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„Geometric morphometric analyses of modern human upper second molars“

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Table of contents

Abstract

Teeth are the most abundant remains in the fossil record. Dental anthropology can provide essential information for taxonomic assessments and phylogenetic reconstruction.

This detailed quantitative study of the crown morphology by means of geometric morphometric methods aims to support further understanding of the complicated outer and inner tooth morphology for the use in comparative anatomy and in human evolution. Our study explores both outer enamel surface (OES) and the enamel-dentin junction (EDJ) in maxillary second molars to assess their morphological variability in a heterogeneous sample of modern human populations from diverse geographical origin and subsistence modes. We examined the EDJ and OES of 38 permanent upper second molars, including teeth from Australian Aboriginals, Avars (a European nomad population with Asiatic background from the 7th and 8th century), middle Europeans, Khoesans, Papuans, Centralafricans, and Eastasians. Additionally, for comparative purposes, we added a group of upper second molars from *Pongo pygmaeus.* We utilized destruction-free micro CT (μCT) to consider high-resolution images of the EDJ and OES. The 3D surfaces were reconstructed, seven true landmarks, 43 semilandmarks (on five ridge curves), and 24 pseudolandmarks (on the cervical, respectively on the crown outline) were collected. Subsequently, Geometric Morphometrics and multivariate statistics were applied to quantify the shape and form of populations. In addition to the morphometric analyses of the EDJ, the cervical and crown outline, and a combined data set, we also evaluated the expression of non-metric dental traits. We hypothesized that, despite different subsistence modes, cultural and geographical backgrounds, there will be a substantial overlap of all populations with regard to molar shape. We supposed that the selective advantages for some specific tooth shape are too weak and therefore random variation is of prime importance. Our results confirm these

assumptions and we found general overlap of all populations regarding to the shape of the EDJ, the combined data set, cervical and crown outline. Furthermore, we expected that, as a result of active functional constraints on tooth shape, there are some regions of the tooth that remain more stable in shape, while others might show a higher variation. Our results of the shape analyses indicate that especially the distal regions of the cervical and also crown outline are variable, while mesial regions stay stable. We assumed that the higher variation in the distal areas is associated with the variability of the lower third molar, which articulates with the upper second molar (especially with the distal cusps) in the case of the bilaterally balanced occlusion.

Although this study could not show any relevant population specific shape pattern of upper second molars, we emphasize the relevance of our results for the taxonomic assignment in comparative analyses with other fossil hominins.

Zusammenfassung

Zähne gehören zu den widerstandsfähigsten Überresten in der Fossilgeschichte. Die rezente Forschung der dentalen Anthropologie liefert unentbehrliche Informationen über die Taxonomie und phylogenetische Rekonstruktion.
Die hier vorliegende quantitative Studie der Zahnmorphologie mittels Geometric Morphometric Methoden hat zum Ziel, zum Verständnis der komplizierten äußeren und inneren Zahnmorphologie für den Einsatz in der vergleichenden Anatomie und in der menschlichen Evolution beizutragen.
Unsere Studie untersucht sowohl Schmelzoberflächen (OES), als auch die Schmelz-Dentin-Grenze (EDJ) von oberen zweiten Molaren, um die morphologische Variabilität in einer sehr heterogenen Stichprobe, bestehend aus modernen menschlichen Populationen unterschiedlicher Subsistenz und geographischer Herkunft, zu erfassen. Wir untersuchten die EDJ und OES von 38 permanenten oberen zweiten Molaren, darunter auch Zähne von australischen Ureinwohnern, Awaren (eine europäische nomadische Bevölkerungsgruppe mit asiatischer Abstammung), Mitteleuropäern, Khoesans, Papuas, Zentralafrikanern und Ostasiaten. Zusätzlich, für Vergleichszwecke, haben wir auch Zähne von *Pongo pygmaeus* einbezogen. Wir nützten die Methode der destruktionsfreien Mikro-CT (µCT) um detaillierte Bilder der OES und der EDJ zu erzeugen. Anschließend wurden 3D-Oberflächen konstruiert, und sieben echte Landmarks, 43 Semilandmarks auf fünf Gratkurven auf der EDJ und 24 Pseudolandmarks auf der Cervixumrisslinie und der Kronenumrisslinie positioniert. Darüber hinaus wurden Geometric Morphometrics und multivariate Statistik angewandt, um die Unterschiede in Form und Gestalt zwischen den Populationen interpretieren zu können. Neben den morphometrischen Analysen von EDJ, kombiniertem Datensatz (EDJ mit der Cervixumrisslinie), Cervixumrisslinie und der Kronenumrisslinie haben wir im Rahmen dieser Arbeit auch die Analyse der nicht-metrischen Merkmalen durchgeführt.

Eine unserer Hypothesen war es, dass trotz unterschiedlicher Methoden der Subsistenz oder differierender kultureller und geographischer Hintergründe

eine generelle Überlappung aller Populationen hinsichtlich der Gestalt und Form gegeben ist. Zusätzlich gehen wir davon aus, dass die selektiven Vorteile für die Entwicklung davon abweichender Zahngestalt zu gering sind und daher die Variabilität eher zufallsbedingt ist. Unsere Ergebnisse bestätigen diese Hypothese. Wir fanden eine starke Überlappung von allen Populationen in Bezug auf alle durchgeführten Gestalt- und Formanalysen. Außerdem haben wir erwartet, dass bestimmte Zahnregionen eher variieren als andere, da die Gestalt eines Zahnes nur gewisse Freiheitsgrade aufweisen kann, wenn eine funktionierende Okklusion gewährleistet werden soll. Die Ergebnisse zeigen, dass vor allem die distalen Bereiche der Cervix- und Kronenumrisslinie variabel sind, während mesiale Regionen stabiler bleiben. Wir gehen davon aus, dass die Variation in den distalen Bereichen mit der Variabilität des unteren dritten Molaren assoziiert ist, da dieser mit dem oberen zweiten Molaren (exakt: mit seinen distalen Höckern) im Fall der bilateral symmetrischen Okklusion artikuliert.

Obwohl in dieser Studie keine populationsspezifischen Zahnmuster in den oberen Molaren identifiziert werden konnten, betonen wir die Bedeutung unserer Ergebnisse für Vergleichsstudien mit anderen fossilen Homininen.

1.0 Introduction

The proportions of the human body fascinated people since the time of the ancient Egyptians. Artisans used such proportions as templates for paintings, and even included precise images of males and females (Slice, 2005). In 1654, the German anatomist Johann Sigismund Elsholtz formalized the scientific measurement of living individuals, today classified as anthropometry (Slice, 2005). It is the science dealing with metrical measurements of the human body to obtain data on morphological variability (Slice, 2005).

Presently, dental morphology can support important claims in phylogeny of hominins and help assign fossil specimens to hominin taxa (e.g., Bailey, 2004; Bailey et al., 2009; Gómez-Robles et al., 2007; Martinón-Torres et al., 2006; Skinner et al., 2008b). The tooth-crown-studies performed in the last three decades concentrated on establishment and standardization of methods for the classification of dental traits. These researches investigated how the expression of dental traits varied within and among modern human populations (e.g., Turner et al., 1991).

Initially with dental research, non-metric traits received a higher focus, for instance the studies undertaken by Hrdlička (1920), Pedersen (1949), or Turner and Scott (1977). Measurements and description of gross dental morphology have long played an important role in the dental research in anthropology (Wolpoff, 1971; Frayer, 1977; Brace et al., 1987; e.g., Bermúdez de Castro and Nicolás, 1996). Investigations by Dahlberg in 1956 brought a huge improvement in methodology, not only by describing standards for dental morphology, but also by creating a classification of many variations, such as Carabelli´s cusp, accessory marginal cusps, or hypocone reduction. As a result of this process, the Arizona State University Dental Anthropology System (ASUDAS) by Turner et al. (1991) was published. Furthermore, there were many other studies performed, which were based on quantification of morphological variation and the description of relative cusp areas (Wood and Abbott, 1983; Wood and

Uytterschaut, 1987; Wood and Engleman, 1988; Bailey et al., 2004; Moggi-Cecchi and Boccone, 2007), or cusp angles (Morris, 1986; Bailey, 2004), respectively on crest lengths (Pilbrow, 2003; 2007). These studies focused only on the outer crown morphology, thus hidden dental traits could not be explored.

The enamel-dentine junction (EDJ) preserves the spatial information of the shape of the membrana praeformativa. The membrana praeformativa is the template for layering of enamel plies during the process of tooth crown formation (Butler, 1956). At the membrana, ameloblasts start to produce enamel protein in the form of a non-mineralized matrix. Afterwards, this protein mineralizes to form the enamel cup (Scott and Turner, 1997). A correspondence between the OES and the EDJ was shown quite early. Weidenreich (1937; 1945) noted this correspondence in cross-sections of a *Homo erectus* molar.
Studies of Kraus in 1952 and Korenhof in 1960 emphasized the lack of totally correspondence between EDJ and OES. Additonally, Corruccini (1987) and Schwartz et al. (1998) claimed the analogy between OES and EDJ but they demonstrate, that not all morphological structures on the EDJ are necessarily present on the OES.

Recent research of Skinner et al. (2008a; 2009) showed strong correlations between EDJ and EOS and assumed that dental traits founded on the EDJ are, in principle, also present on the OES. In the case of serious occlusal wear affecting the OES, the EDJ still might provide valuable information about the morphology of the unworn OES, at least to a certain degree. The discrepancies between OES and EDJ are caused by some additional enamel deposition in particular regions (Skinner et al., 2008a; 2009). Also Ortiz et al. (2012) described a strong correlation between Carabelli´s cusp expression at the EDJ and the OES. Altogether, the spatial congruence of the EDJ and OES topography in large regions of a tooth suggests, that the EDJ can be used as a fairly good indicator for the outer crown morphology, if the latter is, for instance, affected by wear, attrition or damage.

If the enamel dentine junction (EDJ) was investigated in earlier studies, the destruction of the teeth was necessarily implicated which is of course an undesirable disadvantage. For a morphometric study of the EDJ by Sakai (1967) or the determination of enamel patterns by Kono (2002), acids were used to remove the enamel from the dentine. Since those studies, enormous technical progress has been achieved. With the modern abilities of digitalization, we are now able to consider tooth morphology destruction-free and in much more detail.

With high-resolution computed tomography (hrCT) visualization of hidden intraosseal and endocranial structures is possible (Spoor et al., 1993). Still, teeth components cannot be visualized in such a high quality as with micro-Computed Tomography (μCT). Olejniczak et al. (2007), as one of the first who used μCT in dental research, showed the potential of μCT as a non-destructive method. The advantage of destruction free μCT scanning is well recognized; it allows investigating the crown surface but also structures below the outer enamel surface. Thereby, the morphology of the enamel-dentine junction can be visualized, offering a major advantage over other approaches.

The use of geometric morphometrics in biology and anthropology has become important and is used as an effective tool in shape analysis (e.g., Bookstein, 1991; 1996; Rohlf and Marcus, 1993; Mitteroecker and Gunz, 2009; Weber & Bookstein 2011). Some studies using geometric morphometrics on teeth have already been published (e.g., Bailey, 2004; Bailey et al., 2009; Gómez-Robles et al., 2007; 2008; 2011; 2012; Martinón-Torres et al., 2006). Yet, detailed morphometric analyses of the EDJ in 3D are still rare, and include just some tooth types. Researches of the last decade have often used occlusal photographs in connection to geometric morphometric techniques. The great advantage of this approach is the simple data acquisition, and thus the large sample size. The major drawbacks of this method are, however, that 1) the possibilities to study geometric information from a 3D object based on 2D projections are quite restricted, 2) slight deviations of the orientation of a tooth during the photographic process can lead to severe measurement errors, and 3) it can only capture the OES. In addition, only very well preserved teeth can be used. By using worn teeth, some authors (e.g., Bailey, 2004; Martinón-Torres et al., 2006, Gómez-Robles et al., 2007) approximated the position of the original cusp´s horn tip as a center of the wear facet, but the accuracy of this procedure on 2D projections has never been fully tested (Benazzi et al., 2012). Hence, localization and digitizing of landmarks and semilandmarks is prone to error in such cases.

In this study, we therefore utilized micro-computed tomography to capture the shape of the EDJ surface and the OES of upper second molars of modern humans from diverse geographical origins which also feature different subsistence strategies (hunter/gatherers, nomads, agriculturists). The resulting data are in 3D and comprise the full volume of teeth, thus also hidden features. Geometric morphometrics were applied to analyse form and shape.

This study focused on the maxillary second molars. The reason is because they are often in a much better preservational condition than first molars are (the latter are affected by attrition or wear much earlier in life). Moreover, we excluded the third molars because of their huge individual variability in the dental morphology. Simultaneously, they are often congenitally missing in some specimens. The standard morphology of the upper second molars has been described and discussed by many authors (e.g., Kraus et al., 1969; Hillson, 1996; Scott and Turner, 1997). In occlusal view (see Fig.1), the second upper molar is showing three major cusps; the paracone, the largest cusp (bucco-mesial cusp), followed by the protocone (mesio-lingual cusp), and the metacone (bucco-distal cusp). These cusps build a triangular form called "trigon". In the middle of the trigon is a deep depression, the fossa centralis. It borders on the mesial side to the mesial marginal ridge and on the distal side to the prominent crista obliqua. The latter represents a connection between the metacone and the protocone. The fourth cusp on upper second molars is the hypocone, situated disto-lingually. It is less prominent than the other three cusps. The hypocone and the metacone are linked by the distal marginal ridge. Separately from the trigon, there is a second fossa, the fossa distalis. Here is the origin of the lingual fissure, which continues lingually.

One goal of this study was the analysis of non-metric dental traits. Particular attention was paid to the hypocone reduction. In general, cusps that are formed phylogenetically (Butler, 1967a; 1967b) and ontogenetically later tend to be more variable in size than cusps that appeared earlier (Corrucini, 1979). Phylogenetically, the hypocone represents the last cuspal addition to maxillary molars during primate evolution and it is most often reduced in size (Swindler, 1976). Furthermore, the hypocone is chronologically the last cusp of molars that calcifies during dental ontogeny (Scott and Turner, 1997). There are several authors that have discussed the consequences of cusp morphogenesis on cusp stability (e.g., Jernvall, 2000; Takahashi et al., 2007). The calcification starts at the mesial cusps and progresses towards the distal cusps, reaching the hypocone last (Kraus and Jordan, 1965). In the recent studies, the high prevalence of hypocone reduction, was found by Gómez-Robles et al. (2012) and also Martinón-Torres et al. (2012). Furthermore Carabelli´s cusp, metaconule and mesial marginal accessory tubercules were also determined on dental material in this study.

Not only cuspal reduction, but also general tooth reduction is an important point to be mentioned here. Several authors (e.g., Brace et al., 1984, 1995, 1991; Calcagno and Gibson, 1991; Frayer, 1978) described the effect of natural selective factors, respectively cultural background, of populations on tooth size. In general, there is a trend that Europeans show a marked tooth size reduction (Brace et al., 1991). The ancestors of modern Europeans were the first using cooking as an obligatory procedure for their survival in the periods of intensive glaciation. Therefore, the lack of selective pressure led to the strongest size reduction in Europeans (Brace et al., 1995). Afterwards, cooking spread also to other areas. From this follows, that the dental reduction is proportional to the time since the populations have been using cooking (Brace et al., 1995). Hence, in Australia, where cooking was established only 35 000 years ago, Australians undergo the teeth reduction for the shortest time span. Therefore they have relatively biggest teeth, according to Brace et al. (1995).

This study aims to support further understanding of the complicated outer and inner tooth morphology for the use in comparative anatomy and in human evolution. We assessed the morphological variability of upper second molars in our geographically heterogeneous sample. We investigated a quantity of shape variation of EDJ, crown, and cervical outlines. Geometric morphometric methods were applied, based on Procrustes superimposition, Principal Component Analysis, and Partial Least Square analysis. For the interpretation of allometry, we used multivariate regression. We hypothesized that, despite different subsistence modes and cultural and geographical backgrounds, there will be a substantial overlap of all populations with regard to molar shape. The reason for this is that selective pressures on tooth shape diminished since extraoral food processing gained importance. For example, cooking was widely used since at least 300,000 years ago (Brace, 1987, 1995; Ragir, 2000; Roebroeks and Villa, 2011; Shahack-Gross et al., 2014) and existed long before regional populations diverged. We thus expect to find random shape variation as the predominant pattern. Yet, there are of course active functional constraints on tooth shape, for instance, to maintain a certain degree of masticatory function still necessary, and to guide the movement of the mandible during the power stroke. We thus expect that there are some regions of the tooth that remain more stable in shape, while others might show a higher shape variation.

The results of this study can be used as a support for further comparative analyses and might be usefully applied to taxonomic studies of fossil teeth.

2.0 Materials

The sample of this study comprises 38 upper second molars of recent modern humans from different geographical backgrounds, including permanent teeth from Australian Aboriginals (n=4), Avars (a European nomad population with Asiatic background from the 7th and 8th century, n=7), middle Europeans (n=6), Khoesan (n=7), Papuans (n=5), Centralafricans (n=6) and Eastasians (n=3). Additionally, for comparative purposes, we added a group of four upper second molars from *Pongo pygmaeus* (see Figure 1). The sample size was rather limited because only well preserved teeth without any pathology could be used. At the same time, only specimens with known cultural and geographical background could be analyzed in this study. With regard to wear, we used only well preserved teeth that corresponded to category one or two of the scheme published by Molnar (1971). These categories were selected because more severe abrasion would affect dentine horn tips and thus the localization of landmarks. Additionally, the abrasion of outer enamel could impede the interpretation of non-metric traits. Teeth that were damaged in the region of the EDJ or tooth cervix were not included in this study. Simultaneously, for comparative purposes in a larger study, also upper and lower premolars from the same specimens had to be present in the jaw, featuring a similar preservational status as mentioned above. In general, both the process of scanning and image reconstruction, respectively the segmentation, as well as the analyses were very time-consuming which explains the rather small sample. To achieve larger groups for comparison, we defined three continental groups: Europeans (middle Europeans and Avars), Australasians (Aboriginals, Papuans and Eastasians), and Africans (Khoesan and Centralafricans).

The dental material of Papuans, Aboriginals, Khoesan and some Centralafricans (C 333, S 128, S 87, S 85, S138, see Table 1), which were used in this study are part of the Rudolf Pöch collection of the Department of Anthropology at University of Vienna. They were collected between the years 1904-1906, and 1907- 1909. Avars originate from a burial site at Csokorgasse in Vienna and are housed at the Department of Anthropology at the University of Vienna. Specimens of middle Europeans and one Centralafrican (MN 735) were

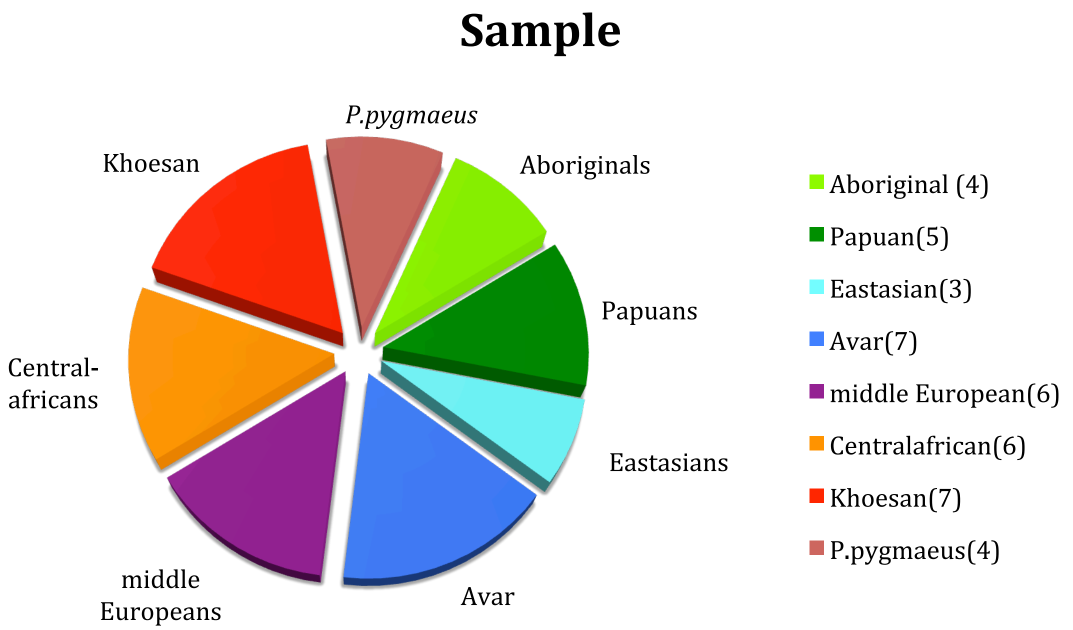

Fig.1: *Sample composition*

allocated for this project from the collection of the Anatomical Institute of Medical University in Vienna and from the Pathologic-Anatomical Museum Narrenturm in Vienna. *Pongo pygmaeus* data were collected on skeletal material provided by the Museum of Natural History in Vienna. Three Eastasian skulls were loaned from Pathologic-Anatomical Museum Narrenturm in Vienna. For a list of individual specimens (see Table 1).

These groups of specimens have been chosen for a detailed comparison of modern human populations because of their different geographical origin and cultural background. Our sample is very heterogeneous and it includes populations with different modes of subsistence such as hunter/gatherers, nomads, and agriculturists. With this sample we hope to have covered a great deal of the possible intraspecific variation in modern human upper second molars.

Table 1: *List of specimens*

The list of specimens included in this study

Collection	Individual	Sex	Age
Khoesan	S 46	Female	14-18
	S 68	Male	25-30
	S 121	Male	25-30
	S 103	Male	12-15
	S 111	Male	20-30
	S 29	Male	30-40
	S 61	Male	25-30
Papuan	CN 5	Male	Adult
	CN 230	Male	Adult
	CN 232	Male	Adult
	CN 236	Male	Mature
	CN 264	Male	cca 30
Avar	CG 495	?	7-8
	CG 502	?	13-15
	CG 569	Male	16-18
	CG 428	Male	16-18
	CG 582	Female	19-25
	CG 498	Female	25-30
	CG 673	Female	19-23
Aboriginal	C54	Male	?
	C55	Male	?
	C56	?	?
	C104	?	Juvenile
European	1103//122.510/1554	Male	22
	19710	?	20
	1149//125.011/1072	Male	Adult
	502//126.804/1171	Male	29
	512//122.199/961	Male	20
	709//122.622/1200	Male	24
Centralafrican	C 333	?	?
	MN 735	?	?
	S 128	?	?

	S 87	?	?
	S 85	?	?
	S 138	?	?
Eastasian	1348//122.342/1383	Male	28
	1340//122.335/1376	Male	36
	1377//122.369/1412	?	?
P. pygmaeus	657	?	Infantil
	658	Female	Adult
	B 5414/800	Female	Adult
	A 28801	Female	Adult

3.0 Methods

3.1 Morphometric analysis

High-resolution µCT-scans of the whole cranium or of isolated maxillae were taken with a Viscom X8060 NDT scanner at the Vienna micro-CT Lab, using the following parameters: 110-140kV, 280-410µA, 1400-2000msec., 0,75mm copper filter, rotation of 360 degrees (1440 images). The resulting isometric voxel resolution was between 25 and 35µm. After the scanning, the raw images were reconstructed using the software XVR-CT and converted into TIFF image stacks. Subsequently, dentine and enamel were separated by virtual segmentation and 3D surfaces were reconstructed using Amira 5.6 (Mercury Computer Systems, Chemsford, MA). For the segmentation, the protocol of the half-maximum height value was applied (Spoor et al., 1993). For the generation of triangulated surfaces the marching-cube algorithm was used and surfaces were smoothened by unconstrained smoothing. All right molars were mirrored to the left side to standardize the sample (left-right tooth asymmetry was not an aspect considered in this study). We created a set of landmarks along the cervical line, and determinated the cervical plane by creating a best-fit plane to these points. Finally we digitally cut the tooth crown at this plane (see Figure 2).

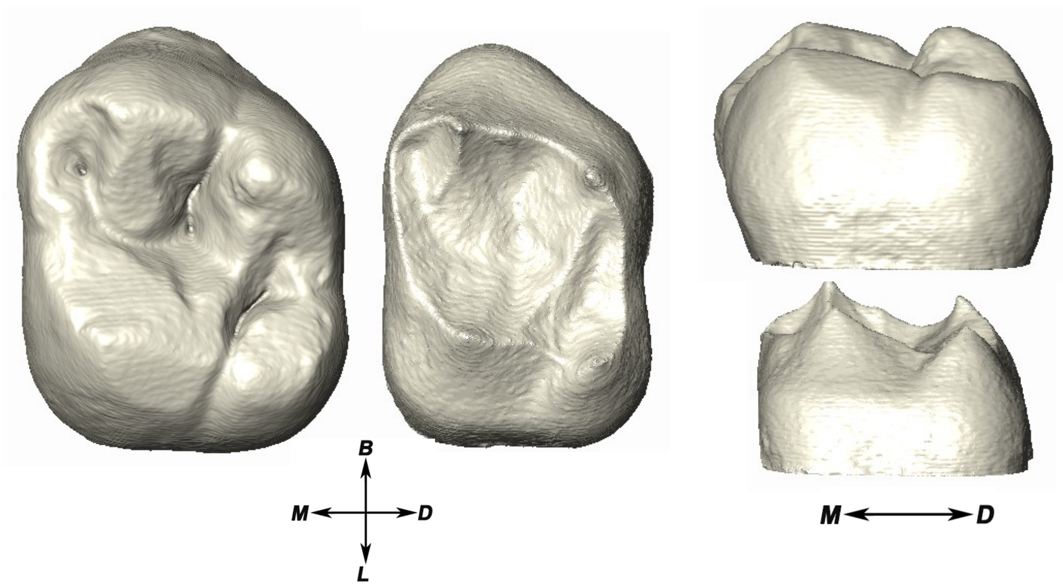

Fig.2*: Enamel and dentine in occlusal view (right) and buccal view (left)*

Seven true landmarks (measuring points that are biologically homologous among specimens, see e.g., Bookstein, 1991; Bookstein 1997; Mitteröcker and Gunz, 2009, Gunz et al., 2005) were identified on the EDJ. Four landmarks were located on the horn tips (in order: 1. paracone, 2. metacone, 3. hypocone, 4. protocone). The fifth and sixth landmarks were positioned on the deepest point of the central and the distal fossa. The seventh landmark marked the lowest point along the marginal edge between protocone and hypocone. Here we had to exclude five individuals (19710, ID-127-622-1200, ID-125-011-1072, Avar 582, and S87) because we were not able to place one or more of the seven true landmarks. The problem in those cases was the severe reduction of the hypocone. Therefore, it was impossible to place the third landmark. Simultaneously, we were not able to identify the curve between second and third landmark, respectively the exact position of landmark number seven (see Figure 3).

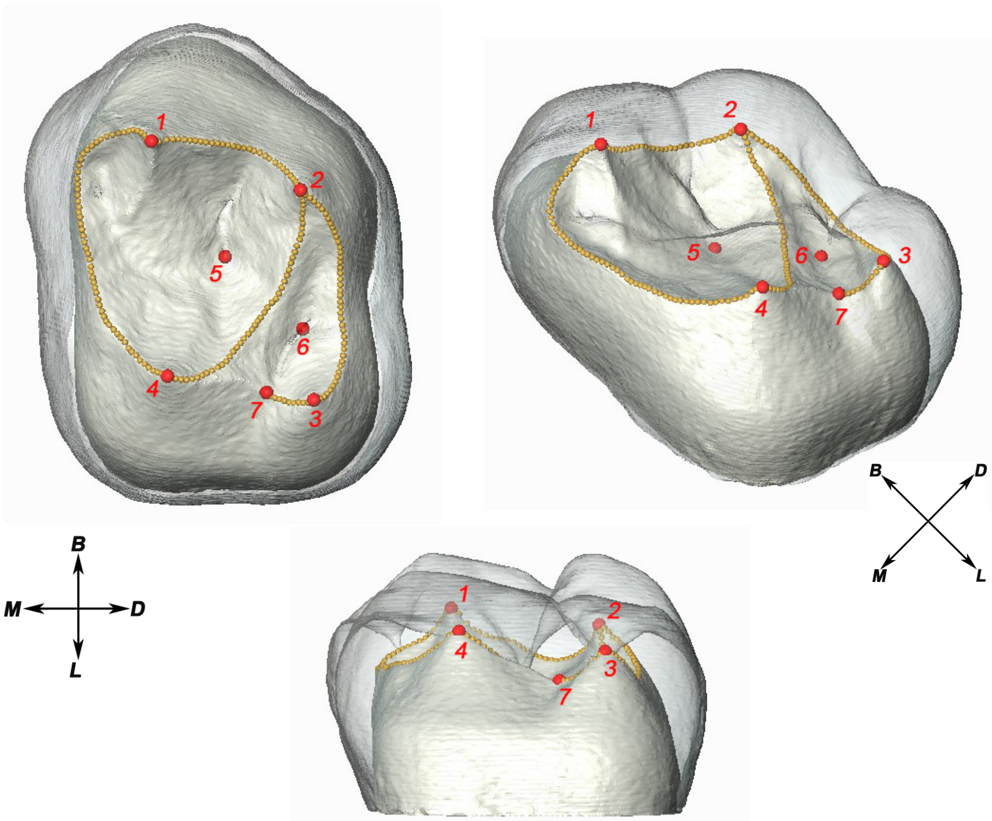

Fig. 3: *Landmarks position (1=paracone; 2=metacone; 3=hypocone; 4=protocone; 5=central fossa; 6=lateral fossa; 7= lowest point along the marginal edge between protocone and hypocone and curves*

After placing seven true landmarks, the ridge curves were determined as indicated in Figure 3 by the yellow spheres. The first curve started at the metacone and expanded along the crista obliqua till it reached the protocone. Other curves were collected clockwise following the respective marginal ridges. The second curve ran from the protocone, along mesial marginal ridge to the paracone. The third curve begun at the paracone and was placed along the buccal marginal ridge, until it reached the metacone. From metacone ran the fourth curve to the hypocone. The fifth curve started at the hypocone and ended at landmark number seven (see Figure 3). Additionally, the landmark set was amended by semilandmarks generated along those ridge curves. We placed in total 43 sliding semilandmarks (geometrically homologous measuring points, Bookstein, 1991; Bookstein, 1997; Gunz et al., 2005) along the five ridge curves using the EVAN ToolBox 1.71. These semilandmarks were projected on the template´s specimen curves (in this case specimen "CN230" was chosen for its excellent preservational status). Furthermore, we warped this template onto other specimens and slid and projected semilandmarks to minimise the bending energy (Bookstein, 1997; Gunz et al., 2005; Mitteröcker and Gunz, 2009).

As a next step, we imported the enamel and dentine surfaces in STL format into Rapidform XOR2 (INUS Technology, Inc., Seoul, Korea) and followed the protocol of Benazzi et al. (2012). The cervical plane, defined above to cut the tooth crown, was oriented parallel to the xy-plane of the Cartesian coordinate system in Rapidform (i.e. corresponding to the plane of the screen). Afterwards we rotated the tooth around the z-axis in such a way so that the mesial margin was aligned parallel to the y-axis of the coordinate system. The cervical outline was assessed using a spline curve as a contour of the tooth cervix at the height of the cervical plane. The crown outline was also captured as a spline curve, namely that of a projected silhouette of the oriented crown to the cervical plane (see Figure 4).

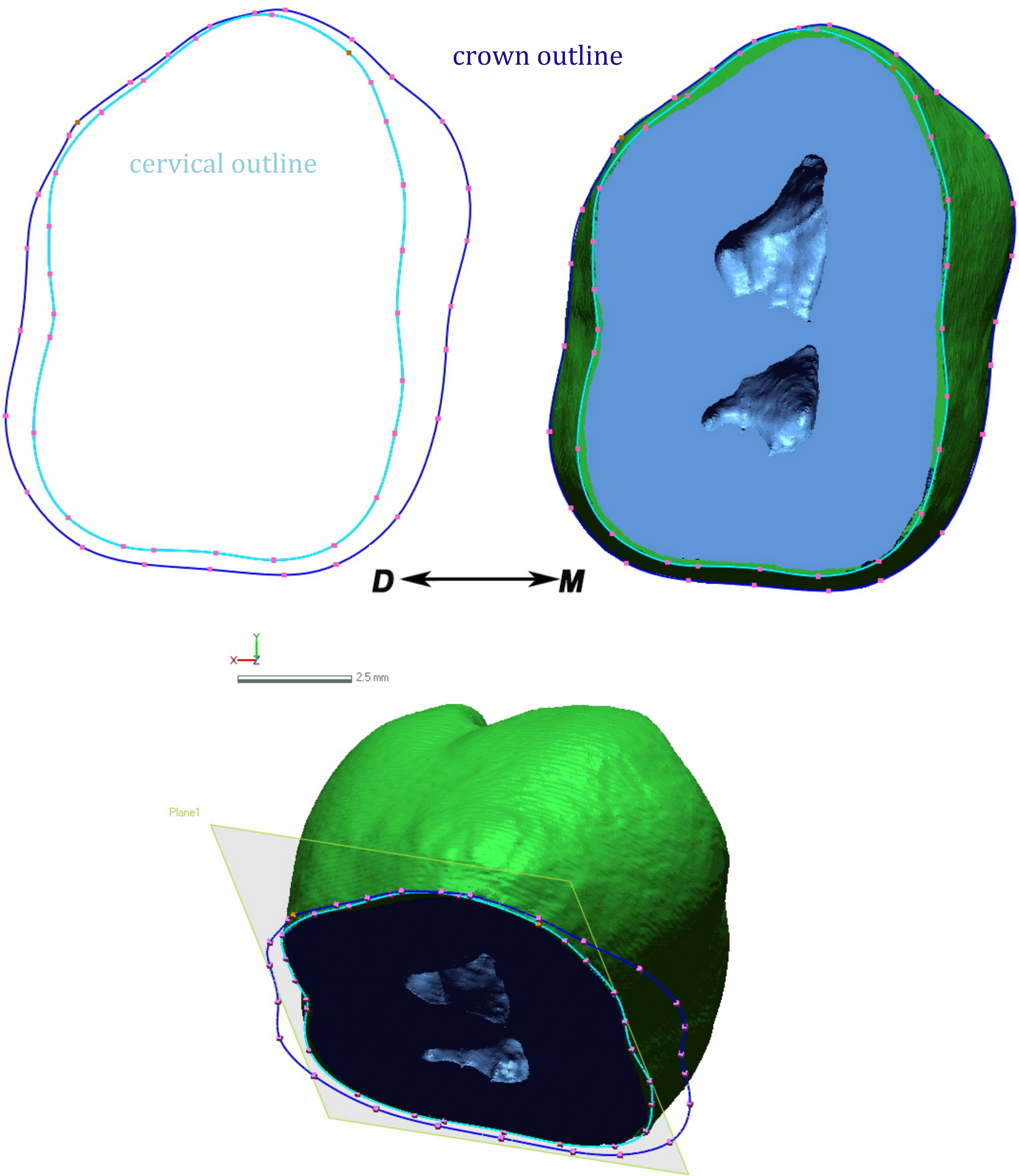

Fig. 4: *Creating outlines in Rapidform XOR2*

After this procedure, both outlines were imported into Rhinocerus 4.0 (Robert McNeel & Associates, Seattle, WA), where they were aligned with the xy-plane of the Cartesian coordinate system (see the protocol of Benazzi et al., 2012). For each curve the centroid and a set of 24 equiangular radii, placed every 15 degrees, were determined. The origin of these radii was in the centroid of the outline, whereby the first radius was parallel to the y-axis and on top, and the other radii followed counterclockwise. Where the radii cut the outline, 24 pseudolandmarks were created and exported. This procedure was used for both outlines (see Figure 5)

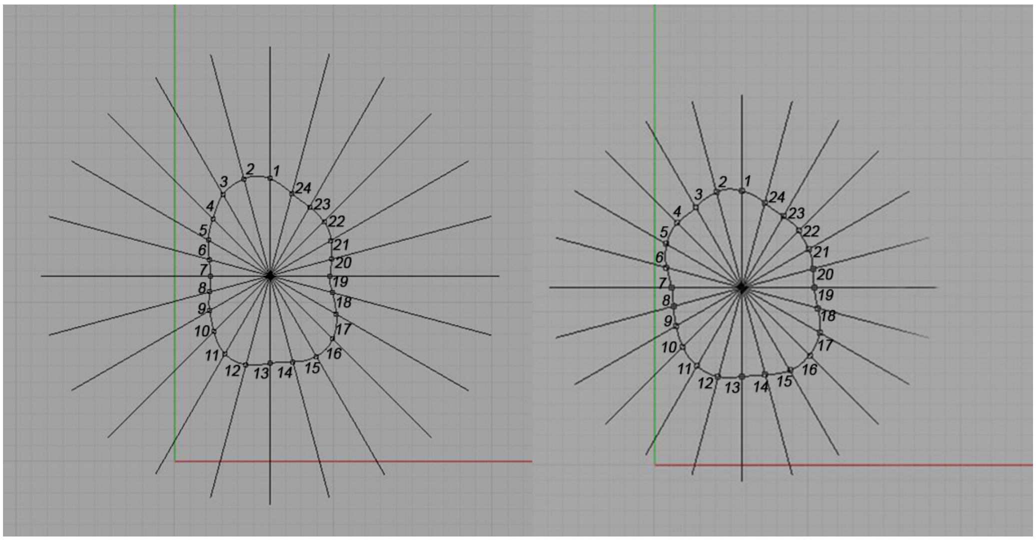

Fig. 5: *Creating pseudolandmarks in Rhinocerus 4.0*

As a result, we had one landmark set on the EDJ containing seven true landmarks and all curve semilandmarks, and two other landmark sets containing the pseudolandmarks of the cervical and crown outlines. But these landmark sets were collected in different coordination systems. However, to appreciate the height of the tooth crown and to be able to analyse the coupled changes of the EDJ and cervical outline, we had to include the latter into the EDJ data set. Hence, we created a combined data set containing coordinates from both datasets using Amira 5.6 and the function “align surface”.

3.2 Statistical analysis

After we completed the data acquisition, we applied General Procrusted Analysis (GPA) and Principal Component Analysis (PCA) to the data.

Generally, the GPA generates shape coordinates using superimposition of landmarks (Mitteröcker and Gunz, 2009). It removes information about location, orientation and size by translating each specimen to a common centroid, scaling them to the same size, and rotating them according to minimization of the sum of squared distances between corresponding points (Rohlf and Slice, 1990). If the natural logarithm of Centroid Size (lnCS) is included, then in the Procrustes form space can be interpreted (Mitteröcker et al., 2004).

Principal component analysis of the covariance matrix of Procrustes shape coordinates (Bookstein, 1991; Rohlf and Marcus, 1993) is the most common method in geometric morphometrics to examine shape variation in the sample. PCA is a method of variable reduction, which results in a low-dimensional space, representing most of the variance in the data (Mitteröcker and Gunz, 2009). GPA as well as PCA was conducted on the following four data sets separately:

- cervical outline
- crown outline
- EDJ
- combined dataset (EDJ and cervical outline)

All analyses were performed for data including the out-group *P. pygmaeus,* and without *P. pygmaeus.* Afterwards, we applied the Sphericity test for two consecutive Eigenvalues after Anderson (1963) adapted by Coquerelle et al. (2011):

$$2N*\ln(a/g)$$

N = sample size
a = arithmetic mean
g = geometric mean (= nth root of the product of n numbers)

If the Anderson´s value for some combination of PCs is less then two, the PC interpretation should be stopped at this stage, because this PC is probably reflecting only noise, and not really shape changes.

Subsequently, Partial Least Square analysis was carried out to determine the correlations between the shapes of EDJ and cervical outline, respectively crown outline, or between both outlines separately. Finally, we evaluated the effect of allometry using a multivariate regression of lnCS on shape.

The GM approach used here allows analysing size separately from shape. For all four tested features (EDJ, combined dataset, cervical outline, and crown outline), the non-parametric Mann-Whitney test was used to determine the differences between modern human groups and *Pongo pygmaeus*. Furthermore, a Kruskal-Wallis test was applied to test potential size differences among the three continental groups of modern humans in all four features. If there was a significant difference, Mann-Whitney tests were used to detect which continental groups differ significantly. The same approach was used also on the population level. In all implemented tests, Monte-Carlo permutation tests were included. Statistical analyses were conducted using the statistical package SPSS 19.0.

3.3 Analysis of non-metric traits

The non-metric trait analysis used the established standards from the Arizona State University Dental Anthropology System (Turner et al., 1991) and was further supported by additional trait descriptions published by several other authors (Kanazawa et al, 1990; Harris and Bailit, 1980; Hillson, 1996; Scott and Turner, 1997).

3.3.1 Hypocone reduction

Reduction of the hypocone (see Figure 6) is quite common in second upper molars (Turner et al., 1991). In this study, we used reference scores from a classification system published by Turner et al. in 1991 to describe the expression of the hypocone. If no hypocone was present and the "*site was smooth*" (Turner et al., 1991, p.18) it corresponded to a score 0. If slight ridging was present, the score was 1. A slight cuspule corresponded to a score 2, and when a small cusp was present the score 3 was assigned. The scores 4 and 5 applied to large and very large cusps. In this study, the teeth contained well developed hypocones (score 4 and 5) equal with standard morphology of second upper molar, and are therefore not discussed further. Because of the hypocone reduction, we excluded following specimens from the EDJ analyses:

- 19710 (middle European) → grade 1 of the hypocone reduction
- ID-127-622-1200 (middle European) → grade 1 of the hypocone reduction
- ID-125-011-1072 (middle European) → grade 0 of the hypocone reduction
- CG 582 (Avar) → grade 0 of the hypocone reduction
- S87 (Centralafrican) → grade 1 of the hypocone reduction

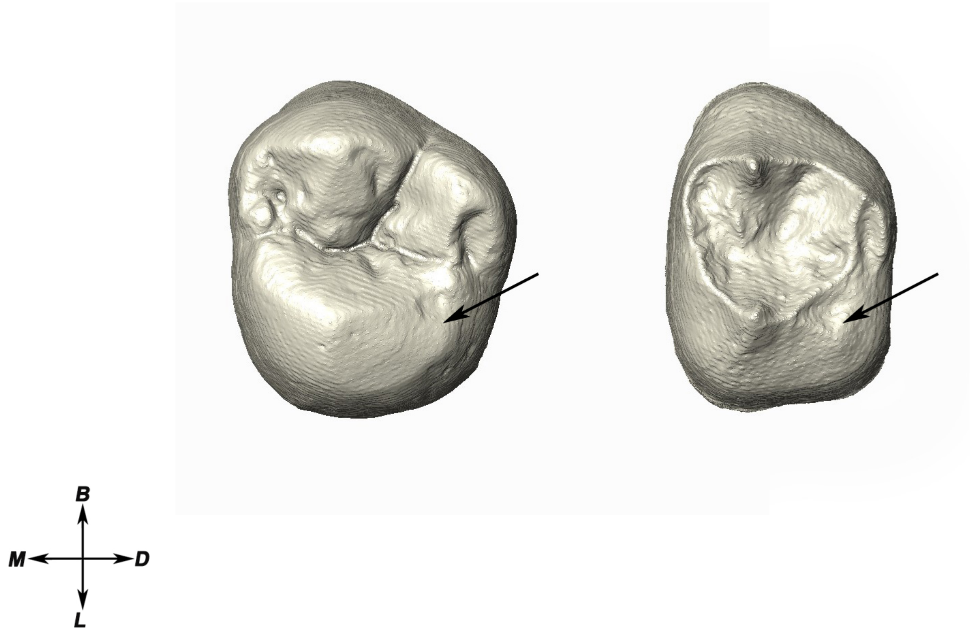

Fig. 6: *hypocone reduction, grade 0 (no hypocone present)*

3.3.2 Carabelli´s cusp

This dental trait is occasionally expressed on the lingual side of the protocone of upper molars (see Figure 7).

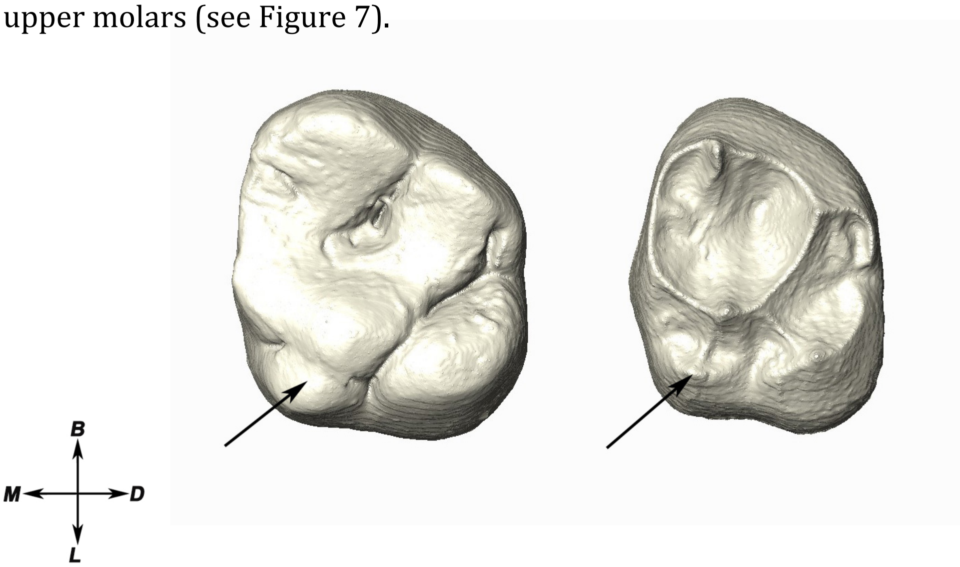

Fig. 7*: Carabelli´s cusp, grade 6 (medium sized cusp)*

The classification we used is based on an eight-step grade system published by Dahlberg in 1956. The manifestation ranges from slight groove or pit to large cusp, which is separated from the protocone. Carabelli´s cusp occurs most often on permanent upper first molars and is less prevalent on second upper molars (Hillson, 1996).

3.3.3 Metaconule

A metaconule represents an accessory cusp placed on the distal marginal ridge between the metacone and the hypocone (see Figure 8). The scaling range was established by Harris and Bailit in 1980 and divides the expression of the metaconule into six stages.

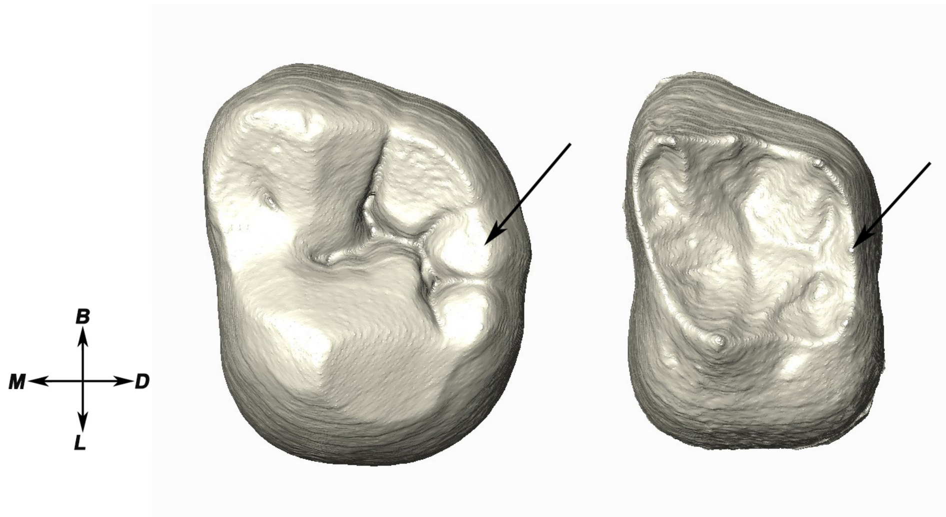

Fig. 8: Metaconule, grade 5 (medium sized cusp)

3.3.4 Mesial marginal accessory tubercule

For the interpretation of mesial marginal accessory tubercules, we used a classification pattern of Kanazawa et al. (1990). They define four accessory tubercules, whereby in our sample we found only two of them: mesial paracone tubercule, and protoconule (see Figure 9).

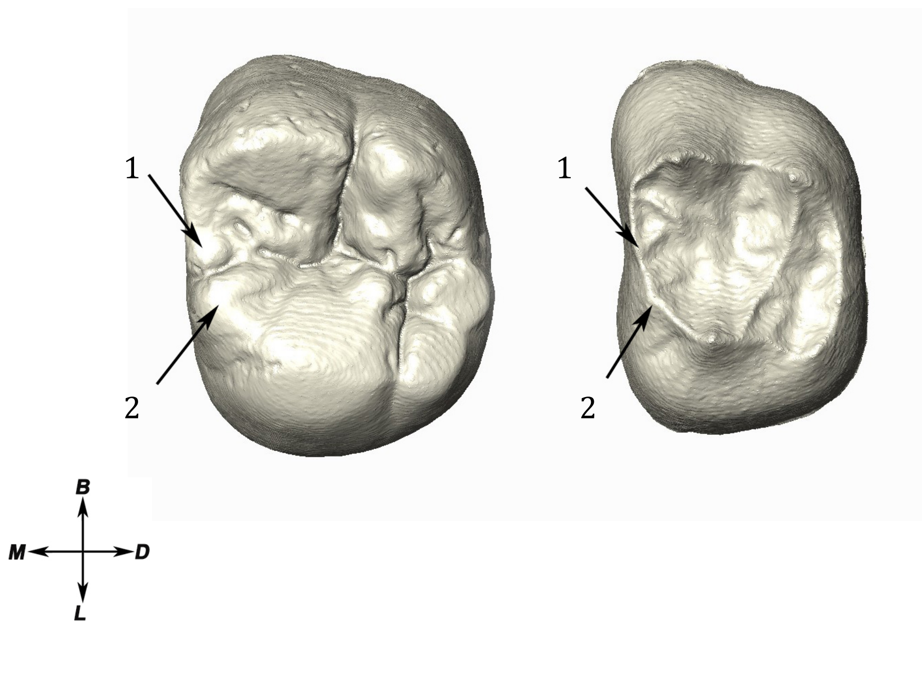

Fig. 9: Paraconule (1) and protoconule (2)

4.0 Results

4.1 Morphometric analysis of shape changes

The inclusion of geographically and culturally diverse recent human populations in our shape analysis allows testing hypotheses (see introduction) about shape variation observed in modern humans. We used Geometric Morphometrics to quantitatively explore the morphological variation of upper second molars of these populations. We examined the shape variation of the EDJ and the cervical, respectively crown outlines, and included qualitative descriptions of the EDJ surface.

The percentages of explained variance for the first four PCs and for all analyses in shape are provided in Table 2 and 3. The results of the sphericity test for two consecutive Eigenvalues after Anderson (1963) for the shape space are shown in Table 4. If the Anderson´s value is less then 2, the PC interpretation should be stopped on this stage, because this PC is probably reflects only noise, and not really shape changes. The results show that, except of the combined dataset, all the first two PC´s can be interpreted. Nevertheless, we decided to display also PC2 of the combined dataset because the result of the Anderson test is still quite high (1.662).

Table 2: *Percentage of explained variance in shape space (P.pygmaeus excluded)*

	combined	EDJ	Cervical outline	Crown outline
PC1	35.91	34.28	40.72	55.80
PC2	16.09	19.20	19.83	16.79
PC3	10.24	10.50	12.11	8.28
PC4	7.96	7.34	10.35	5.25

Table 3: *Percentage of explained variance in shape space (P.pygmaeus included)*

	combined	**EDJ**	**Cervical outline**	**Crown outline**
PC1	38.96	30.50	45.79	56.66
PC2	13.05	16.33	16.20	14.47
PC3	9.94	11.35	10.90	8.64
PC4	7.84	9.79	9.50	6.82

Table 4: *Sphericity test after Anderson (1963) for shape space*

	EDJ	combined	cervical	crown
PC 1 - PC2	**2.734**	**5.182**	**4.812**	**12.947**
PC 2 - PC3	**2.965**	1.662	**2.290**	**4.655**
PC 3 - PC4	1.047	0.526	0.232	1.955

4.1.1 Combined dataset

For the combined dataset of the EDJ together with the cervical outline, PC1 accounts for 35.91 % and PC2 for 16.08 % of the total variance. Positive scores correspond to a squared molar shape and a broader mesio-distal diameter (see Figure 10). Additionally the deepest points of both fossae are closer together and the fossa lateralis is reduced in size in positive values. However, all four tips are well developed, only the protocone appears to be relatively reduced in comparison to the other three cusps. With increasing positive scores of PC1, the paracone seems to be fixed in its relative position. The hypocone and the metacone, against it, move disto-buccally, and the protocone mesio-lingually. Additionally, we identified a clockwise rotation of the cervical outline relative to the EDJ surface. Negative scores of PC1 are associated with a relative expansion of the bucco-lingual diameter, caused partly by a mesio-lingual displacement of the hypocone, but also by a buccal enlargement of the paracone. This results in a more skewed shape of the molars featuring a prominent trigon. The paracone and the protocone are placed more buccally, and the metacone and the hypocone more lingually, which results in a shorter crista obliqua. Also in the negative scores of PC1, the mesial marginal ridge becomes higher in lingual view.

The positive scores of PC2 correspond to an increase of the total tooth mass. While the four main cusps and the deepest points of the fossae keep relatively constant relations to each other, the enlargement is rather caused by a higher body of the crown (see Figure 10), resulting in a larger distance of the cervical outline from the occlusal surface. Regarding negative scores of PC2, the tooth becomes shorter in the bucco-lingual dimension. Additionally, the crown height is reduced and the tooth appears flatter. Because the metacone and the hypocone become more pronounced, the molar changes its appearance in mesio-distal direction and looks broader.

As expected, we found a large overlap of all continental groups. A quite similar shape variability is present in all populations and we cannot define some special shape patterns typifying any certain continental group. There is no clustering even on the population level. The specific shape changes that we observe are not characterising particular populations or continental groups, but rather appears throughout all tested groups or populations. Despite this general overlap of continental groups, there is a slight separation of a few European specimens in the PC plot, where those teeth tend to be of a more elongated ellipsoid shape in the bucco-lingual diameter (see Figure 10). However, these results are based on a quite small sample size.

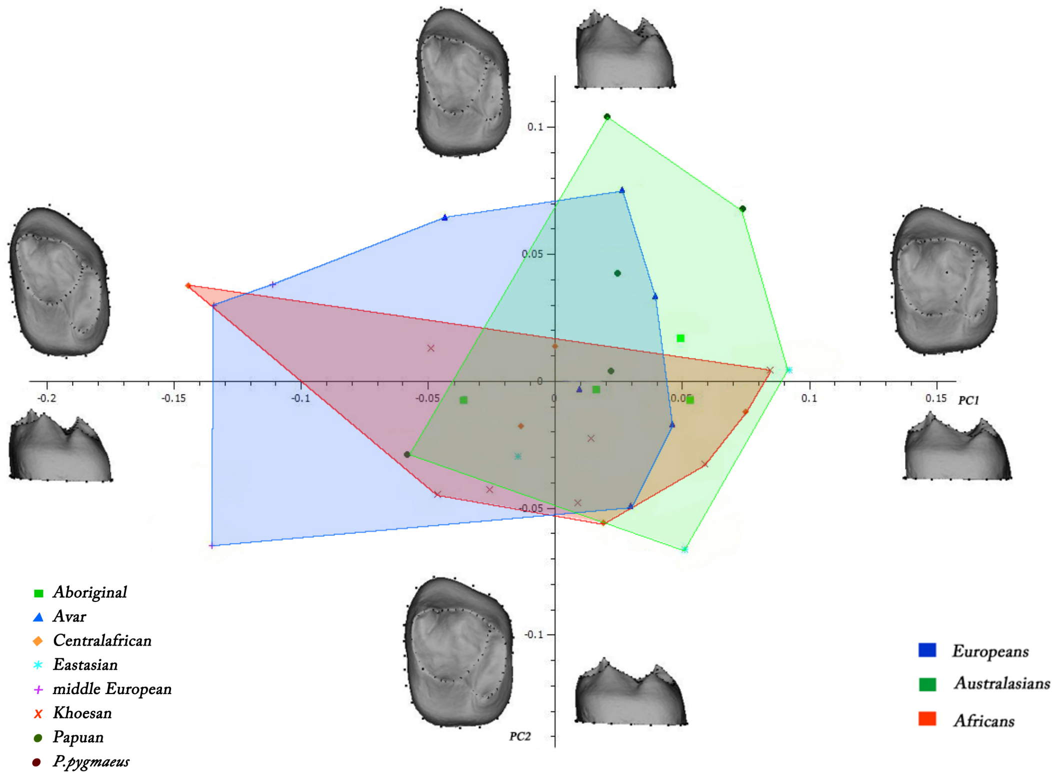

Fig. 10: *Plot of the first two PCs in shape space for the combined dataset*

Based on the results of the combined dataset, we emphasize that the bucco-lingual diameter is a fairly variable feature that leads to molars with a more rhomboid or a more squared appearance.

4.1.2 EDJ data set

The first PC of the EDJ dataset accounts for 34.27 % of total variance. Positive values of PC1 (see Figure 11) correspond to a more disto-lingual position of the hypocone, whereas the paracone is located more bucco-mesially. The molars become more elongated in the paracone-hypocone axis. At the same time the metacone and the protocone are closer together and the crista obliqua seems to be shorter and flatter. Additionally, in comparison with the fossa centralis, the fossa lateralis expands. On the other side, negative scores of PC1 correspond to squared shaped molars with a prominent protocone. The fossa lateralis becomes smaller. PC2 explains 19.20 % of the variance in the data. The changes associated with increased positive PC2 values are represented by a disto-buccal sliding of the hypocone, as well as by a buccal movement of the paracone. This results in a shorter distal marginal curve. Finally, along positive PC2 values the central fossa becomes deeper. The negative scores of the PC2 are connected with the lingual sliding of the hypocone, as well as with an enlargement of the fossa lateralis.

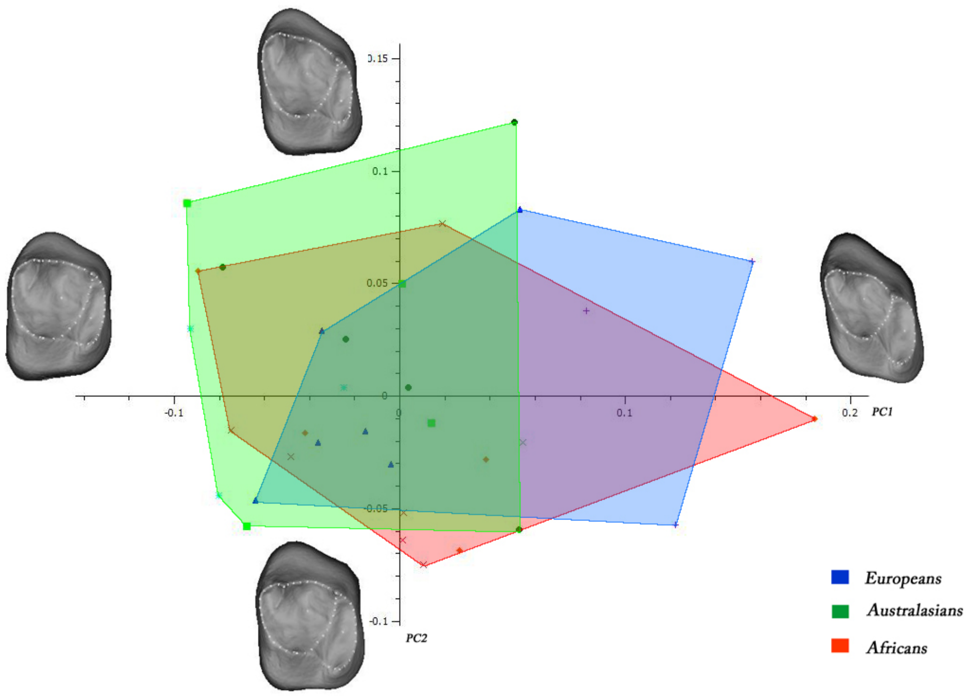

Fig. 11: *Plot of the first two PCs in shape space for the EDJ, for the legend see Fig.10*

To sum it up, the analysis reveals general overlapping of all continental groups and confirmed our hypothesis, respectively our results from the analysis of the combined dataset. The specimens are homogeneously distributed in shape space and there is no pattern to separate the continental groups or populations. However, it is noteworthy that there is a slight clustering of Europeans (representing ellipsoid shape), which are generally plotting at the positive half of the first PC.

4.1.3 Cervical outline

The first PC accounts for 40.72 % of the total variance. In positive scores of PC1 (see Figure 12), the buccal cervical pseudolandmarks slide to the lingual direction. This results in a shortening of the bucco-lingual diameter, or in a relative broadening in the mesio-distal axis. This leads to a more squared contour of the molars. Negative values of PC1 are associated with a distinct constriction on the mesial and distal margin of the cervical outline. That results into an elongation of the tooth in the bucco-lingual axis. The second PC explains 19.84 % of the variance in the data. Along the positive scores, the area of the hypocone appears much more enlarged. Additionally, there is a slight embayment of the cervical outline between hypocone- and metacone-area. With increased negative scores of PC2, the cervical outline is basically oval, but with a dominant metacone area (see Figure 12).

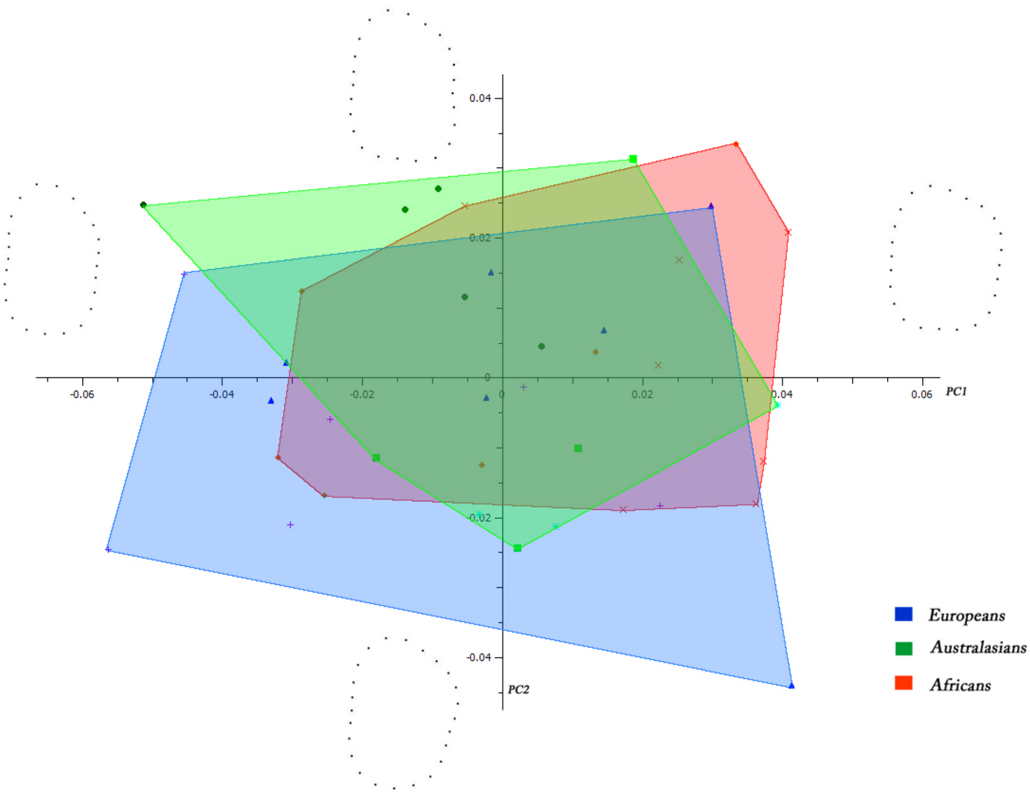

Fig. 12: *Plot of the first two PCs in shape space for the cervical outline, for the legend see Fig. 10*

The analysis of the cervical outline revealed a clear distribution of the individuals across the whole shape space of PC1 and PC2. Similarly to previous analyses, we identified general overlapping of all groups, not only on the continental but also on the population level. Therefore these findings again confirmed our prior hypothesis, that despite so different forms of subsistence and habitat, there is no clear difference among the populations. The identified variation seems rather at random. The cervical outline is a relatively stable feature of the upper second molars. The distribution and the large overlap of the explored groups confirmed the presumption of homogeneity in the cervical outline. The mesial part of the cervical outline did not change at all and the whole variance is mainly concentrated in distal areas, namely those of the hypocone and the metacone. This is on the other hand in agreement with our second hypothesis, i.e., that some regions of the tooth are more stable, but other tend to show more variation.

4.1.4 Crown outline

PC1 of the crown outline explains 55.80 % of the total variance in the sample. Positive scores of this PC (see Figure 13) correspond with a broader mesio-distal diameter and a distinct rounded shape of the tooth silhouette. In negative values of PC1 there appears again a slight embayment on the mesial margin of the crown outline. This results in an elongation of the outline in the bucco-lingual axis. Along positive values of PC2, which accounts for 16.79 % of the total variance, the area of the paracone and the hypocone appears to be reduced and roundish. Synchronously, the diameter in the paracone-hypocone axis becomes obviously shortened (see Figure 13). On the contrary, negative scores of PC2 showed a slight extension of the cervical outline in the paracone-hypocone axis, resulting in more skewed outline shapes.

Again, also the analysis of the crown outline showed that there is a considerable overlap of the continental groups. Most of the specimens are located in the central area of the plot. Although the continental groups generally overlap, on the population level, there is a noticeable trend for clustering. Papuans and middle Europeans (plotting on the negative half of PC1) represent rather populations with elongated crown shape in the bucco-lingual axis. In contrast, Khoesan (distributed in positive half of PC1) have teeth with a rather rounded crown outline.

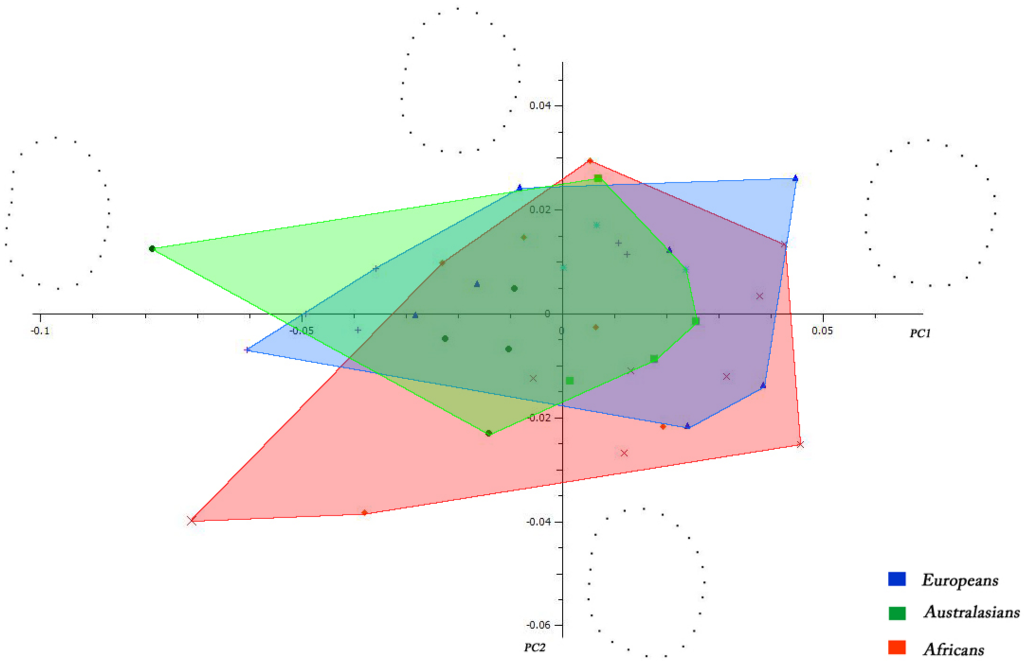

Fig. 13: *Plot of the first two PCs in shape space for the crown outline, for the legend, see Fig. 10*

4.1.5 Shape analysis including the *P.pygmaeus*

The entire set of shape analyses mentioned above, were repeated including the four *P. pygmaeus* specimens. In the analysis of the combined dataset, *P. pygmaeus* plotted on the negative half of the PC1. Therefore, the upper second molars of *P.pygmaeus* have typically a squared shape with four well developed horn tips. Additionaly, the molars are mesio-distally broad and the crown is flat (see Figure 14). EDJ shape analysis reveals similar findings as mentioned above using the combined dataset (see Figure 15). Cervical outline of *P.pygmaeus* is squared shaped, with a relatively prominent area of the paracone (see Figure 16). On the other hand the crown outline appears relatively rounded (see Figure 17).

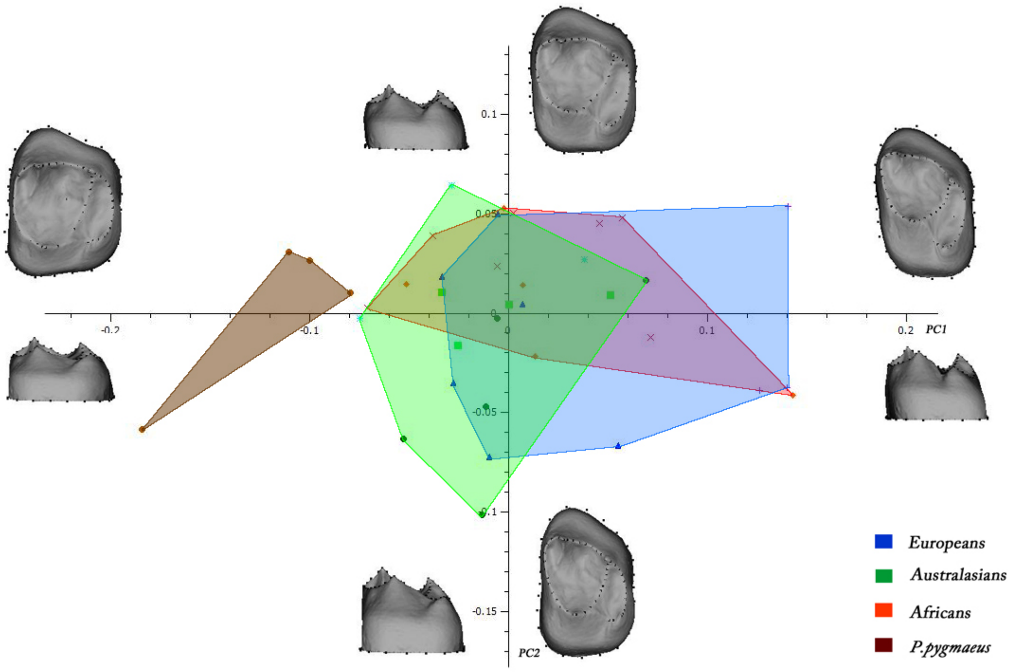

Fig. 14: *Plot of the first two PCs in shape space for the combined dataset, including P.pygmaeus, for the legend, see Fig. 10*

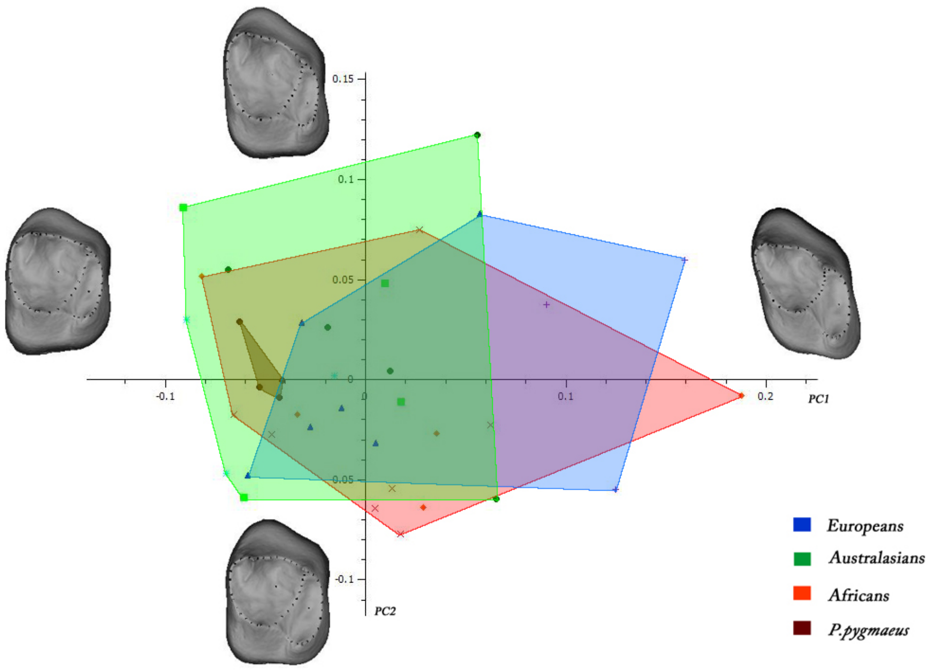

Fig. 15: *Plot of the first two PCs in shape space for the EDJ including, P.pygmaeus, for the legend, see Fig. 10*

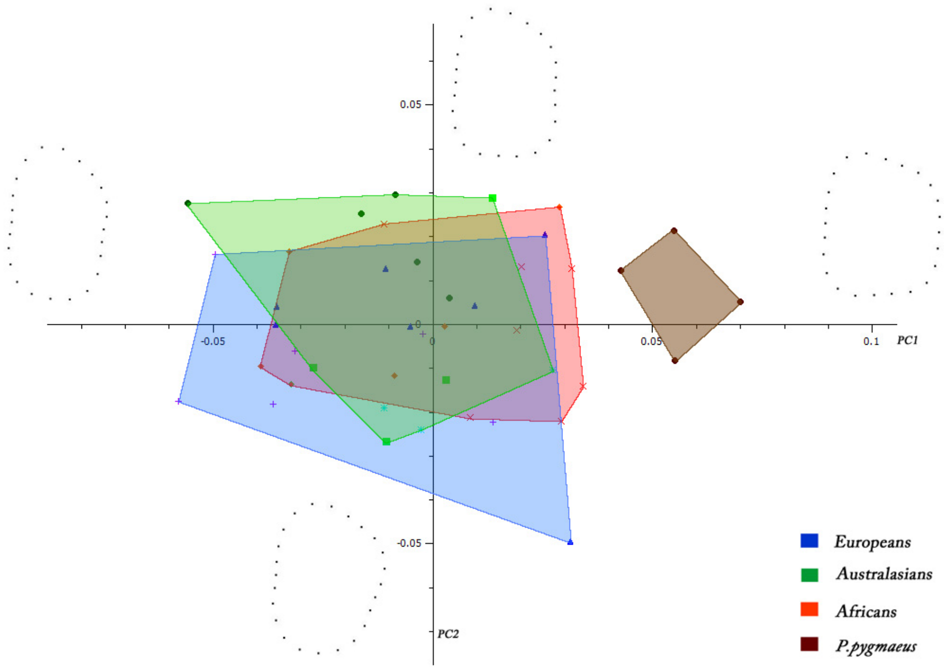

Fig. 16: *Plot of the first two PCs in shape space for the cervical outline, including P.pygmaeus, for the legend, see Fig. 10*

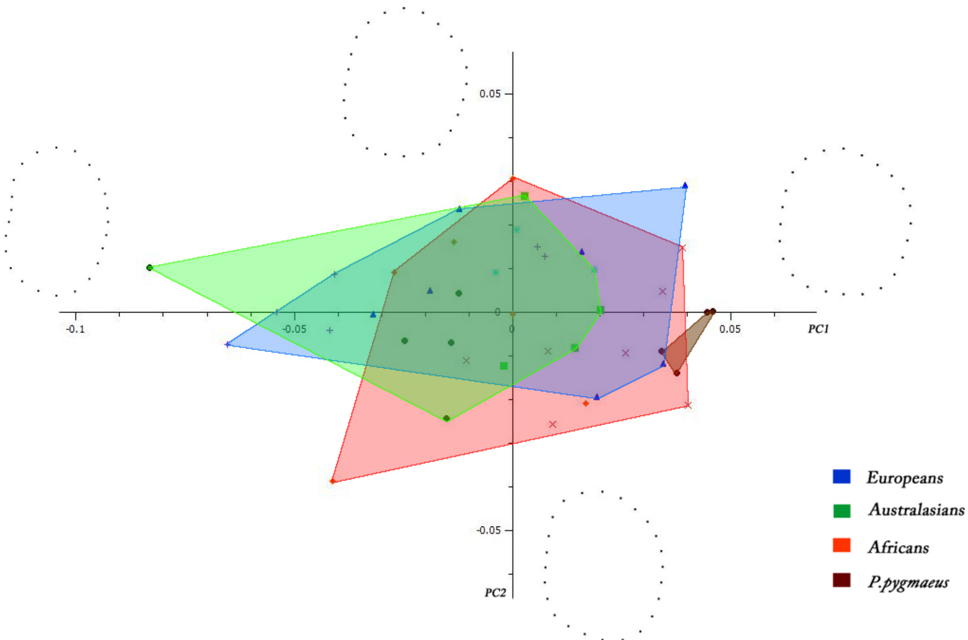

Fig. 17: *Plot of the first two PCs in shape space for the crown outline, including P.pygmaeus, for the legend, see Fig. 10*

The extended graphic of the cervical outline (see Figure 16) and the combined data set (see Figure 14) shows a slight separation of *P. pygmaeus* from the modern human populations. However, in the case of the crown outline (see Figure 17) *P. pygmaeus* is localised fairly near (respectively partly overlaps with) the modern human populations. In the EDJ shape analysis (see Figure 15) *P. pygmaeus* (with shape patterns similar to the recent human populations) falls completely into the range of variation of modern *Homo sapiens.* Therefore, we have to emphasize that the shape variation of *Pongo* in general could considerable overlap with the variation of modern humans. For a confirmation of this statement, a bigger sample of orang-utans would be required.

4.2 Morphometric analysis in form space

To interpret the aspect of size, we included the natural logarithm of Centroid Size (lnCS) to the Procrustes shape space. This allowed us to observe the sample in form space (see Methods). We analysed again the EDJ, the combined data set, and the cervical, respectively crown outlines. The percentage of explained variance in form space for the first four PCs are shown in Table 5, respectively 6.

Table 5: *Percentage of explained variance in form space (P.pygmaeus excluded)*

	Combined dataset	EDJ	Cervical outline	Crown outline
PC1	40.81	40.87	76.94	81.15
PC2	17.57	17.56	9.28	9.97
PC3	10.84	12.55	4.66	3.48
PC4	6.52	6.62	2.97	1.53

Table 6: *Percentage of explained variance in form space (P.pygmaeus included)*

	Combined dataset	EDJ	Cervical outline	Crown outline
PC1	54.92	53.93	86.03	87.86
PC2	12.33	12.87	5.31	6.05
PC3	7.43	8.42	2.57	2.11
PC4	4.93	5.05	1.84	1.30

However, in all data sets a clear separation of the continental groups was not possible. Only *P. pygmaeus*, if size is included, is clearly separated from the modern human populations with regard to all four features (see Figure 18, 19, 20 and 21). The continental groups are overlapping and only weak trends are noticeable. By all four tested features, the Europeans primarily occupied the positive area of PC1, while *P. pygmaeus* is located in the negative area of PC1. The widest range of distribution in form space appears with Australasians because they seem to be very variable in size.

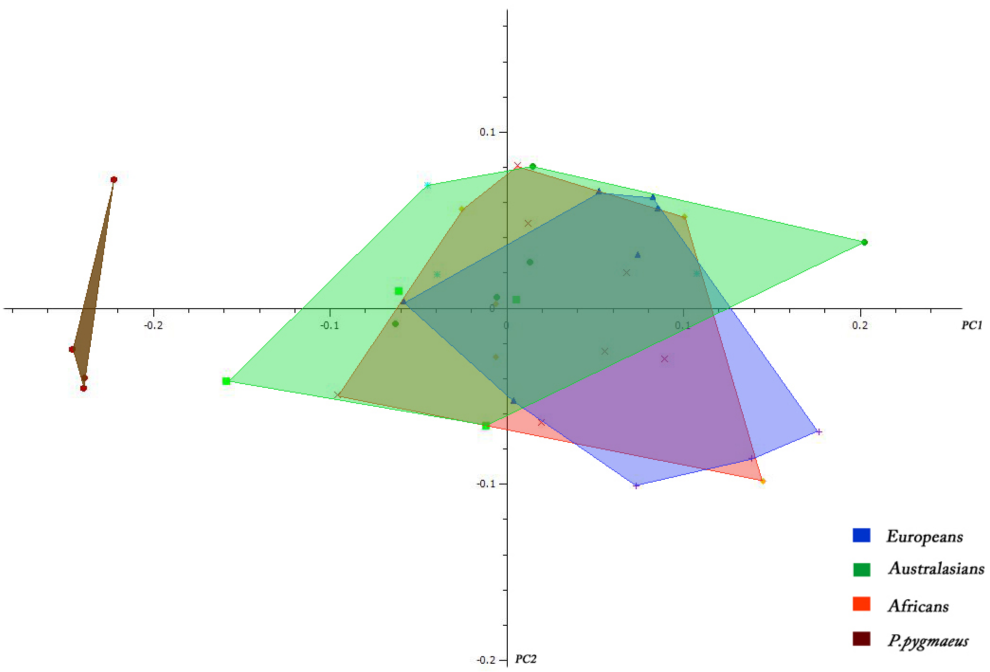

Fig. 18: *Plot of the first two PCs in form space for the combined dataset, for the legend, see Fig. 10*

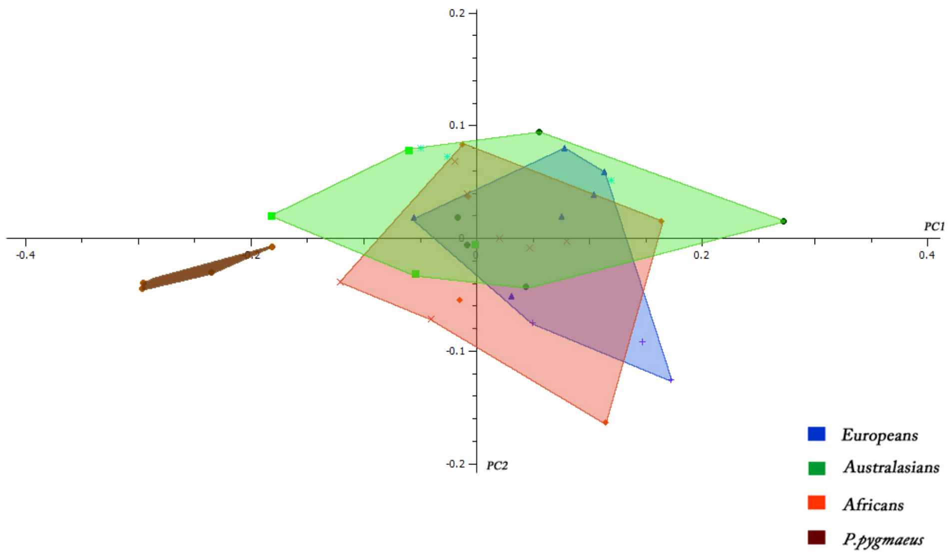

Fig. 19: *Plot of the first two PCs in form space for the EDJ, for the legend, see Fig. 10*

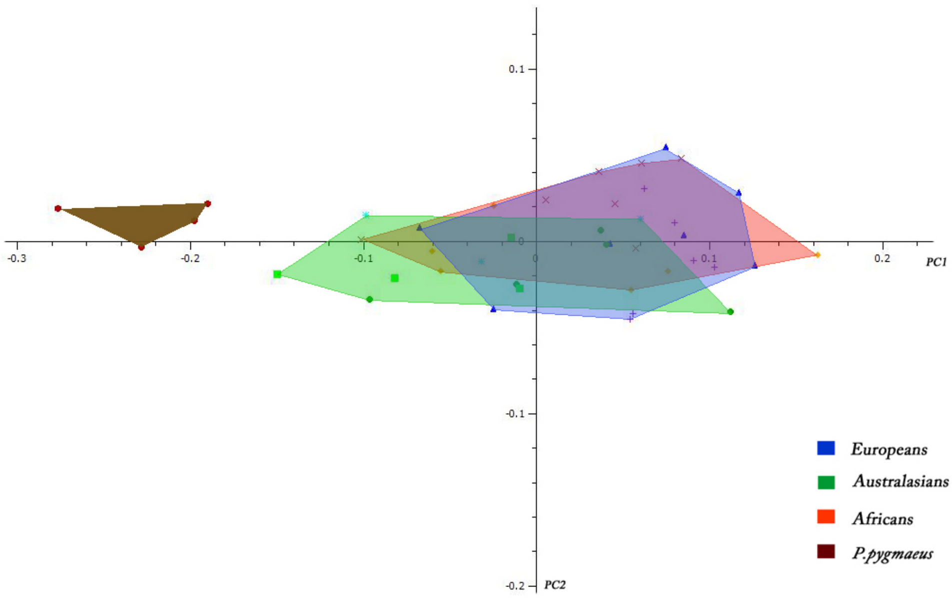

Fig. 20: *Plot of the first two PCs in form space for the cervical outline, for the legend, see Fig. 10*

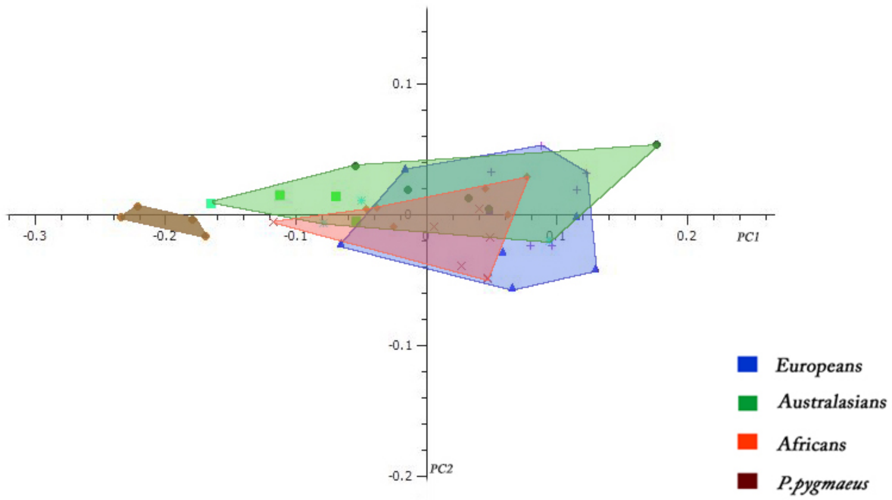

Fig. 21: *Plot of the first two PCs in form space for the crown outline, for the legend, see Fig. 10*

4.2.1 Size

Size plays an important role, not only in the EDJ but also in the outline and combined analyses. The combined dataset, the EDJ, the crown, and the cervical outline showed in a Mann-Whitney test (with Monte-Carlo-permutation, n=10000) that molars of the *P. pygmaeus* group are significantly larger ($p<0.001$) in the size than molars of modern humans (see Table 7 and 8).

Additionally, from the lnCS values of the combined dataset (for Europeans and Avars), we computed the percentage of the tempo of the size reduction for the time period of approximately 1200 years. Consequently, the size reduction accounted for 0.80 % per 1200 years.

Table 7: *lnCS values (combined dataset, EDJ, cervical and crown outline) for the continental groups*

Continental group	Cervical outline	Crown outline	Combined dataset	EDJ
Australasiens	3.21357	3.31566	3.60680	3.10812
African	3.16458	3.27861	3.57721	3.10409
European	3.13184	3.22599	3.54951	3.04489
P.pygmaeus	3.40738	3.48945	3.80415	3.35502
Total	3.19157	3.29299	3.60460	3.11813

Table 8: *lnCS values (combined dataset, EDJ, cervical and crown outline) for the populations*

Population	Cervical outline	Crown outline	Combined dataset	EDJ
Papuans	3.17676	3.25975	3.56873	3.05365
Australian Aboriginals	3.25798	3.39386	3.66982	3.18963
Khoesan	3.16150	3.27528	3.58289	3.12554
Avar	3.14060	3.24346	3.55892	3.05549
European	3.12163	3.20562	3.53070	3.02372
Centralafrican	3.16818	3.28251	3.56927	3.07407
Eastasian	3.21574	3.30461	3.58622	3.09025
P.pygmaeus	3.40738	3.48945	3.80416	3.35502
Total	3.19157	3.29299	3.60461	3.11813

Within modern humans, size was examined on the population and on the continental group level (see Figure 22 and 23). On the continental group level, there is a tendency towards larger teeth in Australasians, followed by Africans and Europeans, regarding to all four tested aspects. The Kruskal-Wallis test (with Monte-Carlo-Permutation, n=10000) shows a significant difference in the crown (p=0.016) and cervical outline (p=0.004) for continental groups. The Mann-Whitney test (with Monte-Carlo-Permutation, n=10000) shows, that the Australasians have significantly a larger cervical (p=0.006) and crown (p=0.011) outlines than Europeans. Synchronously, Africans represent a group with significantly larger crown outlines than Europeans (Mann-Whitney test with Monte-Carlo permutation, n=10000; p=0.008). Also on the population level, we can see a similar pattern as in the continental groups (see Figure 23). However, in this case the non-parametric Kruskal-Wallis test (with Monte-Carlo-permutation, n=10000) showed significant difference in size only for the crown outline (p=0.007). The Mann-Whitney test (with Monte-Carlo-Permutation, n=10000) reveals, that Australian Aboriginals have significantly bigger crown outlines in comparison to all populations except Eastasians. Additionally, Khoesans and Centralafricans have significantly bigger crown outlines than Europeans. The results are summarised in Table 9.

Table 9: *The results of the Mann-Whitney test (with Monte-Carlo-Permutation, n=10000) for the crown outline*

Population	**Significance**	**Mean Rank**
Aboriginal vs. Papuan	p = 0.03	**7.25** vs. 3.20
Aboriginal vs. Khoesan	p = 0.044	**8.75** vs. 4.43
Aboriginal vs. Avar	p = 0.013	**9.25** vs. 4.14
Aboriginal vs. European	p = 0.009	**8.50** vs. 3.50
Aboriginal vs. Centralafrican	p = 0.009	**8.50** vs. 3.50
Khoesan vs. European	p = 0.007	**9.57** vs. 4.00
Centralafrican vs. European	p =0. 009	**9.17** vs. 3.83

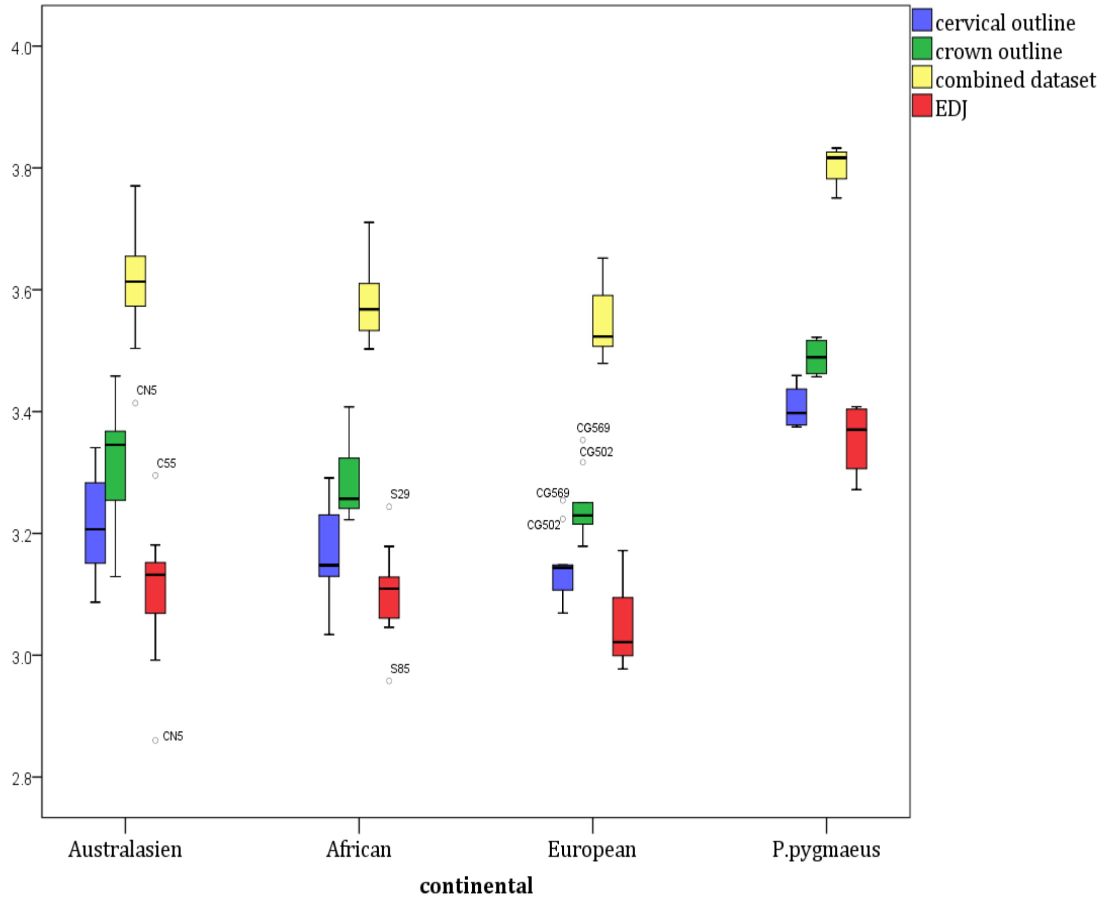

Fig. 22: *lnCS distribution on the continental level*

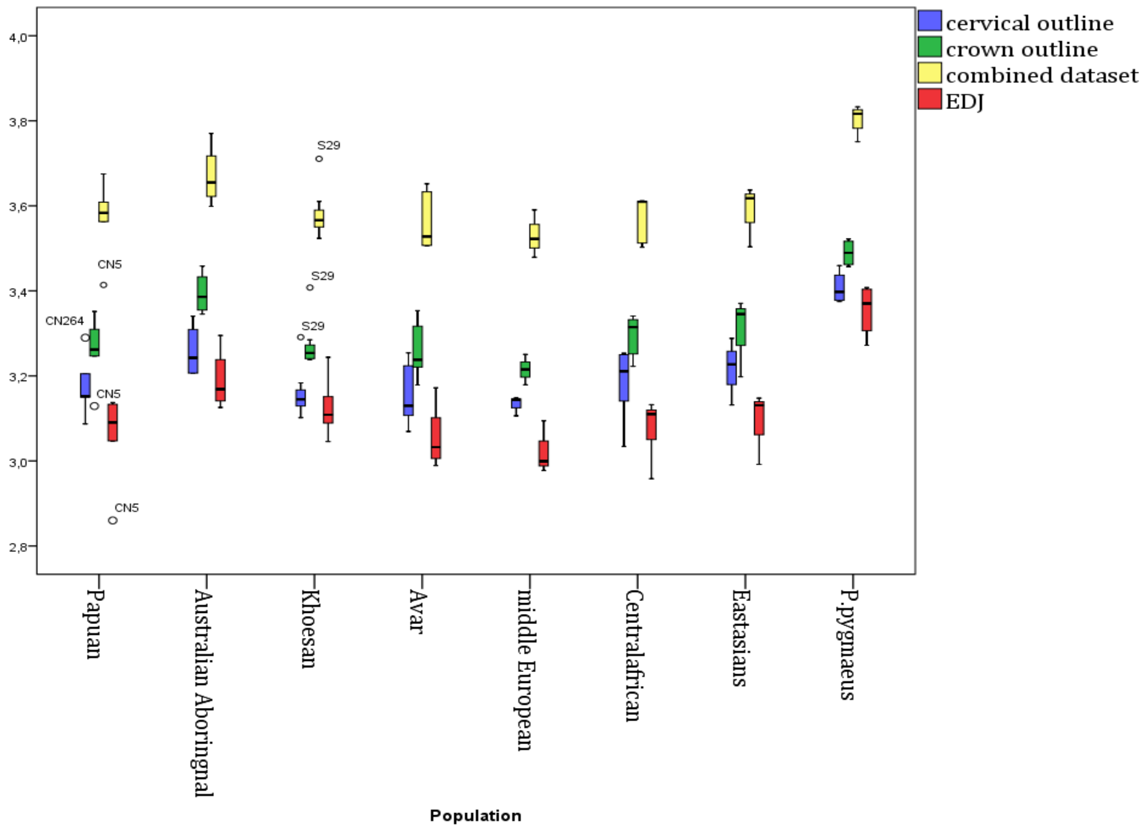

Fig. 23: *lnCS distribution on the population level*

4.3 Partial least square analysis

To detect how the three tooth traits in upper second molars covary with each other, thus between EDJ and cervical outline, EDJ and crown outline, respectively both outlines together, we used Two Block Partial Least Analysis (Rohlf and Corti, 2000; Bookstein et al., 2003). The analysis of the EDJ and the cervical outline showed a pairwise correlation of the left and right first singular warps r=0.80, explaining 72.63 % of the total squared covariance. If the EDJ is elongated in the paracone-hypocone axis (corresponding to positive scores), and additionally a well pronounced paracone area is present (see Figure 24), then the cervical outline has also an ellipsoid shape and is elongated in the bucco-lingual diameter. On the other hand, if the EDJ has a squared shape (corresponding to the negative scores), then the cervical outline is relatively shortened in the bucco-lingual diameter. The PLS of the EDJ and the cervical outline shows general overlap on the continental level, however there is a noticeable trend of separation of middle Europeans from the other populations. However, it has to be mentioned, that the sample size of the middle Europeans was very small (n=3) for this analyses.

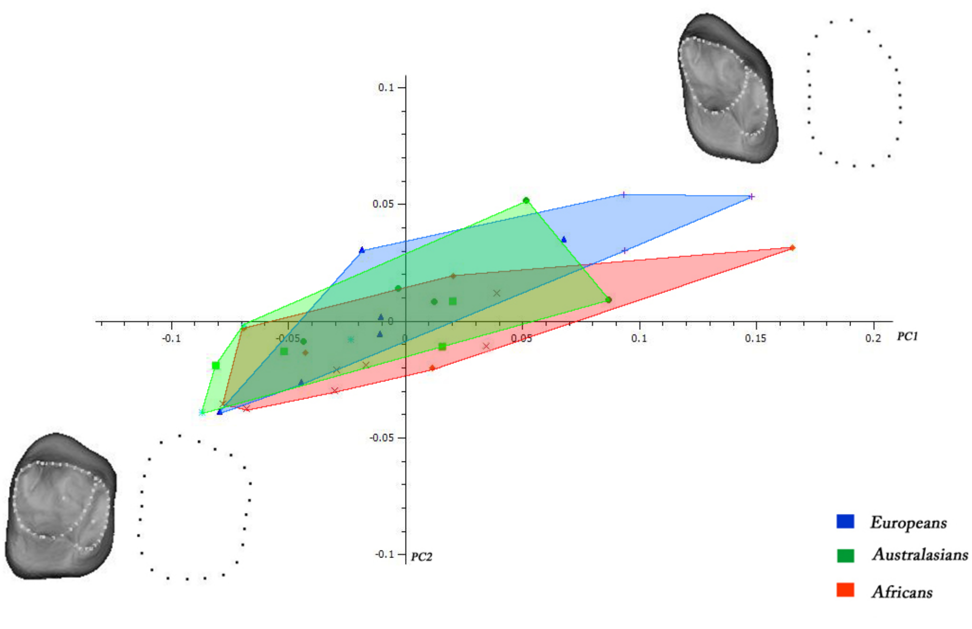

Fig. 24: Partial Least Square analysis of the EDJ and the cervical outline, for the legend, see Fig. 10

We can identify a similar pattern for EDJ and crown outline (pairwise correlation r=0.84, 85.85 % of the total squared covariance explained). If the EDJ has a rhomboid shape (in positive scores), the crown outline becomes ellipsoid. On the contrary, a squared shape (in negative scores) of the EDJ is linked with a round crown outline (see Figure 25). Again, there is not any noticeable clustering on a continental, respectively on a population level. Only the slight separation of the middle Europeans is detectable, but as mentioned above, the sample size of the middle Europeans was very small (n=3).

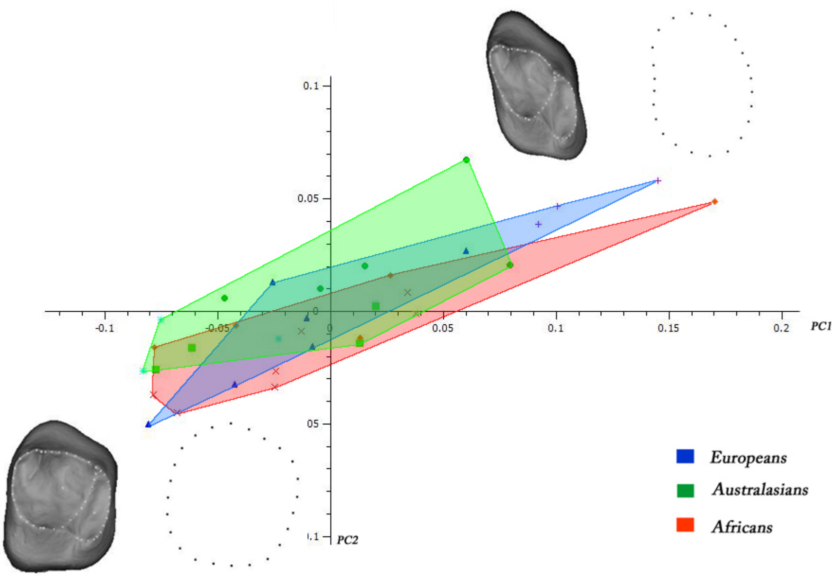

Fig. 25: Partial Least Square analysis of the EDJ and the crown outline, for the legend, see Fig. 10

Finally, the strongest correlation was observed in the cervical and crown outline (pairwise correlation r=0.92 %, 87.09 % of the total squared covariance explained). Here, the squared shape of the cervical outline is associated with a roundish shape of the crown outline (in positive scores). On the other hand, in negative scores, an ellipsoid shape of the cervical outline with a mesial embayment correlates with a bucco-lingual elongation and mesial embayment of the crown outline (see Figure 26).

Partial least square analysis (PLS) of the cervical and the crown outline showed a general overlap of all populations of the modern humans, and hence no peculiar pattern.

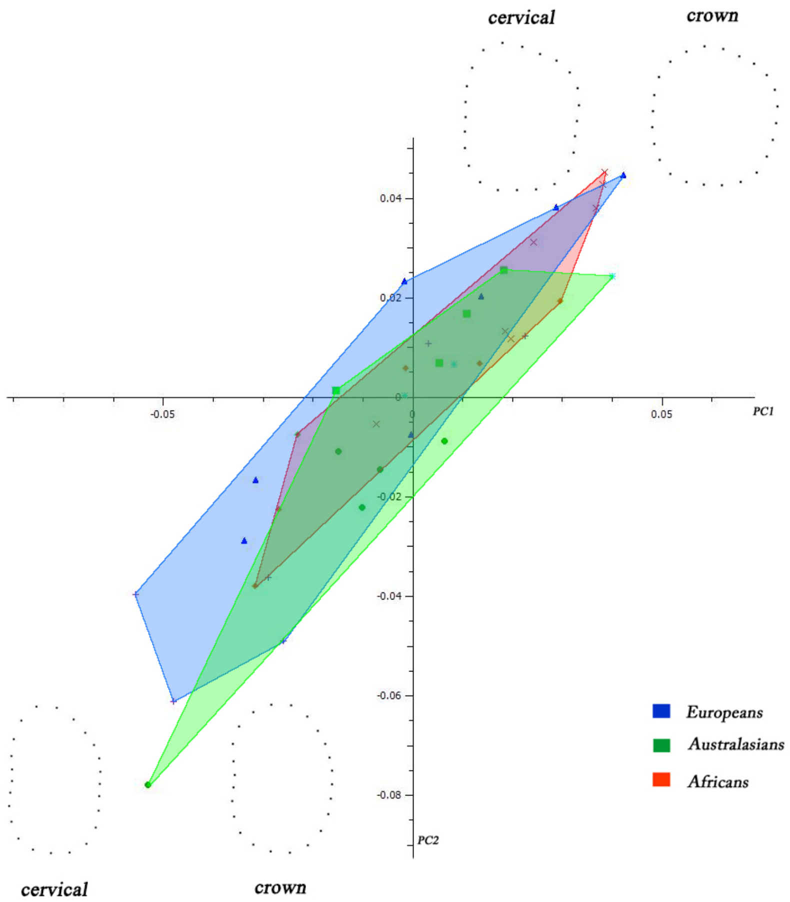

Fig. 26: *Partial Least Square analysis of the cervical and the crown outline, for the legend, see Fig. 10*

4.4 Allometry

The multivariate regression of the natural logarithm of centroid size on EDJ shape reveals a relatively small allometric effect, only 5.96% of the EDJ total shape variance can be explained by size. Larger molars tend to have a square shape and a well developed hypocone, whereas small molars show an ellipsoid shape with an elongation in the paracone-hypocone axis. At the same time, there can be a reduction of the hypocone observed. Having larger or smaller upper second molars could potentially be also a matter of sex. However, the combined dataset in a Mann-Whitney test (with Monte-Carlo permutation, n=10000) showed no significant difference (p=0.119) in size between males (n=20) and females (3). Five individuals were excluded from the combined dataset (hypocone reduction) and by remaining 10 specimens, the sex could not be determined.

4.5 Qualitative analysis of dental traits

One aim of this study was the analysis of non-metric dental traits represented in our sample. In a relatively high frequency we observed a hypocone reduction. The hypocone reduction is common in modern humans (Turner et al., 1991; Hillson, 1996), and we could identify it in 10 cases out of 38 thus in 26.32%. Elevated frequencies of hypocone reduction occurred in the European continental group, namely in four middle Europeans and three Avars (see Table 9). In five cases, specimens had to be even excluded from the EDJ analyses (marked with * in Table 9) because the hypocone was either completely missing, or only a slight ridging was visible. In all of these five individuals, the hypocone was reduced on both, the EDJ and the OES. Otherwise, in specimens CN 236, CG 495, and CG502, a small hypocone was present on both surfaces. In the case of the middle European specimen 502//126.804/1171 and the Centralafrican C 333, a reduced hypocone was visible on the EDJ, but there was any evidence of hypocone on OES.

Carabelli´s cusp can appear on the lingual side of the protocone and is less common in second molars, then in first molars (Hillson, 1996). It was identified only in two specimens in our sample (5.26 %) (see Table 10). In the Eastasian 1377//122.369/1412, Carabelli´s cusp was visible only on the OES, but not on the EDJ. In the other specimen (Centralafrican S 128), it appeared on both surfaces.

A metaconule was expressed in four specimens (10.52%)(see Table 11). It is particularly common in the Austrialian-Sahul populations (Bridsell, 1993; Scott and Turner, 1997) this is also partially reflected in our results. Furthermore, if a metaconule was present, it was visible in all cases on both surfaces, i.e. on the EDJ and on the OES.

Finally, we observed accessory mesial marginal cusps, especially in the Avar population (see Table 12). Only in CG 498 (Avar), these accessory cusps could be clearly identify on both surfaces. In other specimens, accessory cusps were visible only on OES, but not on the EDJ.

The qualitative analysis of the *P.pygmaeus* molars shows, that these upper second molars typically have a squared shape, and the occlusal enamel is markedly wrinkled (see Figure 27).

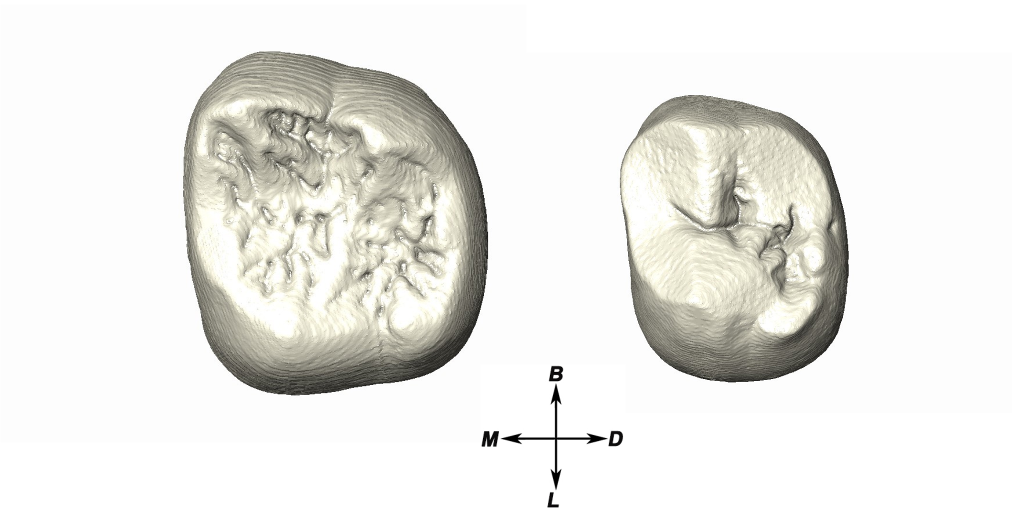

Fig. 27: *P. pygmaeus (left) with characteristic enamel wrinkling and modern H.sapiens (right)*

Table 9: *Specimens featuring a hypocone reduction*

*Those marked with * were excluded from the EDJ analyses*

Individuals	**Score of hypocone reduction, Turner (1991)**
CN 236 (Papuan)	3, small cusp
CG 495 (Avar)	2, slight cuspule
CG 502 (Avar)	2, slight cuspule
CG 582 (Avar) *	0 side is smooth, no hypocone
19710 (middle European) *	1, slight ridging
1149//125.011/1072 (middle European) *	0, side is smooth, no hypocone
502//126.804/1171 (middle European)	2, slight cuspule
709//122.622/1200 (middle European) *	1, slight ridging
C 333 (Centralafrican)	3, small cusp
S 87 (Centralafrican) *	1, slight ridging

Table 10: *Carabelli´s cusp expression*

Individuals	**Score of Carabelli´s cusp expression, Dahlberg (1956)**
S 128 (Centralafrican)	6, medium sized cusp
1377//122.369/1412 (Eastasian)	3, small Y-shaped depression

Table 11: *Metaconule expression*

Individuals	**Score of metaconule expression, Harris and Bailit (1980)**
CN 232 (Papuan)	5, medium sized cusp
C 104 (Aboriginal)	4, small cusp
1103//122.510/1554(middle European)	3, small cuspule
S 85 (Centralafrican)	3, small cuspule

Table 12: *Mesial marginal accessory tubercule expression*

Individuals	**Mesial marginal cusps, Kanazawa et al., (1990)**
CG 582 (Avar)	mesial paracone tubercule
C55 (Aboriginal)	mesial paracone tubercule
CG 498 (Avar)	mesial paracone tubercule
CG 498 (Avar)	protoconule

5.0 Discussion

The aims of this study were to provide both metrics for the EDJ and non-metrics for the OES and EDJ of second upper molars of recent human populations with different geographical origin and quite diverse subsistence strategies such as hunter/gatherers, nomads or agriculturists. We used Geometric Morphometrics to quantitatively explore the morphological variation of upper second molars of these populations. We examined the shape variation of the EDJ and the cervical, respectively crown outlines, and included qualitative descriptions of the EDJ surface.

There are several morphometric studies on upper molars (Kondo and Yamada, 2003; Kondo et al., 2005; Harris and Dinh, 2006; Gómez- Robles et al., 2007; Takahashi et al., 2007; Gómez- Robles et al., 2012; Bailey et al., 2014), on lower molars (Skinner et al., 2008a, Skinner et al., 2008b), and also on the lower and upper premolars (Martinón-Torres et al., 2006; Gómez-Robles et al., 2011), which describe the morphological variability of the human fossil record. However, in recent research, the detailed geometric morphometric analyses of the EDJ (in 3D) regarding maxillary second molars of modern human populations were still missing.

5.1 Shape analyses

In our shape analysis, we provided a general overview of the shape variation observed in modern humans. The results of all four tested aspects (EDJ, combined data set, cervical and crown outline) showed generally a wide overlap of all continental groups. Therefore, we can confirm our prior hypothesis that, despite different subsistence modes, respectively cultural and geographical backgrounds, a substantial overlap of all populations occurs. This is likely a consequence of the weak selective forces in operation. When extra-oral food preparation techniques gained importance (around 300 000- 400 000 years ago), lesser need of intracavital food fractioning or chewing led to the reduction of natural selective forces (e.g., Ragir, 2000; Lucas, 2004; Roebroeks and Villa, 2011; Shahack-Gross et al., 2014; Zink et al., 2014). Thus, we emphasize, that the random shape variation discovered in this study is of prime importance. In all performed analyses, we were not able to identify any specific shape pattern regarding continental groups. However, it is noteworthy, that if we consider data on a population level, we find a weak trend for clustering in some cases. In the case of the crown outline shape analysis, this concerns Europeans, which plot only on the negative side of PC1 while Khoesans are found on the positive one. Quite contrary to this result, the distribution of Aboriginals and Papuans extends across the entire range of variability of the whole sample.

The unique combined dataset allowed us appreciating the whole height of the tooth crown and thus we could analyse the coupled changes of the EDJ and the cervical outline. Square shaped molars are rather flat, while ellipsoid molars have clearly a higher crown. Additionally, we can observe a clockwise rotation of the cervical outline relative to the EDJ surface. We would like to stress the enormous potential of this methodology, to the contrary of 2D approaches, which can capture only the OES.

Based on the results of the combined dataset and the EDJ shape analysis, we emphasize, that the bucco-lingual diameter is a quite variable feature that leads to molars with a more rhomboid or a more squared appearance. Gómez-Robles et al. (2007) and Bailey (2004) described that modern humans developed primitive square shaped crowns while *H. neanderthalensis* showed rather rhomboid shapes. These results contradict our own, because we find this whole range of variation in our sample comprising only modern humans. Further investigations are needed in this direction. Nevertheless, such morphological differences could potentially be used in taxonomic assessments.

Similarly to the above mentioned analyses, we identified also in cervical outlines a general overlap of all groups, not only on the continental, but also on the population level. The whole variance of the cervical outline is mainly concentrated in distal areas, namely those of the hypocone and the metacone.

The variation of the crown outline has been in the center of interest in a number of studies of taxonomic assessment (e.g., Bailey et al., 2014; Gómez-Robles at al., 2011). Benazzi et al. (2012) describes the crown outline as a more reliable parameter for taxonomic assessments than the cervical outline. The morphological variability of both outlines observed in our analyses appeared mainly in the distal areas of the teeth, while the mesial part of the teeth kept their shape relatively stable. We find higher variation (in the context of distal reduction) in the distal areas. This was also observed in upper premolars (Gómez-Robles et al., 2011) or upper second and third molars (Gómez-Robles et al., 2012), which is corroborating our results. Looking at mandibular molars, Corruccini and Potter (1981) suggested that distal cusps showed higher asymmetry then the mesial ones. Scott and Turner (1997) argued, that increased asymmetry in distal cusps is probably due to environmental and developmental factors.

The occlusal mechanisms necessary for functional dentitions of course have an impact on the spatial arrangement of tooth cusps. Generally, following the concept of bilaterally balanced occlusion, each tooth has contact with two counterparts in the opposing dental arch (Kraus et al., 1969). The lingual cusps of maxillary teeth and the buccal cusps of mandibulary teeth are functionally supporting cusps. The upper buccal, respectively lower lingual cusps are guiding cusps (Kraus et al., 1969). However, our results show that distal regions of the upper second molars are more variable then mesial regions. While the mesial cusps are articulating into the central fossa of the lower M_2, the distal cups articulate with the interproximal marginal ridge of the third molar (see Figure 28 and 29). As known, the third molar is anatomically the most variable and unpredictable molar (e.g., Kraus et al., 1969; Hillson, 1996; Scott and Turner, 1997). Third molar crowns have highly irregular morphology, comprising all possible molar types. The roots are also variable and the expression varies from one fused root to three single roots. Finally, these molars have very fluctuating eruption times (often associated with eruption failures), or they are even congenitally missing (e.g., Kraus et al., 1969; Hillson, 1996; Scott and Turner, 1997). Therefore, the interaction of the distal regions of upper second molars with those shapewise unpredictable lower third molars could be a reason why we found more variability in the distal region rather than in the mesial one.

Fig. 28: *Symmetrical occlusion in lingual view, arrows indicate cusp tips which articulate into central fossa, adapted from Kraus et al. (1969)*

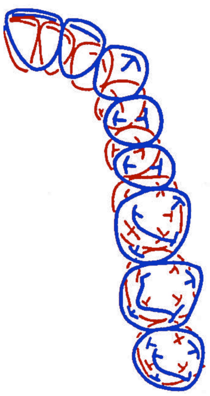

Fig. 29: *Normal Occlusion, maxillary teeth = blue, mandibulary teeth=red, adapted from Hillson (1996)*

5.2 Partial Least Squared Analysis

According to our investigations, currently there is no study, which examined how individual tooth traits in upper second molars covary with each other.
Our PLS analyses showed that EDJ, cervical and crown outline correlate quite highly. The square shape of the molars is linked with an also squared cervical outline and a roundish crown outline without any protrusions. With these results, we are in the agreement with Gómez-Robles et al. (2007), however they examined upper first molars.

5.3 Size

The relative size of the teeth was also a point of interest in our study. A significant reduction of the whole masticatory apparatus characterizes the evolution of *Homo* (e.g., Brace, 1967; McHenry, 1994; Wood and Collard, 1999; Zink et al., 2014). There are several researches (e.g., Frayer, 1978; Calcagno and Gibson, 1991), which claim an impact of natural selective factors on tooth size. The maximum of the tooth size reduction occurs in the Late Pleistocene (75,000-100,000 years ago) with 1 % in 2000 years and this process was maintained by the adaptation of new food processing techniques such as earth ovens (Brace et al., 1991). This non-dental food preparation technique played an important role in the tooth size reduction as the lesser need of intracavital food fractioning or chewing led to the reduction of natural selective forces (e.g., Brace et al., 1991; Zink et al., 2014). Brace et al. (1991) argued that earth ovens represent cultural adaptation in the periods of intensive glaciation and therefore were used for the first time in the northern temperature zone. Europeans, as the descendants of these populations underwent the dental reduction for the longest time period. To the contrary, the idea of earth ovens for cooking spread to Australia ca. 30,000 years ago (Bowler et al., 1970; Brace et al., 1991) and would mean, that Australian Aboriginals were the last starting the process of dental reduction and therefore should have the biggest teeth. However, while some of the argumentation for a smaller dentition in the presence of cooking is convincing, it remains very questionable how glaciation and northern and southern habitats really would impact on tooth size. For instance, the first anatomically modern humans were present in Africa, also around the time, that Brace et al. considers (100-75 kya), and glaciation played no role there. Subsequently, these people migrated to Eurasia and Australia, but we have no indication, if they arrived earlier in the harsh environments of glacial Europe than in the warm climates of Australia. Nevertheless, in our results, the Australasians (Austral Aboriginals, Papuans and East Asians) developed the biggest upper second molars. On the contrary, Europeans (middle Europeans and Avars) have indeed the smallest. Similar findings were also published in other studies of world populations, for example by Hanihara and Ishida (2005). After the adaptation of earth ovens, there were new tendencies in food processing. New techniques such as pounding or grinding, and finally usage of pottery, were developed (Brace et al., 1991). Especially pottery usage induced a further relaxation of the selective forces and the dental reduction accelerated to the end of the Pleistocene to 1 % per 1000 years (Brace et al., 1991; Brace, 1995). With our results of lnCS of the combined data set, we can approximately confirm this suggested tempo of dental reduction, because our Europeans possess teeth, which are approximately 0.80 % (based on average lnCS for both populations) smaller in size than the molars of the Avars, which lived about 1,200 years earlier. We suppose, that if our sample of the Europeans would include more females (Avars included two females, Europeans included none), the estimated tempo of the dental reduction could be quite similar (1%) to the one mentioned above.

5.4 Allometry

The multivariate regression of natural logarithm of centroid size on the EDJ shape variables reveals a very small allometric effect, which accounts only for 5,96% of the observed variation. As a result most of the morphological variability encountered cannot be interpreted in terms of allometry. In general, there is a discrepancy between published opinions, i.e. how strong the effect of allometry would be on tooth morphology in human evolution. Some authors assume that there are not any allomeric effects at all, which would have had an impact on the dentition during hominin evolution (Wood et al., 1983; Bailey and

Lynch, 2005). In contrast, Martinón-Torres et al. (2006) or Gómez-Robles et al. (2007; 2008) emphasized significant allometric effects (but using quite different samples including Australopithecines, and 2D data). In our study, larger molars general tend to have a more squared shape, whereas small molars rather develop an ellipsoid shape. Similar results were described in the morphological study of upper first molars from Gómez-Robles et al. (2007).

5.5 Sexual dimorphism

Generally, sexual dimorphism in tooth size is less pronounced in humans, than in other primates, and it is more concentrated in frontal dentitions (Hillson, 1996). Still, differences in crown diameters have been used as a method of the sex determination in several studies (e.g., Garn et al., 1977; De Vito and Saunders, 1990; Bermudéz de Castro et al., 1993). Kondo et al. (2005) claimed that the percentage of the sexual dimorphism in crown diameters of upper molars ranged from 3.4 – 6.4%. He emphasized, that the sexual dimorphism is high for some cusps, but only moderate for the whole crown of the molars. Probably the Y-chromosome affects the sexual dimorphism of tooth size, because it promotes the growth of both, enamel and also dentine (Alvesalo, 2013). The complex twin study of Hughes and Townsend (2013) showed that the variation in crown size is affected by strong genetic contributes. Although, there is evidence in the literature, that male teeth are larger than female teeth (e.g., Garn et al., 1977; Hillson, 1996), we could not prove this with our data. The combined dataset in a Mann-Whitney test (with Monte-Carlo permutation, n=10000) showed no significant difference (p=0.119) in size between males and females.

5.6 Non-metric trait expression

Dental traits are extremely variable within and between populations (Scott and Turner, 1997). Butler (1956) suggested that growth and folding of the membrana praeformativa is responsible for the crown morphology. He mentioned, that the composition of the crown features is already present on the membrana praeformativa, before the calcification process begins. Additionally, Butler (1956) also argued, that the EDJ contains the complete original information about this membrane. This led him to the assumption, that the traits expressed on the OES are the result of the EDJ shape in combination with additional variable effects of the enamel deposition process. However, a certain lack of correspondence between EDJ and OES surfaces were discovered in several later studies (e.g., Korenhof, 1960; Corruccini, 1987; Schwartz et al., 1998). These authors argued, that on the EDJ there are some rudimentary traits, which are not necessarily present on the OES too. However, recent researches based on μCT data (Skinner et al., 2008; 2009; Bailey et al., 2011) underline the correspondence and predictive relationship between EDJ and OES.

Regarding our results, we agree with Skinner et al., (2008; 2009), who claimed the general analogy between EDJ and OES. Despite of this, in few cases (see Results, Subsection 4.5), the OES lacked the expression of some corresponding traits on the EDJ. Therefore, we concluded that there is a concordance between the EDJ and OES to a certain degree.

To be more specific, one of the dental traits identified in our sample was the hypocone reduction. This in agreement with Scott and Turner (1997), who suggested, that especially Europeans tend to show a hypocone reduction. They argued, that the frequency of the total hypocone loss in Europeans amounts about 20-35 %. In our study the partial hypocone loss in the European continental group accounts for 30% (total hypocone loss ca. 14 %). As mentioned above, the hypocone represents the last cuspal addition to the tooth relief (ontogenetically, but also phylogenetically), and therefore it is the most often reduced cusp in molars (Corrucini, 1979; Kraus and Jordan, 1965). This hypothesis was pick up also by other authors. Jernvall (2000) stated, for instance, that the earlier termination of the cusp morphogenesis could lead into higher rates of the cusp reduction in distal molars.

Recent studies of Gómez-Robles et al. (2012), but also Martinón-Torres et al. (2012), described a relatively high prevalence of hypocone reduction (only three cusped upper molars) in modern *H. sapiens*. We found the hypocone reduction in several Avars (warrior nomads of the 7th and 8th century with Asian background) and in middle Europeans. Since these last mentioned specimens with a total reduced hypocone (five specimens, 14% of the whole sample size), had to be excluded from the EDJ shape analyses for technical reasons (see Methods), the effect of the hypocone reduction could be interpreted only by means of qualitative description. This explains, why the hypocone reduction is not noticeable in our EDJ shape analyses.

Carabelli's cusp was only expressed in two specimens in our study. The expression of this dental trait in modern human was assessed in several other studies. Harris (2007) described a strong correlation between Carabelli's cusp and crown size. He assumed, that the longer growth period of teeth (linked with larger crown size) could be a crucial factor for the higher expression of Carabelli's cusp. In contrast to this, Garn et al. (1966) did not find any coherence between tooth size and the expression of Carabelli´s trait. Hunter et al. (2010) stated, that the expression of Carabelli's cusps depends on the interrelation between tooth size and intercuspal areas. The highest prevalence of this trait is found in larger molars featuring relatively small areas. We found Carabelli´s cusp in one Central African and one Eastasian individual. However, these two individuals are average-sized. Unfortunately, our small sample size prohibited a definite statistics for Carabelli´s cusps correlating with size. Despite a huge amount of published literature about this topic, there is still no clarity in evolutionary history about Carabelli's trait in upper molars, and further research is required.

Last but not least, we found the metaconule, an accessory cusp, in four specimens in our sample. Interestingly, two of these specimens belong to the Australasian continental group and the other two are European, respectively African. Several authors reported the metaconule expression. Kanazawa et al. (1992) described the high frequencies found in Australians (36.4%). Also Bridsell (1993) observed that this trait is extraordinarily often present in

Australian Aboriginals. This would support our findings, however, due to our small sample size we can neither confirm nor reject any hypothesis.

5.7 Pongo pygmaeus

The outer occlusal surfaces of the *P.pygmaeus* teeth possess a multitude of little ridges known as wrinkles or crenulations (Swindler, 2002). The molars have four cusps, and the EDJ morphology is very similar to modern humans. Therefore, in our shape analyses, *P.pygmaeus* is localised fairly near (cervical outline, combined dataset), or partly (crown outline), respectively completely, overlaps (EDJ) with modern human populations. Regarding size, we showed that molars of the *P. pygmaeus* group are significantly larger ($p<0.001$) than molars of modern humans.

5.8 Summary

The results of our shape and form analyses of second upper molars of recent human populations can be useful for taxonomic purposes in comparative studies dealing with fossil hominins. The study also accentuates the importance of geometric morphometric techniques to exactly analyze the dental morphological variation in modern humans. Due to the huge potential of this methodology, there is an open field for future researches. These studies should also focus on other tooth groups (currently there is a lack of research on frontal dental material, such as incisors or canines) and try to answer suggested questions in the framework of taxonomic and phylogenetic investigations. Nevertheless, additional data should be gathered from larger samples of dental material.

6.0 Conclusion

The usage of modern digitalization techniques such as destruction-free µCT allows detailed research on the OES and underlying EDJ, to understand the complex dental morphology for comparative, taxonomic, and phylogenetic purposes.

Our analyses of shape and form of the human second upper molar could not identify any relevant population specific patterns in the tested tooth traits (EDJ, combined dataset, cervical and crown outline). Therefore, we confirmed our prior hypothesis. Despite quite different populations (geographically, culturally, lifestyle, subsistence strategies), we are not able to separate them based on shape or form. This is likely due to weak selective advantages for any specific tooth shape or form. The reason behind could be the ubiquitous acceptance of non-dental food preparation techniques (chopping, slicing, cooking, pottery usage), which led to a lesser need of intracavital food fractioning or chewing, and therefore to a reduction of the selective pressure. Furthermore, we expected that, as a result of active functional constraints on tooth shape, there would be some regions of the tooth that remain more stable in shape, while others might show a higher variation. Especially, the analyses of the cervical and the crown outline demonstrated that distal regions of molars exhibit more variation than mesial regions. We argue, that the occlusal mechanisms, necessary for a functional dentition, have an impact on the spatial arrangement of tooth cusps. Therefore, we suppose, that the occlusal interaction of distal cusps of M^2s with the very variable lower third molar affects the occurrent variation, which was found mainly in those distal regions of upper second molars. The multivariate regression of natural logarithm of centroid size on the EDJ shape variables reveals a very small allometric effect. We therefore support the notion, that most of the morphological variability cannot be interpreted in terms of allometry. Furthermore, our results show, that Australasians have significantly larger teeth than Europeans. These results are in agreement with the current literature.

Our study has demonstrated the great potential of this methodology. Future research requires the inclusion of larger samples and other dental classes, and should concentrate on quantification of the specific morphological variability between and within different dental classes. Finally, we emphasize the relevance of our results for the taxonomic assignment in comparative analyses with other fossil hominins.

7.0 Acknowledgements

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9.0 Attachment

Lebenslauf

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