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RESEARCH ARTICLE

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Variation of 3D outer and inner crown morphology in modern human mandibular premolars

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Abstract

Objectives: This study explores the outer and inner crown of lower third and fourth premolars (P_3, P_4) by analyzing the morphological variation among diverse modern human groups.

Materials and Methods: We studied three-dimensional models of the outer enamel surface and the enamel–dentine junction (EDJ) from μCT datasets of 77 recent humans using both an assessment of seven nonmetric traits and a standard geometric morphometric (GM) analysis. For the latter, the dental crown was represented by four landmarks (dentine horns and fossae), 20 semilandmarks along the EDJ marginal ridge, and pseudolandmarks along the crown and cervical outlines.

Results: Certain discrete traits showed significantly different regional frequencies and sexual dimorphism. The GM analyses of both P_3s and P_4s showed extensive overlap in shape variation of the various populations (classification accuracy 15–69%). The first principal components explained about 40% of shape variance with a correlation between 0.59 and 0.87 of the features of P_3s and P_4s. Shape covariation between P_3s and P_4s expressed concordance of high and narrow or low and broad crowns.

Conclusions: Due to marked intragroup and intergroup variation in GM analyses of lower premolars, discrete traits such as the number of lingual cusps and mesiolingual groove expression provide better geographic separation of modern human populations. The greater variability of the lingual region suggests a dominance of functional constraints over geographic provenience or sex. Additional information about functionally relevant aspects of the crown surface and odontogenetic data are needed to unravel the factors underlying dental morphology in modern humans.

KEYWORDS
enamel–dentine junction, geometric morphometrics, occlusion, outline, teeth

1 | INTRODUCTION

Dental anthropological studies have investigated lower premolars in terms of general morphology (Hillson, 1996; Irish & Scott, 2016; Kraus, Jordan, & Abrams, 1969; Nelson & Ash, 2010; Scott & Irish, 2017; Scott, Turner, Townsend, & Martinón-Torres, 2018), nonmetric traits (Kraus & Furr, 1953; Ludwig, 1957; Sakai, 1967; Scott & Irish, 2017; Wood & Green, 1969), metrics (Bermúdez de Castro & Nicolás, 1996; Wood & Uytterschaut, 1987), and biomechanics (Benazzi, Grosse, Gruppioni, Weber, & Kullmer, 2014). This information has also been used for the assessment of hominin taxonomy (Bailey, 2002; Bailey & Hublin, 2013; Bailey & Lynch, 2005; Bermúdez de Castro & Nicolás, 1996; Gómez-Robles et al., 2008; Martinón-Torres et al., 2006; Martinón-Torres & Bermúdez de Castro, 2016; Pan et al., 2016; Weber et al., 2016; Wood & Uytterschaut, 1987).

Standardized systems for the description of premolar nonmetric traits have been established (Alt, 1997; Alt, Rösing, & Teschler-Nicola, 1998; Hillson, 1996; Kraus et al., 1969; Kraus & Furr, 1953; Lease, 2016; Ludwig, 1957; Nelson & Ash, 2010; Scott & Irish, 2017; Scott & Turner, 1997; Turner, Nichol, & Scott, 1991). These systems revealed that relative to other tooth classes, lower premolars show a high morphological variability of nonmetric traits. In particular, mandibular third premolars (P_3) were found to exhibit a large variety of features that are difficult to score because of their extensive morphological range (e.g., cusp number, ridges, and grooves pattern). Kraus et al. (1969) even considered the P_3 as one of the most variable teeth in the entire dentition. From a functional standpoint, premolars occupy an intermediate position between the anterior and posterior dentition. As such, the P_3 resembles in some aspects a canine, while the lower fourth premolar (P_4) possesses some characteristics of a small molar (Kraus et al., 1969; Nelson & Ash, 2010). Mandibular premolar heterogeneity was also described among different modern human populations. The Arizona State University Dental Anthropology System (ASUDAS; Turner et al., 1991) has been employed in various studies to assess population affinities based on dental phenotype (Irish & Scott, 2016; Scott et al., 2018; Scott & Irish, 2017; Scott & Turner, 1997). Lower premolar crown variation was described in terms of lingual cusp number and the presence of odontomes. Population differences were only significant for odontomes, a generally rare trait in humans (Scott & Irish, 2017; Scott & Turner, 1997). Other studies explored population differences in the number of lingual cusps and fissure patterns in the P_4 (Bailey & Hublin, 2013; Ludwig, 1957) and expression of the central ridge in the P_3 (Hanihara, 2008).

Metric variables such as buccolingual and mesiodistal crown diameters, crown height, and derived indices have been used to characterize premolar dimensions. In spite of methodological heterogeneity, premolar size differences were observed among various human populations (Ashar et al., 2012; Hanihara & Ishida, 2005; Harris & Rathbun, 1991; Hillson, 1996; Pilloud, Hefner, Hanihara, & Hayashi, 2014; Rathmann et al., 2017). The largest teeth were found among Australasians (Australia, Melanesia, and Micronesia), followed by Africans and Native Americans, while continental Asians and Europeans possessed the smallest teeth. Brace, Rosenberg, and Hunt (1987) recorded a secular trend of dental size reduction in modern humans during the Holocene.

Most of the studies focusing on mandibular premolars relied on the outer enamel surface (OES). The internal structures of teeth, such as the pulp cavity or the enamel–dentine junction (EDJ), are usually more difficult to access. However, a major advantage of using the EDJ is that it is less exposed to wear and damage than the OES. Importantly, the EDJ is the primary developmental structure of the dental crown. Thus, the EDJ is less variable than the OES, even though they show a high morphological concordance (Bailey, Skinner, & Hublin, 2011; Fornai, Bookstein, & Weber, 2015; Guy, Lazzari, Gilissen, & Thiery, 2015; Morita, 2016; Morita et al., 2014; Olejniczak et al., 2007; Skinner et al., 2010; Skinner, Wood, et al., 2008). Nowadays, nondestructive imaging techniques such as μCT can be used to virtually expose the dentine (see Braga, 2016 for a comprehensive review), avoiding physical removal of the enamel cap (Sakai, 1967). Consequently, the number of studies analyzing the internal three-dimensional (3D) morphology of teeth has increased rapidly in recent years (Bailey et al., 2011; Fornai et al., 2016; Fornai et al., 2015; Hershkovitz et al., 2018; Macchiarelli, Bayle, Bondioli, Mazurier, & Zanolli, 2013; Ortiz, Bailey, Hublin, & Skinner, 2017; Pan et al., 2016; Skinner, Gunz, Wood, & Hublin, 2008; Skinner, Wood, et al., 2008; Skinner, Wood, & Hublin, 2009; Weber et al., 2016; Zanolli et al., 2018).

Martinón-Torres et al. (2006), Gómez-Robles et al. (2008), Pan et al. (2016), and Weber et al. (2016) used geometric morphometric (GM) methods to investigate the discriminant power of lower premolar morphology in hominin taxonomy. Although these studies included the 2D or 3D shape and size of recent human premolars, they did not systematically analyze variation among geographically diverse modern human populations. Thus far, GM methods have only been applied in studies of dental morphological variability in modern human molars (Morita et al., 2014; Polychronis, Christou, Mavragani, & Halazonetis, 2013). Moreover, 3D shape and size variation of the EDJ in modern human lower premolars have never been explored. Similarly, a standardized system for the description of nonmetric traits suitable for both EDJ and OES—and applicable to both lower premolars—has yet to be established.

Our study investigates mandibular premolars using both quantitative and qualitative approaches. Our main goals are (a) to assess morphological variation within and between geographically diverse modern human groups from five continents using GM, (b) to identify the main trends of shape variation in P_3s and P_4s, and to study their covariation, (c) to investigate size and to explore allometry, (d) to develop a catalog of discrete traits that can be applied to both EDJ and OES of both lower premolars, so as to evaluate their congruence as well as population differences, and (e) to compare the GM approach with one based on discrete traits and test whether they yield comparable results.

2 | MATERIALS AND METHODS

2.1 | Study sample

Our sample included P_3s and P_4s from 77 recent modern humans from diverse geographic regions and with different subsistence patterns (hunter/gatherers, nomads, agriculturalists, and post-industrial-revolution; see Table 1). The specimens were from Africa (*n* = 19, including 12 Khoesan and seven other Sub-Saharans), Southeast Asia (*n* = 13, including six Papuans, six Indonesians, and one Chinese), the Middle East (*n* = 5, Bedouins), America (*n* = 16, including one Native North American, 10 Native South Americans and five Tierra del Fuegians), and Europe (*n* = 24, of which eight were Avars—8th century warrior nomads originally migrated from Asia— and 16 were modern

TABLE 1 List of materials

Institution	Catalog number	Origin	Region	Age (years)	Sex	Analyses P_3	W	Analyses P_4	W
UNIVIE[a]	S103	Khoesan	Kuruman District	12–15	M	✓	2	CER, CRO, DT	2
UNIVIE	S111	Khoesan	Kuruman District	20–30	M	✓	1	✓	1
UNIVIE	S121	Khoesan	Kuruman District	25–30	M	✓	2	✓	2
UNIVIE	S126	Khoesan	Middledrift	Adult	–	✓	3	✓	3
UNIVIE	S16	Khoesan	Nooitegedagt	9–13	F	✓	2	✓	2
UNIVIE	S23	Khoesan	Blinkfontain	15–20	F	✓	2	✓	2
UNIVIE	S29	Khoesan	Nooitegedagt	30–40	M	✓	3	✓	2
UNIVIE	S4	Khoesan	Groot Kibi	30–40	F	✓	3	✓	3
UNIVIE	S46	Khoesan	Kalahari	14–18	F	✓	2	✓	2
UNIVIE	S5	Khoesan	Groot Kibi	30–40	F	✓	2	✓	2
UNIVIE	S68	Khoesan	Gordonia District	25–30	M	CER, CRO, DT	3	✓	2
UNIVIE	S97	Khoesan	Kuruman District	12–15	F	CER, CRO, DT	3	✓	2
NhM, Narrenturm[b]	1,291–122.421/1464	Sub-Saharan	Congo	Adult	M	✓	2	✓	2
NhM, Narrenturm	MN735	Sub-Saharan	Africa	Adult	–	✓	3	✓	3
UNIVIE	S138	Sub-Saharan	Ramah	Adult	–	✓	2	✓	2
UNIVIE	S81	Sub-Saharan	Bameda	Adult	–	CER, CRO, DT	2	✓	2
UNIVIE	S85	Sub-Saharan	Cameroon	Adult	–	✓	2	EDJ	2
UNIVIE	S86	Sub-Saharan	Cameroon	Adult	–	N/A	–	✓	2
UNIVIE	S87	Sub-Saharan	French Guinee	Adult	–	✓	2	✓	2
UNIVIE	CS428	Avar	Austria	16–18	M	✓	3	✓	3
UNIVIE	CS495	Avar	Austria	7–8	–	✓	1	✓	1
UNIVIE	CS498	Avar	Austria	25–30	F	✓	2	✓	2
UNIVIE	CS502	Avar	Austria	13–15	–	✓	2	✓	2
UNIVIE	CS541	Avar	Austria	19–30	F	✓	3	✓	2
UNIVIE	CS569	Avar	Austria	16–18	M	✓	2	✓	2
UNIVIE	CS582	Avar	Austria	19–25	F	✓	2	✓	2
UNIVIE	CS654	Avar	Austria	3–5	–	✓	1	✓	1
NhM, Narrenturm	19,710	Central European	–	20	–	✓	2	✓	2
NhM, Narrenturm	ID_120_074_711	Central European	Czech Republic	6	M	✓	2	N/A	–
CACB[c]	ID_120_120_997	Central European	Europe	7	F	✓	2	N/A	–
CACB	ID_122_032_749	Central European	Europe	17	F	CER, CRO, DT	2	✓	2
NhM, Narrenturm	ID_122_199_961	Central European	Austria	20	M	✓	2	✓	2
NhM, Narrenturm	ID_122_510_1554	Central European	Italy	22	M	CER, CRO, DT	2	✓	2
NhM, Narrenturm	ID_122_511_1555	Central European	Italy	Adult	F	CER, CRO, DT	2	✓	2
NhM, Narrenturm	ID_125_011_1072	Central European	Greece	Adult	M	✓	2	✓	2
NhM, Narrenturm	ID_125_028_1089	Central European	Europe	10	F	✓	2	N/A	–
NhM, Narrenturm	ID_125_213_1015	Central European	Germany	46	F	✓	3	✓	2
NhM, Narrenturm	ID_126_804_1171	Central European	Czech Republic	29	M	✓	2	✓	2
NhM, Narrenturm	ID_127_622_1200	Central European	Czech Republic	24	M	CER, CRO, DT	2	CER, CRO, DT	2
CACB	ID-120-080-717	Central European	–	10	M	N/A	–	✓	1
NhM, Narrenturm	ID-120-123/1043	Central European	Austria	10	M	✓	1	✓	2
NhM, Narrenturm	ID-125-415/1124	Central European	Austria	6	M	✓	1	✓	1
NhM, Narrenturm	ID-300-510/578	Central European	–	11	F	✓	1	✓	1
UNIVIE	CN220	Papuan	Morobe	Adult	M	✓	2	N/A	–
UNIVIE	CN230	Papuan	Siar	Adult	M	✓	3	✓	2
UNIVIE	CN232	Papuan	Siar	Adult	M	✓	2	✓	2

(Continues)

TABLE 1 (Continued)

Institution	Catalog number	Origin	Region	Age (years)	Sex	Analyses P_3	W	Analyses P_4	W
UNIVIE	CN236	Papuan	Siar	Mature	M	✓	2	✓	2
UNIVIE	CN264	Papuan	East New Britain	30	M	✓	3	N/A	–
UNIVIE	CN5	Papuan	Madang	Adult	M	✓	2	✓	2
NhM[d]	1,365	South East Asia	Java	Adult	F	✓	2	✓	2
NhM	1,368	South East Asia	Celebes	Adult	F	✓	2	✓	2
NhM	1,370	South East Asia	Celebes	Adult	F	N/A	–	✓	2
NhM	2,583	South East Asia	South China	Adult	F	✓	2	✓	2
NhM, Narrenturm	1,340–122.335/1376	South East Asia	Java	36	M	✓	2	✓	2
NhM, Narrenturm	1,348–122.342/1383	South East Asia	Java	28	M	✓	2	✓	2
NhM, Narrenturm	1,377–122.369/1412	South East Asia	Sulawesi	Adult	M	✓	2	✓	2
NhM	793	American	South America	Adult	M	✓	2	✓	3
NhM	806	American	Chile	Adult	M	N/A	–	✓	2
NhM	964	American	USA	Juvenil	F	✓	2	✓	2
NhM	1,453	American	Chile	Adult	M	✓	3	N/A	–
NhM	1,525	American	Peru	Adult	–	N/A	–	✓	2
NhM	3,537	American	Costa Rica	Adult	F	N/A	–	✓	3
NhM	5,041	American	Brazil	Adult	–	✓	3	✓	2
NhM	5,385	American	Argentina	Adult	–	✓	3	✓	3
NhM	5,443	American	Brazil	Juvenil	F	✓	2	✓	2
NhM	6,321	American	Brazil	Adult	–	✓	3	✓	3
NhM	15,353	American	Argentina	Adult	M	✓	2	✓	2
NhM	6,030	Tierra del Fuego	Tierra del Fuego	Adult	M	✓	3	✓	2
NhM	6,033	Tierra del Fuego	Tierra del Fuego	Adult	–	✓	2	✓	2
NhM	6,034	Tierra del Fuego	Tierra del Fuego	Adult	F	✓	3	✓	3
NhM	6,035	Tierra del Fuego	Tierra del Fuego	Adult	–	✓	3	✓	3
NhM	6,038	Tierra del Fuego	Tierra del Fuego	Adult	M	✓	3	✓	3
TAU[e]	BLZ_004	Bedouin	Israel	Adult	–	✓	1	✓	1
TAU	BLZ_014	Bedouin	Israel	Adult	–	✓	3	✓	3
TAU	BLZ_026	Bedouin	Israel	Adult	–	✓	2	✓	2
TAU	BLZ_037	Bedouin	Israel	Adult	–	✓	2	✓	2
TAU	EAR_H298	Bedouin	Israel	Adult	–	✓	3	✓	3

Abbreviations: N/A, not available; W, Wear stage after Molnar (1971); ✓, all analyses.
Analyses of P_3 and P_4 were performed separately for enamel–dentin junction (EDJ), cervical outline (CER), crown outline (CRO), and discrete traits (DT).
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Central Europeans). Information about the collections can be found in Bondy-Horowitz (1930), Abel (1933), Kiernberger (1955), and Pacher (1961). The grouping of our populations is in line with the major subdivisions of humanity in Scott et al. (2018). We created an additional group for Bedouins owing to the striking divergence of their dental traits from geographically adjacent populations. Although they are West Asians living in the Levant, they are genetically more closely related to Europeans based on the migration patterns of modern humans during the Early and Middle Holocene (Skoglund et al., 2012). We are reporting results on both continental and population level. Using continental groups allowed us to achieve larger sample sizes. Breaking down the sample into different populations allowed us to capture signals that may be obscured by continental pooling. Both P_3s and P_4s were represented in 65 of the 77 individuals. Thus, we examined 142 teeth in total.

These dental specimens did not exceed Molnar's (1971) wear Stage 3 "exposing small dentine patches". This guaranteed that, in case the EDJ was already affected by wear, the horn tips could be confidently reconstructed using virtual approaches. Carious teeth, independent of their state of dental treatment, were excluded. While this sample may appear small in comparison to traditional dental studies based on the OES, obtaining suitable specimens for μCT is difficult. Ancient tooth collections are generally heavily worn and thus problematic to quantify by morphometric methods. Juvenile teeth that have not yet reached the oral cavity would be ideal but often are severely cracked or broken, probably owing to their lower degree of mineralization (Dean & Scandrett, 1995; Sandholzer, Baron, Heimel, & Metscher, 2014). In recent collections, the presence of caries and dental treatments made these teeth unusable for our purposes.

2.2 | μCT and surface reconstruction

All teeth were scanned at the Vienna μCT Lab, Austria, using a custom-built VISCOM X8060 scanner. Scan parameters were adjusted for the individual scans: voxel size 25–50 μm, 110–140 kV, 280–410 mA, 1,400–2000 ms, and 0.75 mm copper filter. The image stacks were imported in Amira 6.7 (www.fei.com) and virtually segmented to isolate the dental crown and separate the dentine from the enamel. We applied a semiautomatic segmentation based on the half-maximum-height value protocol proposed by Spoor, Zonneveld, and Macho (1993). Afterwards, we generated 3D virtual surface models for both the OES and the EDJ.

We analyzed lower premolars of the left side. When these were not usable, we virtually mirrored the right premolar. As directional asymmetry in human premolar form has not been reported (Frederick & Gallup, 2007; Hershkovitz, Livshits, Moskona, Arensburg, & Kobyliansky, 1993; Kegley & Hemingway, 2007; Kieser & Becker, 1989; Mayhall & Saunders, 1986; Moskona, Vainder, Hershkovitz, & Kobyliansky, 1996), this procedure ensures a larger sample size without introducing a bias.

2.3 | Landmark sampling

For the EDJ, we used both landmarks and curve semilandmarks along the marginal ridge following the protocol proposed by Weber et al. (2016). Four landmarks were placed on the horn tips and on the deepest points of the mesial and distal fossae, and 20 curve semilandmarks were identified on the marginal ridge (Figure 1; for intraobserver and interobserver error assessment see Supporting Information Figure S1). Sliding of the semilandmarks was carried out in the EVAN Toolbox (ET; www.evan-society.org), which uses the bending energy technique (Gunz & Mitteroecker, 2013; Gunz, Mitteroecker, & Bookstein, 2005; Perez, Bernal, & Gonzalez, 2006). Eighteen teeth with slightly worn buccal horn tips were virtually reconstructed by extrapolating the curvature of the preserved marginal ridge (Supporting Information Figure S2). The reconstructed portions of the dentine horns had an average height of 0.53 mm (*SD* ±0.24 mm).

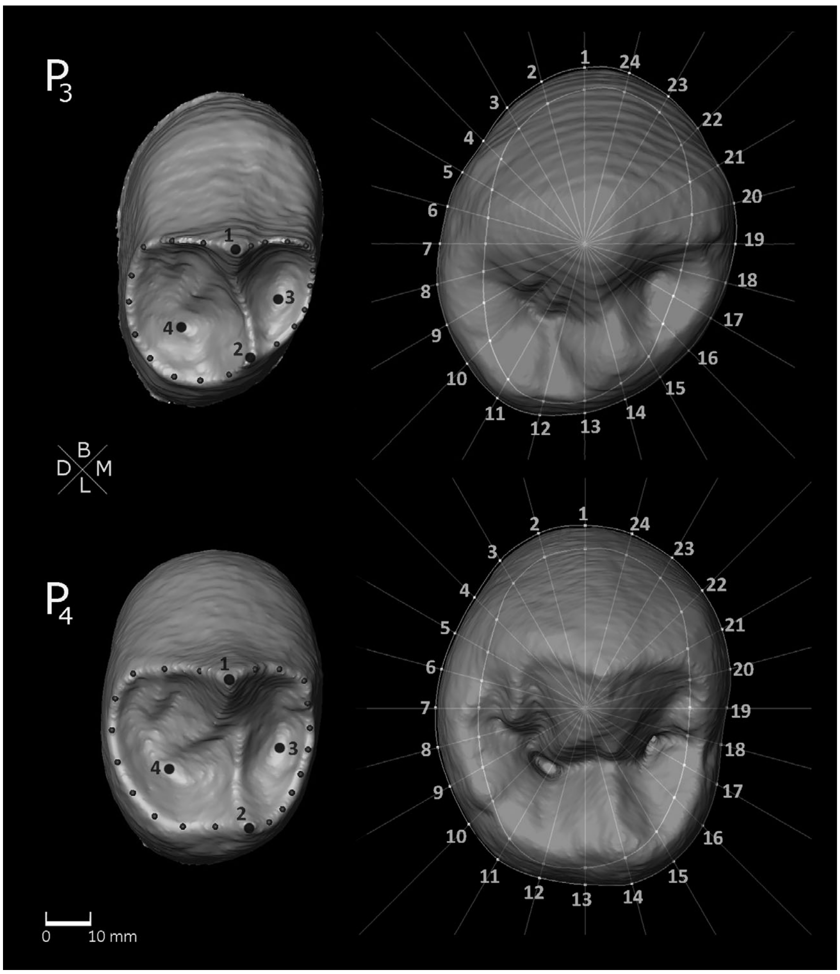

FIGURE 1 P_3 and P_4 occlusal view of the EDJ (left) including real landmarks (1—buccal horn tip; 2—lingual horn tip; 3—deepest point of the mesial fossa; 4—deepest point of the distal fossa) and curve semilandmarks, and OES (right) including pseudolandmarks

Crown and cervical outlines were sampled according to Benazzi et al. (2012). A best-fit plane of the cervical margin was computed and the 3D models representing both the EDJ and the OES were reoriented so that the cervical plane was parallel to the *x*–*y* plane of the virtual environment. The models were rotated until the buccal aspect of the marginal ridge was parallel to the *x*-axis. The cervical outline was the contour of the tooth surface at the cervical plane. The crown outline was the silhouette of the crown as seen in occlusal view in the oriented crown. Interproximal contact facets were corrected manually with a spline curve. The outlines were then intersected by 24 equiangularly spaced radii originating from their centroids. Pseudolandmarks were collected at the intersections of the radii with each outline (Figure 1).

In 10 teeth, the state of preservation or the degree of wear made it impossible to collect all variables. Nevertheless, these teeth were included in some of the analyses depending on the preserved features (as specified in Table 1).

2.4 | Geometric morphometric analyses

The landmark coordinates of the EDJ occlusal aspect, cervical outline, and crown outline were treated separately. In addition, the EDJ and cervical outline landmark configurations were combined (hereafter, "combined analysis"), thus representing also the relative crown height. Size, position, and orientation of the landmark configurations were standardized by a generalized Procrustes analysis. Size was taken into account by augmenting the shape coordinates with the natural logarithm of centroid size (lnCS). This converts shape space into form space and is the standard size measure in studies of landmark data (Mitteroecker & Bookstein, 2009; Mitteroecker & Gunz, 2009; Mitteroecker, Gunz, Bernhard, Schaefer, & Bookstein, 2004; Mitteroecker, Gunz, Windhager, & Schaefer, 2013). The Procrustes shape coordinates were analyzed by a principal component analysis (PCA). For a comprehensive overview of GM techniques, see Slice (2005) and Bookstein (2014, 2018). A general review of GM in dental anthropology can be found in Rizk, Grieco, Holmes, and Hlusko (2013).

We used canonical variates analyses (CVA) on the scores of the first seven principal components (PCs; explaining ~90% of the total variance) to check for the accuracy of the group classification implied by the PCs. Further multivariate statistical analyses on landmark data explored shape covariation between P_3s and P_4s by examining the latent variables produced by partial least squares (PLS; Bookstein, 2018). A multivariate regression was performed to analyze the effect of allometry. This was done by regressing the EDJ shape variables against lnCS. Additionally, lnCS was compared between groups by the distribution-free Kruskal–Wallis test. Sex differences in lnCS were investigated with the Mann–Whitney *U* test. Correlation between P_3 and P_4 lnCS was calculated using Pearson's correlation coefficient. The difference in lnCS between P_3s and P_4s was tested via Wilcoxon signed rank test. The Bedouin subsample was excluded from some of the statistical analyses due to its small sample size.

2.5 | Qualitative description

To describe the EDJ of lower premolars we assessed the following seven traits: number of occlusal ridges, manifestation of the transverse ridge, extension of the transverse ridge, number of lingual cusps, relative position of the main lingual cusp, independence of the main lingual cusp, and marginal ridge. These traits are described below in detail (see also Figure 2, as well as Supporting Information Figure S3). This list was based on previous research on the OES (Hillson, 1996; Kraus et al., 1969; Kraus & Furr, 1953; Ludwig, 1957; Nelson & Ash, 2010; Sakai, 1967; Scott & Irish, 2017; Scott & Turner, 1997; Turner et al., 1991). The features scored are present on both the EDJ and the OES. Thus, our catalog is conceived for external as well as internal crown aspects and is usable for both mandibular premolar types. We were able to assess these qualitative traits on 141 EDJ and 117 OES models. Since the literature is not consistent regarding premolar cusp nomenclature (Butler, 2000; Kraus et al., 1969; Scott & Turner, 1997; Wood & Uytterschaut, 1987), in this text, we refer to the dentine horns and other structures based on their anatomical position (i.e., buccal, lingual, mesial, distal, distolingual, and mesiolingual).

1. Number of occlusal ridges

The surface of the EDJ always expresses one main ridge, referred to as the transverse or median ridge. The transverse ridge runs between the buccal and the lingual horn tips. Accessory ridges originating independently from the buccal marginal ridge and running roughly parallel to the transverse ridge may occur.

[1A] Transverse ridge only.

[1B] Transverse ridge plus distal ridge.

[1C] Transverse ridge plus mesial ridge.

[1D] Transverse ridge plus mesial and distal ridges.

2. Manifestation of the transverse ridge

The transverse ridge may appear as one single ridge from the buccal to the lingual horn tips or can be bifurcated. Slight splitting at the buccal or lingual horn tips should also be regarded as a bifurcation. In contrast to parallel independent accessory ridges (see Trait 1), a bifurcated transverse ridge always originates directly at the buccal or lingual horn tip.

[2A] Single transverse ridge.

[2B] Bifurcated transverse ridge.

3. Extension of the transverse ridge

The transverse ridge may be continuous from the buccal to the lingual horn tip or may be interrupted by a central groove (the latter condition is referred to as "two triangular ridges" by Kraus et al., 1969).

[3A] Continuous transverse ridge visible throughout its whole course from the buccal to the lingual cusp. The mesial and distal fossae are not joined and form two distinct depressions.

[3B] Ridge interrupted by a central groove that connects the mesial and distal fossae.

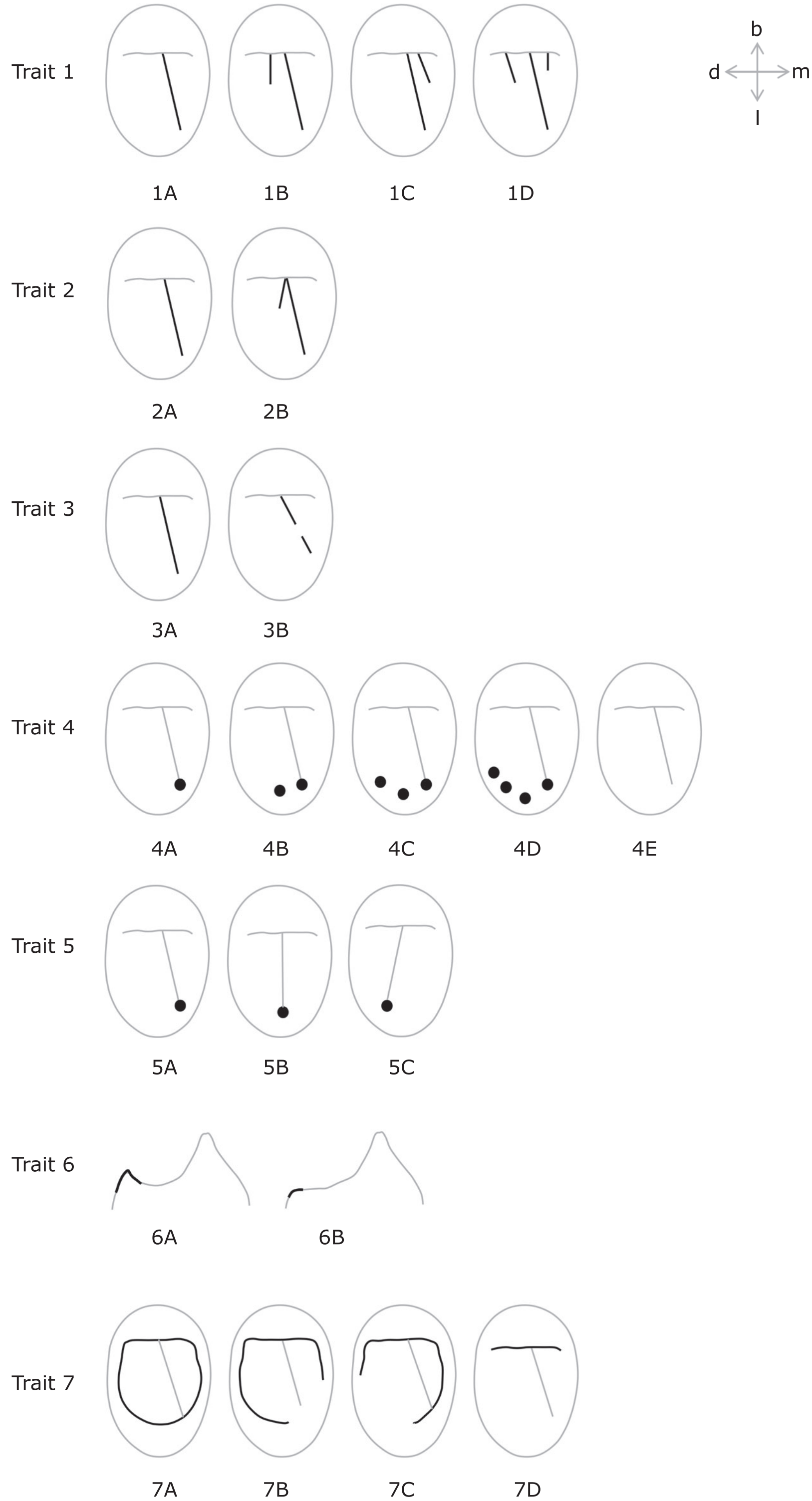

FIGURE 2 Illustrations of features

4. Number of lingual cusps

On the EDJ, the lingual aspect might express a variable number of dentine horns, while the buccal aspect expresses always one major horn tip. If a pointed lingual apex cannot be found, an elevation between the transverse ridge and the lingual marginal ridge can be considered. If neither can be seen, the cusp is absent.

[4A] One lingual cusp (i.e., two-cusped premolar).

[4B] Two lingual cusps (i.e., three-cusped premolar).

[4C] Three lingual cusps (i.e., four-cusped premolar).

[4D] Four lingual cusps (i.e., five-cusped premolar).

[4E] Absent (i.e., single-cusped premolar).

5. Relative position of the main lingual cusp

The relative position of the main lingual dentine horn varies with respect to the buccolingual axis of the tooth (namely, the axis perpendicular to the buccal cusp ridge). The relative position of the lingual cusp can therefore be:

[5A] mesial.

[5B] medial.

[5C] distal.

6. Independence of the main lingual cusp

The horn tip of the lingual cusp can be independent or fused with the transverse ridge.

[6A] Independent lingual cusp.

[6B] Main lingual cusp fused with the transverse ridge.

7. Marginal ridge

The EDJ marginal ridge is formed by four segments running from the buccal horn tip to the mesial edge (mesiobuccal margin); from the lingual horn tip to the mesial edge (mesiolingual margin); from the buccal horn tip to the distal edge (distobuccal margin); from the lingual horn tip to the distal edge (distolingual margin). Sometimes, all segments are clearly visible; otherwise, some of the segments can be weakly expressed or completely smooth. We distinguish the following cases.

[7A] All four segments well expressed along their entire lengths.

[7B] Mesiolingual margin either missing or fading and difficult to see.

[7C] Distolingual margin either missing or fading and difficult to see.

[7D] More than one segment missing or difficult to see.

3 | RESULTS

3.1 | GM analyses

The dominant finding in all our geometric morphometric analyses is the extensive overlap among human populations in both shape space and form space. No separation occurs among the major continental or population groups in any of the 16 PC analyses. As expected, size is the main determinant of variance along PC1 in form space, but the populations are indistinguishable. The variances explained by the first three principal components are given in Table 2 for both premolar types in shape space.

Figure 3a shows the results of the shape space analysis of the combined dataset (EDJ surface plus cervical outline) for P_3s. The plots for the other landmark configurations (i.e., cervical outline, crown outline, and EDJ only) and for all P_4 landmark configurations show similarly overlapping populations (combined P_4 plot see Supporting Information Figure S4). Shape variation as shown by the warps is discussed for the first two PCs for all analyses in the following two sections. This will highlight the general pattern of shape variation in mandibular premolars.

TABLE 2 Percentage of explained variance in shape space

Analysis	PC1	PC2	PC3
P_3 enamel dentine junction surface	33.5	18.7	9.4
P_3 cervical outline	47.0	25.5	12.0
P_3 crown outline	44.1	16.8	11.8
P_3 combined set	34.9	26.4	9.2
P_4 enamel dentine junction surface	33.7	17.0	9.5
P_4 cervical outline	45.5	29.5	11.0
P_4 crown outline	47.9	18.0	12.6
P_4 combined set	44.3	23.6	8.5

Abbreviation: PC, Principal component.

3.2 | P_3 shape variation

In the combined analysis, variation along PC1 accounted for narrow and high dentinal crowns versus broad and low ones. PC2 reflected the relative position of the lingual horn with respect to the buccal horn in the mesiodistal direction. Additionally, PC2 reflects the proportion of the distal fossa relative to the mesial fossa.

For the EDJ, variation along PC1 affected the fossae proportions and PC2 reflected the position of the lingual relative to the buccal horns.

The P_3 cervical outline was generally elliptical. Shape changes along PC1 were associated with a mesialward versus distalward position of the lingual aspect relative to the buccal ridge. Along PC2, P_3 cervical outline varied from more elliptical to bean-shaped.

P_3 crown outline was either circular or bean-shaped along PC1 and varied in the mesiodistal position of the lingual aspect along PC2.

3.3 | P_4 shape variation

Similarly to P_3s, the combined dataset showed that the dentinal crown of P_4s can be either relatively high and narrow or low and broad. P_4s also varied in terms of position of the lingual horn with respect to the buccal horn in the mesiodistal direction, and relative proportions of the mesial and distal fossae (Supporting Information Figure S4).

The EDJ shape changed along PC1 owing to the relative proportions of the mesial and distal fossae and horn height. A relatively smaller and distobuccally compressed mesial fossa was associated with a pronounced lingual horn tip. Conversely, a large and distobuccally expanded fossa was associated with a short lingual horn tip. Shape changes along PC2 reflected the distance between the deepest points of the fossae.

Shape variation along PC1 for the cervical outline expressed a mesiodistal shearing of the lingual aspect with respect to the buccal ridge. Along PC2, the cervical outline varied from circular to bean-shaped.

As in P_3s, the P_4 crown presented a round-to-bean-shaped outline, with varying mesiodistal position of the lingual aspect.

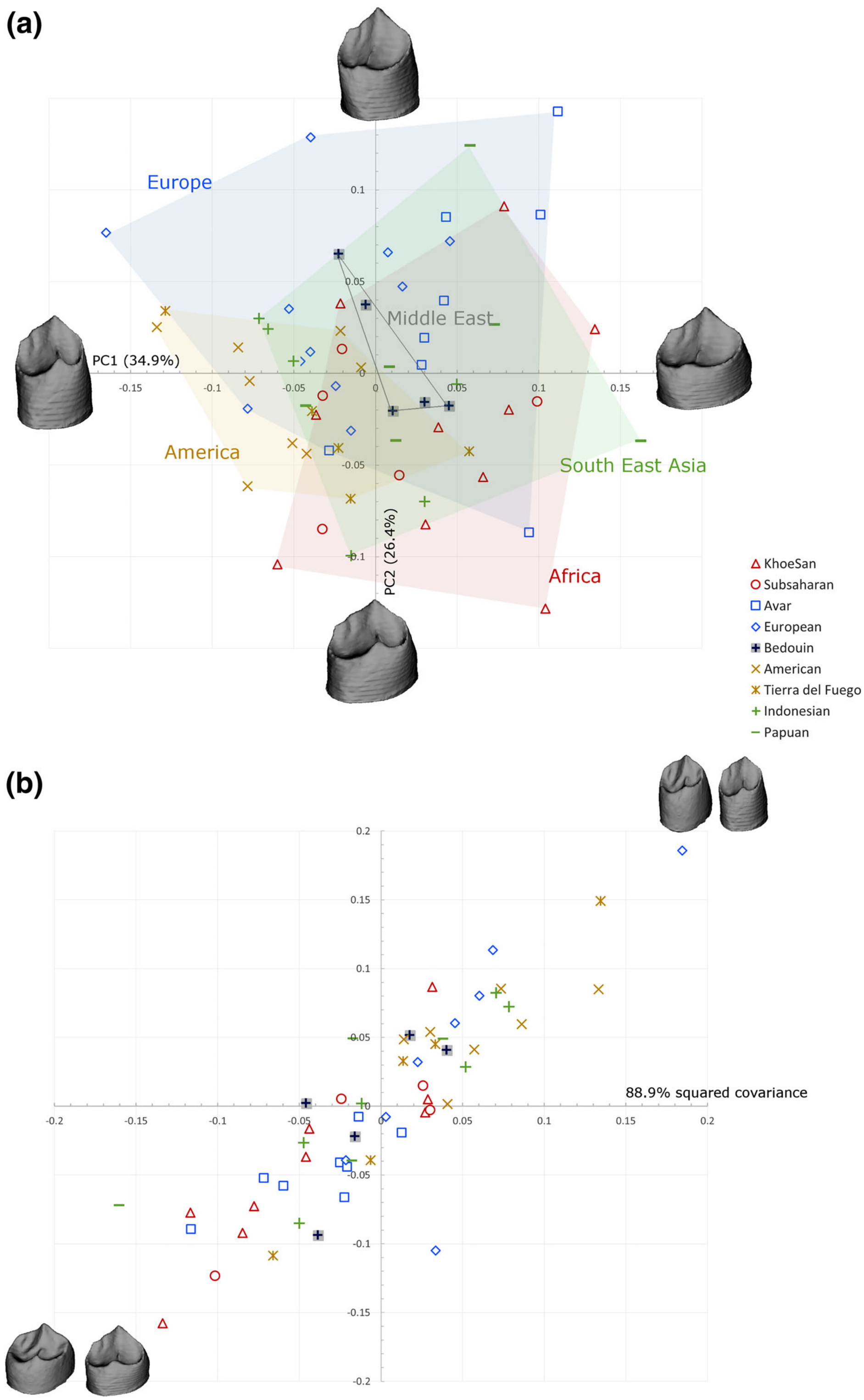

FIGURE 3 (a) PC1-2 plot for the combined dataset in P_3 and (b) partial least-squares plot for P_3 versus P_4 combined dataset including TPS warps (the warping shows the real shape variation at the extremities of the range of distribution)

3.4 | P_3–P_4 covariation

The 2B-PLS analyses showed that the correlation between the first pair of latent variables for P_3 and P_4 for the different traits ranged between 0.59 and 0.87 (Table 3). Pairwise correlation for the combined datasets (EDJ and cervical outline) of P_3 and P_4 showed the highest value (r_1 = 0.87).

The percentage of total squared covariance ranged from 62% to 90%. Again, there was no grouping of the specimens at continental or population level (combined dataset Figure 3b). In none of the PLS

TABLE 3 Partial least-squares results for P_3 and P_4

Correlation of singular warps	P_4 EDJ	P_4 CER	P_4 CRO	P_4 combined
P_3 EDJ	0.61			
P_3 CER		0.59		
P_3 CRO			0.60	
P_3 combined data				0.87
% of total squared covariance	**P_4 EDJ**	**P_4 CER**	**P_4 CRO**	**P_4 combined**
P_3 EDJ	62.3			
P_3 CER		81.9		
P_3 CRO			89.8	
P_3 combined data				88.9

Abbreviations: CER, cervical outline; CRO, crown outline; EDJ, enamel–dentin junction.

analyses were males separated from females (Supporting Information Figure S5). Thus, the observed shape variation did not seem to be related to geographic origin or to sex.

The expression of the EDJ marginal ridge also showed a high covariation between P_3 and P_4 (r_1 = 0.61 and 62.3% of the total squared covariance). This owed mainly to a correlated degree of mesiodistal relative expansion. Mesiodistally narrow EDJ marginal ridges tended to have shorter or absent lingual horns, whereas broad marginal ridges possessed well-expressed lingual horns.

A similar pattern of covariation was observed for the P_3 and P_4 cervical and crown outlines, which varied from elliptical to more circular (cervical outlines: r_1 = 0.59 and 81.9% of the total squared covariance; crown outlines: r_1 = 0.60 and 89.8% of the total squared covariance).

3.5 | Classification accuracy

CVA plots showed equally large overlaps for all GM datasets of both tooth types for all continental groups (note that the Middle East sample was omitted due to its small sample size). Confusion matrices showed low classification accuracies ranging between 15.4% and 69.2%. Considering all features in both P_3 and P_4, classification accuracy was highest for Southeast Asians (P_3 = 54.2%; P_4 = 47.7%), followed by Native Americans (P_3 = 48.1%; P_4 = 43.3%). Highest classification accuracy was achieved with the combined dataset for P_3s (53.2%) and with the cervical outline for P_4s (46.7%). Overall, classification rates for P_3 and P_4 are very similar (P_3 = 43.8%; P_4 = 42.4%). Detailed scores for each feature and each population are presented in Supporting Information Table S1.

3.6 | Size and allometry

Centroid size accounts for the size of the dental crown as represented by the landmarks in the various configurations used. Thus, centroid size is a 3D measure for the EDJ and the combined dataset. In the latter, height is included, and thus this analysis best reflects overall size of the dentinal crown.

The continental groups were significantly different ($p < .05$) for crown outlines of both premolars (Supporting Information Figure S6a). The same was true for the P_3 cervical outline. On the population level, none of the investigated features reached statistical significance (Supporting Information Table S2 and Figure S6b). Averaging all four investigated datasets, Europeans were 2.0% smaller, Native Americans 0.7% smaller, Bedouins 0.6% smaller, and Southeast Asians 0.2% larger than Africans (see *z*-scores in Supporting Information Table S3). The larger size of the Southeast Asian premolars was particularly driven by the Indonesians rather than the Papuans. We found no significant difference for lnCS of all investigated features between male and female P_3s and P_4s (Supporting Information Table S2). Similarly, no significant differences between P_3s and P_4s lnCS were found based on the EDJ and combined datasets. On the contrary, cervical and crown outlines yielded a significant result ($p < .001$). The most pronounced difference was observed in Bedouins, with P_4s being about 3% larger than P_3s (Supporting Information Table S3). The correlation between P_3s and P_4s lnCS was strong (r = 0.875) and highly significant ($p < .001$; see Figure 4).

Average size reduction for lnCS from the 8th century Avars to modern Central Europeans of the isolated features (EDJ, CER, and CRO) was about 2.0% in P_3s and 1.0% in P_4s. However, based on the combined dataset, that is including crown height, Avars were smaller than recent Europeans. The associated morphological shape changes showed that the dentinal crowns of Avars tended to be low and broad whereas those of modern Europeans were high and narrow (Supporting Information Figure S7).

The multivariate regression of the combined dataset on lnCS showed that only a low percentage of shape variance in this sample was explained by size (P_3s, 3.62%; P_4s, 8.16%). The observed size-related shape changes referred mainly to cusp height and relative proportions of mesial to distal fossae. Premolars with smaller mesiodistal dimensions had relatively larger mesial fossae with a medially placed lingual horn, whereas mesiodistally larger premolars showed an expanded distal fossa and a more mesially placed lingual horn.

3.7 | Nonmetric traits

The scores for the nonmetric traits are presented in Tables 4 and 5 for the entire sample and by continental groups. Overall, we found a high variability in P_3s. Thus, in P_3s several traits differed significantly between the groups, both at a continental level and at a population level ($p < .005$; Supporting Information Table S4). In P_4s, none of the trait frequency differences reached statistical significance among groups, neither at a continental nor at a population level.

In P_3s, accessory ridges occurred frequently, mainly distally. Native Americans had the highest rate (69%) of accessory distal ridges. In P_4s, a single transverse ridge was most common. In both premolars, about half of the sample showed a bifurcated transverse ridge, which in most cases ran continuously from the buccal to the

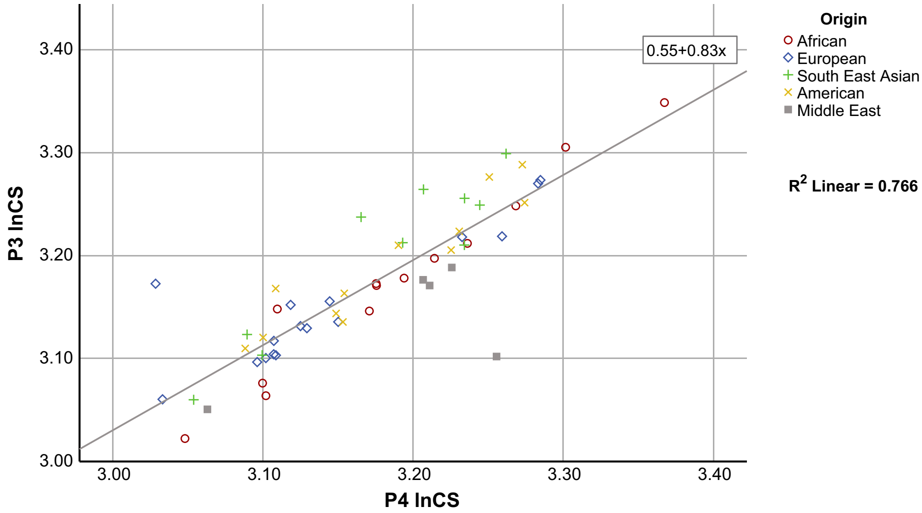

FIGURE 4 Covariation of P_3–P_4 of the natural logarithm of centroid size (lnCS)

TABLE 4 Highest prevalence for each trait

Trait	P_3—Expression		Prevalence (%)	P_4—Expression		Prevalence (%)
EDJ 1	B	Transverse ridge plus distal ridge	47.90	A	Transverse ridge only	57.10
EDJ 2	B	Bifurcated transverse ridge	54.90	A	Single transverse ridge	52.90
EDJ 3	A	Continuous transverse ridge	81.70	A	Continuous transverse ridge	74.30
EDJ 4	A	One lingual cusp	49.30	B	Two lingual cusps	50.00
EDJ 5	A	Mesial	69.00	A	Mesial	74.30
EDJ 6	A	Independent lingual cusp	59.20	A	Independent lingual cusp	97.10
EDJ 7	A	Complete marginal ridge	45.10	A	Complete marginal ridge	97.10
OES 1	B	Transverse ridge plus distal ridge	47.60	A	Transverse ridge only	54.20
OES 2	B	Bifurcated transverse ridge	58.70	B	Bifurcated transverse ridge	59.30
OES 3	A	Continuous transverse ridge	81.30	A	Continuous transverse ridge	71.20
OES 4	A	One lingual cusp	43.80	B	Two lingual cusps	59.30
OES 5	A	Mesial	62.50	A	Mesial	74.60
OES 6	A	Independent lingual cusp	71.90	A	Independent lingual cusp	100.00
OES 7	A	Complete marginal ridge	40.60	A	Complete marginal ridge	94.90

Abbreviations: EDJ, enamel–dentine junction; OES, outer enamel surface.

lingual cusp tips. The Bedouin sample, although small, showed a very high frequency of additional distal ridges and bifurcations.

The number of lingual cusps (OES)/dentine horns (EDJ) varied from zero to four. They were absent in 7% of P_3s but absent only in 2.8% of P_4s. Most Europeans and Native Americans P_3s expressed one lingual horn tip. In all continental groups, except Europeans, P_4s most commonly showed two lingual horns. The most common position of the main lingual cusp was mesial (which agrees with the EDJ analysis, as illustrated by PC2 in Figure 3a). The lingual horn tip was always well defined in P_4s, whereas it was often small and less discernible in P_3s.

In the P_4s, typically the marginal ridge was well defined in all continental groups. Conversely, in 45% of the P_3s, the marginal ridge was missing, at least in its mesial aspect. This manifestation of the marginal ridge correlated significantly ($p < .001$) with the occurrence of a mesiolingual groove on the OES, a P_3 characteristic described by Kraus et al. (1969), which was present in 42% of the specimens in our P_3 sample. The frequency of the mesiolingual groove significantly differed among the groups on both continental and population level (Supporting Information Table S4). This trait was very common in the European sample and especially in the Papuans and Bedouins, whereas it was less frequent in the American and African samples (Table 6). We detected no effect of sex in the expression of the mesiolingual groove. A single odontome was observed in a P_4 from Tierra del Fuego (TF_6035).

The number of lingual horns (trait 4) was the only trait that was sexually dimorphic. Both sexes equally expressed a single horn tip, while the presence of two horns was more likely to occur in males and three horns in females. This difference was significant in P_4s (EDJ: p = .011; OES: p = .041). Although not statistically significant, the same pattern was observed in P_3s (Figure 5).

TABLE 5 List of discrete traits scored for both P_3s and P_4s

	Africa (*n* = 18)					Europe (*n* = 23)					South East Asia (*n* = 12)					America (*n* = 13)					Middle East (*n* = 5)				
Trait EDJ P_3	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
(1) Number of ridges	38.9	50.0	0.0	11.1		47.8	39.1	0.0	13.0		58.3	33.3	0.0	8.3		7.7	69.2	0.0	23.1		20.0	60.0	0.0	20.0	
(2) Manifestation of ridge	44.4	55.6				56.5	43.5				50.0	50.0				30.8	69.2				20.0	80.0			
(3) Extension of ridge	77.8	22.2				73.9	26.1				91.7	8.3				92.3	7.7				80.0	20.0			
(4) Number of lingual cusps	33.3	33.3	33.3	0.0	0.0	60.9	26.1	8.7	0.0	4.3	41.7	25.0	16.7	0.0	16.7	61.5	23.1	7.7	7.7	0.0	40.0	20.0	0.0	0.0	40.0
(5) Position of lingual cusp	72.2	22.2	5.6			52.2	34.8	13.0			66.7	33.3	0.0			100.0	0.0	0.0			60.0	40.0	0.0		
(6) Independence of lingual cusp	77.8	22.2				69.6	30.4				41.7	58.3				46.2	53.8				20.0	80.0			
(7) Marginal ridge	38.9	16.7	27.8	16.7		43.5	17.4	13.0	26.1		33.3	33.3	0.0	33.3		76.9	0.0	15.4	0.0		20.0	80.0	0.0	0.0	

	Africa (*n* = 18)					Europe (*n* = 21)					South East Asia (*n* = 11)					America (*n* = 16)					Middle East (*n* = 5)				
Trait EDJ P_4	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
(1) Number of ridges	55.6	16.7	5.6	22.2		52.4	19.0	4.8	23.8		81.8	18.2	0.0	0.0		46.7	20.0	6.7	26.7		60.0	0.0	0.0	40.0	
(2) Manifestation of ridge	44.4	55.6				47.6	52.4				54.5	45.5				60.0	40.0				0.0	100.0			
(3) Extension of ridge	72.2	27.8				71.4	28.6				81.8	18.2				86.7	13.3				40.0	60.0			
(4) Number of lingual cusps	16.7	66.7	16.7	0.0	0.0	52.4	28.6	14.3	0.0	4.8	18.2	63.6	18.2	0.0	0.0	46.7	53.3	0.0	0.0	0.0	40.0	40.0	0.0	0.0	20.0
(5) Position of lingual cusp	77.8	16.7	5.6			66.7	33.3	0.0			81.8	18.2	18.2			73.3	26.7	0.0			80.0	20.0	0.0		
(6) Independence of lingual cusp	100.0	0.0				95.2	4.8				100.0	0.0				100.0	0.0				80.0	20.0			
(7) Marginal ridge	100.0	0.0	0.0	0.0		90.5	0.0	9.5	0.0		100.0	0.0	0.0	0.0		100.0	0.0	0.0	0.0		100.0	0.0	0.0	0.0	

Letters A, B, C, D, and E refer to the category of expression as detailed in the methods section. Numbers indicate percentage (%) of observed cases.
Abbreviation: EDJ, enamel–dentine junction.

TABLE 6 Mesiolingual groove prevalence (%) for the different populations

KhoeSan (*n* = 11)	Sub-Saharan (*n* = 7)	Avar (*n* = 8)	European (*n* = 15)	Papuan (*n* = 6)	Indonesian (*n* = 6)	American (*n* = 9)	Tierra del Fuego (*n* = 5)	Bedouin (*n* = 5)
27	14	50	60	100	33	11	0	80

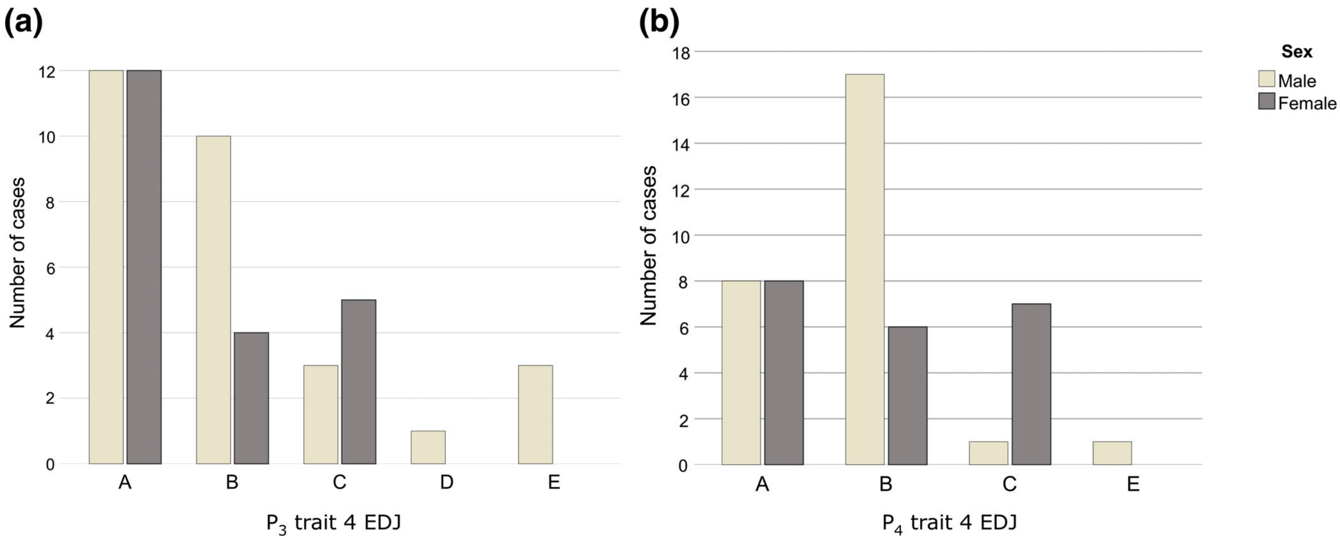

FIGURE 5 Sexual dimorphism in Trait 4. Note that two horns are more frequent in males and three horns in females

Of the seven traits investigated separately for EDJ and OES, four were positively correlated between P_3 and P_4 ($p < .005$; Supporting Information Table S4), namely the number of dentine horns (EDJ, trait 4), accessory occlusal ridges (OES, trait 1), and the expression of the marginal ridge (both EDJ and OES, trait 7). Correlation between trait expression on the EDJ and on the OES was positive for all homologous traits (between 0.595 and 0.919), with the exception of trait 4 in P_3s (0.460), and highly significant ($p < .001$) for both premolar types.

4 | DISCUSSION

This study provides extensive information on modern human mandibular premolar morphological variation, and combines and compares both qualitative and state-of-the-art quantitative approaches. We investigated the morphological variation of both the EDJ and OES in a geographically diverse sample of modern human lower premolars based on landmarks and semilandmarks as well as nonmetric traits. Our findings provide a basis for further comparative studies either within modern humans or in a paleoanthropological context.

Our study showed that the modern human populations we analyzed are too variable to be distinguishable based on the shape of the dentinal crown (classification accuracy of only 43%). Conversely, we found evidence that certain discrete traits discriminate better with respect to geographic origin and sex. Overall, discrete traits are less variable in P_4s than in P_3s, and P_4 cusps and ridges are usually better defined than in P_3. Our knowledge about the genetic background of dental trait expression is still limited. The effects of natural selection, genetic drift, gene flow, and mutation on crown morphology remain unresolved (Hlusko, 2016; Hlusko, Schmitt, Monson, Brasil, & Mahaney, 2016; Scott et al., 2018). Incisor winging and Carabelli's cusp in molars are the traits that have received the most attention in this regard (summarized in Scott et al., 2018; but see Mizoguchi, 1985, 1993, 2013).

Premolar traits indicative of geographic origin are the mesiolingual groove, accessory, and marginal ridge forms as well as cusp number. These observations parallel previous findings for antagonists in the upper jaws (Burnett, 1998; Scott & Irish, 2017). The number of lingual cusps was the only sexually dimorphic trait in premolars we found; this appears to be a novel finding. The small size of some of our subgroups might explain why only some of the discrete traits reached statistical significance. Taking together the results from the shape analyses and the analysis of the discrete traits, we observed greater shape variation in P_3s than in P_4s. This is in accordance with the findings reported by other authors (Hillson, 1996; Kraus et al., 1969; Kraus & Furr, 1953; Ludwig, 1957; Nelson & Ash, 2010; Scott & Turner, 1997; Turner et al., 1991).

Depending on the metric dataset, the correlation between the first pair of latent variables for P_3 and P_4 ranged from 0.59 to 0.87. Bermúdez de Castro and Nicolás (1996) found comparable results using premolar crown area. Our GM shape study also revealed that both premolars vary together between two different configurations, being either high and narrow or low and broad. Moreover shape variation seems to affect individual regions of the lower premolars to differing extents. This supports the idea that some regions may be more strongly canalized than others (Siegal & Bergman, 2002; Waddington, 1957). For both lower premolars, the buccal aspect of the dentinal crown is less variable, while the lingual aspect varies extensively, both in the number of cusps as well as in their height and relative position.

The scope of this study was to investigate the morphological variation of premolars across different human populations, thus emphasizing existing similarities and dissimilarities. The nature of the data measured here does not allow us to unfold the reasons behind this variation. However, one possible way of interpreting this differential degree of variation of buccal and lingual dentine horns would be in functional terms. Premolars are positioned between the anterior and posterior dentition, but only the lower P_3 engages directly with the anterior dentition. In normal occlusion, the buccal cusp of P_3 moves through the interproximal embrasure of the upper canine and first premolar, leading Kraus et al. (1969) to consider them a "functional entity." The mesiobuccal ridge of the P_3 contacts the upper canine and participates in ripping rather than crushing or grinding. The buccal cusps of the mandibular premolars engage the buccal cusp slopes of the maxillary premolars until the upper lingual cusps and lower buccal cusps reach their maximum intercuspation (Figure 6).

Biomechanical analyses of P_4, using 3D finite element analysis (FEA) to evaluate occlusal loading conditions, suggest that the mesiobuccal and the distobuccal aspects of the crown experience high tensile stresses, whereas the lingual aspect does not (Benazzi et al., 2014). This indicates that shape changes in the buccal cusp of mandibular premolars would have stronger functional implications than shape changes in the lingual cusp. Consequently, the lingual crown aspect of lower premolars may be less constrained. This also suggests that upper premolars should be less variable than lower premolars since both cusps of the upper premolars engage directly in occlusion, and not just one (Alt et al., 1998; Hillson, 1996; Kraus et al., 1969; Scott et al., 2018; Scott & Turner, 1997). The absence of population-specific large-scale shape differences in our data might thus indicate that the occlusal functional constraints outweigh any other factors. It is likely that the largely overlapping shape variation of the different populations was influenced neither by genetic drift nor by adaptations to particular environmental or dietary conditions, although there was potentially enough time for such effects to manifest. In fact, genetic studies show that the populations considered separated between 10 and over 100 millennia ago (Duggan & Stoneking, 2014; Henn, Cavalli-Sforza, & Feldman, 2012; Mallick et al., 2016; Schlebusch et al., 2012).

We need more data on the genomics of odontogenesis to understand the mechanisms behind the population similarities in premolar gross shape. Also, measurements of form may involve more details on the functionally relevant aspects of the crown, for instance, wear facets on the OES, rather than conventional landmarks and curves on the EDJ. The occlusal fingerprint analysis (OFA; Benazzi et al., 2014; Kullmer et al., 2009), an approach for the quantification of the occlusal aspect (i.e., occlusal compass; DeVreugd, 1997; Maier & Schneck, 1981), or finite element methods (review in Dogru, Cansiz, & Arslan, 2018) could help to elucidate this further.

Bermúdez de Castro and Nicolás (1996) suggested that the P_3 is under the influence of factors affecting the size and shape of the canines. Yet, canine size is rather variable and differs significantly between the sexes (Staka & Bimbashi, 2013). Nevertheless, we found no evidence suggesting that P_3 or P_4 size variation is related to sex. On the other hand, we observed significant size differences at the level of continental groups for the outlines. We also found a nonsignificant

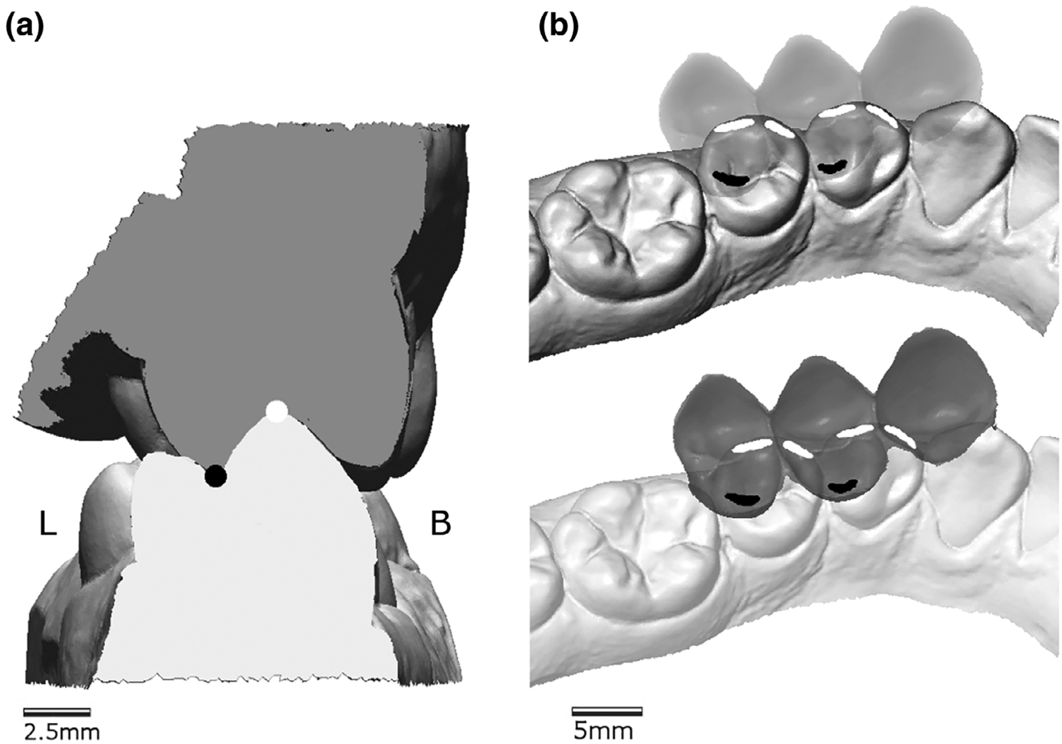

FIGURE 6 Intercuspation of the upper and lower dentition, visualizing points of contact: (a) coronal section of the distal aspect of the left P_4s in maximum intercuspation and (b) 3D area of contact points (black = upper lingual cusps engaging with the distal fossa and midpoint of the distal marginal ridge, white = lower buccal cusps engaging with the buccal cusp slopes)

trend for size differences at the population level, but our data sample might have been too small to reach significance. Our data are generally compatible with the previously described pattern of large teeth in Southeast Asians and small ones in Europeans, with moderately sized teeth in Africans and Americans (Ashar et al., 2012; Hanihara & Ishida, 2005; Harris & Rathbun, 1991; Hillson, 1996; Pilloud et al., 2014; Rathmann et al., 2017). We found no sexual dimorphism in any of the size analyses. Size explains only a very small fraction of shape variance in both premolars. Such small allometric effects have already been observed in other studies (Bailey & Lynch, 2005; Martinón-Torres et al., 2006; Weber et al., 2016). Our finding regarding size reduction over time, in this case from 8th century Avars to contemporaneous Middle Europeans, for cervical and crown outlines matches well the Holocene size reduction rate of 1% per 1,000 years suggested by Brace et al. (1987). In this case, 2D outline measurements can be considered to some extent analogous to the traditional mesiodistal or buccolingual diameters. In contrast, when the whole dentinal crown is considered, thereby including relative crown height, a different phenomenon was observed. Premolars of recent Central Europeans tended to be higher and narrower than those of Avars, resulting in a slightly increased size of Europeans.

Although our observations mainly focused on the EDJ, the features described can also be observed at the OES. Our results for the discrete traits confirm previous research that the general morphology of both surfaces is highly correlated (Bailey et al., 2011; Fornai et al., 2015; Guy et al., 2015; Morita, 2016; Olejniczak et al., 2007; Skinner, Wood, et al., 2008). Working with the EDJ can be advantageous to detect small features (e.g., accessory cusps, tubercles, and crests) since they are usually recognized more easily on the EDJ than on the OES. An additional advantage is that the EDJ is affected by wear later than the OES so that the inclusion of moderately worn teeth is still possible. This is crucial when working with archeological or palaeontological material.

5 | CONCLUSIONS

We found interpopulation differences in some discrete traits of the human mandibular premolars, namely, in the well-represented mesiolingual groove (42% of the total sample) and in the expression of different occlusal ridges. Moreover, the number of lingual cusps proved to be a sexually dimorphic feature. This emphasizes the relevance of classical odontological approaches to the biological understanding of dental remains. Based on our outcomes, we recommend the incorporation of the mesiolingual groove into the ASUDAS standards. We stress the importance of P3 lingual cusp number, included in the ASUDAS but seldomly used in the literature. The nonmetric trait catalog developed here is, to the best of our knowledge, the first published for both mandibular premolars inner and outer morphology and may serve as a reference for future studies.

Our complementary data source based on geometric morphometrics using landmarks and curve semilandmarks at the EDJ did not yield a comparable separation. Instead, we found these same samples to overlap completely on the principal component ordinations of their shape variability.

The GM approach, however, allowed detection of general patterns of shape variability which indicate that P_3 and P_4 crowns covary from low and broad to high and narrow. Moreover, the buccal aspect of both lower premolars seems to be less variable than the lingual aspect. Canalization of the buccal aspect of lower premolars might be explained by functional constraints acting on dental crowns for tight occlusion and efficient mastication. Conversely, our results do not support any effect of genetic drift or adaptation to particular environmental and dietary conditions on premolar shape. Such observation should be evaluated using approaches designed to capture the functional aspects of the dental crown in combination with genetics. Additionally, future research might explore covariation of mandibular premolars with the neighboring lower canines and molars, as well as with the upper antagonists—the maxillary canines and premolars. In such study designs, newly developed techniques based on a more sophisticated approach to surface form than semilandmarks (Bookstein, 2018; Currie, 2018) might yield an additional source of information.

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ETHICAL STATEMENT

The authors declare no financial interests. The data that support the findings of this study are available from the corresponding author upon request. This study contains digital image data of 23 South African and Papuan specimens from the Rudolf Pöch collection. The data was acquired prior to the collections' current closure for provenance research as to the contexts of colonial injustice. The Department of Evolutionary Anthropology states: “The Department of Evolutionary Anthropology at the University of Vienna is fully aware of the highly problematic acquisition circumstances regarding indigenous remains procured by the Austrian anthropologist Rudolf Pöch (1870–1921) during his Oceania and South Africa expeditions between 1904 and 1909. These collections held by the Department of Evolutionary Anthropology have since been closed for a thorough provenance research as to the contexts of colonial injustice."

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SUPPORTING INFORMATION

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

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