0.074
	Crown outline	32/27	1.072	0.283	32/11	2.157	**0.030**	6.78	0.237
udm2	**Dentinal crown**	22/33	1.898	0.057	22/22	2.325	**0.020**	10.97	0.052
	Cervical outline	23/37	3.740	< **0.001**	23/22	3.485	< **0.001**	18.45	**0.002**
	Crown outline	23/37	2.934	**0.003**	23/22	3.235	**0.001**	16.58	**0.005**

Table 4. Results of the Mann–Whitney U test and Kruskal–Wallis test. Differences in dental sizes (expressed by the logarithm of Centroid Sizes) for the deciduous upper and lower second molars (udm2 and ldm2, respectively) between the Baka and the rest of the comparative sample, between the Baka and the Europeans, and among all populations across the sample (B = Baka; n-B = non-Baka; E = Europeans; N = number of individuals).

tendency of sub-Saharan African populations to show larger permanent teeth than Asians or Europeans[30], which was confirmed by our results. According to Hanihara and Ishida[30], the modern aboriginal Australians show the largest dental diameters, followed by a group of average-sized dentitions of sub-Saharan Africans, Native Americans, and Southeast Asians. Europeans and populations of the Near East showed the smallest dentition. We observed the smallest dm2s sizes in Europeans and Bedouins, followed by Egyptians, Southeast Asians, and sub-Saharan Baka Pygmies. South Americans showed the largest dm2 crowns. It is worth nothing that our results use lnCS rather than linear distances, a different measure of size, depending also on the landmark configuration[26]. The dental size of the Baka and other Pygmies was studied also in relation to their body size and developmental patterns[19,20]. The results for the Baka's dm2s match previous findings on permanent molars which are larger than in any other non-Pygmy population[20], including the Bantu[18]. However, the Baka's dm2s do not show sex-related size variation that was previously reported for the permanent molars[18].

Early dental eruption timing has not only been found in the Baka's dm2s[3] but also in other sub-Saharan populations' permanent[45–48] and deciduous teeth[49]. According to Lam et al. (2015)[50], the earlier onset of tooth

		Prevalence (%)						
ldm2	Trait expression	Baka (n = 32)	Europe (n = 11)	Southeast Asia (n = 3)	Egypt (n = 7)	South America (n = 2)	Bedouins (n = 4)	Complete sample
Entoconulid	0–2	88	100	100	100	100	75	34
	3–5	12	–	–	–	–	25	
Metaconulid	0,1,1A	75	82	67	72	50	75	54
	2–4	25	18	33	28	50	25	
udm2	Trait expression	Baka (n = 23)	Europe (n = 22)	Southeast Asia (n = 5)	Egypt (n = 6)	South America (n = 2)	Bedouins (n = 2)	Complete sample
Hypocone	0–3	4	50	40	33	100	–	100
	4–6	96	50	60	67	–	100	
Carabelli's cusp	0–3	83	95	100	83	100	100	60
	4–7	17	5	–	17	–	–	

Table 5. Prevalence and degree of expression of the deciduous upper and lower second molars' (udm2 and ldm2, respectively) discrete dental traits, observed on the outer enamel surface. *n* number of individuals.

eruption can be associated with postnatal factors such as an increased rate of weight gain during the first three months of life. It is worth noting that, contrary to the Sua Pygmies, the Baka's weight gain during the first years of infancy does not differ from that of the Bantu[3]. However, considering that the time of dm2 formation partially overlaps with the time of growth decrease in the Baka, and the dm2s' size is not negatively affected by the decrease in body growth, we can assume no or only a very low correlation between odontogenesis and body growth in the Baka. Romero et al.[18] and Ramirez Rozzi[19] underline this finding stating that short stature is likely an adaptation not related to dental shape and size variation, and is genetically inherited by the Baka's ancestors[51]. Furthermore, no clear genetic pathway affecting both the teeth and the somatic growth that could explain the phenomena we observed in the Baka has yet been found[19]. Earlier tooth eruption has been associated with a lower degree of root growth[52], although root development and morphology in the Baka have not been studied yet, therefore the mechanisms prompting an early eruption of their teeth remain unknown.

In conclusion, despite the Baka's long-lasting geographical isolation[3,51], very low levels of genetic admixture with non-Pygmy neighboring populations[51,53], specific growth pattern, and dental eruption timing, we did not find a morphological difference distinguishing the Baka from other world populations based only on their dental morphology. As expected, we also did not observe any significant shape differences between sexes. Yet, the Baka's ldm2s can be particularly narrow and elongated with respect to the rest of the sample analyzed, and udm2s also clearly exceed the range of low and squared-crown variation in the sample, despite the fact that they are less variable than the ldm2s. Outstandingly, differential patterns of variation of udm2s and ldm2s in Central Europeans and the Baka might reflect their different population history reaching back to the migration of the European ancestors out of Africa[17], a topic that deserves further attention. Still, assigning an individual tooth to a certain population based on its shape alone remains impossible.

Methods

On the usage of the term 'pygmy'. In spite of the fact that term pygmy has been used by some as pejorative, its etymology is not dubious nor derogatory, deriving from the Greek pygmē 'a cubit', the measure of length from the elbow to the extreme of the middle finger (for an historical account of the usage of the term pygmy see Ballabriga, 1981[54]; Bahuchet, 1993[55]; Ramirez Rozzi, 2015[56]; 2021[57]). From a scientific standpoint, population genetics has shown that all human groups living in the African Equatorial forest usually referred to as Pygmies, shared a common ancestor which split from non-Pygmy groups around 65–50,000 years ago[51,53]: The term pygmy identifies groups of humans based on their geographic distribution, morphology, adaptation to the environment, growth pattern, and cultural and social traits: the Pygmies have a distinct population history which deserves to be acknowledged. In fact, adopting the term 'Pygmy', the Baka distinguish themselves from the Nzime, a group of Bantu ethnicity living in the surroundings of the Bosquet area. Some among the detractors of the term Pygmy have suggested the use of the term 'twa' (meaning 'dwarf') as an alternative. However, the Baka strongly oppose to defining themselves as such since non-Pygmy neighbor populations use it with disparaging intent. Others have proposed 'hunters-gatherers', but this term accounts only for the subsistence strategy thereby clouding all of the other distinctive traits of the Pygmies. The Pygmies have faced centuries of oppression which have led them to hide their identity to escape persecution[58]. Thus, referring to the Baka as Pygmies, is not only proper in scientific and anthropological terms, but is also ethically correct.

Sample. Our sample consists of 60 upper and 59 lower modern human deciduous second molars (Table 1). Of these, 55 teeth, including 23 udm2s and 32 ldm2s, are from 39 Baka individuals. The Baka were collected by F.V.R.R. after the Baka children naturally shed and donated them with the consent of their families. The rest of our sample was composed of individuals from different geographical regions, including Europe (udm2 = 22; ldm2 = 11), Africa (udm2 = 6; ldm2 = 7), Asia (udm2 = 5; ldm2 = 3), the Near East (udm2 = 2; ldm2 = 4), and South America (udm2 = 2; ldm2 = 2), which is quite a large sample considering the paucity of infant and juvenile

specimens in osteological collections. More so, the meta-data for the comparative sample is often incomplete since the individuals come from archaeological collections (Table 1). Other limitations of the sample size are the wear stage and state of preservation of the teeth. Dental specimens showing a moderate abrasion of the horn tips (stages 3 and 4) were virtually reconstructed (see *µCT acquisition and data segmentation* below) before data collection. To achieve a successful virtual reconstruction of the EDJ surface, wear cannot exceed stage 4[23], thus specimens showing higher degree of wear or with extensive decay in the occlusal area were either excluded from the sample or used only for the analyses of the cervical and crown outlines (see below). The heavy wear, the poor state of preservation, and occasional dental treatment made a large number of the Baka dental collection unusable for our study. In fact, the Baka's traditional dental treatments are quite invasive and entail the drilling of large cavities later filled with natural substances[9]. The Baka teeth were naturally shed and thus were in function approximately until the age of 11 which explains their advanced degree of wear. In some cases, the molars were kept by the Baka children or their families until the next visit of F.V.R.R., which likely led to the poor state of preservation of the teeth at the moment of image data acquisition (i.e., for the presence of numerous and deep cracks).

µCT acquisition and data segmentation. The dental datasets were imaged at four different facilities. The Baka teeth were scanned mainly at the Plateforme Imageries du vivant, Université de Paris, using a Micro CT-scanner PerkinElmer, Quantum FX (voxel size 20-40/30-59/10-20 µm, 90 kV, 16 mA) and at the Hard Tissue Research Unit, College of Dentistry (NYU), with a SCANCO Scantron 40 Micro-CT scanner (voxel size 12 µm, 70 kV, 275 mA, 200 ms). The comparative sample was scanned at the Centre de Microtomographie of the Université de Poitiers, with a VISCOM X8050-16 (voxel size 24–60 µm, 95–110 kV, 0.5 mA), and at the Vienna Micro-CT Lab, Austria, with a VISCOM X8060 NDT scanner (voxel size 21–60 µm, 110–140 kV, 280–410 mA, 1400–2000 ms, 0.75 mm copper filter). X-ray images were taken from 1440 different angles. Using filtered back-projection in VISCOM XVR-CT 1.07 software, these data were reconstructed as 3D volumes with a color depth of 16,384 grey values.

The µCT data were then imported into Amira software (www.fei.com) and virtually segmented to separate the enamel from the dentine, pulp, and the surrounding material (i.e., air, alveolar bone). In case of a slight abrasion of the dentinal horn tips, the specimens were virtually reconstructed by using the "brush" tool and extending the contours of the existing dentine into the empty area. In case both left and right dm2s were available from one individual, we preferred the left ones among our whole sample. However, if better preserved, we used the right tooth after virtual mirroring to the left side. Since there is no scientific evidence indicating the existence of directional asymmetry in human dentition, we assume that the choice of left teeth should not affect the results of this study[59–61].

Reorientation and outline collection. After segmentation, the surface models of the crowns were consistently reoriented in Geomagic Design X 64 (www.3dsystems.com) following an established protocol[62–64]. The crown and cervical outlines were collected from the reoriented surface models and projected onto the cervical plane. Afterwards, the outlines were split into 24 segments by as many equiangular radial vectors originating from the centroid of the outline area, using Rhinoceros 6 (www.rhino3d.com). Twenty-four pseudo-landmarks were placed at the point of intersection of the radii and the outline.

Landmark collection on the enamel-dentine junction. To collect the landmarks on the EDJ, we followed established protocols[62–64]. For the ldm2s, we placed a total of eight landmarks (LM) on the five main horn tips and three at the deepest points between metaconid and entoconid, protoconid and hypoconid, and between hypoconid and hypoconulid. Afterwards, 23 curve semilandmarks (sLM) were placed to represent the EDJ marginal edge. To ensure homology, we traced the EDJ occlusal edge by creating a spline curve ignoring all the accessory cusps. For the udm2s, seven LMs were placed on the four horn tips, and on the deepest points of the central fovea and distal fossa, and the deepest point of the lingual marginal ridge of the hypocone. The EDJ marginal edge was resampled by 47 sLMs. The LM collection was carried out in the EVAN Toolbox 1.75 (www.evan-society.org), which uses the bending energy technique for sliding the sLM[65–67].

Geometric Morphometric analysis. The geometric morphometric analyses were performed using the EVAN Toolbox separately for each set of landmarks, resulting in four different analyses per tooth type, namely: (1) cervical outlines; (2) crown outlines; (3) EDJ and (4) dentinal crown, combining the landmark configuration of the EDJ with the cervical outline. First, the landmark configurations had to be normalized via General Procrustes Analysis (GPA)[65,68,69]. The translation and rotation of the landmark sets were not necessary for the outline configurations since they were manually aligned. We run the principal component analysis (PCA) on the Procrustes shape coordinates and visualized the shape changes along the principal components by means of warpings, using the Thin-Plate Spline technique[70,71]. The analyses of the size were carried out using the natural logarithm of Centroid Size (lnCS). The lnCS of the combined EDJ and cervical outline is an accurate measure of the dentinal crown size since it embeds information about the crown's height, too. We explored shape covariation between udm2s and ldm2s as well as between different features of the same dental types by means of the 2-block partial least squares analysis (2B-PLS). Using R Studio (www.r-project.org), we performed the analysis of variance on Procrustes shape coordinates of larger subsamples, i.e., the Baka and the Europeans, to assess their degree of variation in the morphological expression of udm2s and ldm2s. In addition, the group mean differences between male and female Baka dentinal crowns were assessed with a permutation test of the Procrustes distances (10,000 random permutations) using a R script written for this purpose. A Mann–Whitney U test was used to test significance in size differences between the Baka and the Europeans, and between the Baka and the

rest of the sample. Another one was performed to the sex-related size differences within the Baka. This analysis could not be carried out on the other populations owing to the lack of associated meta-data. These tests have been carried out using PAST 4.03 (www.softpedia.com), as well as the Kruskal–Wallis test used to assess the size differences on populational level across the whole sample. Moreover, we performed a multivariate regression to analyze size-related shape variation of the dentinal crown.

Non-metric traits. The non-metric traits were evaluated based on the Arizona State University Dental Anthropology System (ASUDAS)[24,72]. Because of the high degree of wear of the Baka sample and root resorption, we focused on four among the most informative dental traits visible on the outer enamel surface, possibly reflecting neutral genetic variation[21,73].

1. Hypocone
   The hypocone is the fourth cusp of the upper molars that forms a separate region of the occlusal aspect, the trigon. Its manifestation varies from grade 0 to 6[24]. In this study, we used dichotomous classes to represent the hypocone degree of expression: none/light (0–3) and moderate/heavy (4–6).
2. Carabelli's cusp
   The Carabelli's cusp is an upper molar's accessory cusp occurring on the protocone (mesio-lingual cusp). This trait has been used as a diagnostic trait for European populations[42], however, according to more recent studies, no differences in prevalence and expression between populations are expected[24]. According to ASUDAS, the expression of the cusp of Carabelli varies between grades 0 to 7, but for this study, we used two categories: none/light (0–4) and moderate/heavy (5–7).
3. Entoconulid
   The entoconulid, or cusp 6, can be found in the distal area of the occlusal aspect of lower molars between the hypoconulid and the entoconid. The manifestation of the entoconulid can be expressed between grades 0 to 5, which are grouped into none/light (0–2) and moderate/heavy (3–5) in our study.
4. Metaconulid
   The metaconulid, or cusp 7, can be found on the lingual aspect of the lower molars between the entoconid and metaconid. Its manifestation can be expressed with 6 grades: 0, 1, 1A, 2, 3, and 4. We dichotomized the scoring values as none/light (0 to 1A) and moderate/heavy (2 to 4).

The prevalence of the non-metric traits was analyzed by $Chí^2$ test using SPSS (www.ibm.com).

Ethical statement. We did not use any physically invasive or destructive methods in our study. Our sample included only teeth obtained from archaeological collections or donated by the Baka children after they naturally shed them, and thus were not extracted. The Baka teeth were collected during field works carried out as part of an international agreement between the French National Research Institute for Development (IRD) and the Ministry of Scientific Research and Technology of Cameroon (Accord-cadre de Coopération en matière de Recherche Scientifique et Technique, 2004). Accordingly, informed consent was obtained from all participants and from both parents of any participants aged under 18.

Data availability

All data generated or analyzed during this study are either included in this published article (and its Supplementary Information file) or available from the corresponding author on reasonable request.

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References

1. Cavalli-Sforza, L. L. *African Pygmies*. (Academic Pr, 1986).
2. Ramirez Rozzi, F. V. Thoughts about responsibility in anthropological research, from a study with the Baka Pygmies. *Ethics Med. Public Health* **18**, 100668 (2021).
3. Ramirez Rozzi, F. V. Diversity in tooth eruption and life history in humans: illustration from a Pygmy population. *Sci. Rep.* **6**, 27405 (2016).
4. Althabe, G. Changements sociaux chez les Pygmées Baka de l'Est-Cameroun. *Cah. DÉtudes Afr.* **5**, 561–592 (1965).
5. Tsuru, D. Diversity of ritual spirit performances among the Baka Pygmies in southeastern Cameroon. *Afr. Study Monogr. Suppl. Issue* **39**, 47–84 (1998).
6. Ramirez Rozzi, F. V. Reproduction in the Baka pygmies and drop in their fertility with the arrival of alcohol. *Proc. Natl. Acad. Sci.* **115**, E6126 (2018).
7. Romero, A., Ramirez Rozzi, F. V., Cuesta-Torralvo, E. & Pérez-Pérez, A. Age-related tooth wear in African rainforest hunter-gatherers. *Am. J. Phys. Anthropol.* **170**, 622–628 (2019).
8. Yamauchi, T., Sato, H. & Kwamura, K. Nutritional status, activity pattern, and dietary intake among the Baka Hunter-Gatherers in the village camps in Cameroon. *Afr. Study Monogr.* **21**, 67–82 (2000).
9. Hattori, S. Utilization of Marantaceae plants by the Baka hunter-gatherers in southeastern Cameroon. *Afr. Study Monogr.* **33**, 29–48 (2006).
10. Ramirez Rozzi, R. F. V., Koudou, Y., Froment, A., Le Bouc, Y. & Botton, J. Growth pattern from birth to adulthood in African pygmies of known age. *Nat. Commun.* **6**, 1–12 (2015).
11. Meazza, C. *et al.* The Enigma of Pygmy short stature. *Endocrinology* **1**, 16 (2006).
12. Diamond, J. M. Why are pygmies small?. *Nature* **354**, 111–112 (1991).
13. Migliano, A. B., Vinicius, L. & Lahr, M. M. Life history trade-offs explain the evolution of human pygmies. *Proc. Natl. Acad. Sci.* **104**, 20216–20219 (2007).
14. Perry, G. H. & Dominy, N. J. Evolution of the human pygmy phenotype. *Trends Ecol. Evol.* **24**, 218–225 (2009).

15. Becker, N. S. A. *et al.* Indirect evidence for the genetic determination of short stature in African Pygmies. *Am. J. Phys. Anthropol.* **145**, 390–401 (2011).
16. Becker, N. S. *et al.* The role of GHR and IGF1 genes in the genetic determination of African pygmies' short stature. *Eur. J. Hum. Genet.* **21**, 653–658 (2013).
17. Pemberton, T. J. *et al.* A genome scan for genes underlying adult body size differences between Central African hunter-gatherers and farmers. *Hum. Genet.* **137**, 487–509 (2018).
18. Romero, A., Ramirez Rozzi, F. V. & Pérez-Pérez, A. Dental size variability in Central African Pygmy hunter-gatherers and Bantu-speaking farmers. *Am. J. Phys. Anthropol.* **166**, 671–681 (2018).
19. Ramirez Rozzi, F. V. & Romero, A. Tooth dimensions and body size in a Pygmy population. *Ann. Hum. Biol.* **46**, 467–474 (2019).
20. Shea, B. T. & Gomez, A. M. Tooth scaling and evolutionary dwarfism: an investigation of allometry in human pygmies. *Am. J. Phys. Anthropol.* **77**, 117–132 (1988).
21. Weber, G. W. & Bookstein, F. L. *Virtual Anthropology: A Guide to a New Interdisciplinary Field* (Springer, 2011).
22. Sardi, M. L. & Ramirez Rozzi, F. V. Developmental connections between cranial components and the emergence of the first permanent molar in humans. *J. Anat.* **210**, 406–417 (2007).
23. Molnar, S. Human tooth wear, tooth function and cultural variability. *Am. J. Phys. Anthropol.* **34**, 175–189 (1971).
24. Scott, G. R. & Irish, J. D. *Human Tooth Crown and Root Morphology* (Cambridge University Press, 2017).
25. Buchegger, L. *Variation of Outer and Inner Crown Morphology in Upper Premolars* (University of Vienna, 2015).
26. Krenn, V. A. *et al.* Variation of 3D outer and inner crown morphology in modern human mandibular premolars. *Am. J. Phys. Anthropol.* **169**, 646–663 (2019).
27. Teplanova, D. *Geometric Morphometric Analyses of Modern Human Upper Second Molars* (University of Vienna, 2015).
28. Halász, V. *Morphological Assessment of Modern Human Upper and Lower First Molars* (University of Vienna, 2019).
29. Hanihara, T. 19 Geographic structure of dental variation in the major human populations of the world. *Anthropol. Perspect. Tooth Morphol. Genet. Evol. Var.* **66**, 479 (2013).
30. Hanihara, T. & Ishida, H. Metric dental variation of major human populations. *Am. J. Phys. Anthropol. Off. Publ. Am. Assoc. Phys. Anthropol.* **128**, 287–298 (2005).
31. Relethford, D. J. H. & Harpending, H. C. Craniometric variation, genetic theory, and modern human origins. *Am. J. Phys. Anthropol.* **95**, 249–270 (1994).
32. Relethford, J. H. Apportionment of global human genetic diversity based on craniometrics and skin color. *Am. J. Phys. Anthropol. Off. Publ. Am. Assoc. Phys. Anthropol.* **118**, 393–398 (2002).
33. Edgar, H. & Lease, L. Correlations between deciduous and permanent tooth morphology in a European American sample. *Am. J. Phys. Anthropol.* **133**, 726–734 (2007).
34. Dahlberg, A. A. Analysis of the American Indian dentition. in *Dental Anthropology* 149–177 (Elsevier, 1963).
35. Hanihara, K. Standard models for classification of crown characters in the human deciduous dentition. *Mimeogr. Assoc. Plaster Casts* (1960).
36. Lease, L. R. *Ancestral Determination of African American and European American Deciduous Dentition using Metric and Non-Metric analysis*. (The Ohio State University, 2003).
37. Saunders, S. R. & Mayhall, J. T. Fluctuating asymmetry of dental morphological traits: new interpretations. *Hum. Biol.* **12**, 789–799 (1982).
38. Gómez-Robles, A. *et al.* A geometric morphometric analysis of hominin upper first molar shape. *J. Hum. Evol.* **53**, 272–285 (2007).
39. Polychronis, G., Christou, P., Mavragani, M. & Halazonetis, D. J. Geometric morphometric 3D shape analysis and covariation of human mandibular and maxillary first molars. *Am. J. Phys. Anthropol.* **152**, 186–196 (2013).
40. Lease, L. R. A descriptive study of African American deciduous dentition. *Dent. Anthropol. J.* **26**, 45–55 (2013).
41. Hanihara, T. Morphological variation of major human populations based on nonmetric dental traits. *Am. J. Phys. Anthropol. Off. Publ. Am. Assoc. Phys. Anthropol.* **136**, 169–182 (2008).
42. Scott, G. R. Population variation of Carabelli's trait. *Hum. Biol.* **11**, 63–78 (1980).
43. Harris, E. F. Carabelli's trait and tooth size of human maxillary first molars. *Am. J. Phys. Anthropol.* **132**, 238–246 (2007).
44. Irish, J. D. Characteristic high-and low-frequency dental traits in sub-Saharan African populations. *Am. J. Phys. Anthropol. Off. Publ. Am. Assoc. Phys. Anthropol.* **102**, 455–467 (1997).
45. Garn, S. M., Sandusky, S. T., Nagy, J. M. & Trowbridge, F. Negro-Caucasoid differences in permanent tooth emergence at a constant income level. *Arch. Oral Biol.* **18**, 609–615 (1973).
46. Hassanali, J. & Odhiambo, J. W. Ages of eruption of the permanent teeth in Kenyan African and Asian children. *Ann. Hum. Biol.* **8**, 425–434 (1981).
47. Blankenstein, R., Cleaton-Jones, P., Maistry, P., Luk, K. & Fatti, L. The onset of eruption of permanent teeth amongst South African Indian children. *Ann. Hum. Biol.* **17**, 515–521 (1990).
48. Reid, D. J. & Dean, M. C. Variation in modern human enamel formation times. *J. Hum. Evol.* **50**, 329–346 (2006).
49. Liversidge, H. Variation in modern human dental development. *Camb. Stud. Biol. Evol. Anthropol.* **10**, 73–113 (2003).
50. Lam, C. U. *et al.* Influence of metabolic-linked early life factors on the eruption timing of the first primary tooth. *Clin. Oral Investig.* **20**, 1871–1879 (2016).
51. Patin, E. *et al.* Inferring the demographic history of African farmers and Pygmy hunter–gatherers using a multilocus resequencing data set. *PLoS Genet.* **5**, 116 (2009).
52. Dean, M. C. & Cole, T. J. Human life history evolution explains dissociation between the timing of tooth eruption and peak rates of root growth. *PLOS ONE* **8**, e54534 (2013).
53. Verdu, P. *et al.* Sociocultural behavior, sex-biased admixture, and effective population sizes in Central African Pygmies and non-Pygmies. *Mol. Biol. Evol.* **30**, 918–937 (2013).
54. Ballabriga, A. Le malheur des Nains. Quelques aspects du combat des grues contre les Pygmées dans la littérature grecque. *Rev. Études Anciennes* **83**, 57–74 (1981).
55. Bahuchet, S. L'invention des Pygmées (Inventing Pygmies). *Cah. DÉtudes Afr.* **33**, 153–181 (1993).
56. Ramirez Rozzi, F. V. Les pygmées: histoire d'une rencontré, origines d'une dénomination, évolution des représentations. *Images Mém.* **45**, 13–20 (2015).
57. Ramirez Rozzi, F. V. Chez les Pygmées Baka de Cameroun. Voyage dans l'altérité. (L'Harmattan, 2021).
58. Bacirongo, I. & Nest, M. *Still a Pygmy*. (Finch Publishing, 2014).
59. Frederick, M. & Gallup, G. Fluctuating dental asymmetry in great apes, fossil hominins, and modern humans: Implications for changing stressors during human evolution. *Acta Psychol. Sin.* **39**, 489–494 (2007).
60. Kegley, A. & Hemingway, J. Assessing fluctuating odontometric asymmetry among fossil hominin taxa through alternative measures of central tendency: effect of outliers and directional components on reported results. *Homo* **58**, 33–52 (2007).
61. Moskona, D., Vainder, M., Hershkovitz, I. & Kobyliansky, E. Bilateral asymmetry in dental discrete traits in human isolates: South Sinai Bedouin tribes. *Anthropol. Anz.* 289–306 (1996).
62. Fornai, C. *et al.* The Qesem Cave hominin material (part 2): a morphometric analysis of dm2-QC2 deciduous lower second molar. *Quat. Int.* **398**, 175–189 (2016).
63. Fornai, C., Bookstein, F. L. & Weber, G. W. Variability of Australopithecus second maxillary molars from Sterkfontein Member 4. *J. Hum. Evol.* **85**, 181–192 (2015).

64. Hershkovitz, I. *et al.* The earliest modern humans outside Africa. *Science* **359**, 456–459 (2018).
65. Bookstein, F. L. *Morphometric tools for landmark data: geometry and biology*. (Cambridge University Press, 1997).
66. Gunz, P., Mitteroecker, P. & Bookstein, F. L. Semilandmarks in three dimensions. in *Modern morphometrics in physical anthropology* 73–98 (Springer, 2005).
67. Gunz, P. & Mitteroecker, P. Semilandmarks: a method for quantifying curves and surfaces. *Hystrix Ital. J. Mammal.* **24**, 103–109 (2013).
68. Gower, J. C. Generalized procrustes analysis. *Psychometrika* **40**, 33–51 (1975).
69. Mitteroecker, P., Gunz, P., Windhager, S. & Schaefer, K. A brief review of shape, form, and allometry in geometric morphometrics, with applications to human facial morphology. *Hystrix Ital. J. Mammal.* **24**, 59–66 (2013).
70. Bookstein, F. L. Principal warps: thin-plate splines and the decomposition of deformations. *IEEE Trans. Pattern Anal. Mach. Intell.* **11**, 567–585 (1989).
71. Bookstein, F. L. Thin-plate splines and the atlas problem for biomedical images. In *Proceedings of the 12th International Conference on Information Processing in Medical Imaging* 326–342 (Springer, 1991).
72. Scott, G. R. & Turner, C. G. Dental anthropology. *Annu. Rev. Anthropol.* **17**, 99–126 (1988).
73. Rathmann, H. & Reyes-Centeno, H. Testing the utility of dental morphological trait combinations for inferring human neutral genetic variation. *Proc. Natl. Acad. Sci.* **117**, 10769–10777 (2020).

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Author contributions

C.F. and F.V.R.R. initiated the project. C.F. and G.W.W. designed and supervised the project. P.G.Š. and C.F. performed the image data processing. P.G.Š collected landmark data. P.G.Š. carried out the analyses supported by C.F. and G.W.W. P.G.Š. wrote the paper with the contribution of C.F., G.W.W. and F.V.R.R. P.G.Š. compiled figures and tables. L.S., J.S. acquired the μCT scans.

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Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to P.G.Š. or C.F.

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Šimková et al. Supplementary Information

Morphological variation of the deciduous second molars in the Baka Pygmies

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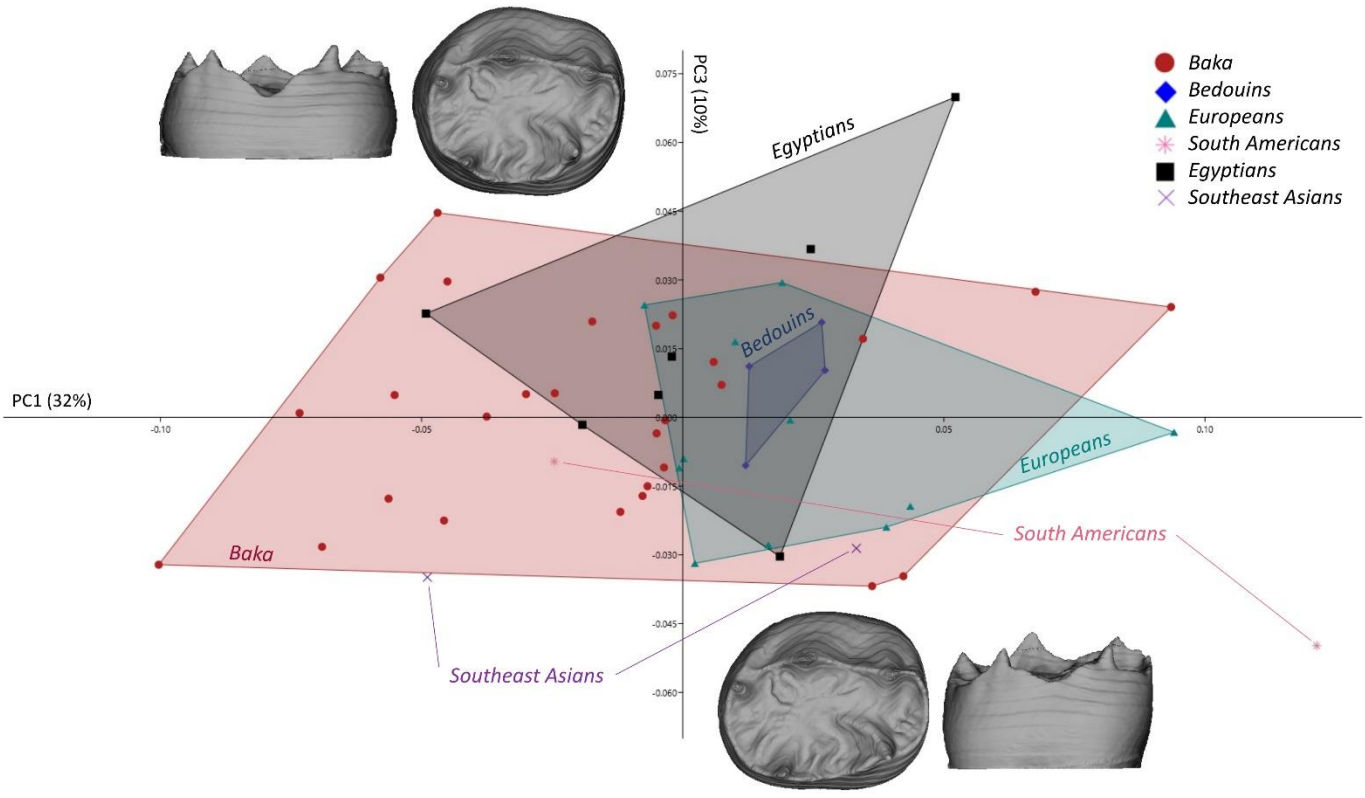
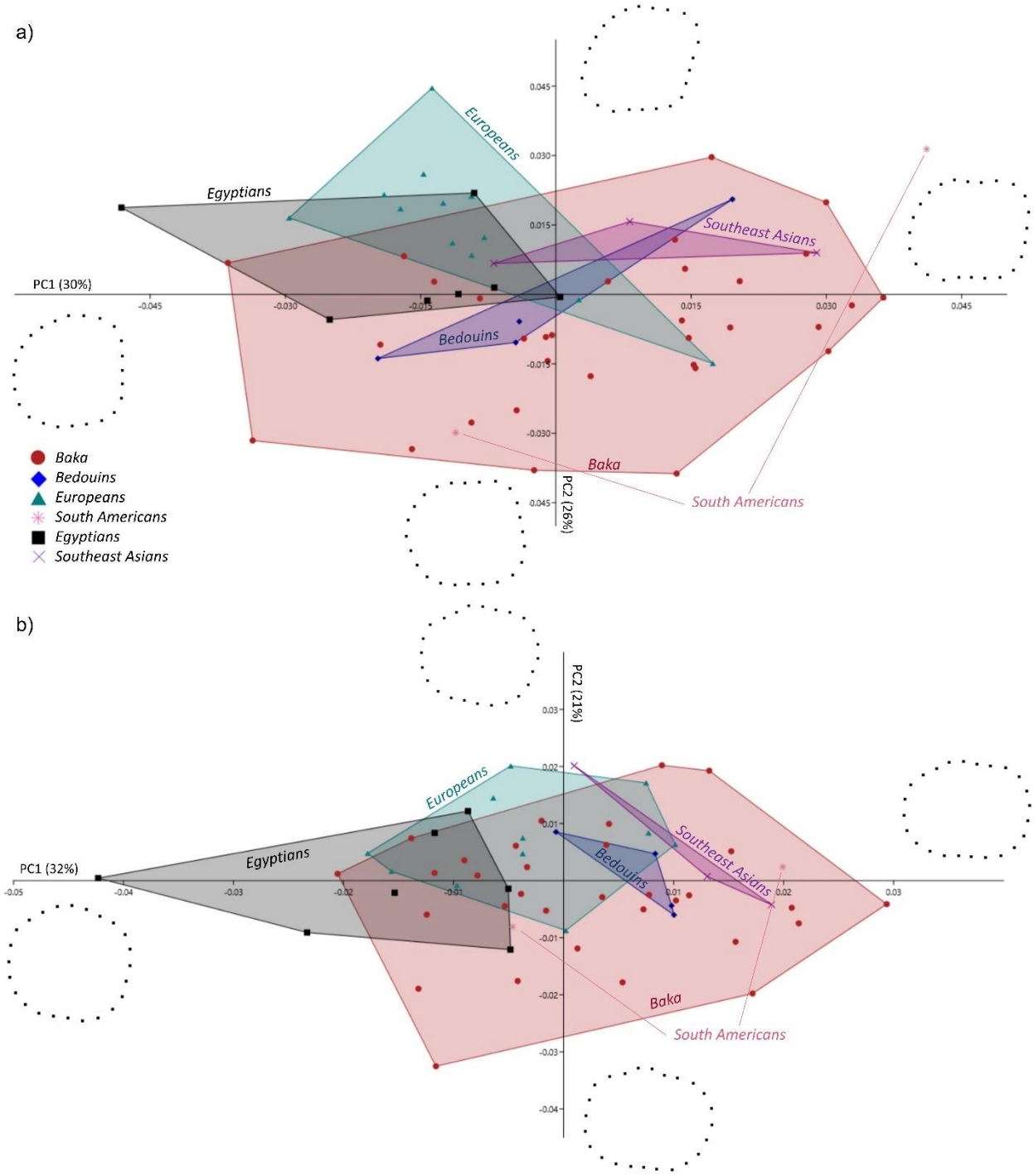
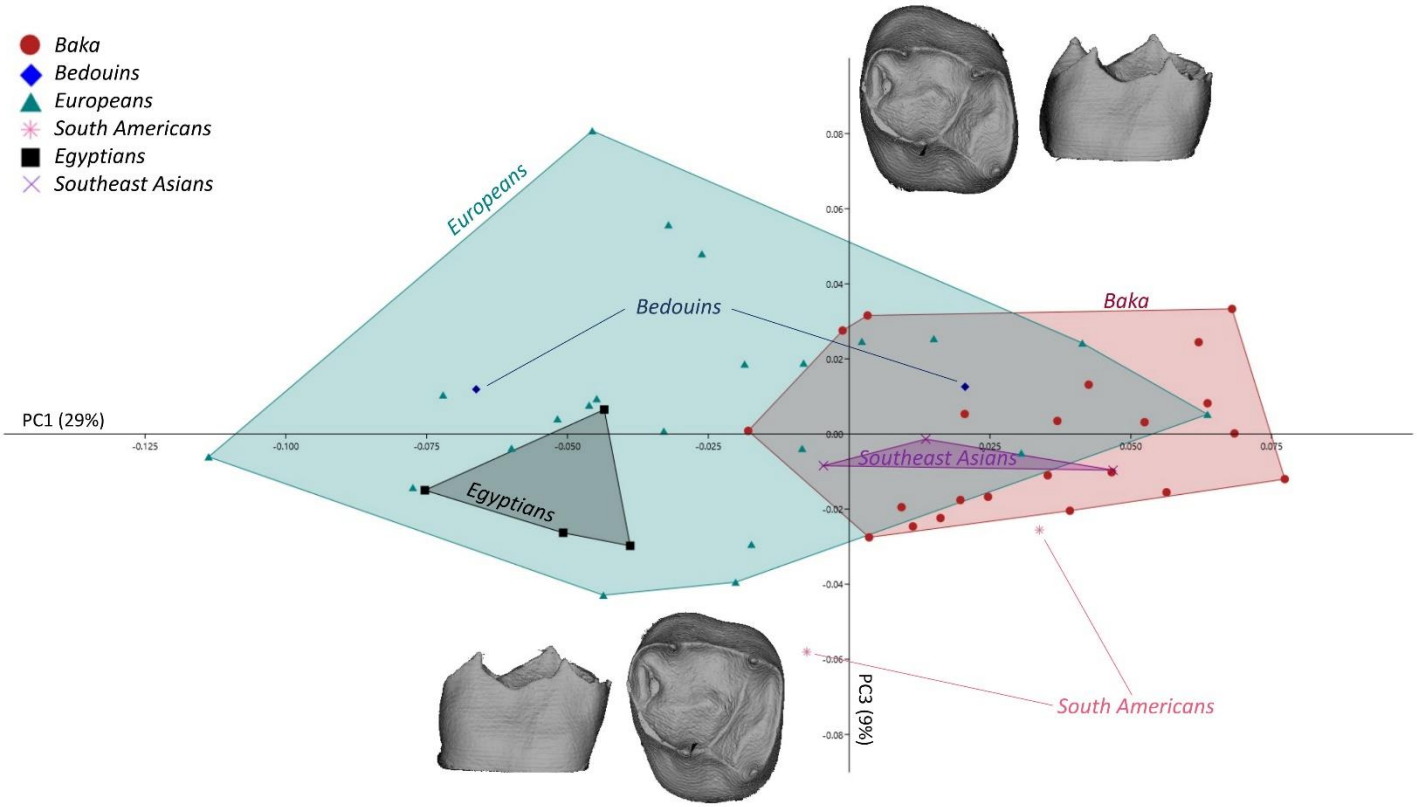


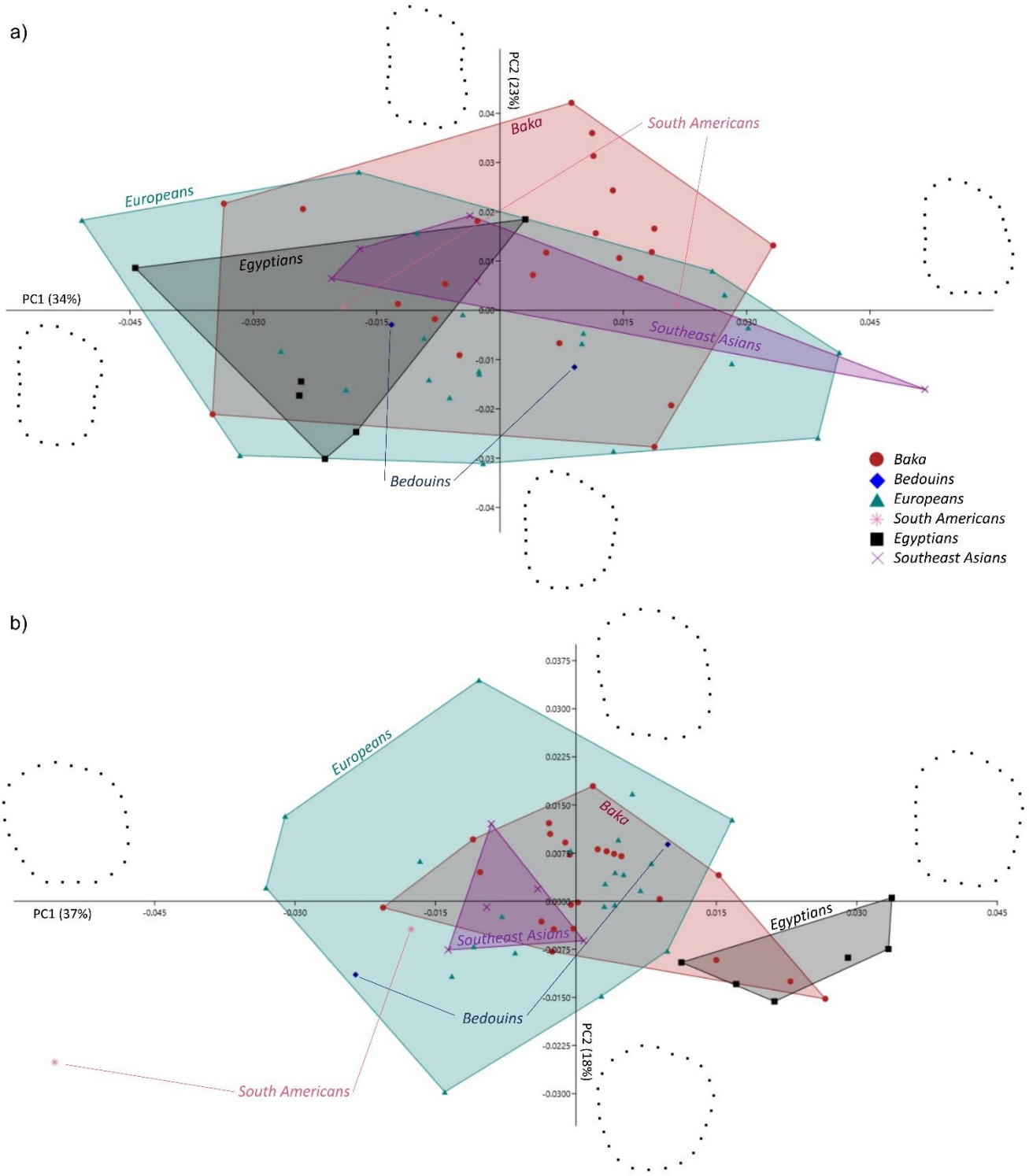
Supplementary Figure S1 PC1 – PC3 plot for the deciduous lower second molars in shape space for the dentinal crown, combining enamel-dentine junction and cervical outline. Along PC3 (explaining 10% of the total variance), the human groups overlap extensively. Shape changes are driven by the relative size of the tooth base in relation to the occlusal aspect, as well as changes in the crown height that increases with decreased relative size of the base (warpings at values ± 0.07). For the description of the results along PC1, see Fig. 1a in the main text.



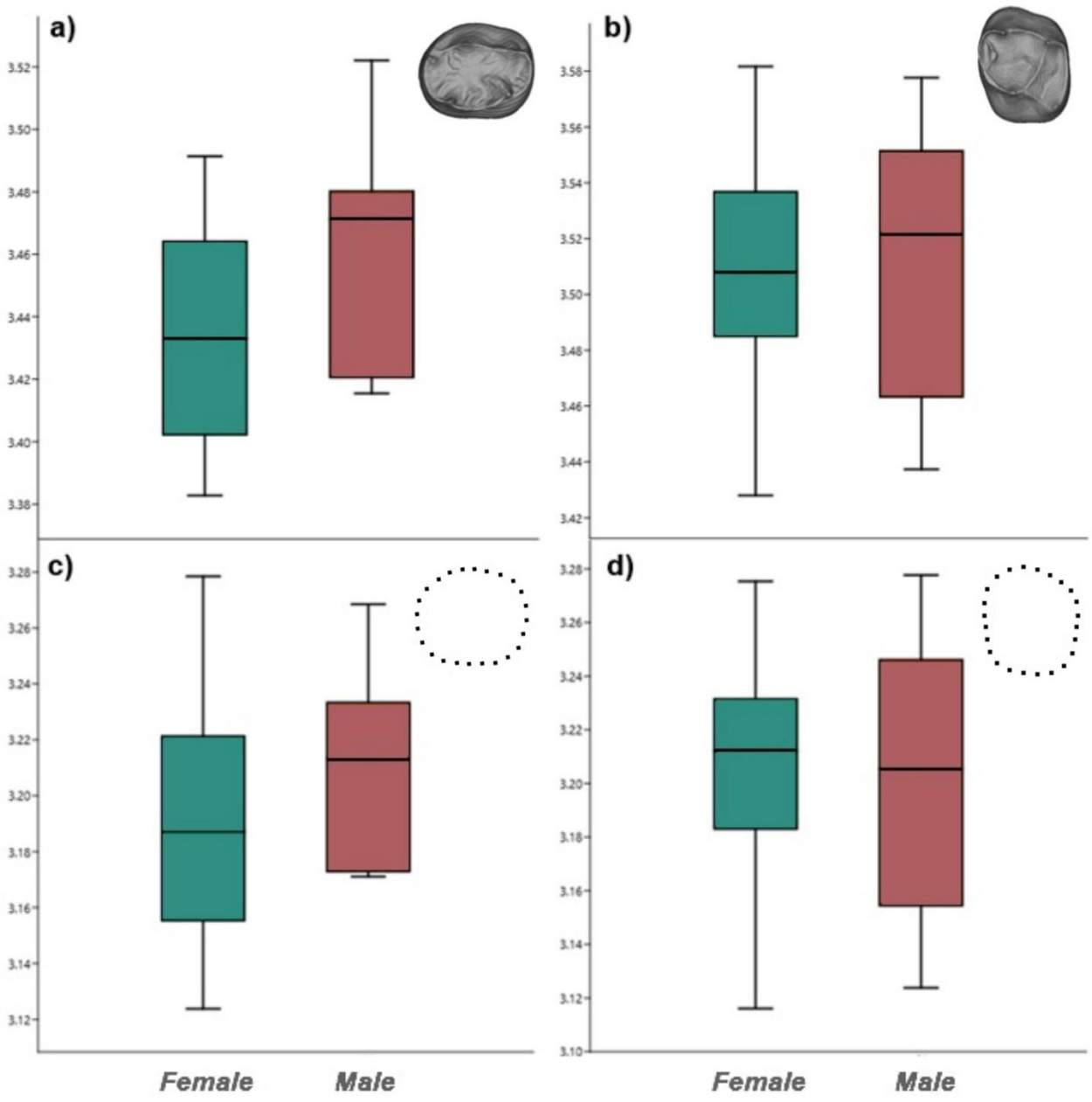
Supplementary Figure S2 PCA plots for the deciduous lower second molars' dental outlines. a) PC1 - PC2 plot in shape space for the cervical outline (warpings at values ± 0.05). The Baka exceed the range of variation of the rest of the sample, for the particularly pronounced hourglass-like shaped cervical outlines; b) PC1 - PC2 in shape space for the crown outline (warpings at values ± 0.05). The Baka show an extreme variation expression in elongated and bucco-lingually constricted crown outlines.



Supplementary Figure S3 PC1 – PC3 plot for the deciduous upper second molars in shape space for the dentinal crown, combining enamel-dentine junction and cervical outline. Along PC3 (explaining 9% of the total variance), the variation is driven mainly by the relative expansion of the trigon with respect to the talon. The reduction of the trigon is associated with observed metacone reduction. The Europeans exceed the range of variation of the rest of the sample in both directions, while the Baka are much less variable and centered around the mean values (warpings at values ± 0.085). The two South American specimens possess particularly reduced talons. For the description of the results for PC1 see Fig. 1b in the main text.



Supplementary Figure S4 PCA plots for the deciduous upper second molars' dental outlines a) PC1 - PC2 plot in shape space for the cervical outline (warpings at values ± 0.05). The human groups overlap extensively; b) PC1 - PC2 plot in shape space for the crown outline (warpings at values ± 0.04). The Europeans are more variable than the Baka and exceed the range of variance of the sample along PC2.



Supplementary Figure S5 Boxplots of the natural logarithm of Centroid Sizes for the Baka's male and female deciduous second molars (dm2s). The comparison of the lnCS distributions for the **a)** lower and **b)** upper dentinal crowns, and **c)** lower and **d)** upper crown outlines, do not reflect marked sexual differences, although a trend for larger male lower dm2s is noticeable.

Supplementary Table S1 Variance of the Procrustes shape coordinates for the Baka's and Europeans' dentinal crowns (combining enamel-dentine junction and cervical outline) of the deciduous upper and lower second molars (udm2s and ldm2s, respectively).

	The Baka	Europeans
ldm2	0.006	0.005
udm2	0.005	0.007

Supplementary Table S2 Results of the Mann-Whitney U test on the Baka's male and female logarithm of Centroid Sizes. Differences in dental sizes between the Baka's male and female dentinal crowns and crown outlines (f = female; m = male; N = number of individuals) are not statistically significant.

	ldm2		udm2	
	Dentinal crown	Crown outline	Dentinal crown	Crown outline
N (f/m)	17/11	18/14	9/13	9/14
Z	1.740	1.234	0.133	0.094
***p*-value**	0.081	0.216	0.893	0.924

2.2 Shape variation of modern human upper premolars

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Contribution: This project was designed and supervised by Prof. Gerhard Weber, with the data collection initially started by Lisa Wurm in 2015. Since 2022, this project was further continued by me, under the joint administration of Prof. Weber and myself. I significantly extended the sample and performed the image data acquisition and processing, as well as landmark collection. Furthermore, I conducted the analyses, created the data visualization, and wrote the paper, with contribution of all co-authors.

PLOS ONE

RESEARCH ARTICLE

Shape variation in modern human upper premolars

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Data Availability Statement: The data used in this study is not freely accessable, since the authors do not have the permission to share this data. The usage of some of the data has been personally requested from third parties and the consent has only been aquired for our group project, without any special access privileges. Consent has to be personally requested and obtained again for every group/researcher that meets the criteria of the third party. The image datasets are stored within the image archive of the Department of Evolutionary Anthropology of the University of Vienna (Anthropologie@univie.ac.at; +43-1-4277-54777;

Abstract

Morphological variation in modern human dentition is still an open field of study. The understanding of dental shape and metrics is relevant for the advancement of human biology and evolution and is thus of interest in the fields of dental anthropology, as well as human anatomy and medicine. Of concern is also the variation of the inner aspects of the crown which can be investigated using the tools and methods of virtual anthropology. In this study, we explored inter- and intra-population morphometric variation of modern humans' upper third and fourth premolars (P3s and P4s, respectively) considering both the inner and outer aspects of the crown, and discrete traits. We worked by means of geometric morphometrics on 3D image data from a geographically balanced sample of human populations from five continents, to analyse the shape of the dentinal crown, and the crown outline in 78 P3s and 76 P4s from 85 individuals. For the study of dental traits, we referred to the Arizona State University Dental Anthropology System integrated with more recent classification systems. The 3D shape variation of upper premolar crowns varied between short and mesio-distally broad, and tall and mesio-distally narrow. The observed shape variation was independent from the geographical origin of the populations, and resulted in extensive overlap. We noted a high pairwise correlation (r1 = 0.83) between upper P3s and P4s. We did not find any significant geographic differences in the analysed non-metric traits. Our outcomes thus suggest that geographical provenance does not play a determinant role in the shaping of the dental crown, whose genesis is under strict genetic control.

Introduction

Dental morphology has been investigated widely in order to study the variation within and between species based on general shape, metric and non-metric traits [1–14]. The crown of the premolars is usually composed of two main cusps (buccal and lingual) each continuing into a root cone [15]. These cones can form two separate roots or be fused into one root. Rarely, a three-

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rooted premolar with one lingual and two buccal roots can be observed [11,15]. By position within the dental row and function, maxillary premolars are intermediate between front and molar teeth. In this respect, third premolars possess some functional characteristics of a canine, while fourth premolars are more engaged in grinding functions comparable to molars [6].

3D morphological variation of upper premolar crowns among modern human populations is poorly investigated since most of the studies focused on the roots and root canals [16,17] also in clinical settings [18–20]. Previous investigations of the human maxillary premolar variation using traditional metric approaches [21] and landmark-based multivariate techniques [22], but on the outer enamel surface (OES), revealed morphological differences that help to distinguish between modern human populations. On the other hand, upper premolar morphology delivered varying results for the purposes of taxonomic classification in paleoanthropological studies using non-metric trait analyses [2,23,24], and geometric morphometric approach delivered significant results only for the P4s [23]. Metric measurements such as bucco-lingual and mesio-distal crown dimensions or crown height have also been used to study premolar size variation between and within populations [3,25,26]. Other studies focused on the occurrence and expressions of non-metric traits such as the maxillary premolar accessory ridges [11,27,28], the premolar mesial and distal accessory cusp [11,29,30], or the essential crests [30] among various modern human populations.

Compared to the OES, the examination of the enamel-dentine junction (EDJ) presents advantages because it represents the primary developmental structure of the crown and is consequently the precursor of the enamel cap's morphology [31–40], naturally resulting in a strong correlation between these two surfaces [31,33,41,42]. Furthermore, the overlying enamel protects the EDJ from early deterioration processes such as abrasion, erosion or chipping. The EDJ thus represents an appropriate alternative to the OES since it also bears relevant taxonomic information [31,37,39,43,44]. Morphometric studies of the EDJ of both permanent and deciduous molars are well represented in the literature [31,33,44–46], while for maxillary premolars, extensive comparative studies are very rare [47], or only focused on the descriptive assessment of the EDJ and OES [48–51]. Currently, 3D geometric morphometric (GM) analyses of the inner tooth crown variation in geographically diverse modern human samples have been published for permanent and deciduous molars [23,33,44,46,47,52], as well as lower premolars [53,54], and central incisors [55]. These studies did not detect geographically-dependent dental shape variation [33,54], thus, in the current study, we applied quantitative approaches to explore inter- and intra-population shape variation and covariation of modern human P3s and P4s, expecting to find similar outcomes. We want to test the hypothesis that the ranges of shape variation of upper premolars in different world populations overlap, meaning that recent human populations cannot be distinguished based on the shape of their upper premolar's dentinal crown. We used high-resolution 3D image data and GMM to investigate a geographically heterogeneous sample consisting of modern human populations from Africa, South America, Europe, the Near East, Southeast Asia and Oceania. Size variation was investigated based on the natural logarithm of Centroid Size. Moreover, we analysed the expression of the non-metric trait on both the EDJ and the OES. Comprehensive knowledge of these factors is essential in biology for taxonomic and evolutionary research, including human genotype-phenotype associations, and is also relevant in the various fields of medicine including forensics and dentistry.

Materials

We included 78 maxillary P3s and 76 maxillary P4s from 85 recent modern humans (Table 1). In particular, our sample comprised individuals from Oceania (n = 15), including the

Table 1. List of third and fourth premolars (P3 and P4, respectively) used in this study. (Wear recorded according to Molnar, 1977; f = female; m = male; + = present; — = absent; N.A. = not available; EDJ = enamel-dentine junction).

Population sample	*Individual*	*Origin*	*Wear*		*Age (years)*	*Sex*	*P3 analyses*		*P4 analyses*	
			P3	*P4*			*EDJ*	*Outlines*	*EDJ*	*Outlines*
Africans [a]	S16	Sub-Saharan	2	1	9–13	N.A.	+	+	+	+
	S29	Sub-Saharan	3	2	30–40	m	+	+	+	+
	S46	Sub-Saharan	2	2	14–18	f	+	+	+	+
	S61	Sub-Saharan	1	2	25–30	m	+	+	+	+
	S68	Sub-Saharan	2	2	25–30	m	+	+	+	+
	S97	Sub-Saharan	2	2	12 15	f	+	+	+	+
	S103	Sub-Saharan	-	2	12 15	m	-	-	+	+
	S111	Sub-Saharan	2	2	20–30	m	+	+	+	+
	S121	Sub-Saharan	2	2	25–30	m	+	+	+	+
	C333	Sub-Saharan	1	1	adult	N.A.	+	+	-	+
	ID_122_421_1464	Sub-Saharan	2	2	adult	m	+	+	+	+
	S85	Sub-Saharan	3	2	adult	N.A.	+	+	+	+
	S87	Sub-Saharan	2	2	adult	N.A.	+	+	+	+
	S128	Sub-Saharan	3	2	adult	N.A.	+	+	+	+
	S138	Sub-Saharan	2	2	adult	N.A.	+	+	+	+
Europeans [a, b]	Cs428	Avar	3	2	16–18	m	+	+	+	+
	Cs495	Avar	1	1	7–8	N.A.	+	+	+	+
	Cs498	Avar	2	2	25–30	f	+	+	+	+
	Cs502	Avar	2	1	13–15	N.A.	+	+	+	+
	Cs541	Avar	3	2	19–30	f	+	+	+	+
	Cs582	Avar	3	2	19–25	f	+	+	-	+
	Cs654	Avar	1	1	3–5	N.A.	+	+	+	+
	ID_120_123_1043	Central European	1	1	10	m	+	+	+	+
	ID_300_510_578	Central European	1	1	11	f	+	+	-	-
	ID_125_028_1089	Central European	1	-	10	f	+	+	-	-
	ID_125_415_1124	Central European	-	1	6	m	-	-	+	+
	ID_120_074_711	Central European	1	1	6	m	+	+	-	+
	ID_122_510_1554	Central European	2	1	22	m	+	+	+	+
	ID_122_511_1555	Central European	2	2	adult	f	+	+	+	+
	Anatomy_19710	Central European	2	2	20	N.A.	+	+	+	+
	ID_126_804_1171	Central European	2	2	29	m	+	+	+	+
	ID_122_199_961	Central European	2	2	20	m	+	+	+	+
Near Easterners [c]	BLZ_483	Bedouin	2	2	N.A.	N.A.	+	+	+	+
	BLZ_506	Bedouin	1	1	N.A.	N.A.	+	+	+	+
	BLZ_515	Bedouin	3	2	N.A.	N.A.	+	+	+	+
	NN1	Bedouin	3	3	N.A.	N.A.	+	+	+	+
	RCEH038	Bedouin	3	-	N.A.	N.A.	+	+	-	-
	AM_23	Natufian	2	2	N.A.	N.A.	+	+	+	+
	AM_56	Natufian	1	1	N.A.	N.A.	+	+	+	+
	AM_67	Natufian	3	2	N.A.	N.A.	+	+	+	+
	AM_69	Natufian	3	3	N.A.	N.A.	+	+	+	+
	AM_101	Natufian	3	2	N.A.	N.A.	+	+	+	+
	Hay_8	Natufian	4	4	N.A.	N.A.	-	+	-	+
	Hay_12	Natufian	1	1	N.A.	N.A.	+	+	+	+
	Hay_19	Natufian	2	2	N.A.	N.A.	+	+	+	+
	Hay_25	Natufian	3	3	N.A.	N.A.	+	+	+	+

(*Continued*)

Table 1. (Continued)

Population sample	*Individual*	*Origin*	*Wear*		*Age (years)*	*Sex*	*P3 analyses*		*P4 analyses*	
			P3	*P4*			*EDJ*	*Outlines*	*EDJ*	*Outlines*
Oceanians [a]	C54	Oceanian	2	2	adult	m	+	+	+	+
	C55	Oceanian	3	3	adult	m	+	+	+	+
	C104	Oceanian	1	1	juvenile	N.A.	+	+	+	+
	S89	Oceanian	2	1	adult	N.A.	+	+	+	+
	NZ_1459	Oceanian	4	4	N.A.	N.A.	-	+	-	+
	NZ_3093	Oceanian	1	-	N.A.	N.A.	+	+	-	-
	NZ_3099	Oceanian	-	3	N.A.	N.A.	-	-	+	+
	NZ_3104	Oceanian	4	3	N.A.	N.A.	-	+	+	+
	NZ_3108	Oceanian	1	-	N.A.	N.A.	+	+	-	-
	CN5	Papuan	-	1	adult	m	-	-	+	+
	CN220	Papuan	1	2	adult	m	+	+	+	+
	CN230	Papuan	2	2	adult	m	+	+	+	+
	CN232	Papuan	2	2	adult	m	+	+	+	+
	CN236	Papuan	3	2	mature	m	+	+	+	+
	CN264	Papuan	2	1	30	m	+	+	+	+
South Americans [b]	793	American	2	3	adult	m	+	+	+	+
	806	American	-	2	adult	m	-	-	+	+
	1169	American	1	1	juvenile	N.A.	-	+	+	+
	2286	American	2	2	N.A.	f	+	+	+	+
	3537	American	2	2	adult	N.A.	+	+	+	+
	5382	American	1	-	juvenile	N.A.	+	+	-	-
	5385	American	2	2	adult	N.A.	+	+	+	+
	5389	American	1	1	infant	N.A.	+	+	+	+
	5443	American	-	1	juvenile	f	-	-	+	+
	6321	American	3	3	adult	N.A.	+	+	+	+
	15353	American	1	2	adult	m	+	+	+	+
	TF_6031	Selk'nam	4	4	adult	N.A.	-	+	-	+
	TF_6034	Selk'nam	4	3	adult	f	-	+	+	+
	TF_6035	Selk'nam	3	3	adult	N.A.	+	+	+	+
	TF_6038	Selk'nam	3	-	adult	m	+	+	-	-
	TF_6040	Selk'nam	3	-	adult	f	+	+	-	-
	TF_6041	Selk'nam	4	3	adult	m	-	+	+	+
	TF_21462	Selk'nam	4	-	N.A.	N.A.	-	+	-	-
Southeast Asians [b]	ID_122_342_1383	Indonesian	2	1	28	m	+	+	+	+
	ID_122_335_1376	Indonesian	3	3	36	m	+	+	+	+
	ID_122_369_1412	Indonesian	3	2	adult	m	+	+	+	+
	1365	Indonesian	4	-	N.A.	f	-	+	-	-
	1368	Indonesian	-	4	N.A.	f	-	-	-	+
	2583	South Chinese	4	4	N.A.	f	-	+	-	+

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[b]Natural History Museum, Vienna

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indigenous people of Papua New Guinea (n = 6), the former diverging from Eurasian populations around 62 to 75 thousand years ago [56–58], South America (n = 19), including the native population of Selk'nam from Tierra del Fuego (n = 7), Europe (n = 17), including Avars from the 8th century (n = 7), sub-Saharan Africa (n = 15), Southeast Asia (n = 6) and the Near East (n = 14) including Bedouins (n = 4) and Natufians (n = 10). All teeth used for the EDJ analyses were free of decay or fillings and did not exceed Molnar's [59] wear stage 3 (slight to moderate wear). Accordingly, populations from various geographical regions, climatic zones, and environments, featuring different life styles, were represented in our sample.

Methods

Scanning & segmentation

All specimens were scanned at the Vienna μCT Lab, Austria, with an industrial Viscom X8060 NDT scanner (scanning parameters: 110–140 kV, 280–410 mA, 1400–2000 ms, 0.75 mm copper filter with a voxel size ranging between 20 and 50 μm). Virtual segmentation of the μCT data was performed in Amira software (www.thermofisher.com) to obtain triangulated surface models of the enamel and dentine. Models were extracted from the left premolars but, if absent or unusable, the right-side premolars were considered (n = 49) since directional asymmetry is not expected in teeth [60]. If this was the case, the right-side datasets were flipped before surface extraction. Slightly worn dentinal horn tips were virtually reconstructed in Amira software by extending the contours of the existing part of the dentinal horn tips (n = 38).

Reorientation of the models and data collection on the cervical and crown outlines

The segmented dental surface models were reoriented in Geomagic Design X 64 (www.3dsystems.com) according to an established protocol [61]. Thus, the best-fit plane of the cervical margin, or cervical plane, was computed and was used to reorient the dataset to set the cervical plane parallel to the x-y plane of the virtual working space. Afterwards, the crown was rotated to align the mesial ridge to the y-axis. Cervical and crown outlines of the reoriented surfaces were collected and projected onto the cervical plane. The outlines were imported into Rhinoceros 6 (www.rhino3d.com) and split into 24 curve segments by equiangular radial vectors that originated from the centroid of the outline. The intersection points between the radii and the outline marked 24 pseudo-landmarks.

Landmark collection on the EDJ

The reoriented surfaces of the dentinal crowns were imported into the free-to-download EVAN-Toolbox software (www.evan-society.org) for further landmark collection. A total of 24 landmarks were placed on the EDJ of each of the premolars. On all premolars, two fixed landmarks were placed on the paracone and protocone horn tips. On the P3s, the remaining two fixed landmarks were placed at the deepest mesial and distal points of the central groove. Since the central groove is not as well defined in P4s, the landmarks were instead placed on the deepest points of the distal and mesial ridges, which are always well visible. Additionally, the marginal ridge of both tooth types was represented by 20 curve semilandmarks. The sliding of the semilandmarks was performed using the bending energy technique [62–64]. Our approach matched that performed on lower premolars in Krenn et al. [54], who validated this technique by assessing the observer error. We do not expect the validity of these protocols to change in the upper premolars.

Geometric morphometric analyses

We performed GM analyses using the EVAN-Toolbox for each set of landmarks (namely, cervical outline, crown outline, EDJ, and dentinal crown) for P3s and P4s, separately. The dentinal crown dataset was created by merging the cervical outline and the EDJ landmark configurations. A General Procrustes Analysis (GPA [65]) was performed for all data sets to normalize the landmark configurations. The resulting Procrustes shape coordinates were analyzed via principal component analysis (PCA). The Thin-Plate Spline technique was used to visualize the shape changes along the principal components (PCs) by means of warping [62,66]. To explore the shape covariation between P3s and P4s, as well as between different aspects within the same dental type (i.e. between the cervical outline and the EDJ), we performed a 2-block partial least squares (2B-PLS) analysis. To analyze the effect of allometry, multivariate regression was performed. The size was investigated by using the natural logarithm of Centroid Size (lnCS [67]). By using the lnCS derived from the dentinal crown dataset (combining the EDJ with cervical outline) we obtained a measure of size representing crown height as well. For testing tooth size differences between populations, we performed Kruskal-Wallis and Mann-Whitney-U tests (in PAST 4.03; www.softpedia.com), since the obtained data was not normally distributed. Permutational Multivariate Analysis of Variance (PERMANOVA) was performed using PAST 4.03 to test for shape differences between the groups (using all PC-scores), allowing for a distribution-free setting using permutational algorithms.

Non-metric traits

The following non-metric traits were considered:

1. Premolar mesial and distal accessory cusps

Accessory mesial or distal cusps originate from a bifurcation on the margin of the sagittal sulcus that is separating the buccal and lingual cusps, forming a free-standing cusp on the OES [11,13,68]. Sakai et al. [69] describe accessory cusps as indistinct on the EDJ, with a protrusion or swelling of the marginal ridge where the cusp could be. We used the following classifications to evaluate the presence of the accessory cusps: absent (0), mesial cusp (1), distal cusp (2), and mesial and distal cusp (3).

2. Maxillary premolar accessory ridges (MxPAR)

Accessory ridges may or may not be present on the lingual lobe segment of the buccal cusp, mesially and distally from the essential crest of both P3s and P4s [11], and can be extended into the central groove. These features are prevalent in both the OES and the EDJ, and their distribution was found to be heterogeneous among populations [28,70]. To represent the expression of these features, we used the following classification: absent (0), mesial accessory ridge (1), distal accessory ridge (2), and mesial and distal accessory ridges (3).

3. Essential crest

The essential crests of the central lobe of the paracone and protocone are usually separated by the sagittal sulcus and central groove. However, if this is not the case, i.e., both essential crests are connected, they create a transverse crest. Additionally, the separated essential crest of the buccal cusp can also be bifurcated. Several studies were able to detect these features on both EDJ and OES [48–51]. In this study, we classified every kind of essential crest expression separately, namely the separated buccal and lingual crests (0), transverse crest (1), and the bifurcated buccal essential crest (2).

Traits 1 and 2 were derived from the Arizona State University Dental Anthropological System [11,12]. For trait 3 (essential crest) we referred to Bailey's [2] buccal essential crest and

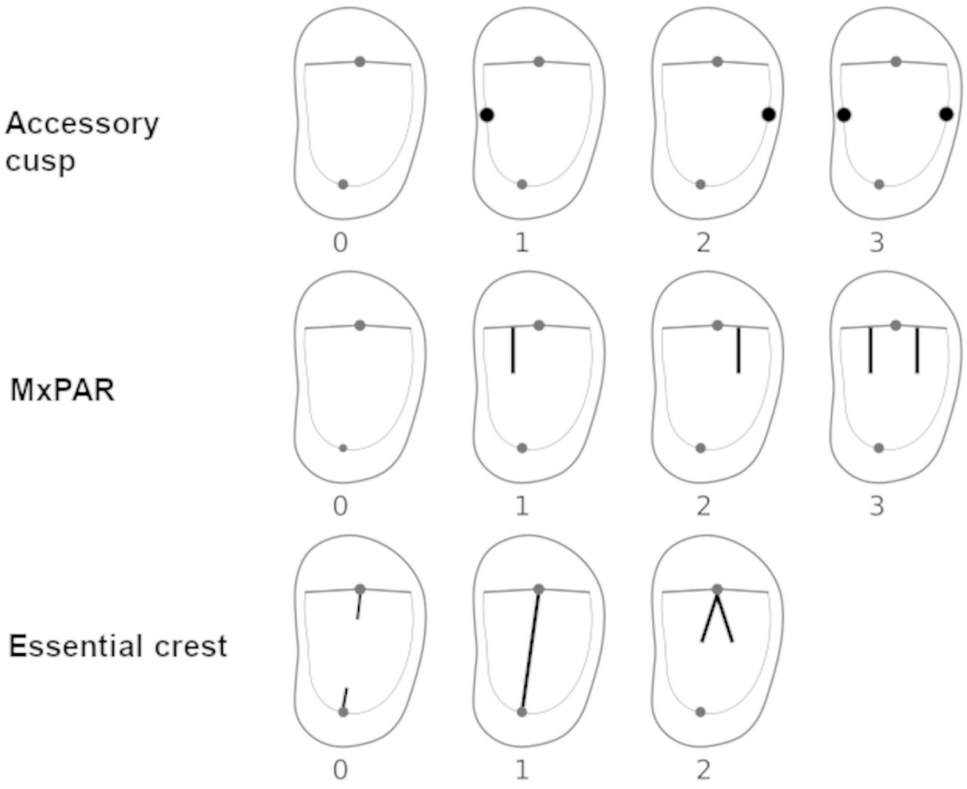

Fig 1. Illustration of scored non-metric traits and their expression categories.

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traverse crest grading system. All traits were scored both on the OES and EDJ of each specimen and evaluated according to their presence and expression (Fig 1), performing a Chí^2 test in PAST 4.03.

Ethics statement

In this study, we used non-invasive μCT (micro computed tomography) data (a three-dimensional X-ray method) from human remains included in the collections of the Department of Evolutionary Anthropology, University of Vienna and the Natural History Museum Vienna, Austria. Since only osteological material was used, no ethical approval or guidance was required as the research did not involve present-day human samples. The specimens were exported to Europe between the 19th century and the beginning of the 20th century, being curated in Austrian institutions for over a century. They were regarded and treated as precious parts of natural history collections and used for research since then. Today, their acquisition raises concerns according to current ethical principles. During the last 15 years, skulls were μCT-scanned at the Vienna μCT Lab of the University of Vienna for the purpose of preservation and fundamental research. Specimens were handled only by highly trained personnel in the most respectful way. No damages or alterations of any kind were caused to the remains.

Results

Shape variation in P3s

Results obtained from the PCA analysis of the P3 dentinal crown (Fig 2A) revealed an extensive overlap of the populations. Variation along PC1 (27% of the total variance explained) was

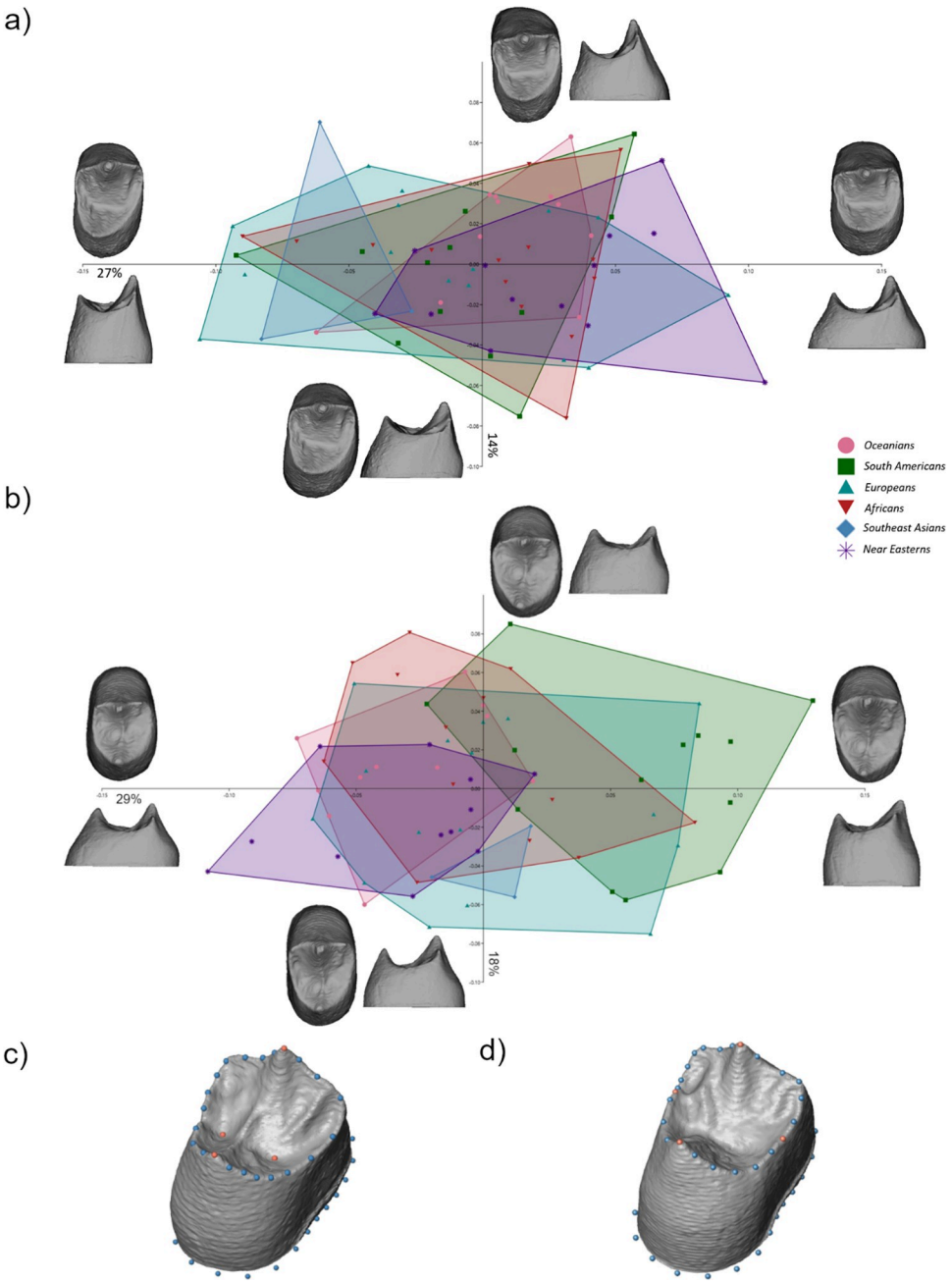

Fig 2. PC1—PC2 plot for third premolars (a) and fourth premolars (b) in shape space for the dentinal crown. The warpings show the real variation in occlusal and distal view at values PC1±0.15/ PC2 ±0.10; c) Landmark configuration capturing the dentinal crown of the P3s; and d) the P4s (orange = landmarks, blue = semi- and pseudolandmarks).

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mainly driven by the expansion of the distal fossa relative to the mesial fossa and the relative mesio-distal broadening of the cervical outline. The shape of the P3 dentinal crown varied from relatively short with a broad base, and rounded lingual occlusal aspect, to tall and mesio-distally narrow crowns. The horn tips shifted towards each other or apart, relative to the expansion and reduction of the cervical aspect of the crown, with broad crowns expressing horn tips closer to each other and narrow crowns the other way around. Shape variation along

PC2 (14%) reflected the relative expansion of the mesio-buccal and mesio-lingual areas of the dentinal crowns with respect to each other. With the expansion of the mesio-lingual side, the relative position of the lingual cusp shifted mesially. Along PC3 (10%), the relative shape changes reflected the expansion of the distal aspect of the tooth as well as the relative position of the lingual cusp. The lingual cusp was more mesially positioned in disto-buccally expanded dentinal crowns, and disto-lingually positioned in disto-lingually expanded crowns.

Shape variation in P4s

The shape of the P4 dentinal crowns (Fig 2B) along PC1 (29%) varied between short with mesio-distally expanded lingual cusp and bucco-lingually elongated crown base to tall with a mesio-distally narrow lingual cusp and bucco-lingually shorter crown base. A narrower occlusal aspect corresponds with a taller buccal cusp, positioned further from the lingual cusp relative to the crown base, while in broader occlusal aspects the cusps are more even and shifted towards each other. We observed a separation of Southeast Asian individuals from South Americans and Oceanians. However, this was probably caused by the small size of the Southeast Asian sample. On the other hand, Near Easterners and South Americans, although partially overlapping, tended to show the opposite shape variation along PC1. Shape variation along PC2 (18%) was mainly driven by the relative bucco-lingual position of the mesial fossa. A taller buccal cusp and relatively buccolingually elongated cervical outline were associated with a lingually shifted mesial fossa and vice versa. The variation along PC3 (14%) reflected the relative buccolingual position of the distal fossa as well as the height of the crown and relative expansion of the cervical outline.

The PCA analyses of the cervical outline, crown outline and EDJ dataset in both P3s and P4s (Supplementary Fig A, B, C, D in S1 File) showed that the populations in our sample overlapped widely and could not be differentiated based on the morphology of the upper premolars crown. Supplementary Table A in S1 File contains percentages of variance explained by the first five PCs in every dataset. Furthermore, our observations were confirmed by insignificant results obtained from the PERMANOVA analyses for P3s' ($p = 0.572$), as well as P4s' ($p = 0.613$) dentinal crowns, EDJ (P3, p = 0.53; p4, p = 0.87), cervical outlines (P3, p = 0.45; P4 = 0.06) and the P3s' crown outlines (p = 0.17) (Supplementary Tables B and C in S1 File). We have found significant results only in the crown outline analysis for P4s (p = 0.03).

Covariation

The 2B-PLS analysis demonstrated a strong pairwise correlation (r1 = 0.83) between P3 and P4 dentinal crowns (Fig 3), which showed concurrent trends of dentinal crown shape variation. For the Singular Warp scores 1 of the dentinal crown analysis (explaining 81% of total covariance), both tooth types covaried between short-crowned with mesio-distally broad base or tall-crowned with mesio-distally narrow base. The occlusal aspect associated with the short and broad crown type was mesio-distally expanded and squared with a broad central groove. Tall- and slender-crowned teeth, on the other hand, showed mesio-distally reduced occlusal aspects and were lingually narrow. Furthermore, higher dentinal horn tips were observed in short-crowned P4s with respect to tall-crowned premolars.

The covariation between the EDJ occlusal aspect and the crown base (represented by the cervical outline) within tooth types was not as high as the covariation between the dentinal crowns of the two different tooth types. Nevertheless, pairwise correlation within the P4s parameters was higher (r1 = 0.56) than within P3s (r1 = 0.46) (Table 2).

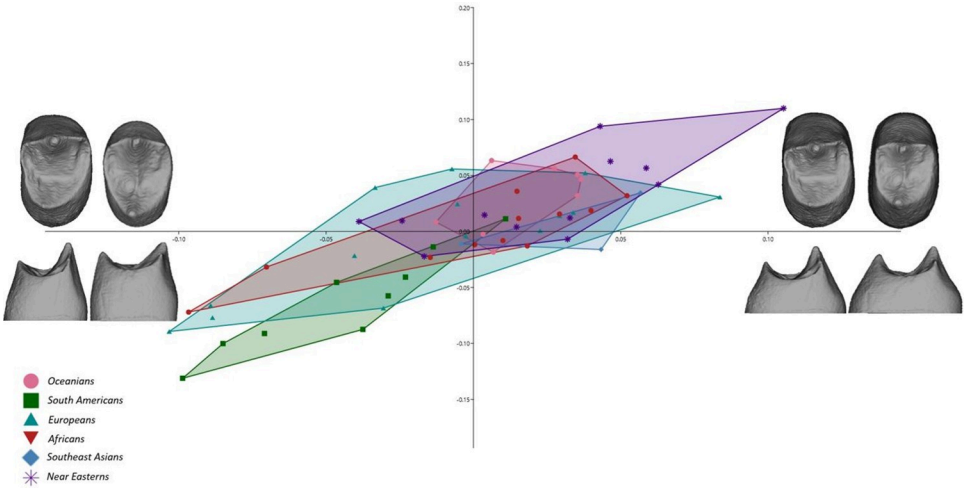

Fig 3. 2B-PLS plot capturing the covariation of upper third and fourth premolar dentinal crowns (dataset combining enamel-dentine junction and cervical outline; the warping shows the real shape variation in occlusal and distal view at the extremities of the range of distribution).

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Size

The lnCS was compared across the sample separately for the dentinal crown, cervical and crown outlines, and EDJ (Fig 4, Supplementary Fig E in S1 File). Kruskal-Wallis test showed significant differences for lnCS between all populations (Table 3). Comparing the groups against each other, the Mann-Whitney-U test showed that the Oceanian teeth were significantly larger than any other group for all features except the EDJ analysis. Similarly, Europeans and Africans were consistently smaller. Supplementary Table D in S1 File shows size differences between sexes in upper premolars, assessed via Mann-Whitney-U test.

Multivariate regression of size on shape for dentinal crowns was performed to analyse allometry and showed that only 1.9% and 2.1% of the total P3, respectively P4 shape variance could be explained by size.

Non-metric traits

Table 4 reports the prevalence of the premolar mesial and distal accessory cusps, accessory ridges and essential crest expressions on both the OES and the EDJ. Mesial and distal accessory cusps were present in only 10% of our sample. None of the Southeast Asian individuals exhibited this feature, and within the European, Near Eastern, South American and African samples, it could only be detected in a few individuals either on the OES or the EDJ of the P3 or P4.

Table 2. Results of the 2B-PLS (single warp score 1) for the upper third and fourth premolars (P3 and P4, respectively). (EDJ = enamel-dentine junction).

	Pairwise correlation (r1)	% of the total covariance
	P3 dentinal crown	P3 dentinal crown
P4 dentinal crown	0.83	81
	P3 cervical outline	P3 cervical outline
P3 EDJ	0.46	57
	P4 cervical outline	P4 cervical outline
P4 EDJ	0.56	70

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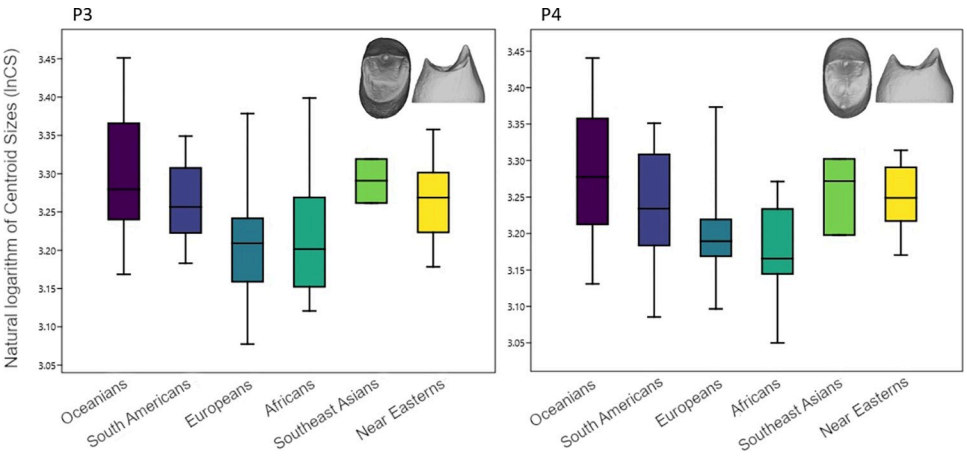

Fig 4. Boxplots of the natural logarithm of centroid sizes from the dentinal crown.

https://doi.org/10.1371/journal.pone.0301482.g004

Various expressions of the accessory cusps on the OES were three times more frequent in P4s (12%) than in P3s (4%). Only five of these specimens expressed also an elevation of the marginal ridge on the EDJ. Interestingly, three accessory cusps were detected on the EDJ of the P3s and another three in the P4s, without any sign of expression on the OES.

MxPARs were present in 55% of P3s and 65% of P4s. In P3s, a distally located accessory ridge occurred commonly on both OES and EDJ. Conversely, most of the P4 specimens expressed accessory ridges in both mesial and distal positions. The highest frequency of expression of accessory ridges in P3s was found in South Americans (50% on the OES, 75% on the EDJ), while in P4s, Europeans possessed most MxPARs (76% on the OES, 70% on the EDJ).

Essential crest expression was homogenously distributed on both surfaces in both tooth types. Approximately 90% of the individuals possessed a simple essential crest, separated by the sagittal sulcus. The prevalence of the transverse crests and bifurcated crest expressions were comparable among the samples in both tooth types on both OES and EDJ.The results of the Chí^2 test performed for every non-metric trait in both tooth types for both surfaces showed no significant difference in trait occurrence.

Discussion

Dental development is generally under strong genetic influence resulting in constrained morphological variation of the dentition [71–73]. The applied 3D methodology delivered fine-scale results about the morphometric variation of premolars' crowns. Maxillary premolars varied mainly in the length-to-breadth ratio of the occlusal aspect as well as the height of the dentinal crown and horn tips. A broader tooth shape is associated with short crowns but higher

Table 3. Results of the Kruskal–Wallis test. Differences in dental sizes (expressed by the logarithm of Centroid Sizes) for the upper third and fourth premolars (P3 and P4, respectively) among all populations across the sample. (EDJ = enamel-dentine junction).

	P3		P4	
	Kruskal-Wallis test	***p*-value**	**Kruskal-Wallis test**	***p*-value**
Cervical outline	21.20	< **0.001**	19.51	< **0.001**
Crown outline	25.54	< **0.001**	14.06	**0.015**
EDJ	17.88	**0.003**	10.30	0.067
Dentinal crown	14.88	**0.01**	16.05	**0.006**

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Table 4. List of non-metric traits scored for the third and fourth premolars (P3 and P4, respectively). (Numbers 0,1,2 and 3 refer to the manifestation (see Methods); n = Number of individuals).

		Prevalence (%)							
P3		Trait expression	Oceania (n = 13)	South America (n = 16)	Europe (n = 16)	Africa (n = 14)	Southeast Asia (n = 3)	Near East (n = 13)	Complete sample
Accessory cusp	Enamel	0	85	100	100	100	100	93	96
		1	7	-	-	-	-	7	3
		2	8	-	-	-	-	-	1
		3	-	-	-	-	-	-	-
	Dentine	0	93	93	93	93	100	100	95
		1	7	7	7	-	-	-	4
		2	-	-	-	7	-	-	1
		3	-	-	-	-	-	-	-
Accessory ridges (MxPAR)	Enamel	0	69	50	44	36	67	39	48
		1	-	-	-	7	-	15	4
		2	8	31	25	43	33	15	25
		3	23	19	31	14	-	31	23
	Dentine	0	69	25	31	57	100	38	45
		1	-	6	6	7	-	8	5
		2	31	25	44	36	-	16	30
		3	-	44	19	-	-	38	20
Essential crest	Enamel	0	77	93	100	72	67	100	88
		1	15	7	-	14	-	-	7
		2	8	-	-	14	33	-	5
	Dentine	0	77	76	93	72	67	100	83
		1	15	12	7	14	-	-	9
		2	8	12	-	14	33	-	8
P4		Trait expression	Oceania (n = 13)	South America (n = 16)	Europe (n = 16)	Africa (n = 14)	Southeast Asia (n = 3)	Near East (n = 13)	Complete sample
Accessory cusp	Enamel	0	62	86	100	93	100	93	88
		1	31	-	-	7	-	-	7
		2	7	7	-	-	-	7	4
		3	-	7	-	-	-	-	1
	Dentine	0	84	93	100	73	100	100	91
		1	8	-	-	27	-	-	7
		2	8	-	-	-	-	-	1
		3	-	7	-	-	-	-	1
Accessory ridges (MxPAR)	Enamel	0	31	47	24	27	34	69	38
		1	8	-	6	13	33	8	8
		2	15	13	29	13	33	15	18
		3	46	40	41	47	-	8	36
	Dentine	0	86	13	30	40	67	54	35
		1	8	-	-	20	-	8	5
		2	8	7	23	7	33	15	14
		3	-	80	47	33	-	23	46
Essential crest	Enamel	0	100	100	93	74	67	92	91
		1	-	-	-	13	-	8	4
		2	-	-	7	13	33	-	5
	Dentine	0	93	100	88	74	100	84	88
		1	-	-	12	13	-	8	7
		2	7	-	-	13	-	8	5

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horn tips positioned closer to each other. On the contrary, narrow teeth displayed a tall crown but lower horn tips more apart from each other.

Based on our analyses of the dentinal crown, it was evident that the populations in our sample could not be characterized or distinguished by their upper premolars crown morphology, despite their different population history. A subtle divergence could be detected visually in the PCA plots for the P4 dentinal crown, separating Southeast Asians from Oceanians and South Americans, but a statistical shape difference between these groups could not be confirmed by the PERMANOVA. Nonetheless, it is interesting that similar observations were made for lower premolars [54]. However, this outcome should be confirmed using a larger sample since our results are based on 3 specimens only. Additionally, the overlap between South American and Near Eastern populations was minor. Prevailing short and broad posterior dental crown types including its extreme expressions, like in the Near Easterners, were already described for African populations [46,54]. As mentioned above, small sample sizes of various populations have to be acknowledged, as well as the different number of sub-samples within geographical populations. Our goal for this study was to create a sample containing as much diversity as possible, thus including more sub-samples from geographical regions. In this sense, our sample composition works against its harmonization, nevertheless we find that these world populations have very similar upper premolar shape variation. Since our research requires dental specimens with minimal signs of wear and abrasion, no dental treatments, and high-resolution scanning, living or recently deceased individuals are not usable for our study. While these are restrictions not in our favour, our approach allowed for an accurate 3D shape analyses of dentinal crowns, which are not accessible with traditional methods.

Compared to the dentinal crown and EDJ analyses, PERMANOVA analysis of the 2D outline datasets, revealed a couple of significant differences in group centroids in five out of sixty pairs of populations (Supplementary Table B in S1 File). Since the cervical and crown outline analyses do not contain 3D information, and thus are more similar to traditional linear measurements that have been proven nonreliable in population variation analyses [14], we suggest using them only in the absence of combined datasets rather than alone.

We interpret the strong covariation between P3 and P4 shapes (r1 = 0.83), both either broad and short, or narrow and tall, as a sign of a strong genetic component during tooth development in an environment with shared genetic influence [72,73]. This high correlation might be surprising, considering that P3s show a rather prominent buccal cusp and share the tearing function with the canines, while the P4s' more even cusps engage in grinding activities, like in molars [8]. These are the first 3D outcomes on upper premolars and they are in agreement with those obtained for the lower premolars using a similar protocol [54], showing the same general pattern of variation. Thus, combining the evidence from our study and that of Krenn et al. [54], the dentinal crowns of both upper and lower P3s and P4s showed an extensive overlap between diverse populations that vary from short and broad to tall and narrow. European and Southeast Asian upper and lower premolars expressed extreme variations of the tall crown variant in both analyses. However, we found the relative cusp position of both cusps in upper P4s rather stable, with changes observable mainly in horn height, while in lower P4s, significant variation of the mesio-distal position of the lingual cusp was observed. The covariation expressed by upper and lower premolars can be explained by the need for morphological compatibility to guarantee stable occlusion and efficient function [6]. Since only the buccal cusp of the lower premolars is engaged at maximum intercuspation of the dentitions, the lingual cusp is less morphologically constrained [6,9,74]. Differently, in upper premolars both cusps are directly involved in occlusion [6], thus a lower degree of morphological variation might be expected. Consequently, the lingual aspect of the buccal cusp (paracone) and both

the lingual and buccal aspects of the lingual cusp (protocone) should be more stable than other parts of the crown [3,6] because they are directly involved in occlusion.

Such constraints in shape variation could also be reflected in more local features. In our non-metric trait analysis, we observed a higher rate of trait expression on the paracone than on the protocone, in the central groove and on the marginal ridges, which would be in line with the predictions by Kraus et al. [6]. However, we also found considerable variation in the lingual facet of the paracone. Therefore, not all of our results support the assumption of higher morphological conservation due to functional constraints. On the other hand, non-metric traits are understood to be under strong genetic control and vary neutrally in comparison to metric traits [14,75], therefore we should not expect them to be strictly related to function only. Our findings were mostly consistent with those of the previous studies on non-metric traits focusing on the OES [2,3,13,21–23,27,28,68,76–78] as well as studies based on OES and EDJ [48–51,69]. However, in contrast to our results, Sakai et al. [69] reported that mesial and distal accessory cusps occurred more often in P3s than in P4s, working, however, only on Japanese dentitions. Overall, the rate of expression of accessory cusps in different human populations is heterogeneous, most of them observed in Oceanian individuals, while Southeast Asians and Europeans showed nearly none. Our findings are in agreement with Xing et al. [49], who found that accessory cusps on the OES are not necessarily associated with elevations on the EDJ. Turner et al. [68] also stated that there is no dentine involvement necessary in the formation of mesial and distal accessory cusps. Uneven enamel disposition can also correspond to accessory cusps, as well as other features displayed on the OES [38,79,80]. Similar patterns can be found in the derived forms of the essential crest that are more common in the African sample. We detected all different manifestations of the essential crest on the EDJ and OES of both tooth types; however, more often in P3s. Accessory ridges are also equally present on the EDJ and OES, where more than half of the P3s in our sample and nearly two-thirds of the P4s possessed an accessory ridge. This is consistent with the results of Burnett et al. [70] and Mihailidis et al. [28], which were, however, solely based on the OES. In agreement with these studies, accessory ridges in our sample were primarily present on the distal aspect of the tooth. In P3s, we observed the highest occurrence of accessory ridges in Europeans, followed by Africans and Near Easterners. In contrast to our work, Burnett et al. [70] found the highest frequency of accessory ridges in North-East Asian or Asian-derived populations and lower frequencies in Africans and Europeans. For the P4s, again, the distribution of accessory ridges in our sample was unequal, while Burnett et al. [70] described a relatively even distribution across the whole sample.

Regarding premolar crown size, Townsend's [81] findings showed a significant sexual dimorphism for both maxillary premolars in the mesiodistal and buccolingual crown dimensions, with females possessing more broad/circular tooth crowns and males more narrow/ellipsoid crowns. We could not verify these results in our study due to the lack of reliable information about sex for most of our individuals. However, with regard to dental size, as represented by the lnCS, we did not find significant differences between males and females for the upper P4s, which parallels the results in Krenn et al. [54] for the lower premolars from a partially overlapping human sample. Conversely, we found significant differences for the upper P3s, but since our sample included more males (n = 26) than females (n = 9) our results should be interpreted with caution because of the uneven composition of the sample. According to Brace et al. [82] and Brace and Mahler [83], there is a gradient in increasing tooth size from north to south of the world. We indeed observed the smallest dental size in Europeans, while the largest teeth of our sample belonged to the Oceanian population. However, we also found African teeth of small size, which would not support Brace's theory, but is in agreement with Hanihara and Ishida [24], who found sub-Saharan African dental sizes close to the average.

Regardless of the unverifiable impact of sex on our results, we can state that static allometry in maxillary premolars is generally quite small in our sample. The effect of size was also small, explaining about 2% of the shape variation within our sample.

Our study generally demonstrated a generally large morphological variation of modern human upper premolars, yet a high correlation between first and second upper premolar. This variation does not seem to be the result of geographical separation in the course of modern human evolution, since most studied populations from different continents, climatic zones and environments did not differ from each other strong enough to be discriminated based on their upper premolar morphology. Our results instead show that the upper premolar morphological variation of recent modern humans is very similar among world populations, and thus, based on our data, we accept the tested hypothesis that the ranges of upper premolar shape variation of various human populations overlap and they cannot be distinguished based on their upper premolar's dentinal crown morphology only. For evolutionary studies, comparing modern humans with archaic hominins or non-human primates, this means that the geographic composition of the comparative modern human sample is of lesser importance. More work is needed to unravel the mechanisms behind this concordance of premolar shape independent of geographical origin.

Supporting information

S1 File. Šimková et al. Supporting information.
(DOCX)

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References

1. Alt KW, Rösing FW, Teschler-Nicola M. Dental anthropology: fundamentals, limits and prospects. Springer Science & Business Media; 2012.
2. Bailey SE. Neandertal dental morphology: implications for modern human origins. Arizona State University; 2002.
3. Hillson S. Dental anthropology. Cambridge University Press; 1996.
4. Irish JD, Nelson GC. Technique and application in dental anthropology. Cambridge University Press; 2008.
5. Kelley MA, Larsen CS. Advances in dental anthropology. Wiley; 1991.
6. Kraus BS, Jordan RE, Abrams L. Dental anatomy and occlusion: A study of the masticatory system. Baltimore: Baltimore, MD: Williams and Wilkins; 1969.
7. Lucas PW. Dental functional morphology: how teeth work. Cambridge University Press; 2004.
8. Nelson SJ, Ash M. Wheeler's dental anatomy, physiology and occlusion. St. Louis: Elsevier; 2010.
9. Scott GR, Turner CG, Townsend GC, Martinón-Torres M. The anthropology of modern human teeth: Dental morphology and its variation in recent and fossil Homo sapiens. Cambridge: Cambridge University Press; 2018.
10. Scott GR, Irish JD. A companion to dental anthropology. John Wiley & Sons; 2015.
11. Scott GR, Irish JD. Human tooth crown and root morphology. Cambridge University Press; 2017.
12. Scott GR, Turner CG. Dental Anthropology. Annu Rev Anthropol. 1988; 17: 99–126. https://doi.org/10.1146/annurev.an.17.100188.000531
13. Scott GR, Turner CG. Anthropology of modern human teeth. Cambridge University Press Cambridge; 1997.
14. Rathmann H, Perretti S, Porcu V, Hanihara T, Scott GR, Irish JD, et al. Inferring human neutral genetic variation from craniodental phenotypes. PNAS Nexus. 2023; 2: pgad217. https://doi.org/10.1093/pnasnexus/pgad217 PMID: 37457893
15. Scott GR, Maier C, Heim K. Identifying and Recording Key Morphological (Nonmetric) Crown and Root Traits. A Companion to Dental Anthropology. 2015. pp. 245–264. https://doi.org/10.1002/9781118845486.ch17
16. Ahmad IA, Alenezi MA. Root and Root Canal Morphology of Maxillary First Premolars: A Literature Review and Clinical Considerations. J Endod. 2016; 42: 861–872. https://doi.org/10.1016/j.joen.2016.02.017 PMID: 27106718
17. de Lima CO, de Souza LC, Devito KL, do Prado M, Campos CN. Evaluation of root canal morphology of maxillary premolars: a cone-beam computed tomography study. Aust Endod J. 2019; 45: 196–201. https://doi.org/10.1111/aej.12308 PMID: 30230115
18. Neelakantan P, Subbarao C, Ahuja R, Subbarao CV. Root and canal morphology of Indian maxillary premolars by a modified root canal staining technique. Odontology. 2011; 99: 18–21. https://doi.org/10.1007/s10266-010-0137-0 PMID: 21271321
19. Özcan E, Çolak H, Hamidi MM. Root and canal morphology of maxillary first premolars in a Turkish population. J Dent Sci. 2012; 7: 390–394. https://doi.org/10.1016/j.jds.2012.09.003
20. Saber SEDM Ahmed MHM, Obeid M Ahmed HMA. Root and canal morphology of maxillary premolar teeth in an Egyptian subpopulation using two classification systems: a cone beam computed tomography study. Int Endod J. 2019; 52: 267–278. https://doi.org/10.1111/iej.13016 PMID: 30225932
21. Morris DH. Maxillary first premolar angular differences between North American Indians and non-North American Indians. Am J Phys Anthropol. 1981; 54: 431–433. https://doi.org/10.1002/ajpa.1330540317 PMID: 7234988
22. Lavelle CLB. A metrical comparison of maxillary first premolar form. Am J Phys Anthropol. 1984; 63: 397–403. https://doi.org/10.1002/ajpa.1330630407 PMID: 6731607
23. Gómez-Robles A, Martinón-Torres M, Bermúdez de Castro JM, Prado-Simón L, Arsuaga JL. A geometric morphometric analysis of hominin upper premolars. Shape variation and morphological integration. J Hum Evol. 2011; 61: 688–702. https://doi.org/10.1016/j.jhevol.2011.09.004 PMID: 22047673
24. Wood BA, Engleman CA. Analysis of the dental morphology of Plio-Pleistocene hominids. V. Maxillary postcanine tooth morphology. J Anat. 1988; 161: 1–35. PMID: 3254883
25. Hanihara T, Ishida H. Metric dental variation of major human populations. Am J Phys Anthropol Off Publ Am Assoc Phys Anthropol. 2005; 128: 287–298. https://doi.org/10.1002/ajpa.20080 PMID: 15838862

26. Pilloud MA, Hefner JT, Hanihara T, Hayashi A. The Use of Tooth Crown Measurements in the Assessment of Ancestry. J Forensic Sci. 2014; 59: 1493–1501. https://doi.org/10.1111/1556-4029.12540 PMID: 25060236

27. Burnett SE. A New Look at Premolar Trait Variation: Maxillary Premolar Accessory Ridges (MxPAR). Dent Anthopology Publ Dent Anthropol Assoc. 1996.

28. Mihailidis S, Scriven G, Khamis M, Townsend G. Prevalence and patterning of maxillary premolar accessory ridges (MxPARs) in several human populations. Am J Phys Anthropol. 2013; 152: 19–30. https://doi.org/10.1002/ajpa.22323 PMID: 23913636

29. Adams D, Swenson VM, Scott GR. Global Distribution of Marginal Accessory Tubercles of the Maxillary Premolars. Dent Anthropol J. 2019; 32: 8–15.

30. Liao W, Xing S, Li D, Martinon-Torres M, Wu X, Soligo C, et al. Mosaic dental morphology in a terminal Pleistocene hominin from Dushan Cave in southern China. Sci Rep. 2019; 9: 1–14.

31. Fornai C, Bookstein FL, Weber GW. Variability of Australopithecus second maxillary molars from Sterkfontein Member 4. J Hum Evol. 2015; 85: 181–192. https://doi.org/10.1016/j.jhevol.2015.05.013 PMID: 26163295

32. Korenhof C. Evolutionary trends of the inner enamel anatomy of deciduous molars from Sangiran (Java, Indonesia). Teeth Funct Evol. 1982; 350–365.

33. Monson TA, Fecker D, Scherrer M. Neutral evolution of human enamel–dentine junction morphology. Proc Natl Acad Sci. 2020; 117: 26183–26189. https://doi.org/10.1073/pnas.2008037117 PMID: 33020281

34. Nager G. Der Vergleich zwischen dem räumlichen Verhalten des Dentin-Kronenreliefs und dem Schmelzrelief der Zahnkrone. Cells Tissues Organs. 1960; 42: 226–250. https://doi.org/10.1159/000141662

35. Olejniczak A J, Martin L B, Ulhaas L. Quantification of dentine shape in anthropoid primates. Ann Anat—Anat Anz. 2004; 186: 479–485. https://doi.org/10.1016/S0940-9602(04)80087-6 PMID: 15646281

36. Sasaki K, Makiguchi H, Yoshino K, Kanazawa E. Morphological Study of the Dentino-enamel Junction of the Upper First Deciduous Molar. Anthropol Sci. 2002; 110: 349–363. https://doi.org/10.1537/ase.110.349

37. Skinner MM, Wood BA, Hublin J-J. Protostylid expression at the enamel-dentine junction and enamel surface of mandibular molars of Paranthropus robustus and Australopithecus africanus. J Hum Evol. 2009; 56: 76–85. https://doi.org/10.1016/j.jhevol.2008.08.021 PMID: 18986683

38. Skinner MM, Evans A, Smith T, Jernvall J, Tafforeau P, Kupczik K, et al. Brief communication: Contributions of enamel-dentine junction shape and enamel deposition to primate molar crown complexity. Am J Phys Anthropol. 2010; 142: 157–163. https://doi.org/10.1002/ajpa.21248 PMID: 20091839

39. Skinner MM, Gunz P, Wood BA, Hublin J-J. Enamel-dentine junction (EDJ) morphology distinguishes the lower molars of Australopithecus africanus and Paranthropus robustus. J Hum Evol. 2008; 55: 979–988. https://doi.org/10.1016/j.jhevol.2008.08.013 PMID: 18824253

40. Skinner MM, Wood BA, Boesch C, Olejniczak AJ, Rosas A, Smith TM, et al. Dental trait expression at the enamel-dentine junction of lower molars in extant and fossil hominoids. Dent Tissue Stud 2D 3D Insights Hum Evol. 2008; 54: 173–186. https://doi.org/10.1016/j.jhevol.2007.09.012 PMID: 18048081

41. Bailey SE, Skinner MM, Hublin J-J. What lies beneath? An evaluation of lower molar trigonid crest patterns based on both dentine and enamel expression. Am J Phys Anthropol. 2011; 145: 505–518. https://doi.org/10.1002/ajpa.21468 PMID: 21312178

42. Guy F, Lazzari V, Gilissen E, Thiery G. To What Extent is Primate Second Molar Enamel Occlusal Morphology Shaped by the Enamel-Dentine Junction? PLOS ONE. 2015; 10: e0138802. https://doi.org/10.1371/journal.pone.0138802 PMID: 26406597

43. Gómez-Robles A, Bermúdez de Castro JM, Arsuaga J-L, Carbonell E, Polly PD. No known hominin species matches the expected dental morphology of the last common ancestor of Neanderthals and modern humans. Proc Natl Acad Sci. 2013; 110: 18196. https://doi.org/10.1073/pnas.1302653110 PMID: 24145426

44. Weber GW, Fornai C, Gopher A, Barkai R, Sarig R, Hershkovitz I. The Qesem Cave hominin material (part 1): A morphometric analysis of the mandibular premolars and molar. Quat Int. 2016; 398: 159–174.

45. Benazzi S, Coquerelle M, Fiorenza L, Bookstein F, Katina S, Kullmer O. Comparison of dental measurement systems for taxonomic assignment of first molars. Am J Phys Anthropol. 2011; 144: 342–354. https://doi.org/10.1002/ajpa.21409 PMID: 21302262

46. Šimková PG, Weber GW, Ramirez Rozzi FV, Slimani L, Sadoine J, Fornai C. Morphological variation of the deciduous second molars in the Baka Pygmies. Sci Rep. 2021; 11: 16480. https://doi.org/10.1038/s41598-021-95524-3 PMID: 34389746

47. Hershkovitz I, Weber GW, Quam R, Duval M, Grün R, Kinsley L, et al. The earliest modern humans outside Africa. Science. 2018; 359: 456–459. https://doi.org/10.1126/science.aap8369 PMID: 29371468

48. Liu W, Schepartz LA, Xing S, Miller-Antonio S, Wu X, Trinkaus E, et al. Late Middle Pleistocene hominin teeth from Panxian Dadong, South China. J Hum Evol. 2013; 64: 337–355. https://doi.org/10.1016/j.jhevol.2012.10.012 PMID: 23465337

49. Xing S, Sun C, Martinón-Torres M, Bermúdez de Castro JM, Han F, Zhang Y, et al. Hominin teeth from the Middle Pleistocene site of Yiyuan, Eastern China. J Hum Evol. 2016; 95: 33–54. https://doi.org/10.1016/j.jhevol.2016.03.004 PMID: 27260173

50. Xing S, Martinón-Torres M, Bermúdez de Castro JM, Wu X, Liu W. Hominin teeth from the early Late Pleistocene site of Xujiayao, Northern China. Am J Phys Anthropol. 2015; 156: 224–240. https://doi.org/10.1002/ajpa.22641 PMID: 25329008

51. Xing S, Martinón-Torres M, Bermúdez de Castro JM, Zhang Y, Fan X, Zheng L, et al. Middle Pleistocene Hominin Teeth from Longtan Cave, Hexian, China. PLOS ONE. 2015; 9: e114265. https://doi.org/10.1371/journal.pone.0114265 PMID: 25551383

52. Fornai C, Benazzi S, Gopher A, Barkai R, Sarig R, Bookstein FL, et al. The Qesem Cave hominin material (part 2): A morphometric analysis of dm2-QC2 deciduous lower second molar. State Art Multidiscip Res Middle Pleistocene Qesem Cave Isr 2015. 2016; 398: 175–189. https://doi.org/10.1016/j.quaint.2015.11.102

53. Davies TW, Delezene LK, Gunz P, Hublin J-J, Skinner MM. Endostructural morphology in hominoid mandibular third premolars: Geometric morphometric analysis of dentine crown shape. J Hum Evol. 2019; 133: 198–213. https://doi.org/10.1016/j.jhevol.2019.06.004 PMID: 31358180

54. Krenn VA, Fornai C, Wurm L, Bookstein FL, Haeusler M, Weber GW. Variation of 3D outer and inner crown morphology in modern human mandibular premolars. Am J Phys Anthropol. 2019; 169: 646–663. https://doi.org/10.1002/ajpa.23858 PMID: 31099892

55. Kato A, Kouchi M, Mochimaru M, Isomura A, Ohno N. A geometric morphometric analysis of the crown form of the maxillary central incisor in humans. Dent Anthropol J. 2011; 24: 1–10.

56. Malaspinas A-S, Westaway MC, Muller C, Sousa VC, Lao O, Alves I, et al. A genomic history of Aboriginal Australia. Nature. 2016; 538: 207–214. https://doi.org/10.1038/nature18299 PMID: 27654914

57. Mallick S, Li H, Lipson M, Mathieson I, Gymrek M, Racimo F, et al. The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. Nature. 2016; 538: 201–206. https://doi.org/10.1038/nature18964 PMID: 27654912

58. Rasmussen M, Guo Xiaosen, Wang Yong, Lohmueller Kirk E., Rasmussen Simon, Albrechtsen Anders, et al. An Aboriginal Australian Genome Reveals Separate Human Dispersals into Asia. Science. 2011; 334: 94–98. https://doi.org/10.1126/science.1211177 PMID: 21940856

59. Molnar S. Human tooth wear, tooth function and cultural variability. Am J Phys Anthropol. 1971; 34: 175–189. https://doi.org/10.1002/ajpa.1330340204 PMID: 5572602

60. Frederick M, Gallup G. Fluctuating dental asymmetry in great apes, fossil hominins, and modern humans: Implications for changing stressors during human evolution. 2007.

61. Benazzi S, Panetta D, Fornai C, Toussaint M, Gruppioni G, Hublin J-J. Technical Note: Guidelines for the digital computation of 2D and 3D enamel thickness in hominoid teeth. Am J Phys Anthropol. 2014; 153: 305–313. https://doi.org/10.1002/ajpa.22421 PMID: 24242830

62. Bookstein FL. Principal warps: Thin-plate splines and the decomposition of deformations. IEEE Trans Pattern Anal Mach Intell. 1989; 11: 567–585.

63. Gunz P, Mitteroecker P, Bookstein FL. Semilandmarks in Three Dimensions. In: Slice DE, editor. Modern Morphometrics in Physical Anthropology. Boston, MA: Springer US; 2005. pp. 73–98. https://doi.org/10.1007/0-387-27614-9_3

64. Gunz P, Mitteroecker P. Semilandmarks: a method for quantifying curves and surfaces. Hystrix Ital J Mammal. 2013; 24: 103–109.

65. Gower JC. Generalized procrustes analysis. Psychometrika. 1975; 40: 33–51.

66. Bookstein FL. Thin-plate splines and the atlas problem for biomedical images. Springer; 1991. pp. 326–342.

67. Mitteroecker P, Gunz P. Advances in geometric morphometrics. Evol Biol. 2009; 36: 235–247.

68. Turner CGI, Nichol CR, Scott GR. Scoring produces for key morphological traits of the permanent dentition: The Arizona State University dental anthropology system. Adv Dent Anthropol. 1991; 13–31.

69. Sakai T, Sasaki I, Hanamura H. A Morphological Study of Enamel-Dentin Border on the Japanese Dentition. J Anthropol Soc Nippon. 1967; 75: 207–223. https://doi.org/10.1537/ase1911.75.207

70. Burnett SE, Hawkey DE, Turner CG II. Brief communication: Population variation in human maxillary premolar accessory ridges (MxPAR). Am J Phys Anthropol. 2010; 141: 319–324. https://doi.org/10.1002/ajpa.21230 PMID: 19953530

71. Brook AH. Multilevel complex interactions between genetic, epigenetic and environmental factors in the aetiology of anomalies of dental development. Int Workshop Oral Growth Dev. 2009; 54: S3–S17. https://doi.org/10.1016/j.archoralbio.2009.09.005 PMID: 19913215

72. Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev. 2000; 92: 19–29. https://doi.org/10.1016/s0925-4773(99)00322-6 PMID: 10704885

73. Tucker A, Sharpe P. The cutting-edge of mammalian development; how the embryo makes teeth. Nat Rev Genet. 2004; 5: 499–508. https://doi.org/10.1038/nrg1380 PMID: 15211352

74. Alt KW, Rösing FW, Teschler-Nicola M. Dental Anthropology—An Introduction. In: Alt KW, Rösing FW, Teschler-Nicola M, editors. Dental Anthropology: Fundamentals, Limits and Prospects. Vienna: Springer Vienna; 1998. pp. 1–3. https://doi.org/10.1007/978-3-7091-7496-8_1

75. Hanihara T. Morphological variation of major human populations based on nonmetric dental traits. Am J Phys Anthropol Off Publ Am Assoc Phys Anthropol. 2008; 136: 169–182. https://doi.org/10.1002/ajpa.20792 PMID: 18257017

76. Delgado-Burbano ME, Scott GR, Turner CG II. The Uto-Aztecan premolar among North and South Amerindians: Geographic variation and genetics. Am J Phys Anthropol. 2010; 143: 570–578. https://doi.org/10.1002/ajpa.21351 PMID: 20623681

77. Johnson KM, Stojanowski CM, Miyar KO, Doran GH, Ricklis RA. New evidence on the spatiotemporal distribution and evolution of the Uto-Aztecan premolar. Am J Phys Anthropol. 2011; 146: 474–480. https://doi.org/10.1002/ajpa.21593 PMID: 21994018

78. Martinón-Torres M, Bastir M, Bermúdez de Castro JM, Gómez A, Sarmiento S, Muela A, et al. Hominin lower second premolar morphology: evolutionary inferences through geometric morphometric analysis. J Hum Evol. 2006; 50: 523–533. https://doi.org/10.1016/j.jhevol.2005.12.004 PMID: 16472839

79. Corruccini RS. The dentinoenamel junction in primates. Int J Primatol. 1987; 8: 99. https://doi.org/10.1007/BF02735159

80. Kono RT, Suwa G, Tanijiri T. A three-dimensional analysis of enamel distribution patterns in human permanent first molars. Arch Oral Biol. 2002; 47: 867–875. https://doi.org/10.1016/s0003-9969(02)00151-6 PMID: 12450518

81. Townsend GC. Intercuspal Distances of Maxillary Pre-molar Teeth in Australian Aboriginals. J Dent Res. 1985; 64: 443–446. https://doi.org/10.1177/00220345850640031001 PMID: 3855896

82. Brace CL, Rosenberg KR, Hunt KD. Gradual change in human tooth size in the late Pleistocene and post-Pleistocene. Evolution. 1987; 41: 705–720. https://doi.org/10.1111/j.1558-5646.1987.tb05847.x PMID: 28564360

83. Brace CL, Mahler PE. Post-Pleistocene changes in the human dentition. Am J Phys Anthropol. 1971; 34: 191–203. https://doi.org/10.1002/ajpa.1330340205 PMID: 5572603

Šimková et al. Supporting information

Shape variation in modern human upper premolars

Table A. Percentage of variance explained by the first five PCs (principal components) as resulting from the analysis of the upper third and fourth premolars' (P3 and P4, respectively) cervical outline, crown outline, the enamel-dentine junction (EDJ) and combined EDJ and cervical outline (dentinal crown).

	P3				P4			
	Cervical outline	Crown outline	EDJ	Dentinal crown	Cervical outline	Crown outline	EDJ	Dentinal crown
PC1	46	39	25	27	53	45	36	29
PC2	25	28	13	14	27	31	22	18
PC3	13	9	10	10	9	8	12	14
PC4	7	8	9	9	5	5	10	9
PC5	2	5	7	7	2	4	4	6
Total % PC1-5	93	89	64	67	96	93	84	76

Table B. Pairwise comparison of the PERMANOVA analysis results (*p*-values) between examined geographical populations' group centroids of all datasets.

Dentinal crown

P3

	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.579	0.975	0.526	0.457	0.410
South America	0.579		0.709	0.823	0.183	0.153
Europe	0.975	0.709		0.763	0.280	0.347
Africa	0.582	0.823	0.763		0.459	0.246
Southeast Asia	0.457	0.183	0.280	0.459		0.171
Near East	0.410	0.153	0.347	0.246	0.171	

P4

	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.840	0.807	0.454	0.433	0.728
South America	0.804		0.740	0.627	0.186	0.158
Europe	0.807	0.740		0.572	0.509	0.715
Africa	0.454	0.627	0.572		0.581	0.172
Southeast Asia	0.433	0.186	0.509	0.581		0.569
Near East	0.728	0.158	0.715	0.172	0.569	

Cervical outline

P3

	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.6654	0.4255	0.1553	0.6341	0.1364
South America	0.6654		0.6456	0.6966	0.6826	0.8068
Europe	0.4255	0.6456		0.3797	0.4054	0.1728
Africa	0.1553	0.6966	0.3797		0.5637	**0.0335**
Southeast Asia	0.6341	0.6826	0.4054	0.5637		0.5687
Near East	0.1364	0.8068	0.1728	**0.0335**	0.5687	

P4

	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.3001	0.5617	0.2244	0.1828	0.3915
South America	0.3001		**0.0014**	0.1071	0.1013	0.0555
Europe	0.5617	**0.0014**		0.1837	0.4417	0.1449
Africa	0.2244	0.1071	0.1837		0.2004	0.2503
Southeast Asia	0.1828	0.1013	0.4417	0.2004		0.4241
Near East	0.3915	0.0555	0.1449	0.2503	0.4241	

Crown outline

P3

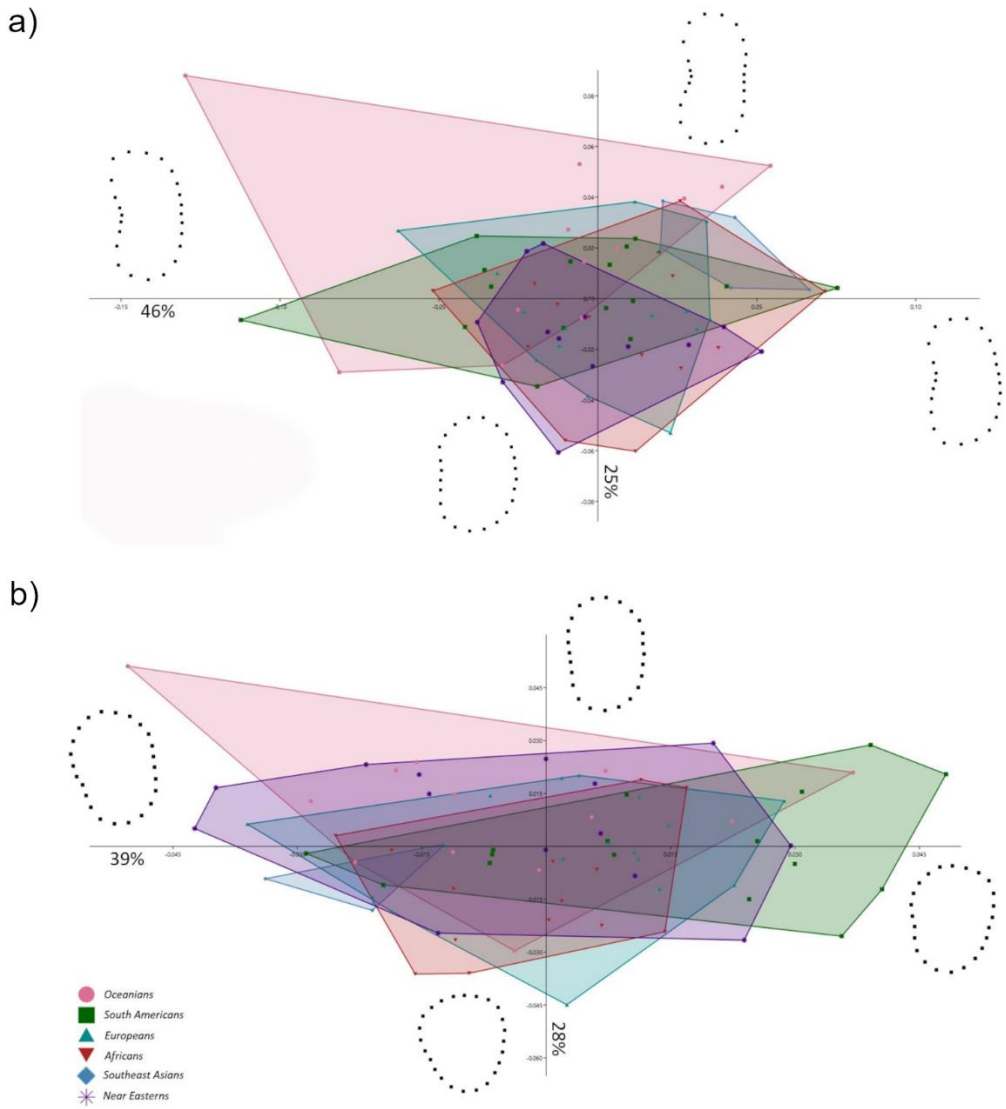
	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		**0.0185**	0.329	0.1369	0.7294	0.4304
South America	**0.0185**		0.302	0.6464	0.7158	0.1842
Europe	0.329	0.302		0.2094	0.6146	0.1076
Africa	0.1369	0.6464	0.2094		0.5283	0.252
Southeast Asia	0.7294	0.7158	0.6146	0.5283		0.3619
Near East	0.4304	0.1842	0.1076	0.252	0.3619	
P4						
	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.911	0.1508	0.0963	0.1016	0.0787
South America	0.911		0.2746	0.0906	0.4864	0.3273
Europe	0.1508	0.2746		**0.0068**	0.1307	**0.0086**
Africa	0.0963	0.0906	**0.0068**		0.2952	0.4801
Southeast Asia	0.1016	0.4864	0.1307	0.2952		0.5197
Near East	0.0787	0.3273	**0.0086**	0.4801	0.5197	
EDJ						
P3						
	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.1729	0.5617	0.2295	0.5241	0.7011
South America	0.1729		0.2665	0.0706	0.1279	0.281
Europe	0.5617	0.2665		0.988	0.7473	0.9594
Africa	0.2295	0.0706	0.988		0.6718	0.6872
Southeast Asia	0.5241	0.1279	0.7473	0.6718		0.7868
Near East	0.7011	0.281	0.9594	0.6872	0.7868	
P4						
	Oceania	South America	Europe	Africa	Southeast Asia	Near East
Oceania		0.8453	0.6065	0.5863	0.4667	0.9161
South America	0.8453		0.6903	0.5115	0.7264	0.6098
Europe	0.6065	0.6903		0.7084	0.8078	0.4025
Africa	0.5863	0.5115	0.7084		0.6685	0.732

Table C. Results of the PERMANOVA analysis. Differences between group centroids in all datasets (Permutation N: 9999).

		Total sum of squares	Within-group sum of squares	*p*-value	F-value
Dentinal crown	**P3**	7574	7026	0.57	0.96
	P4	8602	7985	0.60	0.95
Cervical outline	**P3**	1987	1856	0.45	1.00
	P4	2918	2663	0.06	1.32
Crown outline	**P3**	2968	2741	0.17	1.19
	P4	3350	3025	**0.03**	1.48
EDJ	**P3**	9213	8531	0.53	0.97
	P4	1.291E04	1.212E04	0.87	0.82

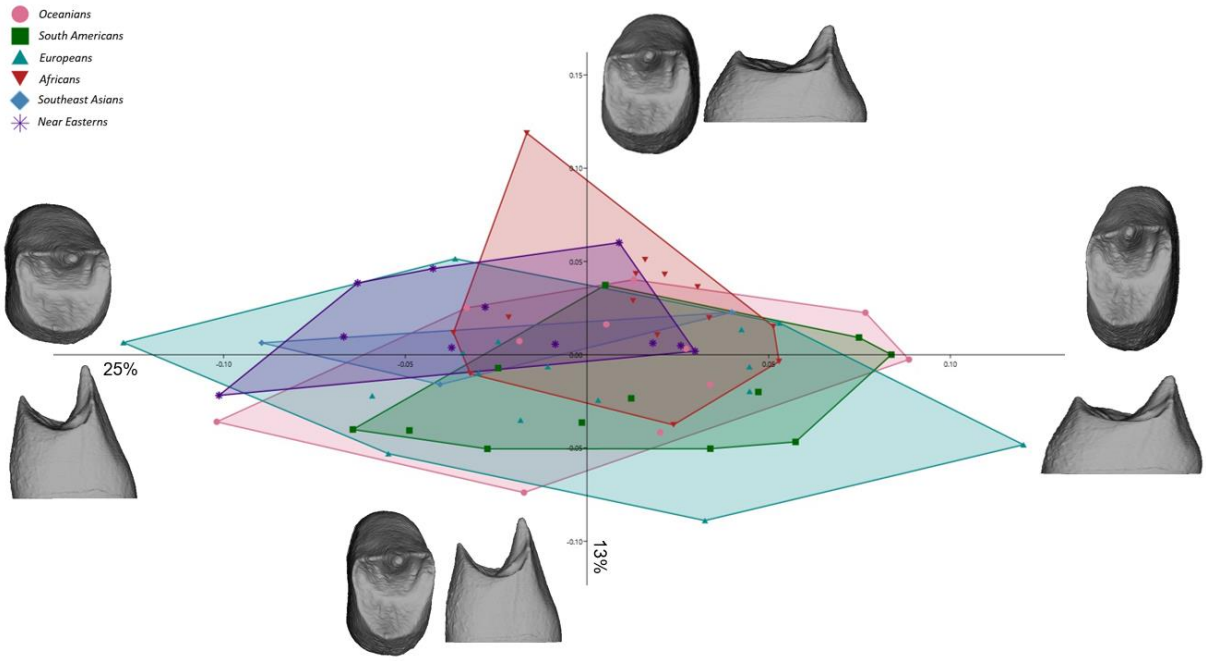
Table D. Results of the Mann–Whitney U test. Differences in dental sizes (expressed by the logarithm of Centroid Sizes) between sexes in upper third and fourth premolars (P3 and P4, respectively).

	P3	**P4**
n (F/M)	9/24	9/26
p-value	**0.03**	0.11
Z	2.14	1.56



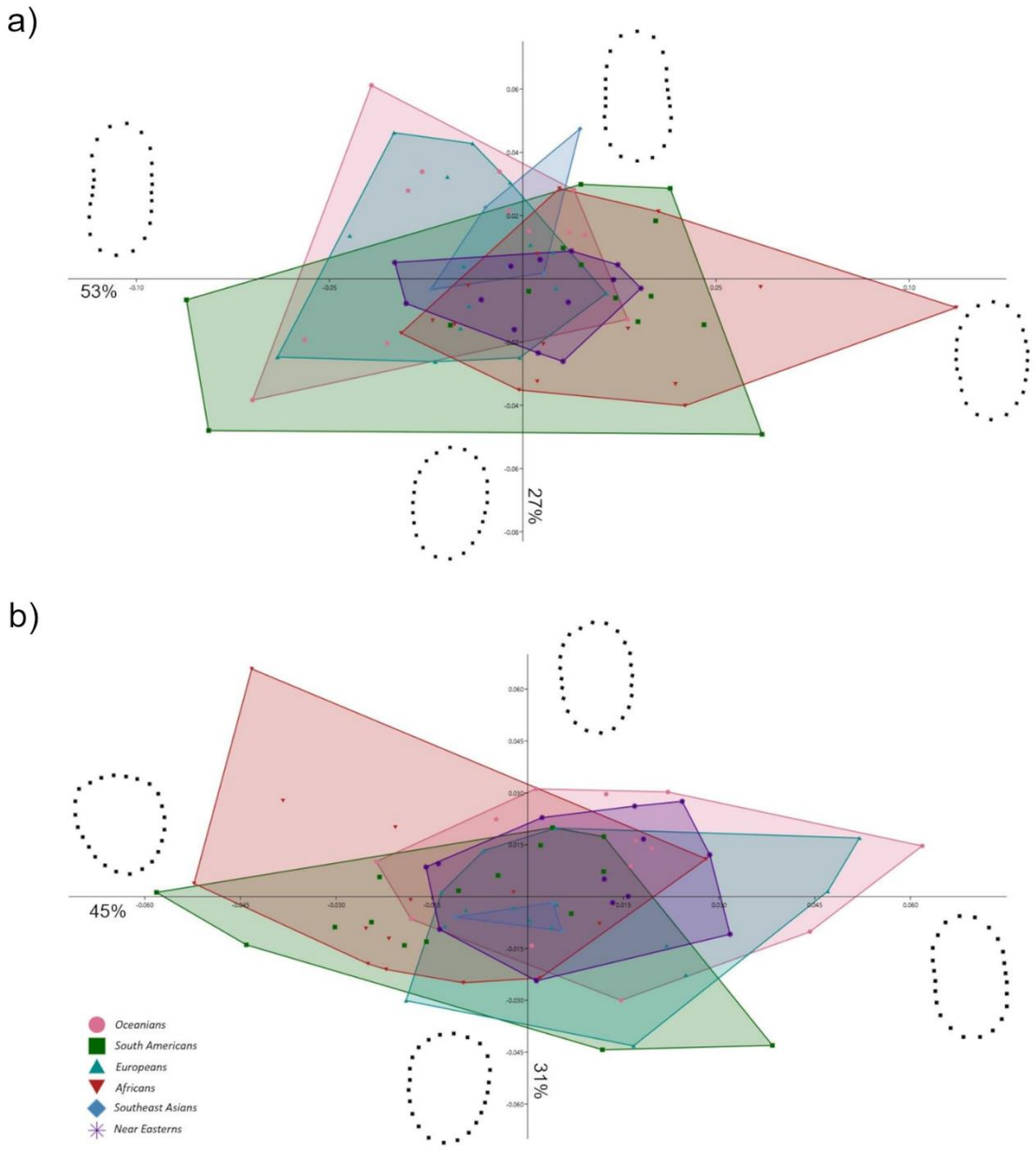
Supplementary Figure A. (a) PC1—PC2 plot for third premolars in shape space for the cervical outline (warpings show the real variation in occlusal view at values±0.15/±0.10) and b) the crown outline (warpings show the real variation in occlusal view at values±0.05/±0.06).

Shape variation of the cervical outline along PC1 (46%) was mainly driven by the expansion of the bucco-mesial and mesio-lingual parts of the outline relatively to each other. Along PC2 (25%), the cervical outline varied mainly between round and hourglass-shaped. Oceanians displayed the hourglass-shaped variant more often with a bucco-mesial expansion, while the cervical outlines of African populations were more rounded and mesio-lingually expanded. Shape variation in crown outline along PC1 (39%) reflected the relative expansion of the bucco-distal side, as well as expansion and reduction of the lingual aspect of the tooth either in the mesial or distal direction. Along PC2 (28%) the variation reflected similar findings like in cervical outline, thus the crown outline varied between hourglass-shaped and mesio-distally broad.



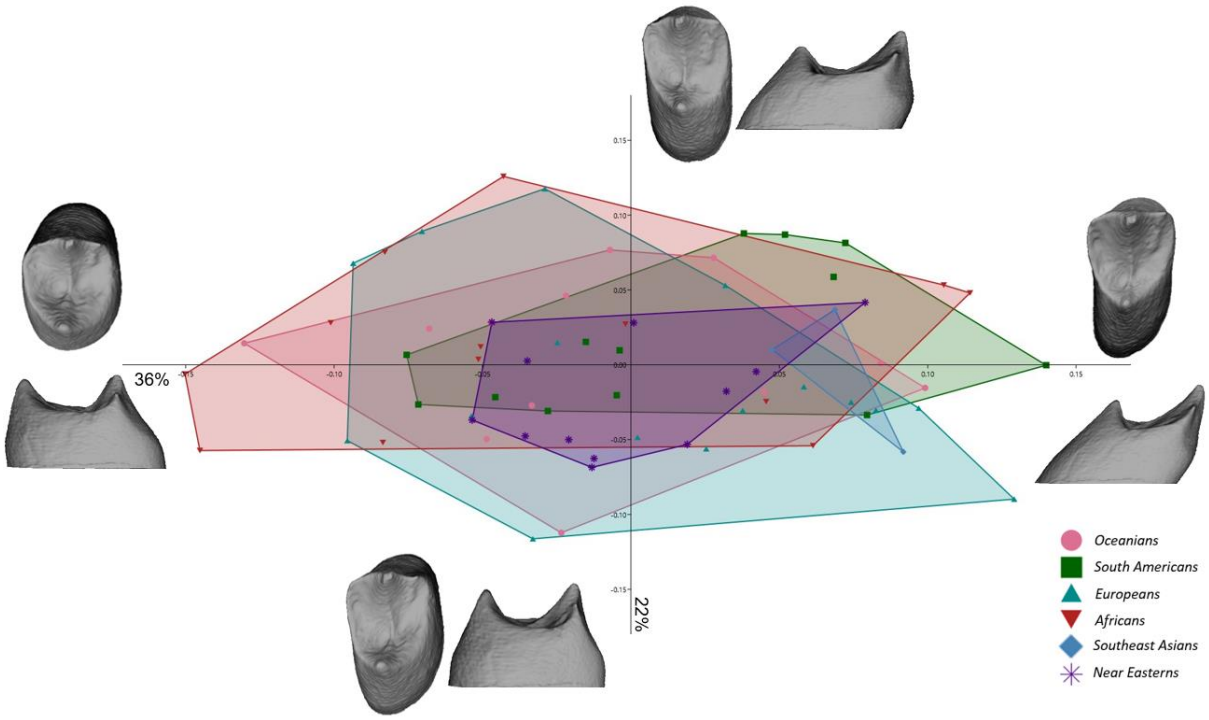
Supplementary Figure B. PC1—PC2 plot for third premolars in shape space for the enamel-dentine junction (warpings show the real variation in occlusal view at values±0.15).

The EDJ variation in P3s along PC1 (25%) mostly consists in the relative mesio-distal expansion of the central groove affecting the whole occlusal part of the tooth. The occlusal aspect varied between mesio-distally broad, bucco-lingually constricted (protocone and paracone are shifted towards each other) with wide central groove, and mesio-distally reduced, with main cusps shifted apart from each other due to the mesio-distal reduction of the central groove. Shape variation along PC2 (13%) was driven by the relative expansion of the distal and disto-lingual sides, resulting in either broad and lingually rounded occlusal aspect, or narrow, lingually convergent occlusal aspect.



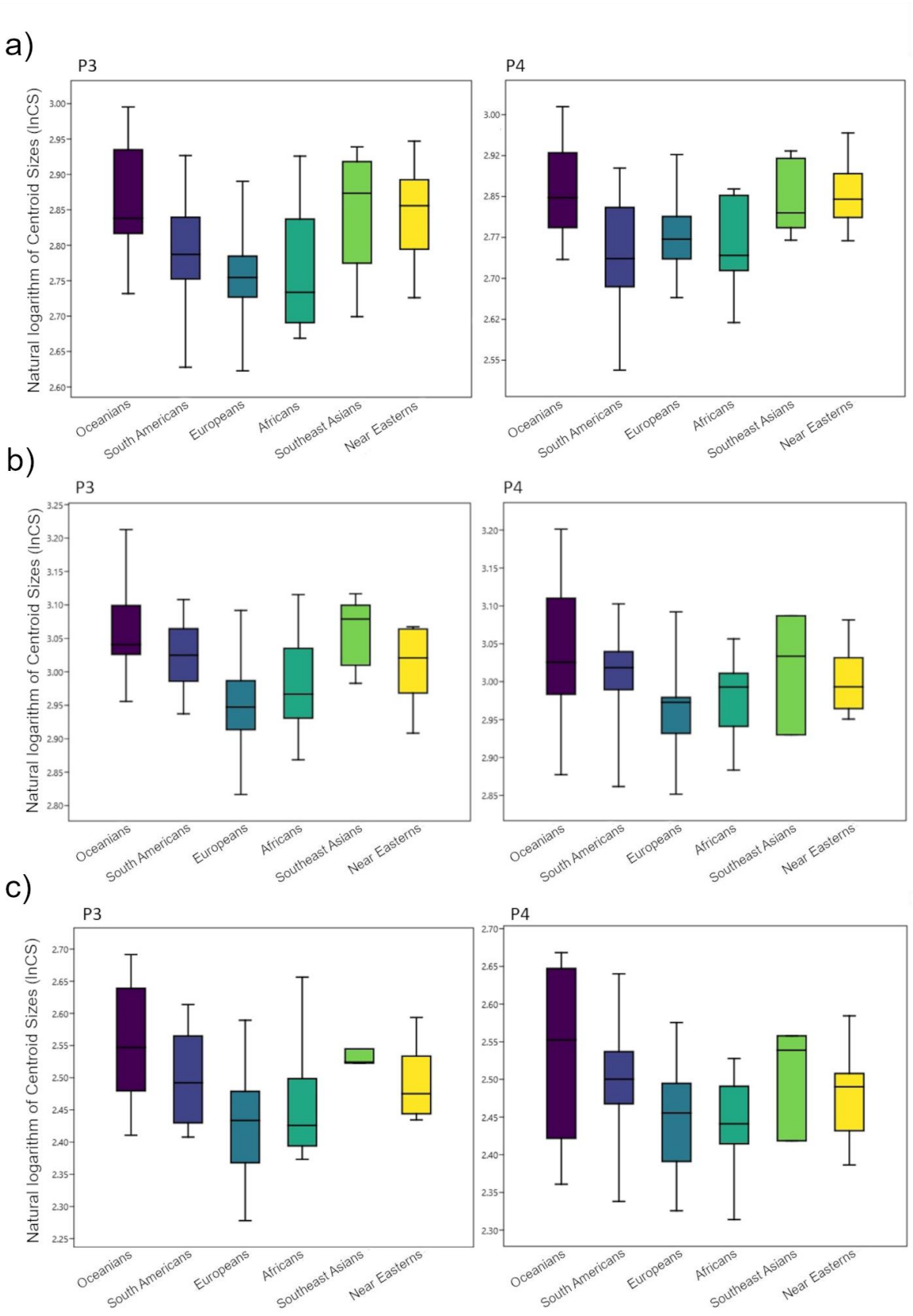
Supplementary Figure C. (a) PC1—PC2 plot for fourth premolars in shape space for the cervical outline (warpings show the real variation in occlusal view at values±0.10/±0.08) and b) the crown outline (warpings show the real variation in occlusal view at values±0.07).

For the P4s cervical outline, PC1 (53%) reflected a relative expansion either on the bucco-distal and mesio-lingual, or mesio-buccal and disto-lingual sides of the outline. Variation along PC2 (27%) consisted in the relative mesio-distal constriction in combination with bucco-lingual expansion, resulting in an hourglass shape of the cervical outline. The shape variation along PC1 (45%) in crown outline reflected the relative mesio-distal constriction and bucco-lingual expansion of the outline, resulting in either hourglass-shaped or rather rounded crown outline. Along PC2 (31%) the shape change reflected relative expansion and reduction of the mesial and distal aspects of the crown outline. Africans show extreme values of the broad, short and bucco-mesially expanded crown outline variation.



Supplementary Figure D. PC1—PC2 plot for fourth premolars in shape space for the enamel-dentine junction (warpings show the real variation in occlusal view at values±0.15).

The P4s' EDJ variation along PC1 (36%) reflected mainly two shape types, namely mesio-distally narrow with higher buccal cusp and pointy lingual aspect, or mesio-distally broad, with even cusps' height and rather rounded lingual aspect. Shape change along PC2 (22%) was driven by the relative position of the lingual cusp shifting either mesially or distally. Mesially positioned buccal cusp was associated with its height increase.

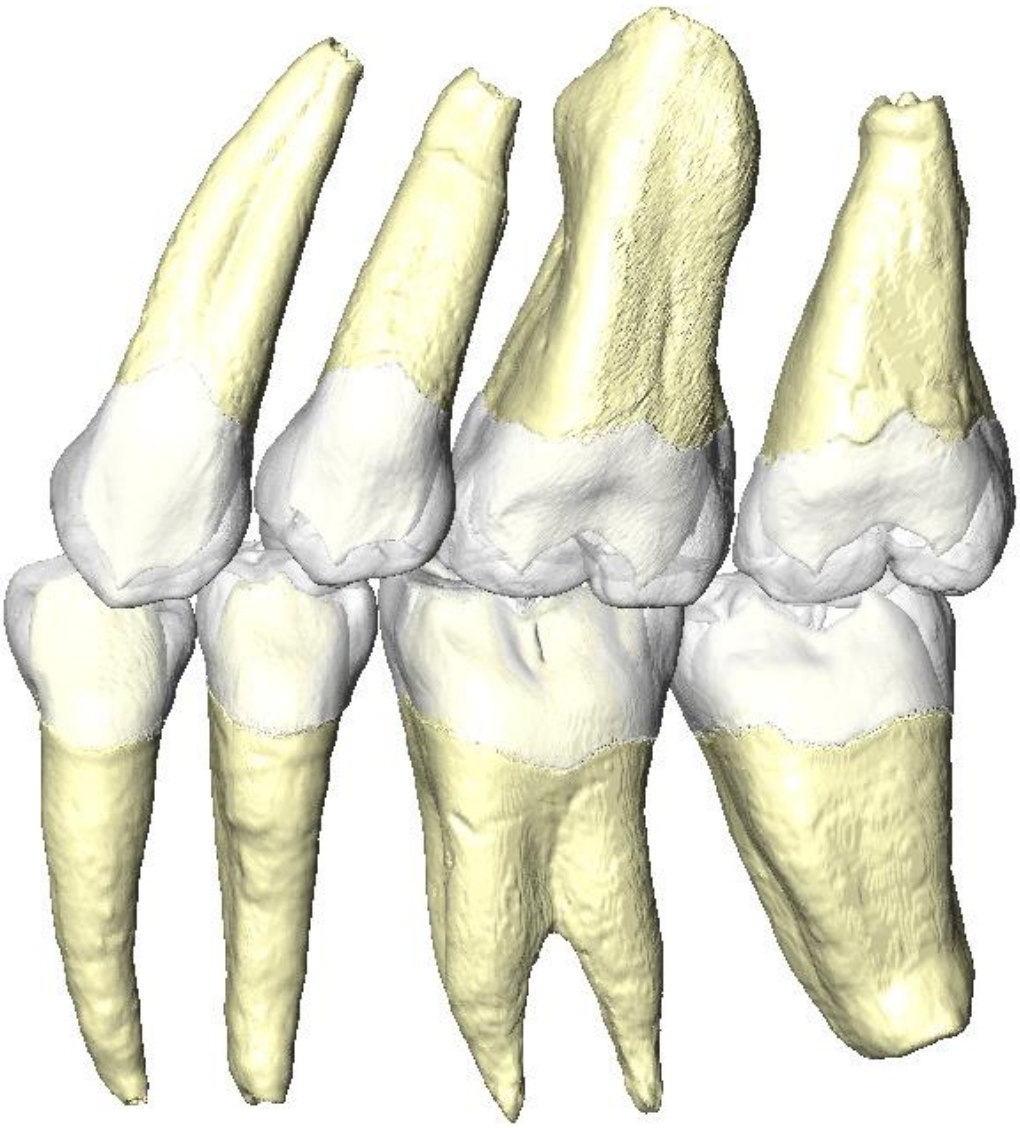


Supplementary Figure E. Boxplots of the natural logarithm of Centroid Sizes from the a) cervical outline, b) crown outline and c) the enamel-dentine junction in third and fourth premolars.

2.3 Connecting crowns: Analyzing morphological covariation in the modern human postcanine dentition

Simkova, P. G., Krenn, V. A., Fornai, C., Wurm, L., Halasz, V., Lidinsky, D., & Weber, G. W. (2024). Connecting crowns: Analyzing morphological covariation in the modern human postcanine dentition. *bioRxiv*, 2024.09.05.611460.
https://doi.org/10.1101/2024.09.05.611460

Contribution: This project was conducted by me, following the design and supervision of Prof. Gerhard Weber, as the final component of this thesis. It brought together data of various tooth types to perform an extensive covariation analysis, including the entire postcanine dentition (third molars excluded). I compiled, edited and harmonized all of the previously existing samples, and performed a further data collection to significantly extend the sample. Additionally, I conducted the image data acquisition and landmark collection on new specimens, performed all covariation analyses, and produced the visualizations. Ultimately, I wrote the paper with contribution of Prof. Weber, Dr. Krenn and Dr. Fornai.



Connecting crowns: Analyzing morphological covariation in the modern human postcanine dentition

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Keywords: premolars, molars, geometric morphometrics, morphological correlation, 3D shape analysis

Abstract

Morphological covariation within the modern human postcanine dentition remains an open field of study. Analysis of covariation patterns of the three-dimensional (3D) shape between different tooth types has been seldom conducted, but it is relevant for the advancement of human biology and evolution, as well as dental anthropology, phylogeny, and medicine. Here, we analysed 3D shape covariation of the postcanine dentition (excluding third molars), both within and between dental arches using geometric morphometrics (GM). Based on high-resolution (µCT) scans of 526 teeth from 136 individuals we found high pairwise correlation in tooth pairs within the dental arches (lower P3 and P4, $r_1 = 0.89$; upper P3 and P4, $r_1 = 0.81$; upper M1 and M2, $r_1 = 0.86$). The correlation values between antagonists varied notably from the highest value detected between upper and lower M1s ($r = 0.9$), to the lowest between upper P4s and lower M1s ($r = 0.58$). Of all analysed tooth types, only the upper M1s showed moderate to high correlation in every pair analysis. Noticeably, unusually high covariation was detected between some of the tooth type pairs that do not articulate in a normal dentition (e.g., lower P3 and upper M2, $r_1 = 0.88$). Furthermore, a relatively high covariation was found in the pairs of lower P4s and M1s ($r_1 = 0.79$), and upper P4s and M1s ($r_1 = 0.77$), which are the only tooth type pairs of the postcanine dentition belonging to different tooth classes (premolars and molars, respectively) and still serving similar masticatory functions.

This study points to the fact that higher morphological integration seems to characterize teeth within the same dental arch rather than between antagonistic teeth. With this study, we provided an overview of pairwise correlations and strength of covariation between different tooth types. This information might inform future studies aimed at understanding developmental, phylogenetic, and functional aspects of the human postcanine dentition, including possible phenotype-genotype associations. However, with this study being the first one performed on a 3D sample of this size, we also report on obstacles and peculiarities that have been determined.

Introduction

Human dental morphology has been a topic of interest in the fields of dental medicine, biological anthropology, and human evolution since the last century, with a particular focus on exploring dental geometry of various tooth types in 3D, an approach emerging in the past two decades (Bailey et al., 2011; Benazzi et al., 2012, 2014; Fornai et al., 2014; Hershkovitz et al., 2018; Krenn et al., 2019; Macchiarelli et al., 2013; Monson et al., 2020; Morita et al., 2014, 2016; Ortiz et al., 2017; Pan et al., 2016; Šimková et al., 2021, 2024; Weber et al., 2016; Zanolli et al., 2018). Since the 3D crown shapes of the human postcanine dentition have never been analyzed in relation to each other, the phenotypic covariation of the actual geometry of human postcanine teeth is still unknown, yet crucial to be connected to the genotype in a further step. Research on developmental genetics has linked dental genotype to phenotype (Hlusko, 2016; Thesleff, 2014), but while the odontogenetic morphological stages are well-documented (Thesleff & Tummers, 2008), the genetic pathways regulating odontogenesis remain under investigation. Quantitative genetics distinguishes populational phenotypic variation effects into genetic, non-genetic, and variation due to covariate effects. Similarly, phenotypic correlations can be broken down into genetic and non-genetic mechanisms, enabling the exploration of genetic covariation patterns underlying phenotypic traits (Hlusko, 2016). The comprehensive study of dental phenotypic variation in 3D instead of traditional linear distances can represent a major step forward in identifying morphological patterns and their underlying genetic mechanisms.

The development of anatomical structures is a coordinated process driven by growth factors, induction processes, signaling molecules and cascades, in which adjacent elements interact in order to create tightly integrated forms. This process, referred to as developmental integration, synchronizes the formation of various tissues, generating cohesive patterns of phenotypic variation throughout growth. Apart from developmental integration, pleiotropy (genes affecting multiple traits; Cheverud, 1996; Hlusko & Mahaney, 2009; Hodgkin, 1998; Wagner & Zhang, 2011) as well as linkage disequilibrium (deterministic association of alleles on multiple loci, with an effect on various traits; Mitteroecker et al., 2012) affect the induction

of phenotypic covariation in populations. The need for a tight link between anatomical elements that co-participate in a particular function (functional integration), leads to correlational selection that can result in linkage disequilibrium, and thus in covariation between structures that are not developmentally linked. However, even with robust selection pressures, the effect on phenotypic covariation is lower in comparison to the influence of developmental integration and pleiotropic effects (Mitteroecker et al., 2012).

More than 2400 genes appear to be involved in odontogenesis (Landin et al., 2012; Sehic et al., 2017), with the majority of related proteins belonging to four signaling pathway families; BMP, FGF, SHH, and WNT (Bei, 2009; Hosoya et al., 2020; Jussila & Thesleff, 2012; Mikkola, 2007; Yuan & Chai, 2019), as well as to the proteoglycans (Chen et al., 2023). Various evolutionary developmental models have been proposed to explain odontogenesis (Butler, 1939; Jernvall, 2000). Kavanagh et al. (2007) significantly contributed to the inhibitory cascade model (which describes how the development of one tooth might influence the adjacent teeth), demonstrating that activator-inhibitor dynamics determine size differences between molars and significantly contribute to metameric variation within a tooth row (Morita et al., 2016). Resulting from both neutral and adaptive processes, various parts of the primate dentition are heritable and vary significantly inter- as well as intra- specifically (Hanihara & Ishida, 2005; Hlusko et al., 2011; Monson et al., 2019, 2020). A high genetic contribution to dental morphological variation in humans has also been underlined by twin studies (Hughes et al., 2014; Townsend et al., 2012). In an experimental study on *Cryptotis parva's* molars, narrow-sense heritability (proportion of total phenotypic variance that is attributed to additive genetic variance alone) of overall crown shape has been determined at h^2=0.34 (in a range between 0 and 1), thus playing an important role in determining the shape of the tooth crown (Polly & Mock, 2018). However, as mentioned above, covariation between morphological traits is not only influenced by shared developmental and genetic factors but also by variations in the growth processes and allele frequencies that underlie it (Mitteroecker et al., 2012). The concept of morphological integration, where phenotypic traits are correlated if they share a developmental pathway or function, and modularity (Wagner & Altenberg, 1996) that explains

the mechanisms behind morphological integration, are the supporting concepts of this approach. Modules are further classified as developmental, functional, genetic or evolutionary (Klingenberg, 2008), and can influence each other, causing phenotypic covariation (Cheverud, 1996). Assessment of the covariation patterns within the human dentition is crucial for understanding odontogenesis and modularity (Hlusko et al., 2011; Stock, 2001). Genetic studies showed that odontogenesis involves hierarchical genetic patterning mechanisms across and between dental arches, as well as within and between tooth types (Jernvall & Thesleff, 2000). One of the possible investigation approaches uses genetic and genomic analyses to identify loci linked to dental variation (Geller et al., 2011; Pillas et al., 2010), though these methods do not clarify the mechanisms behind morphological variation.

Functional occlusion is key for efficient food breakdown and it relies heavily on the complementary 3D topography of the occluding dental surfaces (antagonists; Nelson & Ash, 2010). Therefore, we can expect the morphological shape correlation between antagonistic teeth to be high, while odontogenetic mechanisms suggest higher shape covariation within dental arches. This study explored the shape covariation between modern human postcanine dentinal crowns (third molars excluded) within and between upper and lower dental arches working by means of geometric morphometrics (GM) and 3D imaging. To our knowledge, no research has yet used comprehensive 3D dental shape data to explore the covariation between both third and fourth premolars, as well as the first and second molars of both jaws. In this project we selected high-resolution (µCT) scans of 526 teeth from 136 individuals for analyses based on the condition of their dentition, ensuring the representation of as many dental types per individual as possible. The focus of our approach was on the enamel-dentine junction (EDJ) that is the primary developmental structure of the crown and is consequently the precursor of the enamel cap's morphology, which naturally results in a strong correlation between EDJ and the outer enamel surface (OES; Bailey et al., 2011; Fornai et al., 2015; Guy et al., 2015; Monson et al., 2020). The EDJ thus represents an appropriate alternative to the OES (Gómez-Robles et al., 2013; Skinner et al., 2008, 2009; Weber et al., 2016) and since the overlying enamel protects the EDJ from early deterioration processes such as abrasion,

erosion or chipping, its examination offers advantages. This approach will enable us to analyze every dental type within and between dental arches in most of the individuals and explore potential differences between human populations.

Materials and Methods

Altogether, this study investigated 526 teeth from the upper and lower postcanine dentitions of 136 individuals from seven geographically different human groups (Egyptians, n = 26, Europeans, n = 123, Near Easterners, n = 69, Oceanians, n = 59, Sub-Saharan Africans, n = 109, South Americans, n = 83, and Southeast Asians, n = 57). In particular, we considered teeth from eight different tooth types, namely 70 lower third premolars (P3s), 74 lower fourth premolars (P4s), 76 upper P3s, 74 upper P4s, 49 lower first molars (M1s), 48 lower second molars (M2s), 59 upper M1s, and 76 upper M2s. We use the term “tooth class” to refer to premolars or molars in general. We relied on the study of osteological specimens from museum collections since they present natural dental wear, rarely show any caries, and mostly no dental intervention such as fillings. Importantly, our study required µCT images which cannot be obtained from living subjects. However, assembling a sample with fully preserved postcanine dentition from osteological collections was challenging because of missing, broken or severely worn teeth, resulting in differing subsample sizes for the various tooth types. For instance, M1s, as the first permanent teeth to erupt, are more prone to wear than other tooth types and are the least represented in our sample.

Scanning and Segmentation

All specimens were scanned at the Vienna µCT Lab in Austria using an industrial Viscom X8060 NDT scanner with parameters of 110–140 kV, 280–410 mA, 1400–2000 ms, and a 0.75 mm copper filter, achieving a voxel size between 20 and 50 µm. Virtual segmentation of the µCT data was conducted using Amira software (www.thermofisher.com) to separate the dental tissues from each other and from the surrounding area, and subsequently generate triangulated surface models of the enamel and dentine. The models were derived from both

the right and left side, but the datasets representing right teeth were flipped prior to surface extraction to avoid later reflection of the landmark coordinates. Teeth are not expected to exhibit directional asymmetry (Frederick & Gallup, 2007), thus we considered this procedure uninfluential on the outcomes of our study. In cases of slightly worn dentinal horn tips (maximal stage 3; Molnar, 1971), virtual reconstruction was performed in Amira using the 'brush' tool by extending the existing contours of the dentinal horn tips.

Reorientation and Data Collection

The segmented 3D surface models were reoriented in Geomagic Design X 64 (www.3dsystems.com) following established protocols according to the dental type. The cervical plane, calculated as the best-fit plane of the cervical margin, was used to reorient the dataset parallel to the x-y plane of the virtual workspace. The crowns were then rotated to align the upper premolars' and molars' mesial ridge parallel with the y-axis, the lower premolars' buccal aspect of the marginal ridge with the x-axis and the lower molars' lingual aspect of the cervical outline with the x-axis. The cervical outlines of the reoriented surfaces were collected and projected onto the cervical plane. These outlines were imported into Rhinoceros 6 (www.rhino3d.com) and divided into 24 segments using equiangular radial vectors originating from the area centroid of the outline. Subsequently, 24 pseudo-landmarks were placed at the points of intersections between the outline and the radial vectors, representing the cervical outline of every tooth type.

Landmark Collection on the EDJ

The reoriented 3D surfaces of the dentinal crowns were imported into the EVAN-Toolbox software (www.evan-society.org) for landmark collection, using configurations suitable to the different dental types (Fig. 1). For all premolars, two fixed landmarks (LMs) were placed on the paracone and protocone horn tips. On both lower premolars (Krenn et al., 2019), as well as, on the upper P3s, two additional LMs were placed at the deepest mesial and distal points of the central groove. In upper P4s, where the central groove is less defined, the landmarks were

instead placed on the deepest points of the distal and mesial ridges (Hershkovitz et al., 2018; Šimková et al., 2024). Additionally, 20 curve semilandmarks (sLMs) were placed along the marginal ridge.

Upper molars were represented by 4 LMs on each of the main cusp's horn tips, and 3 more LMs placed at the deepest points of the central fossa, distal fossa and on the lowest point between the hypocone and the protocone along the marginal ridge of the EDJ (Hershkovitz et al., 2018). Forty-three sLMs were used to capture the curve of the EDJ in both upper molars.

On lower molars, LMs were placed on the tips of the main horns, including the hypoconulid in the LM1s, as well as on the lowest point of the lingual and buccal marginal ridge, and the deepest point of the central fossa (Weber et al., 2016). The marginal occlusal ridge was represented by 47 sLMs in the LM1s and 22 sLMs in the LM2s.

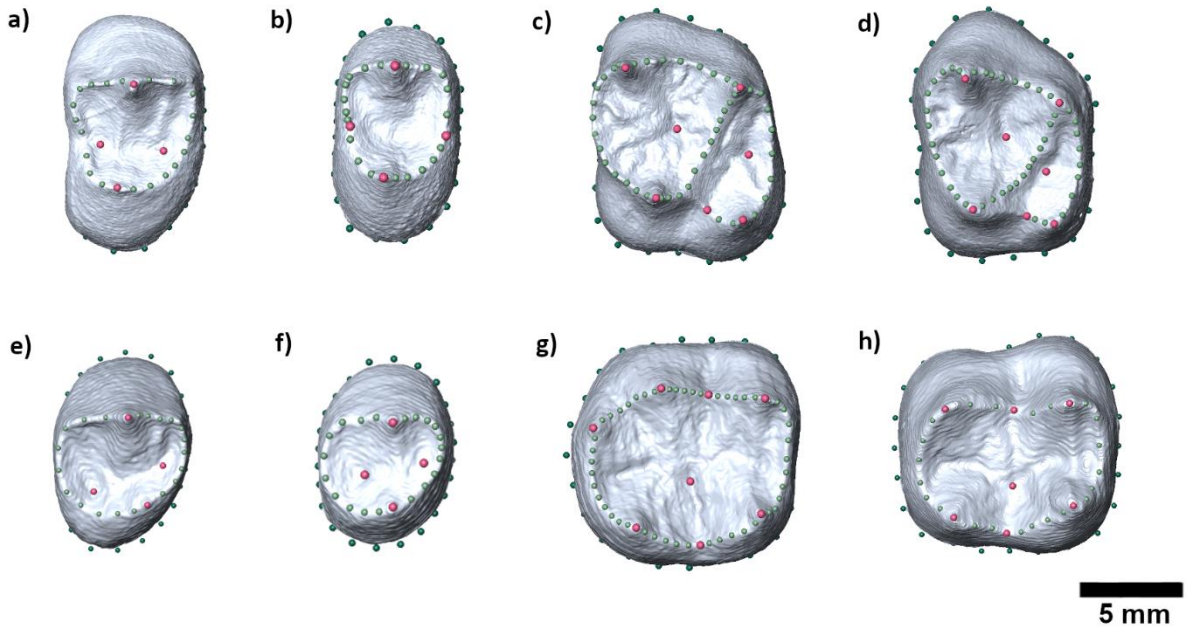

Fig. 1 Landmark configurations of the dentinal crown dataset of upper third **(a)** and fourth **(b)** premolars, upper first **(c)** and second **(d)** molars, lower third **(e)** and fourth **(f)** premolars, and lower first **(g)** and second **(h)** molars. Fixed landmarks = pink; semilandmarks = light green; pseudo-landmarks = dark green.

Geometric Morphometric Analyses

The shape of the dentinal crowns was analyzed in the EVAN-Toolbox 1.75 software (www.evan-society.org) using the landmark configuration representing both the cervical outline and the EDJ. For each of the subsamples, General Procrustes Analysis (GPA; Gower, 1975) was conducted to normalize the landmark configurations. The obtained Procrustes shape coordinates were then analyzed using a 2-block Partial Least Squares (2B-PLS) analysis to explore shape covariation and pairwise correlation between different dental types of the postcanine dentition within and between the jaws. In this study, PLS Mode A (a symmetric relation between blocks; Rosipal & Krämer, 2006) was employed. Consequently, we did not report on the RV coefficient, as its accuracy can in this case be compromised by factors related to sample size and the number of variables considered (Adams, 2016). Instead, we present the total squared covariance and the pairwise correlations, which provide a standardized measure easy to compare.

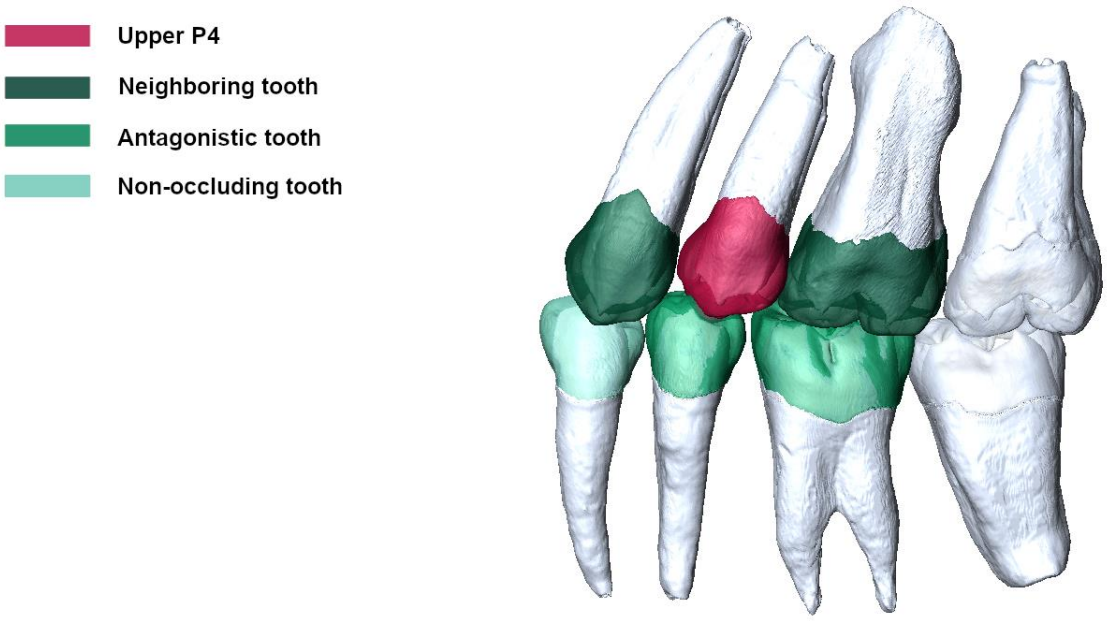

Fig. 2 The postcanine dentition (third molars excluded) in occlusion. The color coding is used to describe the specific relationship of the upper fourth molar (chosen as an example) to its neighboring tooth types.

Results

The percentages of total covariance according to the Singular Warp Score 1 and pairwise correlations are listed in Table 1 and Table 2. We found a high pairwise correlation (Table 2) in all pairs of the same tooth class within the dental arch (lower P3s and P4s, $r_1 = 0.89$; upper P3s and P4s, $r_1 = 0.81$; upper M1s and M2s, $r_1 = 0.83$), except for the lower M1s and M2s that expressed a moderate pairwise correlation of 0.73. The correlation values between antagonists varied notably from very high values calculated for the pair of upper/lower M1s ($r_1 = 0.90$) and upper M1s/lower M2s ($r_1 = 0.82$), through moderate values in upper/lower P3s ($r_1 = 0.71$) and lower P4/upper P3 ($r_1 = 0.70$), to low in upper/lower P4s ($r_1 = 0.65$), upper/lower M2s ($r_1 = 0.64$) and upper P4/lower M1s ($r_1 = 0.58$). Moderate to low correlation values were detected between non-neighboring tooth types located in the same jaw, as well as tooth types

that do not touch in full occlusion at all, with most of the relatively high values detected in those tooth pairs that included a lower premolar. An unexpectedly high pairwise correlation was found in the pair of lower P3 and upper M2 (r_1 = 0.88). A relatively high pairwise correlation was also found in the pairs of lower P4s and M1s (r_1 = 0.79), and upper P4s and M1s (r_1 = 0.77), which are the only pair of adjacent postcanine teeth belonging to different dental classes, yet serving similar functional purposes during mastication (Kraus et al., 1969). Additionally, the correlation between the upper P4 and M2 (r_1 = 0.78) was as strong as with upper M1s. We detected a coherent pattern in the overall shape change of the crowns, consistent in all tooth types, namely the change between tall and narrow crowned teeth versus short and broad crowned (shape-types described by Pfneiszl (2024)). A thorough description of the actual 3D morphological changes of the covarying occluding or neighboring tooth type pairs can be found in the caption of Figure 3 and in the Supplementary Information (Supplementary Fig. S1-S15).

Table 1. Percentages of total squared covariance for the Singular Warp Score 1, resulting from 2-block Partial Least Squares Analyses. (L = lower; U = upper; P3 = third premolars: P4 = fourth premolars; M1 = first molars; M2 = second molars)

	LP3	LP4	LM1	LM2	UP3	UP4	UM1	UM2
LP3		85	86	62	64	67	82	80
LP4			86	66	58	61	74	57
LM1				60	55	45	70	42
LM2					52	58	65	62
UP3						79	46	63
UP4							59	64
UM1								62
UM2								

Table 2. Pairwise correlation values resulting from 2-block Partial Least Squares Analyses. (L = lower; U = upper; P3 = third premolars: P4 = fourth premolars; M1 = first molars; M2 = second molars)

	LP3	LP4	LM1	LM2	UP3	UP4	UM1	UM2
LP3	1	0.89	0.76	0.74	0.71	0.75	0.77	0.88
LP4		1	0.79	0.75	0.70	0.65	0.73	0.44
LM1			1	0.73	0.61	0.58	0.90	0.50
LM2				1	0.63	0.66	0.82	0.64
UP3					1	0.81	0.67	0.70
UP4						1	0.77	0.78
UM1							1	0.83
UM2								1

Regarding geographical variation in our sample, we found a great overlap of morphological expressions across populations with larger and partially also smaller sub-sample sizes (e.g., Figure 3). However, the analyses that revealed a complete or partial separation between populations, are also those with the smallest sample sizes (see Supplementary Information).

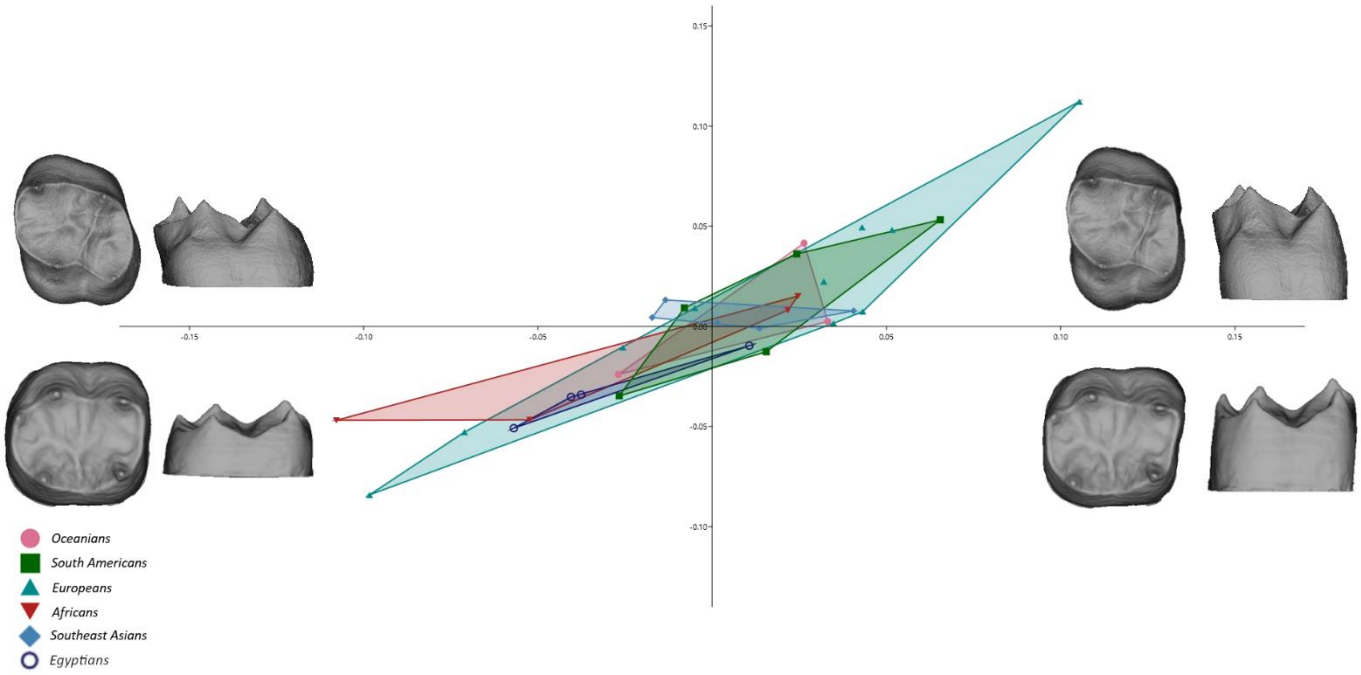

Fig. 3 2B-PLS plot for the covariation of upper and lower first molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution. The covariation analysis between antagonistic M1s showed that the main shape change followed the pattern between low and broad crowns versus tall and narrow crowns. In upper M1s, the hypocone was variably expanded relative to the trigon, while lower M1s showed a mesio-distal and bucco-lingual expansion of the cervical outline relative to the occlusal aspect, slightly stronger on the mesial part. The occlusal aspect of the lower M1 remained rather stable, showing more rounded mesial and distal aspects, with the relative expansion of the talon.

Discussion

In previous studies, high pairwise correlation has been reported for premolars in the lower jaw (r_1 = 0.87; Krenn et al., 2019), as well as in the upper jaw (r_1 = 0.83; Šimková et al., 2024), indicating that the morphology of both upper and lower premolars varies in a concordant manner, with the crown shape-type being either tall and narrow or low and broad. The current

study included analyses of extended samples with more individuals from additional human populations which confirmed the results of previous studies. Furthermore, agreeing with the findings of Buchegger et al. (2016), we found that the third highest correlation within this tooth class occurred between lower P3s and upper P4s which do not occlude in normal occlusion (Table 2). Surprisingly, correlations between antagonistic upper and lower P3s and P4s were in contrast lower than expected. This can probably be explained by the significant variability that lower premolars exhibit on the lingual crown side, due to differences in mesial and distal fossae expansion, affecting their lingual cusp positioning (Krenn et al., 2019). Hence, the non-occluding lingual cusp is free to vary, whereas the buccal aspect is more stable, being the most canalized region of this tooth (Siegal & Bergman, 2002). This distinguishes them from their upper antagonists, which are overall more stable and less variable.

Interestingly, P4s covaried more tightly with M1s within the same dental arch, rather than with their antagonists of the same tooth class. Premolars are located between the anterior teeth and the molars and serve distinct functions. In normal occlusion, the buccal cusp of the lower P3 slides across the interproximal embrasure of the upper canine and third premolar, leading Kraus et al. (1969) to consider them a “functional entity.” Unlike P4s, P3s participate in tearing rather than crushing or grinding food. Hlusko and Mahaney (2009) and Hlusko et al. (2011) discovered sub-modularity in baboon maxillary post-canine dentitions, with significant genetic correlations between premolar and molar sizes, and higher correlations within each tooth class. We obtained compatible results based on the shape covariation between human maxillary premolars and molars, reporting a comparable shape covariation pattern, as well as a similar correlation value between upper P4s with both upper M1s (r_1 = 0.77) and upper M2s (r_1 = 0.78). Given their distinct functional roles, it is not unexpected to find a lower pairwise correlation between upper P3s and molars. Still, comparable to the P4s, the covariation patterns between upper P3s and both upper molars exhibit a strong morphological resemblance, as the upper molars feature similar morphologies and high correlations with each other (Table 2). Furthermore, Hlusko et al. (2011) reported high genetic correlations between tooth size measurements of upper P3s and P4s for mice and monkeys (length r = 0.84; width

r = 0.92), which aligns with our results for the morphological correlation of dentinal crowns in humans. Similarly, for upper molars in a baboon sample, high to moderate values were reported (length r = 0.97; mesial width r = 0.88; distal width r = 76), in agreement with our results for shape correlation in human molars (Hlusko et al., 2011).

In the mandibular postcanine dentition, we observed lower correlation values within the molars than within premolars, and noteworthy, lower than between P4s and M1s. In general, this high pairwise correlation between P4s and M1s in both upper and lower dental arches would support the field theory expressed by Butler (1939). He claimed that all teeth within the postcanine dentition derive from one type of tooth germs under the influence of incomplete pleiotropy and different effects of morphogens on various tooth types. However, it is important to keep in mind that this model stands on primarily phenotypic data, with inconclusive results in subsequent studies aimed at testing Butler´s hypothesis (Henderson & Greene, 1975; Lombardi, 1975). On the other hand, since P4s and M1s are both engaged in the same function during mastication (Kraus et al., 1969), functional modularity might exert an influence on the morphological covariation of these two dental types as well.

In her study on first molars, Halász (2019) observed a high morphological correlation between the occlusal surfaces of the upper and lower M1s only (r_1 = 0.85), but a much lower correlation when considering the whole dentinal crown (r_1 = 0.61) which was analyzed in the current study. Since the pairwise correlation between the upper and lower first molars has been the highest in our study (r_1 = 0.90), we cannot replicate her results, possibly due to the impact of the sample size on the analyses (n = 18 in Halász's study; n = 31 in the current study). However, the high correlation of occlusal surfaces of antagonistic teeth is notable, given that these tissues develop following distinct odontogenic pathways. This suggests that apart from developmental integration and pleiotropy, functional integration might underlie the selection of certain trait combinations (correlational selection; Mitteroecker et al., 2012). In contrast to other connective tissue surfaces of the body such as osseous articulations, the only way for antagonistic teeth

to alter their shape after initial formation is through wear; this, however, did not affect our analyses of the EDJ (see Introduction and Methods).

Furthermore, our 3D morphological data unraveled a pattern in pairwise correlation values within the postcanine dentition: all of the possible tooth type pairs which included upper M1s showed a high or moderate pairwise correlation value, never lower than $r_1 = 0.67$ (non-occluding pair of lower P4 and upper M1), distinguishing upper M1s from the rest of the postcanine dentition. Unlike the other tooth types analyzed in this study, upper M1s do not only correlate strongly in morphology with other teeth in the same dental arch but show high values of morphological covariation with their antagonists and non-occluding tooth types as well. Morita et al. (2016) found M1s exhibiting the least variation in size among upper molars. They considered them the most stable tooth type in morphological terms and suggested upper M1s exhibiting inherent odontogenetic potential. Even though our study is not investigating the inhibitory cascade model (Kavanagh et al., 2007; Morita et al., 2016), our results on the 3D morphology of the upper M1s support the particular status of the upper M1s. Moreover, in the field of dental medicine, first molars are considered pivotal in maintaining the stability of the dental arches throughout growth and development. They play a crucial role in distributing masticatory forces and loads, ensuring the proper function of the stomatognathic system (Slavicek, 2002). Further research is essential to determine whether this is an effect of functional modularity in human evolution, particularly with regard to the upper M1 serving as a key structural pillar for the whole postcanine dentition.

In this study, both the occlusal aspects and the cervical margin of the 3D dentinal crowns were demonstrated by landmark data, capturing the height of the crown as one of the variables in our analysis. Thus, not only the morphology of the occlusal surfaces played a distinctive role in our analyses, but also the general shape-type (see Fig. 3; defined according to the proportions of the crown as tall and narrow or low and broad (Pfneiszl, 2024)) of the dentinal crown was considered. According to Pfneiszl (2024), 87% of individuals from a geographically diverse sample expressed one shape-type consistently across their entire postcanine dentition, thus exhibiting either tall and narrow or low and broad teeth. We detected high

pairwise correlations in some non-neighboring and non-occluding tooth type pairs (Supplementary Table S1), highlighting the shared influence of the overall shape-type of the crown. Most of these analyzed tooth type pairs included either the lower premolars or lower M2s, which are known for their morphological variability. Our analysis points to the fact that lower premolars and M2s often exhibited a complex morphological covariation with other teeth, however, this signal was largely masked by the dominant effect of overall crown shape-type. The most extreme value was found between the lower P3s with the upper M2s (r_1 = 0.88), which covaried almost only with regard to the overall shape-type, while expressing no greater shape changes of the occlusal aspects along the Singular Warp Score 1. On the other hand, distinct tooth types that showed more complex morphological covariation of the dentinal crowns, and thus were not mainly affected by the signal of their shape-type alone, showed low pairwise correlation values (lower P4 and upper M2; r_1 = 0.44). Methodological limitations, including spatial arrangement of the measurements or geometric dependencies between size-corrected measurements, like Procrustes shape coordinates, may have contributed to these findings by influencing correlation patterns (Mitteroecker, 2009). In related as well as unrelated tooth pairs, particularly if they include an extremely variable tooth type, the general dominance of the overall crown shape-type (tall/narrow vs. low/broad) may lead to a suppression of the signals for the actual shape variation of the occlusal aspect. Confounding factors, possibly affecting our analyses, such as the mode of capturing the geometry of the tooth crowns, need to be considered to fully understand these results. However, since this study is to our knowledge the first one considering morphological covariation in the human postcanine dentition in 3D, obstacles in methodology could be expected and acquire our further attention and tuning.

Nevertheless, given how important teeth are in many fields of natural sciences and medicine, we have relatively little knowledge of odontogenesis. Aspects of cusp pattern development in the interplay of genetic and epigenetic factors still need to be unravelled. Knowledge about the morphological covariation between and within various tooth classes might help supporting future genetic studies. Through our quantitative and qualitative description of the dentinal

crown shape covariation of postcanine teeth from various modern human populations, we laid the morphological groundwork for further research on genotype-phenotype associations that is essential to understand odontogenetic processes in mammals, including the genus *Homo*.

Conclusions

This study extends our understanding of the morphological covariation of human postcanine dentition by analyzing 3D dentinal crown shapes across a geographically diverse sample of modern humans. The results show that upper first molars are a key player in the dental covariation patterns, while developmental integration within dental arches is also evident. Notably, compatibility between antagonistic teeth does not appear to be the primary factor influencing morphological relationships within the postcanine dentition. Furthermore, our results suggest that factors like a shared crown shape-type may dominate the observed covariation patterns, particularly in morphologically variable tooth types like lower premolars. These findings highlight the need for further study of odontogenetic processes and the interplay of genetic and epigenetic factors in tooth development and evolution.

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References

Adams, D. C. (2016). Evaluating modularity in morphometric data: Challenges with the RV coefficient and a new test measure. *Methods in Ecology and Evolution*, *7*(5), 565–572. https://doi.org/10.1111/2041-210X.12511

Bailey, S. E., Skinner, M. M., & Hublin, J.-J. (2011). What lies beneath? An evaluation of lower molar trigonid crest patterns based on both dentine and enamel expression. *American Journal of Physical Anthropology*, *145*(4), 505–518. https://doi.org/10.1002/ajpa.21468

Bei, M. (2009). Molecular genetics of tooth development. *Current opinion in genetics & development*, *19*(5), 504–510.

Benazzi, S., Fornai, C., Buti, L., Toussaint, M., Mallegni, F., Ricci, S., Gruppioni, G., Weber, G. W., Condemi, S., & Ronchitelli, A. (2012). Cervical and crown outline analysis of worn Neanderthal and modern human lower second deciduous molars. *American Journal of Physical Anthropology*, *149*(4), 4. https://doi.org/10.1002/ajpa.22155

Benazzi, S., Grosse, I. R., Gruppioni, G., Weber, G. W., & Kullmer, O. (2014). Comparison of occlusal loading conditions in a lower second premolar using three-dimensional finite element analysis. *Clinical Oral Investigations*, *18*(2), 2. https://doi.org/10.1007/s00784-013-0973-8

Buchegger, L., Krenn, V., Bookstein, F., Fornai, C., & Weber, G. (2016). *Covariation in Upper and Lower Premolars in Modern Humans*. 58.

Butler, P. M. (1939). *Studies of the Mammalian Dentition.–Differentiation of the Post-canine Dentition*. *109*, 1–36.

Chen, J., Sun, T., You, Y., Lin, B., Wu, B., & Wu, J. (2023). Genome-wide identification of potential odontogenic genes involved in the dental epithelium-mesenchymal interaction during early odontogenesis. *BMC Genomics*, *24*(1), 163. https://doi.org/10.1186/s12864-023-09140-8

Cheverud, J. M. (1996). Developmental integration and the evolution of pleiotropy. *American Zoologist*, *36*(1), 44–50.

Fornai, C., Benazzi, S., Svoboda, J., Pap, I., Harvati, K., & Weber, G. W. (2014). Enamel thickness variation of deciduous first and second upper molars in modern humans and Neanderthals. *Journal of human evolution*, *76*, 83–91.

Fornai, C., Bookstein, F. L., & Weber, G. W. (2015). Variability of Australopithecus second maxillary molars from Sterkfontein Member 4. *Journal of Human Evolution*, *85*, 181–192. https://doi.org/10.1016/j.jhevol.2015.05.013

Frederick, M., & Gallup, G. (2007). *Fluctuating dental asymmetry in great apes, fossil hominins, and modern humans: Implications for changing stressors during human evolution*.

Geller, F., Feenstra, B., Zhang, H., Shaffer, J. R., Hansen, T., Esserlind, A.-L., Boyd, H. A., Nohr, E. A., Timpson, N. J., Fatemifar, G., Paternoster, L., Evans, D. M., Weyant, R. J., Levy, S. M., Lathrop, M., Smith, G. D., Murray, J. C., Olesen, J., Werge, T., … Melbye, M. (2011). Genome-Wide Association Study Identifies Four Loci Associated with Eruption of Permanent Teeth. *PLOS Genetics*, *7*(9), e1002275. https://doi.org/10.1371/journal.pgen.1002275

Gómez-Robles, A., Bermúdez de Castro, J. M., Arsuaga, J.-L., Carbonell, E., & Polly, P. D. (2013). No known hominin species matches the expected dental morphology of the last common ancestor of Neanderthals and modern humans. *Proceedings of the National Academy of Sciences*, *110*(45), 45. https://doi.org/10.1073/pnas.1302653110

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 1.

Guy, F., Lazzari, V., Gilissen, E., & Thiery, G. (2015). To What Extent is Primate Second Molar Enamel Occlusal Morphology Shaped by the Enamel-Dentine Junction? *PLOS ONE*, *10*(9), e0138802. https://doi.org/10.1371/journal.pone.0138802

Halász, V. (2019). *Morphological assessment of modern human upper and lower first molars* [Masterthesis]. University of Vienna.

Hanihara, T., & Ishida, H. (2005). Metric dental variation of major human populations. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *128*(2), 2.

Henderson, A., & Greene, D. (1975). Dental field theory: An application to primate evolution. *Journal of Dental Research*, *54*(2), 344–350.

Hershkovitz, I., Weber, G. W., Quam, R., Duval, M., Grün, R., Kinsley, L., Ayalon, A., Bar-Matthews, M., Valladas, H., Mercier, N., Arsuaga, J. L., Martinón-Torres, M., Bermúdez de Castro, J. M., Fornai, C., Martín-Francés, L., Sarig, R., May, H., Krenn, V. A., Slon, V., … Weinstein-Evron, M. (2018). The earliest modern humans outside Africa. *Science*, *359*(6374), 6374. https://doi.org/10.1126/science.aap8369

Hlusko, L. J. (2016). Elucidating the evolution of hominid dentition in the age of phenomics, modularity, and quantitative genetics. *Annals of Anatomy-Anatomischer Anzeiger*, *203*, 3–11.

Hlusko, L. J., & Mahaney, M. C. (2009). Quantitative genetics, pleiotropy, and morphological integration in the dentition of Papio hamadryas. *Evolutionary Biology*, *36*, 5–18.

Hlusko, L. J., Sage, R. D., & Mahaney, M. C. (2011). Modularity in the mammalian dentition: Mice and monkeys share a common dental genetic architecture. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *316*(1), 21–49.

Hodgkin, J. (1998). Seven types of pleiotropy. *The International journal of developmental biology*, *42*(3), 501–505.

Hosoya, A., Shalehin, N., Takebe, H., Shimo, T., & Irie, K. (2020). Sonic hedgehog signaling and tooth development. *International journal of molecular sciences*, *21*(5), 1587.

Hughes, T., Townsend, G., Pinkerton, S., Bockmann, M., Seow, W., Brook, A., Richards, L., Mihailidis, S., Ranjitkar, S., & Lekkas, D. (2014). The teeth and faces of twins: Providing insights into dentofacial development and oral health for practising oral health professionals. *Australian Dental Journal*, *59*(s1), s1. https://doi.org/10.1111/adj.12101

Jernvall, J. (2000). Linking development with generation of novelty in mammalian teeth. *Proceedings of the National Academy of Sciences*, *97*(6), 6. https://doi.org/10.1073/pnas.050586297

Jernvall, J., & Thesleff, I. (2000). Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mechanisms of Development*, *92*(1), 19–29. https://doi.org/10.1016/S0925-4773(99)00322-6

Jussila, M., & Thesleff, I. (2012). Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. *Cold Spring Harbor perspectives in biology*, *4*(4), a008425.

Kavanagh, K. D., Evans, A. R., & Jernvall, J. (2007). Predicting evolutionary patterns of mammalian teeth from development. *Nature*, *449*(7161), 7161. https://doi.org/10.1038/nature06153

Klingenberg, C. P. (2008). Morphological integration and developmental modularity. *Annual review of ecology, evolution, and systematics*, *39*(1), 115–132.

Kraus, B. S., Jordan, R. E., & Abrams, L. (1969). *Dental anatomy and occlusion: A study of the masticatory system.* Baltimore, MD: Williams and Wilkins.

Krenn, V. A., Fornai, C., Wurm, L., Bookstein, F. L., Haeusler, M., & Weber, G. W. (2019). Variation of 3D outer and inner crown morphology in modern human mandibular premolars. *American journal of physical anthropology*, *169*(4), 4.

Landin, M. A., Shabestari, M., Babaie, E., Reseland, J., & Osmundsen, H. (2012). Gene Expression Profiling during Murine Tooth Development. *Frontiers in Genetics*, *3*. https://www.frontiersin.org/articles/10.3389/fgene.2012.00139

Lombardi, A. V. (1975). A factor analysis of morphogenetic fields in the human dentition. *American Journal of Physical Anthropology*, *42*(1), 99–104.

Macchiarelli, R., Bayle, P., Bondioli, L., Mazurier, A., & Zanolli, C. (2013). From outer to inner structural morphology in dental anthropology: Integration of the third dimension in the visualization and quantitative analysis of fossil remains. In G. R. Scott & J. D. Irish (Hrsg.), *Anthropological Perspectives on Tooth Morphology: Genetics, Evolution, Variation* (S. 250–277). Cambridge University Press; Cambridge Core. https://doi.org/10.1017/CBO9780511984464.011

Mikkola, M. L. (2007). Genetic basis of skin appendage development. *Seminars in cell & developmental biology*, *18*(2), 225–236.

Mitteroecker, P. (2009). The developmental basis of variational modularity: Insights from quantitative genetics, morphometrics, and developmental biology. *Evolutionary biology*, *36*, 377–385.

Mitteroecker, P., Gunz, P., Neubauer, S., & Müller, G. (2012). How to Explore Morphological Integration in Human Evolution and Development? *Evolutionary Biology*, *39*(4), 536–553. https://doi.org/10.1007/s11692-012-9178-3

Molnar, S. (1971). Human tooth wear, tooth function and cultural variability. *American Journal of Physical Anthropology*, *34*(2), 2.

Monson, T. A., Boisserie, J., Brasil, M. F., Clay, S. M., Dvoretzky, R., Ravindramurthy, S., Schmitt, C. A., Souron, A., Takenaka, R., & Ungar, P. S. (2019). Evidence of strong stabilizing effects on the evolution of boreoeutherian (Mammalia) dental proportions. *Ecology and Evolution*, *9*(13), 7597–7612.

Monson, T. A., Fecker, D., & Scherrer, M. (2020). Neutral evolution of human enamel–dentine junction morphology. *Proceedings of the National Academy of Sciences*, *117*(42), 26183–26189. https://doi.org/10.1073/pnas.2008037117

Morita, W., Morimoto, N., & Ohshima, H. (2016). Exploring metameric variation in human molars: A morphological study using morphometric mapping. *Journal of Anatomy*, *229*(3), 3. https://doi.org/10.1111/joa.12482

Morita, W., Yano, W., Nagaoka, T., Abe, M., Ohshima, H., & Nakatsukasa, M. (2014). Patterns of morphological variation in enamel–dentin junction and outer enamel surface of human molars. *Journal of Anatomy*, *224*(6), 669–680. https://doi.org/10.1111/joa.12180

Nelson, S. J., & Ash, M. (2010). *Wheeler's dental anatomy, physiology and occlusion*. Elsevier.

Ortiz, A., Bailey, S. E., Hublin, J.-J., & Skinner, M. M. (2017). Homology, homoplasy and cusp variability at the enamel–dentine junction of hominoid molars. *Journal of Anatomy*, *231*(4), 585–599. https://doi.org/10.1111/joa.12649

Pan, L., Dumoncel, J., de Beer, F., Hoffman, J., Thackeray, J. F., Duployer, B., Tenailleau, C., & Braga, J. (2016). Further morphological evidence on South African earliest Homo lower postcanine dentition: Enamel thickness and enamel dentine junction. *Journal of Human Evolution*, *96*, 82–96. https://doi.org/10.1016/j.jhevol.2016.05.003

Pfneiszl, J. N. (2024). *An investigation on the relationship between tooth crown proportions and dentine and pulp volume*. https://utheses.univie.ac.at/detail/72097

Pillas, D., Hoggart, C. J., Evans, D. M., O'Reilly, P. F., Sipilä, K., Lähdesmäki, R., Millwood, I. Y., Kaakinen, M., Netuveli, G., Blane, D., Charoen, P., Sovio, U., Pouta, A., Freimer, N., Hartikainen, A.-L., Laitinen, J., Vaara, S., Glaser, B., Crawford, P., … Jarvelin, M.-R. (2010). Genome-Wide Association Study Reveals Multiple Loci Associated with Primary Tooth Development during Infancy. *PLOS Genetics*, *6*(2), e1000856. https://doi.org/10.1371/journal.pgen.1000856

Polly, P. D., & Mock, O. B. (2018). Heritability: The link between development and the microevolution of molar tooth form. *Historical Biology*, *30*(1–2), 53–63.

Sehic, A., Tulek, A., Khuu, C., Nirvani, M., Sand, L. P., & Utheim, T. P. (2017). Regulatory roles of microRNAs in human dental tissues. *Gene*, *596*, 9–18. https://doi.org/10.1016/j.gene.2016.10.009

Šimková, P. G., Weber, G. W., Ramirez Rozzi, F. V., Slimani, L., Sadoine, J., & Fornai, C. (2021). Morphological variation of the deciduous second molars in the Baka Pygmies. *Scientific Reports*, *11*(1), 1. https://doi.org/10.1038/s41598-021-95524-3

Šimková, P. G., Wurm, L., Fornai, C., Krenn, V. A., & Weber, G. W. (2024). Shape variation in modern human upper premolars. *PLOS ONE*, *19*(4), e0301482. https://doi.org/10.1371/journal.pone.0301482

Skinner, M. M., Gunz, P., Wood, B. A., & Hublin, J.-J. (2008). Enamel-dentine junction (EDJ) morphology distinguishes the lower molars of Australopithecus africanus and Paranthropus robustus. *Journal of Human Evolution*, *55*(6), 6. https://doi.org/10.1016/j.jhevol.2008.08.013

Skinner, M. M., Wood, B. A., & Hublin, J.-J. (2009). Protostylid expression at the enamel-dentine junction and enamel surface of mandibular molars of Paranthropus robustus and Australopithecus africanus. *Journal of Human Evolution*, *56*(1), 1. https://doi.org/10.1016/j.jhevol.2008.08.021

Slavicek, R. (2002). *The masticatory organ: Functions and dysfunctions*. GAMMA Medizinisch-wissenschaftliche Fortbildung-AG.

Stock, D. W. (2001). The genetic basis of modularity in the development and evolution of the vertebrate dentition. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *356*(1414), 1414. PubMed. https://doi.org/10.1098/rstb.2001.0917

Thesleff, I. (2014). Current understanding of the process of tooth formation: Transfer from the laboratory to the clinic. *Australian dental journal*, *59*, 48–54.

Thesleff, I., & Tummers, M. (2008). *Tooth organogenesis and regeneration* (20614625). Harvard Stem Cell Institute, Cambridge (MA); PubMed. http://europepmc.org/abstract/MED/20614625

Townsend, G., Bockmann, M., Hughes, T., & Brook, A. (2012). Genetic, environmental and epigenetic influences on variation in human tooth number, size and shape. *Odontology*, *100*(1), 1. https://doi.org/10.1007/s10266-011-0052-z

Wagner, G. P., & Altenberg, L. (1996). Perspective: Complex adaptations and the evolution of evolvability. *Evolution*, *50*(3), 967–976.

Wagner, G. P., & Zhang, J. (2011). The pleiotropic structure of the genotype–phenotype map: The evolvability of complex organisms. *Nature Reviews Genetics*, *12*(3), 204–213.

Weber, G. W., Fornai, C., Gopher, A., Barkai, R., Sarig, R., & Hershkovitz, I. (2016). The Qesem Cave hominin material (part 1): A morphometric analysis of the mandibular premolars and molar. *State of the art of the multidisciplinary research at the Middle Pleistocene Qesem Cave, Israel, 2015*, *398*, 159–174. https://doi.org/10.1016/j.quaint.2015.10.027

Yuan, Y., & Chai, Y. (2019). Regulatory mechanisms of jaw bone and tooth development. *Current topics in developmental biology*, *133*, 91–118.

Zanolli, C., Martinón-Torres, M., Bernardini, F., Boschian, G., Coppa, A., Dreossi, D., Mancini, L., Martínez de Pinillos, M., Martín-Francés, L., Bermúdez de Castro, J. M., Tozzi, C., Tuniz, C., & Macchiarelli, R. (2018). The Middle Pleistocene (MIS 12) human dental remains from Fontana Ranuccio (Latium) and Visogliano (Friuli-Venezia Giulia), Italy. A comparative high resolution endostructural assessment. *PLOS ONE*, *13*(10), e0189773. https://doi.org/10.1371/journal.pone.0189773

Supplementary Information

Connecting crowns: Analyzing morphological covariation in the modern human postcanine dentition

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Detailed results of the PLS analyses

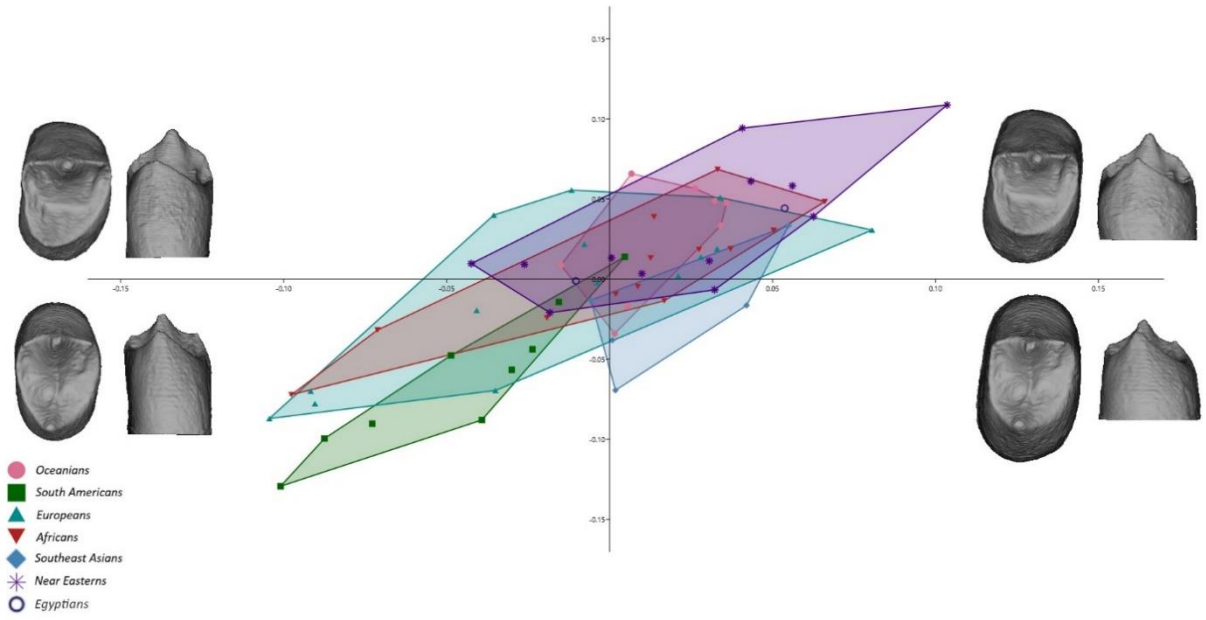
As described in “Material and Methods” of the main text, we performed 2-block Partial Least Squares (2B-PLS) analyses (PLS Mode A; relation between blocks is symmetric) to determine the covariation between tooth types of the human postcanine dentition.

Apart from the morphological changes on the enamel-dentine junction, we observed a general pattern of shape change in dentinal crowns of every tooth type. The tooth types covaried between tall and narrow crowned, and low and broad crowned. Despite a general overlap of the variation between populations, we detected a partial or complete separation of the South American sample from other populations in some of the analyses (Supplementary Fig. S2, S10, S12). However, due to the rather small sample size used in these analyses, and recent literature that points towards the fact that the geographical origin of a modern human individual cannot be detected solely based on the 3D morphological analysis of their dentinal crown (Halász, 2019; Krenn et al., 2019; Šimková et al., 2021, 2024), we consider these findings morphological tendencies.

A. Morphological covariation of the maxillary teeth

Upper P3 – P4

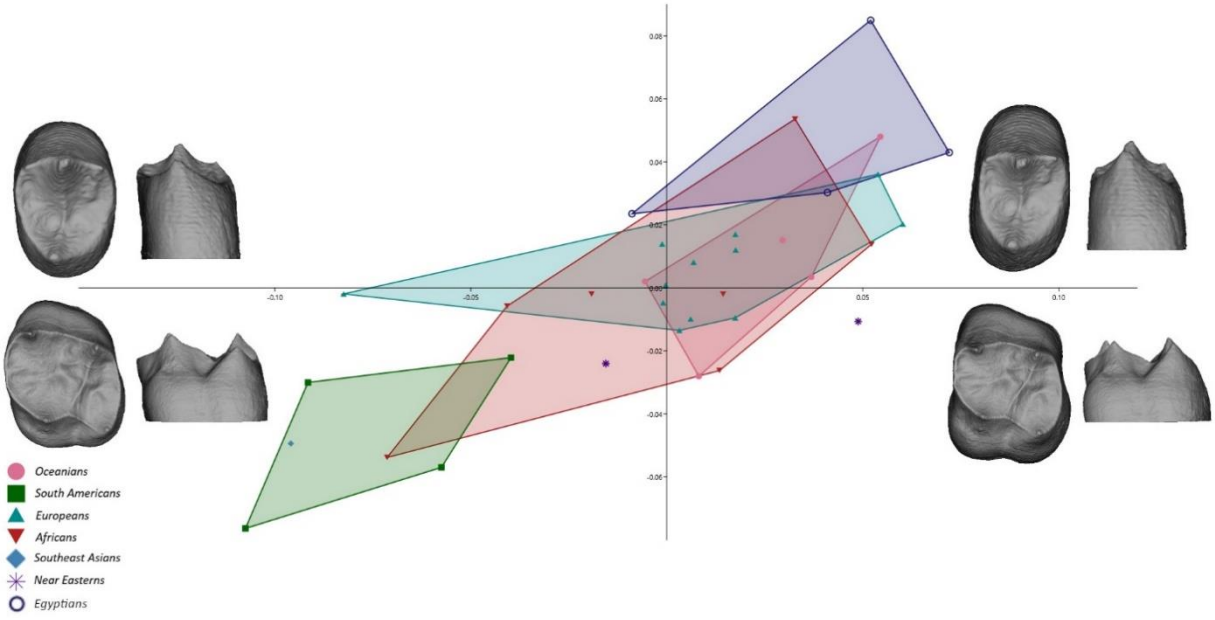
Both tooth types varied between short-crowned with a mesio-distally broad base and tall-crowned with a mesio-distally narrow base. The occlusal aspect of the short, broad crown type was mesio-distally expanded and square-shaped with a broad central groove. Conversely, tall and slender-crowned teeth had a mesio-distally narrow occlusal aspect with reduced lingual side. Additionally, short-crowned P4s exhibited higher dentinal horn tips compared to tall-crowned premolars.



Supplementary Fig. S1 2B-PLS plot capturing the covariation of upper third and fourth premolars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Upper P4 – M1

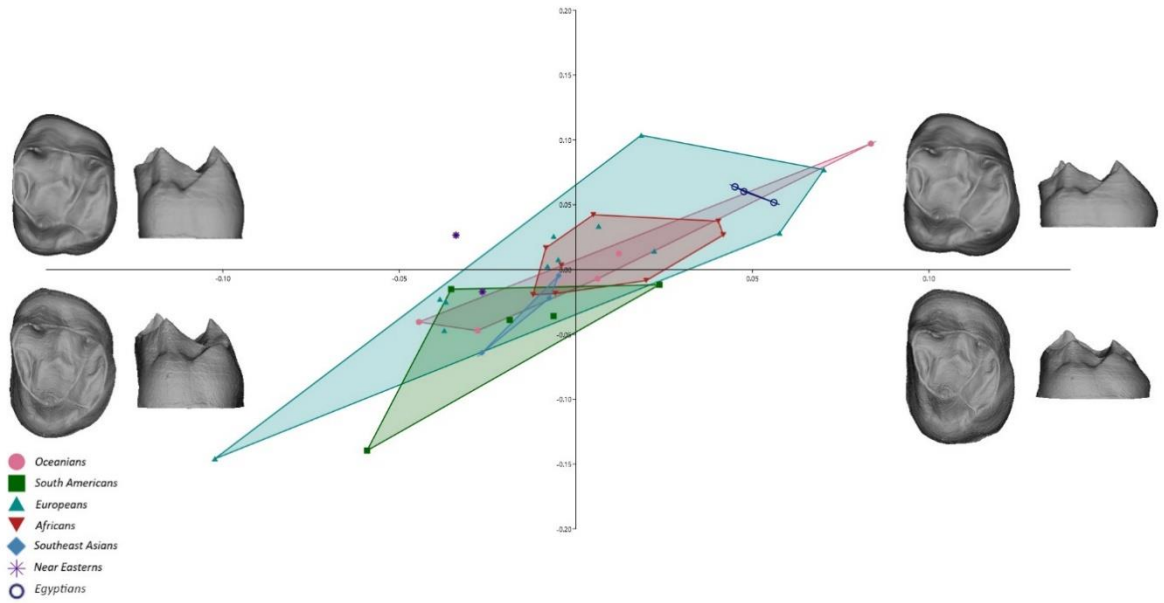
Main shape changes of both teeth occurred in the morphology of the occlusal aspect, as well as in the change of its size relatively to the base of the tooth. Both dental types varied between large-based with a constricted occlusal aspect and small-based with expanded occlusal aspect. In the latter, the premolar's buccal part of the occlusal aspect expanded mesio-distally and buccally, with higher buccal cusp relatively to the lingual cusp. The occlusal lingual side was mesio-distally reduced relatively to the buccal part. The molars showed a variable relationship between the trigon and the talon, with a relative expansion and reduction of the hypocone.



Supplementary Fig. S2 2B-PLS plot capturing the covariation of upper fourth premolars' and first molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Upper M1 – M2

The upper M1s and M2s covaried in the same manner. Their morphology varied across the sample between low-crowned with expanded base and relatively large hypocone in comparison to the trigon, and tall-crowned with reduced base, with bucco-lingually expanded trigon and relatively reduced hypocone.

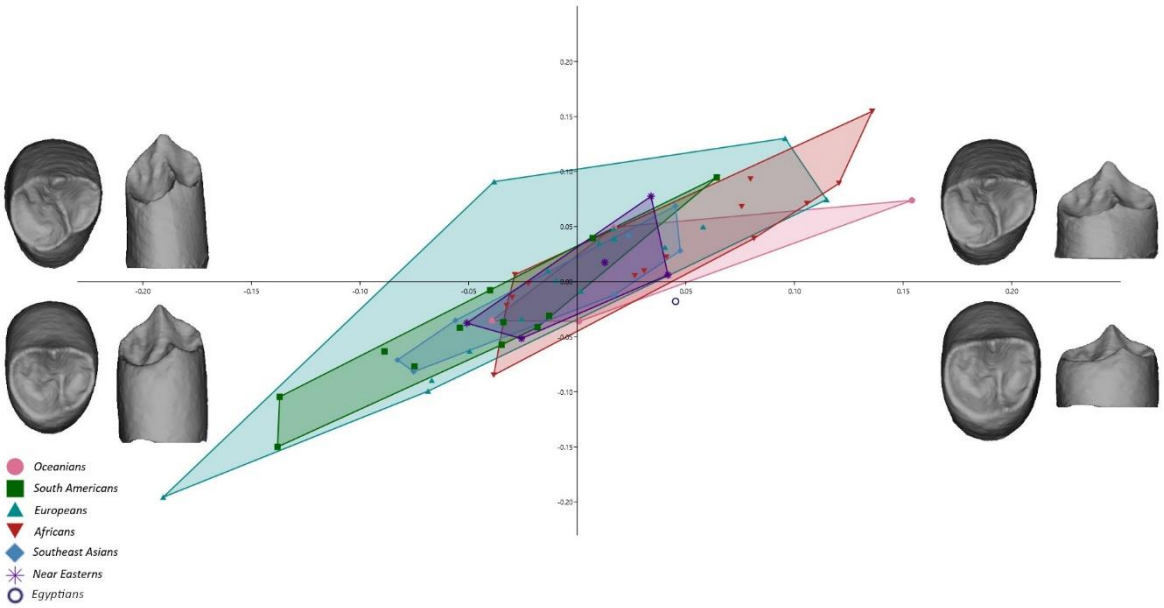


Supplementary Fig. S3 2B-PLS plot capturing the covariation of upper first and second molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

B. Morphological covariation of the mandibular teeth

Lower P3- P4

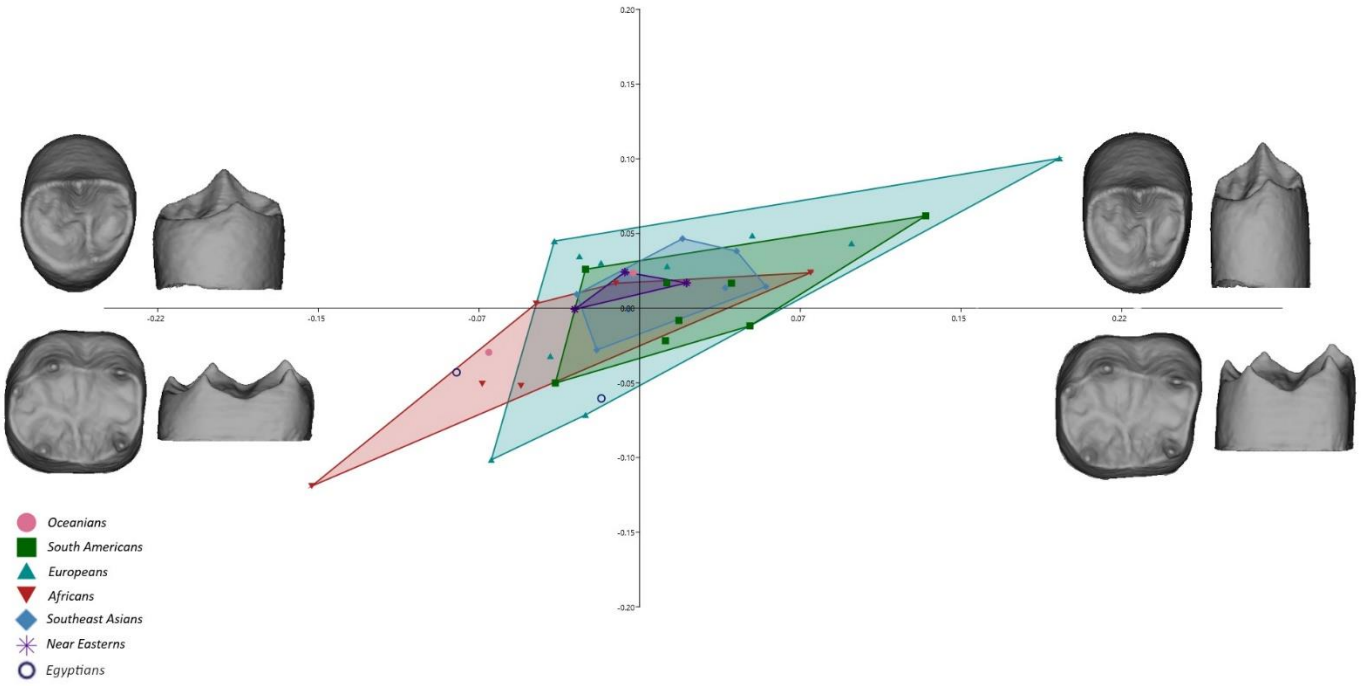
Similar to upper jaw, the covariation analysis between lower premolars reflected shape change in both tooth types mainly between short, broad- crowned teeth with expanded, squared occlusal aspect, resulting from disto-lingual shift of the lingual cusp, and tall, narrow- crowned teeth with mesially shifted lingual cusp and relatively smaller mesial fovea of the central fossa in comparison to the distal fovea.



Supplementary Fig. S4 2B-PLS plot capturing the covariation of lower third and fourth premolars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Lower P4 – M1

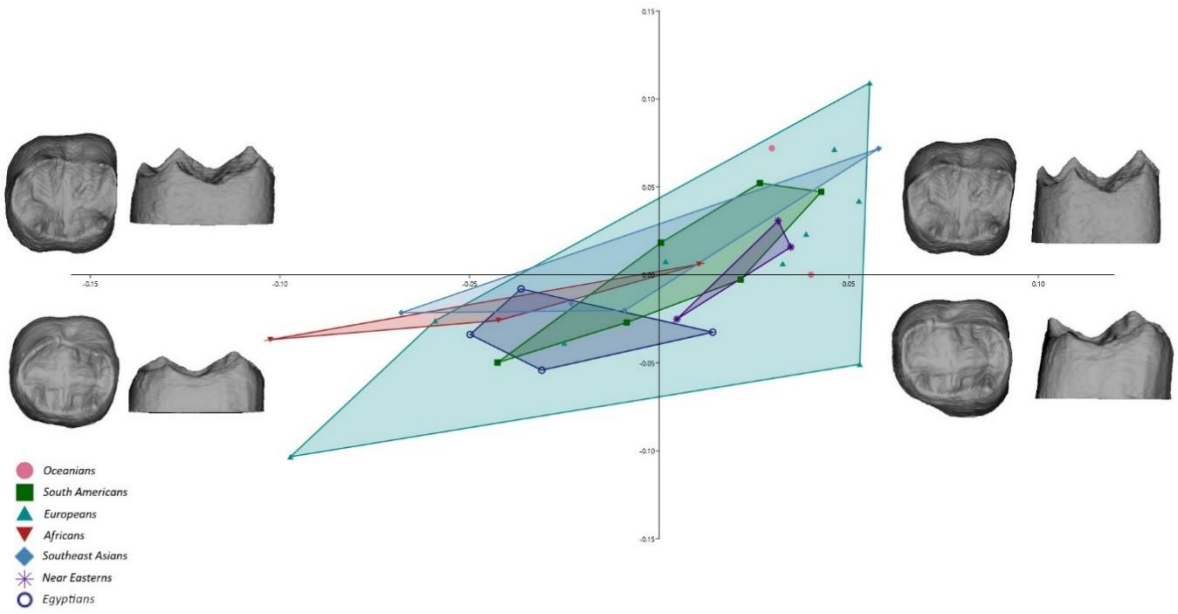
Covariation analysis of lower P4s' and M1s' dentinal crowns, reflected main shape change between tall and narrow crowns, and short and broad crowns in both dental types. The expansion and reduction of the base was relatively consistent with the expansion and reduction of the occlusal aspect. Tall- crowned P4s featured relatively bigger increase in height of the buccal cusp in comparison to the short- crowned teeth.



Supplementary Fig. S5 2B-PLS plot capturing the covariation of lower fourth premolars' and first molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Lower M1 – M2

The crowns of both tooth types varied mainly between short and broad crowns, with short cusps, and a barrel shaped base and occlusal aspect, and tall crowns with tall cusps, bucco-lingually constricted narrow base and occlusal aspect, and disto-lingually reduced occlusal aspect in M2s.

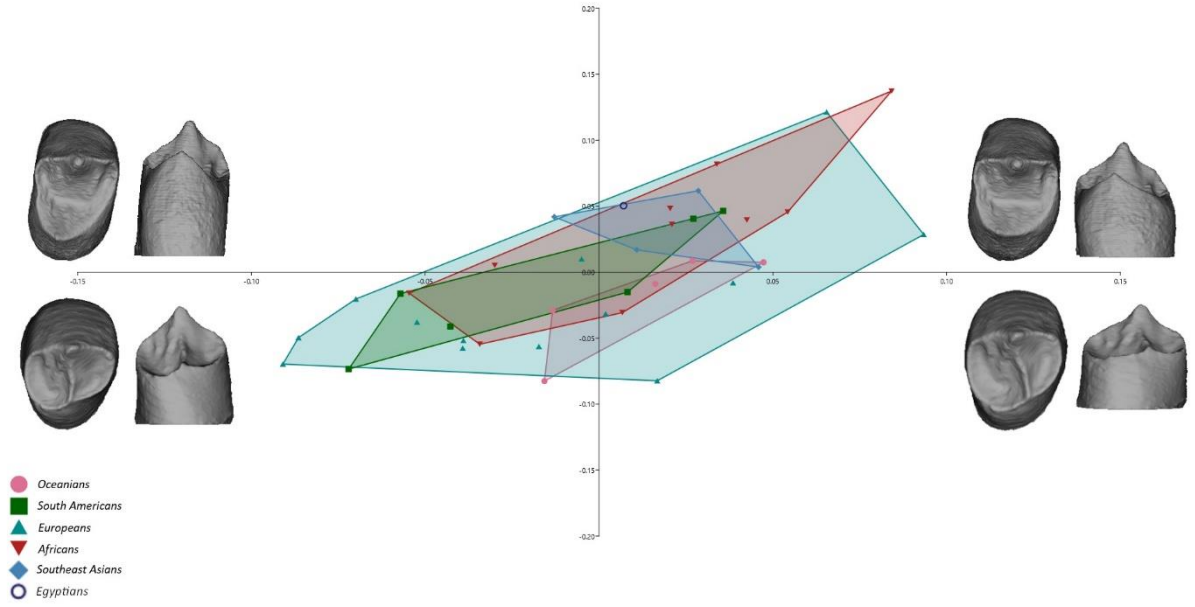


Supplementary Fig. S6 2B-PLS plot capturing the covariation of lower first and second molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

C. Morphological covariation between antagonists

Lower P3 – Upper P3

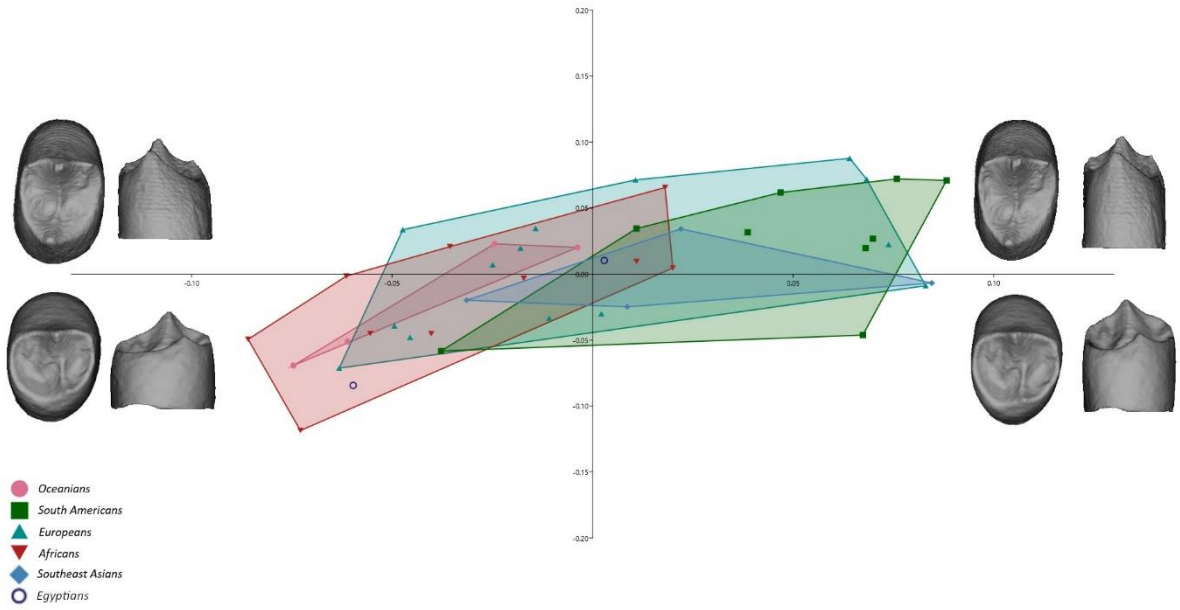
The crowns of antagonistic P3s covaried mainly between tall and narrow-crowned, and short and broad-crowned. The first type showed reduction of the distal and disto-lingual aspect in the upper P3, as well as in lower P3s that additionally showed a relatively taller buccal cusp and lingual shifting of the lingual cusp.



Supplementary Fig. S7 2B-PLS plot capturing the covariation of upper and lower third premolars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Lower P4 – Upper P4

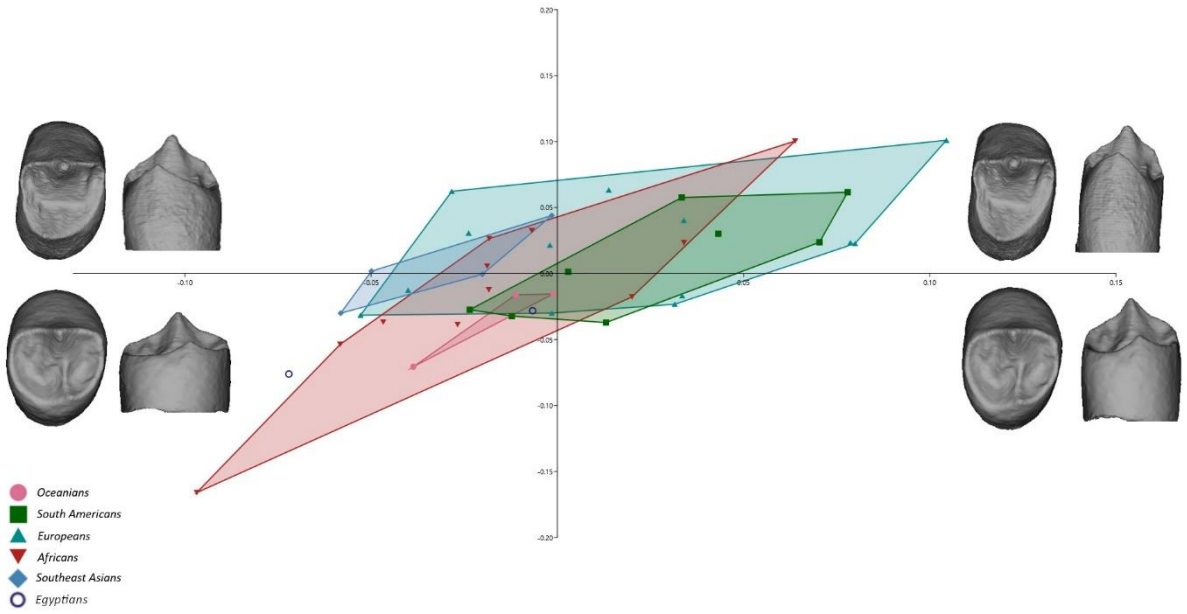
Similar to P3s, in P4s shape changes occurred mainly between tall and narrow-crowned and short and broad-crowned teeth. The buccal cusp of the lower P4s was variably expanded with respect to the rest of the crown.



Supplementary Fig. S8 2B-PLS plot capturing the covariation of upper and lower fourth premolars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Upper P3 – Lower P4

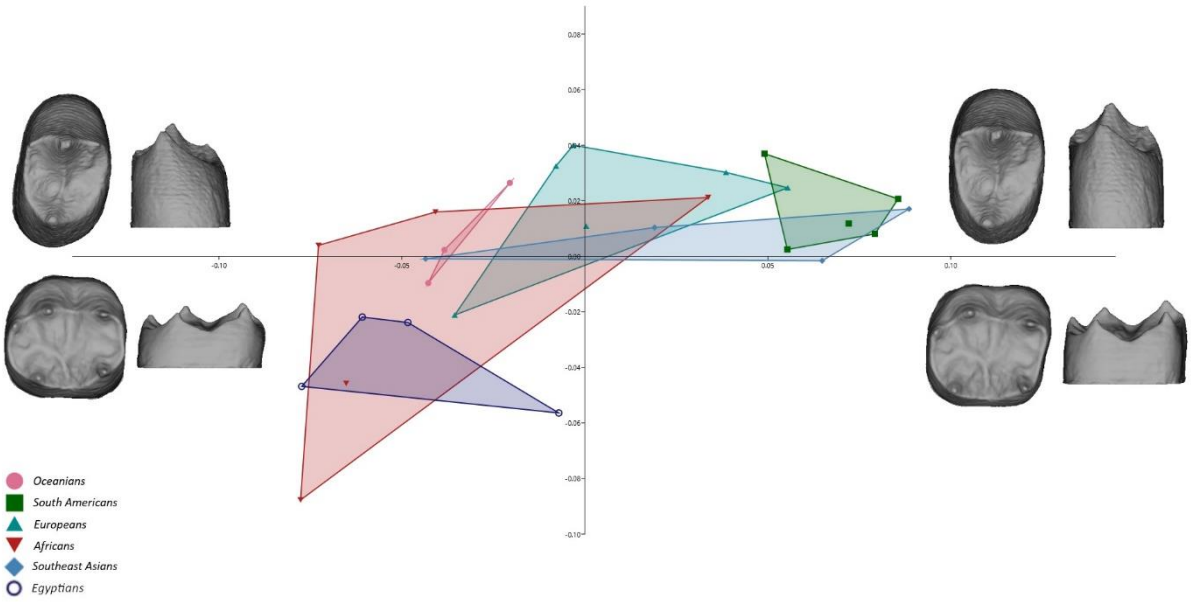
The premolars covaried mainly between tall and narrow-crowned to short and broad-crowned, the latter presenting with expanded distal side of the occlusal aspect creating a squared shape in both P3s and P4s. Tall and narrow crowned lower P4s featured a taller buccal cusp and disto-lingually shifted lingual cusp.



Supplementary Fig. S9 2B-PLS plot capturing the covariation of upper third and lower fourth premolars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Upper P4 – Lower M1

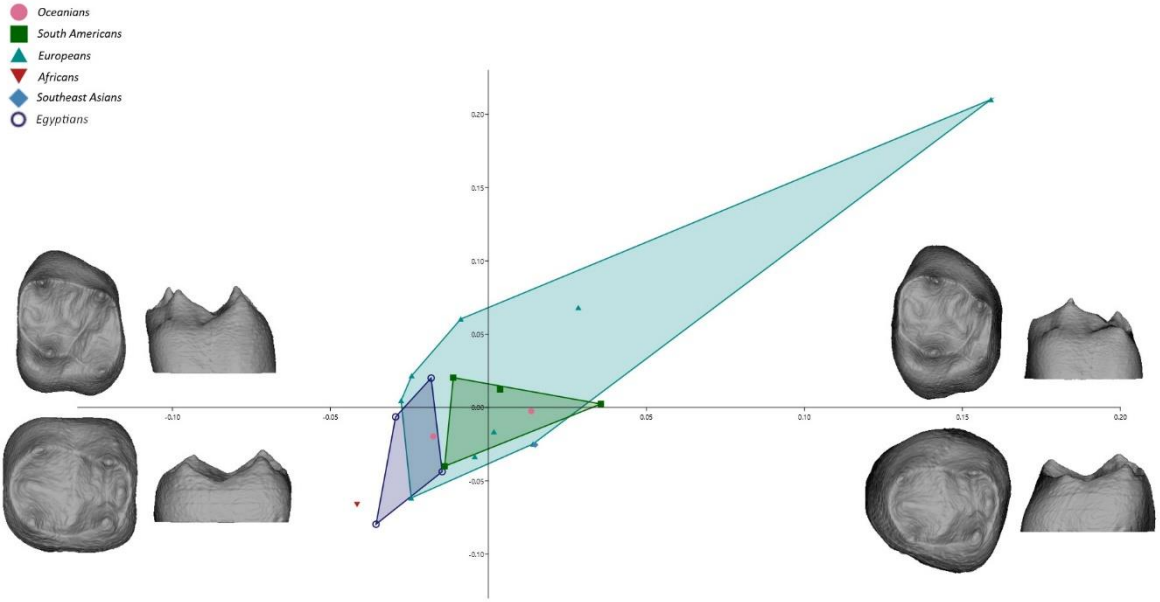
The morphological covariation occurred mainly in the height of the dental crowns and in their basal relative size. Shape variation of the occlusal aspect was mild and driven by the mesio-distal shifting of the lingual cusp in P4s, and relative inclination of the molar horn tips either towards or away from each other.



Supplementary Fig. S10 2B-PLS plot capturing the covariation of upper fourth premolars' and lower first molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Upper M1 – Lower M2

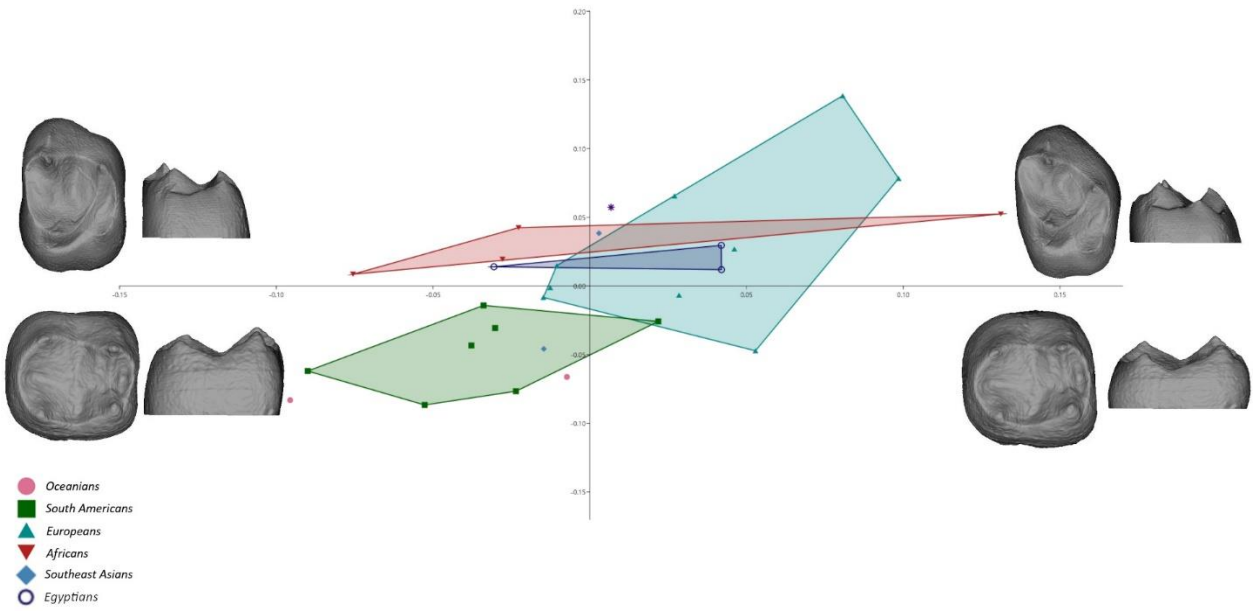
The shape changes were evident in the marked relative expansion and reduction of the distal and disto-lingual aspect of the dentinal crown reflected in the general presentation of the crown as broader or narrower.



Supplementary Fig. S11 2B-PLS plot capturing the covariation of upper first and lower second molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Lower M2 – Upper M2

The antagonistic M2s covaried mainly in the height of the crown and shape of the crown base. Tall crowns were mesio-distally constricted in upper M2s, featuring a relatively reduced base in comparison to the occlusal aspect that was more squared, while tall lower molar showed a bucco-lingually constricted base and expanded distal part of the occlusal aspect. Short crowns presented a large base relative to the occlusal aspect, mesio-distally expanded in upper molars, with lingually shifted hypocone. Lower molars showed a bucco-lingually, as well as mesio-lingually expanded base, featuring a reduction of the distal part of the occlusal aspect.

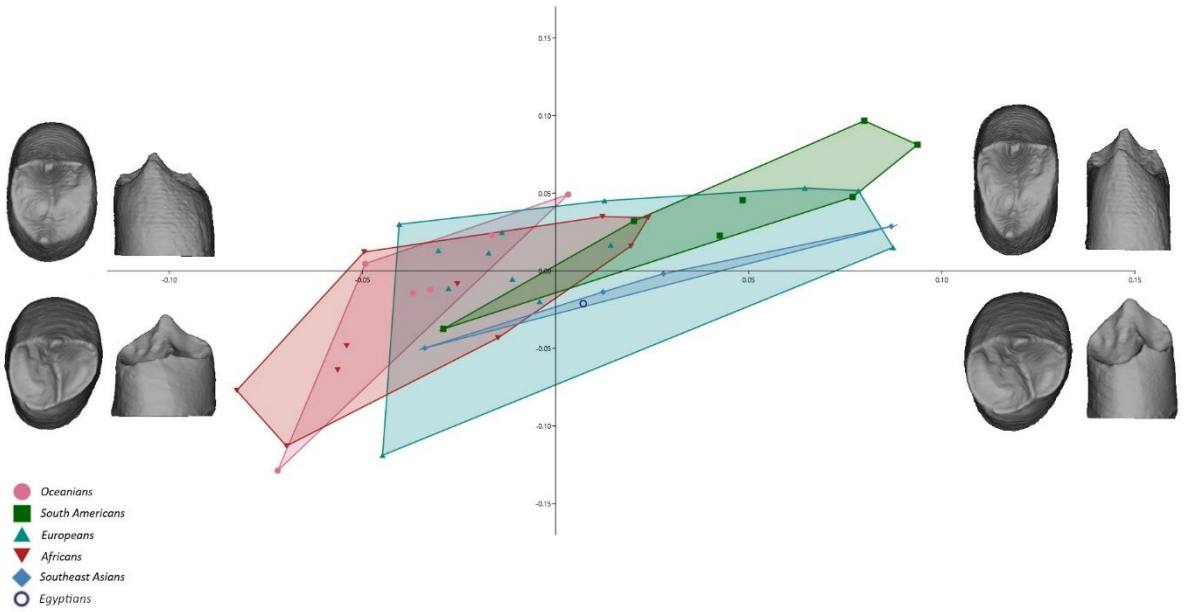


Supplementary Fig. S12 2B-PLS plot capturing the covariation of upper and lower second molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

D. Morphological covariation between non-occluding tooth types

Lower P3 – Upper P4

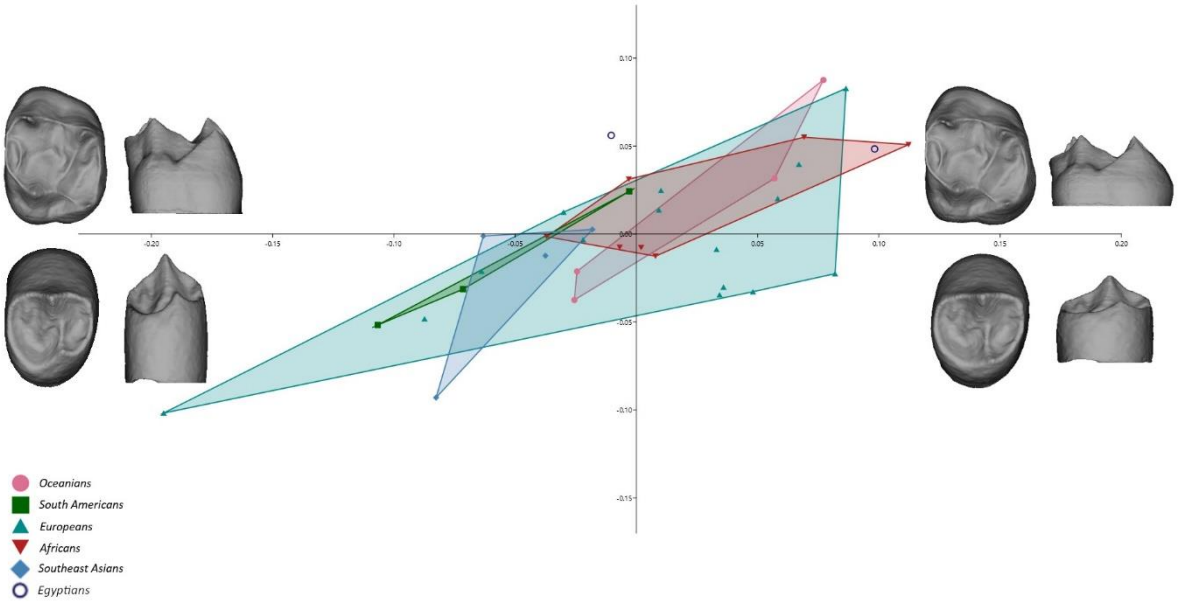
The results of the 2B-PLS analysis showed morphological covariation between lower P3s and upper P4s to be mainly between tall and narrow-crowned type and short and broad type of the crowns. In the upper premolars, the occlusal aspect varied from squared in short crowns, to narrow and pointy, due to the lingual shifting of the lingual cusp in taller crowns. In the lower premolars, the position of the lingual cusp varied between mesially and lingually shifted, resulting in varying expansion of the mesial fossa.



Supplementary Fig. S13 2B-PLS plot capturing the covariation of lower third and upper fourth premolars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Lower P4 – Upper M1

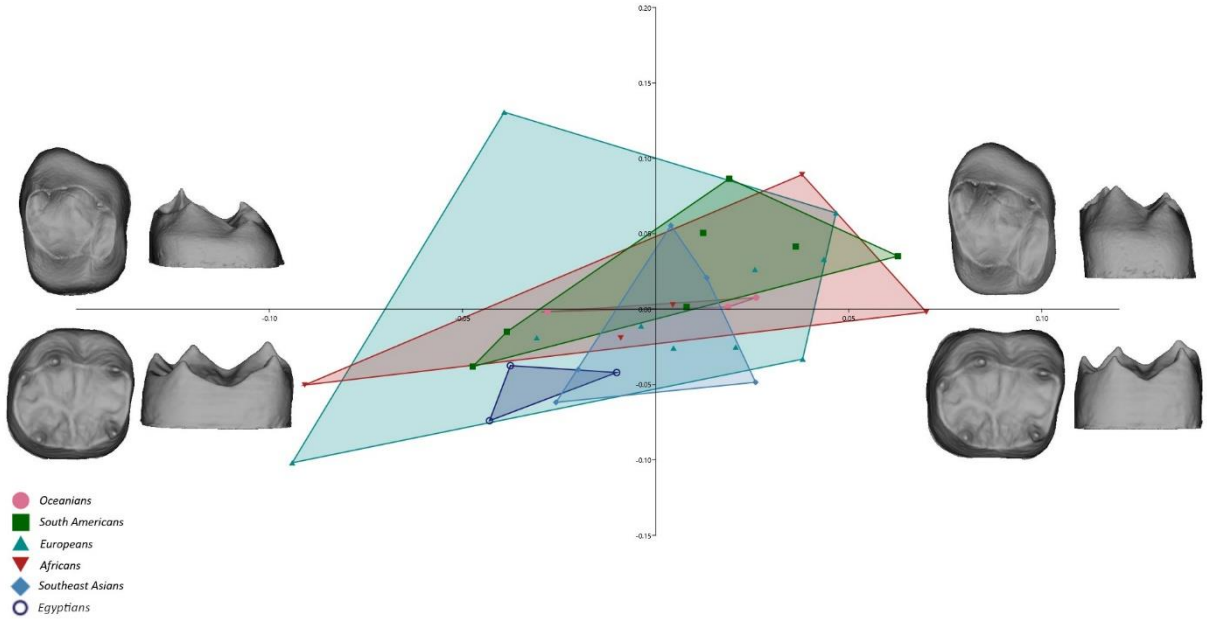
Lower P4s' and upper M1s' crowns covaried between tall and narrow with relatively reduced mesio-lingual side of the occlusal aspect in premolars, and disto-lingual side in molars, and short and broad crowns with relative expansion of the same aspects.



Supplementary Fig. S14 2B-PLS plot capturing the covariation of lower fourth premolars' and upper first molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

Lower M1 – Upper M2

Both tooth types covaried between tall crowns with narrow occlusal aspects and lingually positioned hypocone/hypoconulid, and short crowns with broad base and buccally positioned hypocone/hypoconulid.



Supplementary Fig. S15 2B-PLS plot capturing the covariation of lower first and upper second molars' dentinal crowns; the warping shows the real shape variation in occlusal and lingual view at the extremities of the range of distribution.

E. Pairwise correlation values indicated by occluding/neighboring and non-occluding/non-neighboring status

Supplementary Table S1. Colormap of the pairwise correlation values resulting from 2-Block Partial Least Squares Analyses. (L = lower; U = upper; P3 = third premolars: P4 = fourth premolars; M1 = first molars; M2 = second molars).

Blue colors indicate occluding or neighboring tooth type pairs; orange colors indicate non-occluding and non-neighboring tooth-type pairs. In both instances, darker colors indicate stronger correlations.

	LP3	LP4	LM1	LM2	UP3	UP4	UM1	UM2
LP3	1	0.89	0.76	0.74	0.71	0.75	0.77	0.88
LP4		1	0.79	0.75	0.70	0.65	0.73	0.44
LM1			1	0.73	0.61	0.58	0.90	0.50
LM2				1	0.63	0.66	0.82	0.64
UP3					1	0.81	0.67	0.70
UP4						1	0.77	0.78
UM1							1	0.83
UM2								1

3. Discussion

The aim of this thesis is to enhance our understanding of 3D dental morphology and variation, specifically within the postcanine dentition. Our research demonstrated that the applied methods effectively allow for a thorough in-depth investigation of shape variation across different tooth types within modern human populations. By combining landmark configurations from various crown structures, we were able to simultaneously analyze shape changes in both the occlusal aspect and the crown base, and thus incorporating crown height into the analysis. Geometric morphometrics (GM) enables the study of objects within shape space, focusing exclusively on shape by removing size as a variable (Mitteroecker & Gunz, 2009). This approach allowed us to isolate and analyze variation attributable solely to shape changes, rather than size differences, within the sample. Through the use of 3D imaging techniques, we were able to visualize and describe real shape variation among individuals. This holistic approach to exploring shape variation across the entire dentinal crown offers a more accurate representation of morphological patterns within the sample, in comparison to examining isolated traits.

In fact, a distinct pattern of morphological variation was observed across all populations in both upper and lower deciduous second molars, as well as in the upper third and fourth premolars. The primary shape variation detected in the dentinal crowns was characterized by two main shape-types: short and broad versus tall and narrow crowns. This pattern has also been identified in previous studies on lower premolar morphological variation (Krenn et al., 2019), as well as in first permanent molars (Halász, 2019). Additionally, Pfneiszl (2024) investigated this variation in a large sample of postcanine teeth. This particular morphological pattern, in combination with shape changes on the occlusal aspect and the crown base, dominated the analyses performed in this thesis.

Our research on the covariation of the postcanine dentition revealed the exact same patterns between different pairs of tooth types, regardless of their specific relationship. This finding supports Pfneiszl's results that 87% of the studied individuals consistently feature one of the two shape-types throughout their whole postcanine dentition. This thesis suggests that the

morphological signal of this particular variation might be one of the strongest phenomena in dental variation, as well as covariation. Shape variation analyses performed in this thesis identified the varying shape-type on the first, respectively second principal component in every Principal component analysis (PCA). This emphasizes that a significant share of shape variation in the investigated samples can be attributed to the shape-type variation.

In the case of the covariation analyses, the results turned out to be very similar, however, we identified shape-type variation among tooth pairs as a limiting factor to our method, overshadowing other morphological variables, such as the shape changes on the occlusal aspect in some of the analyses (see Chapter 2.3). This led to unexpectedly high pairwise correlation values between unrelated tooth type pairs. We hypothesize that since the occlusal aspects of unrelated teeth are not as strongly influenced by developmental and functional integration factors as antagonistic teeth, the most significant signal of covariation lies in the shape-type of the teeth. Consequently, the identification of shape changes on the occlusal aspect is limited. This hypothesis requires further investigation, possibly by removing the variable of crown height from the analysis to concentrate solely on the morphological covariation of the occlusal surfaces.

In general, the analyses revealed a considerable range of morphological variation within each tooth type sample. Remarkably, despite this extensive variation, we observed significant overlap in morphological expressions across subsamples of different geographical populations. We were able to identify the primary morphological directions in which the analyzed tooth types vary most significantly, while also capturing a range of shape variations that accurately reflect the diversity present in modern human dentition.

Even though we did not observe significant separations of different populations based on their dental morphology, neither in our variation nor covariation research, some populations showed tendencies towards a certain shape-type or even its extreme expressions. Sub-Saharan African, Egyptian, and Oceanian specimens more frequently exhibited the short and broad shape-type and showed exclusively this one when exceeding the range of variation of the rest

of the sample. On the other hand, South Americans and Europeans tended to show extreme expressions of the tall and narrow shape-type.

3.1 Does the sample size matter?

Concerning the non-metric trait analyses performed in Chapters 2.1 and 2.2, we observed two main limiting factors influencing each other as well as the outcomes of our analyses: the sizes of our samples, and the wear status of the teeth. In Chapter 2.1 we examined the prevalence of two non-metric traits for each tooth type (upper and lower deciduous second molars) on the outer enamel surface (OES) of the crowns. All four traits represented an accessory cusp formation, as these types of traits could still be identified even in dental crowns with moderate wear. However, since one of the inherent limitation factors of the non-metric trait analysis itself is the subjectivity of trait scoring (Irish & Scott, 2015; Scott & Turner, 1997), wear might obscure the process of accurate scoring even more. Furthermore, wear played a significant role in the compilation of the sample, particularly when collecting deciduous teeth. Due to the limited availability of infant individuals in skeletal collections, most of the specimens derived from mixed dentitions of juvenile individuals. As a result, the deciduous second molars had been in function for a long time and displayed corresponding wear. This led to an imbalance in the number of specimens across different population subsamples, resulting in a relatively small sample size compared to previous studies on non-metric dental traits (Scott, 1980; Scott & Irish, 2017).

In Chapter 2.2 the data collection conditions improved in comparison to Chapter 2.1, since this study focused on the upper permanent premolars. The sample size increased, was more balanced, and was easier to collect, since upper premolars are present in most of the young adults and are typically in a relatively good condition. Additionally, moderate wear could be more easily virtually reconstructed. This allowed us to perform non-metric trait analyses on both, the OES and the enamel-dentine junction (EDJ) on a larger sample.

Although we primarly focused on traits suitable for the reconstruction of population affinities (e.g. Cusp 6, Cusp 7; Rathmann & Reyes-Centeno, 2020), we did not find significant results

for most of the investigated non-metric traits, except for the hypocone in the upper deciduous second molars. Due to the limited sample size, we only categorized the hypocones into two groups: small to moderate and large to massive, with the hypocone present in 100% of the sample. According to Rathmann et al. (2023) the investigation of non-metric dental traits has proven to be the most reliable morphological method for studying affinities between human populations. However, our approach is fundamentally different, as we focus on studying the 3D shape of teeth in detail. This imposes particular constraints to the selection of samples. In this context, the non-metric trait analysis in our research was more of a by-product than the primary objective. Consequently, our samples are relatively small and may not be impactful for non-metric trait analyses.

On the other hand, this thesis demonstrates that it is not possible to determine the geographical origin of an individual, solely based on 3D dental morphology using geometric morphometrics. We observed substantial overlap in the dental morphology of all geographical populations across all studied tooth types, with partial or complete separations only in cases of some rather small population subsamples. As the overall sample size increased and subsample sizes became more balanced, the overlap between different populations became even more pronounced. We were able to detect clear tendencies toward extreme shape variation in some populations (e.g. the Baka), exceeding the range of variation of the rest of the sample. However, our research showed that it was not possible to separate any of the populations represented by at least 6 individuals from the rest of the sample (see Chapter 2.1).

This has been previously observed by Krenn et al. (2019) on lower premolars in a relatively large sample, as well as on upper and lower first molars (Halász, 2019). Furthermore, our investigation of the upper third premolars (Chapter 2.2), which built upon Buchegger's (2015) preliminary study performed on a smaller sample, revealed that significantly extending the sample did not lead to noticeable changes in the PCA results. This suggests that the methods we applied reliably detected the principal components, thus the original variables, capturing the most variance within the sample. Increasing subsample size thus only modulates the extant

of overlap between different populations and increases the chance to detect potential extreme variations.

It is thus worth noting that the effects of increasing sample size differ between non-metric trait analysis and 3D geometric morphometric shape analysis. Increasing the sample size in geometric morphometric analysis often reveals greater overlap between populations, reflecting the true variation within the data. In contrast, in non-metric trait analysis, a larger sample size tends to highlight statistically significant differences between groups.

3.2 Does the geographical origin matter in modern human sample composition?

As mentioned above, we observed an extensive overlap in morphological variation across all populations studied, regardless of their geographical origin. To challenge our approach, we included populations with a history of isolation. The Baka, Khoesan, and the indigenous populations of Papua New Guinea, Oceania or Tierra del Fuego have lived in complete isolation for thousands of years, with very low levels of genetic admixture with other populations. Some of these groups diverged from Eurasian populations at the minimum of 50,000 years ago. Nevertheless, our results suggest that the occasionally detected deviations of shape variation from other populations occur primarily due to the unbalanced sizes of subsamples.

Dental morphology investigated by means of GM has been successfully used in taxonomic studies (e.g., Benazzi et al., 2011, 2012; Fornai et al., 2016; Gómez-Robles et al., 2007, 2011, 2012; Hershkovitz et al., 2018; Martinón-Torres et al., 2006; Sarig et al., 2021; Weber et al., 2016; Zanolli et al., 2018), often including modern human specimens as comparative sample. However, the overlap of shape variation observed in this thesis, aligns with previous studies on different modern human tooth types (Buchegger, 2015; Halász, 2019; Krenn et al., 2019). This shows that while hominin taxa can be quite well distinguished based on their 3D dentinal crown morphology, it is not possible to replicate this within a geographically diverse sample of modern humans only. Therefore, we interpret extreme shape variation expressions or partial separations between groups as tendencies rather than definitive morphological distinctions.

Our findings thus contribute to a possibly more effective approach to sample composition in future taxonomic studies, since we showed that the geographical diversity of a modern human subsample is not of big importance in such cases.

To summarize, we presented three studies that have built upon the integration of GM approach with 3D virtual imaging techniques, allowing for a detailed and accurate investigation of morphological variation and covariation within the modern human postcanine dentition. Our methods are easily reproducible and adaptable for future research involving distinct samples. Furthermore, our investigation of the covariation patterns within the postcanine dentition produced novel results, marking the first of their kind. These findings raise new questions and present challenges in this relatively unexplored corner of dental morphology and development.

4. References

Al-Jasser, N. M., & Bello, L. L. (2003). Time of eruption of primary dentition in Saudi children. *The Journal of Contemporary Dental Practice*, *4*(3), 3. PubMed.

Bailey, S. E., Skinner, M. M., & Hublin, J.-J. (2011). What lies beneath? An evaluation of lower molar trigonid crest patterns based on both dentine and enamel expression. *American Journal of Physical Anthropology*, *145*(4), 505–518. https://doi.org/10.1002/ajpa.21468

Benazzi, S., Coquerelle, M., Fiorenza, L., Bookstein, F., Katina, S., & Kullmer, O. (2011). Comparison of dental measurement systems for taxonomic assignment of first molars. *American Journal of Physical Anthropology*, *144*(3), 3. https://doi.org/10.1002/ajpa.21409

Benazzi, S., Fornai, C., Buti, L., Toussaint, M., Mallegni, F., Ricci, S., Gruppioni, G., Weber, G. W., Condemi, S., & Ronchitelli, A. (2012). Cervical and crown outline analysis of worn Neanderthal and modern human lower second deciduous molars. *American Journal of Physical Anthropology*, *149*(4), 4. https://doi.org/10.1002/ajpa.22155

Benazzi, S., Grosse, I. R., Gruppioni, G., Weber, G. W., & Kullmer, O. (2014). Comparison of occlusal loading conditions in a lower second premolar using three-dimensional finite element analysis. *Clinical Oral Investigations*, *18*(2), 2. https://doi.org/10.1007/s00784-013-0973-8

Bookstein, F. L. (1986). Size and shape spaces for landmark data in two dimensions. *Statistical science*, *1*(2), 181–222.

Bookstein, F. L. (1989). Principal warps: Thin-plate splines and the decomposition of deformations. *IEEE Transactions on pattern analysis and machine intelligence*, *11*(6), 6.

Bookstein, F. L. (1991a). *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press. https://books.google.at/books?id=amwT1ddIDwAC

Bookstein, F. L. (1991b). *Thin-plate splines and the atlas problem for biomedical images*. 326–342.

Bookstein, F. L. (1997). *Morphometric tools for landmark data: Geometry and biology*. Cambridge University Press.

Boyde, A. (1964). The structure and development of mammalian enamel [Ph. D. thesis]. *London: The London Hospital Medical College*.

Buchegger, L. (2015). *Variation of outer and inner crown morphology in upper premolars*.

Burroughs, R. W. (2019). Phylogenetic and Developmental Constraints Dictate the Number of Cusps on Molars in Rodents. *Scientific Reports*, *9*(1), 1. https://doi.org/10.1038/s41598-019-47469-x

Corruccini, R. S. (1987). The dentinoenamel junction in primates. *International Journal of Primatology*, *8*(2), 99. https://doi.org/10.1007/BF02735159

Cunningham, C., Scheuer, L., & Black, S. (2016). *Developmental juvenile osteology*. Academic press.

Davies, T. W., Delezene, L. K., Gunz, P., Hublin, J.-J., & Skinner, M. M. (2019). Endostructural morphology in hominoid mandibular third premolars: Geometric

morphometric analysis of dentine crown shape. *Journal of Human Evolution*, *133*, 198–213. https://doi.org/10.1016/j.jhevol.2019.06.004

Dryden, I., & Mardia, K. (1998). *Statistical analysis of shape*. Wiley.

Fornai, C., Benazzi, S., Gopher, A., Barkai, R., Sarig, R., Bookstein, F. L., Hershkovitz, I., & Weber, G. W. (2016). The Qesem Cave hominin material (part 2): A morphometric analysis of dm2-QC2 deciduous lower second molar. *State of the art of the multidisciplinary research at the Middle Pleistocene Qesem Cave, Israel, 2015*, *398*, 175–189. https://doi.org/10.1016/j.quaint.2015.11.102

Fornai, C., Benazzi, S., Svoboda, J., Pap, I., Harvati, K., & Weber, G. W. (2014). Enamel thickness variation of deciduous first and second upper molars in modern humans and Neanderthals. *Journal of human evolution*, *76*, 83–91.

Gómez-Robles, A., Bermúdez de Castro, J. M., Martinón-Torres, M., Prado-Simón, L., & Arsuaga, J. L. (2012). A geometric morphometric analysis of hominin upper second and third molars, with particular emphasis on European Pleistocene populations. *Journal of Human Evolution*, *63*(3), 512–526. https://doi.org/10.1016/j.jhevol.2012.06.002

Gómez-Robles, A., Martinón-Torres, M., Bermúdez de Castro, J. M., Prado, L., Sarmiento, S., & Arsuaga, J. L. (2008). Geometric morphometric analysis of the crown morphology of the lower first premolar of hominins, with special attention to Pleistocene Homo. *Journal of Human Evolution*, *55*(4), 627–638. https://doi.org/10.1016/j.jhevol.2008.03.011

Gómez-Robles, A., Martinón-Torres, M., Bermúdez de Castro, J. M., Prado-Simón, L., & Arsuaga, J. L. (2011). A geometric morphometric analysis of hominin upper premolars. Shape variation and morphological integration. *Journal of Human Evolution*, *61*(6), 6. https://doi.org/10.1016/j.jhevol.2011.09.004

Gómez-Robles, A., Martinón-Torres, M., De Castro, J. B., Margvelashvili, A., Bastir, M., Arsuaga, J. L., Pérez-Pérez, A., Estebaranz, F., & Martínez, L. M. (2007). A geometric morphometric analysis of hominin upper first molar shape. *Journal of Human Evolution*, *53*(3), 3.

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 1.

Gunz, P., & Mitteroecker, P. (2013). Semilandmarks: A method for quantifying curves and surfaces. *Hystrix, the Italian journal of mammalogy*, *24*(1), 1.

Gunz, P., Mitteroecker, P., & Bookstein, F. L. (2005). Semilandmarks in three dimensions. In *Modern morphometrics in physical anthropology* (S. 73–98). Springer.

Guy, F., Lazzari, V., Gilissen, E., & Thiery, G. (2015). To What Extent is Primate Second Molar Enamel Occlusal Morphology Shaped by the Enamel-Dentine Junction? *PLOS ONE*, *10*(9), e0138802. https://doi.org/10.1371/journal.pone.0138802

Halász, V. (2019). *Morphological assessment of modern human upper and lower first molars* [Masterthesis]. University of Vienna.

Hanihara, T. (2008). Morphological variation of major human populations based on nonmetric dental traits. *American Journal of Physical Anthropology*, *136*(2), 169–182. https://doi.org/10.1002/ajpa.20792

Hanihara, T., & Ishida, H. (2005). Metric dental variation of major human populations. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *128*(2), 2.

Hershkovitz, I., Weber, G. W., Quam, R., Duval, M., Grün, R., Kinsley, L., Ayalon, A., Bar-Matthews, M., Valladas, H., Mercier, N., Arsuaga, J. L., Martinón-Torres, M., Bermúdez de Castro, J. M., Fornai, C., Martín-Francés, L., Sarig, R., May, H., Krenn, V. A., Slon, V., … Weinstein-Evron, M. (2018). The earliest modern humans outside Africa. *Science*, *359*(6374), 6374. https://doi.org/10.1126/science.aap8369

Hillson, S. (1996). *Dental anthropology*. Cambridge University Press.

Irish, J. D., & Scott, G. R. (2015). *A companion to dental anthropology*. John Wiley & Sons.

Jernvall, J., Åberg, T., Kettunen, P., Keränen, S., & Thesleff, I. (1998). The life history of an embryonic signaling center: BMP-4 induces p21 and is associated with apoptosis in the mouse tooth enamel knot. *Development*, *125*(2), 161–169.

Jernvall, J., Kettunen, P., Karavanova, I., Martin, L. B., & Thesleff, I. (1994). Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: Non-dividing cells express growth stimulating Fgf-4 gene. *The International journal of developmental biology*, *38*(3), 463–469.

Jernvall, J., & Thesleff, I. (2012). Tooth shape formation and tooth renewal: Evolving with the same signals. *Development*, *139*(19), 3487–3497.

Kato, A., Kouchi, M., Mochimaru, M., Isomura, A., & Ohno, N. (2011). A geometric morphometric analysis of the crown form of the maxillary central incisor in humans. *Dental Anthropology Journal*, *24*(1), 1–10.

Kenessey, D. E., Stojanowski, C. M., & Paul, K. S. (2024). Evaluating predictions of the patterning cascade model of crown morphogenesis in the human lower mixed and permanent dentition. *PLOS ONE*, *19*(6), e0304455. https://doi.org/10.1371/journal.pone.0304455

Kraus, B. S., Jordan, R. E., & Abrams, L. (1969). *Dental anatomy and occlusion: A study of the masticatory system.* Baltimore, MD: Williams and Wilkins.

Krenn, V. A., Fornai, C., Wurm, L., Bookstein, F. L., Haeusler, M., & Weber, G. W. (2019). Variation of 3D outer and inner crown morphology in modern human mandibular premolars. *American journal of physical anthropology*, *169*(4), 4.

Landin, M. A., Shabestari, M., Babaie, E., Reseland, J., & Osmundsen, H. (2012). Gene Expression Profiling during Murine Tooth Development. *Frontiers in Genetics*, *3*. https://www.frontiersin.org/articles/10.3389/fgene.2012.00139

Lele, S. R., & Richtsmeier, J. T. (2001). *An invariant approach to statistical analysis of shapes*. Chapman and Hall/CRC.

Liversidge, H. M., & Molleson, T. (2004). Variation in crown and root formation and eruption of human deciduous teeth. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *123*(2), 2.

Lovell, N. C., & Haddow, S. (2007). Nonmetric Traits of Deciduous Dentitions from Bronze Age Tell Leilan, Syria. *International Journal of Dental Anthropology*, *11*, 1–21.

Lucas, P. W. (2004). *Dental functional morphology: How teeth work*. Cambridge University Press.

Macchiarelli, R., Bayle, P., Bondioli, L., Mazurier, A., & Zanolli, C. (2013). From outer to inner structural morphology in dental anthropology: Integration of the third dimension in the visualization and quantitative analysis of fossil remains. In G. R. Scott & J. D. Irish (Hrsg.),

Anthropological Perspectives on Tooth Morphology: Genetics, Evolution, Variation (S. 250–277). Cambridge University Press; Cambridge Core. https://doi.org/10.1017/CBO9780511984464.011

Martin, R., & Saller, K. (1957). Lehrbuch der. *Anthropologie*, *1*(3), 433–476.

Martinón-Torres, M., Bastir, M., Bermúdez de Castro, J. M., Gómez, A., Sarmiento, S., Muela, A., & Arsuaga, J. L. (2006). Hominin lower second premolar morphology: Evolutionary inferences through geometric morphometric analysis. *Journal of Human Evolution*, *50*(5), 523–533. https://doi.org/10.1016/j.jhevol.2005.12.004

Mitteroecker, P., & Gunz, P. (2009). Advances in Geometric Morphometrics. *Evolutionary Biology*, *36*(2), 235–247. https://doi.org/10.1007/s11692-009-9055-x

Mitteroecker, P., Gunz, P., Windhager, S., & Schaefer, K. (2013). A brief review of shape, form, and allometry in geometric morphometrics, with applications to human facial morphology. *Hystrix, the Italian Journal of Mammalogy*, *24*(1), 1.

Monson, T. A., Fecker, D., & Scherrer, M. (2020). Neutral evolution of human enamel–dentine junction morphology. *Proceedings of the National Academy of Sciences*, *117*(42), 26183–26189. https://doi.org/10.1073/pnas.2008037117

Morita, W., Yano, W., Nagaoka, T., Abe, M., Ohshima, H., & Nakatsukasa, M. (2014). Patterns of morphological variation in enamel–dentin junction and outer enamel surface of human molars. *Journal of Anatomy*, *224*(6), 669–680. https://doi.org/10.1111/joa.12180

Ortiz, A., Bailey, S. E., Hublin, J.-J., & Skinner, M. M. (2017). Homology, homoplasy and cusp variability at the enamel–dentine junction of hominoid molars. *Journal of Anatomy*, *231*(4), 585–599. https://doi.org/10.1111/joa.12649

Pan, L., Dumoncel, J., de Beer, F., Hoffman, J., Thackeray, J. F., Duployer, B., Tenailleau, C., & Braga, J. (2016). Further morphological evidence on South African earliest Homo lower postcanine dentition: Enamel thickness and enamel dentine junction. *Journal of Human Evolution*, *96*, 82–96. https://doi.org/10.1016/j.jhevol.2016.05.003

Rathmann, H., Perretti, S., Porcu, V., Hanihara, T., Scott, G. R., Irish, J. D., Reyes-Centeno, H., Ghirotto, S., & Harvati, K. (2023). Inferring human neutral genetic variation from craniodental phenotypes. *PNAS Nexus*, *2*(7), pgad217. https://doi.org/10.1093/pnasnexus/pgad217

Rathmann, H., & Reyes-Centeno, H. (2020). Testing the utility of dental morphological trait combinations for inferring human neutral genetic variation. *Proceedings of the National Academy of Sciences*, *117*(20), 20. https://doi.org/10.1073/pnas.1914330117

Rohlf, F. J., & Corti, M. (2000). Use of two-block partial least-squares to study covariation in shape. *Systematic biology*, *49*(4), 740–753.

Rohlf, F. J., & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, *39*(1), 1.

Sakai, T., Sasaki, I., & Hanamura, H. (1967). A Morphological Study of Enamel-Dentin Border on the Japanese Dentition. *Journal of the Anthropological Society of Nippon*, *75*(5), 5. https://doi.org/10.1537/ase1911.75.207

Salazar-Ciudad, I., & Jernvall, J. (2002). A gene network model accounting for development and evolution of mammalian teeth. *Proceedings of the National Academy of Sciences*, *99*(12), 8116–8120.

Sarig, R., Fornai, C., Pokhojaev, A., May, H., Hans, M., Latimer, B., Barzilai, O., Quam, R., & Weber, G. W. (2021). The dental remains from the early upper Paleolithic of Manot cave, Israel. *Journal of human evolution*, *160*, 102648.

Sarkar, L., & Sharpe, P. T. (1999). Expression of Wnt signalling pathway genes during tooth development. *Mechanisms of development*, *85*(1–2), 197–200.

Schaefer, M., Black, S., & Scheuer, L. (2009). Chapter 2—The Dentition. In M. Schaefer, S. Black, & L. Scheuer (Hrsg.), *Juvenile Osteology* (S. 67–95). Academic Press. https://doi.org/10.1016/B978-0-12-374635-1.00002-3

Scott, G. R. (1980). Population variation of Carabelli's trait. *Human biology*, 63–78.

Scott, G. R., & Irish, J. D. (2017). *Human tooth crown and root morphology*. Cambridge University Press.

Scott, G. R., & Pilloud, M. A. (2018). Dental morphology. *Biological anthropology of the human skeleton*, 257–292.

Scott, G. R., & Turner, C. G. (1997). *Anthropology of modern human teeth*. Cambridge University Press Cambridge.

Sehic, A., Tulek, A., Khuu, C., Nirvani, M., Sand, L. P., & Utheim, T. P. (2017). Regulatory roles of microRNAs in human dental tissues. *Gene*, *596*, 9–18. https://doi.org/10.1016/j.gene.2016.10.009

Tafforeau, P., & Smith, T. M. (2008). Nondestructive imaging of hominoid dental microstructure using phase contrast X-ray synchrotron microtomography. *Journal of human evolution*, *54*(2), 272–278.

Thesleff, I. (2003). Epithelial-mesenchymal signalling regulating tooth morphogenesis. *Journal of cell science*, *116*(9), 1647–1648.

Thesleff, I., & Tummers, M. (2008). *Tooth organogenesis and regeneration* (20614625). Harvard Stem Cell Institute, Cambridge (MA); PubMed. http://europepmc.org/abstract/MED/20614625

Tucker, A., & Sharpe, P. (1999). Molecular genetics of tooth morphogenesis and patterning: The right shape in the right place. *Journal of dental research*, *78*(4), 826–834.

Weber, G. W. (2015). Virtual anthropology. *American journal of physical anthropology*, *156*, 22–42.

Weber, G. W., & Bookstein, F. L. (2011). *Virtual anthropology: A guide to a new interdisciplinary field*.

Weber, G. W., Fornai, C., Gopher, A., Barkai, R., Sarig, R., & Hershkovitz, I. (2016). The Qesem Cave hominin material (part 1): A morphometric analysis of the mandibular premolars and molar. *State of the art of the multidisciplinary research at the Middle Pleistocene Qesem Cave, Israel, 2015*, *398*, 159–174. https://doi.org/10.1016/j.quaint.2015.10.027

Zanolli, C., Martinón-Torres, M., Bernardini, F., Boschian, G., Coppa, A., Dreossi, D., Mancini, L., Martínez de Pinillos, M., Martín-Francés, L., Bermúdez de Castro, J. M., Tozzi, C., Tuniz, C., & Macchiarelli, R. (2018). The Middle Pleistocene (MIS 12) human dental remains from Fontana Ranuccio (Latium) and Visogliano (Friuli-Venezia Giulia), Italy. A comparative high resolution endostructural assessment. *PLOS ONE*, *13*(10), e0189773. https://doi.org/10.1371/journal.pone.0189773