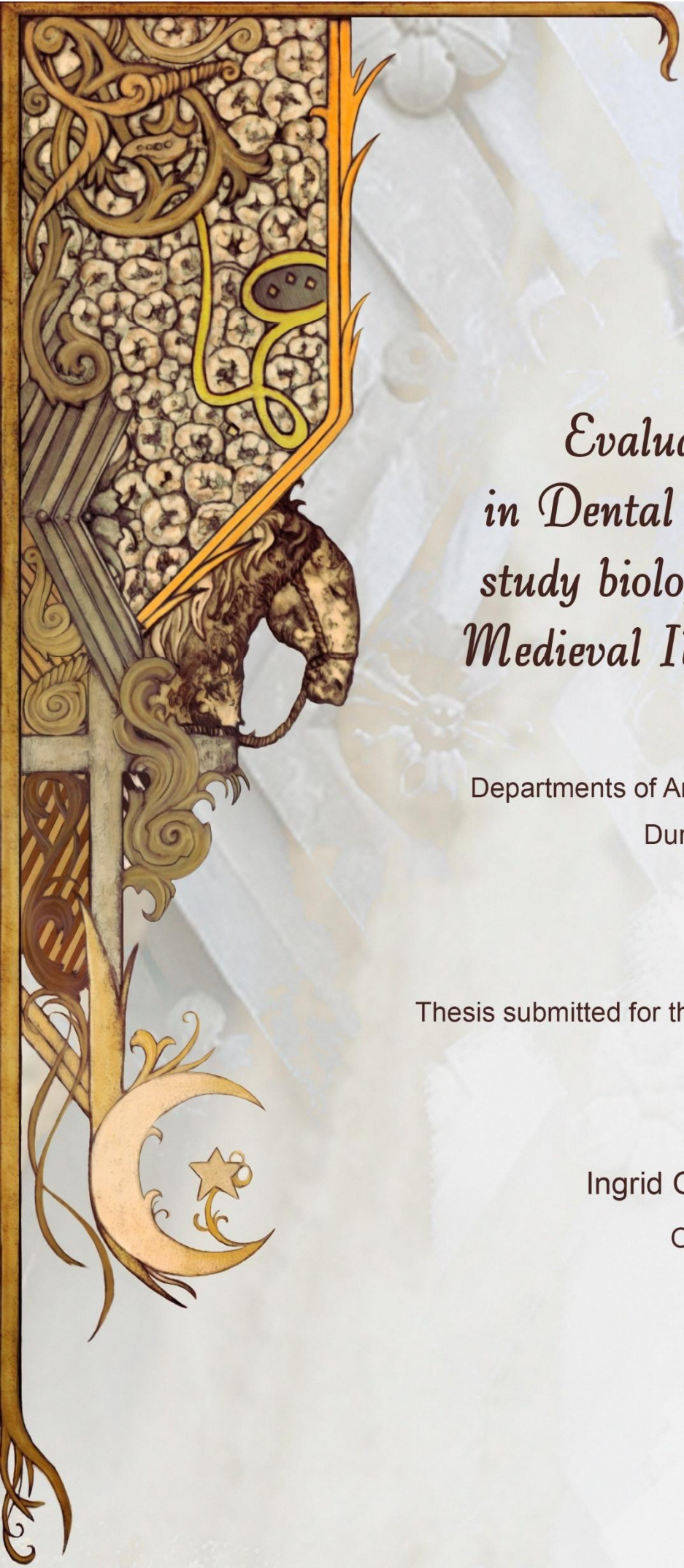
Evaluating methods in Dental Anthropology to study biological affinities in Medieval Iberian Populations

GRUESO-DOMINGUEZ, INGRID



Evaluating methods in Dental Anthropology to study biological affinities in Medieval Iberian Populations

Departments of Anthropology and Archaeology
Durham University

Thesis submitted for the degree of Doctor of Philosophy

Ingrid Grueso Dominguez

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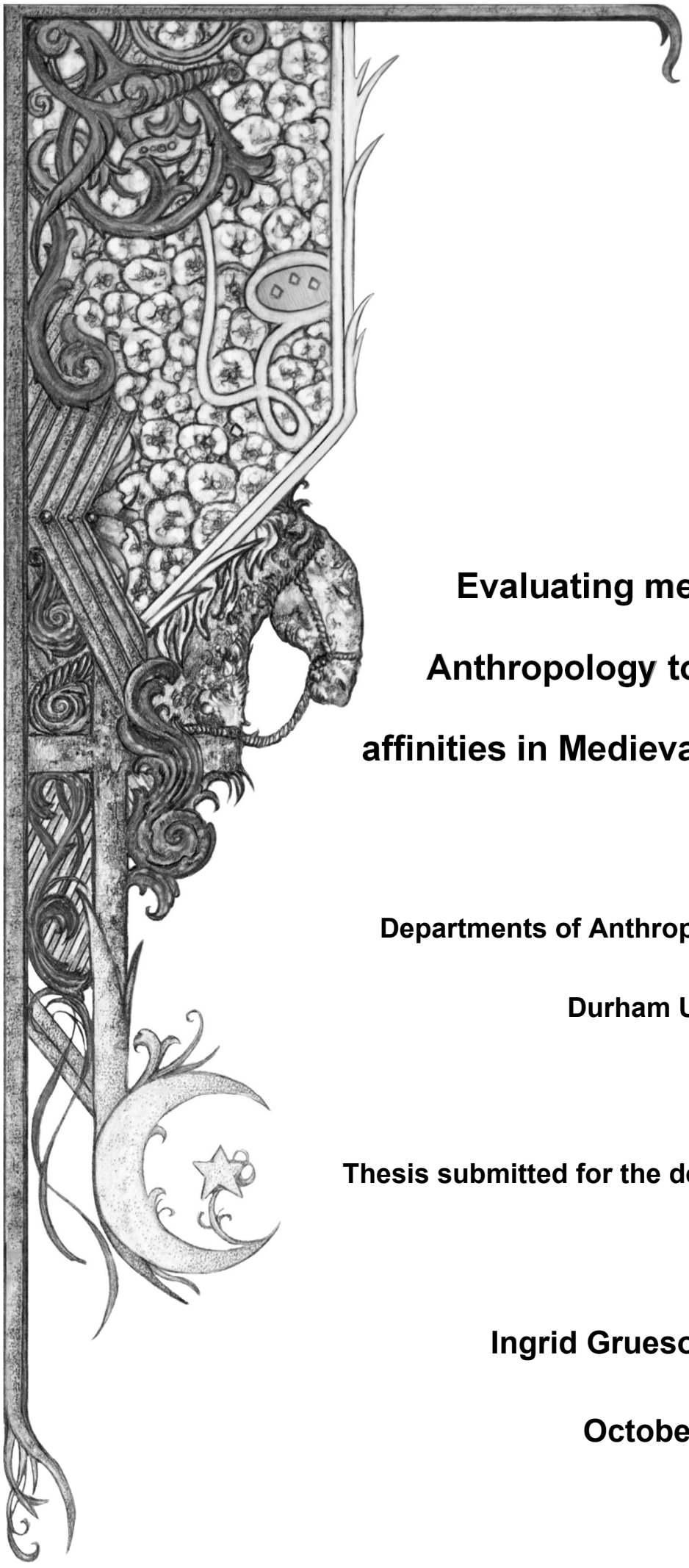
Dental anthropologists have used dental morphology and measurements to infer biological relatedness among species, populations, and sometimes even family members, for nearly a century. The rapid development of new technologies in the last 15 years has resulted in sophisticated methods. These new methods have allowed us to observe teeth and dental structures which we could not study without destroying other human remains or that we could not access because they were kept at museums overseas. While many researchers have embraced the new methods, few studies have examined what they bring to dental anthropology, whether they improve on traditional methods, and when it is best to use them. I compared four methods to estimate biological relatedness in terms of the information they yield, their reliability, and the ease of application: metric variables, nonmetric dental traits included in the Arizona State University system, and 2-Dimensional and 3-Dimensional geometric morphometrics. To make this comparison, I used three archaeological medieval samples from different cultural contexts (one Christian and two Muslim) in the Iberian Peninsula. I aimed to infer whether there are biological differences among the samples and if so, which samples are biologically closer to one another. I chose these samples because traditionally, historians have thought that the Muslim entry to the Iberian Peninsula occurred in very low numbers and that the vast majority of Muslims that lived in the Iberian Peninsula during the Middle Ages were local people converted to Islam, so I tested if this was observed when comparing samples with different cultural backgrounds. Of the four methods, 2-Dimensional geometric morphometrics and nonmetric dental traits found the most significant differences between populations. These significant differences were between the Christian and the Muslim samples, although one of the Muslim samples, whose cultural background was Christian, was geographically closer to the Christian sample. The fact that there are significant differences between the Muslim and the Christian samples suggests that the Islamic arrival to the Iberian Peninsula was made in larger numbers than it was thought, even if there was an admixture with the local population. I also found that 3-Dimensional geometric morphometrics was difficult to apply, more time-consuming, and provided results that were not consistent with the results obtained with the other methods. The results of 3-Dimensional geometric morphometrics could have improved if used in the enamel-dentine junction of CT-scans of the teeth, but that requires a great deal of money, time, and skills. Researchers aiming to study the biological relatedness of archaeological samples should choose their methods taking into account the samples used, their degree of expertise, and their availability of resources if they want to avoid results that do not necessarily reflect such relatedness.



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List of Abbreviations

ANOVA – Analysis of Variance

ASU System – Arizona State University Dental Anthropology System

BL – Buccolingual

CA – Crown area

CI – Crown index

CVA – Canonical Variates Analysis

DFA – Discriminant Function Analysis

EDJ – Enamel-dentine junction

GMM – Geometric Morphometrics

GPA – General Procrustes Analysis

LM1 – First lower molar

LM2 – Second lower molar

MD – Mesiodistal

Micro-CT - Microtomography or micro-computed tomography

MMD – Smith's Mean Measure of Divergence

MS - Mean sum of squares

PC – Principal component

PCA – Principal Component Analysis

SMA – Santa María la Real – Christian sample in Christian territory

SNA – San Nicolás de Ávila – Muslim sample in Christian territory

SS – Sum of squares

UCI – Upper central incisor

ULI – Upper lateral incisor

UM1 – First upper molar

UM2 – Second upper molar

UM3 – Third upper molar

XAR – Xarea – Muslim sample in Muslim territory

✞ - Christian sample

☪ - Muslim sample

Statement of Copyright

Acknowledgements

When it takes as long as 10 years to complete a PhD, you certainly meet many people along the way, many of whom help you in one way or another.

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To my parents, because they made me who I am. To my husband, because he has always cherished me for how I am. And to my children, because since I set eyes on them, I have always wanted to improve.

“I used to think that they were things the wonderful folk of the stories went out and looked for, because they wanted them, because they were exciting and life was a bit dull, a kind of a sport, as you might say. But that's not the way of it with the tales that really mattered, or the ones that stay in the mind. Folk seem to have been just landed in them, usually – their paths were laid that way, as you put it. But I expect they had lots of chances, like us, of turning back, only they didn't. And if they had, we shouldn't know, because they'd have been forgotten”

J.R.R. Tolkien, The Two Towers

1. Introduction

Dental Anthropology is a subfield of Biological Anthropology that focuses on studying the morphology, development, pathology, and other characteristics of hominin dentition (Scott and Turner II, 1997). Teeth are useful sources of information in archaeological and palaeontological human remains for four main reasons: they are usually well preserved, easy to observe, highly variable, and their variation is heritable to a certain extent (Scott, 2008). The hard layer of enamel that covers the crown allows teeth to be preserved in the fossil and archaeological records, even when the taphonomic conditions mean that no other bone remains (Scott and Turner, 1997; White and Folkens, 2005; Scott, 2008; Irish et al., 2013). The observability of teeth allows dental anthropologists to study them without causing any damage to the tissue and to observe them in live individuals (Scott and Turner II, 1997; White and Folkens, 2005; Scott, 2008). Because tooth morphology and size vary across and within hominins and in human populations, they can be used to discriminate species and populations (Scott and Turner II, 1997; White and Folkens, 2005; Scott, 2008). Finally, tooth morphology and development are strongly linked to genes. Because they are formed inside the alveoli, their structure is very stable, even if teeth suffer attrition, abrasion and demineralisation (Scott and Turner II, 1997; Ramírez Rozzi, 2002; White and Folkens, 2005; Scott, 2008; Irish et al., 2013)

My research focuses on human teeth and it has two principal aims:

1. To evaluate which of four methods is best to estimate biological relatedness between skeletal samples in terms of the information they yield, their reliability and the ease of application
2. To examine whether there are observable biological differences between three medieval skeletal samples excavated from different religious contexts within the Iberian Peninsula

The first question is exploratory and is based on methodology, because methods to compare populations using teeth vary from relatively simple measurements to the use of complicated and usually expensive technology. It is important to explore which methods provide more informative results with fewer resources, and under what circumstances it a method provides adequate information. This will help future researchers in dental anthropology.

The second question explores whether there are biological differences between Muslim and Christian samples that lived in different cultural contexts in the Iberian Peninsula. To address this question, I chose a Christian sample from a Christian territory (Santa María la Real - SMA), a Muslim sample from a Christian territory (San Nicolás de Ávila - SNA), and a Muslim sample from a Muslim territory (Xarea - XAR). This question is based on two competing hypotheses:

a) Biological continuity: the Muslim migrants made very little biological contribution to the population during 800 years of cohabitation. According to this hypothesis, sustained by traditional historians during the 20th century, the Muslim entry from Syria, and the North and West of Africa, to the Iberian Peninsula was in low numbers, and the vast majority of Muslims that lived in the Iberian Peninsula during the Middle Ages were local people converted to Islam (Sánchez-Albornoz, 1977, as cited in García Sanjuán, 2017; Arié, 1989).
b) Migration: this hypothesis supports that the Muslim arrival happened in higher numbers and in a similar way to population migration, leading to an admixture of locals and migrants through years of coexistence (Bosch et al., 2001; Adams et al., 2008).

If the biological continuity hypothesis is correct, I predict either no significant differences between my samples, or differences only between XAR (☪) and the other two samples, based on their geographical distance. In contrast, if the migration hypothesis is

correct, I predict differences between SMA (✞) and the other two samples, and that the distance between SMA (✞) and SNA (☪) will be smaller than the distance between SMA (✞) and XAR (☪).

To provide background, this chapter reviews the political and social context of the Middle Ages in the Iberian Peninsula. Then, Chapter 2 reviews dental anatomy and morphology, including information about discriminating populations using teeth measurements and morphology. Chapter 3 provides information on the archaeological samples and dental casts and Chapter 4 reviews the methods I used. The results are split into four chapters, one for each of the methods used: metric variables, nonmetric traits through the ASU (Arizona State University) system, 2D GMM (Geometrics Morphometrics) and 3D GMM. The Discussion and Conclusions follow.

Although I have tried to avoid it, in some parts of this thesis I use words traditionally used in History books to explain Spanish history. These words include “conquest”, “invasion”, “reconquest”, and “*Reconquista*”. I believe, however, that this sort of vocabulary, even if convenient, is sometimes West-centred and Christian-centred and not necessarily accurate in describing what happened. Muslims arrived in the Iberian Peninsula like many other peoples had done before, but not all these arrivals are termed an “invasion”. Muslims made the peninsula their home for many centuries, and that undeniably changed the Iberian Peninsula linguistically, architecturally, and culturally.

1.1. The Middle Ages in the Iberian Peninsula

The Middle Ages in the Iberian Peninsula began with the Arab Conquest in 711 and ended in 1492, with the end of the Christian *Reconquista,* the name given to the warlike effort to regain control of the Iberian Peninsula. During those centuries, Muslim peoples of

different backgrounds and from various territories from the Mediterranean Littoral, including the North of Africa and the Middle East (Berber, Syrian, Qays, Yemeni, *Marīni, Ŷundi, etc.)*, arrived in the Iberian Peninsula in different waves. In 710, a large part of the Iberian Peninsula was controlled by the Visigothic Christian king Rodrigo, when a troop of roughly 400 Berber soldiers (originally from the North of Africa, what it is today Morocco to Libya) commanded by *Ŷazīrat Tarīf* successfully penetrated it. This easy arrival encouraged *Mūsā bin Nuṣayr*, the governor of *Ifrīqiya*, a territory that is now part of Tunisia, to enter the peninsula when he took the North of Morocco. He sent a freed Berber, *Tāriq b. Ziyād*, with over 7,000 Berber and Syrian soldiers, to Gibraltar, where another 5,000 Berber soldiers soon joined them. In 712, *Mūsā bin Nuṣayr* entered the Peninsula with 18,000 Arab, Qays (originally from Al Aridhah, in today's Saudi Arabia) and Yemeni soldiers. By the end of 713, *Mūsā* and *Tāriq* had conquered all the territory up to the Ebro Valley, to the northeast of the Iberian Peninsula (Figure 1.1) (Arié, 1989).

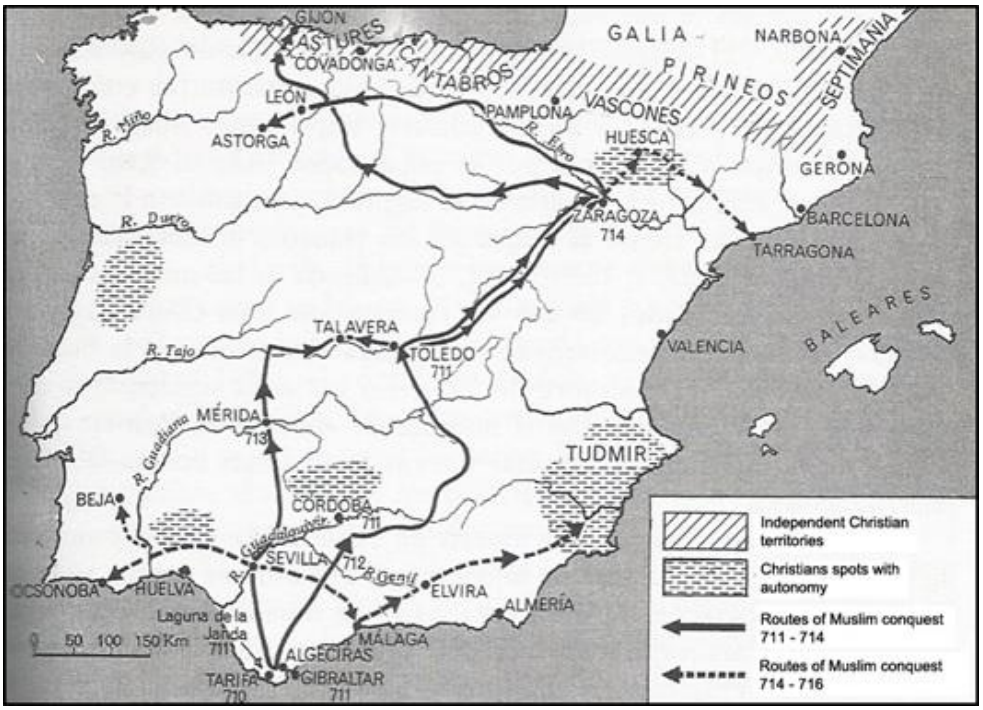


Figure 1.1 Map of the entry routes of Muslims to the Iberian Peninsula from 711 to 716 A.D. (sourced from Arié, R., 1989, p.15)

Within a few years, the Muslims spread through most of the Iberian Peninsula and reached Gaul, in the south of France, where they were defeated for the first time in the Battle of Poitiers in 734. The occupied territory was called *Al-Andalus* and was ruled by a succession of governors designated by Damascus. The Visigoth nobles retreated to the Kingdom of Asturias, a region in the north of the Iberian Peninsula, from where they initiated the *Reconquista* (Arié, 1989).

The Muslim Conquest was not always warlike; many territories were peacefully transferred from the Visigoths to the Muslims through pacts that gave the former owner rights to work the land in exchange for a tax for the new governors, sometimes adding a requirement of faith conversion to Islam for the former owner of the land (Acién Almansa, 2009). Most of the time, this conversion was not mandatory, and Jews and Christians were allowed to keep their faith. However, since non-Muslims were not citizens with full rights, many converted to Islam to avoid higher taxes (Arié, 1989). During the eighth century, more Muslims arrived in the Iberian Peninsula, many of whom were *Ŷundis* (originally from the Levant or *Bilad al-Sham*, which today is part of Israel, Palestine, Lebanon, Jordan and Syria). The many different tribal origins of the Muslims resulted in confrontations, with the Syrians being against the Berbers and the Qays against the Kalbis (the Kalbis were originally from central North Africa) (Arié, 1989).

In 750, the ruling family of the Arab Empire in Syria, the Omeyas, were slaughtered after political conflicts in the capital, Damascus. The late Caliph's grandson managed to escape and fled with some supporters to *Al-Andalus*, where he arrived in 755. Soon, he allied with the *Ŷundis*, Yemenis and Berbers and fought the Qays in Seville, and he was proclaimed Emir of Cordova (Figure 1.2) (Arié, 1989). During his first six years as Emir, *Abd al-Rahmān ibn Mu'āwiya* pacified *Al-Andalus,* and a new wave of Muslim settlers arrived in the Iberian Peninsula from Syria. Berbers and enslaved people from across the world went to the peninsula to be part of his army (Arié, 1989). His successors continued to pacify their

territory, fighting the Christians at the borders annually during the summer military campaigns (Arié, 1989). The people of Cordova, with a Christian background, although mostly converted to Islam, did not always accept Arab rules, keeping their currency, language and the Roman calendar (Larochelle, 2007). These cultural differences caused several waves of civil uprisings, which ended in 912 when *'Abd al-Rahmān III* gained control of the territory, becoming Caliph of Cordova in 929 (Arié, 1989).

Figure 1.2 Map of the Iberian Peninsula at the time of the Emirate of Cordova, circa 780 A.D. (sourced from Arié, R., 1989, p.22)

After 929, the *jihad*, understood as the war against other faiths, became more important than it had been until then. The Caliph became the head of the religious community (Arié, 1989; Robledo, 1998; Cabellos, 2007). To prove their faith in Allah, Muslims became less tolerant of Christians, and fights between both communities became more frequent and violent. After the siege of Barcelona in 985, the Christian kingdoms of *León, Castilla, Navarra*, and *Pamplona* sued for peace and became vassals of the Caliphate (Figure 1.3) (Arié, 1989; Castro Hernández, 2012).

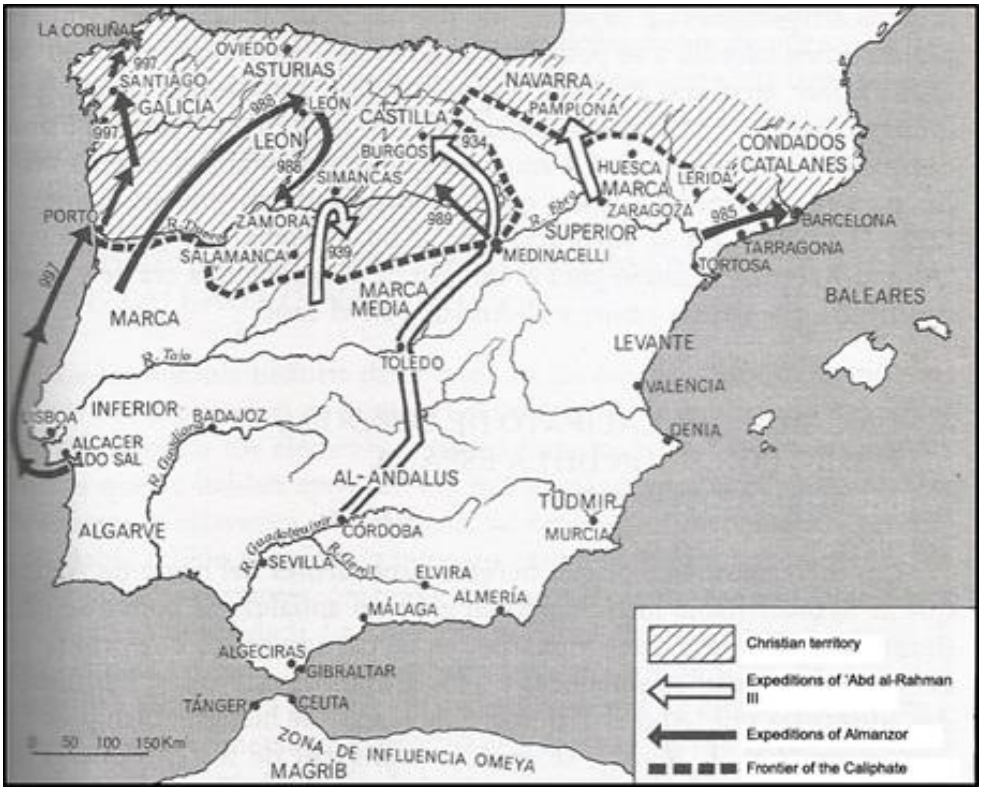


Figure 1.3 Map of the Iberian Peninsula at the time of the Caliphate of Cordova, circa 929 A.D. (sourced from Arié, R., 1989, p.25)

In 1008, a civil war between the Muslims sank the Caliphate into chaos, with civil uprisings across the territory. This led to the execution of the then Caliph and his steward. In 1031, the noble families cancelled the Caliphate, and a Board of Noblemen started to rule the territory, leading to the *Taifas* kingdoms, small states divided according to their tribal backgrounds which competed with each other (Figure 1.4) (Arié, 1989). The tribes involved in the *Taifas* kingdoms were: *Ŷahwari, 'Abbadi* (Yemeni), *Hūdi* (Arab), *Banū Sumādih* (Yemeni), *Berber, Aftasi, Birzali* (*Zenat*), *Hammūdi*, and *Zīri* (*Sanhāŷi*). To support the wars among them, each *Taifas* kingdom paid the neighbouring Christian kingdoms a tribute for help, giving the Christians an opportunity to retake some territory. However, once the Muslim noblemen realised this, they requested assistance from the sultan of the North

of Africa. He arrived in Algeciras, in the south of the peninsula, in 1090, bringing together the *Taifas* (Arié, 1989).

Figure 1.4 Map of the Iberian Peninsula at the time of the first period of *Taifas* kingdoms, circa 1031 A.D. (sourced from Arié, R., 1989, p.29)

This unity did not last, however, and in 1143 the *Taifas* kingdoms were fighting again (Figure 1.5). The Christian kingdoms took advantage of the situation again, gaining territory. In 1227 *Al-Andalus* was hit by a devastating drought and left in poor economic conditions. This weakness allowed a nobleman, *Muhammad b. Yūsuf b. Naṣr*, to start a rebellion against his peers and be proclaimed a Sultan of the remaining territory of *Al-Andalus* (Arié, 1989).

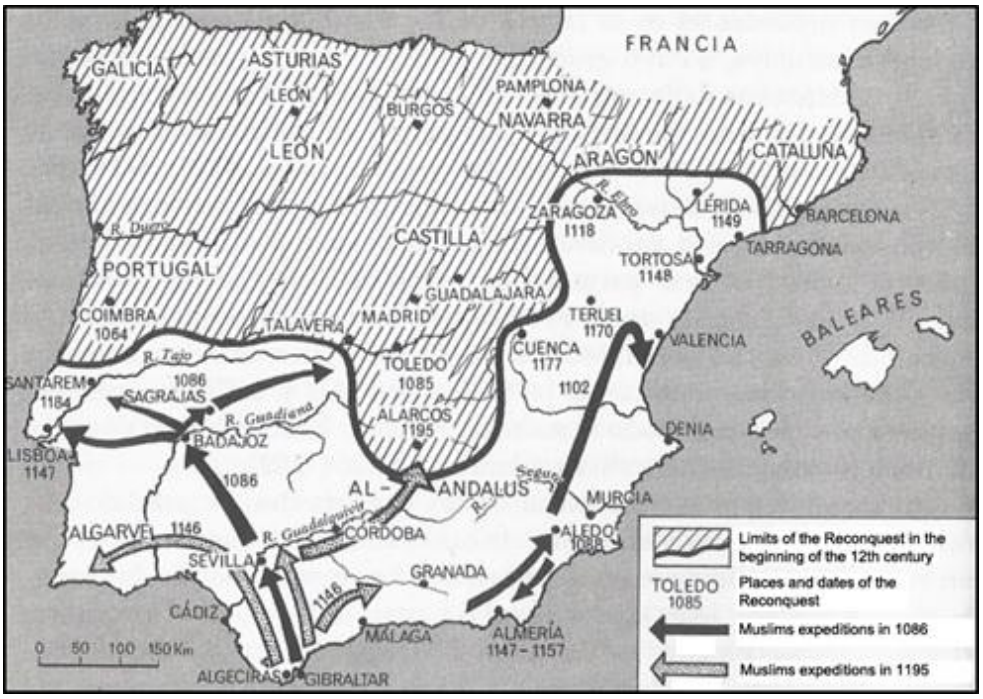


Figure 1.5 Map of the Iberian Peninsula at the time of the second period of Taifas kingdoms, circa 1143 A.D. (sourced from Arié, R., 1989, p.34)

Muhammad b. Yūsuf b. Naṣr managed to become Sultan after being helped by the Christian King *Fernando III*. *Nasr* took the city of Granada and established the capital of the *Nasri* kingdom there. The *Reconquista* kept gaining territory for the Christians, and *Nasr* became a vassal of *Fernando III* (Arié, 1989). After *Nasr's* death, his son, *Muhammad II*, took control of the kingdom and asked for help from the *Marīni* tribe of Morocco to fight the Christians. The *Marīni* entered the peninsula but took the remaining territory of *Al-Andalus* for themselves. When *Muhammad II* realised this, he allied with the Christians to control the *Marīni.* Fights between Christians and Muslims continued, and in 1333 more reinforcements were sent from Morocco to support the *Marīni.* In 1469 the Christian kingdoms managed to unite through the marriage of the king of *Aragón* and the queen of *Castilla*, forming what would become the Kingdom of Spain in 1479. The King and Queen started a very aggressive war against the remaining *Al-Andalus*, the Kingdom of Granada. In 1492 the Muslim king, *'Abū 'Abd Allāh Muhammad*, surrendered the city of Granada to the Queen and King, which ended the *Reconquista*. For years, Muslims were allowed to keep their

horses and arms, faith, mosques, judges and traditions. However, later, Muslims were expelled from the Iberian Peninsula, and the territory was re-populated by Christians from the north of Spain (Arié, 1989).

Figure 1.6 Map of the Iberian Peninsula at the time of the *Nasri* kingdom, circa 1238 A.D. (sourced from Arié, R., 1989, p.38)

1.2. Biological relatedness in the Iberian Peninsula

Traditionally, and especially during the 20th century, historians' views of the Middle Ages in the Iberian Peninsula did not consider Muslims as full-fledged inhabitants. Instead, they were seen as "invaders" that arrived in the Peninsula in limited numbers and without their families, did not mingle with the people already living there, and were "expelled" after years of fights, abandoning the Peninsula again (Sánchez-Albornoz, 1977, as cited in

García Sanjuán, 2017; Arié, 1989). This view also implies that the Muslim's success in their conquest was due to the conversion of the local population rather than the movement of people into the Peninsula (Sánchez-Albornoz, 1977, as cited in García Sanjuán, 2017).

Few researchers have attempted to explore the question of biological continuity in the Iberian Peninsula using archaeological remains, and the available results are contradictory. Cabellos (2007) used nonmetric dental traits to analyse differences among an extensive series of archaeological samples, comprising chronologies from the 2nd century (Romans) to the 17th century, from across the Iberian Peninsula. Her study has some limitations, such as small sample sizes (as few as eight individuals for one of the samples, with the largest sample being 150 individuals), with very few teeth preserved in some cases. There were significant differences among almost all of the samples, but the interpretation of these results was problematic because these differences did not seem to be linked to geographic, chronological or cultural differences (Cabellos, 2007). The only significant difference that could be interpreted was between a late medieval and a Roman population. The Muslim samples were more distant from one another than from the Christian and Roman samples. These results seem to support that there is biological continuity in the population of the Iberian Peninsula, and that there is little biological contribution from the Muslim populations.

Prevedorou et al. (2010) conducted an isotopic analysis on two individuals from the Iberian Peninsula, dated to the 8th century and buried in a *maqbara* with Islamic rituals. The researchers chose these two individuals as they showed intentional dental modifications, which have never been found in Christian individuals from the Iberian Peninsula. The isotopic analysis confirmed that these two individuals had spent part of their childhood away from where they were buried, probably in the north of Africa. Interestingly, these individuals were male and female, supporting the hypothesis that the Muslim arrival to the Peninsula was similar to population migration, with families and traders following the armies. This would go against the traditional view, in which the Muslim arrival was mainly formed by

soldiers. However, throughout history there have been other examples of families settling in with soldiers, as shown in the Roman fort of Vindolanda in England (Greene, 2013).

A genetic study of an early medieval *maqbara* from Pamplona, in the North of Spain, revealed many individuals with North African origins, with approximately a third of individuals being female, and up to two-thirds male (Fontecha, 2013, as cited in De Miguel Ibáñez, 2020). This study also showed that some individuals with dental modifications did not have an African origin, with both of their parents being inhabitants of the Iberian Peninsula. According to Fontecha 2013 (as cited in De Miguel Ibáñez, 2020), this suggests that early medieval population were formed of both migrants and local people. This again supports the hypothesis of population migration.

Genetic studies of modern populations have found traces of a North African lineage in Iberian populations, compatible with the arrival of North Africans in the 8th century, which supports the hypothesis of a migration that left a noticeable, although small, demographic contribution (Bosch et al., 2001; Adams et al., 2008). This lineage is seen better in the western Iberian Peninsula than in the eastern, including west Andalusia, Extremadura, Portugal, Leon, Northwest Castile, and Galicia (Adams et al., 2008). More recent and thorough research on the genetics of the modern population identifies fractions of north-west African ancestry, which can represent up to 11% of the lineage, and supports an admixture of Europeans and North Africans in the 9th to 12th centuries (Bycroft et al., 2019). This also supports the hypothesis that a large group of people migrated from the North of Africa to the Iberian Peninsula, especially during the first centuries of the Muslim arrival, followed by admixture of these migrant populations with the local inhabitants.

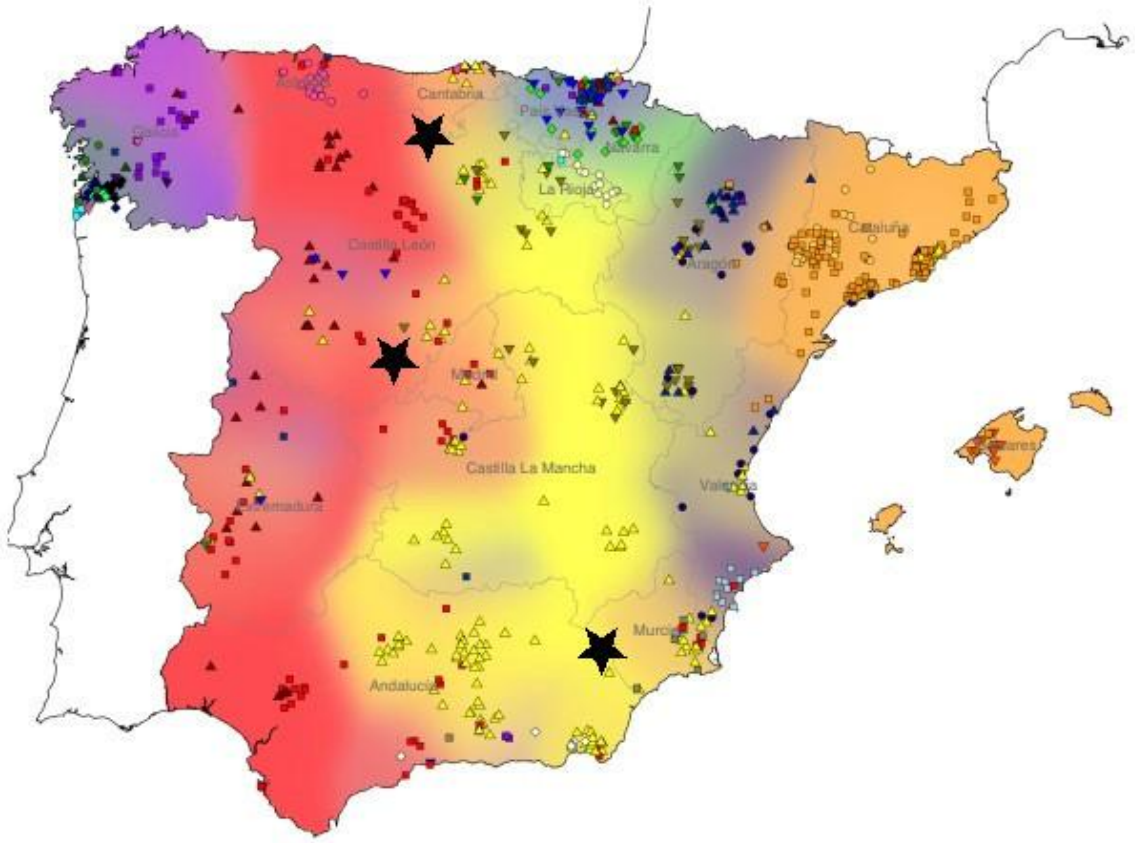


Figure 1.7 Map of the Iberian Peninsula with the clusters inferred in Bycroft et al. 2019, and the background coloured in accordance with the spatial densities of the clusters (see Bycroft et al., 2019, for the Methods). I added the black stars to mark the location of the samples I studied.

In the latter genetic study of the modern Iberian population (Bycroft et al., 2019), genetic differences are spread along the West-East axis of the Peninsula, with the North-South axis showing fewer differences among individuals (Figure 1.7). In Figure 1.7, the colours of the background indicate the spatial densities of clusters of populations that are genetically close to each other, therefore, the same colour indicate that clusters of genetically close populations are spread through that area. The authors related the North-South pattern to the expulsion of the Muslims in later centuries, during and after the *Reconquista*, when Christian migrants from the North moved towards the South after the North Christian kingdoms gained the territory. It was common practice during this period to repopulate the expanded border territory with people from the Christian areas, as seen in other border towns in Portugal (MacRoberts et al., 2020).

The samples I analyse in this study date from before the end of the *Reconquista*, so the territories had not yet been repopulated by Christians. Therefore, instead of predicting the West-East pattern that follows the modern population distribution, I predict differences between the North and the South of the Peninsula (hence my prediction that there will be differences between SMA (✞) and XAR (☪)). If SNA (☪) is biologically closer to XAR (☪) than to SMA (✞), then the results will not support the hypothesis of biological continuity, as both Muslim samples would differ from the Christians, indicating a different ancestry which could be due to an influx of migrant population.

2. Teeth, and the information they can provide

2.1. Dental anatomy

During our lives, we have two sets of teeth: the deciduous and the permanent dentitions. The deciduous dentition starts developing in the sixth week of intrauterine life. It consists of 20 teeth, which erupt through the gum into the mouth sequentially, starting in the sixth month after birth. The permanent dentition comprises 32 teeth and begins to develop around the fourth month after birth, erupting sequentially between the sixth and the 17th years, although the third molar is highly variable and it may erupt much later or even not erupt at all (Kraus et al., 1969; Ubelaker, 1978; Ash and Nelson, 2004; Al Qahtani et al., 2010).

Both deciduous and permanent teeth have two parts: the crown and the root (Scott, 2008). In general, the crown remains over the gum and the root inside the pocket in the bone where tooth development begins, the alveolus. The boundary between the root and the crown is called the cementoenamel junction (Ash and Nelson, 2004).

A tooth is formed of four tissues: enamel, cementum, dentine, and pulp (Scott, 2008) (Figure 2.1). The enamel is the external layer of the crown. It is very hard, formed by calcified prisms of hydroxyapatite produced by cells called ameloblasts (Kraus et al. 1969). These prisms zigzag from the most internal part of the enamel in the enamel-dentine junction towards the outer surface of the enamel, producing three different structures: Hunter-Schreger bands, cross striations and Retzius striae (Figure 2.2). Hunter-Schreger bands occur because of the difference between the layouts of the groups of prisms, whereas the cross striations are observed inside the prisms as perpendicular lines. The Retzius striae are microscopic grooves crossing the surface of the enamel prisms, and in the outer layer of enamel, they form convexities called perikymata (Ramírez Rozzi, 2002).

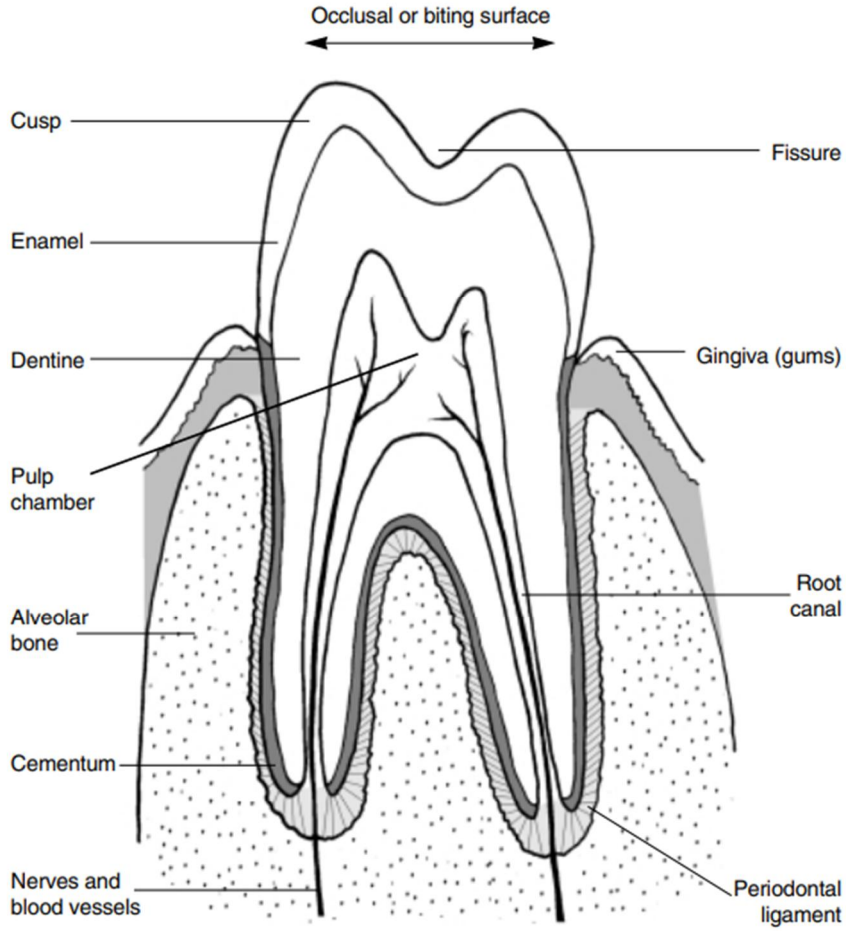


Figure 2.1 Dental anatomy. Cross-section of a lower molar tooth (sourced from Rowson, J., and Slaney, A., 1996, pp.20)

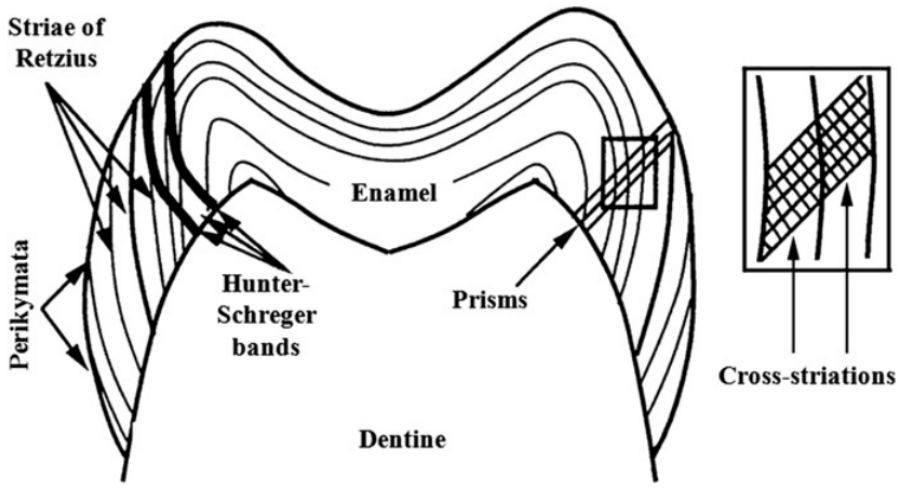


Figure 2.2 Cross-section of the enamel layer of a human crown, illustrating the Hunter-Schreger bands, cross striations and Retzius striae (sourced from Bromage et al., 2012, adapted from Ramírez Rozzi (1999)).

The cementum is a hard tissue, although not as hard as the enamel. It covers the root and fixes it to the alveolus in addition to the periodontal ligaments. The thinnest part of the cementum is the cervicoenamel junction (Rowson and Slaney, 1996; Rodríguez Cuenca, 2003; White and Folkens, 2005; Scott, 2008). The layer under the enamel and the cementum is the dentine, which covers the tooth's pulp cavity. It is formed by cells called odontoblasts, and it is not directly innervated by blood vessels (Rowson and Slaney, 1996; White and Folkens, 2005).

The only soft tissue of the tooth is in the pulp cavity, in which there is blood, lymphatic vessels and nerves. It is wider inside the crown and narrower in the roots, shaping the root canal and opening to the alveolar bone in the apex through the apical foramen (Rodríguez Cuenca, 2003; White and Folkens, 2005).

2.2. Dental morphology

Different tooth types (i.e., incisors, canines, premolars and molars) have different morphology, including crown shape and number of roots. In the deciduous dentition, the dental formula (for a single quadrant) includes two incisors, one canine and two molars. In contrast, in the permanent dentition, there are two incisors, one canine, two premolars and three molars.

A tooth has five faces, which we use to describe where cusps, ridges, grooves and cones are located (Figure 2.3). The surface facing the walls of the mouth is considered labial for incisors and canines and buccal for premolars and molars. The lingual surface faces the tongue, whereas mesial and distal faces are between the teeth, facing respectively the middle part of the mouth and the far extreme of the maxillae. The occlusal surface faces the opposite teeth, forming the biting or chewing surface (Rowson and

Slaney, 1996; Scott and Turner, 1997; Rodríguez Cuenca, 2003; White and Folkens, 2005; Scott, 2008).

The crowns are formed by cusps, which have lobes and ridges (Figure 2.4). The roots have cones that are separated by inter-radicular projections (Figure 2.5). When there are only developmental grooves instead of inter-radicular projections the cones are called radicals (Scott and Turner, 1997; White and Folkens, 2005; Scott, 2008).

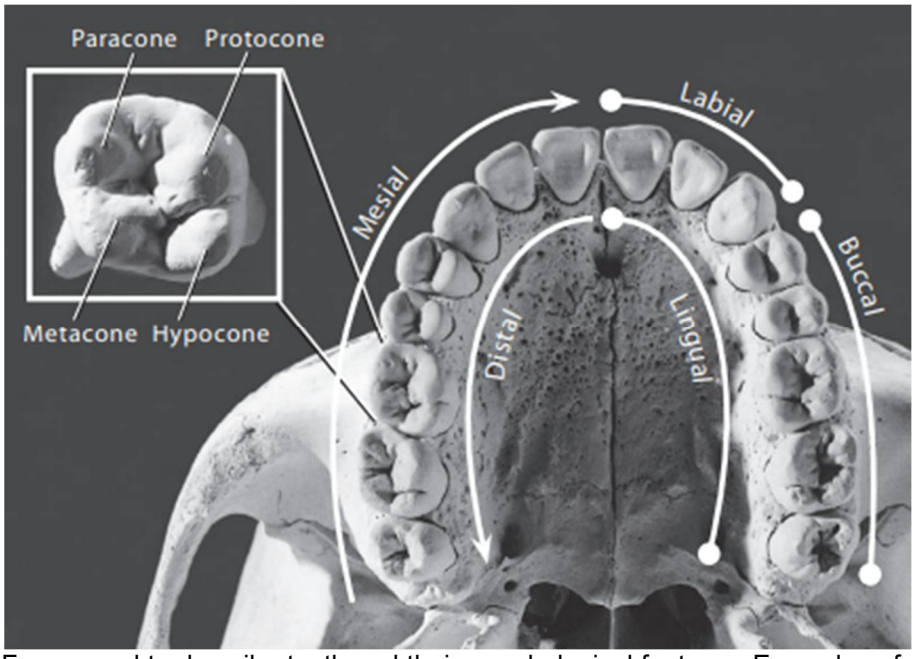


Figure 2.3 Faces used to describe teeth and their morphological features. Examples of cusps in an upper molar (sourced from White and Folkens, 2005, pp.132)

Incisors and canines are characterised by crowns with one cusp and roots with one cone, although the crown shapes differ significantly (Rodríguez Cuenca, 2003; White and Folkens, 2005). Premolars are more variable in the number of cusps and cones. In the mandible, the first premolar is usually formed by one lingual cusp and one buccal cusp. In contrast, the second mandibular premolar often has two lingual cusps and one buccal cusp. In the maxilla, premolars typically have two cusps; one lingual and one buccal (Scott, 2008). In the roots, the number of cones and radicals is flexible: first premolars frequently have two

or three cones, while second premolars usually have one cone formed by two radicals (Scott, 2008).

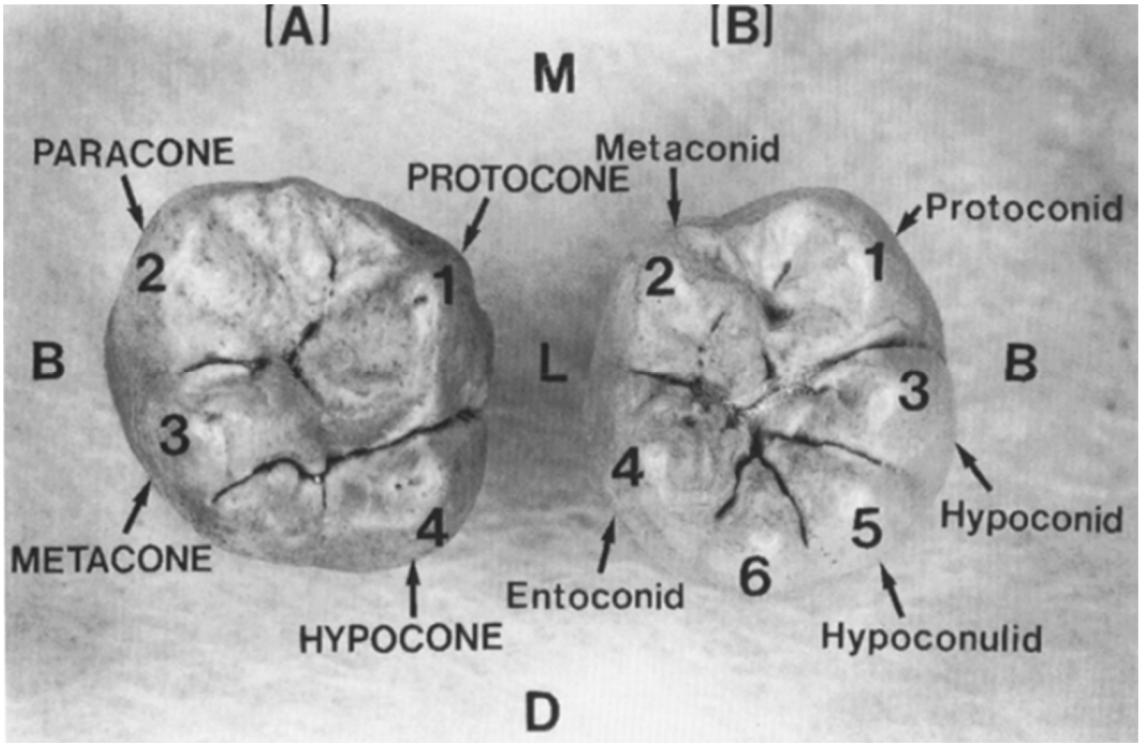


Figure 2.4 Cusp numbers and names for upper and lower molars (sourced from Scott and Turner II, 1997, pp. 18). [A] Upper right first molar with four major cusps; [B] Lower right first molar with five named major cusps and one additional supernumerary cusp – n. 6; [M] Mesial; [D] Distal; [B] Buccal; [L] Lingual

The crown and roots of the molars are far more complex. For the upper molars, the crown is usually formed of four cusps: protocone, paracone, metacone and hypocone (Figure 2.4) (Rodríguez Cuenca, 2003; White and Folkens, 2005; Scott, 2008). Roots have three cones (one lingual and two buccal). Lower molars have crowns with five cusps: protoconid, metaconid, hypoconid, entoconid and hypoconulid (although the hypoconulid or cusp 5 may vary from a well-developed cusp to not being present) (Figure 2.4). Their roots have two cones, one distal and one mesial (Rodríguez Cuenca, 2003; White and Folkens, 2005; Scott, 2008).

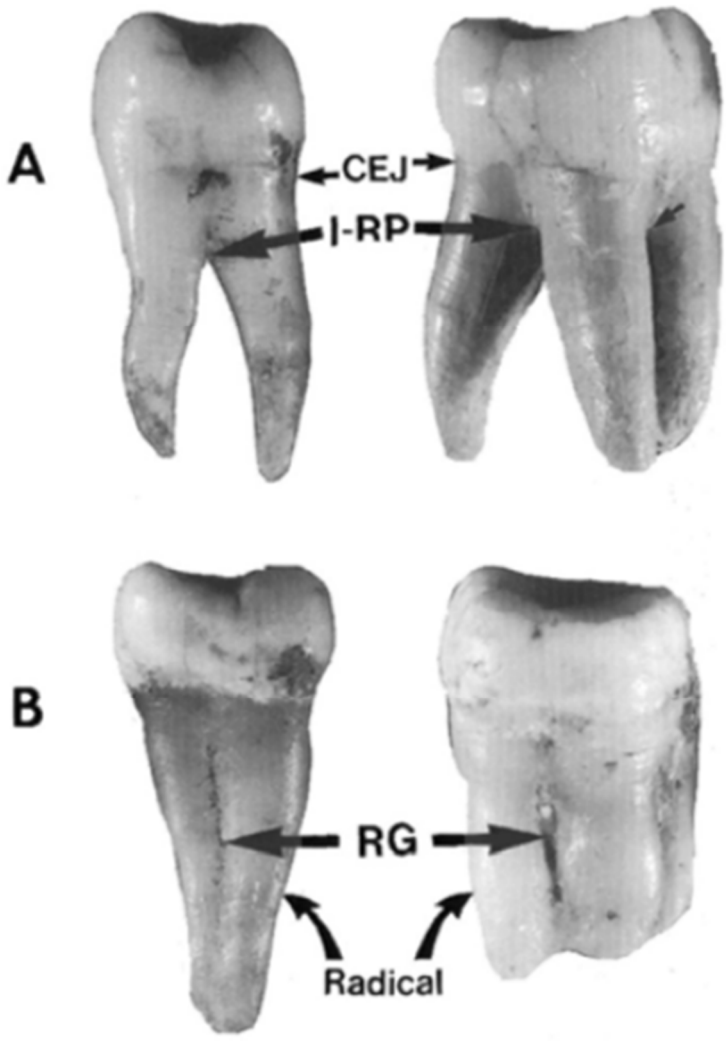


Figure 2.5 Examples of different tooth roots (sourced from Scott and Turner II, 1997, pp. 23). [A] Premolar and molar with roots separated by inter-radicular projections (I-RP), showing different cones; [B] Premolar and molar with radicals instead of cones, separated by developmental grooves (RG); [CEJ] Cementoenamel junction

2.3. Metric variables applied to the study of teeth

Metric variables, which in the past were seen as unreliable (Townsend and Brown, 1978), are now accepted as a proxy for genetics in the study of population affinity and said to yield very similar results to other methods, such as dental nonmetric traits (Rathmann et al., 2017). Dental metric variation has been proved to explain around 31% of neutral genetic

differences (genetic variants that do not confer advantages or disadvantages to the bearer) between populations (Rathmann et al., 2017). This has been calculated from single nucleotide polymorphisms and short tandem repeats (Rathmann et al., 2017).

The following sections give information on the type of measurements used, their application, and the difficulties they entail.

2.3.1 Common measurements used

The most commonly used metric variables are the mesiodistal and the buccolingual diameters (Townsend and Brown, 1978; Harris, 1997; Hanihara and Ishida, 2005; Brook et al., 2009; Martinón-Torres et al., 2012; Nelson and Fitzpatrick, 2013; Xing et al., 2015; Rathmann et al., 2017; Stewart, 2021), with some authors also studying crown indices or areas (Lukacs, 1983; Cabellos, 2007; Martinón-Torres et al., 2008). There are also studies based on cusp diameters and cusp areas (Kondo et al., 2005; Martín-Albaladejo et al., 2017). There are even studies that compare samples through the additions of the mean crown areas of the teeth observed (Lukacs, 1983).

The disparity of measurements found in the literature complicates the use of metric variables, as it is difficult to compare studies. Townsend and Brown (1978) found that the buccolingual diameter has a slightly higher degree of heritability than the mesiodistal diameter, so I included it in this study. I also included the mesiodistal diameter, the crown area and the crown index, as they have been widely used by other authors.

2.3.2 Application of metric variables

A large range of studies has used metric variables to infer biological affinity (i.e., the degree of morphological resemblance that indicates a common ancestry) in ancient human populations. Townsend and Brown (1978) studied a sample of Australian Aboriginals from

Yuendumu, in the North of Australia, and found that around 64% of the size variability was due to genetic factors. They observed that certain prenatal factors, such as the age of the mother and the birth order, had an impact on tooth size (Townsend and Brown, 1978). Lukacs (1983) studied the teeth of an Early Neolithic site in Baluchistan, in Southeast Pakistan, and compared them to other Neolithic populations from Iraq and Israel, inferring biological affinities that corresponded with chronological and cultural similarities.

Biological affinities in the fossil record have been also studied through metric variables. Late Pleistocene hominin fossils from the Xujiayao site (dated around 124,000 to 71,000 years BP) were compared with fossils from similar geographic areas, including *Homo neanderthalensis*, fossil and modern *Homo sapiens* and Pleistocene hominins from Asia (Xing et al., 2015). The authors observed that the teeth from Xuijayao are close in size to the individuals analysed from the Early and Middle Pleistocene from East Asia (Xing et al., 2015). Another study compares the Sima de los Huesos sample to individuals of *Homo erectus, Homo antecessor, Homo heidelbergensis, Homo neanderthalensis*, early and contemporary *Homo sapiens*, and to measurements taken by other authors of *Australopithecus sp*. and *Paranthropus sp*., showing a decrease in size from the early to the late *Homo* specimens (Martín-Albaladejo et al., 2017).

On a wider scale, dental measurements have been used to study the variability of very large geographic groups (Hanihara and Ishida, 2005). A higher degree of diversity found was in Sub-Saharan Africa than elsewhere, which concurs with results obtained in other studies that used other genetic proxies, such as craniometric variables. Another large-scale study provided results that indicated a gradient of size decrease from China to Europe (Brook et al., 2009). In that study, an ancient Roman sample was found to have the smallest teeth, which the authors attributed to poor nutrition, high lead ingestion and pathological conditions, as this would not be consistent with the hypothesis of dental size reduction as

an effect of the time of introduction of agriculture (i.e., populations in which the agriculture was introduced earlier would have a smaller dental size) (Brook et al., 2009).

On a more local scale, metric variables were used to infer kinship and familiar relationships within four Anglo-Saxon cemeteries in Cambridgeshire and Kent (Stewart, 2021). The results helped to infer family relationships between some individuals who were buried nearby or in the same areas of the cemeteries, and to rule-out biological kinship in other individuals who were buried together or in proximity. This was validated through some analyses that used mitochondrial DNA to study maternal relationships (Stewart, 2021).

2.3.3 Difficulties in applying metric variables

One of the issues with metric variables is that they are not well defined, and there are no standard methods to study them (Bailey, 2007). This means that it is difficult to compare results across studies, because the measurements and indices may differ, and because the statistical analysis used to compare dentitions may also differ. In addition, tooth size is affected by sexual dimorphism (Harris, 1997; Brook et al., 2009), which may complicate studies of archaeological samples where the sex ratio is unbalanced or when the sex of the individuals is unknown. This has been addressed by some authors by selecting samples with a balanced sex composition, but this is sometimes not possible (Brook et al., 2009).

Another issue when trying to compare the results obtained with metric variables with those obtained in previous studies is that the sample size of the collections in which metric variables have been used vary greatly. Whilst some authors used sample sizes as small as four (Xing et al., 2015) or 16 individuals (Lukacs, 1983), others have used larger samples of 127 individuals (Townsend and Brown, 1978), or very large samples of over 400 individuals (Brook et al., 2009). The sample size in my study varied from one collection to another, as usually happens with archaeological collections. SNA had a large proportion of

female individuals, whereas SMA had just four females. To correct the effects of the different sex ratios in the samples, I ran a second set of analyses on individuals sexed as male only. This also gave very similar sample sizes across the collections.

Finally, some authors found that metric variables could provide different results to those obtained with other methods, such as nonmetric dental traits (Hanihara and Ishida, 2005). The affinities between populations in East Asia differed when using metric methods and nonmetric dental traits (Hanihara and Ishida, 2005). For example, dental nonmetric trait frequencies in Chinese and Japanese populations were similar to those in Northeast Asian populations, whereas the metric variables indicated that Chinese and Japanese populations are closer to populations from Southeast Asia. In the same research, the authors found that the Ainu population was similar to Northeast Asian populations when using metric traits, whereas with nonmetric traits the Ainu and Northeast Asian populations are classified in different groups (Hanihara and Ishida, 2005). Therefore, although dental nonmetric and metric traits have both proved to be useful to infer biological relatedness among populations (Rathmann et al., 2017), Hanihara and Ishida (2005) propose that metric traits are more reliable on a worldwide scale than on a smaller, regional scale.

2.4. Nonmetric dental traits

Nonmetric dental traits are structures that rise (cusps, lobes, ridges, projections and tubercles) or sink (lines and grooves) in the crowns and roots of teeth at specific locations and that can be categorised as present, absent, or intermediate if present (Scott and Turner, 1997; Scott, 2008). Because the number of accessory cusps, ridges and lobes in the molars is very variable, as is their development, a high number of nonmetric dental traits is based on the crowns of upper and lower molars (Rodriguez Cuenca, 2003; Scott, 2008). Nonmetric dental traits correlate with neutral genomic data (Hubbard et al., 2015; Rathmann et al.,

2017). Therefore, they can be used as a proxy for biological relatedness, although only part of the dental variation can be explained through neutral genetic differences, and the remaining variation must have other causes, such as the type of diet and food preparation methods (Rathmann et al., 2017).

In the following sections, I give an insight into how the ASU system works, its applications, the difficulties usually found when using it and its future directions.

2.4.1 ASU System

Although some researchers had previously described a few dental traits, Hrdlička (1920) was the first author to standardise the recording of nonmetric dental traits (Turner II et al., 1991; Scott, 2008; García Sívoli, 2009). Hrdlička, whose methods in anthropology are now questioned due to his racist and unethical practices in Native American remains and his links to American eugenics laws, described a gradation for the incisor shovel shape (Hrdlička, 1920). A shovel-shaped incisor, or shoveling, happens when the crown has marginal lingual ridges, giving the incisor an appearance similar to a scoop or a shovel. In Hrdlička's gradation of the trait, he included pictures and descriptions of intermediate variation from absent to present shoveling. This allowed other researchers to observe intermediate variation in their dental collections. Dahlberg developed the method further in 1956, producing plaster plaques with casts showing different degrees of development for several nonmetric dental traits (Turner II et al., 1991; Scott, 2008; García Sívoli, 2009). In 1961, Hanihara developed some casts for nonmetric dental traits in deciduous dentition, including shoveling, Carabelli's trait and protostylid (Turner II et al., 1991; Scott, 2008; García Sívoli, 2009). In 1970, Turner developed two new plaques with standardised degrees of expression for two more nonmetric dental traits (Scott, 2008). This was the beginning of the Arizona State University Dental Anthropology System – the ASUDAS or ASU system (Turner II et al., 1991), which is a set of standardised crown and root nonmetric

traits in several plaster plaques with casts, giving detailed information about the scoring procedures for the different grades (Figure 2.6) (Turner et al., 1991; Scott and Turner, 1997; Scott, 2008; García Sívoli, 2009).

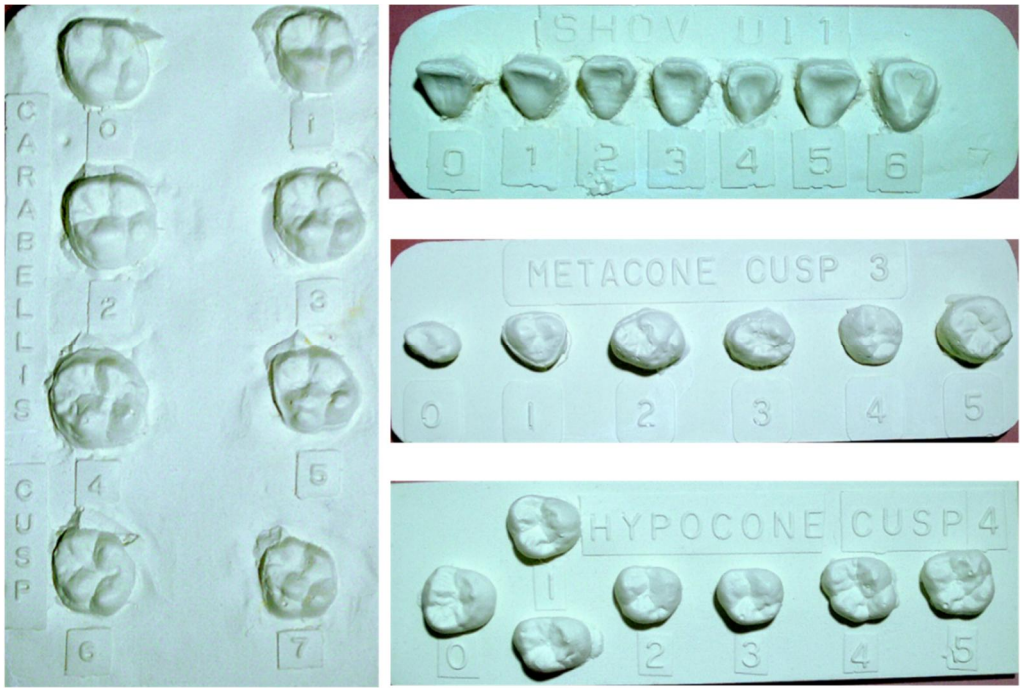


Figure 2.6 Examples of ASU system plaques with the casts representing the variation of the nonmetric traits: Carabelli's, shoveling, metacone and hypocone

Although more than 100 nonmetric dental traits have been described for the teeth, only 39 are standardised in the ASU system (Table 2.1) (Scott and Turner II, 1997; García Sívoli, 2009). These standardised nonmetric traits were selected by Turner II and colleagues in 1991 based on the following characteristics (Turner II et al., 1991; Irish et al., 2013):

- Easily observed
- High persistence, even with intense dental wear
- Low or no sexual dimorphism
- Slow evolution
- High potential for the study of the affinity of biological populations

The ASU standards include 20 plaster casts with some of the dental traits standardised, showing different degrees of development, and a procedure to score the features according to their size and shape (Turner II et al., 1991; Scott and Turner II, 1997; Scott, 2008). The advantage of the ASU traits versus other morphological characters (dental or not) used in phylogenetic studies (i.e., studies of evolutionary relationships) is that they have been extensively studied, and their heritability, correlation and scoring replicability are well understood (Irish et al., 2014). In addition, a lack of standards of reference is linked to intraobserver error as high as 20% for some other nonmetric traits, supporting the use of the ASU system over nonmetric traits that lack a standardised reference (Sofaer et al., 1972).

Table 2.1 Nonmetric Dental Traits described in the Arizona State University Dental Anthropology System (based on Turner et al., 1991, and Scott and Turner, 1997). LM1 – Lower Molar 1 / LM2 – Lower Molar 2 / UCI – Upper Central Incisor / ULI – Upper Lateral Incisor / UM1 – Upper Molar 1 / UM2 – Upper Molar 2 / UM3 – Upper Molar 3

		Reference teeth	**Trait**	**Description**	**Plaque**	**Citations**	**Observations**
Incisors	Crown	Upper and lower central Incisors	Winging	Mesiolingual rotation of the central incisors	No	Enokin and Dahlberg (1958), Turner (1970)	In some cases, overcrowding might cause the rotation.
		Upper Incisors	Labial Convexity	Degree of convexity of the labial surface of the upper incisors	Yes	Nichol et al. (1984)	This trait is inversely correlated with double-shoveling. The focal tooth is UI1.
			Interruption Groove	Grooves crossing the cingulum	No	Turner (1967)	This trait is related to the tuberculum dentale. The focal tooth is UI2.
		Upper lateral Incisor	Peg-shaped Incisor	Smaller size and change in the general crown morphology	No	Turner et al. (1991)	

		Reference teeth	Trait	Description	Plaque	Citations	Observations
Incisors and Canines	Crown	Upper Incisors, Canines and lower Incisors	Shoveling	Distal and mesial marginal ridges in the lingual surface	Yes	Hrdlička (1920), Dahlberg (1956), Scott (1973)	Correlates in the different teeth, so when studying populations, one kind of tooth has to be chosen. The focal tooth is UI1.
		Upper Incisors and Canine	Tuberculum Dentale	Ridges and tubercles in the lingual surface	Yes	Nichol and Turner (1986), Turner et al. (1991)	The trait presents more variability at the lateral incisor. The focal tooth is UI2.
Incisors, Canines and Premolars	Crown	Upper Incisors, Canine and first Premolar, and lower Incisors	Double-shoveling	Distal and mesial marginal ridges in the labial surface	Yes	Dahlberg (1956), Turner and Laidler Dowda (1979)	This trait is inversely correlated with labial convexity. The focal tooth is UI1.
Incisors, Canines, Premolars and Molars	Root	All teeth	Radical Number	Grooves in roots, forming radicals	No	Turner (1967)	Maximum variation in molars
Incisors, Premolars and Molars	Root and Crown	Upper lateral Incisors, lower central Incisors, upper and lower second Premolars and upper and lower third Molars	Congenital Absence	Agenesis of the teeth	No	Montagu (1940)	This trait should not be considered in individuals younger than 20 years old.
Canines	Crown	Upper Canines	Canine Mesial Ridge	Mesiolingual ridge larger than the distolingual	Yes	Turner and Dale Klausner (1979)	This feature is frequently asymmetrical
		Upper and lower Canines	Canine Distal Accessory Ridge	Ridge located between the median and the distal ridges	Yes	Scott (1973, 1977)	Only observable in unworn teeth. Moreover, it presents a high degree of sexual dimorphism.

		Reference teeth	Trait	Description	Plaque	Citations	Observations
	Root	Lower Canines	Canine Root Number	Presence of two roots	No	Turner (1967)	Rare feature, the maximum frequency of 10%
Premolars	Crown	Upper Premolars	Premolar Mesial and Distal Accessory cusps	Small accessory cusps separated from the lingual and buccal cusps	No	Turner (1967)	This trait should be considered only in unworn teeth.
			Tricuspid Premolars	Three cusps	No	Turner et al. (1991)	Only found, and in a low proportion, among the Amerindian populations.
		Upper first Premolar	Distosagittal Ridge	Ridge from the buccal cusp to the distal occlusal border	No	Morris et al. (1978)	Only found, and in a low proportion, among the Amerindian populations.
		Upper and lower Premolars	Odontome	Peg-shaped projection on the occlusal face	No	Pedersen (1949) and Alexandersen (1970)	Very rare feature
		Lower Premolars	Premolar Lingual Cusp Variation	Multiple lingual cusps	Yes	Scott (1973)	The focal tooth is LP2
	Root	Upper Premolars	Premolar Root Number	Variation in root number from 1 to 3	No	Turner (1967, 1981)	
		Lower Premolars	Tomes' Root	Groove in the mesial roots surfaces	Yes	Turner and Herzog (1979)	
Premolars and Molars	Crown	Upper Premolars and Molars	Enamel Extensions	Extensions of the enamel from the cervicoenamel junction towards the roots	No	Pedersen (1949)	Enamel pearls might also be present. The focal tooth is UM1.
Molars	Crown	Upper Molars	Metacone	Distobuccal cusp (cusp 3)	Yes	Turner and Kaschner (1978)	The focal tooth is UM3
			Hypocone	Distolingual cusp (cusp 4)	Yes	Larson (1978)	The focal tooth is UM2
			Metaconule	Distal cusp (cusp 5), between the metacone and the hypocone	Yes	Turner and Warner (1977)	The focal tooth is UM1

	Reference teeth	Trait	Description	Plaque	Citations	Observations
		Carabelli's Trait	Tubercle on the lingual surface of the protocone (mesiolingual cusp – cusp 1)	Yes	Dahlberg (1956)	The focal tooth is UM1
		Parastyle	Tubercle on the buccal surface of the paracone (mesiobuccal cusp – cusp 2)	Yes	Katich and Turner (1974)	This trait might also be found in metacone. The focal tooth is UM3.
	Upper third Molar	Peg-shaped Molar	Smaller size and change in the general crown morphology	No	Turner et al. (1991)	
	Lower Molars	Cusp Number	Differences in the number of cusps	No	Gregory (1916) and Turner (1967)	
		Hypoconulid	Distal cusp (cusp 5)	Yes	Turner and Warner (1977)	Focal tooth is LM2, although it also can be observed in LM1.
		Entoconulid	Distal cusp (cusp 6), between the hypoconulid and the entoconid (distolingual cusp or cusp 4)	Yes	Turner (1970)	Cusp 6 only can be scored when cusp 5 is present. The focal tooth is LM1.
		Metaconulid	Cusp (cusp 7) between the metaconid (mesiolingual cusp or cusp 2) and entoconid	Yes	Turner (1970)	The focal tooth is LM1
		Protostylid	Tubercle on the buccal surface of the protoconid (mesiobuccal cusp – cusp 1)	Yes	Dahlberg (1956)	The focal tooth is LM1
		Groove Pattern	Pattern in which the cusps contact	No	Gregory (1916), Hellman (1928) and Jørgensen (1955)	Also called Dryopithecus Y pattern, important among hominoids

	Reference teeth	Trait	Description	Plaque	Citations	Observations
		Distal Trigonid Crest	Buccolingual crest that links the protoconid and the metaconid	No	Hrdlička (1924), Hanihara (1961)	Most common in deciduous dentition. The focal tooth is LM1.
	Lower first Molar	Anterior Fovea	Groove linking the protoconid and the metaconid between the marginal and the median ridges	Yes	Turner and Chilton (1979)	The focal tooth is LM1, although it can be observed in the LM3.
		Deflecting Wrinkle	Variations in the form of the medial ridge on the metaconid	Yes	Seybert and Turner (1975)	This trait should be considered only in unworn teeth.
	Lower third Molar	Torsomolar Angle	Rotation lingually or buccally of the molar	No	Neiberger (1978)	Common in Amerindian populations
Root	Upper Molars	Upper Molar Root Number	Variation in root number from 1 to 4	No	Turner (1967)	
	Lower Molars	Lower Molar Root Number	Variation in root number from 1 to 3	No	Turner (1967)	The focal tooth is LM2

Although the ASU system has helped to standardise recording of dental nonmetric traits, its accuracy depends on the ability and expertise of the observer and on the complexity of the crown (e.g., several traits expressed in the same crown might create problems in assessing one of them) (Scott and Turner II, 1997). Therefore, it is essential to consider interobserver error when comparing the results obtained by different researchers (Irish, 2000). The presence of wear in teeth also affects the results when evaluating dental traits, even though the traits were selected to avoid this (Burnett et al., 2013). Furthermore, the ASU system might not be helpful when working with fossil species, as it does not cover the whole hominin variation (Gómez-Robles et al. 2007).

Another characteristic of the ASU system is that it is usual to dichotomise the results of the observations as ‘present’ or ‘absent’ and then compare the frequencies of occurrence

to establish differences and similarities among populations. This is usually done using a Mahalanobis D2 statistic (Irish, 2006; Irish et al., 2007) or a mean measure of divergence matrix (Scott and Turner, 1997; Irish, 1997; Irish, 1998; Sciulli, 1998; Cucina et al., 1999; Irish, 2000; Kitagawa, 2000; Guatelli-Steinberg et al., 2001; Irish and Guatelli-Steinberg, 2003; Irish, 2005; Bollini et al., 2006; Irish, 2006; Coppa et al., 2007; Cabellos, 2007; Edgar, 2007; Leblanc et al., 2008; Martinón-Torres et al., 2012; Lee and Zhang, 2013; Carter et al., 2014; Irish et al., 2014). This means that, when using the ASU system, we first assign a degree of expression to every tooth, then categorise the results into “absent” and “present”, choosing a threshold between the two categories. The advantage of this dichotomy is that if a feature shows a high frequency of higher degrees of expression in a population, it can be considered as ‘present’ at a higher threshold so that differences among groups become more evident (Martinón-Torres et al., 2012). For example, one study comparing *Homo heidelbergensis* with *Homo sapiens,* considered characters such as labial convexity, dental tubercle, shovel shape, metacone, hypocone, Carabelli’s trait, parastyle, premolar lingual cusp and hypoconulid, to be present in individuals if the trait scored above stage 3 (Martinón-Torres et al., 2012). However, according to the ASU system procedures, most of these characters are regarded as present at stage 1 (Turner II et al., 1991). As a result, differences between the *Homo heidelbergensis* sample of *Sima de los Huesos* and the *Homo sapiens* samples analysed become more evident for some traits, such as labial convexity, than they were before adjusting the level of presence (Martinón-Torres et al., 2012).

2.4.2 Application of nonmetric dental traits

Nonmetric dental traits have been widely used to study the biological relatedness between samples and populations of many different contexts, and with a variety of aims (Scott et al., 1983; Irish, 1997; Scott and Turner II, 1997; Sciulli, 1998; Irish, 2000; Kitagawa,

2000; Guatelli-Steinberg et al., 2001; Bollini et al., 2006; Irish and Konigsberg, 2007; Leblanc et al., 2008; Lee and Zang, 2013). They have been proved to be particularly useful to observe the biological affinity of human populations that inhabited the same geographic area in different periods, lived in different regions during the same period, or to explore population movements. They have proved helpful in comparisons among different hominin species (Martinón-Torres et al., 2008; Martinón-Torres et al., 2012; Irish et al., 2013; Martinón-Torres et al., 2013; Carter et al., 2014; Irish et al., 2014). For example, in a study comparing the dental sample from the *Sima de los Huesos* with specimens of Neanderthals, *Homo heidelbergensis* and fossil and contemporary *Homo sapiens*, the authors used nonmetric traits to infer biological affinities. They conclude that the remains from *Sima de los Huesos* are more similar to those of Neanderthals than any of the other samples, whilst the most different samples were Neanderthals and modern *H. sapiens*. (Martinón-Torres et al., 2012; Martinón-Torres et al., 2013).

Another study of nonmetric dental traits in samples from the Pleistocene, and that compared them to recent populations in Africa and worldwide, found that samples from North Africa were more similar to samples from Europe, and samples from South Africa, instead of showing similarity with the samples from Europe, were morphologically closer to samples from Australia and Melanesia (Irish, 1998). The author linked these results to a retention of ancestral characters. This question was further explored by Irish and Guatelli-Steinberg (2003), where the authors included data from African Plio-Pleistocene fossil samples and many recent populations. Their results showed that gracile fossil hominins were closer to South African modern populations than to any other modern population, which supported the hypothesis of an African human origin (Irish and Guatelli-Steinberg, 2003). Another study whose results supported the out-of-Africa hypothesis observed that the Kaprina samples analysed were more similar to modern Africans and Australians than to modern Europeans (Stringer et al., 1997).

Nonmetric dental traits have also been used to study biological relatedness among human groups in populations of Native Americans (Scott et al., 1983; Sciulli, 1998; Bollini et al., 2006; Leblanc et al., 2008). Scott et al. (1983) found that, although the Pima peoples were initially thought to be closely related to the Yuma, they were more closely related to the Hopi than to the Yuma, which is supported also by linguistic data. Natives of the Ohio Valley were found to have a conservative dental morphology, although three nonmetric dental traits showed significant and consistent differences among the populations, with higher frequencies in older populations, associated with the introduction of crops of maize and beans around 1,000 A.D. (Sciulli, 1998). The author ascribed this change in the frequency of dental traits to either gene flow or selective forces due to a change in diet (Sciulli, 1998). A change in diet may affect the frequency of dental traits by adding or removing selective pressures on certain morphologies (Sciulli, 1998). For example, harder food will require teeth with a larger occlusal surface, such as molars with more cusps, so individuals with accessory cusps will be more successful and the frequencies of molars with accessory cusps will be higher in the population. In contrast, softer food will remove this selective pressure, so the frequencies of molars with accessory cusps may decrease. Diet, or any other environmental factor, may also affect the frequency of dental traits by selecting for phenotypes that also happen to have certain dental traits (Sciulli, 1998). In those cases, the change in the frequency of the dental trait is a secondary effect of the selective pressures on another characteristic.

Araucanian remains were analysed, and the results indicated that they were biologically close to other populations from Northwest Argentina, and closer to the Sinodonty pattern, found in populations in East Asia, than to European and African populations (Bollini et al., 2006). Leblanc et al. (2008) studied remains from the cultures of the *Basketmakers* and the *Mimbres*, and observed that the individuals initially grouped within the *Basketmaker* population were biologically differentiated along a geographic West-

East axis, with the Eastern *Basketmaker* different to any other population analysed in the study and the *Mimbres* in an intermediate position between samples from the north and the south of the area included in the analysis (Leblanc et al., 2008).

Many African populations have also been studied through nonmetric dental traits (Irish, 1997; Irish, 2000; Guatelli-Steinberg et al., 2001; Irish and Konigsberg, 2007). The dental morphology of samples from across the African continent, dating from prehistory to the 20th century, was observed to differentiate the populations from the North of Africa from the sub-Saharan populations (Irish, 1997). Other study focused in the Iberomaurusians and the African Nubians, including other populations from the North of Africa to widen the comparison (Irish, 2000). The results indicated biological continuity between the Iberomaurusian samples and samples from the same geographic area but later periods, and a remarkable difference with the Nubian samples (Irish, 2000). A comparison of aboriginal inhabitants of the Canary Islands with Berbers, Bedouins, Carthaginians, three populations of Egyptians and three populations of Nubians, found that aboriginals from the Canary Islands were more closely related to the Carthaginians, although the Arabs and the Berbers are closer geographically (Guatelli-Steinberg et al., 2001). Also in Africa, a study by Irish and Konigsberg (2007) tried to replicate the results of a previous study that had used craniometric variables to compare the archaeological population of Jebel Moya, from Sudan and dated between 500 B.C. and 400 A.D., with other nearby populations from different chronologies, including samples from Egypt, Nubia, Kenya, Cameroon, Ethiopia, Ghana, Congo, Gabon and Nigeria (Mukherjee et al., 1955). The results of the comparison of dental traits were consistent with those obtained using craniometric variables (Jebel Moya was different but in an intermediate place in relation to the other samples) but also differed, since the craniometric results showed a Sub-Saharian link and the dental traits a Northwest link (Irish and Konigsberg, 2007).

Nonmetric dental traits have been applied to study populations in Asia (Lee and Zang, 2013; Kitagawa, 2000). A study comparing 52 populations across 8 geographic areas from China and Mongolia with a chronology between 770 BC and 420 AD. found differences that were not only biological and geographical but also cultural and linguistic (Lee and Zang, 2013). Studies in Asian populations have also included information for the deciduous dentition, as for example the one carried out by Kitagawa (2000). This study distributed nearly 1,500 individuals from Japan into two groups, which was consistent with what had been previously observed with the permanent dentition (Kitagawa, 2000).

In Oceania, a study that used nonmetric dental traits to compare the remains from a cemetery with other samples with different chronologies indicated that the first settlers of Palau were closely related to peoples from Micronesia, Southeast Asia and Polynesia (Nelson and Fitzpatrick, 2013). In Europe, studies of nonmetric dental traits have explored biological relatedness among populations in the Western Mediterranean, from Majorca and Catalonia (García Sívoli, 2009) and the Iberian Peninsula (Cabellos, 2007; Scott et al., 2013). Biological similarities between the Basque population and other European populations were explored through nonmetric dental traits, showing that the tooth morphology of the Basque groups had little differences to that of other populations from the North of the Iberian Peninsula, and in general, was similar to the European group (Scott et al., 2013). However, a cluster analysis placed the Basque sample as an outlier to the western European dentition (Scott et al., 2013).

Cabellos (2007) carried out a study in the Iberian Peninsula that addressed similar aims to this study. She used 28 nonmetric dental traits and four metric variables to compare samples from a variety of periods and cultural contexts, such as Romans from the 2nd century, Visigoths, Medieval Muslims, Medieval Christians, and postmedieval samples to the 17th century. The results supported the hypothesis of biological continuity in the Iberian Peninsula, with nearly all the distances between samples small but statistically significant

(Cabellos, 2007). There was a disparity between the results obtained with nonmetric dental traits and with metric variables: with the former, the largest distance was between one of the Muslim samples and the Roman sample, followed by the distance between the Romans and the Visigoth, whilst in the latter, there were statistically significant differences between the Romans and all the other samples (Cabellos, 2007).

Less common but also interesting are studies that use nonmetric dental traits to infer family relationships in archaeological remains, establishing kinship from a biological perspective (family relationships that are not based on biological relatedness cannot be inferred through nonmetric dental traits). For example, family relations have been explored in 29 individuals from the site Huaca Loro, in Peru, dated around 1200 A.D., although the analysis did not provide any significant results (Corruccini and Shimada, 2002). Likewise, a study trying to establish family relationships in the cemetery of Jebel Mutawwaq, in Jordan, dated between 3500-2000 BC, revealed a very high frequency of certain nonmetric dental traits, such as Carabelli cusp, protostilid and shoveling, which the authors interpreted as a high degree of biological relatedness (Cabellos et al., 2002).

Nonmetric dental traits may also be a useful tool to examine variation in human groups after historical events (Cuccina et al., 1999; Desideri and Eadies, 2002; Irish, 2005; Irish, 2006; Coppa et al., 2007; Edgar, 2007). Edgard (2007) studied the biological affinity of African Americans with other populations after the African Slave Trade and concluded that early populations of African Americans were biologically closer to populations from the West of Africa, but later populations diverged from this and had more affinity with European populations (Edgar, 2007). The Neolithic Revolution has also been thoroughly studied using nonmetric dental traits. Cuccina et al. (1999) observed biological differences between three samples dating from the Neolithic, Copper Age, and Early Bronze Age, from the Trentino alpine region of Italy. Their results indicated that the differences between the populations were very small, and most of them were not statistically significant (Cuccina et al., 1999).

For the same period, but in Western Switzerland, eight individuals dated from the Middle Neolithic to the Bell Beaker period were studied with nonmetric dental traits, finding differences between both samples (Desideri and Eades, 2002). A much larger-scale study centred on the Italian Peninsula compared individuals dated from the Upper-Paleolithic to the Middle Ages (Coppa et al., 2007). The results indicated that a population substitution happened during the Neolithic, since the Upper Palaeolithic-Mesolithic individuals were clearly distinct from the other populations (Coppa et al., 2007).

The same historical period has been studied by Irish (2005) in Nubian samples, observing a discontinuity between the peoples of the Late Palaeolithic and the Neolithic populations, since large significant distances were found between the sample from the Late Palaeolithic (dated between 12000 and 10000 BC) and all the others. This suggests that the Neolithic in this area involved a large population substitution (Irish, 2005). A similar study was carried out in samples pre- and post-Neolithic in Egypt, where there were small distances between the samples, and few significant differences were found (Irish, 2006).

2.4.3 Difficulties in applying nonmetric dental traits

As happened with the metric variables, sample size may be an issue when studying nonmetric dental traits. In palaeontological studies, the number of individuals is often too small to apply statistical methods. Therefore, the researchers compare the morphology observed without statistical analysis. For example, in a study of dental samples from the hominin remains from Dmanisi, Georgia, dating circa 1.8 million years, the authors compared the dental nonmetric traits observed to those typically seen in other species of hominins. They concluded that the presence of certain nonmetric traits made the dental morphology of the Dmanisi remains closer to *Australopithecus* and *H. habilis* than to *H. erectus* and *H. ergaster* (Martinón-Torres et al., 2008).

Other authors have addressed the small sample size by comparing not only the traits present/absent but differences in the number of states of presence. Irish et al. (2013) analysed three dental traits in only two individuals of *Au. sediba* and compared the results with *Au. africanus*, *Au. afarensis*, *H. habilis* and *Paranthropus*. Although their results aligned with other obtained with other skeletal structures, like the hand, the foot or the spine, were heavily criticised by Carter et al. (2014), since according to the ASU system, a larger number of states of presence cannot be directly translated into greater genetic distance. Small changes in the analysis parameters and the design of the experiment may lead to large differences in the results (Carter et al., 2014). Sometimes the analyses carried out with small sample sizes provide results that are difficult to interpret (Desideri and Eades, 2002)

Another difficulty when studying nonmetric dental traits is that it may be challenging to discriminate closely related populations. García Sívoli (2009) solved this issue in the same way that Irish et al. (2013, 2014) dealt with the small sample size: comparing the frequencies of the states of presence. He found that doing this provided him more information in the comparison of Talayotic and Late-Roman Majorcan populations (García Sívoli 2009). However, more information is needed to understand if comparing frequencies improves the results relative to presence/absence scoring.

An issue usually faced when using nonmetric dental traits is the number of traits chosen, and their suitability. The number of traits used by different authors is varied, and sometimes they do not restrict to the focal tooth (i.e., a trait is observed in other teeth in addition to the focal tooth). For example, there can be found studies that analyse less than 20 dental traits (Scott et al., 1983; Kitagawa, 2000; Cabellos et al., 2002), studies that use around 20 to 30 dental traits (Cuccina et al., 1999; Irish, 2000; Guatelli-Steinberg et al., 2001; Corruccini and Shimada, 2002; Bollini et al., 2006; Cabellos, 2007; Edgard, 2007; Leblanc et al., 2008; Lee and Zang, 2013; Scott et al., 2013), and studies that use over 30 dental traits (Irish, 1997; Sciulli, 1998; Irish, 2005; Coppa et al., 2007; Irish and Konigsberg,

2007). The number of traits chosen affects not only to the reliability of the results, but also to the ability of the researchers to compare their results with previously published studies. According to Rathmann and Reyes-Centeno (2020), a higher number of traits provide better results up to 16 traits, and over that number the reliability of the results will not increase.

2.4.4 Further Directions

New technologies have allowed new ways of scoring nonmetric dental traits, and some researchers have studied the traits in the enamel-dentine junction of three-dimensional models instead of in the outer enamel surface of the teeth (Skinner et al., 2008; Skinner et al., 2009; Martínez de Pinillos et al., 2014; Martinón-Torres et al., 2014). Skinner and colleagues (2008) used surface models of teeth from eight species of hominoids (both extinct and extant) obtained through microtomography (micro-CT) to compare the degree of development of four nonmetric dental traits (cusp 6, cusp 7, protostylid and trigonid crest pattern) in the lower molars at the enamel-dentine junction and the outer enamel surface. The authors observed that these nonmetric dental traits developed at the enamel-dentine junction, and it was not the enamel deposition which determined the presence of the traits (Skinner et al., 2008). Moreover, they noted that occasionally the enamel deposition masked the degree of development of the nonmetric traits, which was better differentiated at the enamel-dentine junction than at the outer enamel surface (Skinner et al., 2008). The degree of development of the protostylid was further observed at the level of the enamel-dentine junction and compared with its appearance at the outer enamel surface, and it was found that the enamel deposition may alter the expression of the trait at the outer enamel surface (Skinner et al., 2009). In addition, the trait was more visible at the enamel-dentine junction in heavily worn teeth, as wear did not affect this layer (Skinner et al., 2009).

Following the same research line, the lower molars of the *Homo heidelbergensis* sample from Sima de los Huesos were observed both at the enamel-dentine junction and

at the outer enamel surface to study differences in the degree of development of the trigonid and talonid crests (Martínez de Pinillos et al., 2014; Martinón-Torres et al., 2014). The authors observed a greater degree of variability of the trigonid crest at the enamel-dentine junction than at the outer enamel surface, as the trait develops in the enamel-dentine junction (Martínez de Pinillos et al., 2014). Likewise, the talonid crest, not yet included in the ASU system, is better observed at the enamel-dentine junction than at the outer enamel surface (Martinón-Torres et al., 2014).

2.5. 2D Geometric Morphometrics applied to the study of teeth

The availability of new technologies and the requirements of remote work have pushed the field of dental anthropology towards the use of new methodologies, with 2D GMM now used extensively. Its application and difficulties when using it are explained in the following sections.

2.5.1 Application of 2D GMM in dental anthropology

2D GMM has been used to study teeth in hominin fossils (Martinón-Torres et al., 2006; Gómez Robles et al., 2007; Gómez Robles et al., 2008; Gómez Robles et al., 2011a; Gómez Robles et al., 2011b; Gómez Robles et al., 2013; Bailey et al., 2014; Gómez Robles et al., 2015; Xing et al., 2019) and even extinct and extant animal species (Evin et al., 2013; Gómez Cano et al., 2013). In animal teeth, Gómez Cano and colleagues (2013) used the analysis of the outline of the crown of 232 first upper molars of extant *Murinae* (rats and mice) to observe differences in the shape of the crowns depending on dietary variations, and to infer the diets of 9 species of extinct *Murinae* from the Iberian Peninsula (Gómez Cano et al., 2013). Evin and colleagues (2013) established which was the most useful parameter to infer the group affiliation of isolated teeth of modern wild and domestic pigs.

They observed nearly 1000 teeth, including upper and lower second and third molars, and collected measurements including size and shape (Evin et al., 2013). The authors concluded that whilst size was a poor indicator of whether the teeth belonged to wild or domesticated pigs, shape was an accurate variable that provided a high degree of statistical confidence (Evin et al., 2013).

2D GMM was used in lower second premolars to examine the morphological variation within different fossil hominin species from the Pliocene and the Lower, Middle and Late Pleistocene, of various geographical areas (Martinón-Torres et al., 2006). The results of this analysis showed a reduction in the occlusal face that forms a gradient from *Australopithecus* to modern *Homo*, as well as differences in the symmetry of the premolars that go from a more asymmetrical pattern, with a more mesial metaconid, in the *Australopithecus* towards more symmetrical premolars in *Homo* (Martinón-Torres et al., 2006). Another interesting finding of this study was that the shape of the outline of the crown was directly related to the degree of development of the cusps, which could allow the use of worn teeth in comparisons of dental morphology (Martinón-Torres et al., 2006). A similar study in fossil hominin species was carried out in the first upper molars to characterise the shape of the occlusal face of crowns (Gómez-Robles et al., 2007). The results showed that *Homo neanderthalensis*, as well as *Homo heidelbergensis*, have a distinctive crown morphology for the first upper molars, with both the protocone and the hypocone displaced towards the distal face, and the hypocone protruding from the occlusal view of the crown (Gómez-Robles et al., 2007).

The same study was carried out using the lower first premolars, where the authors found clear differences in the shape of the premolars between early hominins (*Australopithecus* and *Paranthropus*) and late hominins (*Homo neanderthalensis*, *Homo heidelbergensis* and *Homo sapiens*), with over 17% of this shape change directly related to a change in size (Gómez-Robles et al., 2008). Similarly, 2D GMM was applied to upper first

and second premolars of 116 and 120 individuals respectively, showing that the fossils from the Pliocene have more asymmetrical premolars with larger lingual cusps, that change into more symmetrical and with smaller lingual cusps premolars in more recent fossils (Gómez-Robles et al., 2011a). Interestingly, the upper premolars are not very useful to discriminate hominin species when applying the ASU system in nonmetric traits (Gómez-Robles et al., 2011a). Another study focused on the variability of the shape and size changes of the first, second and third lower molars throughout the fossils (Gómez-Robles et al., 2015). The results show fewer differences in the morphology of the first lower molars than in the morphology of second and third lower molars throughout the studied species (Gómez-Robles et al., 2015). However, it is more difficult to discriminate isolated second and third molars and classify them under the correct species than doing the same with first molars (Gómez-Robles et al., 2015). A reduction in size is also observed throughout the chronological line, although it does not always follow a linear pattern, as for example the molars belonging to *Homo heidelbergensis* from Sima de los Huesos appear to be smaller in size than the molars from *Homo neanderthalensis* (Gómez-Robles et al., 2015).

2D GMM has been useful in assessing whether isolated teeth have a morphology consistent with a given fossil species, in combination with other techniques (Xing et al., 2019). Four teeth (an upper central incisor, two upper first premolars and an upper first molar) from Tongzi, in China, were observed and compared with teeth belonging to individuals from the Pleistocene and the Holocene from Asia, Europe and Africa, using not only 2D GMM but also metric variables and nonmetric dental traits (Xing et al., 2019). The results obtained were consistent, and indicated that these teeth, dated around 170,000-240,000 years BP and initially assigned to *Homo erectus*, have a morphology that do not fit the morphology observed in *Homo erectus*, and are therefore closer to archaic *Homo sapiens* (Xing et al., 2019).

Deciduous dentition has also been studied with 2D GMM. The upper second molars of 2 *Homo erectus* (more likely to belong to *Homo heidelbergensis*), 17 *Homo neanderthalensis*, 17 archaic *Homo sapiens* and 80 modern *Homo sapiens* were observed and compared through 2D GMM, proving to be useful to discriminate between *Homo neanderthalensis* and *Homo sapiens*, regardless of whether archaic or modern, which is valuable in fossil assemblages where teeth are isolated (Bailey et al., 2014).

Studies comparing the results obtained through these new techniques with those obtained through traditional methods, while using archaeological samples, as this one, are very rare. Bernal (2007) measured the mesiodistal and buccolingual diameters of 35 upper second molars from North Patagonia, West-Central, and Northeast Argentina, and compared this to a landmark configuration of four landmarks and 79 outline semilandmarks. To observe how both methods capture information about size and shape, the author used the centroid size and the crown index (Bernal, 2007). She observed that, whilst the two methods produced a similar level of information for size, for shape GMM was much more informative than the crown index, capturing a higher level of detail, especially regarding the crown contour (Bernal, 2007). She concluded that GMM would be more appropriate for studies of modern human groups, as the level of variation among groups is smaller (Bernal,2007).

2.5.2 Difficulties in the use of 2D GMM

The main difficulty in the use of 2D GMM lies in choosing the appropriate set of landmarks. Most of the published studies use a combination of sliding semilandmarks (around 30 to 40) and landmarks (around 4 to 8) (Martinón-Torres et al., 2006; Gómez-Robles et al., 2007; Gómez-Robles et al., 2008; Gómez-Robles et al., 2011a; Gómez-Robles et al., 2015). However, there are also studies where the comparison is done exclusively through a few equidistant semilandmarks (Bailey et al., 2014). The set of

landmarks used in GMM must have enough landmarks as to capture the range of variation, without adding variables that do not provide further information.

An interesting observation when applying 2D GMM to dental morphology lies in the set of landmarks chosen to observe the development of a trait that may be absent in some individuals. To observe the development of the fifth cusp in the lower second molars of 129 individuals belonging to 10 different hominin species, a study used six different sets of landmarks/semilandmarks to see whether using a different configuration of points would have an impact on the results (Gómez-Robles et al., 2011b). The results showed that combining semilandmarks with landmarks common to all the individuals and a maximum of one landmark within the potentially absent trait gave the most accurate results in the comparison of the individuals (Gómez-Robles et al., 2011b). Some of the landmark configurations failed to identify differences among the species that are easily observed without GMM (Gómez-Robles et al., 2011b).

2.6. 3D Geometric Morphometrics applied to the study of teeth

Shortly after 2D GMM started to be applied to dental morphology, researchers began to explore the use of 3D GMM. This methodology has been applied especially to fossil remains in studies of hominin evolution, although animal teeth morphology has been explored with 3D too. Its application, difficulties and future directions are explained in the following sections.

2.6.1 Application of 3D GMM in dental anthropology

In animal teeth, Skinner and Gunz (2010) explored the enamel-dentine junction morphology of 55 chimpanzee and bonobo lower molars in relation to the presence of the

cusp 6 scored at that same level. 3D GMM has also proved to be useful in the study of the evolution of hominoids because it allows comparison of the root morphology of fossil teeth that are still in position, without damaging the remains to access the roots. A study that compared 69 hominoid specimens, including different species of apes, with two fossil specimens of the genera *Khoratpithecus* and *Ouranopithecus*, analysed the roots of first and second lower molars (Emonet et al., 2012). The results showed distinct root morphology for the different groups of extant hominoids, which are differentiated with little overlapping (Emonet et al., 2012).

An early study using 3D GMM in human teeth compared the differences observed in the lingual surface of 38 human upper central incisors from different geographic areas when they were scored with the ASU system and when 3D GMM was applied to them at the level of the outer enamel surface and at the level of the enamel-dentine junction (Kato et al., 2011). To compare the results of the two-3D GMM analyses, the authors first visually scored the morphology of the incisors and placed them into two groups: weak degree of shoveling and strong degree of shoveling. They proceed then to landmark the 3D models at the outer enamel surface and at the enamel-dentine junction and observed whether the discrimination between “weak” and “strong” shoveling was maintained (Kako et al, 2011). The results of this analysis show that, whilst there were differences between the “weak” and the “strong” groups when applying 3D GMM in the outer enamel surface, these differences were lost when the methodology was applied to the enamel-dentine junction (Kako et al, 2011). This result does not concur with other studies in which the enamel-dentine junction had proved to be useful to predict the degree of development of nonmetric traits (Skinner et al., 2008; Skinner et al., 2009; Martínez de Pinillos et al., 2014; Martinón-Torres et al., 2014; Skinner et al., 2016).

Many institutions now systematically scan the fossil remains that they curate, sometimes even publishing these scans in freely available repositories (for example, the 3D

Scanning Frontier program of the Smithsonian Institution Digitization Office, in the Smithsonian Museum). This, alongside the fact that 3D GMM allows the study of complex dental morphology of fossil remains without having to handle them directly, has made it a popular tool in studies of hominin evolution. Early Pleistocene remains from Eritrea, in West Africa, consisting of an upper lateral incisor, a lower central incisor, and a lower first or second molar crown, were analysed and compared with remains of *Homo erectus*, *Homo antecessor*, *Homo heidelbergensis*, *Homo neanderthalensis* and modern *Homo sapiens* through dental nonmetric traits, metric variables and 3D GMM (Zanolli et al., 2014). The molar crown, which was found in a site with a high concentration of Acheulean stone tools, was compared to other remains through 3D GMM at the enamel-dentine junction (Zanolli et al., 2014). The molar morphology does not overlap with the crown morphology of any of the other samples, although seems closer to the morphologies of the second molars of *Homo neanderthalensis* and *Homo sapiens* than to the first molars (Zanolli et al., 2014).

Dental fossil remains from other famous archaeological sites, such as Sterkfontein Member 4, have also been analysed using 3D GMM to infer the identity of the Australopithecine species that lived on that site (Fornai et al., 2015). In that study, the authors observed 46 upper second molars from Sterkfontein Member 4 and one from Makapansgat assigned to *Australopithecus africanus* and *Australopithecus prometheus*, and compared them to individuals of *Paranthropus robustus*, early *Homo*, *Homo neanderthalensis*, and modern *Homo sapiens*, using a combination of landmarks and semilandmarks at the outer enamel surface and the enamel-dentine junction, as well as a set of semilandmarks for the crown and the cervical outlines (Fornai et al., 2015). The results indicated a clear separation of *Homo neanderthalensis* and *Homo sapiens* along the second principal component, and *Homo sapiens* was also clearly distinct from *Australopithecus* and *Paranthropus* within the first principal component, but *Australopithecus* and *Paranthropus* overlapped and there was no differentiation between

them (Fornai et al., 2015). Interestingly, the results obtained were consistent regardless of the set of landmarks used and the area observed, although the differentiation among species was more marked when the comparison was done at the level of the enamel-dentine junction (Fornai et al., 2015).

3D GMM can be helpful when isolated deciduous teeth are found in fossil assemblages, since it allows us to compare their morphology to other teeth for various species and assess the resemblances. A study of a single deciduous lower second molar found in Qesem Cave, Israel, and dated around 420,000-220,000 years BP, applied 3D GMM to the enamel-dentine junction and the crown and cervical outlines (Fornai et al., 2016). The results of this study placed the tooth closer to *Homo neanderthalensis* than to *Homo sapiens* for both the cervical outline and the enamel-dentine junction, which concurs with the results of previous analyses with other methods (Fornai et al., 2016). The crown outline does not yield such consistent results, as it places the individual in an intermediate place between *Homo neanderthalensis* and *Homo sapiens* (Fornai et al., 2016).

Following on with studies of biological resemblance between hominin fossil remains, Skinner and colleagues published a study in 2016 around the dental remains found in the cave of Mala Balanica, in Serbia. Three lower molars (first, second and third), dated around 400,000 years BP, were examined at the enamel-dentine junction and compared to 141 individuals of *Homo erectus*, *Homo neanderthalensis* and archaic and modern *Homo sapiens* (Skinner et al., 2016). The results of this analysis concur with previous analyses on the enamel-dentine junction (Skinner et al., 2008; Skinner et al., 2009; Martínez de Pinillos et al., 2014; Martinón-Torres et al., 2014), proving that comparing the morphology of this dental region is useful in taxonomic studies (Skinner et al., 2016). In particular, the morphology of the three molars analysed in this study falls in between the morphology observed in *Homo erectus* and the one observed in modern *Homo sapiens* (Skinner et al., 2016). Another study using 3D GMM is the analysis of a third upper molar and a first and a

second upper premolars dated around 60,000 BP from the cave of Marillac, in France, comparing them to other teeth belonging to *Homo neanderthalensis* at the level of the enamel-dentine junction (Garralda et al., 2020). Although the aim of this analysis was not to carry out an exhaustive taxonomic study but to do a comprehensive characterisation of the dental remains found at the site, the GMM analysis revealed that the morphology of the studied teeth at the enamel-dentine junction fits within the morphology of other Eurasian Neandertals (Garralda et al., 2020).

2.6.2 Difficulties and Further Directions

The limiting factor in 3D GMM is data acquisition. All analyses that use 3D GMM to explore differences in dental morphology use a micro-CT to acquire their data (Skinner and Gunz, 2010; Kako et al., 2011; Emonet et al., 2012; Zanolli et al., 2014; Fornai et al., 2015; Fornai et al., 2016; Skinner et al., 2016; Garralda et al., 2020). In this study I used a structured-light scanner, which also helped me to assess whether limiting the resources (i.e., equipment) could affect the results. Another important issue is the lack of homology of the sets of landmarks used, for example when teeth with different levels of wear are used and the landmarks are placed in positions that are sensitive to wear, or when landmarks are placed in traits that may not be present in all the teeth, and the difficulty in placing the landmarks. However, the problems linked to the sets of landmarks found when working with 3D GMM will be very similar to those reviewed in 2D GMM.

To tackle some of the issues with 3D GMM, another methodology is being developed, which may give better results when comparing human teeth: diffeomorphic surface matching (Braga et al., 2019). This new methodology solves the issue of lack of homology between landmarks, commonly found in teeth when dental nonmetric traits may be absent in some teeth and highly developed in others (Braga et al., 2019). It is especially interesting in studies that focus on the outer enamel surface, where the lack of homologous points is

larger (for example, due to wear). Diffeomorphic surface matching is based on the matching of the surfaces in a continuous way, without relying upon point positions (Braga et al., 2019). This new methodology, although not yet widely used, has given more accurate results than 3D GMM when observing lower molars of *Australopithecus africanus*, *Paranthropus robustus* and early *Homo sp*. found in the sites of Sterkfontein and Swartkrans, in South Africa (Braga et al., 2019). Diffeomorphic surface matching has been used to characterise the morphology of teeth belonging to species with little fossil remains, where the taxonomic relationships with other species are unclear, as for example with the postcanine teeth of *Homo luzonensis* found in Callao Cave, in Philippines, for which diffeomorphic surface matching was used to analyse its resemblance to *Homo erectus* (Zanolli et al., 2022). Interestingly, some studies that aim to characterise dental fossils now combine the use of 3D GMM with diffeomorphic surface matching (Pan et al., 2022). Using a battery of methods that include metric variables, ASU system, 3D GMM and diffeomorphic surface matching, Pan and colleagues (2022) studied the six teeth of the individual from Gongwangling of Lantian, in Central China, and compared them with teeth belonging to African and Asian *Homo erectus*, *Homo antecessor*, *Homo heidelbergensis*, *Homo neanderthalensis* and modern *Homo sapiens*. The results obtained using these methods are not entirely consistent, as the analyses done with metric variables and the ASU system place the fossils taxonomically closer to Early Pleistocene *Homo erectus*, and those based on shape place them closer to Middle Pleistocene *Homo erectus*. These results leave the door open to further analyses of the shape of dental remains belonging to *Homo erectus*, to assess variations in the specimens from East Asia (Pan et al., 2022).

3. Materials and Individuals

This chapter summarises information about the ASU Dental Anthropology System casts used and the archaeological samples studied.

3.1. ASU Dental Casts

The ASU Dental Anthropology System describes and standardises 39 dental traits, 19 of which are modelled in plaster casts to show their different degrees of expression (Turner et al., 1991; Scott and Turner, 1997; Scott, 2008). I included 13 of these casts, choosing traits that were not asymmetrical, were not too sensitive to wear and were not correlated with other traits (Table 3.1). I excluded all the dental traits for which there are no casts and 6 of the traits for which there are casts, but which were not appropriate for the study because they did not meet the criteria (Table 3.2).

Table 3.1: Dental traits included in this study

Focus teeth	Trait	Description
Upper Central Incisor	Shoveling	Distal and mesial marginal ridges on the lingual surface
	Double-shoveling	Distal and mesial marginal ridges on the labial surface
Upper Lateral Incisor	Tuberculum Dentale	Ridges and tubercles in the lingual surface
First Upper Molar	Metaconule	Distal cusp (cusp 5), between the metacone and the hypocone
	Carabelli's Trait	Tubercle on the lingual surface of the protocone (mesiolingual cusp – cusp 1)
Second Upper Molar	Hypocone	Distolingual cusp (cusp 4)
Third Upper Molar	Parastyle	Tubercle on the buccal surface of the paracone (mesiobuccal cusp – cusp 2)
	Metacone	Distobuccal cusp (cusp 3)
First Lower Molar	Entoconulid	Distal cusp (cusp 6), between the hypoconulid and the entoconid (distolingual cusp or cusp 4)
	Metaconulid	Cusp (cusp 7) between the metaconid (mesiolingual cusp or cusp 2) and entoconid
	Protostylid	Tubercle on the buccal surface of the protoconid (mesiobuccal cusp – cusp 1)

	Anterior Fovea	Groove linking the protoconid and the metaconid between the marginal and the median ridges
Second Lower Molar	Hypoconulid	Distal cusp (cusp 5)

Table 3.2 Dental casts excluded from this study

Trait	Reason to exclude
Labial Convexity	Inversely correlated with, and more difficult to quantify than, double-shoveling
Mesial Canine Ridge	Tends to be asymmetrical (Turner et al., 1991), which is a problem when quantifying archaeological collections, in which it is rare to have both antimeres
Canine Distal Accessory Ridge	Only observable in unworn teeth
Premolar Lingual Cusp Variation	The variation lies in the number of cusps, so it would be difficult to observe using GMM as there would be no homologous landmarks
Tome's Root	It is not possible to study root traits without invasive methods
Deflecting Wrinkle	Only observable in unworn teeth

3.2. Archaeological Samples

I studied 836 teeth belonging to 188 individuals from three different archaeological sites from the Iberian Peninsula (Table 3.3). The majority of individuals in my samples were over 12 years of age, and the sample size for the decidual dentition was very small. Thus, I restricted my research to the permanent dentition in individuals of all ages. I collected information from both antimeres when they were available but excluded the antimere with a lower degree of expression of the dental trait from the final analysis for the nonmetric analyses, as recommended in the standards (Turner et al., 1991; Scott and Turner, 1997).

Table 3.3 Summary of sites and individuals included from each site

	SMA (Santa Maria la Real – Christians – North of IP)	SNA (San Nicolas de Avila – Muslims – Centre of IP)	XAR (Xarea – Muslims – South of IP)
Females	4	51	12
Males	18	23	12
Unknown	13	34	21
Total	35	108	45

The skeletal samples are all from cemeteries of medieval date (10th to 15th century). Their cultural environments and their religious adscription differ, with two samples coming from a Christian environment (SMA and SNA) and one from a Muslim environment (XAR), whereas one sample is Christian (SMA) and two are Muslims (SNA and XAR). Their geography is also diverse; on the west-east axis, two are in the centre and one in the east of the Iberian Peninsula, whilst on the north-south axis, one is in the north, another in the centre and the third in the south (Figure 3.1).

Figure 3.1: Map of the Iberian Peninsula with the location of the three archaeological samples studied (map made in ArcGIS)

3.2.1 Santa María la Real (SMA)

This skeletal sample comes from the monastery *Santa María la Real*, in *Aguilar de Campóo,* in the northern region of *Palencia*, and is currently curated in the Complutense University of Madrid. The construction of the monastery, which belonged to the Monastic

Order of the Premonstratensians, started during the 11th century, and it was modified over time until the 18th century. It was finally abandoned in the 19th century. The archaeological excavation of the remains, which occurred between 1988 and 1991, identified six different levels, all related to the construction phases dated between the 11th and 13th centuries (Matesanz, 1993). There is a remarkable *antemortem* tooth loss in this archaeological population, which may be related to a high proportion of mature and older individuals. There is also a very high prevalence of *postmortem* tooth loss, probably linked to the difficult conditions of the archaeological excavation, since many of the tombs were flooded (Matesanz, 1993; Grueso *et al.*, 2009; Grueso *et al.*, 2011). The sex and age distribution of this collection is known (Grueso, 2009) (Table 3.4).

Table 3.4 Sex and age distribution of individuals in the SMA skeletal sample (Grueso, 2009)

	Child (0-12)	Young (12-20)	Adult (20-40)	Mature (40-60)	Elder (60+)	Total
Females	0	0	6	1	0	7
Males	1	5	24	12	1	43
Unknown	7	3	6	9	2	27
Total	8	8	36	22	3	77

The demography of this sample, alongside the fact that they were buried in a Christian monastery, led to the conclusion that they may have been monks, since similar distributions are seen in other monastic environments (Du Souich *et al.*, 1990). Given the significant *postmortem* tooth loss and the relatively old age of the individuals, I included only 35 of the 77 individuals, with a total of 91 teeth (Tables 3.5 and 3.6). Only four of the individuals were female, and most were adults between 20 and 40 years of age (Table 3.5). Nearly all of the teeth included were molars, as there were no upper central incisors with a score of wear under four, and only five lateral incisors that met the wear requirements (Table 3.6). Most of the teeth included were in degrees of wear 2 or 3 (Table 3.6).

Table 3.5 Sex and age distribution of individuals of SMA included in the analyses

	Child (0-12)	Young (12-20)	Adult (20-40)	Mature (40-60)	Elder (60+)	Total
Females	0	0	4	0	0	4
Males	1	5	11	1	0	18
Unknown	3	2	4	4	0	13
Total	4	7	19	5	0	35

Table 3.6 Summary of teeth from the SMA population included in this study

	Wear				Total
	Score 1	Score 2	Score 3	Score 4	
Upper Central Incisor	0	0	0	0	0
Upper Lateral Incisor	0	2	3	0	5
Upper First Molar	3	5	4	2	14
Upper Second Molar	4	8	2	3	17
Upper Third Molar	3	8	0	0	11
Lower First Molar	2	2	7	6	17
Lower Second Molar	3	7	12	5	27
No. of Teeth	15	32	28	16	91

3.2.2 San Nicolás de Ávila (SNA)

This skeletal sample comes from the largest Muslim cemetery, or *maqbara*, found in the Castilian territory, and is currently partially curated in the Complutense University of Madrid. It was discovered in 1999, and more than 3000 individuals were recovered, radiocarbon dated to the 10th to 14th centuries (Barrio and Trancho, 2008; Barrio et al., 2009; Barrio and Trancho, 2014; Barrio, 2015).

The *maqbara* was outside the city walls of *Ávila*, which had been a border area since the Muslim conquest of 711, finally falling within the Christian territories after 1085. The archaeological site's chronology indicates that the people buried in this cemetery were Muslims who lived during the period after 1085 when the site was within the Christian territory. The graves were oriented east-west or northeast-southwest, and the bodies were lying on their right side, with the head pointing to *Mecca*, the sacred Arab city of Makkah, and with little or no grave goods, as per the Muslim tradition. Some funerary steles, which

are scarce in *maqbaras*, were found, and they were simple, with little decoration or engraved with quotes from the *Quran* (Barrio, 2015).

Of the 600 individuals curated in the Complutense University of Madrid, 341 have been analysed in previous studies and as such their sex and age have been estimated (Barrio and Trancho, 2014; Barrio, 2015). The sex and age distribution shows a younger population than SMA, with a high proportion of female individuals (Table 3.7).

Table 3.7 Sex and age distribution of individuals in the SNA archaeological population (Barrio and Trancho, 2014; Barrio, 2015)

	Child (0-12 yr)	Young (12-20 yr)	Adult (20-40 yr)	Mature (40-60 yr)	Elder (60+ yr)	Total
Females	13	18	61	48	3	143
Males	2	9	39	44	3	97
Unknown	39	16	17	29	0	101
Total	54	43	117	121	6	341

I recorded data for all the teeth with a score of dental wear of up to 4, with a total of 528 teeth belonging to 108 individuals (Tables 3.8 and 3.9). Nearly half of the individuals were female and 34 individuals were of unknown sex (Table 3.8). Most of the individuals were children or young people under 20 years of age (Table 3.8). Most of the teeth (over 400) had very little wear, in scores 1 or 2, and only 16 were scored with a grade 4 (Table 3.9). The second molars (upper and lower) accounted for nearly half of the teeth included (Table 3.9).

Table 3.8 Sex and age distribution of individuals of SNA included in the analyses

	Child (0-12)	Young (12-20)	Adult (20-40)	Mature (40-60)	Elder (60+)	Total
Females	8	13	28	2	0	51
Males	2	8	10	3	0	23
Unknown	20	10	4	0	0	34
Total	30	31	42	5	0	108

Table 3.9 Summary of teeth from the SNA population included in this study

	Wear				Total
	Score 1	Score 2	Score 3	Score 4	
Upper Central Incisor	11	17	21	7	56
Upper Lateral Incisor	2	19	7	1	29
Upper First Molar	21	24	33	3	81
Upper Second Molar	29	82	5	1	117
Upper Third Molar	28	44	1	0	73
Lower First Molar	22	23	26	4	75
Lower Second Molar	22	66	9	0	97
No. of Teeth	135	275	102	16	528

3.2.3 Xarea (XAR)

The skeletal sample of Xarea comes from a *maqbara* excavated in 1995 in *Vélez-Rubio, Almería*, in what was part of Granada's Kingdom from 1232 until 1492, with brief Christian governance of the area from 1436 to 1447 (Robledo, 1998). The population comprises 229 individuals, radiocarbon dated from the middle 13th to early 15th centuries (Robledo, 1998; Robledo and Trancho, 2001). Therefore, these individuals were Muslims who lived in a Muslim territory. The graves were oriented northeast-southwest, and the bodies were lying on their right side, with the head pointing to *Mecca* and no grave goods. Nineteen funerary steles were also excavated during the archaeological works (Robledo, 1998).

Table 3.10 Sex and age distribution of individuals in the XAR archaeological population (Robledo, 1998)

	Child (0-12)	Young (12-20)	Adult (20-40)	Mature (40-60)	Elder (60+)	Total
Females	0	4	32	50	1	87
Males	0	5	46	52	3	106
Unknown	32	1	3	0	0	36
Total	32	10	81	102	4	229

These 229 individuals are curated in the Complutense University of Madrid, where they have been studied (Robledo, 1998). The sex and age distribution shows a slightly older population than SNA, with an approximately even sex ratio (Table 3.10).

I collected information for all the teeth with a wear score of up to 4, with a total of 214 teeth belonging to 45 individuals (Tables 3.11 and 3.12). The number of female and male individuals was well balanced, but nearly the same number of the individuals were of unknown sex (Table 3.11). There were as many children under 12 years of age as adults up to 40, whereas there was only one individual in the range of 12 to 20 years (Table 3.11). Most of the teeth analysed scored 2 or 3 for wear (Table 3.12) There were as many upper central incisors as upper lateral incisors, and for this population the balance between the numbers of the different teeth was good, with only the lower molars being over-represented (Table 3.12).

Table 3.11 Sex and age distribution of individuals of XAR included in the analyses

	Child (0-12)	Young (12-20)	Adult (20-40)	Mature (40-60)	Elder (60+)	Total
Females	0	0	8	4	0	12
Males	0	0	6	6	0	12
Unknown	17	1	3	0	0	21
Total	17	1	17	10	0	45

Table 3.12 Summary of teeth from the XAR population included in this study.

	Wear				Total
	Score 1	Score 2	Score 3	Score 4	
Upper Central Incisor	5	6	9	6	26
Upper Lateral Incisor	3	18	5	1	27
Upper First Molar	4	8	11	6	29
Upper Second Molar	6	13	10	1	30
Upper Third Molar	6	12	5	0	23
Lower First Molar	6	10	15	9	40
Lower Second Molar	4	15	17	3	39
No. of Teeth	34	82	72	26	214

4. Methods

4.1. Osteological methods

I collected information about the sex and age of the individuals from previous osteological reports (Barrio, 2015; Grueso, 2009; Robledo, 1998). These reports used standard variables such as the degree of synostosis of cranial sutures, morphological changes of the pubic symphysis and height of the dental crowns to estimate the age of skeletal remains (Table 4.1), and differences in the cranium, mandible, ilium and pubis to establish the sex (Table 4.2).

Table 4.1 Methods used for the estimation of the age of the individuals

Sample	Reference	Adults/Non-adults	Methods
SMA	Grueso, 2009	Adults	Cranial sutures (Meindl and Lovejoy, 1985)
			Pubic symphyseal development (Suchey et al., 1988)
			Height of the dental crowns (Walker and Shapiro, 1992)
		Non-adults	Dental development (Ubelaker, 1989)
SNA	Barrio, 2015	Adults	Pubic symphyseal development (Suchey et al., 1988)
			Cranial sutures (Olivier, 1960; Masset, 1989)
			Dental Wear (Miles, 1962; Smith, 1984)
		Non-adults	Dental development (Ubelaker, 1989)
			Long bone length (Fazekas and Kósa, 1978)
			Degree of epiphyseal fusion (Brothwell, 1981)
XAR	Robledo, 1998	Adults	Cranial sutures (Meindl and Lovejoy, 1985)
			Pubic symphyseal development (Suchey et al., 1988)
			Height of the dental crowns (Walker and Shapiro, 1992)
		Non-adults	Dental development (Ubelaker, 1989)
			Degree of epiphyseal fusion (Pacciani and Chiarelli, 1993)
			Long bone length (Ubelaker, 1989)

Table 4.2: Methods used for the estimation of the sex of the individuals

Sample	Reference	Methods
SMA	Grueso, 2009	Differences in cranium and mandible (Ferembach et al., 1980, Bass, 1987; Brothwell, 1987)
SNA	Barrio, 2015	Differences in ilium and pubis (Ferembach et al., 1980) Differences in cranium and mandible (Ferembach et al., 1980) Discriminant functions for bones length (López-Bueis et al., 1996, 2000; Trancho et al., 1996, 1997, 2000, 2012; Barrio et al., 2006)
XAR	Robledo, 1998	Differences in ilium and pubis (Phenice, 1969; Rivero, 1985; Anderson,1990) Differences in cranium and mandible (Ferembach et al., 1980, Bass, 1987; Brothwell, 1987) Discriminant functions for bones length developed ad-hoc

I also collected information about the degree of dental wear, measured the buccolingual and mesiodistal diameters, calculated the crown area and the crown index, and studied the chosen nonmetric dental traits.

4.1.1 Dental wear

As established in chapter 3 (Materials), I only included teeth with a low degree of dental wear. I scored dental wear on a scale of 1-8, following Smith's method (1984) (Figure 4.1). I discarded all teeth with a score of 5 or higher to prevent wear from having an effect on my data collection, either through altering the metric values, impacting the visibility of nonmetric traits, or affecting the homology of the landmarks.

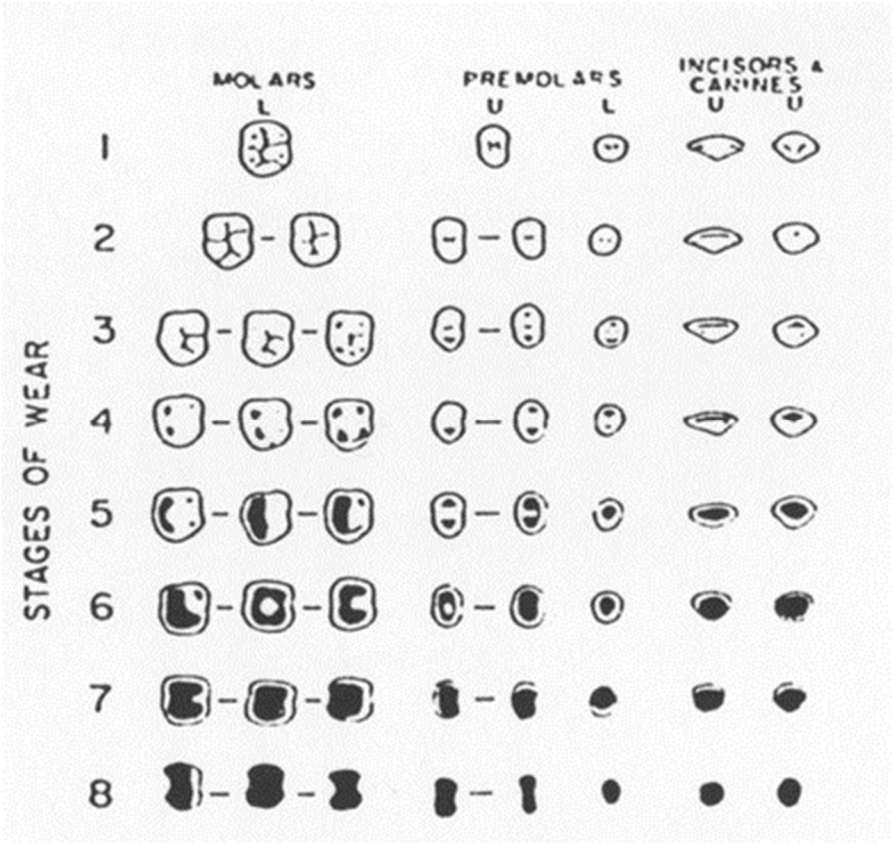


Figure 4.1 Diagrams from Smith (1984) to score dental wear

4.1.2 Metric variables

I took measurements using an electronic calliper to the nearest 0.01 mm. I measured the mesiodistal and buccolingual diameters of crowns following the method described by Lefèvre (1973). For incisors, the mesiodistal diameter is the maximum width, and the buccolingual diameter is the maximum thickness. For molars, the mesiodistal diameter is the maximum distance between the mesial and the distal faces on a parallel plane to the occlusal face, using the mesial face as reference (Figure 4.2a). The buccolingual diameter for molars is the maximum distance between the buccal and the lingual faces on a parallel plane to the occlusal face, using the flattest face as reference (this is usually the buccal face for upper molars and the lingual face for lower molars) (Figure 4.2b). This method has low interobserver error, can be used both in loose and *in situ* teeth, and has low sensitivity to

wear (Lefèvre, 1973; Martinón-Torres et al., 2008; Martinón-Torres et al., 2012). Some authors use the method described by Bailey and Lynch (2005) to apply a correction for interproximal dental wear (Martinón-Torres et al., 2006). However, this correction requires taking a photograph of the occlusal view and reconstructing the original crown margin over the digital image, to then take the measurements over the reconstructed crown margin. I measured the teeth directly using a calliper, so I did not apply this correction.

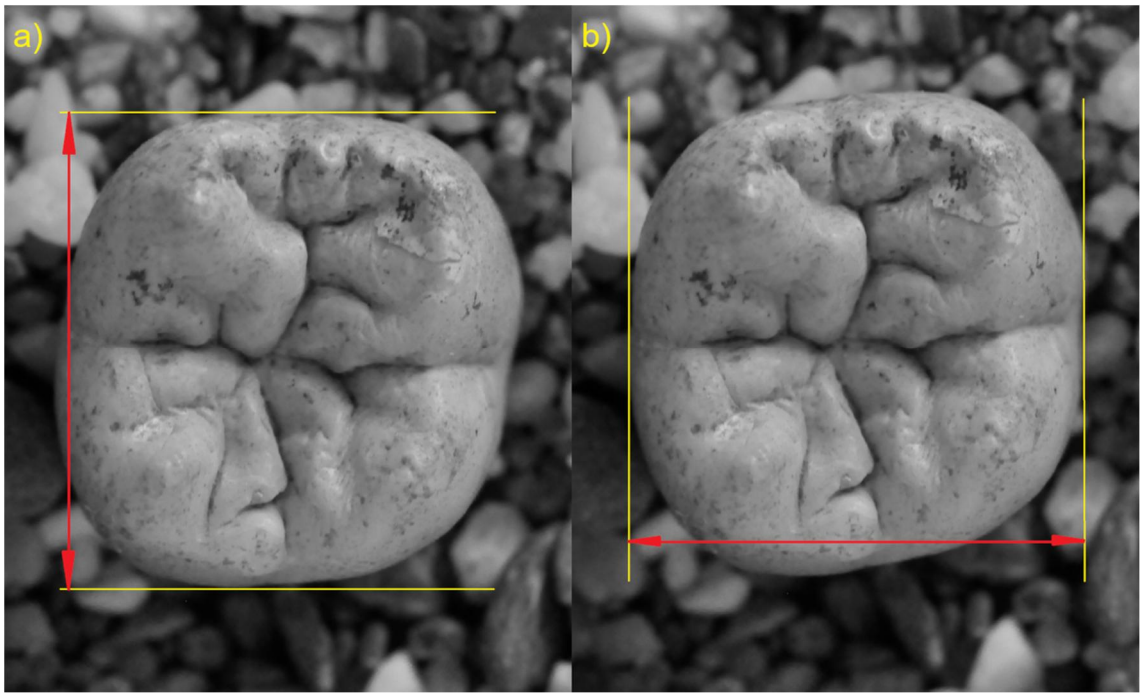


Figure 4.2 Measurements taken following Lefèvre (1973): (a) Mesiodistal diameter, and (b) Buccolingual diameter

I used these measurements to calculate the crown area, as an indicator of the size of the crown, and the crown index, which gives information about the crown proportions (Cabellos, 2007; Kondo et al., 2005):

$$Crown\ area = Mesiodistal\ diameter \times Buccolingual\ diameter$$

$$Crown\ index = \frac{Buccolingual\ diameter}{Mesiodistal\ diameter} \times 100$$

4.1.3 Nonmetric Dental Traits

I evaluated the development of the chosen dental traits by comparison with the ASU dental casts and followed the standard procedures (Turner et al., 1991; Scott and Turner, 1997). I assessed each trait in the “focus teeth”, as recommended by Scott and Turner (1997). The traits can be seen sometimes in different teeth; however, the focus teeth are those which are most useful for the study of biological affinities using dental traits. For example, although it is possible to observe the hypocone in first and second upper molars, the focus tooth is the second upper molar because its presence is nearly constant in first upper molars.

Table 4.3 List of dental traits observed and scores regarded as absence

Dental trait	Focus tooth	Absence
Shoveling	UCI	0
Double-shoveling	UCI	0
Tuberculum Dentale	ULI	0-1
Metaconule	UM1	0
Carabelli's trait	UM1	0-1
Hypocone	UM2	0-1
Parastyle	UM3	0
Metacone	UM3	0-1
Entoconulid	LM1	0
Metaconulid	LM1	0
Protostylid	LM1	0
Anterior Fovea	LM1	0
Hypoconulid	LM2	0

After assessing each tooth against the dental casts and giving it a score for each dental trait, I dichotomised the results into "absent" and "present", choosing the cut-off to dichotomise the results for a trait based on the degree of development that the trait had in the studied samples (Table 4.3). For example, if most of the individuals scored 2 or more for a given trait, I considered that trait "absent" in individuals that scored 0 and 1, and "present" in individuals that scored 2 or more. I based this threshold on my own observation

of the samples. This is a standard practice when using the ASU system (Cabellos, 2007; Martinón-Torres et al., 2012).

4.2. Statistical analysis

I set the statistical significance level (α) at 0.05

4.2.1 Descriptive statistics

I used R© (R Core Team, 2019) for all statistical tests (unless specified). I calculated the mean, median, variance and standard deviation for each metric variable. Additionally, I looked at the minimum and maximum values to detect mistakes in data acquisition or processing.

4.2.2 Normality

I used the Shapiro-Wilk normality test and a histogram to determine whether the metric variables were normally distributed. I used a Quantile-Quantile (Q-Q) plot to detect outliers.

When the population distribution for a given tooth was not normal, I identified the outliers in the Q-Q plot and excluded them from the analysis. If this did not achieve a normal distribution, I included the outliers and transformed the data for the three samples to Naperian logarithm. If any of the distributions were still not normal, I excluded the outliers in the transformed data. This procedure resolved the situation in all cases.

4.2.3 Homoscedasticity

I assessed the homoscedasticity (homogeneity of variances) of the metric variables using the Bartlett test and the Fligner-Killeen test. I assumed data were homoscedastic only when both tests were non-significant. The Bartlett test is robust but more sensitive to deviations from normality, and although I checked for normality, combining this test with the Fligner-Killeen test, which is very robust in non-normal distributions, improved the inference of homoscedasticity.

4.2.4 Analysis of Variance

I used an Analysis of Variance (ANOVA) to test for statistically significant differences among the metric variables of the samples when their variances are homogeneous. ANOVA tests whether the mean differences are larger among groups than within groups. To test for differences in the upper central incisors, for which I only had data for SNA (○) and XAR (○), I used a Student's t-Test, a special case of ANOVA when there are only two groups for comparison.

When the ANOVA was significant, I carried out a post hoc analysis with Tukey's test, which compares the mean of all the samples (as if they were a single population) to the mean of each sample, establishing among which samples there are significant differences.

4.2.5 Kruskal-Wallis analysis of ranks

I used the nonparametric Kruskal-Wallis test to test for significant differences among the metric variables of the samples for variables that did not have homogeneous variances.

If the Kruskal-Wallis test showed significant differences among the samples, I used pairwise Mann-Whitney tests to establish where these differences lay. The Mann-Whitney test is a nonparametric test that, like the Kruskal-Wallis test, depends on the sums of ranks.

4.2.6 Smith's Mean Measure of Divergence (MMD)

I used Smith's Mean Measure of Divergence (MMD) in the package AnthropMMD (Santos, 2018) in R© v3.6.1 (R Core Team, 2019) to examine the biological distances among the samples using the observed dental nonmetric traits. Smith's MMD is a multivariate statistical analysis that provides information on phenetic likenesses among samples, estimating their dissimilarity, with lower values indicating more similarity (Guatelli-Steinberg et al., 2001; Irish, 2000; Irish, 2005; Irish, 2006; Irish, 2010). Smith's MMD is often used to infer biological likeliness using dental nonmetric traits (Irish, 1997; Irish, 1998; Cucina et al., 1999; Irish, 2000; Guatelli-Steinberg et al., 2001; Hallgrímsson et al., 2004; Irish, 2005; Bollini et al., 2006; Cabellos, 2007; Coppa et al., 2007; Edgar, 2007; Irish and Konisberg, 2007; García-Sívoli, 2009; Martinón-Torres et al., 2012; Sołtysiak and Bialon, 2013; Carter et al., 2014; Irish et al., 2014; Irish, 2016). An alternative statistical analysis is Mahalanobis D^2. These provide comparable results and Smith's MMD is less sensitive to missing values than Mahalanobis D^2, although it requires that the traits are not correlated (Irish, 2010; Nikita, 2015).

Smith's MMD depends on sample size, and trait frequencies should not be too low (≤ 0.05) or too high (≥ 0.95) to discriminate populations (Green & Suchey, 1976; Harris and Sjøvold, 2004; Irish, 2005; Irish 2006; Cabellos 2007). Either Asconmbe or Freeman and Tukey corrections can be used to correct this, and I chose the Freeman and Tukey formula, which is the most widely used (Harris and Sjøvold, 2004; Irish, 2005; Irish 2006; Cabellos 2007). To get a more robust result with Smith's MMD, it is advisable to exclude traits that are non-discriminatory among the populations (because all the studied populations have

them in high or low expression) (Harris and Sjøvold, 2004; Irish, 2010). I chose to exclude traits with an overall negative measure of divergence, and also used only those with a minimum of five individuals per population.

To visualise the data as a hierarchical cluster, I chose Ward's algorithm of minimum variance in AnthropMMD (Santos, 2018) in R© (R Core Team, 2019). This method uses the minimum variance among the groups for each cluster to create the hierarchical cluster.

4.3. Geometric morphometrics (GMM)

GMM allow us to compare the shape of different objects through the various configurations of groups of equivalent points called landmarks and semilandmarks defined in a Cartesian axis in 2 or 3 dimensions (O'Higgins, 2000; Richtsmeier et al., 2002; Slice, 2005; Slice, 2007; Baab et al., 2012). Shape is the geometric information that is not affected by position, scale and orientation (Kendall, 1977, as cited in Zelditch et al., 2004; O'Higgins, 2000; Slice, 2005; Slice, 2007).

Variation in shape can be visualised in the thin-plate spline, which is a grid whose squares deform to represent different shapes. It illustrates the amount of deformation required to go from the shape of a specimen to another, or to the mean shape. The amount of energy required to deform the grid from the reference shape to a given shape is called the bending energy (Bookstein, 1991; Bookstein, 1997).

Landmark types differ depending on how they are described and their reliability: Type I landmarks are anatomically defined, and are more reliable, as they are easy to find across a range of specimens. These landmarks are usually where different tissues or bones meet, for example the *Nasion* (middle point of the nasofrontal suture) of the human skull would be a type I landmark. Type II landmarks are geometrically defined, for example a maximum

curvature. In the human skull, the *Basion* (middle point on the anterior margin of the foramen magnum) would be considered a landmark type II. Type III or semilandmarks are landmarks that can be placed following a reference but lack a specific location. An example of landmark type III would be the *Euryon* (most lateral point placed on the side of the skull) in the human skull (Bookstein, 1991; O'Higgins, 2000).

The equivalence of landmarks, understood as biological homology, has been debated by Macleod (2001), who argues that biological homology applies to structures, rather than to mathematically defined points. However, as explained by Gunz et al. (2005), "homology" in GMM does not refer to biological or evolutive homology but to geometric homology, which is related to the variation in a structure's location. Bookstein (1997) resolves the lack of homology in certain structures by defining semilandmarks in outline shapes (i.e., curves). The semilandmarks are a series of points whose location is relative to one another in accordance with a defined rule (for example, a number of equidistant points between two defined positions), and their use reduces the bending energy of deformation when sliding. Sliding helps to minimise shape differences caused by the different disposition of landmarks along the curve from one specimen to another (Bookstein, 1997; Gunz et al., 2005).

4.3.1 Image acquisition

To acquire images of the archaeological remains, I used a Nikon® D90 digital reflex camera fitted with a 60 mm microlens (AF-S Micro Nikon® 60 mm) and attached to a Kaiser® photographic stand with four light bulbs and a grid baseboard. I placed the teeth on the stand in a sand bed and orientated them with the mesial face looking upwards, the distal face backwards, and the occlusal face horizontal and parallel to the camera, which I levelled using a spirit level, to avoid lateral parallax. To avoid vibration of the camera whilst taking the photographs, I used a wireless camera trigger. As with the casts, I placed the

scale at the occlusal plane level, took pictures of both antimeres and mirror-imaged the left antimeres with Adobe Photoshop®.

I took additional pictures of the buccal surface of the parastyle and protostylid traits. Similarly, for Carabelli's trait, I took additional pictures of the lingual surface, with the teeth lying on the buccal surface and the occlusal face looking upwards, and the scale placed on the lingual plane.

For 3D analysis, I tried to use a Next Engine 2000® laser surface scanner at the highest scanning resolution to scan the archaeological remains. I then processed the scans with Scanstudio HD Pro® and Geomagic® software, as recommended by Slizewski and co-workers (2010). However, the crown enamel reflects the laser and produces a vibration effect on the scan, complicating the use of laser scanners. Furthermore, I had limited access to the archaeological collections, so I needed a faster scanner. I had to test many different scanners, as their different characteristics affected the scan obtained, and some of them, which proved to be very quick in the data acquisition, produced poorly detailed scans insufficient to observe the dental surface. I also tried photogrammetry, but again the results were not sufficiently detailed. For all the above reasons, I chose a structured light surface scanner PicoScan® to scan the teeth. I processed the scans with Mephisto Process® software. The differences between laser and structured light scanners are that while laser scanners use a laser beam to collect a cloud of points with defined x, y and z coordinates, structured light scanners project a plane of light over a target placed on the turntable after scanning a calibration board and collect information about the bending of the light beans (Errickson et al., 2017).

4.3.2 Landmarking

To carry out GMM analysis in 2D, I used several sets of landmarks for the crown shape of each focus tooth, to establish whether the results differed when using different landmarks. My aim when I chose my sets of landmarks was to be able to observe the impact in the crown shape of the different dental traits, and that is the reason why some sets only differ from one another in one or two landmarks. I used a different number of sets of landmarks in each focus tooth, depending on the shape changes that I was trying to capture. For example, for LM1 I used five sets of landmarks (Table 4.4), whereas for LM2 I only used two (Table 4.5). I based most of the combinations of landmarks and semilandmarks along the outline of the crown from the occlusal view and the main cusps in previous research in 2D GMM, carried out by other authors in molars and premolars of extinct and extant mammals (Gómez Cano et al., 2013; Martinón-Torres et al., 2006; Gómez Robles et al., 2007; Gómez Robles et al., 2008; Gómez Robles et al., 2011a; Gómez Robles et al., 2011b).

In LM1 (Table 4.4; Figure 4.3) I wanted to observe the shape of the outline of the crown, along with the position of the main cusps (protoconid, hypoconid, entoconid and metaconid), and determine whether including landmarks for the main cusps would provide more information to differentiate between the populations than using just the outline. I also wanted to explore whether the position of the landmarks for the main cusps differs between crowns with a well-developed metaconulid and those without a metaconulid. I included a landmark set without landmarks for the main cusps (LM1 Outline) to see whether adding the cusps would be more informative or the same results could be achieved with fewer landmarks. I created the landmark set LM1 Anterior Fovea to explore changes in the region of the anterior fovea. However, to prevent issues with lack of homology, I avoided including landmarks that were not present in all individuals. Therefore, I landmarked the outline, the main cusps, and the most mesial point of the central groove. The reasoning behind this last

landmark was that teeth with a well-developed anterior fovea would have the most mesial point of the central groove at a more central location than teeth without an anterior fovea, for which the central groove would finish close to the mesial face. Moreover, landmarking the central groove and not the anterior fovea allowed me to add all the landmarks in teeth in which the anterior fovea was not expressed.

Table 4.4 Sets of 2D landmarks for Lower Molar 1, with the set name, and the number, type and description of landmarks.

Landmark set	Number	Type	Description
LM1	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the protoconid
	42	II	Tip of the hypoconid
	43	II	Tip of the entoconid
	44	II	Tip of the metaconid
LM1 Outline	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
LM1 Anterior Fovea	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the protoconid
	42	II	Tip of the hypoconid
	43	II	Tip of the entoconid
	44	II	Tip of the metaconid
	45	III	Most mesial point of the central groove
LM1 Entoconulid	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the protoconid
	42	II	Tip of the hypoconid
	43	II	Tip of the entoconid
	44	II	Tip of the metaconid
	45	II	Tip of the hypoconulid
LM1 Protostylid Buccal	1-30	III sliding	Outline of the buccal face of the crown, starting from the mesial cementoenamel junction and finishing at the distal cementoenamel junction

I chose the LM1 Entoconulid (Table 4.4; Figure 4.3) landmark set to observe the development of the entoconulid, by placing a landmark in the tip of the hypoconulid in addition to the semilandmarks for the outline and the landmarks for the main cusps. The rationale for this is that when an entoconulid is present, the hypoconulid will be further away from the entoconid. The larger the entoconulid, the further apart the hypoconulid and the

entoconid will be. Although the hypoconulid is a dental trait, and as such is susceptible to not being present in all molars, lower first molars without it are uncommon (Scott and Turner, 1997). Finally, I tried to observe the development of the protostylid with the landmark set LM1 Protostylid, which included 30 semilandmarks for the outline of the buccal face, as the protostylid would be difficult to observe from an occlusal view, and therefore it is unlikely that the landmarks placed in the occlusal face are affected by a different degree of expression of the protostylid.

Table 4.5 Sets of 2D landmarks for Lower Molar 2, with the set name, and the number, type and description of landmarks.

Landmark set	Number	Type	Description
LM2 Hypoconulid	1-40	III sliding	Outline of the crown, starting from the most mesial point and following towards the lingual face
	41	II	Tip of the protoconid
	42	II	Tip of the hypoconid
	43	II	Tip of the entoconid
	44	II	Tip of the metaconid
LM2 Outline	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face

I created the LM2 Hypoconulid landmark set (Table 4.5; Figure 4.3) to explore changes in this dental trait. I expected that the greater the degree of development of the hypoconulid, the greater the distance there would be between the hypoconid and the entoconid. Logically, the additional cup should be seen in the outline of semilandmarks. Following the same logic as with LM1 Outline, I used a set of landmarks excluding the landmarks for the cusps (LM2 Outline) to see whether I obtained the same results with fewer landmarks.

Figure 4.3 2D Landmarks and semilandmarks before sliding, for: (a) LM1, (b) LM1 Anterior Fovea, (c) LM1 Entoconulid, (d) LM1 Protostylid Buccal, and (e) LM2 Hypoconulid. The sets for the outlines include the same semilandmarks that are in the above sets

I included two landmark sets for the upper central incisors and only one for the lateral incisors (Table 4.6; Figure 4.4). The UCI Shoveling landmark set captured information about the outline of the crown from the occlusal and lingual perspectives, adding landmarks for the mesial and distal ends of the occlusal and lingual face, to capture variation related with the development of the shoveling dental trait. I used the same landmark set, excluding the landmarks for the distal and mesial ends of the faces, for UCI Double-Shoveling and ULI Tuberculum Dentale, as adding more landmarks could have resulted in more noise and was unlikely to capture details for double-shoveling or for tuberculum dentale.

Table 4.6 Sets of 2D landmarks for Upper Central and Lateral Incisor, with the set name, and the number, type and description of landmarks.

Landmark set	Number	Type	Description
UCI Shoveling	1-30	III sliding	Outline of the crown, starting from the mesiobuccal corner and continuing towards the distobuccal corner
	31	III	Inner mesial end of the occlusal face
	32	III	Inner distal end of the occlusal face
	33	III	Inner mesial end of the lingual face
	34	III	Inner distal end of the lingual face
UCI Double-Shoveling	1-30	III sliding	Outline of the crown, starting from the mesiobuccal corner and continuing towards the distobuccal corner
ULI Tuberculum Dentale	1-30	III sliding	Outline of the crown, starting from the mesiobuccal corner and continuing towards the distobuccal corner

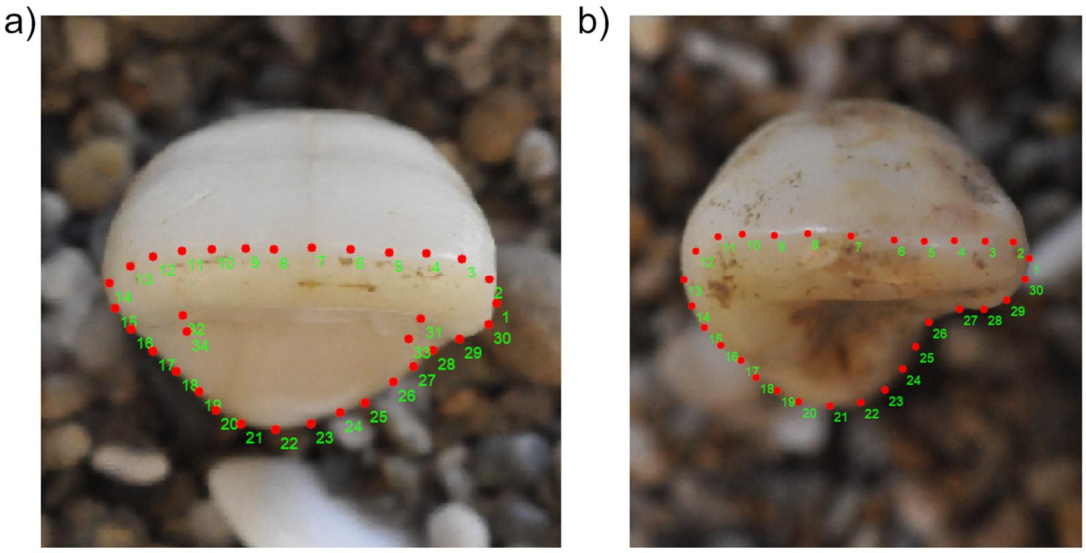


Figure 4.4 2D Landmarks and semilandmarks before sliding for: (a) UCI Shoveling, and (b) ULI Tuberculum Dentale.

I used three different landmark sets for the upper first molars (Table 4.7; Figure 4.5). The UM1 landmark set included 40 semilandmarks for the outline of the crown, and one landmark for each main cusp. I designed this set of landmarks to observe the general change in morphology of the crown, and to see changes in the outline as a consequence of the development of the metaconule and Carabelli's cusp. The rationale for using this set of landmarks to study the development of the metaconule was that a well-developed metaconule would result in a larger gap between the metacone and the hypocone. The development of Carabelli's cusp would not necessarily affect the position of the cusps, but in teeth with a very well-developed Carabelli's cusp, the protocone would be further from the outline of the crown that in teeth without a Carabelli's cusp. The UM1 Outline landmark set is an attempt to get similar information with fewer landmarks, relying solely on the shape of the crown. For the third landmark set for the upper first molars, I used a set of landmarks placed on the outline of the lingual face, similarly to the protostylid in the lower first molars, since capturing the development of Carabelli's cusp from the occlusal face would be more difficult.

Table 4.7 Sets of 2D landmarks for Upper Molar 1 with the set name, and the number, type and description of landmarks.

Landmark set	Number	Type	Description
UM1	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the protocone
	42	II	Tip of the hypocone
	43	II	Tip of the metacone
	44	II	Tip of the paracone
UM1 Outline	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
UM1 Carabelli Lingual	1-30	III sliding	Outline of the lingual face of the crown, starting from the mesial cementoenamel junction and finishing in the distal cementoenamel junction

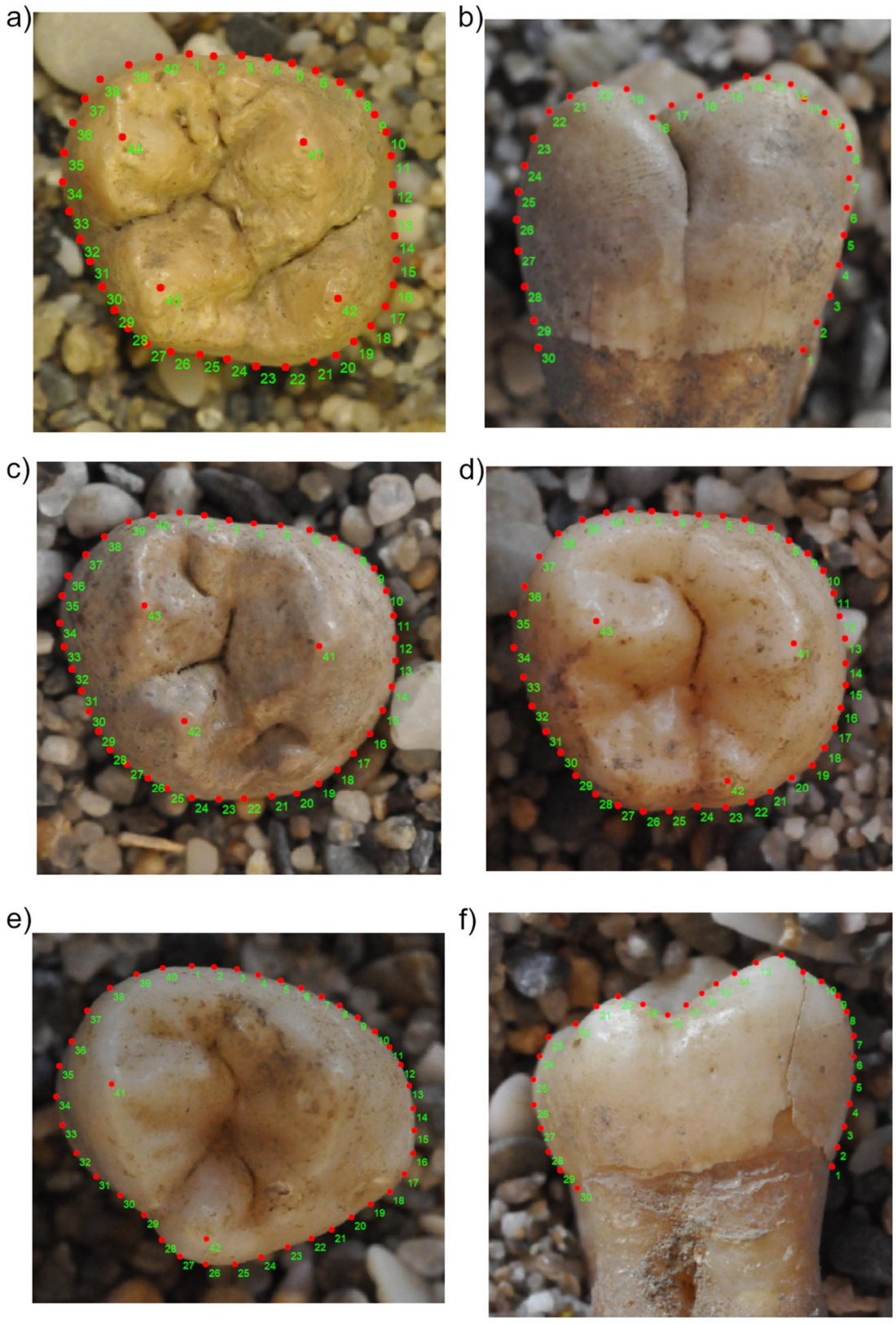


Figure 4.5 2D Landmarks and semilandmarks before sliding for: (a) UM1, (b) UM1 Carabelli Lingual, (c) UM2 Hypocone, (d) UM3 Metacone, (e) UM3 Parastyle, and (f) UM3 Parastyle Buccal. The sets for the outlines include the same semilandmarks that are in the above sets.

I devised two landmark sets to study the upper second molars (Table 4.8; Figure 4.5). The UM2 Hypocone landmark set comprises the outline of the crown from the occlusal face and landmarks for three of the main cusps. I expected that a tooth with a well-developed hypocone would have a buccodistal metacone, whereas a tooth without hypocone would present the metacone in a more distal position. The UM2 Outline landmark set is again focused exclusively on the outline, to observe whether fewer landmarks would provide the same results as a landmark set that included the cusps.

Table 4.8 Sets of 2D landmarks for Upper Molar 2, with the set name, and the number, type and description of landmarks.

Landmark set	Number	Type	Description
UM2 Hypocone	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the protocone
	42	II	Tip of the metacone
	43	II	Tip of the paracone
UM2 Outline	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face

In the UM3 landmark set (Table 4.9; Figure 4.5) I wanted to observe the shape of the outline of the crown, along with the position of the main cusps, and test whether including landmarks for the main cusps would provide more information to differentiate between the populations than when using just the outline. In the UM3 Metacone landmark set, the outline and three of the main cusps (protocone, hypocone, paracone) are included, with the rationale that in addition to changes in the outline when the metacone is developed, the position of the other three cusps may also change. For example, the tip of the paracone may be displaced distally when the metacone is absent. As with other teeth, I included the UM3 Outline landmark set to determine whether fewer landmarks would give similar results. I chose the UM3 Parastyle landmark set to determine whether the development of the parastyle could be captured from the occlusal view, including landmarks for the paracone and the metacone in addition to the outline, as a well-developed parastyle could displace the paracone and the metacone further from the outline. Finally, following the same logic

as for the protostylid and for Carabelli's cusp, I included a set of landmarks for the outline of the buccal face, to see whether the parastyle could be observed that way.

Table 4.9 Sets of 2D landmarks for Upper Molar 3, with the set name, and the number, type and description of landmarks.

Landmark set	Number	Type	Description
UM3 Metacone	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the protocone
	42	II	Tip of the hypocone
	43	II	Tip of the paracone
UM3 Outline	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
UM3 Parastyle	1-40	III sliding	Outline of the crown, starting from the most mesial point and continuing towards the lingual face
	41	II	Tip of the paracone
	42	II	Tip of the metacone
UM3 Parastyle Buccal	1-30	III Sliding	Outline of the buccal face of the crown, starting from the mesial cementoenamel junction and finishing in the distal cementoenamel junction

I converted images into TPS files using TPSUtil© v1.78 (Rohlf, 2019) and digitised the landmarks using TPSDig© v1.4 (Rohlf, 2004), adding the scale. Once digitised, I uploaded the files again to TPSUtil© v1.78 (Rohlf, 2019) to make the sliders file. After marking the sliders, I exported the file to TPSRelW© v1.70 (Rohlf, 2019) to superimpose the variables (details follow in section 4.3.3), minimising the bending energy to place the points. I also obtained the Centroid Size (details follow in section 4.3.3) for use as a covariate when exploring allometry (details follow in section 4.3.4). I saved all the coordinates into a new TPS file for analysis in MorphoJ© v1.07a (Klingenberg, 2011).

Many of the scans lost detail during postprocessing, making them useless and limiting my sample size for the 3D GMM analyses. This is a common problem, as postprocessing entails overlapping scans from different angles, so the overlap of different scans affects the texture of the final product. It does not pose a problem in long bones but can be for smaller structures such as teeth. The landmarking process was also more complicated for 3D GMM than for 2D GMM, and the time required from scanning to analysis was considerable, so I

chose to focus on those teeth for which the 2D analyses showed biological differences among the samples, to compare the results of the different methods (Table 4.10 and Figure 4.6). The landmarks I chose consisted of the tips of the main cusps, and the outline of the occlusal rim, to try to capture the different shapes of the crown without adding too much noise caused by dental wear.

Table 4.10 Sets of 3D landmarks for Lower and Upper Molars, with the set name, and the number, type and description of landmarks.

Landmarks Set	Number	Type	Description
LM1 and LM2	1	II	Tip of the protoconid
	2	II	Tip of the metaconid
	3	II	Tip of the hypoconid
	4	II	Tip of the entoconid
	5	III sliding	Mesial limit of the crown, in front of the central groove
	6-20	III sliding	Taking landmark 5 as a starting point and marking towards the buccal face, outline of the occlusal rim
UM1 and UM2	1	II	Tip of the protocone
	2	II	Tip of the paracone
	3	II	Tip of the hypocone
	4	II	Tip of the metacone
	5	III sliding	Mesial limit of the crown, in front of the central groove
	6-20	III sliding	Taking landmark 5 as a starting point and marking towards the lingual face, outline of the occlusal rim

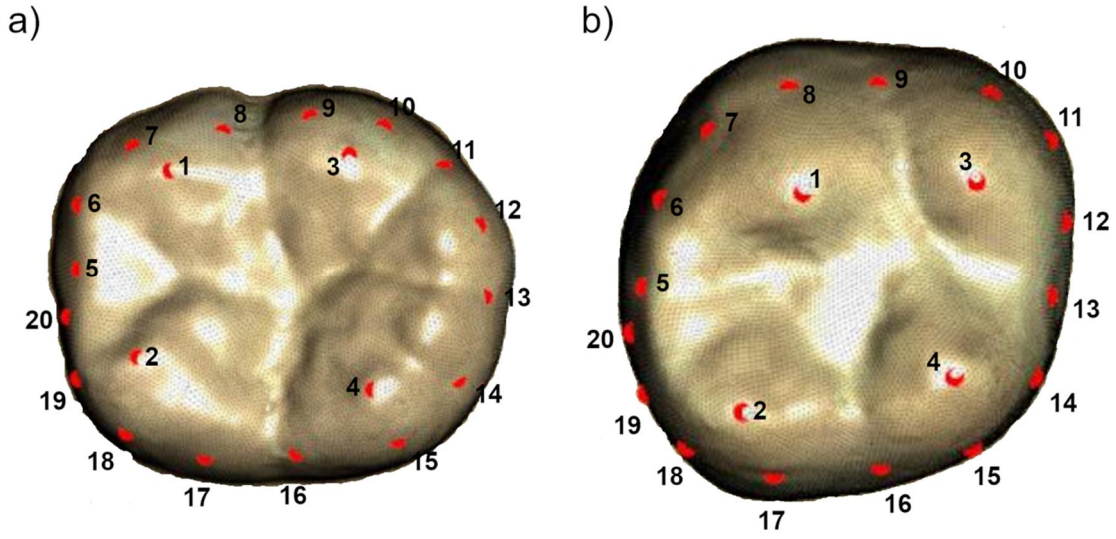


Figure 4.6 3D Landmarks, before sliding, for: (a) LM1 and LM2, and (b) UM1 and UM2

I loaded the PLY files into R© v3.6.1 (R Core Team 2019) and digitised the landmarks using Geomorph© v.3.1.0 (Adams et al., 2019). Once landmarked, I marked the sliders and used the *gpagen* function to superimpose the data (section 4.3.3), minimising the bending energy to place the points. I saved the coordinates obtained in this process into a TPS file, downloading the coordinates for the centroid size as I had done in 2D, to conduct analysis in MorphoJ© v1.07a (Klingenberg, 2011).

4.3.3 Generalized Procrustes Analysis

The first step in MorphoJ© was to perform a Generalized Procrustes Analysis (GPA). The data were already superimposed (in TPSRelW© for 2D and Geomorph© for 3D) but MorphoJ© does not allow further analysis until it has superimposed the data. Superimposition is required to subtract information about size, location and rotation to compare shape. GPA is a least-squares method formulated by Gower in 1975 (Gower, 1975; Slice, 2005). It scales all the objects in the sample to a common size by dividing by centroid size, which is the square root of the sum of squared distances of all the landmarks from their centroid, and measures the dispersion of landmarks; in a more dispersed configuration, the centroid size is larger (Slice, 2007; Viscosi and Cardini, 2011; Plomp, 2012; Mitteroecker et al., 2013; Klingenberg, 2016).

After scaling, GPA estimates location and rotation by minimising the sum of squared distances between pairs of points (Gower, 1975; Adams et al., 2004; Strand Viðarsdóttir and Cobb, 2004; Slice, 2005; Sheets et al., 2006; Slice, 2007; Plomp, 2012).

After GPA, remaining differences among the individuals in the sample are due to shape.

4.3.4 Allometry

Allometry is defined as the statistical relationship between size and shape, seen as the variation of traits explained by the variation in size (Mosimann, 1970; Mitteroecker et al., 2013; Klingenberg, 2016). Application of a size correction to the sample aims to remove the effects of size to study shape change not attributable to variation in size. To test the effects of allometry in each set of landmarks, I used a regression in MorphoJ© (Klingenberg, 2011), including a permutation test of 10,000 rounds. When the regression was significant, I corrected for the size effect by using the residuals of the regression instead of the Procrustes coordinates in subsequent analyses.

4.3.5 Principal Components Analysis

Principal Components Analysis (PCA) is a multivariate statistical analysis that reduces the dimensionality of a dataset and extracts a set of new uncorrelated variables with eigenvalues that account for decreasing amounts of variation (Rohlf, 1999; Klingenberg and Zaklan, 2000; Singleton 2005; Mitteroecker et al., 2013). PCA is useful to visualize the shape differences alongside principal axes of variation in the tangent space (Strand Viðarsdóttir et al., 2002). It is also useful to reduce the degrees of dimensionality in a dataset by using a limited number of the Principal Components (PC) scores in subsequent analysis instead of the Procrustes coordinates, choosing only the first few principal components or retaining all with non-zero eigenvalues (Sheets et al., 2006; Kovarovic et al., 2011; Owen, 2013). However, excluding too many PCs may result in the loss of valuable information, so the number of variables used must be well-chosen (Kovarovic et al., 2011).

I carried out the PCA in MorphoJ© v1.07a (Klingenberg, 2011) and in the package Geomorph© v.3.1.0 (Adams et al., 2019) of R© v3.6.1 (R Core Team 2019) for all the 2D and 3D datasets.

4.3.6 Canonical Variate Analysis

Canonical Variate Analysis (CVA) is a multivariate statistical analysis that produces a set of uncorrelated variables that maximise the separation of predefined groups (Klingenberg, 2011). It is an ordination technique commonly used in an exploratory way to visualise differences in the distribution of known groups, by measuring the differences between groups scaled by the variation within groups, which is called the Mahalanobis distance (Klingenberg and Monteiro, 2005; Mitteroecker and Bookstein, 2011; Cooke and Terhune, 2015).

CVA can also be based on the Procrustes distance, examining the distance among the means of each group, and considering the distribution of specimens around the mean (Klingenberg and Monteiro, 2005; Cooke and Terhune, 2015). Mahalanobis distance can be influenced by small and irregular sample sizes, whilst Procrustes distance responds poorly to variation that does not have a uniform direction (Klingenberg and Monteiro, 2005; Cooke and Terhune, 2015).

I carried out CVA in MorphoJ© v1.07a (Klingenberg, 2011) for all the 2D and 3D sets of landmarks, assessing the reliability of the results with a permutation test with 10,000 iterations. I report results for both the Mahalanobis and the Procrustes distances and considered a difference between groups as significant when both analyses were significant.

4.3.7 Discriminant Function Analysis

Discriminant Function Analysis (DFA) with cross-validation is a multivariate statistical analysis that constructs linear functions that maximise between- to within-group variance to test whether the functions predict differences between two groups (Kovarovic et al., 2011; Mitteroecker and Bookstein, 2011). It supplements the tests with cross-validation, validating the functions by randomly leaving out a single specimen and determining the possibilities

of correctly classifying the specimen based on the distribution of the remaining sample, then repeating the procedure for the rest of specimens (Kovarovic et al., 2011; Mitteroecker and Bookstein, 2011; Plomp, 2013).

DFA results can be affected by small sample sizes, when the number of variables exceeds the size of any of the groups, and when there are outliers (Kovarovic et al., 2011; Mitteroecker and Bookstein, 2011). The accuracy of DFA is as important as its significance, as the percentage of individuals correctly classified is affected by the number of groups (Kovarovic et al., 2011). For example, 34% correct classification with three groups means that the discriminant function is as effective as randomly classifying them, whilst 34% of correct classification with eight groups means that the discriminant function helps to assign the individuals to a given group.

I performed DFA with cross-validation in MorphoJ© v1.07a (Klingenberg, 2011) in all the sets of 2D and 3D landmarks, using the Procrustes coordinates or the regression residuals when the test for allometry was significant. I assessed the reliability of the results with a permutation test of 10,000 iterations and considered the discrimination significant when both the Mahalanobis and the Procrustes distances were significant.

I also performed DFA in the package Geomorph© v.3.1.0 (Adams et al., 2019) of R© v3.6.1 (R Core Team 2019) using the principal components scores, to assess the percentage of correctly classified individuals when reducing the number of principal components used, avoiding the correct classification of individuals due to over-fitting. This allowed me to observe the number of PCs needed to obtain an overall reliability of 50% in the classification of individuals with the discriminant function, to assess the accuracy of the discriminant function and infer how different the samples are. Discriminant functions that require a large number of PCs to correctly classify individuals usually occur when the samples are alike. Likewise, discriminant functions that provide a poor rate of reliability are usually a consequence of very similar samples. I choose the threshold point of 50% as I

wanted a noticeable increased accuracy in comparison with a random classification of groups. Because I had three samples, randomly classifying the individuals would have resulted in a 34% of individuals being put correctly in their sample, if my sample sizes were the same. However, my samples had very different sizes, with SNA being much larger than XAR and SNA. That means that there would be a greater probabilities of the individuals of SNA being classified correctly by chance. Thus, I selected a larger percentage than 34%.

4.3.8 Interobserver and Intraobserver errors

To test the robustness of the results, I investigated the replicability of the landmarking. After landmarking all the teeth in my samples, I chose two sets of landmarks for LM1 (LM1 and LM1 Anterior Fovea) and one for UM2 (UM2 Hypocone) and used R© v3.6.1 (R Core Team 2019) to select 10 individuals from each sample randomly. I then landmarked the subsamples following the same procedure that I had follow for landmarking them the first time. I also sent the pictures for the subsamples, with basic instructions that included the tables with the landmark descriptions, to Dr Kimberly Plomp, who landmarked the pictures using her equipment and TPSDig© v1.4 (Rohlf, 2004). Once I had the coordinates for her landmarks, I carried out a GPA and a PCA in MorphoJ© v1.07a (Klingenberg, 2011) to visualise any differences between my first and my second rounds of landmarks, and between my first round and Dr Plomp's round of landmarks.

I quantified the interobserver and intraobserver error with a Procrustes ANOVA, which is a test that quantifies the amount of shape variation due to different factors (for example, to differences between the individuals in the samples and differences between the observer or the number of observations) and estimates the probabilities of the variation (Fruciano, 2016). I did this with the interobserver error and the intraobserver error separately. I obtained the Procrustes sums of squares (SS), the Procrustes mean squares (MS), the degrees of freedom (df), the F statistic (F) and the associated p-value. I then used the MS

to follow the method described in Fruciano (2016), which is based in Fisher RA (1958) “Statistical methods for research workers”, to obtain an intraclass correlation coefficient (R) by dividing the amount of measurement error by the total variation in the sample. The closer that R is to 1, the smaller the measurement error is in relation with the total amount of variation, which allows us to quantify the error (Fruciano, 2016).

5. Results: Dental Metric Variables

This chapter presents the results from the analyses performed to compare the three archaeological samples using four metric variables: mesiodistal diameter, buccolingual diameter, crown area, and crown index.

Table 5.1 Summary of sites and individuals included from each site.

	SMA (Santa Maria la Real – Christians – North of IP)	SNA (San Nicolas de Avila – Muslims – Centre of IP)	XAR (Xarea – Muslims – South of IP)
Females	4	51	12
Males	18	23	12
Unknown	13	34	21
Total	35	108	45

The results obtained seemed to indicate that the unbalanced sex ratio among the samples introduced a bias due to the sexual dimorphism of the metric variables. To check if this was the case, I conducted post-hoc exploratory analyses including only the individuals that were sexed as males and excluding female and unknown individuals.

5.1. Mesiodistal Diameter

The mean and the median mesiodistal diameters were strikingly similar for all three samples across all the teeth regardless of whether all the individuals were included or only male individuals (Tables 5.2 and 5.3).

Table 5.2 Descriptive statistics for mesiodistal diameter (mm) for all the individuals.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	17	10.94	10.98	0.19	0.44	10.22	11.79
	SNA	75	11.01	10.96	0.29	0.53	9.71	12.26
	XAR	40	10.93	10.93	0.34	0.59	9.52	12.56
LM2	SMA	27	10.57	10.51	0.24	0.49	9.66	11.33
	SNA	97	10.59	10.55	0.43	0.66	8.85	12.11
	XAR	39	10.45	10.51	0.40	0.63	9.20	11.90

UCI	SMA	---	---	---	---	---	---	---
	SNA	56	8.42	8.39	0.23	0.48	7.63	9.51
	XAR	26	8.43	8.39	0.23	0.48	7.67	9.57
ULI	SMA	5	6.58	6.58	0.03	0.17	6.35	6.76
	SNA	29	6.72	6.77	0.28	0.53	5.60	7.63
	XAR	27	6.62	6.57	0.39	0.62	5.62	7.87
UM1	SMA	14	10.14	10.12	0.24	0.49	9.51	11.30
	SNA	81	10.19	10.18	0.32	0.56	8.78	11.39
	XAR	28	10.24	10.26	0.65	0.81	9.00	12.00
UM2	SMA	17	9.42	9.23	0.44	0.67	8.15	10.63
	SNA	117	9.44	9.38	0.54	0.74	7.29	11.62
	XAR	30	9.48	9.67	0.59	0.77	7.61	11.25
UM3	SMA	11	8.09	8.29	1.31	1.15	5.64	10.30
	SNA	73	8.73	8.64	0.73	0.86	6.76	11.16
	XAR	23	8.78	8.92	0.55	0.74	7.30	9.88

Note: There are no data for SMA in UCI because there were no upper central incisors for this population

Table 5.3 Descriptive statistics for mesiodistal diameter (mm) for males.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	8	11.04	11.03	0.07	0.26	10.58	11.46
	SNA	6	10.97	11.17	0.18	0.42	10.21	11.31
	XAR	8	10.77	10.84	0.17	0.41	10.00	11.24
LM2	SMA	12	10.65	10.62	0.22	0.47	9.70	11.32
	SNA	17	10.65	10.81	0.66	0.81	9.62	11.81
	XAR	11	10.43	10.79	0.75	0.87	9.20	11.90
UCI	SMA	---	---	---	---	---	---	---
	SNA	7	8.21	8.16	0.08	0.28	7.90	8.66
	XAR	6	8.59	8.29	0.54	0.74	7.82	9.57
ULI	SMA	2	6.56	6.56	0.08	0.29	6.35	6.76
	SNA	6	6.44	6.29	0.46	0.67	5.60	7.45
	XAR	6	6.73	6.79	1.12	1.06	5.62	7.87
UM1	SMA	11	10.12	10.18	0.12	0.34	9.71	10.75
	SNA	9	10.19	10.29	0.25	0.50	9.51	10.96
	XAR	6	10.01	9.62	1.06	1.03	9.31	12.00
UM2	SMA	15	9.43	9.23	0.50	0.71	8.15	10.63
	SNA	19	9.68	9.46	0.88	0.94	8.23	11.62
	XAR	5	9.31	9.54	2.15	1.47	7.61	11.25
UM3	SMA	9	8.36	8.31	0.81	0.90	7.00	10.30
	SNA	21	8.88	8.82	0.80	0.90	7.44	10.56
	XAR	5	9.19	9.33	0.43	0.66	8.33	9.82

Note: There are no data for SMA in UCI because there were no upper central incisors for this population

There was no need to transform the data or delete individuals as there was no significant departure from normality (Table 5.4). Only the upper lateral incisors (one test) and the first upper molar (both tests) showed significant homoscedasticity (Table 5.4), so a Kruskal-Wallis test was used for these teeth rather than an ANOVA.

There was no departure from normality for the analyses in males, and only the upper central incisors and first upper molar showed significant homoscedasticity for one test (Table 5.5), so the Kruskal-Wallis test was used for these teeth.

Table 5.4 Results of Shapiro-Wilk normality and homoscedasticity tests for mesiodistal diameter for all the individuals. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.97	0.81	1.80 (0.41)	0.81 (0.67)
	SNA	0.98	0.14		
	XAR	0.97	0.31		
LM2	SMA	0.96	0.40	3.05 (0.22)	2.65 (0.27)
	SNA	0.99	0.79		
	XAR	0.98	0.78		
UCI	SMA	---	---	---	---
	SNA	0.97	0.14	0.00 (0.99)	0.27 (0.60)
	XAR	0.94	0.17		
ULI	SMA	0.95	0.75	6.49 (**0.04**)	4.30 (0.12)
	SNA	0.98	0.83		
	XAR	0.94	0.12		
UM1	SMA	0.92	0.20	6.99 (**0.03**)	7.81 (**0.02**)
	SNA	0.99	0.66		
	XAR	0.95	0.18		
UM2	SMA	0.94	0.27	0.39 (0.82)	0.85 (0.66)
	SNA	0.99	0.48		
	XAR	0.94	0.10		
UM3	SMA	0.92	0.30	2.80 (0.25)	0.46 (0.79)
	SNA	0.99	0.82		
	XAR	0.95	0.35		

Table 5.5 Results of Shapiro-Wilk normality and homoscedasticity tests for mesiodistal diameter for males. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.98	0.97	1.58 (0.45)	0.52 (0.77)
	SNA	0.80	0.05		
	XAR	0.93	0.53		
LM2	SMA	0.95	0.63	4.14 (0.13)	5.39 (0.07)
	SNA	0.89	0.05		
	XAR	0.91	0.28		
UCI	SMA	---	---	---	---
	SNA	0.93	0.58	4.20 (**0.04**)	0.70 (0.40)
	XAR	0.85	0.16		
ULI	SMA	---	---	1.89 (0.39)	5.43 (0.07)
	SNA	0.96	0.80		
	XAR	0.81	0.07		
UM1	SMA	0.93	0.39	8.66 (**0.01**)	1.51 (0.47)
	SNA	0.96	0.75		
	XAR	0.75	0.02		
UM2	SMA	0.93	0.31	3.87 (0.59)	3.79 (0.15)
	SNA	0.96	0.59		
	XAR	0.96	0.82		
UM3	SMA	0.91	0.29	0.54 (0.76)	0.79 (0.67)
	SNA	0.97	0.77		
	XAR	0.90	0.41		

There were no significant differences in the mesiodistal diameters of teeth across the three samples, in all the individuals (Table 5.6) and in only male individuals (Table 5.7).

Table 5.6 Comparison of mesiodistal diameter across samples, using ANOVA or Kruskal-Wallis, as appropriate, for all the individuals. Significant p-values are in bold red.

Tooth	ANOVA				Kruskal-Wallis	
	df	df residuals	F (p)	Student's T (p)	df	Chi-squared (p)
LM1	2	129	0.30 (0.74)	---	---	---
LM2	2	160	0.77 (0.47)	---	---	---
UCI	---	---	---	-0.10 (0.92)	---	---
ULI	---	---	---	---	2	1.17 (0.56)
UM1	---	---	---	---	2	0.16 (0.92)
UM2	2	161	0.04 (0.96)	---	---	---
UM3	2	104	2.77 (0.07)	---	---	---

Table 5.7 Comparison of mesiodistal diameter across samples, using ANOVA or Kruskal-Wallis, as appropriate, for males. Significant p-values are in bold red.

Tooth	ANOVA			Kruskal-Wallis	
	df	df residuals	F (p)	df	Chi-squared (p)
LM1	2	19	1.11 (0.35)	---	---
LM2	2	37	0.36 (0.70)	---	---
UCI	---	---	---	1	0.51 (0.48)
ULI	2	11	0.18 (0.84)	---	---
UM1	---	---	---	2	2.62 (0.27)
UM2	2	36	0.46 (0.64)	---	---
UM3	2	32	1.70 (0.20)	---	---

5.2. Buccolingual Diameter

The means and medians of the buccolingual diameter for the different samples were also relatively homogeneous but less so than for the mesiodistal diameter (Table 5.8). The range of variation in SNA (☪) is quite extensive for UM3, where the minimum is 57% of the maximum, as opposed to a value of 82% for SMA (☥) and 71% for XAR (☪). UM3 also has a large variance.

Table 5.8 Descriptive statistics for buccolingual diameter (mm) for all the individuals.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	17	10.25	10.39	0.24	0.49	8.81	10.94
	SNA	75	10.12	10.14	0.27	0.52	8.50	11.36
	XAR	40	10.31	10.17	0.32	0.57	9.45	11.61
LM2	SMA	27	9.86	9.85	0.16	0.40	8.64	10.38
	SNA	97	9.50	9.54	0.34	0.58	7.95	11.22
	XAR	39	9.79	9.81	0.33	0.57	8.44	11.41
UCI	SMA	---	---	---	---	---	---	---
	SNA	56	7.13	7.14	0.14	0.37	6.37	7.83
	XAR	26	7.03	7.08	0.39	0.63	5.32	7.94
ULI	SMA	5	6.01	6.17	0.40	0.63	5.06	6.72
	SNA	29	6.34	6.37	0.17	0.42	5.23	6.89
	XAR	27	6.48	6.45	0.30	0.55	5.42	7.50
UM1	SMA	14	10.82	10.82	0.29	0.54	9.92	12.04
	SNA	81	10.73	10.76	0.16	0.40	9.51	11.60
	XAR	28	10.98	10.96	0.42	0.65	9.55	12.38

UM2	SMA	17	10.99	10.86	0.56	0.75	9.84	12.20
	SNA	117	10.74	10.74	0.43	0.66	9.30	12.26
	XAR	30	11.08	11.06	0.72	0.85	9.45	13.51
UM3	SMA	11	10.25	10.26	0.35	0.59	9.16	11.21
	SNA	73	10.16	10.03	0.93	0.96	8.10	14.26
	XAR	23	10.40	10.43	0.87	0.93	8.88	12.56

When only the males were observed, the means and medians remained homogeneous (Table 5.9). The range of variation in SNA (♀) for UM3 is smaller in males than in both sexes combined but remains the same for SMA (♂) and XAR (♀). The most considerable variances among males are for UM2 and UM3 for XAR (♀).

Table 5.9 Descriptive statistics for buccolingual diameter (mm) in males.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	8	10.55	10.49	0.05	0.22	10.32	10.94
	SNA	6	10.10	10.13	0.02	0.14	9.86	10.27
	XAR	8	10.43	10.17	0.48	0.69	9.78	11.61
LM2	SMA	12	9.92	10.02	0.13	0.36	9.18	10.30
	SNA	17	9.54	9.50	0.70	0.83	8.34	11.22
	XAR	11	9.66	9.93	0.60	0.77	8.44	11.41
UCI	SMA	---	---	---	---	---	---	---
	SNA	7	6.94	7.09	0.12	0.34	6.51	7.34
	XAR	6	7.33	7.30	0.20	0.45	6.77	7.83
ULI	SMA	2	6.25	9.25	0.45	0.67	5.77	6.01
	SNA	6	6.51	6.51	0.06	0.23	6.12	6.78
	XAR	6	6.59	6.44	0.34	0.58	5.95	7.50
UM1	SMA	11	10.94	10.86	0.27	0.52	10.28	12.04
	SNA	9	10.76	10.86	0.15	0.39	9.87	11.19
	XAR	6	11.13	10.84	0.65	0.81	10.35	12.38
UM2	SMA	15	11.03	10.86	0.59	0.77	9.84	12.20
	SNA	19	11.07	11.04	0.48	0.69	10.00	12.26
	XAR	5	11.02	11.00	1.68	1.30	9.45	12.80
UM3	SMA	9	10.26	10.26	0.43	0.66	9.16	11.21
	SNA	21	10.30	10.36	0.75	0.87	8.52	12.19
	XAR	5	11.07	11.60	2.27	1.51	8.88	12.56

LM1, LM2, UCI, and UM3 showed a significant departure from normality for SMA (♂) and XAR (♀) (Table 5.10). For UM3, transformation to Naperian logarithm resolved this.

For LM1, LM2 and UCI, the normality test remained significant after transformation, so outliers in a Q-Q (Quantile-Quantile) plot were identified and excluded from analyses.

Table 5.10. Results of Shapiro-Wilk normality and homoscedasticity tests for buccolingual diameter for both sexes. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.96	0.73	4.27 (0.12)	3.96 (0.14)
	SNA	0.98	0.31		
	XAR	0.95	0.24		
LM2	SMA	0.94	0.14	10.62 (**<0.001**)	5.29 (0.07)
	SNA	0.99	0.47		
	XAR	0.99	0.87		
UCI	SMA	---	---	---	---
	SNA	0.98	0.57	0.28 (0.60)	0.97 (0.33)
	XAR	0.96	0.36		
ULI	SMA	0.96	0.83	2.54 (0.28)	1.60 (0.45)
	SNA	0.94	0.13		
	XAR	0.97	0.70		
UM1	SMA	0.96	0.72	10.30 (**<0.001**)	5.24 (0.72)
	SNA	0.98	0.45		
	XAR	0.97	0.50		
UM2	SMA	0.95	0.46	3.44 (0.18)	0.94 (0.62)
	SNA	0.99	0.65		
	XAR	0.96	0.25		
UM3	SMA	0.98	0.95	2.88 (0.24)	2.47 (0.29)
	SNA	0.97	0.11		
	XAR	0.97	0.62		

In males only, there was no departure from normality for any teeth in any population (Table 5.11). Only two teeth (LM1 and LM2) had significant results for the homoscedasticity tests, so I used a Kruskal-Wallis test instead of an ANOVA for these teeth.

Table 5.11 Results of Shapiro-Wilk normality and homoscedasticity tests for buccolingual diameter for males. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.90	0.31	14.54 (**<0.001**)	6.17 (0.05)
	SNA	0.94	0.63		
	XAR	0.86	0.12		

LM2	SMA	0.90	0.14	7.59 (**0.02**)	4.05 (0.13)
	SNA	0.93	0.26		
	XAR	0.94	0.55		
UCI	SMA	---	---	---	---
	SNA	0.87	0.19	0.36 (0.55)	0.03 (0.86)
	XAR	0.90	0.35		
ULI	SMA	---	---	3.32 (0.19)	4.98 (0.08)
	SNA	0.94	0.63		
	XAR	0.94	0.62		
UM1	SMA	0.93	0.44	3.30 (0.19)	3.10 (0.21)
	SNA	0.87	0.12		
	XAR	0.89	0.31		
UM2	SMA	0.95	0.49	3.17 (0.21)	3.21 (0.20)
	SNA	0.95	0.45		
	XAR	0.99	0.98		
UM3	SMA	0.98	0.97	4.08 (0.13)	2.31 (0.32)
	SNA	0.97	0.78		
	XAR	0.93	0.57		

There were significant differences across samples in buccolingual diameter for the LM2 and the UM2 when both sexes were included (Table 5.12). Tukey posthoc analysis showed no significant differences for UM2 (Table 5.13). The Mann-Whitney test showed significant differences for LM2 between SNA (☪) and both XAR (☪) and SMA (☩), but XAR (☪) and SMA (☩) were not significantly different (Table 5.14).

Table 5.12. Comparison of buccolingual diameter across both sexes of the samples, using ANOVA or Kruskal-Wallis, as appropriate. Significant p-values are in bold red.

Tooth	ANOVA				Kruskal-Wallis	
	df	df residuals	F (p)	Student's T (p)	df	Chi-squared (p)
LM1	2	114	2.39 (0.10)	---	---	---
LM2	---	---	---	---	2	16.90 (**<0.001**)
UCI	---	---	---	-0.50 (0.62)	---	---
ULI	2	58	2.02 (0.14)	---	---	---
UM1	---	---	---	---	2	3.36 (0.19)
UM2	2	161	3.15 (**0.05**)	---	---	---
UM3	2	103	1.14 (0.32)	---	---	---

Table 5.13. Results of Tukey posthoc analysis for buccolingual diameter for both sexes. Significant p-values are in bold red.

Tooth	Samples	Difference (p)	Lower	Upper
UM2	SNA-SMA	-0.24 (0.38)	-0.68	0.19
	XAR-SMA	0.09 (0.90)	-0.42	0.60
	XAR-SNA	0.33 (0.06)	-0.01	0.68

Table 5.14 Results of pairwise Mann-Whitney posthoc analysis for buccolingual diameter for both sexes. Significant p-values are in bold red.

Tooth	Samples	W (p)
LM2	SNA-SMA	1875.50 (**<0.001**)
	XAR-SMA	599.00 (0.22)
	XAR-SNA	1371.00 (**0.01**)

In males only, the Kruskal-Wallis was significant only for LM1 (Table 5.15). Post-hoc Mann-Whitney tests showed significant differences for LM1 between SNA (☪) and SMA (✞), with no differences between SNA (☪) and XAR (☪), as for the analyses of both sexes (Table 5.16).

Table 5.15 Comparison of buccolingual diameter across male individuals of the samples, using ANOVA or Kruskal-Wallis, as appropriate. Significant p-values are in bold red.

Tooth	ANOVA				Kruskal-Wallis	
	df	df residuals	F (p)	Student's T (p)	df	Chi-squared (p)
LM1	---	---	---	---	2	6.54 (**0.04**)
LM2	---	---	---	---	2	4.05 (0.13)
UCI	---	---	---	-1.79 (0.10)	---	---
ULI	2	11	0.40 (0.68)	---	---	---
UM1	2	23	0.81 (0.46)	---		
UM2	2	36	0.02 (0.99)	---	---	---
UM3	2	32	1.54 (0.23)	---	---	---

Table 5.16 Results of pairwise Mann-Whitney posthoc analysis for buccolingual diameter for males. Significant p-values are in bold red.

Tooth	Samples	W (p)
LM1	SNA-SMA	48.00 (**<0.001**)
	XAR-SMA	43.00 (0.28)
	XAR-SNA	22.00 (0.85)

5.3. Crown Area

The mean crown areas were very similar across samples for LM1 and UCI when all the individuals were included, with larger differences for the other teeth, especially for UM2 and UM3 (Table 5.17).

Table 5.17 Descriptive statistics for crown area (mm2) for all the individuals.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	17	112.23	113.84	69.70	8.35	90.04	121.65
	SNA	75	111.59	110.52	104.25	10.21	82.54	136.66
	XAR	40	112.82	111.59	101.11	10.06	91.58	139.29
LM2	SMA	27	104.23	105.58	58.60	7.66	83.46	116.26
	SNA	97	100.91	99.10	137.93	11.74	70.36	131.68
	XAR	39	102.43	104.20	114.50	10.70	78.41	135.78
UCI	SMA	---	---	---	---	---	---	---
	SNA	56	60.11	59.90	31.51	5.61	48.60	69.92
	XAR	26	59.38	58.51	50.28	7.09	47.24	74.93
ULI	SMA	5	39.63	40.17	22.97	4.79	33.29	45.43
	SNA	29	42.62	42.11	20.82	4.56	35.77	51.51
	XAR	27	43.12	41.70	48.64	6.97	32.55	57.45
UM1	SMA	14	109.79	107.56	78.48	8.86	94.34	123.17
	SNA	81	109.46	109.85	80.34	8.96	89.58	130.18
	XAR	28	112.75	112.25	195.52	13.98	85.95	148.56
UM2	SMA	17	103.81	101.77	169.24	13.01	83.13	129.69
	SNA	117	101.63	100.22	149.61	12.23	79.54	136.88
	XAR	30	105.36	105.15	207.49	14.40	71.91	144.00
UM3	SMA	11	83.30	83.47	233.87	15.29	57.08	115.46
	SNA	73	89.15	87.23	253.46	15.92	59.78	159.14
	XAR	23	91.59	95.35	188.30	13.72	65.12	118.12

In males only, the means and medians were not very homogeneous, with larger differences for UM3 than for any other teeth (Table 5.18).

Table 5.18 Descriptive statistics for crown area (mm2) for males.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	8	116.40	115.80	11.43	3.38	112.80	121.70
	SNA	6	110.70	111.60	20.37	4.51	103.70	116.20
	XAR	8	112.40	107.70	111.34	10.55	101.80	130.50
LM2	SMA	12	105.65	106.89	53.26	7.30	94.38	116.26
	SNA	17	102.05	103.78	254.00	15.94	81.31	131.68

	XAR	11	101.25	104.90	262.04	16.19	78.41	135.78
UCI	SMA	---	---	---	---	---	---	---
	SNA	7	57.08	58.59	20.22	4.50	51.51	62.35
	XAR	6	63.16	59.38	78.32	8.85	54.74	74.93
ULI	SMA	2	41.03	41.03	38.63	6.22	36.64	45.43
	SNA	6	41.97	42.42	21.52	4.64	36.40	47.90
	XAR	6	44.77	44.20	109.33	10.46	34.96	57.45
UM1	SMA	11	110.80	109.20	56.98	7.55	102.40	123.20
	SNA	9	109.63	112.12	51.65	7.19	94.06	116.52
	XAR	6	111.84	105.94	353.65	18.81	99.29	148.56
UM2	SMA	15	104.35	101.77	190.08	13.79	83.13	129.69
	SNA	19	107.46	101.79	224.63	14.99	87.16	136.88
	XAR	5	104.17	104.94	811.19	28.48	71.91	144.00
UM3	SMA	9	86.19	83.81	197.01	14.04	64.12	115.46
	SNA	21	91.81	93.67	214.64	14.65	63.42	115.10
	XAR	5	102.12	109.27	321.10	17.92	73.97	118.12

LM1, UM2 and UM3 showed a significant departure from normality for SMA (✞) and SNA (☪) when all individuals were included, which remained after transforming the data to Naperian logarithm (Table 5.19). Therefore, I identified the outliers from the raw data in a Q-Q plot and excluded them from analyses. LM2 and UM1 were significantly homoscedastic, so I used a Kruskal-Wallis test instead of an ANOVA for these teeth.

Table 5.19 Results of Shapiro-Wilk normality and homoscedasticity tests for crown area for both sexes. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.91	0.10	4.73 (0.09)	2.87 (0.24)
	SNA	0.98	0.15		
	XAR	0.95	0.11		
LM2	SMA	0.95	0.23	6.28 (**0.04**)	6.34 (**0.04**)
	SNA	0.99	0.83		
	XAR	0.97	0.30		
UCI	SMA	---	---	---	---
	SNA	0.97	0.28	1.94 (0.16)	0.38 (0.54)
	XAR	0.95	0.27		
ULI	SMA	0.98	0.95	4.78 (0.09)	4.32 (0.12)
	SNA	0.96	0.27		
	XAR	0.94	0.13		
UM1	SMA	0.93	0.27	9.43 (**0.01**)	3.16 (0.21)

	SNA	0.99	0.66		
	XAR	0.93	0.07		
UM2	SMA	0.93	0.19	3.26 (0.20)	0.04 (0.98)
	SNA	0.98	0.06		
	XAR	0.94	0.08		
UM3	SMA	0.93	0.46	0.23 (0.89)	0.07 (0.97)
	SNA	0.99	0.67		
	XAR	0.98	0.85		

In males only, only UM1 for XAR (C⋆) showed a significant departure from normality, which was corrected after identifying one outlier and excluding it from the analysis (Table 5.20). LM1, LM2 and ULI gave significant results for one of the homoscedasticity tests, so I used a Kruskal-Wallis test for these teeth.

Table 5.20 Results of Shapiro-Wilk normality and homoscedasticity tests for crown area for males. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.87	0.14	8.81 (**0.01**)	1.56 (0.46)
	SNA	0.97	0.86		
	XAR	0.88	0.18		
LM2	SMA	0.92	0.28	7.11 (**0.03**)	6.07 (0.05)
	SNA	0.91	0.09		
	XAR	0.94	0.51		
UCI	SMA	---	---	---	---
	SNA	0.88	0.24	2.22 (0.14)	0.40 (0.53)
	XAR	0.82	0.09		
ULI	SMA	---	---	2.55 (0.28)	9.30 (**0.01**)
	SNA	0.93	0.57		
	XAR	0.79	0.05		
UM1	SMA	0.88	0.12	0.22 (0.90)	0.75 (0.69)
	SNA	0.88	0.14		
	XAR	0.81	0.10		
UM2	SMA	0.94	0.34	4.41 (0.11)	3.66 (0.16)
	SNA	0.93	0.17		
	XAR	0.97	0.89		
UM3	SMA	0.92	0.43	0.35 (0.84)	1.01 (0.60)
	SNA	0.95	0.40		
	XAR	0.89	0.36		

There were no significant differences among the crown areas of the samples, regardless of whether all the individuals were included (Table 5.21) or only males (Table 5.22).

Table 5.21 Comparison of crown area across both sexes of the samples, using ANOVA or Kruskal-Wallis, as appropriate. Significant p-values are in bold red.

Tooth	ANOVA				Kruskal-Wallis	
	df	df residuals	F (p)	Student's T (p)	df	Chi-squared (p)
LM1	2	128	0.39 (0.68)	---	---	---
LM2	---	---	---	---	2	2.71 (0.26)
UCI	---	---	---	0.51 (0.62)	---	---
ULI	2	58	0.77 (0.47)	---	---	---
UM1	---	---	---	---	2	0.86 (0.65)
UM2	2	158	1.92 (0.15)	---	---	---
UM3	2	103	1.36 (0.26)	---	---	---

Table 5.22 Comparison of crown area across male individuals of the samples, using ANOVA or Kruskal-Wallis, as appropriate. Significant p-values are in bold red.

Tooth	ANOVA				Kruskal-Wallis	
	df	df residuals	F (p)	Student's T (p)	df	Chi-squared (p)
LM1	---	---	---	---	2	3.48 (0.18)
LM2	---	---	---	---	2	1.32 (0.52)
UCI	---	---	---	-1.52 (0.17)	---	---
ULI	---	---	---	---	2	0.04 (0.98)
UM1	2	22	1.36 (0.28)	---	---	---
UM2	2	36	0.18 (0.84)	---	---	---
UM3	2	32	1.83 (0.18)	---	---	---

5.4. Crown Index

The mean crown indices were very heterogeneous across all teeth and samples for all the individuals, and the variance for UM3 SMA (☥) was particularly high (Table 5.23). In males only, the means were still quite heterogeneous, and the variances of UM3 for SNA (☪) and XAR (☪) were particularly high, as were those of UM2 and ULI SNA (☪) (Table 5.24).

Table 5.23 Descriptive statistics for crown index (%) for all the individuals.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	17	93.78	94.21	17.09	4.13	84.99	101.70
	SNA	75	92.00	92.20	13.80	3.72	83.23	107.99
	XAR	40	94.48	93.90	36.85	6.07	83.78	111.29
LM2	SMA	27	93.37	93.37	18.82	4.34	86.00	100.88
	SNA	97	89.71	89.74	12.38	3.52	81.05	97.61
	XAR	39	93.82	94.24	27.60	5.25	82.22	107.91
UCI	SMA	---	---	---	---	---	---	---
	SNA	56	84.75	84.89	20.14	4.49	72.77	94.67
	XAR	26	83.57	86.03	58.95	7.68	59.91	92.96
ULI	SMA	5	91.26	94.35	73.68	8.58	76.90	99.41
	SNA	29	95.03	94.02	99.26	9.96	76.46	118.69
	XAR	27	98.26	100.16	57.16	7.56	83.38	112.39
UM1	SMA	14	106.90	107.70	30.87	5.56	95.40	117.70
	SNA	81	105.57	105.72	24.89	4.99	95.14	122.10
	XAR	28	107.62	105.94	54.45	7.38	95.87	127.18
UM2	SMA	17	116.80	115.70	57.05	7.55	100.90	129.10
	SNA	117	114.30	112.60	83.49	9.14	100.90	156.20
	XAR	30	117.20	115.90	81.59	9.03	105.30	139.20
UM3	SMA	11	128.80	125.80	341.01	18.47	108.80	179.40
	SNA	73	116.90	114.50	94.80	9.74	100.50	144.00
	XAR	23	118.90	118.30	108.56	10.42	106.20	144.40

Table 5.24 Descriptive statistics for crown index (%) for males.

Tooth	Population	N	Mean	Median	Variance	SD	Minimum	Maximum
LM1	SMA	8	95.65	95.03	10.40	3.23	92.32	101.70
	SNA	6	92.20	90.72	15.46	3.93	88.27	99.51
	XAR	8	96.78	94.42	28.30	5.32	89.67	103.29
LM2	SMA	12	93.22	93.08	15.52	3.94	86.00	100.31
	SNA	17	89.66	88.81	23.90	4.89	81.05	95.90
	XAR	11	92.77	92.34	12.85	3.58	86.22	97.72
UCI	SMA	---	---	---	---	---	---	---
	SNA	7	84.49	83.61	7.17	2.68	81.27	88.22
	XAR	6	85.66	84.58	23.89	4.89	80.50	92.96
ULI	SMA	2	95.14	95.14	36.47	6.04	90.87	99.41
	SNA	6	101.97	104.13	116.39	10.79	86.31	116.07
	XAR	6	98.96	98.13	97.94	9.90	84.63	110.68
UM1	SMA	11	108.10	108.70	24.66	4.97	100.70	117.70
	SNA	9	105.80	103.60	33.43	5.78	96.90	114.90
	XAR	6	111.70	110.20	73.72	8.59	103.20	127.20
UM2	SMA	15	117.20	115.70	55.41	7.44	100.90	129.10
	SNA	19	115.10	114.00	101.92	10.10	101.40	140.90

	XAR	5	119.00	116.30	29.13	5.40	113.80	125.50
UM3	SMA	9	123.30	124.80	71.23	8.44	108.80	133.60
	SNA	21	116.60	114.50	109.89	10.48	101.70	142.60
	XAR	5	120.50	118.10	224.82	14.99	106.60	144.40

When including all individuals, LM1, UCI, and UM2 showed a significant departure from normality for either SNA (G) or XAR (G), which I could not resolve through transformation to Naperian logarithm (Table 5.25). Therefore, I identified outliers in the data before the transformation in a Q-Q plot and excluded them from analyses. UM3 showed a significant departure from normality for the three samples, but transformation to Naperian logarithm resolved this. UM2 still showed departure from normality after excluding outliers and I resolved this by transforming the remaining data to Naperian logarithm. LM1, LM2 and UM1 were significantly heteroscedastic, so I used a Kruskal-Wallis test to compare samples for these variables.

Table 5.25 Results of Shapiro-Wilk normality and homoscedasticity tests for crown index for both sexes. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.96	0.63	21.35 (**<0.001**)	16.19 (**<0.001**)
	SNA	0.99	0.77		
	XAR	0.96	0.23		
LM2	SMA	0.95	0.23	9.66 (**<0.001**)	7.79 (**0.02**)
	SNA	0.99	0.74		
	XAR	0.99	0.97		
UCI	SMA	---	---	---	---
	SNA	0.99	0.78	0.71 (0.40)	0.37 (0.54)
	XAR	0.94	0.15		
ULI	SMA	0.86	0.22	1.92 (0.38)	1.92 (0.38)
	SNA	0.97	0.60		
	XAR	0.96	0.38		
UM1	SMA	0.98	0.95	6.79 (**0.03**)	4.05 (0.13)
	SNA	0.98	0.30		
	XAR	0.95	0.24		
UM2	SMA	0.93	0.70	2.24 (0.33)	1.11 (0.57)
	SNA	0.98	0.09		
	XAR	0.94	0.10		

UM3	SMA	0.93	0.41	0.72 (0.70)	0.57 (0.75)
	SNA	0.97	0.07		
	XAR	0.93	0.09		

In contrast with what happened when all the individuals were included, when only male individuals were included in the analysis, there was no departure from normality for any teeth in any population, and all of them were homoscedastic (Table 5.26).

Table 5.26 Results of Shapiro-Wilk normality and homoscedasticity tests for crown index for males. Significant p-values are in bold red.

Tooth	Population	Normality		Homoscedasticity	
		w	p	Bartlett test K-squared (p)	Fligner-Killeen test chi-squared (p)
LM1	SMA	0.92	0.43	1.66 (0.44)	1.34 (0.51)
	SNA	0.83	0.12		
	XAR	0.84	0.08		
LM2	SMA	0.99	0.99	1.28 (0.53)	3.18 (0.20)
	SNA	0.90	0.07		
	XAR	0.94	0.49		
UCI	SMA	---	---	---	---
	SNA	0.89	0.30	1.77 (0.18)	2.37 (0.12)
	XAR	0.93	0.55		
ULI	SMA	---	---	0.36 (0.84)	0.31 (0.86)
	SNA	0.97	0.90		
	XAR	0.94	0.64		
UM1	SMA	0.95	0.71	2.13 (0.35)	0.87 (0.65)
	SNA	0.96	0.77		
	XAR	0.90	0.36		
UM2	SMA	0.95	0.59	2.70 (0.26)	2.08 (0.35)
	SNA	0.95	0.35		
	XAR	0.84	0.17		
UM3	SMA	0.94	0.58	1.83 (0.40)	0.67 (0.72)
	SNA	0.95	0.38		
	XAR	0.91	0.44		

In the analyses that included both sexes, UM2, LM1, and LM2 showed significant differences across samples (Table 5.27). Tukey posthoc analysis revealed significant differences between XAR (C) and SNA (C) for UM2 (Table 5.28). Mann-Whitney posthoc

tests showed significant differences between XAR (☪) and SNA (☪) for LM1 and LM2 and between SNA (☪) and SMA (☩) for LM2 (Table 5.29).

Table 5.27 Comparison of crown index across both sexes of the samples, using ANOVA or Kruskal-Wallis, as appropriate. Significant p-values are in bold red.

Tooth	ANOVA				Kruskal-Wallis	
	df	df residuals	F (p)	Student's T (p)	df	Chi-squared (p)
LM1	---	---	---	---	2	6.87 (**0.03**)
LM2	---	---	---	---	2	26.43 (**<0.001**)
UCI	---	---	---	-0.69 (0.49)	---	---
ULI	2	58	1.75 (0.18)	---	---	---
UM1	---	---	---	---	2	1.81 (0.40)
UM2	2	155	4.98 (**0.01**)	---	---	---
UM3	2	103	2.40 (0.10)	---	---	---

Table 5.28 Results of Tukey posthoc analysis for crown index for both sexes. Significant p-values are in bold red.

Tooth	Samples	Difference (p)	Lower	Upper
UM2	SNA-SMA	-0.03 (0.11)	-0.07	0.01
	XAR-SMA	0.00 (0.99)	-0.04	0.05
	XAR-SNA	0.04 (**0.02**)	0.00	0.07

Table 5.29 Results of pairwise Mann-Whitney posthoc analysis for crown index for both sexes. Significant p-values are in bold red.

Tooth	Samples	W (p)
LM1	SNA-SMA	837.50 (**0.03**)
	XAR-SMA	342.00 (0.97)
	XAR-SNA	1128.50 (**0.04**)
LM2	SNA-SMA	1909.00 (**<0.001**)
	XAR-SMA	490.00 (0.64)
	XAR-SNA	973.00 (**<0.001**)

In males only, the comparison did not show any significant differences among the samples for any of the teeth (Table 5.30).

Table 5.30 Comparison of crown index across male individuals of the samples. Significant p-values are in bold red.

Tooth	ANOVA			
	df	df residuals	F (p)	Student's T (p)
LM1	2	19	2.06 (0.15)	---
LM2	2	37	3.00 (0.06)	---
UCI	---	---	---	-0.52 (0.62)
ULI	2	11	0.38 (0.69)	---
UM1	2	23	1.65 (0.21)	---
UM2	2	36	0.50 (0.61)	---
UM3	2	32	1.28 (0.29)	---

6. Results: Nonmetric Dental Traits

This chapter presents the results and discussion of the analyses performed to compare the archaeological samples using the classic nonmetric dental method.

Table 6.1 Summary of sites and individuals included from each site.

	SMA (Santa Maria la Real – Christians – North of IP)	SNA (San Nicolas de Avila – Muslims – Centre of IP)	XAR (Xarea – Muslims – South of IP)
Females	4	51	12
Males	18	23	12
Unknown	13	34	21
Total	35	108	45

Smith's Mean Measure of Divergence (MMD) showed that the closest samples were SNA (☪) and XAR (☪), both similarly distanced from SMA (✞). Moreover, the differences between the Muslim samples and the Christian sample were significant (Table 6.2).

Table 6.2 Results of Smith's Mean Measure of Divergence analysis (MMD values over diagonal and in bold red if significant, standard deviations below diagonal).

	SMA	SNA	XAR
SMA	----	0.26	0.25
SNA	0.08	----	0.10
XAR	0.13	0.07	----

All the frequencies for each variable within each group are very high, with lower frequencies in SNA than in the other samples for most of the traits (Table 6.3)

Table 6.3 Number of individuals and relative frequencies for each variable within each group.

Traits	SMA		SNA		XAR	
	n	Freq	n	Freq	n	Freq
Shoveling	--	--	34	0.82	9	1.00
Double Shoveling	--	--	37	0.11	11	0.27
Tuberculum dentale	4	1.00	18	0.94	6	1.00
Metaconule	7	0.86	51	0.69	13	0.92
Carabelli's trait	7	0.86	49	0.69	12	0.91
Hypocone	10	0.90	75	0.81	9	1.00
Parastyle	8	0.00	49	0.08	6	0.17
Metacone	8	1.00	49	0.98	6	1.00
Entoconulid	7	0.00	43	0.35	14	0.50
Metaconulid	11	0.46	50	0.22	15	0.33
Protostylid	13	0.69	49	0.37	14	0.64

Anterior Fovea	4	1.00	29	0.97	12	0.83
Hypoconulid	15	0.53	60	0.35	10	0.40

When I excluded traits with an overall negative measure of divergence and used only those with a minimal number of five individuals per population, only four out of the thirteen traits were useful to compare the samples. In order of discriminatory power, highest to lowest, these were entoconulid, protostylid, hypocone and metaconulid (Table 6.4).

Table 6.4 Overall measure of divergence for each variable useful to compare the samples.

	Overall Measure of Divergence
Entoconulid	1.93
Protostylid	0.36
Hypocone	0.12
Metaconulid	0.03

Hierarchical cluster analysis of the distance matrix using Ward's algorithm of minimum variance shows that the three archaeological samples can be divided into two clusters, one including both Muslim samples and the other the Christian sample (Figure 6.1).

When the exclusion method is not applied, and therefore traits with an overall negative measure of divergence are included, none of Smith's Mean Measure of Divergence results are significant. In addition, the closest samples are XAR (☪) and SMA (✞), with a negative MMD value between them, which does not have biological sense. Likewise, when traits with an overall negative measure of divergence are excluded, only traits with at least 10 individuals per group are retained. The only significant result is between SMA (✞) and SNA (☪), and the MMD value between XAR (☪) and SMA (✞) is negative. Therefore, the results are susceptible to the parameters chosen.

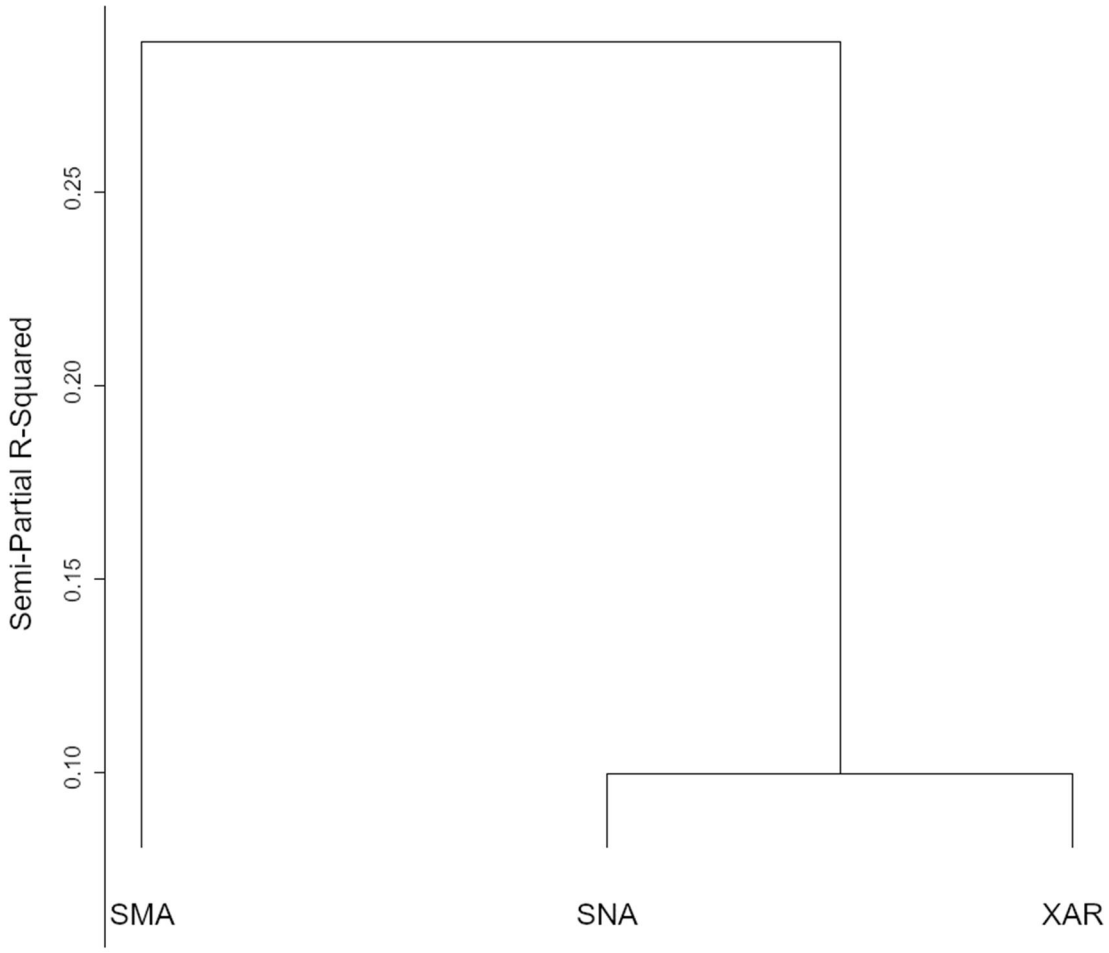


Figure 6.1 Hierarchical clustering of the three archaeological samples using Ward's algorithm

Examples of variability in the samples are shown in figures 6.2 (lower first molars), 6.3 (lower second molars), 6.4 (upper lateral incisors), 6.5 (upper first molars), 6.6 (upper second molars) and 6.7 (upper third molars).

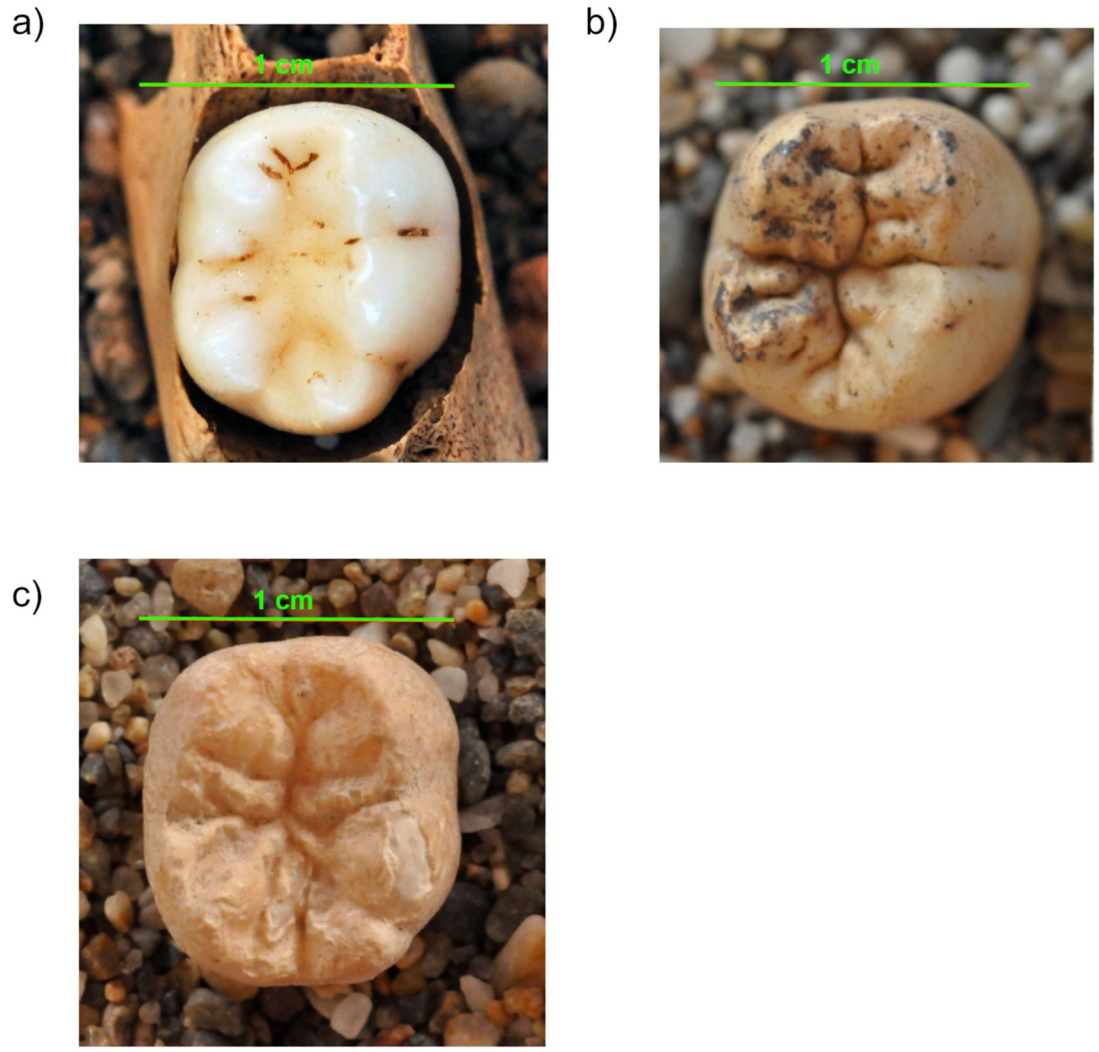
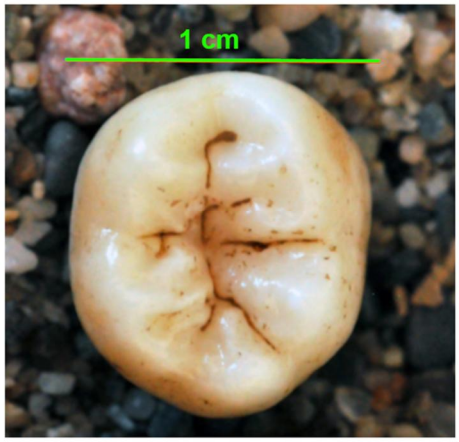


Figure 6.2 Examples of the variability in lower first molars for: (a) SMA, (b) SNA, and (c) XAR. The dental traits in these molars were scored: (a) Anterior Fovea 3 – Protostylid 1 – Metaconulid 1 – Entoconulid 0; (b) Anterior Fovea 4 – Protostylid 1 – Metaconulid 0 – Entoconulid 1; (c) Anterior Fovea 2 – Protostylid 0 – Metaconulid 0 – Entoconulid 2

a)



b)

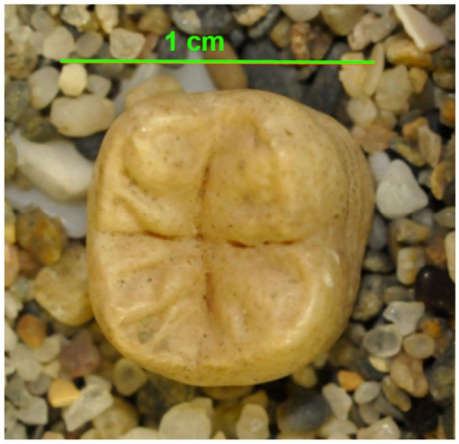


Figure 6.3 Examples of the variability in lower second molars for: (a) SMA, and (b) SNA. The dental traits in these molars were scored: (a) Hypoconulid 4; (b) Hypoconulid 0

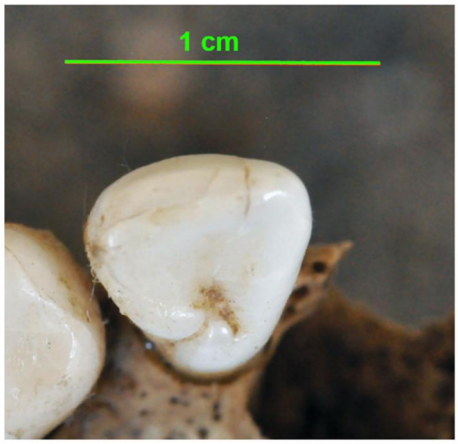


Figure 6.4 Example of an upper lateral incisor from SMA, for which the Tuberculum Dentale was scored 4

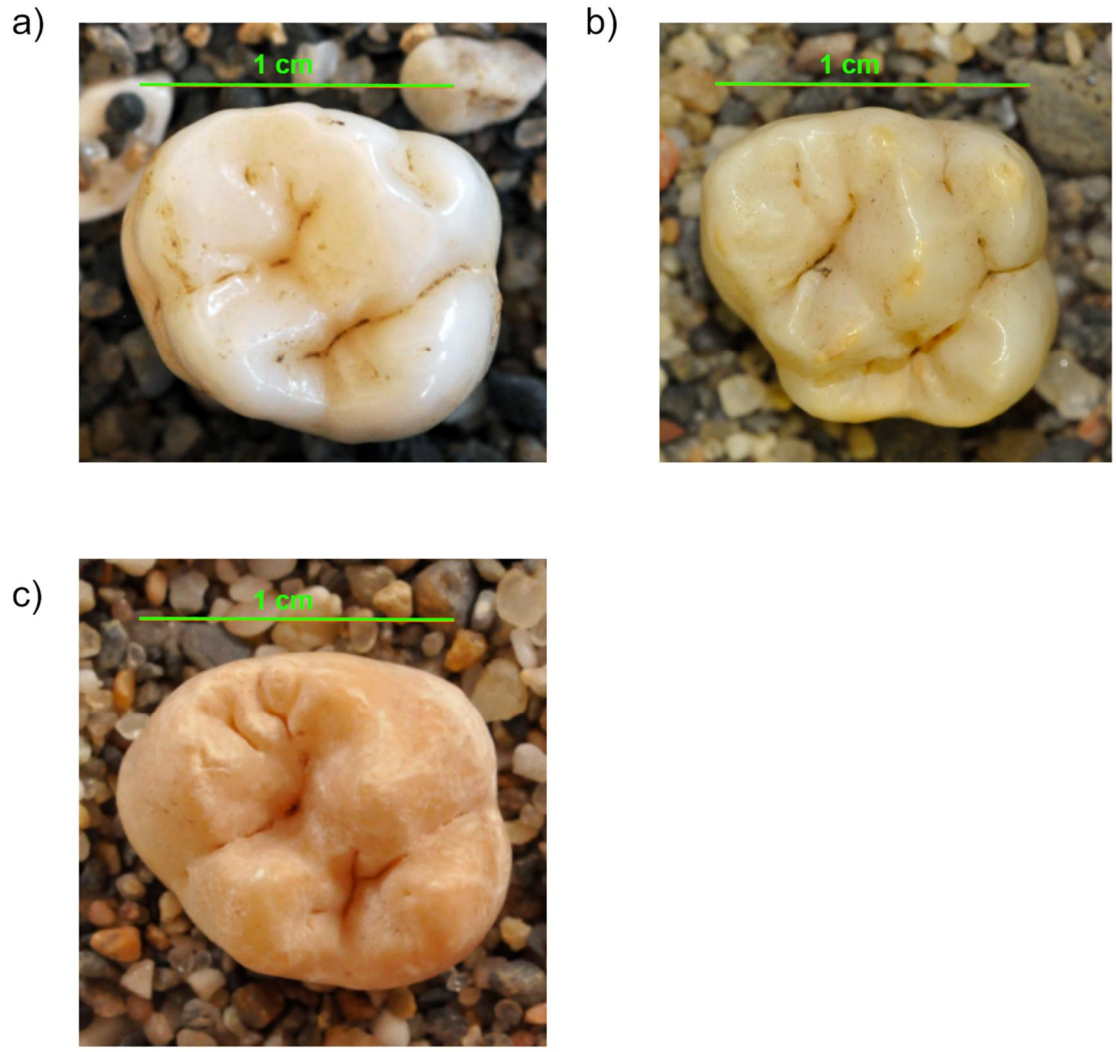


Figure 6.5 Examples of the variability in upper first molars for: (a) SMA, (b) SNA, and (c) XAR. The dental traits in these molars were scored: (a) Carabelli's cusp 6 – Metaconule 1; (b) Carabelli's cusp 7 – Metaconule 5; (c) Carabelli's cusp 2 – Metaconule 2

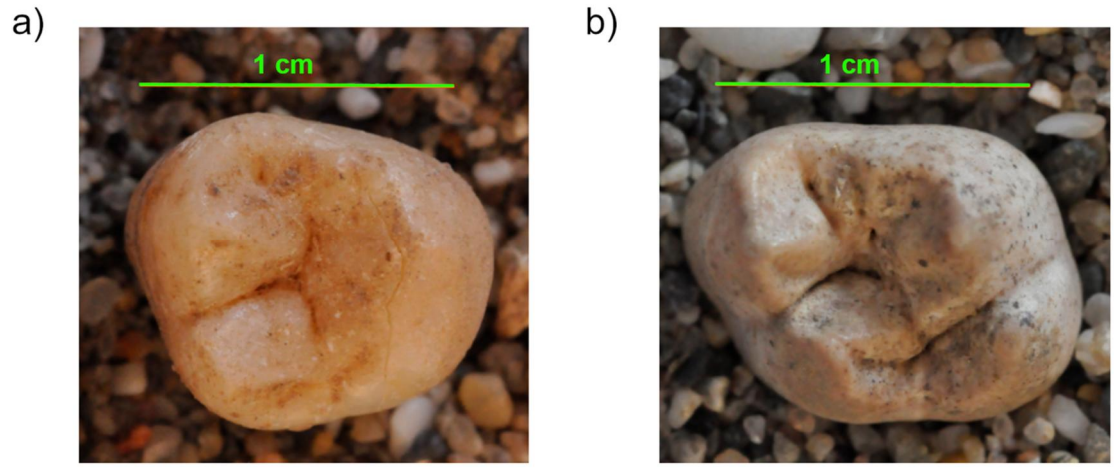


Figure 6.6 Examples of the variability in upper second molars for: (a) XAR, and (b) SNA. The dental traits in these molars were scored: (a) Hypocone 1; (b) Hypocone 5

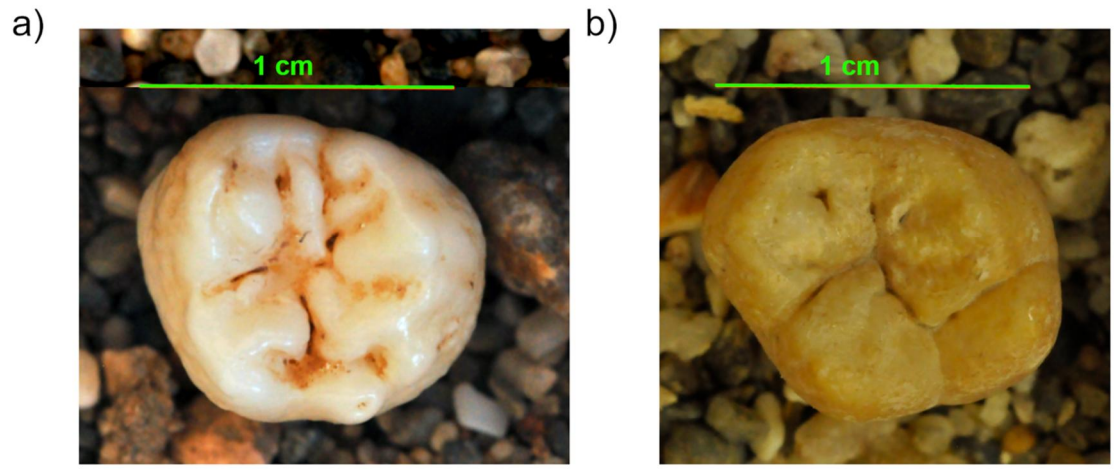


Figure 6.7 Examples of the variability in upper third molars for: (a) SMA, and (b) XAR. The dental traits in these molars were scored: (a) Metacone 4 – Parastyle 0; (b) Metacone 5 – Parastyle 1

7. Results: 2D Geometric Morphometrics

This chapter presents the results from the analyses performed to compare the archaeological samples using 2D GMM.

Table 7.1 Summary of sites and individuals included from each site.

	SMA (Santa Maria la Real – Christians – North of IP)	SNA (San Nicolas de Avila – Muslims – Centre of IP)	XAR (Xarea – Muslims – South of IP)
Females	4	51	12
Males	18	23	12
Unknown	13	34	21
Total	35	108	45

7.1. Interobserver Error

In the Principal Component Analyses, there was good overlap between the landmarks placed by another observer and me in the three chosen sets of landmarks, with UM2 Hypocone showing the greatest degree of overlap (Figure 7.1). The specimens I landmarked had, for LM1 and LM1 Anterior Fovea, a greater range of distribution over PCs 1 and 2 than those landmarked by Dr Plomp.

Procrustes ANOVA showed that the differences among individuals were greater than the differences among observers, although both differences were too tight to assume that the sets of landmarks had a good repeatability (Table 7.2).

Table 7.2 Results of Procrustes ANOVA quantifying interobserver measurement error. For each effect the procrustes sums of squares (SS) and the procrustes mean squares (MS) are provided

Set of landmarks	Effect	SS	MS	df	F	p
LM1	Individual	0.1275	0.000052	2436	2.16	<0.0001
	Interobserver	0.0609	0.000024	2520		
LM1 Anterior Fovea	Individual	0.1478	0.000059	2494	2.16	<0.0001
	Interobserver	0.0707	0.000027	2580		
UM2 Hypocone	Individual	0.2358	0.000099	2378	2.91	<0.0001
	Interobserver	0.0838	0.000034	2460		

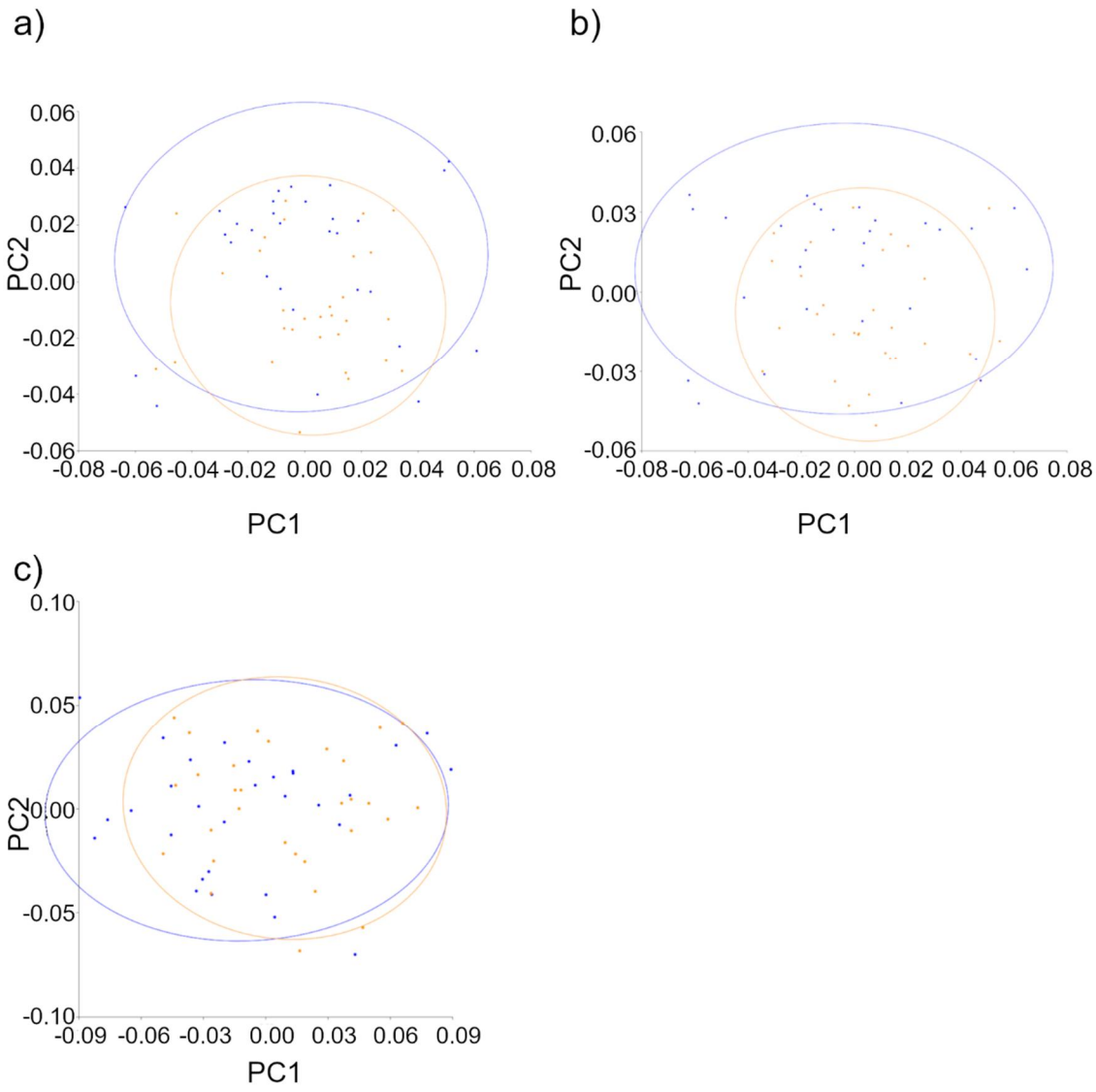


Figure 7.1 PCA for the chosen individuals grouped along their principal component axes (PCs 1 and 2) for: (a) LM1, (b) LM1 Anterior Fovea, and (c) UM2 Hypocone. Orange = landmarked by Dr Kimberly Plomp; blue = landmarked by me

The intraclass correlation coefficient (R), was low for all cases (Table 7.3), not reaching 0.5 in any of the landmark sets, with UM2 Hypocone showing slightly higher repeatability, as the PCA seemed to indicate.

Table 7.3 Intraclass correlation coefficient (R) for interobserver error for the three sets of landmarks

Set of landmarks	R
LM1	0.37
LM1 Anterior Fovea	0.37
UM2 Hypocone	0.49

7.2. Intraobserver Error

The PCA revealed very good overlap between my two rounds of landmarks, with UM2 Hypocone again showing a larger degree of overlap (Figure 7.2). The Procrustes ANOVA confirmed the PCA results (Table 7.4), with the UM2 Hypocone showing clear greater differences among individuals, which were larger than the differences between the observations. The differences for the individuals were also larger than the differences for the observations in LM1 and LM1 Anterior Fovea, although as in the interobserver error, the distances between the differences were small.

Table 7.4 Results of Procrustes ANOVA for quantifying intraobserver measurement error. For each effect the procrustes sums of squares (SS) and the procrustes mean squares (MS) are provided.

Set of landmarks	Effect	SS	MS	df	F	p
LM1	Individual	0.1618	0.000066	2436	3.13	<0.0001
	Intraobserver	0.0534	0.000021	2520		
LM1 Anterior Fovea	Individual	0.1894	0.000076	2494	3.19	<0.0001
	Intraobserver	0.0614	0.000024	2580		
UM2 Hypocone	Individual	0.2913	0.000122	2378	8.12	<0.0001
	Intraobserver	0.0371	0.000015	2460		

The intraclass correlation coefficient, which gives information about the measurement error in relation to the total amount of variation showed that the set of landmarks UM2 Hypocone had the greatest repeatability, nearly 0.8 (an intraclass correlation coefficient of 1 would mean that there is no intraobserver error) (Table 7.5). The correlation coefficient for the other sets of landmarks was 0.52, which shows a poor repeatability, although it is better than for interobserver error.

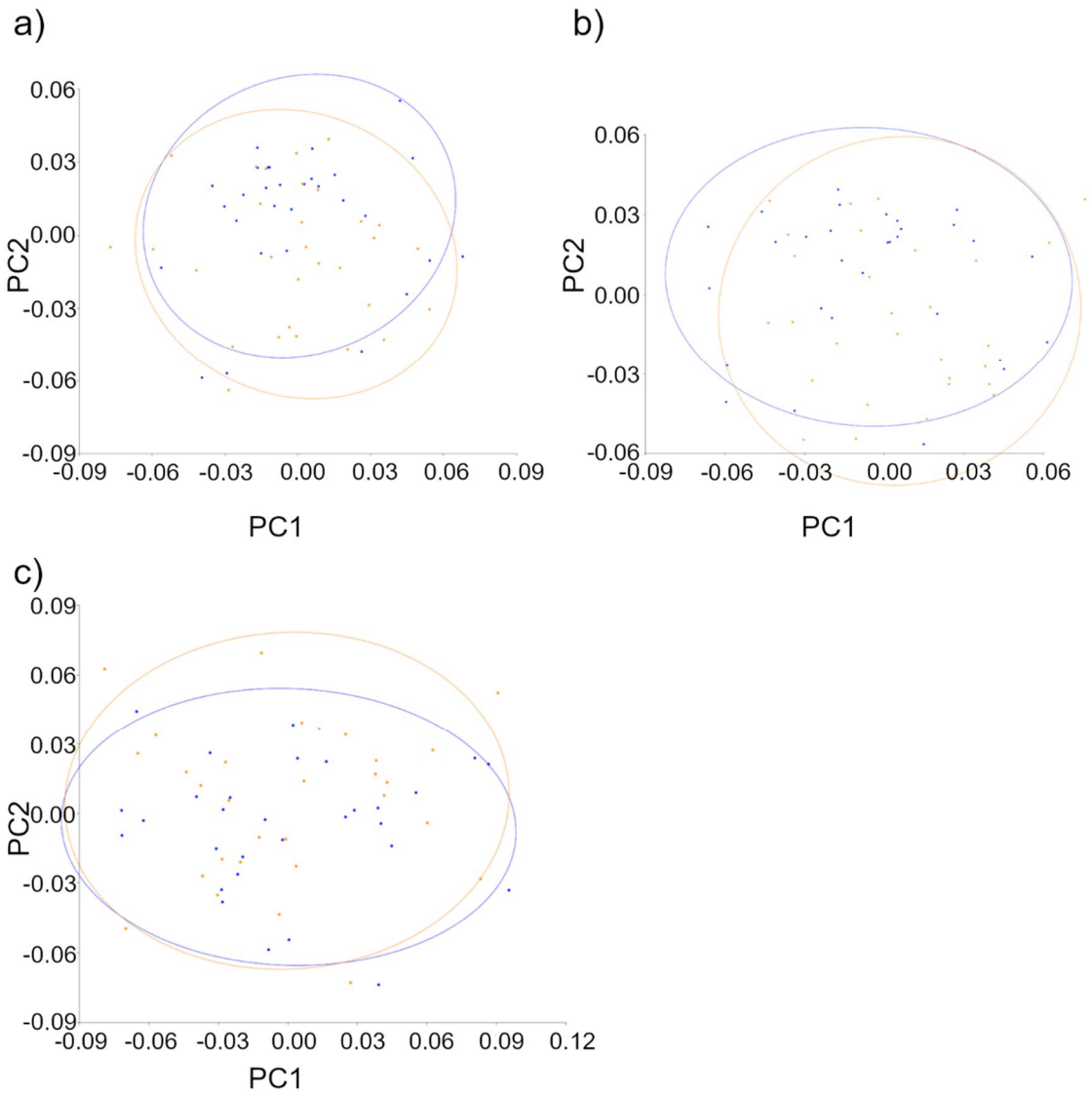


Figure 7.2 PCA for the chosen individuals grouped along their principal component axes (PCs 1 and 2) for: (a) LM1, (b) LM1 Anterior Fovea, and (c) UM2 Hypocone. Blue = First round of landmarks; Orange = Second round of landmarks

Table 7.5 Intraclass correlation coefficient (R) for the intraobserver error for the three sets of landmarks

Set of landmarks	R
LM1	0.52
LM1 Anterior Fovea	0.52
UM2 Hypocone	0.78

7.3. Lower Molar 1

As described in the Methods (Chapter 4), I studied four traits of LM1 (using three landmark configurations), and two additional landmark configurations for the outline of the crown and the outline and cusps. The results of all the allometry analyses were significant but the percentage shape change predicted by the size was very low (the highest value is 3% for the LM1 Protostylid buccal) (Table 7.6). Given that the allometry was significant, although small, I used the residuals from the regressions instead of the raw data in subsequent analyses.

Table 7.6 Results of regression analysis to explore allometry in Lower Molar 1. Significant p-values are in bold red.

	R-squared	p
LM1	0.0236	**<0.001**
LM1 Outline	0.0180	**0.03**
LM1 Anterior Fovea	0.0201	**0.01**
LM1 Entoconulid	0.0255	**<0.001**
LM1 Protostylid Buccal	0.0307	**<0.001**

For all the sets of landmarks except the LM1 Protostylid Buccal the number of principal components (PCs) was 76-86 and the first five PCs explained <75% of shape variation (Table 7.7). The LM1 Protostylid Buccal, however, was defined by fewer PCs (56) and the first five explained more of the shape variation (86%). The landmark configuration LM1 Outline, which does not include information for the cusps, explained shape variation better than LM1 with fewer PCs.

Table 7.7 Results of PCA for Lower Molar 1

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
LM1	1	0.0006	23.48	23.48	84
	2	0.0005	17.62	41.10	
	3	0.0003	12.36	53.46	
	4	0.0003	9.44	62.90	
	5	0.0002	6.63	69.53	
	1	0.0004	31.06	31.06	76

LM1 Outline	2	0.0003	20.30	51.35	
	3	0.0001	9.45	60.80	
	4	0.0001	7.13	67.93	
	5	0.0001	6.46	74.39	
LM1 Anterior Fovea	1	0.0009	27.98	27.98	86
	2	0.0005	14.14	42.12	
	3	0.0004	12.86	54.98	
	4	0.0003	8.59	63.57	
	5	0.0002	6.81	70.38	
LM1 Entoconulid	1	0.0006	20.30	20.30	86
	2	0.0006	19.26	39.56	
	3	0.0004	13.05	52.61	
	4	0.0003	9.93	62.53	
	5	0.0002	7.52	70.05	
LM1 Protostylid Buccal	1	0.0028	38.31	38.31	56
	2	0.0018	23.98	62.29	
	3	0.0011	15.27	77.56	
	4	0.0004	4.93	82.49	
	5	0.0003	3.91	86.40	

Shape variation in LM1 and LM1 Outline (the same landmark configuration as LM1 except for the landmarks in the cusps) affected the development of the protoconid (cusp 1) and the hypoconulid/entoconulid (cusps 5 and 6) most (Figure 7.3 a and b). These changes explained up to 50% of shape variation (Table 7.7). The biggest shape variation in LM1 Anterior Fovea was defined by PC1, in which landmark 45 migrated from a mesial situation on the edge of the crown to a more central position on the occlusal side, between the protoconid and the metaconid (cusp 2) (Figure 7.3 c). This suggested differences in the development of this dental trait between the samples, explaining up to 27% of shape variation when the position of the trait was included in the landmark configurations (Table 7.7).

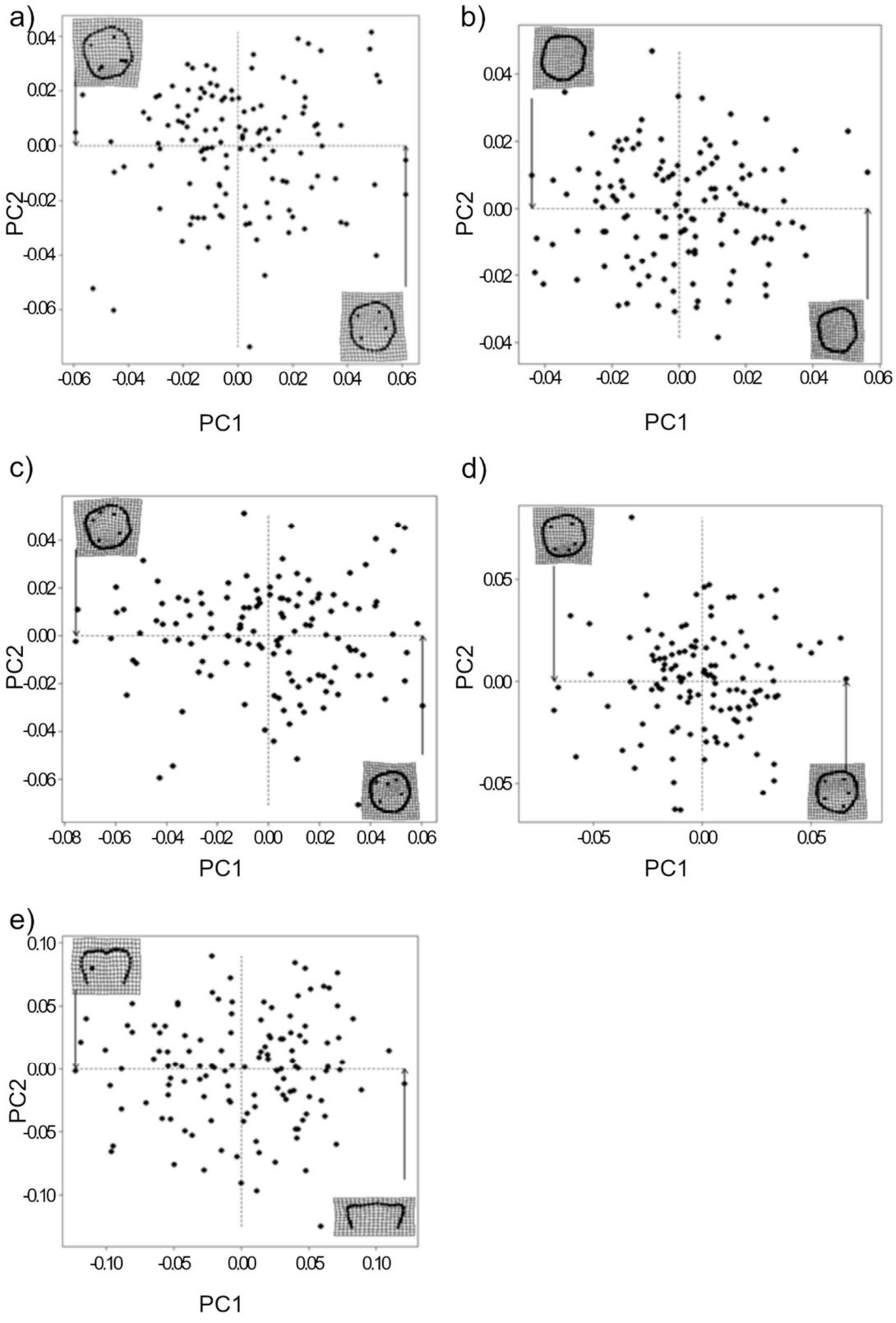


Figure 7.3 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) LM1, (b) LM1 Outline, (c) LM1 Anterior Fovea, (d) LM1 Entoconulid, and (e) LM1 Protostylid Buccal

In addition, 20% of the shape variation in the entoconulid landmark configuration was defined by a change in the development of the distal surface of LM1 (Figure 7.3 d). It is not clear whether this change is an effect of the development of the hypoconulid, or whether it responded to the entoconulid, given that a larger distal surface was not directly linked to a visible sixth cusp. Although this shape variation was the largest (PC1) for LM1 and LM1 Outline, the percentage of the variation explained was larger in LM1 Outline, so the extra landmarks for the cusps may not make the landmark configuration more informative (Table 7.7).

For the LM1 Protostylid Buccal, the largest shape variation (almost 40%) was explained by the wear of the occlusal face, making this landmark configuration inadequate for studying biological relatedness, as it relies on an area that is too vulnerable to wear (Figure 7.3 e).

CVA showed differences between SMA (✞) and XAR (☪) and SNA (☪), and the landmark set that best reflected these differences was LM1 Entoconulid, followed by LM1 Anterior Fovea and LM1 (Figure 7.4). Not using landmarks in the cusps, as in LM1 Outline, seems to reduce the differences among the samples, as the samples are closer in the graph for this landmark set than in the graph for LM1, which is based on the same landmarks except for the cusps. SMA (✞) was in the negative side of CV1 in the sets of landmarks LM1, LM1 Outline, and LM1 Anterior Fovea, whereas for LM1 Entoconulid was in the positive side of CV1, set of landmarks for which SNA (☪) and XAR (☪) were in the negative side of CV1 (Figure 7.4).

More Procrustes distances were significant than Mahalanobis distances were (Table 7.8). Given that DFA tends to overestimate the differences among groups, I only considered differences where both distances are significant.

There were significant differences between SMA (☥) and XAR (☪) for both LM1 Anterior Fovea and LM1 Entoconulid (Table 7.8, Figure 7.4 c and d). The DFA for LM1 Protostylid Buccal also showed significant differences between SMA (☥) and SNA (☪) and between SNA (☪) and XAR (☪) but given that the largest shape variation for this landmark configuration was determined by wear, this result probably does not reflect biological differences among these samples.

The Procrustes distance for all the landmark configurations showed that the closest samples were SNA (☪) and XAR (☪), with SMA (☥) being further from both (but closer to SNA (☪) than to XAR (☪)) (Table 7.8). In contrast, the Mahalanobis distance showed that although SNA (☪) and XAR (☪) were still the closest samples, the biggest difference was between SMA (☥) and SNA (☪), with SMA (☥) and XAR (☪) in an intermediate position (Table 7.8).

Table 7.8 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Lower Molar 1. Significant p-values are in bold red

		Mahalanobis Distance		Procrustes Distance	
		SMA	SNA	SMA	SNA
LM1	SNA	14.57 (0.24)		0.02 (**0.03**)	
	XAR	7.31 (0.07)	3.58 (0.48)	0.03 (**<0.001**)	0.01 (**0.03**)
LM1 Outline	SNA	8.49 (0.11)		0.01 (0.16)	
	XAR	6.18 (0.44)	3.04 (0.46)	0.01 (0.14)	0.01 (0.20)
LM1 Anterior Fovea	SNA	17.07 (0.41)		0.02 (**0.03**)	
	XAR	7.37 (**0.03**)	3.47 (0.69)	0.03 (**0.01**)	0.02 (**0.01**)
LM1 Entoconulid	SNA	13.64 (0.45)		0.02 (0.10)	
	XAR	7.90 (**0.02**)	3.84 (0.66)	0.03 (**0.01**)	0.02 (**0.02**)
LM1 Protostylid Buccal	SNA	4.78 (**0.02**)		0.05 (**<0.001**)	
	XAR	13.52 (0.15)	2.92 (**<0.001**)	0.02 (0.83)	0.04 (**<0.001**)

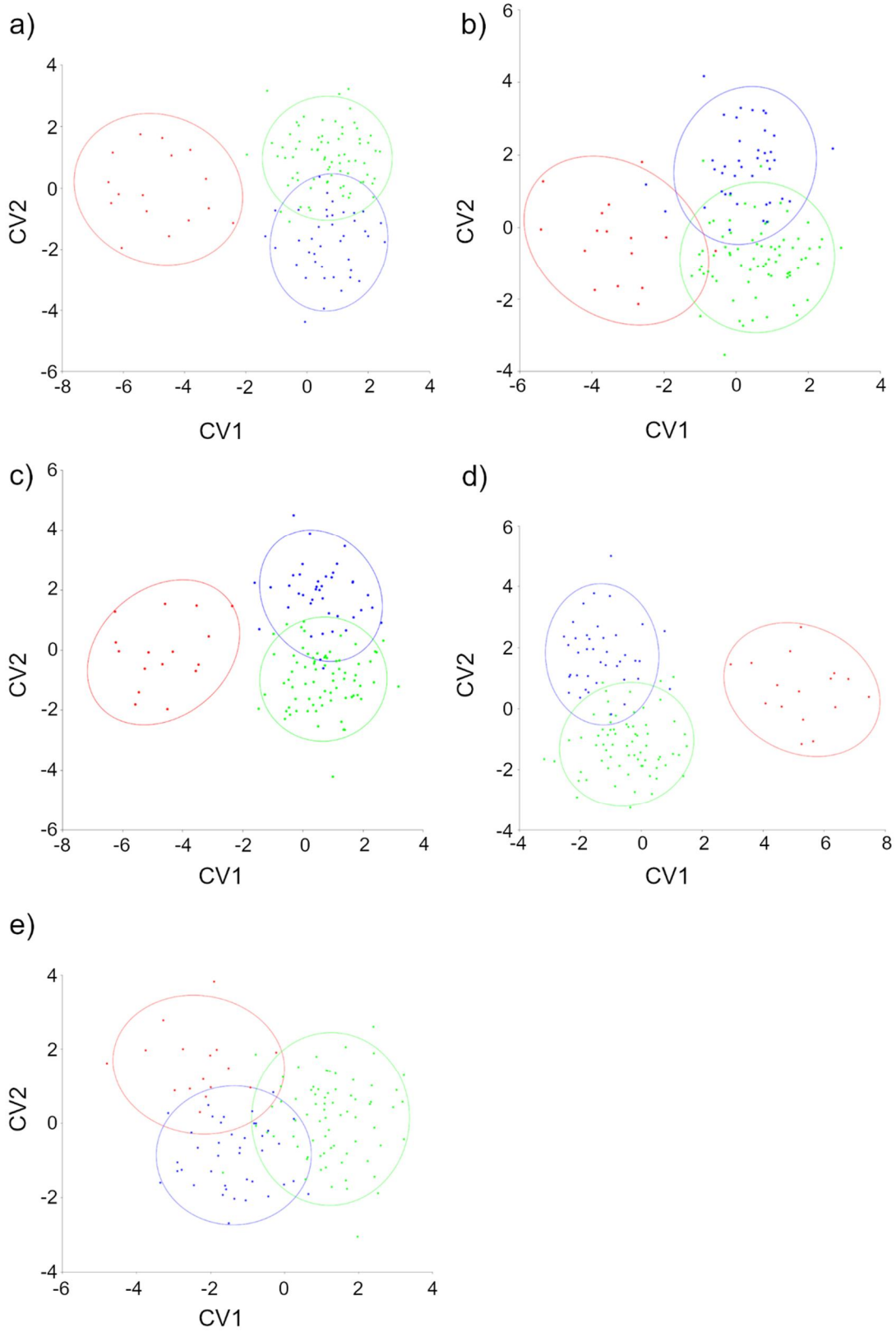


Figure 7.4 CVA with specimens grouped along their canonical variate axes (CVs 1 and 2) for: (a) LM1, (b) LM1 Outline, (c) LM1 Anterior Fovea, (d) LM1 Entoconulid, and (e) LM1 Protostylid Buccal. Red = SMA; green = SNA; blue = XAR

The lowest number of PCs needed to obtain >50% reliability was for the landmark configuration of LM1 Anterior Fovea where 8 PCs correctly identified 75% of the individuals of SMA (✞) and 65% of XAR (☪) (Table 7.9). For LM1 Entoconulid, 16 PCs were needed, and the percentage of individuals correctly classified was 44% for SMA (✞), 51% for SNA (☪) and 58% for XAR (☪). Including the cusps in the landmark configuration entailed a much better capacity to differentiate the samples with a lower number of PCs, as the results for LM1 and LM1 Outline suggest (the percentage of individuals correctly classified was slightly higher in LM1 than LM1 Outline but the number of PCs needed was noticeably lower).

Table 7.9 Percentage correct classification of specimens according to cross-validation of DFA for Lower Molar 1, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	LM1	LM1 Outline	LM1 Anterior Fovea	LM1 Entoconulid	LM1 Protostylid Buccal
lowest number of PCs needed to obtain an overall reliability >50%	11	35	8	16	15
SMA	63%	44%	75%	44%	38%
SNA	49%	55%	51%	51%	64%
XAR	53%	40%	65%	58%	43%

7.4. Lower Molar 2

I only studied one dental trait for LM2 (Hypoconulid), although I added a landmark configuration for the outline of LM2 to compare the differences in the results when landmarks for the cusps were not included in the analysis.

Both landmark configurations showed significant allometry, although the percentage of the shape change affected by the size was very small (<1.5% for all landmark sets, Table 7.10). Therefore, as with LM1, I used the residuals of the regressions rather than the raw data for subsequent analyses.

Table 7.10 Results of regression analysis to explore allometry in Lower Molar 2. Significant p-values are in bold red

	R-squared	p
LM2 Hypoconulid	0.0143	**0.03**
LM2 Outline	0.0146	**0.03**

The percentage of shape explained by the first PC was very similar in both landmark sets, with the cumulative shape variation explained by the first five PCs being practically identical (Table 7.11). The number of PCs needed to describe the shape variation fully was slightly lower for LM2 Outline, the landmark configuration that did not include the cusps.

Table 7.11 Results of PCA for Lower Molar 2

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
LM2 Hypoconulid	1	0.0009	26.10	26.10	84
	2	0.0007	19.69	45.78	
	3	0.0005	14.47	60.25	
	4	0.0003	8.03	68.28	
	5	0.0002	7.12	75.40	
LM2 Outline	1	0.0003	24.39	24.39	76
	2	0.0003	23.22	47.61	
	3	0.0001	11.16	58.77	
	4	0.0001	8.82	67.58	
	5	0.0001	7.91	75.49	

The most important shape variation in the LM2 Hypoconulid (26% of the total shape variation) was related to the development of the hypoconid and the location of its cusp, which would be associated with the development of the hypoconulid; a mesial position of the hypoconid was linked to greater development of the hypoconulid, whereas in teeth where the hypoconulid was absent the cusp of the hypoconid tended to be more distal (Figure 7.5 a). In addition, the greater development of the metaconid seemed to be the second most important feature affecting the shape variation.

For LM2 Outline, the feature most affecting shape variation was the development of the metaconid (24% of the shape variation), followed by the development of the protoconid

and the buccodistal face of the teeth (Figure 7.5 b). This meant that the landmark configuration that included the cusps described the variation associated with the hypoconulid better than that without the cusps. However, in terms of the general shape variation, both landmark configurations explained similar percentages of shape variation, and LM2 Outline did so with fewer principal components than LM2 Hypoconulid.

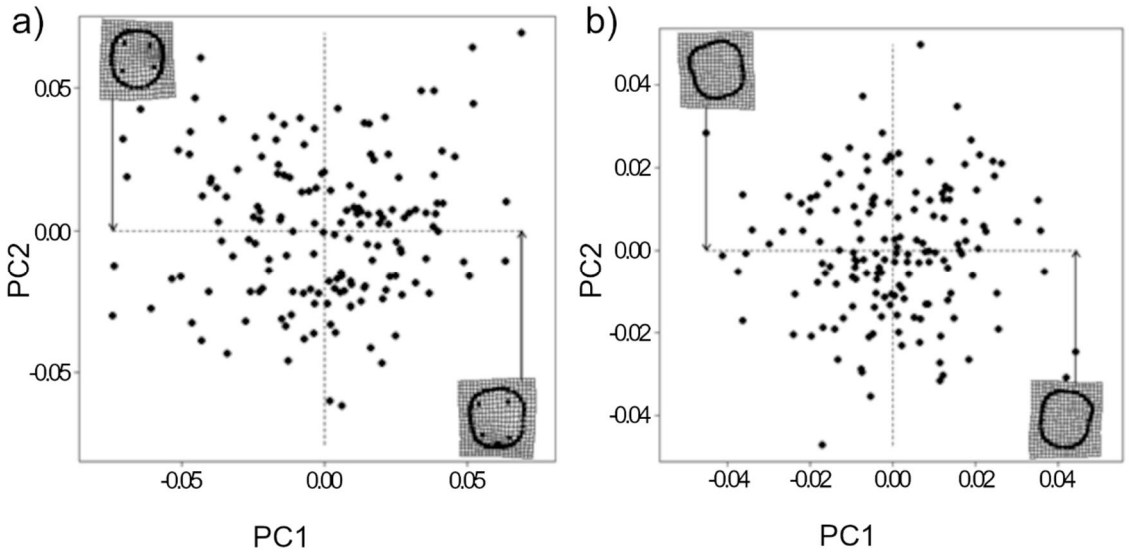


Figure 7.5 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) LM2 Hypoconulid and (b) LM2 Outline

The three archaeological samples overlapped and there were no evident differences between them, although the SMA (✞) sample overlapped less than the two others did, especially for LM2 Hypoconulid, the landmarks set that included the cusps (Figure 7.6). For both sets of landmarks, SMA (✞) was distributed over the negative side of CV1, whereas XAR (☪) was mostly in the positive side of CV1, and SNA (☪) was directly in between of both samples (Figure 7.6).

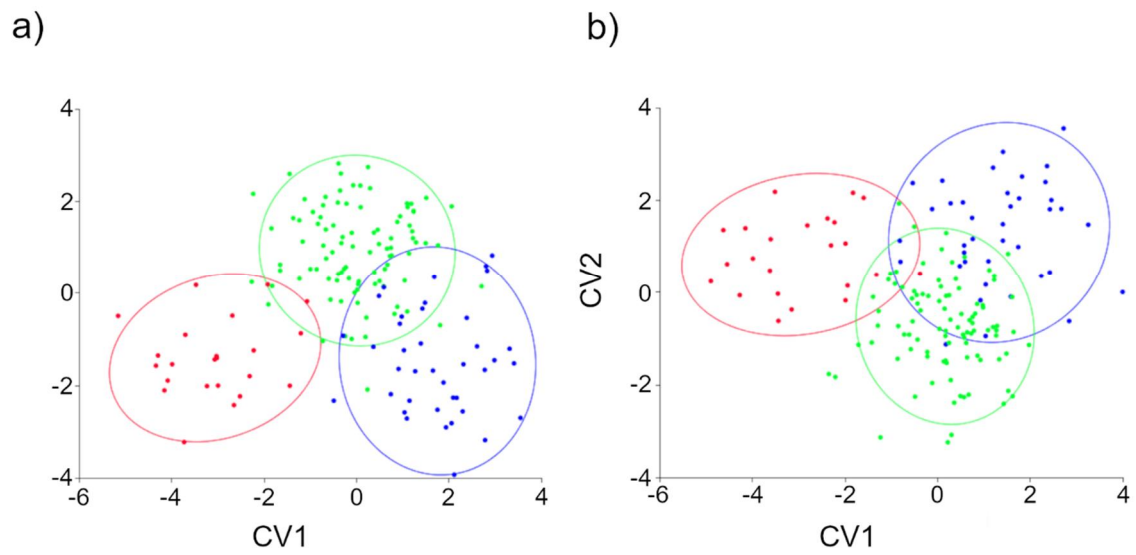


Figure 7.6 CVA with specimens grouped along their canonical variate axes (CVs 1 and 2) for: (a) LM2 Hypoconulid, and (b) LM2 Outline. Red = SMA; green = SNA; blue = XAR

As with LM1, there was a discrepancy between the results of the Mahalanobis and the Procrustes distances, so I only took values of the distances into account when both analyses were significant (Table 7.12). For both distances, the LM2 Hypoconulid was significant for SMA-SNA and SMA-XAR, which means that SMA (✞) was biologically further from the other two samples, which were closer to one another.

Table 7.12 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Lower Molar 2. Significant p-values are in bold red

		Mahalanobis Distance		Procrustes Distance	
		SMA	SNA	SMA	SNA
LM2 Hypoconulid	SNA	5.26 (**0.03**)		0.02 (**0.05**)	
	XAR	7.11 (**0.03**)	3.31 (0.11)	0.03 (**<0.001**)	0.02 (**<0.001**)
LM2 Outline	SNA	4.84 (**0.01**)		0.01 (0.15)	
	XAR	7.24 (0.09)	2.72 (0.26)	0.01 (**0.02**)	0.01 (0.06)

LM2 Hypoconulid provided much more powerful discrimination than LM2 Outline, as the overall rate of reliability was higher than 50% with just 6 PCs, whereas LM2 Outline required 44 PCs to reach this criterion (Table 7.13). When the landmark configuration included the cusps, 61% of the individuals from SMA (✞) could be correctly classified with

the first 6 PCs, as could 59% of the individuals from XAR (Ꞓ). The shape variation observed with LM2 Hypoconulid was more related to the development of the hypoconulid than the shape variation observed with LM2 Outline, which was related to the metaconid and the protoconid. Therefore, even if both sets of landmarks provided similar information in terms of the general shape variation, the fact that LM2 Hypoconulid was more affected by the hypoconulid than by other cusps made it more suitable to find differences between populations, as neither the metaconid nor the protoconid are defined nonmetric dental traits in the ASU system.

Table 7.13 Percentage correct classification of specimens according to cross-validation of DFA for Lower Molar 2, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	LM2 Hypoconulid	LM2 Outline
Lowest number of PCs needed to obtain overall reliability >50%	6	44
SMA	61%	46%
SNA	46%	39%
XAR	59%	53%

7.5. Upper Central Incisor

I studied shoveling and double-shoveling in the UCI, with two different landmark configurations. Both sets of landmarks had significant results in the regression to test for allometry, with a high percentage of the shape fix by size (33% for shoveling and 13% for double-shoveling) (Table 7.14). Therefore, I used the residuals of the regression instead of the raw data for the rest of the analyses.

Table 7.14 Results of regression analysis to explore allometry in Upper Central Incisor. Significant p-values are in bold red

	R-squared	p
UCI Shoveling	0.3288	**<0.0001**
UCI Double-Shoveling	0.1332	**<0.0001**

For UCI shoveling there were fewer PCs than dimensions in shape space, which could be a consequence of the small sample size (Table 7.15). As such, the results for the UCI shoveling have limited reliability. The PCA for the landmark configuration double-shoveling gave 56 PCs to describe the 100% of the shape variation, with the first 5 PCs accruing over 90% of this variation (Table 7.15).

Table 7.15 Results of PCA for Upper Central Incisor

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
UCI Shoveling	1	0.0052	38.41	38.41	46
	2	0.0035	25.67	64.09	
	3	0.0020	14.46	78.54	
	4	0.0008	6.07	84.61	
	5	0.0007	5.21	89.82	
UCI Double-Shoveling	1	0.0053	59.95	59.95	56
	2	0.0017	18.90	78.85	
	3	0.0007	7.51	86.35	
	4	0.0003	3.43	89.78	
	5	0.0003	3.15	92.93	

The shape variation in the landmark configuration UCI shoveling relied mostly on its contour, going from an incisor with a shape similar to a scalene triangle to one closer to an equilateral triangle (Figure 7.7 a). This change in shape accrued 64% of the total variation. For the landmark set UCI double-shoveling 78% of the shape variation fell in the outline, with changes from a thicker to a flatter shape (i.e., reduced buccolingual distance) (Figure 7.7 b). None of the landmark configurations collected information on variation in the specific dental trait to which they were related, and their shapes changes were limited to their contour.

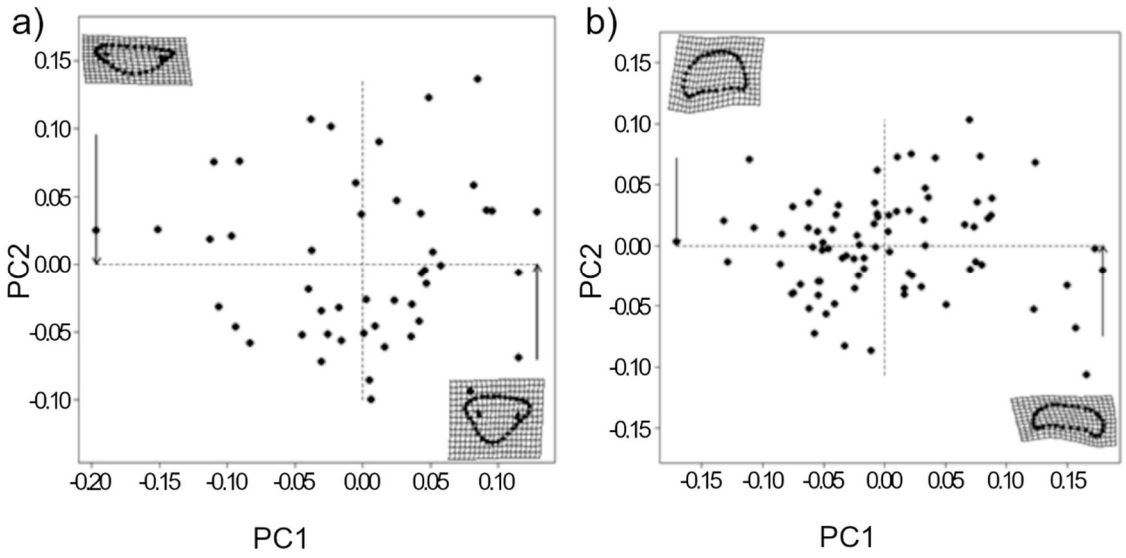


Figure 7.7 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) UCI Shoveling and (b) UCI Double-Shoveling

Given that there were no upper central incisors for the SMA (♱) population, I did not perform a CVA. The DFA showed significant differences between SNA (☪) and XAR (☪) for UCI Double-Shoveling but not for UCI Shoveling (Table 7.16), which differs from the patterns of the CVA (Figure 7.10). The number of PCs needed for an overall reliability of >50% was low in both cases, with 3 for Double-Shoveling and 4 for Shoveling, although the correct classification of individuals was higher for UCI Double-Shoveling, being 73% for XAR (☪) and 82% for SNA (☪) (Table 7.17). This reinforces the results of the DFA, where both distances were significant. In contrast, the power of discrimination of the four first PCs for UCI Shoveling was quite low, not reaching 60% for SNA (☪) and being only 50% for XAR (☪) (the expected result if we assigned individuals to a group at random).

Table 7.16 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Upper Central Incisor. Significant p-values are in bold red

		Mahalanobis Distance	Procrustes Distance
		SNA	SNA
UCI Shoveling	XAR	2.58 (**<0.001**)	0.03 (0.48)
UCI Double-Shoveling	XAR	4.93 (**<0.001**)	0.10 (**<0.001**)

Table 7.17 Percentage correct classification of specimens according to cross-validation of DFA for Upper Central Incisor, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	UCI Shoveling	UCI Double-Shoveling
lowest number of PCs needed to obtain overall reliability >50%	4	3
SNA	59%	82%
XAR	50%	73%

7.6. Upper Lateral Incisor

For the ULI, I only studied the tuberculum dentale, using one landmark configuration. The allometry observed in the ULI was significant and 46% of shape variation was predicted by the size (with p-value < 0.0001). Therefore, I used the residuals of the regression instead of the raw data for the rest of the analyses.

As for UCI Shoveling, there were fewer PCs (n = 51) than dimensions in shape space (Table 7.18). This may be a consequence of the small sample size and means the results for the ULI Tuberculum Dentale have limited reliability. The first five PCs accrued 92% of the shape variation.

Table 7.18 Results of PCA for Upper Lateral Incisor

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
ULI Tuberculum Dentale	1	0.0047	43.72	43.72	51
	2	0.0030	27.65	71.37	
	3	0.0011	10.49	81.86	
	4	0.0008	7.07	88.93	
	5	0.0004	3.93	92.86	

The shape went from an incisor similar to a scalene triangle to an equilateral triangle (Figure 7.8). This may represent the development of the tuberculum dentale effectively,

although these results should be interpreted cautiously as there are fewer PCs than dimensions in shape space.

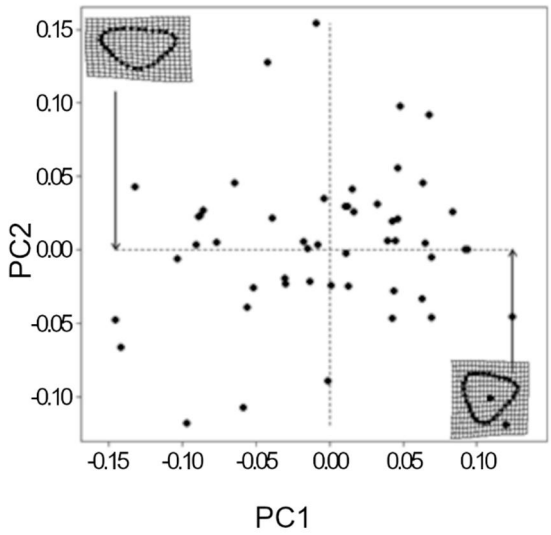


Figure 7.8 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for ULI Tuberculum Dentale

The samples were well separated in both analyses, although the low number of individuals may affect their distribution, especially for SMA (☥) (Figure 7.9). SMA (☥) differed from the other two samples for both CV1 and CV2, being negative for both CVs, whilst SNA (☪) and XAR (☪) showed more marked differences in CV1 (Figure 7.9).

There were no significant differences among the samples for ULI Tuberculum Dentale (Table 7.19).

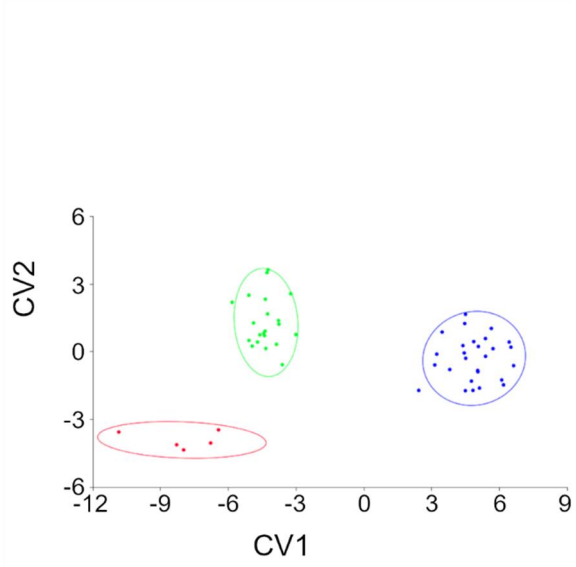


Figure 7.9 CVA with specimens grouped along their canonical variate axes (CVs 1 and 2) for ULI Tuberculum Dentale Red = SMA ; green = SNA; blue = XAR

Table 7.19 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Upper Lateral Incisor. Significant p-values are in bold red

		Mahalanobis Distance (p)		Procrustes Distance (p)	
		SMA	SNA	SMA	SNA
ULI Tuberculum Dentale	SNA	3.30 (0.72)		0.05 (0.38)	
	XAR	6.96 (0.10)	8.90 (0.30)	0.04 (0.46)	0.06 (**0.01**)

At least 22 PCs were needed to discriminate the individuals with an overall reliability of >50%, with a maximum reliability of 63% for XAR (C) and a minimum of 47% for SNA (C) (Table 7.20). Given the low number of individuals, these results should be interpreted cautiously.

Table 7.20 Percentage correct classification of specimens according to cross-validation of DFA for Upper Lateral Incisor, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	ULI Tuberculum Dentale
lowest number of PCs needed to obtain overall reliability >50%	22
SMA	60%
SNA	48%
XAR	63%

7.7. Upper Molar 1

For UM1 I studied two different dental traits, metaconule and Carabelli's trait, represented by three landmark configurations: two for the occlusal face and one for the lingual face. To these, I added two additional landmark configurations for the outline of the crown and the outline and cusps. Allometry did not have a significant effect on shape variation in UM1 and UM1 Outline (Table 7.21), so I used the raw data in subsequent analyses. In UM1 Carabelli Lingual, however, size had a significant impact on shape, although this effect explains only 6% of shape variation. Given the significant result, I used the residuals for the regression in analyses of UM1 Carabelli Lingual.

Table 7.21 Results of regression analysis to explore allometry in Upper Molar 1. Significant p-values are in bold red

	R-squared	p
UM1	0.0103	0.25
UM1 Outline	0.0082	0.35
UM1 Carabelli Lingual	0.0589	**<0.0001**

For UM1 there were 84 PCs, with the first five explaining 68% of the shape variation (Table 7.22). UM1 Outline, without landmarks in the cusps, was defined by 76 PCs, and the first five PCs explained 82% of the shape variation. The number of PCs that described the shape variation of the landmark configuration UM1 Carabelli Lingual is much lower, 56, and the first five PCs explained the 85% of the shape variation. Without any other information, this would make UM1 Carabelli Lingual the most informative landmark set to explore shape variation, followed by UM1 Outline.

Table 7.22 Results of PCA for Upper Molar 1

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
UM1	1	0.0010	26.45	26.45	84
	2	0.0005	14.15	40.60	
	3	0.0004	10.47	51.07	
	4	0.0003	8.67	59.74	
	5	0.0003	8.18	67.92	

UM1 Outline	1	0.0015	50.53	50.53	76
	2	0.0003	11.81	62.34	
	3	0.0002	7.55	69.89	
	4	0.0002	6.52	76.41	
	5	0.0002	5.94	82.35	
UM1 Carabelli Lingual	1	0.0029	44.47	44.47	56
	2	0.0012	19.12	63.59	
	3	0.0008	12.21	75.80	
	4	0.0003	4.98	80.78	
	5	0.0003	4.31	85.10	

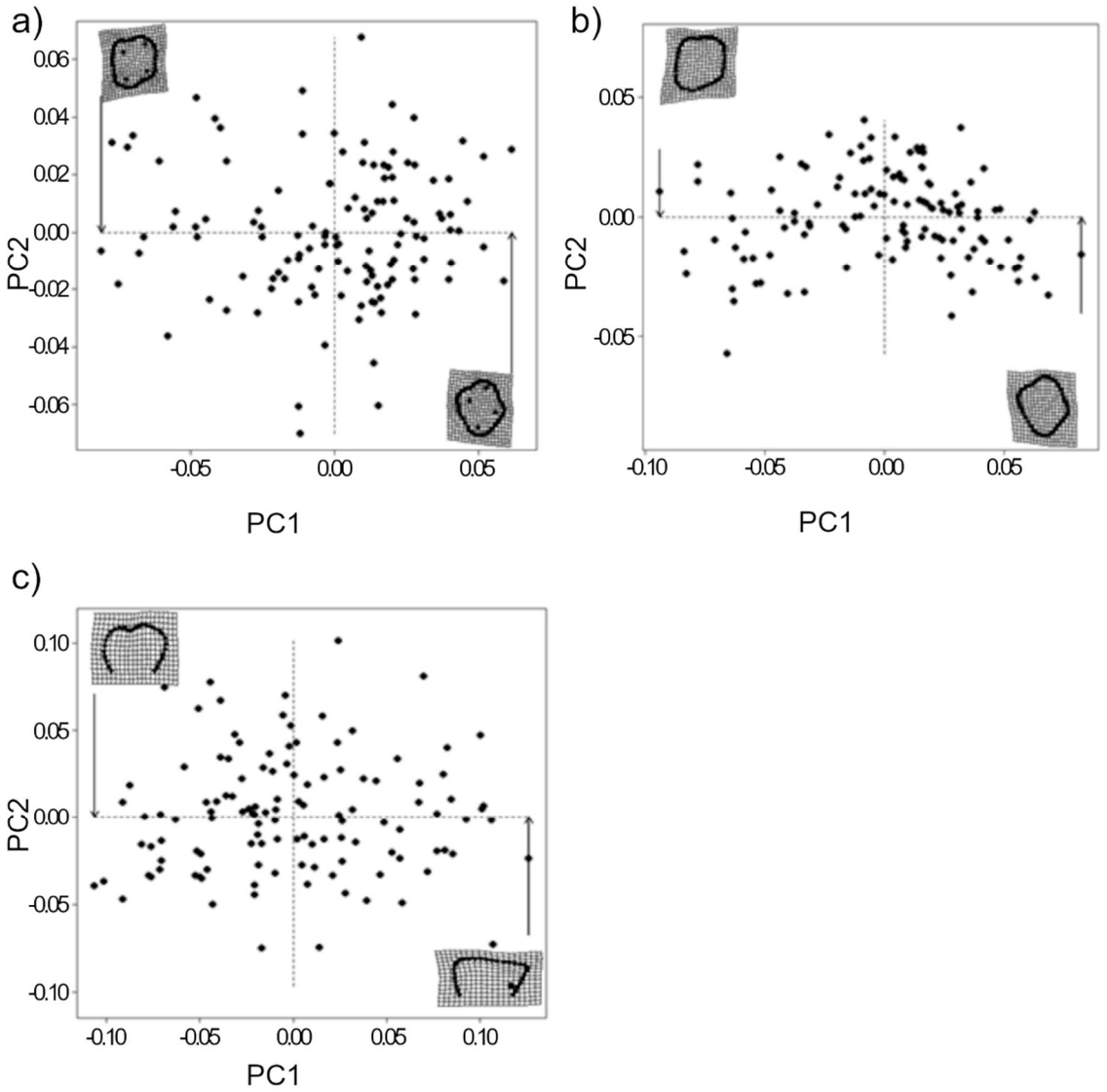


Figure 7.10 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) UM1, (b) UM1 Outline and (c) UM1 Carabelli Lingual

For UM1, 40% of shape variation relied mainly on the development of the metaconule, observed as change in the position of metacone, and the degree of development of protocone, that went from a mesiolingual position to a more mesial situation (Figure 7.10 a). The 62% shape variation for UM1 Outline was very similar to that for UM1, although the shape change of the paracone was also visible (Figure 7.10 b).

The 63% shape variation in the UM1 Carabelli Lingual landmark configuration was due to dental wear, so was not related to biological affinities among the samples (Figure 7.10 c).

The results of the CVA showed that SMA (✞) and XAR (☪) were the most morphologically distinct samples, both in different sides of CV1 in UM1 and in UM1 Outline (Figure 7.11 a and b). SMA (✞) and SNA (☪) were closer but also on different sides of CV2 for UM1 and UM1 Outline.

For UM1 Carabelli Lingual, the samples were closer than for the rest of the landmark sets, and overlapped, but this set of landmarks was very sensitive to dental wear, so these results do not reflect the degree of biological relatedness (Figure 7.11 c).

UM1 was significant for Mahalanobis and Procrustes distances (Table 7.23), which supported the differences between SMA (✞) and XAR (☪) (Figure 7.11 a). As with the lower second molars, the set of landmarks that included the cusps was affected by the development of a dental trait, which made this set of landmarks more suitable to find differences between the populations than the UM1 Outline landmark set, which did not include the cusps. The results for the Mahalanobis and the Procrustes distances were not consistent for UM1 and UM1 Outline. While SMA (✞) and SNA (☪) were furthest apart based on the Mahalanobis distance, SMA (✞) and XAR (☪) were furthest apart based on the Procrustes distance. The results for UM1 Carabelli Lingual were not reliable given that the shape variation is due to dental wear.

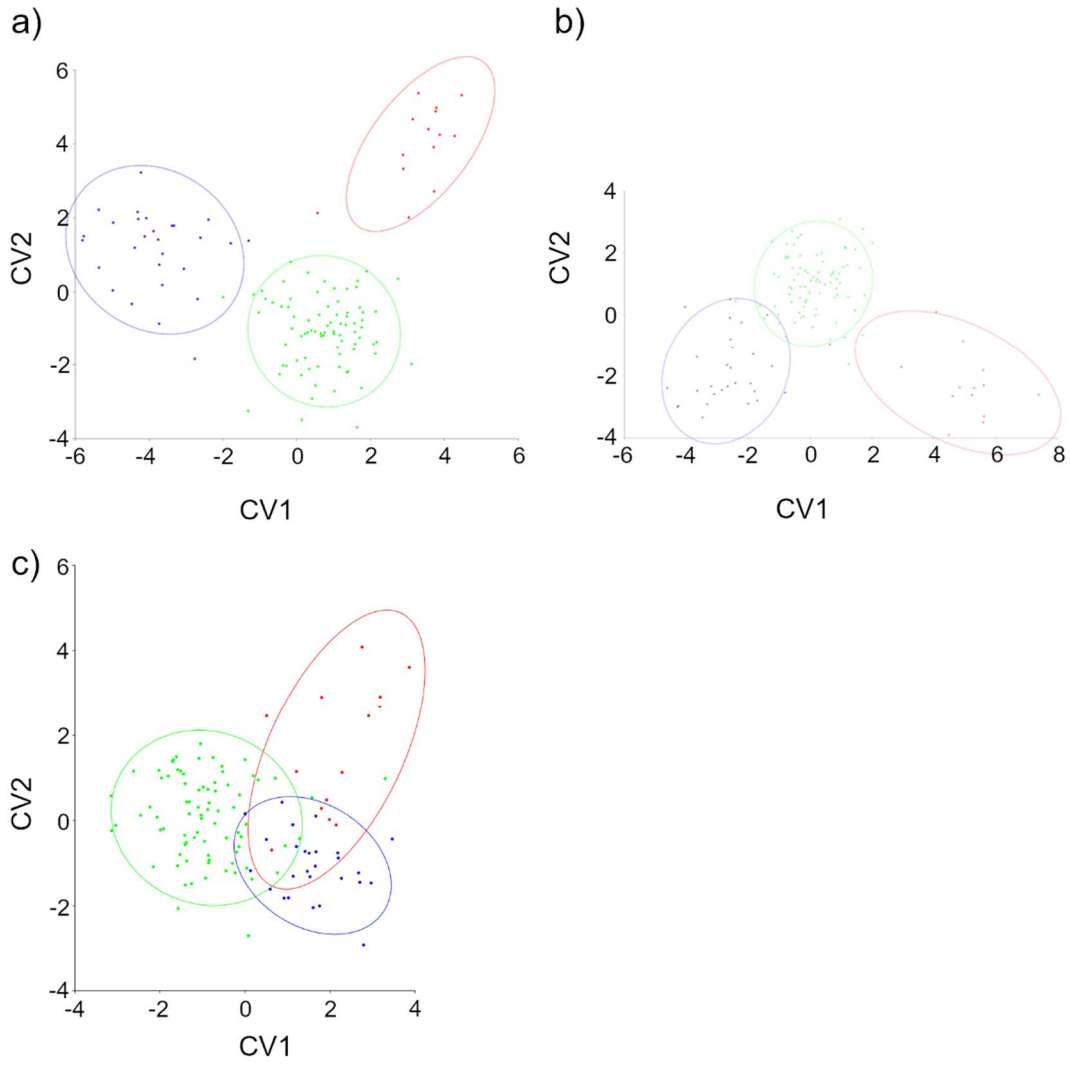


Figure 7.11 CVA with specimens grouped along their canonical variate axes (CVs 1 and 2) for: (a) UM1, (b) UM1 Outline, and (c) UM1 Carabelli Lingual. Red = SMA; green = SNA; blue = XAR

Table 7.23 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Upper Molar 1. Significant p-values are in bold red

		Mahalanobis Distance (p)		Procrustes Distance (p)	
		SMA	SNA	SMA	SNA
UM1	SNA	10.84 (0.16)		0.05 (**<0.001**)	
	XAR	6.41 (**<0.001**)	5.17 (0.13)	0.06 (**<0.001**)	0.01 (0.42)
UM1 Outline	SNA	7.61 (0.10)		0.05 (**<0.001**)	
	XAR	5.72 (0.07)	4.67 (**0.03**)	0.06 (**<0.001**)	0.02 (0.10)
UM1 Carabelli Lingual	SNA	3.45 (0.46)		0.06 (**<0.001**)	
	XAR	4.14 (0.79)	2.77 (0.14)	0.02 (0.76)	0.06 (**<0.001**)

The minimum number of PCs to obtain an overall reliability >50% was 4 for the set of landmarks UM1 Outline and 10 for UM1, and the population that is most easily classified in both cases was SMA (✞), with >70% of individuals correctly assigned (Table 7.24). For both landmark configurations, 50% of SNA (☪) individuals were correctly classified, but the individuals of XAR (☪) were more easily classified with UM1 Outline, with 10% more individuals correctly classified. This doesn't necessarily mean that UM1 Outline discriminates between populations better than UM1, as the results from the DFA for UM1 Outline were not significant and those for UM1 were significant. Instead, the fact that the discriminant functions for UM1 Outline require fewer principal components than the discriminant functions for UM1 to correctly assign a larger percentage of individuals may be an effect of the disproportionate sample sizes (Morrison, 1969).

Table 7.24 Percentage correct classification of specimens according to cross-validation of DFA for Upper Molar 1, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	UM1	UM1 Outline	UM1 Carabelli Lingual
lowest number of PCs needed to obtain an overall reliability >50%	10	4	3
SMA	79%	71%	50%
SNA	49%	49%	73%
XAR	43%	54%	33%

7.8. Upper Molar 2

I only studied one dental trait for UM2 (hypocone), although I added a landmark configuration for the outline of UM2 to compare the results when the cusps were not included in the analysis.

Both landmark configurations showed significant allometry, although the percentage of the shape change affected by the size was very small (3.4% for UM2 Hypocone and 2% for UM2 outline, Table 7.25). Therefore, I used the residuals of the regressions rather than the raw data for subsequent analyses.

Table 7.25 Results of regression analysis to explore allometry in Upper Molar 2. Significant p-values are in bold red

	R-squared	p
UM2 Hypocone	0.0337	**<0.0001**
UM2 Outline	0.0173	**0.03**

For UM2 Hypocone there were 82 PCs, with the first five explaining 78% of the shape variation (Table 7.26). UM2 Outline, without landmarks in the cusps, was defined by 76 PCs, and the first five PCs explained the 89% of the shape variation. The first two PCs explained 49% of the shape variation for UM2 Hypocone and 76% for UM2 Outline. In view of these results, the landmark configuration UM2 Outline is more informative than UM2 Hypocone.

Table 7.26 Results of PCA for Upper Molar 2

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
UM2 Hypocone	1	0.0018	33.65	33.65	82
	2	0.0008	15.66	49.31	
	3	0.0007	13.19	62.50	
	4	0.0004	8.14	70.64	
	5	0.0004	7.37	78.01	
UM2 Outline	1	0.0023	52.93	52.93	76
	2	0.0009	19.65	72.59	
	3	0.0003	7.01	79.60	
	4	0.0002	5.21	84.81	
	5	0.0002	3.95	88.75	

The most striking shape change for both sets of landmarks was the development of the hypocone, linked to the development of the paracone (Figure 7.12). There was little difference between the landmark sets, although UM2 Hypocone confirmed that the position of the cusps changed (Figure 7.12 a). A larger and more developed hypocone resulted in a square-like UM2, with a more prominent paracone. When the hypocone was less developed, the paracone was smaller too, and its cusp was more buccal.

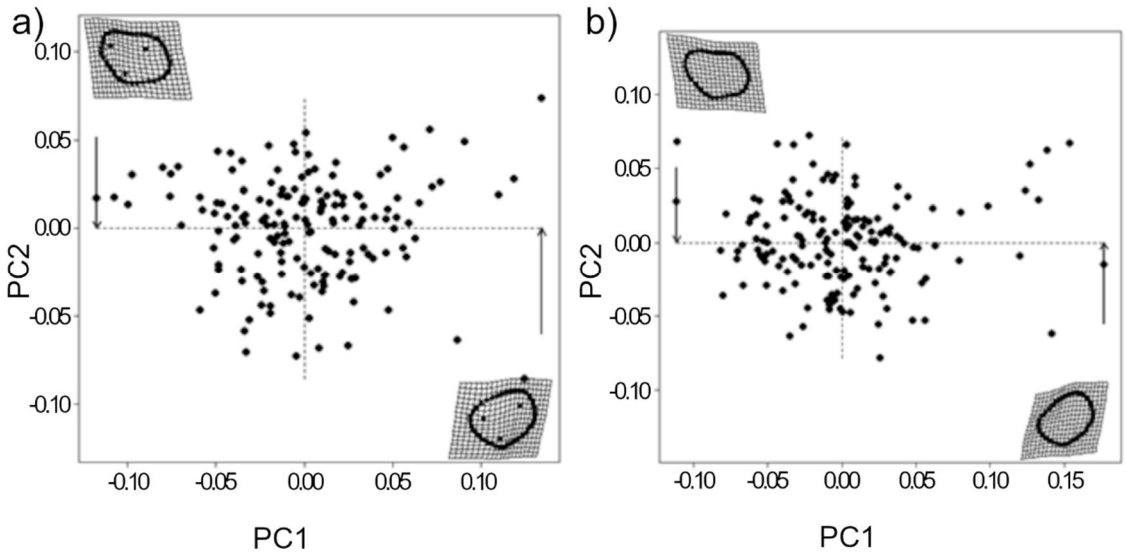


Figure 7.12 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) UM2 Hypocone, and (b) UM2 Outline

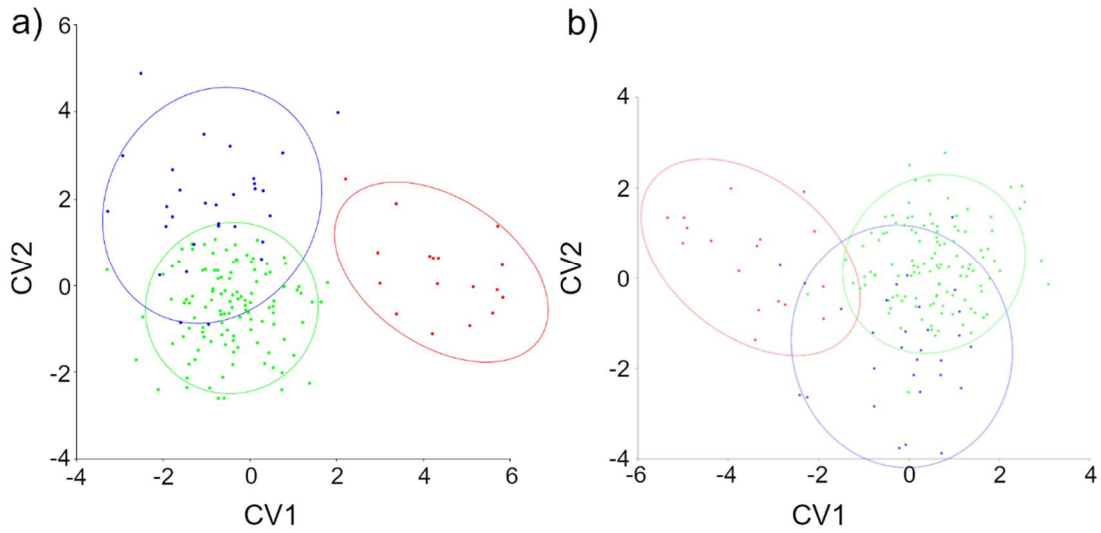


Figure 7.13 CVA with specimens grouped along their canonical variate axes (CVs 1 and 2) for: (a) UM2 Hypocone, and (b) UM2 Outline. Red = SMA; green = SNA; blue = XAR

UM2 Hypocone seemed to be more helpful than UM2 outline in differentiating among the samples (Figure 7.13 a). For UM2 Hypocone, SMA (✞) was slightly different from the other samples for CV1, although the samples were still very close. In both sets of landmarks SMA (✞) was on a different side of CV1 that the other samples, being positive for UM2

Hypocone and negative for UM2 Outline, with the other samples distributed over both sides of CV1 (Figure 7.13)

For UM2 Hypocone there were significant differences between SMA (☥) and SNA (☪) and SMA (☥) and XAR (☪), whereas the only significant difference for UM2 Outline was between SMA (☥) and SNA (☪) (Table 7.27). For both Mahalanobis and Procrustes the closest samples were SNA (☪) and XAR (☪), while there was no consensus for SMA-SNA and SMA-XAR.

Table 7.27 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Upper Molar 2. Significant p-values are in bold red

		Mahalanobis Distance (p)		Procrustes Distance (p)	
		SMA	SNA	SMA	SNA
UM2 Hypocone	SNA	7.10 (**<0.001**)		0.06 (**<0.001**)	
	XAR	7.56 (**<0.001**)	2.58 (0.75)	0.06 (**<0.001**)	0.02 (0.31)
UM2 Outline	SNA	5.50 (**<0.001**)		0.05 (**<0.001**)	
	XAR	6.43 (0.08)	2.03 (0.98)	0.04 (**<0.001**)	0.02 (0.24)

The minimum number of PCs to obtain overall reliability >50% was 6 for UM2 Hypocone and 4 for UM2 Outline (Table 7.28). However, the ability to assign individuals to the correct population was higher with UM2 Hypocone than for UM2 Outline for individuals from SMA (☥) and XAR (☪), but similar for SNA (☪). Again, the landmark configuration with landmarks for the cusps was better at discriminating among the populations than the landmark configuration with landmarks only for the outline, even if the latter was able to explain the shape variation with fewer principal components.

Table 7.28 Percentage correct classification of specimens according to cross-validation of DFA for Upper Molar 2, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	UM2 Hypocone	UM2 Outline
lowest number of PCs needed to obtain overall reliability >50%	6	4
SMA	82%	71%
SNA	45%	46%
XAR	50%	40%

7.9. Upper Molar 3

In UM3 I studied the metacone, with one set of landmarks, and the parastyle, with two. I also included a set for the outline of the molars, which did not include the cusps.

The landmark configurations UM3 Metacone and UM3 Parastyle Buccal showed significant allometry with a small effect (<4.5% for both sets, Table 7.29). Hence, I used the residuals instead of the raw data. Neither UM3 Outline nor UM3 Parastyle had significant allometry, so I used the raw data for these landmark sets in subsequent analyses.

Table 7.29 Results of regression analysis to explore allometry in Upper Molar 3. Significant p-values are in bold red

	R-squared	p
UM3 Metacone	0.0449	**<0.001**
UM3 Outline	0.0028	0.89
UM3 Parastyle	0.0124	0.24
UM3 Parastyle Buccal	0.0210	**0.04**

The landmark configuration UM3 Parastyle Buccal explained the shape variation with the lowest number of PCs, 56, with 81% of the variation explained by the first five PCs (Table 7.30). For the other three landmark sets, the number of PCs was higher than for UM3 Parastyle Buccal (76 for UM3 Outline, 80 for UM3 Parastyle and 82 for UM3 Metacone), and for both UM3 Metacone and UM3 Parastyle the shape variation explained with the first five PCs was 84%. The first five PCs of UM3 Outline explained >91% of shape variation, with >76% explained with just two PCs.

Table 7.30 Results of PCA for Upper Molar 3

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
UM3 Metacone	1	0.0043	47.08	47.08	82
	2	0.0012	13.00	60.08	
	3	0.0009	10.10	70.18	
	4	0.0008	8.46	78.64	
	5	0.0005	5.60	84.24	
UM3 Outline	1	0.0025	52.81	52.81	76
	2	0.0011	23.89	76.70	

	3	0.0003	6.69	83.39	
	4	0.0002	5.00	88.39	
	5	0.0001	3.03	91.41	
UM3 Parastyle	1	0.0020	31.85	31.85	80
	2	0.0014	23.55	55.40	
	3	0.0008	12.53	67.94	
	4	0.0006	10.15	78.08	
	5	0.0004	6.55	84.63	
UM Parastyle Buccal	1	0.0020	29.43	29.43	56
	2	0.0014	20.28	49.71	
	3	0.0008	12.49	62.20	
	4	0.0007	10.33	72.53	
	5	0.0006	8.97	81.49	

The most striking shape change for UM3 Metacone was the development of the metacone, noted by the displacement of the hypocone from a central position towards the lingual face (Figure 7.14 a). Additionally, the protocone went from a more mesiolingual position to align with the paracone. The landmark set UM3 Outline showed the same changes for the outline, although these were more difficult to see because there is no information for the cusps (Figure 7.14 b).

For UM3 Parastyle the paracone and the metacone moved towards the distal face, with an enlargement of the mesiolingual side (Figure 7.14 c). Hence, variation in the dental trait parastyle did not seem to be an important influence on the shape, at least for the two first PCs (55% of shape variation), other than a minor change in the buccal surface of the paracone that pulled the paracone slightly from the edge of the occlusal face towards the centre.

UM3 Parastyle Buccal moved from a bigger paracone, close to the metacone, to both cusps being more separated and similar in size. It would be difficult to link this to the development of the dental trait parastyle, and it must be very sensitive to wear (Figure 7.14 d).

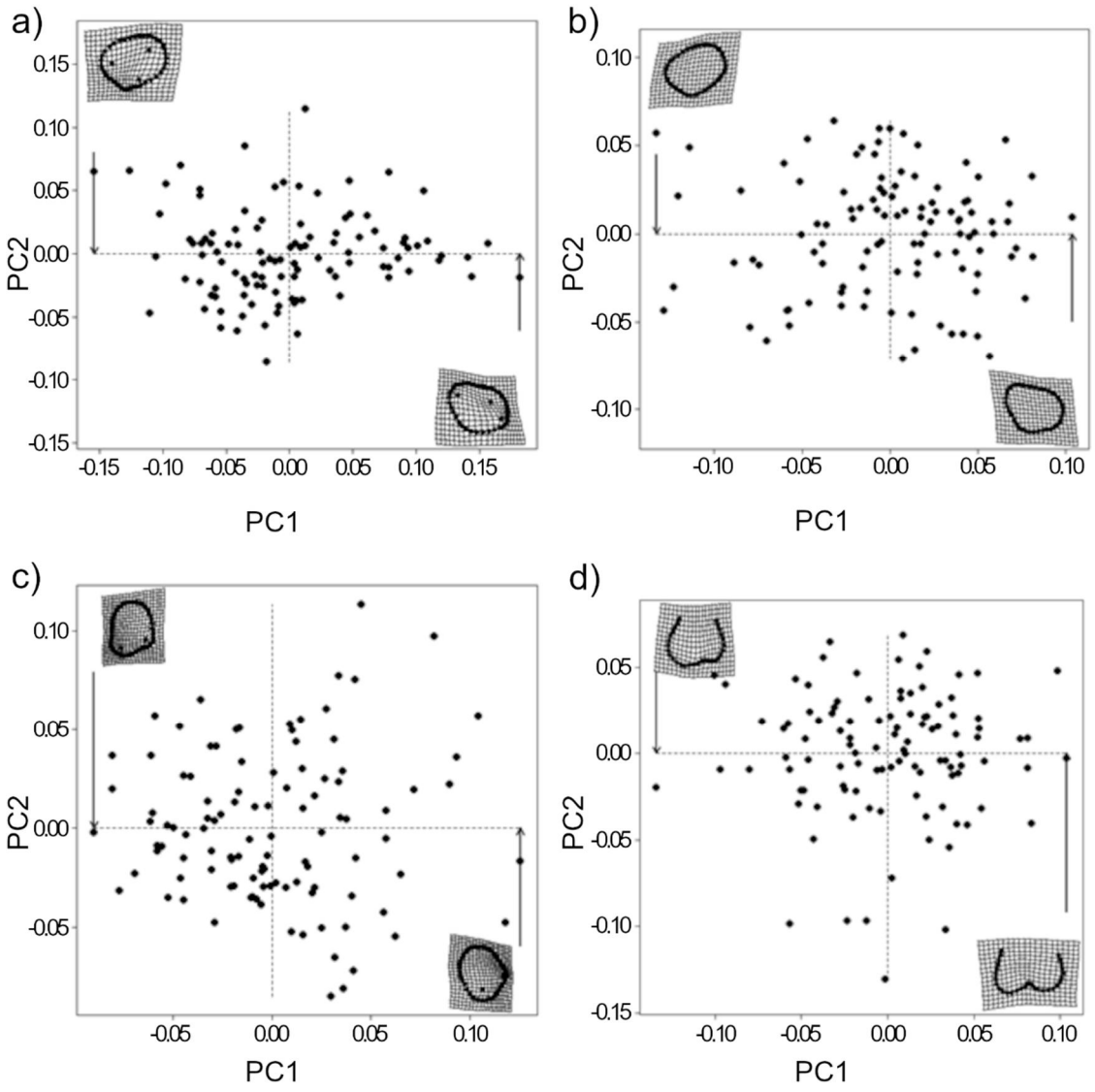


Figure 7.14 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) UM3 Metacone, (b) UM3 Outline, (c) UM3 Parastyle, and (d) UM3 Parastyle Buccal

There was a tendency for SMA (☥) to be slightly separated from SNA (☪) and XAR (☪). This was most noticeable in UM3 Metacone, followed by UM3 Parastyle and UM3 Outline (Figure 7.15). Although SMA (☥) was not in a different quadrant of the graphs than the other samples, it was on a different side of CV1 for UM3 Metacone (Figure 7.15).

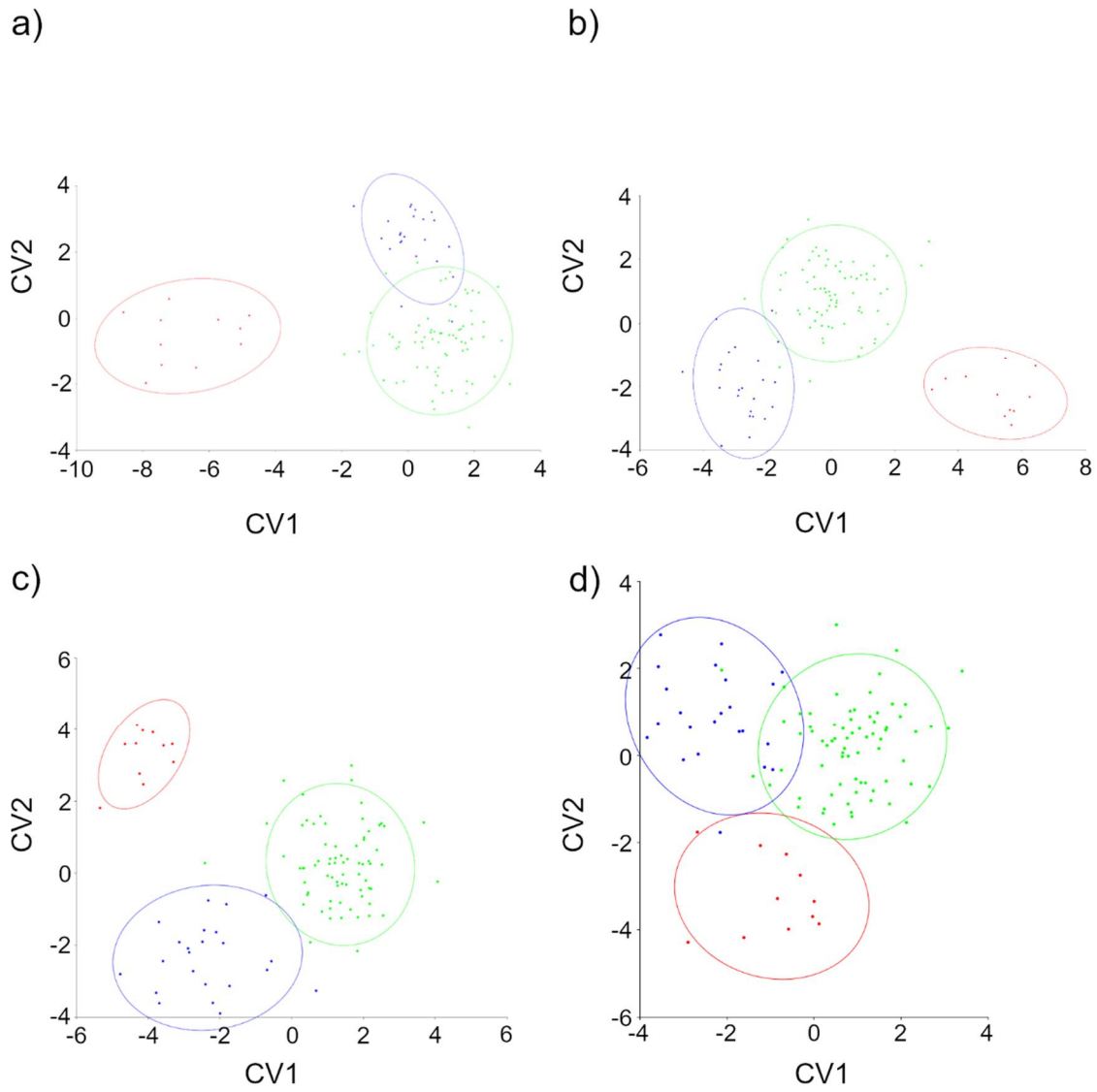


Figure 7.15 CVA with specimens grouped along their canonical variate axes (CVs 1 and 2) for: (a) UM3 Metacone, (b) UM3 Outline, (c) UM3 Parastyle, and (d) UM3 Parastyle Buccal. Red = SMA; green = SNA; blue = XAR

There was no significant difference among the samples for any of these sets of landmarks and the results for the Mahalanobis and the Procrustes distances are not consistent (Table 7.31).

Table 7.31 Results of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means for Upper Molar 3. Significant p-values are in bold red

		Mahalanobis Distance (p)		Procrustes Distance (p)	
		SMA	SNA	SMA	SNA
UM3 Metacone	SNA	14.15 (0.87)		0.42 (0.09)	

	XAR	4.51 (0.12)	3.52 (1.00)	0.04 (0.30)	0.01 (0.95)
UM3 Outline	SNA	8.14 (0.78)		0.03 (0.19)	
	XAR	4.49 (0.11)	4.39 (0.65)	0.02 (0.62)	0.01 (0.56)
UM3 Parastyle	SNA	21.25 (0.31)		0.04 (**0.04**)	
	XAR	4.76 (**0.04**)	5.08 (0.64)	0.02 (0.90)	0.03 (0.09)
UM3 Parastyle Buccal	SNA	4.74 (0.29)		0.04 (**0.01**)	
	XAR	4.19 (0.41)	3.52 (0.07)	0.06 (**0.01**)	0.03 (0.09)

The number of PCs needed to correctly classify the individuals using UM3 Metacone and UM3 Outline was 7 but the reliability of the classification was quite low, ranging 30-55% (Table 7.32). UM3 Parastyle was more reliable, with 82% for SMA (♱) and 73% for SNA (☪), however, the number of PCs needed was 15. UM3 Parastyle Buccal had a reliability of 73% for SMA (♱) with just 6 PCs, but the shape variation for this set of landmarks could be highly related to wear and must be interpreted carefully. For this tooth, there were no differences in the results when using a set of landmarks with just the outline or another including the tips of the main cusps. However, this may be a consequence of the lack of differences among the upper third molars of the populations.

Table 7.32 Percentage correct classification of specimens according to cross-validation of DFA for Upper Molar 3, with the lowest number of PCs needed to obtain an overall rate of reliability over 50%

	UM3 Metacone	UM3 Outline	UM3 Parastyle	UM3 Parastyle Buccal
lowest number of PCs needed to obtain an overall reliability > 50%.	7	7	15	6
SMA	45%	55%	82%	73%
SNA	44%	42%	73%	50%
XAR	30%	43%	39%	61%

8. Results: 3D Geometric Morphometrics

This chapter presents the results from the analyses performed to compare the archaeological samples using 3D GMM. As described in the Methods (Chapter 4), I only included four teeth in the 3D geometric morphometric analyses. These were the teeth that showed the best results in the 2D discriminant function analyses, to aid the comparison between techniques: Lower Molar 1 and 2 and Upper Molar 1 and 2.

Table 8.1 Summary of sites and individuals included from each site.

	SMA (Santa Maria la Real – Christians – North of IP)	SNA (San Nicolas de Avila – Muslims – Centre of IP)	XAR (Xarea – Muslims – South of IP)
Females	4	51	12
Males	18	23	12
Unknown	13	34	21
Total	35	108	45

I chose a single landmark set for each tooth, with a combination of landmarks in the cusps and sliding semilandmarks in the outline of the crown. None of the landmark configurations showed significant allometry (Table 8.2), so I used the raw data for all analyses.

Table 8.2 Results of regression analysis to explore allometry in all the 3D sets of landmarks.

	R-squared	p
LM1	0.0359	0.22
LM2	0.0296	0.29
UM1	0.0930	0.26
UM2	0.0542	0.66

For all the landmark sets, there were fewer PCs than dimensions in shape space (Table 8.3). This may be a consequence of the small sample size, as many of the scans either could not be processed or were not suitable for landmarking. Hence, the results for the 3D analyses have limited reliability. The first five PCs for the lower molars explained

only 62% of the shape variation, but for the upper molars the first five PCs explained 80% of the shape variation for UM1 and 84% for UM2.

Table 8.3 Results of PCA for all the sets of 3D landmarks

Tooth	PC	Eigenvalue	% Variance explained	Cumulative % variance explained	Total number of PCs
LM1	1	0.0037	23.70	23.70	34
	2	0.0019	12.54	36.24	
	3	0.0017	10.70	46.94	
	4	0.0012	7.72	54.66	
	5	0.0010	6.74	61.39	
LM2	1	0.0030	21.36	21.36	39
	2	0.0019	13.36	34.73	
	3	0.0015	10.88	45.61	
	4	0.0014	9.82	55.43	
	5	0.0009	6.36	61.79	
UM1	1	0.0044	26.06	26.06	13
	2	0.0040	23.38	49.44	
	3	0.0024	14.42	63.85	
	4	0.0015	8.78	72.63	
	5	0.0013	7.61	80.24	
UM2	1	0.0070	29.66	29.66	14
	2	0.0060	25.21	54.87	
	3	0.0026	11.06	65.93	
	4	0.0023	9.68	75.61	
	5	0.0019	8.01	83.62	

For LM1 the most noticeable change in shape was that the outline of the crown narrowed buccolingually from negative to positive values of PC1, and the crown went from a shape closer to a square to a more oval shape (Figure 8.1 a). The cusps, which were closer to the outline towards the negative boundary of PC1, moved towards the centre and higher on the positive side. None of these changes seemed to be the effect of the development of a particular nonmetric trait, but they may be due to dental wear, with more worn molars on the negative side of PC1.

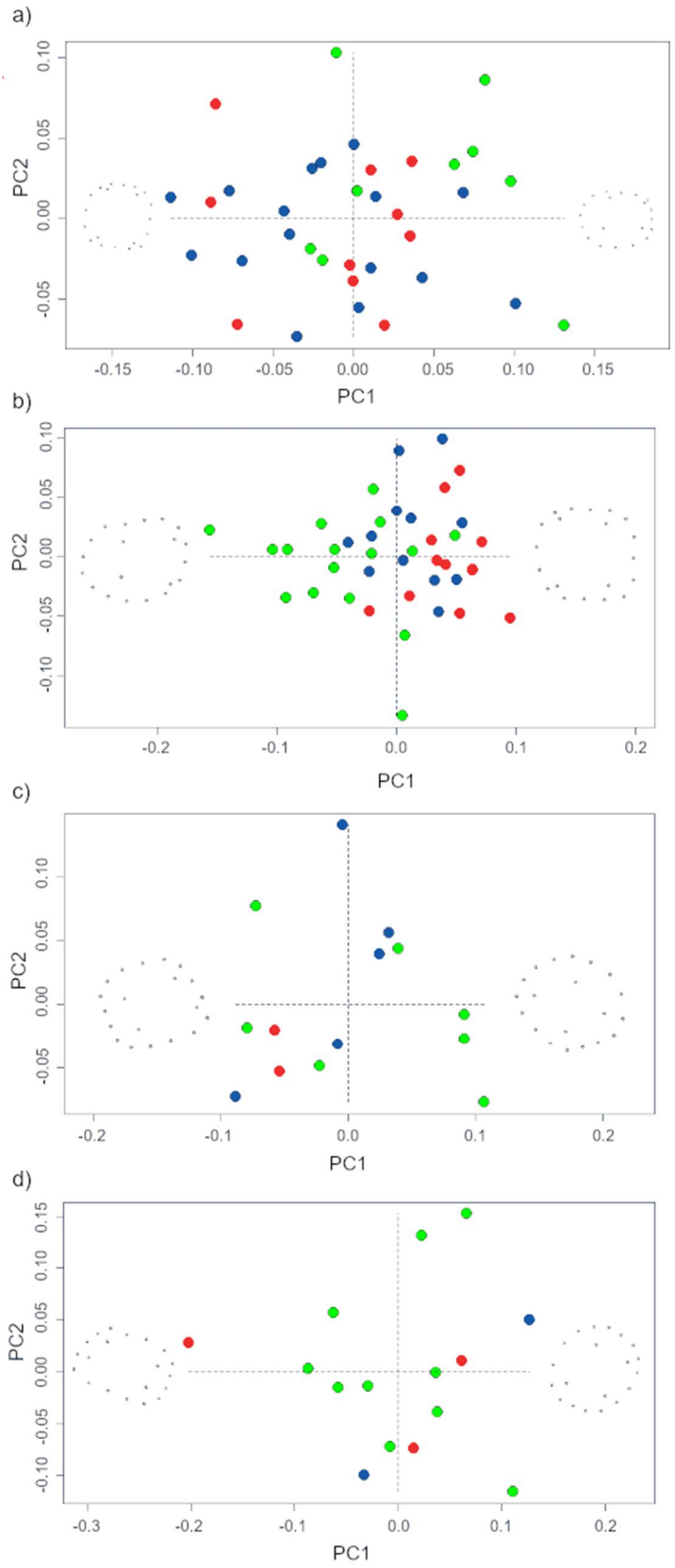


Figure 8.1 Procrustes shape variables in tangent space along their principal component axes (PCs 1 and 2) for: (a) LM1, (b) LM2, (c) UM1, and (d) UM2. Red = SMA; green = SNA; blue = XAR.

LM2 changed in the opposite way along PC1, being narrower buccolingually and with higher cusps closer to the centre on the negative side and with a square shape and lower cusps closer to the outline on the positive side (Figure 8.1 b). The extreme of the negative side of PC1 showed more space in the crown distally, which could represent an enlarged hypoconulid. Likewise, the metaconid seemed to be expanded.

In UM1 the protocone and the paracone were closer to the outline of the crown and all the cusps were low, with the paracone being the lowest, on the negative side of PC1 (Figure 8.1 c). The shape of the crown did not change much along PC1 but the protocone and paracone moved towards the centre on the positive side. The metacone was much higher on the positive extreme of PC1. Changes in dental traits such as Carabelli's trait and the metaconule were not represented in PC1.

UM2 narrowed buccolingually on the negative side of PC1, with the cusps further away mesiodistally than buccolingually (Figure 8.1 d). On the positive side of PC1, the cusps were slightly lower, and the crown was rounded, with the protocone and the paracone further towards the mesial ridge. The hypocone barely changed on PC1.

The samples were well defined in LM1 and LM2 and overlapped in UM1 and UM2 (Figure 8.3). This overlap was most likely a consequence of the small number of specimens. For both LM1 and LM2, SNA (☪) and XAR (☪) were on the positive side of CV2, whilst SMA (✞) remained in the negative side of the axis for both teeth (Figure 8.3 a and b).

There were significant differences between SNA (☪) and XAR (☪) for both LM1 and LM2, although the results for the Mahalanobis and the Procrustes distances were not fully consistent (Table 8.4). The results of the discriminant function analysis do not concur with the patterns observed in the canonical variate analyses, probably due to the small number of specimens.

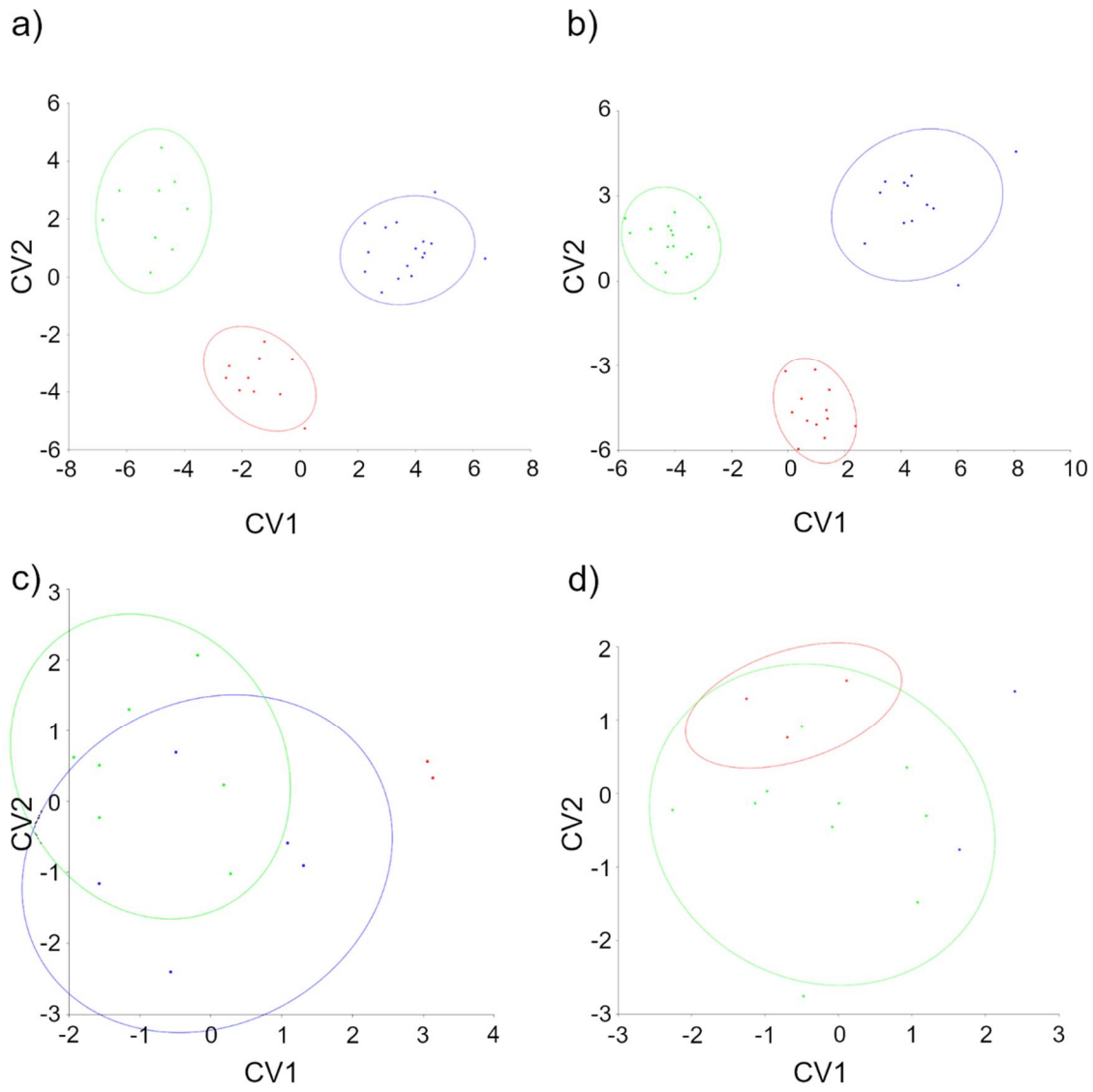


Figure 8.2 CVA for the archaeological samples with individuals grouped along their canonical variate axes (CVs 1 and 2) for: (a) LM1, (b) LM2, (c) UM1, and (d) UM2. Red = SMA; green = SNA; blue = XAR

Table 8.4 Results for all the 3D sets of landmarks of DFA for the Mahalanobis distances between centroids and the Procrustes distances between the means. Significant p-values are in bold red

		Mahalanobis Distance (p)		Procrustes Distance (p)	
		SMA	SNA	SMA	SNA
LM1	SNA	3.78 (0.05)		0.08 (**0.02**)	
	XAR	4.75 (0.05)	5.11 (**<0.001**)	0.04 (0.64)	0.09 (**<0.001**)
LM2	SNA	4.29 (0.07)		0.09 (**<0.001**)	
	XAR	3.59 (0.28)	5.74 (**0.01**)	0.06 (**0.03**)	0.07 (**<0.001**)
UM1	SNA	2.04 (0.35)		0.13 (0.14)	
	XAR	1.33 (0.57)	1.49 (0.60)	0.12 (0.25)	0.08 (0.28)

UM2	SNA	1.66 (0.67)		0.08 (0.80)	
	XAR	0.54 (0.60)	2.02 (0.83)	0.13 (0.67)	0.09 (0.85)

The number of PCs needed to correctly classify the individuals was very low in LM2 and UM1, with just one PC. However, the reliability of the classification was quite poor for XAR (Ꞩ), ranging 33-40%, and middling for SNA, ranging 57-69% (Table 8.5). LM1 had reliability of 50-66%, and the number of PCs needed to achieve this was 9. UM2 was not at all useful to classify the individuals.

Table 8.5 Correct classification of specimens according to cross-validation of DFA for all the 3D sets of landmarks, with the lowest number of PCs needed to obtain an overall reliability over 50%

	LM1	LM2	UM1	UM2
lowest number of PCs needed to obtain an overall reliability >50%	9	1	1	4
SMA	50%	83%	100%	0%
SNA	66%	69%	57%	30%
XAR	63%	33%	40%	0%

9. Discussion

In this thesis, I used four methods in seven focal teeth from three archaeological samples (Table 9.1) to address two aims:

1. To evaluate which of four methods is best to estimate biological relatedness between skeletal samples in terms of the information they yield, their reliability and the ease of application
2. To examine whether there are observable biological differences between three medieval skeletal samples excavated from different religious contexts within the Iberian Peninsula

Table 9.1 Summary of teeth, samples and methods used in this thesis

Sample	SMA	Santa Maria de la Real (Christian)
	SNA	San Nicolas (Muslim)
	XAR	Xarea (Muslim)
Tooth	UCI	Upper Central Incisor
	ULI	Upper Lateral Incisor
	LM1	Lower Molar 1
	LM2	Lower Molar 2
	UM1	Upper Molar 1
	UM2	Upper Molar 2
	UM3	Upper Molar 3
Method	BL, MD, CA, CI	Metric variables
	ASU system	Nonmetric dental traits
	2D GMM	Two-dimensions geometric morphometric
	3D GMM	Three-dimensions geometric morphometric

9.1. Comparing the methods

The results obtained using the different methods are generally consistent, except for 3D GMM: metric variables, nonmetric dental traits and 2D GMM found significant differences between SMA (✞) and SNA (☪) in at least one tooth (Table 9.2; Figure 9.1). 3D

GMM provided very different results, finding significant differences between SNA (☪) and XAR (☪) in two teeth. Metric variables also found significant differences between SNA (☪) and XAR (☪), but only before correcting for the unbalanced sex ratio. Nonmetric dental traits and 2D GMM provided the most similar results, with significant differences between SMA (✞) and SNA (☪) and between SMA (✞) and XAR (☪) for the same teeth. 2D GMM also found differences between SMA (✞) and XAR (☪) in two additional teeth.

The accuracy of a method is measured by how close the results provided by it are to the real values. Because the DNA in these samples has not been analysed, I do not know how accurate the methods are. However, if we understand precision as obtaining similar results with different methods, the most precise methods are nonmetric dental traits using the ASU system and 2D GMM, immediately followed by metric variables. However, the nonmetric dental traits and 2D GMM may provide results that are different to 3D GMM because the former methods capture different aspects of the dental morphology than the latter method, which affects the ability of the methods to differentiate my samples.

Table 9.2 Teeth for which there were significant differences between samples for each method. In brackets are teeth for which the significant differences disappeared when individuals were split by sex. Symbols refer to the religious contexts of the samples.

	SMA (✞) – SNA (☪)	SMA (✞) – XAR (☪)	SNA (☪) – XAR (☪)
Metric	LM1, (LM2)		(LM1), (LM2), (UM2)
Nonmetric	LM1, UM2	LM1, UM2	
2D GMM	LM2, UM2	LM1, LM2, UM1, UM2	
3D GMM			LM1, LM2

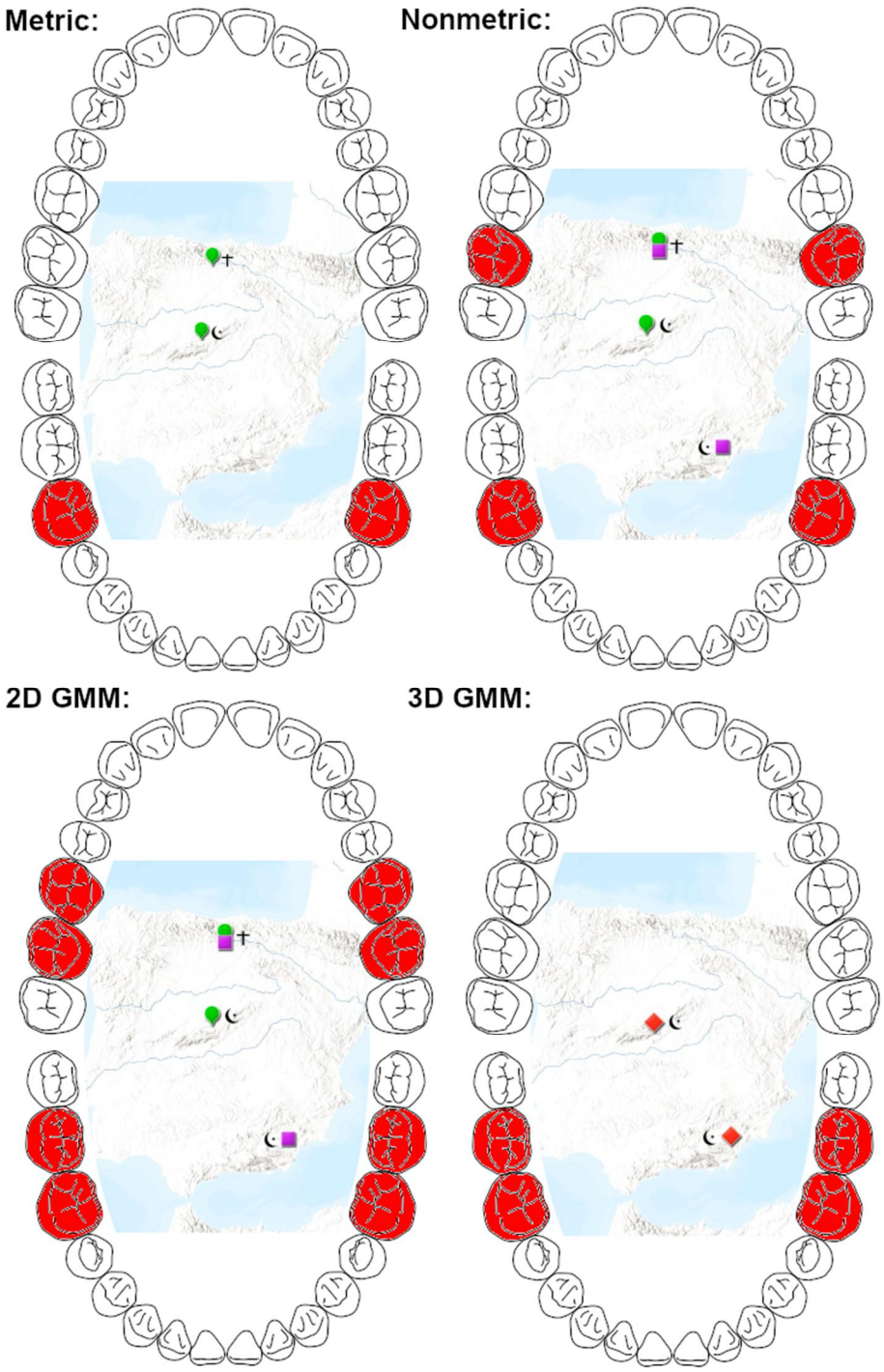


Figure 9.1 Diagram with the results of each method. In red are teeth where I found significant differences. In each map, samples marked with the same shapes and colours are significantly different using that method. Moon and star and cross symbols refer to the religious contexts of the samples.

9.1.1 Metric variables

The results obtained using metric variables were heavily affected by the unbalanced sex ratio of the samples. Most of the significant differences observed with this method disappeared when I excluded the females from the analyses, except the differences between SMA (☥) and SNA (☪) identified for the buccolingual diameter of LM1, which remained the same. Because teeth can be sexually dimorphic in size, the results of the comparison of samples with an unbalanced sex ratio may be affected (Kieser, 1990; Hillson, 1996; Prieto Carrero, 2002). Even if size differences between sexes are very small, their statistical effects can be large (Hillson, 1996; Harris, 1997). The size effect was clearly seen in the results for the crown area, which is an indicator of the size of the crown (Kondo et al., 2005). Thus, the minimum values of the crown area of SMA (☥) were larger than in SNA (☪) and XAR (☪) for most teeth, but there were no significant differences between the different samples. The effect of sex in the results of the analyses of metric variables poses a problem in the study of archaeological samples, where there is little control of the sex composition. To avoid obtaining results affected by the sex ratio of the samples, I excluded the females from the analyses of the dental metric variables.

The significant differences that remained after excluding the females were between SMA (☥) and SNA (☪) and could be linked to their biological relatedness to European and African populations, respectively. European populations have broader molars than African populations (Hillson, 1996), so the differences observed in my samples for LM1 may be due to SMA (☥) being biologically more similar to European populations and SNA (☪) and XAR (☪) being closer to African populations. According to Hillson (1996), the buccolingual diameter may be altered by occlusal attrition. Therefore, it could be argued that the differences observed between SMA (☥) and SNA (☪) are not linked to genetics but to other population differences, such as different food preparation techniques (Alt and Pichler, 1998)

or food consumption (Cabellos, 2007, Brook et al., 2009). The differences could also be linked to other cultural factors, such as the mothers' ages, which according to Townsend and Brown (1978) may affect tooth size. It is also possible that the differences were due to the demography of the population. For example, even if all the teeth are below the wear score of 4, more teeth in SNA (☪) had a wear score of 1 or 2 than in SMA (✞), which had higher wear in general. More worn teeth have smaller buccolingual diameters (Hillson, 1996). However, in this instance, the buccolingual diameter of SMA (✞) is larger, and not smaller, than the buccolingual diameters in the other two samples and especially larger than in SNA (☪).

9.1.2 Nonmetric Dental Traits

Although only four traits were useful in finding significant differences between the samples, the Smith's Mean Measure of Divergence (MMD) shows that there are significant differences between the Christian (SMA) and the Muslim (SNA and XAR) samples. In contrast, the distance between SNA (☪) and XAR (☪) is shorter and non-significant. This result was obtained after excluding traits with an overall negative measure of divergence, and that were observable in less than five individuals per population. Therefore, only four of the 13 traits (entoconulid, protostylid, hypocone and metaconulid) could be used to identify differences among my samples. The focal teeth for these traits are LM1 (entoconulid, protostylid, and metaconulid) and UM2 (hypocone). LM1 was the only tooth in which I found significant differences using metric variables, which means that both metric and nonmetric methods detect differences in the samples, although nonmetric dental traits is potentially more sensitive to such differences.

The results using nonmetric dental traits show some morphological continuity between the samples, but with differences that reflect genetic differences among them and that correspond with their cultural adscription (i.e., the Christian sample and the Muslim

samples may be genetically different). Samples that are biologically close and conservative tend to show small distances in a small number of nonmetric traits, so it is not uncommon that few traits can be used to find differences between samples, even if data have been collected for a much larger number of traits (Sciulli, 1998). Likewise, it is common to observe biological continuity in samples that are geographically or chronologically close (Sciulli, 1998; Irish, 2006; Cabellos, 2007; García Sívoli, 2009; Scott et al., 2013). In my study, the results of the MMD analysis show small distances between samples, although the distance between the Muslim samples is less than half that those between the Muslim samples and the Christian sample, unlike the pattern found in a similar study in the Iberian Peninsula (Cabellos, 2007). In her study, Cabellos (2007) found small but significant differences between nearly all the samples analysed, being the largest distances between one of the Muslim samples and the Roman sample. However, her results using metric variables identified significant differences between the Romans and all the other samples (Cabellos, 2007). The lack of precision of her methods made her results difficult to interpret.

I found little similarity in the frequencies of presence of the dental traits in my samples with respect to the frequencies observed in other studies for similar samples, although this is most likely caused by my sample sizes being noticeably smaller than the samples used for such studies (Scott and Turner II, 1997; Irish et al., 2020). XAR's entoconulid (0.5) and metaconulid (0.33) frequencies are similar to the frequencies seen in samples from the West of Africa in Scott and Turner II (1997) (0.44 for both), but those are the only similarities found with the samples from Scott and Turner II (1997). The frequencies of presence for each dental trait in SNA (☪) and XAR (☪) are also not similar to those in Berber and Kabyle samples studied by other authors (Irish et al., 2020). It would be interesting to compare the frequencies of presence in my study populations to the frequencies in well-studied populations from the Mediterranean Littoral, including the North of Africa and the Middle East. Ideally, the trait frequencies obtained in this study should be compared to samples

from similar locations and chronology, like those studied in Cabellos (2007), but unfortunately, those frequencies have not been published.

9.1.3 2D GMM

In 2D GMM, there are significant differences between SMA (☥) and SNA (☪) for two sets of landmarks in two teeth, LM2 and UM2, and significant differences between SMA (☥) and XAR (☪) for five sets of landmarks in four teeth, LM1 (two sets of landmarks), LM2, UM1, and UM2. This seems to indicate that there are more differences between SMA (☥) and XAR (☪) than between SMA (☥) and SNA (☪), so SMA (☥) and XAR (☪) could be more distant biologically than SMA (☥) and SNA (☪) are. However, the same teeth provided significant and not significant differences depending on the set of landmarks used, which indicates how dependent these differences are on the sets of landmarks chosen, affecting the reliability of the results. For LM1, the tooth for which the metric variables and the nonmetric traits found differences between SMA (☥) and SNA (☪), two of the sets of landmarks revealed differences between two samples: SMA (☥) and XAR (☪). The third set of landmarks, which revealed differences between SMA (☥) and SNA (☪), LM1 Protostylid Buccal, cannot be considered from the perspective of biological relatedness, as the landmarks are placed alongside the perimeter of the occlusal face viewed from the buccal face and are strongly susceptible to wear. The two sets of landmarks revealing significant differences are the LM1 Anterior Fovea and the LM1 Entoconulid, but only the former is linked to a change in position of the landmark marking the dental nonmetric trait, whilst the latter indicates a general shift in the shape of the crown, not necessarily linked to the dental trait. Nevertheless, the distances between the samples must be very small, as the lowest number of PCs needed for any of them to determine the samples with at least a 50% of reliability is 8 PCs for LM1 Anterior Fovea.

The fact that several sets of landmarks of LM1 are useful in discriminating the samples is consistent with the results obtained with the metric and nonmetric methods. This reveals that some of the sets of landmarks used effectively measure changes in the crown shape. However, it also exposes how the results may differ from one set of landmarks to the other. Interestingly, the samples for which I found differences in LM1 with the other methods were not SMA (☥) and XAR (☪), but SMA (☥) and SNA (☪). For LM2, one of the sets of landmarks chosen (the one with information for the position of the cusps) also had discriminatory power, positioning SMA (☥) biologically further from SNA (☪) and XAR (☪), which were closer together. None of the traits used in LM2 with the ASU system detected significant differences in these samples. However, 2D GMM revealed that the differences among the samples rely on the development of the hypoconid and the metaconid, and to a certain extent, the protoconid.

UCI Double-Shoveling found significant differences between XAR (☪) and SNA (☪), and with this set of landmarks, 82% of the individuals from SNA (☪) were correctly classified with as few as three PCs. However, given that the upper central incisor was not helpful in differentiating the samples in any of the other methods, and that the set of landmarks used only collects information about the contour of the crown, the variation may be a consequence of the different dental wear observed in the samples rather than a reflection of their actual biological variances.

Neither ULI nor UM3 helped discriminate the samples at all. Of the three sets of landmarks chosen for UM1, just one (UM1) could be used to differentiate between SMA (☥) and XAR (☪), with no differences found between SNA (☪) and SMA (☥) or XAR (☪). UM1 differentiates SMA (☥) the most, assigning 79% of individuals to their correct group when the DFA includes 10 PCs. This reveals differences among the samples in the development of the metaconule and the protocone. UM2 is useful to differentiate between SMA (☥) and SNA (☪), and SMA (☥) and XAR (☪), for the UM2 Hypocone set of landmarks. UM2 Outline

is also helpful in discerning SMA (☥) and SNA (☪). With only 6 PCs in the DFA, UM2 Hypocone can help assign 82% of individuals of SMA (☥) to their group. This set of landmarks reflects the development of the hypocone and the paracone, giving, as a result, a crown with a square-like shape.

In summary, 2D GMM found differences in LM1 and UM2, like metric and nonmetric methods. However, for LM1 the differences lie between SMA (☥) and XAR (☪) instead of SMA (☥) and SNA (☪), and for UM2 there are also differences between SMA (☥) and XAR (☪), apart from the differences between SMA (☥) and SNA (☪) also found with the other methods. In addition, 2D GMM found differences in LM2 (SMA (☥) and SNA (☪)) and UM1 (SMA (☥) and XAR (☪)). The method also found differences between SNA (☪) and XAR (☪), but only in UCI. Therefore, this method is precise, as it provides results that are consistent with the results observed with the other methods, and it is more informative. Whether this information is accurate (that is, whether it corresponds to real differences between the samples) should be confirmed by DNA analyses.

A key point for consideration before applying 2D GMM in analyses of biological relatedness is that the results vary depending on the sets of landmarks chosen. In this study, only one set of landmarks that did not include the tips of the cusps found significant differences for the samples (UM2 Outline), whereas several sets of landmarks that included the cusps were useful for discriminating the samples. This means that if I had chosen sets of landmarks with only sliding semilandmarks for the outline, as some authors do (Bailey et al., 2014), I would have missed most of the differences detected among the samples, and would only have found differences in UM2 between SMA (☥) and SNA (☪). However, samples that differ more between them may not be that sensitive to the landmark configurations used to compare them. Landmark configurations used by other authors in teeth include 30-45 sliding semilandmarks and four to eight landmarks (Martinón-Torres et al., 2006; Gómez-Robles et al., 2007; Gómez-Robles et al., 2008; Gómez-Robles et al.,

2011a; Gómez-Robles et al., 2011b; Gómez-Robles et al., 2015), which is similar to the landmark configurations for which I found significant differences. My results corroborate those obtained by Gómez-Robles et al., (2011b), where the most accurate results were obtained with landmark configurations that included semilandmarks and landmarks common to all individuals and a maximum of one landmark for a trait that was missing in some individuals.

9.1.4 3D GMM

3D GMM gave noticeably different results to those obtained with other methods. It showed significant differences between SNA (ɢ) and XAR (ɢ) for LM1 and LM2, a result only similar to those obtained with metric variables before correcting the imbalanced sex of the samples. This may indicate that this method is not precise. However, it would be a mistake to criticise a particular method for lack of precision without considering all the variables involved, for example, the type of data acquisition (e.g., if scanning, with what kind of scanner), the degree of expertise of the researcher, or the conditioning aspects of the sample (e.g., degree of wear). For example, using a micro-CT scanner to acquire the scans and landmarking in the enamel-dentine junction instead of in the outer enamel surface could have provided more precise, and more accurate results, as seen in other studies (Skinner and Gunz, 2010; Zanolli et al., 2014; Fornai et al., 2015; Fornai et al., 2016; Skinner et al., 2016).

Differences between the results using different methods may not be a matter of precision, but rather the fact that the methods gather information for a different morphology, for which the samples vary differently. For example, 3D GMM may capture morphology related to dental wear, because it collects information for the height of the main cusps. Looking at the LM1 and LM2 of SNA (ɢ) and XAR (ɢ) in my study, where 3D GMM finds differences, we see differences in wear. For SNA (ɢ), 60% of LM1 and 90.7% of LM2 had

a dental wear of 1 or 2. However, for XAR (☪), 40% of LM1 and 48.8% of LM2 had a dental wear of 1 or 2. 3D GMM may capture these differences.

9.1.5 Choosing a method

The sensitivity of the methodology based on metric variables to the sex distribution of the samples poses a problem. Differences found could be attributed to biological distance, even if they are partially or entirely explained by sexual dimorphism, as in this study. This could limit the use of the dental metric variables for archaeological remains, where it is not unusual to find samples with a heterogeneous sexual distribution or for which there are many individuals of unknown sex. In addition, comparing the results for metric dental traits with other published studies is difficult, even if the samples are similar in chronology and cultural context (Cabellos, 2007), given the disparity of variables and statistical methods used (univariate, multivariate, and with or without size correction). Many published studies do not consider the sex ratio of the populations studied or the sexual dimorphism in each variable (Lukacs, 1983; Cabellos, 2007; Brook et al., 2009) and do not apply a size correction or multivariate statistical analyses, as suggested by Harris (1997). In contrast, data acquisition using metric traits is very affordable, as the only material or tool needed is an accurate calliper (electronic to 0.01 mm), which does not require major investment. However, this method requires direct access to the teeth (data cannot be acquired remotely) and involves a certain degree of expertise to take the measures correctly and avoid measurement errors. Taking measures in teeth that are still in the maxilla or the mandible increases the difficulty of data acquisition, as neighbouring teeth make it more difficult to obtain some measures. However, to take measures in loose teeth the researcher must be experienced enough to orientate the teeth correctly. Lastly, the calliper may damage the enamel or the adjacent bone if not handled carefully, so some samples are not made available to external researchers wishing to take measurements.

The results of the ASU system of dental nonmetric traits correlate well with neutral genomic data and can be used as a proxy to study biological relatedness, although only part of the dental variation can be explained through genetics (Hubbard et al., 2015; Rathmann et al., 2017). Nonmetric dental traits are helpful in comparing hominin species (Martinón-Torres et al., 2008; Martinón-Torres et al., 2012; Irish et al., 2013; Martinón-Torres et al., 2013; Carter et al., 2014; Irish et al., 2014), studying biological relatedness among human groups (Scott et al., 1983; Irish 1997; Sciulli, 1998; Irish, 2000; Kitagawa, 2000; Guatelli-Steinberg et al., 2001; Bollini et al., 2006; Cabellos, 2007; Irish and Konigsberg, 2007; Leblanc et al., 2008; García Sívoli, 2009; Lee and Zhang, 2013; Nelson and Fitzpatrick, 2013; Scott et al., 2013), inferring family relationships in archaeological remains (Corruccini and Shimada, 2002; Cabellos et al., 2004), and studying changes within a population after historical milestones (Cucina et al., 1999; Desideri and Eades, 2002; Irish, 2005; Irish, 2006; Coppa et al., 2007; Edgar, 2007). However, the results obtained when studying dental nonmetric traits depend greatly on the number of dental traits studied and the parameters used in the analysis, such as the strategy for trait selection (Santos, 2017; Rathmann and Reyes-Centeno, 2020). When studying nonmetric dental traits using the ASU system, a larger number of dental traits will capture more genetic variation up to a certain number – 16, according to Rathmann and Reyes-Centeno (2020). Over that number, adding more dental traits will not add further information. The trait combination also affects the reliability of the results (Rathmann and Reyes-Centeno, 2020). In this study, I could include only 13 traits, although I avoided correlating traits that could affect the results. In addition, dental nonmetric traits often provide better results when studying large geographic regions (Scott and Turner II, 1997). In terms of the difficulty of assessing the scores of the traits, the ASU system requires a certain level of expertise (Scott and Turner II, 1997). In terms of resources, access to the dental casts and the teeth that one wants to study is necessary, although most of the institutions housing archaeological collections have a set

of ASU dental casts. Dental wear will also affect the results, as more worn teeth result in lower scores (Burnett et al., 2013).

In my study, nonmetric traits were more informative than metric variables, they showed more significant differences between the samples and these differences lay in one more tooth. According to Rathmann et al. (2017), dental metric variables and nonmetric traits can be used in biological distance studies, and both methods give strong and similar results (with slightly better results in nonmetric traits). My study supports this conclusion that although both methods can be used to discriminate between different samples, the ASU system provides slightly better results. Other studies, however, reach different conclusions. For example, in Cabellos (2007), the nonmetric dental traits used provided significant differences between almost all the samples, whereas the metric traits differentiated the Roman sample from most of the others, which had more recent chronology. Whilst the results with nonmetric dental traits in Cabellos (2007) were difficult to interpret, the results with metric variables seemed to be more accurate.

2D GMM provided more significant differences between my samples, but these were in line with the differences observed with the metric and nonmetric methods. The interobserver error analysis suggests that the repeatability of the sets of landmarks chosen is low, although the intraobserver error provides better results, with an intraclass variation coefficient of 0.8 for UM2 Hypocone. Because the interobserver error was particularly high, it would be interesting to explore it further, to see whether there are ways to reduce it, and how it affects the comparison of the samples. The differences observed between the samples for some teeth may not be a consequence of the different crown morphologies but of the difficulty of landmarking accurately and following the descriptions of the landmarks. Likewise, because the samples are very similar to one another, the effect of the measurement error is larger than if there were more differences among the samples. Therefore, there may not be a problem with the repeatability of the sets of landmarks but

with the similarity of the samples. This raises the question of whether it is appropriate to compare crown morphology with 2D GMM instead of using the ASU system because the placement of the landmarks seems to be as subjective as assigning the score of the nonmetric traits, and the space for improvement is narrow. The great advantage of 2D GMM is that it can be used even when there is no access to the skeletal remains, as long as there is access to an adequate photographic record. In addition, it is inexpensive and does not require a high degree of expertise. However, it is more time-consuming than assessing the dental nonmetric traits following the ASU system. Besides, any methodology that relies on comparing the outer structure of the teeth will compromise results in older individuals, where the teeth have higher degrees of wear. A way to solve this problem is by observing the morphology in the enamel-dentine junction, either through direct observation or 3D GMM, instead of analysing the outer layer of enamel (Skinner et al., 2008; Skinner et al., 2009; Skinner and Gunz, 2010; Skinner et al., 2016). This allows us to capture morphological differences without being affected by wear, attrition, or uneven deposits of enamel (Skinner et al., 2008; Skinner et al., 2009; Skinner and Gunz, 2010; Skinner et al., 2016). The issue then becomes the requirement of using microtomography, which is very expensive and inaccessible to many researchers. The use of such methods also makes it more challenging to collect data outside the laboratory (Errickson et al., 2017).

Comparing the results obtained with metric variables and with 2D GMM, the latter captures higher levels of detail, which is useful in studies in which the samples used are biologically close (i.e., studies of intra-specific variation) (Bernal, 2007; Evin et al., 2013). Other studies that compare the application of 2D GMM with metric variables and nonmetric dental traits show that the former provides more detailed and informative results than those obtained with other methods (Xing et al., 2019), as observed in this study. Other studies have compared results using 2D or 3D GMM (Cardini, 2014; Fruciano, 2016; Buser et al., 2018). Cardini (2014) analysed the different results obtained in 3D structures when using

2D and 3D GMM, concluding that results obtained with 2D GMM were more reliable in flat structures and that their inaccuracy increased with more spheric structures. Therefore, even if 2D GMM can be used in spherical structures, the results obtained using 2D GMM in comparisons of species that are less distinctive (assuming they are used in inter-specific comparisons) are poorer than when using 3D GMM (Cardini, 2014). However, the measurement error when applying 2D GMM to 3D structures is generally small (Cardini, 2014). Studies that explored the differences when using different methodologies further support these conclusions (Pečnerová et al., 2015; Fruciano, 2016; Buser et al., 2018), with one study finding large differences between the application of 2D and 3D GMM that increased with the volume of the structures (Buser et al., 2018). If using 2D images to study 3D structures can introduce error in the results, this suggests that 3D GMM provides more accurate results.

3D GMM may provide very good results in teeth when using microtomography and applied to the enamel-dentine junction. However, as my results show, if not applied to the enamel-dentine junction, 3D GMM may produce inaccurate results by measuring morphology other than that we want to focus on. For example, it may provide information related to differences in dental wear. As observed by Fruciano (2016), the acquisition of 3D data is a source of error and varies with the device and techniques used to capture the data, so the use of 3D GMM may be problematic when using a structured-light scanner and applied to the outer enamel layer. The lack of precision of 3D GMM in this analysis is more likely linked to how the data were acquired than to the adequacy of the methodology, and seems to indicate that the error introduced by the acquisition of 3D data is larger than the error introduced by using 2D data to study 3-dimensional objects such as human teeth. Thus, if I had carried out the 3D data acquisition using different equipment, such as a CT or a micro-CT, I could have obtained better results, more consistent with the results obtained with the other methods, and more accurate than the results obtained with the scanner that

I used (Errickson et al., 2017). In addition, 3D GMM is a very time-consuming method (it was the most time-consuming in this study), expensive (the equipment requirements make this methodology very expensive), and the degree of expertise required is very high, as it requires the researcher to set up the scanner, align the scans, clean the images, etc., in addition to finding and describing suitable landmarks. This study shows that using 3D GMM in teeth when there are no means of acquiring the data to the highest detail does not improve the results of the comparison but indeed worsens them, whilst being more expensive and requiring more time and skills.

Based on my results, the most suitable methods to infer biological relatedness are the ASU system and 2D GMM. The latter provides better results but is very sensitive to the landmark configuration chosen and the similarity between the samples. The former is very standardised and well-studied, although it is also sensitive to the exclusion strategy and the traits chosen. In addition, 2D GMM allows us to make comparisons in fewer focal teeth, whereas to study a larger number of dental traits it is necessary to study a larger range of teeth. The choice of method must be based on several criteria, including the precision and accuracy of the method, the sample composition, the camera, scanner or equipment used, the landmark configurations, the degree of expertise of the researcher for each method, and the accessibility of the samples. Taking into account the precision of the methods used in this research, the fact that 2D GMM detects differences in more teeth may indicate that it is more appropriate to use it to infer biological relatedness in samples that are biologically close and show more subtle morphological differences, such as the samples analysed in this study, even if it requires more time and resources than using the ASU system. However, the sets of landmarks that included the tips of the main cusps were more useful for differentiating among the populations, so it would not be advisable to restrict the landmark configuration to the outline of the crown.

9.2. Biological and archaeological interpretation of the results

The consistency of the metric, nonmetric, and 2D GMM results seems to indicate that there are actual biological differences between the Christian (SMA) and the Muslim samples (SNA and XAR). Interestingly, the distances obtained with 2D GMM between the samples are slightly smaller between SMA (✞) and SNA (☪) than between SMA (✞) and XAR (☪). This supports the hypothesis of population migration from various parts of the Mediterranean Littoral, including the Middle East and the North of Africa, to the Iberian Peninsula.

There are some examples of well-studied Muslim burials in the Iberian Peninsula (Rascón-Pérez, 2003; Roca de Togores Muñoz, 2007; Barrio, 2015; Molero-Rodrigo, 2017; De Miguel Ibáñez, 2020), but few researchers have attempted to study their biological relatedness to other contemporary populations. Inskip (2013) studied activity patterns and gendered division of work in Muslim and Christian populations and found significant differences between them. However, as these differences are linked to activity patterns rather than inherited genetic traits, they are not identifiers of biological ancestry but of cultural linkage; activity patterns are related to daily activities that do not necessarily depend on a genetic relation. In other words, individuals living within a cultural context may adopt the day-to-day activities involved in that environment even if they come from a different group.

De Miguel Ibáñez (2007; 2020) studied a Muslim cemetery, the *Maqbara* of Pamplona, in the North of the Iberian Peninsula, dated to the 8th century and in which 177 individuals were found with the expected demography of an average pre-vaccine population (sex balanced, with a high number of children and subadults and a limited number of mature individuals). She found several individuals with intentional cosmetic dental modifications, for whom she inferred an African origin (De Miguel Ibáñez, 2007). This archaeological

population's DNA has been studied in depth (Fontecha, 2013, as cited in De Miguel Ibáñez, 2020), and two-thirds of the male individuals studied showed a high percentage of North African genes on the Y chromosome. Likewise, the female individuals' mitochondrial DNA shows that some have a North African genetic linkage, others correspond to the local population, and some others are of unknown origin (Fontecha, 2013, as cited in De Miguel Ibáñez, 2020). A study of isotopes found that some of the female individuals of unknown origin had most likely migrated from the North of Africa (Prevedorou et al., 2010). One of the most remarkable findings concerning this *maqbara* was that one or both parents of some of the individuals with dental manipulations had a North African origin, whilst others were undoubtedly local with no traces of North African origins (Fontecha, 2013, as cited in De Miguel Ibáñez, 2020; De Miguel Ibáñez, 2020). Therefore, it appears that during the early Muslim occupation of Pamplona, individuals from both sexes coming from North Africa settled in what had been Christian territory until then and mixed with the local population, part of which converted to Islam and adopted their culture, lifestyle and traditions (De Miguel Ibáñez, 2020).

The findings in De Miguel Ibáñez (2020) are similar to my results, which indicate that SNA (☪) and XAR (☪) are closer between them than to SMA (☩), from which both are distinct. Because SMA is the Christian sample, and SNA and XAR are the Muslim samples, these significant biological differences, even if small, indicate that the ancestry of the Christian and Muslim samples was slightly different, which contradicts the traditional idea that most of the Muslim individuals that lived in the Iberian Peninsula in the Middle Ages were Christians converted to Islam (Sánchez-Albornoz, 1977, as cited in García Sanjuán, 2017; Arié, 1989). Likewise, the distances obtained here through nonmetric traits are smaller for SMA (☩) and SNA (☪) than for SMA (☩) and XAR (☪), which indicates that the immigrant Muslim population mixed with the local population, which converted to Islam, before the Christians retook the territory. Traces of Muslim presence in the Iberian

Peninsula are evident at many different levels, from language and architecture to traditions, musical instruments and place names (Gil Cuadrado, 2002); it is obvious that Muslims and Christians were near each other for many centuries, sometimes fighting over the land and simply cohabiting at other times (Gil Cuadrado, 2002; Lázaro Pulido 2009). Therefore, the idea of admixtured populations, although unpopular for many years (Sánchez-Albornoz, 1977, as cited in García Sanjuán, 2017; Arié, 1989), seems plausible.

Another interesting finding in this study is that there are no differences among the samples within the West-East axis of the Iberian Peninsula (the differences only occur along the North-South axis). These results do not concur with studies of genetic differences in modern populations (Bycroft et al., 2019). However, this is probably because my samples date from before 1492, when the entire Muslim territory was taken by the Christians, which is the event that Bycroft et al. (2019) identified as prompting the genetic differences found along the West-East axes in the modern population.

Finally, although there are significant differences between the Christian and the Muslim samples, the distances among the samples are small. This most likely reflects admixture between migrated and local peoples, which resulted in some degree of biological continuity, as observed by other researchers (Cabellos, 2007). These results, alongside those of genetic and anthropological studies (Bosch et al., 2001; Adams et al., 2008; Fontecha, 2013, as cited in De Miguel Ibáñez, 2020; Bycroft et al., 2019; De Miguel Ibáñez, 2020), show that the biological continuity that some historians have claimed for a long time (Sánchez-Albornoz, 1977, as cited in García Sanjuán, 2017; Arié, 1989) is not due to a lack of peoples migrating from North Africa, but to cohabitation among migrants and locals that resulted in an admixture of the population.

10. Conclusions

This study had two aims:

1. To evaluate which of four methods is best to estimate biological relatedness between skeletal samples in terms of the information they yield, their reliability and the ease of application
2. To examine whether there are observable biological differences between three medieval skeletal samples excavated from different religious contexts within the Iberian Peninsula

Regarding the first aim, the methods that provided the most significant differences for my samples were the study of nonmetric dental traits through the ASU system and the study of crown morphology through 2D GMM. Whereas the ASU system requires fewer resources, it is sensitive to the statistical analyses used (i.e., the strategies for trait exclusion). 2D GMM is sensitive to the sets of landmarks chosen and requires more resources, both in terms of equipment and time. However, 2D GMM found significant differences in more teeth. The ASU system requires the scoring of a large number of traits (ideally around 16), and thus many different types of teeth (to observe each dental trait on its focal tooth), whereas 2D GMM allows us to identify similarities and differences between samples with fewer teeth. For example, if I had only looked at LM2 and UM1 in this study, I would still have found differences between SMA (☥) and SNA (☪) and XAR (☪) with 2D GMM, but I would not have found differences with the ASU system. Therefore, according to my results, the method that provides more information and presents fewer issues for evaluating biological differences between my samples is 2D GMM.

This study has provided a number of insights regarding the appropriate use of methods in dental anthropology. For example, it shows that studies aiming to infer biological affinities

in archaeological samples through metric variables should include a correction for sex to prevent misleading results affected by sexual dimorphism. Because such a correction in archaeological collections is not always possible (for example, in collections with a high number of loose teeth, or where the sex of the individuals could not be inferred), using metric variables may be problematic and compromise the reliability of the results. Another important lesson is that 3D GMM should be avoided unless data acquisition is going to be carried out with the appropriate equipment, which means equipment that provides information for the dental surface and structure to a very high level of detail, such as that obtained with micro-CT. Scans acquired with other tools, even if they initially appear suitable, may lead to deceptive results.

Taking the time to find, describe and observe the sets of landmarks to apply GMM, has to be a paramount part of the design of a study. The sets of landmarks chosen will define the success when analysing the differences in the morphology of the crowns. Choosing a set of landmarks that does not capture enough information, or that includes a number of landmarks that are not present in all the individuals, will provide inaccurate results. Therefore, when using 2D GMM it is important to consider that those that include the main cusps may be more useful in differentiating among the populations than those that only have landmarks for the outline of the crown. Ideally, in studies of crown morphology, the sets should include a combination of fixed landmarks and an outline of sliding semilandmarks. Finally, this study has shown that the methods chosen should be tailored to the samples used, the degree of expertise of the person collecting and analysing the data, and the availability of resources in terms of equipment and time. A method to study the biological relatedness of archaeological samples, used under the wrong circumstances, will provide results that do not necessarily reflect such relatedness.

With respect to my second aim, I observed differences between the Christian (SMA) and the Muslim samples (SNA and XAR), even if one of the Muslim samples (SNA) was in

Christian territory, and geographically closer to the Christian sample (SMA) than to the other Muslim sample (XAR). These results are interesting because they do not support the hypothesis maintained by traditional historians that the Muslim arrival to the Iberian Peninsula was in small numbers and meaningless from a demographic point of view (the biological continuity hypothesis). Instead, it aligns with more recent studies (Fontecha, 2013, as cited in De Miguel Ibáñez, 2020; De Miguel Ibáñez, 2020) that indicate that the Muslim arrival was in larger numbers than initially thought and that there was an admixture of the populations (the migration hypothesis). The small distances between the samples reflect previous findings, which have been interpreted as supporting the biological continuity hypothesis (Cabellos, 2007). However, an alternative explanation for the small distances is migration followed by population admixture. Given the limited number of samples and individuals studied, to better understand the biological relatedness of the medieval population across the Iberian Peninsula and the accuracy of the methods that I have assessed, further analyses should be carried out, revisiting samples already studied with different methods and including analyses of DNA to assess their accuracy.

This study contributes to future research in biological relatedness through dental anthropology by providing insight into the suitability and effectiveness of four different methods, widely used in this discipline. It will assist other researchers in designing their studies and prevent inaccurate results by adapting their methods to their requirements and the characteristics of their samples. I have also improved our understanding of the demographic composition of the Iberian Peninsula in the Middle Ages, which will hopefully help to break down myths still present in the way in which Spanish History is taught, and to cherish the Spanish multicultural past.

11. References

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