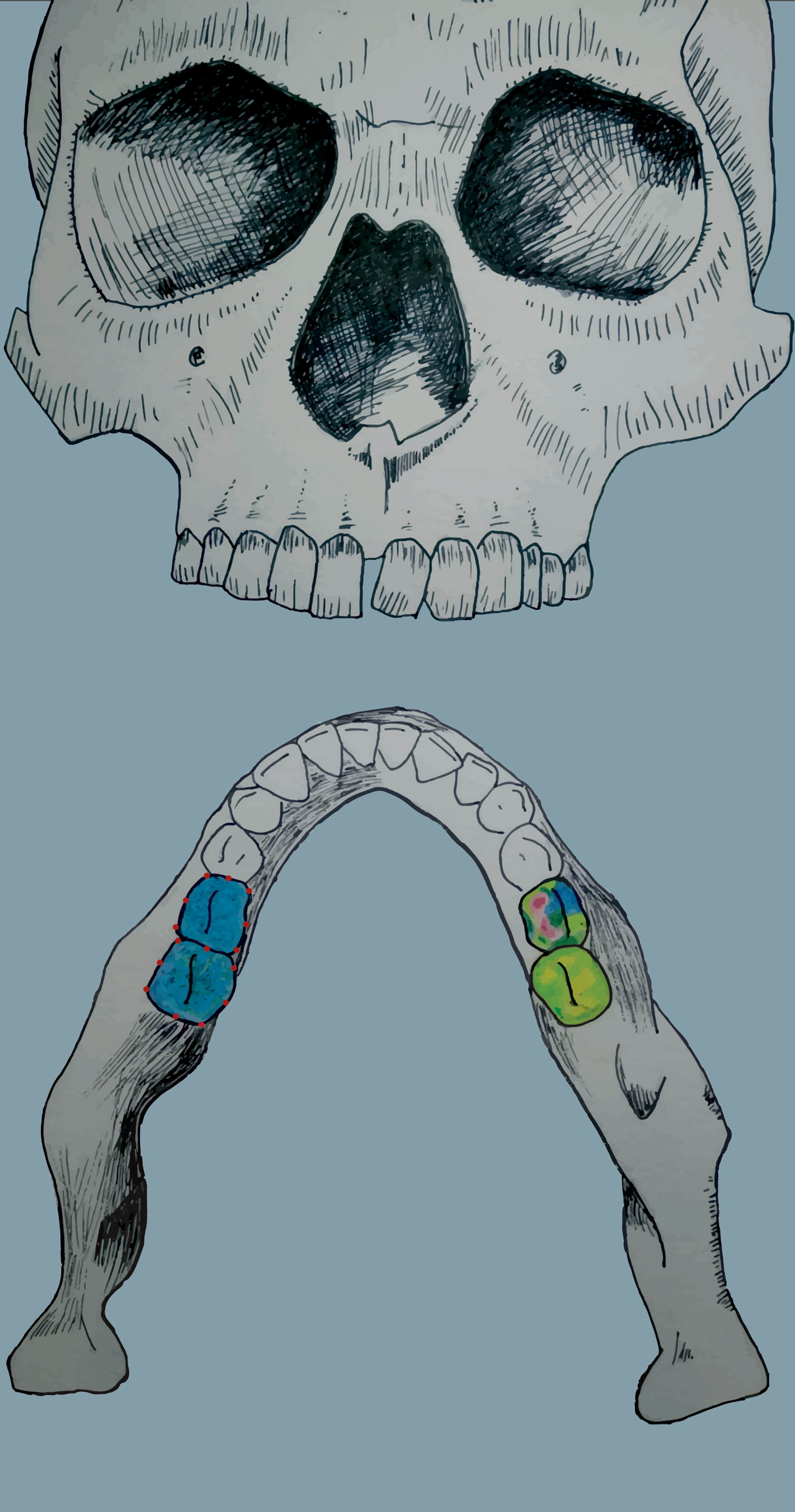
UNIVERSITAT DE BARCELONA

Geometric morphometrics and topographic analyses of dental wear in modern human populations

Elisabeth Cuesta Torralvo



GEOMETRIC MORPHOMETRICS AND TOPOGRAPHIC ANALYSES OF DENTAL WEAR IN MODERN HUMAN POPULATIONS

Elisabeth Cuesta Torralvo

Doctoral Thesis 2020

GEOMETRIC MORPHOMETRICS AND TOPOGRAPHIC ANALYSES OF DENTAL WEAR IN MODERN HUMAN POPULATIONS

Dissertation by

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A mis padres, mi yaya Pepa y Nala

A Juan, a mis amigos

“Show me your teeth and

I’ll tell you who you are”

George Cuvier

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Abstract

Dental wear is a natural, complex, physiological process of gradual enamel tissue loss that occurs during an individual's life span and provides information about dietary habits, food processing techniques and cultural practices. Many studies have characterised dental wear in human populations by using observer-dependent, qualitative methods. In contrast, more objective, quantitative approaches have been scarcely used. This thesis aims to assess differences in molar morphology among modern human populations and the effect of dental wear on shape by using novel quantitative methods, such as the percentage of dentine exposure (PDE), 3D geometric morphometrics (GM) and dental topography. The PDE of mandibular permanent first molars recorded in a known-age Baka Pygmy forager population showed an close relationship between wear and age, no sexual dimorphism in wear patterns and reduced PDE values of ≈4% for a foraging population relying on Underground Storage Organs (USO) consumption, likely due to culture-specific dietary proclivities that influenced dental wear rates. Three-dimensional (3D) dental crown analyses (GM and dental topography) carried out in maxillary and mandibular permanent first and second molars of the Coimbra International Exchange known age-at-death skull collection showed significant regressions between the morphometric variables and age-at-death, with a significant portion of the overall shape variation attributed to anatomical traits (e.g. cusp and groove patterns) independently of wear, while another significant portion attributed to the loss of dental crown height with age. The 3D-GM PCA procedure applied to the repeated measurement test showed an intra-observer methodological error <5%. Dental topographic analysis of the Portuguese sample showed negative correlations with age in all analysed teeth, with surface curvature (DNE), complexity (OPCR) and crown relief (RFI) scores decreasing with age. Dental topography procedures applied to maxillary permanent molars of Central African populations with distinct dietary habits (Pygmy foragers and Bantu-speaking agriculturalists) and distinct degrees of dental wear patterns showed DNE and occlusal relief (OR) scores that also decreased with wear, while ambient occlusion (PCV) and OPCR increased with wear. The Pygmy foragers showed higher OPCR and DNE values than the Bantu-speaking agriculturalists. These variables also proved to effectively distinguish between foragers and agriculturalists in the PC analysis. This research has also shown that it is important to pay attention to the different cropping methods used in the quantification of RFI and OR since the different methodological perspectives may cause distinct methodological errors.

Resumen

El desgaste dental es un proceso fisiológico, natural y complejo de pérdida gradual del esmalte que se produce durante la vida de un individuo y que proporciona información sobre los hábitos alimentarios, las técnicas de procesamiento de alimentos y las prácticas culturales. Muchos estudios caracterizaron el desgaste en poblaciones humanas mediante métodos cualitativos dependientes del observador. En cambio, pocos estudios utilizaron métodos objetivos y cuantitativos. Esta tesis pretende evaluar las diferencias morfológicas de los molares en poblaciones humanas modernas, así como el efecto del desgaste en la forma del diente mediante métodos cuantitativos novedosos, como el porcentaje de exposición de dentina (PDE), la morfometría geométrica (MG) en 3D y la topografía dental. El PDE de los primeros molares inferiores de una población de cazadores recolectores de pigmeos Baka de edad conocida mostró una relación significativa entre desgaste y edad, sin dimorfismo sexual en los patrones de desgaste y valores reducidos de PDE del ~ 4% para una población cazadora recolectora dependiente del consumo de *Underground Storage Organs* (USO), probablemente debido a las particularidades culturales relacionadas con la dieta que influyeron en el desgaste. Los análisis 3D (MG y topografía dental) en primeros y segundos molares superiores e inferiores de la colección de *Trocas Internacionais* de cráneos de Coímbra, con edad de la muerte conocida, mostraron regresiones significativas entre las variables morfométricas y la edad de la muerte, con una parte de la variación de la forma del diente atribuida a su anatomía (p. ej. patrones de cúspides y surcos) e independiente del desgaste, y otra, a la pérdida de altura de la corona con la edad. El ACP de la MG en 3D aplicada a la prueba de medición repetida mostró un error metodológico intraobservador del <5%. El análisis de la topografía de la muestra portuguesa mostró correlaciones negativas con la edad, con valores de curvatura (DNE), complejidad (OPCR) y relieve de la corona (RFI) que disminuyen con la edad. Los análisis de topografía en molares superiores de poblaciones centroafricanas con dietas distintas (pigmeos cazadores recolectores y agricultores de habla bantú) y distintos grados de desgaste mostraron valores de DNE y relieve oclusal (OR) que disminuían con el desgaste, mientras que la oclusión ambiental (PCV) y OPCR aumentaba. Los cazadores recolectores mostraron además valores de OPCR y DNE más altos que los agricultores. Estas variables fueron efectivas para distinguir entre ambas dietas en el análisis de CP. También se demostró la importancia de prestar atención a los métodos de corte utilizados en la cuantificación de RFI y OR, ya que pueden causar errores metodológicos.

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2D/3D	Two/Three-dimensions
AIC	Akaike information criterion
BCO	Basin cut off
CEJ	Cemento-enamel junction
CI	Confidence interval
CS	Centroid size
CV	Coefficient of variation
DNE	Dirichlet normal energy
DE	Dentine exposure
EEC	Entire enamel cap
GPA	General Procrustes Analysis
GIS	Geographic Information Systems
GM	Geometric morphometrics
OA	Outline area
OPC	Orientation patch count
OPCR	Orientation patch count rotated
OR	Occlusal relief
PC	Principal component
PCA	Principal Component Analysis
PCV	Portion de ciel visible
PDE	Percent of dentine exposure
RFI	Relief index
RMA	Reduced Major Axis
SA	Surface area
SD	Standard deviation
SEM	Scanning electron microscopy
SQ	Shearing quotient
SR	Shearing ratio
TME	Technical measurement error
TOA	Total occlusal area
USO	Underground storage organ

INTRODUCTION

1. Human dentition

1.1. Tooth anatomy

Teeth are hard and resistant structures that are mainly used for obtaining, cutting, and crushing items of food for efficient digestion. Because teeth are also important to communicate effectively, missing teeth can affect the ability to speak properly. Additionally, they are crucial to maintain the structure of the face and support the facial muscles. Each tooth consists of a **crown** and one or more **roots**. The crown is the part that is visible in the mouth and the root is the part embedded in both the upper and lower jaws. The crown and root join at the **cemento-enamel junction (CEJ)** or cervical line, which is embraced by the **gingiva** or gum, a soft connective tissue covered by a mucous membrane (Hillson, 2002; Scheid and Weiss, 2012).

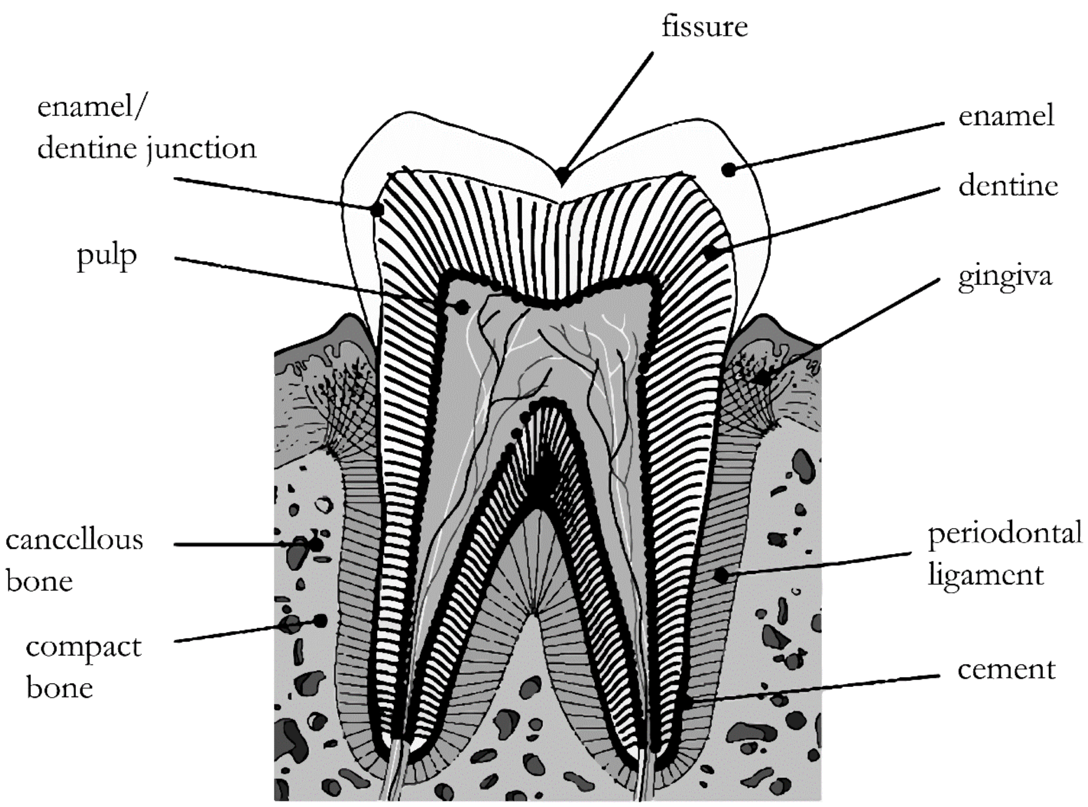


Figure 1. Dental anatomy of a human molar that shows the distribution of the tooth tissues. From Lucas (2004).

Tooth is composed of three hard and mineralized tissues, the enamel, the cementum, and the dentine; and a soft tissue component, the pulp (Figure 1). The **enamel** is the protective outer surface layer of the crown and is mostly comprised of the mineral calcium hydroxyapatite, which makes the enamel the hardest and denser substance in the human body. This tissue develops from the enamel organ (ectoderm) and is a product of the specialized epithelial cells, the ameloblasts. The **cementum** is the external and thin layer that covers the tooth root and consists of calcium hydroxyapatite and collagen. This tissue gives attachment to the periodontal ligaments that hold the tooth firmly in place within the alveolar bone. It develops from the dental sac (mesoderm), produced by cementoblasts. Underneath the enamel and the cementum, there is the **dentine**, a dense tubular tissue that forms the major bulk of the tooth and mainly consists of apatite crystals of calcium and phosphate. The dentine is formed by the odontoblasts, a highly specialized cell line originated from dental papilla (mesoderm). The **pulp** is a soft connective tissue, not mineralized or calcified, that enervates and contains containing a rich supply of blood vessels and nerves. It occupies a cavity located in the center of the tooth and communicates with the periodontal ligament via a hole or holes in the apex of the tooth root. The portion of the pulp in the crown is called the pulp chamber and the portion in the root is called the pulp/root canal developing the dental papilla (Nelson and Ash, 2010; Scheid and Weiss, 2012).

1.2. Characteristics of the human dentition

Humans have **heterodont** teeth as we have four different tooth classes: incisors, canines, premolars, and molars (Figure 2). Besides, like most mammals, humans have a **diphyodont** type of dentition, meaning they have two successive sets of functional teeth during the human life span: the deciduous or primary dentition and the permanent or secondary dentition (Nelson and Ash, 2010; White *et al.*, 2012). The **deciduous (primary or 'milk')** dentition is the first set of teeth to erupt at about 6-12 months of age until 25-33 months of age. It consists of a total of 20 teeth, 10 in each jaw, and with the dental formula 2102/2102. This indicates that each half of the upper and lower jaws has two incisors (central and lateral), one canine, and two molars (first and second). At the age of 6, the deciduous teeth begin to be replaced by the **permanent teeth**. This process continues until the age of 12-13, when the second molar erupts, or until the age of 18-25 years when the third molar is expected to erupt. There are 32 permanent teeth, 16 in each jaw, and with the **dental formula 2123/2123**. Therefore, each half of the upper jaw and the lower jaw has two incisors (central and lateral), one canine, two premolars, and three molars (first, second and third) that will remain in place and functional until death (Figure 2). The incisors and canines are often referred to as the anterior teeth, while those behind the canine are called posterior teeth (White *et al.*, 2012).

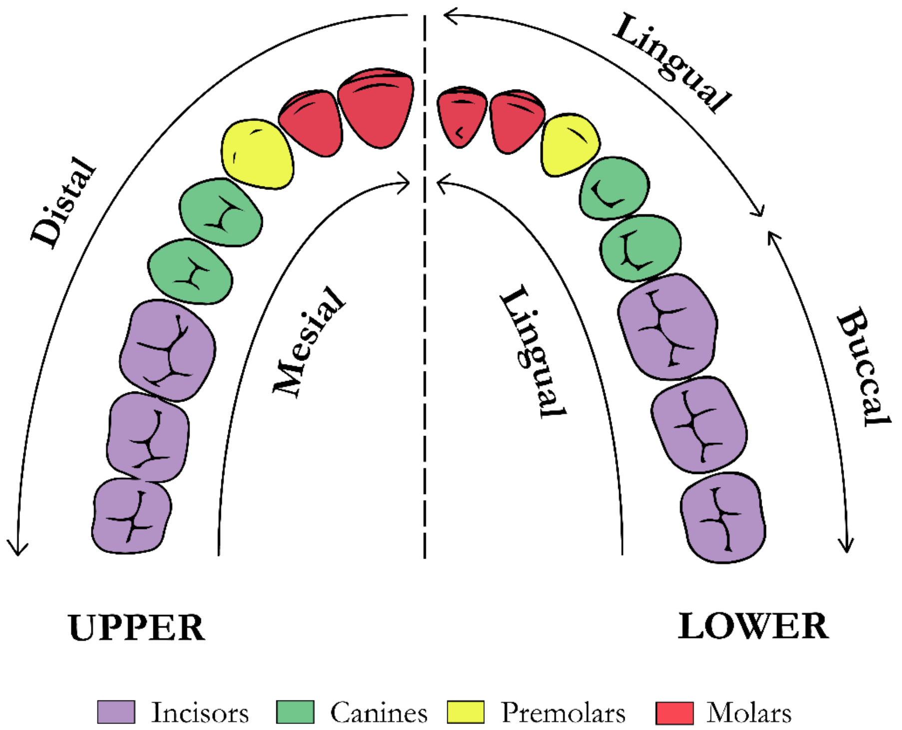


Figure 2. Representation of the upper (left) and lower (right) permanent human dentition.

Incisors, one central and one lateral, are spatulate teeth placed in the front of the mouth. While upper and lower central incisors are next to the midline, lateral incisors connect with the canines on their distal side. Incisors have one toot and their crowns are flat and blade-like, with sharp biting surfaces that help to cut and shear food into small chewable pieces. They also enable to articulate speech and help to support the lip. Newly emerged permanent incisor teeth present **mamelons**, three rounded labial lobes which quickly wear off with age. Generally, the upper incisors are bigger, their crowns have deeper fossae and more pronounced marginal ridges and *cingulum* on their lingual aspect compared to the lower incisors. Besides, the upper central incisors have the tooth's largest mesiodistal distance in comparison to any other teeth (Lucas, 2004; Nelson and Ash, 2010).

Canines are the only teeth in the dentition with a single cusp and the longest teeth in the dental arch. Generally, these conical-shaped teeth assist the incisors in biting. The upper and lower canines have similar morphologies, but the upper ones are larger and longer than the lower ones. Canine roots are longer and larger than other roots in the same dentition (Lucas, 2004). Behind each canine there are two **premolars**, which can tear and pierce the food as well as help the grinding of the molars during mastication. Premolar crowns are round, shorter than canine crowns, and smaller than molar crowns. They usually have two cusps, but they may present a variation in the number of cups from one to three cusps. They have a transitional form between the anterior and the posterior teeth (Lucas, 2004; Scheid and Weiss, 2012). Typically, premolars have one root, except for the upper first premolars that usually have two roots. The first, second, and third **molars** are the most posterior and strongest teeth in the dental arch. Besides, these square-crowned **bunodont** teeth usually have four or five cusps that play an important role in the mastication of food (chewing and grinding). Molars also maintain the vertical dimension of the face and the continuity within the dental arches. Typically, molars have three roots in the upper molars, while the lower molars have two roots. However, the number of roots in molars may vary (Nelson and Ash, 2010; Scheid and Weiss, 2012).

1.2.1. Morphology of the upper and lower permanent molars

In humans, as in all other primates, the morphology of the molars derives from a primitive triangular or **tribosphenic** structure (Simpson, 1936; see also Dean, 1992). In upper molars, this structure is called the **trigon** and is formed by three major cusps: the **paracone** and **metacone** on the buccal side, and the **protocone** on the lingual side (Figure 3). A fourth cusp is added onto the modified original triangle of cusps, the **hypocone** that is placed on a distal platform: the **talon**. In the lower molars the triangle of cusps, the **trigonid**, is mirror-imaged, with the **paraconid** and **metaconid** on the lingual side, and the **protoconid** on the buccal side. An additional platform, the **talonid**, is added to the distal part of the molar. This structure is formed by two or three cusps: the buccal **hypoconid**, the lingual **hypoconulid**, and the **entoconid**, in the bucco-distal side (Hillson, 2002; Lucas, 2004).

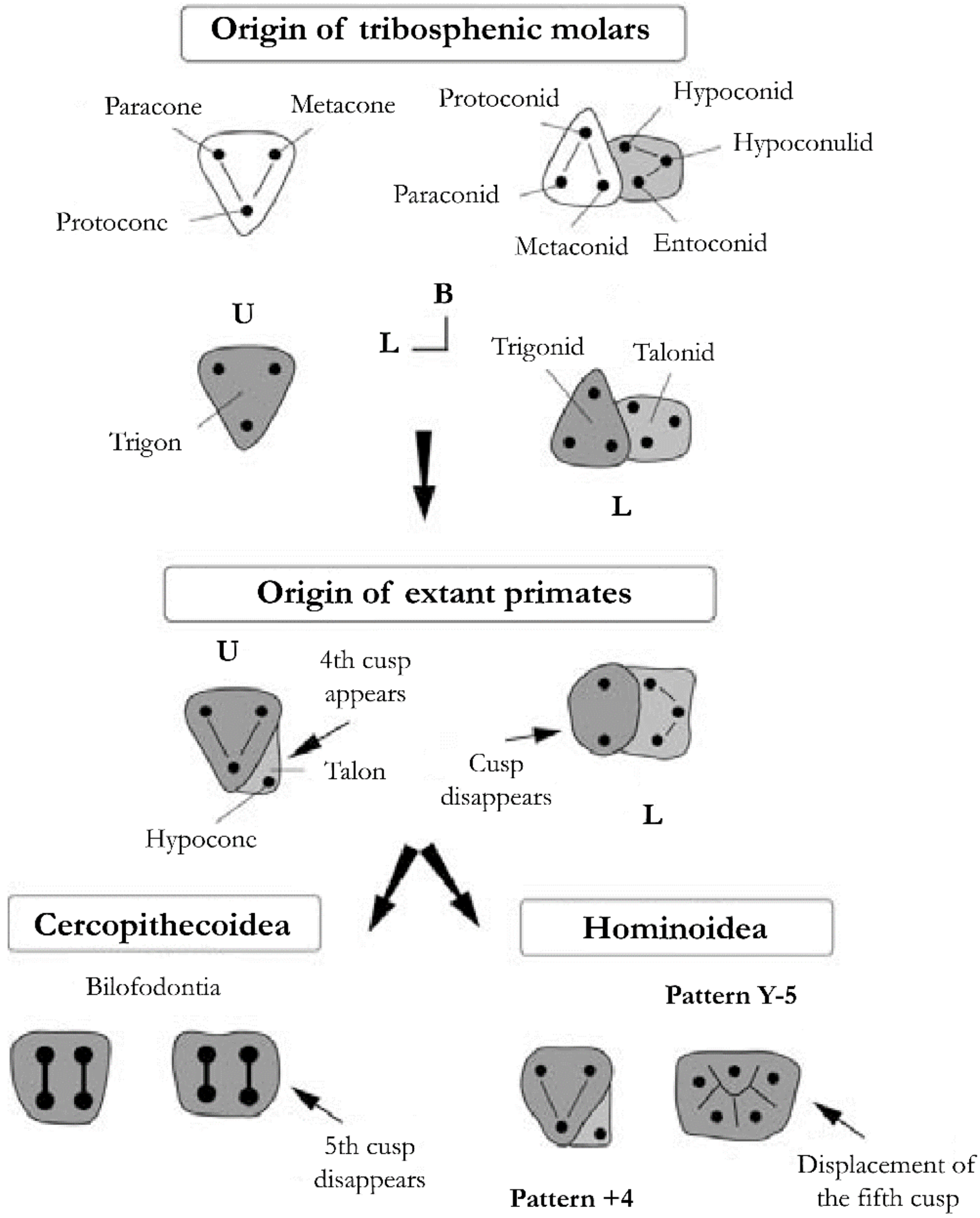


Figure 3. Origin of the *Hominoidea* upper and lower molars from the primitive triangular or tribosphenic structure. Note the archaic upper molars have a structure called trigon, to which is added a distal platform, the talon, where we can find a fourth cusp, the hypocone, as we can observe in the extant *Hominoidea* upper molars. The archaic lower molars have a trigonid added to a talonid in the distal part of the molar. Note a cusp disappears during the origin of extant primates and finally, there is a displacement of the fifth cusp that forms the characteristic pattern Y-5 of the *Hominoidea* lower molars. In the case of the *Cercopithecoidea*, molars become bilophodont, with a fifth cusp that disappears. U: upper; L: lower; B: buccal; M: mesial. Modified from Butler (1978); Dean (1992) and Lucas (2004).

The **upper first molar** is normally the largest tooth in the maxillary arch. From an occlusal view, the tooth is larger buccolingually than mesiodistally. It has four well-developed functioning cusps, two buccal (paracone and metacone), and two lingual (protocone and hypocone), arranged in a rhomboid shape (Figure 4). The protocone is almost always the largest and highest cusp and the hypocone is the smallest. Sometimes there is an extra small, non-functional supplemental cusp, the tubercle of Carabelli, found lingual to the metacone cusp. This trait has been used to distinguish among populations since its frequency of presence greatly varies (Scott, 1980). The upper first molars exhibit five developmental grooves on its occlusal surface: the central, buccal, distal oblique, lingual, and, sometimes, the transverse groove of the occlusal ridge. Most frequently these molar teeth have three well-formed roots, two placed buccally and one lingually, being the lingual one the longest and the distobuccal one the smallest of the three (Nelson and Ash, 2010).

The **upper second molar** is slightly smaller than the first molar, but its buccolingual diameter is about the same. Depending on their occlusal anatomy, there are two types of upper second molars: (i) four cusped type with a rhomboidal occlusal design that resembles the upper first molar, although the rhomboidal outline is more extreme, with developmental grooves as well-marked as in the first molar; and (ii) three cusped type with heart-shaped occlusal aspect, resembling a typical upper third molar shape. The hypocone or distolingual cusp is small and poorly developed compared to the first upper molar, being the other three cusps the ones that predominate. The well-formed roots of this tooth are as long as those of the first molar (Scheid and Weiss, 2012).

The **upper third molar** shows great morphological variability in size, contour, and relative position compared to the other molar teeth. The crown is smaller than that of the first and second molars. From an occlusal view, the third molars are quite wrinkled due to numerous supplemental grooves, ridges or cusps (Jordan *et al.*, 1992). Besides, its design is similar to the heart-shaped type of second molars. The hypocone is very small and poorly developed in most cases, and it may be absent. The roots are usually shorter, and this tooth tends to have fused roots.

The **lower first molar** is the largest tooth in the mandibular arch, especially on its mesiodistal dimension. It most often has five well-developed cusps: two buccal (protoconid and hypoconid), two lingual (metaconid and entoconid), and one buccodistal (hypoconulid). The protoconid (mesiobuccal cusp) is the largest, widest, and highest of all the cups, and the hypoconulid (buccodistal cusp) is the smallest. The lingual cusps are larger than the buccal cusps. Usually, the morphology of the lower first molar presents an occlusal **Y-5 pattern** (Figure 4), but **+4** and **×4** patterns have also been found in lower first molars with 4 cusps, as it happens in the molars of Western Eurasia populations (Scott and Turner, 1997). The occlusal aspect of this tooth shows four developmental grooves (central, mesiobuccal, distobuccal, and lingual) as well as three major fossae (central, mesial, and distal). It has two long, separated, and well-developed roots, one mesial and one distal, which are broad buccolingually (Scott and Irish, 2017).

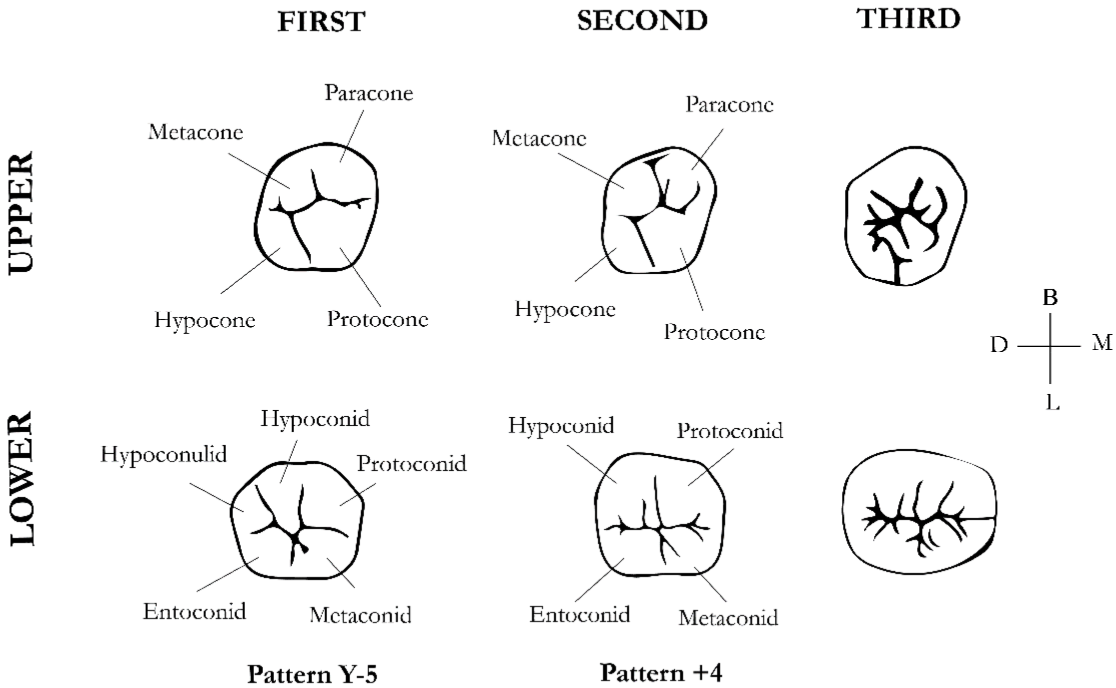


Figure 4. Differences in the morphology of the upper and lower first, second, and third upper and lower molars. Note the differences in the number of cusps and dental cusp patterns. B: buccal; L: lingual; M: mesial; D: distal. Based on Scheid and Weiss (2012).

The **lower second molar** is usually smaller than the first molar, but it is not uncommon to find lower second molar crowns larger than the first molar crowns. The most common occlusal **pattern** in this tooth is **+4**, with four well-developed cusps, two buccal and two lingual. As on the first molar, the protoconid is wider mesiodistally than the hypoconid. On rare occasions, the occlusal Y-5 pattern, with 5 cusps, can be observed on the second lower molar in some human populations (Scott and Turner, 1997). The lower second molars have three main grooves (central, buccal and lingual) and three main fossae (central, mesial and distal). The tooth has two well-developed roots, one mesial and one distal. These roots are broad buccolingually, but they are not as broad as those of the first molar, nor are they as widely separated (Scott and Irish, 2017).

Finally, among human populations, the **lower third molar** exhibits considerable variation in size and shape (Hattab *et al.*, 1999). They tend to be smaller and more crenulated than the other lower molars. Its occlusal design is highly variable for showing from four cusps like the lower second molars to five cusps like the lower first molars, or even more than five cusps, showing several small tubercles roughened by multiple grooves (Scheid and Weiss, 2012). As on the lower first and second molars, the lingual cusps are often larger and longer than the buccal ones. The lower third molars have two very curved roots that often tend to be fused (Nelson and Ash, 2010).

2. Dental wear

The **wear of teeth** is an inevitable, physiological, and cumulative dynamic process of gradual enamel tissue loss that results in the gradual exposure of dentine during an individual's life span as a result of three distinct and interacting mechanisms: attrition, abrasion, and erosion (Schmidt and Watson, 2020).

2.1. Attrition, abrasion, and erosion

Attrition is the result of direct tooth-to-tooth contacts without the presence of food (Kaidonis, 2008). It occurs between neighbouring teeth (interproximal wear) or between opposing teeth (occlusal wear), and it produces flat and shiny wear facets at the contact surfaces between teeth on the interproximal and occlusal surfaces (Hillson, 2002). Abrasion is the loss of tooth substance caused by the constant friction of dental enamel surfaces with abrasive, foreign particles (e.g. food abrasiveness and other objects held in the mouth). It is considered the principal wear mechanism and it is strongly correlated with age (Kaidonis, 2008; Kaidonis *et al.,* 2012). The most common abrasion process is caused by tooth-food-tooth contacts during mastication. The abrasive action of food particles on the occlusal surface eventually causes exposed dentine (Kaidonis, 2008). Moreover, food storage and food preparation techniques may introduce external abrasives (sand, grit, dust, or ash) to food that can also influence dental wear in modern human populations (Molnar, 1971; 1972; Prinz, 2004; Watson, 2008; Fiorenza *et al.,* 2018). Finally, erosion is caused by the extended exposure to intrinsic or extrinsic acids and other chemical agents, causing a gradual dissolution of the enamel surface structure (Kaidonis, 2010; Kaidonis *et al.,* 2012). Dental wear is often a combination of the three mechanisms, each with distinct intensity and duration, resulting in a broad range of different dental wear patterns (Smith, 1984; Hillson, 2002; Kaidonis, 2008).

The wear mechanisms above mentioned result from normal tooth use during food **mastication or chewing**. Mastication is a well-known and complex biomechanical process in which food is crushed and ground by teeth in two alternating phases (Figure 5): **phase I** or puncture-crushing phase, when the cusps of the molars slide past one another in a shearing action, to end up in a centric occlusion and then the lingual surfaces of the upper molar cusps become in contact with the buccal surfaces of the lower molar cusps -the repeated vertical movements help to chop the food, producing blunting wear on the entire tooth surface; and **phase II** or chewing phase, when the teeth move lingually of the centric position, grinding the food with the lingual surfaces of the buccal lower molar cusps against the buccal surfaces of the lingual upper molar cusps –the lateral movements produce oblique wear facets on the mandibular buccal and maxillary lingual cusps that wear on both faces of cusp slopes, while the remaining cusps wear only on one face (Hiiemae and Kay, 1972; Kay and Hiiemae, 1974a; Smith, 1984; Hillson, 2002).

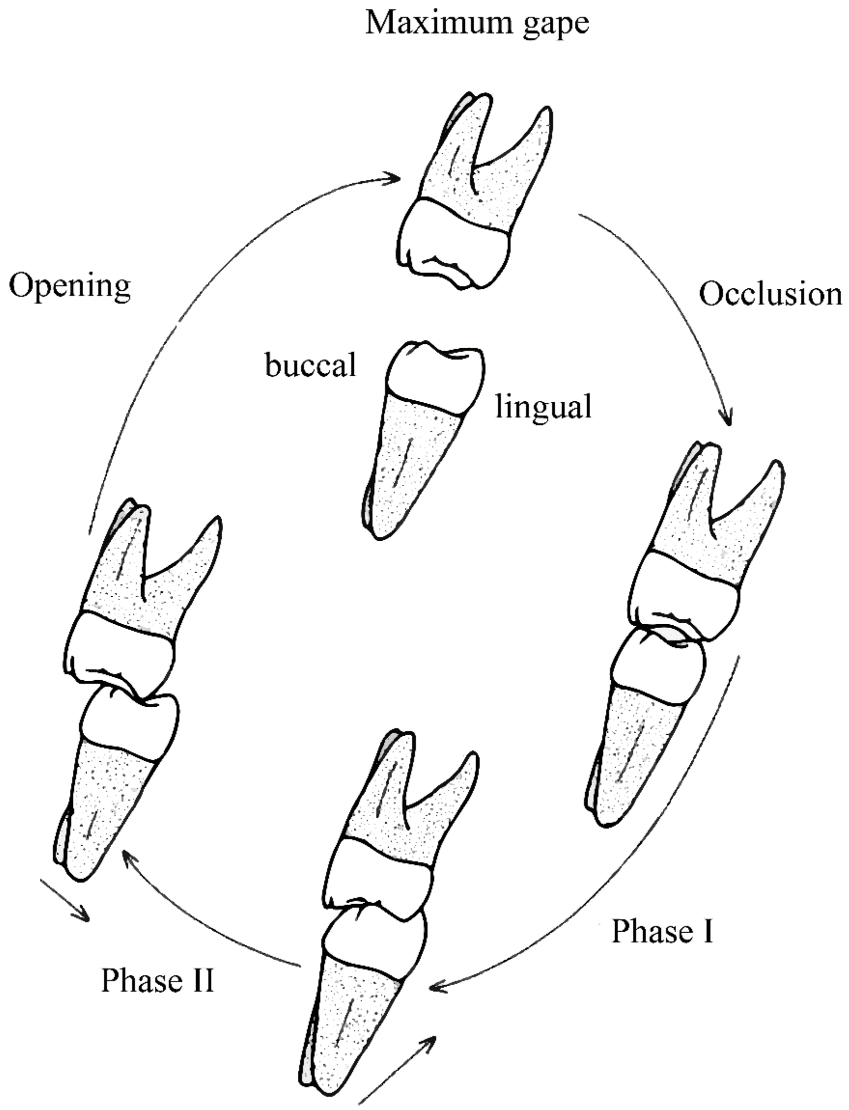


Figure 5. The chewing cycle in molars. In Phase I, the lower molars shift into a centric occlusion with the upper molars reaching maximum intercuspation. This is followed by phase II, the teeth move out of occlusion, followed by the jaw opening to maximum gape. Adapted from Hillson (2002).

On the other hand, wear produced by non-alimentary tooth use is categorized as **non-masticatory dental wear**. This type of wear is usually related to economic and cultural-related activities and it mostly affects the teeth on the anterior part of the dental arcade that, apart from biting, can be used as a third hand or as a tool for holding objects, processing and manipulating materials as it has been reported in numerous studies carried out in fossils hominins (Lozano *et al.,* 2008; Lozano *et al.,* 2017) and prehistoric (Molnar, 1972; Larsen, 1985) and modern human populations (Brown and Molnar, 1990; Berbesque *et al.,* 2012).

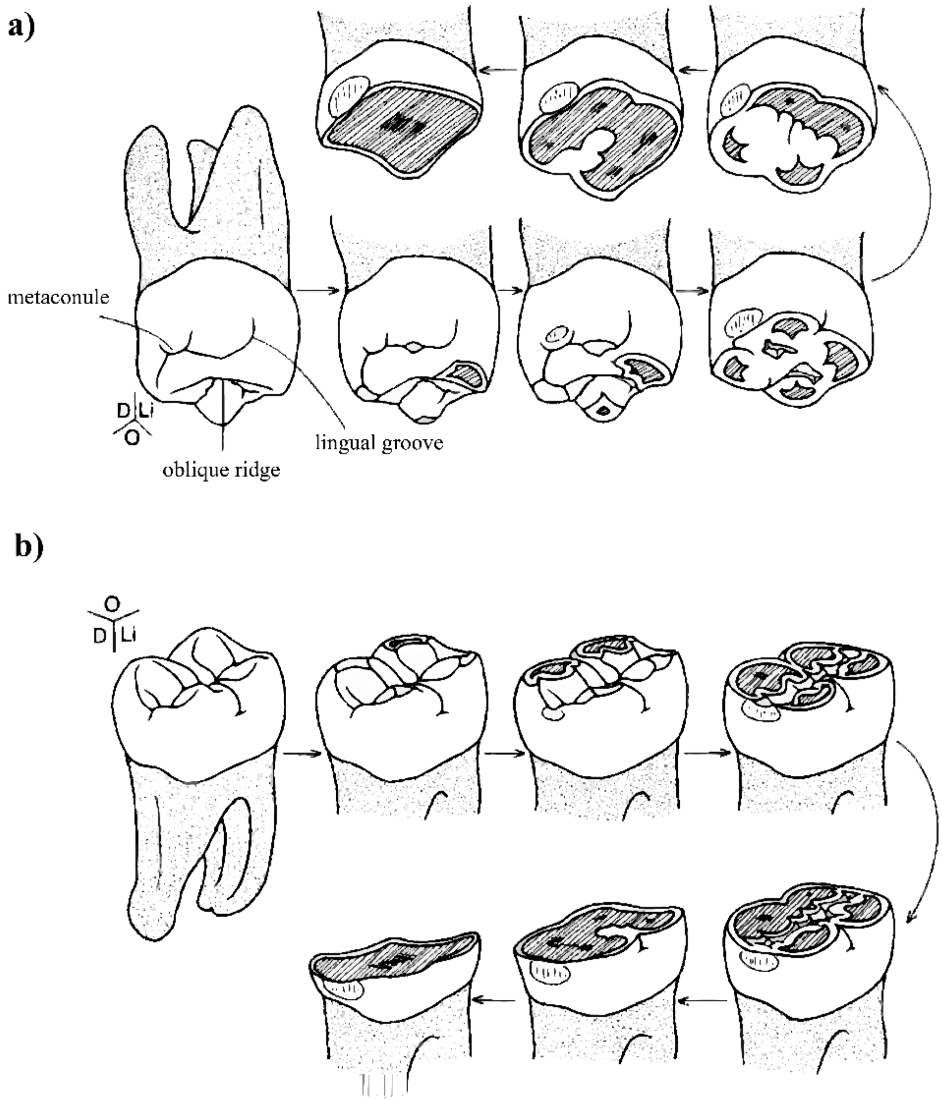


Figure 6. Permanent molar wear stages viewed from the distal-lingual-occlusal corner; (a) in the upper molars, the mesiolingual cusp wears first, followed by the buccal and distolingual cusps; (b) in the lower molars, the mesiobuccal cusp wears down first, followed by the distobuccal and lingual cusps. Adapted from Hillson (2002).

It is important to note that different categories of teeth may wear down at different rates. The mechanism of tooth wear in the upper and lower molars is well known (Smith, 1984; Hillson, 2002). It starts with the loss of occlusal enamel, initially resulting in a dot of dentine exposure. The lingual cups of the upper molars and the buccal cusps of the lower molars wear more rapidly than the rest of the cusps (Figure 6). During the wear process, interproximal wear facets also appear at the contact point between teeth. The three roots of the upper molars and the double roots of the lower molars are usually locked firmly into their sockets, and it is common for the roots to remain *in situ* whilst the crown fractures away.

2.2. Techniques for the study of dental wear

Dental wear is one of the most important subjects of research in the field of dental anthropology and its study can provide an enormous amount of information about an individual's life and lifestyle. Dental wear has been used as an indicator of dietary habits and food processing techniques (Larsen, 1997; Kaidonis *et al.*, 2012; Górka *et al.*, 2015, 2016) but also as a tool for the reconstruction of cultural habits in the production of crafts or in food preparation (Molnar, 1971, 1972; Smith, 1984; Fiorenza and Kullmer, 2013). The short-term effect of dietary abrasiveness on enamel surfaces and the long-term process of enamel loss could be registered using micro- or macrowear techniques, respectively; and either using qualitative or quantitative methods (Lucas, 2004; Romero and De Juan, 2012).

2.2.1. Dental microwear

During mastication, both **extrinsic** particles (e.g. dust, clay, ash or sand) incorporated to food items during food preparation or storage, and **intrinsic** food particles (e.g. silicon-based phytoliths) found in plant tissues, seed coverings or cereals, may interact with the enamel surface of a tooth during food breakdown. Food particles harder than the enamel on the Mohs scale of mineral hardness (range 1–10) may cause microscopic tooth-wear, often referred to as dental microwear (Pérez-Pérez *et al.,* 1994; Galbany *et al.,* 2009; Romero and De Juan, 2007, 2012). This interaction is responsible for the microscopic marks on the enamel, such as striations or scratches and pits, which requires the use of a Scanning Electron Microscopy (SEM) for observation. Striations or scratches, features defined for having a length:width ratio >4:1, can be found on both the occlusal and buccal enamel surface, whereas pits, features defined as having a length:width ratio smaller than 4:1, can be only observed on the occlusal enamel surface (Teaford, 1994; Pérez-Pérez *et al.*, 1999, 2003; Schmidt, 2010).

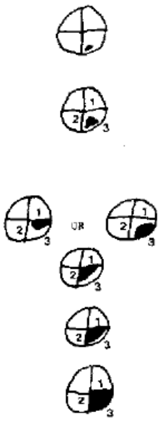
Dental microwear has proved to be a reliable indicator of dietary habits in extinct non-human primate species (Galbany and Pérez-Pérez, 2004; Galbany *et al.*, 2004, 2009) and fossil hominins (Pérez-Pérez *et al.*, 2003; Estebaranz *et al.*, 2009, 2012; Martínez *et al.*, 2016), but also in modern human populations (Mahoney, 2006a, b; Romero *et al.*, 2012; Salazar-García *et al.*, 2016).

2.2.2. Dental macrowear

Dental macrowear is the loss of dental hard tissues as viewed at the macroscopic level (Schmidt, 2010). Macrowear studies have been practiced for more than one hundred years and, therefore, many different methods of study have been developed. These can be divided into (i) qualitative and (ii) quantitative scoring techniques.

(i) Qualitative dental wear studies

Assessing and recording the degree of tooth wear started in the latter half of the 19th century when Broca (1879) developed the first systematic method to classify occlusal dental wear into five categories, whereby 0 represented no wear, and 4 represented a completely worn out tooth crown. This method was the precursor of many other classification systems. For instance, Murphy (1959) proposed an eight-stage system, graded from *a* to *h*, supported by a series of diagrams that illustrated the levels of dentine exposure on the occlusal surface of a tooth. This method was successfully applied in Australian Aborigines but created difficulties when applied to other populations (Smith, 1984). Molnar (1971) developed a dental wear scoring system also including eight stages but grouping individual teeth into three categories: incisor and canines, premolars and molars, so dental wear was classified depending on the number of dentine exposure patches (8 categories), the direction of surface wear (8 categories) and the form of the occlusal surface of a tooth (6 categories). This study aimed to show if dental wear could distinguish among populations since dental wear is highly related to certain aspects of culture such as diet, food preparation techniques and tool usage. For this purpose, Molnar (1971) compared the dental wear of three Indian populations from North America: hunter-gatherer populations from California, and agriculturalist populations from the Southwest and the Valley of Mexico. The results showed significant differences between sexes and among populations. Another ordinal dental wear scoring system was proposed by Scott (1979), also applied to three collections of Amerind skeletal samples. This system (Figure 7) divided the molar occlusal surface into four sections that were separately scored on a 1-10 scale, based upon the amount of enamel present in each quadrant. The sum of the scores of four sections was used as the score for the whole tooth crown ranging from 4 to 40. While scores from 1 to 4 indicated the removal of enamel from the occlusal surface, scores from 5 to 10 represent the amount of dentine exposure relative to the amount of enamel present in the quadrant. The main disadvantage of this method is that it can only be applied to the molar teeth. The incisors, canines and premolars must be scored using other methods. Compared to other methods, it may seem a time-consuming technique, but it offers a detailed description of molar wear.



Score	Description
0	No information available (tooth not occluding, unerupted, antemortem or postmortem loss, etc.)
1	Wear facets invisible or very small
2	Wear facets large, but large cusps still present and surface features (crenulations, noncarious pits) very evident. It is possible to have pinprick size dentine exposures or "dots" which should be ignored. This is a quadrant with *much* enamel.
3	Any cusp in the quadrant area is rounded rather than being clearly defined as in 2. The cusp is becoming obliterated but is not yet worn flat.
4	Quadrant area is worn flat (horizontal) but there is no dentine exposure other than a possible pinprick sized "dot."
5	Quadrant is flat, with dentine exposure one-fourth of quadrant or less. (Be careful not to confuse noncarious pits with dentine exposure.)
6	Dentine exposure greater: more than one-fourth of quadrant area is involved, but there is still much enamel present. If the quadrant is visualized as having three "sides" (as in the diagram) the dentine patch is still surrounded on all three "sides" by a ring of enamel.
7	Enamel is found on only two "sides" of the quadrant.
8	Enamel on only one "side" (usually outer rim) but the enamel is thick to medium on this edge.
9	Enamel on only one "side" as in 8, but the enamel is very thin—just a strip. Part of the "edge" may be worn through at one or more places.
10	No enamel on any part of quadrant—dentine exposure complete. Wear is extended below the cervicoenamel junction into the root.

Figure 7. Scott's (1979) classification of dental wear (from Scott, 1979: 214).

One of the most commonly used methods for scoring dental wear was developed by Smith (1984), who produced a simplified version of Murphy's (1959) classification system to score dental wear in populations of modern and prehistoric hunter-gatherers and agriculturalists to test the hypothesis that there are differences in the patterns of tooth wear associated to differences in dietary subsistence patterns and food preparation techniques (Figure 8). As with Molnar's method (Molnar, 1971), Smith's system (Smith, 1984) also grouped individual teeth into three categories: incisors and canines, premolars and molars.

Qualitative methods for the characterisation of dental wear in human populations, using ordinal scales, are largely observer-dependent (Scott, 1979; Smith, 1984), with a lack of precision and standardisation in terminology (Bardsley, 2008), contrary to quantitative methods (Hillson, 2002).

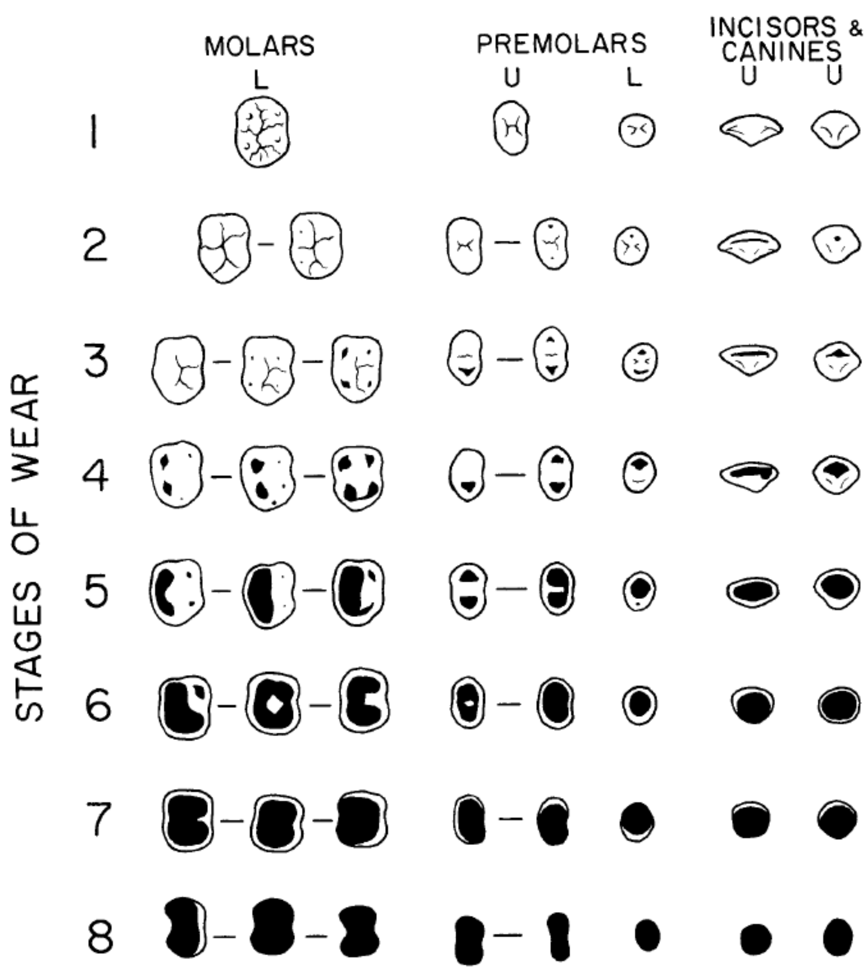


Figure 8. Smith's (1984) dental classification of dental wear (from Smith, 1984: 46).

(ii) Quantitative dental wear studies

Quantitative methods tend to rely on physical measurements for scoring dental wear, most frequently using ratio scale metrics, offering a more objective and precise approach to dental wear characterisation than qualitative methods. In addition, the quantitative proxies reduce interobserver measurement errors in recording tooth crown changes in wear through time (Górka *et al.,* 2016). For instance, quantitative measurement has typically relied on determining the depth of the groove, area of facet, the height of crown and cups (Walker *et al.,* 1991; Mays, 2002; Benazzi *et al.,* 2008) or the percentages of dentine exposure areas (Clement and Hillson, 2012; Górka *et al.,* 2015, 2016).

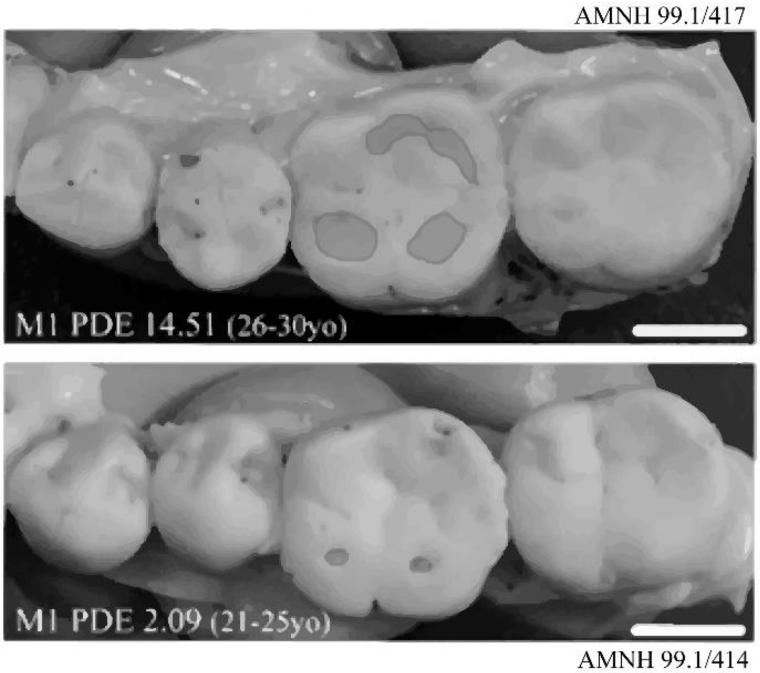


Figure 9. Occlusal view of high-resolution replicas (lower P3–M2) of Tigara hunter-gatherers. Solid outlines delineate dentine exposed regions on first molars. Individual PDE values and age-range in years are also shown. Mesial: left; lingual: top. Scale bar: 5 mm. From Górka *et al.* (2016).

In the particular case of the quantification of the dentine exposure areas to assess dental wear, photographs of the dental occlusal surface have successfully provided a great resolution of the degree of dentine exposure (or percentage of dentine exposure, PDE) (Behrend, 1977; see also Burnett, 2015). For instance, Walker (1978) calculated wear rates by quantifying the overall area of exposed dentine from photographs of molars of the prehistoric island and mainland populations along the Santa Barbara channel. The same method has been successfully applied by other researchers in different modern human populations. For instance, Deter (2009) used it to quantify the degree in dentine exposure in North American archaeological samples of modern human foragers and agriculturalists. Recent studies have recorded ratios of dentine exposure in modern human populations controlling for the individual variability due to age and sex related-factors showing that PDE is age-dependent and a lack of sexual dimorphism is commonly archived. In this context, Clement and Hillson (2012) focused on the dentition (anterior and postcanine) of known-sex modern Igloolik Eskimo population and Górka *et al.* (2016) on first lower molars among Alaskan Tigara foragers from Point Hope (Figure 9).

Nevertheless, the development of high-resolution scanning techniques has also allowed for the implementation of 3D procedures than can be implemented in the quantification of dental shape changes with wear (e.g. Fiorenza *et al.,* 2018; Ungar and M'Kirera, 2003; M'Kirera and Ungar, 2003; Pampush *et al.*, 2016a; Berthaume *et al.*, 2018) and the change of shape with functional use, such as the Geometric Morphometrics (GM) and the dental topography.

3. Geometric morphometrics

Morphometrics is the branch of morphology that quantitatively characterises the biological **form**, a concept that encompasses **size** and **shape** components. Morphometric analyses can be used to quantify shape, shape variation and its covariation with other variables (Bookstein, 1991; Dryden and Mardia, 1998).

The first use of morphometric approaches to depict biological forms can be traced back to the artwork (e.g. pictures and statues) of the ancient Egyptian, Roman, and Greek civilizations. Indeed, Egyptian artists used squared grids that allowed them to control figure's proportions (Slice, 2005). This procedure was later employed by great and versatile artists of the Renaissance, such as Leonardo da Vinci (1452-1519) and Albrecht Dürer (1471-1528), who were particularly notable for their systematic geometric studies of the human body. Specifically, Dürer's study of human proportions and the use of transformation grids to describe facial variation inspired similar work by D'Arcy Wentworth Thompson (1860-1948) in his book *On Growth and Form* (Figure 10). Published in 1917, this fundamental book introduces the biological shape variation on a Cartesian transformation grid for the first time. The problem with Dürer's and Thompson's approaches was that their deformation grids were drawn by hand, without any reference to a formal algorithm (Mitteroecker and Gunz, 2009). It was not until the 19th and 20th centuries that the "fathers of biometry" Francis Galton (1822-1911), Karl Pearson (1856-1936), and Ronald Fisher (1890-1962) started using statistical approaches to analyse biological variation.

In the 1960s and 1970s, biometricians began using **traditional morphometrics**, or multivariate morphometrics (Marcus, 1990; Reyment, 1991), that describe shape variation within and among groups typically applying multivariate statistical methods to sets of morphological variables. These variables usually correspond to linear distance measurements (e.g. lengths, widths, perimeters, and areas) but also angles, and ratios (Marcus, 1990; Rohlf and Marcus, 1993). However, traditional morphometrics may have certain limitations. Linear distances often overlap, making it difficult to describe local shape changes. These measurements are usually highly correlated with size, so extracting the information about shape might also be difficult, and there is no agreement on which size correction methods should be applied (Adams *et al.,* 2004). Besides, the homologies of linear distances are not easy to assess. In addition, graphical representations of shape from linear distances are usually difficult to generate since geometric relationships among the variables are not retained (Adams *et al.,* 2004). In the 1980s, the many limitations of traditional morphometrics led to the development of a new morphometric approach based on geometry, the geometric morphometrics, causing an authentic methodological revolution.

a) **b)**

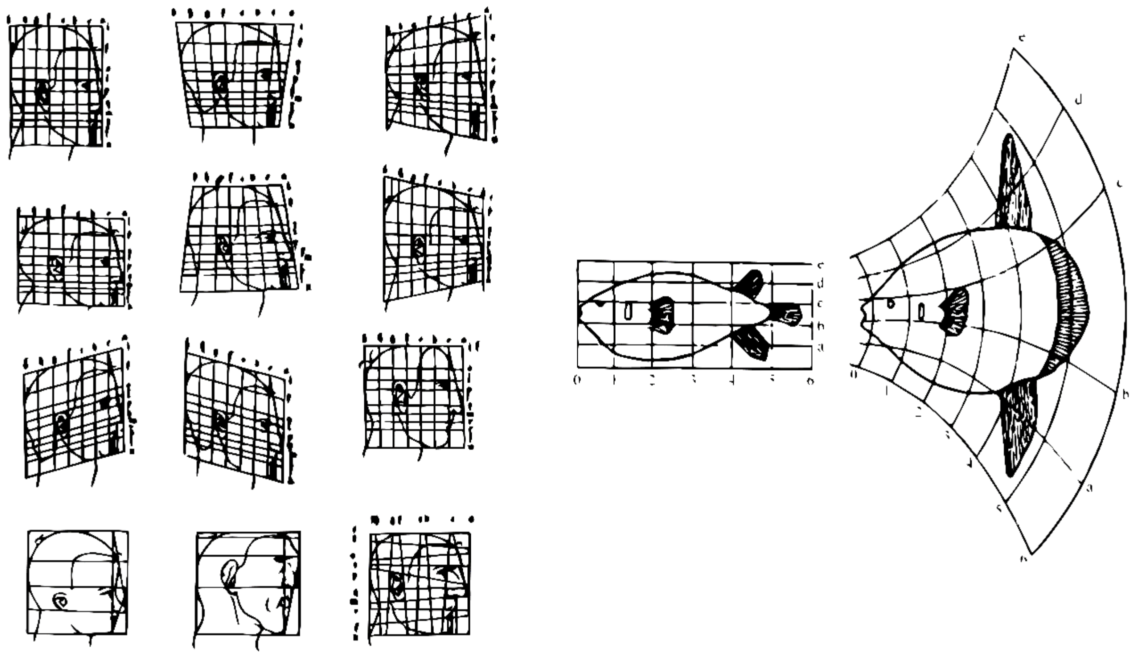


Figure 10. (a) Albrecht's Dürer's sketches of human facial proportions, from *Vire Bücher von Menchlicher Proportion* (1524); (b) D'Arcy Wentworth Thompson's example of a species of fish *Diodon* being geometrically transformed into another species, *Orthagoriscus*, from his book *On Growth and Form* (1917).

Geometric morphometrics (GM) has been defined as the combination of geometry and biology. Unlike traditional morphometrics, GM begins with the collection of Cartesian coordinates of biologically definable landmarks located in a two-dimensional (x, y) or three-dimensional (x, y, z) space whose configuration allows capturing shape variation of biological structures (Rohlf, 1990; Bookstein, 1991; Rohlf and Marcus, 1993; O'Higgins, 2000; Adams *et al.,* 2004; Zelditch *et al.,* 2004).

3.1. Types of landmarks

According to Dryden and Mardia (1998), a **landmark** is a point of correspondence on each specimen that matches between and within populations; or, equivalently, homologous anatomical loci easily recognizable on every specimen studied (Zelditch *et al.,* 2004). Landmarks selection is important since they capture the shape of the structure in study, offering some valuable information about their function, development, or evolution as well as statistically detect shape differences between groups (Rohlf and Marcus, 1993; Zelditch *et al.*, 2004; Webster and Sheets, 2010). Landmarks can be classified into three categories. **Type I** (Bookstein, 1991) **or anatomical landmarks** (Dryden and Mardia, 1998) are usually the easiest and most reliable points to locate. Their homology is supported by a biological significance, such as the juxtapositions (i.e. tissues, sutures, veins, among others). As they are surrounded in all directions by surfaces, this type of landmarks allows you to identify the direction of forces that may affect a structure and/or recognize the effects of processes that may cause landmarks movements (Zelditch *et al.*, 2004). **Type II** (Bookstein, 1991) **or mathematical landmarks** (Dryden and Mardia, 1998) correspond to points of minimum or maximum curvature, for instance a tooth tip or the end of a bony process. Their homology is supported only by geometric, not anatomical, evidence. These landmarks lack information from surrounding tissues (in at least one direction) so one cannot distinguish between several possible directions in which forces might be affecting the position of the landmarks (Zelditch *et al.,* 2004). **Type III landmarks** (Bookstein, 1991) are the most difficult points to locate. They are defined as the extremes of curvature or points away from some structure, like the endpoints of diameters, a centroid, or the intersection between landmarks. Both type II and III landmarks do not carry as much biological information in terms of homology compared to type I landmarks, but they may be useful to include geometric information in regions that are poorly characterised by the use of fixed landmarks, such as irregular or curvilinear areas. Such surfaces may require the use of **semi-landmarks** (or sliding semi-landmarks) or **pseudo-landmarks**, type III landmarks that quantify shape variation along surfaces, curves or outlines (Bookstein, 1991; Bookstein, 1997; Gunz *et al.*, 2005; Gunz and Mitteroecker, 2013). Despite the definitions of both semi-landmarks and pseudo-landmarks are often unclear and interchangeable, semi-landmarks may refer to the points whose initial position is relative to landmarks with biological homology and pseudo-landmarks are automatically placed without reference to anatomically defined landmarks (Goswami *et al.*, 2019). Semi-landmarks or pseudo-landmarks can be used in combination with fixed landmarks to obtain a better quantification of the shape of a structure (Adams *et al.,* 2013).

3.2. Procrustes superimposition

In geometric morphometrics, shape is defined as "all the geometric information that remains when the effects of the position, scale and rotation are removed from an object" (Kendall, 1977). Two or more objects have the same shape if they can be translated, scaled and rotated to each other until there are no differences between them. This can be accomplished using the Procrustes superimposition or partial least squares algorithm called **General Procrustes Analysis** (GPA; Gower 1975; Rohlf and Slice, 1990), considered as the first step of the morphometric analysis (Kendall, 1984; Bookstein, 1986; Rohlf and Marcus, 1993; Dryden and Mardia, 1998; O'Higgins, 2000; Zelditch *et al.*, 2004). In Greek mythology, the name Procrustes refers to a giant who fit his victims to a bed by stretching their limbs or chopping them off, minimizing the difference between his victims and the bed. Unlike the mythological Procrustes, who modified the shape of his victims, GPA superimposes the configurations of landmarks in all specimens to obtain a common coordinate system, without altering the shape. It also minimizes the differences between the specimens as well as separates the size component from the shape using three steps: **translation**, **scaling**, and **rotation** (Figure 11). First, the center of each configuration of landmarks (or **centroid**) is translated to the origin. To remove the size component, the landmark configurations are scaled so that they share a common centroid size of 1 by dividing each coordinate of each landmark by the centroid size of that configuration. The **centroid size** is the square root of the summed squared distances of each landmark to the centroid, whose location is obtained by averaging the x and y coordinates of all landmarks (Zelditch *et al.,* 2004; Klingenberg, 2016). Finally, the landmark configurations are rotated to minimize the total sums-of-squares distances between corresponding landmarks from all specimens (Rohlf and Slice, 1990; Bookstein *et al.*, 1999; Rohlf, 1999; Adams *et al.,* 2004; Zelditch *et al.*, 2004; Slice, 2005; Mitteroecker and Gunz, 2009; Adams *et al.,* 2013). For this purpose, all centered and scaled landmark configurations are rotated to one arbitrary configuration to achieve an optimal alignment. This step determines the **mean shape (consensus)** onto which all specimens are rotated. At this point, the mean shape is recalculated, and it will be used as an updated template for the next iteration.

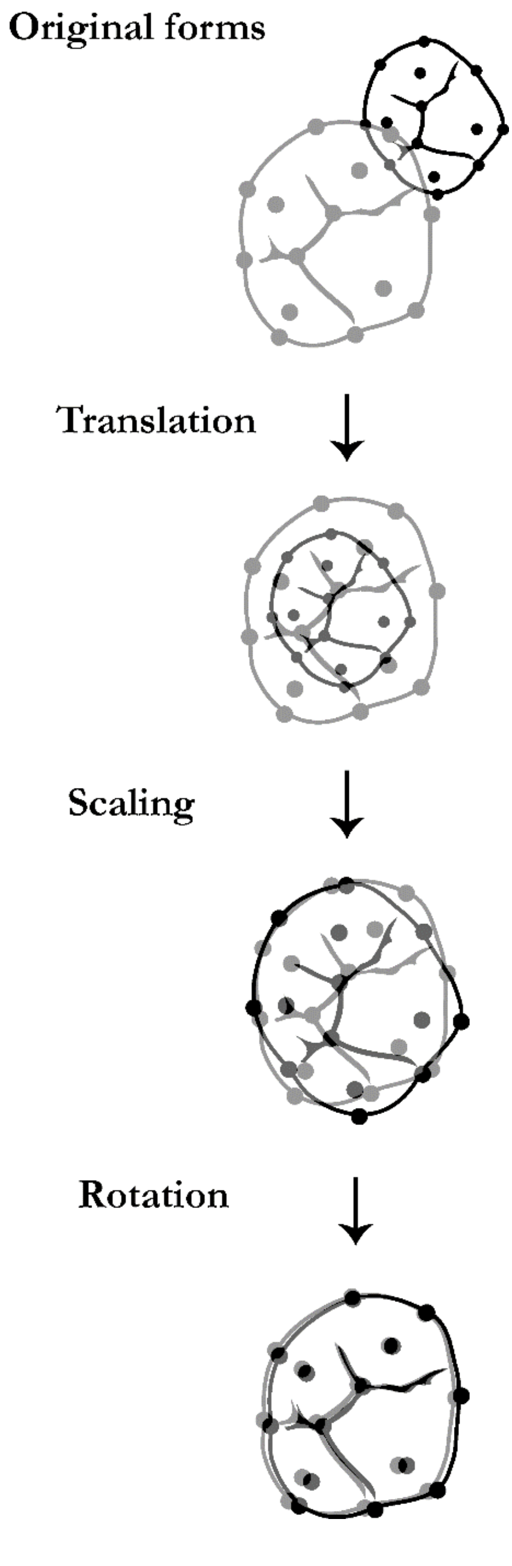


Figure 11. Steps of the General Procrustes Analysis (GPA): translation to a common origin, scaling to a centroid size of 1 and, rotation to minimize the total sums-of-squares of Euclidean distances among the homologous landmarks. An upper molar diagram (occlusal view) is used to illustrate the GPA steps.

This process is repeated until the sum of the Procrustes distances cannot be reduced further. In other words, until the mean shape does not change significantly within an iteration (Rohlf and Slice, 1990; Dryden and Mardia 1998; Rohlf, 1999; Zelditch *et al.,* 2004). The **Procrustes distance** is defined as the square root of the sum of squared differences between the coordinates of corresponding landmarks (Rohlf and Slice, 1990; Dryden and Mardia, 1998), and indicates the similarity (or dissimilarity) in shape between two landmark configurations.

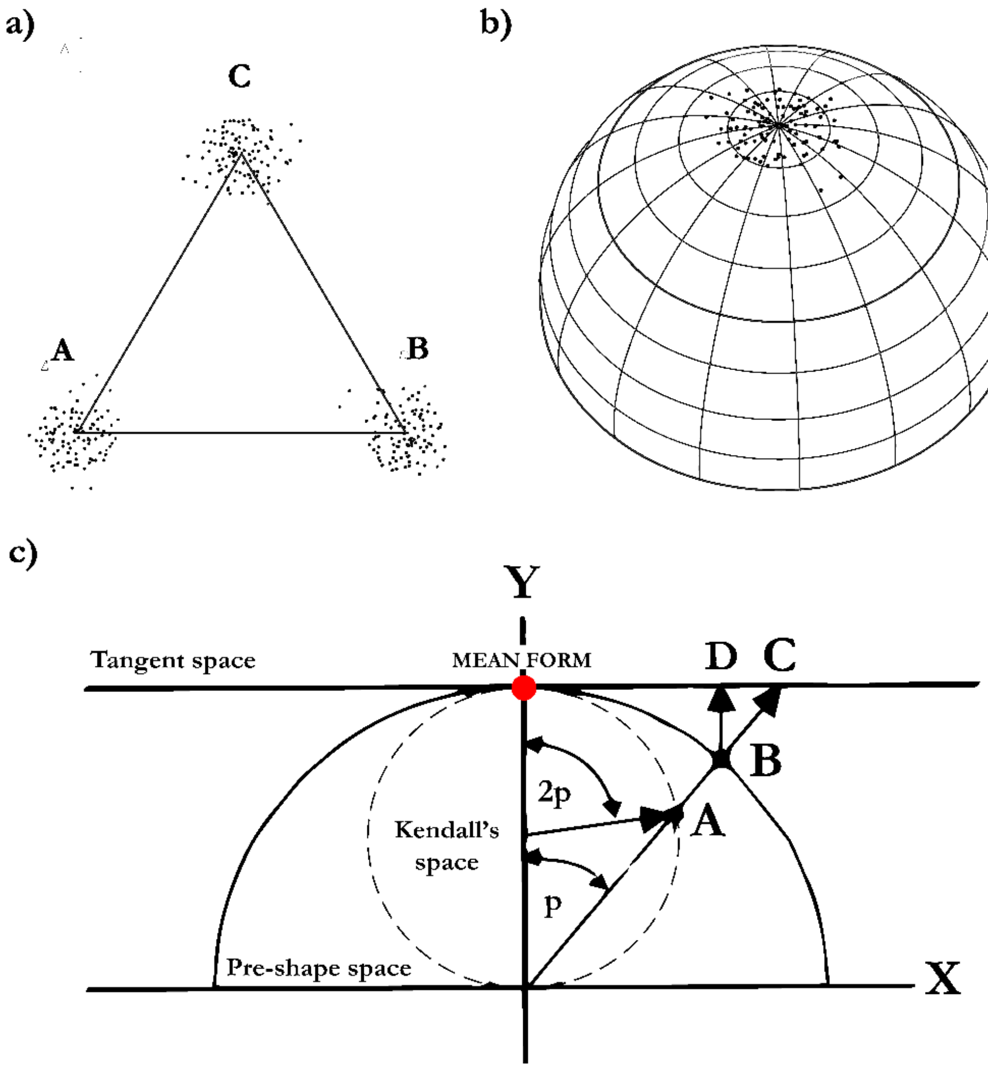


Figure 12. (a) The vertices of one hundred random triangles with an equilateral triangle as the mean (consensus) form; (b) the shapes of these triangles are isotropically distributed in Kendall's shape space (Mitteroecker and Gunz, 2009); (c) diagram of a cross-section of Kendall's shape space, hemisphere of pre-shape space aligned to the reference (red dot), and Euclidean tangent space. Procrustes distance is the angle p in radians. Point A represents the position of a shape in Kendall's shape space and point B is the corresponding position in the hemisphere. Point C is the stereographic projection of point A onto the tangent space and point D is the orthogonal projection of point B onto tangent space (Rolf, 1999; Slice, 2001).

After the superimposition, the coordinates of the resulting centered, scaled, and rotated landmarks are known as **Procrustes shape coordinates**, that describe the location of each specimen in a curved space called **Kendall's shape space** and allow visualizing the changes in the shape to analyse its variability (Rohlf, 1999; Slice, 2001; Adams *et al.*, 2013). The dimensions of this shape space are defined by the number of landmarks and dimensions, and they are calculated with the equation: pk-k-k(k-1)/2-1 (p: number of landmarks; k: dimensions). The superimposition results in a loss of the number of dimensions (or degrees of freedom). For 2D configurations, the number of dimensions obtained is 2p-4 (4 degrees lost) and for 3D configurations, the number of dimensions is calculated as 3p-7 (7 degrees lost). Kendall's shape space is curved, non-linear, or non-Euclidean (Figure 12). Since the data must be linear, it is unsuitable to conduct further multivariate analyses of shape variation. To overcome this limitation, data must be projected into a Euclidean, linear space that is tangent to the mean configuration (Dryden and Mardia, 1993, 1998; Rohlf, 1999; Kent and Mardia, 2001). In the case of semi-landmarks, these points are iteratively slid along their tangent vectors until their positions minimize the shape difference among specimens. This can be accomplished by minimizing the bending energy or the Procrustes distance between each specimen and the mean shape (Bookstein, 1997; Bookstein *et al.*, 1999; Gunz *et al.,* 2005).

Some dental studies have carried out geometric morphometric analysis in two-dimensions and three-dimensions, mostly to explore dental shape and functional anatomy. In the particular case of three-dimensional geometric morphometrics (3D-GM), dental studies have been conducted in non-human primates' species (Singleton *et al.*, 2011; Cooke, 2011; Nova Delgado *et al.*, 2015b) and fossil hominins (see Skinner *et al.*, 2008, 2009b). However, only a few studies were focused on modern human populations. For instance, to quantify shape variation and covariation of maxillary and mandibular first permanent molar occlusal surfaces in a Greek sample (Polychronis *et al.*, 2013), to investigate also the potential shape covariation of the maxillary and mandibular permanent first molar occlusal surfaces and the craniofacial complex at different developmental stages in a Greek sample (Polychronis and Halazonetis, 2014) or to assess sexual dimorphism in premolars of Australian populations (Yong *et al.*, 2018). Nevertheless, to our knowledge, no 3D geometric morphometrics analyses have been used as a tool to explore dental wear in modern human populations.

4. Dental topography

Dental topography analysis has become a popular method for quantifying functional aspects of tooth shape. Early studies of tooth shape and function did not account for wear-related shape changes and have been limited to unworn and slightly worn teeth (Berthaume *et al.,* 2020). Some of these studies include the quantification of the **shearing quotient (SQ)** and derivatives thereof, such as the **shearing ratio (SR)** (Winchester *et al.*, 2014; Boyer *et al.*, 2015), metrics that measure the relative lengths of shearing crests. They have been successfully used and widely for dietary reconstruction and study of chewing efficiency in primates (e.g. Kay and Hylander, 1978; Sheine and Kay, 1982; Kay and Covert, 1984; Anthony and Kay, 1993; Winchester *et al.*, 2014; Boyer *et al.*, 2015). The results have shown that folivores/insectivores, with high-cusped and relatively long-crested teeth, adapted to shearing and slicing, have higher SQ values and therefore greater chewing efficiencies. In contrast, frugivores/omnivores/hard-object feeders, with flatter, more rounded occlusal surfaces and relatively short crested teeth, adapted to grinding and crushing, have lower SQ values and, therefore, lower chewing efficiency (see Sheine and Kay, 1977, 1982; Kay and Sheine, 1979; Bunn *et al.*, 2011; Winchester *et al.*, 2014; Ledogar *et al.,* 2013; Allen *et al.*, 2015; Boyer *et al.,* 2015). These results suggested that primates with diets that were difficult to process (e.g. insect chitin or cellulose-rich leaves) developed sharp cusps and long shearing crests, which allowed them to process food more efficiently (Sheine and Kay, 1977, 1982). Despite the successful results of these studies, the quantification of SQ and SR has disadvantages. First of all, they require a large number of carefully chosen landmarks, which may increase inter-observer error. Moreover, when teeth wear down, the landmarks used to measure shearing crest length become obliterated. This makes it difficult to calculate both SQ and SR and limit their quantification to relatively unworn teeth with prominent shearing crests (Bunn *et al.*, 2011). For these reasons, and with the development of advanced scanning techniques, a new dental topography approach based on shape descriptor metrics overcame these limitations.

Dental topography analysis became important in the early 2000s as a method that quantifies whole tooth shape using Geographic Information Systems (GIS) technologies (Ungar and Williamson, 2000). The idea of using a GIS approach is that tooth surfaces can be modelled as three-dimensional landscape surfaces; tooth cusps are treated as mountains and fissures are treated as valleys (Figure 13) (Zuccotti *et al.*, 1998; Jernvall and Selänne, 1999; Ungar and Williamson, 2000). This technique has been limited to measurements of slope, angularity and relief index of individual cusps (Ungar and Williamson, 2000). Later, additional software and techniques have been developed (e.g. Surfer Manipulator, Morphotester, MolaR, CloudCompare) that have led to the incorporation of newly non-GIS topographic analyses (see Boyer, 2008; Evans *et al.*, 2007; Bunn *et al.*, 2011; Berthaume *et al.*, 2019a).

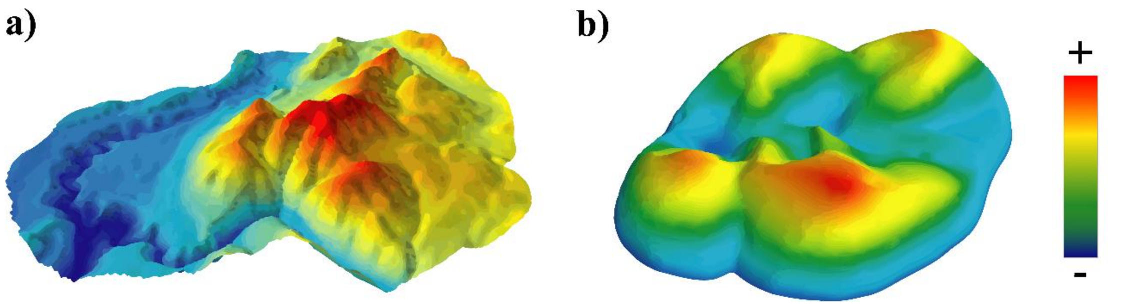


Figure 13. Display of 3D digital elevation models. (a) Rasterized landscape topographic elevation model using ArcGIS software (University of Washington) and (b) non-rasterized elevation map of an upper first molar of a Babinga pygmy; Central Africa (# 18450; Musée de l´Homme, Paris; mesh from AF1-9 cast, University of Alicante repository). Warmer and cooler colours for elevation maps indicate higher and lower height.

Dental topography metrics include, among others, curvature (DNE, Dirichlet Normal Energy; Bunn *et al.*, 2011), occlusal relief (OR; Ungar and M'Kirera, 2003), crown Relief Index (RFI; Boyer, 2008), complexity (OPC and OPCR, Orientation Patch Count Rotated; Evans *et al.*, 2007; see also Evans and Jernvall, 2009; Winchester, 2016; for further details), and ambient occlusion (PCV; *portion de ciel visible*; Berthaume, 2016a) (Figure 14). During the last two decades, dental topography has been widely used to study the correlations between tooth shape and function and dietary behaviours in mammals (Zuccotti *et al.,* 1998; Evans, 2013). Studies carried out among non-human primates (e.g. Boyer, 2008; Bunn *et al.*, 2011; Godfrey *et al.,* 2012; Winchester *et al.,* 2014; Allen *et al.,* 2015) generally have shown higher dental topographic values that indicate a more insectivorous/folivorous/fibrous diet, while lower dental topographic values indicate a more omnivorous/frugivorous/harder diet (Boyer, 2008; Bunn *et al.*, 2011; Godfrey *et al.*, 2012; Winchester *et al.*, 2014; Allen *et al.*, 2015). Dental topography has been also used to describe and attribute a primate fossil to a new species (Boyer *et al.*, 2012), explore the complexity of the enamel-dentine junction and enamel surface (Skinner *et al.*, 2010) or give information for reconstructing dietary niches (Berthaume and Schroer, 2017).

Unlike previous methods, the topographic approach does not depend on specific manually set landmarks, so the inter-observer measurement error is then reduced (Bunn *et al.*, 2011; Winchester *et al.,* 2014). Moreover, dental topography allows working equally well on unworn and worn teeth (Ungar and Williamson, 2000; M'Kirera and Ungar, 2003; Ungar and M'Kirera, 2003; Berthaume *et al.,* 2018), allowing researchers to explore the interaction of dental topography with wear in primates (e.g. M'Kirera and Ungar, 2003; Dennis *et al.*, 2004; Bunn and Ungar, 2009; Pampush *et al.*, 2016b, 2018; Ungar *et al.*, 2018) and fossil hominins (Ungar, 2004; Berthaume *et al.*, 2018). However, this research line has not yet been explored in modern human populations.

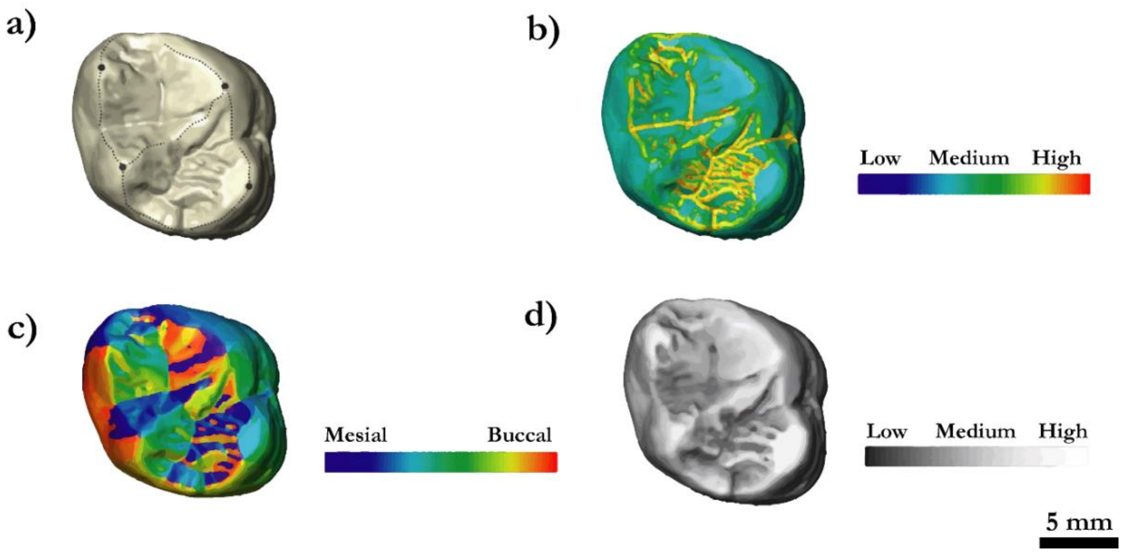


Figure 14. Occlusal views of a second upper molar of *Gorilla gorilla* (MRAC-27755) displaying (a) shearing crests for the calculation of SQ (shearing quotient), and morphometric maps showing (b) DNE (Dirichlet normal energy); (c) OPC (orientation patch count); and (d) PCV (*portion de ciel visible*). Warmer and cooler colours for DNE and OPC maps indicate higher and lower curvatures, and surface orientation patches, respectively. Brighter areas indicate higher PCV values (wear facets become whiter). Scale bar: 5 mm. Adapted from Berthaume *et al.* (2020).

4.1. Dirichlet Normal Energy (DNE)

Dirichlet normal energy (DNE) was introduced in dental topography by Bunn *et al.* (2011) as a metric that quantifies the variability in occlusal surface curvature, regardless of position, size and orientation (Figure 14b). As a continuous function, DNE is equivalent to measuring the sum of squares of principal curvatures across the surface (Evans, 2013;

Winchester, 2016). When quantifying DNE, a percent of data, usually 0.1% area × energy, can be discarded. This corresponds to "noisy" polygons or "artefacts" (e.g. sharp points/edges) that produce energy values out of proportion to the overall surface (Winchester, 2016). DNE increases with both convex and concave surfaces, reflecting greater dental characteristics such as taller and sharper cusps and crenulated surfaces as well as deeper and more acutely angled basins. On the contrary, short, bulbous cusps produce low DNE values (Bunn *et al.,* 2011; Winchester *et al.,* 2014; Winchester, 2016). Most studies carried out on non-human primates show that DNE is a useful tool to distinguish dental morphologies by dietary categories and to track dietary fibre content in great apes. High DNE values indicate insectivory/folivory/high fibre diets and lower DNE values suggest omnivory/frugivory/low fibre diets (Bunn *et al.*, 2011; Godfrey *et al.,* 2012; Ledogar *et al.*, 2013; Winchester *et al.,* 2014; Winchester, 2016; Berthaume and Schroer, 2017). Moreover, DNE can be also used for teeth with different dental morphologies across different wear stages (Pampush *et al.,* 2016a).

4.2. Relief index (RFI) and occlusal relief (OR)

The relief index (RFI), originally introduced by Ungar and Williamson (2000), measures the overall relief of a tooth crown as the ratio between the 3D crown surface area of a tooth and its 2D outline projected area (Figure 15). Two versions of RFI have been developed depending on the surface cropping method used. On one hand, the relief index proposed by Ungar and M'Kirera (2003), referred as occlusal relief (OR), takes into account only the tooth cropped at the lowest point of the occlusal basin (basin cut off, BCO; see also Berthaume *et al.,* 2020). This measure focuses on the tooth surfaces most likely to be involved in mastication and it is calculated as a simple ratio between the 3D surface area (SA) of the cropped tooth and its 2D planimetric footprint or outline area (OA):

$$\mathrm{OR} = \frac{\mathrm{SA}}{\mathrm{OA}} \tag{1}$$

This calculation provides a ratio between the relative height of the cusps (SA) and the size of the tooth (OA) (Pampush *et al.,* 2018). On the other hand, the modification of RFI proposed by Boyer (2008) takes into account the entire enamel cap (EEC) in the 3D area summation by cropping along the cemento-enamel junction (CEJ). For allomeric reasons, Boyer's (Boyer, 2008) formulation transforms the original calculation into the natural log of the ratio of the square roots of the 3D surface area (SA) of the enamel crown and its 2D outline projected area (OA) of the crown oriented in the occlusal view:

$$\mathrm{RFI} = \ln\left(\frac{\sqrt{\mathrm{SA}}}{\sqrt{\mathrm{OA}}}\right) \quad (2)$$

This metric indicates relatively taller crowned/cusped teeth have higher RFI and OR values and lower crowns/cups have lower RFI and OR values (Ungar and M'Kirera, 2003; Boyer, 2008). Crown relief has been briefly investigated in fossil hominins (Berthaume *et al.,* 2018) and modern human populations (Górka, 2016). On the contrary, during the last two decades this metric has been widely explored on primates, showing to be a very useful metric to group teeth among different dietary categories (e.g. Ulhaas *et al.*, 2004; Boyer, 2008; Boyer *et al.*, 2010; Godfrey *et al.,* 2012; Winchester *et al.*, 2014; Allen *et al.,* 2015). For instance, those with hypsodonty or high crowns/cusps, like folivores and insectivores, show higher RFI and OR values than those with brachydonty or lower crowned/cusped molars, like frugivores and hard-object feeders (M'Kirera and Ungar, 2003; Ulhaas *et al.*, 2004; Boyer, 2008; Winchester *et al.,* 2014; Allen *et al.,* 2015; Berthaume *et al.*, 2020). Both RFI and OR ratios can be greatly affected by tooth wear since the cusp height decreases as wear accrues (Evans, 2013). Therefore, it is convenient to take into account dental wear when exploring crown relief (Pampush *et al.,* 2016b; Berthaume *et al.,* 2018).

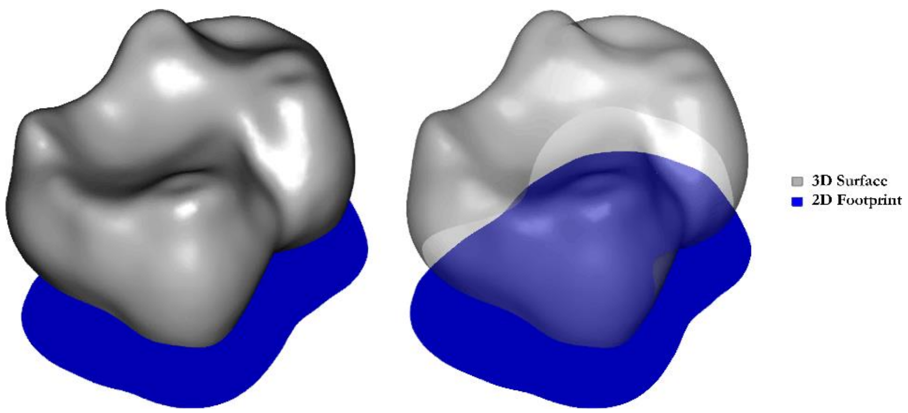


Figure 15. 3D surface view of mantled howler monkey lower first molar (in grey) with 2D surface footprint projected below (in blue). Adapted from Pampush *et al.* (2016b).

4.3. Orientation Patch Count Rotated (OPCR)

The Orientation Patch Count (OPC) was first introduced by Evans *et al.* (2007) to quantify dental complexity of cheek tooth-rows in carnivores and rodents. The occlusal surface of a tooth is divided into patches by grouping contiguous areas with the same orientation as a joint 'patch' (Figure 14c). However, Evans and Jernvall (2009) modified the OPC metric into the Orientation Patch Count Rotated (OPCR) to make the result less sensitive to tooth orientation and, therefore, increase the robusticity of the approach. OPCR accomplishes this by successively rotating the tooth eight times across a total arc of 45° (5.625° per rotation), calculating OPC at each new rotation. The mean of these eight OPC calculations provides an OPCR value. Essentially, OPCR can be considered a measure of the number of features or "tools" (e.g. cusps, crests, crenulations, and cutting edges) on the occlusal surface involved in the chewing process. An occlusal surface with more tools is more efficient at chewing foods with structural fibres and register higher OPCR values (Evans *et al.*, 2007; Berthaume *et al.,* 2018).

Several studies have proved dental complexity is a useful indicator of dental-dietary adaptation in different groups of mammals, such as carnivores, rodents or bats (Evans *et al.,* 2007; Evans and Jernvall, 2009; Santana *et al.,* 2011, Pineda-Munoz *et al.*, 2017). Generally, herbivores and insectivores have teeth with a complex occlusal surface (high OPCR values) since they are adapted to consume tough foods that are difficult to process. Therefore, differences in food processing and eating habits would demand different "tools". However, within primates, OPCR appears to be a poor indicator of diet, showing overlap among species with different dietary categories (e.g. Winchester *et al.*, 2014; Guy *et al.,* 2013; Berthaume *et al.*, 2018) probably because there is a low level of variation in dental complexity within primates compared to other mammals (Boyer *et al.,* 2010).

4.4. Portion de ciel visible (PCV)

Ambient occlusion, quantified through PCV (*portion de ciel visible*), is a recently introduced dental topographic metric (Berthaume, 2016a; Berthaume *et al.,* 2018) and it has been used in different studies in primates (Berthaume *et al.*, 2019a, b) and fossil hominins (Berthaume *et al.*, 2018; Berthaume *et al.*, 2019a). PCV has been used to quantify morphological wear resistance (Berthaume *et al.,* 2018). It is a visualization method for making 3D objects appear more realistic by measuring how exposed a surface is to ambient lighting. Nevertheless, this surface will be more or less exposed to the light depending on which direction the light is coming from. Moreover, dental topography studies only consider light coming from the positive z-direction, which in the case of teeth it is the occlusal direction. If a tooth is oriented in this way, the areas of the tooth that are more exposed to ambient light (e.g. cusps tips, crests, or blade edges) are more likely to contact the food/bolus during a chewing cycle, thus have higher PCV values, and are more likely to experience wear than areas that are less exposed to ambient light (e.g. basins, sides of enamel caps or enamel fissures) (Berthaume *et al.*, 2019a, b). Teeth that are less exposed to ambient light have lower PCV values and are more wear-resistant (Figure 16; see also Figure 14d).

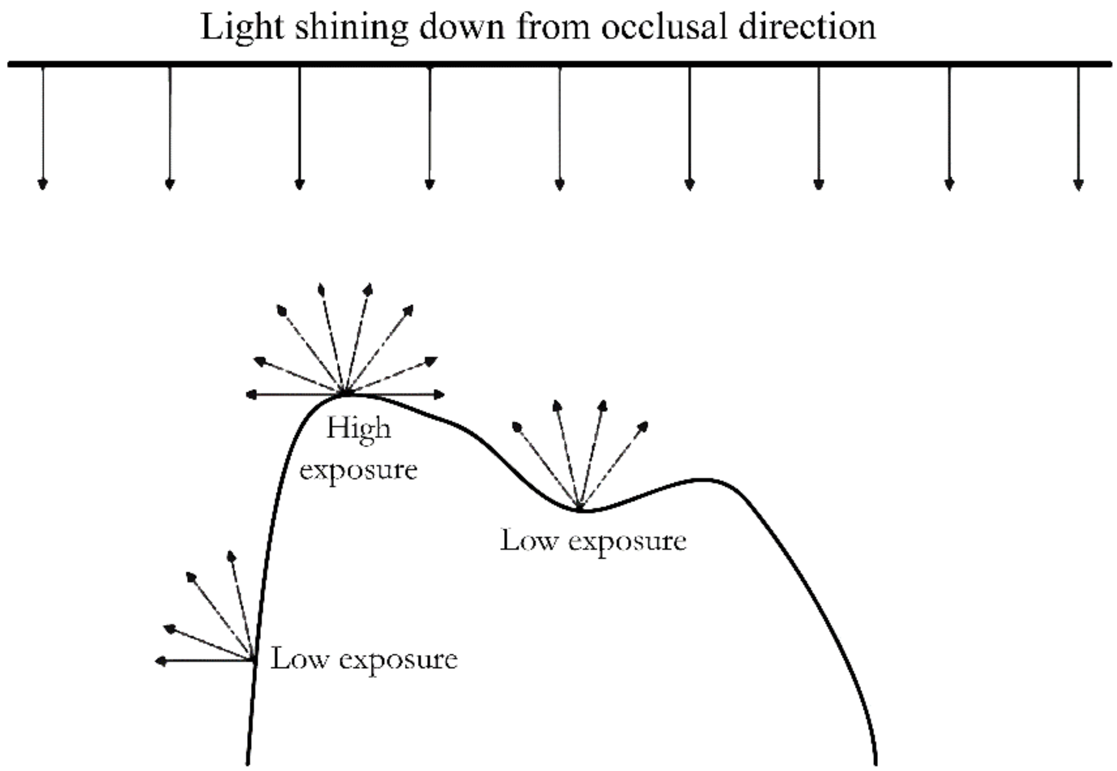


Figure 16. Light shining from the superior direction onto the occlusal surface of the tooth. Areas of the teeth that are more exposed to ambient light are more likely to contact with food, grit, and/or occluding tooth during mastication, making them more likely to experience wear than areas less exposed to ambient light. Teeth that are less exposed to ambient light have lower PCV values and are more wear resistant. From Berthaume *et al.* (2018).

A strong relationship between crown height and PCV was found in South African hominins, such as *Homo naledi*, *Paranthropus robustus*, and *Australopithecus africanus* (Berthaume *et al.,* 2018) but also within primates, such as platyrrhines and prosimians (Berthaume *et al.,* 2019b). In the latter study, average ambient occlusion would predict dietary categories in primates. Molars with taller crowns/cusps, such as folivores and insectivores, have lower PCV values than molars with relatively lower crowns/cusps, such as frugivores and hard-object feeders, since they have basins that are hidden from ambient light. Finally, PCV appears to be useful at predicting what parts of the tooth will experience wear once wear facets have formed (Berthaume *et al.,* 2019b).

OBJECTIVES and JUSTIFICATION

The work presented in this PhD dissertation focuses on the study of dental wear in modern human populations. Previous researchers have mainly applied subjective, qualitative methods to characterise dental wear, while dental wear quantitative studies of modern human populations are scarce, mostly limited to the quantification of the depth of grooves, the heights of dental crowns and cusps or the percentage of dentine exposure areas. This PhD research has been an opportunity to analyse differences in molar morphology among modern human populations and the effect of dental wear on shape, in an attempt to provide new insights of a yet poorly unexplored area of anthropological studies, through the application of novel quantitative techniques, such as three-dimensional geometric morphometrics and dental topographic approaches.

The general objective of this research can be broken down into specific objectives that are addressed in the chapters shown in this PhD dissertation.

CHAPTER 1. Age-related tooth wear in African rainforest hunter-gatherers

1. Analyse the long-term effect of USO (Underground Storage Organs) consumption on the wearing down of the first lower permanent molars of a known-age Baka Pygmy forager population with well-documented diet from southern Cameroon.

CHAPTER 2. Three-dimensional proxies for dental wear characterisation in a known age-at-death skeletal collection

2. Standardise scanning and three-dimensional (3D) meshes methodologies to obtain morphological and topographic data on molar teeth useful for among population comparisons.
3. Evaluate the efficiency and reliability of 3D proxies and metrics to describe shape changes with age and wear in modern human populations.
4. Explore three-dimensional pseudo-landmark-based morphometrics techniques for dental wear characterisation to investigate age-dependent shape differences in first and second permanent maxillary and mandibular molars of the Coimbra International Exchange known age-at-death skull collection.

CHAPTER 3. Dental topography and wear in Central African foragers and agriculturalist populations

6. Conduct dental topographic analyses to explore the association between dental topography and wear in first, second and third upper permanent molars in distinct hunter-gatherer and agriculturalist populations from Central Africa.
7. Evaluate if 3D topographic metrics may be used to detect changes in occlusal molar surface curvatures and complexity in relation to dental wear among populations showing distinct dietary regimens.

Further standardisation and hypothesis-testing analyses are still needed for determining the validity of 3D methods as proxies for characterising the morphological and/or topographical molar shape changes in relation to dental wear among populations. The scarcity of 3D analyses on worn teeth, most frequently discarded in dental research, justifies this attempt to characterise molar shape in relation to molar crown height loss.

CHAPTERS

CHAPTER 1

Age-related tooth wear in African rainforest hunter-gatherers

Abstract

Central African small-scale foragers subsist primarily on hunting game activities and wild plant-food gathering. Starch-rich tubers are underground storage organs (USOs) and staple food resources in savanna and tropical rainforests. However, little is known about the USO-eating on tooth wear behaviour in living hunter-gatherers. Here, we report age and sex-dependent tooth wear rates in forest-dwelling Baka Pygmies with well-documented wild yam tuber based-diet to explore the long-term impact of USO mechanical hardness and abrasiveness on the wearing down of the teeth. Percentages of dentine exposure (PDE) of permanent first molars (M_1) were recorded using *in vivo* high-resolution replicas of Baka individuals (aged 8-33 yeas) inhabiting Le Bosquet district in Cameroon (western Africa). Regression and covariance analyses were used to test the effect of individual aging by sex on PDE ratios. We found strong increase of PDE controlled for age among Baka individuals. No evidence of sexual dimorphism in wearing patterns suggests similar sex-related dietary and masticatory demands during growth. Overall, greatest dentine exposure values ≈4% denote unexpected slow wear down rates for foraging diets relying on USO consumption. The lower molar wear with age found in Baka Pygmies contrast with extensive wear-rates for savanna-dwelling foragers, reflecting differences in thermal processing techniques affecting fracture toughness and grittiness of mechanically challenging foods. Our findings reveal that culture-specific dietary proclivities influence tooth wearing among foraging behaviours with important implications in hominin dietary versatility and abrasive stress on chewing surfaces.

1.1. Introduction

Tooth wear refers to the loss of enamel and the gradual exposure of the underlying dentine as a physiological result of mastication. Diet-related abrasion is the predominant tooth wear mechanism, largely determined by the enamel resistance on working surfaces to food µm-scale indenter particulates (Gügel *et al.*, 2001; Romero *et al.*, 2012; Lucas *et al.*, 2013). Tissues of many edible monocotyledon plants, including various cereal species, contain phytoliths, hydrated silicon dioxide (opal), capable to abraded enamel during tooth-food-tooth contacts (Gügel *et al.*, 2001; Piperno, 2006; Lucas *et al.*, 2014). Other extraneous gritty quartz (crystalline silica) contaminants from grinding, roasted or airborne processing techniques, are potential wear agents >2.5 times harder than dental enamel (Newesely, 1993; Lucas *et al.*, 2013). Accordingly, the type and amount of abrasives influence the rate of enamel abrasion (Smith, 1984; Gügel *et al.*, 2001).

Tooth enamel wear is also an age-dependent process. However, tooth wear reports based on known-chronological age from living hunter-gatherers are scarce. Studies conducted on foragers retaining traditional diets from Brazil (Vieira *et al.*, 2015) and Greenland (Davies and Pederson, 1955) found a common pattern of extremely rapid and greater postcanine enamel loss rates relative to age than groups exposed to a diet based on less abrasive and more refined carbohydrate-rich foods. Other studies on Australian aborigines from native settlements provided a longitudinal source of tooth wear data on a transitional diet during dental development (Richards and Brown, 1981; Molnar *et al.*, 1983; Molnar and Molnar, 1990). A significant age-wear correlations has been shown and the rapid molar-wear rate and cusp height reduction was attributed to gritty contaminants introduced by maintained traditional cooking methods, since the bulk of their diet was obtained from low-abrasive modem western foods (Molnar *et al.*, 1983). Therefore, the age-diet dependence should have a very strong effect on tooth wear behaviour amongst populations in relation to different ecological and dietary proclivities (Tomenchuk and Mayhall, 1979; Richards and Brown, 1981).

Ethnographic and ecological data indicate that underground storage organs (USOs) such as roots and tubers are major sources of food year-round and supply over 50% the nutritional requirements for small-scale African foragers from semi-desert (Marlow and Berbesque, 2009; Crittenden *et al.*, 2017) and rainforest habitats (Heymer, 1986; Sato *et al.*, 2012), that consumed either raw or briefly thermally processed foods (Dominy *et al.*, 2008; Marlow and Berbesque, 2009). Likewise, the greater diversity of edible USO-bearing species in savanna environments notably contribute to include in the diet more species (65%) edible without cooking than in rainforest (<10%) habitats (Laden and Wrangham, 2005). Most USOs are highly resistant to fracture when raw and digging-derived outer tunic quartz particles potential enamel indenters (Dominy, 2012; Lucas *et al.*, 2014). While roasting significantly reduces tuber toughness and masticatory effort, increasing also starch digestibility and energy gain (Zink *et al.*, 2014), overall the mechanical challenge of energy-rich geophytes for human molars is still excessive (Dominy *et al.*, 2008).

A reliance on USO-bearing plant consumption has been suggested as far-reaching adaptive shift for early Pleistocene hominin radiation (Laden and Wrangham, 2005). Particularly, effectiveness of meat and starch-rich tuber dry roasting should largely impact on evolutionary apomorphic and cranio-dental changes in *early Homo* (Lucas, 2004; Zink *et al.*, 2014; Zink and Lieberman, 2016). Nonetheless, softened mechanically processed foods do not necessarily reflect nonabrasive potential for enamel (Romero *et al.*, 2012) and differences in foraging behaviour determine specific wear patterns (Richards and Brown, 1981; Galbany *et al.*, 2014). Yet molar wear signatures for African early *Homo* reflect dietary versatility including mechanically challenging foods during fallback episodes (Ungar and Scott, 2009, Martínez *et al.*, 2016). However, the USO abrasive effect on tooth wear-behaviour among modern foragers is poorly understood and should greatly contribute to delineate wear-related dietary histories for early *Homo*.

Currently, tooth-wear studies on known-age African foragers are limited and the impact of USO consumption on wearing patterns remains to be elucidated. For instance, dental physiological reports conducted on San (van Reenen, 1966), Hadza from savanna-woodlands (Berbesque *et al.*, 2012; Crittenden *et al.*, 2017) and rainforest-dwelling Pygmies (Heymer, 1986; Walker and Hewlett, 1990) are mainly based on estimated individual age from life-history events or long-term demographic data. Otherwise, most studies have used simple ordinal scales based upon dentin exposure to record the extent of molar wear according to age-cohorts. Because tooth wear occurs slowly as a progressive development with age, scoring scales result in a lack of finer-grained quantitative detail resolution of enamel loss (Richards and Brown, 1981; Molnar *et al.*, 1983; Górka *et al.*, 2015, 2016). Further, the food-tooth interaction by which dental tissue is lost remains overestimated unless individual age is independently known. Here, we conduct the first quantitative analysis of tooth wear based on dentine exposure ratios in living known-age Baka Pygmy hunter-gatherer population from southern Cameroon (Central Africa). We chose to analyse Baka Pygmies because their dental eruption timing (Ramirez-Rozzi, 2016) and growth patterns (Ramirez-Rozzi, 2018; Ramirez-Rozzi *et al.*, 2015) have been accurately stablished. Further, forest-based foraging activities, based on bushmeat and wild yam tubers (*Dioscorea* spp.) supply the bulk of the Baka diet, and culinary practices are well-documented for the Baka (Vallois and Marquer, 1976; Heymer, 1986; Sato *et al.*, 2012). Dietary behaviour is expected to result in specific intra-population dental wear patterns which might provide new insights on the tooth-effect of USOs consumption for the evolutionary hominin adaptations.

1.2. Material and methods

1.2.1. Study population

The Baka Pygmies are semi-nomadic hunter-gatherers living in equatorial African rainforest areas (Vallois and Marquer, 1976; Ramirez-Rozzi, 2018). Among western Pygmies, Baka groups show limited effective dispersal ranges probably reinforcing their genetic isolation (Verdu *et al.*, 2010). The Baka subsistence economy is based primary on hunting and foraging activities (Vallois and Marquer, 1976; Sato *et al.*, 2012). Foraging activities supply the bulk of the diet among Baka Pygmies mainly focused on gathering wild yam tubers (*Dioscoreaceae*), specially the *Dioscorea praehensilis* species, providing more than 60% of their estimated energy intake. Animal protein from small and medium sized hunted mammals provided ≈15%-20% of energy intake. Wild nuts, fish, honey or insect resources play a complementary role (Sato *et al.*, 2012). Overall, yam tubers and bushmeat occupied more than 90% of food weight in diet composition with few seasonal differences (Heymer, 1986; Hayashi, 2008; Sato *et al.*, 2012). Exchanges of products with their Bantu-speakers neighbours and practicing slash-and-burn farming influenced their livelihood driven by important social changes, especially since 1950s-1960s with government-led sedentarisation programs and missionaries' influences (Hayashi, 2008; Gallois *et al.*, 2015). Nevertheless, Baka continue to be highly dependent on wild resources from forest camps (Sato *et al.*, 2012; Hagino and Yamahuci, 2016). The exchanges are limited mainly to manioc or plantain produced by farmers for meat and honey and, commercially packaged foods are rarely consumed due to geographic isolation and cost (Hayashi, 2008; Ramirez-Rozzi, 2018).

1.2.2. Sample size

In the Baka village of Moango-le-Bosquet (Lomié District, Southeast Cameroon, Figure 1.1a), we recruited 96 Baka individuals (32 males, 64 females), aged from 8 to 33 years, during fieldworks carried out from 2007 to 2017 (Figure 1.1b). Only individuals that had the first mandibular permanent molar (M_1) fully erupted and in occlusion were included. Individuals with oral pathologies or developmental anomalies were discarded. We focused our study on the M_1 because it is the first permanent tooth to erupt in the Baka Pygmies and is in full occlusion at ≈5 years in both sexes (Ramirez-Rozzi, 2016). Further, the M_1s are expected to show higher occlusal wear with age than any other permanent tooth (Molnar *et al.*, 1983; Smith, 1984).

Individual chronological ages were obtained from birth records held by nursing assistants in the health centre at Monago-le-Bosquet (see Ramirez-Rozzi *et al.*, 2015; Ramirez-Rozzi, 2016, 2018 for further details). Baka individuals with unrecorded birth were not included. Participants were nonliterate and provided their oral informed consent for the study. This study obtained approval of the Centre National de la Recherche Scientifique, Agence National de la Recherche (France) and the French Institut de Recherche et Développement (IRD) and was carried out as part of the international agreement between the IRD and the Ministry of Scientific Research and Technology of Cameroon.

1.2.3. Data collection

High-resolution dental moulds of mandibular teeth were first made with hydrophobic polyvinylsiloxanes (Coltène-Whaledent®) using impression trays (Figure 1.1c). This material, that provides detailed and high-quality impressions of the tooth crown, was applied using a dispenser with a thin tip to remove air bubbles before contact with the enamel surface. Dental replicas were produced from moulds using Feropur PR-55 (Feroca®) polyurethane resin (see Romero *et al.*, 2013; Romero *et al.*, 2018, Figure 1.1d). To avoid air bubble formation during the polymerization of the resin, samples were centrifuged (1 min, 1000 rpm).

Figure 1.1. (a) Baka village of Moango-le-Bosquet (Lomié District, Southeast Cameroon); (b) Baka individuals selected for the study during the fieldwork carried out in 2017; (c) high-resolution dental moulds of mandibular teeth obtained with hydrophobic polyvinylsiloxanes (Elodie Lewo, fieldwork carried out in 2011); and (d) dental replica produced from moulds using polyurethane resin (Jean-Blaise Etoa, fieldwork carried out in 2017).

Digital images (3872×2592 pixels) of M_1 occlusal crown surface replicas were obtained with a digital single-lens reflex camera (Sony αA230 10.2MP) with a focal distance fixed at 50cm. Crown cervical lines were oriented perpendicular to the focal point of the camera using a levelling device and a millimetre scale was placed near the occlusal plane. Calibrated images were edited using Adobe Photoshop® CS5 to enhance image contrast and dentine areas resolution. Total occlusal area (TOA) and dentin exposure visible as depressed occlusal enamel areas were outlining (Galbany *et al.*, 2014; Górka *et al.*, 2016) (Figure 1.2). Measurements (in mm2) were recorded using SigmaScan Pro® software (SPSSTM, Chicago, IL). When several spots of dentin were present, each one was measured separately (Górka *et al.*, 2016) and the summed areas accounted for total dentine exposure (DE). The percentage of dentine exposure (PDE) was computed as (DE/TOA)*100 (Galbany *et al.*, 2014). Measurements were registered two times with at least 4-week interval in twenty-five randomly selected molars to evaluate the technical measurement error (TME). The TME values of TOA (1.771%) and DE (0.308%) were less than 5% indicating that the methods is highly precise and repeatable (Górka et al., 2016).

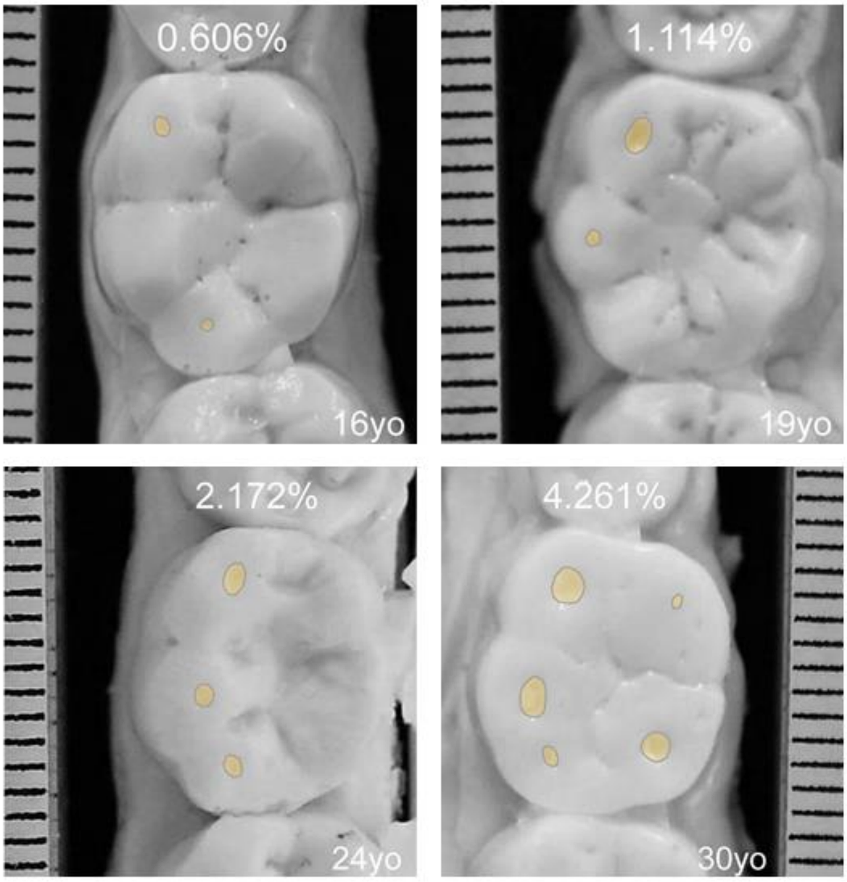


Figure 1.2. Occlusal view of mandibular first molars (M_1) showing the percent of dentine exposure (PDE) changes with individual age (in years) for Baka Pygmy foragers. Dentine exposed regions are highlighted. Note non-broad contact areas. Mesial: top; buccal: left. Scale in millimetres.

1.2.4. Statistical analyses

We tested arcsine-transformed molar PDE as independent linear and quadratic functions of log-transformed individual age covariate separately conducted for Baka males and females (Galbany *et al.*, 2014; Richards and Brown, 1981). Analyses of covariance (ANCOVA) were also used as needed to estimate the relationship and differences (homogeneity of slopes) between PDE on individual aging by sex. The Akaike Information Criterion (AIC) was used to test the best fit regression models (Galbany *et al.*, 2014). Descriptive (mean ± SD; standard deviation) and statistical analyses were conducted using PAST 3 (Hammer *et al.*, 2001). The significance level was set at α=0.01.

1.3. Results

We found a strong linear and quadratic relationship between tooth wear derived from PDE and chronological age as a predictor variable in Baka Pygmies (r^2 = 0.7, p < 0.0001; PDE = 0.792±1.085, 8-33 age range, N = 96). PDE values were also highly correlated with age for both Baka males (r^2 = 0.8, p < 0.0001; 8-33 age range, N = 32) and females (r^2 > 0.6, p< 0.0001; 10-30 age range, N = 64) individuals (Table 1.1 and Figure 1.3). Baka males (PDE = 1.036±1.252) generally had teeth that appeared to be more worn with age than females (PDE = 0.669±0.979). Likewise, the test for homogeneity of slopes ($F_{1,93}$ = 0.674, p = 0.413; ANCOVA) revealed non-sexual dimorphic trends in enamel loss with aging. The statistically best model following smallest AIC values corresponds to linear predictions (see Table 1.1).

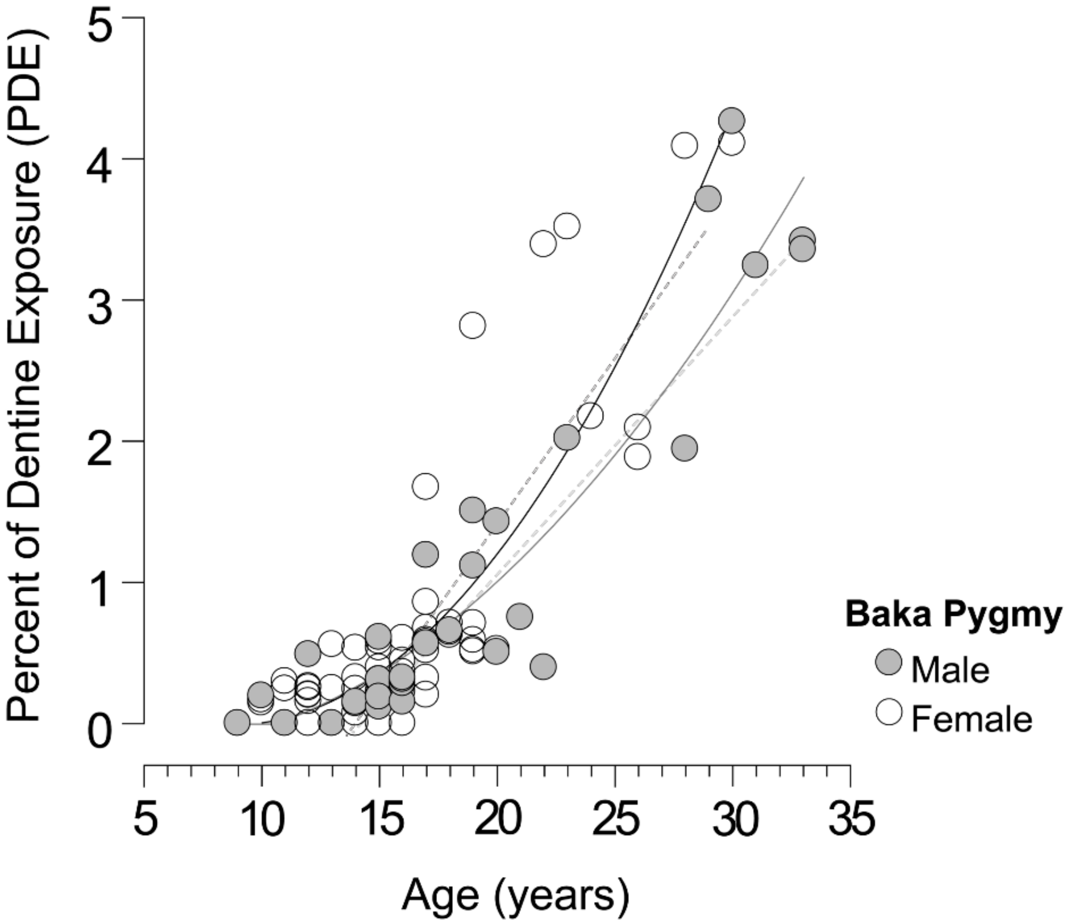


Figure 1.3. Age-related change in percent of dentine exposure (PDE) controlling for sex in Baka Pygmies. Linear (dashed lines) and quadratic (continuous lines) regression models (see Table 1.1). Note the increase of PDE values with age for both sexes.

Table 1.1. Regressions results for predicted percent of dentine exposure (PDE) in molar-teeth with age for Baka Pygmies.

Model	**Sex†**	***N***	***r*²‡**	**F**	***p***	**AIC§**	**Equation**
Linear	Male	32	0.811	128.8	<0.0001	6.596	PDE = 3.444 × age − 3.449
	Female	64	0.647	113.9	<0.0001	9.797	PDE = 3.836 × age − 3.921
	All	96	0.720	242.6	<0.0001	11.969	PDE = 3.624 × age − 3.670
Quadratic	Male	32	0.848	81.394	<0.0001	7.747	PDE = 3.801 × age^2 − 5.996 × age + 2.317
	Female	64	0.710	74.893	<0.0001	10.597	PDE = 8.479 × age^2 − 16.924 × age + 8.683
	All	96	0.759	146.58	<0.0001	12.761	PDE = 4.764 × age^2 − 8.167 × age + 3.545

Note. Dental wear values on first permanent molars (M_1) are expressed as Percent of Dentine Exposure (PDE) [total dentine exposure /total crown area*100].

†Male age range (8-33 years), female age range (10-30 years).

‡Coefficient of determination (r^2) differs significantly from zero at $p < 0.01$.

§Akaike Information Criterion (AIC).

Moreover, dentine exposure evidence is commonly detected at ages greater than 16 years, inducing significant intra-population differences in molar wearing patterns between age-ranged individuals ($F_{1,93} = 16.640$, $p < 0.001$; ANCOVA). Baka Pygmies between 8 and 16 years (PDE = 0.208±0.181, 0-0.6 in range; *N* = 56) wear down their molars at a lower linear association ($r^2 = 0.157$, $p = 0.002$) than older individuals ($r^2 = 0.666$; $p < 0.001$) showing heaviest rates of wear (PDE = 1.608±1.284, 0.20-4.26 in range; 17-33 individual ages; *N* = 40)

1.4. Discussion

We provide the first model of age-related tooth wear progression in Central African Baka Pygmy foragers. A strong linear association of molar wearing with aging was found in agreement with early reports on living foraging groups from artic (Davies and Pedersen, 1955; Tomenchuk and Mayhall, 1979) and more temperate climates (Richards and Brown, 1981; Molnar *et al.*, 1983; Vieira *et al.*, 2015). However, Baka Pygmies show surprisingly lower tooth wear rates than expected for foraging diets (Molnar *et al.*, 1983; Górka *et al.*, 2015).

We found that Baka individual age was able to explain 70% of tooth wear variability. Nonetheless, maximum PDE values no greater than 5% found in older ≈30 aged individuals correspond mainly to buccal cusps removal and moderately to large spots of dentine exposure (wear stages 3-4; Smith, 1984). No straightforward comparison is possible between PDE rates obtained in Baka Pygmies and qualitative wear patterns previously reported among other African Pygmy foragers (Heymer, 1986; Walker and Hewlett, 1990). Likewise, this negligible tooth wear is in complete contrast to the usual pattern of abrasion found among bush-dwelling Hadza (Northern Tanzania) at early ages, since ≈50% of the maxillary molars at estimated individual age ≥18 years exhibit flat surfaces with considerable exposed dentine (Crittenden *et al.*, 2017). Consequently, the low molar-wear rates with age among the Baka Pygmies compared to the Hadza dentition (Berbesque *et al.*, 2012; Crittenden *et al.*, 2017) might be mainly caused by differences in mechanical and physical properties of chewed foods.

Abrasive wear is likely due to repetitive loading of both phytolith-rich plant foods and exogenous grit (Lucas *et al.*, 2014). However, while enamel loss increases with silica content, the abrasive potential estimates for opal-phytoliths are way below those of siliceous grit and dust, >10 times higher for enamel abrasively (Newesely, 1993; Gügel *et al.*, 2001). Raw meat-consumption is not hard enough to scratch enamel surfaces when compared to plant foods (Lucas, 2004; Romero *et al.*, 2013) and the abrasive effect for enamel of animal-based foods is only dependent on silica-based grit adhered during meat roasting that Baka directly grill on the fire (Vallois and Marquer, 1972). Otherwise, Baka Pygmies consumed foraged plant foods with reduced etching particles compared to Bantu harvested foods (Romero *et al.*, 2013), since no phytoliths exist in yam and manioc starch grains from tropical environments (Piperno, 2006). Further, yam-like tubers, especially the annual plant *Dioscorea praehensilis*, are soaked in water for a few days before being eaten for detoxification, and boiled in pottery pots or cooked in hot ashes wrapped in leaves (Vallois and Marquer, 1972; Heymer, 1986; Sato *et al.*, 2012). In contrast, *Vigna frutescens* tubers are consumed by Hadza preferably raw or briefly open air roasting (Marlowe and Berbesque, 2009) and, flour derived from baobab hard seeds open-rock grinding incorporate high amounts of grit-soil particles as potential factors in Hadza dental abrasion (Berbesque *et al.*, 2012). However, Baka Pygmies grinding tubers and seeds to produce flour using wood mortars. Other harvested fruits are cooked to obtain porridges (Vallois and Marquer, 1976; Heymer, 1986). Overall, mechanical and thermal food processing in Baka culture appears to contribute to ingest generally less abrasive and tenderer foods (Dominy *et al.*, 2008; Romero *et al.*, 2013; Zink *et al.*, 2014).

Moreover, tooth-eruption and life-history events in comparison to worn surfaces are considered particularly relevant factors (Molnar *et al.*, 1983; Molnar and Molnar, 1990). Minimal tooth wear found among Baka Pygmies indicates significant implications in terms of the individual's age and suggest that wearing patterns could be also attributed to inter-individual masticatory function and dietary changes that occur during periods of growth and development. In this context, our findings reveal no evidence of sexual dimorphism of PDE rates controlling for age, suggesting equal enamel loss process and bite dynamics during individual growth affecting both males and females. Full lower-upper first molar occlusion occurs in the Baka between 5.2-5.5 years; with negligible sex differences in age ranges (Ramirez-Rozzi, 2016). Lack of gender-based differences in molar wear patterning is similarly reported among other foraging populations (Richards and Brown, 1981; Crittenden *et al.*, 2017). Otherwise, despite documented sexual division in subsistence activities (Vallois and Marquer, 1976; Gallois *et al.*, 2015), Baka males and females consume similar foods and acquire equal daily energy intakes (Sato *et al.*, 2012). Further, Baka children participate in the same food procurement activities than adults (Gallois *et al.*, 2015; Hagino and Yamahuci, 2016). However, we found significant increases in wear values up to age 16 years. In the Baka at Le Bosquet, first pregnancy occurs at 16 years and adult size reached at around 20 years of age for both sexes when body muscle and fat increase (Ramirez-Rozzi *et al.*, 2015; Ramirez-Rozzi, 2018). As Baka move into adolescence they are considered socio-culturally adults and their social role changes to include execution of the main part of subsistence-related activities (Vallois and Marquer, 1976; Gallois *et al.*, 2015), which require the increase of food intake for physical development (Hagino and Yamahuci, 2016). Thereby, greater metabolic demands and occlusal loading during adulthood might be sufficiently explaining age-related trends of the wear rates observed.

Meat and starch-rich USOs are considered keystone resources for early *Homo* and their mechanical processing involved adaptive smaller masticatory features (Lucas, 2004; Laden and Wrangham, 2005). Cheek-teeth occlusal microwear evidence suggests that *H. ergaster* might have relied more on hard-brittle foods (Ungar and Scott, 2009). Further, the highly abraded buccal enamel support also the consumption of a wide range of abrasive food items including mechanically demanding USOs (Martínez *et al.*, 2016). However, our findings challenge the view about the long-term abrasive impact of USOs consumption on enamel damage with important implications about cooking methods on hominin tooth wear behaviours (Laden and Wrangham, 2005; Dominy *et al.*, 2008; Zink and Lieberman, 2016).

Unprocessed raw edible tubers are too tough force-limited foods for human consumption (Dominy *et al.*, 2008; Zink and Lieberman, 2016). Roasting significantly decrease both tuber toughness and fracture stress for chewing (Zink *et al.*, 2014; Zink and Lieberman, 2016), while food acquisition and processing may actually introduce gritty contaminants that encourage tooth wear (Dominy *et al.*, 2008; Romero *et al.*, 2012). Previous buccal microwear findings denote that Baka Pygmies mainly consume foods with reduced abrasiveness (Romero *et al.*, 2013). Thence, Baka cooking modes in which both meat and USOs resources are thermally processed should also impact in reducing enamel abrasive foreign materials causing slower rates of dentine exposure. Instead, minimally processed grit-laden foods appear to be the major cause of higher enamel wearing for

Hadza foragers (Berbesque *et al.*, 2012). On the basis of these findings, we suggest that tooth wear behaviour is culturally specific among African foragers relying on USO-based diets and certain patterns are unique to particular food processing. Therefore, non-cooked mechanically processed foods (sliced meat and pounded USOs) causing abrasive effects on enamel wear within early *Homo* species (Ungar and Scott, 2009; Martínez *et al.*, 2016) should not be dismissed and may have favoured adaptive selection for smaller jaws and teeth (Zink and Lieberman, 2016). Because the mechanical properties of foods differently impact on occlusal morphology as the enamel wears down (Smith, 1984; Ungar and Scott, 2009), further studies are needed to explore dental topographic changes with wear among known-age living foraging groups.

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CHAPTER 2

Three-dimensional proxies to dental wear characterisation in a known age-at-death skeletal collection

Abstract

Dental wear is a function of age-at-death in human skeletal populations. However discrete scoring proxies of dentine exposure areas have shown to largely depend on dietary life-history and cultural practices. In addition, dental wear greatly limits research on dental morphological variability since unworn teeth are scarce in osteo-archaeological repositories. Age at death is seldom known and actual trends in dental crown loss are generally assumed to be age-dependent. We applied three-dimensional (3D) dental crown continuous metrics (geometric morphometrics and topographic shape descriptors) to explore the association of first and second permanent maxillary (UM1 and UM2) and mandibular (LM1 and LM2) molar wear with age in the Coimbra International Exchange known age-at-death skull collection. Results are indicative of significant regressions between the morphometric variables and age-at-death, though showing coefficients of determination of 1.4–23.9%. The precision percentages for determining age-at-death from dental crown shape varied from 31.8% to 45.3%, while a significant portion of the overall shape variation of the molar teeth studied could be attributed to anatomical traits independently of dental wear, since modern human populations display a great variability in cusp patterns and molar teeth relative size.

2.1. Introduction

Dental crown surface morphology is greatly modified by wearing processes during an individual's life span (Smith, 1984; Fiorenza *et al.*, 2018, Schmidt and Watson, 2020). The wear of teeth is an irreversible long-term dynamic process of enamel tissue loss and gradual dentine exposure due to well-defined processes of attrition during tooth-to-tooth contacts, abrasion caused by silica-based particles through food-tooth contacts, either intrinsic to the ingested foods or exogenous contaminants, and erosion caused by food chemical agents (Richards and Brown, 1981; Kaidonis *et al.*, 2012). Dental wear is an age-related process greatly dependent on the abrasiveness of ingested foods in relation to dietary habits and food processing techniques (Larsen, 1997; Kaidonis *et al.*, 2012). Qualitative methods for the characterisation of dental wear in human populations, using discrete scoring scales, are largely observer-dependent (Scott 1979; Smith 1984). Instead, quantitative methods, measuring cusp heights (Walker *et al.*, 1991; Mays, 2002; Benazzi *et al.*, 2008) or the percentages of dentine exposure areas (Górka *et al.*, 2016), reduce interobserver measurement errors in recording tooth crown shape changes through wear. Despite the rates of dental wear mostly depend on dietary habits (Smith, 1984; Benazzi *et al.*, 2008; Fiorenza *et al.*, 2011, 2018; Clement and Hillson, 2012), interpopulation comparisons are difficult to make when individual chronological ages remain unknown. The assessment of age from dental wear scores is based mainly on compiled skeletal collections of known age-at death (Mays, 2002; Benazzi *et al.*, 2008). However, discrepancies between known and estimated ages may arise, especially in individuals showing extreme stages of wear (Millard and Gowland, 2002). Further, discrete classifications of dental wear might only be reliable for clustering specimens into selected age groups (Alayan *et al.*, 2018).

Teeth are geometrically complex structures and two-dimensional (2D) methods can only partially measure wearing processes and crown shape changes over time (Smith, 1984; Benazzi *et al.*, 2008). Three-dimensional (3D) methods quantify dental morphology of the whole crown surface, including overall crown shape and complexity (Ulhaas *et al.*, 2004; Benazzi *et al.*, 2011; Evans, 2013). For instance, 3D Geometric Morphometrics (3D-GM) has been applied to investigate occlusal surface covariation (Polychronis *et al.*, 2013) and sexual dimorphism (Yong *et al.*, 2018) of cheek teeth. However, no comparable studies have used pseudolandmark-based 3D-GM shape variation for characterising both unworn and worn out molar crowns of known-aged individuals. Landmark-free topographic metrics have established interactions between dietary related signals and tooth function among non-human Primates (Boyer, 2008; Winchester *et al.*, 2014; Berthaume and Schroer, 2017). Dental topography measures include OPCR (Orientation Patch Count Rotated; Evans *et al.*, 2007), DNE (Dirichlet Normal Energy; Bunn *et al.*, 2011), and RFI (crown Relief Index; Boyer, 2008). OPCR reflects the complexity of a dental crown surface by assessing the diversity of the orientation of enamel patches (Evans *et al.*, 2007; Pampush *et al.*, 2016a). Higher cusped teeth are expected to show greater OPCR values than lower crested occlusal surfaces (Winchester *et al.*, 2014; Pampush *et al.*, 2016a). DNE quantifies surface bending, with sharpened occlusal surfaces showing higher DNE values than low cusped ones (Bunn *et al.*, 2011). RFI provides a ratio of 3D crown surface area to the 2D-projection dental crown size (Boyer, 2008), with cusped crown surfaces showing high RFI values (Ulhaas *et al.*, 2004; Boyer, 2008). Topographic measures on unworn or slightly worn teeth have provided a phylogeny independent measure of diet-related dental functionality (Evans, 2013; Berthaume and Schroer, 2017). Nevertheless, reports on the interaction of dental topography with wear are scarce (Pampush *et al.*, 2016b, 2018; Berthaume *et al.*, 2018). No previous studies in modern humans have documented how enamel wear progression affects topographic features and shape descriptors or how individual age and wear sequences covariate with dental crown shape and complexity metrics. Likewise, no standardised, 3D quantitative wear assessment parameters (related to aging processes) is yet available.

The aim of the present study is to evaluate 3D pseudo-landmark based morphometrics and topographic metrics to assess the significance of cheek teeth cusp shape changes with wear in a known sex and age-at-death, well-documented modern human osteo-archaeological population. Testing dental methodological approaches is important for archaeological studies, especially if researchers want to analyse how a biological structure changes through the life of the individual, as is the case of dental crown shape with age and dental wearing processes.

2.2. Material and methods

2.2.1. Dental sample

Eusébio Tamagnini assembled the *International Exchange Skull Collection* between 1932 and 1942, housed at the Department of Life Science (University of Coimbra, Portugal). The Collection consists of 1,075 identified skulls of known sex and age-at-death individuals from 6 to 109 years old, most of them born in Portugal between 1817 and 1924 and inhumed at the *Conchada* cemetery in Coimbra (Rocha, 1995; Cunha and Wasterlain, 2007). Along the 19th and 20th centuries this Portuguese population experienced slow modernization and a strong duality between urban centres and rural areas (Weisensee and Jantz, 2011). Most documented individuals were from a low socioeconomic class and deceased at varying towns, as well as at hospitals (see Santos, 2000; for further details). Overall, their nutritional intake was poor; their daily intake greatly consisted of maize bread, green and dried vegetables and potatoes, with reduced intake of fish (usually sardine and salt codfish) and bacon (Correia, 1951). We selected 411 first and second permanent maxillary (UM1 and UM2) and mandibular (LM1 and LM2) molars, preferably left side (65.2%) depending on preservation, from 142 crania and associated mandibles of both sexes (69 males, 73 females). We included a minimum of five individuals of each sex and cross-sectional six-year age-group intervals, from 6 to 65 years of age (Table 2.1), and discarded teeth with pathological conditions or dental crown damage. The dental crown sample included broad changes in crown shape with age, ranging from unworn molars to all degrees of dentine exposure (Smith, 1984).

Table 2.1. Samples analysed by tooth-type and sex.

Tooth		Female [a]	Male [b]	All
Upper M1	UM1	54	51	105
Upper M2 [c]	UM2	47	50	97
Lower M1	LM1	55	54	109
Lower M2 [c]	LM2	49	51	100
Total		205	206	411

[a] Age-ranged of the female sample from 7 to 65 years old.
[b] Age-ranged of the male sample from 6 to 65 years old.
[c] Samples including individuals with ages ≥11 years old.

2.2.2. 3D models acquisition

The selected teeth were cleaned with pure acetone and ethanol, using a cotton bud, to remove preservativers, dirt or any other particle attached to the enamel surface. We obtained silicone-base moulds (Affinis® regular body, Coltène-Whaledent Corp., Switzerland) from the original tooth crowns and produced high-resolution polyurethane replicas (Feropur PR-55, Feroca® Composites, Spain) (Figure 2.1a, b). The samples were centrifuged (1 min, 1000 rpm) to avoid air bubble formation during the polymerization of the resin. This tooth replication technique allowed for the obtention of high-quality dental copies without damaging the original samples. The 3D digital models of the upper and lower molars were obtained using a structured light scanner (HP DAVID® SLS-2) from twelve fused scans at 30º rotation intervals covering 360º and 60 µm resolution, saved as triangulated polygonal meshes (.ply format) (Figure 2.1c).

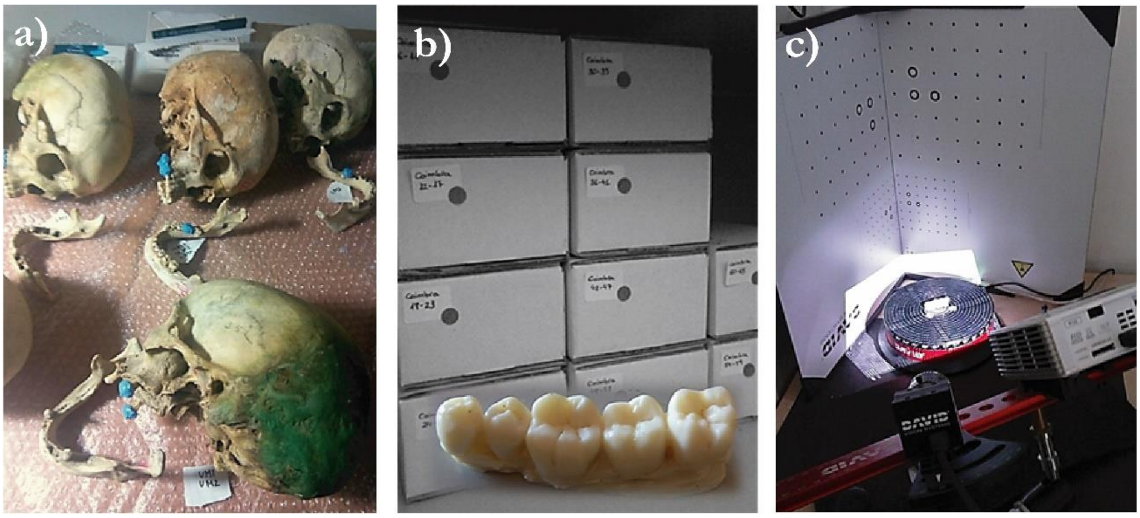

Figure 2.1. (a) Obtention of the silicone-base moulds from the original samples of the Coimbra Exchange skull collection, held at the Department of Life Science (University of Coimbra, Portugal); (b) high-resolution polyurethane replica of an upper left P3-M2, held at the University of Barcelona; and (c) structured light scanner (HP DAVID® SLS-2) scanning a sample.

We then processed the 3D meshes with the Geomagic® 2014 software (3D Systems, Rock Hill, USA) to isolate teeth by cropping the dental crowns along the cemento-enamel junction (CEJ) and the interproximal facets. The surface cropping method used provides topographic information of the entire dental crown, rather than only of a dental crown cap when cropping at the lowest point of the occlusal enamel basin, particularly when teeth are highly worn (Berthaume *et al.*, 2018). We then re-triangulated and slightly smoothed meshes to generate homogenous polygon face sizes retaining a fine-scale surface geometry. A conservative smoothing prevented inconsistencies in topographic metrics (Spradley *et al.* 2017), showing insignificant effects on mesh distances and volumetric measurements (Veneziano *et al.*, 2018). Finally, the edited 3D tooth models were mirror-imaged to the left side when necessary to standardise spatial position for the morphometric analysis. The meshes were oriented to maximize crown projection with the occlusal plane perpendicular to the Z-axis (Pampush *et al.*, 2016a) using the open source system MeshLab (Cignoni *et al.*, 2008; http://www.meshlab.net/).

2.2.3. Geometric Morphometrics

3D-GM techniques capture molar shape variation from 3D coordinate data (Polychronis *et al.*, 2013; Yong *et al.*, 2018). We used a pseudo-landmark approach to characterise dental crown shape from the processed meshes using the Geomorph package (Adams and Otárola-Castillo, 2013) for R statistical computing environment, since cusp tips and dental crown grooves are not preserved in heavily worn teeth (Dykes and Pilbow, 2019). We defined a landmark configuration (Bookstein, 1991), irrespective of dental wear, including six type II landmarks along the CEJ and two type III landmarks on the proximal and distal rims of the occlusal crown surface (Table 2.2, Figure 2.2). Two dental crowns lacking dentine exposure were used to build representative templates of upper (UM1 and UM2) and lower (LM1 and LM2) molars. We choose a 4,800 pseudo-landmarks configuration for characterising the dental crown surface topography after testing varying pseudo-landmark densities (600, 1,200, 2,400 and 4,800). Templates with a low number of pseudo-landmarks showed more variable and larger triangles on the dental crown surface than those with a larger number, which seemed to better replicate the steeper slopes of the lateral surfaces (Figure 2.3). Then, Geomorph *buildtemplate* command was used to derive the 4,800 pseudo-landmark meshes that were placed onto the dental crown using the *digitsurface* function that superimposed the 4,800 points of the template onto the scanned dental crowns using the 8 defined landmarks configuration as spatial reference (Figure 2.4). The XYZ coordinates of the pseudolandmarks were computed minimizing the distances between the template points and the superimposed cloud points. The computed pseudo-landmark coordinates (.NTS file format) are representative of the overall surface shape of the analysed tooth and can be considered as type I true landmarks (Adams and Otárola-Castillo, 2013).

Table 2.2. Definition of landmarks selected for the GM analysis of molar crown shapes.

Type*	Landmark	Definition
1	II	Disto-Lingual landmark on the CEJ, tangent at 45º angle between the distal rim (landmark 8) and landmark 2 in occlusal view
2	II	Lingual landmark on the CEJ, between landmarks 1 and 3 at the lingual groove
3	II	Mesio-Lingual landmark on the CEJ, tangent at 45º angle between the mesial rim (landmark 4) and landmark 2
4	III	Mesial point, on the occlusal rim, between landmarks 3 and 5 in occlusal view
5	II	Mesio-Buccal landmark on the CEJ, tangent at 45º between the mesial rim (landmark 4) and landmark 6
6	II	Buccal landmark on the CEJ, between landmarks 5 and 7 at the buccal groove
7	II	Disto-Buccal landmark at the CEJ, between landmark 6 and the distal rim (landmark 8) in occlusal view
8	III	Distal point, on the occlusal rim, between landmarks 1 and 7 in occlusal view

* Landmark type classification according to Bookstein (1991) defined in Geomorph (Adams and Otárola-Castillo, 2013).

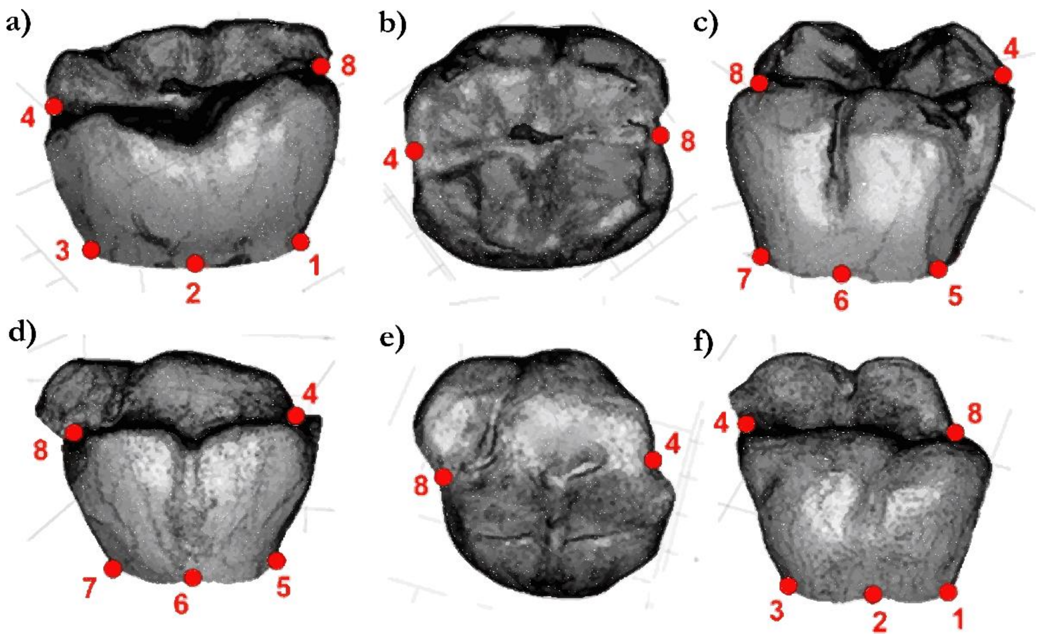

Figure 2.2. Landmark configuration used in the GM analyses. Lower molar: (a) lingual side, (b) occlusal view and (c) buccal side. Upper molar: (d) buccal side, (e) occlusal view and (f) lingual side. Landmark 1 is place in a disto-lingual position in both teeth and the following landmarks are set consecutively in a clockwise direction in occlusal view. Landmarks 1, 2 and 3 placed on the lingual side, while 5, 6 and 7 located on the buccal sides (all at the CEJ). Landmark 4 is proximal and 8 is distal, both on the occlusal margin.

We used a Generalized Procrustes Analysis (GPA) to rotate, scale and superimpose the landmark configurations of all specimens using least-squares estimates for standardising size within the multivariate analysis (Slice, 2007). The GPA was performed using the *gpagen* function of the Geomorph library, which computes the centroid size (CS) as the square root of the sum of the squared distances of all landmarks from their centroid (Bookstein, 1991).

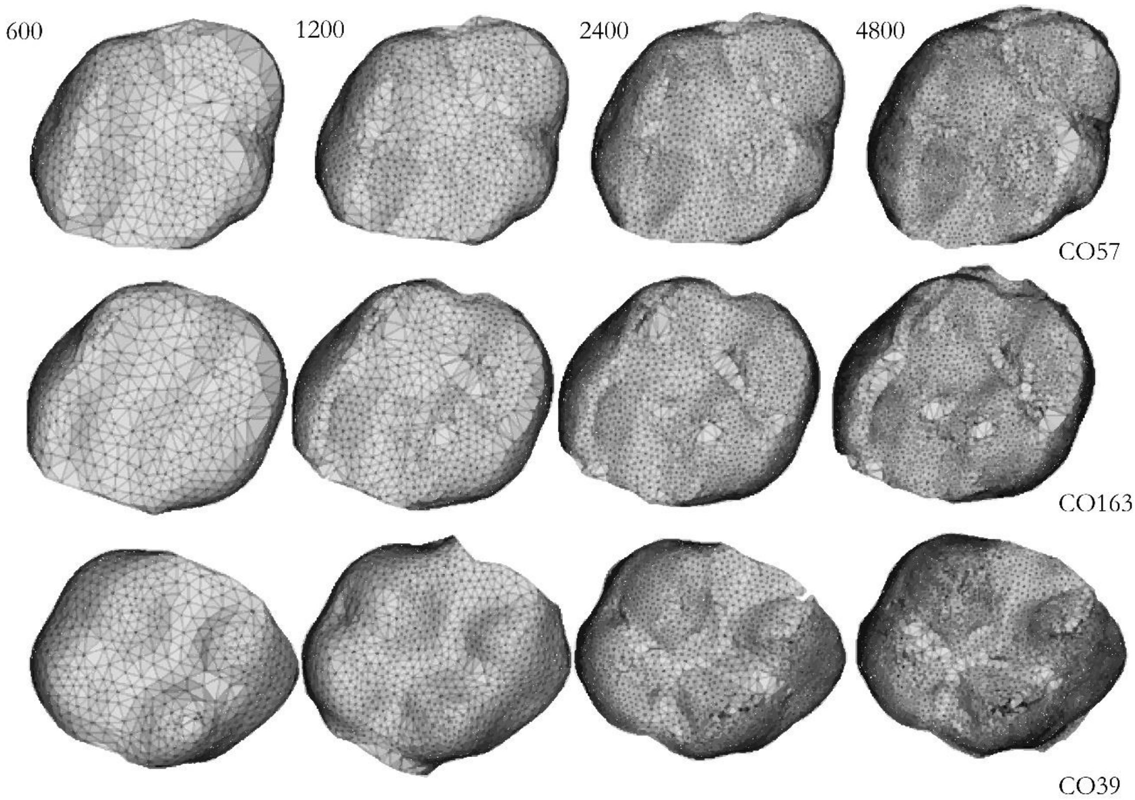

Figure 2.3. Templates comparisons for the upper left M1 molars using 600, 1200, 2400, and 4800 points configurations to test which template configuration best reflected dental crown topography. A better triangulation is observed when the number of points increases, more closely reflecting the actual surface topography. Three specimens (CO57, CO163, CO39) from the *International Exchange Skull Collection*, University of Coimbra (Portugal) were used in the comparison.

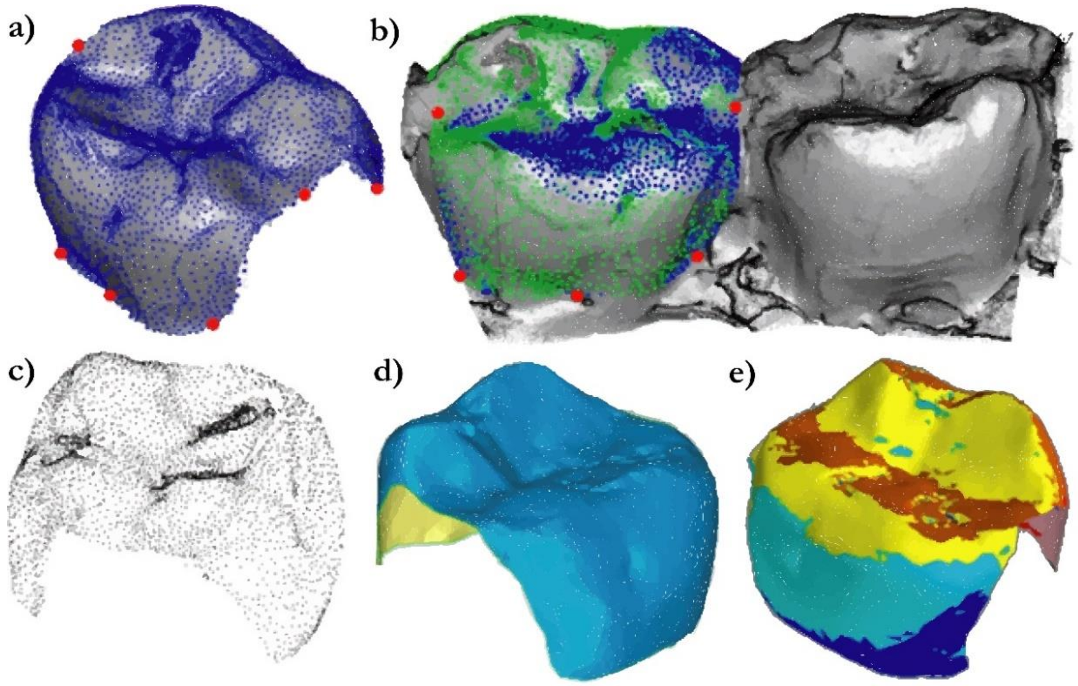

Figure 2.4. Standardised procedure to generate the 4,800 pseudo-landmarks configuration onto the analysed tooth using the template (blue dots in a). The derived pseudo landmarks (green dots in b) were superimposed onto the dental crown mesh using the eight-landmark configuration (red dots in a and b). (a) Landmarks (red) and pseudo-landmarks (blue) on the template; (b) superimposition of the template landmarks (blue) onto the 3D mesh to generate the pseudo-landmarks (green) on the studied specimen; (c) point cloud mesh of the pseudo-landmarks derived after de superimposition; (d) 3D surface derived from the 4,800 landmark configurations used for the topographic analysis; and (e) image derived from the OPCR topographic analysis.

2.2.4. Topographic metrics

We also characterised age-related changes in dental crown shape using topographic metric algorithms in MorphoTester (Winchester, 2016), including OPCR, DNE, RFI (see Figure 2.5 for further details), and the complementary size (in mm^2) metrics of 3D surface areas (3D-Area) and 2D projected crown surfaces (2D-Area). As a novel approach, we use the 4,800 pseudo-landmark 3D-GM derived coordinates for each tooth to record the topographic metrics by transforming the 3D coordinate point clouds into surface meshes (.ply format). We measured OPCR from the non-rasterized 3D polygonal mesh surfaces with a minimum patch size count of 5 polygons and averaging the oriented patch values rotated eight times between 0° and 45° (Winchester, 2016). DNE values were obtained using a 0.01% (99.9th percentile) energy output (Berthaume *et al.*, 2018) and RFI was computed as the ratio of 3D area to the projected 2D planimetric surface area of the occlusal table (Pampush *et al.*, 2018).

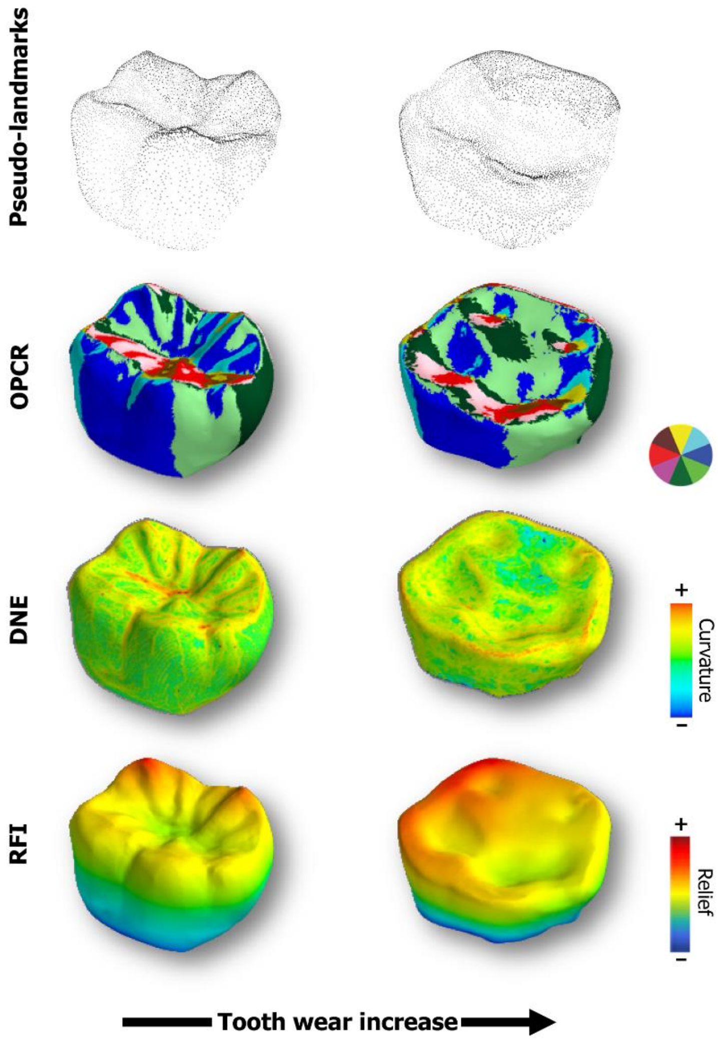

Figure 2.5. 3D models of the LLM1 of specimens 145 (left) and 232 (right) of the Coimbra Exchange skull collection showing the 4,800 pseudo-landmark configuration (top row), the dental crown complexity (OPCR) and curvature (DNE) views (second and third rows respectively) derived from MorphoTester, and the crown surface elevation(bottom row) used to compute the RFI.

2.2.5. Statistical analyses

We first calculated the intra- and interobserver measurement error for the proxies used by three different observers. The repeatability of the procedure was tested by making 5 repeated measurements, with at least two-day interval, of a sample of 10 upper first molars (UM1) with different degrees of dentine exposure. We measured the percentage of intra- and interobserver errors as the coefficient of variation (CV) of the standard error of the repeated measurement (Singleton, 2002), and estimated the significance of the inter-repetition and interobserver models using a Mixed Linear model with repeated measurements and Student t-test comparisons in IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA). The Kolmogorov-

Smirnov (K-S) test analysed the normality of the variable distributions. A Principal Components Analysis (PCA) was performed to calculate patterns of molar shape variations, summarizing the complex 4,800 points multidimensional data into a number of eigenvectors that are linear combinations of the landmark displacements (O'Higgins, 2000; Zelditch *et al.*, 2004; Webster and Sheets, 2010; Klingenberg, 2011). The covariation of molar shape with age and size (CS) was tested with a Procrustes ANOVA test using the Geomorph *procD.lm* function (Adams and Otárola-Castillo, 2013). We derived linear regression models of molar crown topography on age using the Reduced Major Axis (RMA) algorithm using PAST 3.0 (Hammer *et al.*, 2001). We used non-parametric, between-group tests (median Yates and Kruskal-Wallis) to detect between-group differences in dental shape ($\alpha = 0.05$).

2.3. Results

2.3.1. Measurement error

The 3D-GM PCA procedure applied to the repeated measures data set showed small average CV values for the first 7 PCs derived: 4.30% for observer 1 (3.26-5.06% range), 4.11% for observer 2 (2.90-4.88% range), and 4.37% for observer 3 (3.41-5.92% range), with an overall average of 4.26% (smaller than the critical 5% error). The mixed-linear repeated measure ANOVA models showed significant among-group differences for the observer-factor for PC1 (F=31.076, p<0.0001) and PC2 (F=9.083, p<0.001), but not for the repeat-factor (PC1: F=0.977, p=0.438; PC2: F=0.793, p=0.541). PC1 showed significant differences between observers 2 and 3 (t=4.298, p<0.0001), while PC2 showed significant differences between observer 1 and both observers 2 (t=5.578, p<0.0001) and 3 (t=6.597, p<0.0001). The greatest dispersion of the repeated measurements was shown for observer 2 for PC1 and for observer 1 for PC2 (Figure 2.6). Overall, the 3D-GM repeated measurement test showed that the methodological error within each observer was non-significant (p>0.05) and smaller than 5%, whereas the researchers may significantly differ in their observations.

Regarding the topographic analysis, the mean CV of the standard error of the repeated topographic metrics was 4.23% for DNE (4.00%, 4.17% and 4.50% for observers 1, 2 and 3, respectively), 2.19% for OPCR (2.13%, 2.15% and 2.28%), and 1.03% for RFI (0.94%, 1.04% and 1.12%). The mixed-linear model did not show significant among-group differences for DNE, either for the observer factor (F=1.659, p=0.195) or for the repetition factor (F=0.252, *p*=0.906). This was also the case for OPCR (observer factor F=1.060, *p*=0.350; repetition factor F=0.222, *p*=0.924). However, significant differences were observed for RFI between observers (F=55.639, *p*<0.0001), though not among repetitions (F=1.139, *p*=0.361). Observer 3 measured significantly larger 3D surface areas (F=19.081, *p*<0.0001), while no significant differences were observed among researchers for the 2D outline projected area (F=1.943, *p*=0.148). Differences in defining the cement-enamel junction would explain the larger RFI values obtained by observer 3. As expected, this was the major source of interobserver error. To prevent biases due to the interobserver error, the maxillary and mandibular teeth were independently measured by observer 2 (lower teeth) and observer 3 (upper teeth).

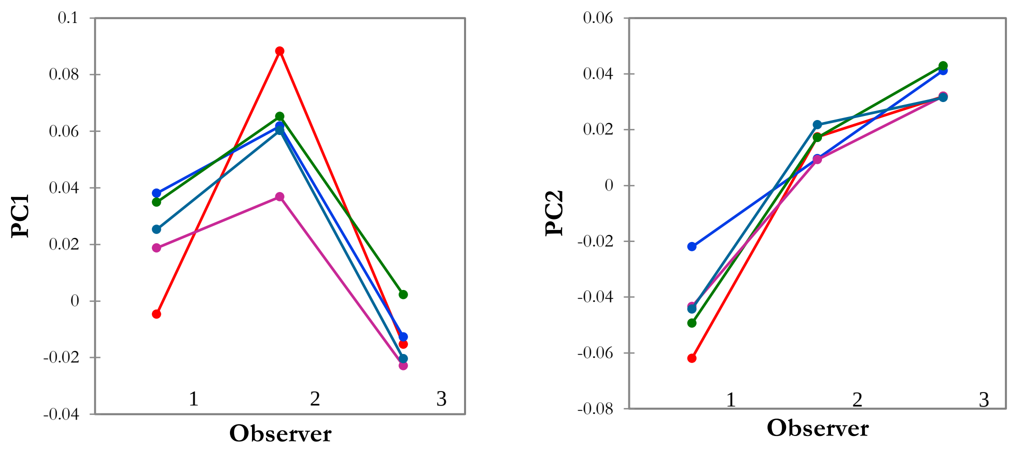

Figure 2.6. Mixed Lineal model with repetitions for the first two Principal Components of the repeated measurement test. The between-observer variability of PC1 (left) and PC2 (right) are shown for the five repeated measurements indicated by the coloured lines (repeat 1, repeat 2, repeat 3, repeat 4, repeat 5)

2.3.2. 3D morphometric analysis of dental crown shape variation

The 3D-GM PCA of dental shape by tooth type (UM1, UM2, LM1 and LM2) yielded a large number of PCs, of which we only considered those explaining more than 1% of total shape variance (Supplementary material): 18 for the UM1 (82.36%), 15 for the UM2 (83.32%), 15 for the LM1 (83.60% of total variance) and 16 for the LM2 (84.84%). The results differed by tooth type depending on both the morphological variability of the tooth considered and on the variation in occlusal wear. Some components showed a clear association to dental crown shape variation, such as the presence of the disto-lingual cusp (hypocone) in the UM1 (PC1, PC3) and UM2 (PC1, PC3, PC11), or with the Y5 cusp pattern in the LM1 (PC11, PC12), or the relative cusp size on the +4 pattern of the LM2 (PC5, PC16). However, most PCs showed distinct patterns of dental crown or cusp height loss caused by dental wear (PC1, PC3 and PC5 for the LM1; PC1 and PC3 for the LM2; PC1, PC4 and PC7 for the UM1; and PC2, PC8, and PC10 for the UM2) (see Supplementary material). The Procrustes regression models of the overall crown shape onto age showed significant associations only for the lower molars (p=0.001 for LM1, p=0.020 for LM2), though not for the upper ones (p=0.312 for UM1, p=0.172 for UM2). Independent linear regression models of the first 3 PCs (accounting for ≈40% of total variance) on age showed statistically significant, negative linear regressions of age on PC1 for the UM1, PC2 for the UM2, and on PC1 and PC2 for both the LM1 and LM2 (Table 2.3, Figure 2.7). None of the correlations between PC3 (<12% of total variance) and age were significant. The Pearson correlation (r) coefficients (ranging from −0.484 to 0.312; Table 2.3) showed that despite the significance of some associations, the PCs explaining the highest percentages of overall shape variation did decisively account for age-at-death.

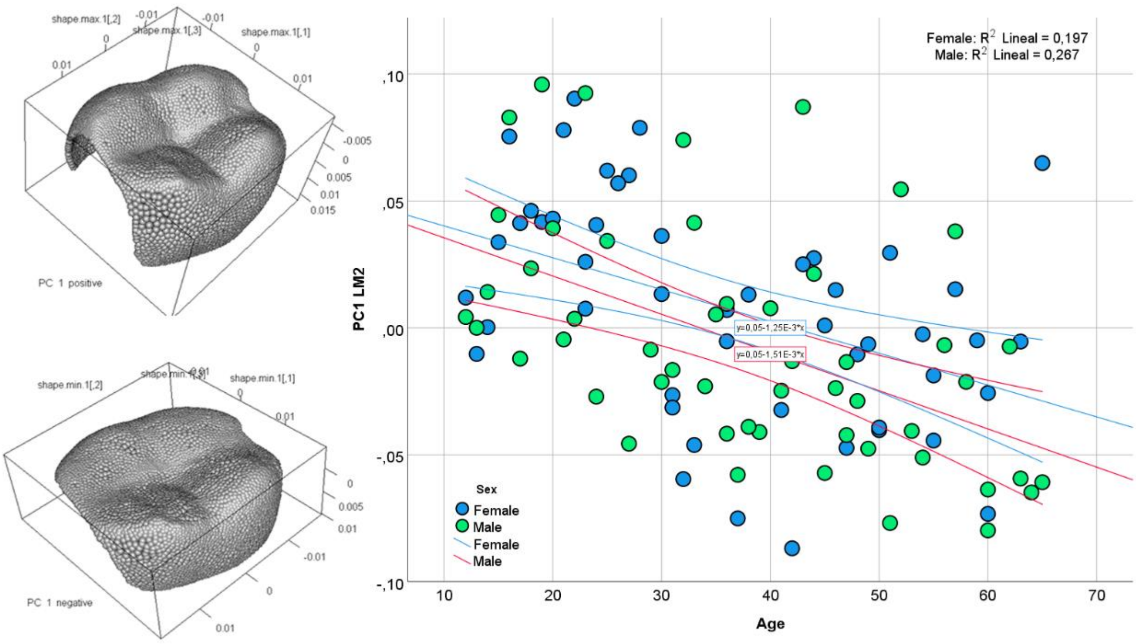

Figure 2.7. Linear regression models between PC1 of the GM analysis of dental crown shape and the age-at-death of all the specimens studied for the LM2 teeth by sex. Images on the Y axis show the landmark configuration for the minimum (bottom, showing a smaller crown height and less marked and rounder cusp tips) and maximum (top, showing taller crowns and more prominent cusp tips) PC1 values.

Table 2.3. Linear regression analyses of the Principal Components explaining the shape variability observed on age.

Tooth	*N*	PCs	Variance (%)	Intercept	95% CI of slope		*r*	R (r^2)	*t*	*P*
UM1	105	PC1	19.99	0.077	−0.002	−0.001	−0.218	0.047	−2.272	0.025*
		PC2	10.56	0.056	−0.005	−0.001	−0.162	0.026	−1.672	0.097
		PC3	9.34	−0.053	0.001	0.004	0.065	0.004	0.670	0.504
UM2	97	PC1	21.51	−0.109	0.002	0.009	0.121	0.014	1.195	0.234
		PC2	12.73	0.083	−0.002	−0.001	−0.304	0.092	−3.116	0.002*
		PC3	10.76	−0.077	0.001	0.006	0.062	0.003	0.609	0.543
LM1	109	PC1	21.14	0.084	−0.002	−0.002	−0.438	0.191	−5.042	0.000*
		PC2	13.46	−0.067	0.001	0.002	0.312	0.097	3.404	0.000*
		PC3	11.73	0.063	−0.005	−0.001	−0.158	0.025	−1.661	0.099
LM2	100	PC1	23.42	0.108	−0.003	−0.002	−0.484	0.234	−5.477	0.000*
		PC2	12.98	0.080	−0.002	−0.001	−0.290	0.084	−3.000	0.003*
		PC3	10.88	0.073	−0.005	−0.001	−0.158	0.025	−1.588	0.115

Confidence interval (CI); Pearson correlation coefficient (*r*); Student t-test for the slope (*t*). Percent variance for the three first PCs by tooth are: 39.88% for UM1, 45.00% for UM2, 46.33% for LM1 and 47.28% for LM2. Statistically significant regressions at $p<.05$ (*).

In addition, the analyses of shape allometry showed significant effects of both the centroid size (CS) and age on shape variation for the lower dentition, though not for the combined effects of size and age (Table 2.4). For the topographic variables (OPCR, DNE, RFI; Table 2.5), the linear regression models with age showed significant correlation coefficients for all the studied teeth and variables except for OPCR of LM2 (Table 2.6). The significant correlation coefficients ranged from −0.477 to −0.198. RFI showed higher absolute correlations in the lower molars, while DNE showed higher absolute correlations in the upper molars (UM1 and UM2) and the first lower molar (LM1). All the correlations were significant and negative, showing that the curvature, complexity and crown relief decreased with age.

Table 2.4. *P*-values of ANOVA of the GM Procrustes shape regression on the Centroid Size and age as factors for each molar tooth studied.

Tooth	CS *P-value*	Age *P-value*	CS×Age *P-value*
UM1	0.152	0.299	0.343
UM2	0.056	0.176	0.121
LM1	0.139	0.001*	0.582
LM2	0.001*	0.037*	0.110

Statistically significant differences at p<0.05 (*)

Table 2.5. Descriptive statistics (mean ± standard error, e_x) of the topographic variables (DNE, OPCR, RF) and the measures of 3D surface area (3D-Area) and the projected outline surface area (2D-Area).

		LM1		LM2		UM1		UM2	
		mean	**e_x**	**mean**	**e_x**	**mean**	**e_x**	**mean**	**e_x**
DNE	Female	148.53	6.05	146.14	3.68	152.64	5.75	138.06	4.03
	Male	145.29	5.61	143.67	4.46	156.40	5.23	140.39	5.62
OPCR	Female	94.01	2.38	86.76	1.81	89.30	2.18	76.93	1.77
	Male	95.94	2.60	87.53	2.51	91.58	2.23	75.91	2.35
RFI	Female	1.93	0.02	1.94	0.02	1.79	0.02	1.80	0.02
	Male	1.92	0.02	1.88	0.02	1.79	0.02	1.79	0.02
3D-Area	Female	164.08	2.65	155.17	2.82	160.72	2.70	145.96	2.62
	Male	170.94	2.59	156.62	3.09	166.59	2.91	150.23	3.01
OArea	Female	84.94	1.15	80.04	1.22	89.63	1.25	81.12	1.38
	Male	89.36	1.28	83.46	1.70	93.16	1.34	83.92	1.64

Table 2.6. Linear regression models of the topographic variables on age for the teeth analysed.

Tooth	*N*	Metric	Intercept	Slope	95% CI of slope		*r*	R^2	*t*	*p*
UM1	105	OPCR	123.88	−0.975	−1.215	−0.811	−0.205	0.042	−2.135	0.035*
	105	DNE	238.18	−2.440	−2.905	−1.913	−0.443	0.196	−5.024	0.000*
	105	RFI	2.031	−0.007	−0.008	−0.005	−0.298	0.089	−3.174	0.001*
UM2	97	OPCR	110.41	−0.931	−1.115	−0.746	−0.214	0.046	−2.142	0.034*
	97	DNE	218.82	−2.182	−2.572	−1.724	−0.527	0.278	−6.058	0.000*
	97	RFI	2.075	−0.007	−0.008	−0.006	−0.391	0.153	−4.147	0.000*
LM1	109	OPCR	132.55	−1.092	−3.153	−0.915	−0.198	0.039	−2.091	0.038*
	109	DNE	235.11	−2.562	−3.12	−1.899	−0.477	0.228	−5.621	0.000*
	109	RFI	2.242	−0.009	−0.010	−0.007	−0.489	0.239	−5.809	0.000*
LM2	100	OPCR	124.74	−1.009	−3.152	−0.848	−0.046	0.002	−0.461	0.645
	100	DNE	214.95	−1.881	−2.208	−1.571	−0.279	0.078	−2.880	0.004*
	100	RFI	2.302	−0.010	−0.012	−0.008	−0.433	0.188	−4.766	0.000*

Confidence interval (CI); Pearson correlation coefficient (*r*); coefficient of determination (r^2); student *t-test* for the slope (*t*). Statistically significant regressions at $p<0.05$ (*).

The multivariate, step-wise linear regression models of all the PCs by tooth type showed higher correlations with age (Table 2.7) than those seen for the individual PCs (Table 2.5), since the step-wise regression selected those components that loaded the most for predicting age. The multivariate Pearson correlation coefficients (*r*) between the step-wise derived PCs and age ranged from 0.564 to 0.658 (Table 2.6), whereas those for the univariate comparison between each PC and age ranged from 0.062 to 0.484 (Table 2.5). Even so, the models did not fully reflect a clear association between age and molar shape, since age was also significantly correlated with the residuals of the regressions, and the percentages of precision of the models predicting age from the PCs ranged from 31.8% to 45.3% (Table 2.6).

Table 2.7. Multivariate step-wise linear regression models of the PCs on age. *r*: Pearson correlation coefficient, R: coefficient of determination (R=r^2) for the regression models and for the residuals, indication the percentage of precision of the model (% precis.).

Tooth	*n*	Significant PCs in the analysis	*r*	r²	r² resid	% precis.
LM1	109	PC1, PC2, PC5, PC6, PC3, PC9	0.658	0.432	0.569	45.3%
LM2	100	PC1, PC2, PC5	0.592	0.350	0.649	39.0%
UM1	105	PC7, PC1, PC17, PC4, PC15, PC5, PC8	0.564	0.318	0.675	31.8%
UM2	97	PC7, PC2, PC6, PC8, PC4, PC14	0.608	0.369	0.631	39.79%

r: Pearson correlation coefficient, r^2: coefficient of determination for the regression models and for the residuals (r^2 resid.), indication the percentage of precision of the model (% precis).

2.4. Discussion

Dental wear is a complex process (Schmidt and Watson, 2020). Although age is a significant factor affecting the loss of occlusal relief and crown height, estimating an individual's age from dental crown morphology does not only depend on dental wear, but also on the variation of dental crown morphology within the sample studied. Overall measures of dental crown topography accounted for a small fraction of the observed variability. Previous studies have found that cropping around the cervical margin is insensitive to observer error in topographic metric values (Boyer, 2008; Bunn *et al.*, 2011). However, our results show that the cropping procedure derived from visual assessment of virtual models is a source of observer error affecting the measurement of 3D surface areas and cropped crown heights. All three topographic metrics showed significant, negative correlations with age, with the highest correlation observed accounted for 27.8% of the total variance (UM2). However, the PCA that explained the highest percentage of dental crown shape variation for this tooth (PC1= 21.5%) mainly reflected the relative size of the hypocone, while the components showing higher weights on age was PC7, only explaining 3.2% of the overall morphometric variation, and PC2 (12.7%). The morphometric and topographic variation of the UM2 tooth clearly reflects the complex nature of dental crown morphology. Overall, although a significant number of PCs can be attributed to dental wear, the results varied by tooth, which suggests that molar shape is not fully obscured by dental wear. Dental morphology is a combination of multiple types of shape variation, and the 3D-GM analysis discriminates them into separate components. The multivariate step-wise regression model efficiently extracted the components that were informative of wear-related variation, thus increasing the correlation with age, although the components selected for each tooth varied and the coefficients of determination were still small. The interpretation of the shape changes of the components was not always straightforward. The complex nature of dental crown shape was not segmented in the same way by the topographic metrics, which represent combined measures of various kinds of curvatures or complexities. For instance, a larger hypocone would likely add to the overall DNE and OPCR metrics, independently from occlusal wear. The same accounts for RFI, since a larger hypocone, for instance, would more significantly increase the 3D surface area than the 2D outline area. However, wear has a long-term effect on dental shape and, eventually, the progressive loss of crown height would overcome the effect of dental morphological variation.

Modern human populations show great variation in cusp patterns (Scott and Irish, 2017). Maxillary molars frequently show four cusps, though the hypocone may vary from well-developed to completely absent, the later more frequently so in the second upper molars. The lower M1 most frequently shows a Y5 cusp pattern but may also show a C6 and/or a C7 additional cusps, as well as a reduction to a +4 cusped pattern, which is most frequent in the lower M2. The large variability of molar cusp pattern in modern human populations, as evidenced by the PCs of the 3D-GM analyses, is likely to affect both dental crown surface and outline areas, as well as the topographic metrics, independently of wear. Despite this, a significant number of PCs reflected dental crown height loss though wear, independently of dental crown morphology. Factors affecting dental wear in modern human populations may relate to food preferences, cultural practices or mechanical demands of chewed particles (Fiorenza *et al.*, 2011, 2018). Further, differences in dental wear ratios among populations may reflect differences in dietary habits, abrasiveness of consumed foods, or food processing techniques. In addition, wear varies between mandibular and maxillary molars, as well as among teeth and between buccal and lingual cusps in mandibular or maxillary molars (Larsen, 1997; Mays, 2002; Benazzi *et al.*, 2011; Kaidonis *et al.*, 2012).

All these being major sources of dental crown shape variation, the morphometric analysis of dental shape in modern humans needs to control them depending on the objective of the proposed research. Dental topographic metrics have proved informative in non-human primates with diverse dietary regimens (Berthaume and Schroer, 2017), showing the expected negative regression slopes (Pampush *et al.*, 2016b) and playing an important role in the characterisation of occlusal wear and cusp height decrease with age (Evans 2013). Topographic measures of dental crown variation have been shown to be a phylogenetically informative metric for stable, low-variable dental morphological patterns of primate species (M'Kirera and Ungar, 2003; Pampush *et al.*, 2016b). However, topographic or morphometric proxies for the characterisation of age-related dental crown shape changes on modern human populations requires controlling for the within population molar morphological variation, in addition to dietary and social factors. More stable molar shape variation in non-human primates and in fossil hominines might show greater age-related covariation with wearing processes.

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Supplementary material

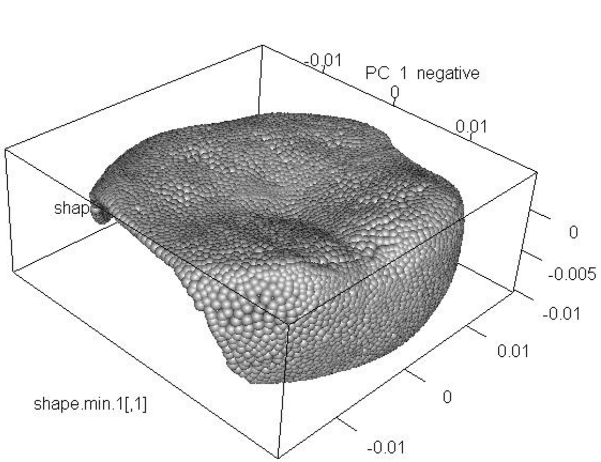
Lower first molar (LM1)

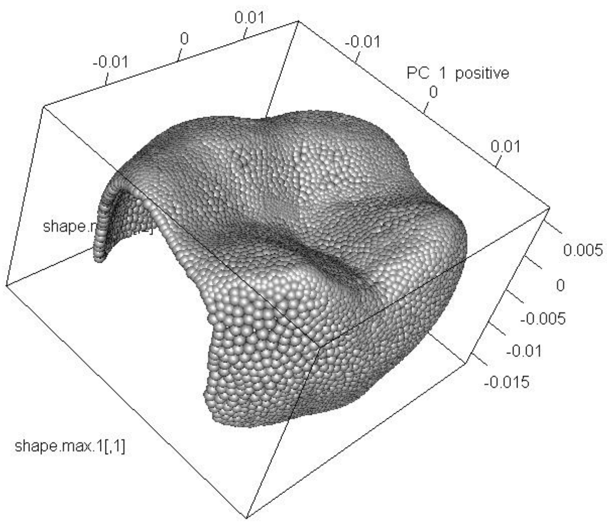
Importance of components:

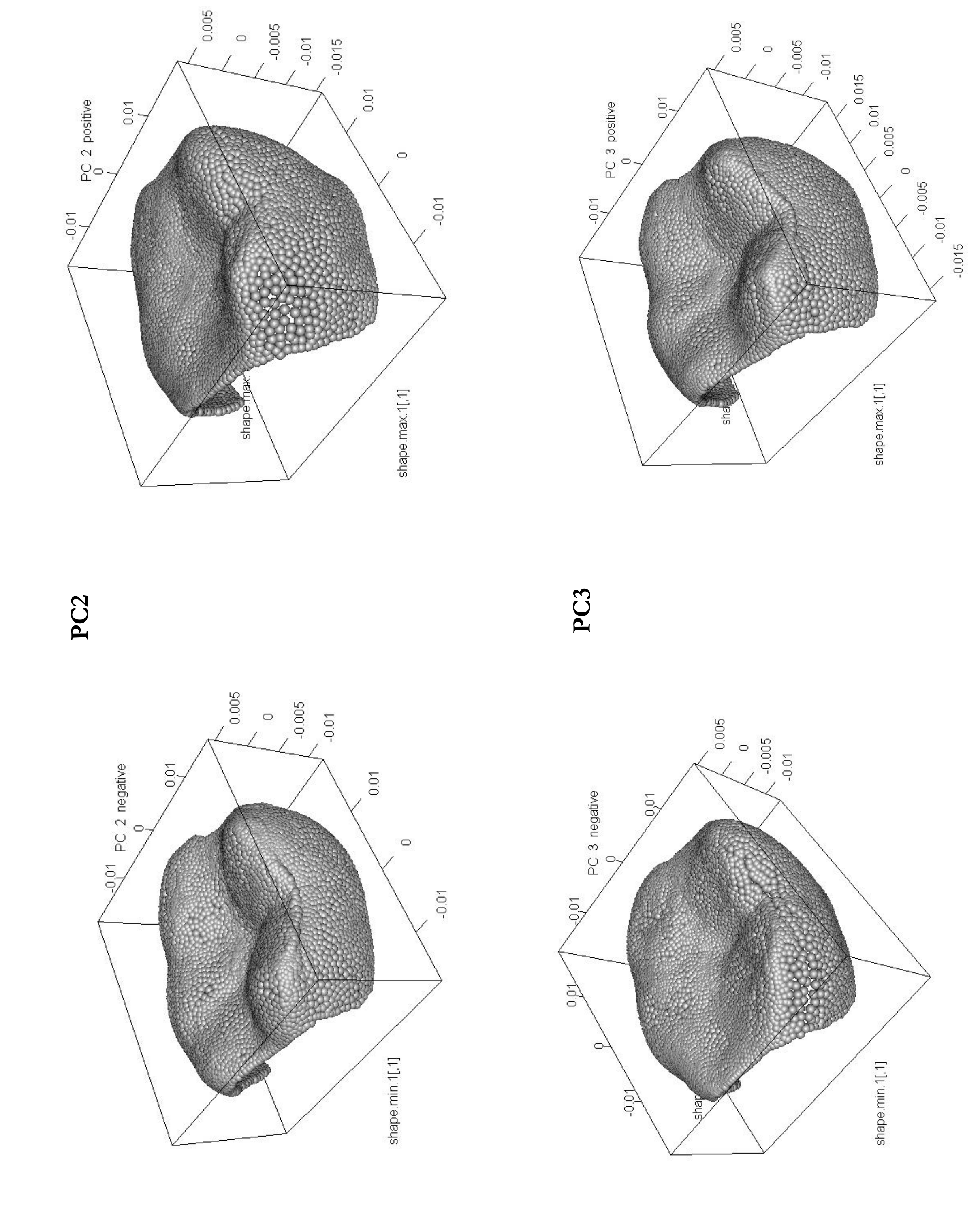
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	0.04126	0.03293	0.03074	0.02542	0.02237	0.01991	0.01784	0.01544	0.01394
Proportion of Variance	0.21139	0.13462	0.11734	0.08021	0.06211	0.04920	0.03953	0.02959	0.02411
Cumulative Proportion	0.21139	0.34601	0.46335	0.54356	0.60568	0.65488	0.69440	0.72399	0.74810

	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17
Standard deviation	0.01314	0.01208	0.01045	0.01017	0.009645	0.009129	0.008825	0.008437
Proportion of Variance	0.02144	0.01812	0.01356	0.01284	0.011550	0.010350	0.009670	0.008840
Cumulative Proportion	0.76954	0.78766	0.80122	0.81405	0.825600	0.835950	0.845620	0.854450

PC1



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PC3
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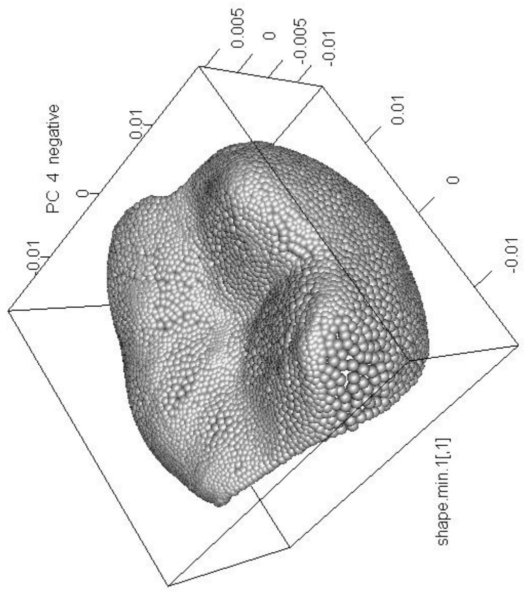
PC4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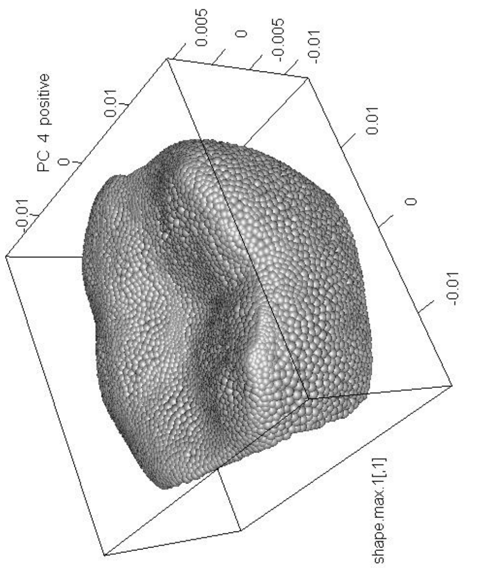
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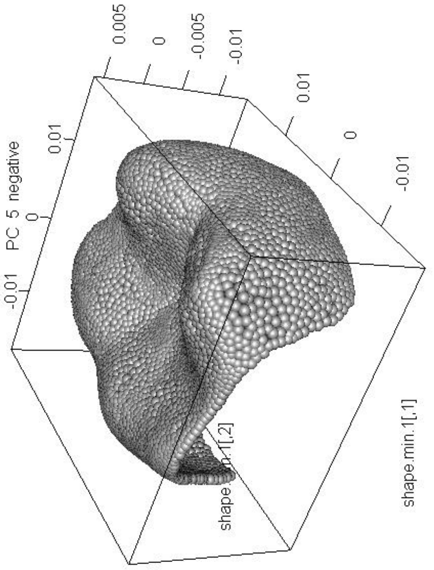
PC5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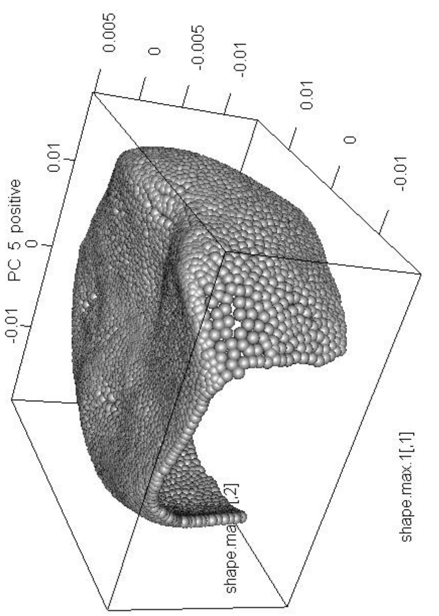
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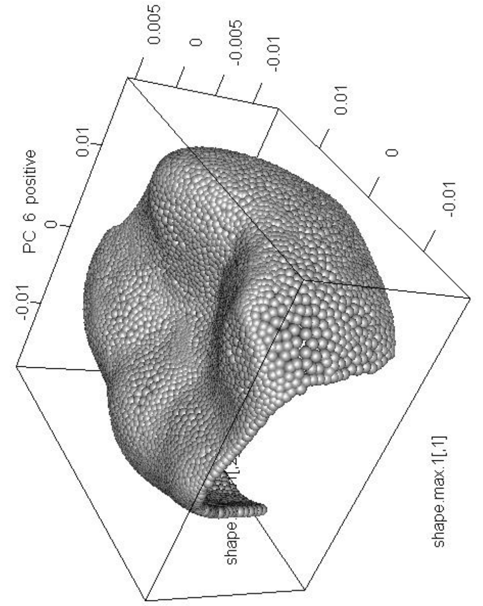
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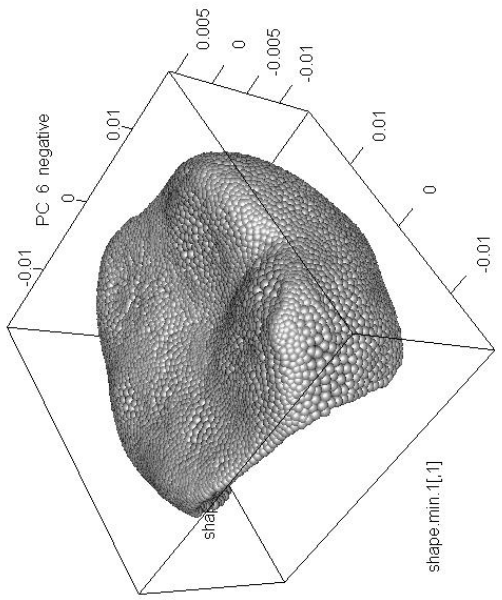
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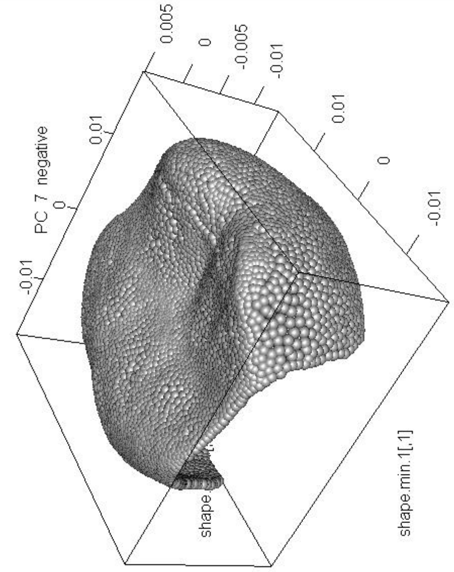
PC6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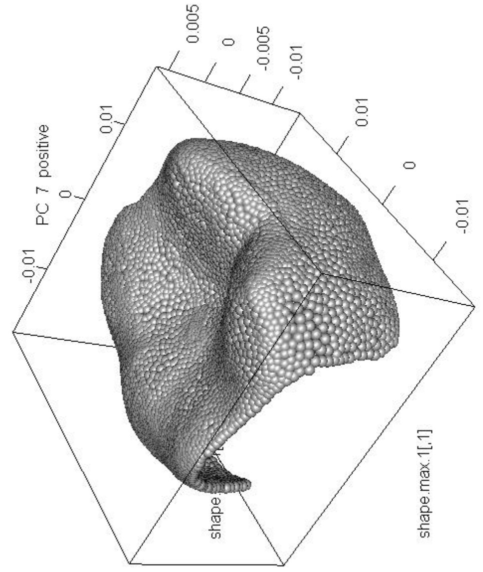
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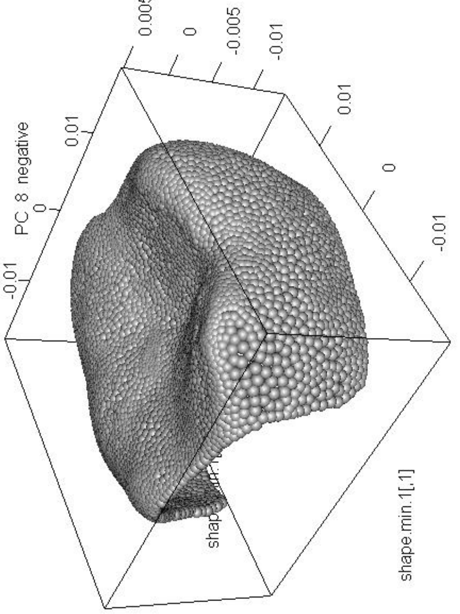
PC7

PC8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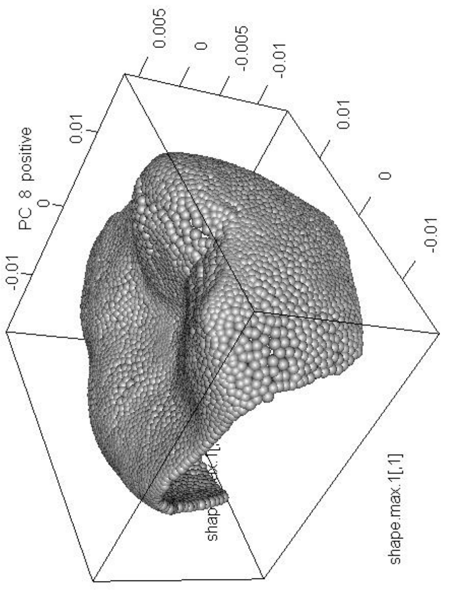
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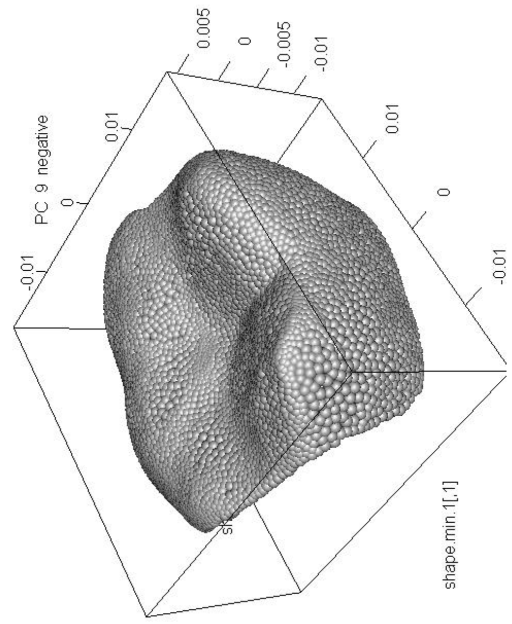
PC9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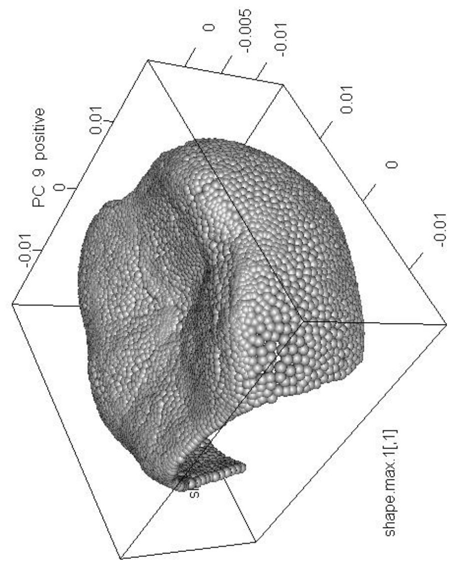
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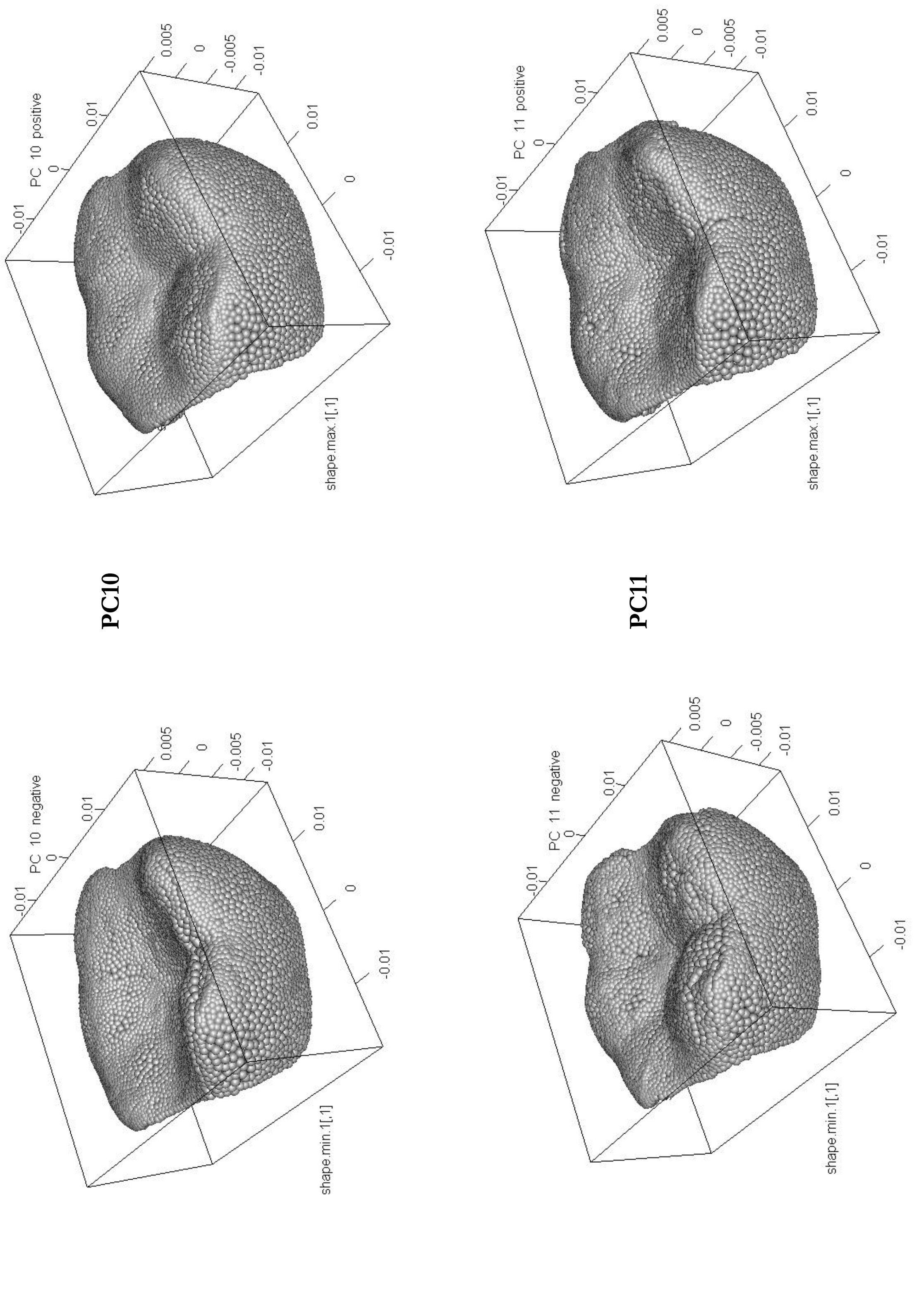
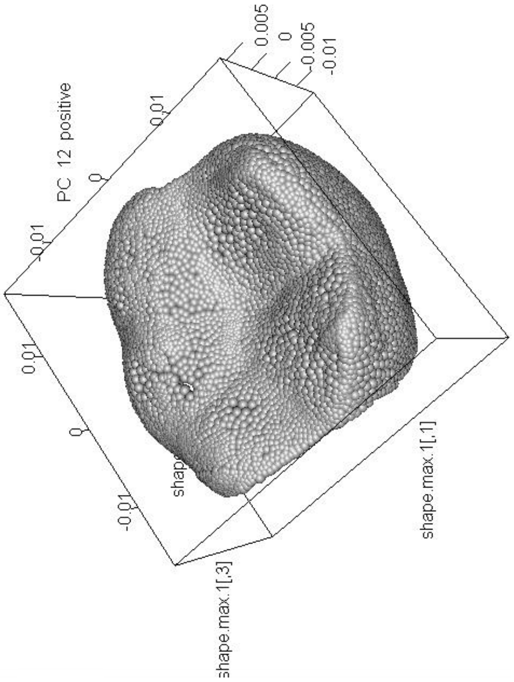
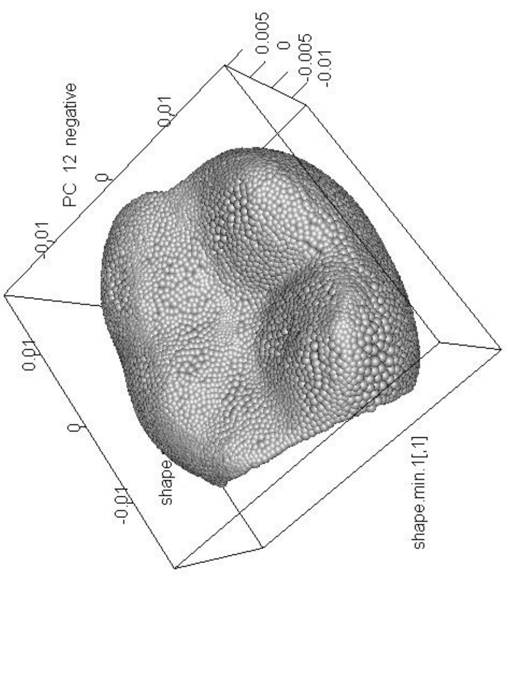
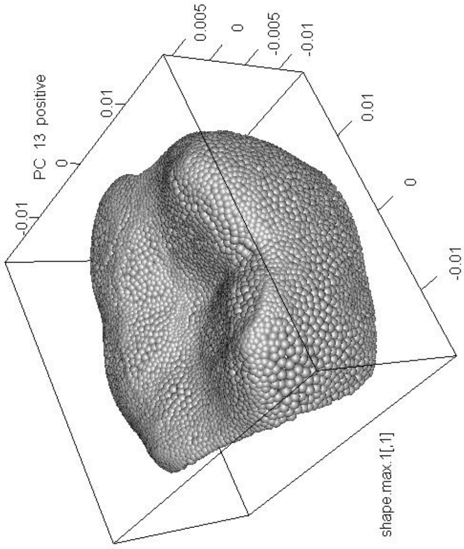
-0.01
shape.max.1[,1]


PC10
PC11
PC 10 positive
PC 10 negative
PC 11 positive
PC 11 negative
shape.max.1[,1]
shape.min.1[,1]
-0.01
0
0.01
0.005
-0.005

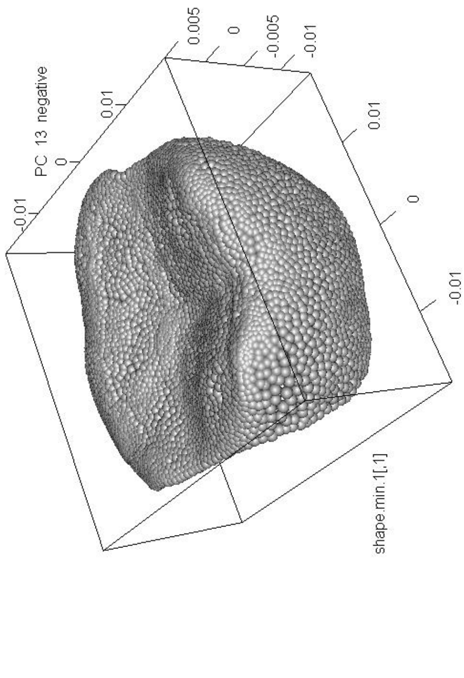

PC 12 positive
shape.max.1[,1]
shape.max.1[,3]
-0.01
0
0.01
0.005
-0.005

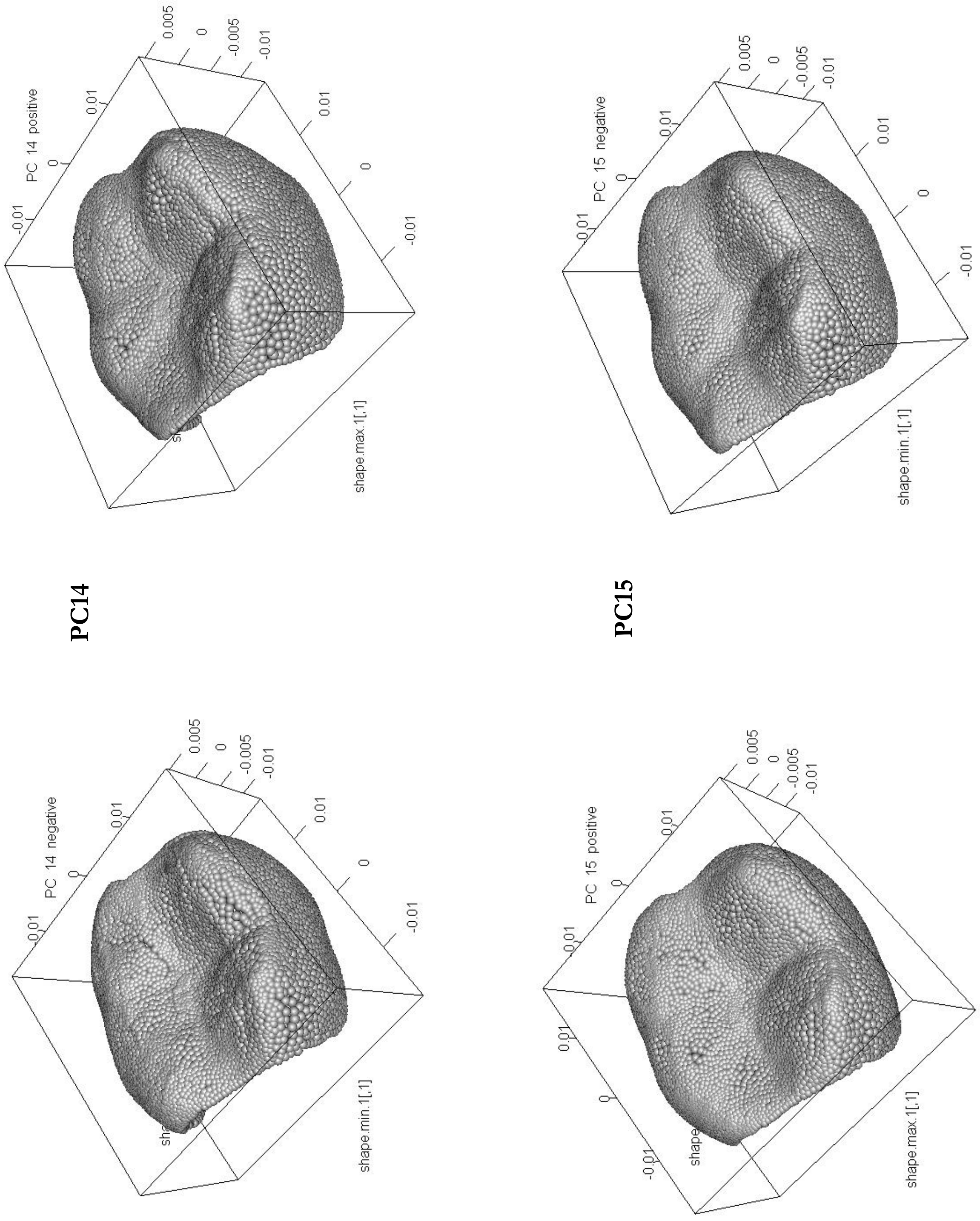
PC12


PC 12 negative
shape.min.1[,1]
-0.01
0
0.01
0.005
-0.005


PC 13 positive
shape.max.1[,1]
-0.01
0
0.01
0.005
-0.005

PC13


PC 13 negative
shape.min.1[,1]
-0.01
0
0.01
0.005
-0.005


PC14
PC 14 positive
PC 14 negative
PC15
PC 15 negative
PC 15 positive
shape.max.1[,1]
shape.min.1[,1]

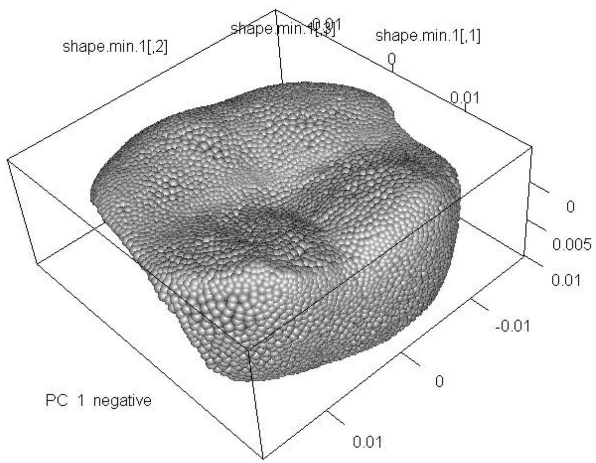
Lower second molar (LM2)

Importance of components:

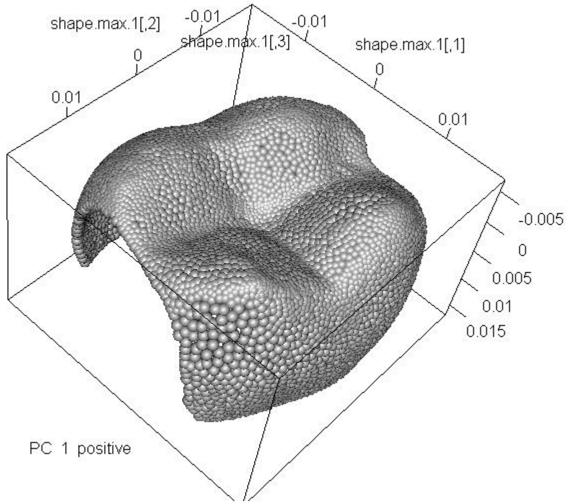
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	0.04461	0.0332	0.0304	0.02951	0.02035	0.01818	0.01635	0.01475
Proportion of Variance	0.23423	0.1298	0.1088	0.10251	0.04873	0.03890	0.03147	0.02562
Cumulative Proportion	0.23423	0.3640	0.4728	0.57527	0.62400	0.66291	0.69438	0.72000

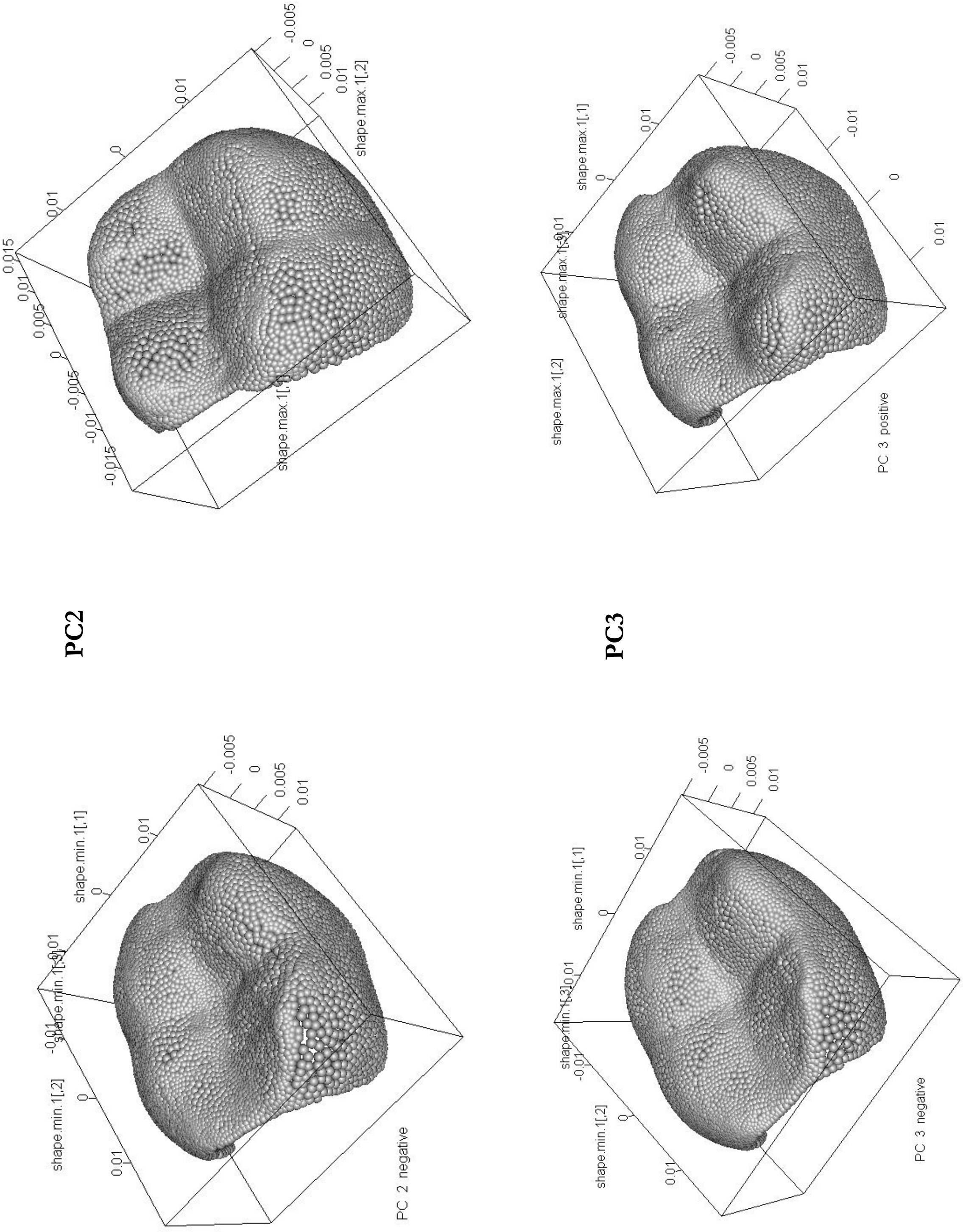
	PC9	PC10	PC11	PC12	PC13	PC14	PC15
Standard deviation	0.01425	0.01359	0.01300	0.01176	0.01082	0.01020	0.009388
Proportion of Variance	0.02391	0.02174	0.01991	0.01627	0.01379	0.01224	0.010380
Cumulative Proportion	0.74391	0.76564	0.78555	0.80183	0.81561	0.82786	0.838230

	PC16	PC17	PC18	PC19	PC20	PC21	PC22
Standard deviation	0.009278	0.008544	0.007794	0.007764	0.007387	0.007118	0.006895
Proportion of Variance	0.010130	0.008590	0.007150	0.007100	0.006420	0.005960	0.005600
Cumulative Proportion	0.848360	0.856960	0.864110	0.871210	0.877630	0.883590	0.889190

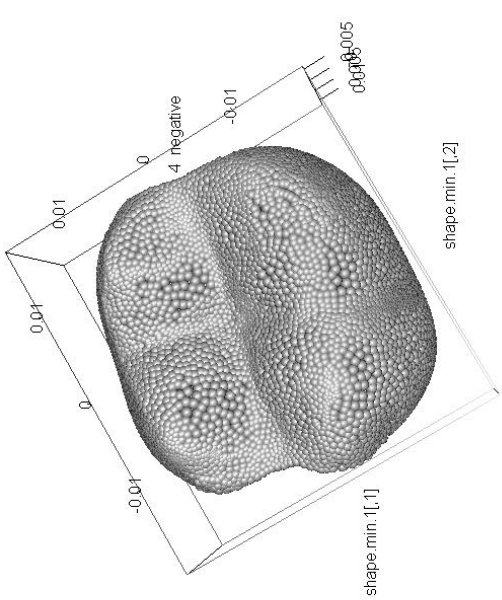


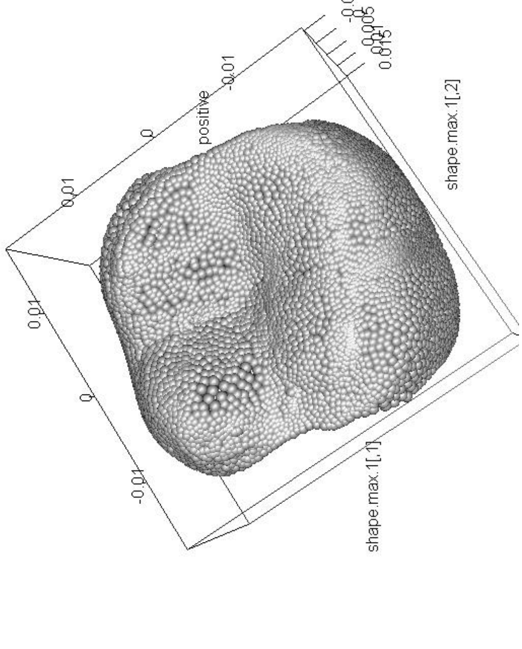
PC1



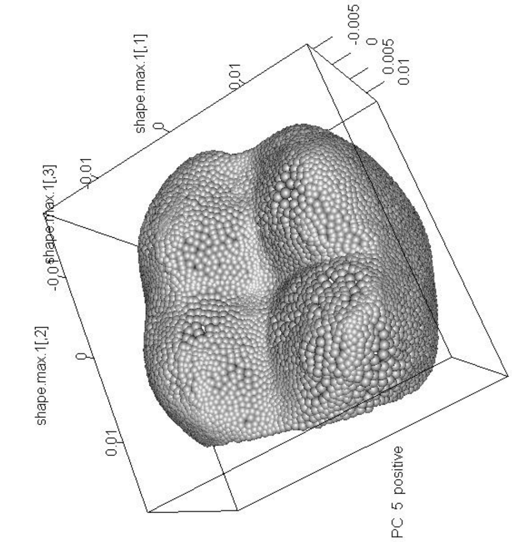

PC2
PC 2 negative
PC3
PC 3 positive
PC 3 negative

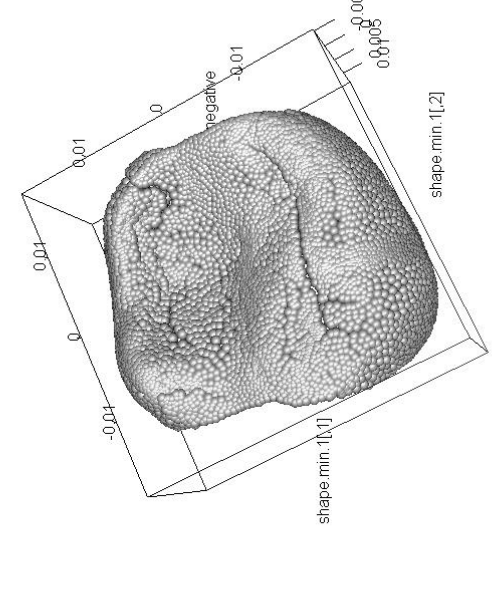
PC4


4 negative
shape.min.1[,1]
shape.min.1[,2]

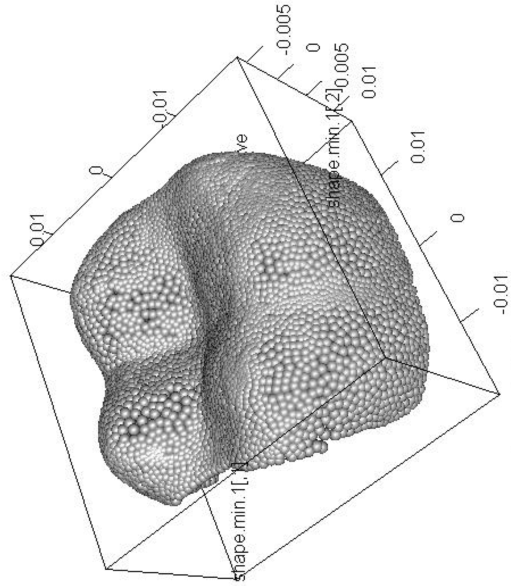

positive
shape.max.1[,1]
shape.max.1[,2]

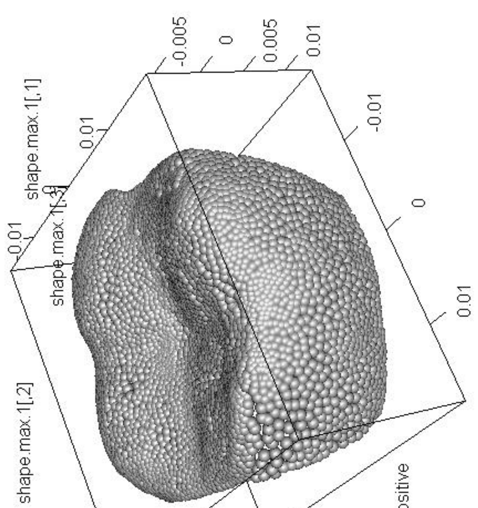
PC5


PC 5 positive
shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]

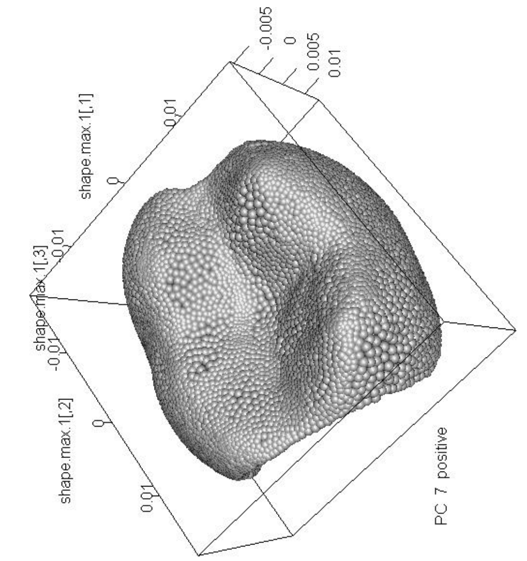

negative
shape.min.1[,1]
shape.min.1[,2]

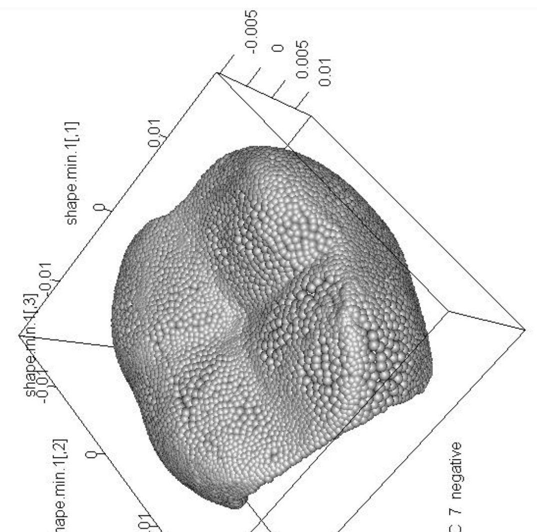
PC6

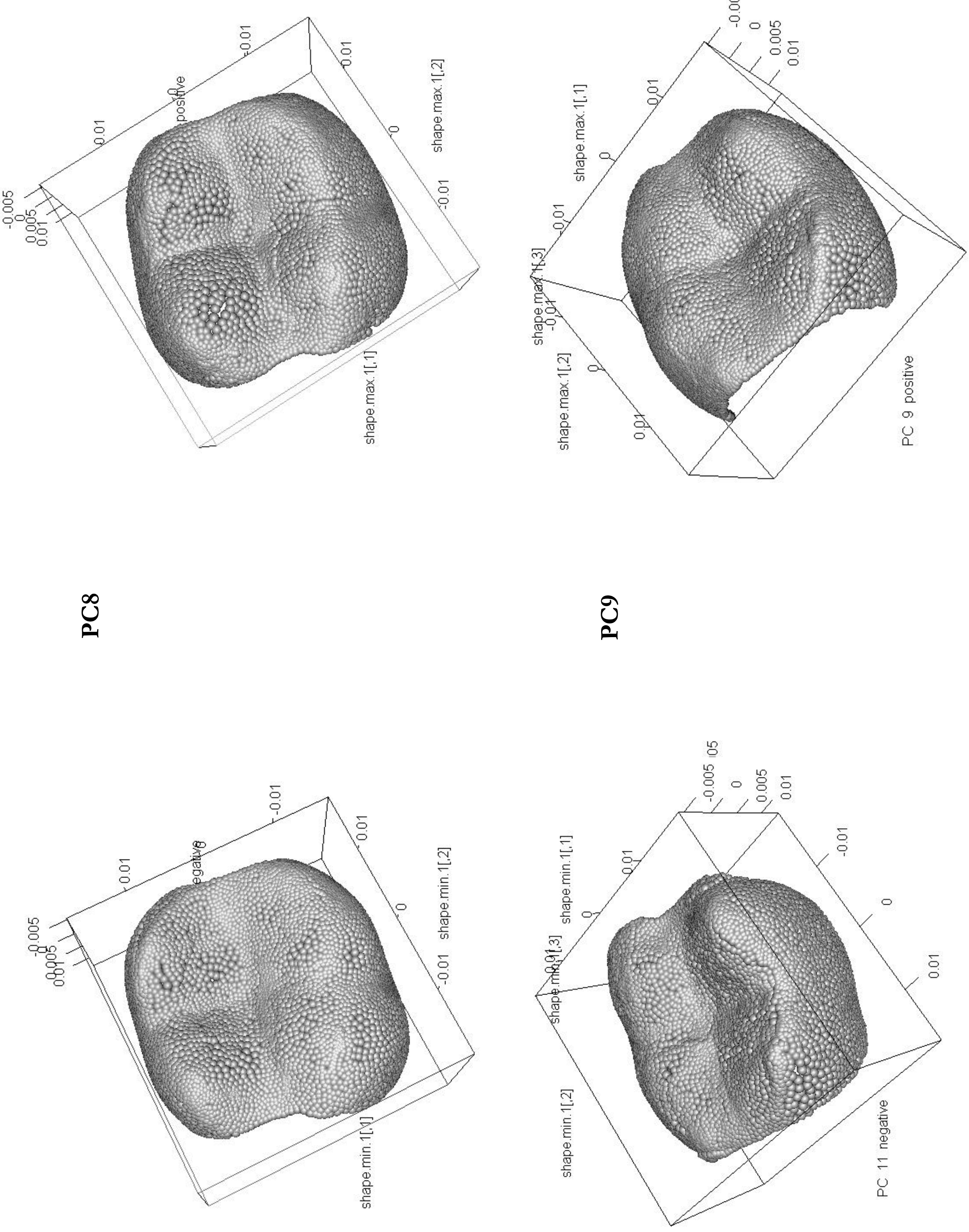


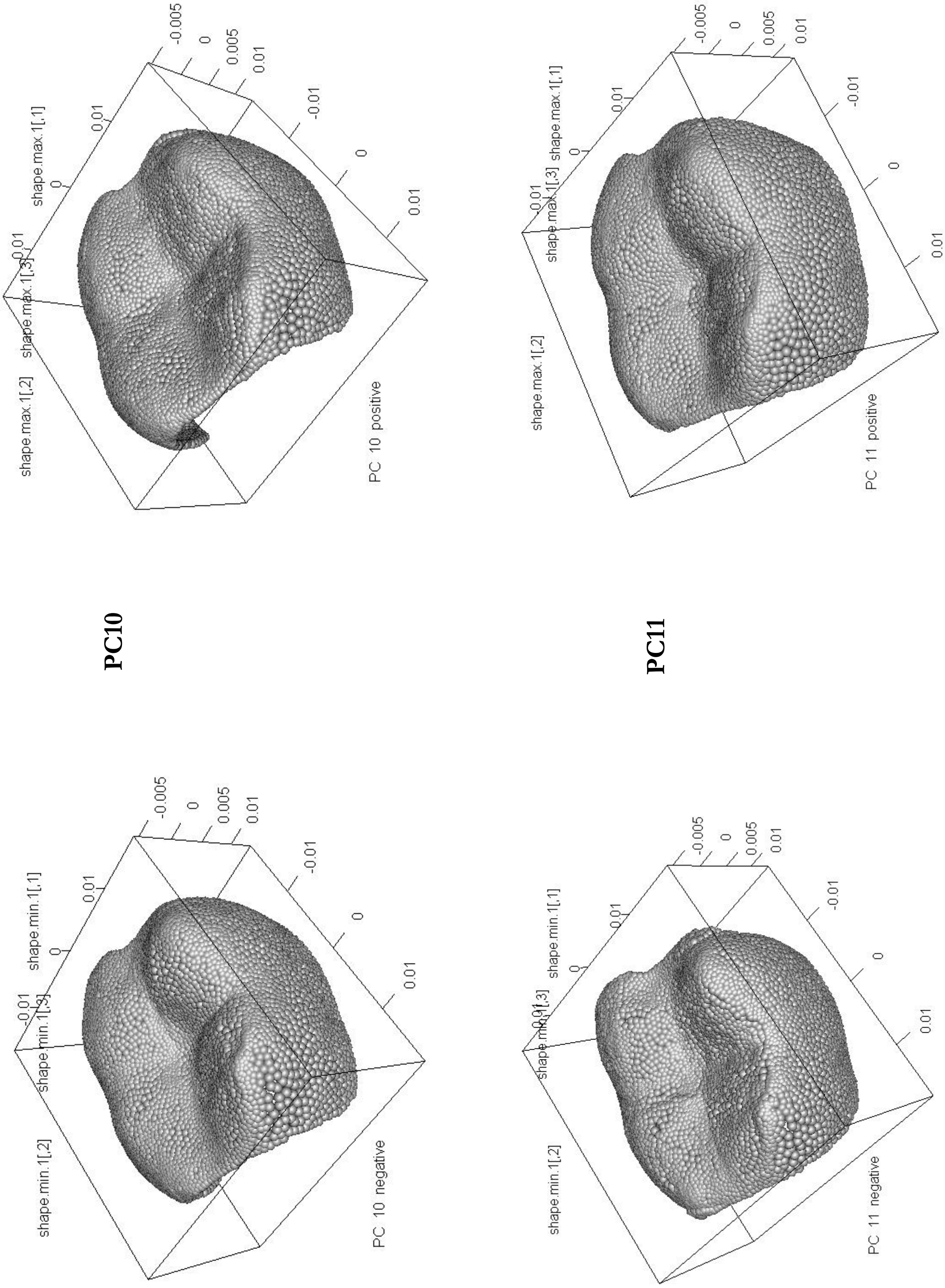


PC7

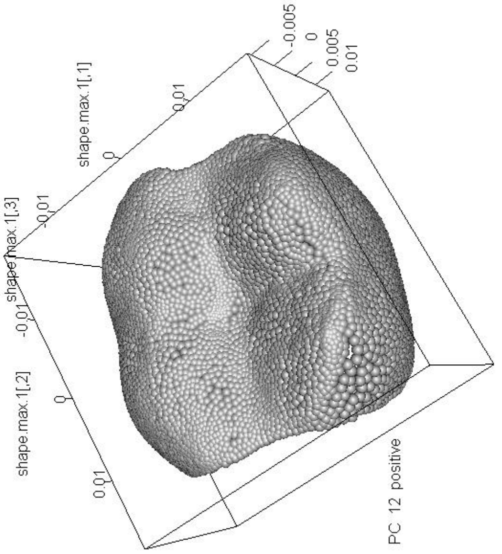


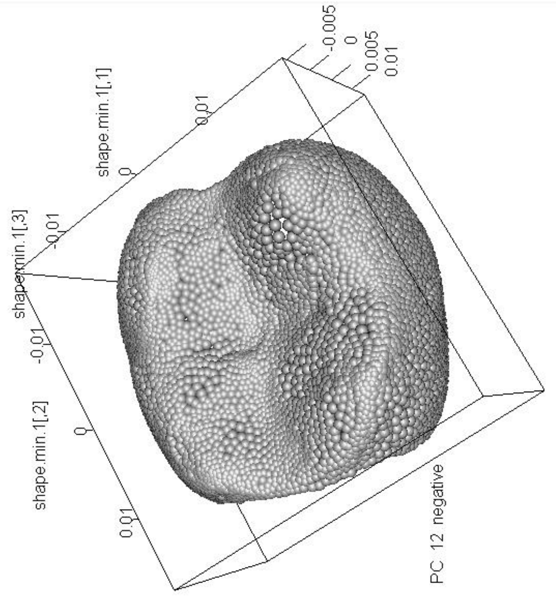



PC8
PC9
positive
negative
PC 9 positive
PC 11 negative

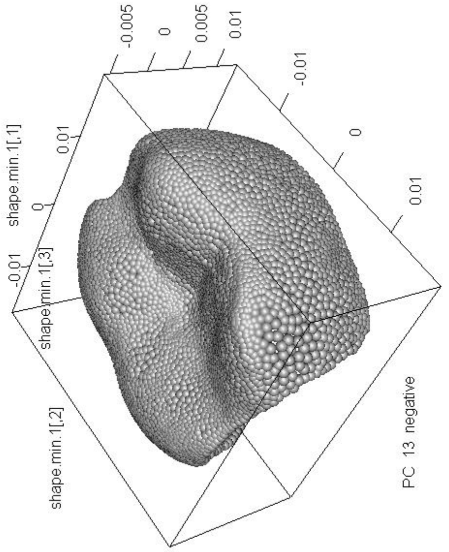

PC10
PC11
PC 10 positive
PC 10 negative
PC 11 positive
PC 11 negative
shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]
shape.min.1[,1]
shape.min.1[,2]
shape.min.1[,3]

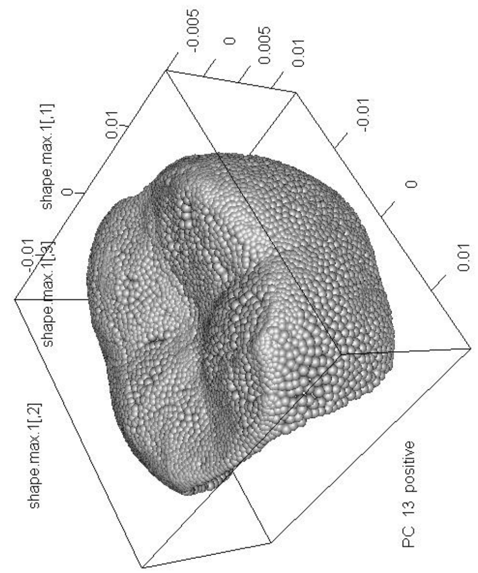
PC10

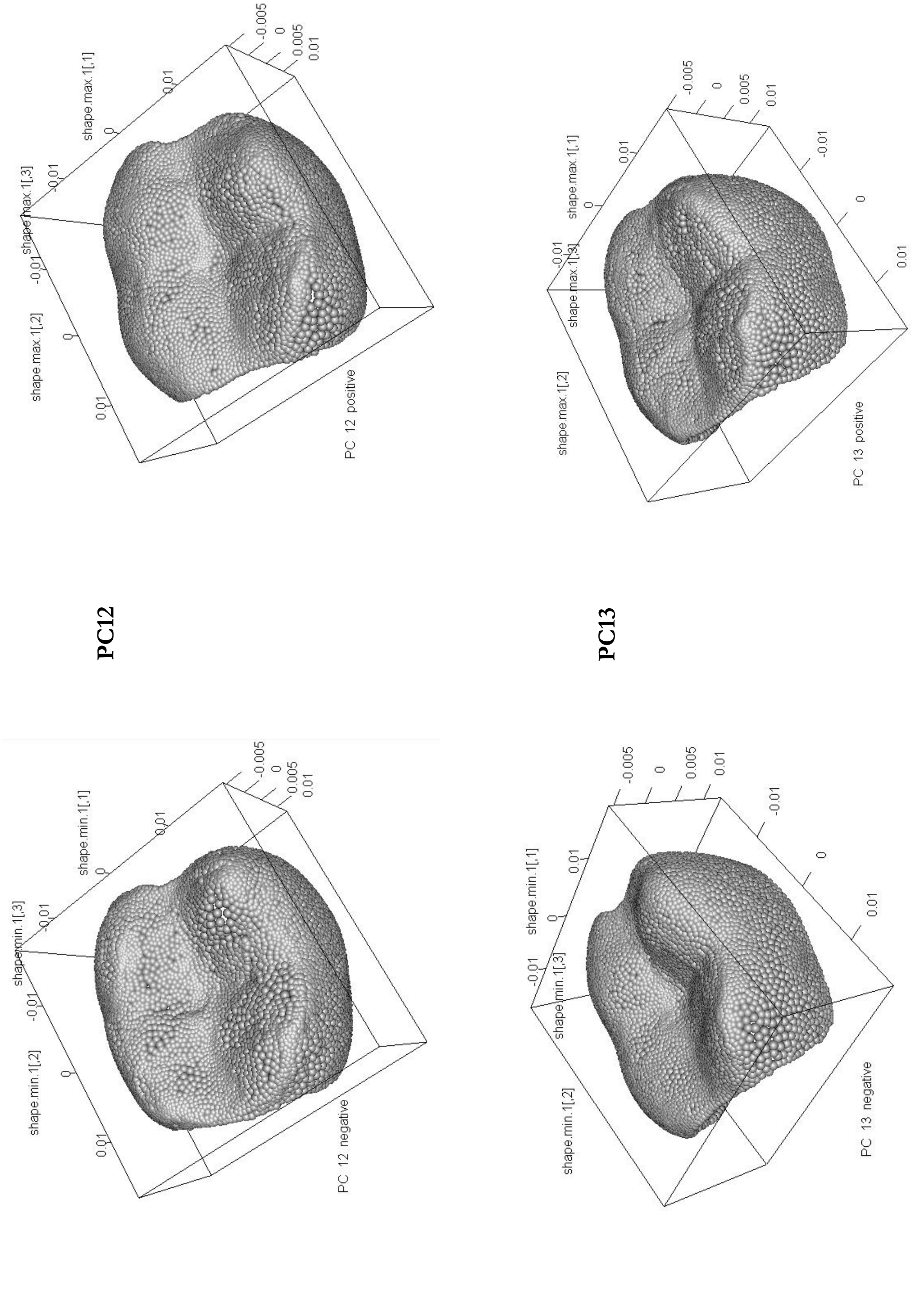

shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]
PC 12 positive


shape.min.1[,1]
shape.min.1[,2]
shape.min.1[,3]
PC 12 negative

PC11

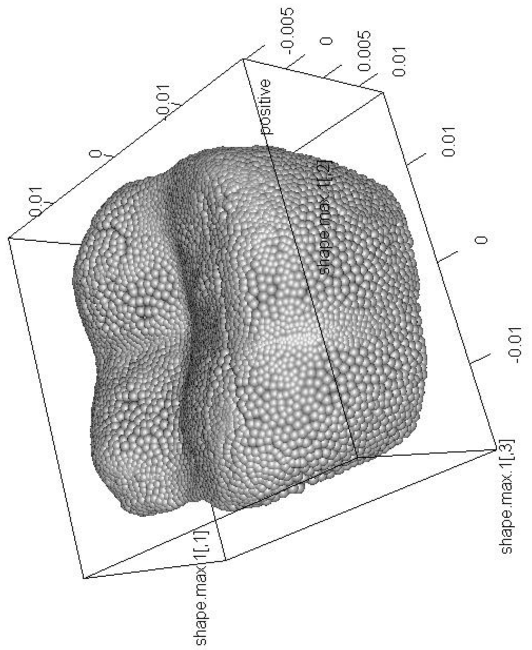

shape.min.1[,1]
shape.min.1[,2]
shape.min.1[,3]
PC 13 negative

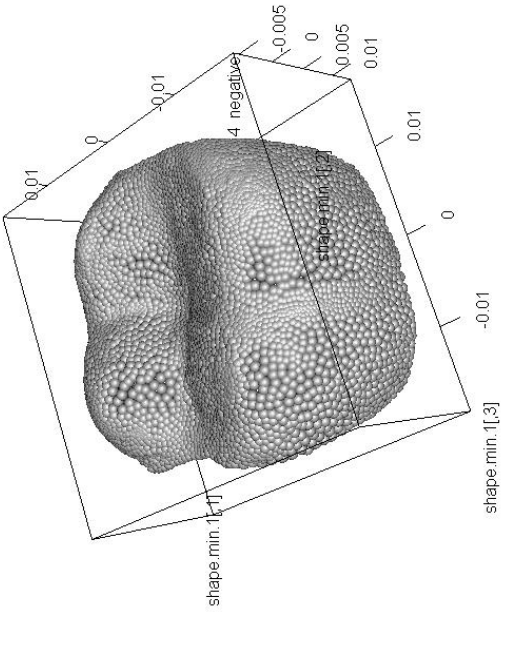

shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]
PC 13 positive



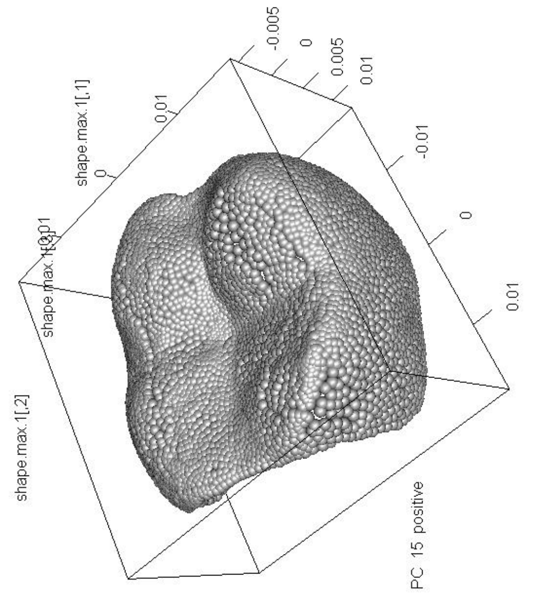
PC12
PC13
PC 12 positive
PC 12 negative
PC 13 positive
PC 13 negative
shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]
shape.min.1[,1]
shape.min.1[,2]
shape.min.1[,3]
-0.01
0
0.01
-0.005
0.005

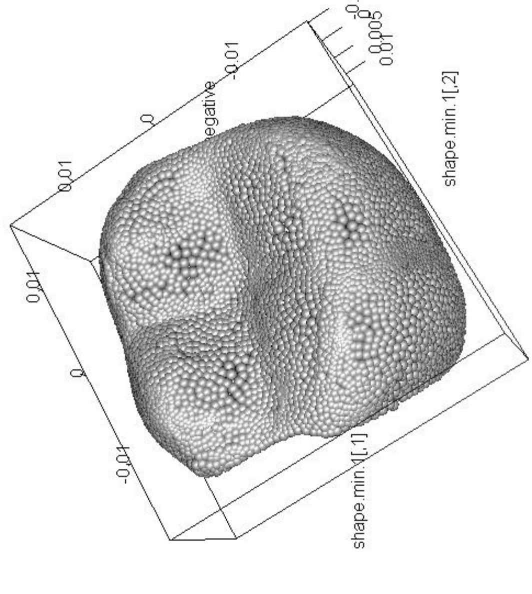
PC14

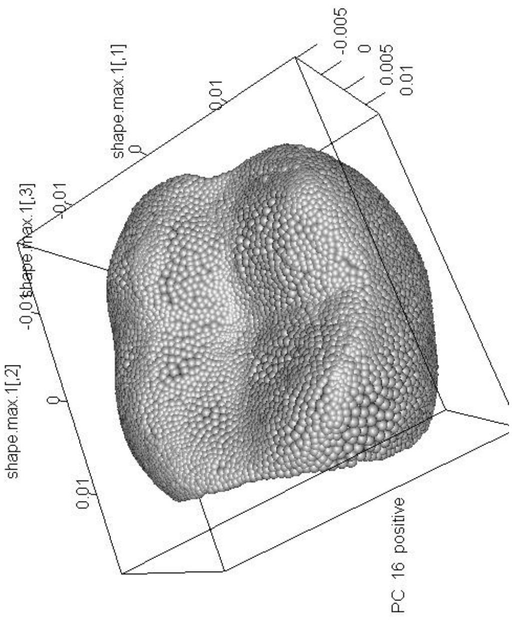

positive
shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]
-0.01
0
0.01
-0.005
0
0.005
0.01


4 negative
shape.min.1[,1]
shape.min.1[,2]
shape.min.1[,3]
-0.01
0
0.01
-0.005
0
0.005
0.01

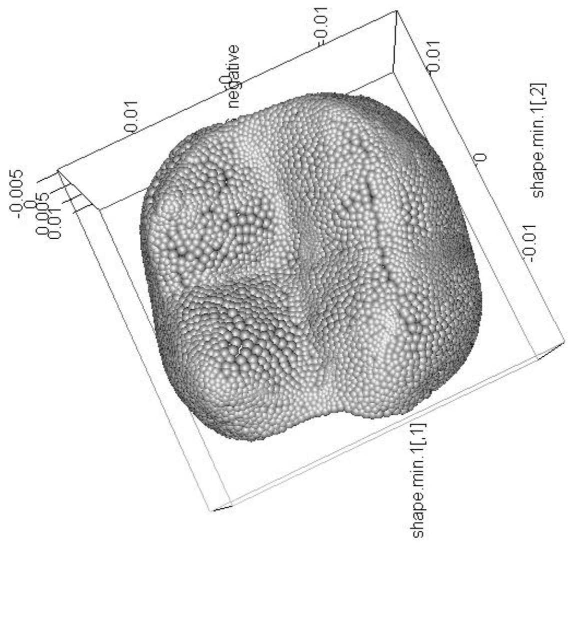
PC15


PC 15 positive
shape.max.1[,1]
shape.max.1[,2]
-0.01
0
0.01
-0.005
0
0.005
0.01


shape.min.1[,1]
shape.min.1[,2]
-0.01
0
0.01


shape.max.1[,1]
shape.max.1[,2]
shape.max.1[,3]
PC 16 positive

PC16

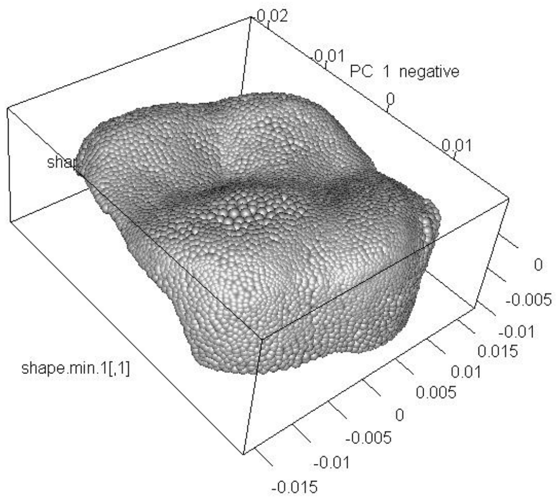

shape.min.1[,1]
shape.min.1[,2]
negative

Upper first molar (UM1)

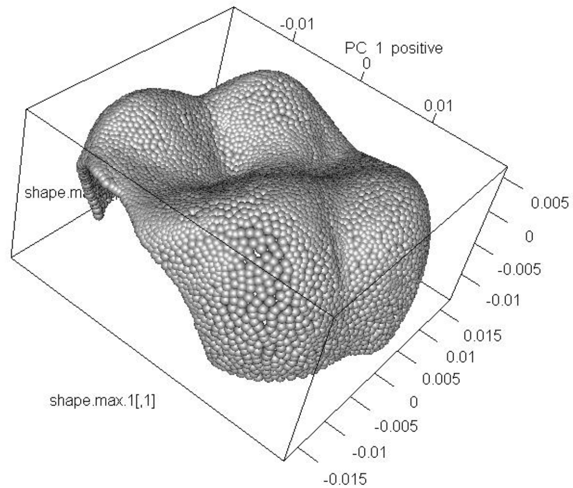
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	0.03688	0.0268	0.02521	0.02427	0.02178	0.01884	0.01635	0.01453
Proportion of Variance	0.19989	0.1056	0.09339	0.08656	0.06967	0.05218	0.03928	0.03103
Cumulative Proportion	0.19989	0.3054	0.39884	0.48541	0.55508	0.60726	0.64653	0.67756

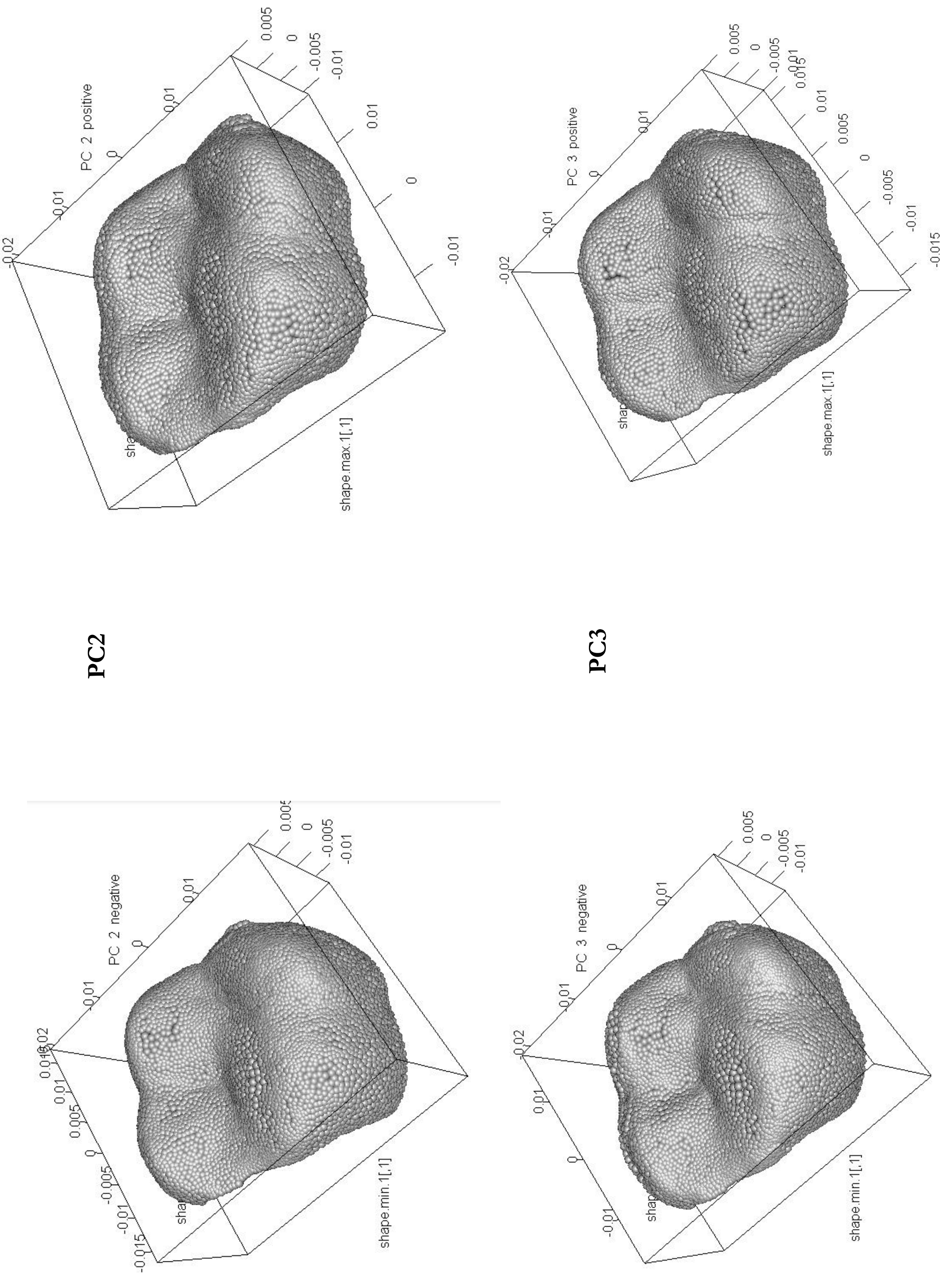
	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
Standard deviation	0.01323	0.01181	0.01137	0.01064	0.01033	0.009623	0.00926	0.008828
Proportion of Variance	0.02572	0.02048	0.01899	0.01664	0.01569	0.013610	0.01260	0.011450
Cumulative Proportion	0.70327	0.72375	0.74274	0.75939	0.77507	0.788680	0.80128	0.812730

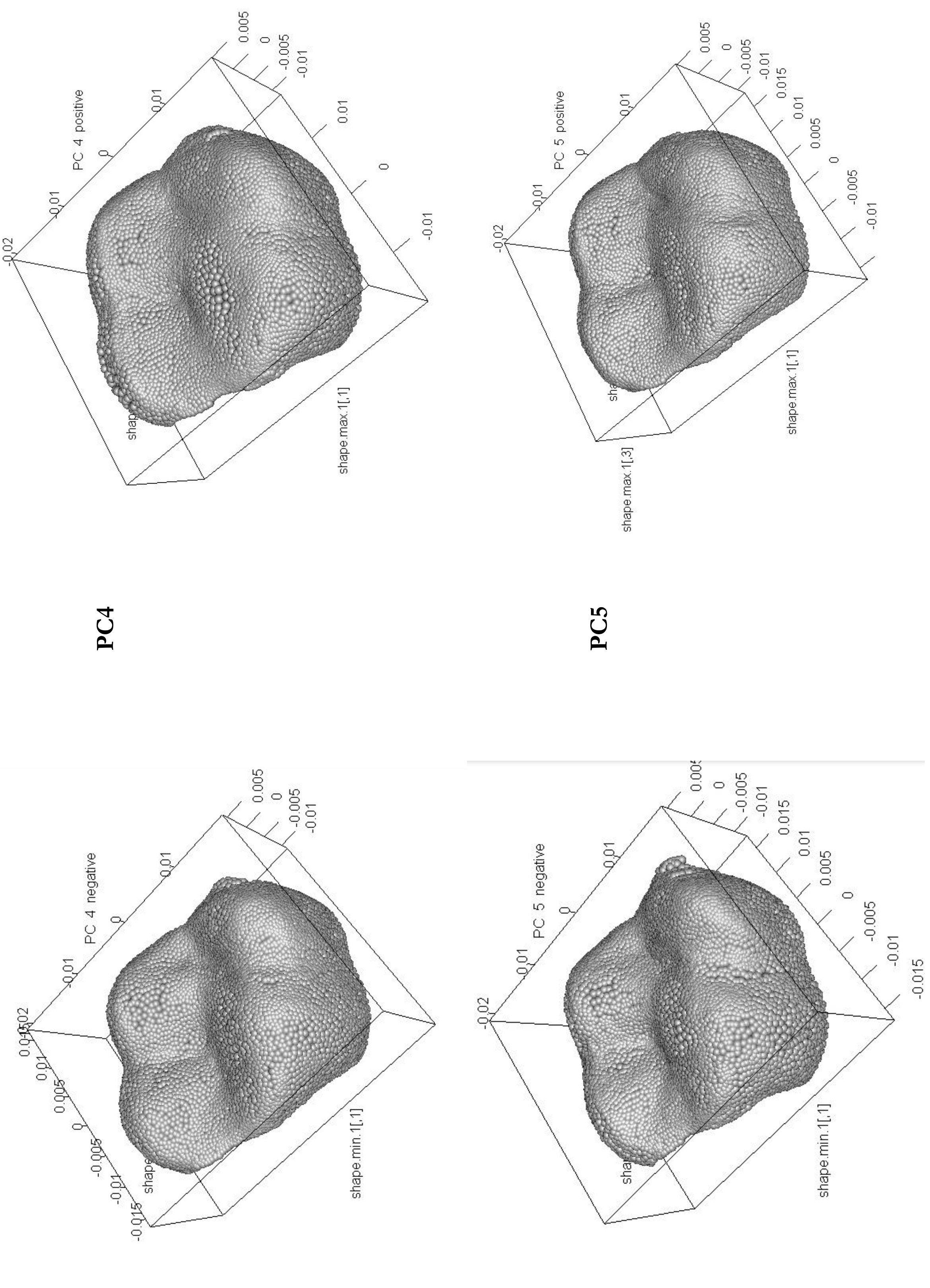
	PC17	PC18	PC19	PC20	PC21	PC22	PC23	PC24
Standard deviation	0.008615	0.008119	0.007895	0.00733	0.00720	0.006844	0.006246	0.00599
Proportion of Variance	0.010900	0.009680	0.009160	0.00789	0.00762	0.006880	0.005730	0.00527
Cumulative Proportion	0.823630	0.833320	0.842480	0.85037	0.85799	0.864870	0.870600	0.87588

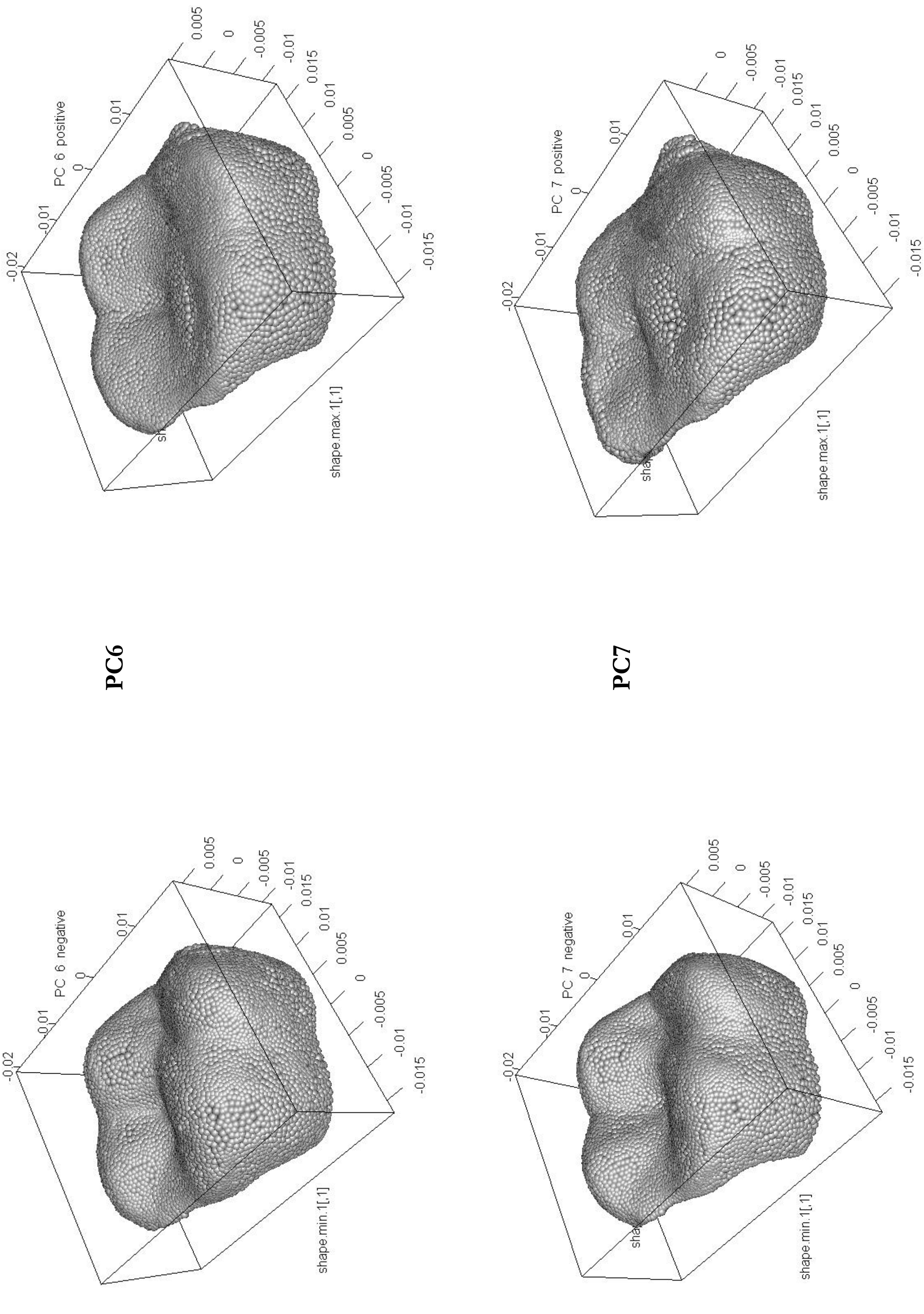


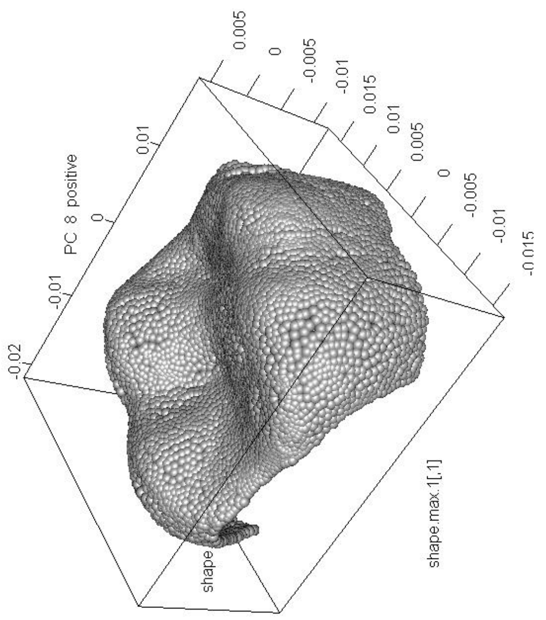
PC1

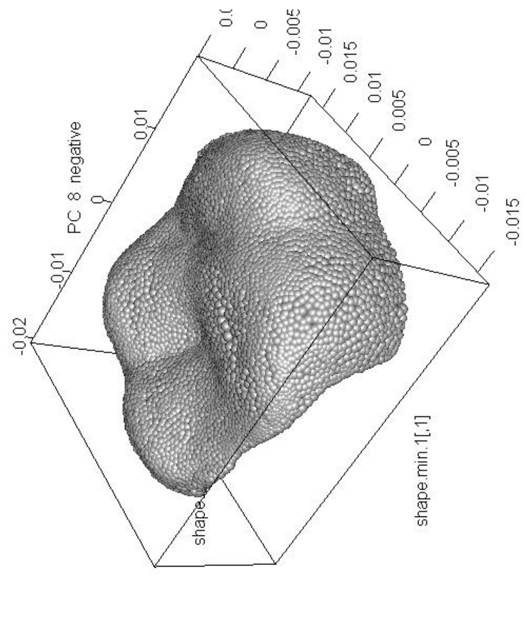



PC2
PC3
PC 2 positive
PC 2 negative
PC 3 positive
PC 3 negative
shape.max.1[,1]
shape.min.1[,1]

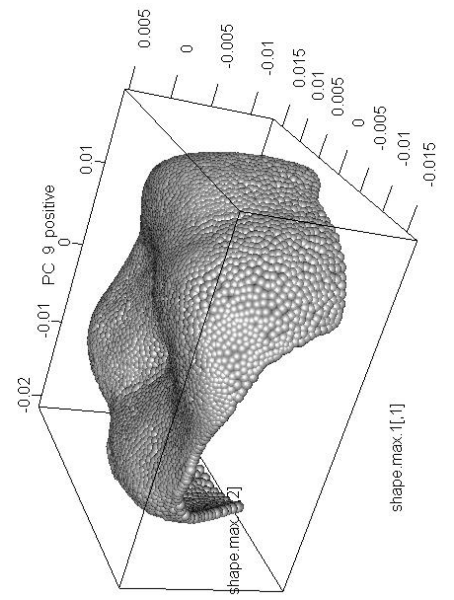

PC 4 positive
shape.max.1[,1]
PC4
PC 4 negative
shape.min.1[,1]
PC 5 positive
shape.max.1[,1]
shape.max.1[,3]
PC5
PC 5 negative
shape.min.1[,1]

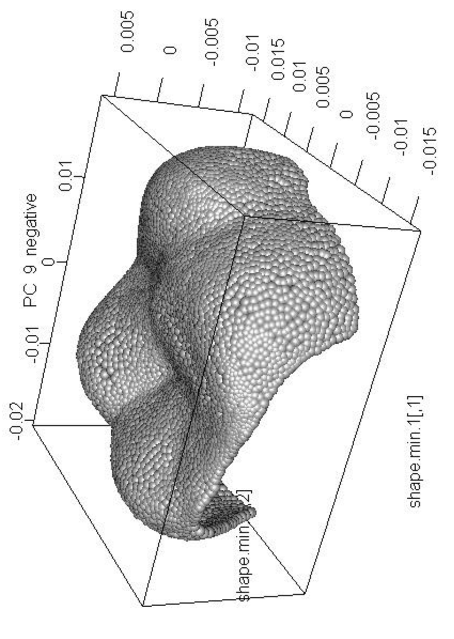

PC6
PC 6 positive
shape.max.1[,1]
PC 6 negative
shape.min.1[,1]
PC7
PC 7 positive
shape.max.1[,1]
PC 7 negative
shape.min.1[,1]


PC 8 positive
shape.max.1[,1]


PC 8 negative
shape.min.1[,1]

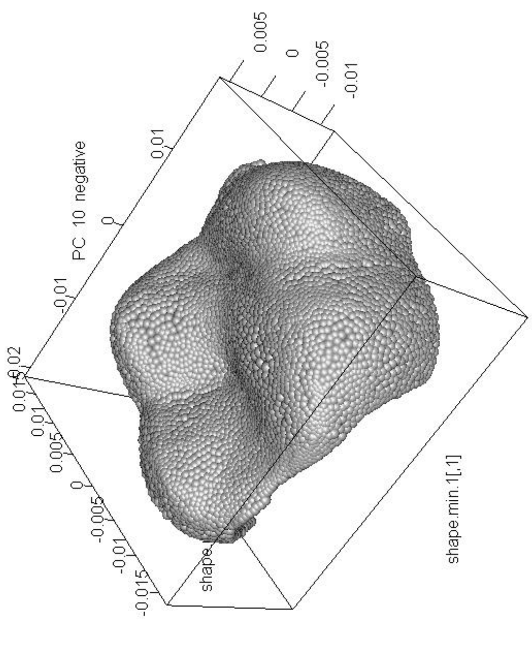
PC8

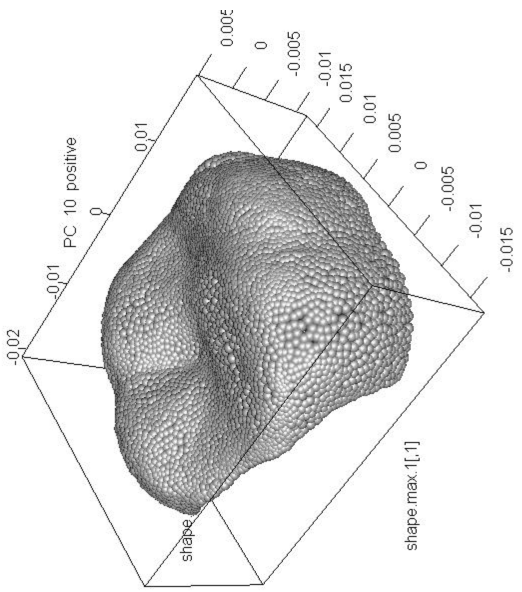

PC 9 positive
shape.max.1[,1]


PC 9 negative
shape.min.1[,1]

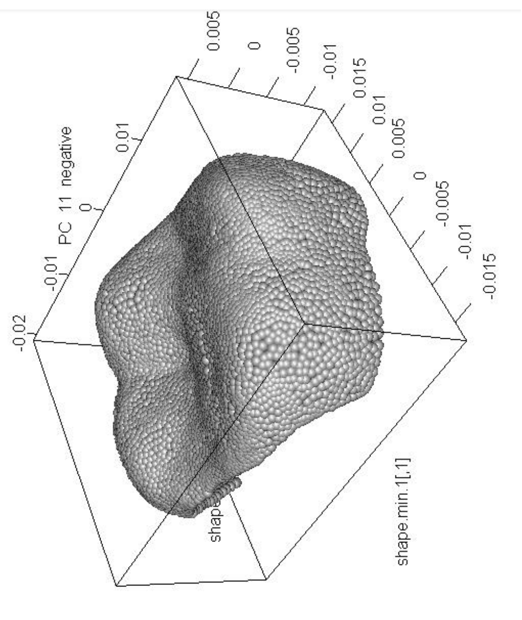
PC9

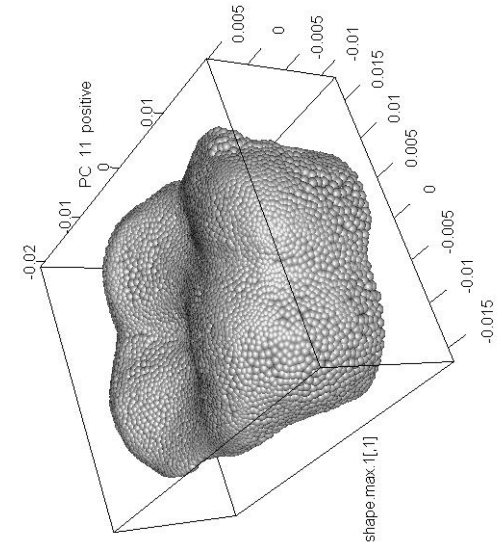
PC10

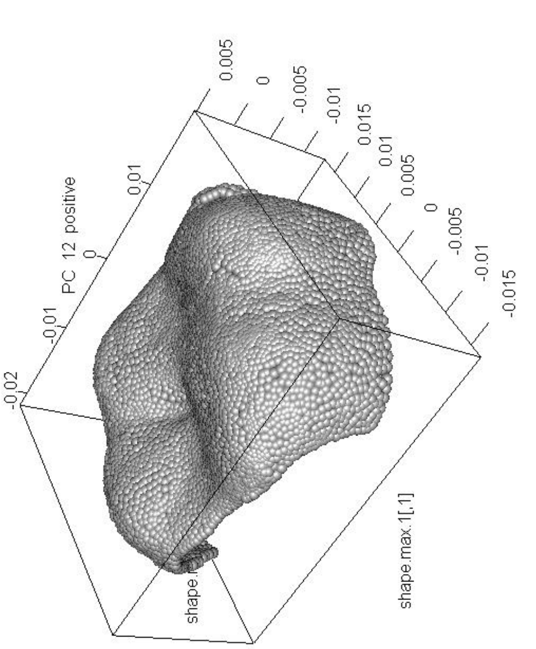

PC 10 negative
shape.min.1[,1]


PC 10 positive
shape.max.1[,1]

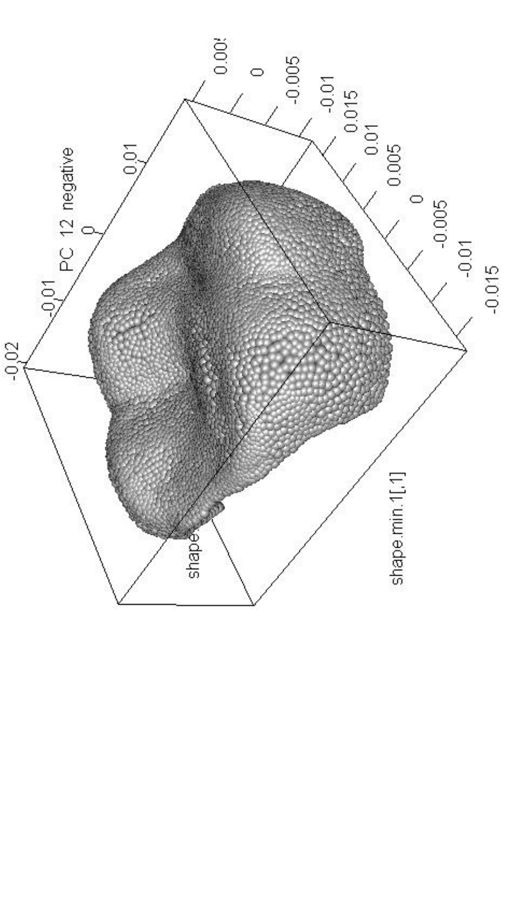
PC11

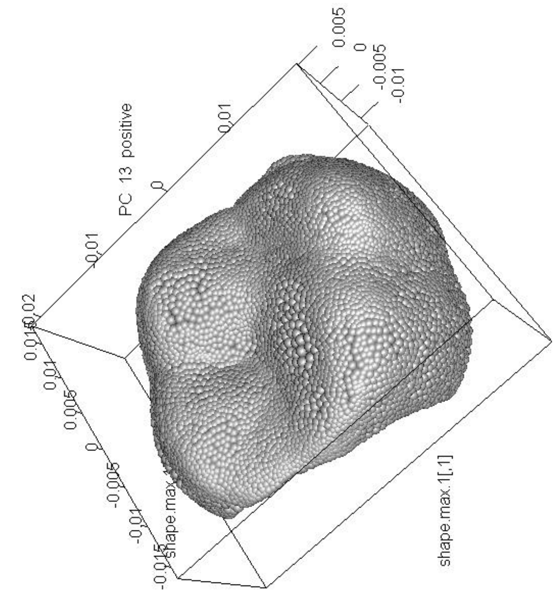

PC 11 negative
shape.min.1[,1]


PC 11 positive
shape.max.1[,1]

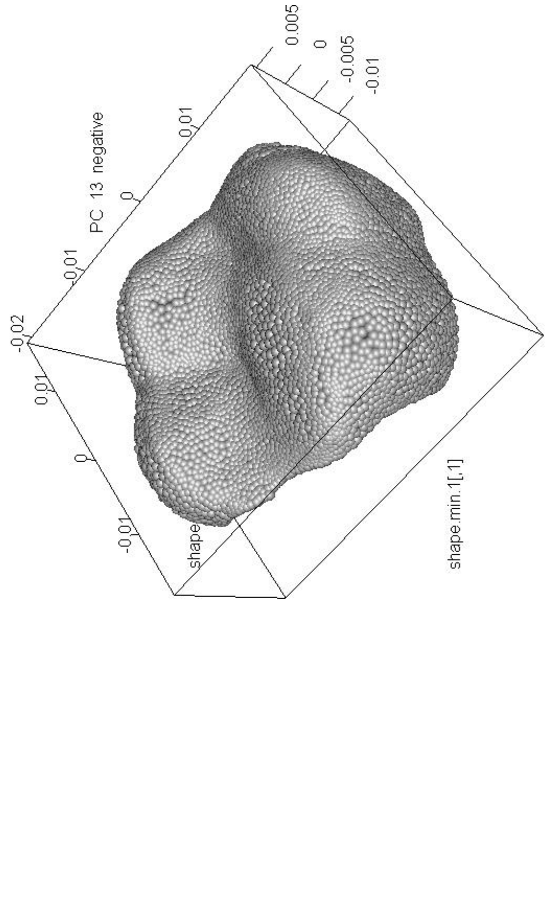

PC 12 positive
shape.max.1[,1]

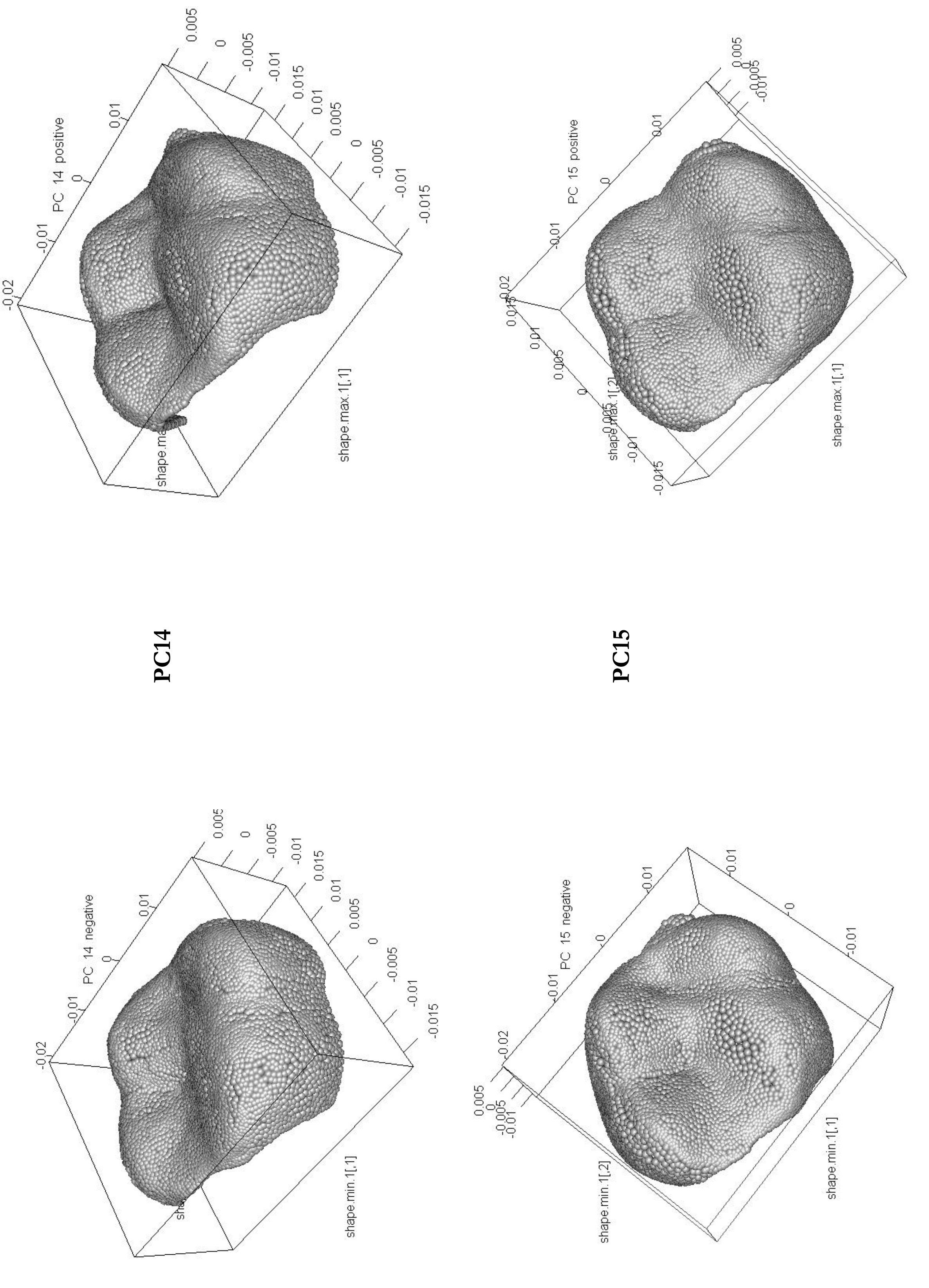
PC12

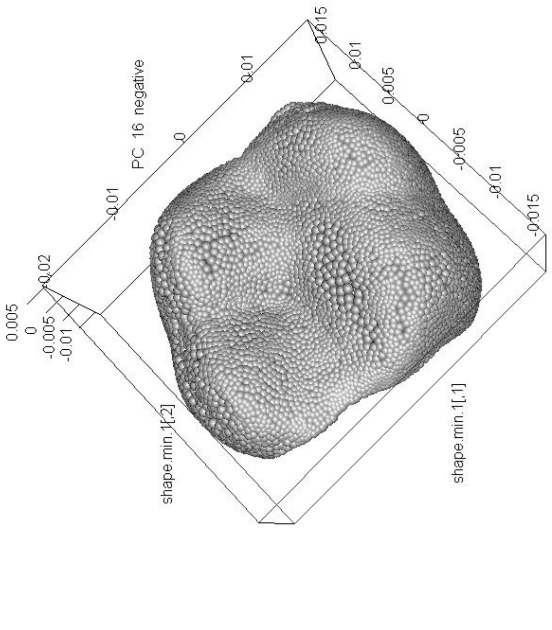

PC 12 negative
shape.min.1[,1]


PC 13 positive
shape.max.1[,1]

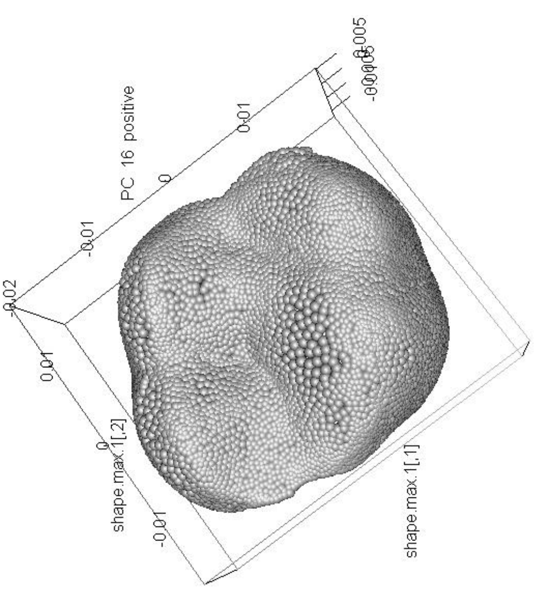
PC13

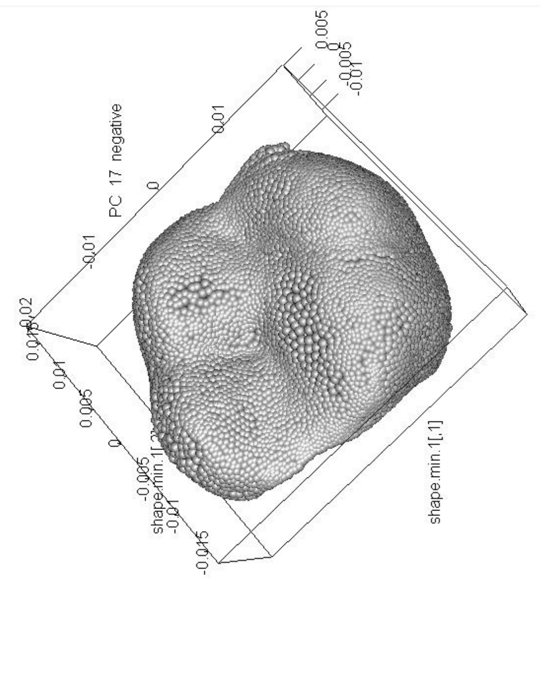

PC 13 negative
shape.min.1[,1]


PC 14 positive
PC14
PC 14 negative
PC 15 positive
PC15
PC 15 negative
shape.max.1[,1]
shape.max.1[,2]
shape.min.1[,1]
shape.min.1[,2]

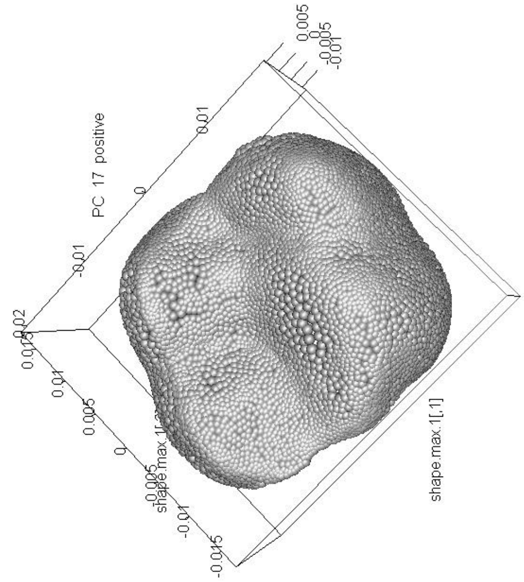

PC 16 negative
shape.min.1[,2]
shape.min.1[,1]

PC16


PC 16 positive
shape.max.1[,2]
shape.max.1[,1]


PC 17 negative
shape.min.1[,1]

PC17

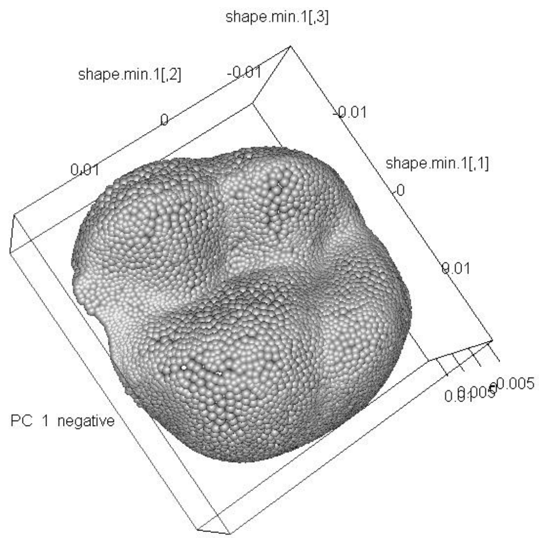

PC 17 positive
shape.max.1[,1]

Upper second molar (UM2)

Importance of components:

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Standard deviation	0.04595	0.03535	0.0325	0.02984	0.02659	0.01974	0.01778	0.01702	0.01558	0.01476	0.01436
Proportion of Variance	0.21510	0.12731	0.1076	0.09071	0.07201	0.03970	0.03222	0.02951	0.02473	0.02220	0.02101
Cumulative Proportion	0.21510	0.34241	0.4500	0.54073	0.61274	0.65244	0.68465	0.71416	0.73889	0.76110	0.78210

	PC12	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC21
Standard deviation	0.01238	0.01151	0.01062	0.01015	0.009781	0.009462	0.009138	0.008223	0.008036	0.008029
Proportion of Variance	0.01560	0.01348	0.01149	0.01050	0.009740	0.009120	0.008510	0.006890	0.006580	0.006570
Cumulative Proportion	0.79771	0.81119	0.82268	0.83318	0.842920	0.852040	0.860550	0.867430	0.874010	0.880570



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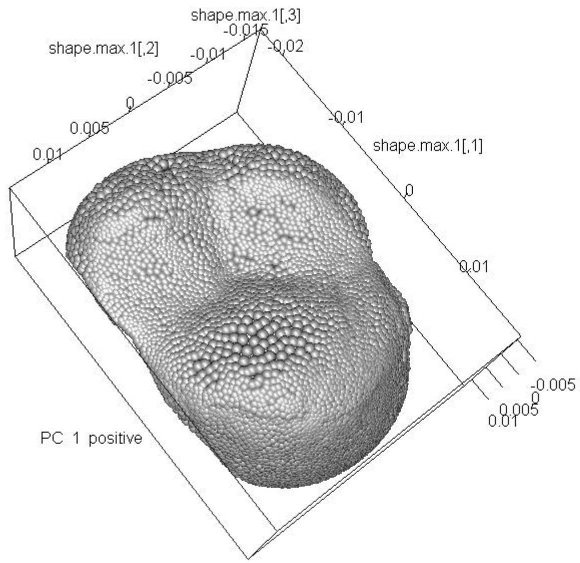

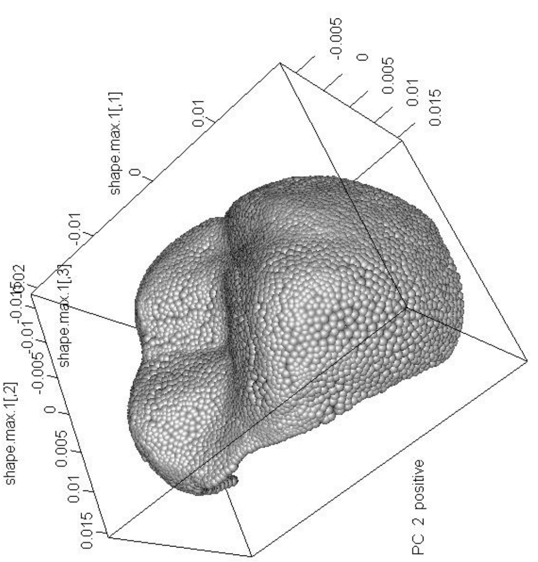
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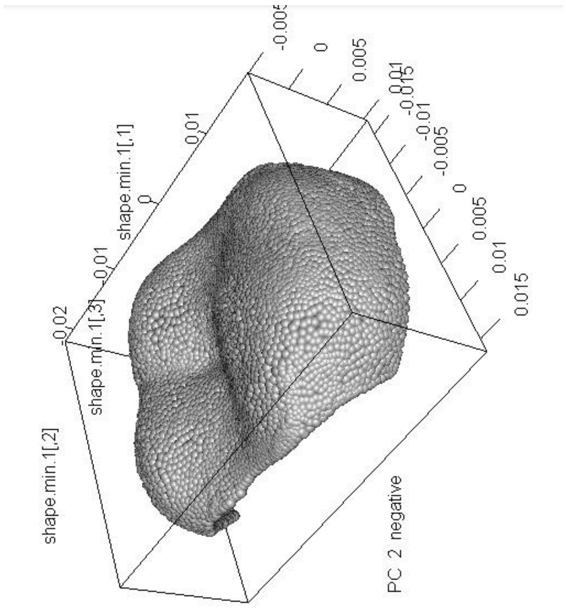
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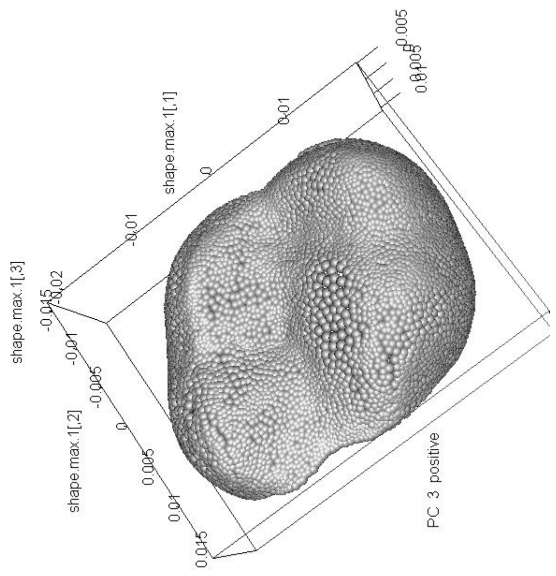
PC2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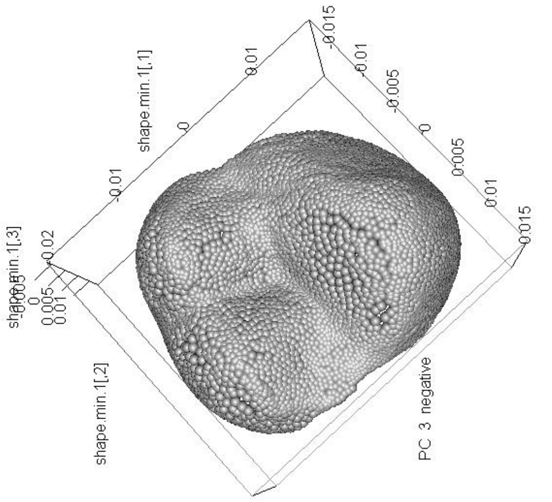
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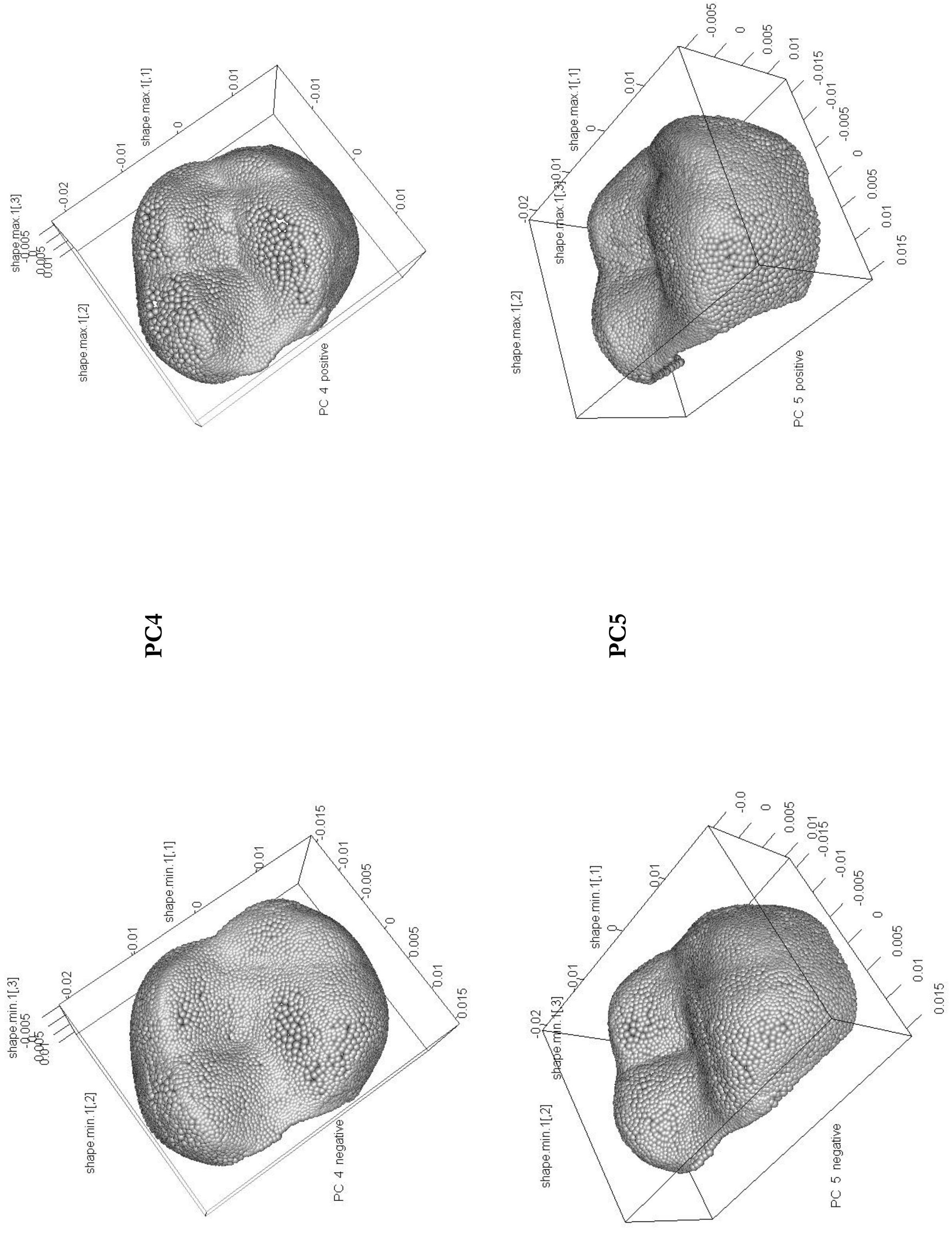
PC3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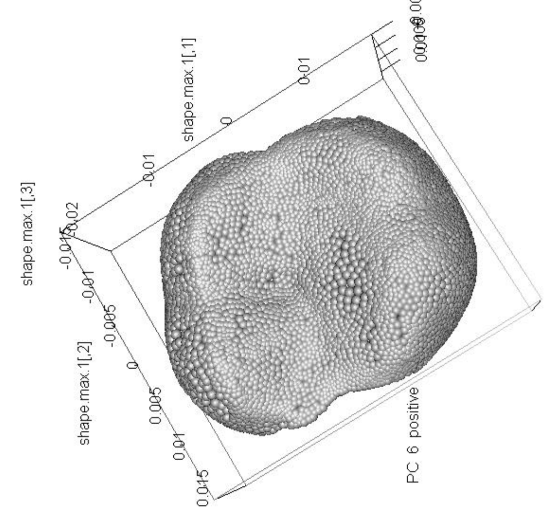
PC4
PC 4 negative
PC 5 positive
PC5
PC 5 negative

PC6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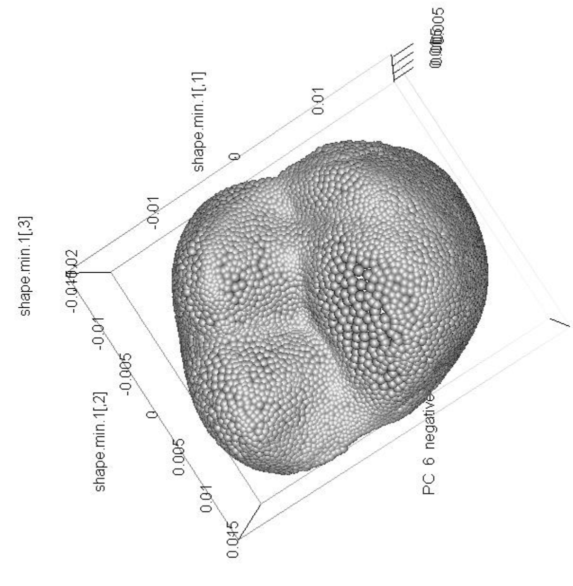
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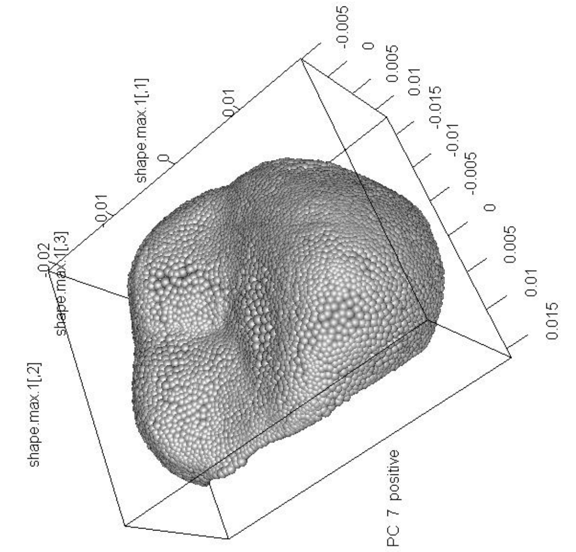
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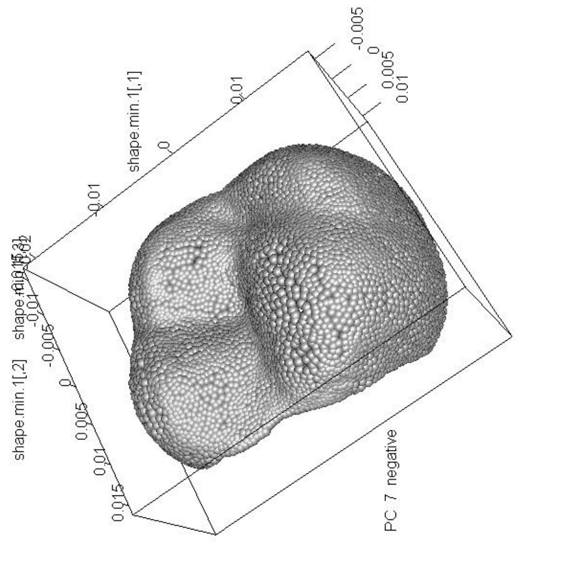
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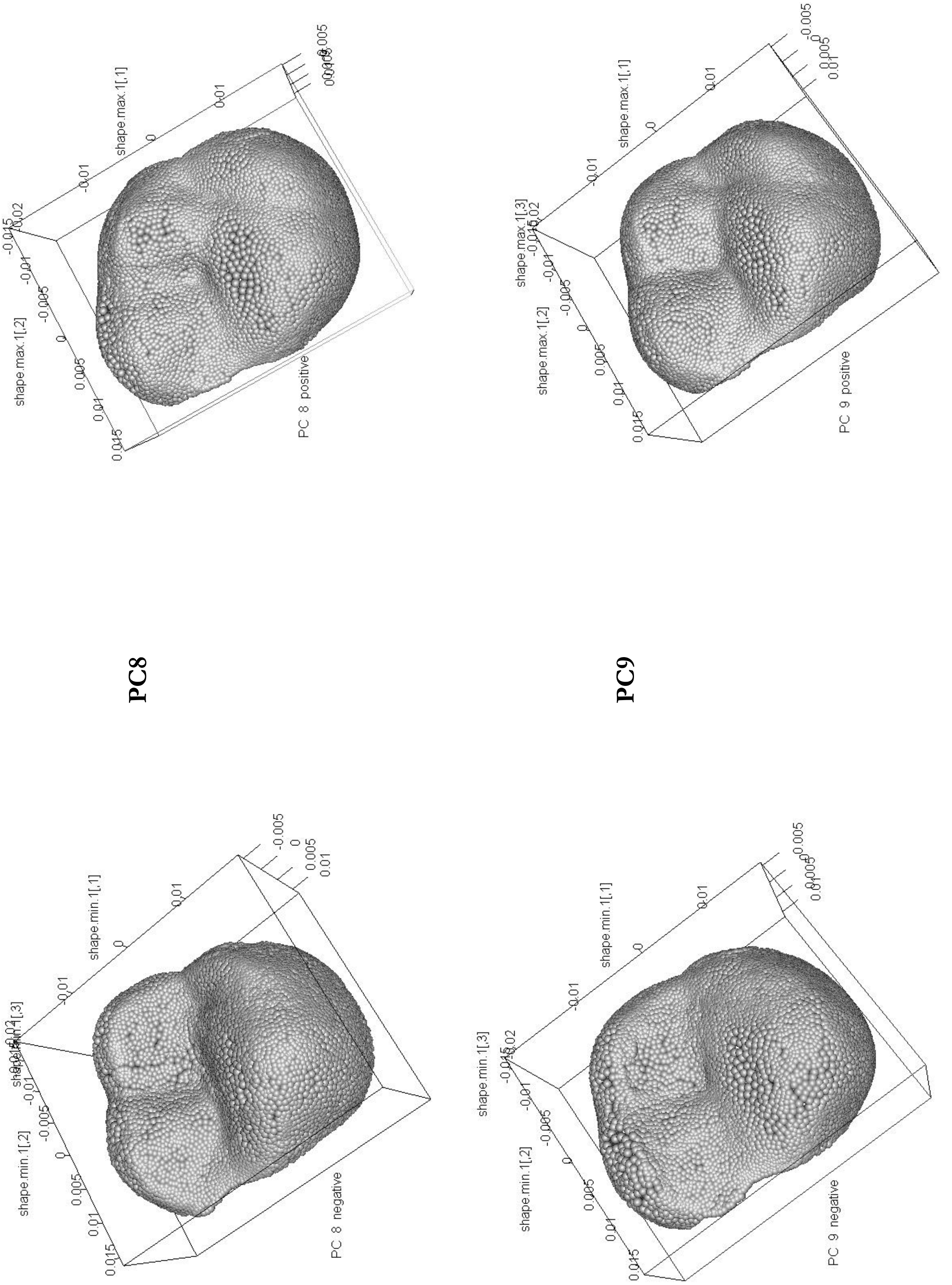
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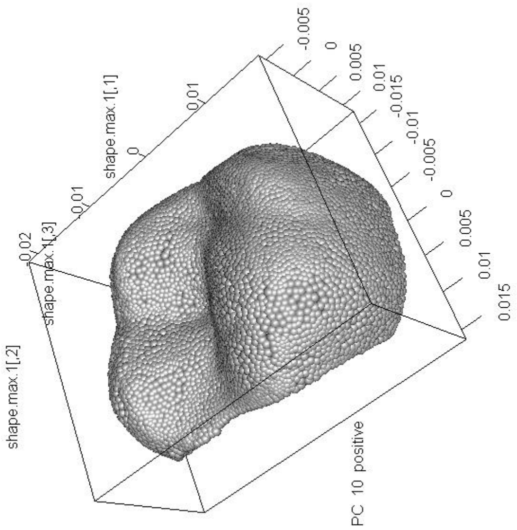
PC8
PC 8 negative
PC 9 positive
PC9
PC 9 negative

PC10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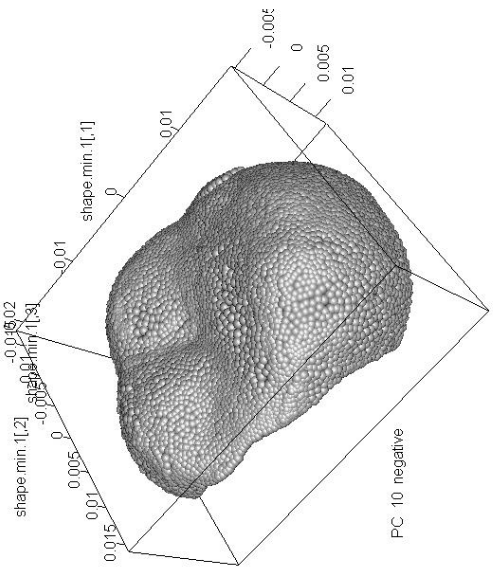
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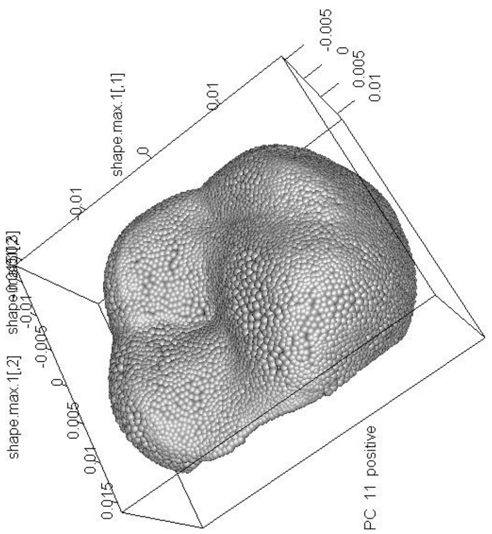
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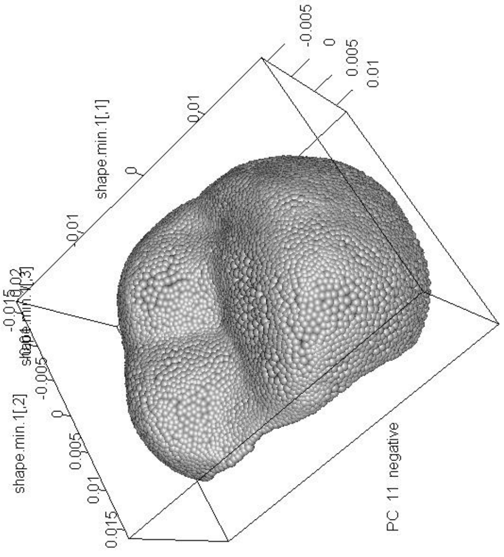
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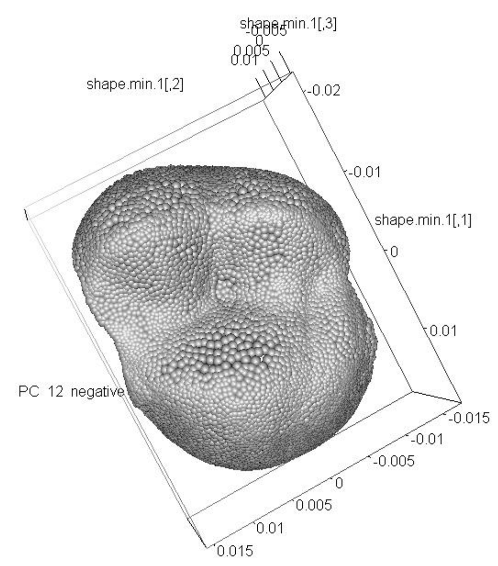
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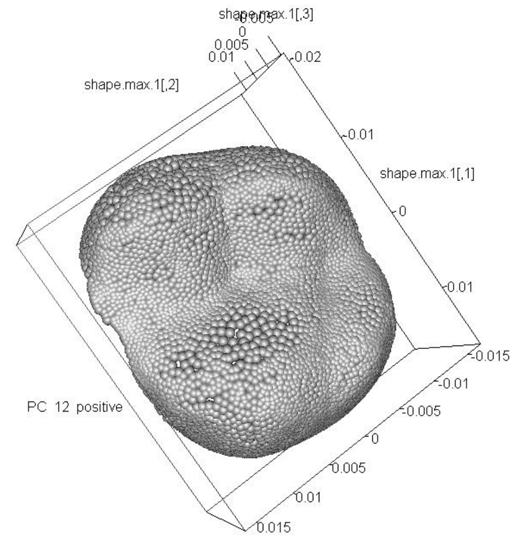
PC12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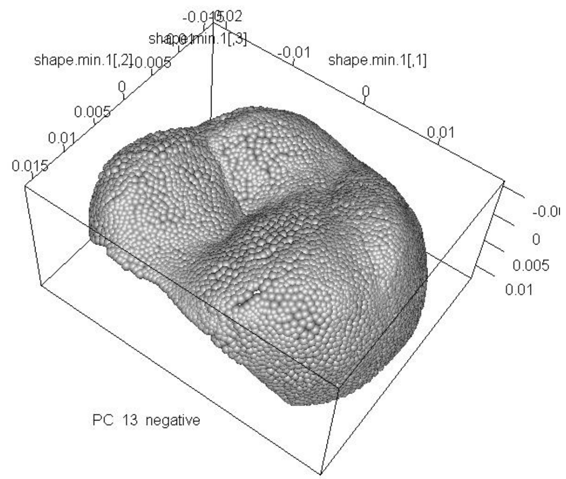
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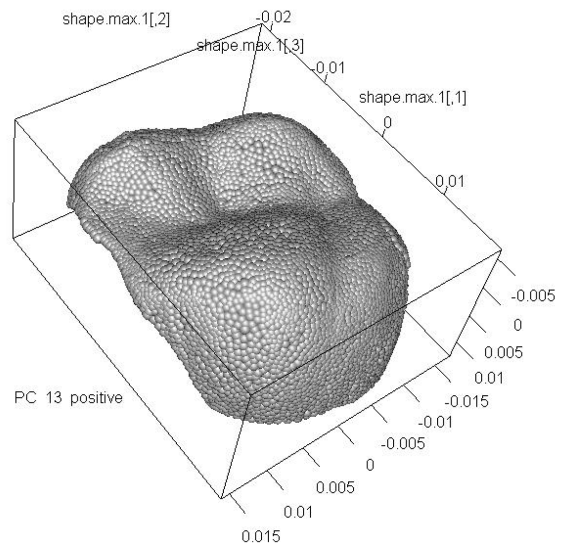
PC 13 negative

PC13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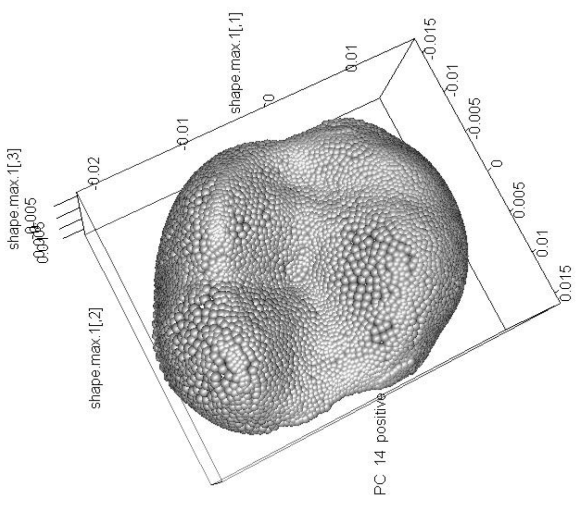
PC 13 positive

PC14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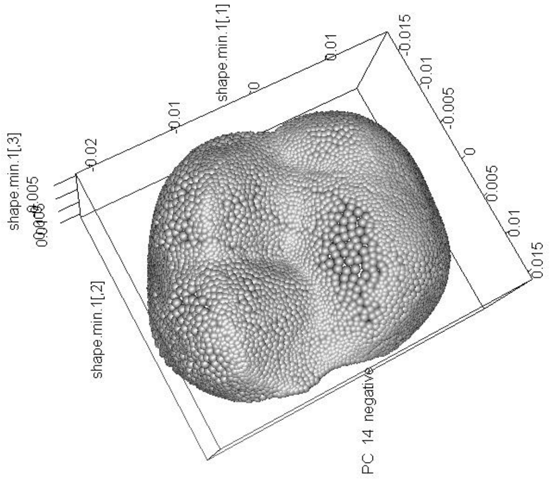
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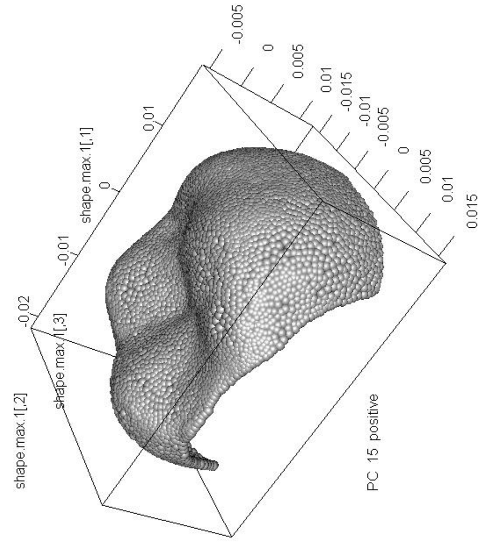
PC15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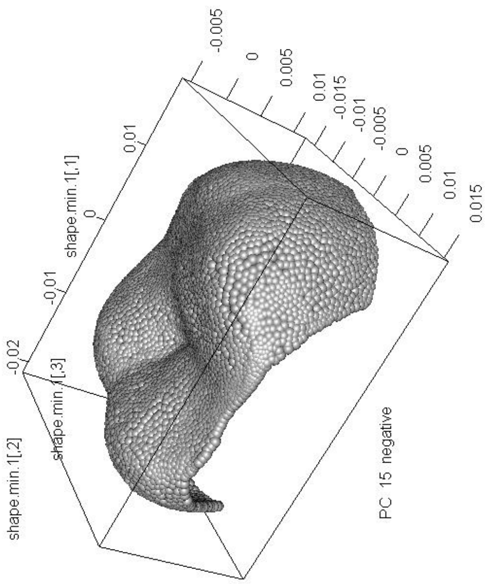
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CHAPTER 3

Dental topography and wear in Central African foragers and agriculturalists

Abstract

Dental wear can be indicative of dietary-related habits, food-processing methods and the abrasive particles content of ingested foods. However, little is known about how dental wear affects topographic features in modern human populations with distinct subsistence strategies. In the present study, we carried out topographic analyses to explore the association between dental topography and wear in Central African hunter-gatherer and agriculturalist populations. Four topographic metrics (curvature, DNE; occlusal relief, OR; dental complexity, OPCR; and ambient occlusion, PCV) were quantified in first, second and third permanent upper (UM1, UM2 and UM3) molars. To explore the differences and interactions between tooth-types, degrees of wear, and dietary groups on these topographic metrics, we conducted ANOVA and a Principal Component Analysis (PCA). The ANOVA results are indicative of significant differences for both the wear stages and dietary-group effects on topographic metrics. No interactions were found between tooth-type and wear, indicating that topographic differences exhibit similar magnitude at different wear stages. OPCR and DNE showed interactions between factors meaning there are differences in dental topography for each dietary group by tooth-type and degree of wear. The PCA results showed that dental topographic metrics can distinguish groups with distinct subsistence strategies, with the first two principal components (PC1 and PC2) accounting for $\approx$100% of the total variance. The PC1 (<80% of the variance) is positively correlated with OPCR and DNE, related to greater associated values in pygmy foragers. The PC2 (<20% of the variance) is positively correlated with OPCR and PCV metrics and negatively correlated with OR. Our findings showed that pygmy foragers are generally associated with higher topographic values compared to the agriculturalist populations, being OPCR and DNE the metrics that best distinguish among Central African populations with distinct dietary specialisation and proving, in this particular case, the effectivity of dental topographic procedures when making dietary approaches.

3.1. Introduction

Dental wear is a natural, complex and unidirectional process of enamel tissue loss and dentine exposure of the occlusal surface as a physiological result from three interacting mechanisms: attrition, due to the action of opposing teeth; abrasion, resulting from the friction of abrasive particles through tooth-food contacts during chewing; and erosion, caused by extended exposure to intrinsic or extrinsic chemical agents (Kaidonis *et al.*, 2012). The wear of the teeth is indicative of dietary-related habits/subsistence strategies, food-processing techniques, and the abrasive properties of the ingested foods among populations (Larsen, 1997; Kaidonis *et al.*, 2012).

In African human populations, most of the studies conducted to characterise dental wear use qualitative scoring methods. These studies often report greater wear for hunter-gatherers compared to agriculturalists, caused by distinct subsistence strategies and food-preparation techniques. For instance, the studies of dental attrition carried out in South African Bantu showed moderate occlusal wear compared to foragers San people (Van Reenen, 1964; Jacobson, 1972). Walker and Hewlett (1990) also reported a lower rate of molar attrition among Bantu farmers individuals compared to the Pygmy foragers, probably due to distinct culinary practices rather than diet. Other qualitative studies on African foragers were carried out to observe sex differences in wear patterns, such as the ones conducted on Hadza (Berbesque, 2012; Crittenden *et al.*, 2017). To minimize inter-observer measurement error of qualitative studies, some authors have investigated tooth wear in African human populations in a quantitative way, based on more objective measurements. For instance, microwear studies conducted on living Hadza foragers (Ungar *et al.*, 2019) and on Central African Pygmy hunter-gatherers, Bantu-speaking farmers and pastoralist populations (Romero *et al.*, 2013). This last study showed differences in buccal striation density, probably as a result of stone-ground foods consumed by farmers but not foragers.

Recently, one quantitative method that has shown to successfully reflect diet-related differences in occlusal morphology is three-dimensional (3D) dental topography (e.g. Winchester *et al.*, 2014; Pampush *et al.*, 2016a; Berthaume *et al.*, 2018). This landmark-free approach quantifies occlusal crown shape and cusp relief (Zuccotti *et al.*, 1998; Ungar and Williamson, 2000; Evans, 2013). It has been applied to fossil hominins (Ungar, 2004; Ungar and Sponheimer, 2011; Berthaume *et al.*, 2018) and extant primates (e.g. Evans *et al.*, 2007;

Bunn *et al.*, 2011; Berthaume and Schroer, 2017; Pampush *et al.*, 2018), showing its effectiveness. Besides, this approach allows the incorporation of worn teeth in the analyses (Ungar and Williamson, 2000; Ungar and M'Kirera, 2003; Dennis *et al.*, 2004; Winchester *et al.*, 2014). To our knowledge, no previous 3D dental topographic studies have been carried out in African modern human populations. Dental topography metrics include orientation patch count rotated (OPCR; Evans and Jernvall, 2009), Dirichlet normal energy (DNE; Bunn *et al.*, 2011), occlusal relief (OR; Winchester, 2016; Berthaume *et al.*, 2019b), and *portion de ciel visible* (PCV; Berthaume, 2016a). Briefly, OPCR describes occlusal complexity by assessing the diversity of orientation of enamel patches (Evans *et al.*, 2007; Evans and Jernvall, 2009; Pampush *et al.*, 2016a). Teeth with more 'tools' such as cusps, crests, and crenulations, will have higher OPCR values and therefore, more complexity (Winchester *et al.,* 2014). DNE was used to quantify occlusal surface curvature and tooth sharpness regardless of the size, position, or orientation (Bunn *et al.*, 2011). Higher DNE values are shown in teeth with sharper cusps (Bunn *et al.*, 2011). OR reflects occlusal relief and tooth height as the ratio of the 3D surface area cropped from the lowest point on the basin to the 2D outline area (Winchester, 2016; Berthaume *et al.*, 2019b). Higher OR values tend to be associated with taller crowned molars (Ungar and M'Kirera, 2003). We also quantified an additional topographic metric derived from the ambient occlusion or *portion de ciel visible* (PCV) which measures how exposed a surface is to ambient lighting and it has been shown to quantify morphological wear resistance. Molars with relatively taller cusps have lower PCV values (Berthaume *et al.*, 2019a).

This study aims to evaluate the use of dental topography metrics to trace changes in cheek teeth complexity with wear in Central Africa populations that present different subsistence strategies and detect which variables would help researchers to distinguish among groups by dietary regimen.

3.2. Material and methods

A sample of 238 well-preserved *in situ* upper postcanine dentition including first (UM1), second (UM2) and third (UM3) molars with variable stages of wearing were selected from individuals of different Central African populations, including Babinga (Central African Republic and Congo) and Babongo (Gabon) western pygmy hunter-gatherers and, Bantu-speaking Bahutu (Rwanda) agriculturalists (Table 3.1). Cranial samples are housed at the

Musée de 1'Homme (Paris) and the Institut Royal des Sciences Naturelles de Belgique (Brussels). Ethnographic and geographical provenience were obtained from museum records. Detailed anthropological descriptions are available for both the pygmy (Marquer, 1972) and the Bahutu (Brabant, 1963) studied samples.

Table 3.1. Teeth analysed by dietary-group and wear-stage.

Dietary-group[a]	Tooth	Slightly worn[b]	Moderately worn[b]	All
Hunter-gatherer	UM1	5	22	27
	UM2	9	10	19
	UM3	6	8	14
Agriculturalist	UM1	20	56	76
	UM2	20	44	64
	UM3	13	25	38
Total		73	165	238

[a]Central African hunter-gatherers (Babinga and Babongo) and agriculturalist (Bahutu) groups.
[b]Type of wear based on Smith (1984) as slight (stages 1 and 2) and moderate (stages 3 to 5).

Babinga and Babongo samples were collected from different African expeditions between the middle of the 19th century and the first half of the 20th century (Marquer, 1972). Ethnographic studies for Babinga camps from the western part of the Congo Basin documented a forest-based subsistence economy and a lack of both plantations and domestic animals (Regnault, 1911). Babinga were essentially hunters (elephant, antelope, or wild pig) and gatherers, mainly wild-yams, also including the consumption of exchange agricultural products (corn, cassava, and bananas) with neighbour farmers, though this practice remained very limited (Regnault, 1911; Demesse, 1980). The Babongo of central Gabon, when first contacted, were also living in hunting camps (Du Chaillu, 1867). At the end of the 1940s, Babongo people shifted to a sedentary lifestyle increasing dependence on agricultural products (Knight, 2003). However, hunting-gathering activities still occupy an important part in their diet (≈40%) to satisfy nutritional requirements (Matsuura, 2006). Finally, the Bahutu crania were recovered in 1960 from a volcanic cave in Ruhengeri (Rwanda) and dated at the beginning of the last century (Brabant, 1963). Hiernaux (1954) documented that the Bahutu dietary regimen is characterised by a relative abundance of agricultural products based mainly on beans, sweet potatoes, millet, cassava and bananas, and a marked deficiency in animal proteins for cultural reasons.

3.2.1. Data acquisition

Original teeth were cleaned using ethanol and air-dried prior to obtaining polyvinylsiloxane-based moulds (PresidentJet regular body, Coltène® Corp.) and, high-resolution non-translucent polyurethane resin (Feroca® Composites, Spain) were produced from moulds following standard procedures (Galbany *et al.*, 2006). Teeth were grouped into both slight and moderate wear categories according to 8 stages of occlusal surface dentine exposure (Smith, 1984). Slightly worn molars with stages 1-2 included unworn teeth or teeth showing no more than one or two pinpoint dentine exposure areas. We grouped as moderately worn those teeth exhibit wear stages 3-5, only showing dentine exposure areas isolated within cusps, either with full cusp removal and/or large areas of exposed dentine. However, teeth showing more than two dentinal areas coalesced (stages >6; Smith, 1984) were not considered.

Three-dimensional (3D) models of each dental replica were obtained using a structured light scanner (DAVID® SLS-2) at a maximum resolution of 0.06 mm and multiple 360° scans automatically aligned and fused. Polygonal meshes were edited using Geomagic® Wrap 2014 (3D Systems, Rock Hill, USA) and MeshLab v.1.3.3 (Cignoni *et al.*, 2008) to isolate molars from the tooth rows as needed, cropping along the interproximal facets and above the lowest point on the occlusal basin (Berthaume *et al.*, 2019b). Meshes were then oriented placing the occlusal surfaces perpendicular to the Z-axis (Pampush *et al.*, 2016a). Meshes were also re-triangulated to generate more homogeneous polygonal faces, slightly smoothed to remove noise, and simplified down to 10,000 polygons following smoothing and decimation protocols for dental topographic metrics (Winchester, 2016; Spradley *et al.*, 2017). Three topographic algorithms (OPCR, DNE, and OR; see Figure 3.1) were first quantified from each mesh using MorphoTester (Winchester, 2016). We measured OPCR with a minimum patch size of 5 polygons as the average number of independently oriented patches counted at eight 45° tooth orientations (Winchester, 2016). DNE was calculated with 1% outlier removal applied (the 99.9th percentile) in energy x area (Winchester, 2016). OR was reported as the ratio of the surface area (3D area of the occlusal surface tooth) divided by the tooth size (2D outline area projected on a plane) (Winchester, 2016). PCV was calculated with *CloudCompare* (http://www.danielgm.net/cc/) and the PCV function was executed. The command 'fit a statistical model on the active scalar field' calculates the average PCV of the mesh (Berthaume *et al.*, 2016a).

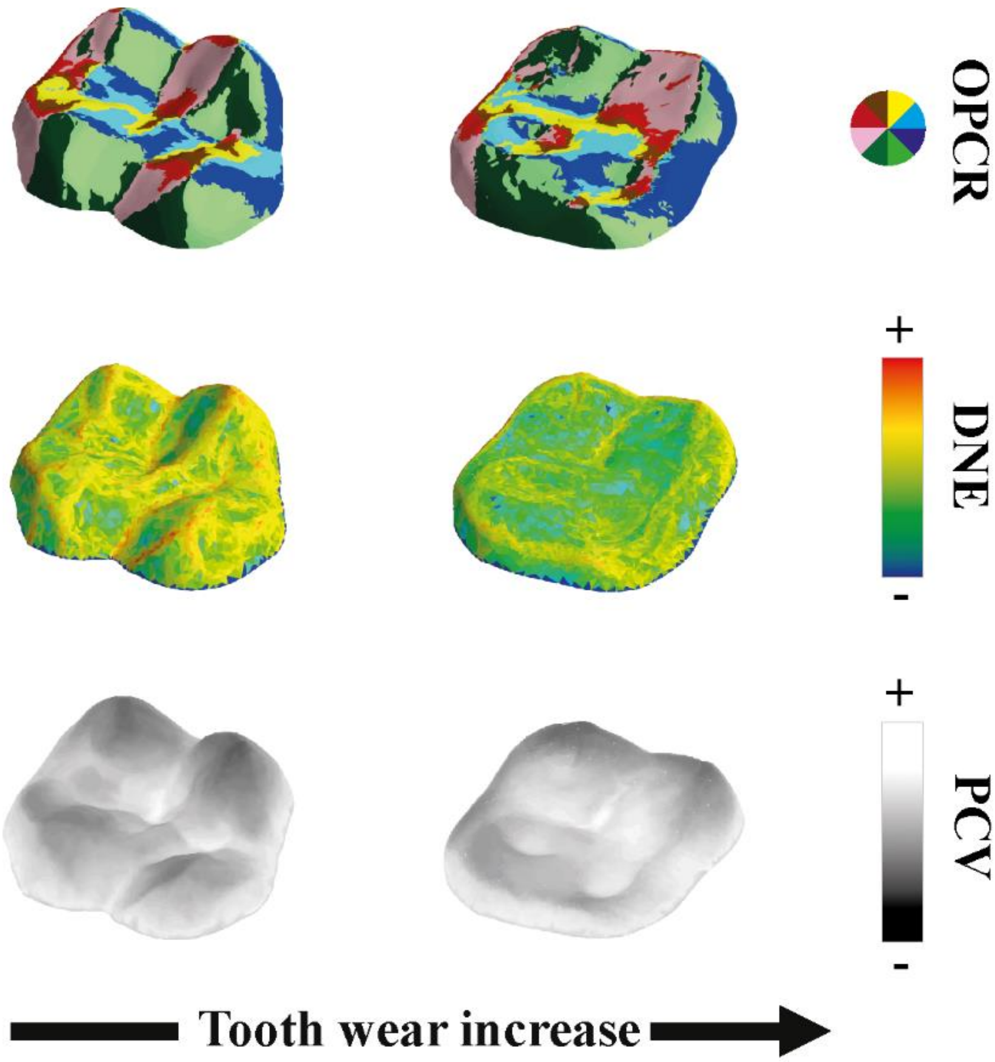

Figure 3.1. Dental complexity (OPCR), curvature (DNE) and portion de ciel visible (PCV) changes with wear in maxillary second molars. Slight (left) and moderate (right) worn crowns. OPCR indicates surface orientation patches (see colour wheel). Warmer and cooler colours for DNE maps indicate higher and lower curvature, respectively. Brighter areas indicate higher PCV values.

3.2.2. Statistical analyses

The Kolmogorov-Smirnov goodness-of-fit test showed non-significant topographic metric differences in distribution and variance (Z=0.676 to 1.107; p >.05). Differences and interactions between tooth-type, wear stages, and dietary groups on topographic metrics was assessed using a three-way ANOVA model. Sources of significant variation were also determined by using single classification ANOVAs. In addition, a Principal Component Analysis (PCA) on the covariance matrix was used to calculate specific patterns from topographic metrics by tooth-type that account for most of the variability observed among dietary groups. Descriptive and statistical analyses were conducted using IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA) and PAST 3.0 (Hammer *et al.*, 2001) with a significance level of $\alpha=0.05$.

3.3. Results

Summary statistics are shown in Table 3.2. Factorial ANOVA results showed significant variation in the models (Table 3.3). Accordingly, significant differences were found in the topographic metrics (OPCR, DNE, OR and PCV) among the wear stages and dietary groups effects. However, only OPCR and DNE were affected by tooth-type. In addition, the lack of interaction between tooth-type and wear indicates that topographic differences showed similar magnitudes at different degrees of wear. Interactions were also found for OPCR and DNE between factors also suggesting significant variations in dental topography for each dietary group by molar position and wearing processes. One-way ANOVAs reflected the significant variations shown in Table 3.4. Overall results revealed differences between the hunter-gatherers and the agriculturalists' dental topography ($p < .05$), especially for dental complexity (OPCR) and curvature (DNE) mean values when comparing dietary specializations for both slight and moderate wear categories.

The PCA results provided strong evidence that dental topographic metrics distinguish groups by dietary regimens (Table 3.5 and Figure 3.2). The first two principal components (PC1 and PC2) for each tooth accounted for ≈100% of the total variance. Topographic metrics with the greatest correlations (Pearson r) showed significant dental shape changes, despite wear, between foragers and agriculturalists. Overall, PC1 (>80% of the variance) showed uniformly high positive values ($r > 0.7$; $p < .01$) for teeth complexity (OPCR) and surface curvature (DNE), which showed great discrimination of pygmy hunter-gatherers. The sample distribution along PC2 (<20% of the variance) was also mainly affected by positive loadings for OPCR ($r > 0.4$; $p < .01$) and PCV ($r > 0.7$; $p < .01$) metrics. By contrast, negative loadings were found for OR ($r \geq -0.6$; $p < .01$), since agriculturalists show high occlusal reliefs compared with those observed in the forager groups.

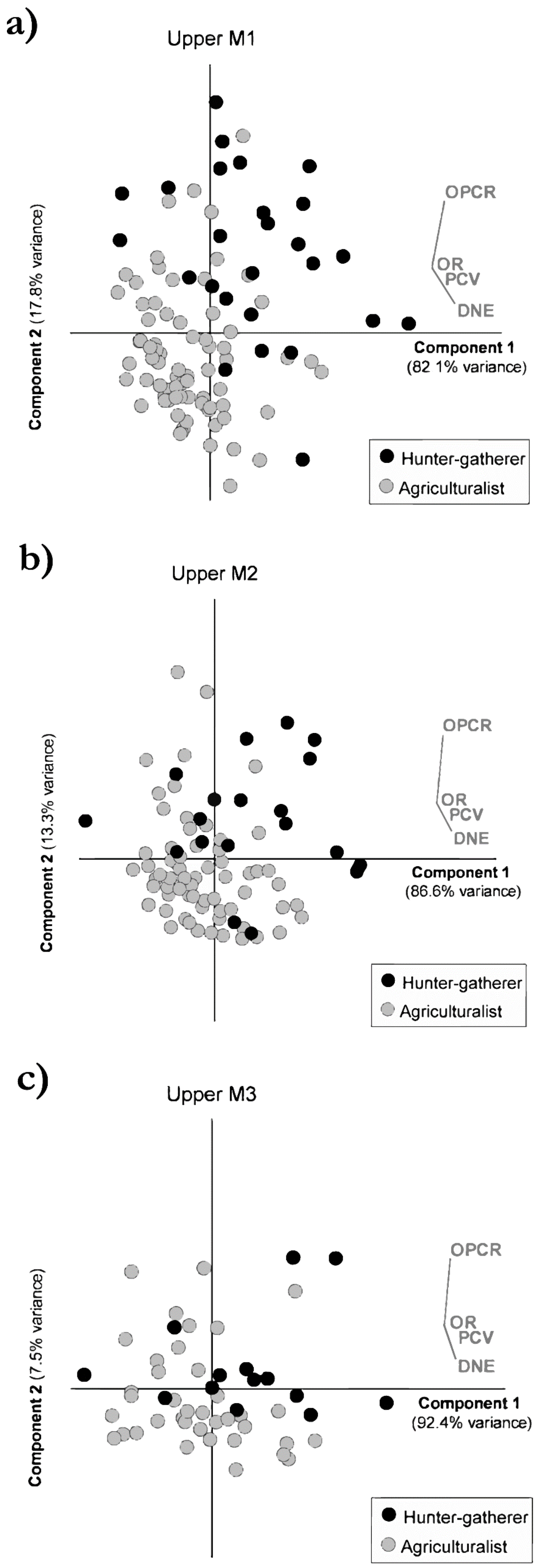

Figure 3.2. Binary plots of the first two principal components (PC1-2) accounting for >95% of total variance by tooth-type and dietary groups. The labelled rays show the unrotated loadings of dental topographic metrics onto PC1 and 2 axes. Note that higher correlation values for OPCR and DNE influence among-groups variability. See methods sections and Table 3.5 for dental topographic correlation details.

Table 3.2. Descriptive statistics of topographic metrics for slightly and moderately worn upper molars among dietary groups.

Tooth	Dietary group[a]	Wear[b]	*N*	OPCR		DNE		OR		PCV	
Upper M1				Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Hunter-gatherer	Slight	5	137.600	28.582	249.855	77.996	1.370	0.060	0.750	0.023
		Moderate	22	154.051	28.607	187.522	58.957	1.277	0.075	0.795	0.015
	Agriculturalist	Slight	20	85.737	14.163	164.911	42.026	1.385	0.092	0.741	0.025
		Moderate	56	101.022	23.704	156.957	34.346	1.321	0.114	0.773	0.026
Upper M2											
	Hunter-gatherer	Slight	9	108.875	25.501	206.709	56.352	1.380	0.094	0.740	0.019
		Moderate	10	121.062	32.049	168.709	54.705	1.245	0.062	0.789	0.017
	Agriculturalist	Slight	20	84.675	13.313	180.956	29.661	1.392	0.057	0.734	0.013
		Moderate	44	84.392	20.092	139.803	30.265	1.301	0.082	0.770	0.027
Upper M3											
	Hunter-gatherer	Slight	6	102.208	18.115	174.249	33.185	1.347	0.038	0.752	0.010
		Moderate	8	122.328	41.921	186.764	73.157	1.257	0.052	0.780	0.022
	Agriculturalist	Slight	13	94.759	14.029	182.906	33.508	1.407	0.071	0.734	0.016
		Moderate	25	81.445	25.140	128.569	36.563	1.303	0.102	0.768	0.027

[a]Central African hunter-gatherers (Babinga and Babongo) and agriculturalist (Bahutu) groups.
[b]Tooth wear scored based on Smith (1984) as slight (stage 1 and 2) and moderate (stage 3 to 5).

Table 3.3. Results of three-way ANOVA model for tooth-type, wear and diet effects on topographic metrics.

Effects	**OPCR**		**DNE**		**OR**		**PCV**	
	F	*p*	*F*	*P*	*F*	*p*	*F*	p
Model	18.378	0.000*	7.361	0.000*	5.414	0.000*	13.701	0.000*
Tooth[a]	11.952	0.000*	3.403	0.000*	0.178	0.837	1.195	0.305
Wear[b]	4.694	0.031*	20.930	0.000*	40.972	0.000*	90.694	0.000*
Diet[c]	84.551	0.000*	27.617	0.000*	6.847	0.009*	13.635	0.000*
Tooth × Wear	0.962	0.384	0.604	0.548	0.496	0.609	0.663	0.516
Tooth × Diet	4.873	0.008*	2.382	0.095	0.201	0.818	0.026	0.974
Wear × Diet	4.087	0.044*	0.140	0.709	0.411	0.522	0.664	0.416
Tooth × Wear × Diet	1.353	0.261	5.873	0.003*	0.308	0.735	0.546	0.580

[a]Tooth-type included upper molars (M1, M2 and M3).

[b]Wear included molars with slightly (stages 1 and 2) and moderate (stage 3 to 5) wear based on Smith (1984).

[c]Dietary categories by hunter-gatherers and agriculturalist groups.

Significant differences at $p < .05$ (*).

Table 3.4. One-way ANOVA to compare slightly and moderately worn upper molars among dietary groups on topographic metrics.

Tooth	Wear[a]	Dietary group[b]	*N*	OPCR		DNE		OR		PCV	
Upper M1				*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
	Slight	Hunter-gatherer	5	34.954	0.000*	11.466	0.003*	0.132	0.720	0.500	0.487
		Agriculturalist	22								
	Moderate	Hunter-gatherer	20	70.194	0.000*	8.133	0.006*	2.754	0.101	12.527	0.001*
		Agriculturalist	56								
Upper M2				*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
	Slight	Hunter-gatherer	9	11.452	0.002*	2.639	0.116	0.207	0.653	1.189	0.285
		Agriculturalist	10								
	Moderate	Hunter-gatherer	20	21.417	0.000*	5.338	0.025*	4.102	0.048*	4.575	0.037*
		Agriculturalist	44								
Upper M3				*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
	Slight	Hunter-gatherer	6	0.967	0.339	0.276	0.606	3.707	0.071	6.209	0.023*
		Agriculturalist	8								
	Moderate	Hunter-gatherer	13	11.431	0.002*	9.149	0.005*	1.479	0.233	1.240	0.274
		Agriculturalist	25								

[a]Tooth wear scored based on Smith (1984) as slight (stage 1 and 2) and moderate (stage 3 to 5).
[b]Central African hunter-gatherers (Babinga and Babongo) and agriculturalist (Bahutu) groups.
Significant differences at $p < .05$ (*).

Table 3.5. Factor loadings of the first two Principal Components (PC1-2) on dental topographic metrics for the teeth analysed.

	Upper M1		**Upper M2**		**Upper M3**	
PCs[a]	PC1	PC2	PC1	PC2	PC1	PC2
% variance	82.115	17.885	86.685	13.315	92.45	7.549
Metric[b]						
OPCR	0.759**	0.652**	0.730**	0.684**	0.872**	0.489**
DNE	0.969**	−0.246*	0.987**	−0.162	0.990**	−0.144
OR	0.199*	−0.673**	0.284**	−0.728**	0.192	−0.713**
PCV	−0.140	0.849**	−0.360**	0.823**	−0.290*	0.788**

[a]PCs: Principal component 1 (PC1) and 2 (PC2); percentage (%) of variance explained.
[b]Correlation (Pearson's *r*) at $p<.05$ (*) and $p<.01$ (**).

3.4. Discussion

We provide the first study of dental wear in Central African foragers and agriculturalists using 3D dental topographic procedures. Our results indicate that the molar teeth of the pygmy foragers exhibit higher dental complexity (OPCR) and curvature (DNE) than the Bantu-speaking agriculturalists, a topography that is characteristic of molars with sharper cusps and notable crests and crenulations (Bunn *et al.*, 2011; Winchester *et al.* 2014). Despite the dental topographic metrics can be studied as independent variables, our results reflect that these variables do show interactions with each other since they may reflect similar adaptations to food processing (Bunn *et al.*, 2011).

All topographic variables analysed showed significant differences between wear stage and dietary specialisations. Differences in tooth wear for the different metrics were expected according to previous dental topographic studies (e.g. Glowacka *et al.*, 2016; Pampush *et al.*, 2016a; Berthaume *et al.*, 2018). As expected, when analysing changes with wear progression, both DNE and OR tend to decrease with wear for both dietary habits and therefore, molars were becoming lower cusped and flatter. Wear caused PCV to increase, turning molars less wear resistant. Wear also caused OPCR to increase significantly, meaning molars were becoming more complex as they wear down. This would suggest that a better functionality could be present at moderate stages of dental wear, not at initial stages. The differences found only for OPCR and DNE by tooth-type may be indicating that these metrics are more likely to reflect the variation in the number of cups in the upper molars of Central Africa modern human populations.

In our PC analysis, both dental complexity and curvature are the topographic metrics that better distinguish between foragers and farmers. This can be observed especially in PC1, which explains >80% of the total variance for these two metrics, which also showed the highest mean values in pygmy hunter-gatherers compared to the agriculturalists. Teeth of pygmy foragers showing both high complexity and curvature values on the occlusal surface, in contrast with the Bantu-speaking farmers, may indicate complex dentitions with sharp cusps that are often related to individuals that require higher shearing forces and more mechanically processing requirements for tough foods (e.g. Pampush *et al.*, 2016a; Thiery *et al.*, 2017; Berthaume and Schroer, 2017; Pineda-Muñoz *et al.*, 2017; Pampush *et al.*, 2018; Berthaume *et al.*, 2018).

Our results showed that dental topography seems to be an effective tool to draw dietary inferences on modern human populations of Central Africa. Besides, it has allowed to detect that both OPCR and DNE may distinguish between Central African foragers and agriculturalists.

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GENERAL DISCUSSION

In this dissertation, I will focus on the objectives previously stated in the quantitative study of dental shape in relation to wear in modern human populations. As no specific research on this topic has yet been published on this matter, not much is known about how dental wear affects dental crown shape of the cheek teeth. As I have mentioned in previous chapters, the assessment of tooth wear in previous anthropological studies has mainly addressed this research from a qualitative perspective, using visual examination and ordinal scales (see Molnar, 1971; Scott, 1979; Smith, 1984) and only a few studies have assessed this aspect quantitatively (see Mays, 2002; Benazzi *et al.,* 2008; Clement and Hillson, 2012; Górka *et al.*, 2015). The study presented in this dissertation focuses on the application of three different, standardised quantitative techniques in order to characterise dental enamel loss in different modern human populations. Below, I will present a general discussion on the main results obtained.

1. Dental wear assessment using 2D techniques

In Chapter 1 we showed the abrasive effect of USO consumption on dental wear rates in a group of Central African Baka pygmy forager population with individuals of known ages and well-documented diet using a two-dimensions procedure, the percentage of dentine exposure (PDE). This technique has proved its effectiveness in previous studies of dental wear in modern human populations, such as the studies carried out in the prehistoric island and mainland populations along the Santa Barbara Channel (Walker, 1978), in an archeological sample of hunter-gatherers and agriculturalists from North America (Deter, 2009), in a known sex modern Igloolik Eskimo population (Clement and Hillson, 2012) and in the Alaskan Tigara foragers from Point Hope (Górka *et al.,* 2016). Our results indicated that dental wear is a natural and physiological age-dependent process. In the particular case of Baka Pygmies, age explained 70% of dental wear variability in the studied sample. This was in accordance with previous studies carried out in forager populations that also showed a strong age-wear relationship. For instance, the Igloolik Eskimo population reported age-wear correlations of between 79-85% (Tomenchuk and Mayhall, 1979), the indigenous Amazon populations from Brazil showed correlations of 65-86% (Vieira *et al.*, 2015), natives from Greenland also reported significant age-wear correlations (Davies and Pedersen, 1955), and the age-wear correlations reported for Australian aboriginals were also significant, 63-94% shown in the study carried out by Richards and Brown (1981), and 39-68% according to Molnar *et al.* (1983).

In our analyses we obtained no differences between men and women in molar wear patterns. This is not consistent with previous indications that Baka foragers show sexual division in subsistence activities (Vallois and Marquer, 1976; Gallois *et al.*, 2015) but consistent with other studies carried out in other hunter-gatherer populations, such as the Australian Aborigines (Richards and Brown, 1981), the Hadza (Crittenden *et al.*, 2017) and the Alaskan Tigara foragers from Point Hope (Górka *et al.*, 2016). This result can be explained by the fact that all Baka individuals eat similar foods regardless of sexual division of labour (Sato *et al.*, 2012).

Our results also showed lower rates of dentine exposure in Baka pygmies younger than 16 years old than those older individuals (≈30 years old) that showed greater values of PDE. Nevertheless, the percentage of dentine exposure of the older individuals was less than <5% of total occlusal surface. Higher tooth wear rates were expected in a foraging population such as the Baka. Molnar *et al.* (1983) showed a percentage of dentin exposure area of 3.4-24.4% in Australian Aborigines in individuals between 11 and 13 years old, while that percentage in individuals between 18 and 21 years old was 16-44.9%. The study carried out in Tigara foragers from Point Hope by Górka *et al.* (2015) reported maximum PDE values no greater than 15% in individuals between 16 and 25 years old, and maximum PDE values of ≈50 in individuals between 36-45 years old. Similarly, bush-dwelling Hadza populations (Northern Tanzania) showed gross wear at early ages, with 45-48% of the upper molars showing flat surfaces with severe/moderate exposed dentine (Crittenden *et al.*, 2017).

Our results confirmed that culture-specific food processing techniques in the Baka Pygmies foragers, in terms of mechanical and thermal food processing, contribute to the ingest generally low-abrasive and tenderer foods (Dominy *et al.*, 2008; Romero *et al.*, 2013; Zink *et al.*, 2014) that reduce dental wear rates. These results open new questions on the variability of dental wear among groups if only the abrasiveness of food items is the sole factor considered. This opens new avenues for research that not only includes tooth shape change with wear, but also food processing techniques or food availability and choice.

Questions about the discrimination efficiency of 2D proxies may also arise. For this reason, an attempt was made to applying 3D methods to the characterisation of dental wear, such as Geometric Morphometrics and dental topography.

2. Dental wear assessment using 3D techniques

2.1. Geometric Morphometrics and Dental Topography approaches

Both techniques, Geometric Morphometrics and dental topography, have been applied in Chapter 2 and 3 of the present dissertation to characterise dental wear in modern human populations and to assess their significance on discrimination tooth morphology in relation to dietary habits. On one hand, Geometric Morphometrics analyses (see Chapter 2) in dental samples have been conducted in both 2D and 3D models to investigate differences in tooth shape among individuals. Most of the GM studies previously carried out were dominated by analyses of fossils hominin teeth (2D studies: Martinón-Torres *et al.*, 2006; Gomez-Robles *et al.*, 2007; Liu *et al.*, 2010 and 3D studies: Skinner *et al.*, 2008, 2009b) and primates (2D studies: White, 2009; Nova Delgado *et al.*, 2016; Gamarra *et al.*, 2016 and 3D studies: Singleton *et al.*, 2011; Cooke, 2011; Nova Delgado *et al.*, 2015). Very few studies focused on the study of dental wear in modern human populations (e.g. Polychronis *et al.*, 2013; Polychronis and Halazonetis, 2014; Yong *et al.*, 2018) and, as far as I know, no studies have applied 3D GM methodologies to investigate dental wear in modern human populations. We have conducted 3D-GM in the Coimbra International Exchange skull collection, which is of great importance because essential data on age-at-death, sex and social structure are available for correlating with dental shape and wear.

Our PCA 3D-GM analysis on dental shape yielded a large number of PCs, from 18 to 16 depending on the molar tooth analysed, of which we only considered those explaining more than 1% of total shape variance. Many of these PCs reflected molar morphological variability, independently of wear. The molar morphology of modern human populations is greatly variable. Dental crown cusp number (3, 4, 5 or 6) and occlusal groove patterns (+, × or Y) may greatly vary in individuals of the same population, as well as between populations from different geographic origin (Scott and Turner, 1997). This variability, affecting both dental crown surface and outline areas, can be observed in upper molar teeth where the disto-lingual cusp (hypocone) may be present, reduced in size, or absent, causing a rhomboidal occlusal design or even a heart-shaped occlusal aspect when the hypocone is absent. This is most frequent in the upper M2 teeth, and even so in the M3. As for the lower molars, the Y5 cusp pattern in the lower M1 teeth, and the relative cusp size of the +4-cusp pattern in the lower M2 teeth, significantly modify molar morphology and topography. This is also the case for the presence/absence of the tubercle of Carabelli in the upper M1 teeth, a common trait among European populations, also present in some African and American Indians populations (Smitha *et al.*, 2018). Many other morphological traits may affect topographic analysis of dental shape: the presence of a cusp 5 in the upper molars, common in Australian and New Guinea, with frequencies around 60% (Scott *et al.*, 2016; Scott and Irish, 2017); the presence of a cusp 6 in the lower molars, very common in Asian and Asian-derived groups (frequency of 40-70%) (Khraisat *et al.*, 2011; Scott and Irish, 2017) and quite high in Australian and Melanesian populations (frequency of 50-70%) (Townsend *et al.*, 1990; Scott and Irish, 2017); the prevalence of cusp 7 in the lower molars of African populations, where frequencies range between 25-40% (Sakuma and Ogata, 1987; Scott and Irish, 2017); or the presence of +4 and ×4 molar crown cusp patterns in the lower first molars of Western Eurasia populations (Scott and Turner, 1997).

Despite the evident effect on molar morphology on the 3D-GM analyses, a significant number of the PCs obtained were clear associations to dental crown or cusp wear, as they showed distinct crown/cusp height loss patterns with age. Since the PCs in a PCA are uncorrelated among each other, the wear-dependent PCs could be discriminated and showed no association to dental morphology. Dental wear patterns on molar teeth can be related to various factors: food preferences, food processing techniques or cultural habits (Larsen, 1997; Kaidonis *et al.*, 2012; Górka *et al.*, 2015, 2016; Fiorenza *et al.*, 2011, 2018). Despite our methodological approach has shown to efficiently detect the loss of occlusal relief and dental crown height with age, as well as molar shape differences in cusp number and groove patterns regardless of wear, GM analyses of dental shape in modern human populations still needs to control the major sources of dental crown shape variation depending on the objective of research. GM procedure requires the definition of a set of homologous landmarks that lack of worn teeth. The use of a pseudo-landmark configuration as an alternative approach may, though, represent a different source of error that needs to be controlled for. Our repeated measurement test showed an intra-observer methodological error smaller than 5%, but the inter-observed measurement error may differ and needs to be quantified.

The topographic metric algorithms applied in Chapters 2 and 3 do not present the methodological error found in the 3D-GM analysis since it is a landmark-free approach. Dental topography metrics (DNE, OPCR, OR/RFI and PCV) have been applied to accomplish a wide range of purposes: to explore the interactions between dietary behaviour and tooth shape among non-human primates (Boyer, 2008; Bunn *et al.*, 2011; Godfrey *et al.,* 2012; Winchester *et al.,* 2014; Allen *et al.,* 2015) and fossil hominins (Berthaume *et al.*, 2018; Berthaume *et al.*, 2019a), to describe and ascribe a primate fossil to a new species (Boyer *et al.*, 2012), to give information for reconstructing dietary niches (Berthaume and Schroer, 2017), to explore the complexity of the CEJ (Skinner *et al.*, 2010), to characterise how occlusal wear and cusp height decrease with age (Evans, 2013), and to investigated the relationship between dental topography and wear in primates (M'Kirera and Ungar, 2003; Dennis *et al.*, 2004; Glowacka *et al.*, 2016; Pampush *et al.*, 2016b, 2018; Ungar *et al.*, 2018) and fossil hominins (Ungar, 2004; Berthaume *et al.*, 2018). Not all the topographic metrics are suitable for all types of analyses and it is difficult to choose which method is suitable for accomplishing a particular task. However, according to the review on dental topography made by Berthaume *et al.* (2020), the use of several topographic metrics will allow exploring all the aspects of tooth shape.

In the context of tooth wear analysis, little is yet known about the topographic variability of molar teeth in modern human populations. In Chapter 2, dental topography methods have been used to characterise age-related changes in dental crown shape using three topographic variables (DNE, OPCR and RFI). Except for OPCR of the lower M2 teeth, all analysed teeth showed a significant negative correlation with age, which indicated that molars wear, measured from surface curvature, complexity and crown relief values decrease with age.

Chapter 3 evaluated dental topography in Central African populations with distinct subsistence strategies (forager in Pygmy groups and agriculturalist in Bantu-speaking populations) and distinct degrees of dental wear patterns. Our analyses of the four topographic metrics (DNE, OR, OPCR and PCV) in the upper permanent molars showed significant differences among wear-stages and dietary specializations. However, a lack of interaction was detected between tooth-type and wear, indicating that the topographic metrics exhibited similar magnitudes at different wear stages, independently from dentin enamel exposure and tooth-type. Dental crown topographic curvature (DNE) and crown relief (OR) metrics tended to decrease with wear scores, as also shown in Chapter 2, reflecting a decrease in cusps height as wear scores increased. In contrast, molar complexity (OPCR) increased, rather than decrease, with wear stages, suggesting that cusp complexity raised as the formation of enamel rims associated with dentine exposure increased. The Ambient Occlusion (PCV) metric also showed higher values in worn that unworn teeth, reflecting that, as molars wore down, the occlusal relief of the dental crown became flatter. This result is consistent with the study carried out in fossil hominins by Berthaume *et al.* (2018).

2.2. Dental topography in hunter-gatherer and agriculturalist populations

Regarding dietary specialisations, we found that dental topographic metrics clearly discriminated between forager and agriculturalist populations. Molar topography of the forager Pygmy populations from Central African showed higher topographic scores compared to the Bantu-speaking agriculturalist populations, especially for complexity (OPCR) and curvature (DNE) variables. The forager individuals analysed had complex molar dentitions, with high and sharp cusps, often related to strong shearing forces and mechanically demanding diets including tough foods (Pampush *et al.*, 2016a; Thiery *et al.*, 2017; Berthaume and Schroer, 2017; Pineda-Munoz *et al.*, 2017; Pampush *et al.*, 2018; Berthaume *et al.*, 2018). Dental complexity (OPCR) and curvature (DNE) also effectively discriminated between foragers and agriculturalists. In primates species with distinct diets, however, while DNE has been shown to be a good dietary discrimination metric (Bunn *et al.*, 2011; Godfrey *et al.,* 2012; Ledogar *et al.*, 2013; Winchester *et al.,* 2014; Winchester, 2016; Berthaume and Schroer, 2017), OPCR has shown to be a poor indicator of diet (Guy *et al.*, 2013; Winchester *et al.,* 2014; Glowacka *et al.*, 2016; Ungar *et al.*, 2016). This also seems to be the case for fossil hominins that showed greatly overlapping OPCR values (Berthaume *et al.*, 2018), perhaps due to the low variability in dental complexity in close related species sharing bunodont molar teeth (Winchester *et al.*, 2014; Berthaume *et al.*, 2018).

Finally, our research has shown that dental topographic procedures have to cope with the dispersion of topographic metrics that may cause the use of different cropping methods used in the quantification of RFI and OR, most frequently done at the lowest point in the occlusal enamel basin. This procedure forces RFI to exclusively reflect the proportion of the enamel cap surface with respect to its projected 2D (indicative of cusp shape), instead of reflecting total crown shape. Despite this cropping point is considered a fixed point in unworn teeth, it should in fact be considered a type II or even III, moving landmark, whose position may greatly vary depending on which fovea or valley is more profound on the occlusal dental crown surface. Perhaps, a more homologous cropping point may be represented by the cemento-enamel junction (CEJ) that takes into account the whole dental crown for cropping the tooth. Topographic metrics of teeth measured using these two cropping methods cannot be directly compared since they may produce significantly different dental topographic measures (Winchester, 2016; Berthaume *et al.*, 2018; Berthaume *et al.*, 2020). While in Chapter 2 (analysis of the correlation of age and topographic metrics in the Coimbra known-age sample) the dental crown CEJ cropping was used to quantify the topographic metrics OPCR, RFI and DNE, in Chapter 3 (analysis of forager and agriculturalist African populations) the lowest-point cap cropping method was applied to quantify OR, RFI and DNE.

A major concern with the lowest-point cap cropping method is that molars can have the deepest basis surfaces at various positions depending both on occlusal morphology and on inclination of mesial or distal cervical margins, which may cause variations in the enamel cropped on different tooth surfaces or the inclusion of tooth roots in the cropped portion (Boyer, 2008). The CEJ cropping method also shows some methodological concerns, the most significant being the definition of the crow-root junction and a cropping plane intersecting the whole CEJ. Despite previous studies have found this method to be insensitive to observer error for topographic metrics (Boyer, 2008; Bunn *et al.*, 2011), defining the CEJ is certainly affected by observer's experience, visualization of the entire CEJ line and the method used to define a plane intersecting it. The visual assessment of the CEJ in virtual models is likely to be affected by inter-observer error that may affect measurements of 3D surface area, projected 2D surface area, and RFI, and perhaps to a lesser extent DNE and OPCR metrics.

CONCLUSIONS

1. As expected, a strong linear association of 70 % between chronological age and wear stages derived from PDE was observed in the lower permanent first molars of the Baka Pygmies forager population from southern Cameroon (Central Africa).
2. No differences between men and women in molar wear patterning, suggesting similar sex-related diets and equal enamel loss processes and bite dynamics during individual growth.
3. The Baka Pygmies showed maximum dentine exposure values not greater than 5% in older ≈30 aged individuals, with lower tooth wear rates than expected for African foragers relying on USO-based diets.
4. The African foragers may have culture-specific dietary tendencies that affect fracture toughness and grittiness of mechanically challenging foods, reducing enamel abrasiveness of ingested materials and reducing dentine exposure rates.
5. Three-dimensional geometric morphometrics (3D-GM) applied to first and second permanent maxillary (UM1 and UM2) and mandibular (LM1 and LM2) molars of the Coimbra International Exchange skull collection showed significant regressions between the morphometric variables and age-at-death.
6. A significant portion of the overall dental shape variation was attributed to the loss of occlusal dental relief and dental crown/cusp height with age. The other portion of total variance reflected morphological variability in dental cusp and groove patterns, independent of dental wear.
7. The repeated measurement test showed an intra-observer error for the 3D pseudo-landmark proxy of less than 5%.
8. This pseudo-landmark configuration method proved to be reliable for characterising dental topography when homologous landmarks were lacking due to dental wear.
9. The dental topographic analysis conducted in the Coimbra known-age reference collection showed significant negative correlations between molar topography and age for all the analysed teeth, indicative that molar surface curvature, complexity and crown relief values decrease with age.
10. The dental topography methods applied to the Central African populations with distinct subsistence strategies and dental wear patterns showed no interactions between tooth-type and wear, signifying that the topographic metrics exhibited similar magnitudes at different wear stages, independently of tooth type and dentine exposure.

11. The dental crown topography of the Central African populations studied showed a tendency of curvature (DNE) and crown relief (OR) values to decrease with wear.
12. The Ambient Occlusion (PCV) showed high values in worn teeth, reflecting that the occlusal relief of the dental crown became flatter as molars wore away. Molar complexity (OPCR) also showed increased values with wear, likely due to the formation of enamel rims associated with the increase of dentine exposure areas.
13. The Central African Pygmy foragers analysed had complex molar dentitions, with high and sharp cusps, since their molar topography exhibited higher dental complexity (OPCR) and curvature (DNE) compared to the Bantu-speaking agriculturalists, showing to effectively discriminate between forager and agriculturalist populations.
14. From a methodological perspective, the topographic methods for the characterising dental crown morphology need to pay attention to the cropping method used for the quantification of RFI and OR.
15. Measuring OR by cropping the dental crown at the lowest point on the occlusal enamel basin is affected by the highly variable positions of this landmark depending on both the degree of occlusal wear and the inclination of the mesial or distal cervical margins.
16. Cropping the dental crown at the cemento-enamel junction (CEJ) to quantify RFI may significantly be affected by the error caused by the observer's assessment of the CEJ to define a plane intersecting it.

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