Ancestral variation in mid-craniofacial morphology in a South African sample

by

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List of Abbreviations

2D: Two-dimensions

3D: Three-dimensions

AA: African ancestry

ANOVA: Analysis of variance

ANS: Anterior nasal spine

BNL: Basion-nasion length

BPH: Basion-prosthion height

CVA: Canonical variate analyses

DF: Discriminant function

DFs: Discriminant functions

DFA: Discriminant function analysis

DKB: Interorbital breadth

EA: European ancestry

EKB: Bi-orbital breadth

GM: Geometric morphometrics

INM: Inferior nasal margin

IOB: Interorbital breadth

MA: Mixed ancestry

MAB: Maxillo-alveolar breadth

MD: Mahalanobis distance

MLR: Multinomial logistic regression

MT: Malar tubercle

NAW: Nasal aperture width

NBC: Nasal bone contour

NHL: Nasal height left

NHR: Nasal height right

NLB: Nasal breadth

NPH: Upper facial height

NT: Nasal trauma

OBB: Orbital breadth

OBHL: Orbital height left

OBHR: Orbital height right

OS: Orbital shape

PC: Principal component

PCA: Principal Component Analysis

SD: Standard deviation

SS: Supranasal suture

SA: South Africa

SAs: South Africans

YRS: Years of age

ZMB: Bimaxillary breadth

ZP: Zygomatic projection

ZS: Zygomaxillary suture shape

ZT: Zygomatic trauma

ZYB: Bizygomatic breadth

AP: Alveolar prognathism

Abstract

Ancestry estimation is a critical component of the demographic profile compiled by forensic anthropologists when unknown skeletal remains are discovered. The mid-craniofacial region is most frequently used to estimate ancestry as this region reflects the genetic and morphological ancestry of an individual. The diverse composition of the South African population makes ancestry estimation problematic, and necessitates the development of reliable, population-specific standards. This study sought to characterise variations in mid-craniofacial shape and size between South Africans of European ancestry (EA), African ancestry (AA) and Mixed ancestry (MA). Metric, nonmetric and geometric morphometric assessments were performed on 392 crania from skeletal collections in South Africa. Variations in mid-craniofacial shape and size were assessed in the orbital, nasal, zygomatic and maxillary regions in two-and three-dimensions. Univariate and multivariate statistical analyses were employed to characterise variation and estimate ancestry in AA, MA and EA individuals. Multivariate analyses suggest that tightly integrated ancestral variations in each component of the mid-craniofacial region are associated with functional, regional and developmental proximities of these regions. Specifically, AA individuals exhibited wider and shorter midfacial regions than EA individuals, who exhibited the narrowest orbital, zygomatic and nasal breadths and the longest upper facial, orbital and nasal heights. EA individuals exhibited inferiorly-angled orbits, elongated nasal apertures and anteriorly projecting nasal bridges. Rounder nasal apertures, less anteriorly projecting nasal bridges and more anteriorly projecting maxillary regions were detected in AA individuals. MA individuals exhibited heterogeneity in terms of craniofacial shape and size, and therefore produced the lowest ancestry estimation accuracies. Overall, nasal and maxillary regions were the most ancestrally diverse regions. Antemortem maxillary tooth loss and midfacial trauma were confounding factors in ancestry estimation accuracies. The lowest ancestry estimation accuracies were yielded by two-dimensional metric (27%-60.2%) and nonmetric (57.1%-82.4%) methods. Metric and geometric morphometric assessments yielded the highest repeatability (≥ 95%) indicating that these methods may be more reliable for use in medicolegal contexts. Geometric morphometric shape assessments yielded the highest ancestry estimation accuracies (75-97.9%), suggesting the presence of three-dimensional shape variations between ancestry groups. These results suggest that a continuum of ancestral variation, with large areas of overlap, exists across South African populations and emphasises the need to develop multivariate ancestry estimation standards which can estimate ancestry reliably.

Chapter One: Introduction

The systematic variation that exists between individuals from different geographic and genetic backgrounds has been expanded by forensic anthropologists to estimate ancestry from skeletonised remains (Sauer & Wankmiller, 2016). When human skeletal remains are discovered, estimations of sex, ancestry, age and stature are essential in constructing a demographic profile of the deceased. Although sex estimation is traditionally the first step in forensic anthropological protocol (Steyn *et al*., 1997), studies have shown that standards for age (Walker, 2005), sex (İşcan & Steyn, 1999; Loth *et al*., 2005) and stature (Dayal *et al*., 2008; Mummert *et al*., 2011) estimation are influenced by ancestry. Due to the high crime rate in South Africa (Seedat *et al*., 2009), a reliable demographic profile of the deceased is essential in preventing a backlog of cases and ensuring that criminal and social justice is served. Ancestry estimation is crucial as it limits the number of missing persons with which the deceased's demographic profile is compared, thereby increasing the likelihood of identification.

Ancestry estimations have traditionally been performed using nonmetric assessments of morphological shape (Hooton, 1946; Van Rooyen, 2010; Hefner and Ousley, 2014) and metric assessments of inter-landmark distances (De Villiers, 1968; İşcan and Steyn, 1999; Stull *et al*., 2014). The methods and standards used to estimate ancestry influence the accuracy of ancestry estimations and therefore, it is important to validate these methods to ensure they are reliable and population-specific. Despite the tremendous scientific value of metric and nonmetric methods, both fail to assess shape variation exclusively from size variation and are limited as they only effectively evaluate variation in two-dimensions. The use of geometric morphometrics in biological anthropology has allowed for shape and size variation to be quantified and visualised in both two-and three-dimensions (Adams *et al*., 2004, Slice, 2007, Hallgrímsson *et al*., 2008). Geometric morphometrics is advantageous in understanding total variation in skeletal material, as well the contribution of each region to the total variation in skeletal elements. This method is statistically complicated, timeous and costly in nature and is therefore not commonly used in forensic cases (Ross *et al*., 2010). However, information obtained using this method can be used to validate metric and nonmetric methods by confirming whether these methods effectively represent osteological variations in different populations.

Numerous studies identify the mid-craniofacial region as the most ancestrally diverse component of the human skeleton as this region closely reflects the genetic and morphological ancestry of an individual (Gill & Gilbert, 1990; Hanihara *et al*., 2003; Hefner, 2003; Hefner, 2009, Gonzalez *et al*., 2011*a*; L'Abbé *et al*., 2011). This region is also vulnerable to physiological and morphological changes associated with age (Akgül & Toygar, 2002; Albert *et al*., 2007), antemortem tooth loss (Reichs *et al*., 2011; Small *et al*., 2016) and even minor, healed antemortem trauma (specifically in the nasal and zygomatic region) (Chelotti, 2013). Tooth loss, age and healed nasal and zygomatic trauma have been shown to influence the reliability of the demographic profile compiled during forensic cases (Steadman and Konigsberg, 2009). The influence of these variables on craniofacial shape and size is yet to be investigated in the context of ancestry estimation in South Africa.

1.1. Defining ancestry

Throughout much of the world, social ideologies of *race* remain a reality in which individuals attribute themselves to social and legislative identities, however, only *ancestry*, a scientifically derived descriptor of biological variation can be estimated by forensic anthropologists (Konigsberg *et al*., 2009; Stull *et al*., 2014). Morphological variations between different ancestry groups, have been attributed to positive assortive mating, geographic distances, adaptations to environmental conditions and social forces which have limited geneflow and increased variation between different population groups (Howells, 1960; Dirkmaat *et al*., 2008; Stull *et al*., 2014). Outdated notions that r*acial* categories represent discrete, identifiable and natural biological groups, have been extensively rejected in the field of biological anthropology (Crawfurd, 1868; Brace and Hunt, 1990; Rhine, 1990; Sauer, 1992; Konigsberg *et al*., 2009). Race was to justify discrimination and prejudice in countries like South Africa, North America and Germany, and thus contemporary research has endeavoured to distance itself from the terminology and methodologies used in these countries. These days, ancestry estimations are exclusively performed to aid in death investigations and the term '*race'* has largely been replaced with *ancestry* or *biological affinity* (Albanese & Saunders, 2006; Stull *et al*., 2014; L'Abbé *et al*., 2013). Research pertaining to ancestry estimation should always consider the social implications of words and categories used, and not confuse *ancestry* estimation with past typological approaches of classification that suggest greater biological grouping than is recognisable scientifically (Dubow, 1995; Albanese and Saunders, 2006). Regardless of the terminology used, the underlying assumption in forensic applications is the same: using nonmetric, metric, or a combination of both methods, it is possible to estimate an individual's ancestry or geographic origin from skeletal remains (e.g. African, European and Asian) (Albanese & Saunders, 2006).

1.2. Forensic application of ancestry estimation

The South African population consists of approximately 56 million people from diverse linguistic, genetic, cultural and geographic backgrounds (*Statistics South Africa, Census 2011: Statistical Release).* The genetic and morphological diversity of the South African population has been influenced by the policies and ideologies pursued during colonial and Apartheid eras (Comaroff, 1991). Various groups of people from Dutch, French, Malaysian, West African, Eastern European, Indian origins etc., have either willingly or forcefully migrated to South Africa (Dubow, 1995; Jacobson *et al*., 2004; Van Rooyen, 2010). Over time, this unique cultural, morphological and genetic amalgamation has produced the South African identity and contributed to unique skeletal features which differ from populations in North America or Europe (L'Abbé *et al*., 2013). While biological anthropologists avoid using race as a biological category, in the subdiscipline of forensic anthropology, estimations of race are frequently requested to aid in the identification of unknown individuals. South Africa may not be unique in its high rate of violent crime or its large number of missing persons, the heterogenous nature of its population certainly is unique, and makes identification of individuals from their skeletal remains complicated (Van Rooyen, 2010). The South African statistical census (2011) identified the three largest ancestry groups in South Africa: "Black Africans"- comprising South Africans of African ancestry (79.2% of the population); "Whites"- comprising South Africans of European ancestry (8.9% of the population) and, "Coloureds"- comprising South Africans of Mixed ancestry (8.9% of the population). Unfortunately, many ancestry estimation standards were developed using European, Asian and sub-Saharan African populations (Pietrusewsky, 2000; Ossenberg, 1976; Hefner, 2003; Wescott & Jantz, 2005; Hefner *et al*., 2012) and it is therefore necessary to test and revise current standards to ensure they are reliable and relevant to South African's of African, European and Mixed ancestry.

1.3. Aims

This study aims to characterise ancestral variations in mid-craniofacial size and shape in a skeletal sample of South Africans of European, African and Mixed ancestry. The ancillary aim of this study is to assess the impact of antemortem maxillary tooth loss on ancestral variations in the craniofacial region.

1.4. Objectives

1. Assess variations in size and shape between ancestry groups using existing metric, nonmetric geometric morphometric methods.
2. Evaluate the influence of antemortem maxillary tooth loss and other confounders (minor, healed mid-craniofacial trauma of the zygomatic and nasal bones) on craniofacial morphology using metric, nonmetric and geometric morphometric methods.
3. Assess the effect of maxillary tooth loss and minor, healed nasal and zygomatic trauma on ancestry estimation accuracies.
4. Determine which methods most reliably estimate ancestry.

Chapter Two: Background

2.1. A brief history of race in biological anthropology

Until the beginning of the 19th century, scientists failed to dispute the hierarchical view that races represented different subspecies (Brace, 2005). In addition, decades of global engagements reinforced the idea of distinct human races rather than one human race (Brace, 2005). Research in this era gave credence to the concept of race through the development of typological and often pseudo-scientific methods of categorisation (Brace, 2005). Despite the effort of scientists to abstract their research from society; words, categories and methodologies developed by biological anthropologists were used in the architecture of segregationist and oppressive political systems in countries like South Africa, Germany and the United States of America (Dubow, 1995; Albanese & Saunders, 2006). Nowadays, the traditional westernised notion that races represent inherent, discrete, biologically identifiable groups has been extensively rejected in biological anthropology, and current research focuses on understanding the nature and causes of inter-individual variation (Sauer, 1992).

2.1.1. Pre-twentieth century conceptions of race

Brace (2005) found no evidence that the concept of race existed in Europe before the Renaissance in the 15th century. As Europeans began to colonise newly discovered lands, the biological disparities between themselves and indigenous populations were emphasised to justify subordination of indigenous people (Dubow, 1995, Jacobson *et al*., 2004). While the term *race* was only recorded after the 16th century, the idea that individuals could be characterised based on appearance was a part of the fabric of colonial society (Hannaford, 1996). The concept of racial classification was formalised by Carl Linnaeus (1707-1778), who described four distinct human subspecies or races: *Homo sapiens europeaus*, *H. sapiens americanus*, *H. sapiens asiaticus,* and *H. sapiens africanus* (Brace, 2005). Each race represented a different geographic region (Europe, America, Asia and Africa) and Linnaeus based his classifications on skin colour, soft tissue morphologies and observed behavioural characteristics (Wheat, 2009). Linnaeus proposed that races were fixed entities with intrinsically determined variations which were essential to their existence (Caspari, 2003). He maintained that each race occupied an inherent position within a hierarchy which favoured Europeans as the most physically and intellectually superior group (Brace, 2005). The methods and categories proposed by Linnaeus were extensively criticised by Johann Friedrich Blumenbach (1752-1840) who maintained the belief that humans shared a single origin, but that morphology and races altered over time due to climatic, nutritional and environmental factors (Wheat, 2009). Blumenbach proposed the existence of five

races (Caucasian, Mongolian, Ethiopian, American and Malayan) which he argued exhibited both intra- and inter-population differences (Brace, 2005). Linnaeus and Blumenbach, were both monogenists, and they believed that humans were a single species comprised of different subspecies or race groups. It was not long before the dominant theory of monogenism (single species theory) was disputed in favour of a polygenism (or the multiple species theory). Samuel Morton (1799-1851) was one of the most outspoken opponents to monogenism, and he maintained that each human race exhibited an independent origin, and thus races were representative of different species (Brace, 2005). Morton measured cranial dimensions of different race groups to develop an "intelligence index" which he used to substantiate the belief that Caucasians and Europeans were intellectually superior to other races. Morton's findings were central in justifying slavery in the Americas and colonialism in Africa (Dobzhansky, 1973; Brace, 2005). Decades of global engagements prior to the 20th century reinforced the idea of distinct human races instead of one distinct human race.

One of the most prominent monogenists before the 20th century was Charles Darwin (1809-1882), who proposed that humans shared a common origin and that race groups were representative of different clines and not discrete biological species (Darwin, 1871). Darwin asserted that variations between geographically separated populations (or races) occurred due to natural selection and speciation, and that morphological variations between population groups did not abruptly terminate but continued from one group to the next (Darwin, 1871). While he was criticised heavily for his extreme, naturalist views (Brace, 2005), his research concerning processes shaping biological variation (evolution) and the concept of grouping people based on geographic origin or ancestry remain at the forefront of population studies within biological anthropology (Brace, 1995).

2.1.2. Twentieth century conceptions of race

By the early 20th century, race became the theoretical foundation underpinning most research in biological anthropology (Caspari, 2009). As Darwin's theories that environmental factors shaped human variation began to gain traction, researchers like Franz Boas (1869-1943) began to challenge the suitability of race in studies of human variation (Brace, 2005). Boas rejected biological determinism (the belief that physical and mental characteristics in humans are inherited and unchanging) and questioned the validity of human races (Caspari, 2003). Craniometric findings by Boas were central in challenging the typological approach to race which were dominant during this era. Ernest Hooton (1887-1954) disagreed with assertations in Boas' research and argued that pure races, in a biological sense, did not exist due to inter-breeding and he maintained that each race represented different

subspecies with separate evolutionary origins (Caspari, 2009). Hooton assembled a list of morphological characteristics which were used to distinguish between different race groups (Wheat, 2009) and this list later became known as the "Harvard List" (Brace, 2005). Hooton attempted to standardise the nonmetric and metric observations included in the "Harvard List", and thus he provided a scoring system of traits which were used by many who disagreed with his typological approach to race (Rhine, 1990; Hefner, 2003).

World War II (1939-1945) marked a fundamental shift in the social and scientific understanding of race. Hooton and his contemporaries renounced Nazi doctrine and the Eugenics practiced in Germany (Brace, 2005). While they renounced the fact that race was used to justify genocide, they did not challenge the existence of the concept. By the mid-20th century, Ashley Montagu emerged as an outspoken critic of the concept of race and suggested the word *race* be substituted by *ethnicity* (Montagu 1963). He recognised that the term race and its typological methodology legitimised and perpetuated scientific racism, as its very origin was rooted in racial discrimination (Brace, 2005). The late 20th century saw the dawning of post-modernism, human rights, and the popularisation of social constructionist definitions of "race" (Littlefield *et al*., 1982). In 1996, the concept of biological race was addressed by the American Association of Physical Anthropology (AAPA) in a statement with the following assertions: all living humans have evolved from a common ancestor; there is no evidence that genetically homogenous or "pure" races exist or existed in the past; and social forces, assortive mating and geographic barriers have functioned to limit gene flow, resulting in conserved biological variations within socially constructed race groups. The political and social climate in the 20th century, fostered introspection and discussion about the validity and reality of race and thus some biological anthropologists began to favour terms like ancestry and ethnicity instead of race (Caspari, 2003).

2.1.3. Contemporary views of race in biological anthropology

Over the past few decades, outdated, westernised notions of race have been extensively rejected by biological anthropologists who now recognise its existence as a social construct (Sauer, 1992). In countries like South Africa (SA), race is no longer a legislative requirement, however, it is perpetuated due to its social, economic and cultural legacy. In 1992, Sauer posed the following question, "If race does not exist, why are forensic anthropologists so good at identifying them?". According to Sauer the answer is simple; estimating race does not give credence to the concept of race because forensic anthropologists interpret morphological variations using a socially constructed labelling system (Sauer, 1992). Moreover, the use of race/ancestry as an exclusion criterion in forensic anthropology is

meant to advance social justice by improving the probability of identification. The connection between social race and cranial morphology can be attributed to genetic and geographic ancestries of different population groups (Dirkmaat *et al*., 2008). Therefore, the morphological differences between races (for example, black and white race groups) originated due to the separate geographic histories of different ancestry groups (for example, African and European groups) (Ousley *et al*., 2009). Social conceptions of race, as well as language and cultural barriers, institutionalised racism and political segregation have influenced population admixture and thus biological variation (Ousley *et al*., 2009). These days, forensic anthropologists recognise the fluidity and environmental sensitivity of morphological variations and emphasize the causes of variation between different population groups (Hefner, 2003; Roseman, 2004; Bastir *et al*., 2006; McDowell, 2012; Freidline *et al*., 2015; Maddux *et al*., 2015; Maddux *et al*., 2017).

As the understanding of the concept of race in biological anthropology has changed, methods used to assess variation have also been reformed. While nonmetric methods remain common among researchers (Gill & Rhine, 1990; Hefner, 2003; Wheat, 2009; Maddux *et al*., 2015), metric assessments of skeletal morphology assume a more prominent role in ancestry estimations in forensic contexts (Dirkmaat *et al*., 2008). Researchers test existing ancestry estimation methods to ensure they are population-specific and reliable for forensic casework (De Villiers, 1968; L'Abbé *et al*., 2011; Mc Dowell *et al*., 2012; L'Abbé *et al*., 2013). Complex statistical frameworks (for example, Discriminant Function analyses and Logistic Regression analyses) and statistical software (Fordisc® and Cranid®) have been developed to assess univariate and multivariate patterns in morphology. The use of three-dimensional analytical frameworks (such as geometric morphometrics) have allowed researchers to detect variations which may be problematic to distinguish or describe using metric or nonmetric methods (Pretorius *et al*., 2006; Maass, 2016; Gillick, 2012; Stull *et al*., 2014).

Although the concept of race has been scientifically refuted, debates regarding the use of racial terminology persist. While some forensic anthropologists recommend that the terminology used in research should reflect that of the law enforcement officials and society in general (e.g. black and white) (İşcan & Steyn, 1999; Konigsberg *et al*., 2009; McDowell, 2012; L'Abbé *et al*., 2013), others prefer terms related to ancestry (e.g. African, European etc.) to distance themselves from the political, social and methodological implications of race (Sauer, 1992; Brace, 1995; Vitek, 2012; Xing *et al*., 2013). Regardless of the terminology used, races are deemed inadequate categories for studying human variation, and ancestry remains a better framework to study the distribution and selection of variation.

2.2. Ancestry for identification in forensic anthropology

While biological anthropologists avoid using race as a biological category, in the subdiscipline of forensic anthropology, estimations of race are frequently requested to aid in the identification of unknown individuals. Correctly estimating ancestry reduces the number of missing persons to which an individual's demographic profile is compared and this significantly improves the likelihood of victim identification. Therefore, it is necessary to ensure that standards used to estimate ancestry are population-specific, reliable and admissible in medicolegal proceedings.

While various court cases in the 1990s were responsible for re-defining scientific standards for court admissible testimonies in the United States of America, the ruling made in the case of *Daubert v Merrel Dow Pharmaceuticals, Inc*. (1993) radically impacted scientific methodologies used in forensic anthropological casework (Christensen and Crowder, 2009). The *Daubert* principles, instructed the judge to focus on the principles and methods used in scientific testimonies, rather than conclusions that were generated (Christensen and Crowder, 2009). These principles stipulated that for methods to be admissible in court they should: be repeatable, have known or quantifiable error rates, have recognised standards and be subject to peer review and publication (Faurie, 2000, Christensen and Crowder, 2009). Recent re-evaluations of some of the most commonly used ancestry estimation techniques have shown that some are less repeatable and reliable than originally reported (Hefner, 2009; Wheat, 2009; Van Rooyen, 2010; Ta'ala *et al*., 2014). These findings emphasise the need to re-visit well-established ancestry estimation standards to ensure that they are repeatable, reliable and effectively approximate variation between different populations. While SA does not have specific rules regulating the admissibility of scientific evidence in court (Faurie, 2000), the *Daubert* principles are effective guidelines which govern evidentiary standards and are recognised as a standard requirement in forensic anthropological research internationally.

2.2.1. Forensic anthropology in South Africa

South Africa (SA) has one of the fastest growing murder rates in Africa (*Statistics South Africa, Victims of Crime Survey 2017: Statistical Release*). The high reported murder rate of 341 per 1 000 000 people (*Statistics South Africa, Victims of Crime Survey 2017: Statistical Release*) and the alarming prevalence of missing women and children (Isaacs, 2017); emphasises the need for rapid and reliable victim identification. The heterogenous composition of the SA population makes identification of individuals from their skeletal remains problematic and necessitates the development of population-specific standards (Van Rooyen, 2010).

2.2.1.1. The South African population

In 1652, the Dutch East India Company established a fuelling station at the Cape of Good Hope (known today as Cape Town) in South Africa (Adhikari, 1992). After some time, Europeans (primarily from Dutch, German and French Huguenot descent) began to settle permanently in the area; displacing many of the Khoekhoe and San inhabitants (Patterson *et al*., 2010). The mid-1800s saw the establishment of a British settlement at the Cape of Good Hope, which resulted in the rapid expansion of the Cape Colony (de Wit *et al*., 2010, Patterson *et al*., 2010). To maintain this expansion, slaves were introduced from various countries like Central Africa, Madagascar, India, Indonesia and Malaysia, and indigenous populations like the Khoekhoe and San were also enslaved (Adhikari, 1992). While marriages between European males and free African and indigenous females were common, it was only after the emancipation of the Khoesan (1828) and slaves (1838) at the Cape colony that various groups (such as Malay, Khoesan, West Africans, Griquas) began to integrate more rapidly (Adhikari, 1992). This admixture was mainly attributed to shared culture and socio-economic status resulting from their position in the lower ranks of Cape Colonial society (Adhikari, 1992). Admixture of the various ancestry groups at the Cape Colony was short lived, as the Apartheid government used legislation to formalise segregation and prevent further population integration in the early 1900s; specifically, between South Africans of European ancestry ("white") and those of African ("black") and Mixed ("coloured") ancestry (Adhikari, 1992). The forced classification of individuals under the *Population Registration Act* (1950), made rigid segregation of race groups possible (Adhikari, 1992). This act required all South Africans to be registered as a "white person", "native or bantu", or "coloured person"; these terms were later extended to include South Africans identified as "Indian" and "Asian" (Posel, 2001, Christopher, 2002). While these categories dehumanised the "native or bantu" group (later identified as "black"), those of mixed descent were grouped as a single entity "coloured" and stripped of all cultural identity. These categories determined every aspect of a person's life: where they could live, what job they could hold, whom they could socialise with, where they could walk and sit, what type of education they could receive and even whom they could marry (Christopher, 2002). Under the *Prohibition of Mixed Marriages Act* (1949) and the *Immorality Amendment Act* (1950), marriage and sex were prohibited between individuals from different races in an attempt to preserve racial "purity" (Adhikari, 2005). Racial segregation of residential areas and public amenities was formalised under the *Group Areas Act* (1950) and the *Separate Amenities Act* (1953). As such, colonial and Apartheid history in SA functioned to influence and conserve morphological and genetic differences between various ancestry groups (Tishkoff *et al*., 2009; de Wit *et al*., 2010; Patterson *et al*., 2010).

Despite the end of Apartheid in 1993, these categories are upheld socially and are thus used for identifying individuals in forensic cases. The SA statistical census (2011) identified the nation's three largest ancestry groups: South Africans of African ancestry, European and Mixed ancestry. These ancestry groups represent social and cultural identities and while group admixture is no longer limited by legislation, many groups still do not intermix, thus conserving variation between ancestry groups (Jacobson *et al*., 2004).

South Africans of "African" ancestry

South Africans of African ancestry are those who self-identify as "Black Africans" (*Statistics South Africa, Census 2011: Statistical Release).* This diverse group represents the largest percentage of the South African population, and contains individuals whose ancestors were a product of the Bantu (linguistic group) migration into Southern Africa, and were not Khoekhoe or San (Jacobson *et al*., 2004), with unique cultural heritages and even designated homelands (McDowell, 2012), and from various ancestral and linguistic subgroups, such as Zulu, Xhosa, Ndebele, Sotho-Tswana, Setswana, Sepedi and Venda. While some have argued that distinct cranial variations occur between different tribal groups in SA (De Villiers, 1968; Franklin *et al*., 2005b; Franklin *et al*., 2007a) their results are questionable as they are often derived from skeletal remains from the Raymond A. Dart Collection of Human Skeletons. In this collection, it has been reported that in some cases when tribal identifications were not reported on the death certificate, this information was inferred from surname or contextual information (Tal and Tau, 1983). South Africans of African ancestry have been seen to be morphologically (İşcan and Steyn, 1999, L'Abbé *et al*., 2011, L'Abbé *et al*., 2013) and genetically distinct (Tishkoff *et al*., 2009) from both North American and other African ancestry groups, emphasising the need to ensure that ancestry estimation standards are population-specific.

South Africans of "European" ancestry

South Africans of European ancestry are those who self-identify as "White" (*Statistics South Africa, Census 2011: Statistical Release)*. This group includes descendants of Dutch, Greek, German, French, Hungarian, Portuguese and British individuals who settled in SA from the 17th century onwards (Steyn *et al*., 2004). Due to founder effects and limited admixture of South Africans of European descent with other SA ancestry groups, variation is conserved within this group and these individuals are morphologically and genetically distinct from European individuals (Steyn *et al*., 2004).

South Africans of "Mixed" ancestry

South Africans of Mixed Ancestry are individuals who self-identify as "Coloured" (*Statistics South Africa, Census 2011: Statistical Release)*. This group is descended largely from Cape slaves (from the East Indies, West Africa and Madagascar), indigenous Khoesan populations and some people of Eastern European, Southern and Eastern Asian, and West African descent (Adhikari, 1992). The "Coloured" group contains three smaller groups, namely, Cape coloureds, Griquas and Cape Malays (Adhikari, 2005). In SA, "Coloured" identity has been forged as a cultural and linguistic independence from the suppressive Apartheid government, and this identity persists today (Adhikari, 2005; Adhikari, 2006). South Africans of Mixed ancestry are a genetically diverse group and are morphologically different from both African-descendent South Africans and European-descendent South Africans (L'Abbé *et al*., 2011; Stull *et al*., 2014; Liebenberg *et al*., 2015b). While studies have investigated morphological variations in the Mixed ancestry group (Van Rooyen, 2010; Stull *et al*., 2014; McDowell *et al*., 2015), they have failed to characterise the extent of shape and size variation in the mid-craniofacial region in individuals of Mixed ancestry. Therefore, characterising mid-craniofacial morphology could assist in the development standards to reliably estimate ancestry of South Africans of African, European and Mixed ancestry.

2.3. Ancestral variation in mid-craniofacial morphology

2.3.1. The structural and functional components of the mid-craniofacial region

The viscerocranium, or facial region of the cranium, is considered the most ancestrally diverse region of the skeleton (Howells, 1960; Gill & Gilbert, 1990; Liebenberg *et al*., 2015a). Paired nasal, zygomatic and maxillary bones, and part of the frontal bone constitute the midfacial region of the cranium (Figure 2.1). Within the first four weeks of prenatal development, the nasal, zygomatic, maxilla and frontal bones develop from neural crest cells, or sclerotome (Cunningham *et al*., 2016). The left and right nasal bones are the first bones to develop from cartilage and the remainder of the facial bones (excluding the frontal bone) develop around the nasal region through intramembranous ossification (Cunningham *et al*., 2016). The growth of the cranial vault and orbital region of the frontal bone follows the rapid pattern of nerve growth (Cunningham *et al*., 2016); while growth in the remainder of the facial region is associated with the development of dentition and muscles of mastication (Enlow and Hans, 1996). Due these patterns of growth, facial regions in young children and infants are proportionally different than in adults (Enlow and Bang, 1965). Orbital growth is limited to the transverse plane by the age of 7 whereas transverse and longitudinal growth in the zygomatic, nasal and maxillary regions persist into adulthood (Enlow and Bang, 1965; Niida *et al*., 1991). Research suggests that variation in the position or shape of ossification centres between different ancestry

groups could explain variation in craniofacial morphology (Enlow and Bang, 1965; Niida *et al*., 1991; Freidline *et al*., 2015).

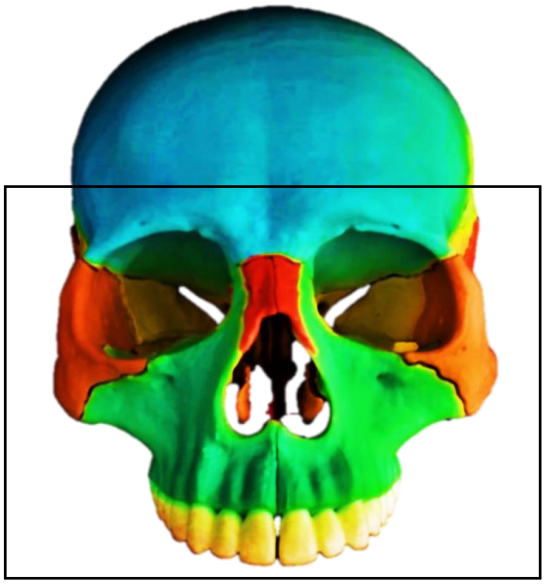

Figure 2.1. Image of a cranium highlighting the mid-craniofacial region. Regions of the mid-craniofacial complex include: orbital region of the frontal bone (blue); zygomatic and nasal bones (orange), maxillae (green).

[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

While several genes have been implicated in human mid-craniofacial variation, bone remodelling occurs due to selective pressures and adaptive requirements of this region. Genetic isolation, heredity, and environmental influences, such as climate and diet, are possible reasons for geographic patterning in mid-craniofacial variations (Harvati & Weaver, 2006, Perez & Monteiro, 2009; Adhikari *et al*., 2016). Variations in major structural units, which comprise the mid-craniofacial region, are best understood in the framework of heterochrony and modularity (Bruner, 2007, Masters, 2008).

2.3.1.1. Heterochrony

Heterochrony refers to changes in the timing or rate of ontogenic or developmental processes (Masters, 2008). Growth and development fluctuate within osteological structures, allowing for shape and size to change in response to selective pressures. The close relationship between shape and size is known as allometry. Sometimes allometric changes are uncoupled and changes in shape are not reflected in size, and vice versa (Bruner, 2007). In the mid-craniofacial region, different allometric trajectories often occur due to variations in growth, allowing for greater adaptability to environmental pressures (Mitteroecker *et al*., 2004; Bastir *et al*., 2006). Freidline *et al*., (2015) found wide maxillary

breadths and prognathic maxillae in Khoesan individuals were associated with an earlier and more rapid growth in lateral and anterior planes of the maxillae. These differences may reflect mechanical adaptations in response to diet and climatic conditions (von Cramon-Taubadel, 2014). Enlow and Hans (1996) found that early growth in the frontal and temporal bones was associated with greater facial widths. Developmental differences in the neurocranium also influence the shape, position and projection of the orbital and maxillary regions (Enlow and Hans, 1996). These studies all suggest that variations in craniofacial morphology between different ancestry groups could be attributed to different growth trajectories and notably, slight growth in one craniofacial region may impact the growth of adjacent region, meaning that the adjacent craniofacial modules may share similar growth trajectories.

2.3.1.2. Modularity and integration

Modularity refers to variations in "modules", or anatomical regions, which are dependent on variation in other regions due to structural or functional relationships (Bruner, 2007). Modularity is often studied in conjunction with *Integration*, which proposes high levels of covariation within adjacent modules (Bruner, 2007). Findings by Bastir and Rosas (2005) and Singh *et al*. (2012) suggest that the entire craniofacial region functions as a module and varies most significantly relative to components of the neurocranium. Tightly integrated morphological shapes and sizes have been detected in the nasal bone, maxillae and zygomatic bones (Hylander *et al*., 1991; Holton and Franciscus, 2008; Holton *et al*., 2013). Integration in mid-craniofacial morphology has been attributed to similar developmental origins of these components and their close regional proximities (Paschetta *et al*., 2010; Cunningham *et al*., 2016). Variations in mid-craniofacial morphologies between ancestry groups are indicative of craniofacial integration as broader maxillae, zygomas and nasal bones, in addition to narrower orbital regions have been detected in African populations; while European populations are known to exhibit narrower maxillae, zygomas and nasal bones relative to slightly wider orbital regions (Gill & Rhine, 1990; Gillick, 2012,). Heuzé *et al*. (2016) suggests that the close morphological relationship between the maxilla and zygoma may be linked to similar developmental origins as both regions are derived from the same neural crest cells. Yet, Mitteroecker and Bookstein (2008) found that enlarged and laterally projecting zygomatic arches were associated with enlarged and prognathic maxillae and that these relationships were mainly related to mastication and force distribution. Thus, genetic, functional and developmental factors are associated with variations in craniofacial morphologies and should remain considerations when interpreting ancestral variations in mid-craniofacial morphology.

2.3.2. Ancestral variations in mid-craniofacial morphology

Some of the most commonly used ancestry estimation standards were developed using three broad reference samples and categories: Africa, European and Asian. In the past, these ancestry groups were referred to as Negroid, Caucasoid and Mongoloid (Howells,1960; Rhine, 1990), however, due to offensive connotations, contemporary forensic anthropologists have adopted geographic-based identifiers to refer to these groups (Hefner, 2003; Gillick, 2012; Xing *et al*., 2013). Standards for African ancestries were developed using reference samples from West Africa, Central Africa, Southern Africa (Khoekhoe, San and Bantu-speaking tribes) and African American populations (Howells,1960; Rhine, 1990; Hefner, 2003). Standards for European and Asian ancestry groups were developed using samples from people of European, Eastern European, Mediterranean, Middle Eastern ancestry and East Asian, North American and Inuit ancestry, respectively (Howells,1960; Rhine, 1990; Hefner, 2003). It is therefore likely these reference samples for African, European and Asian ancestries may not be representative of three genetically and morphologically discrete ancestry groups; but rather, each sample is likely to be comprised of several distinct, yet overlapping, ancestry groups. In SA, the three largest ancestry groups are comprised of individuals of African, European and Mixed descent, however, these groups are genetically and morphologically different from other European, African, and Mixed ancestry populations. Therefore, standards developed internationally may not be practical in distinguishing between South Africans of European, African, and Mixed ancestry.

2.3.2.1. Ancestral variations in South Africans of African, European and Mixed ancestry

Mid-craniofacial variations in South Africans (SAs) of African and European ancestry reported by De Villiers (1968), İşcan & Steyn (1999), L'Abbé *et al*. (2011), Mc Dowell *et al*. (2012) and L'Abbé *et al*. (2013) closely resemble those reported by Gill & Gilbert (1990), Rhine (1990) and Hefner (2003) for African and European reference samples. Generally, medium-to-wide facial breadths and low facial heights have been reported in SAs of African ancestry (De Villiers, 1968; İşcan & Steyn, 1999; L'Abbé *et al*., 2013). Whereas, medium to narrow facial breadths and medium to high facial heights have been recorded in SAs of European ancestry (İşcan & Steyn, 1999; L'Abbé *et al*.,2013). More laterally projecting zygomas, in addition to more prognathic maxillae and wider, hyperbolic to parabolic-shaped palates have also been noted in SAs of African ancestry compared to those of European ancestry (De Villiers, 1968, İşcan & Steyn, 1999; L'Abbé *et al*.,2013). The nasal region (comprising the nasal bone and the nasal portion of the maxilla) is recognised as the most ancestrally diverse region in African and European descendent SAs (McDowell, 2012). SAs of Mixed ancestry are a morphologically heterogenous group and tend to classify indeterminately using African, European and

Asian using standards developed internationally (L'Abbé *et al*., 2011, McDowell, 2012). SAs of Mixed ancestry are more morphologically and genetically similar to SAs of African ancestry, yet findings by Van Rooyen (2010); McDowell (2012) and Maass (2016) suggest that this group is morphologically and genetically distinct enough to estimate ancestry. This emphasises the need to develop reliable standards for South Africans of Mixed ancestry as this may be fundamental in ensuring the identification of missing persons.

2.3.2.2. The impact of age at death, antemortem maxillary tooth loss and antemortem trauma of mid-craniofacial variation

Generally, studies pertaining to ancestral variation in mid-craniofacial morphology exclude individuals who are older than 65 years of age at death, who have severe antemortem tooth loss (more than 6 teeth lost), or who have signs of antemortem trauma in regions of interest (Gillick, 2012, McDowell, 2012, Stull *et al*., 2014). Although this is common practice in anthropological research, forensic anthropologists cannot exclude victims whom they are attempting to identify that do not fulfil these criteria. Age has been associated with the remodelling of the mid-craniofacial region and Sarnäs and Solow (1980) and Mendelson and Wong (2012) found that older individuals exhibited taller nasal and facial heights relative to wider nasal breadths and narrower maxillary regions. The process of aging is often accompanied by tooth loss, gingival recessions, loss of periodontal ligament attachments (associated with periodontal disease), increased bone porosity and increased osteoclast activity (Natto *et al*., 2014). South Africa has a high incidence of edentulism in Mixed ancestry groups (51.6%); followed by European (16.2%) and African ancestry groups (6.3%) (van Wyk & van Wyk, 2004). A study by Friedling and Morris (2007) showed that individuals who identified themselves "Coloured" (Mixed ancestry) were more likely to extract teeth due to peer pressure and fashion rather than medical or accidental reasons. Furthermore, dental modifications were detected most frequently in young MA males (15-19 years old) who chose to extract their maxillary incisors (Friedling & Morris, 2007). Although tooth extraction may be considered a cultural practice in this group, tooth extraction is more common in lower income groups, and therefore, socio-economic status is a critical aspect in understanding the frequency of this dental modification (Friedling & Morris, 2007). As such, further research is required to investigate the extent to which this cultural and socio-economic practice may influence ancestral variations.

In addition to aging and tooth loss, midfacial trauma is also associated with a remodelling of the mid-craniofacial region. While studies have focussed on the mechanics of craniofacial trauma (Kieser *et al*., 2009; Chelotti, 2013) and the causes of facial trauma in past (Wu *et al*., 2011; Chelotti, 2013; Cohen

et al., 2014) and present populations (Lee, 2009; Chrcanovic, 2012; Geldenhuys *et al*., 2016), none have addressed the impact of craniofacial trauma on ancestry estimation. Steadman and Konigsberg (2009) refer to a forensic anthropological case in which the ancestral variation was ambiguous in a North American male of Caucasian or European ancestry, due to severe antemortem, zygomatic and nasal trauma and tooth loss (Steadman and Konigsberg, 2009). This ambiguous aspect of the demographic profile made this individual difficult to identify and may have been avoidable if researchers had information about how confounding factors, like tooth loss and midfacial trauma, influenced ancestral variations. The high rate of tooth-loss in South Africa and its unique cultural and socioeconomic patterning, emphasise the need to test standards in individuals with craniofacial trauma and tooth-loss, as these factors may require the use of different standards to achieve accurate ancestry estimations.

2.4. Ancestry estimation methods

2.4.1. Nonmetric methods

From as early as the 17th and 18th century, nonmetric cranial traits have been used to assess variation between different population groups (Morton, 1839; Hefner, 2003; De Villiers, 1968). Nonmetric ancestry estimation involves the visual evaluation of skeletal traits scored on an ordinal scale relative to shape or size (Rightmire, 1972); for example, nasal aperture width can be scored as either narrow, medium or broad (Hefner, 2009). Shape and size categories are associated with specific ancestry groups, allowing for an ancestry estimation to be made (Hefner, 2009). Ancestry estimation standards initially existed as percentage frequencies of trait occurrences in different populations (Hooton, 1946; Hefner and Ousley, 2006), however more recently, forensic anthropologists have used multivariate statistics to assess ancestry (L'Abbé *et al*., 2011; Mc Dowell *et al*., 2012; Hefner & Ousley, 2014). The longevity of nonmetric assessments amongst biological anthropologists is based on their ease of use, rapid results, and applicability to fragmentary, incomplete and poorly preserved remains (Corruccini, 1974). Despite this, concerns have been raised regarding the subjectivity, repeatability and scientific validity of nonmetric methods (Wheat, 2009; Van Rooyen, 2010; Vitek, 2012) and whether they are consistent with the *Daubert* criteria for court admissible evidence (Dirkmaat *et al*., 2008).

Hefner (2009) sought to improve the repeatability and subjectivity of this method by including illustrations of standard trait variations associated with African, Asian and European ancestry groups. Yet, Hefner's illustrations (2009) yielded poor repeatability and low ancestry estimation accuracies in South Africans of African, European and Mixed ancestry (L'Abbé *et al*., 2011). Notably, South Africans

of European ancestry primarily fell into European categories for traits such as nasal bone contour, anterior nasal spine and inferior nasal margin; while South Africans of African and Mixed ancestry fell into African and Asian categories (L'Abbé *et al*., 2011, Van Rooyen, 2010). Findings reported by L'Abbé *et al*. (2011) suggested that nonmetric methods were particularly useful in estimating ancestry between African and European ancestry groups whereas Mixed ancestry individuals classified more frequency as African than European. These standards do not accurately estimate the ancestry for SAs of African, Mixed, or European ancestry, and questions have emerged whether nonmetric traits developed in North American populations are applicable to the heterogeneous South African population (L'Abbé *et al*., 2011; McDowell *et al*., 2015). Further studies are necessary to characterise shape variations amongst South African populations to ensure reliable ancestry estimations are made.

2.4.2. Metric methods

Metric ancestry estimation involves the analysis of morphological variation through the measurement of distances between formally defined landmarks. Although these landmarks are well-defined and constant across all populations (Buikstra & Ubelaker, 1994), distances between landmarks are highly variable, allowing for estimation of ancestry (Pietrusewsky, 2000). Postcranial and cranial ancestral variations have been studied metrically in North American (Spradley *et al*., 2008; Ta'ala *et al*., 2014; L'Abbé *et al*., 2013), Malawian (Msamati *et al*., 2005; Igbigbi and Ebite, 2010), Ugandan (Igbigbi & Nanono-Igbigbi, 2003), Brazilian (Urbanová *et al*., 2014), South Indian (Kanchan *et al*., 2014) and South African populations (L'Abbé *et al*., 2013; Stull *et al*., 2014; Liebenberg *et al*., 2015a; McDowell *et al*., 2015). Mid-craniofacial variation in orbital aperture (De Villiers, 1968), nasal aperture (Mc Dowell *et al*., 2012) and prognathism (L'Abbé *et al*., 2013) have been assessed metrically in SAs of African and European ancestry. Currently, no studies have assessed metric variation in the entire mid-craniofacial region in SAs of African, European and Mixed ancestral descent. It is imperative that such a study be undertaken to understand the extent of size variation between ancestry groups as this could be fundamental in ensuring reliable metric ancestry estimations.

The conservative nature of continuous variation combined with the empirical nature of measurements makes metric data suitable for both univariate and multivariate analyses (Pietrusewsky, 2000). Traditionally, cranial indices have been used to analyse metric variation between morphological features (Mosimann, 1970), however, this univariate method fails to consider the multiple variables which are known to contribute to human variation (Giles & Elliot, 1962; Liebenberg *et al*., 2015b). Discriminant functions were developed on North American populations, considering multiple

variables when assessing ancestry (Giles & Elliot, 1962). When these functions were tested in SA samples, low accuracies were obtained, indicative of the need for population-specific discriminant functions (İşcan & Steyn, 1999). Discriminant functions developed for SAs of African and European descent, increased ancestry estimation accuracies to between 86% and 92%, in males and females, respectively (İşcan & Steyn, 1999). However, this sample should be expanded and should also include SAs of Mixed Ancestry. While computational software, like FORDISC 3.0® and Cranid®, have been developed to estimate ancestry, these databases do not currently include modern SAs of African, European and Mixed ancestry. Furthermore, FORDISC 3.0® poorly assigns ancestry to SAs of European and African descent (L'Abbé *et al*., 2013) and this database needs to be expanded to include contemporary SAs of European, African and Mixed ancestry, as such an expansion could significantly impact victim identification in South African forensic anthropological cases.

2.4.3. Geometric morphometric methods

While metric and nonmetric methods fail to disregard size variation and quantify shape in three-dimensions (3D), geometric morphometrics allows for the analysis of biological shape variation and its co-variation in two-and three-dimensions (Adams *et al*., 2004). Information regarding the relative positions of landmark co-ordinates on bones can be recorded and visualised using multivariate analyses of landmark configurations (Adams *et al*., 2004). The most prominent morphometric method, known as Generalised Procrustes Superimposition (GPS), is based on the least-squares estimation of translation, rotation and scaling parameters which optimally align sets of landmark coordinates for pairs of specimens (Pimental, 1992; Slice, 2007). As depicted in Figure 2.2, 2D or 3D landmark co-ordinates are centred and scaled to the same mean centroid size, and co-ordinates are then rotated around the mean centroid to minimise the differences between all co-ordinates. The resulting landmarks, referred to as Procrustes co-ordinates, can be used to assess the distances (Procrustes distances) between landmarks co-ordinates. Once scaled into a common co-ordinate system, landmark co-ordinates can be statistically analysed using ordination methods (like Principal Component Analyses), or multivariate statistical analyses (like Discriminant Function Analyses or Canonical Variate Analyses) to detect and quantify group differences (Slice, 2007). Geometric morphometrics has been expanded to analyse the effect of size on shape (allometry), different types of structural asymmetries (static or ontogenic), and modularity/integration of structural units (Klingenberg and McIntyre, 1998; Klingenberg *et al*., 2003; Klingenberg, 2016). Therefore, this method allows landmark configurations (shape differences) to be compared and quantified between different structures (Slice, 2007; Ross *et al*., 2010).

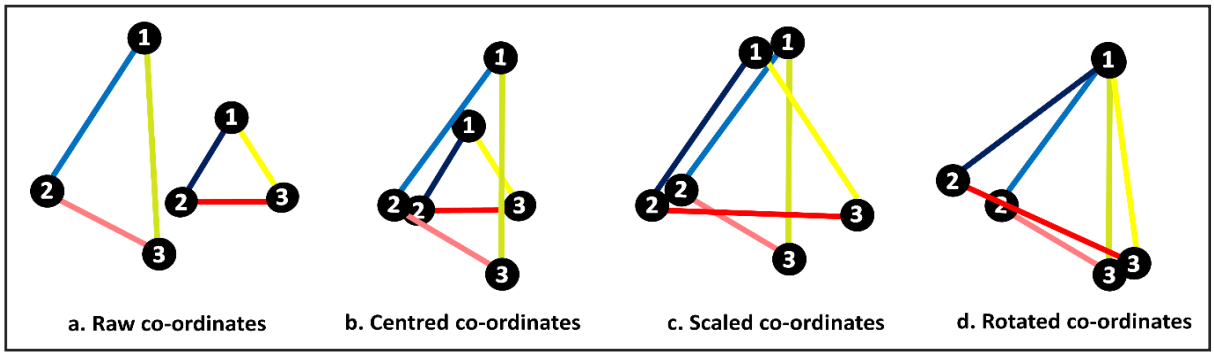

Figure 2.2. Generalised Procrustes Superimposition steps used to transform landmarks into Procrustes shape co-ordinates. a. Landmarks co-ordinates are captured in 2D or 3D. b. Landmark configurations are centred around a mean centroid. c. Co-ordinates are then scaled to the same mean centroid size. d. Co-ordinates are then rotated to minimise the differences between all co-ordinates.

Geometric morphometrics requires costly equipment and is timeous, therefore it is not commonly used in forensic cases (Ross *et al*., 2010). Another disadvantage of this method is the susceptibility of superimposed landmarks to the "Pinocchio Effect" (Webster & Sheets, 2010; Klingenberg, 2016). The Pinocchio effect occurs when large variance differences at one or two landmarks are distributed over many landmarks by least-squares rotation, thus providing spurious representations of landmark variations (Webster & Sheets, 2010). Despite this concern, Procrustes superimposition (GPS) is considered one of the most statistically robust analytical frameworks for three-dimensional co-ordinate data (Adams *et al*., 2004; Rohlf & Slice, 1990). Geometric morphometric analyses are advantageous for determining whether shape and size variations (assessed nonmetrically and metrically) are morphologically realistic and are relatively objective (Adams *et al*., 2004). Additionally, they are useful for characterising osteological variation, especially in relatively heterogeneous ancestral populations, as in South Africa.

Geometric morphometrics has been used to analyse shape variation in North Americans (Kimmerle *et al*., 2008, Ross *et al*., 2010), Northern Indians (Saini *et al*., 2011), South Americans (Perez *et al*., 2007, Perez and Monteiro, 2009) and South Africans (Franklin *et al*., 2005a; Xing *et al*., 2013; Mc Dowell *et al*., 2012). Shape variation in the orbital aperture has been seen between South Africans of African and European descent in the internal and lateral aspect of the lower orbital margin (Masters, 2008; Xing *et al*., 2013). A South African geometric morphometric study on the nasal aperture found that individuals of Mixed ancestry were more morphologically similar to those of African ancestry (McDowell *et al*., 2015) whereas a study on the cranium and facial region found South Africans of Mixed ancestry were more similar to those of European ancestry (Stull *et al*., 2014). Both studies used

different landmarks and analysed different regions of the cranium. Additionally, studies have assessed variation in the cranial vault (Stull *et al*., 2014) and nasal aperture (Mc Dowell *et al*., 2012; McDowell *et al*., 2015) but currently, no study has assessed relative shape and size variation using geometric morphometrics in the entire mid-craniofacial region of South Africans of African, European and Mixed ancestry.

Metric, nonmetric and geometric morphometric methods will be used to evaluate variations in shape and size in South Africans of African, European and Mixed ancestry. It is imperative that such a study be undertaken as this data could be used to determine whether standards used to estimate ancestry are reliable within a South African population, and furthermore, these findings could be fundamental in informing forensic anthropological protocol.

Chapter Three: Materials and Methods

3.1. Materials

3.1.1. Sample selection

Crania were sampled from the Raymond A. Dart Collection of Human Skeletons (University of Witwatersrand), the Kirsten Collection (Stellenbosch University) and the University of Cape Town Human Skeletal Collection (University of Cape Town). Acquisition of cadaveric remains by these collections, is legislatively permitted and regulated by the National Health Act (Act No. 61 of 2003) (and prior to that, the Human Tissue Act (Act No. 65 of 1983)) concurrently, ethical approval from the University of Cape Town was not required for this study.

Skeletal collections are commonly used as samples for forensic anthropological research; however, public perception of body donation, along with cultural and religious beliefs regarding death, burial and the afterlife, have introduced demographic biases into skeletal collections worldwide (L'Abbé *et al*., 2005; Komar and Grivas, 2008). For instance, Muslim communities have expressed objection towards body and organ donation, citing concerns about how this may influence one's afterlife (Hayward and Madill, 2003). Therefore, these individuals are less likely to be represented in skeletal collections. Similarly, individuals from African communities are not inclined to participate in body donation due to "ancestor reverence" and the belief that the body and soul should be preserved as a unit after death (L'Abbé *et al*., 2005). Da Silva (2006) found that most individuals of African and Mixed ancestry donated to the University of Cape Town were unclaimed at the time of death. This contrasted with individuals of European ancestry, whose bodies were largely bequeathed by themselves or their families. Komar and Grivas (2008), cite a study by Wilson *et al*., (2007) which reported that individuals who donated their bodies before death were generally older at death and came from better socioeconomic circumstances than those whose bodies were donated by family members or a medicolegal authority after their death. Despite the scientific value of skeletal collections, they share a major limitation: cadaveric collections are often not accurate analogues for characterising variation in living populations. This is because institutional acquisition practices have introduced biases associated with sex, age at death, year of birth, ancestry and socio-economic status into each collection (Wood *et al*., 1992; Komar & Buikstra, 2008). Although using a forensic sample was considered, this sample may have provided an unbalanced representation of population variation, introducing biases associated with demographics, age and socio-economic status. To reduce these biases, crania were sampled from different skeletal collections (representing different regions in South Africa) and as far as possible, covered a wide range of ages within each ancestry group.

3.1.2. Sample acquisition

3.1.2.1. The Raymond A. Dart Collection of Human Skeletons

The Raymond A. Dart Collection of Human Skeletons, commonly referred to as the Dart collection, is housed at the School of Anatomical Sciences at the University of Witwatersrand, Johannesburg. The Dart Collection is the largest and oldest cadaveric skeletal assemblage in South Africa, housing approximately 2605 cadaveric skeletons (Dayal *et al*., 2009). This collection was established in the 1920s and comprises various indigenous and immigrant populations from Southern Africa, Europe and Asia (Dayal *et al*., 2009). Males represent 71% of the cadaveric skeletal collection (Dayal *et al*., 2009). This collection is skewed in its representation of South Africans of African (76%), European (15%), Mixed (4%) and Indian (0.3%) ancestry (Dayal *et al*., 2009). Recorded ages at death range from the first year to over 100 years of age; however, most individuals died between the ages of 20 and 70 (Dayal *et al*., 2009). Collection procedures based on availability of remains, have affected the demographic composition of the Dart collection (Dayal *et al*., 2009). Remains accessioned before 1958, and large proportions subsequently, were derived from unclaimed bodies from state hospitals in South African (Tal & Tau, 1983; Dayal *et al*., 2009).

3.1.2.2. The University of Cape Town Human Skeleton Collection

The University of Cape Town (UCT) Human Skeletal Collection is housed in the Department of Human Biology at UCT. This collection was established in 1913, but the first cadaveric skeleton was accessioned in the 1940s (Gibbon, 2017. *Personal Communication*, 6 September). Acquisition of cadaveric remains occurs primarily through donations by donors themselves or their families (Gibbon, 2017. *pers. comm*., 6 September). The assemblage consists of 351 cadaveric skeletons, of which males (63%) represent a large proportion (Gibbon, 2017. *pers. comm*., 6 September). Low diversity is evident within the female assemblage, with 75% of females representing those of European ancestry (Gibbon, 2017. *pers. comm*., 6 September). This collection is skewed towards an older majority, as 87% of individuals were over the age of 50 years at the time of death (Gibbon, 2017. *pers. comm*., 6 September). Most cadaveric remains in this collection were acquired from state hospitals and retirement centres within the Western Cape Province (Da Silva, 2006). The UCT collection is skewed in its representation of European (66%), Mixed (23%) and African (11%) ancestry groups (Gibbon, 2017. *pers. comm*., 6 September); this is different from other South African skeletal collections.

3.1.2.3. The Kirsten Skeletal Collection

The Kirsten Skeletal Collection is housed in the Department of Anatomy and Histology at the University of Stellenbosch, Cape Town. This collection was established in 1957 with the acquisition of embalmed cadavers from the Anatomy Departments at the University of Witwatersrand and the University of Pretoria. Upon the completion of dissections, the embalmed remains were macerated and accessioned into the Kirsten Collection (Alblas *et al*., 2018). It was only in 1960 that the Kirsten Collection received its first donor from the Western Cape (Alblas *et al*., 2018). While the Kirsten Collection may be the youngest research collection in South Africa, over 1016 individuals are accessioned at this collection (Alblas *et al*., 2018). Approximately 30-40 skeletons are accessioned annually, most of which are from unclaimed individuals from hospitals in the Cape Town metropolitan (specifically the northern suburbs) and surrounding rural towns in the Western Province (Alblas *et al*., 2018). Although many unclaimed remains are from hospitals which service low socio-economic areas (Alblas *et al*., 2018), one cannot definitively make this statement regarding all unclaimed individuals in this collection. The Kirsten Collection is skewed in its representation Mixed (60%); African (17%) and European (12%) ancestries (Alblas *et al*., 2018). Males comprise 62% of the collection with most males being of Mixed ancestry (Alblas *et al*., 2018). Many individuals in this collection were between the ages of 40-60 at the time of death and 54% of the collection died between 1970 and 1981 (Alblas *et al*., 2018). Unlike the Dart and UCT Skeletal Collections', this collection represents a mid-late 20th century sample of individuals.

3.1.3. Standardising terminology

Demographic information for each individual was obtained from accession registers at skeletal collections. This information was originally obtained from the Medical Certificate of Cause of Death (*Births and Deaths Registration Act*; Act no. 51 of 1992) for each donor. Information pertaining to ancestry was largely self-declared by donors and was recorded differently in accession registers at different skeletal collections, under headings of "race", "ancestry" or "ethnicity". While the Kirsten Collection used racial affiliations (e.g. Black, White and Coloured); the University of Cape Town Skeletal Collection used ancestral affiliations (e.g. European and African). The Dart Collection largely used tribal and ethnic affiliations (e.g. Zulu, Pedi and Xhosa) to refer to those who legislatively identified as "Black". However, it has been reported that in some cases when tribal identifications were not reported on the death certificate, this information was inferred from surname or contextual information (Tal & Tau, 1983). Although different terminologies were used in each collection, the ancestral populations from which remains were derived are homogenous. Due to subjectivity and political connotations associated with race and tribal categories, for the purposes of this study,

ancestral categories were applied to the data. Individuals identified in accession registers as "Black" or based on tribal affiliations will be identified as of "African ancestry", while "White" individuals will be identified as "European ancestry" and individuals identified as "Coloured" will be referred to as "Mixed ancestry".

3.1.4. Sample summary

This study collected data from 402 crania accessed from skeletal collections within South Africa. Only crania from adult South Africans for whom sex, age and ancestry were recorded in accession registers at each skeletal collection, were selected. Sex and ancestry distribution of the sample prior to analysis is given in Table 3.1.

Exclusion criteria

- Crania with no mandibles present, which may have resulted in unreliable nonmetric assessments of prognathism.
- Crania with severe macroscopic pathologies or antemortem and/or post-mortem trauma, which may have resulted in disturbance or absence of landmarks of interest.
- Individuals younger than 18 years at death, to minimise the effect craniofacial growth and development on craniofacial morphology (Mc Dowell *et al*., 2012).
- Individuals older than 75 years at death. Craniofacial changes occur in individuals older than 60 years (Albert *et al*., 2007), however, a limit of 75 years was selected to assess the effect of age on ancestral variation.

Table 3.1. Summary of study sample, according to sex and ancestry.

	Ancestry			
Sex	African	Mixed	European	**Total**
Male	54	126	50	230
Female	58	70	44	172
Total	112	196	94	**402**

3.2. Data collection

Metric, nonmetric and geometric morphometric methods were selected to analyse size and shape variation of the entire craniofacial region and each of its components (orbital, nasal, zygomatic and maxillary regions). To lessen observer bias, demographic information for each cranium was unknown until statistical analyses were performed. Data pertaining to the occurrence of antemortem nasal and zygomatic trauma were collected and analysed. Reporting the extent of these variations was beyond the scope of this study but where-relevant to mid-craniofacial variation, results from trauma analyses will be reported and discussed.

3.2.1. Observer agreement

To test intra-observer agreement, 30 crania were randomly selected, and data were re-collected using the same metric, nonmetric and geometric morphometric methods. This was performed 3 months subsequent to the completion of data collection. For interobserver agreement testing, a Masters student from the University of Cape Town, used the same methods to collect data from 30 randomly selected crania. Demographic information pertaining to each cranium was concealed from both observers during data collection.

3.2.2. Metric data

Twelve cranial measurements (Table 3.2. and Figure 3.1) were collected in millimetres, using digital and spreading callipers (for basion-nasion length). Measurements were repeated three times for each cranium and an average value was calculated. Where possible, standardised landmark and measurement definitions reported by Howells (1973) were used; otherwise definitions were taken from Buikstra and Ubelaker (1994). Measurements included: orbital breadth (OBB), orbital height (OBH); interorbital breadth (DKB), bi-orbital breadth (EKB), upper facial height (NPH), nasal height (NH), nasal breadth (NLB), bizygomatic breadth (ZYB), bimaxillary breadth (ZMB), basion-nasion length (BNL) and basion-prosthion length (BPL). Where applicable, measurements were taken from both sides to determine if there were significant size differences between left and right sides. If so, sides were analysed separately and if not, measurements for both sides were averaged for further analysis.

3.2.3. Nonmetric data

Eleven nonmetric traits (Hefner, 2003; Van Rooyen, 2010; L'Abbé *et al*., 2011) were selected from the mid-craniofacial region. These included: nasal bone contour (NBC), nasal aperture width (NAW), anterior nasal spine (ANS), inferior nasal margin (INM), zygomaxillary suture shape (ZS), supranasal suture (SS), interorbital breadth (IOB), orbital shape (OS), malar tubercle (MT), zygomatic projection (ZP) and alveolar prognathism (AP). Trait definitions, variations and drawings were compiled into reference sheets (Appendix A). To reduce the influence of observer bias on subsequent observations; ancestral associations for each trait variant were excluded from illustrations and descriptions used in data collection. It was acknowledged that the ordinal nature of the nonmetric traits may have resulted in an observer predicting ancestry based on order of variants, therefore an independent observer randomly assigned alphabetical characters to each variant. This method has been shown to reduce observer bias associated with successive assessments of nonmetric traits (Wheat, 2009).

3.2.5. Geometric morphometric data

3.2.5.1. Landmark selection

As recommended by Webster and Sheets (2010), landmarks representing discrete anatomical loci, which were easily identified, present on all crania and accurately reflected regional morphologies were selected for this study. Thirty-six landmarks were selected to provide morphological information about the functional units of the mid-craniofacial region. These units included the orbital region of the frontal bone, zygomatic bones, nasal bones and maxillae. Positions and definitions of landmarks are given in Table 3.3 and Figure 3.2 and correspond to those used in metric assessments.

Landmarks can be classified into three categories: Type I, Type II and Type III. Type I landmarks include points where juxtaposed structures meet (e.g. sutures) (Bookstein, 1991), while, Type II landmarks are found at points of maximum geometric curvature (e.g. sharpest point on a canine) (Bookstein, 1991) and Type III landmarks (inclusive of semi-landmarks) include finite points which can be defined in relation to another structure (e.g. most lateral point on a structure). Barbeito-Andrés *et al*. (2012) found that Type III landmarks resulted in poor repeatability and therefore, landmarks in this study were mainly Type I and Type II.

3.2.5.2. Landmark digitisation

Crania were stabilised on a wooden block using modelling clay and selected landmarks were then marked on the bone. This allowed all landmarks to be marked and digitised without moving the skull. Landmark markings were erased before inter/intra-observer digitisation. Three-dimensional Cartesian co-ordinates of the selected landmarks were collected using a Microscribe G2® 3D digitizer with a cited accuracy of 0.23mm (Immersion Corp, San Jose, California, 2002). The configuration of landmarks was digitised three times and digitisation error was evaluated by calculating the mean Euclidean distances between consecutive repeats. If detected error was greater than 1.0 mm, the configuration of landmarks was re-digitised until distances between successive digitisations were less than 1.0mm; as suggested by Terhune *et al*. (2007), von Cramon-Taubadel (2009) and Smith *et al*. (2013).

Table 3.2. Descriptions of mid-craniofacial measurements used in this study. Measurements were taken using sliding callipers unless otherwise stated. (Numbers correspond to illustrations in Figure 3.1.).

	Measurements	**Abbreviation**	**Description**
1	Orbital breadth (L and R) [1]	OBB	The distance from ectoconchion to dacryon, approximating the longitudinal axes of the orbit.
2	Orbital height (L and R) [1]	OBH	The distance between the upper and lower borders of the orbit. Perpendicular to the long axis which dissects the orbit.
3	Interorbital breadth[2]	DKB	The distance from dacryon to dacryon across the nasal space
4	Bi-orbital breadth[1]	EKB	The distance from the left ectoconchion to the right ectoconchion.
5	Nasion-prosthion height[1]	NPH	The distance from nasion to prosthion.
6	Nasal height (L and R)[2]	NH	The average height from nasion to nariale on either side of the nasal aperture
7	Nasal breadth[1]	NLB	The distance between the anterior edges of the widest points on the nasal aperture.
8	Bizygomatic breadth[2]	ZYB	The maximum distance from one zygion to zygion along the same coronal plane.
9	Bimaxillary breadth[1]	ZMB	The distance across the maxilla from one anterior zygomaxillare to another.
10	Maxillo-alveolar breadth[2]	MAB	The maximum breadth across the alveolar border of the maxilla, measured from ectomolare to ectomolare.
11	Basion-nasion length[1]	BNL	The distance from basion to nasion (measured using spreading callipers).
12	Basion-prosthion length[1]	BPH	The distance from basion to prosthion (measured using spreading callipers).

** Descriptions of landmarks and measurements: Howells (1973)[1]; Buikstra and Ubelaker (1994)[2]; (L- Left; R-Right).*

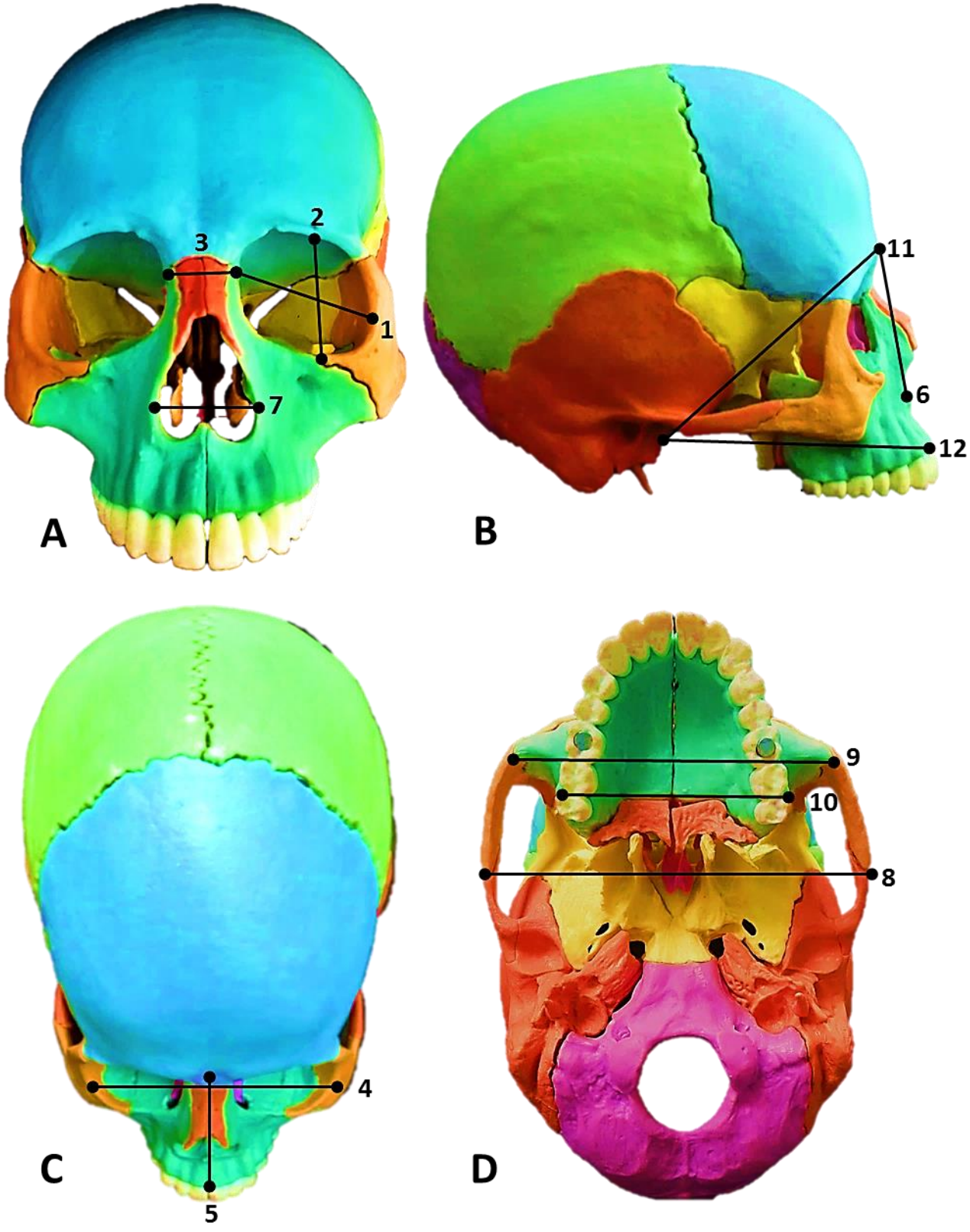

Figure 3.1. Anterior, lateral, superior and inferior views of the cranium showing measurements used in this study.

Table 3.3. Descriptions of mid-craniofacial landmarks used in this study (Numbers correspond to landmarks on Figure 3.2.).

Number	Landmark	Type	Description
1	*Glabella*[2]	II	Most anterior midline point of the supraorbital ridge on the frontal bone (usually above the frontonasal suture on the median sagittal plane.
2	*Nasion*[1]	I	Point of intersection between the frontonasal suture and internasal suture, aligned with the midsagittal plane.
3 and 21	*Infranasion*[1]	I	Point of intersection between the nasofrontal, nasomaxillary and maxillofrontal sutures.
4 and 22	*Dacryon*[1]	I	Point of intersection of sutures from frontal, maxillary and lacrimal bones the frontolacrimal and lacrimomaxillary suture.
5 and 23	*Superior orbitale*[1]	III	Most anterior, superior midpoint of the orbital margin.
6 and 24	*Frontomalare orbitale*[1]	I	Point where the zygomaticofrontal suture crosses the orbital margin.
7 and 25	*Ectoconchion*[1]	II	Intersection of the most anterior surface of the lateral border of the orbit and a line bisecting the orbit along its long axis.
8 and 26	*Zygo-orbitale*[2]	I	Point of intersection between the zygomaxillary suture and the inferior orbital margin.
9 and 27	*Frontomalare temporalis*[1]	II	The point of intersection between the frontozygomatic, zygomaticoshpeniod and sphenofrontal sutures.
10 and 28	*Jugale*[2]	II	The midpoint in the notch between the temporal and frontal process of the zygomatic bone.
11 and 29	*Zygotemporale superior*[1]	II	Most superior point on the zygomaticotemporal suture.
12 and 30	*Zygotemporale inferior*[1]	II	Most inferior point on the zygomaticotemporal suture.
13 and 31	*Zygion*[2]	III	Point on zygomatic arch furthest from corresponding point on opposite side on the same coronal plane.
14 and 32	*Zygomaxillare*[1]	II	Most inferior, anterior point on the zygomaxillary suture.
15 and 33	*Ectomolare*[1]	II	The most lateral point on outer surface of alveolar border of maxilla (often found at 2nd molar).
16	*Prosthion*[1]	II	Most prominent anterior point on the maxillary alveolar process above the septum between the central incisors.
17	*Subspinale*[1]	II	The point where the inferior edge of the nasal spine becomes the anterior edge of the maxilla.
18 and 34	*Nariale*[1]	II	The lowest point on inferior margin of nasal aperture, lateral to the nasal spine.
19 and 35	*Alare*[1]	II	Most lateral point on nasal aperture, taken perpendicular to the nasal height.
20 and 36	*Nasomaxillare*[1]	II	Most inferior point on the nasomaxillary suture.

** Descriptions of landmarks: Howells (1973)[1]; Buikstra and Ubelaker (1994)[2].*

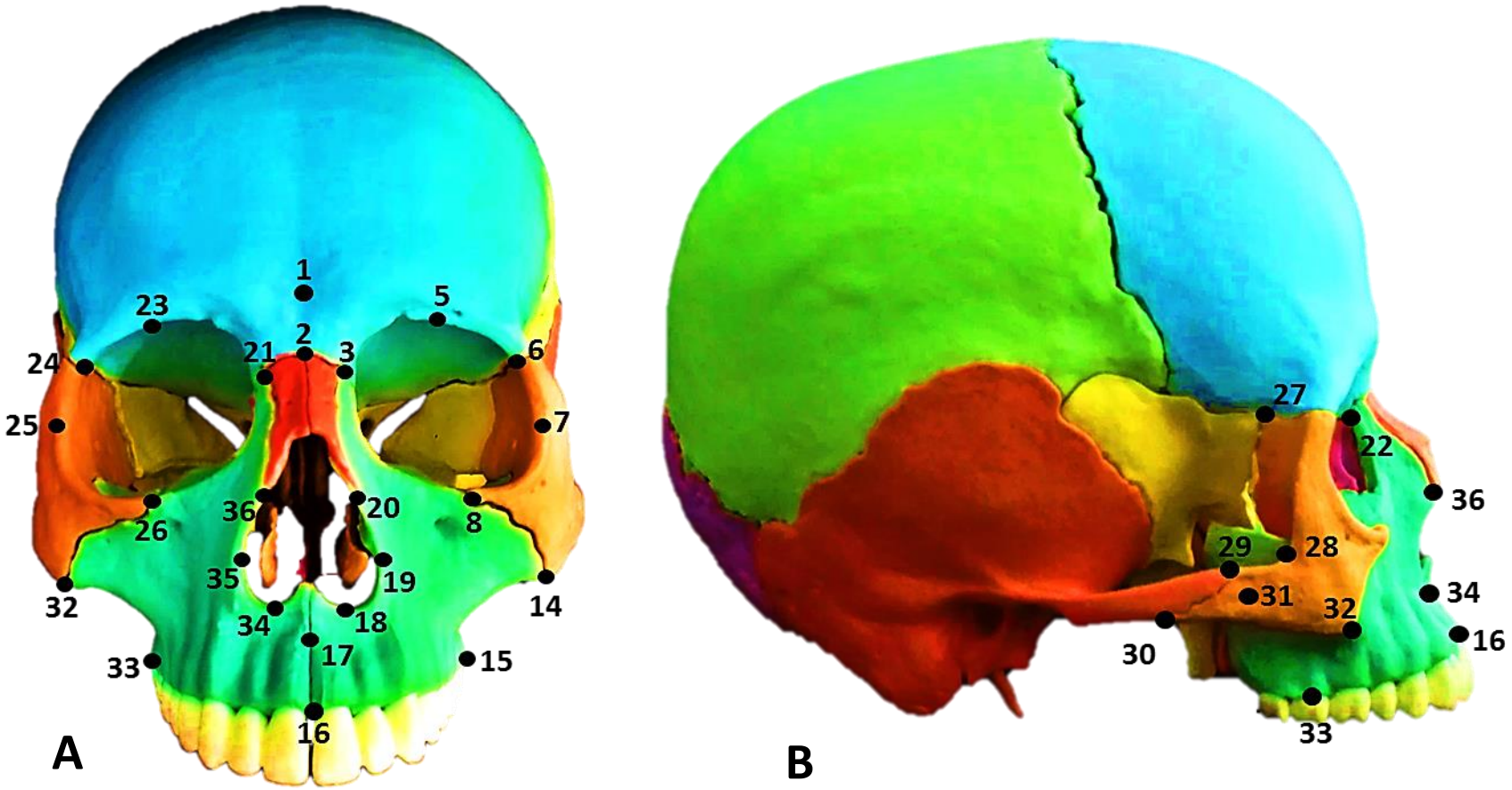

Figure 3.2. Anterior and lateral view of the cranium depicting craniofacial landmarks digitised in this study. Points 4, 9,10,11,12 and 13 are not depicted in the figure.

[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

3.2.6. Antemortem maxillary tooth loss

The number of maxillary teeth lost antemortem, lost post-mortem or antemortem were recorded for each cranium.

3.2.7. Antemortem mid-craniofacial trauma

Only individuals with partially or completely healed fractures on the nasal and zygomatic bone were included in this study. Nasal and zygomatic bone fractures were recorded as present or absent.

3.3. Statistical analyses

Unless otherwise stated, analyses were computed using IMB SPSS 24.0.0.0® software and a p-value ≤ 0.05 was deemed significant for all analyses.

3.3.1. Observer agreement analysis

3.3.1.1. Metric data

To compare the differences and means between independently repeated measurements, Bland-Altman plots (Altman and Bland, 1983; Bland and Altman, 1986) were generated using GraphPad Prism 5®. Average bias was calculated by averaging the values calculated from subtracting the measurements of one observation from the measurements of another observation. If the average bias was close to zero, both methods produced similar results. Conversely if the value was further from zero, the error between the repeats was higher (Mantha *et al*., 2000; Chhapola *et al*., 2015). If more than 75% of repeated measurements fell within the calculated 95% limit of agreement, the results were considered repeatable (Mantha *et al*., 2000; Chhapola *et al*., 2015).

3.3.1.2. Nonmetric data

Cohen's Kappa statistic was used to assess inter-and intra-observer agreement (Cohen, 1960). Although controversy has surrounded the use and interpretation of Kappa statistics, studies largely favour descriptive standards developed by Landis and Koch in 1977 (Table 3.4) (Banerjee *et al*., 1999; Van Rooyen, 2010; Hefner *et al*., 2012; McHugh, 2012).

Table 3.4. Standards for evaluating Kappa statistics (Landis and Koch, 1977).

Scale	Strength of agreement
$K < 0.00$	Less than chance/poor
$0.00 < K < 0.20$	Slight
$0.21 < K < 0.40$	Fair
$0.41 < K < 0.60$	Moderate
$0.61 < K < 0.80$	Substantial
$0.81 < K < 1.00$	Almost precise

3.3.1.3. Geometric morphometric data

A Procrustes ANOVA using MorphoJ® software was used to quantify measurement error (Klingenberg, 2011). This method is equivalent to using the two-factor ANOVA model developed by Palmer and Strobeck (1986) and is commonly used in analysing measurement error (Klingenberg and McIntyre, 1998; Klingenberg *et al*., 2003; Freidline *et al*., 2015; Leamy *et al*., 2015). The Procrustes ANOVA quantifies the among individual variation; directional asymmetry, fluctuating asymmetry (accounted for by calculating 'side*specimen interaction') and error between repeats (Fruciano, 2016). This approach allows for the quantification of measurement error in mean squares for different terms of the ANOVA and therefore one can determine the relative contribution of each component to the total variation in the sample (Fruciano, 2016). Generally, measurement error relative *to* the degree of specimen*side (Ind*side interaction) variation or fluctuating asymmetry in the dataset is assessed because fluctuating asymmetry represents a very small component of phenotypic variation and is influenced by measurement error (Klingenberg *et al*., 2002). Currently, there are no recognised standards for defining *acceptable* error in geometric morphometrics (Sholts *et al*., 2011). The percentage contribution of each factor to the total variation was calculated and if the variation accounted for by "error between repeats" comprised more than 5% of the variation in the sample, the observer agreement was deemed unacceptable (Muñoz-Muñoz & Perpiñán, 2010; Al Shahrani, 2012).

3.3.2. Demographic analyses

3.3.2.1. Preliminary analyses

Descriptive statistics were computed for demographic variables and a Shapiro-Wilk test for normality was used to test distribution of continuous variables (Shapiro & Wilk, 1965). Appropriate results were displayed, and relevant tests were performed depending on whether the variables in question were parametric or nonparametric. For parametric data, the Grubbs' Test (Grubbs, 1950; Grubbs, 1969) was used to detect possible outliers, while boxplots were used to evaluate for nonparametric data for outliers.

3.3.2.1. Sample demographics

Associations between and within covariates (age at death, date of birth and teeth present) were investigated. Possible sampling bias was also investigated.

Categorical covariates

Associations between the categorical variables and nonmetric traits were investigated using Chi-squared and Fisher's Exact tests. The degree of association between variables and covariates was evaluated using Cramér's V or Phi coefficients (ϕ_c) and effect sizes were interpreted using standards from Cohen (1988) (Table 3.5.)

Table 3.5. Effect size index for Cramér's V and Phi coefficient (ϕ_c) (Cohen, 1988).

Effect size	Strength of association
0.10-0.29	Weak
0.30-0.49	Intermediate
0.50-0.99	Strong

Continuous covariates

Univariate ANOVA tests were used to investigate the association between continuous covariates (age at death, year of birth and tooth loss) and sex, ancestry and sex-ancestry groups. To determine between which groups differences occurred, a Fisher's Least Significance Difference test (LSD) was used for post-hoc testing. Nonparametric data, were assessed for differences using a Kruskal Wallis ANOVA (Kruskal and Wallis, 1952). If differences were detected Dunn's post-hoc tests (Zar, 1999) were computed. Either Pearson or Spearman rank ordered correlations were used to test the correlation between continuous covariates and craniofacial measurements.

3.3.3. Metric data analyses

Descriptive statistics were computed for all measurements in ancestry and sex-ancestry groups and Shapiro-Wilk tests for normality were used to evaluate distribution of metric variables (Shapiro and Wilk, 1965). The Grubbs' Test (Grubbs, 1950; Grubbs, 1969) was used to detect possible outliers in parametric data.

Paired t-tests were computed to determine whether there were significant differences between left and right measurements of orbital breadth, orbital height and nasal height. No significant size differences between left and right were detected for measurements of orbital breadth ($t\ (391)$ =-0.79; p=0.43) and therefore these measurements were averaged for further analyses.

3.3.3.1. Size differences between ancestry and sex-ancestry groups

Univariate ANOVA tests were used to evaluate measurement size differences between ancestry and sex-ancestry groups. To determine between which groups differences occurred (comparing fewer than four factors), a Fisher's Least Significance Difference test (LSD) was used for post-hoc testing. Pearson correlations were used to test correlations between continuous covariates and craniofacial measurements.

The effect of tooth loss on size

Due to the nonparametric distribution of tooth loss, data were split into 3 tooth loss groups to allow for relationship between tooth loss, age at death and craniofacial size to be assessed (Table 3.6). The associations between measurements and tooth loss categories were evaluated using Pearson correlations, and Univariate ANOVA tests were used to evaluate the effect of tooth loss on size differences between ancestry and sex-ancestry groups.

Table 3.6. Criteria for tooth loss groups

Category	Number of teeth present
A	*0-5*
B	*6-10*
C	*11-16*

The effect of age at death size

Bivariate Pearson correlations were computed between age at death and measurements for ancestry and sex-ancestry groups. When significant correlations were detected, simple linear regression analyses between age and those measurements were conducted. Furthermore, if measurements were found to be associated with age at death, and tooth loss categories, the covariation of these factors were also investigated using linear regression models.

3.3.3.2. Multivariate size analyses between ancestry and sex-ancestry groups

3.3.3.2.1. Principal Component Analysis

Principal component analyses (PCA) (Kaiser, 1960) were computed to identify possible size and shape patterns in the metric data. In comparison to the ANOVA, which tests the differences between individual variables; PCA reduces the number of variables considered while maximising inter-individual/between-group variation. To obtain the most reliable representation of variation expressed in the sample, a selection of meaningful variables was identified to provide information about horizontal, vertical and anterior-posterior size and shape of the mid-craniofacial region.

Additionally, care was taken to ensure that variables selected did not measure similar aspects of the craniofacial region (e.g. NHL and NHR). As a result, left and right orbital and nasal heights were averaged owing to small size differences detected in t-tests. The variables included were: OBB, OBH, DKB, EKB, NPH, NLH, NLB, ZYB, ZMB, MAB, BNL and BPL. Before performing this analysis, data were standardised to negate possible bias resulting from differences in the variation in magnitude of measurements (Ginter, 2008). Standardisation was performed by subtracting the mean and dividing by the standard deviation of the data set of each variable.

Firstly, eigenvalues and percentage total variance for each of the 12 possible Principal Components (PCs) (using Varimax rotation) were computed. The Kaiser or eigenvalue-one criterion (Kaiser 1960) was applied to determine how many PCs were to be retained for further analysis. This criterion stipulates that only PCs with eigenvalues greater than 1, account for more variance than can be by explained by a single variable (Rourke *et al.*, 1994). PC scores were interpreted by examining the component loadings for each standardised variable (Kaiser, 1960). The first PC usually reflects size variation (when all scores are positive); while the remaining PCs (with negative and positive scores) usually represent shape variation (Jolicoeur and Mosimann, 1960; Pimental, 1992). The loadings of each variable represent the relationship between the PC and variable, and variables that have large scores for a particular component are considered most important since they make the greatest contribution to the variance expressed by that component; conversely, variables with low PC scores are usually excluded as they do not contribute significantly to the overall variance observed (Ginter, 2008). Once the relevant PCs were selected for this study, their loadings for each variable were plotted with loadings organised into *a priori* subgroups (ancestry, sex-ancestry, nasal and zygomatic trauma presence and tooth loss groups) to facilitate investigating patterns of variation.

3.3.3.3. Ancestry estimation

3.3.3.3.1. Discriminant function analysis

A stepwise linear discriminant function analysis (DFA), also known as a canonical variate analysis (CVA), was performed using 12 metric variables (OBB, OHB, DKB, EKB, NPH, NLH, NLB, ZYM, ZMB, MAB, BNL, BPL) as predictors of membership in three ancestry groups (African, Mixed, European). The sample satisfied assumptions of linearity, normality, multicollinearity and singularity, allowing for the DFA to be performed (Tabachnick and Fidell, 2012). Standardised scores calculated for PCA were used to reduce bias associated with differing magnitudes of measurements (Ginter, 2008). Discriminant Functions (DFs) were generated for the entire sample and the sample split by sex. DFA were also performed to determine whether size differences due to tooth loss, and nasal and zygomatic trauma

were large enough to distinguish between individuals. To determine which craniofacial region/s best represented variation between ancestry groups, the following regions and measurements were used:

- Orbital region: DKB, EKB, OBH and OBB
- Nasal region: DKB, NLB, NLH, NPH and BNL
- Zygomatic regions: ZYB and ZMB
- Maxillary region: ZMB, MAB and BPL

A discriminant function was constructed by assigning a discriminant score to each individual. Sectioning points (SP) were generated using mean discriminant scores for each analysis (Barker and Barker, 1984; Rourke *et al.*, 1994). The predictive formula/function was constructed using unstandardized discriminate coefficients, while standardised (Fischer's) coefficients were used to compare the relative importance of the independent variables in the model. The discriminant function was built as follows:

$$P = a1 \times x1 + a2 \times x2 + \ldots + an \times xn + b$$

Where '$a1$' to 'an' are the discriminant coefficients, '$x1$' through 'xn' are the discriminating variables (i.e. measurements significant in the formula) and 'b' is the constant (Gapert *et al.*, 2009).

To assign the case to an ancestry group, the product P was compared to the sectioning point derived by the discriminant function for each group. Leave-one out-cross-validation (LOOCV) was used for the DFA, which involves the consecutive removal of one individual from the sample after the Discriminant Function (DF) is created, and then using all the remaining individuals to attempt to classify the removed individual (Ousley and Jantz, 2012). LOOCV provides a prediction estimate of the DF while combatting optimistic bias and overfitting of the data, thus providing a more realistic estimate (Ousley and Jantz, 2012; Krüger *et al*., 2015).

3.3.4. Nonmetric data analyses

3.3.4.1. Differences in nonmetric trait occurences between ancestry and sex-ancestry groups

Frequency distributions of nonmetric trait scores were computed for individuals of African, European and Mixed ancestry. Owing to the sensitivity of percentage trait frequencies towards sample size, it was deemed that only ancestry groups were large enough to compare results with statistical power. However, to determine whether further investigation into the effect of sex on trait variations

according to ancestry groups was required, trait variants according to sex groups were also compared. Data were submitted to Chi-squared and Fischer-exact testing to detect associations between sex, ancestry groups and trait variants.

3.3.4.2. Ancestry estimation

3.3.4.2.1. Trait frequencies

Frequency distributions of trait variances, in the entire sample were compared to determine how effectively these traits distinguished between ancestry groups (Hefner, 2003; Hooton, 1946). If 4 or more traits led to a classification in the same direction, an ancestry estimation was made. Furthermore, each nonmetric variable was tested individually to determine its suitability in estimating ancestry, within the sample.

3.3.4.2.2. Multivariate analyses

Multinomial logistic regression (MLR) was used to determine which of the nonmetric variables were collectively most accurate in estimating ancestry for the entire sample and the sample split by sex (Tabachnick and Fidell, 2012). Nonmetric traits were coded as strings in ascending numeric values with the first category corresponding to the lowest value (Van Rooyen, 2010). Backwards stepwise regression was used to determine which of the variants were the best predictors of ancestry (Vitek, 2012). This method of stepwise regression begins with all variables included in the analysis, and tests them one by one for statistical significance ($p< 0.05$), and then excludes any that are not significant. This process continues until the variables have been optimized for maximum accuracy in the final model; additionally, variables which are highly correlated with others or redundant in their low significance levels, are excluded (Tabachnick and Fidell, 2012).

While MLR overcomes many of the restrictive assumptions of ordinal least squares regression, it requires the assumption that there is only one regression equation for each category (e.g. ancestry) except the last (McCullagh, 1980). The last categories probability can be predicted as 1- the second last categories probability. The ability to make this assumption was tested using the test of parallel line assumptions (Banerjee *et al*., 1999). Wald statistics were used to as an indicator of significance of the independent variable with degrees of freedom and standard error (Van Rooyen, 2010). MLR is similar to a DFA, which is also used to predict group membership, however, a DFA can only be used with continuous variables or dichotomous categorical variables labelled using binary groups (0 or 1)

(Tabachnick and Fidell, 2012; Vitek, 2012). Therefore, in instances where the independent variables have multiple categories, such as nonmetric traits, MLR requires fewer assumptions and is more statistically robust than a DFA (Tabachnick and Fidell, 2012).

MLR predicts group membership by using the log odds ratio of the dependent variable (a transformation of the raw value of the dependent), and by performing a maximum likelihood estimation, the dependent variable is transformed into a logit variable, allowing ordinal regression to estimate the Log odds of a trait occurring (McCullagh, 1980). The equation for Log-odds is:

$$Log(odds) = A + B_1(X_1) + B_2(X)_2 + B_3(X_3) \ldots$$

Log odds represents the probability of the dependent variable (e.g. ancestry), A is the constant and B is the B-coefficient for variable X, where X represents every variable significantly contributing to the model.

3.3.5. Geometric morphometric analyses

Shape differences were interpreted using wire frames and vector diagrams. Visualisation of craniofacial shape variations at extremes of PC axes was performed by warping the 3D scanned surface of a teaching skull (Remo, 2017) using Landmark ® Software (Wiley, 2005). Warps provide a reliable representation of the extreme variations in the sample because visualised changes are strongly reminiscent of the extreme shape variations in the analysis (e.g. the contrast between the orbits, nasal height and zygomatic breadth), other aspects however, are unrealistic (e.g. cranial flexion of the vault, shape of the anterior maxilla at the incisors) due to over-extension of semi-landmarks (Kulemeyer *et al*., 2009; Drake and Klingenberg, 2010). Therefore, morphs were interpreted with caution, especially in regions where no skull landmarks were digitised as extreme extension of semi-landmarks may have resulted in spurious conclusions (Wiley *et al*., 2005).

3.3.5.1. Preliminary analyses

Raw landmark co-ordinates were entered into the MorphoJ® programme (Klingenberg, 2011). Before the main analysis could be performed, preliminary refining of the data set was required. This included mitigating the influence of asymmetry, removing outliers (based on the criterion of standard distances (Flurry, 1997)) and splitting the data into mid-craniofacial regions (orbital, nasal, zygomatic and maxillary) for subsequent analyses. Klingenberg *et al*. (2002) defines 2 types of bilateral symmetry

known as matching and object symmetry. Matching symmetry occurs when two separate structures exist as mirror images of each other on each side of the body (e.g. left and right femora) and, object symmetry occurs when symmetry exists along the midline of the same structure (e.g. the human skull). Due to the presence of bilateral object symmetry in the mid-craniofacial region, shape variation may be broken into symmetric and asymmetric components. Therefore, by explicitly accounting for symmetry using procedures outlined by Klingenberg *et al*. (2002), symmetric inter-individual variation is separated from the asymmetric intra-individual variation. This procedure compares original shapes of the specimens to their respective mirror-image copies, thereby making it possible to partition shape variation into components of symmetry and asymmetry (Klingenberg *et al*., 2002). Failure to account for the symmetric nature of the cranium could result in ill-conditioned co-variance matrices and unreliable conclusions (Weisensee and Jantz, 2011; Small, 2016). Analysing the asymmetric component of shape variation (intra-individual variation) was beyond the scope of this study and therefore, only the symmetric component was extracted for analysis.

A Generalised Procrustes Analysis (GPA) using least-squares superimposition was used to align all specimens into a common co-ordinate system. A GPA fixes non-shape related variation which arises due to the specimen's position, size and rotation (Rohlf and Slice, 1990). This is achieved through translation of landmark configurations (until they all have a common origin/centroid), scaling co-ordinate configurations (until they all have the same centroid size) and rotating configurations (until the square root of the sum of squared Euclidean distances between landmarks is minimum) (Webster and Sheets, 2010).

Although extracting shape information from the raw co-ordinates theoretically removes variation in size, the shape data may still contain a component of size-related variation because of allometry (Klingenberg, 2016). If this is not corrected for, this minor variation in size may influence the separation of groups in PCA and CVA (Mitteroecker *et al*., 2004; Gonzalez *et al*., 2011b, Klingenberg *et al*., 2012). Therefore, correcting for the effect of size (allometry) using a multivariate regression of the Procrustes co-ordinates on log centroid size allowed the data to be primed for shape analysis. (Klingenberg, 2016; Small, 2016). Pooled within-group variances were used in the regression analysis to eliminate the possible effect of within-group variation before comparing the groups. Furthermore, a permutation test with 10 000 permutations was used to evaluate the significance of the regression results. Size correction by using the residuals from multivariate regression of shape on size is widely used in morphometric studies (Mitteroecker *et al*., 2004; Weisensee and Jantz, 2011; Singh *et al*., 2012; Freidline *et al*., 2015; Leamy *et al*., 2015).

3.3.5.2. Shape variations between sexes

Size-corrected Procrustes residuals were submitted to a Principal Component Analysis (PCA) to explore shape variation. In agreement with convention, PCA graphs depicting 90% probability ellipses for variation in each sex were generated (Paschetta *et al*., 2010; Smith *et al*., 2013; Maass, 2016). The covariation of shape with age at death and maxillary tooth loss, was assessed using multivariate regression analyses. All regression analyses were performed using pooled-within-group variances to eliminate the possible effect of within-group variation prior to comparing groups. A permutation test with 10 000 permutations was used to evaluate the significance of each regression analysis.

3.3.5.3. Shape variations between ancestry and sex-ancestry groups

A Canonical Variate Analysis (CVA) using size-corrected Procrustes residuals, was used to explore the shape differences occurring between ancestry and sex-ancestry groups. Using parameters discussed above, multivariate regression analyses were performed to assess the covariation of shape with age and antemortem, maxillary tooth loss. Two-block partial least squares (PLS) analysis was employed to assess the effects of trauma on mid-craniofacial morphology in different ancestry groups (Klingenberg, 2009). PLS decomposes a matrix of covariances between two landmark configurations into pairs of axes (one axis for each configuration), which function as shape features, allowing the maximal covariance of each landmark to be compared (Klingenberg, 2009, Drake and Klingenberg, 2010). PLS analysis yields shape features which account for the covariation between parts as opposed to the overall variation in the entire structure (similar to a PCA)(Goswami and Polly, 2010). The RV coefficient, which was computed using PLS analyses, is analogous to the Pearson correlation coefficient and was used to measure the strength of covariation between the two sets of data.

Pairwise DFA and Mahalanobis distances (MDs) were computed to assess similarity between ancestry and sex-ancestry groups. MD denotes the distance between individuals from one group in comparison to the mean of another and is expressed in terms of the standard deviation of the latter group (Klingenberg, 2011). This give us an indication of the similarity or dissimilarity between different groups. MD in this study was calculated using MorphoJ® and does not represent the distance squared, which is sometimes reported. The impact of tooth loss on ancestry estimation was assessed by performing a DFA after correcting for the effect of tooth loss on shape. A multivariate regression of Procrustes shape on tooth loss, was used to mitigate shape differences associated with tooth loss and the Procrustes residuals from this analysis were thus used in the DFA (Small, 2016, Klingenberg, 2016). The reliability of the DFA and classification accuracy of ancestry based on the derived functions were tested using a LOOCV with a permutation of 10 000 iterations.

Chapter Four: Results

Mid-craniofacial variation in size and shape between ancestry and sex-ancestry groups will be reported. Size variations will be conveyed using metric data and shape variations will be conveyed using nonmetric and geometric morphometric data. The impact of tooth loss and age on ancestral variations and ancestry estimations will also be reported. Including individuals with minor or healed, antemortem nasal and zygomatic trauma may have impacted mid-craniofacial morphology detected in this study. A detailed assessment of the impact of trauma on mid-craniofacial variation was not the primary focus of this study, therefore, results will be reported in Appendices E and F and will be addressed briefly in the Discussion chapter.

4.1. Observer agreement

4.1.1. Metrics

The differences between inter-and intra-observer repeats of measurements (and quantitative assessments of tooth loss) on 30 crania were not significant, and absolute mean differences between sets of measurements were below 1 mm (Appendix C: Table 1). Smaller differences were detected between measurements taken by the same observer, versus those taken by two different observers (Appendices C and D). Mean differences of one or two repeats for measurements of bi-orbital breadth (EKB), nasal height left (NHL), nasal breadth (NLB), bizygomatic breath (ZYB), maxilla-alveolar breadth (MAB) and basion-prosthion length (BPL) extend slightly below the 95% confidence intervals (Figure 4.1 and Appendix C: Figures 1-3). Although these would be considered outliers by convention, the absolute mean differences were below 1 mm and were not deemed significant enough to warrant further measurement.

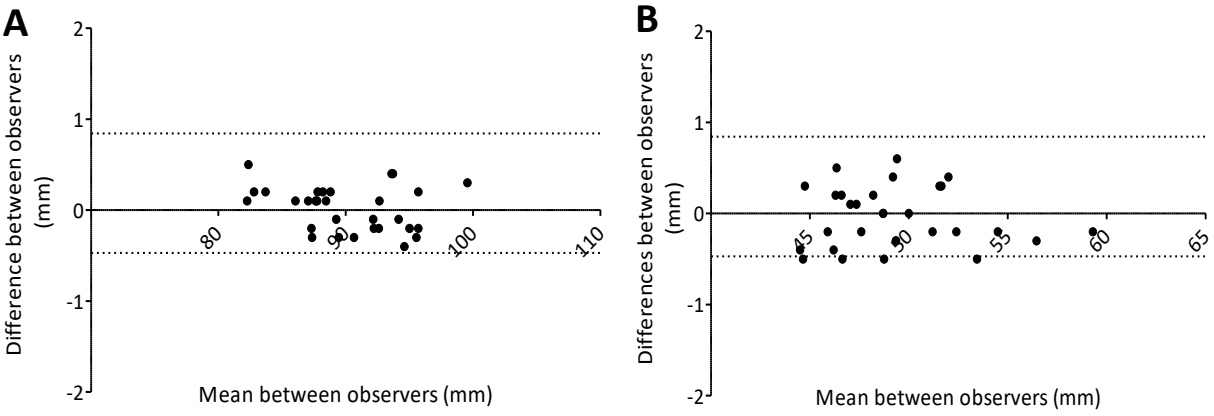

Figure 4.1. Examples of Bland Altman plots showing inter-observer agreement. The dotted lines represent 95% limits of confidence of differences between observers. **A.** Good agreement between repeated measurements of maxillo-alveolar breadth (MAB). **B**. Good agreement but showing four outliers for repeated measurements of nasal height left (NHL).

4.1.2. Nonmetrics

All nonmetric traits except inferior nasal margin (INM), zygomaxillary suture shape (ZS), orbital shape (OS), malar tubercle (MT), zygomatic projection (ZP) and alveolar prognathism (AP), yielded good interobserver agreement (Table 4.1). Overall, intra-observer assessments yielded better repeatability (except for inferior nasal margin (INM)) than inter-observer assessments. Assessments with *poor* or *slight* agreement (INM, MT and ZP) were excluded from further analyses (Table 4.1).

Table 4.1. Observer agreement analysis of nonmetric traits and categorical covariates ($p \leq 0.0001$).

Trait	Interobserver (K_c)	Agreement	Intra-observer (K_c)	Agreement
Nasal bone contour (NBC)	0.510	Moderate	0.569	Moderate
Nasal aperture width (NAW)	0.550	Moderate	0.665	Substantial
Anterior nasal spine (ANS)	0.682	Substantial	0.731	Substantial
Inferior nasal margin (INM)	**- 0.129**	**Poor**	**0.208**	**Fair**
Zygomaxillary suture shape (ZS)	0.281	Fair	0.423	Moderate
Supranasal suture (SS)	0.562	Moderate	0.554	Moderate
Interorbital breadth (IOB)	0.409	Moderate	0.525	Moderate
Orbital shape (OS)	0.372	Fair	0.522	Moderate
Malar tubercle (MT)	**0.182**	**Slight**	0.434	Moderate
Zygomatic projection (ZP)	**0.068**	**Slight**	0.444	Moderate
Alveolar prognathism (AP)	0.378	Fair	0.590	Moderate

(K_c)- Cohen's *K* statistic (intra-observer)

4.1.3. Geometric morphometrics

A Procrustes ANOVA showed good repeatability for inter- and intra-observer repeats. The percentage contribution of measurement error (*Error 1*) to the total variance in the sample was below 5% (Table 4.2). For both observers, measurement error relative to fluctuating asymmetry was slightly higher than anticipated, most likely due to the small sample size used for error testing (n=30) (Figure 4.2). Intra-observer assessments yielded better repeatability than inter-observer assessments. Centroid size yielded, lower variances and better repeatability than Procrustes shape.

Table 4.2. Centroid size and Procrustes shape ANOVA tests for observer agreement testing.

Effect	Effect contribution (%)	SS	MS	df	*F*	*p*
Inter-observer agreement						
Centroid size						
Individual	99	9735.46	335.76	29	103.40	<.0001
Error1	**1**	**97.40**	**3.25**	**30**		
Procrustes Shape ANOVA						
Individual	60.3	0.24	1.62×10^{-4}	1508	6.54	<.0001
Side	26.7	0.006	7.17×10^{-5}	49	2.89	<.0001
*Ind * Side*	**9.2**	**0.04**	**2.48×10^{-5}**	**1421**	**2.46**	**<.0001**
Error1	**3.8**	**0.03**	**1.02×10^{-5}**	**3030**		
Intra-observer agreement						
Centroid size						
Individual	99,24	9713.64	334.95	29	130.97	<.0001
Error1	**0,76**	**76.73**	**2.56**	**30**		
Procrustes Shape ANOVA						
Individual	59,2	0.24	$1,61 \times 10^{-4}$	1508	7.01	<.0001
Side	30,0	0.004	$8,14 \times 10^{-5}$	49	3.55	<.0001
*Ind * Side*	**8,4**	**0.03**	**$2,29 \times 10^{-5}$**	**1421**	**3.54**	**<.0001**
Error 1	**2,4**	**0.02**	**$6,47 \times 10^{-6}$**	**3030**		

- *Individual*: inter-individual variation
- *Side*: variation due to directional asymmetry
- *Ind * side*: variation due to fluctuating asymmetry
- *Error1*: variation among repeats

'SS' – sum of squares. *'MS'* – mean squares. *'df'* – degrees of freedom. *'F'* – *F* statistic. *'p'* – P-value.

**Measurement error (Error 1) is relative to the Ind*side interaction.*

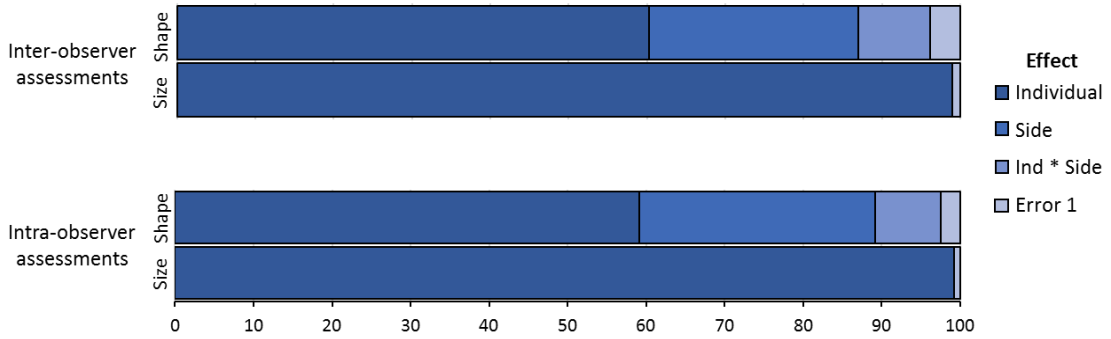

Figure 4.2. Percentage contribution of each effect (Individual, Side, Ind * Side and Error1) to the total variance in Procrustes shape and centroid size for inter-and intra-observer assessments ($p \leq 0.0001$).

4.2. Demographic analyses

4.2.1. Preliminary analyses

All numerical variables, excluding antemortem tooth loss, age at death and year of birth, EKB, BNL and MAB, were normally distributed. Grubb's test and box plots, revealed outliers in measurements from ten individuals born prior to the 1900s. Due to the desired forensic application of this study, these individuals were excluded to ensure the sample more closely resembled a population born in the 20th century. A summary of the adjusted study sample is given in Table 4.3.

Once individuals were excluded, distribution of the data were retested and only tooth loss, age at death and year of birth were not normally distributed (except when data was grouped according to sex, ancestry and sex ancestry). Grubbs test was performed again, and no significant outliers were detected ($p \leq 0.05$). No differences were detected between the number of left and right maxillary teeth lost ($t \leq 0.0001$, $p = 1.00$). Therefore, only tooth loss groups were considered in further analyses.

Table 4.3. Summary of adjusted study sample, according to sex and ancestry.

	Ancestry			
Sex	African	Mixed	European	**Total**
Male	56 (14.3%)	126 (32.1%)	44 (11.2%)	226 (57.7) %
Female	49 (12.5%)	70 (17.9%)	47 (12%)	166 (43.2%)
Total	105 (26.8%)	196 (50%)	91 (23.2%)	**392**

4.2.2. Sample distribution

In general, more individuals of Mixed ancestry (MA) than African ancestry (AA) or European ancestry (EA) were sampled in this study. This reflects demographic bias at skeletal collections in the Western Cape (University of Cape Town Human Skeletal Collection and the Kirsten Collection) which formed the primary sample of this study.

4.2.2.1. Demographic data according to ancestry

More males (57.7%) than females (43.2%) were sampled (Table 4.3). All covariates were significantly associated with ancestry groups ($p \leq 0.0001$) (Table 4.4). Individuals of Mixed ancestry (MA) comprised 50% of the sample; while European (EA) and African ancestry (AA) individuals comprised (23.2%) and (26.8%) of the sample, respectively.

4.2.2.1.1. Antemortem tooth loss

EA individuals had significantly fewer teeth than MA and AA individuals ($p \leq 0.0001$), and MA individuals had significantly fewer teeth than AA individuals ($p=0.001$) (Table 4.4).

4.2.2.1.2. Age at death and year of birth

Due to the skewed nature of skeletal collections used in this study, most EA individuals sampled were older at death (58±9 years) than MA or EA individuals ($p \leq 0.0001$) (Table 4.4). EA individuals were born earlier than AA and MA individuals; with all EA individuals born before 1962 ($p \leq 0.0001$) (Table 4.4). Age at death and tooth loss were associated in EA ($F=3.33$; $p=0.04$) and MA ($F=10.56$; $p \leq 0.0001$) individuals. Generally, EA (61±6.8 years) and MA (53±11.2 years) individuals with fewer than 6 teeth present, were older at the time of death. Furthermore, most EA individuals sampled in this study were born in the early-20th century, while AA and MA individuals were largely born in the mid-late 20th century.

Table 4.4. Demographic data, according to ancestry.

		Ancestry				
		African n =105 (26.8%)	*Mixed* n =196 (50%)	*European* n=91 (23.2%)	F	*p-value*
Teeth present[(a)]	Median (IQR)	14 (6)	8 (12)	1 (11)	**66.29***	**≤0.0001**
Age [Yrs.] [(b)]	Mean (SD)	45 (12)	47 (13)	58 (9)	**35.43**	**≤0.0001**
	(Range)	20-71	18-75	28-74		
YOB [Yr.] [(c)]	Mean (SD)	1939 (19)	1939 (17)	1930 (12)	**11.88**	**≤0.0001**
	(Range)	1939-1988	1901-1981	1902-1962		

F statistic from Univariate ANOVA, *H-statistic from Kruskal Wallis ANOVA.

a) Teeth present: Europeans < Mixed and African ancestry ($p<0.0001$); Mixed < African ancestry ($p=0.001$)

b) Age: Mixed and African ancestry < European ancestry (*$p<0.0001$)*

c) YOB: Europeans < Mixed and African ancestry ($p<0.0001$)

'YOB'-Year of birth. 'Age'-Age at death. 'Teeth'-teeth present. '*n*' – number of individuals. '*SD*' – standard deviation. '*IQR'* – interquartile range. 'Yrs.*'* – years. '*F*' – *F* statistic. '*p*' – P-value.

4.2.2.2. Demographic data according to sex-ancestry

All covariates were significantly associated with sex-ancestry groups ($p \leq 0.0001$) (Table 4.5). MA males represented the largest group sampled (32.1%), and EA males (11.2%) represented the smallest group sampled (Table 4.5).

4.2.2.2.1. Antemortem tooth loss

AA males and females sampled had the most teeth present ($p \leq 0.01$), while MA females had more teeth than EA females ($p=0.02$) (Table 4.5). Some outliers were detected for AA males however, these individuals fell within the normal range of variation, as one would expect the number of maxillary present to vary in a population (Table 4.5).

4.2.2.2.2. Age at death and year of birth

The sampled EA males and females were significantly older than other sex-ancestry groups ($p \leq 0.03$) (Table 4.5). Due to the limitations of skeletal collections, the EA sample is only representative of middle-aged and elderly individuals, as no EA individuals under the age of 38 were sampled (Table 4.5). Age at death and tooth loss were associated in EA females ($F=4.01$; $p=0.03$), MA males ($F=7.08$; $p=0.01$) and MA females ($F=5.0$; $p=0.009$) and those with fewer than 6 teeth were older at the time of death. Investigating a secular trend was beyond the scope of this study, and year of birth was excluded as a covariate in further analyses.

Table 4.5. Demographic data, according to sex-ancestry groups.

		Sex-ancestry							
		AA males *n*=56 *(14.3%)*	*AA females* *n*=49 *(12.5%)*	*MA males* *n*=126 *(32.1%)*	*MA females* *n*=70 *(17.9%)*	*EA males* *n*=44 *(11.2%)*	*EA females* *n*=47 *(12.0%)*	F	*p-value*
Teeth present[(a)]	Median (IQR)	15 (4)	12 (7)	8 (11)	7.5 (13)	3 (12)	0.5 (10.8)	**70.32**	**<0.0001**
Age [Yrs] [(b)]	Mean (SD)	46 (13)	44 (11)	49 (12)	43 (13)	58 (8)	58 (9)	**16.51**	**<0.0001**
	(Range)	20-71	24-66	75-20	18-72	38-74	44-72		
YOB [Yr] [(c)]	Mean (SD)	1942 (20)	1935 (17)	1937 (18)	1942 (17)	1931 (12)	1928 (13)	**6.97**	**<0.0001**
	(Range)	1988-1903	1965-1905	1979-1901	1981-1903	1955-1905	1902-1962		

F statistic from Univariate ANOVA; **H* statistic from Kruskal Wallis ANOVA

a) Teeth present: EA and MA males and females < AA males and females ($p<0.01$). EA females < MA females ($p=0.02$); AA females< AA males *($p=0.02$).*
b) Age: MA and AA males < EA males ($p<0.03$); AA females < EA females ($p<0.0001$); MA females < MA males ($p=0.005$).
c) YOB: EA males < AA and MA males ($p=0.04$*;* EA females < AA and MA females *($p<0.02$)*; MA males < MA females *($p=0.04$)*; AA males < AA females ($p=0.04$).

'YOB'-Year of birth. 'Age'-Age at death. 'Teeth'-teeth present. '*n*' – number of individuals. '*SD*' – standard deviation. 'IQR*'* – interquartile range. 'Yrs.*'* – years. '*F*' – *F* statistic. '*p*' – P-value.

4.3. Size variation

4.3.1. Metric Analyses

4.3.1.1. Craniofacial size variation between ancestry groups

Significant differences in measurements between ancestries were detected for all measurements except basion-nasion length (BNL). Although mean sizes (except BNL) between ancestries were significantly different, large overlaps in ranges were present. Outliers were detected for all measurements (Figures 4.3-4.5) (excluding OBB, NHL and ZMB), however, none of these were considered significant enough to warrant exclusion (Grubbs, 1969).

Orbital region

EA individuals exhibited narrower orbital breadths (OBB) than AA (p=0.02) and MA individuals ($p \leq 0.0001$) (Figure 4.3). EA individuals had longer orbital heights, followed by AA and MA individuals, however, significant differences between ancestry groups were not conserved for left and right orbital heights due to minor asymmetries (Figures 4.3 a-c). EA individuals had the narrowest interorbital breadths (DKB) and bi-orbital breadths (EKB) (Figures 4.4); while AA individuals had the widest ($p \leq$ 0.001). EA individuals exhibited larger orbits and smaller nasal bridges than AA and MA individuals.

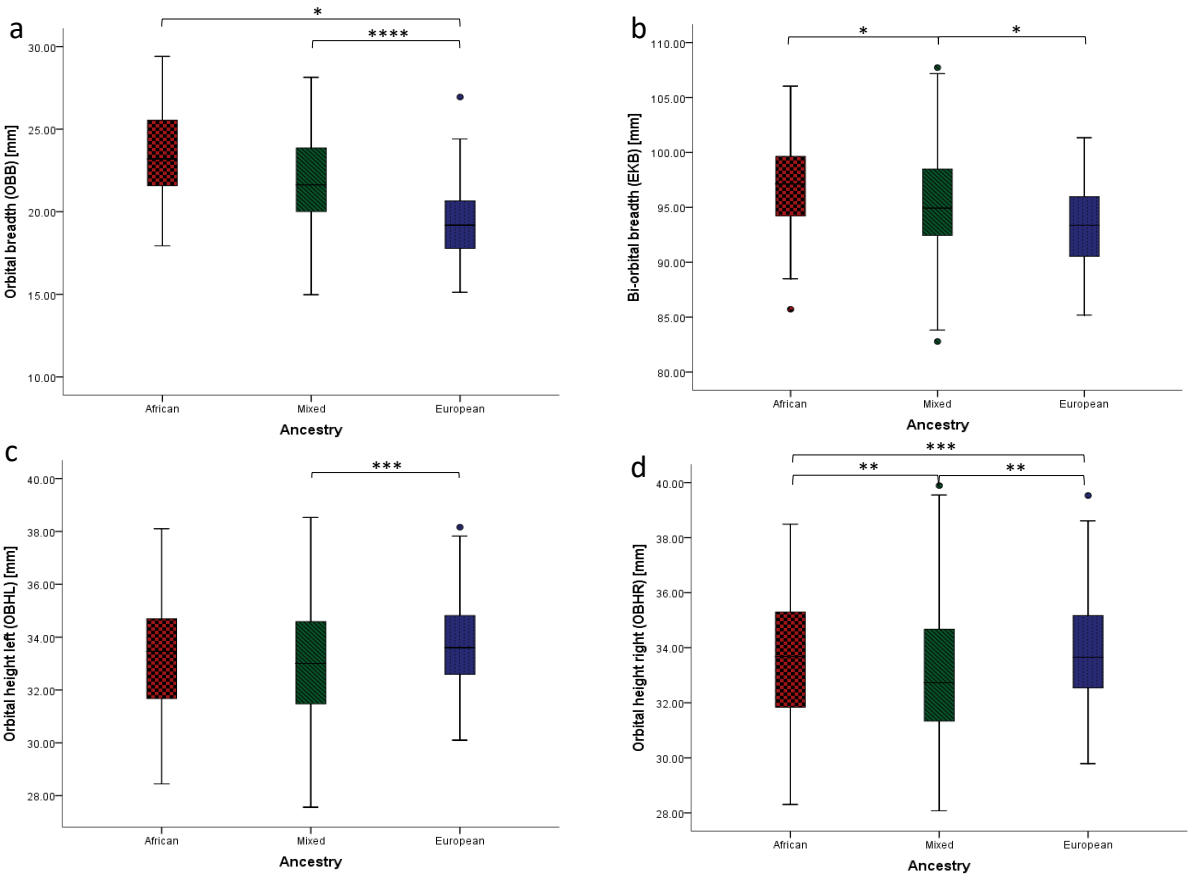

Figure 4.3. Box plots depicting means and confidence intervals of measurements in the orbital region for ancestry groups. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

Nasal region

EA individuals exhibited longer facial heights (NPH) and narrow nasal breadths (NLB), compared to the wider nasal breadths (NLB) and more compressed facial heights (NPH) observed in AA and MA individuals (Figures 4.4. c-d and 4.5 a). EA individuals had the largest mean nasal heights, with the NHL being slightly larger than NHR (Figure 4.4 c-d). Overall, AA individuals had larger facial heights, nasal heights and wider nasal apertures than MA individuals.

Zygomatic region

EA individuals ($p \leq 0.0001$) followed by MA individuals ($p < 0.04$) exhibited relatively less wide zygomatic breadths (ZYB and ZMB) than AA individuals (Figure 4.6. b-c).

Maxillary region

EA individuals had narrower maxillo-alveolar breadths (MAB) than AA and MA individuals; although MA individuals had significantly narrower MAB than AA individuals (Figure 4.5 d). AA individuals ($p < 0.0001$), followed by MA individuals ($p < 0.0001$) exhibited more anteriorly projecting/prognathic maxillae than EA individuals (BPL) (Figure 4.5 f).

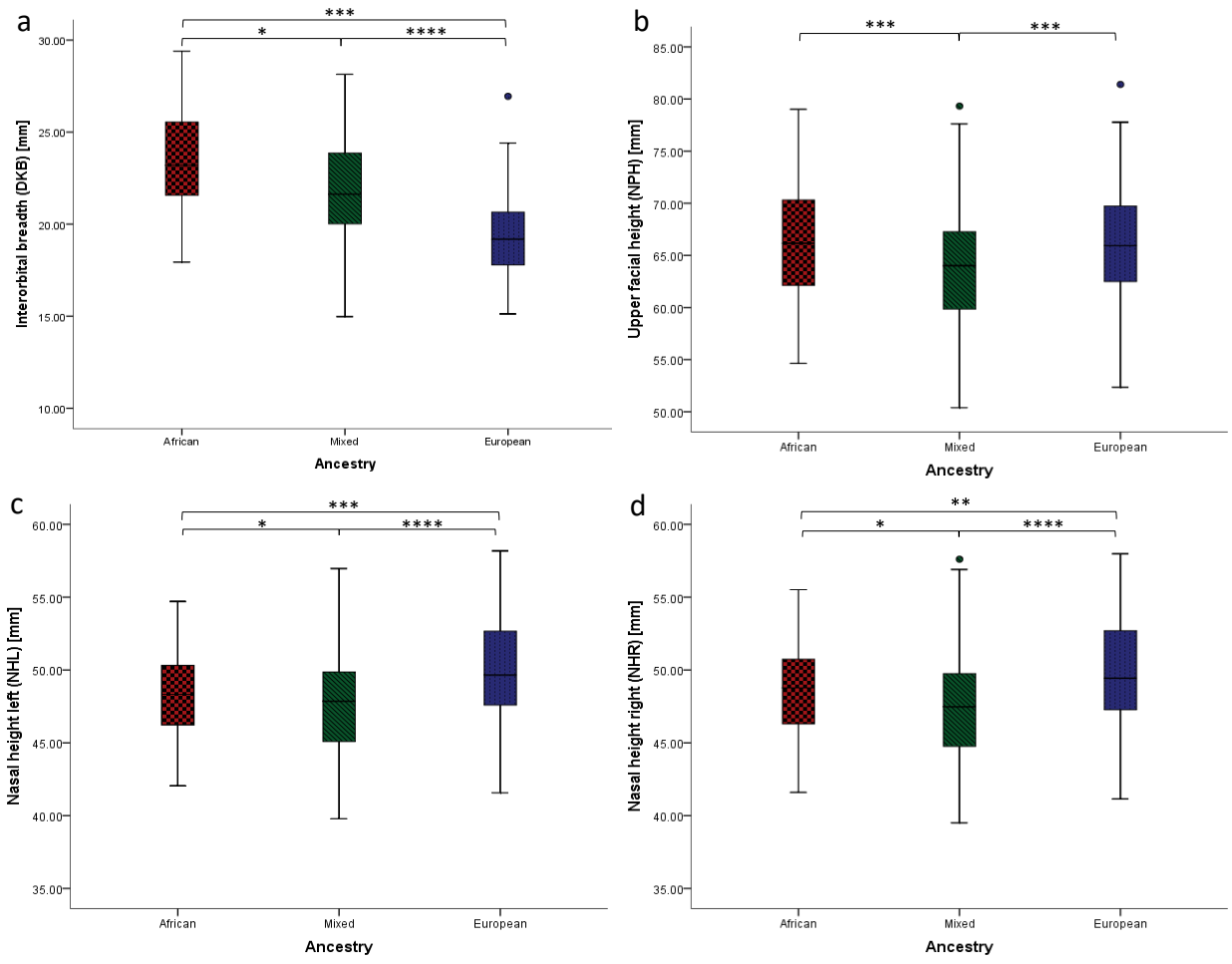

Figure 4.4. Box plots depicting means and confidence intervals of orbital and nasal measurements for ancestry groups. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

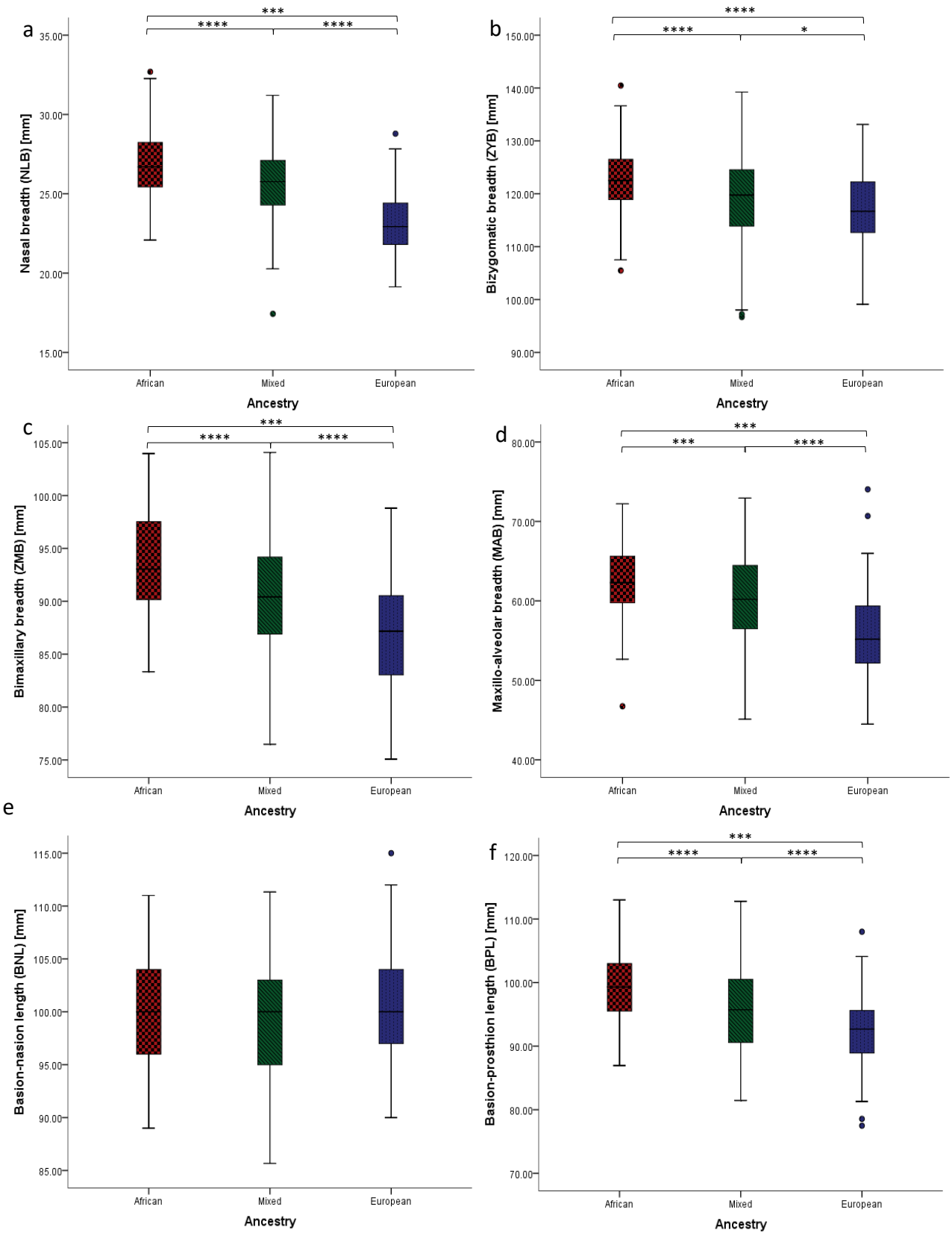

Figure 4.5. Box plots depicting means and confidence intervals of nasal, maxillary and zygomatic measurements for ancestry groups. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.3.1.1.1. The effect of tooth loss on size

Ancestry distributions for tooth loss categories are given in Table 4.6. The general trend was for individuals with fewer than 6 teeth present to have smaller mean measurements than all other groups (Figures 4.6-4.7).

Table 4.6. Antemortem maxillary tooth loss categories based on the number of teeth present, organised according to ancestry.

Category	Teeth present	***Ancestry***			
		AA	*MA*	EA	*Total*
A	*0-5*	14	77	49	**140**
B	*6-10*	12	43	17	**72**
C	*11-16*	79	76	25	**180**
Total		**105**	**196**	**91**	**392**

Orbital region

A positive correlation between interorbital breadth (DKB) and tooth loss was seen in MA individuals (r=0.14; p=0.05) (Figure 4.6 a).

Nasal region

Upper facial height (NPH) increased in EA and MA individuals, as the number of teeth present increased (r≥0.29; p≤0.05) (Figure 4.6 b). Nasal height (NHL and NHR) increased slightly in MA individuals, relative to the increased number of teeth present (r≥0.32; p≤0.05) (Figure 4.6 c-d). When more than 10 teeth were present, NPH was larger in EA individuals, followed by AA and then MA individuals (Figure 4.6 b). However, when fewer than 6 teeth were present, MA and EA individuals exhibited significantly smaller NPH than AA individuals (p≤0.001). Therefore, in EA and MA individuals NPH may not be a reliable determinant of ancestry when more than 10 teeth are lost antemortem (Figure 4.6 b). Notably, in EA individuals the effect of tooth loss was more distinct as NPH size was most drastically reduced. AA individuals with fewer than 6 teeth exhibited larger DKB, NHL and NHR than those with more than 6 teeth, this is contrary to trends in other ancestry groups but is most likely because few AA individuals exhibited severe tooth loss.

Zygomatic regions

In MA individuals, ZMB increased in size relative to the increase in number of maxillary teeth present (r=0.20; p=0.006) (Figure 4.6 e).

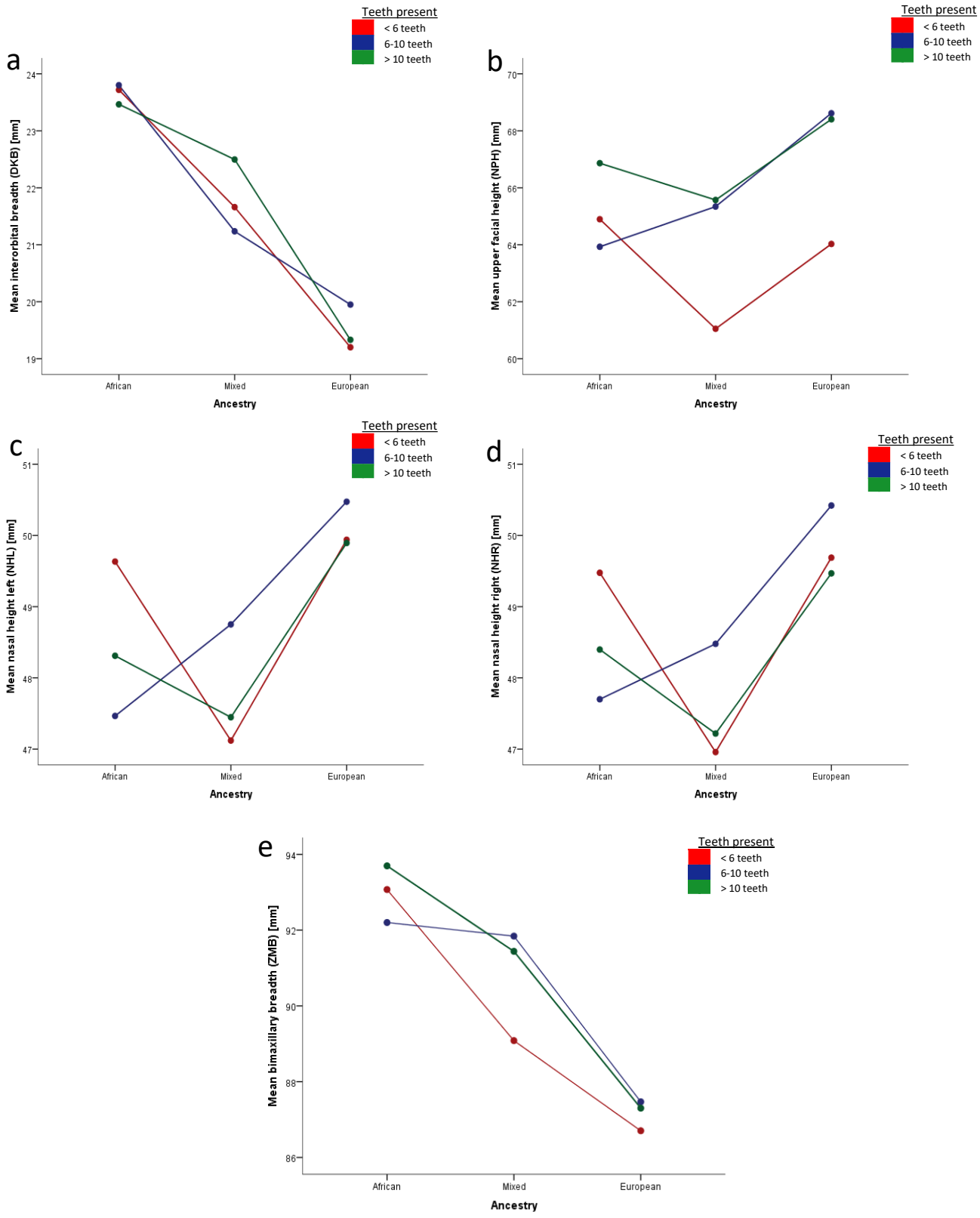

Figure 4.6. Mean plots of measurements associated with tooth loss groups assessed according to ancestry. (a) Interorbital breadth (DKB). (b) Upper facial height (NPH). (c) Nasal height left (NHL) and (d) Right (NHR). (e) Bimaxillary breadth (ZMB).

Maxillary region

MAB size increased in AA, EA and MA individuals ($r \geq 0.36$; $p \leq 0.002$), relative to the increase in teeth present. Although size differences between tooth loss groups were most evident in MAB (Figure 4.7a), the significant differences between ancestry groups were conserved, i.e. AA individuals had the largest MAB, followed by MA, then EA individuals (Figure 4.7 a). In EA and MA individuals, BPL ($r \geq 0.21$; $p \leq 0.03$) increased relative to the increase in number of teeth present (Figure 4.7 b).

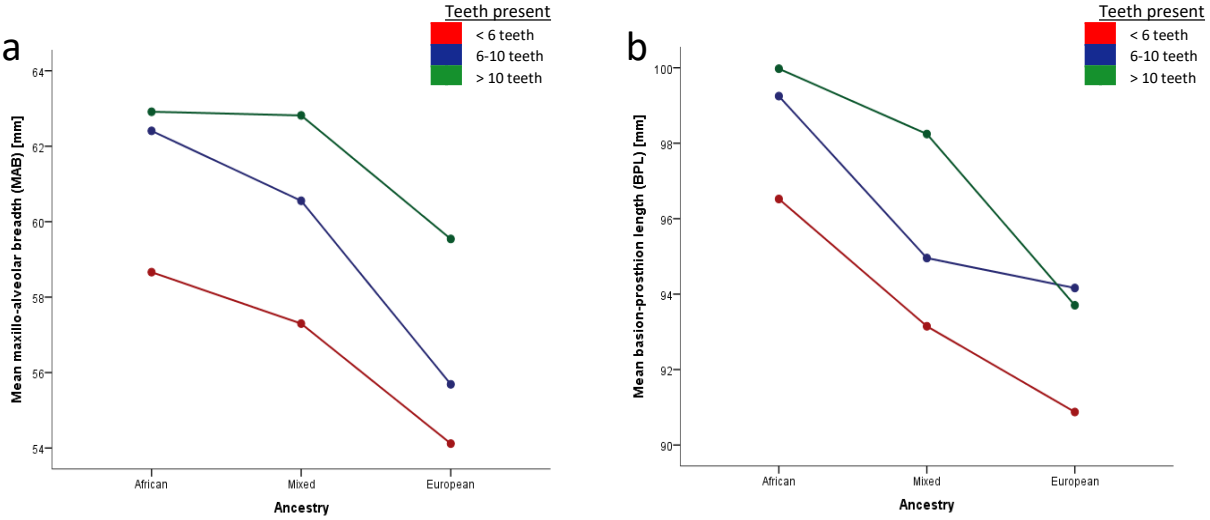

Figure 4.7. Mean plots of maxilla-alveolar breadth (MAB) and basion-prosthion length (BPL) for tooth loss groups, assessed according to ancestry.

4.3.1.1.2. The effect of age on size

Associations were detected between age at death and OBB (r=0.20; p<0.0001); OBHL (r=0.14; p<0.007); DKB (r=-0.19; p<0.0001); NHL (r=0.14; p=0.005); NHR (r=0.13; p=0.01); MAB (r=-0.27; p<0.0001) and BNL (r=0.13; p=0.01). Consequently, linear regression analyses were computed to investigate the relationship between these variables and age at death.

Orbital region

OBB (r=0.22; p=0.002) and OBHL (r=0.15; p=0.03) increased in MA individuals, relative to increased age at death. (Figure 4.8). However, age at death only accounted for a small amount of variation in OBB (R^2=0.04) and OBHL (R^2=0.02) (Figures 4.8 b-c). In EA individuals, DKB decreased relative to the increase in age at death (r=-0.25; p=0.03); however, this only accounted for a small amount of variation in DKB (R^2=0.003) (Figure 4.8 c). Due to the negative correlation between DKB and teeth present, the relationships between these variables was investigated. A decrease in DKB (r=-0.04.; p=0.009) relative to increased age at death, was only detected in EA individuals with fewer than 6 teeth present, indicating that tooth loss is a cofactor contributing to the relationship between age at death and interorbital breadth (Figure 4.8 d).

Nasal region

In MA individuals, nasal height (NHL and NHR) increased relative to the increase in age at death (r=0.14; p=0.04); however, this relationship accounted for a small amount of the variation in nasal height (R^2=0.02) (Figure 4.9 a-b).

Maxillary region

In EA individuals, MAB decreased relative to the increase in age at death (r=-0.23; p=0.03) (Figure 4.9 c); however, this relationship only contributed slightly to the size variation in MAB (R^2=0.01). Narrower MAB were only detected in older EA individuals with fewer than 6 teeth present (r=-0.45 p=0.001), indicating that tooth loss and age at death both impact and MAB (Figure 4.9 d).

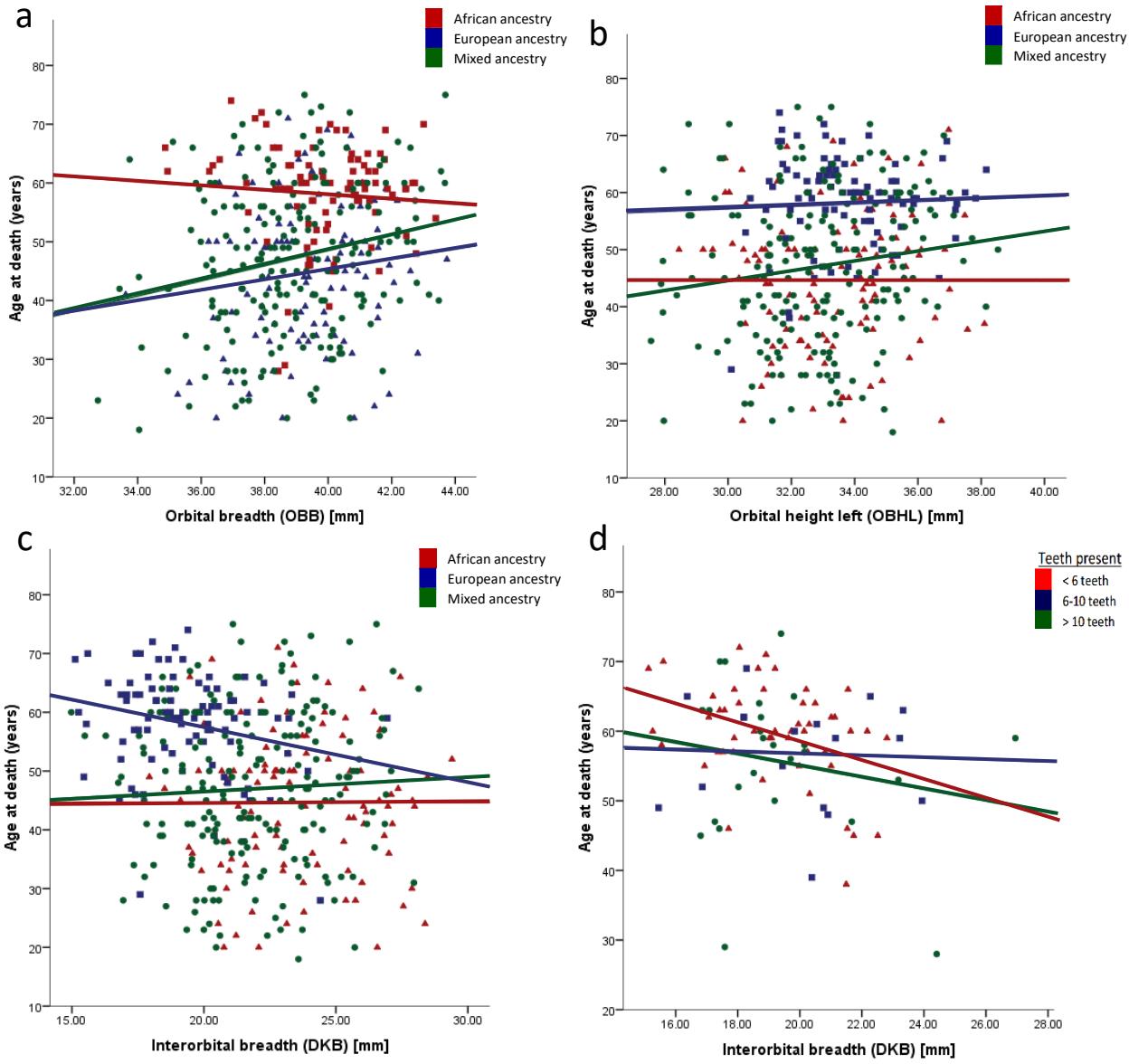

Figure 4.8. Scatter plots of the relationships between measurements in the orbital region and age at death. The relationship between (a) OBB, (b) OBHL, (c) DKB and age at death for different ancestry groups. (d) DKB and age for different tooth loss groups in EA individuals.

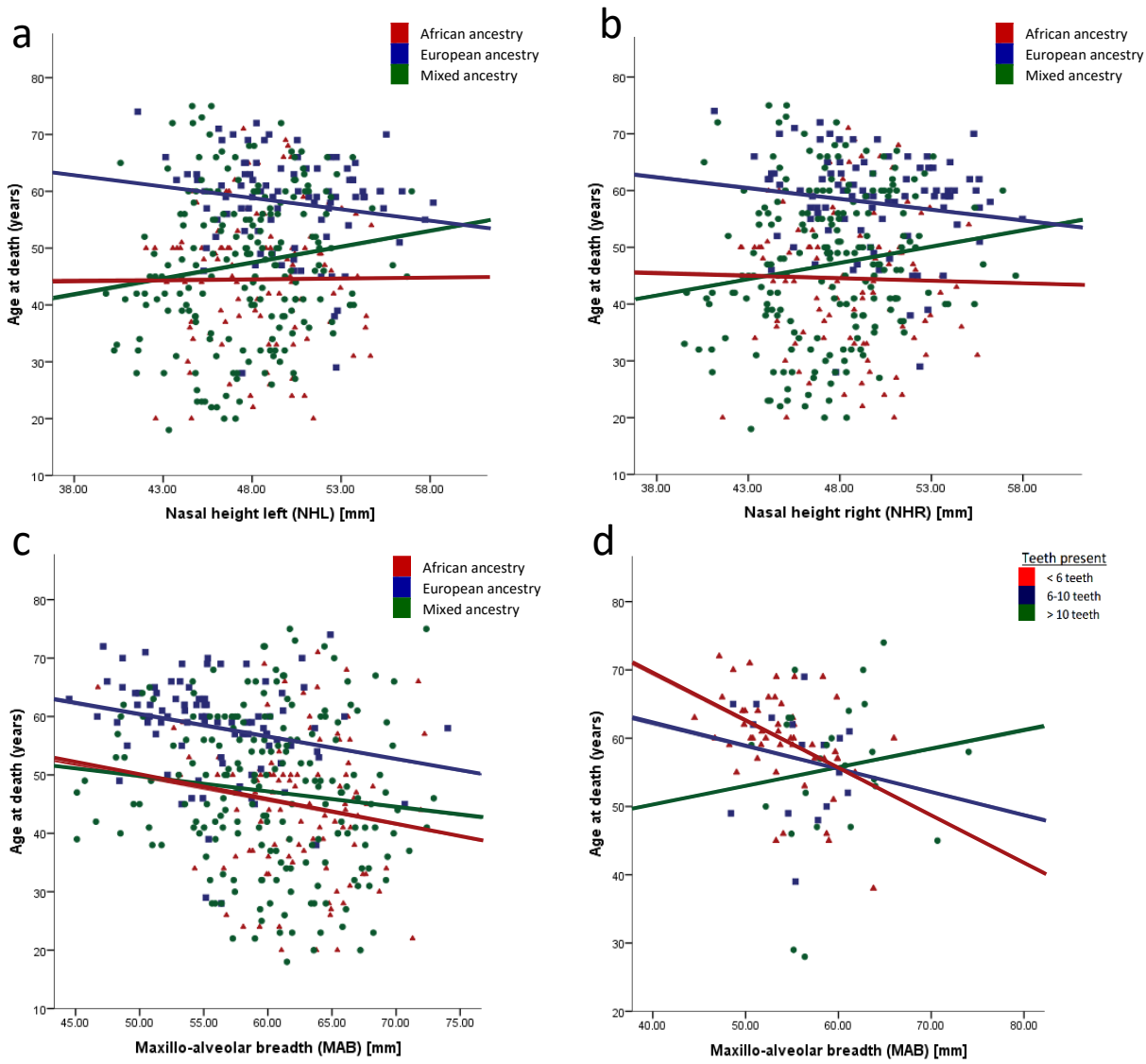

Figure 4.9. Scatter plots of the relationships between measurements in the nasal region and age at death. (a) NHL and age; (b) NHR and age; (c) MAB and age; (d) MAB and age in EA individuals for different teeth categories.

4.3.1.2. Craniofacial size variation between sex-ancestry groups.

Size differences between sex-ancestry groups were detected for all measurements except OBHL, OBHR and BNL (Figures 4.10-4.12). Furthermore, size trends detected between ancestry groups, except for OBB, OBHL, OBHR, ZYB and BNL were the same between sex-ancestry groups (Figures 4.3-4.5). All measurements, except orbital height and nasal breadth were significantly larger in males than females ($F \geq 2.97$; $p \leq 0.01$).

Orbital region

Size differences between males and females of different ancestries were only detected in OBB, DKB and EKB ($F \geq 2.97$; $p \leq 0.01$) (Figures 4.10-4.11). Only EA females, exhibited wider OBB than MA females ($p \leq 0.0001$). When the sample was split according to sex, AA individuals had longer orbital heights, followed by EA and MA individuals, however significant differences between ancestry groups were not conserved for left and right orbital heights due to minor asymmetries (Figures 4.10 b-c). EA individuals had the narrowest interorbital breadths (DKB) and bi-orbital breadths (EKB) (Figures 4.10 d and 4.11 a); while AA individuals had the widest ($p \leq 0.001$).

Nasal region

NLB were only different between EA (F=11.79; p=0.003) and MA (F=8.80; p=0.003) males and females, respectively (Figure 4.12 a). BNL was larger in EA males than MA males, but no differences were detected between females of different ancestries (Figure 4.12 e).

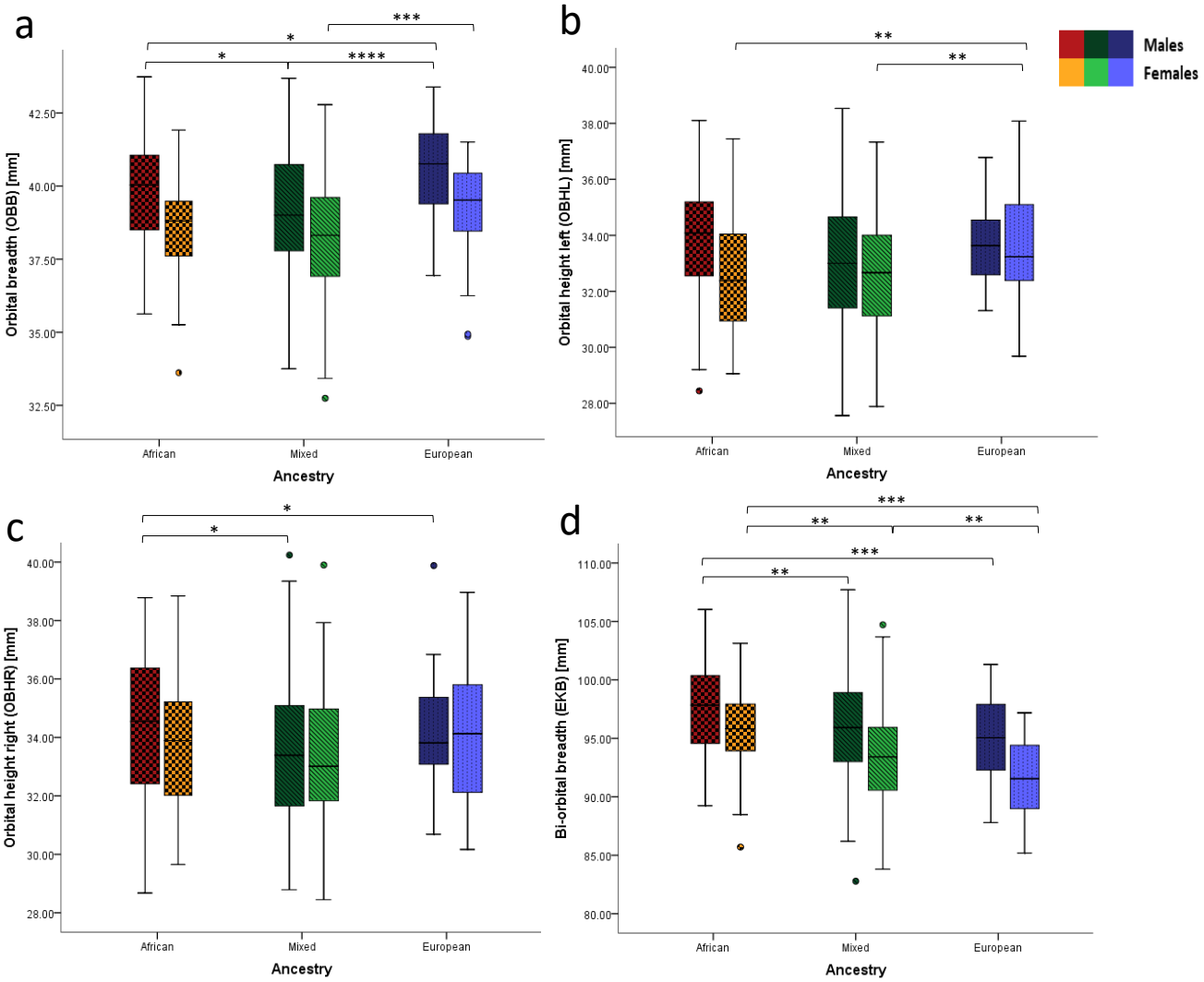

Figure 4.10. Box plots of means and confidence intervals of measurements in the orbital region for sex-ancestry groups. Significance bars show differences between ancestry groups for males and females. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

Zygomatic region

Significant size differences were detected in ZYB measurements between AA and MA individuals, and AA and EA individuals *(p*≤0.0001) (Figure 4.12 b-c). While ZMB measurements were significantly different between females from all ancestry groups *(p*≤0.0001), only AA and MA males exhibited significantly different ZMB *(p*≤0.0001) (Figure 4.12C).

Maxillary region

Size differences in MAB were detected between all sex-ancestry groups (*F*≥17.0; *p*≤0.0001) (Figure 4.12 d). Size trends in MAB between sex-ancestry groups were the same those detected between ancestry groups. AA males exhibited larger BPL than MA and EA males (*p*≤0.0001). BPL were larger in AA females followed by MA and AA females (*p*≤0.002) (Figure 4.12 d).

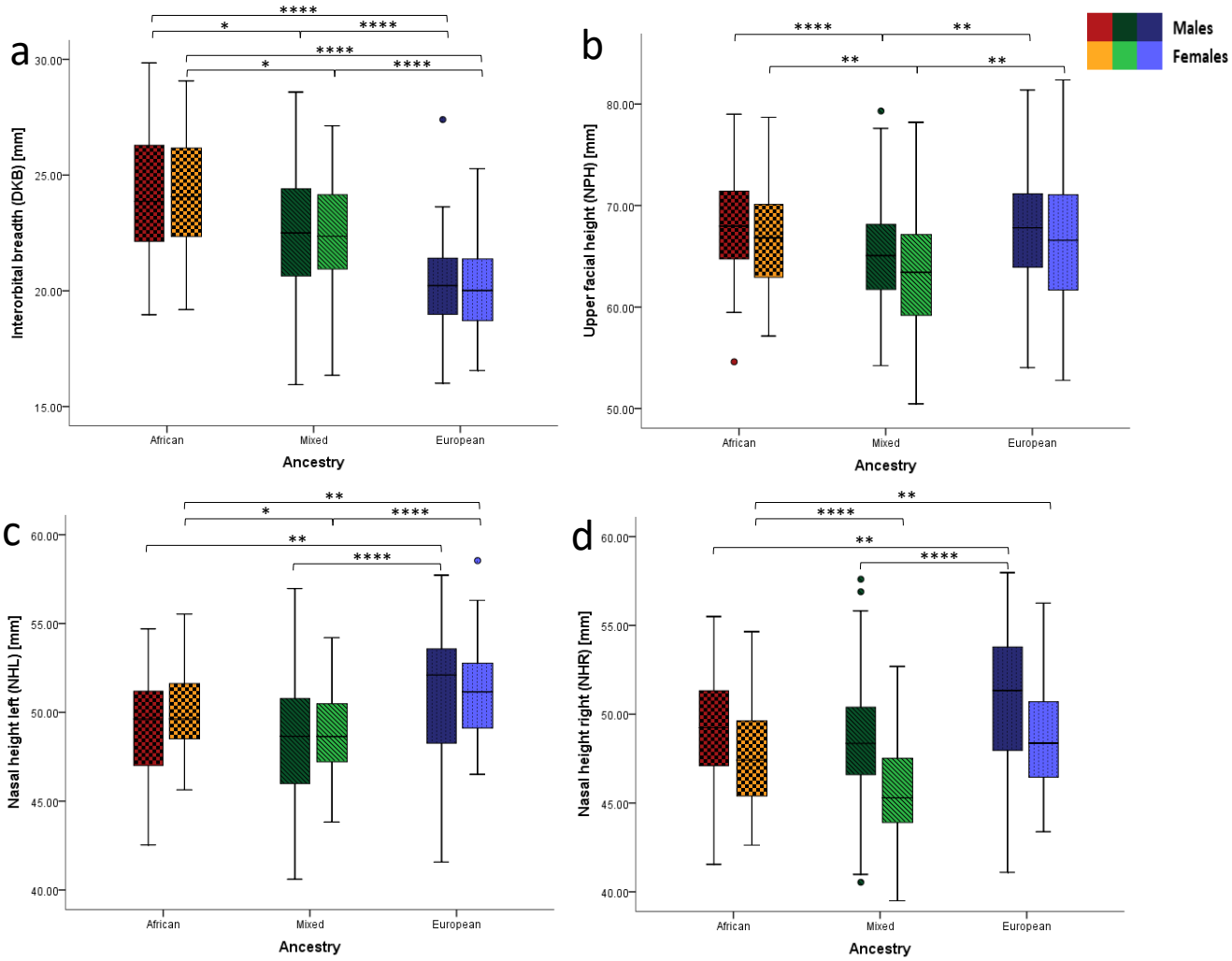

Figure 4.11. Box plots of means and confidence intervals of measurements in the nasal region for sex-ancestry groups. Significance bars show differences between ancestry groups for males and females. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

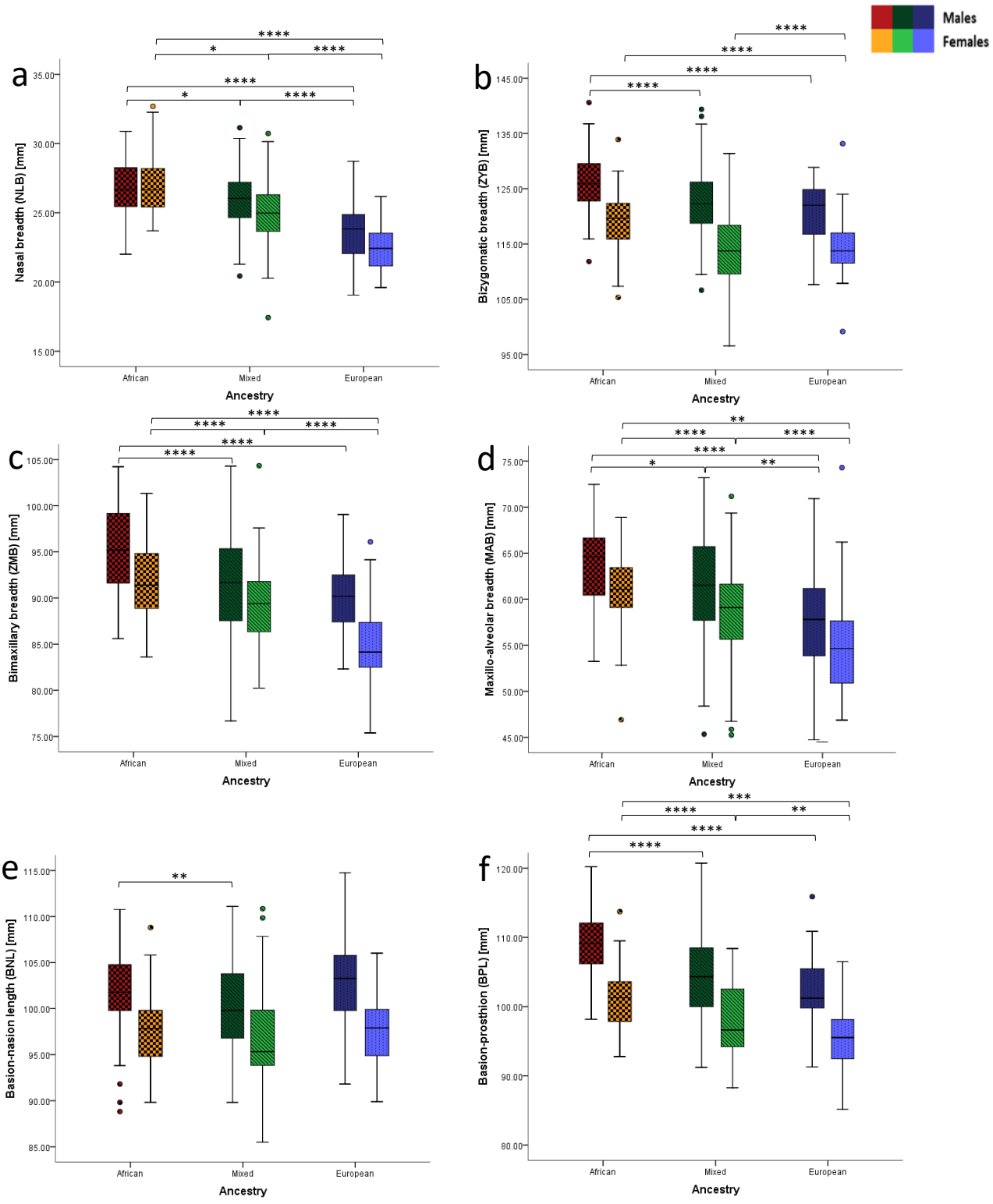

Figure 4.12. Box plots of means and confidence intervals for measurements in the nasal, zygomatic and alveolar region for sex-ancestry groups. Significance bars show differences between ancestry groups for males and females. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

4.3.1.2.1. The effect of tooth loss on size

In EA and MA males and females, NPH (r>0.29; p<0.05) and MAB (r>0.36; p<0.002) were associated with tooth loss. In MA females ZMB (r=0.30; p<0.01) and BPL (r=0.40; p<0.0001) were also associated with tooth loss; and in MA males BPL was also associated with tooth loss (r=0.21; p<0.0001). Generally, those with fewer than 6 teeth had smaller NPH, MAB, ZMB and BPL; the largest differences were detected in MAB and NPH (Figures 4.13 a-b). This trend was not seen in NPH measurements in AA males, because NPH were noted in AA males with fewer than 6 teeth present than in those more than 6 teeth present.

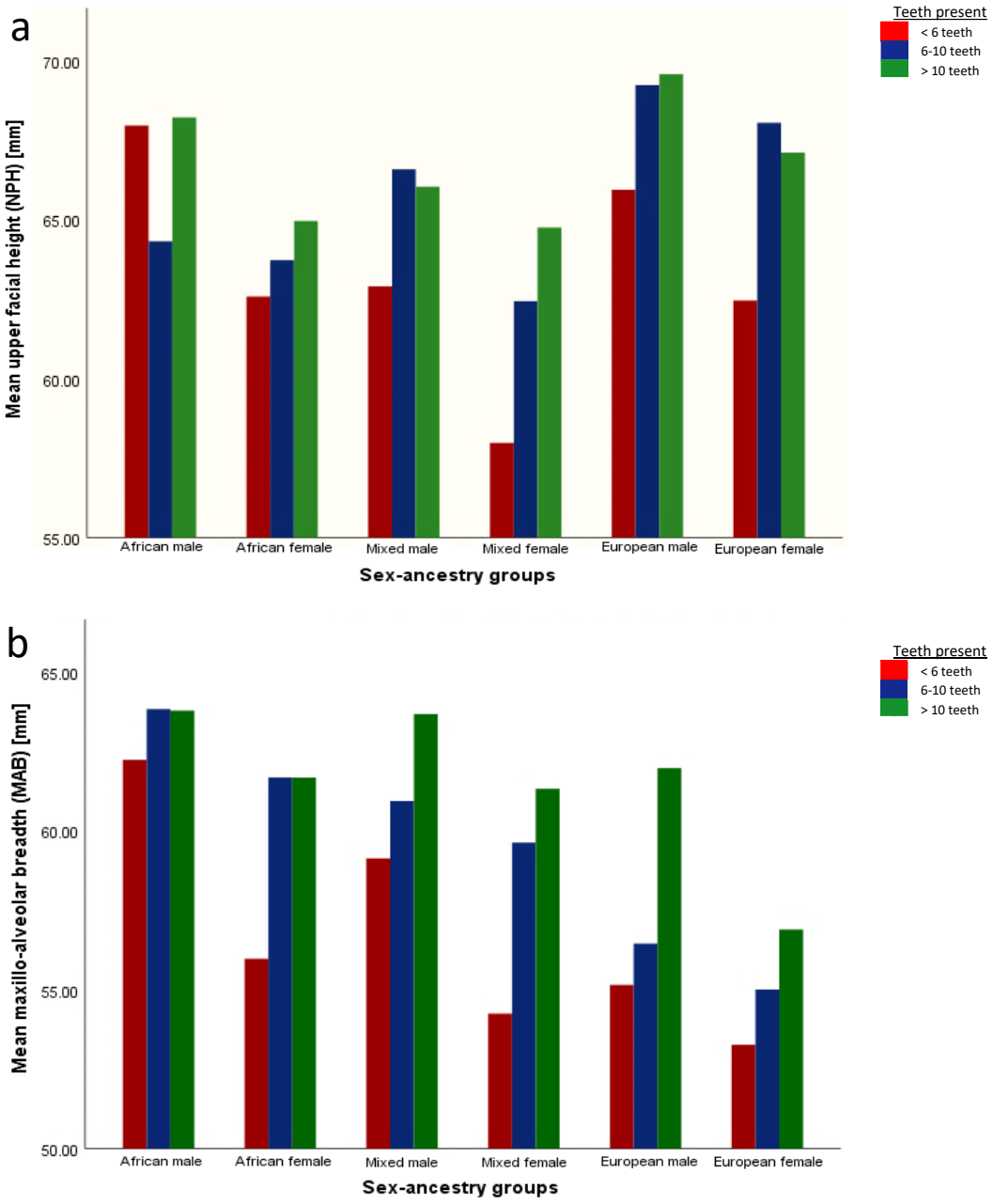

Figure 4.13. Differences in mean upper facial height and maxilla-alveolar breadth measurements between tooth loss categories, for sex-ancestry groups.

4.3.1.2.2. The effect of age on size

Orbital region

In MA males, OBHL increased relative to the increase in age at death (r=0.21; p=0.02), this relationship accounted for minimal variation in OBHL (R^2=0.05). In MA females, OBB size increased relative to the increase in age at death (r=0.24; p=0.04); this relationship accounted minimal variation in OBB (R^2=0.06). In EA females, DKB (r=-0.27; p=0.05) decreased relative to the increase in age at death, this relationship accounted for minor variation in DKB (R^2=0.03) and was only detected when fewer than 6 teeth were present (r=-0.36; p=0.05).

Maxillary region

In AA, EA and MA females MAB (r≥-0.26; p≤0.05) decreased relative to the increase in age at death, these relationships explained some of the variation in MAB (R^2≤0.12). These trends were only detected in females with fewer than 6 teeth present (r≥-0.39; p≤0.04).

4.3.1.3. Principal Component Analysis (PCA)

A series of PCA were performed to evaluate multivariate relationships between measurements for ancestry and sex-ancestry groups. No significant patterns of variation were detected for groups split by tooth loss and trauma (Appendix G: Figures a-c).

4.3.1.3.1. PCA between ancestry groups

A PCA produced 12 Principal Components (PCs) of which only PC 1 (λ=4.91) and PC 2 (λ=2.16) qualified for selection and accounted for 58.95% of the total observed variance in the sample. PC 1 accounted for 40.95% of the total variance and represented craniofacial size variation (Table 4.7).

When PC 1 and 2 scores were plotted, partial separation between AA and EA individuals occurred on PC 1, while MA individuals showed no significant separation from any group (Figure 4.14). PC 1 mainly represented variables pertaining to the width in the orbital, zygomatic, nasal and maxillary bones (ZMB, NLB, EKB, MAB, DKB, ZYB), as well as the anterior projection (BPL and BNL) of the mid-craniofacial region; implying that size variation in the sample is most significantly associated with these variables. Variable loading indicated that ZMB, NLB, EKB, MAB and DKB were most variant in size between ancestry groups (Table 4.7).

AA individuals were mostly in PC 1 (+), indicative that AA individuals have larger facial widths and more anteriorly projecting maxillary and nasal regions than EA individuals who were in PC 1 (-) (Figure 4.14). MA individuals were more heterogenous in facial width and anterior facial projection, as individuals fell in both negative and positive PC 1. Minor separation between AA and EA individuals occurred on PC 2, however, the MA group overlapped with both; therefore, there was no distinction between MA individuals and AA and EA individuals.

Table 4.7. Eigenvalue coefficients for the first principal component.

Measurements	Eigenvalue coefficients
ZMB	0.80
NLB	0.76
EKB	0.74
MAB	0.73
DKB	0.73
BPL	0.72
ZYB	0.70
BNL	0.42

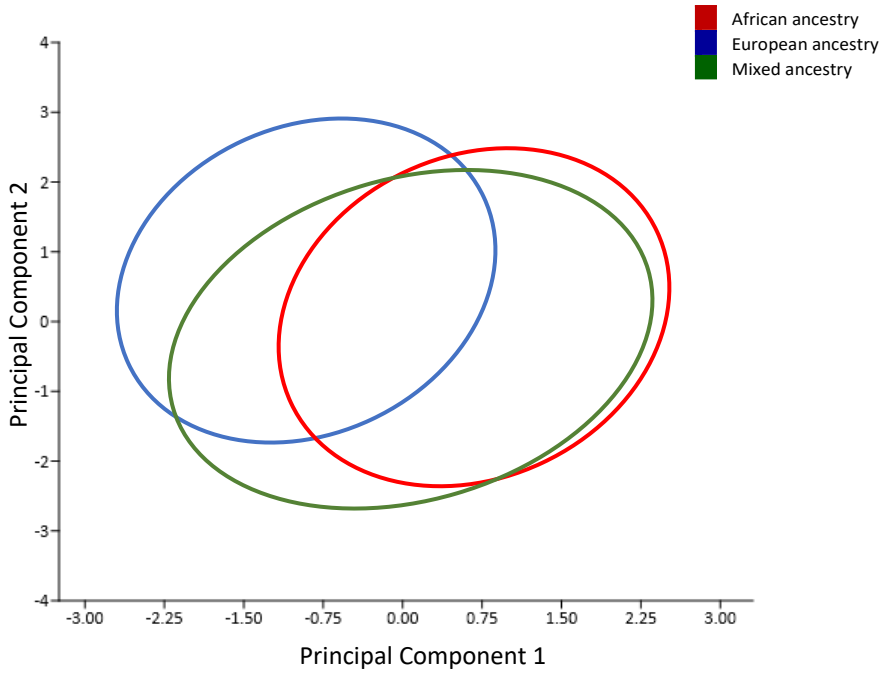

Figure 4.14. Scatter plot PC1 and PC2 scores for African, European and Mixed ancestry individuals, showing 95% confidence ellipses

4.3.1.3.2. PCA between sex-ancestry groups

When PC 1 and 2 scores were plotted for sex-ancestry groups, minor separation was evident between males and females of each ancestry and females had lower PC 2 scores than males (Figure 4.15). PC 2 accounted for 18% of the total variance and represented the variation in mid-craniofacial shape (Table 4.8). PC 2 mainly represented variables pertaining to breadth and height in the orbital region, breadth in the zygomatic region, height in the nasal and upper facial region and anterior projection of the maxilla. This implies that shape is most variable in these regions in the sample. Variable loading indicates that NLH, NPH, OBB and OBL are most significantly represented in shape variation in the sample (Table 4.8). Overall, shape and size in the mid-craniofacial region appear to be more heavily loaded in the orbital and nasal region of for both ancestry and sex-ancestry groups (Tables 4.7-4.8).

Table 4.8. Eigenvalue coefficients for the second principal component.

Measurements	Eigenvalue coefficients
NLH	0.85
NPH	0.76
OBB	0.69
OBH	0.67
BNL	0.59
EKB	0.36
ZYB	0.37

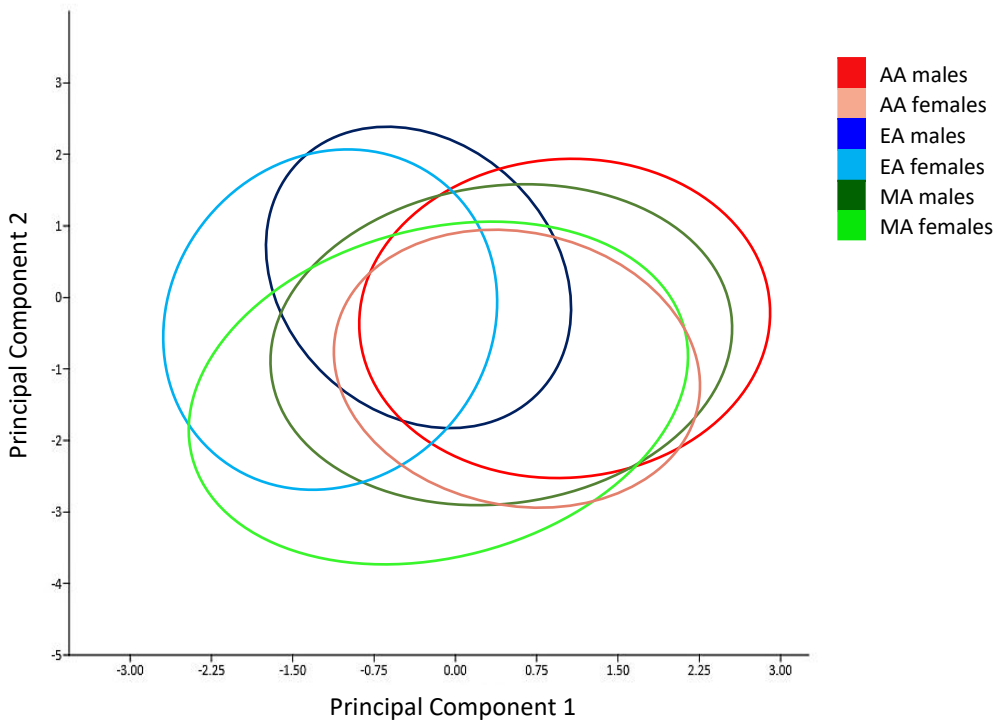

Figure 4.15. Scatter plot PC1 and PC2 scores for sex-ancestry groups, showing 95% confidence ellipses.

4.3.3. Ancestry estimation

Linear-stepwise discriminant function analyses, were performed to estimate ancestry from mid-craniofacial measurements (OBB, OHB, DKB, EKB, NPH, NLH, NLB, ZYM, ZMB, MAB, BNL, BPL).

4.3.3.1. Ancestry estimation in the total sample

Discriminant functions with strong associations between measurements and ancestry groups are given in Appendix H: Table 1 ($F \geq 0.47$; $X^2 \leq 39.75$; $p \leq 0.0001$). Function 1 which comprised measurements pertaining to the entire mid-craniofacial region yielded the highest average ancestry estimation accuracies (60.2%) (Table 4.9). Ancestry estimation accuracies from the zygomatic and maxillary regions were the lowest. Group centroids (Appendix H: Table 1) and ancestry estimation accuracies (Table 4.9), indicate that Functions 1-5 failed to distinguish between MA and AA individuals, and were more accurate in distinguishing between AA and EA individuals.

The most heavily weighted (i.e. ancestrally variant) measurements in Function 1 were NLB and DKB; while EKB and DKB were the most weighted in Function 2 (Appendix H: Tables 1 and 4). NLB and DKB measurements were most heavily weighted in Function 3 (nasal region) (Appendix H: Tables 1 and 4). Function 4 (zygomatic region), contained only ZMB, which suggests that ZYB (the other zygomatic measurement) was not significantly different between ancestry groups (Appendix H: Tables 1 and 4). In Function 5, ZMB was most heavily weighted variable (Appendix H: Table 1).

Table 4.9. Ancestry estimation accuracies from leave-one-out cross validations (LOOCV) of discriminant functions.

Functions	Ancestry estimation accuracies			
	Total (%)	**African (%)**	**Mixed (%)**	**European (%)**
Function 1: Total craniofacial region	**60.2**	60	**50**	**82.4**
Function 2: Orbital region	54.1	56.2	41.8	78.0
Function 3: Nasal region	58.7	**62.9**	47.4	78.0
Function 4: Zygomatic region	43.4	56.2	27.0	63.7
Function 5: Maxillary region	46.9	61.0	31.6	63.7
-Bold values represent the highest ancestry estimation ancestries for each column.				

4.3.3.2. Ancestry estimation in sex-pooled groups

Discriminant functions with strong associations between measurements and ancestry groups were generated for males ($F \geq 0.50$; $X^2 \leq 34.76$; $p \leq 0.0001$) and females, respectively ($F \geq 0.47$; $X^2 \leq 35.11$; $p \leq 0.0001$) (Appendix H: Tables 2-3). For males and females, Function 1 yielded the highest ancestry estimation accuracies (Table 4.10). Ancestry estimation accuracies were lowest in MA individuals. In MA males, the lowest accuracies were generated from the zygomatic region (15.9%), while the maxillary region generated the lowest accuracies in MA females (34.3%) (Figure 4.10). In agreement with findings in the total sample, functions generated for sex-ancestry groups did not effectively distinguish between MA and AA individuals, but more accurately distinguished between AA and EA individuals (Table 4.10). The DF for females yielded better ancestry estimation accuracies than those for the total sample, suggesting that sex-specific DFs may be better applied to females.

For both males and females, the most heavily weighted (ancestrally variant) measurement in Function 1 was NLB (Appendix H: Tables 2-3 and 5-6). Function 2 in females contained only DKB, suggesting other orbital measurements were less effective at differentiating between ancestry groups (Appendix H: Tables 2-3 and 5-6). For both males and females, the most heavily weighted measurements in Function 3 were NLB and DKB (Appendix: H: Tables 2-3). Similar to Function 4 for the total sample, Function 4 for males only included ZMB, while in females, both ZYB and ZMB were included (Appendix H: Tables 2-3 and 5-6). This suggests that ZYB is more ancestrally diverse in females. In females, Function 5 only included ZMB, in contrast to Function 5 in males, which included MAB and BPL and this suggests that MAB is a poor determinant of ancestry in the maxillary region in females, while ZMB, fails to differentiate between ancestry groups in males (Appendix A: Tables 2-3).

Table 4.10. Ancestry estimation accuracies from LOOCV of discriminant functions for males and females.

Function	Ancestry estimation accuracies							
	Total		**AA**		**MA**		**EA**	
	Males (%)	*Females (%)*	*Males (%)*	*Females (%)*	*Males (%)*	*Females (%)*	*Males (%)*	*Females (%)*
Function1: Total craniofacial region	**59.7**	**65.1**	53.6	61.2	**56.3**	**57.1**	**77.3**	**80.9**
Function 2: Orbital region	53.5	54.8	57.1	**65.3**	43.7	35.7	**77.3**	72.3
Function 3: Nasal region	53.5	**65.1**	58.9	61.2	46.0	**57.1**	68.2	**80.9**
Function 4: Zygomatic region	35.8	62.7	60.7	61.2	15.9	51.4	44.4	**80.9**
Function 5: Maxillary region	46.5	52.4	**67.9**	61.2	31	34.3	63.6	70.2

-Bold values represent the highest ancestry estimation ancestries for each column.

4.3.3.3. The effect of tooth loss on ancestry estimation

This study previously reported craniofacial size was significantly reduced when fewer than 6 teeth were present, therefore the ancestry estimation accuracies were evaluated in those with fewer than 6 teeth (Table 4.11). Functions 1-3 yielded greater ancestry estimation accuracies in those with fewer than 6 teeth, suggesting these DFs accounted for size differences which were associated with tooth loss (Table 4.11). The zygomatic and maxillary regions yielded the lowest ancestry estimation accuracies in those with fewer than 6 teeth. Due to issues of statistical power, classification accuracies associated with tooth loss were not assessed for sex-ancestry groups. Statistical limitations of DFA, prevented the effect of age at death on ancestry estimations from being determined.

Table 4.11. Ancestry estimations in those with fewer than 6 teeth present at death.

Functions	Ancestry estimation accuracies	
	Total (%)	**< 6 teeth present (%)**
Function 1: Total craniofacial region	**60.2**	**67.1**
Function 2: Orbital region	54.1	59.3
Function 3: Nasal region	58.7	57.9
Function 4: Zygomatic region	43.4	37.1
Function 5: Maxillary region	46.9	44.3
-Bold values represent the highest ancestry estimation ancestries for each column.		

4.3.4. Summary

AA individuals exhibited the widest orbital, zygomatic and nasal breadths, and shortest upper facial, orbital and nasal heights. In contrast, EA individuals exhibited the narrowest orbital, zygomatic and nasal breadths, and the longest upper facial, orbital and nasal heights. Diverse and heterogenous size variations were detected in MA individuals, who more frequently clustered with AA individuals. PCA revealed size differences between ancestry groups were most heavily associated with facial breadths and anterior projection of the maxilla. Interorbital and nasal breadths were the most ancestrally variant features in PCA. The highest ancestry estimation accuracies were yielded from DFs in the orbital, nasal and total mid-craniofacial regions. DFs more successfully estimated ancestry in AA and EA individuals, than MA individuals. Slight size differences in orbital, nasal and zygomatic measurements were detected between sex-ancestry groups. Ancestry estimations increased when the sample was split according to sex-ancestry. Tooth loss and age at death were associated with smaller measurements in the maxillary, nasal and orbital region.

4.4. Shape variation

4.4.1. Nonmetric analysis

Associations were detected between all nonmetric traits and ancestry groups (χ^2=78.79; p≤0.0001; v≥0.18; p≤0.0001). Frequency distributions were examined to determine which traits occurred most frequently in AA, EA and MA individuals (Appendix I: Tables 1-8). No significant associations were detected between sex and nonmetric trait occurrences (χ^2≤10.9; p≥0.10).

Orbital region

EA individuals primarily exhibited narrow and intermediate interorbital breadths (IOB) and AA individuals exhibited intermediate and wide IOB; while MA individuals exhibited intermediate to narrow IOB but showed no strong affinity towards any particular variant (Figure 4.16 a). Inferiorly angled (square) orbital shapes (OS) occurred with high frequency in EA individuals (87.9%). This trait also occurred with high frequency in MA and AA individuals, together with elongated (rectangular orbits) (Figure 4.16 b and Appendix I: Table 2). AA, EA and MA groups sampled in this study showed no particular affinity towards any specific supranasal suture (SS) shape variants (Figure 4.17a).

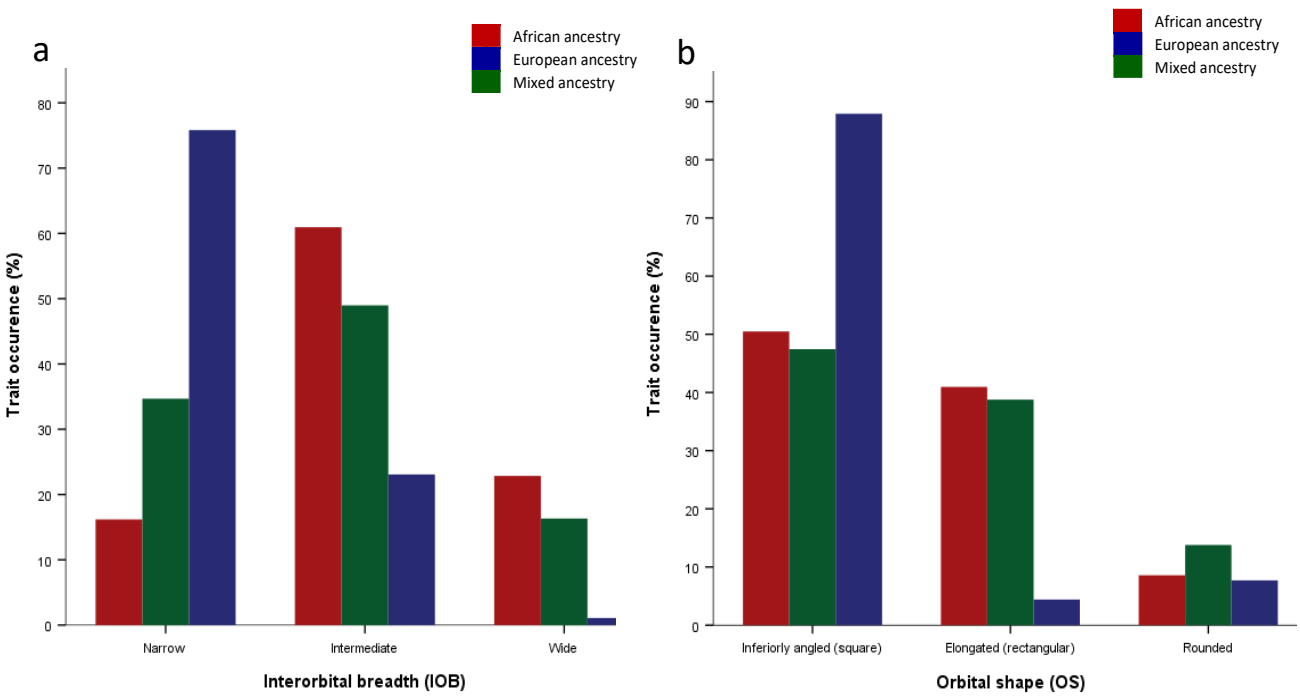

Figure 4.16. Frequency distributions of nonmetric trait variants in the orbital region for different ancestry groups. a. Interorbital breadth (IOB), **b**. Orbital shape (OS).

Nasal region

Oval and low/round nasal bone contours (NBC) were mainly seen in MA individuals; while steep, steepled and semi-triangular (vaulted) NBC were more frequently detected in EA individuals (Figure 4.17 b and Appendix I: Table 4). EA individuals had more narrow/bell-shaped NAW than AA individuals who had wider NAW. MA individuals primarily exhibited wide NAW however, some exhibited bell-shaped NAW (Figure 4.17c) (Appendix I: Table 5).

Zygomatic region

All three ancestry groups exhibited high frequencies of smooth ZS, while EA individuals also exhibited angled ZS with equal frequencies (Figure 4.17 d and Appendix I: Table 6).

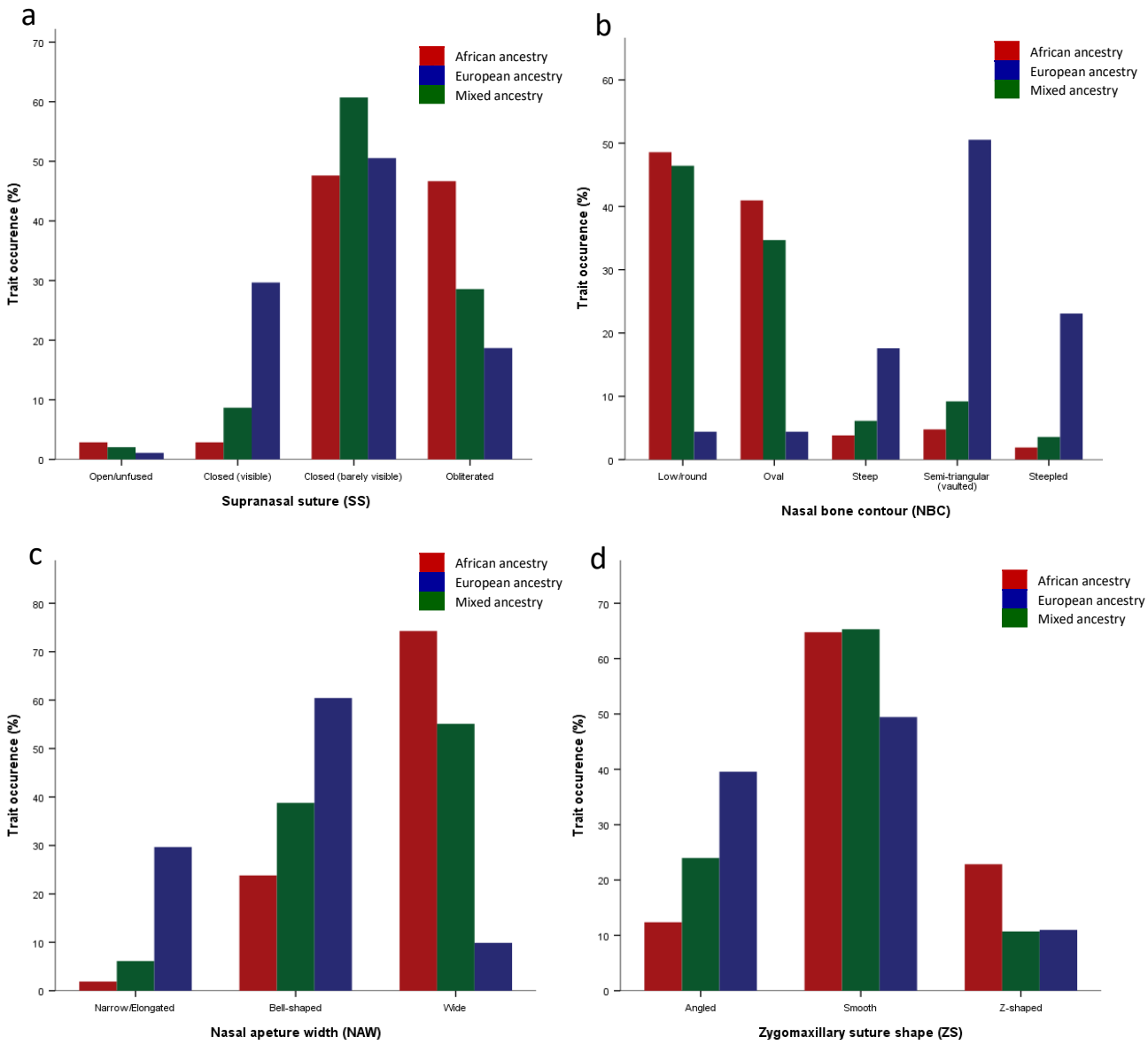

Figure 4.17. Frequency distributions of nonmetric trait variants in the orbital, nasal and zygomatic regions for different ancestry groups. a. Supranasal suture (SS), **b**. Nasal bone contour (NBC), **c**. Nasal aperture width (NAW), **d**. Zygomaxillary suture shape (ZS).

Maxillary region

EA individuals primarily had medium and long/sharp anterior nasal spines (ANS) and African individuals had short (rounded) and dull ANS. Similar frequencies of ANS variants were detected in MA individuals (Figure 4.18 b and Appendix L: Table 7). EA individuals exhibited high frequencies of orthognathism and AA individuals exhibited high frequencies of prognathism. MA individuals showed no affinity towards prognathism or orthognathism (Figure 4.18 a).

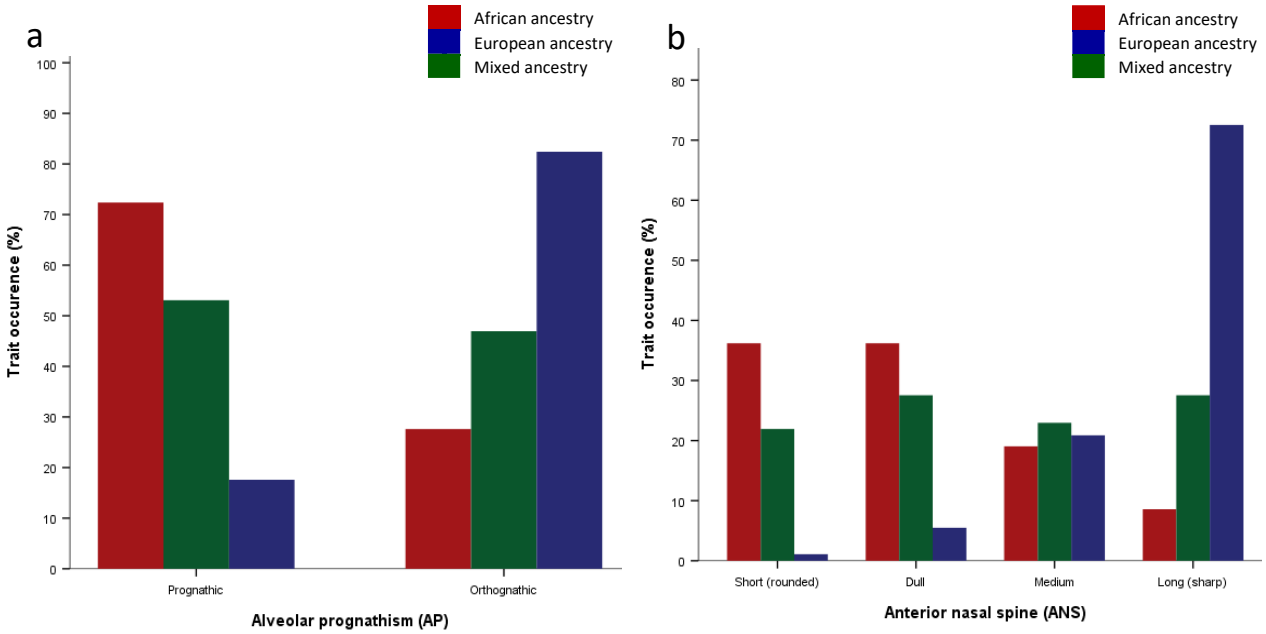

Figure 4.18. Frequency distributions of nonmetric trait variants in the maxillary region for different ancestry groups. a. Alveolar prognathism, **b**. Anterior nasal spine.

4.4.1.1. The effect of tooth loss on nonmetric traits

Significant associations between maxillary tooth loss and nonmetric traits were only observed in ANS ($p \leq 0.0001$) and AP ($p \leq 0.0001$). Long (sharp) ANS observed in each ancestry (AA (35.7%), EA (83.7%), and MA (39%)) were mainly detected in those with fewer than 6 teeth. Similarly, in assessments of AP, AA (63.6%), EA (89.8%) and MA (63.6%) individuals with fewer than 6 teeth exhibited orthognathic maxillary regions.

4.4.2. Geometric morphometric analyses

4.4.2.1. The influence of toothloss and age on craniofacial shape

To understand the effect of tooth loss and age at death on craniofacial shape, these variables were evaluated using variances pooled by sex, ancestry and sex-ancestry. These analyses were performed first ensure that they remained a consideration when interpreting craniofacial variation. The same variations associated with maxillary tooth loss and age at death were detected for sex, ancestry and sex-ancestry groups, and thus, only the results for ancestry groups were graphically represented.

4.4.2.1.1. The effect of maxillary tooth loss on shape

While a significant relationship between tooth loss and craniofacial shape was detected (p<0.0001), this relationship was minor and accounted for less than 3.5% of the variance observed in the sample. Regression scores became more positive as the number of teeth present increased, meaning that individuals with fewer teeth present, exhibited more negative regression scores (Figure 4.19). More EA individuals exhibited tooth loss and characteristic variations associated with this ancestry may have disproportionally influenced shape variations associated with tooth loss.

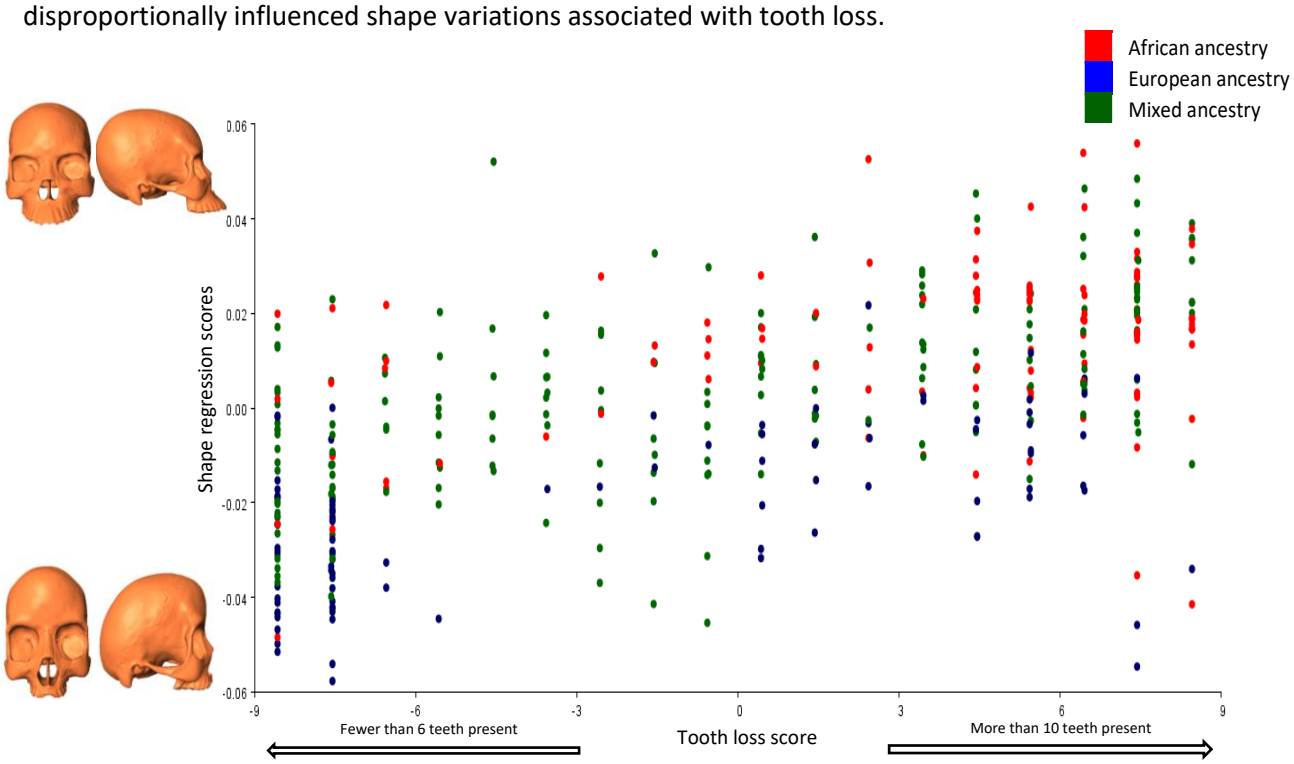

Figure 4.19. Multivariate regression analysis of the relationship between tooth loss and mid-craniofacial shape, with variances pooled by ancestry. 3D warped models of extreme craniofacial variation for positive regression scores (associated with more teeth present) and negative regression scores (associated with fewer teeth present).

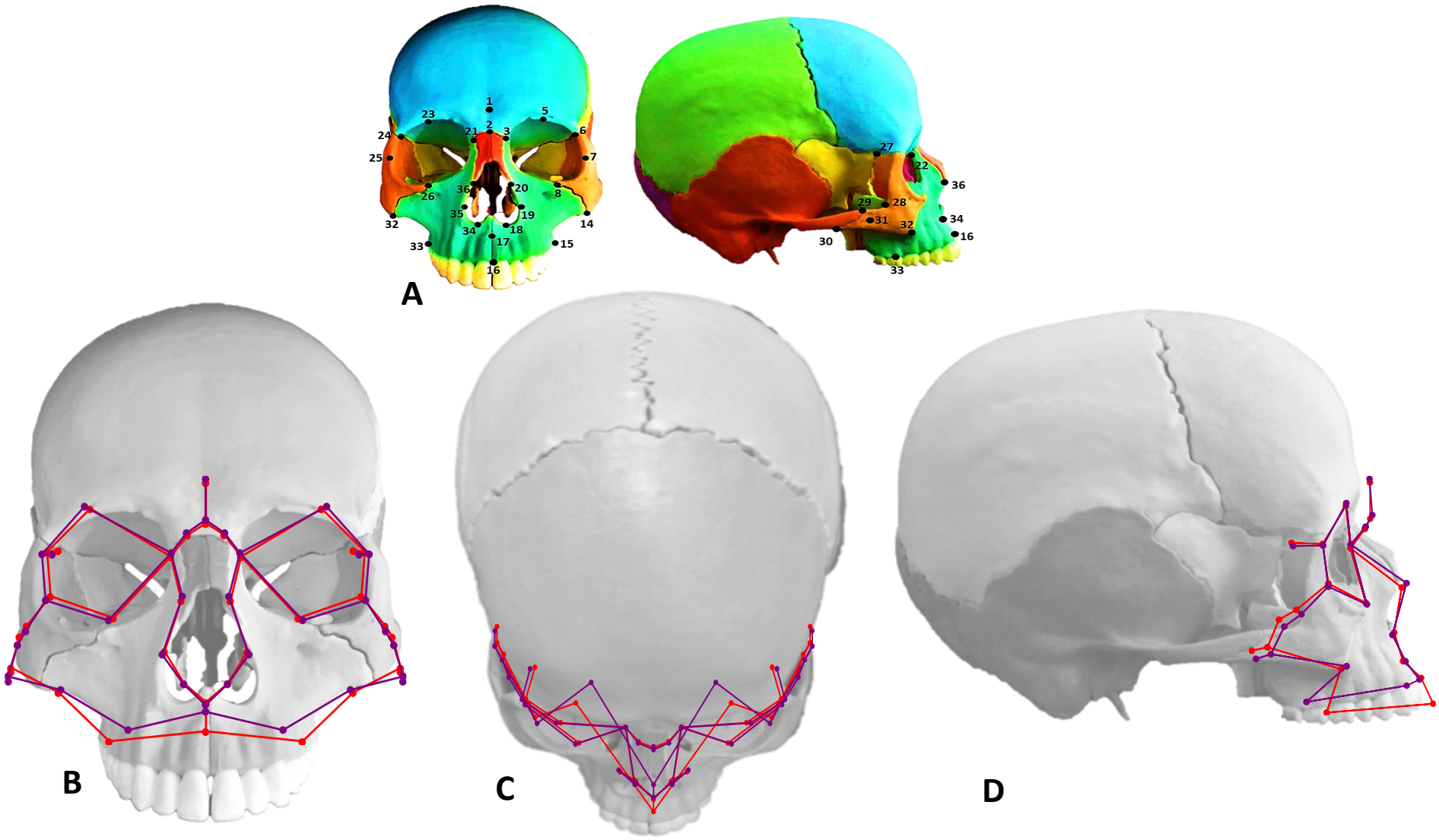

Figure 4.20. The relationship between tooth loss and mid-craniofacial shape. Mean shape of those with fewer teeth in purple; mean shape of those with more teeth in red. A. Illustrations of landmark locations are included and correspond to definitions in Table 3.3. Views of shape change from regression analyses include: B. Anterior; C. Superior and D. Lateral. [40X magnification of differences for visualisation]
[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

Shape variations associated with tooth loss are illustrated in Figure 4.20 and Appendix J: Figure 1. Those with fewer teeth exhibited narrower and more concaved maxillary regions and slightly wider orbital and zygomatic regions (Figure 4.20). More superiorly located glabella and nasion landmarks; and medial and superiorly located dacryon landmarks were associated with the presence of fewer teeth (Figure 4.20 and Appendix J: Figure 1). The zygomatic region was more inferiorly positioned while the maxillary region was more superiorly located, meaning that the facial lengths would have been shorter in those with fewer teeth (Figure 4.20). The alveolar region of the maxilla was more orthognathic in individuals with fewer teeth present.

4.4.2.1.2. The effect of age at death on shape

Significant relationships were detected between age at death and craniofacial shape ($p \leq 0.0001$) and this relationship explained less than 2.1% of the shape variation in the sample. Regression scores were more positive as age at death increased (Figure 4.21). Shape variations are illustrated in Figures 4.21-4.22 and Appendix J: Figure 2. As age at death increased, frontomalare temporalis landmarks were more posterior and inferiorly located, while zygion landmarks were more anterior, inferior and laterally located. Zygomatic regions appeared slightly wider as age at death increased. Variations associated with age at death were most evident in the maxillary region (Figure 4.22). As age at death increased, narrower and more posteriorly positioned the maxillary regions were noted (Figure 4.22). Overall, age at death contributed very little to the total observed craniofacial variation in this sample.

Figure 4.21. Multivariate regression analysis of the relationship between age and mid-craniofacial shape, with variances pooled according to ancestry. 3D warped models of extreme craniofacial variation for positive regression scores (associated with more teeth present) and negative regression scores (associated with fewer teeth present).

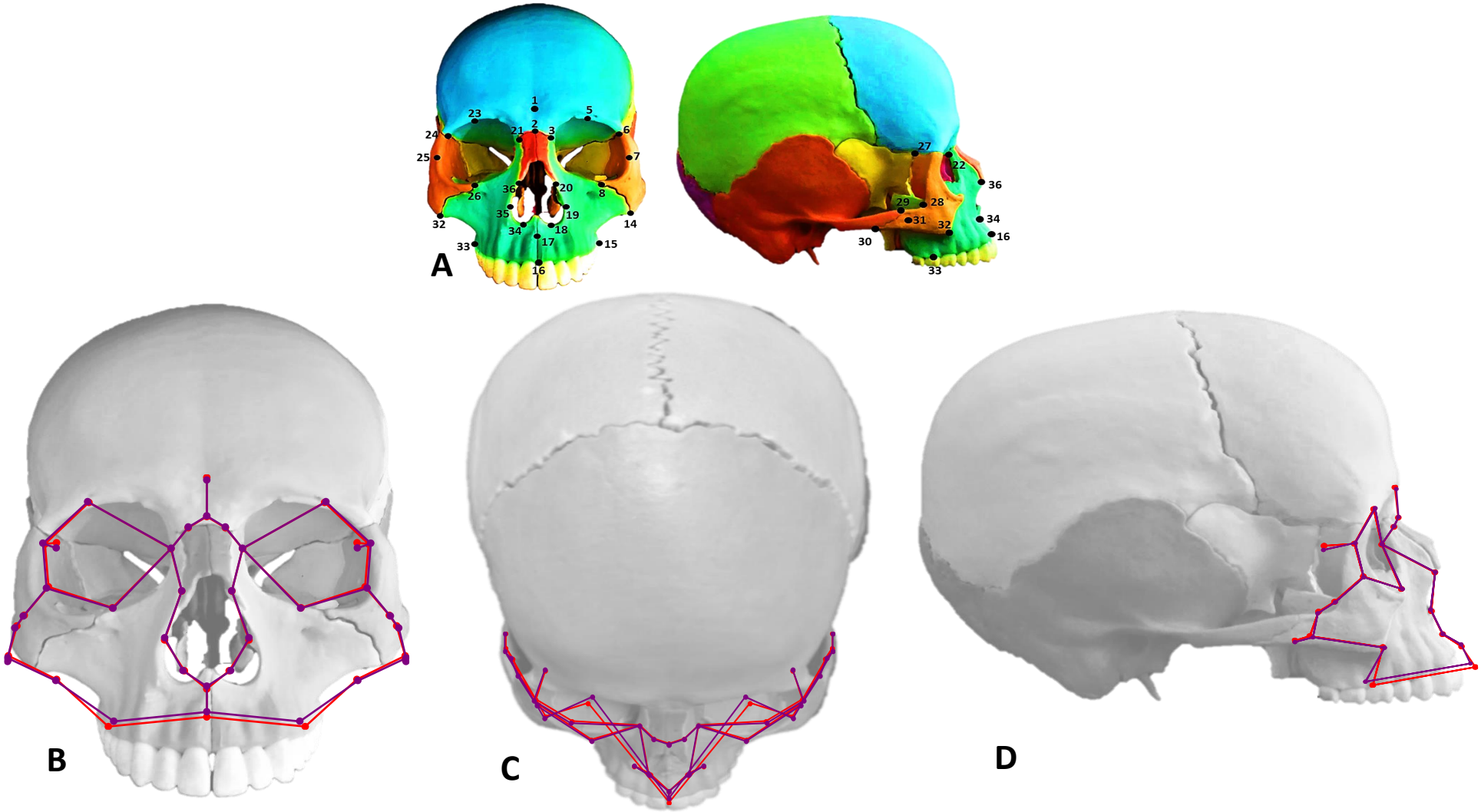

Figure 4.22. The relationship between age at death and mid-craniofacial shape. Mean shape of those with higher ages at death in purple; mean shape of those with lower ages at death in red. A. Illustrations of landmark locations are included and correspond to definitions in Table 3.3. Views of shape change from regression analyses include: B. Anterior; C. Superior and D. Lateral.

[40X magnification of differences for visualisation]
[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

4.4.2.2. Shape variation between sex groups

Differences between males and females were assessed first to determine whether differences seen between sex-ancestry groups were consistent with those seen between sex-groups. A PCA of size corrected Procrustes residuals (using pooled within-sex variances) produced 52 PCs, of which only PC 2 showed slight separation between the sexes and accounted for 10.9% of the total observed variance in the sample (Figure 4.23).

Shape differences between the sexes are illustrated in Figure 4.24 and Appendix J: Figure 3. Males exhibited shorter facial heights; in addition to more anteriorly projecting upper faces and posteriorly projecting maxillary regions (Figure 4.24). Males exhibited less anteriorly projecting nasal apertures and zygomas. Additionally, wider facial breadths, despite narrower nasal apertures were detected in males, while females exhibited rounder and shorter nasal apertures (Figure 4.24). Nasal apertures comprised a larger proportion of mid-craniofacial height, but a smaller proportion of the midfacial width in males (Figure 4.24).

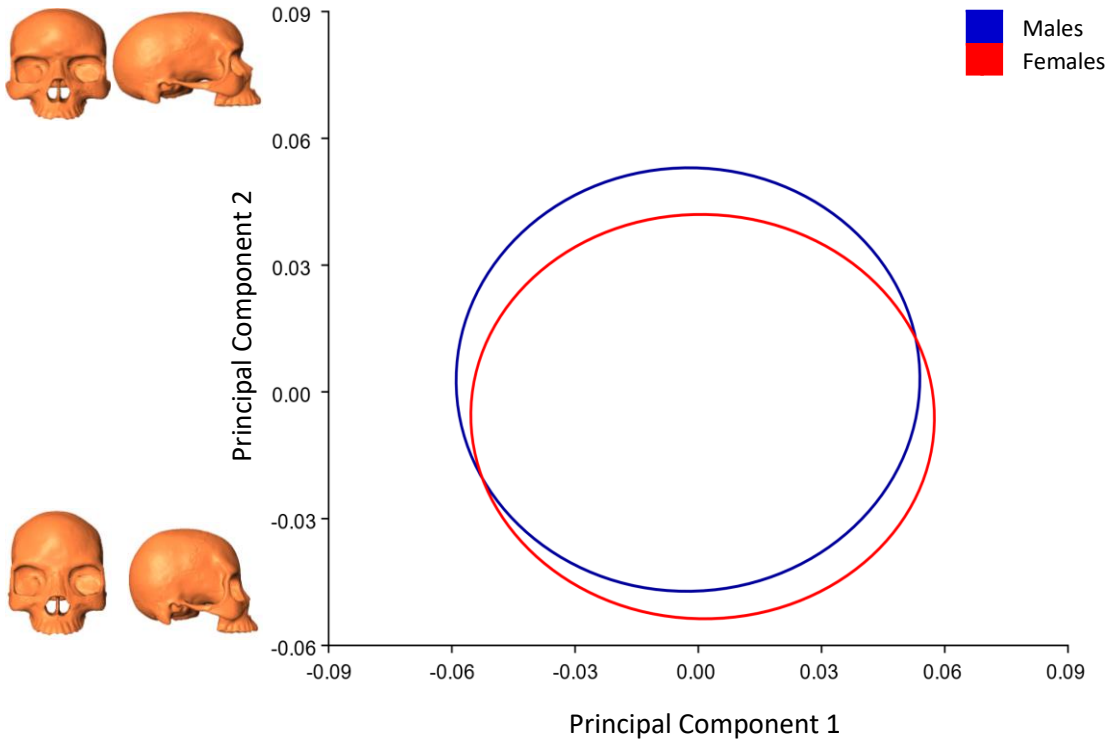

Figure 4.23. Plot of PC 1 and 2 for PCA of mid-craniofacial shape variation showing 90% confidence ellipses for males and females. 3D warped models of extreme craniofacial variation for PC scores (males) and negative PC scores (females) are included.

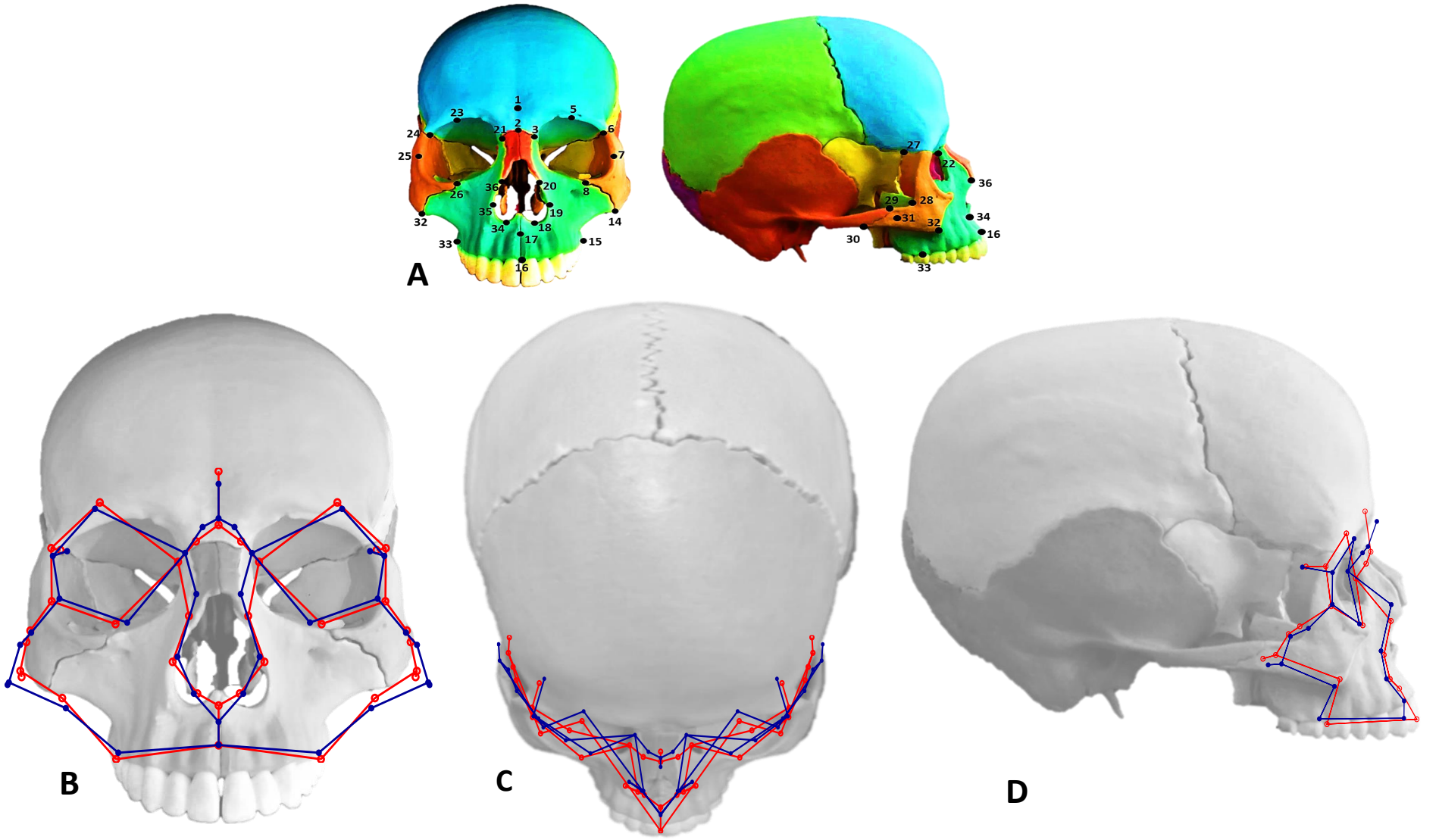

Figure 4.24. Mid-craniofacial shape variation between males and females. Mean shape of males in blue; mean shape of females in red. A. Illustrations of landmark locations are included and correspond to definitions in Table 3.3. Views of shape change from PCA include: B. Anterior; C. Superior and D. Lateral. [5X magnification of differences for visualisation]

[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

4.4.2.3. Shape variation between ancestry groups

A CVA produced 2 CVs, which both showed significant separation of ancestry groups (p<0.0001). CV1 accounted for 72% of the observed variance and separated EA individuals from AA and MA individuals. The CV2 accounted for 28% of the observed variance and separated AA individuals from MA individuals (Figure 4.25).

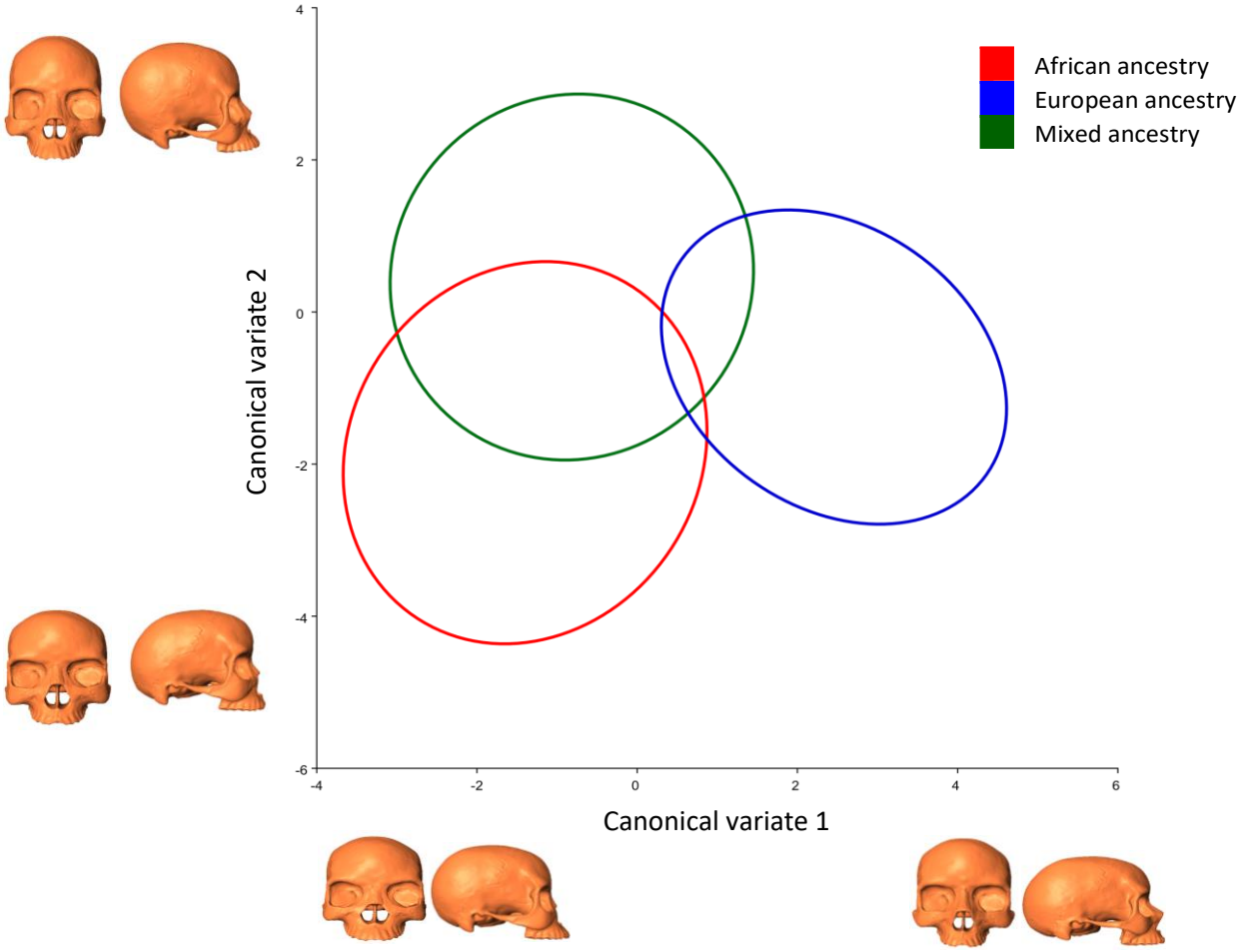

Figure 4.25. CVA of total craniofacial shape variation, showing CV 1 and 2 and 90% confidence ellipses for African, European and Mixed ancestries. 3D warped models of extreme craniofacial variation for CV1 and CV2 are included. African ancestry in red, European ancestry in blue, Mixed ancestry in green

Differences on CV 1

Shape differences between EA, MA and AA individuals are illustrated in Figure 4.26 and Appendix J: Figure 4. EA individuals exhibited narrower facial breadths and longer facial heights than AA and MA individuals. EA individuals had narrower interorbital and orbital breadths, longer orbital heights and more diagonally angled inferior orbital margins (Figure 4.26). In EA individuals, more inferior and anterior nasal bridges, which projected forward beyond the nasal aperture and more posterior, narrow and elongated nasal apertures were detected (Figure 4.26).

Compared to AA and MA individuals, EA individuals also exhibited more superior and posterior frontomalare temporalis landmarks and more posterior, superior and medial jugale landmarks (Appendix J: Figure 4). Narrower zygomatic breadths, less anterior and superior-inferiorly elongated zygomas were detected in EA individuals, compared to AA and MA individuals (Figure 4.26). Relative to MA and AA individuals, EA individuals exhibited more orthognathic maxillae, narrower maxillary breadths, more anteriorly projecting anterior nasal spines. AA and MA individuals has larger distances between the anterior nasal spine and anterior alveolar maxillary margin (prosthion) (Figure 4.26).

Differences on CV 2

Shape differences between the AA individuals and MA individuals (CV 2) are illustrated in Figure 4.26 and Appendix J: Figure 5. Fewer shape differences were detected between AA and MA groups than between EA individuals, and these two ancestry groups (Figure 4.26). Compared to MA individuals, AA individuals exhibited slightly narrower, more posteriorly sunken orbits. AA individuals had more rounded nasal apertures and slightly wider and more anteriorly projecting inferior nasal bridges than MA individuals (Figure 4.26). AA individuals had slightly wider zygomas than MA individuals. AA individuals also exhibited slightly narrower maxillary breadths, more elongated maxillary lengths, less anteriorly projecting anterior nasal spines and more anteriorly projecting maxillae than MA individuals (Figure 4.26).

Compared to AA and MA individuals, EA individuals exhibited narrower interorbital distances and orbital margins. Larger distances between dacryon and infranasion landmarks were detected in AA and MA individuals (Appendix J: Figure 5). EA individuals also had more elongated and posteriorly located orbits.

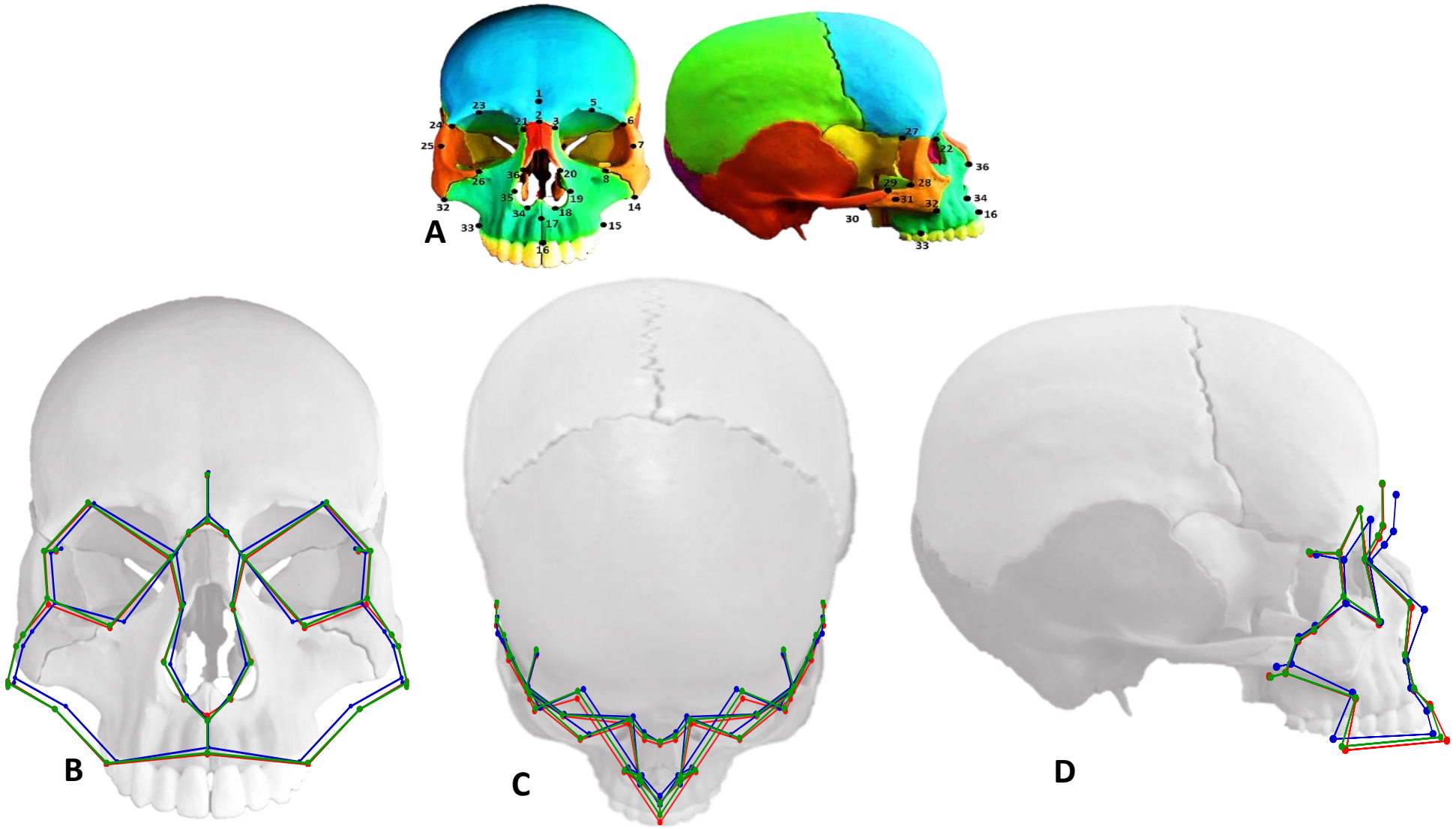

Figure 4.26. Mid-craniofacial shape variation between European, Mixed and African ancestry groups. Mean shape of African ancestry group in red; mean shape of Mixed ancestry groups in green and mean shape of European ancestry group in blue. A. Illustrations of landmark locations are included and correspond to definitions in Table 3.3. Views include: B. Anterior; C. Superior and D. Lateral. [5X magnification of differences for visualisation]
[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

4.4.2.4. Shape variation between sex-ancestry groups

A CVA of Procrustes residuals, produced 5 CVs of which only CV 1 and 2 produced significant separation of sex-ancestry groups ($p \leq 0.0001$). CV 1 accounted for 59.2% of observed variance and separated individuals according to ancestry groups (Figure 4.27). CV 2 accounted for 23.7% of the observed variance and primarily separated males from females, within each ancestry group (Figure 4.27). Shape differences are illustrated in Figure 4.28.

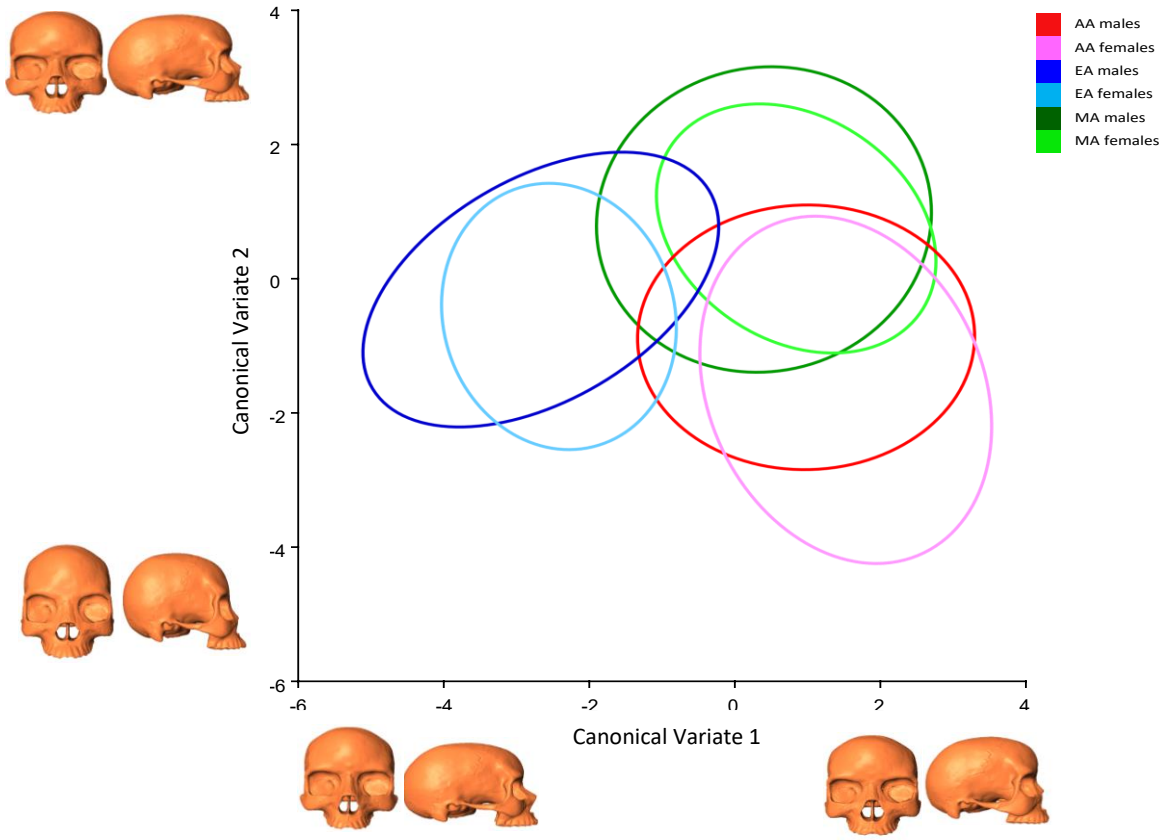

Figure 4.27. CVA of mid-craniofacial shape variation, between sex-ancestry groups, showing CV1 and 2, and 90% confidence ellipses for sex-ancestry groups.

Differences detected on CV 1 between sex-ancestry groups were identical to those reported between ancestry groups (Figure 4.26). On CV 2 poor separation was detected between EA males and females (p=0.22) and MA males and females (p=0.21). Differences between AA males and females were similar to those reported between sex groups. AA males exhibited shorter facial heights; in addition to more anteriorly projecting upper faces and posteriorly projecting maxillary regions (Figure 4.27). Wider facial breadths and narrower nasal apertures were detected in AA males, while AA females exhibited rounder and shorter nasal apertures (Figure 4.27). In AA males, nasal apertures comprised a larger percentage of craniofacial height, but a smaller percentage of the craniofacial width (Figure 4.27).

4.4.3. Ancestry estimation

4.4.3.1. Ancestry estimation from frequency distributions of nonmetric traits

Ancestry estimations were performed using seven nonmetric traits (SS was excluded due to a lack of standards). Despite work by van Rooyen (2010) in South African populations, standards for trait associations have only been validated for African, European and Asian ancestry groups (Hefner, 2003; Byers, 2015). Therefore, ancestry was only estimated for AA and EA individuals, while MA individuals were evaluated to determine in which groups (African, European or Asian) these individuals would classify

4.4.3.1.1. Ancestry estimation in the total sample

EA individuals exhibited higher estimation accuracies (71%) than AA individuals (60.7%) (Table 4.12). More MA individuals classified as African (39.8%) and Asian (25.5%) ancestry than European (16.3%) ancestry (Table 4.12). The maxillary region yielded the highest average ancestry estimation accuracy (71.1%), followed by the nasal (62.6%) and orbital regions (60.6%) (Appendix I: Table 9). The zygomatic region (11.7%) yielded the poorest ancestry estimation accuracies.

NBC, ANS, IOB and AP were the best determinants of ancestry in EA individuals whereas NAW and AP performed best in AA individuals (Appendix I: Table 9). Overall, the least reliable traits for ancestry estimation were ZS, OS and IOB (Appendix I: Table 9). Zygomaxillary suture shape (ZS) variants associated with Asian ancestry groups were seen with high frequencies in AA, EA and MA individuals, suggesting this trait may not be ancestrally variant in South African individuals. All ancestry groups exhibited orbital shapes associated with European ancestries (Appendix I: Table 9). Heterogenous ancestral variations were detected in MA individuals who classified most frequently as African (NBC, NAW and AP), then Asian (ANS, ZS and IOB) and European (OS) (Appendix I: Table 9).

Table 4.12. Ancestry estimations using 7 nonmetric traits.

International ancestry estimation categories [a]	**Classification frequencies for ancestry groups**[b]		
	AA (%)	**MA (%)**	**EA (%)**
African	**60.7**	**39.8**	2.2
European	5.7	16.3	**71**
Asian	5.7	25.5	4.4
Indeterminate	27.6	18.4	15.4

- Bold values represent the likely ancestry estimations, based on the highest trait frequencies.
a) Nonmetric traits and ancestry estimation standards by Hefner (2003) and Byers (2015).
b) Frequencies computed for ancestry groups in this study.

4.4.3.1.2. Ancestry estimations in sex-pooled groups

For AA and EA individuals, ancestry was more reliably estimated in females than males (Table 4.13). NBC variations associated with Asian and African ancestry groups were more frequently detected in AA individuals (Appendix I: Table 10). EA females had high frequencies of NAW variations associated with Asian ancestry groups (Appendix I: Table 10).

MA males and females classified most frequently as African from assessments of NBC, NAW and AP; as European in OS and Asian in ANS and ZS (Table 4.13 and Appendix I: Table 1). IOB trait variants frequently observed in MA individuals were associated with European and Asian ancestry groups, while MA males classified more frequently as Asian (Appendix I: Table 11). Although no significant associations between trait variants and sex were noted, certain traits appear to be reliable in ancestry estimations in males (NBC and IOB) than females (Appendix I: Table 11).

Table 4.13. Classification frequencies in AA, EA and MA males and females, using frequency distributions.

International ancestry estimation categories for nonmetric traits [a]	**Classification frequencies for sex-ancestry groups** [b]					
	AA (%)		**MA (%)**		**EA (%)**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*
African	**57.1**	**65.3**	**40.5**	**38.6**	2.3	2.1
European	5.4	6.1	15.6	17.9	**79.5**	**76.6**
Asian	6.8	4	25.5	25.7	4.5	4
Indeterminate	30.7	24.6	13.7	17.3	18.3	18.6
-Bold values represent the most probable classification						

4.4.3.2. Ancestry estimation from multinomial logistic regression analyses of nonmetric traits

Owing to the relationship between tooth loss, ANS and AP; the number of teeth present was included as a co-variate in the backwards-stepwise multinomial logistic regression (MLR) model applied to eight nonmetric traits.

4.4.3.2.1. Ancestry estimation in the total sample

The most statistically significant nonmetric traits for assessing ancestry were NBC, NAW, ANS, SS and OS (Appendix I: Tables 11 and 12). This model had an accuracy of 54.3% in AA, 76% in MA and 82.4% in EA individuals (Table 4.14). Overall, the MLR model yielded greater ancestry estimation accuracies, suggesting that nonmetric data is best assessed using multivariate statistical modelling.

Table 4.14. Total ancestry estimation accuracies from univariate and multivariate analyses of nonmetric assessments

Ancestry groups	Ancestry estimation accuracies	
	Frequency distributions (%)[a]	**MLR (%)[b]**
AA	60.7	54.3
MA	NA	76
EA	71	82.4

a- Calculated using univariate analyses
b- Calculated using multivariate analyses

4.4.3.2.2. Ancestry estimation in sex-pooled groups

In males, the most statistically significant nonmetric traits for assessing ancestry were NBC, NAW, ANS, SS and OS (Appendix I: Table 13). This model had an accuracy rate of 57.1% in AA, 79% in MA and 84.1% in EA males (Table 4.15). In females, the most statistically significant variables in estimating ancestry were NBC, NAW and AP (Appendix I: Table 14). The formula generated for females yielded accuracies of 63.3% in AA, 68.6% in MA and 87.2% in EA individuals (Table 4.15). This suggests that knowing the sex of an individual before ancestry estimation could significantly improve estimation accuracy and may require the implementation of a different formula.

Table 4.15. Ancestry estimation accuracies for males and females using frequency distributions and multinomial logistic regression

Sex-ancestry groups	**Ancestry estimation accuracies**	
	Frequency distributions (%)[a]	**Regression model (%)[b]**
African ancestry males	57.1	57.1
African ancestry females	65.3	63.3
Mixed ancestry males	NA	79
Mixed ancestry females	NA	68.9
European ancestry males	79.5	84.1
European ancestry females	76.6	87.2

a- Calculated using univariate analyses
b- Calculated using multivariate analyses

4.4.3.3. Ancestry estimation using geometric morphometrics

Ancestry estimations from three-dimensional shape data were performed using pairwise DFA of size corrected Procrustes residuals for the total sample and sex-ancestry groups. Ancestry estimations were performed for each component of the mid-craniofacial region (orbital, nasal, zygomatic and maxilla). Owing to the small effect of age at death on craniofacial shape, it was decided not to analyse the effect of this variable. The influence of tooth loss on ancestry estimations was assessed by correcting for the effect of this variable on shape variation, and then re-assessing the data using DFA.

4.4.3.3.1. Ancestry estimations in the total sample

Total craniofacial analyses yielded the highest ancestry estimation accuracies, followed by the maxilla region, orbital region, nasal region and lastly zygomatic region (Table 4.16). Comparison of Mahalanobis distances between ancestry groups revealed that AA and EA groups were most different from each other ($p \leq 0.0001$), while and MA and AA groups were most similar ($p \leq 0.01$) (Appendix K: Tables 1 and 2). EA and MA individuals were most similar in the zygomatic and maxillary shape yielding, highest ancestry estimation accuracies (Appendix K: Table 1). AA and MA individuals were most similar in the nasal and orbital regions, yielding the lowest accuracies (Appendix K: Table 1) ($p \geq 0.08$). MA individuals yielded the lowest ancestry estimation accuracies in all regions except the nasal and zygomatic bones (Table 4.16).

Table 4.16. Highest ancestry estimation accuracies from leave-one-out cross validations (LOOCV) of classification accuracies from pairwise comparisons.

Craniofacial regions	Ancestry estimation accuracies (%)			
	Total	**AA**	**MA**	**EA**
Total	**93.8**	92.3	**91.3**	**97.8**
Orbital	87.9	88.6	86.2	89.0
Nasal	83.8	88.6	82.7	80.2
Zygomatic	81.3	80	83.7	80.2
Maxilla	86.0	**93.3**	82.3	92.3

-Bold values represent the highest ancestry estimation ancestries for each column.

4.4.3.3.2. Ancestry estimations in a sex-pooled groups

Females yielded higher ancestry estimation accuracies, than males (Table 4.17 and Appendix K: Tables 4-5). LOOCV tests yielded significant differences in all craniofacial elements, except the orbital region for males and the nasal region in females ($p=0.08$). AA and EA individuals were most different from each other while and MA and AA males most similar as seen in Appendix K: Tables 3-10 ($p \leq 0.01$). The highest ancestry estimation accuracies were from maxillary and total craniofacial analyses. The lowest ancestry estimation accuracies were detected between AA and MA males in the orbital and nasal region, and these groups were more morphologically similar in this region (Table 4.17). MD indicate that MA individuals were less morphologically different from EA individuals than AA individuals (Appendix K: Table 6)

Table 4.17. Highest ancestry estimation accuracies from LOOCV of classification accuracies from pairwise comparisons of sex-ancestry groups.

Craniofacial regions	Ancestry estimation accuracies (%)							
	Total		AA		MA		EA	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Total	79.6	89.8	78.6	81.6	87.3	**94.3**	72.8	93.6
Orbital	**83.3**	87.9	76.8	85.7	**89**	88.6	84.1	89.4
Nasal	78	84.5	80.4	87.8	78.6	87.1	75	78.7
Zygomatic	80.3	79.2	80.4	77.6	78.6	85.7	81.8	74.5
Maxilla	86.3	**96**	**87.5**	**95.7**	84.9	**94.3**	**86.4**	**97.9**
-Bold values represent the highest ancestry estimation ancestries for each column.								

4.4.3.3.3. The effect of tooth loss on ancestry estimation between ancestry groups

Once tooth loss was corrected for using multivariate regression of Procrustes shape on tooth loss, ancestry estimation accuracies improved in the orbital region (for AA individuals); nasal region (for AA and MA individuals), maxillae (all ancestries) and zygomatic regions (for EA individuals) (Table 4.18). Ancestry estimation accuracies from total craniofacial analyses decreased once tooth loss was corrected for. Comparison of Mahalanobis distances before and after correcting for tooth loss, only yielded different associations between ancestry groups in total craniofacial shape, where MA and EA individuals were deemed more similar than MA and AA individuals (Appendix N: Table 8).

Table 4.18. Highest ancestry estimation accuracies from LOOCV of classification accuracies from pairwise comparisons after correcting for tooth loss.

Craniofacial regions	Ancestry estimation accuracies for ancestry groups (%)		
	AA (%)	MA (%)	EA (%)
Total	91.4	89.3	93.4
Orbital	88.6	85.7	85.7
Nasal	89.5	82.1	79.1
Zygomatic	79	89.3	93.2
Maxilla	91.4	83.2	80.2

4.3.4. Summary

While similar shape variations were detected using nonmetric and geometric morphometric methods, more shape differences associated with tooth loss and age at death were detected using geometric morphometrics. AA individuals exhibited wider interorbital, nasal, zygomatic and maxillary regions. Rounder nasal apertures and less anteriorly projecting nasal bones were detected in AA individuals. EA individuals exhibited narrower and less anteriorly projecting zygomatic, nasal, zygomatic and maxillary regions. Inferiorly-angled orbits and anteriorly projecting nasal bridges were detected in EA individuals. MA individuals exhibited diverse shape variations, with features similar to EA (orbital and maxillary shape) and AA individuals (nasal and zygomatic shape). Shorter craniofacial heights, and more orthognathic and concave maxillae were associated with tooth loss. Ancestry estimation was most accurate in EA and AA individuals and MA individuals had the lowest accuracies, due to heterogenous shape variation in this group. Orbital and nasal regions yielded the highest ancestry estimation accuracies for males, while the maxilla yielded the highest ancestry estimation accuracies for females. Correcting for tooth loss improved ancestry estimation accuracies, indicative that tooth loss influences ancestral variation in craniofacial shape.

Chapter Five: Discussion

While it is acknowledged that the inclusion of individuals with minor/healed nasal and zygomatic trauma may have impacted the findings of this study, a detailed assessment of the impact of trauma on mid-craniofacial variation did not form the main body of work of this study. Therefore, where trauma may be relevant in understanding mid-craniofacial variations detected in the sample, results reported in Appendices E and F will briefly be discussed.

5.1. Limitations of this study

5.1.1. Demographic biases at skeletal collections

Due limitations of the skeletal collections used in this study, more individuals of Mixed ancestry (MA) than African ancestry (AA) or European ancestry (EA) were sampled. This reflects demographic bias at the primary collections sampled in this study (the University of Cape Town Human Skeletal Collection and the Kirsten Collection). Most EA individuals sampled were older than 65 years, while AA and MA individuals were between the age of 25 and 60. Da Silva (2006) found that most individuals of African (AA) and Mixed ancestry (MA) donated to the University of Cape Town were unclaimed at the time of death, while individuals of European ancestry (EA) were largely bequeathed by themselves or their families. Komar and Grivas (2008) cite a study by Wilson *et al*., (2007) which showed that individuals who donated their bodies prior to death were generally older at death and came from better socioeconomic circumstances than those whose bodies are donated by family members or a medicolegal authority subsequent to their death. Based on this pattern of variation and the acquisition practices at skeletal collections, it is possible that crania from those of AA and MA were from unclaimed individuals (possibly from a lower economic background), while crania from EA individuals were likely bequeathed by themselves or their families (representing individuals from a higher economic background). This information could not be confirmed in accession registers, however, if this was the case, differences seen between different ancestry groups may not be representative of inherent differences between ancestry groups but rather age and socio-economic disparities. Furthermore, it is acknowledged that this sample may not represent the full extent of variation within each ancestry group, but rather a small group of individuals within each group. What is problematic is that these biases can neither be identified nor quantified in individuals and therefore, caution is required when interpreting variation.

Most crania sampled from UCT skeletal collection and the Dart collection were from individuals who lived in the early-mid 20th century, while those from the Kirsten Collection were primarily from the late 20th century. Individuals born prior to the 1900s were excluded from this study as they may have not provided an all-encompassing representation of variation in a modern South African population. Using individuals from these collections to develop standards for contemporary populations requires the assumption of uniformitarianism, which states that the forces and processes that shaped variation in the past are still active in contemporary populations (Cameron, 1993). This theory is flawed in its application to human variation in South Africa due to the influence of social, cultural, historical and political forces, which affected population movement and admixture. No secular trends in craniofacial morphology have been detected (Moore-Jansen, 1989; Jantz and Meadows Jantz, 2000; Weisensee and Jantz, 2011) and this suggests that factors other than age at death are more central in shaping mid-craniofacial variation.

5.1.2. Including individuals with craniofacial trauma

Due to the unequal representation of crania from various ancestry groups in skeletal collections, individuals with minor, healed antemortem nasal and zygomatic fractures were included in this study. No significant separations based on trauma (nasal and zygomatic) presence were yielded from discriminant functions (DFs) of craniofacial shape and size (Appendices E and F). Consequently, those with-and without- trauma were included as one group for all analyses. However, the inability to distinguish between individuals with-and without-trauma does not necessarily mean that trauma fails to influence morphology. In fact, because nasal-and zygomatic- trauma were detected more frequently in MA individuals relative to AA and EA individuals; it is plausible that this sampling disparity influenced the apparent morphological variations that were associated with midfacial trauma. When evaluating ancestral variation in craniofacial shape and size, it is important to recognise that differences seen between ancestry groups may be due to disparities in trauma occurrence and not necessarily intrinsic differences between ancestry groups. While exploring this connection is beyond the scope of the study, it will remain a consideration when discussing ancestral variations.

In South Africa, higher incidences of craniofacial trauma have been correlated with lower socio-economic status (Bamjee *et al*., 1996; Seedat *et al*., 2009). Therefore, the fact that more MA and AA individuals exhibit trauma is further evidence that these individuals may be from a lower socio-economic status than EA individuals in the sample. Little is known about the incidence, aetiology and severity of antemortem craniofacial fractures in individuals accessioned in cadaveric collections in

South Africa. Understanding the cause of craniofacial trauma and the extent of plastic deformation in the craniofacial region may be central in understanding how trauma influences facial morphology (Lee, 2009). Another factor to be considered is the age at which craniofacial trauma was experienced, as trauma experienced in childhood may have impacted development and growth differently than trauma experienced in adulthood. While this study only included adults with minor nasal and zygomatic trauma, it is possible that differences in the severity of trauma and disparities in the age at which trauma occurred influenced morphology in different ways.

5.1.3. Limited information about antemortem maxillary tooth loss

No information about the aetiology or date of antemortem tooth loss can be found in accession registers at skeletal collections, thus researchers tend to speculate based on patterns in living populations. In most living populations, antemortem tooth loss has been attributed to poor dental health, periodontal disease, poor diet, smoking and cultural practices (Bodic *et al*., 2005; Friedling and Morris, 2007; Williams and Slice, 2010). A relationship between age at death and maxillary tooth loss was detected in EA and MA individuals; namely, those with fewer than 6 teeth present were older than 58 years at death. AA individuals sampled in this study had significantly more teeth than MA and EA individuals and this could be because AA individuals, were younger than EA individuals at the time of death. While age may explain why tooth loss was detected less frequently in AA individuals, it does not explain the high frequency of severe of antemortem tooth loss in MA individuals. Extraction of maxillary incisors has been identified as a cultural practice most commonly performed in South African MA communities of a lower socio-economic status (Friedling and Morris, 2007). Individuals from other South African ancestry groups who live in the same communities also tend to practice the same types of tooth extraction (Friedling and Morris, 2005). It is possible that varied tooth loss in different ancestry groups may have uniquely impacted mid-craniofacial morphology, therefore, further studies are required to explore these differences. This study sought to determine the influence of antemortem tooth loss on mid-craniofacial variation and ancestry estimation. Therefore, biases associated with culture and socio-economic status remain important considerations when interpreting tooth loss and its effects on craniofacial variation in different ancestry groups.

5.2. Ancestral variation in mid-craniofacial morphology

Various genetic and environmental factors are implicated in shaping mid-craniofacial morphology. In general, regional and global craniofacial shape variations noted between ancestry groups were consistent with size variations. While cranial variation may correlate with genomic differences between population groups, the pattern of variation in localised structures (e.g. elements of the facial region), implicates both genetic and environmental factors (Martínez-Abadías *et al*., 2006). Climate (Noback *et al*., 2011, Perez and Monteiro, 2009), diet (Paschetta *et al*., 2010) and ontogeny (Freidline *et al*., 2015, Mitteroecker *et al*., 2004) have been associated with craniofacial variation between spatially and temporally separated groups. This study found that antemortem tooth loss; nasal-and zygomatic-trauma and age at death influenced craniofacial morphology, suggesting that traditional ancestry estimation standards may not reliably estimate ancestry in aged individuals or those with severe antemortem tooth loss or facial trauma.

In general, craniofacial variations seen in SA's of AA and EA confirm those reported by Rhine (1990), İşcan and Steyn (1999) and Stull *et al*. (2014). EA individuals exhibited narrower facial breadths, longer facial heights and less prognathic maxillary regions than AA and MA individuals. AA individuals exhibited the widest facial breadths, shortest facial heights and most prognathic maxillary regions. MA individuals tended to neither AA or EA extremes of variation but shared similarities with both ancestry groups. Multivariate analyses of shape and size showed that MA individuals exhibited similar patterns of variation to AA individuals and therefore, ancestry estimations were most problematic in MA individuals, who classified more frequently as AA than EA. The genetic and morphological similarities between AA and MA individuals have been attributed to the political history of South Africa (Adhikari, 2006) and its location on a major trade route between the 15th and 19th century (de Wit *et al*., 2010). The historical record shows that MA individuals are largely descendant of Cape slaves (from the East Indies, West Africa and Madagascar etc.), indigenous Khoesan populations and some people of Eastern European, Southern and Eastern Asian, and West African descent, while AA individuals are primarily the product of Bantu-speaking linguistic groups into Southern Africa. In South Africa, the political climate has been a crucial factor limiting the admixture of different ancestry groups (Adhikari, 2006). Under the Apartheid government, legislation was used to formalise segregation and prevent further population admixture, specifically between individuals of European ancestry ("white South Africans") and those of African and Mixed ancestry (Adhikari, 1992; Stull *et al*., 2014). A Genome-Wide Association study confirmed contributions from Khoesan (32-34%), Bantu-speaking Africans (20-36%), European (21-28%) and Asian (9-11%) groups to the genetic diversity within modern South Africans of

Mixed ancestry (de Wit *et al*., 2010). While MA individuals arguably exhibit the greatest genetic continental admixture of any population (Tishkoff *et al*., 2009), the question remains to what extent does this diversity contribute to mid-craniofacial morphology? While the political climate in South Africa may offer an explanation for mid-craniofacial similarities between MA and AA individuals, the potential socio-economic bias introduced into this sample due to acquisition practices at skeletal collections remains an important consideration: MA and AA individuals are most likely representative of a lower socio-economic status and have exhibited varied degrees of antemortem tooth loss and craniofacial trauma, both of which influence mid-craniofacial morphology.

In agreement with Bernal *et al*. (2006), Martínez-Abadías *et al*. (2006), Gonzalez *et al*. (2011b), Freidline *et al*. (2015) tightly integrated shape variations in different craniofacial elements were detected between different ancestry groups. Specifically, laterally projecting zygomas, rounded nasal apertures, flattened nasal bridges and more prognathic maxillae in AA individuals were contrasted with medially positioned zygomas; longer and narrower nasal apertures; vaulted and steep nasal bridges and more orthognathic maxillae in EA individuals. The close morphological relationships between the nasal bone, maxillae and zygoma have been attributed to similar developmental origins of these components and their close regional proximity (Paschetta *et al*., 2010; Cunningham *et al*., 2016). Genetic, functional and developmental factors have been associated with integration in craniofacial morphology (Cheverud, 1982). Bastir *et al*. (2006) suggested that ancestral variation in craniofacial shape is the result of different growth trajectories in ancestry groups. Therefore, slight growth in one craniofacial component may have marked effects on adjacent components (Bastir *et al*., 2006), meaning that adjacent regions may share similar growth trajectories and thus conserved morphologies. Another possible reason for conserved shape in adjacent craniofacial regions is the widespread distribution of forces linked to biting and muscle movements, which influence spatial morphology in regions that are closely associated with one another (Hylander *et al*., 1991; Paschetta *et al*., 2010). Mitteroecker and Bookstein (2008) found a correlation between enlarged zygomatic arches and wider, more prognathic maxillae; and they suggested that this was likely due to masticatory requirements of the maxilla. Findings that tooth loss influenced shape in all craniofacial regions suggests that the change in bone mass and density, associated with alveolar resorption, impacts distribution of mechanical force during mastication (Reichs *et al*., 2011). This is further evidence that the integration of craniofacial features may be linked to mechanical force distribution in the mid-craniofacial region. Despite the integration in craniofacial morphology, it is important to characterise ancestral variation in each region as forensic anthropologists analyse fragmented and complete remains metrically and non-metrically to estimate ancestry.

5.2.1. Orbital region

EA individuals exhibited the narrowest bi-orbital (EKB) and interorbital breadths (DKB), followed by MA and then AA individuals. DKB were heavily weighted predictors of ancestry in DF analyses of the total craniofacial and orbital region. While McDowell *et al*. (2015) found that AA individuals exhibited the widest DKB, Masters (2008) found no differences in DKB and EKB between Africans, Europeans and Asians. Individuals of African ancestry sampled by Masters (2008) were representative of Sotho and Zulu tribes in South Africa, while the European individuals were from Germany, Switzerland, Italy and France. As such, the Europeans sampled were not representative of South Africans of EA (Masters, 2008), as significant craniometric differences have been detected between South Africans of EA and other populations of European descendent (İşcan and Steyn, 1999; Franklin *et al*., 2007b; L'Abbé *et al*., 2013). Conserved variation South Africans of EA, has been attributed to population bottlenecks in early European settlers in South Africa, and historical policies aimed at preventing population admixture (İşcan and Steyn, 1999). The unique genetic and morphological composition of South Africans of European ancestry emphasises the necessity to development population-specific standards for ancestry estimation in South Africa.

EA individuals exhibited wider orbital breadths (OBB) than both MA and AA individuals and therefore, in EA individuals, the orbits comprised a larger proportion of the width in the orbital region. De Villiers (1968) found that individuals of African ancestry had taller orbital heights relative to shorter orbital breadths. Masters (2008) and Xing *et al*. (2013) have since refuted De Villiers' conclusions, citing shorter orbital heights in individuals of African ancestry relative to those of European and Asian ancestry. Unique postnatal ontogenic trajectories between different population groups have been suggested as a cause of size variation in the orbital region. These trajectories implicate genetic variations as the primary cause of morphological differences between ancestry groups (Mitteroecker *et al*., 2004; Masters, 2008; Freidline *et al*., 2015). Orbital size does not vary in isolation but rather relative to the increase in facial breadth and height (Masters, 2008). This suggests a delicate relationship between components of the mid-craniofacial region, and that differences between ancestry groups do not reflect absolute size but the relative size of craniofacial components. Furthermore, while differences in orbital breadth and height were detected, these were very small and would not be reliable in a forensic context due to immense inter-individual variation within ancestry groups.

Ancestral variations in the orbital region were most effectively identified between AA, MA and EA individuals using geometric morphometrics (GM). This suggests that two-dimensional (2D) metric and nonmetric methods may not be sensitive enough to detect minor differences in orbital shape and size. Inferiorly angled (square) orbits are traditionally associated with European ancestries (Rhine, 1990), but nonmetric assessments yielded similar frequencies of rectangular-shaped and inferiorly angled (square) orbits in MA and EA individuals, indicating that this trait may not reliably distinguish between these two groups. Orbital shape is nonmetrically assessed from an anterior position, therefore, Husmann and Samson (2011) suggest that while this may traditionally be considered a three-dimensional (3D) assessment, its evaluation occurs in two-dimensions and may be distorted by the way the observer positions the cranium. Nonmetric assessments of orbital shape yielded low interobserver repeatability, intimating that this method may not be sensitive enough to detect actual variations in the angle or curvature of the orbits. This provides further evidence that GM assessments more effectively analyse 3D characteristics of orbital shape that cannot be described or quantified using the naked eye.

The orbital region yielded the lowest ancestry estimation accuracies from metric (54.1%) and nonmetric (49.7%) assessments, while GM assessments yielded the highest accuracies (88.6%). Using GM, more square-shaped orbits and diagonally angled orbital rims were detected in EA individuals, compared to more rectangular orbits in AA and MA individuals. Gillick (2012) and Xing *et al*. (2013) employed geometric morphometric methods and found that lateral and inferior orbital borders were highly variable between African, European and Asian ancestry groups. Similarly, Rubin and DeLeon (2017) found that inferior orbital curvature was the most ancestrally informative region between African, European and Asian groups. GM methods capture information pertaining to the multivariate and multidimensional nature of shape variation, suggesting that orbital variation is best understood via these frameworks. Specifically, inferiorly angled orbits in EA individuals were associated with narrower, less anteriorly projecting maxillae and zygomas whereas wider orbits in AA individuals were associated with wider and more prognathic maxillae. These ancestrally conserved shape variations have been associated with analogous developmental origins of adjacent regions (Xing *et al*., 2013) and reflect the functional requirements of the mid-craniofacial complex (Oyen *et al*., 1996; Oettlé *et al*., 2017).

Nonmetric ancestry estimations using frequency distributions of DKB (64.9%) and orbital shape (OS) (52.3%) yielded the lowest average accuracies. When nonmetric assessments of orbital shape were considered in relation to a MLR model, orbital shape was a significant predictor of ancestry, suggesting that this trait effectively characterizes ancestry when assessed relative other elements of the mid-craniofacial region. While multivariate and 3D analyses better distinguished between ancestry groups, ancestry estimation accuracies in this region were lower than those by Hefner (2003), Masters (2008), Gillick (2012), Husmann and Samson (2011), Rubin and DeLeon (2017). This result could be because these studies compared variation between individuals from Asian, African and European ancestry, while the present study compared variation between South Africans of Mixed, African and European ancestry groups, who may have less morphologically distinct orbital shapes.

Ancestral variations in orbital shape and size in AA and EA individuals were influenced by sex, suggesting that sex-specific standards may improve ancestry estimation accuracies in these groups. Regardless of sex, MA individuals yielded poor ancestry estimation accuracies, which suggests that orbital morphologies in this group may be more heterogenous. In general, males exhibited larger orbital dimensions than females and sex-specific DFs yielded higher ancestry estimation accuracies in AA and EA individuals. İşcan and Steyn (1999) developed population-specific DFs which also yielded better ancestry estimatons when sex was known, this suggests that sexual dimorphism contributes to mid-craniofacial variaiton between individuals. DKB and EKB size differences between ancestry groups were the also seen when the sample was split by sex. OBB were significantly different between AA, EA and MA males and only MA and EA females had different OBB. This suggests that unless sex can be confidently estimated, OBB may not be a reliable representative of ancestry. Similair to L'Abbé *et al*. (2011), nonmetric assessments of DKB were more reliable in distinguishing between EA and AA males than females. MLR analyses showed that orbital shape was a better predictor of ancestry in males than females. Husmann and Samson (2011) found evidence that orbital shape differences between males and females of different ancestry groups were statistically significant, but so minor that their use would be impractical in forensic contexts. In general, this study found that sex-specific DFs and MLR analyses yielded heigher ancestry estimation accuracies.

Asymmetry was detected in measurements pertaining to the orbital height. Humans are traditionally considered bilaterally paired organisms but developmental instabilities arising from a series of external and internal stresses are thought to disturb normal developmental pathways, resulting in minor asymmetries (Klingenberg and McIntyre, 1998; Leamy *et al*., 2015). Asymmetrical chewing

patterns and asymmetry in the brain have been proposed as potential reasons for mid-craniofacial asymmetry (Pirttiniemi, 1998). While some of the asymmetry in the craniofacial region may be attributed to developmental stresses, asymmetrical tooth loss and craniofacial trauma may also explain some of the irregularities noted in mid-craniofacial morphology.

Confounding factors in ancestral variation in the orbital region

Antemortem tooth loss and age at death

In MA individuals, age accounted for 2% and 4% of the variation in orbital height and breadth, respectively. Approximately 94% of the growth in the orbital region is completed by age 7, while the remaining 6% is thought to occur during childhood, however, this growth is restricted to the transverse plane of the orbit (Waitzman *et al*., 1992). The interorbital region and orbital heights develop and complete growth early during development and only orbital breadth dimensions change with age. This change occurs through the deposition of bone on the lateral surfaces and the remodelling of bone on the medial surfaces of the orbit (Waitzman *et al*., 1992). In MA individuals, slight increases in OBB and OBH were detected relative to increased age at death, and these changes correlate with patterns of bone deposition noted on lateral surfaces relative to increase of age (Waitzman *et al*., 1992). Tooth loss has been implicated in changing masticatory force dispersal in the orbital region (Paschetta *et al*., 2010) and in EA individuals, both age at death and tooth loss were associated with decreased interorbital breadths. Findings suggest that both tooth loss and age result in osteological changes which alter orbital size. While age has been correlated with decreased craniofacial size (Albert *et al*., 2007), it would appear that changes in age influence the ratio of elements in the craniofacial region rather than the absolute size of each element. Williams (2008) and Williams and Slice (2010) found that inferior and superior orbital curvatures were influenced by sex, ancestry and age. These results were not detected in GM assessments and this could because this study used different landmarks and failed to evaluate curvatures using semi-landmarks. Further research is required to track craniofacial growth and aging trajectories in African, European and Mixed ancestry groups to understand how differing trajectories may influence ancestral variations in individuals of different ages.

Mid-craniofacial trauma

Nasal trauma was associated with wider and less anteriorly projecting apertures and wider interorbital regions, which tapered inferiorly (Appendix E: Figure 2). Since more than 50% of MA individuals in the sample exhibited nasal trauma (Appendix E: Table 1), it is possible that morphological variations in this group may be due to the presence of trauma and not inherent variations in MA individuals.

Furthermore, shape and size variations associated with nasal trauma may have influenced morphological variation between ancestry groups possibly resulting in low ancestry estimation accuracies. Shape differences associated with zygomatic trauma (ZT) were only detected using GM analyses. In those with ZT, superior and lateral landmarks in the orbital region appeared more laterally and posteriorly sunken, while inferior orbital landmarks were more anteriorly located, resulting in more rectangular-shaped orbits (Appendix F: Figures 1-2). Zygomatic bones and the orbital regions of the frontal bone are closely associated, therefore, trauma to the zygoma may have impacted force distribution in both these regions, possibly influencing in orbital shape and size. More research is required to explore the impact of nasal or zygomatic trauma on force distribution during mastication and speech. Due to limitations at skeletal collections, it was not possible to determine when an individual experienced trauma, and thus it is not clear whether the impact of trauma on orbital morphology was due to long-term or short-term changes.

5.2.2. Nasal region

Rounder, wider nasal, maxillary and zygomatic regions in AA individuals contrasted with the narrower, elongated nasal, maxillary and zygomatic regions in EA individuals. Research has suggested that conserved midfacial morphologies within ancestry groups may be associated with developmental differences between these groups (Bastir *et al*., 2006, Freidline *et al*., 2015) and functional demands on the cranium (Hylander *et al*., 1991, Paschetta *et al*., 2010). It has been suggested that ancestral of variations in nasal dimensions are associated with physiological adaptations to climatic conditions (Rhine, 1990; Roseman, 2004; Harvati and Weaver, 2006, Noback *et al*., 2011; Holton *et al*., 2013). While associations have been detected between nasal size and shape (Roseman, 2004; McDowell, 2012) only nasal size has been correlated with climatic adaptations. This suggests that variation in nasal shape and size are uncoupled and that variations in nasal shape are most likely not due to climatic adaptations (Noback *et al*., 2011). In general, those whose ancestors subsisted in colder climates (e.g. Europe, Siberia, Canada) exhibit more narrow and elongated nasal apertures than those whose ancestors lived in more hot and humid climates (e.g. Sub-Saharan Africa, Australia and India) (Roseman, 2004; Harvati and Weaver, 2006; Noback *et al*., 2011; Holton *et al*., 2013). This study found that EA individuals, whose ancestors were European migrants to SA exhibited narrower and longer nasal apertures than AA individuals who exhibited shorter, wider nasal apertures and are mainly descendants of Bantu-speaking linguistic groups who migrated to SA (De Villiers, 1968). Findings by Maddux *et al*. (2017) suggest that ancestral variations in nasal apertures can most likely be attributed to genetic and geographic proximities, which change morphology more rapidly than physiological adaptations to climatic conditions. While unique genetic histories may explain variations in nasal

apertures between AA and EA individuals sampled in this study, the heterogenous composition of variation in MA individuals suggests that both population history and natural selection function together to shape variation in mid-craniofacial morphology. Similar genetic histories (Tishkoff *et al*., 2009; de Wit *et al*., 2010) and geographic origins (Posel, 2001) between AA and MA individuals could explain the similarities detected in nasal aperture morphology. Although morphological variations in the nasal aperture may be shaped by centuries of climatic selection, the degree of inter-and intra-population variation suggests that the nasal aperture is adaptable and influenced by population mobility and genetic admixture.

Compared to other craniofacial regions, the nasal region yielded the highest average ancestry estimation accuracies from metric (58.7%), nonmetric (68.6%) and GM assessments (88.6%). McDowell (2012) also showed that nasal heights and breadths were most different between AA and EA individuals, and that MA individuals were more similar in size to AA individuals. The combination of longer NPH and longer NAW were seen in EA individuals, in contrast to MA and AA individuals who had rounder nasal apertures and shorter NPH (McDowell, 2012). Poor ancestry estimation accuracies from metric DFs in the nasal region were most likely skewed by MA individuals (47.4%) who had lower accuracies than AA (62.9%) and EA individuals (78%). This indicates that size in the nasal region is more distinct between AA and EA individuals, while MA individuals are more heterogenous and morphologically similar to both AA and EA individuals.

Discrete nasal aperture shapes proposed by Hooton (1946) and Rhine (1990) for African and European ancestry groups were not detected between South Africans of AA and EA. Wide nasal apertures, associated with African ancestries, were more frequently observed in AA (74.3%) and MA (55.1%) individuals. In EA individuals, rounded/bell-shaped NAW (characteristic of Asian ancestries) were detected more frequently (60.4%) than elongated/narrow NAW (29.7%) (characteristic of European ancestries). Similarly, L'Abbé *et al*. (2011) observed significant overlap in shape variations between African and Mixed ancestry groups. GM confirmed narrow and more elongated nasal apertures in EA individuals; wider and more rounded nasal apertures in AA individuals. MA individuals had slightly narrower nasal apertures than AA individuals and slightly shorter nasal heights and apertures than EA individuals. EA individuals exhibited more inferior, steep-sloped nasal bridges which projected forward beyond the nasal aperture, while AA individuals exhibited the widest and least anteriorly projecting nasal apertures. Gillick (2012) and McDowell (2012) reported similar

findings for AA and EA samples and suggested that nasal region is the most ancestrally variant component of the mid-craniofacial region.

Nonmetric assessments confirmed these findings as higher frequencies of oval and low/round NBC were noted in AA (41-46.8%) and MA individuals (34.7-46.4%) and more semi-triangular (50.5%), steepled (23.1%) and steep (17.6%) NBC were noted in EA individuals. NBC variations in EA individuals closely mirrored standards developed by Hefner (2003) and Hooton (1946) for European ancestries, while AA and MA individuals in this sample exhibited NBC which were characteristic of African and Asian ancestries. Van Rooyen (2010), reported similar findings in SA's of AA and MA and suggests that NBC may be unsuitable for ancestry estimation in MA and AA groups. While NBC contour was a crucial component of the MLR equation used to estimate ancestry, it seems it may be more useful in distinguishing between EA individuals and those of AA and MA. The heterogeneity in nasal bone shape, size and projection between AA and MA individuals may be representative of genetic and mid-craniofacial morphological similarities between these two groups.

GM assessments of shape and metric assessments of size were influenced by sex, however, there was no evidence that nonmetric assessments were influenced by sex. Metric DFs yielded higher ancestry estimation accuracies in females (65.1%), than males (53.5%) and the DFs for females included nasal height, nasal breadth, and interorbital breadth, suggesting that these were the most ancestrally variant measurements in the nasal region. Similarly, İşcan and Steyn (1999), found that nasal height and nasal breadth were the best predictors of ancestry when sex was known. Nonmetric assessments of nasal bone and aperture shape showed no significant trait occurrences associated with sex, which is contradictory to L'Abbé *et al*. (2011), who suggested that nonmetric assessments of NAW and DKB were only reliable when sex was known. Geometric morphometric shape analyses showed that males had slightly wider, less anteriorly projecting nasal bridges and apertures than females. GM analyses yielded higher ancestry estimation accuracies in females (87.8%) than males (80.4%), indicating that physiological differences associated with sexual dimorphism and intrinsic variations associated with ancestry function together to shape nasal morphology in the mid-craniofacial region.

Confounding factors in ancestral variation in the nasal region

Antemortem tooth loss

Geometric morphometric results confirmed that tooth loss most significantly impacted upper facial and nasal heights, while nasal breadths and projections were largely unaffected. Similar to Small (2016), more anteriorly projecting nasal bridges and nasal apertures were associated with tooth loss, however, these observations were relative to other craniofacial landmarks and most likely reflect a general decrease in size in other craniofacial elements, rather than an absolute size increase in the nasal region. While these findings suggest that shape differences in individuals with tooth loss can be attributed to alveolar resorption, differences may also reflect sampling disparities as more MA and EA individuals exhibited severe antemortem tooth loss. While inherent variations within these groups (e.g. wider and rounder nasal apertures in MA individuals and anteriorly projecting nasal bridges in EA individuals) may influence shape variations associated with tooth loss, Small (2016) suggests that this should not be a concern as there is no reason to believe that different populations would respond differently to tooth loss. While this may be true for edentate individuals sampled by Small (2016), disparities in the types and numbers of teeth lost in individuals sampled in this study may have uniquely influenced shape variations. After correcting for the effect of tooth loss on mid-craniofacial shape, ancestry estimation accuracies in the nasal region improved by 1-2%. This, in addition to the absence of correlations between tooth loss and nonmetric traits, suggests that tooth loss had such a small effect on nasal shape that it failed to influence ancestry estimation accuracies.

Metric analyses revealed smaller facial heights in MA and EA individuals who had experienced severe antemortem tooth loss. The absence of this correlation in AA individuals in this sample may be because the AA individuals sampled were generally younger than MA and EA individuals sampled and exhibited less severe antemortem tooth loss. Regardless, one can extrapolate size variations associated with tooth loss to other populations as biological processes associated with alveolar resorption should be conserved in different populations (Small *et al*., 2016). Twelve months after tooth extraction, alveolar height decreases by 44%, which significantly diminishes masticatory force and results in further resorption of bone in areas, like the zygoma and maxilla, where shape and size are regulated by mastication (Bodic *et al*., 2005). The smaller nasal height in MA individuals is due to more superiorly positioned inferior margins of the nasal aperture, likely in response to severe alveolar resorption in the anterior maxilla. Higher frequencies of maxillary incisor extraction, noted as a cultural practice in South Africans of MA (Friedling and Morris, 2007), may explain why facial and nasal heights were significantly smaller in MA individuals. The close proximity between the alveolar ridge

and nasal bone suggests that this area may be sensitive to alterations associated with sever atrophy of the alveolar ridge and the resultant differences in masticatory loading. While findings by Small (2016) suggest that upper facial height reductions associated with tooth loss may influence ancestry estimations, this study found no evidence to support this in those with fewer than 6 teeth present at death. Since many individuals of EA and MA sampled in this study exhibited severe antemortem tooth loss, it is possible that DFs generated may not represent ancestral variation in individuals of MA or EA, but rather a subset of individuals exhibiting varying degrees of tooth loss. While tooth loss may influence masticatory forces and craniofacial morphologies, more research is required into how these forces may be distributed when differential tooth loss occurs.

Age at death

The effect of age at death significantly influenced metric assessments of nasal morphology, however, these variations were not detected when the sample was split according to sex. This could be because splitting the sample by sex reduced the sample size and the relationship between age and sex was no longer significant. Alternatively, it is possible that the limited age distributions in males and females sampled in this study restricted the significance of linear regression analyses between age and nasal dimensions. Differences between ancestry groups may not be artefactual as Akgül and Toygar (2002), Williams (2008) and Mendelson and Wong (2012) showed that facial heights and nasal heights increased with age. Sarnäs and Solow (1980) and Mendelson and Wong (2012) found that larger nasal and facial heights and breadths were associated with upper facial growth in older individuals. Notably, these differences were not detected in AA or EA individuals, which may be because these samples were skewed in their representation of younger and older individuals, respectively, thus preventing the construction of a significant linear regression model. Aging has been correlated with wider nasal breadths (Sarnäs and Solow, 1980, Behrents, 1985, Akgül and Toygar, 2002, Mendelson and Wong, 2012), but like Williams (2008) this study found no evidence of this relationship. This could once again be associated with the fact that age distribution in this sample was skewed by biases at skeletal collections, specifically, EA individuals were significantly older at death than MA and AA individuals. Therefore, while these findings agree with those in the literature, they should be approached with caution as sample disparities and biases may have influenced these results.

Bilateral asymmetry

Asymmetries detected in nasal heights, may be associated with unilateral occurrences of tooth loss, asymmetrical mastication and developmental instabilities that influence growth. Holton *et al*. (2012) found evidence that septal deviations resulted in nasal height asymmetries, and while septal deviations may be embryologically derived, they can also be a consequence of nasal trauma (Owens, 2007).

Mid-craniofacial trauma

While minor differences in nasal shape and size were associated with zygomatic trauma (Appendix F: Figure 2), nasal trauma was associated with flatter and less anteriorly projecting nasal bridges, wider nasal breadths and slightly longer nasal heights (Appendix E: Figures 1-2). Since nasal breadths were heavily weighted in DFs, it is possible that size differences associated with nasal trauma may have contributed to low ancestry estimation accuracies in MA individuals (Appendices E: Tables 2-3). While no associations between NBC and NAW traits and nasal trauma were detected, it is possible that nonmetric traits, which were uncharacteristic of certain ancestry groups, were seen more frequently due to trauma. For example, while steep-angled nasal bridges may be characteristic of European ancestries, it is possible that individuals of EA with nasal trauma have flatter and wider nasal bridges (Appendix E: Figure 2) which are characteristic of African ancestries, and these associations would influence ancestry estimations. AA and MA individuals exhibited significant co-occurrences of nasal and zygomatic trauma and further research is required to determine how co-occurrence of these factors may influence craniofacial variations and ancestry estimation accuracies

5.2.3. Zygomatic region

GM analyses showed that narrower bizygomatic breadths and more medially positioned zygomas were evident in EA individuals compared to AA individuals. Bizygomatic (ZYB) and zygomaticomaxillary breadths (ZMB) were heavily weighted in metric DFs. İşcan and Steyn (1999) and L'Abbé *et al*. (2013) yielded similair findings in SA populations, suggesting that ZYB are ancestrally variant. MA individuals exhibited more intermediately positioned zygomas and bizygomatic breadths than EA and AA individuals. However, MA individuals were more morphologically similar to AA individuals than EA individuals and these similarities could be attributed to shared genetic histories between these two groups (Tishkoff *et al*., 2009; de Wit *et al*., 2010). Genomic differences between population groups cannot exclusively explain ancestral variations in the zygoma, as rapid adaptation through epigenetic mechanisms have occurred as a result of environmental factors; such as climate and diet (Chen *et al*.,

2011). Research has shown that individuals of African ancestry, whose ancestors subsisted in warmer climates, exhibited wider ZYB and more anteriorly projecting zygomas than those of European ancestry, whose ancestors subsisted in colder climates, exhibited smaller ZYB and retracted zygomas (Kato *et al*., 1997; Chen *et al*., 2011; Freidline *et al*., 2015). While it has been proposed that these differences may be due to dietary requirements varying between different geographic regions, contemporary South African populations generally tend to experience similar dietary requirements and this seems to be an unlikely cause of zygomatic variation. Modern South Africans of AA, MA and EA originate and subsist in the same country and experience similar climatic conditions, therefore, the conserved ancestral variation in zygomatic morphology suggests that genetic differences between population groups are conserved and significantly contribute to ancestral variations (L'Abbé *et al*., 2013; Oettlé *et al*., 2017).

Kato *et al*. (1997), Freidline *et al*. (2015) and Heuzé *et al*. (2016) have detected close morphological integration between zygomatic, nasal, maxillary and orbital morphologies in different population groups. One example of this is the relationship between the laterally located zygoma and the centrally located nasal aperture. In AA individuals, wider zygomas were associated with wider nasal apertures, compared to more medial zygomas and narrower apertures in EA individuals. Lateral expansion of nasal walls in Khoesan and African populations has been associated with posterior migration of the zygomatic bone, which enlarges vertically and laterally (Kato *et al*., 1997; Freidline *et al*., 2015). In European populations, elongated nasal aperture have been associated with facial flatness and narrower zygomas (Kato *et al*., 1997). Although wider zygomas are developmentally associated with wider nasal apertures (which are known to represent climatic, geographic and genetic variations (Dean, 1988; Friess *et al*., 2002; Freidline *et al*., 2015; Maddux *et al*., 2017)), higher and wider zygomatic bones in Asian and Inuit populations are associated with narrower nasal apertures (Chen *et al*., 2011). Research has shown that during growth, the nasal aperture increases in size vertically and horizontally and that the zygomatic bones move posteriorly and expands vertically and laterally (Freidline *et al*., 2015). It has been suggested that different ontogenic trajectories between different ancestry groups may explain variation in zygomatic shapes and sizes (Freidline *et al*., 2015). While certain areas tend to vary together, a degree of modularity exists among components of the craniofacial skeleton suggesting that components can vary independently and thus can evolve separately (Heuzé *et al*., 2016).

EKB in EA individuals were generally wider than ZYB, but in AA individuals, EKB were generally narrower than ZYB; these findings were comparable to Kato *et al*. (1997). GM analyses confirmed these findings as more anterior and laterally projecting zygomas, medially located ectoconchion landmarks (lateral orbital margins) and anterior, inferior and laterally positioned maxillae were noted in AA individuals. EA individuals exhibited more posterior and medially projecting zygomas, laterally located ectoconchion landmarks (lateral orbital margins) and posterior, superior and medially positioned maxillae. Heuzé *et al*. (2016) suggests that the close morphological relationship between the maxilla and zygoma may be linked to similar developmental origins as both regions are derived from the same neural crest cells. Mitteroecker and Bookstein (2008) found that enlarged and laterally projecting zygomatic arches were associated with enlarged and prognathic maxillae, and that these relationships were mainly related to masticatory force distribution. While ancestral variations may be genetically and developmentally associated, mechanical requirements of mastication and speech on the maxilla are fundamental in sustaining zygomatic shape and size (Heuzé *et al*., 2016). Therefore, genomic differences between population groups cannot exclusively explain ancestral variations in the zygoma, as the pattern of craniofacial integration implicates forces associated with speech, mastication and climatic conditions in shaping morphology.

Zygomaxillary suture shape (ZS), yielded the lowest ancestry estimation accuracies (11-12.3%) with all ancestry groups exhibiting high frequencies of "smooth" shaped sutures, which are traditionally associated with Asian ancestry groups (Rhine 1990). L'Abbé *et al*. (2011) and Hefner (2009) also detected non-specific patterning of ZS in SA and North American samples, respectively. Thererfore, ZS as defined by Rhine (1990) appears to be an unreliable indicator of ancestry in various population groups, suggesting that the three-category ancestry estimation system (i.e. African, European, Asian categories) has limited value when assessing a suture pattern which may be more complex than can be assessed with the naked eye. While Sholts and Warmlander (2012) suggested that ZS was influenced by mechanical remodelling due to diet, activity patterns and health status, Maddux *et al*. (2015) refuted these suggestions and argued ancestral variations in suture morphology are associated with population-specific growth and developmental trajectories. Hefner (2009) found an association between nonmetric traits in the nasal and orbital region and ZS, however in the present study, ZS failed to feature in the MLR. The exclusion of this trait from the multivariate analysis of nonmetric data could either suggest no significant association between ZS and ancestry, or it could reveal that variations in ZS are more sensitive and complex than can be assessed visually.

Ancestral variations in zygomatic shape and size were detected in this study, however when this region was assessed in isolation, ancestry was not estimated reliably. Specifically, in the mid-craniofacial region, the zygoma yielded the lowest ancestry estimation accuracies from GM (80%), metric (43.4%) and nonmetric assessments (11%). Zygomatic measurements were heavily weighted in metric DFs, similair to DFs generated by İşcan and Steyn (1999). This suggests that the zygomatic region best represents ancestral variation when assessed in a multivariate model. Furthermore, zygomatic variation is best represented in 3D, as GM assessment yielded the highest ancestry estimation accuracies (80%). Good ancestry estimation accuracies detected in MA individuals (83.7%) may not necessarily be due to inherent differences within the MA group, but rather because of high frequencies of zygomatic trauma (Appendix F) and antemortem tooth loss, which will be discussed below. While zygomatic shape appears to be ancestrally variant when assessed in 3D, the zygoma may be influenced by external factors thereby reducing the reliability of this region for the estimation of an individual's ancestry.

Generally, females yielded higher ancestry estimation accuracies from metric (62.7%) and geometric morphometric (79.3%) assessments of zygomatic morphology, suggesting that sex-specific standards may improve ancestry estimation accuracies. While all ancestry groups were significantly different in ZYB, in the sample split by sex, only AA males and females were significantly larger than MA and EA males and females, respectively. Therefore, the absence of significant size differences between MA and EA males and females could be because minor size differences were no longer significant in such smaller samples, or that sexual dimorphism was less prominent in these ancestry groups. Bimaxillary breadth measurements were significantly different between females of different ancestry groups, unlike trends detected in males, where only AA males were different in size to EA and MA males. DFs in females included bizygomatic and bimaxillary breadths, while in males, only bimaxillary breadth was included in the DF; suggesting that females were more ancestrally variant in zygomatic sizes than males. Lefevre *et al*. (2013) found that wider ZYB and ZMB in males were associated with higher levels of testosterone, suggesting that prior knowledge of sex may improve ancestry estimations in females.

Confounding factors in ancestral variation in the zygomatic region

Antemortem tooth loss

Similar to Small (2016), this study found tooth loss was associated with more lateral and inferiorly positioned zygomas. When fewer than 6 teeth were present, ZMB decreased in MA females. While the absence of these differences in EA individuals who exhibited severe antemortem tooth loss could

be indicative of varied tooth loss between MA and EA individuals, it is more likely that these differences are artefactual as small size differences were associated with antemortem tooth loss. Research has shown that atrophy in medial pterygoid and masseter muscles occur because of severe tooth loss and that this results in reduced bite force in edentulous individuals (Newton *et al*., 1993). Therefore, if this were the case, one may expect reduced zygomatic dimensions to be associated with tooth loss, but this was not the case, suggesting that shape variations may be relative to maxillary and nasal shape differences associated with tooth loss. Small (2016) found that variations detected in the zygoma relative to the entire craniofacial region were not detected in analyses of the zygomatic bones in isolation, suggesting that variations detected in the zygoma were most likely a reflection of morphological changes in the nasal and maxillary regions. After correcting for tooth loss using a multivariate regression of Procrustes shape on tooth loss, GM ancestry estimations using the zygoma in isolation were improved in EA and MA individuals. Minor shape variations associated with tooth loss were detected in the zygomatic bones in isolation and these effects were most severe in EA and MA individuals, most likely because EA and MA individuals experienced more severe antemortem tooth loss. Therefore, these results suggest that while tooth loss influences zygomatic shape, these changes are relative to total craniofacial morphology and are minor in the zygomatic bone in isolation.

Age at death

No significant associations between zygomatic shape and size, and age at death were detected. While, Williams (2008), Williams and Slice (2010) and Mendelson and Wong (2012) have found no significant shape and size variations due to aging, Richard *et al*. (2009) observed decreased anterior projection in the zygomatic bone relative to increased age. Thus, while the present study detected no significant age-related variations, this may not necessarily mean that no differences existed between these individuals. While these findings may indicate the absence of age-related differences in zygomatic morphology, they may also be because this study failed to sample enough individuals from different age ranges or that the landmarks and traits assessed failed to effectively represent areas of the zygoma which are influenced by aging. Further studies should employ more comprehensive curvature analyses to evaluate zygomatic bone shape and size in 3D to determine the extent of variation in these bones.

Mid-craniofacial trauma

Nasal and zygomatic trauma influenced zygomatic morphology, suggesting that the zygomatic bones are unreliable for ancestry estimation in the presence of nasal and zygomatic trauma (Appendices E-F). While the association between nasal trauma and wider zygomatic breadths seen in metric

(Appendix E: Tables 2-3) and GM assessments (Appendix E: Figures 1-2) indicate that nasal trauma inadvertently results in wider zygomas, it is more likely that the difference in zygomatic breadth reflect the size difference in the more closely related nasal aperture rather than the zygomatic region. More medial and inferior zygomas were evident in those with zygomatic trauma, suggesting that these differences occur in response to deformation in the zygomatic arch (Appendix F: Figure 2). These findings suggest that bizygomatic and bimaxillary breadths may not be reliable estimators of ancestry in the presence of zygomatic trauma, while bizygomatic breadths may not reliable in the presence of nasal trauma (Appendices E and F: Tables 2-3).

5.2.4. Maxillary region

Wider maxilla-alveolar breadths and more hyperbolic maxillae, as reported by Rhine (1990) and Gillick (2012), were detected in AA individuals compared to EA individuals. Overall, variations in maxillary shape and size in MA individuals were more similar to AA than EA individuals. While these differences may be due to genetic and population histories between these three ancestry groups (de Wit *et al*., 2010), they may also reflect the sample biases at skeletal collections used in this study. Freidline *et al*. (2015) suggest that differences in maxillary width and projection may be due to dietary requirements in different population groups. For example, Khoesan individuals (width wider maxillary breadths and prognathic maxillae), exhibited more early and rapid anterior and lateral growth in the maxilla than Inuit individuals (with narrower and more orthognathic maxillae) (Freidline *et al*., 2015). While South Africans of AA, EA and MA may not necessarily have different dietary and masticatory requirements, it is possible that ancestral adaptations to these factors are still perpetuated in craniofacial morphology. Mitteroecker and Bookstein (2008) found that that enlarged and laterally projecting zygomatic arches were associated with enlarged and prognathic maxillae, and that these relationships were mainly related to mastication and force distribution. The nasal bone and maxilla are closely related in morphological shape (Holton and Franciscus, 2008; Holton *et al*., 2013) mainly due to similar developmental origins associated with embryology, and the close regional proximity of these bones (Cunningham *et al*., 2016). Freidline *et al*. (2015) found that wide maxillary breadths and prognathic maxillae in Khoesan individuals were associated with and earlier and more rapid growth in lateral and anterior planes of the maxillae. While mechanical adaptations provide a potential mechanism influencing maxillary variations in contemporary South African populations, differences in maxillary growth rates between different ancestry groups propose a better explanation for the causes of this variation (Freidline *et al*., 2015).

AA individuals exhibited larger basion-prosthion lengths and more prognathic maxillae than MA and then EA individuals, in agreement with İşcan and Steyn (1999) and L'Abbé *et al*. (2013). Lesciotto *et al*. (2016) found that facial heights influenced assessments of prognathism, and that alveolar prognathism was best understood using GM shape analyses. GM analyses suggest that variations in prognathism occur due to the relative position of basion (more anteriorly projecting in EA individuals than AA individuals) and prosthion landmarks (more anteriorly projecting in AA than EA individuals). Nonmetric assessments of orthognathism were more frequently seen in EA individuals, while prognathism was detected in AA individuals. More anteriorly projecting nasal spines were seen in EA individuals, while MA and AA individuals showed no affinity towards any shape variants, indicating this trait is poor determinant of ancestry in AA and MA individuals. Notably, anteriorly projecting nasal spines were not evident in geometric morphometric analyses indicating that while they appeared more anteriorly projecting in nonmetric assessments in EA individual. This was most likely relative orthognathic maxilla and the nasal spine. These findings suggest that alveolar prognathism is better represented in shape than size, and in 3D as opposed to 2D.

While significant ancestral variations occurred in maxillary shape and size, when this region was used in isolation it yielded the lowest ancestry estimation accuracies in metric (31.6%), and nonmetric (36.2%) assessments, however, good ancestry estimation accuracies were yielded from GM analyses (93.3%). GM is a more sensitive technique and can detect minor variations in maxillary shape which may not be detectable through simple 2D assessments. GM yielded high ancestry estimations in AA (92.3%), EA (92.3%) and MA (82.3%) individuals, suggesting that this region is the most reliable region to estimate ancestry for all ancestry groups in this study. Females yielded higher ancestry estimation accuracies than males from metric (52.4%) and GM assessments (95.8%), suggesting that sexual dimorphism could mask ancestral variation in the maxilla. The potential influence that tooth loss and age had on ancestral variations will be discussed below. Overall, geometric morphometric assessments of shape yielded the highest ancestry estimation accuracies in the maxillary region, intimating that maxillary shape is most variant in 3D.

Confounding factors in ancestral variation in the maxillary region

Antemortem tooth loss and age at death

Research has shown that resorption in the maxilla occurs rapidly with the increase in age, this generally results in a narrower and less prognathic maxilla (Enlow and Hans, 1996). The alveolar section of the maxilla was more posteriorly located and projected less anteriorly than the upper face

in those with fewer teeth, resulting in a more orthognathic maxilla, with a more concaved appearance (similar to findings by (Small, 2016)). The maxillary alveolus and mid-cheek region (below the orbital region) are most prone to age-related alveolar resorption, which may have inevitably resulted in tooth loss and reduced maxillary size (Enlow and Hans, 1996). In EA individuals, tooth loss and age functioned as covariates, which were both associated with smaller maxillary breadths and less anteriorly projecting maxillae. EA individuals had medium to long, sharp anterior nasal spines, which are known to be servery impacted by tooth loss (Small, 2016). Long sharp anterior nasal spines were mainly detected in individuals with fewer than 6 teeth present, suggesting that anterior nasal spines (associated with EA groups) may have been anteriorly projecting due to severe resorption of alveolus in the maxilla. While these findings suggest that both age at death (Bastir *et al*., 2006; Albert *et al*., 2007) and tooth loss (Reichs *et al*., 2011, Small *et al*., 2016) impact maxillary breadths, disparities in the sample used in this study may have skewed results. Specifically, due to biases at skeletal collections used in this study, EA individuals in the sample were significantly older and had experienced more severe antemortem tooth loss than both MA and AA individuals. Therefore, the absence of large maxillary shape and size differences in MA and AA individuals could be because these ancestry groups were slightly younger and experienced less antemortem tooth loss. Regardless, changes associated with tooth loss agreed with findings by Small (2016). Once the effect of tooth loss was corrected for using GM, ancestry estimation accuracies improved in MA individuals, but worsened in EA individuals, suggesting that tooth loss contributed to inter-group variations detected in this study.

Mid-craniofacial trauma

Variations associated with nasal and zygomatic trauma were assessed with geometric morphometrics (Appendices E-F). Specifically, more superior, narrow and posteriorly projecting maxillary regions were detected in those with mid-facial trauma. The differences may be due to trauma-related deformation in the nasal and zygomatic regions, which impact the functional stability of the craniofacial complex and result in minor shape variations (Lee, 2009). It is more likely that these variations are due to "The Pinocchio effect", which happens due to large differences of variances at one or two landmarks being distributed over many landmarks by least-squares rotation, thus providing spurious representations of landmark variations (von Cramon-Taubadel *et al*., 2009). Thus, it is possible that major variations in the nasal region were erroneously represented in average landmark distributions yielded during generalised least squares superimpositions, and therefore, geometric morphometric representations of the effect of trauma on shape should be approached with caution.

5.3. Evaluating the metric, nonmetric and geometric morphometric ancestry methods

5.3.1. Repeatability

In general, geometric morphometric and metric methods yielded the highest levels of observer agreement. Geometric morphometric methods yielded slightly lower levels of agreement than desired, however, this may because a smaller sample size was used (*n*=30) for observer testing. Since GM observer error was assessed relative to the total variation in the sample, a smaller sample may have resulted in less inter-individual variation, and thus lower levels of agreement. Nonmetric assessments yielded the poorest repeatability, with inferior nasal margin, malar tubercle and zygomatic projection performing the worst. Concerns regarding the repeatability of nonmetric assessments in forensic contexts have been raised by various studies including those by Wheat (2009), Vitek (2012) and Van Rooyen (2010). Poor repeatability has been attributed to vague trait definitions (Hefner, 2003), unrealistic two-dimensional standards (Caple and Stephan, 2017), perspective distortion due to positioning of the cranium (Rubin and DeLeon, 2017) and varied observer education (Vitek, 2012). Nonmetric methods fail to satisfy *Daubert* principles of repeatability and precision, and are generally avoided in forensic contexts (Byers, 2015). Nonmetric ancestry estimations may be useful in identifying individuals of unique ancestral origins, which may not be effectively represented in discriminant functions (e.g. South Africans of Mixed ancestry).

5.3.2. Accuracy

The lowest ancestry estimation accuracies were yielded from 2D metric (27%-60.2%) and nonmetric methods (57.1%-82.4%). These results suggest that that using both metric and nonmetric methods may improve ancestry estimation accuracies. Geometric morphometric assessments yielded the highest ancestry estimations (75-97.9%), indicating that the complexity of ancestral variation is best understood in three-dimensions. Nonmetric ancestry estimation accuracies were similar to L'Abbé *et al*. (2011), despite the inclusion of individuals with severe tooth loss and craniofacial trauma. This suggests that perceptions of nonmetric traits may not be influenced by these cofactors, which are known to influence 3D assessments of shape. These findings agree with the assertion by Hefner (2003), that ancestry estimations occur in the eye of the forensic anthropologists before trait scoring occurs. Overall, no traits were deemed population-specific in that they exclusively occurred in one population, and therefore, one cannot assume complete separation of groups based on the distribution of a single trait. Geometric morphometric analyses revealed significant morphological variations between ancestry groups, however, the overlapping variation in facial measurements and

non-specific occurrences of nonmetric traits suggest that multivariate models generated for each method may provide better ways of estimating ancestry.

DFs from metric data yielded poorer ancestry estimation accuracies than those presented by İşcan and Steyn (1999). Unlike İşcan and Steyn (1999), this study included morphologically diverse MA individuals, which may have reduced the accuracy of DFs generated in this study. In general, measurements in the nasal region yielded higher ancestry estimation accuracies for AA, MA and EA South Africans (McDowell, 2012). Findings suggest that a larger sample size of MA, AA and EA individuals is needed to improve the accuracy from DFs. Measurements pertaining to nasal and zygomatic breadths should be excluded when nasal or zygomatic trauma occurs. Forensic anthropologists are likely to utilise computational programmes (e.g. FORDISC® and CRANID®) or discriminant functions (e.g. İşcan and Steyn (1999) and Giles and Elliot (1962)) when estimating ancestry and none of these presently include MA individuals or consider the effects of tooth loss, trauma or age. Findings suggest that separate DFs may be required to ensure accurate ancestry estimations when tooth loss occurs, however large reference samples with differential tooth loss would be required to established standards. Tooth loss impacts shape and size variation in the maxilla, suggesting that discriminant functions which account for variations in the maxilla may yield higher ancestry estimation accuracies, in the presence of antemortem tooth loss. Correcting for tooth loss resulted in higher ancestry estimation accuracies in AA and MA individuals, suggesting that ancestry estimation accuracies may significantly be improved if tooth loss is considered.

Generally, ancestry estimation accuracies were improved when the sample was split by sex. In males, the orbital region yielded higher ancestry estimation accuracies, while the nasal, zygomatic and maxillary regions yielded higher ancestry estimation accuracies in females. Therefore, estimating sex prior to ancestry improved ancestry estimation accuracies as different discriminant functions for males and females would better represent nuances in ancestral variation. This study showed that while ancestry estimations were possible due to distinct morphological variations, extrinsic and intrinsic factors (such as tooth loss, trauma, age and sex) limited the ability to estimate ancestry.

Chapter Six: Conclusion

Metric, nonmetric and geometric morphometric methods were used to characterise ancestral variations in South Africans of African, European and Mixed ancestry. In general, AA individuals exhibited shorter facial and nasal heights; wider nasal, maxillary and zygomatic breadths; more prognathic maxillae and less anteriorly projecting nasal bridges. EA individuals exhibited narrower orbital, nasal and maxillary breadths; longer facial and nasal heights; inferiorly-angled orbits; anteriorly projecting nasal bridges and more orthognathic maxillae. MA individuals exhibited heterogeneity in terms of craniofacial shape and size, but more closely resembled variations in AA individuals. Similar developmental trajectories and genetic lineages, in addition to functional requirements of the mid-craniofacial region were used to explain population variants detected in this study. Overall, nasal and maxillary regions were the most ancestrally diverse regions, yet, these regions were most influenced by confounding factors such as antemortem tooth loss and antemortem mid-craniofacial trauma. Tooth loss was found to contribute to ancestral variations in EA individuals as the majority of EA individuals exhibited severe antemortem tooth loss. More research is required to investigate the impact of tooth loss and alveolar resorption on craniofacial morphology. Tooth extraction is considered a cultural or social-economic indicator in South Africans of "Mixed" ancestry, therefore, information pertaining to the impact of tooth loss and alveolar resorption in this group may be beneficial in the development of more reliable ancestry estimation standards.

The lowest ancestry estimation accuracies were yielded by two-dimensional metric (27%-60.2%) and nonmetric methods (57.1%-82.4%). Geometric morphometric shape assessments yielded the highest ancestry estimation accuracies (75-97.9%), suggesting the presence of three-dimensional shape variations between ancestry groups. Generally, ancestry estimation accuracies were improved when the sample was split by sex, suggesting that sexual dimorphism masks ancestral variations in mid-craniofacial morphology. Correcting for tooth loss resulted in higher ancestry estimation accuracies in AA and MA individuals, intimating that ancestry estimation accuracies may be significantly improved if tooth loss is considered. Tooth loss impacts shape and size variation in the maxilla, suggesting that discriminant functions which account for variations in the maxilla may yield higher ancestry estimation accuracies in the presence of antemortem tooth loss. These results confirm the continuum of ancestral variation in South African populations and emphasise the need to develop multivariate ancestry estimation standards which can estimate ancestry reliably in South Africans of African, European and Mixed ancestry.

References

AAPA (American Association of Physical Anthropology). 1996. Statement on Biological Aspects of Race. *American Journal of Physical Anthropology*. 101(4): 569-570.

Adams, D.C., Rohlf, F.J. and Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*. 71(1): 5-16.

Adhikari, K., Fuentes-Guajardo, M., Quinto-Sánchez, M., Mendoza-Revilla, J., Chacón-Duque, J.C., Acuña-Alonzo, V., Jaramillo, C., *et al.* 2016. A genome-wide association scan implicates DCHS2, RUNX2, GLI3, PAX1 and EDAR in human facial variation. *Nature Communications*. *7*: 11616.

Adhikari, M. 1992. The sons of ham: slavery and the making of coloured identity. *South African Historical Journal*. 27(1): 95-112.

Adhikari, M. 2005. Contending approaches to coloured identity and the history of the coloured people of South Africa. *History Compass*. 3(1): 1-6.

Adhikari, M. 2006. Hope, fear, shame, frustration: Continuity and change in the expression of coloured identity in white supremacist South Africa, 1910–1994. *Journal of Southern African Studies*. 32(3): 467-487.

Akgül, A.A. & Toygar, T.U. 2002. Natural craniofacial changes in the third decade of life: A longitudinal study. *American Journal of Orthodontics and Dentofacial Orthopedics.* 122(5): 512-522.

Al Shahrani, I.S.A. 2012. *3D geometric morphometric analysis of tooth shape in hypodontia*. Ph.D. Thesis. Newcastle University. (Unpublished).

Albanese, J. & Saunders, S.R. 2006. Is it possible to escape racial typology in forensic identification? In *Forensic Anthropology and Medicine*. A. Schmitt, E. Cunha, J. Pinheiro, Eds. New York: Humana Press. 283-316.

Albert, A. M., Ricanek JR, K. & Patterson, E. 2007. A review of the literature on the aging adult skull and face: Implications for forensic science research and applications. *Forensic Science International*. 172(1): 1-9.

Alblas, A., Greyling, L.M. & Geldenhuys, E.-M. 2018. Composition of the Kirsten Skeletal Collection at Stellenbosch University. *South African Journal of Science*. 114(1-2): 1-6.

Altman, D.G. & Bland, J.M. 1983. Measurement in Medicine: The Analysis of Method Comparison Studies. *The Statistician.* 32(3): 307-317.

Bamjee, Y., Lownie, J.F., Cleaton-Jones, P.E. & Lownie, M.A. 1996. Maxillofacial injuries in a group of South Africans under 18 years of age. *The British Journal of Oral & Maxillofacial Surgery*. 34(4): 298-302.

Banerjee, M., CapozzolI, M., McSweeney, L. & Sinha, D. 1999. Beyond kappa: A review of interrater agreement measures. *Canadian Journal of Statistics*. 27(1): 3-23.

Barbeito-Andrés, J., Anzelmo, M., Ventrice, F. & Sardi, M.L. 2012. Measurement error of 3D cranial landmarks of an ontogenetic sample using computed tomography. *Journal of Oral Biology and Craniofacial Research*. 2(2): 77-82.

Barker, H.R. & Barker, B.M. 1984. *Multivariate analysis of variance (MANOVA): a practical guide to its use in scientific decision-making*. Alabama: University of Alabama Press.

Bastir, M. & Rosas, A. 2005. Hierarchical nature of morphological integration and modularity in the human posterior face. *American Journal of Physical Anthropology*. 128(1): 26-34.

Bastir, M., Rosas, A. & O'Higgins, P. 2006. Craniofacial levels and the morphological maturation of the human skull. *Journal of Anatomy*. 209(5): 637-654.

Behrents, R. G. 1985. *Growth in the aging craniofacial skeleton*. Center for Human Growth and Development. Michigan: University of Michigan.

Bernal, V., Perez, S.I. & Gonzalez, P.N. 2006. Variation and causal factors of craniofacial robusticity in Patagonian hunter-gatherers from the late Holocene. *American Journal of Human Biology.* 18(6): 748-765.

Bland, J.M. & Altman, D.G. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, 327(8476): 307-310.

Boas, F. 1912. Changes in the bodily form of descendants of immigrants. *American Anthropologist*. 14(3): 530-562.

Bodic, F., Hamel, L., Lerouxel, E., Baslé, M.F. & Chappard, D. 2005. Bone loss and teeth. *Joint Bone Spine*. 72(3): 215-221.

Bookstein, F.L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge.

Brace, C.L. & Hunt, K.D. 1990. A nonracial craniofacial perspective on human variation: A(ustralia) to Z(uni). *American Journal of Physical Anthropology*. 82(2): 341-360.

Brace, C.L. 1995. Region does not mean "race"—reality versus convention in forensic anthropology. *Journal of Forensic Science*. 40(2): 171-175.

Brace, C.L. 2005. *"Race" is a four-letter word: the genesis of the concept.* Oxford: Oxford University Press.

Bruner, E. 2007. Cranial shape and size variation in human evolution: structural and functional perspectives. *Child's Nervous System*, 23(12): 1357-1365.

Buikstra, J.E. & Ubelaker, D.H. 1994. *Standards for data collection from human skeletal remains*. Arkansas Archaeological Survey Research Series.

Byers, S.N. 2015. *Introduction to Forensic Anthropology*. Routledge, New York: Prentice Hall.

Cameron, D.W. 1993. Uniformitarianism and Prehistoric Archaeology. *Australian Archaeology*. 36(1): 42-49.

Caple, J. & Stephan, C.N. 2017. Photo-Realistic Statistical Skull Morphotypes: New Exemplars for Ancestry and Sex Estimation in Forensic Anthropology. *Journal of Forensic Sciences*. 62(3): 562-572.

Caspari, R. 2003. From types to populations: A century of race, physical anthropology, and the American Anthropological Association. *American Anthropologist*. 105(1): 65-76.

Caspari, R. 2009. 1918: Three perspectives on race and human variation. *American Journal of Physical Anthropology*. 139(1): 5-15.

Chelotti, K. L. 2013. *Temporal analysis of craniofacial trauma in prehistoric California's Central Valley*. MA(Anth). Thesis. (Unpublished).

Chen, T., Hsu, Y., Li, J., HU, J., Khadka, A., Wang, Q. & Wang, D. 2011. Correction of zygoma and zygomatic arch protrusion in East Asian individuals. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics.* 112(3): 307-314.

Cheverud, J.M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution*. 36(3): 499-516.

Chhapola, V., Kanwal, S. K. & Brar, R. 2015. Reporting standards for Bland–Altman agreement analysis in laboratory research: a cross-sectional survey of current practice. *Annals of Clinical Biochemistry*. 52(3): 382-386.

Chrcanovic, B.R. 2012. Factors influencing the incidence of maxillofacial fractures. *Oral and Maxillofacial Surgery*. 16(1): 3-17.

Christensen, A.M. & Crowder, C.M. 2009. Evidentiary standards for forensic anthropology. *Journal of Forensic Sciences*. 54(6): 1211-1216.

Christopher, A.J. 2002. 'To define the indefinable': Population Classification and the Census in South Africa. *Area*. 34(4): 401-408.

Cohen, H., Sarie, I., Medlej, B., Bocquentin, F., Toledano, T., Hershkovitz, I. & Slon, V. 2014. Trauma to the skull: A historical perspective from the southern Levant (4300BCE-1917CE). *International Journal of Osteoarchaeology*. 24(6): 722-736.

Cohen, J. 1960. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*. 20(1): 37-46.

Cohen, J. 1988. *Statistical power analysis for the behavioural sciences*. New Jersey: Hilsdale.

Comaroff, J.A.J.L. 1991. *Christianity, Colonialism and Consciousness in South Africa of Revelation and Revolution*. Chicago: University of Chicago.

Corruccini, R.S. 1974. An examination of the meaning of cranial discrete traits for human skeletal biological studies. *American Journal of Physical Anthropology*, 40(3): 425-445.

Crawfurd, J. 1868. On the Classification of the Races of Man According to the Form of the Skull. *Transactions of the Ethnological Society of London*, 6: 127-134.

Cunningham, C., Scheuer, L. & Black, S. 2016. *Developmental Juvenile Osteology*. Rev. 2nd ed. London: Academic Press.

Darwin, C. 1871. *The Descent of Man*. New Jersey: Princeton University Press.

Dayal, M. R., Steyn, M. & Kuykendall, K. L. 2008. Stature estimation from bones of South African whites. *South African Journal of Science*. 104(3-4): 124-128.

Dayal, M.R., Kegley, A.D., Štrkalj, G., Bidmos, M.A. & Kuykendall, K.L. 2009. The history and composition of the Raymond A. Dart Collection of human skeletons at the University of the Witwatersrand, Johannesburg, South Africa. *American Journal of Physical Anthropology*, 140(2): 324-335.

De Villiers, H. 1968. *The skull of the South African Negro: a biometrical and morphological study*. Johannesburg: Witwatersrand University Press.

De Wit, E., Delport, W., Rugamika, C.E., Meintjes, A., Möller, M., Van helden, P.D., Seoighe, C. & Hoal, E.G. 2010. Genome-wide analysis of the structure of the South African Coloured Population in the Western Cape. *Human Genetics*. 128(2): 145-153.

Dean, M.C. 1988. Another look at the nose and the functional significance of the face and nasal mucous membrane for cooling the brain in fossil hominids. *Journal of Human Evolution*. 17(7) :715-718.

Dirkmaat, D. C., Cabo, L. L., Ousley, S. D. & Symes, S. A. 2008. New perspectives in forensic anthropology. *American Journal of Physical Anthropology*. 137(47): 33-52.

Dobzhansky, T. 1973. Nothing in biology makes sense except in the light of evolution. *American Biology Teacher*. 35(3): 125-129.

Drake, A.G. & Klingenberg, C.P. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. *The American Naturalist*. 175(3): 289-301.

Dubow, S. 1995. *Scientific racism in modern South Africa*. Cambridge: Cambridge University Press.

Enlow, D.H. & Hans, M.G. 1996. *Essentials of Facial Growth*. Pennsylvania: W.B Saunders Company.

Enlow, D.H. and Bang, S. 1965. Growth and remodelling of the human maxilla. *American Journal of Orthodontics.* 51: 446–464.

Faurie, A. 2000. *The admissibility and evaluation of scientific evidence in court*. LLM. Thesis. University of Cape Town. (Unpublished).

Flurry, B. 1997. *A first course in multivariate statistics*. New York: Springer.

Franklin, D., Freedman, L. & Milne, N. 2005a. Sexual dimorphism and discriminant function sexing in indigenous South African crania. *HOMO-Journal of Comparative Human Biology*. 55(3): 213-228.

Franklin, D., Freedman, L. & Milne, N. 2005b. Three-dimensional technology for linear morphological studies: a re-examination of cranial variation in four southern African indigenous populations. *HOMO - Journal of Comparative Human Biology*. 56(1): 17-34.

Franklin, D., Freedman, L., Milne, N. & Oxnard, C.E. 2007a. Geometric morphometric study of population variation in indigenous southern African crania. *American Journal of Human Biology*. 19(1): 20-33.

Franklin, D., Oxnard, C.E., O'Higgins, P. & Dadour, I. 2007b. Sexual dimorphism in the subadult mandible: quantification using geometric morphometrics. *Journal of Forensic Sciences*. 52(1): 6-10.

Freidline, S.E., Gunz, P. & Hublin, J.J. 2015. Ontogenetic and static allometry in the human face: contrasting Khoisan and Inuit. *American Journal of Physical Anthropology*, 158(1): 116-131.

Friedling, L.J. & Morris, A.G. 2005. The frequency of culturally derived dental modification practices on the Cape Flats in the Western Cape. *South African Dental Journal*. 60(3): 97-99.

Friedling, L.J. & Morris, A.G. 2007. Pulling teeth for fashion: dental modification in modern day Cape Town, South Africa. *South African Dental Journal*. 62(3): 106-113.

Friess, M., Marcus, L.F., Reddy, D.P. & Delson, E. 2002. The use of 3D laser scanning techniques for the morphometric analysis of human facial shape variation. *BAR International Series*. 1049: 31-35.

Fruciano, C. 2016. Measurement error in geometric morphometrics. *Development Genes and Evolution*. 226(3): 139-158.

Gapert, R., Black, S. & Last, J. 2009. Sex determination from the foramen magnum: discriminant function analysis in an eighteenth and nineteenth century British sample. *International Journal of Legal Medicine.* 123(1): 25-33.

Geldenhuys, E.-M., Burger, E.H., Alblas, A., Greyling, L.M. & Kotzé, S.H. 2016. The association between healed skeletal fractures indicative of interpersonal violence and alcoholic liver disease in a cadaver cohort from the Western Cape, South Africa. *Alcohol*. 52: 41-48.

Giles, E. & Elliot, O. 1962. Race identification from cranial measurements. *Journal of Forensic Sciences*. 7(2): 147-157.

Gill, G.W. & Gilbert, B.M. 1990. Race identification from the midfacial skeleton: American Blacks and Whites. In *Skeletal attribution of race: Methods for forensic anthropology.* Rev. 4th ed. Edited by Gill, G. & Rhine, S. University of New Mexico, Albuquerque: Maxwell Museum of Anthropological Papers. 47-53.

Gilllick, H. 2012. *Ancestry determination using geometric morphometrics*. Msc(Res). Thesis. University of Dundee. (Unpublished).

Ginter, J.K. 2008. *A bioarchaeological study of mid-Holocene communities in the Eastern Cape, South Africa: the interface between foraging and pastoralism*, Ph.D. Thesis. University of Toronto. (Unpublished).

Goswami, A. & Polly, P.D. 2010. Methods for studying morphological integration and modularity. *Quantitative Methods in Paleobiology*. 16: 213-43.

Gonzalez, P.N., Bernal, V. & Perez, S.I. 2011a. Analysis of sexual dimorphism of craniofacial traits using geometric morphometric techniques. *International Journal of Osteoarchaeology*. 21(1): 82-91.

Gonzalez, P.N., Perez, S. I. & Bernal, V. 2011b. Ontogenetic allometry and cranial shape diversification among human populations from South America. *The Anatomical Record*. 294(11): 1864-1874.

Gould, S.J. 1978. Morton's ranking of races by cranial capacity. Unconscious manipulation of data may be a scientific norm. *Science*. 200(4341): 503-509.

GraphPad Software. 2010. GraphPad Prism for Windows, Version 5.00. San Diego: CA.

Grubbs, F.E. 1950. Sample criteria for testing outlying observations. *The Annals of Mathematical Statistics*. 1: 27-58.

Grubbs, F.E. 1969. Procedures for detecting outlying observations in samples. Technometrics. 11(1): 1-21.

Hallgrímsson, B., Zelditch, M.L., Parsons, T.E., Kristensen, E., Young, N.M. & Boyd, S.K. 2008. Morphometrics and Biological Anthropology in the Post-Genomic Age. In *Biological Anthropology of the Human Skeleton*. Rev. 2nd ed. Edited by Katzenburg, M.A. & Saunders. New Jersey: Wiley-Liss.

Hanihara, T., Ishida, H. & Dodo, Y. 2003. Characterization of biological diversity through analysis of discrete cranial traits. *American Journal of Physical Anthropology.* 121(3): 241-251.

Hefner, J. & Ousley, S. 2006. Morphoscopic traits and the statistical determination of ancestry II. *Proceedings of the 58th Annual Meeting of the American Academy of Forensic Sciences*. 20-25.

Hefner, J.T. & Ousley, S.D. 2014. Statistical classification methods for estimating ancestry using morphoscopic traits. *Journal of Forensic Sciences*. 59(4): 883-890.

Hefner, J.T. 2003. *Assessing Nonmetric Cranial Traits currently used in forensic determination of ancestry*. Ph.D. Thesis. University of Florida.

Hefner, J.T. 2009. Cranial nonmetric variation and estimating ancestry. *Journal of Forensic Sciences*, 54(5): 985-995.

Heuzé, Y., Kawasaki, K., Schwarz, T., Schoenebeck, J.J. & Richtsmeier, J.T. 2016. Developmental and Evolutionary Significance of the Zygomatic Bone. *The Anatomical Record.* 299(12): 1616-1630.

Holton, N., Yokley, T. & Butaric, L. 2013. The morphological interaction between the nasal cavity and maxillary sinuses in living humans. *The Anatomical Record*. 296(3): 414-426.

Holton, N.E. & Franciscus, R.G. 2008. The paradox of a wide nasal aperture in cold-adapted Neandertals: a causal assessment. *Journal of Human Evolution*. 55(6): 942-951.

Holton, N.E., Yokley, T.R. & Figueroa, A. 2012. Nasal septal and craniofacial form in European- and African-derived populations. *Journal of Anatomy*. 221(3): 263-274.

Hooton, E.A. 1946. *Up from the Ape*. New York, The Macmillan Company.

Howells, W.W. 1960. The distribution of man. *Scientific American*. 203(3): 112-129.

Howells, W.W. 1973. Cranial variation in man: A study by multivariate analysis of patterns of difference among recent human populations. *Peabody Museum of Archaeology and Ethnology*. Cambridge: Harvard University. 67: 163-190.

Husmann, P.R. & Samson, D.R. 2011. In the eye of the beholder: sex and race estimation using the human orbital aperture. *Journal of Forensic Sciences*. 56(6): 1424-1429.

Hylander, W.L., Picq, P.G. & Johnson, K.R. 1991. Masticatory-stress hypotheses and the supraorbital region of primates. *American Journal of Physical Anthropology*. 86(1): 1-36.

Igbigbi, P.S. & Ebite, L.E. 2010. Orbital index of adult Malawians. Internet *Journal of Forensic Medicine and Toxicology*. 11(1): 29-36.

Igbigbi, P.S. & Nanono-Igbigbi, A. M. 2003. Determination of sex and race from the subpubic angle in Ugandan subjects. *The American Journal of Forensic Medicine and Pathology*. 24(2): 168-172.

Inquests Act, No 58 of 1959. Republic of South Africa. Pretoria: *Government Gazette.* 1959: 58.

Isaacs, L. 2017. Fikile Mbalula concerned over apparent escalation of missing persons. Eyewitness News. 13 April 2017. Available: http://ewn.co.za/2017/05/11/fikile-mbalula-concerned-over-apparent-escalation-of-missing-persons. [Accessed on 24 January 2018].

İşcan, M.Y. & Steyn, M. 1999. Craniometric determination of population affinity in South Africans. *International Journal of Legal Medicine*. 112(2): 91-97.

İşcan, M.Y. & Steyn, M. 2013. *The Human Skeleton in Forensic Medicine*. Springfield: Charles C. Thomas Publisher.

Jacobson, C.K., Amoateng, A.Y. & Heaton, T.B. 2004. Inter-racial marriages in South Africa. *Journal of Comparative Family Studies*. 35(3): 443-458.

Jantz, R.L. & Meadows Jantz, L. 2000. Secular change in craniofacial morphology. *American Journal of Human Biology*. 12(3): 327-338.

Jolicoeur, P. & Mosimann, J.E. 1960. Size and shape variation in the painted turtle. A principal component analysis. *Growth.* 24(4): 339-354.

Kaiser, H.F. 1960. The application of electronic computers to factor analysis. *Educational and Psychological Measurement*. 20(1): 141-151

Kanchan, T., Krishan, K., Gupta, A. & Acharya, J. 2014. A study of cranial variations based on craniometric indices in a South Indian population. *Journal of Craniofacial Surgery.* 25(5): 1645-1649.

Kato, K., Ogata, T., Vidal, H., Manabe, Y., Kitagawa, Y., Oyamada, J. & Rokutanda, A. 1997. The internal orbital facial breadth and middle facial breadth in Mongoloid crania from Peru and east Asia, with special reference to their significance as a racial criterion. *Okajimas Folia Anatomica Japonica*. 74(4): 115-124.

Kieser, J., Whittle, K., Wong, B., Waddell, J.N., Ichim, I., Swain, M., Taylor, M. & Nicholson, H. 2009. Understanding craniofacial blunt force injury: a biomechanical perspective. *In Forensic Pathology Reviews*. M. Tsokos, Eds. New York: Humana Press. 39-51.

Kimmerle, E. H., Ross, A. & Slice, D. 2008. Sexual dimorphism in America: Geometric morphometric analysis of the craniofacial region. *Journal of Forensic Sciences.* 53(1): 54-57.

Klingenberg, C. 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution*. 226(3): 113-137.

Klingenberg, C.P. & Mcintyre, G.S. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution*. 52(5): 1363-1375.

Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*. 11(2): 353-357.

Klingenberg, C.P., Barluenga, M. & Meyer, A. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution*. 56(10): 1909-1920.

Klingenberg, C.P., Duttke, S., Whelan, S. & Kim, M. 2012. Developmental plasticity, morphological variation and evolvability: a multilevel analysis of morphometric integration in the shape of compound leaves. *Journal of Evolutionary Biology*. 25(1): 115-129.

Klingenberg, C.P., Mebus, K. & Auffray, J.C. 2003. Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible? *Evolution and Development*. 5(5): 522-531.

Komar, D.A. & Buikstra, J. E. 2008. *Forensic anthropology: contemporary theory and practice*. United States of America: Oxford University Press.

Komar, D.A. & Grivas, C. 2008. Manufactured populations: what do contemporary reference skeletal collections represent? A comparative study using the Maxwell Museum documented collection. *American Journal of Physical Anthropology*. 137(2): 224-33.

Krüger, G.C., L'Abbé, E.N., Stull, K.E. & Kenyhercz, M.W. 2015. Sexual dimorphism in cranial morphology among modern South Africans. *International Journal of Legal Medicine*. 129(4): 869-875.

Kruskal, W.H. & Wallis, W.A. 1952. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*. 47(260): 583-621.

Kulemeyer, C., Asbahr, K., Gunz, P., Frahnert, S. & Bairlein, F. 2009. Functional morphology and integration of corvid skulls–a 3D geometric morphometric approach. *Frontiers in Zoology.* 6(1): 1-14.

L'Abbé, E.N., Loots, M. & Meiring, J.H. 2005. The Pretoria Bone Collection: A modern South African skeletal sample*. HOMO - Journal of Comparative Human Biology*. 56(2): 197-205.

L'Abbé, E.N., Van Rooyen, C., Nawrocki, S. P. & Becker, P. J. 2011. An evaluation of non-metric cranial traits used to estimate ancestry in a South African sample. *Forensic Science International*. 209(1-3): 195-201.

L'Abbé, E.N., Kenyhercz, M., Stull, K.E., Keough, N. & Nawrocki, S. 2013. Application of Fordisc 3.0 to explore differences among crania of North American and South African blacks and whites. *Journal of Forensic Sciences*. 58(6): 1579-1583.

Landis, J.R. & Koch, G.G. 1977. The measurement of observer agreement for categorical data. *Biometrics*. 33(1): 159-174.

Leamy, L.J., Klingenberg, C.P., Sherratt, E., Wolf, J.B. & Cheverud, J.M. 2015. The genetic architecture of fluctuating asymmetry of mandible size and shape in a population of mice: Another look. *Symmetry*. 7(1): 146-163.

Lee, K.H. 2009. Interpersonal Violence and Facial Fractures. *Journal of Oral and Maxillofacial Surgery*. 67(9): 1878-1883.

Lefevre, c. E., lewis, g. J., perrett, d. I. & penke, l. 2013. Telling facial metrics: facial width is associated with testosterone levels in men. *Evolution and Human Behaviour.* 34(4): 273-279.

Lesciotto, K., Cabo, L. & Garvin, H. 2016. A morphometric analysis of prognathism and evaluation of the gnathic index in modern humans. *HOMO-Journal of Comparative Human Biology*. 67(4): 294-312.

Liebenberg, L., L'Abbé, E.N. & Stull, K.E. 2015a. Population differences in the postcrania of modern South Africans and the implications for ancestry estimation. *Forensic Science International*. 257: 522-529.

Liebenberg, L., Stull, K. E., L'Abbé, E.N. & Botha, D. 2015b. Evaluating the accuracy of cranial indices in ancestry estimation among South African groups. *Journal of Forensic Sciences*. 60(5): 1277-1282.

Littlefield, A., Lieberman, L., Reynolds, L.T., Azevêdo, E.S., Beals, K.L., Brace, C., Garn, S.M., Gloor, P., Jensen, A.R. & Kelso, J. 1982. Redefining race: The potential demise of a concept in physical anthropology. *Current Anthropology*. 23(6): 641-655.

Livingstone, F.B. & Dobzhansky, T. 1962. On the non-existence of human races. *Current Anthropology*. 3(3): 279-281.

Loth, S.R., Patriquin, M. L. & Steyn, M. 2005. Metric analysis of sex differences in South African black and white pelves. *Forensic Science International.* 147(2-3): 119-130.

Maass, P. 2016. *A statistical shape analysis of the neurocranium and long bones*. Ph.D. Thesis University of Cape Town. (Unpublished).

Maddux, S.D., Butaric, L.N., Yokley, T.R. & Franciscus, R.G. 2017. Ecogeographic variation across morphofunctional units of the human nose. *American Journal of Physical Anthropology*. 162(1): 103-119.

Maddux, S.D., Sporleder, A.N. & Burns, C.E. 2015. Geographic variation in zygomaxillary suture morphology and its use in ancestry estimation. *Journal of Forensic Sciences*. 60(4) 966-973.

Mantha, S., Roizen, M.F., Fleisher, L.A., Thisted, R. & Foss, J. 2000. Comparing methods of clinical measurement: reporting standards for Bland and Altman analysis. *Anesthesia & Analgesia*. 90(3): 593-602.

Martin, R. & Saller, K. 1957. *Lehrbuch der* Anthropologie. Stuttgart: Gustav Fischer.

Martínez-Abadías, N., González-José, R., González-Martín, A., Van Der Molen, S., Talavera, A., Hernández, P. & Hernández, M. 2006. Phenotypic evolution of human craniofacial morphology after admixture: a geometric morphometrics approach. *American Journal of Physical Anthropology*. 129(): 387-398.

Masters, M. P. 2008. *Modern variation and evolutionary change in the hominin eye orbit*. Ph.D. Thesis. Ohio State University.

McCullagh, P. 1980. Regression models for ordinal data. *Journal of the Royal Statistical Society. Series B (Methodological)*: 109-142.

McDowell, J. L. 2012. *Nasal aperture shape and its application for estimating ancestry in modern South Africans*. Msc Anatomy. Thesis. University of Pretoria.

McDowell, J.L., Kenyhercz, M.W. & L'Abbé, E.N. 2015. An evaluation of nasal bone and aperture shape among three South African populations. *Forensic Science International*: 252: 189-196.

McDowell, J.L., L'Abbé, E.N. & Kenyhercz, M. W. 2012. Nasal aperture shape evaluation between black and white South Africans. *Forensic Science International*. 222(1-3): 397-202.

McHugh, M. L. 2012. Interrater reliability: the kappa statistic. *Biochemia Medica*. 22(3): 276-282.

Mendelson, B. & Wong, C.-H. 2012. Changes in the Facial Skeleton with aging: implications and clinical applications in facial rejuvenation. *Aesthetic Plastic Surgery*. 36(4): 753-760.

Mitteroecker, P. & Bookstein, F. 2008. The evolutionary role of modularity and integration in the hominoid cranium. *Evolution*. 62(4): 943-958.

Mitteroecker, P., Gunz, P., Bernhard, M., Schaefer, K. & Bookstein, F. L. 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. *Journal of Human Evolution*. 46(6): 679-698.

Montagu, A. 1963. *The Concept of Race*. London: Collier-Macmillan.

Moore-Jansen, P.H. 1989. *A multivariate craniometric analysis of secular change and variation among recent North American populations*. Ph.D. Thesis. University of Tennessee.

Morton, S. 1839. *Crania Americana, or a comparative view of the skulls of various aboriginal nations*, Philadelphia: J. Dobson.

Mosimann, J.E. 1970. Size allometry: size and shape variables with characterizations of the lognormal and generalized gamma distributions. *Journal of the American Statistical Association*. 65(330): 930-945.

Moss, M.L. & Young, R.W. 1960. A functional approach to craniology. *American journal of physical anthropology*. 18(4): 281-292.

Msamati, B., Igbigbi, P. & Manda, J. 2005. The sub-public angle in adult indigenous Malawian subjects. *East African Medical Journal*. 7(4): 195-202.

Mummert, A., Esche, E., Robinson, J. & Armelagos, G. J. 2011. Stature and robusticity during the agricultural transition: evidence from the bioarchaeological record. *Economics & Human Biology.* 9(3): 284-301.

Muñoz-Muñoz, F. & Perpiñán, D. 2010. Measurement error in morphometric studies: comparison between manual and computerized methods. *Annales Zoologici Fennici*. 2010. 47(1): 46-56.

National Health Act, No 61 of 2003. Republic of South Africa. Pretoria: *Government Gazette.* 2003: 61.

Natto, Z.S., Aladmawy, M., Alasqah, M. & Papas, A. 2014. Factors contributing to tooth loss among the elderly: A cross sectional study. *Singapore Dental Journal.* 35: 17-22.

Newton, J.P., Yemm, R., Abel, R.W. & Menhinick, S. 1993. Changes in human jaw muscles with age and dental state. *Gerodontology*. 10(1): 16-22.

Niida, S., Yamamoto, S. and Kodama, H. 1991. Variation in the running pattern of trabeculae in growing human nasal bones. *Journal of Anatomy* 179: 39–41.

Noback, M.L., Harvati, K. & Spoor, F. 2011. Climate-related variation of the human nasal cavity. *American Journal of Physical Anthropology*. 145(4): 599-614.

Oettlé, A.C., Demeter, F.P. & L'Abbé, E.N. 2017. Ancestral variations in the shape and size of the zygoma. *The Anatomical Record*. 300(1): 196-208.

Ossenberg, N.S. 1976. Within and between race distances in population studies based on discrete traits of the human skull. *American Journal of Physical Anthropology*. 45(3): 701-715.

Ousley, S., Jantz, R. & Freid, D. 2009. Understanding race and human variation: why forensic anthropologists are good at identifying race. *American Journal of Physical Anthropology*. 139(1): 68-76.

Ousley, S.D. & Jantz, R.L. 2012. Fordisc 3 and statistical methods for estimating sex and ancestry. In *A companion to forensic anthropology*. D. Dirkmaat, Eds. New Jersey: Blackwell Publishing Ltd. 311-329.

Owens, L.S. 2007. Craniofacial trauma in the prehispanic Canary Islands. *International Journal of Osteoarchaeology*. 17(5): 465-478.

Oyen, O. J., Melugin, M. B. & Indresano, A. T. 1996. Strain gauge analysis of the frontozygomatic region of the zygomatic complex. Journal of Oral and Maxillofacial Surgery. 54(9): 1092-1095.

Palmer, A.R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual review of Ecology and Systematics*. 17(1): 391-421.

Paschetta, C., De Azevedo, S., Castillo, L., Martínez-Abadías, N., Hernández, M., Lieberman, D. E. & González-José, R. 2010. The influence of masticatory loading on craniofacial morphology: a test case across technological transitions in the Ohio Valley. *American Journal of Physical Anthropology.* 141(2) 297-314.

Patterson, N., Petersen, D. C., Van Der Ross, R. E., Sudoyo, H., Glashoff, R. H., Marzuki, S., Reich, D. & Hayes, V. M. 2010. Genetic structure of a unique admixed population: implications for medical research. *Human Molecular Genetics*. 19(3): 411-419.

Perez, S.I. & Monteiro, L.R. 2009. Nonrandom factors in modern human morphological diversification: A study of craniofacial variation in Southern South American populations. *Evolution*. 63(4): 978-993.

Perez, S.I., Bernal, V. & Gonzalez, P.N. 2007. Morphological differentiation of aboriginal human populations from Tierra del Fuego (Patagonia): implications for South American peopling. *American Journal of Physical Anthropology.* 133(4): 1067-1079.

Pietrusewsky, M. 2000. Metric analysis of skeletal remains: methods and applications. In *Biological Anthropology of the Human Skeleton*. M. Anne Katzenbcrg & Shelley R. Saunders, Eds. Rev. 2nd ed. New York: Wiley-Liss. 375-415.

Pimental, R. 1992. An Introduction to ordination, principal components analysis and discriminant analysis. In *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationals*. J.T. Sorensen and R. Foottit, Eds. 11-28.

Pirttiniemi, P. 1998. Normal and increased functional asymmetries in the craniofacial area. *Acta Odontologica Scandinavica.* 56(6): 342-345.

Posel, D. 2001. Race as Common Sense: Racial Classification in Twentieth-Century South Africa. *African Studies Review*. 44(2): 87-113.

Pretorius, E., Steyn, M. & Scholtz, Y. 2006. Investigation into the usability of geometric morphometric analysis in assessment of sexual dimorphism. *American Journal of Physical Anthropology*. 129(1): 64-70.

Reichs, K. M., Huber, C. D., Lippnig, W. R., Ulm, C., Watzek, G. & Tangl, S. 2011. Atrophy of the residual alveolar ridge following tooth loss in an historical population. *Oral Diseases*. 17(1): 33-44.

Remo, J. 2017. Human Skull, licensed under CC Attribution (2017) [Online]. SketchFab. Available: https://sketchfab.com/models/92fb3fbacbbf4fd8805d6c5f463e25e8. [Accessed 15 August 2017].

Rhine, S. (1990). Non-metric skull racing. In *Skeletal attribution of race: Methods for forensic anthropology. G.* Gill & S. Rhine, Eds. Rev. 4th ed. Albuquerque: University of New Mexico, Maxwell Museum of Anthropological Papers. 9-20.

Richard, M.J., Morris, C., Deen, B.F., Gray, L. & Woodward, J.A. 2009. Analysis of the anatomic changes of the aging facial skeleton using computer-assisted tomography. *Ophthalmic Plastic & Reconstructive Surgery.* 25(5): 382-386.

Rightmire, G. 1972. Cranial measurements and discrete traits compared in distance studies of African Negro skulls. *Human Biology*. 2(2): 263-276.

Rohlf, F.J. & Slice, D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*. 39(1): 40-59.

Roseman, C.C. 2004. Detecting interregionally diversifying natural selection on modern human cranial form by using matched molecular and morphometric data. *Proceedings of the National Academy of Sciences of the United States of America*. 101(35): 12824-12829.

Ross, A.H., Slice, D.E. & Williams, S.E. 2010. Geometric Morphometric tools for the classification of human skulls. National Criminal Justicce reference Service. Office of Justice Programs, U.S. Department of Justice, https://www.ncjrs.gov/App/Publications/abstract.aspx?ID=253252. [Accessed on 12 March 2018]

Rourke, N.O., Hatcher, L. & Stepanski, E.J. 1994. *A step-by-step approach to using the SAS system for univariate and multivariate statistics*. New York: SAS Institute.

Rubin, K.M. & Deleon, V.B. 2017. Ancestral variation in orbital rim shape: a three-dimensional pilot study. *Journal of Forensic Sciences*. 62(6): 1575-1581.

Saini, V., Srivastava, R., Rai, R.K., Shamal, S.N., Singh, T.B. & Tripathi, S.K. 2011. An osteometric study of Northern Indian populations for sexual dimorphism in craniofacial region. *Journal of Forensic Sciences*. 56(3): 700-705.

Sarnäs, K.-V. & Solow, B. 1980. Early adult changes in the skeletal and soft-tissue profile. *The European Journal of Orthodontics*. 2(1): 1-12.

Sauer, N.J. & Wankmiller, J. C. 2016. The Assessment of Ancestry and the Concept of Race. In *Handbook of Forensic Archaeology and Anthropology.* S. Blau & D.H. Ubelaker, Eds. Walnut Creek, California: Left Coast Press.

Sauer, N.J. 1992. Forensic anthropology and the concept of race: If races don't exist, why are forensic anthropologists so good at identifying them? *Social Science & Medicine*. 34(2): 107-111.

Schlebusch, C.M., Skoglund, P., Sjödin, P., Gattepaille, L. M., Hernandez, D., Jay, F., Li, S., De Jongh, M., Singleton, A. & Blum, M.G. 2012. Genomic variation in seven Khoe-San groups reveals adaptation and complex African history. *Science*. 338(6105): 374-379.

Seedat, M., Van Niekerk, A., Jewkes, R., Suffla, S. & Ratele, K. 2009. Violence and injuries in South Africa: prioritising an agenda for prevention. *The Lancet*. 374: 1011-1022.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*. 52(3/4): 591-611.

Sholts, S., Flores, L., Walker, P. & Wärmländer, S. 2011. Comparison of coordinate measurement precision of different landmark types on human crania using a 3D laser scanner and a 3D digitiser: implications for applications of digital morphometrics. *International Journal of Osteoarchaeology.* 21(5): 535-543.

Sholts, S.B. & Warmlander, S.K. 2012. Zygomaticomaxillary suture shape analyzed with digital morphometrics: reassessing patterns of variation in American Indian and European populations. *Forensic Science International*. 217(1-3): 234-240.

Slice, D. E. 2007. Geometric morphometrics. *Annual Review of Anthropology*. 36(): 261-281.

Small, C. 2016. *Sexual dimorphism in white South African crania*. Ph.D. Thesis. University of Johannesburg.

Small, C., Brits, D. & Hemingway, J. 2016. Assessing the effects of tooth loss in adult crania using geometric morphometrics. *International Journal of Legal Medicine*. 130: 233-243.

Smedley, A. & Smedley, B.D. 2005. Race as biology is fiction, racism as a social problem is real: Anthropological and historical perspectives on the social construction of race. *American Psychologist*. 60(1): 16-26.

Smith, H.F., Ritzman, T., Otárola-Castillo, E. & Terhune, C.E. 2013. A 3-D geometric morphometric study of intraspecific variation in the ontogeny of the temporal bone in modern Homo sapiens. *Journal of Human Evolution*. 65(5): 479-489.

Spradley, M. K., Jantz, R. L., Robinson, A. & Peccerelli, F. 2008. Demographic change and forensic identification: problems in metric identification of Hispanic skeletons. *Journal of Forensic Sciences.* 53(1): 21-28.

Statistics South Africa, Census 2011: Statistical Release (Revised). 2011. Pretoria, South Africa.

Statistics South Africa, Victims of Crime survey 2017: Statistical Release. 2017. Pretoria: South Africa.

Steadman, D.W. & Konigsberg, L.W. 2009. Multiple points of similarity. In *Hard Evidence: Case Studies in Forensic Anthropology*. D.W. Steadman, Eds. New Jersey: Prentice Hall.

Steyn, M. & İşcan, M.Y. 1998. Sexual dimorphism in the crania and mandibles of South African whites. *Forensic Science International*. 98(1-2): 9-16.

Steyn, M., Meiring, J.H. & Nienaber, W. C. 1997. Forensic anthropology in South Africa: a profile of cases from 1993 to 1995 at the Department of Anatomy, University of Pretoria. *South African Journal of Ethnology*. 20(1): 23-26.

Steyn, M., Pretorius, E. & Hutten, L. 2004. Geometric morphometric analysis of the greater sciatic notch in South Africans. *HOMO-Journal of Comparative Human Biology*. 54: 197-206.

Stull, K.E., Kenyhercz, M.W. & L'Abbé, E.N. 2014. Ancestry estimation in South Africa using craniometrics and geometric morphometrics. *Forensic Science International*. 245: 206-213.

Ta'ala, S.C., Berg, G.E., Hefner, J.T., Spradley, K., Mann, R.W., Manabe, J., Byrd, J.E., *et al.,* 2014. Metric ancestry estimation from the postcranial skeleton. In *Biological affinity in forensic identification of human skeletal remains: Beyond black and white*. G.E. Berg, S.C. Tala, Eds. Florida: CRC Press.

Tabachnick, B.G. & Fidell, L.S. 2012. *Using Multivariate Statistics*. United States of America: Ally & Bacon/Pearson Education.

Tal, H. & Tau, S. 1983. Statistical survey of the human skull in the Raymond Dart collection of skeletons. *South African Journal of Science*. 79: 215-217.

Van Rooyen, C. 2010. *Evaluating standard non-metric cranial traits used to determine ancestry on a South African sample*. Msc Anatomy. Thesis. University of Pretoria.

Van Wyk, P.J. & Van Wyk, C. 2004. Oral health in South Africa. *International Dental Journal*. 54(6): 373-377.

Vance, V.L., Steyn, M. & L'Abbé, E.N. 2011. Nonmetric sex determination from the distal and posterior humerus in black and white South Africans. Journal of Forensic Sciences. 56(3): 710-714.

Vitek, C. L. 2012. A critical analysis of the use of non-metric traits for ancestry estimation among two North American population samples. MSc. Thesis. University of Tennessee.

Von Cramon-Taubadel, N. 2009. Congruence of individual cranial bone morphology and neutral molecular affinity patterns in modern humans. *American Journal of Physical Anthropology*. 140(2), 205-215.

von Cramon-Taubadel, N., Frazier, B.C. and Lahr, M.M. 2007. The problem of assessing landmark error in geometric morphometrics: theory, methods, and modifications. *American Journal of Physical Anthropology*, 134(1): 24-35.

Von Cramon-Taubadel, N. 2014. Evolutionary insights into global patterns of human cranial diversity: population history, climatic and dietary effects. Journal of Anthropological Science, 92(4): 43-77.

Waitzman, A.A., Posnick, J.C., Armstrong, D.C. & Pron, G.E. 1992. Craniofacial skeletal measurements based on computed tomography: Part II. Normal values and growth trends. *The Cleft Palate-Craniofacial Journal*. 29(2): 118-128.

Walker, P.L. 2005. Greater sciatic notch morphology: Sex, age, and population differences. *American Journal of Physical Anthropology*. 127(4): 385-391.

Webster, M. & Sheets, H.D. 2010. A practical introduction to landmark-based geometric morphometrics. *Quantitative Methods in Paleobiology*. 16: 168-188.

Weisensee, K.E. & Jantz, R.L. 2011. Secular changes in craniofacial morphology of the Portuguese using geometric morphometrics. *American Journal of Physical Anthropology*. 145(4): 548-559.

Wescott, D. & Jantz, R. 2005. Assessing craniofacial secular change in American blacks and whites using geometric morphometry. In *Modern morphometrics in Physical Anthropology.* D. Wescott, & R. Jantz, Eds. Boston: Springer. 231-245.

Wheat, A.D. 2009. *Assessing ancestry through nonmetric traits of the skull: a test of education and experience*. MSc. Thesis, Texas State University San Marcos.

Wiley, D.F. 2005. *Landmark*. University of California, Davis: Institute for Data Analysis and Visualisation (IDAV)

Wiley, D.F., Amenta, N., Alcantara, D.A., Ghosh, D., Kil, Y.J., Delson, E., Harcourt-Smith, W., Rohlf, F. J., *et al*., 2005. *Visualization*. VIS 05. IEEE, 2005. 431-438.

Williams, S. E. & Slice, D. E. 2010. Regional shape change in adult facial bone curvature with age. *American Journal of Physical Anthropology*. 143(3): 437-447.

Williams, S. E. 2008. *Is aging only skin deep? Assessing change in facial bone curvature with age*. Ph.D. Thesis. University of Florida.

Wood, J.W., Milner, G.R., Harpending, H.C., Weiss, K.M., Cohen, M.N., Eisenberg, L.E., Hutchinson, D.L., Jankauskas, R., Cesnys, G. & Česnys, G. 1992. The osteological paradox: problems of inferring prehistoric health from skeletal samples [and comments and reply]. *Current anthropology*. 33(4): 343-370.

Wu, X.-J., Schepartz, L. A., Liu, W. & Trinkaus, E. 2011. Antemortem trauma and survival in the late Middle Pleistocene human cranium from Maba, South China. *Proceedings of the National Academy of Sciences.* 108: 19558-19562.

Xing, S., Gibbon, V., Clarke, R. & Liu, W. 2013. Geometric morphometric analyses of orbit shape in Asian, African, and European human populations. *Anthropological Science*. 121(1): 1-11.

Zar, J. H. 1999. Biostatistical Analysis, Pearson Education India.

Appendices

Appendix A: Nonmetric trait descriptions, illustrations and variable codes.

Nasal bone contour (NBC)

The contour of the nasal bones and frontal processes of the maxilla, approximately 1cm below nasion (Hefner, 2003).

No	Variant	Ancestral association	Code
1	*Low and rounded* nasal bone contour	African	**E**
2	*Oval contour* with elongation superiorly, projecting anteriorly from the midface. This variant lacks steep walls and presents as a circular shape. Shape can be described as quinoset-hut shaped	Asian	**C**
3	*Steep lateral walls* and a broad and flat superior surface (7mm or more). The superior surface can be described as a plateau.	European	**A**
4	*Semi-triangular (vaulted)*, steep sided lateral walls and narrow superior surface plateau.	European	**D**
5	*Steepled lateral walls*, triangular cross section, lacking superior surface plateau	European	**B**

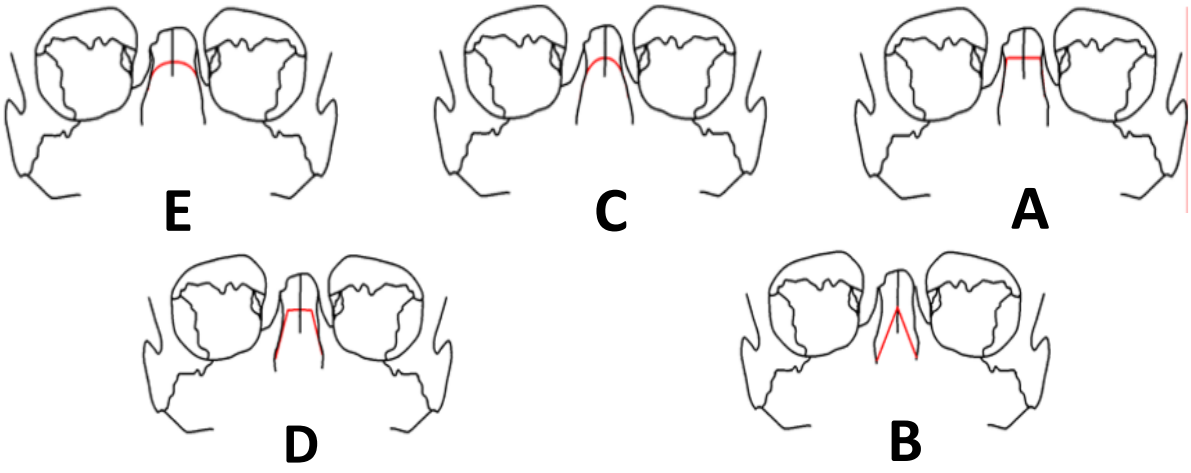

Image taken and adapted from Van Rooyen (2010) and Hefner (2003)

Nasal aperture width (NAW)

The width of the nasal opening relative to the viscerocranium (Hefner, 2003).

No	Variant	Ancestral association	Code
1	*Long a*nd teardrop shaped nasal aperture (when viewed anteriorly) and constricted superior margin and inferior lateral projection (viewed in profile).	European	B
2	The greatest lateral projection of the nasal aperture on the inferior nasal margin, coupled with superior constriction, resulting in a *bell shaped* nasal aperture when viewed anteriorly.	Asian	C
3	The *wide nasal aperture* dominates the face with the greatest lateral projection near the horizontal midline.	African	A

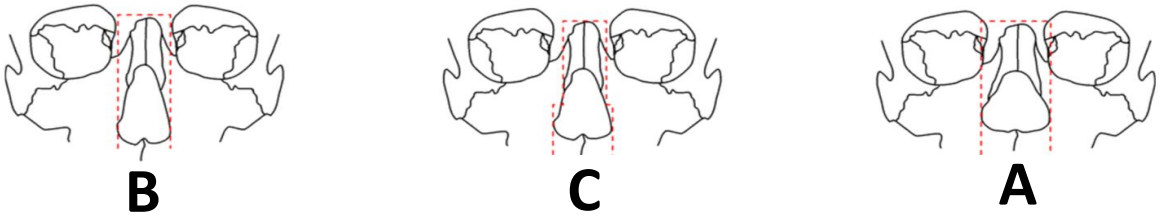

Image taken and adapted from Van Rooyen (2010) and Hefner (2003)

Anterior nasal spine.

Projection of the spine with reference to a midsagittal line from prosthion to nasion (Hefner, 2003).

No	Variant	Ancestral association	Code
1	*Short (rounded)* -defined as minimal to-no projection of the anterior nasal spine.	African	A
2	*Dull*- anterior nasal spine is one which does not transect the midsaggital line running parallel to the face superiorly from prosthion.	Asian	D
3	*Medium*-nasal spine which projects to prosthion, but which neither extends beyond it, nor terminates in a sharp anterior point.	European	B
4	*Long (sharp*)- anterior nasal spine projects beyond prosthion, and is characterized by a sharp anterior termination.	NA	C

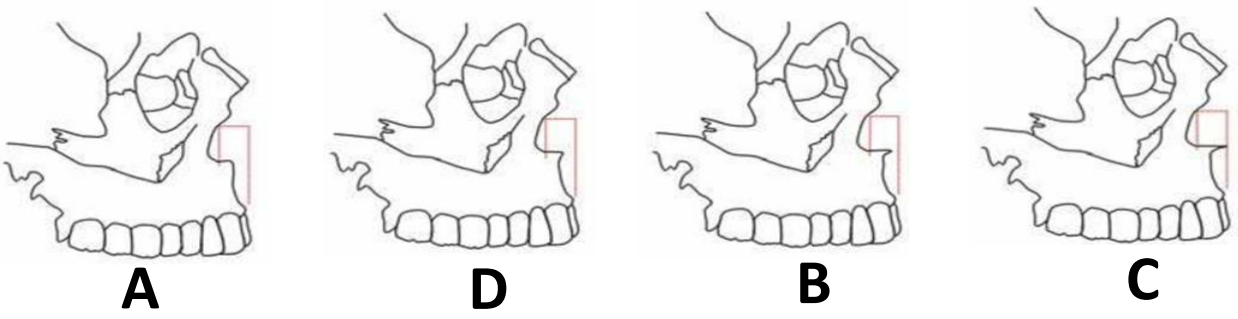

Image taken and adapted from Van Rooyen (2010) and Hefner (2003)

Inferior nasal margin

The most inferior part of the nasal aperture, which, when combined with the lateral alae, constitutes the transition from the nasal floor to the vertical portion of the maxilla (superior to the dentition) (Hefner, 2003).

No	Variant	Ancestral association	Code
1	*Guttered* - Gradual sloping of nasal floor from posterior to anterior. The slope originates where the vomer inserts into the maxillary bone and terminates at the vertical surface of the maxilla.	African	D
2	*Incipient Guttering*- Sloping commences more anteriorly, but is less than 1.	African	A
3	*Straight*- Immediate transition from nasal floor to vertical maxilla, absence of a nasal sill.	Asian	B
4	*Partial sill*- Weak but present ridge of vertical bone, spanning between the two alae.	European	C
5	*Sill*- Pronounced ridge, preventing a smooth transition from the nasal floor to the vertical portion of the maxilla.	European	E

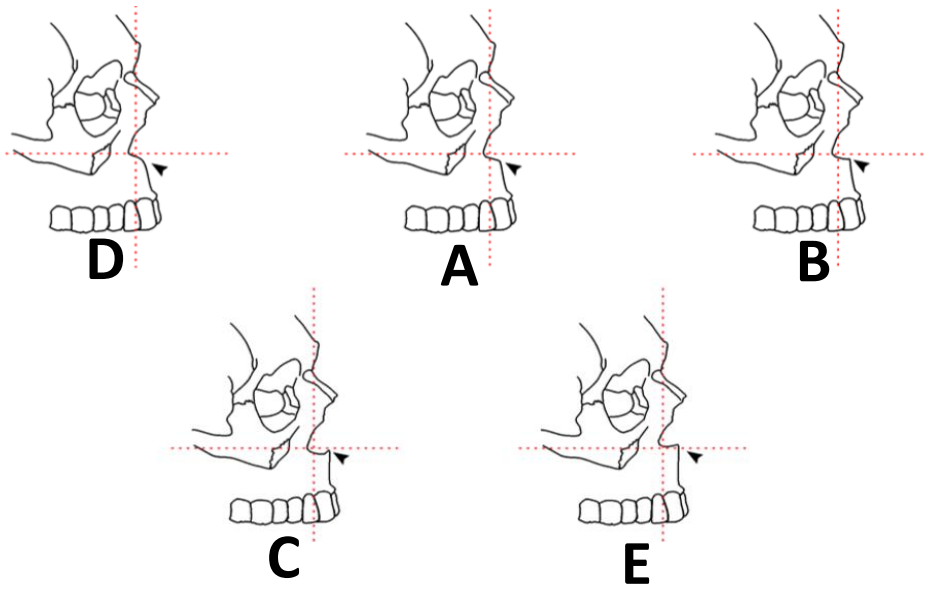

Image taken and adapted from Van Rooyen (2010) and Hefner (2003)

Zygomaxillary suture shape

The shape of the suture between the zygomatic bone and the maxilla (Hefner, 2003).

No	Variant	Ancestral association	Code
1	*Angled*- Zygomaxillary suture has the greatest lateral projection at or near the midline of the suture.	Asian	A
2	*Smooth*- Zygomaxillary suture has the greatest lateral projection at or near the inferior end of the suture.	African	C
3	*Z -shaped*-Zygomaxillary suture is characterised by a zig-zagged appearance.	European	B

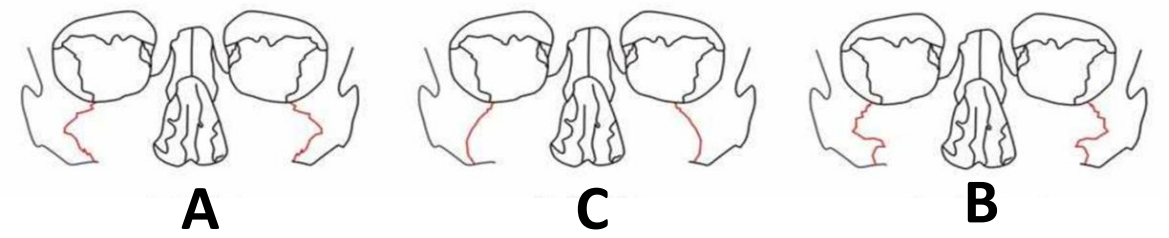

Image taken and adapted from Hefner (2003)

Supranasal suture

The state of the suture which represents the site of fusion of the nasal portion of the frontal suture (Hefner, 2003, Van Rooyen, 2010).

No	Variant	Ancestral association	Code
1	Open and unfused nasal portion of the frontal suture	NA	C
2	Closed but visible frontal suture.	NA	A
3	Closed and barely visible frontal suture	NA	B
4	Completely obliterated suture	NA	D

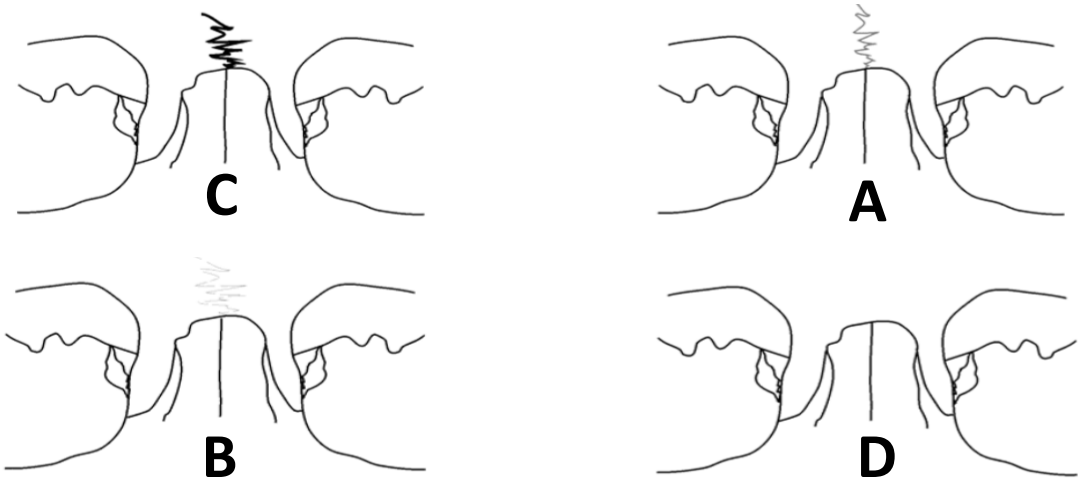

Image taken and adapted from Van Rooyen (2010) and Hefner (2003)

Interorbital breadth

The distance from dacryon to dacryon across the nasal bridge, scored relative to the width of the face (Hefner, 2003).

No	Variant	Ancestral association	Code
1	Narrow relative to facial width	European	B
2	Intermediate relative to facial width	Asian	C
3	Wide relative to face width	African	A

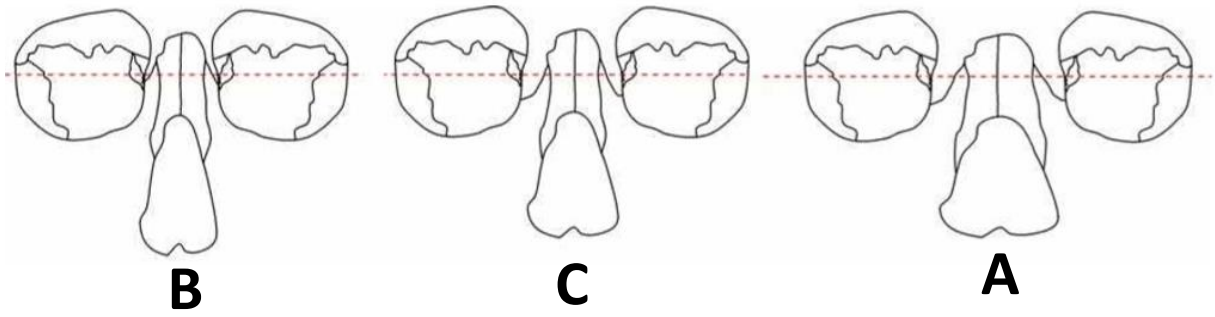

Image taken and adapted from Hefner (2003)

Orbital shape

The shape of the orbits as described by Byers (2015).

No	Variant	Ancestral association	Code
1	Smaller orbital height than width, resulting in a rectangular or square-like shape of the orbits. Orbits tend to be angled inferiorly.	European	C
2	Orbits appear elongated and rectangular	African	B
3	Orbits appear round	Asian	A

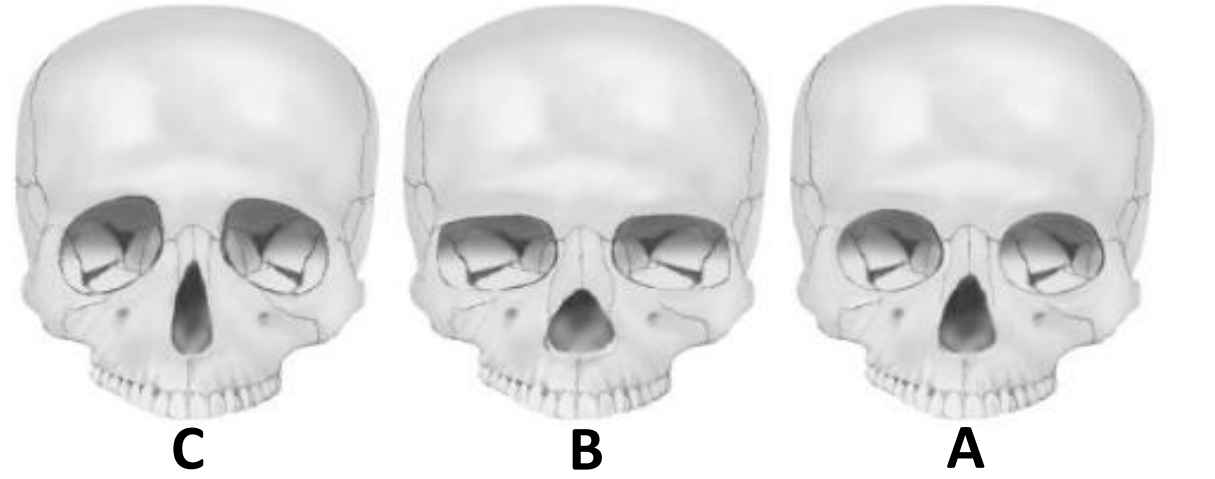

Image taken and adapted from Byers (2015)

Malar tubercle

A transparent ruler is placed at the intersection of the zygomaxillary suture and the inferior margin of the malar tubercle and the degree of protrusion of the tubercle beyond the ruler's edge is scored (Hefner, 2003).

No	Variant	Ancestral association	Code
1	Not present	African	A
2	Very small tubercle (<2mm)	Asian	D
3	Medium protrusion (2-4mm)	European	C
4	Pronounced tubercle on inferior margin of zygoma and maxilla	NA	B

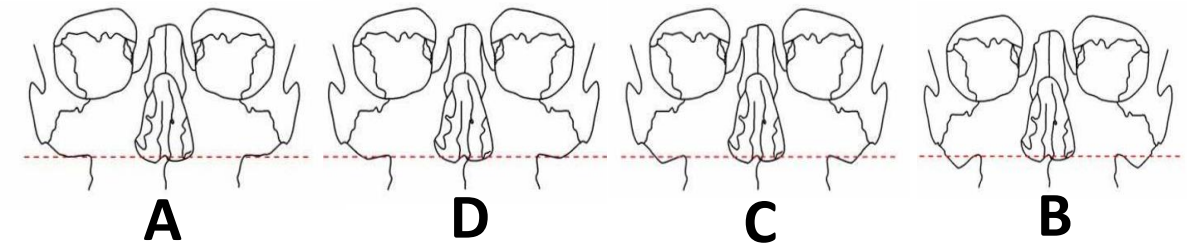

Image taken and adapted from Hefner (2003)

Alveolar prognathism

The degree of protrusion of the alveolar border of the maxilla. Articulate the maxilla with the mandible to determine the whether the maxillas alveolar border projects beyond the mandibles alveolar spine/menton (Van Rooyen, 2010).

No	Variant	Ancestral association	Code
1	Alveolar border does not project in front of alveolar spine/menton of the mandible. Mild projection due to incisors might occur.	European/African	B
2	Projection of alveolar border beyond anterior nasal spine/ menton of the mandible.	African	A

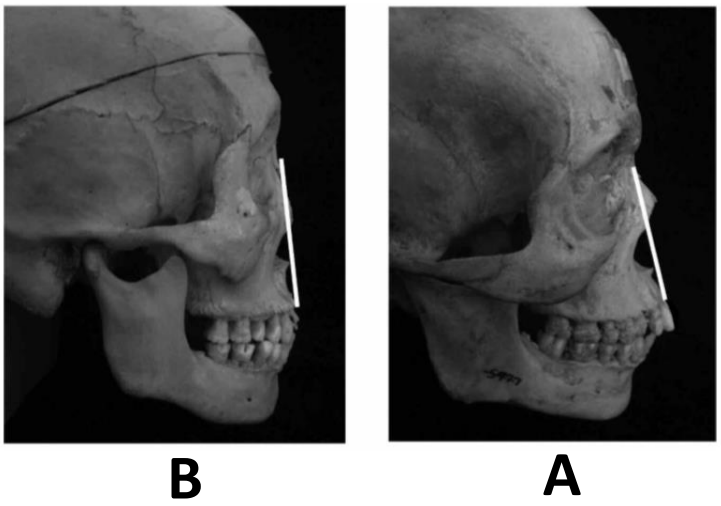

Image taken and adapted from İşcan *and Steyn (2013)*

Zygomatic projection

The skull is held at the occipital region, a pencil is placed across the nasal aperture and the distance between the zygomatic bones and the pencil is evaluated (L'Abbé *et al*., 2011, Van Rooyen, 2010).

No	Variant	Ancestral association	Code
1	Zygoma are positioned posteriorly relative to the opening of nasal aperture in the vertical plane. A finger can be inserted between the zygomatic bone and the pencil, in a non-projecting zygoma or retreating zygoma. Both variants are scored the same.	European/African	A
2	Zygoma on are on the same vertical plane as opening of nasal aperture. In a projecting zygoma the observer is unable to insert a finger between the zygomatic bone and the pencil.	Asian	B

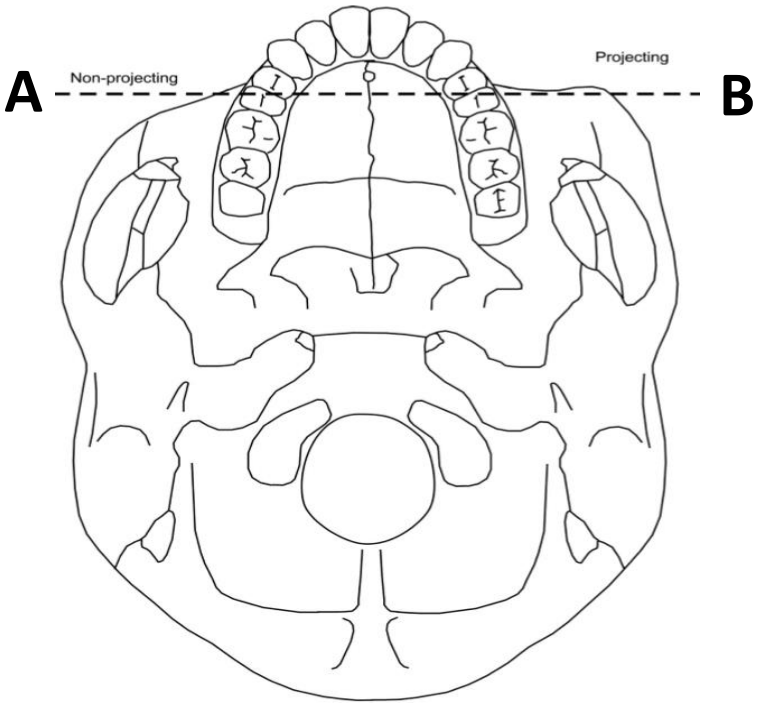

Image taken and adapted from İşcan *and Steyn (2013)*

Appendix C: Bland Altman observer agreement outcomes and plots for inter-observer agreement testing

Table 1. Bland Altman observer agreement outcomes.

Parameter		**Inter-observer agreement**				***Intra-observer agreement***			
		Bias	*SD*	*LLD*	*ULD*	*Bias*	*SD*	*ULD*	*LLD*
Orbital breadth	OBB	0.19	0.34	-0.47	*0.84*	*0.06*	*0.30*	*-0.52*	*0.64*
Orbital height	OBH	0.003	0.28	-0.54	*0.54*	*0.15*	*0.47*	*-0.76*	*1.06*
Interorbital breadth	DKB	0.09	0.27	-0.43	*0.61*	*0.06*	*0.26*	*-0.47*	*0.58*
Bi-orbital breadth	EKB	0.15	0.34	-0.51	*0.81*	*0.14*	*0.32*	*-0.49*	*0.78*
Nasion-Prosthion height	NPH	0.05	0.29	-0.52	*0.62*	*0.01*	*0.27*	*-0.54*	*0.51*
Nasal height left	NHL	-0.04	0.33	-0.69	*0.60*	*0.02*	*0.28*	*-0.52*	*0.57*
Nasal height right	NHR	0.07	0.29	-0.50	*0.64*	*0.003*	*0.26*	*-0.50*	*0.51*
Nasal breadth	NLB	-0.003	0.35	-0.68	*0.67*	*-0.02*	*0.23*	*-0.46*	*0.43*
Bizygomatic breadth	ZYB	-0.07	0.33	-0.71	*0.58*	*0.07*	*0.28*	*-0.48*	*0.61*
Maxillo-alveolar breadth	MAB	0.05	0.33	-0.59	*0.69*	*-0.007*	*0.25*	*-0.51*	*0.49*
Bimaxillary breadth	ZMB	0.02	0.24	-0.46	*0.50*	*-0.02*	*0.17*	*-0.34*	*0.31*
Basion-Nasion length	BNL	0.12	0.32	-0.51	*0.75*	*0.02*	*0.17*	*-0.31*	*0.34*
Basion-Prosthion length	BPL	0.02	0.02	-0.43	*0.48*	*-0.02*	*0.17*	*-0.35*	*0.31*
Tooth loss*	TL	0.00	0.00	0.00	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*

Units (mm)

**Tooth loss is a continuous covariate and is not measured in millimetres.*

SD (standard deviation of bias). LLD (lower 95% limit of agreement). ULD (upper 95% limit of agreement)

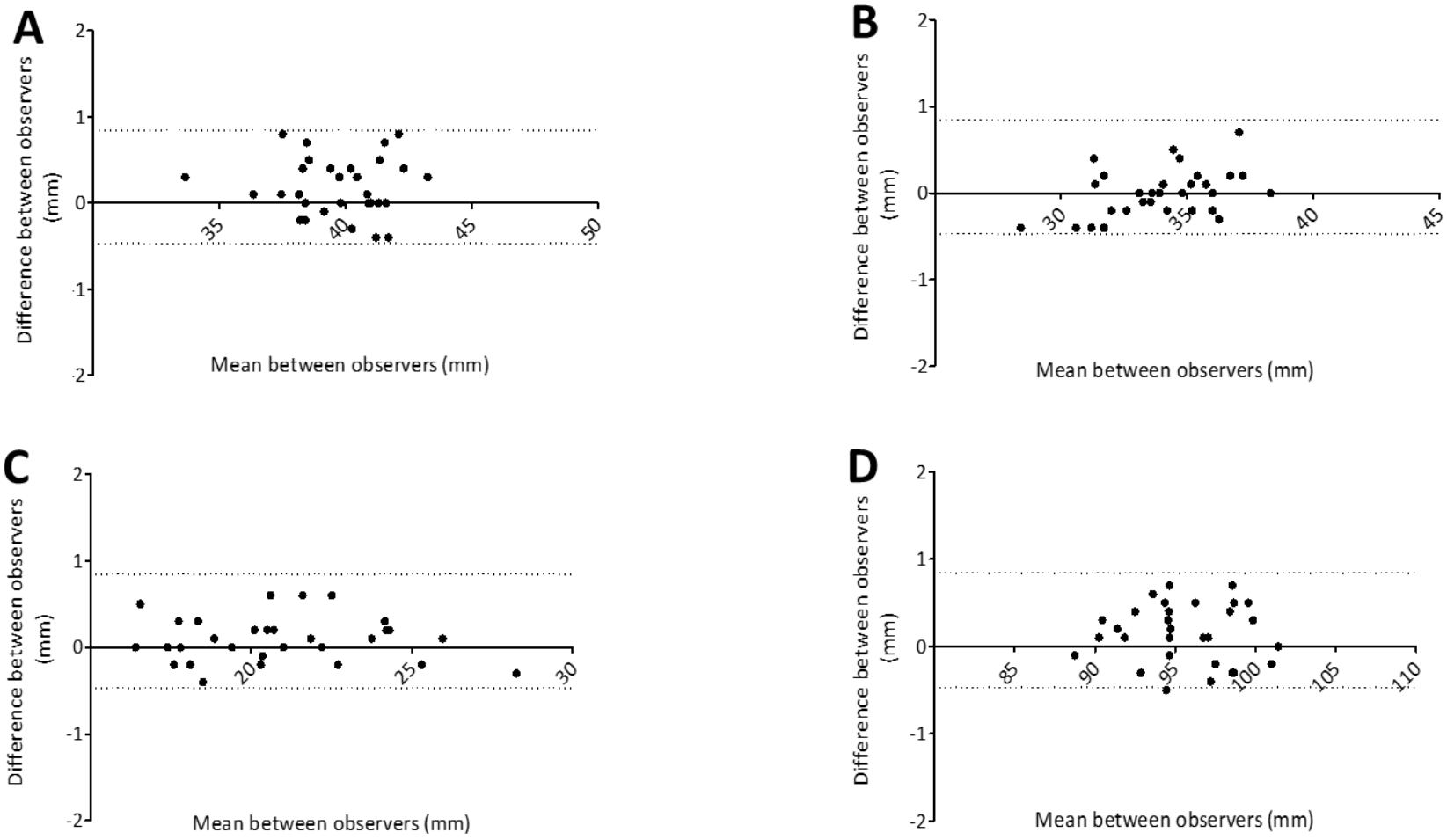

Figure 1: Bland Altman plots of the differences between inter-observer repeats*. The dotted lines represent 95% limits of confidence of differences between observers.* ***A****. Orbital Breadth.* ***B****. Orbital height.* ***C****. Interorbital Breadth.* ***D****. Bi-orbital breadth.*

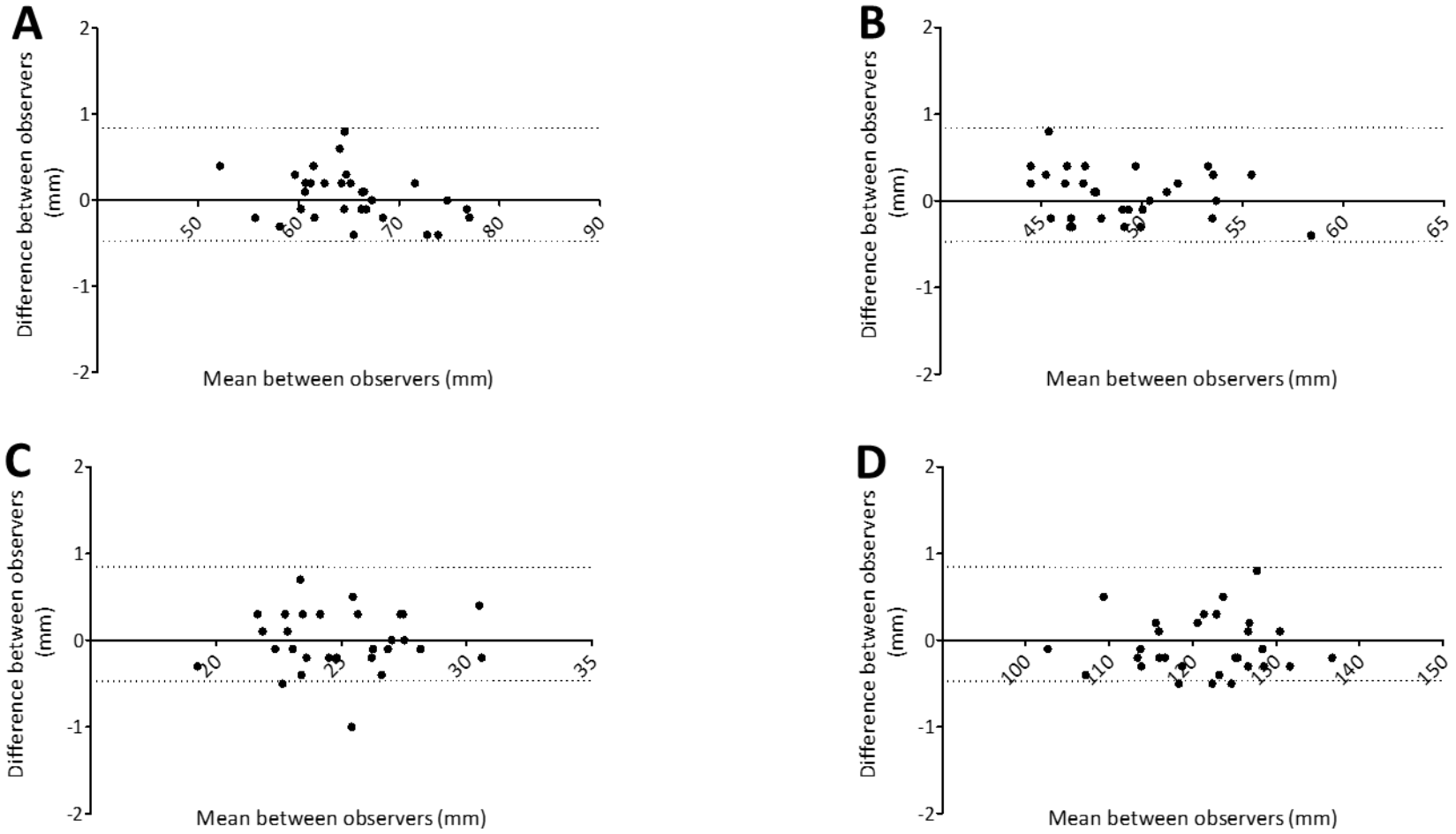

Figure 2: Bland Altman plots of the differences between inter-observer repeats. Dotted lines represent 95% limits of confidence of differences between observers. **A**. Upper facial height. **B**. Nasal height right. **C**. Nasal breadth. **D**. Bizygomatic breadth.

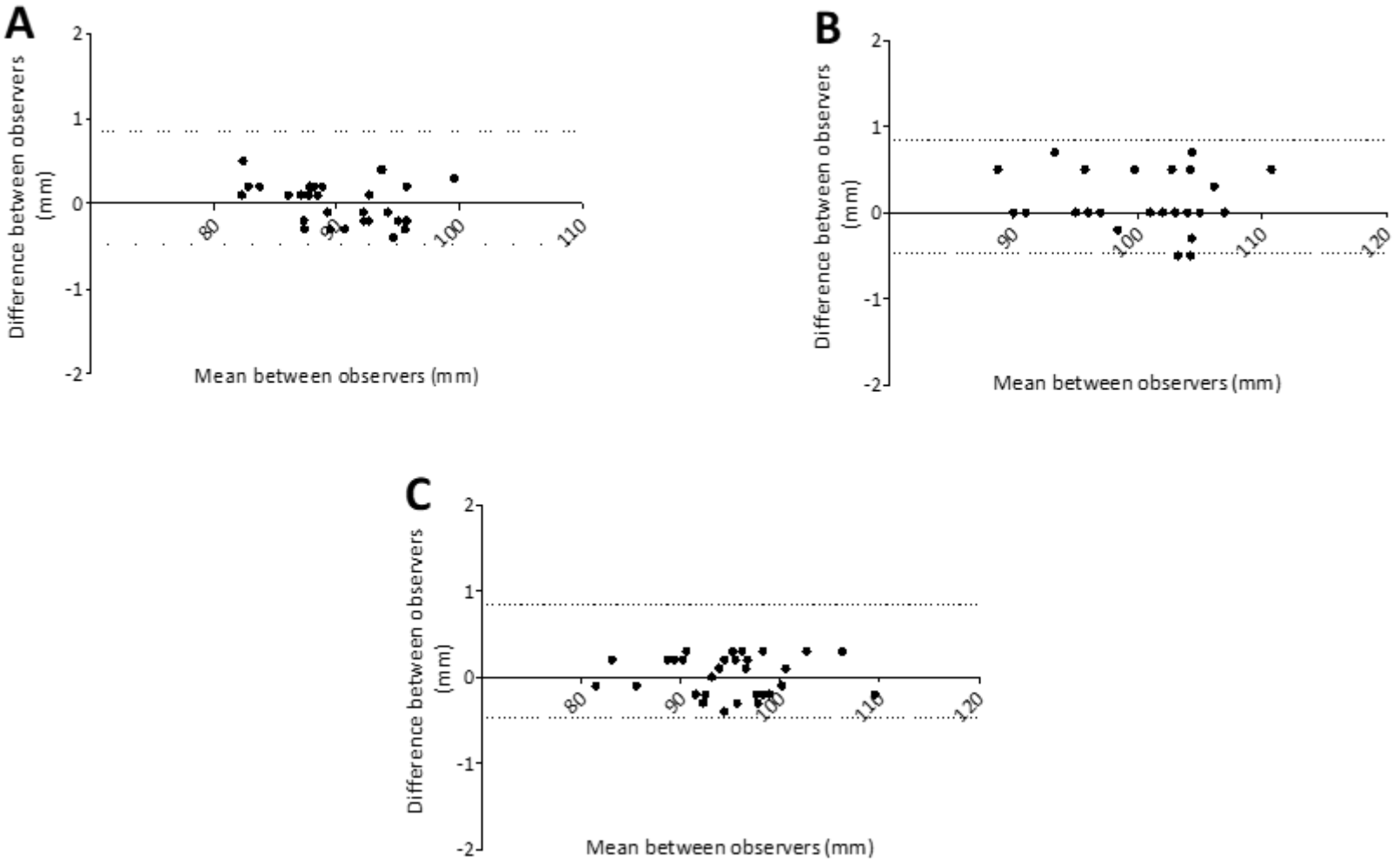

Figure 3: Bland Altman plots of the differences between inter-observer repeats. Dotted lines represent 95% limits of confidence of differences between observers. **A**. Bimaxillary breadth. **B.** Basion-Nasion length. **C.** Basion-Prosthion-length.

Appendix D: Bland Altman plots for intra-observer agreement testing

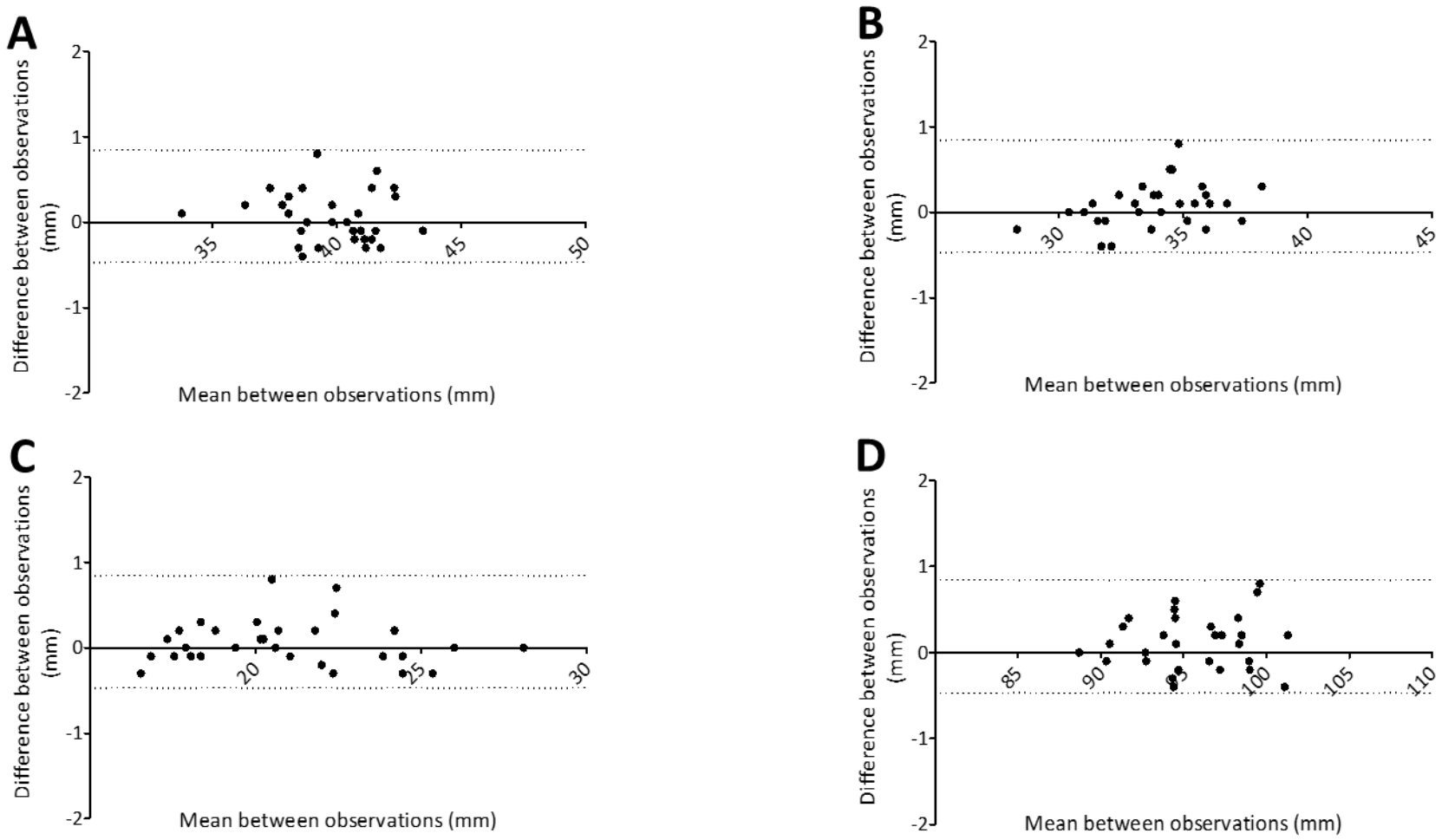

Figure 1: Bland Altman plots of the differences between intra-observer repeats. The dotted lines represent 95% limits of confidence of differences between observers. **A**. Orbital Breadth. **B**. Orbital height. **C**. Interorbital Breadth. **D**. Bi-orbital breadth.

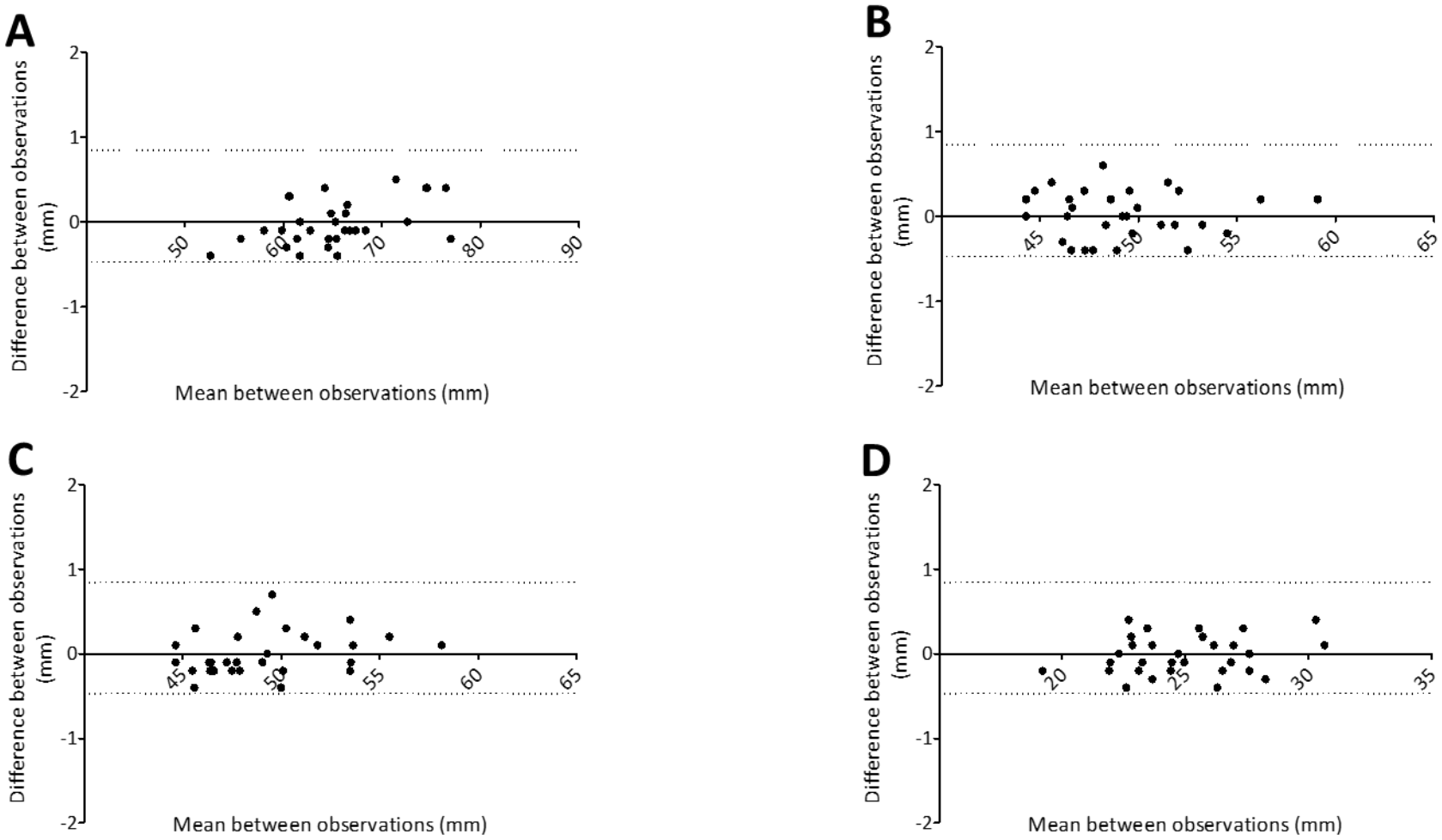

Figure 2: Bland Altman plots of the differences between intra-observer repeats. Dotted lines represent 95% limits of confidence of differences between observers. **A**. Upper facial height. **B**. Nasal height Left. **C**. Nasal height right. **D**. Nasal breadth.

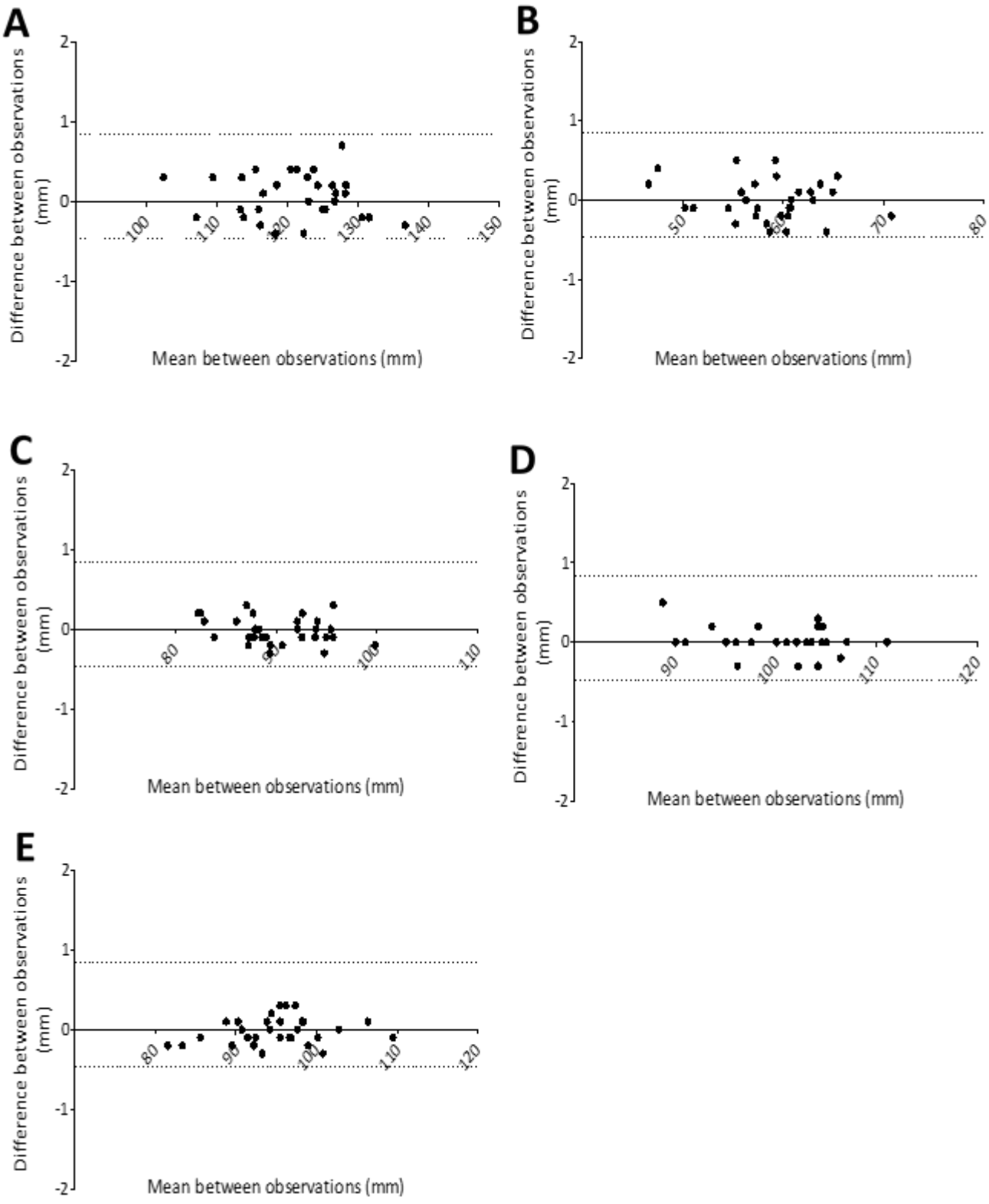

Figure 3: Bland Altman plots of the differences between intra-observer repeats. Dotted lines represent 95% limits of confidence of differences between observers. **A**. Bizygomatic breadth. **B**. Bimaxillary breadth. **C**. Maxillo-alveolar breadth. **D.** Basion-Nasion length. **E.** Basion-Prosthion-length.

Appendix E: The influence of nasal trauma on craniofacial morphology

The effect of nasal trauma on craniofacial size

As seen in Table 1, nasal trauma(NT) occurred more frequently in the sample than zygomatic trauma(ZT). More MA individuals (58.2%) exhibited NT, than EA (40%) and AA individuals (23.1%). Notably, males (54.3%) exhibited NT more frequently than females (31.9%).

Table 1. Frequencies of nasal and zygomatic trauma occurrence in ancestry groups

		Ancestry				
		African n =105 (26.8%)	*Mixed* n =196 (50%)	*European* n=91 (23.2%)	$F/\chi 2$	*P*
Nasal trauma[(b)]	n (%)	42 (40)	114 (58.2)	21 (23.1)	**32.43**	≤**0.0001**
Zygomatic trauma[(c)]	n (%)	16 (15.2)	59 (30.1)	5 (5.5)	**35.45**	≤**0.0001**

'χ^2' from Chi^2 tests for categorical associations

a) Effect size: *v*= 0.3*; p*<0.0001

b) Effect size: *v*= 0.3*; p*<0.0001

'*n*' – number of individuals. 'χ^2'- Chi-squared value. '*p*' – P-value.

As seen in Tables 2-3, size differences of 2.75mm ± 1.25mm were detected in ZYB measurements between AA individuals with-and without- nasal trauma (*t*=2.17, *p*=0.03). Between EA individuals with-and without-NT, size differences of 2.22mm ± 1.25mm in EKB (*t*=2.4, p=0.02); 1.76mm ± 0.85mm in NHL (*t*=2.07, *p*=0.04); 2.03mm ± 0.86mm in NHR (*t*=2.36, *p*=0.02); 1.02mm ± 0.48mm in NLB (*t*=2.13; *p*=0.04) were detected. All the above measurements were larger in those with NT (Tables 2-3).

Table 2. Differences in mean measurements (mm) between ancestry groups (with nasal trauma).

	Ancestry				
Measurement	**African (*n*=42)** ***Mean (SD)***	**European (*n* =21)** ***Mean(SD)***	**Mixed (*n* =114)** ***Mean(SD)***	***F***	***p-value***
EKB [a]	97.37 (4.0)	94.95 (3.8)	95.21 (4.6)	**4.145**	**0.02**
NHL [b]	48.45 (3.2)	51.38(4.0)	47.73 (3.5)	**9.908**	**<0.0001**
NHR [c]	48.56 (3.2)	51.32 (3.8)	47.57 (3.5)	**10.676**	**<0.0001**
NLB [d]	26.84 (2.4)	23.84 (1.9)	25.72 (2.1)	**13.727**	**<0.0001**
ZYB [e]	124.54 (6.4)	118.79 (5.9)	119.34 (7.3)	**9.232**	**<0.0001**

F statistic from Univariate ANOVA for parametric data between ancestry groups.

- Bold values are significant at an α-level < 0.05.

- Measurements: EKB-Bi-orbital breadth; NHL-Nasal height left; NHR-Nasal height right; NLB-Nasal breadth; ZYB-Bizygomatic breadth; BNL- Basion-Nasion length

LSD post-hoc analysis:

a) EKB: AA mean > EA (*p*=0.03) and MA means (p=0.006).

b) NHL: EA mean > AA (*p*=0.002) and MA means (*p*<0.0001).

c) NHR: EA mean > AA (*p*=0.003) and MA means (*p*<0.0001).

d) NLB: EA mean < MA (*p*<0.0001) and AA means (*p*<0.0001). MA mean < AA mean (*p*=0.004).

e) ZYB: AA mean > EA (*p*=0.002) and MA means (*p*<0.0001)

Table 3. Differences in mean measurements (mm) between ancestry groups (without nasal trauma).

	Ancestry				
	African (*n*=63)	**European (*n* =70)**	**Mixed (*n* =82)**		
Measurement	***Mean (SD)***	***Mean(SD)***	***Mean(SD)***	***F***	***p-value***
EKB [a]	96.94(3.8)	93.24 (3.8)	95.26 (4.6)	**18.64**	**<0.0001**
NHL [b]	48.39(3.1)	50.03 (3.5)	47.60 (3.4)	**16.60**	**<0.0001**
NHR [c]	48.46(3.1)	49.76 (3.5)	47.39 (3.4)	**16.01**	**<0.0001**
NLB [d]	26.84(2.2)	23.05 (2.0)	25.69 (2.3)	**78.59**	**<0.0001**
ZYB [e]	122.89(6.4)	117.42 (6.0)	119.29 (7.8)	**15.63**	**<0.0001**
BNL	100.29(4.9)	100.50(4.9)	99.37(5.2)	2.06	0.13

F statistic from Univariate ANOVA for parametric data between ancestry groups.

- Bold values are significant at an α-level < 0.05.

- Measurements: EKB- Bi-orbital breadth; NHL-Nasal height left; NHR-Nasal height right; NLB-Nasal breadth; ZYB-Bizygomatic breadth; BNL- Basion-Nasion length

LSD post-hoc analysis:

a) EKB: AA mean > EA mean (p<0.0001) and MA (p=0.001). EA mean < MA mean (p<0.0001).
b) NHL: MA mean < AA mean (p=0.05) and EA mean (p<0.0001). AA mean < EA mean (p=0.001).
c) NHR: MA mean < AA mean (p=0.008) and EA mean (p<0.0001). AA mean < EA mean (p=0.007).
d) NLB: EA mean < AA mean (p<0.0001) and MA mean (p<0.0001). MA < AA mean (p<0.0001).
e) ZYB: EA mean < AA mean (p<0.0001) and MA mean (p=0.04). MA < AA mean (p<0.0001).

The effect of nasal trauma on craniofacial shape

A weak correlation was detected between craniofacial shape and NT (*RV*=0.02; *p*=0.04) and PLS 1 accounted for 100% of the covariance between these variables. When assessed relative to NT, ancestry groups showed no affinity towards negative or positive changes in shape variation (Figure 1). AA and EA individuals clustered on the shape and trauma axes (Figure 1), indicating that the effect of NT in these groups was more similar than in MA individuals. More MA individuals (58.2%) exhibited NT and characteristic shape variations within this group may have disproportionally influenced, shape differences associated with NT.

Slightly more individuals with NT were positively located on the PLS 1 shape score axis (Figure 1). This weak association between shape and nasal trauma indicates that NT was not associated with extreme of shape variations in this sample (Figure 1). While minor shape differences associated with NT were detected, visualisation of these differences required magnification by a factor of 10 (Figure 1). Nasal bridges were superiorly wider, inferiorly more medial and less anteriorly projecting in those with NT. Wider nasal apertures and more lateral and superior orbits were detected in those with NT (Figure 2). Zygomatic arches were more medial, superior and posteriorly positioned relative to NT (Figure 2). The maxilla was less prognathic, narrower and more steeply-angled posteriorly in those with NT (Figure 2). DFA yielded poor separation based on NT ($p \geq 0.09$) and therefore, the those with and without NT were considered a single group for further analyses.

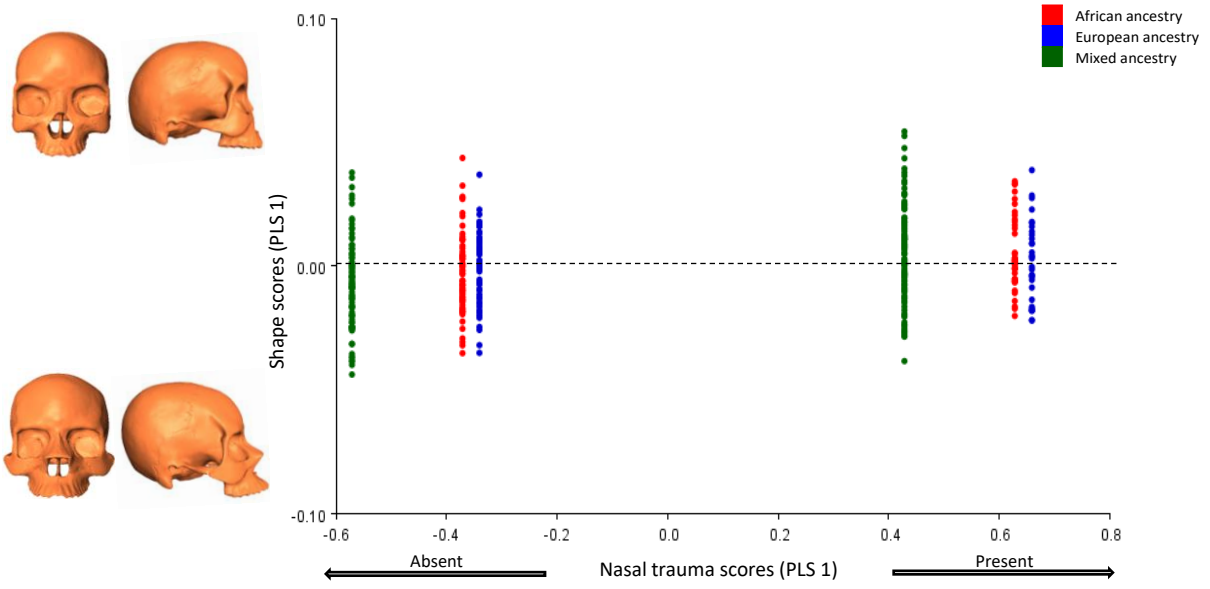

Figure 1. PLS analysis of the relationship between nasal trauma and craniofacial shape, with variances pooled by ancestry. 3D warped models of extreme craniofacial variation for PLS 1 (+) and PLS 1 (-), representative of changes associated with nasal trauma presence and absence respectively, are included.

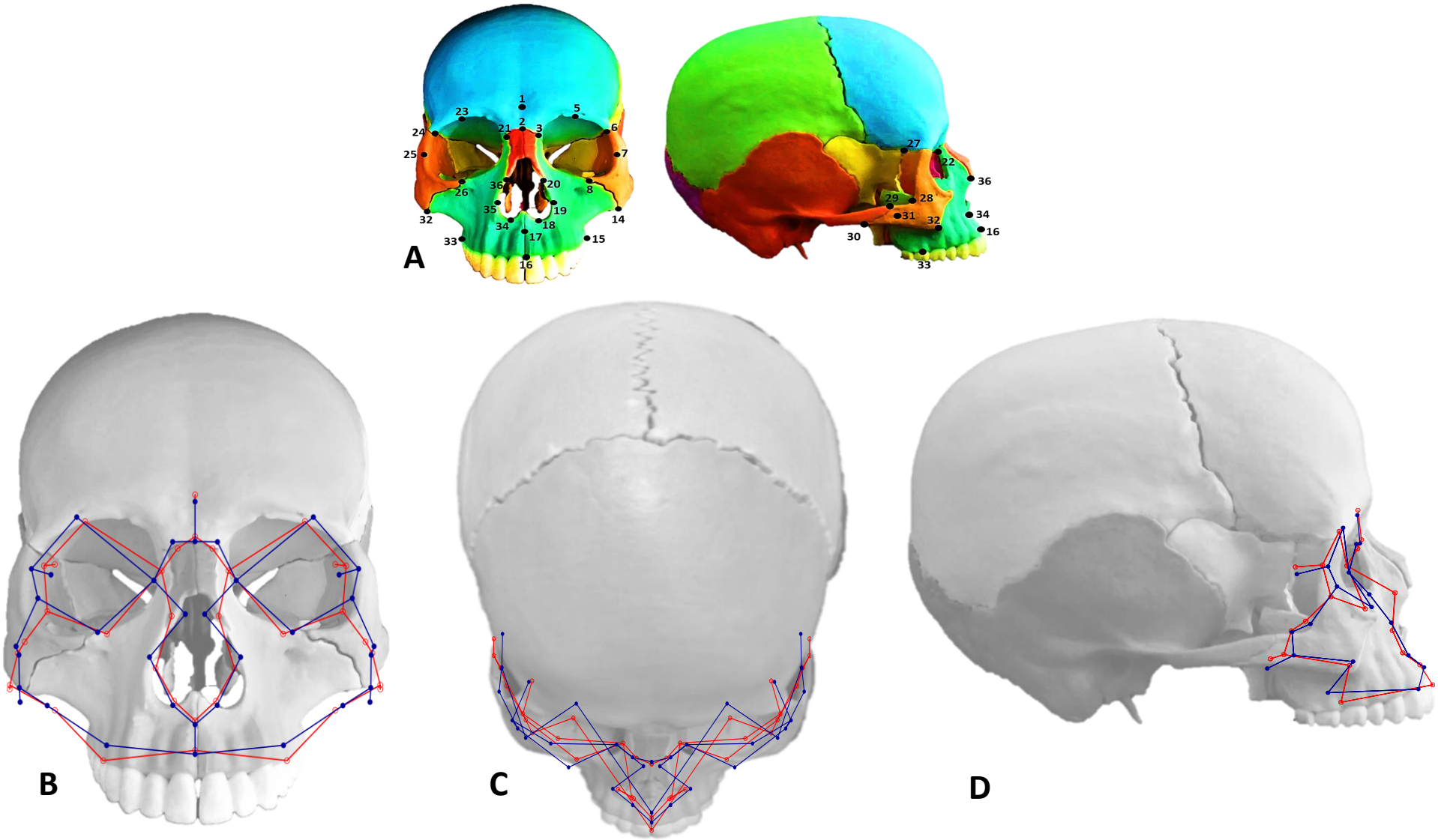

Figure 2. The effect of nasal trauma on total craniofacial shape variation. Mean shape of those with no nasal trauma in red; mean shape of those with nasal trauma in blue. **A**. Illustrations of landmark locations are included and correspond to definitions in Table 3.3. Views of shape change from PLS included are: **B**. Anterior; **C**. Superior and **D**. Lateral. [10X magnification of differences for visualisation]
[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

Appendix F: The influence of zygomatic trauma on craniofacial morphology

The effect of zygomatic trauma on craniofacial size

As seen in Tables 1-2, in MA individuals, mean size differences of 0.81mm ± 0.34mm in NLB (t= 2.39; p=0.02); 2.93mm ± 1.20mm in ZYB (t= -2.45; p=0.02) and 1.73mm ± 0.82mm in ZMB (t= -2.11; p=0.04) were detected between those with and without ZT. All the above measurements, excluding NLB, were slightly smaller in those with ZT (Tables 1-2)

Table 1. Differences in mean measurements (mm) between ancestry groups (with zygomatic trauma).

	Ancestry				
	African (*n*=16)	**European (*n* =5)**	**Mixed (*n* =59)**		
Measurement	***Mean (SD)***	***Mean(SD)***	***Mean(SD)***	***F***	***p-value***
NLB [a]	25.59 (4.2)	23.69 (1.9)	26.26 (2.3)	**3.54**	**0.03**
ZYB [b]	123.03 (8.1)	118.51 (10.3)	117.23(8.0)	***3.17***	***0.05***
ZMB [c]	93.10 (4.3)	87.46 (4.9)	89.39 (5.9)	**3.27**	**0.04**

F statistic from Univariate ANOVA for parametric data between ancestry groups.
- *Bold values are significant at an α-level < 0.05.*
- *Italicised, bold values are not conventionally considered significant, but reflect a strong correlation between variables.*
- *Measurements: NLB-Nasal breadth; ZYB- Bizygomatic breadth; ZMB-Bimaxillary breadth*

LSD post-hoc analysis:

a) NLB: EA mean < MA (*p*=0.01) and AA means (*p*=0.01).
b) ZYB: AA mean > MA mean (*p*=0.01).
c) ZMB: AA mean> MA *(p*=0.02*)*.and EA means*(p*=0.05*)*.

Table 2. Differences in mean measurements (mm) between ancestry groups (without zygomatic trauma).

	Ancestry				
	African (*n*=89)	**European (*n* =86)**	**Mixed (*n* =137)**		
Measurement	***Mean (SD)***	***Mean(SD)***	***Mean(SD)***	***F***	***p-value***
NLB [a]	26.89 (2.2)	23.01 (1.9)	25.44 (2.2)	**73.14**	**<0.0001**
ZYB [b]	122.85(6.1)	117.36(5.8)	120.18(7.6)	**14.63**	**<0.0001**
ZMB [c]	93.50(4.93)	86.99(5.0)	91.12(5.5)	**39.13**	**<0.0001**

F statistic from Univariate ANOVA for parametric data between ancestry groups.
[a] - Results after controlling for age
- *Bold values are significant at an α-level < 0.05.*
- *Measurements: NLB-Nasal breadth; ZYB- Bizygomatic breadth; ZMB-Bimaxillary breadth*

LSD post-hoc analysis:

a) NLB: EA mean < MA (*p*<0.0001) and AA means (*p*<0.0001). MA mean < AA mean (*p*<0.0001).
b) ZYB: AA mean > EA mean (*p*<0.0001) and MA means (*p*=0.004). ME mean > EA mean (*p*=0.003)
c) ZMB: AA mean > MA *(p*<0.0001*)*.and EA means *(p*<0.0001*)*. MA mean > EA mean (*p*<0.0001)

The effect of zygomatic trauma on craniofacial size

A weak correlation was detected between ZT and craniofacial shape (*RV*=0.02; *p*=0.004) and PLS 1 accounted for 100% of the covariance between these variables. Slightly more individuals with ZT were positively located on the PLS 1 shape score axis, indicating that ZT was not a major factor contributor to shape variation (Figure 1). AA and EA individuals clustered on shape and trauma axes (Figure 1), suggesting the effect of ZT in these groups was more similar than in MA individuals.

Glabella landmarks were slightly more inferior and anteriorly positioned in those with ZT (Figure 2). Wide superior nasal bridges which tapered off inferiorly to be narrower and more superiorly positioned were associated with ZT (Figure 2). Those with ZT exhibited more inferior and wide nasal apertures (Figure 2). In those with ZT, superior and lateral landmarks in the orbital region appeared more laterally and posteriorly sunken, while inferior orbital landmarks were more anteriorly located, resulting in more rectangular-shaped orbits. Narrower facial breadths with less laterally projecting zygomas were detected in those with ZT. Zygomaxillare, ectomolare and prosthion appeared more medial, superior and posteriorly located in response to ZT. Nasospinale was more inferior and anteriorly located, resulting in a more orthognathic and posteriorly positioned maxilla. DFA yielded poor separation based on ZT ($p \geq 0.07$). Therefore, those regardless of ZT, the sample was considered a single group in further analyses.

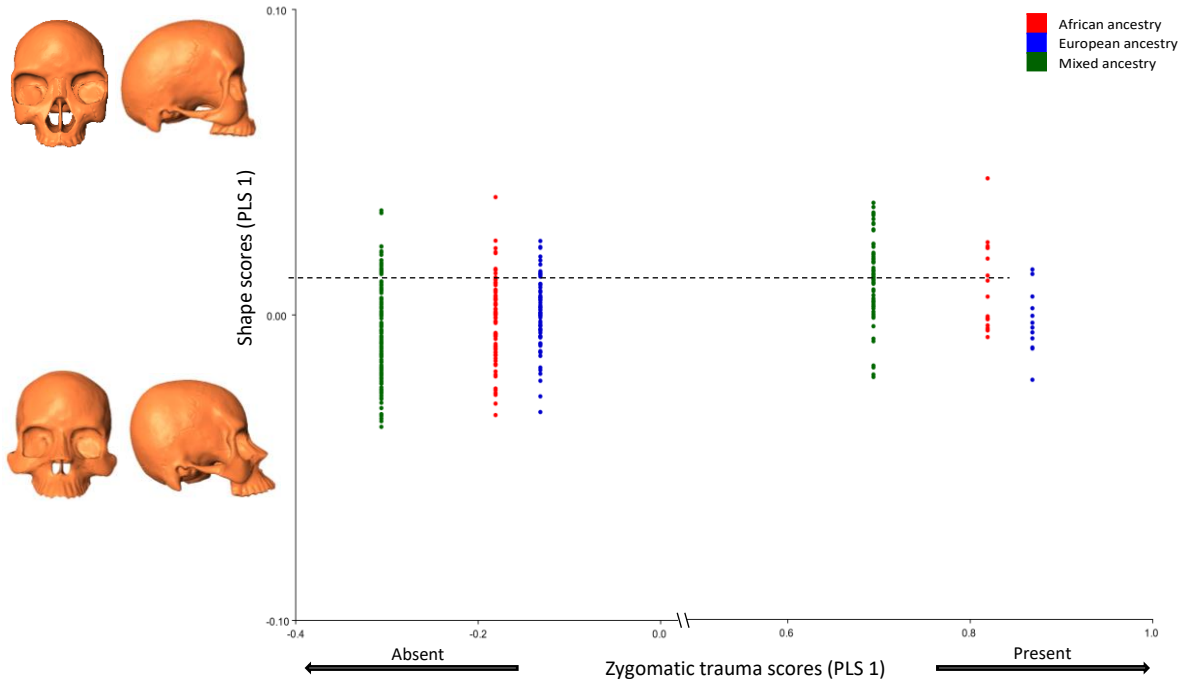

Figure 1. PLS analysis of the relationship between zygomatic trauma and craniofacial shape, with variances pooled by ancestry. 3D warped models of extreme craniofacial variation for PLS 1 (+) and PLS 1(-) representative of changes associated with zygomatic trauma presence and absence respectively.

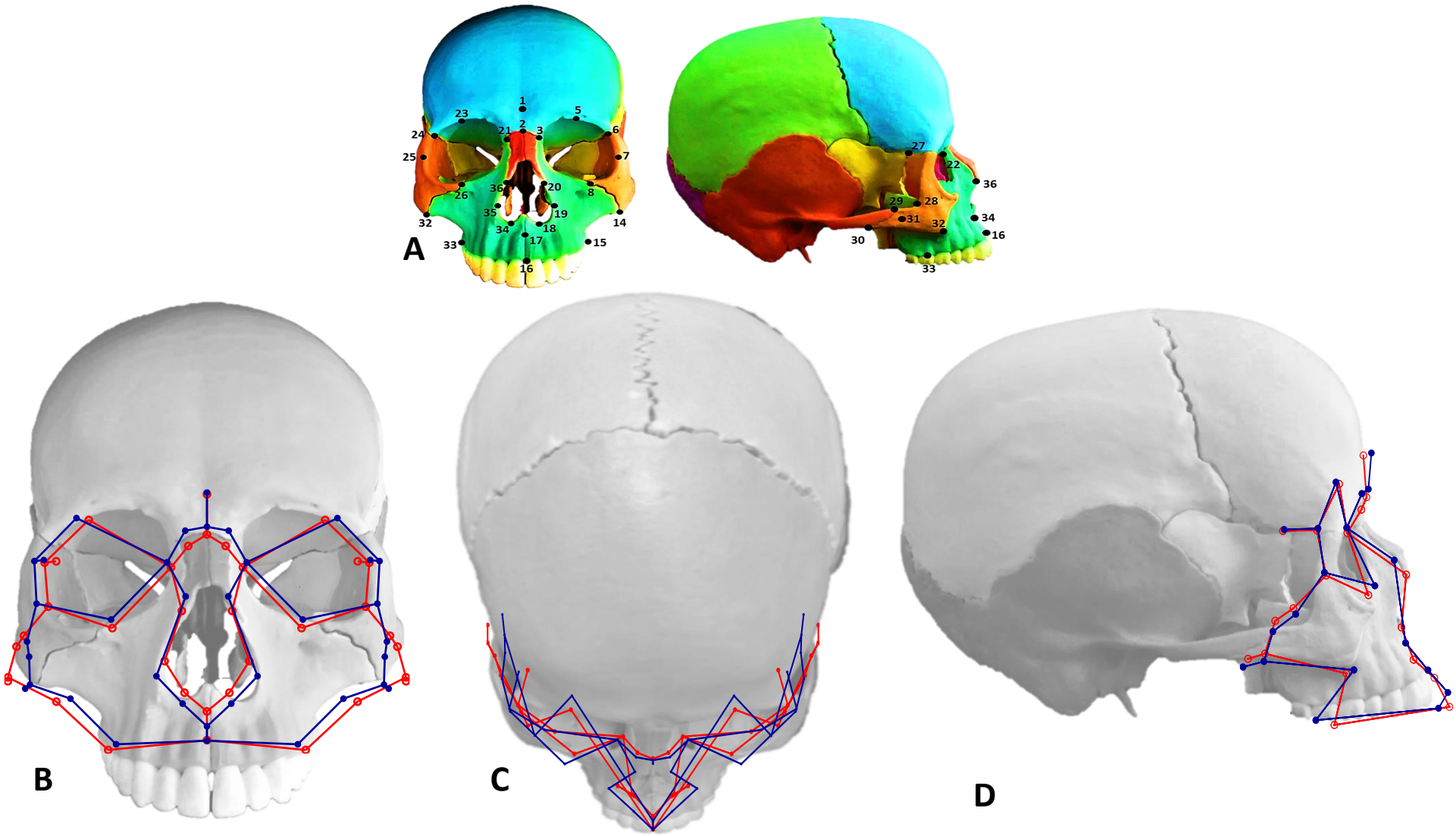

Figure 2. The influence of zygomatic trauma on mid-craniofacial shape variation. Mean shape of those without zygomatic trauma in red; mean shape of those with zygomatic trauma in blue. **A**. Illustrations of landmark locations are included and correspond to definitions in Table 3.3. Views of shape change from PLS include are: **B**. Anterior; **C**. Superior and **D**. Lateral. [10X magnification of differences for visualisation]

[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)

Appendix G: Scatter plots of Principal Component scores for metric data

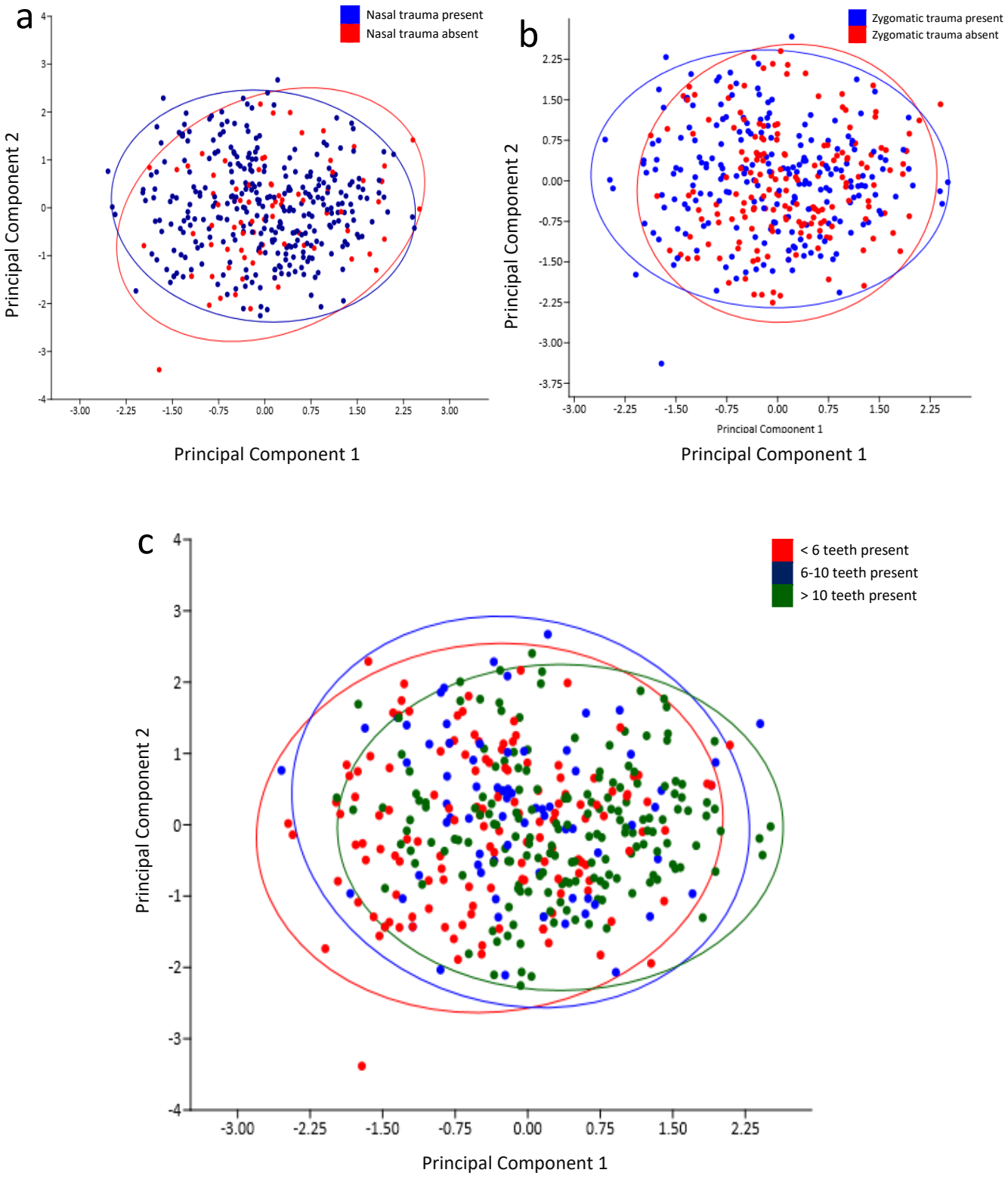

Figure 1. Scatter plot of PC1 and PC2 scores. (a) Individuals with and without nasal trauma (a), Individuals with and without zygomatic trauma, (c) tooth loss groups.

Appendix H: Discriminant function analyses

Table 1. Discriminant functions, sectioning points and centroids generated from step-wise discriminant function analysis for ancestry estimation.

Functions and variables	Unstandardized coefficients	Group centroids	Classification accuracy
Function 1: Craniofacial region			**60.2%**
Orbital breadth (OBB)	-0.162	A=0.893	
Orbital height (OBH)	0.117	M=0.277	
Interorbital breadth (DKB)	0.123	E=-0.1627	
Nasal height (NLH)	-0.160		
Nasal breadth (NLB)	0.303		
Maxillo-alveolar breadth (MAB)	0.062		
Basion-prosthion length (BPL)	0.022		
Constant	-0.6006		
*Sectioning points**	A=0.0571 B=3.585		
Function 2: Orbital region			**54.1%**
Orbital breadth (OBB)	-0.326	A=0.661	
Interorbital breadth (DKB)	0.236	M=0.152	
Bi-orbital breadth (EKB)	0.149	E=-0.1092	
Constant	-0.6556		
*Sectioning points**	A=0.4065 B=0.0214		
Function 3: Nasal region			**58.7**
Interorbital breadth (DKB)	0.183	A=0.849	
Upper facial height (NPH)	0.050	M=0.232	
Nasal height (NLH)	-0.207	E=-0.1478	
Nasal breadth (NLB)	0.332		
Constant	-0.5664		
*Sectioning points**	A=0.5405 B=0.0421		
Function 4: Zygomatic region			**43.4**
Bimaxillary breadth (ZMB)	0.196	A=0.571	
Constant	-17.745	M=0.014	
*Sectioning points**	A=0.2925 B=-0.3380	E=-0.690	
Function 5: Maxillary region			**46.9**
Maxillo-alveolar breadth (MAB)	0.068	A=0.672	
Bimaxillary breadth (ZMB)	0.100	M=0.019	
Basion-prosthion length (BPL)	0.057	E=-0.815	
Constant	-18.594		
*Sectioning points**	A=0.3455 B=-0.3980		

**A discriminant score is higher than sectioning point A classifies as African ancestry, lower than sectioning point B classified as European ancestry. A discriminant score between A and B classifies as Mixed ancestry*

Table 2. Discriminant functions, sectioning points and centroids generated from step-wise discriminant function analysis for males.

Functions and variables	Unstandardized coefficients	Group centroids	Classification accuracy
Function 1: Craniofacial region			**59.7%**
Orbital breadth (OBB)	-0.157	A=0.686	
Interorbital breadth (DKB)	0.146	M=0.233	
Upper facial height (NPH)	0.021	E= -1.539	
Nasal height (NLH)	-0.140		
Nasal breadth (NLB)	0.257		
Maxillo-alveolar breadth (MAB)	0.079		
Constant	-2.988		
*Sectioning points**	A=0.4595 B=-0.653		
Function 2: Orbital region			**53.5%**
Orbital breadth (OBB)	-0.146	A=0.562	
Interorbital breadth (DKB)	0.372	M=0.094	
Constant	-0.2432	E=-0.985	
*Sectioning points**	A=0.328 B=-0.4455		
Function 3: Nasal region			**53.5%**
Upper facial height (NPH)	0.069	A=1.044	
Nasal height (NLH)	-0.220	M=0.347	
Nasal breadth (NLB)	0.284	E=-1.605	
Interorbital breadth (DKB)	0.204		
Constant	-5.560		
*Sectioning points**	A= 0.6955 B=-0.6290		
Function 4: Zygomatic region			**35.4%**
Bimaxillary breadth (ZMB)	0.195	A=0.572	
Constant	-17.980	M=-0.105	
*Sectioning points**	A= 0.2335 B= -0.2655	E=-0.426	
Function 5: Maxillary region			**46.5%**
Maxillo-alveolar breadth (MAB)	0.090	A=0.659	
Basion-prosthion length (BPL)	0.121	M=-0.048	
Constant	-16.190	E=-0.700	
*Sectioning points**	A= 0.3055 B=-0.3740		

**A discriminant score is higher than sectioning point A classifies as African ancestry, lower than sectioning point B classified as European ancestry. A discriminant score between A and B classifies as Mixed ancestry*

Table 3. Discriminant functions, sectioning points and centroids generated from step-wise discriminant function analysis for females.

Functions and variables	Unstandardized coefficients	Group centroids	Classification accuracy
Function 1: Craniofacial region			**65.1%**
Interorbital breadth (DKB) *Nasal height (NLH)* *Nasal breadth (NLB)* *Constant* *Sectioning points**	0.175 -0.182 0.365 -0.4232 A= 0.6955 B= 0.09325	A=1.044 M=0.347 E=-0.1605	
Function 2: Orbital region			**54.8%**
Interorbital breadth (DKB) *Constant* *Sectioning points**	0.404 -0.8605 A=0.4455 B=-0.4485	A=0.840 M=0.043 E=-0.940	
Function 3: Nasal region			**65.1%**
Nasal height (NLH) *Nasal breadth (NLB)* *Interorbital breadth (DKB)* *Constant* *Sectioning points**	0.175 0.635 0.175 -0.4232 A= 0.6955 B= -0.6290	A=1.044 M=0.347 E=-1.605	
Function 4: Zygomatic region			**62.7%**
Bimaxillary breadth (ZMB) *Bizygomatic breadth (ZYB)* *Constant* *Sectioning points**	0.268 -0.059 -16.943 A= 0.4460 B= -0.3980	A=0.649 M=0.243 E=-1.039	
Function 5: Maxillary region			**52.4%**
Bimaxillary breadth (ZMB) *Constant* *Sectioning points**	0.230 20.330 A= 0.4305 B= -0.4145	A=0.739 M=0.122 E=-0.951	

**A discriminant score is higher than sectioning point A classifies as African ancestry, lower than sectioning point B classified as European ancestry. A discriminant score between A and B classifies as Mixed ancestry*

Table 4. Standardised canonical discriminant function coefficients from stepwise discriminant function analyses.

Functions and variables	Standardized coefficients
Function 1: Craniofacial region	
Nasal breadth (NLB)	0.656
Nasal height (NLH)	-0.526
Maxillo-alveolar breadth (MAB)	0.336
Orbital breadth (OBB)	-0.322
Interorbital breadth (DKB)	0.311
Orbital height (OBH)	0.245
Basion-prosthion length (BPL)	0.137
Function 2: Orbital region	
Orbital breadth (OBB)	-0.647
Interorbital breadth (DKB)	0.596
Bi-orbital breadth (EKB)	0.632
Function 3: Nasal region	
Interorbital breadth (DKB)	0.461
Upper facial height (NPH)	0.274
Nasal height (NLH)	-0.682
Nasal breadth (NLB)	0.717
Function 4: Zygomatic region	
Bimaxillary breadth (ZMB)	1.00
Function 5: Maxillary region	
Maxillo-alveolar breadth (MAB)	0.364
Bimaxillary breadth (ZMB)	0.510
Basion-prosthion length (BPL)	0.361

Table 5. Standardised canonical discriminant function coefficients from stepwise discriminant function analyses for males.

Functions and variables	Standardized coefficients
Function 1: Craniofacial region	
Orbital breadth (OBB)	-0.307
Interorbital breadth (DKB)	0.372
Upper facial height (NPH)	0.180
Nasal height (NLH)	-0.452
Nasal breadth (NLB)	0.542
Maxillo-alveolar breadth (MAB)	0.410
Function 2: Orbital region	
Orbital breadth (OBB)	-0.285
Interorbital breadth (DKB)	0.944
Function 3: Nasal region	
Upper facial height (NPH)	0.352
Nasal height (NLH)	-0.707
Nasal breadth (NLB)	0.597
Interorbital breadth (DKB)	0.519
Function 4: Zygomatic region	
Bimaxillary breadth (ZMB)	1.00
Function 5: Maxillary region	
Maxillo-alveolar breadth (MAB)	0.626
Basion-prosthion length (BPL)	0.549

Table 6. Standardised canonical discriminant function coefficients from stepwise discriminant function analyses for females.

Functions and variables	Standardized coefficients
Function 1: Craniofacial region	
Interorbital breadth (DKB)	0.434
Nasal height (NLH)	-0.535
Nasal breadth (NLB)	0.780
Function 2: Orbital region	
Interorbital breadth (DKB)	1.00
Function 3: Nasal region	
Nasal height (NLH)	-0.535
Nasal breadth (NLB)	0.780
Interorbital breadth (DKB)	0.434
Function 4: Zygomatic region	
Bimaxillary breadth (ZMB)	1.167
Bizygomatic breadth (ZYB)	-0.370
Function 5: Maxillary region	
Bimaxillary breadth (ZMB)	1.00

Appendix I: Nonmetric trait frequencies, regression models and ancestry estimation accuracies

Table 1. Trait frequencies for interorbital breadth (IOB) variants in ancestry groups.

IOB		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Narrow	*European*	2	1.9	27	29.7	12	6.1
Intermediate	*Asian*	25	23.8	**55**	**60.4**	76	38.8
Wide	*African*	**78**	**74.3**	9	9.9	**108**	**55.1**

*Associations of traits with ancestries from Hefner (2003), referencing Hooton (1946).

Table 2. Trait frequencies for orbital shape (OS) variants in ancestry groups.

OS		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Inferiorly angled (square)	*European*	**53**	**50.5**	**80**	**87.9**	**93**	**47.3**
Elongated (rectangular)	*African*	**43**	**41**	4	4.4	**76**	**38.8**
Rounded	*Asian*	9	8.6	7	7.7	27	13.8

*Associations of traits with ancestries from Byers (2015)

Table 3. Trait frequencies for supranasal suture (SS) variants in ancestry groups.

SS		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Open/unfused	*NA*	3	2.9	1	1.1	4	2.0
Closed (visible)	*NA*	3	2.9	**27**	**29.7**	17	8.7
Closed (barely visible)	*NA*	**50**	**47.6**	**46**	**50.5**	**119**	**60.7**
Obliterated	*NA*	**49**	**46.7**	17	18.7	**56**	**26.8**

*Associations of traits with ancestries from Hefner (2003), referencing Hooton (1946).

Table 4. Trait frequencies for nasal bone contour (NBC) variants in ancestry groups.

NBC		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196	
Variant	Ancestral associations*	n	%	n	%	n	%
Low/Round	*African*	**51**	**48.6**	4	4.4	**91**	**46.4**
Oval	*Asian*	**43**	**41.0**	4	4.4	**68**	**34.7**
Steep	*European*	4	3.8	**16**	**17.6**	12	6.1
Steepled	*European*	2	1.9	**21**	**23.1**	7	3.6
Semi-triangular (vaulted)	*European*	5	4.8	**46**	**50.5**	18	9.2

*Standards for trait associations with ancestries, from Hefner (2003), referencing Hooton (1946).

Table 5. Trait frequencies for nasal aperture width (NAW) variants in ancestry groups.

NAW		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Narrow/elongated	*European*	2	1.9	**27**	**29.7**	12	6.1
Bell-shaped	*Asian*	25	23.8	**55**	**60.4**	**76**	**38.8**
Wide	*African*	**78**	**74.3**	9	9.9	**108**	**55.1**

*Standards for trait associations with ancestries, from Hefner (2003), referencing Hooton (1946).

Table 6. Trait frequencies for zygomaxillary suture shape (ZS) variants in ancestry groups.

ZS		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Angled	*African*	13	12.4	**36**	**39.6**	47	24.0
Smooth	*Asian*	**68**	**64.8**	**45**	**49.5**	**128**	**65.3**
Z-shaped	*European*	24	22.9	10	11.0	21	10.7

*Associations of traits with ancestries from Hefner (2003), referencing Hooton (1946).

Table 7. Trait frequencies for anterior nasal spine (ANS) variants in ancestry groups.

ANS		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Short (rounded)	*African*	**38**	**36.2**	1	1.1%	**43**	**21.9**
Dull	*Asian*	**38**	**36.2**	5	5.5	**54**	**27.6**
Medium	*European*	20	19	**19**	**20.9**	**45**	**23.0**
Long (sharp)	*NA*	9	8.6	**66**	**72.5**	**54**	**27.6**

*Associations of traits with ancestries from Hefner (2003), referencing Hooton (1946).

Table 8. Trait frequencies for alveolar prognathism (AP) variants in ancestry groups.

AP		Ancestry					
		African (n=105)		European (n=91)		Mixed (n=196)	
Variant	Ancestral associations*	n	%	n	%	n	%
Orthognathic	*European*	29	27.6	**75**	**82.4**	**92**	**46.9**
Prognathic	*African*	**76**	**72.4**	16	17.6	**104**	**53.1**

*Associations of traits with ancestries from Hefner (2003), referencing Hooton (1946).

Table 9. Ancestry estimations using frequency distributions for each nonmetric trait.

International ancestry estimation categories for nonmetric traits [a]	Classification frequencies for ancestry groups [b]		
	AA (%)	**MA (%)**	**EA (%)**
Nasal bone contour (NBC)			
African	**48.6**	**46.4**	4.4
European	10.5	18.9	**91.2**
Asian	41	34.7	4.4
Nasal aperture width (NAW)			
African	**74.3**	**55.1**	9.9
European	1.9	6.1	29.7
Asian	23.8	38.3	**60.4**
Anterior nasal spine (ANS)			
African	**36.2**	21.9	1.1
European	19	23	**93.4**
Asian	21.9	**27.6**	23
Zygomaxillary suture shape (ZS)			
African	12.3	24	36.6
European	22.9	10.7	11
Asian	**64.8**	**65.3**	**49.4**
Interorbital breadth (IOB)			
African	22.9	16.3	1.1
European	16.1	34.7	**75.8**
Asian	**70**	**49**	23.1
Orbital shape (OS)			
African	41	38.3	4.4
European	**50.5**	**47.4**	**58.9**
Asian	8.5	13.8	7.7
Alveolar prognathism (AP)			
African	**72.4**	**53.1**	17.6
European	27.6	46.9	**82.4**
Asian	*NS*	*NS*	*NS*

- Bold values represent the likely ancestry estimations, based on the highest trait frequencies.
- NS: No standards
a) Nonmetric traits and ancestry estimation standards by Hefner (2003) (referencing Hooton (1946)) and Byers (2015).
b) Frequencies computed for ancestry groups in this study.

Table 10. Ancestry estimations for sex-ancestry groups, using frequency distributions for each nonmetric trait.

International ancestry estimation categories for nonmetric traits [a]	Classification frequencies for sex-ancestry groups [b]					
	AA (%)		MA (%)		EA (%)	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*
Nasal bone contour (NBC)						
African	**44.1**	**54.3**	**39.8**	**60**	7.0	2.1
European	11.8	8.7	24.6	8.6	**88.7**	**97.9**
Asian	**44.1**	37	36.5	31.4	9.3	0
Nasal aperture width (NAW)						
African	**74.6**	**73.9**	**57.9**	**50**	9.3	10.4
European	1.7	2.2	5.6	42.9	**44.2**	16.7
Asian	23.7	23.9	36.5	7.1	**46.5**	**72.9**
Anterior nasal spine (ANS)						
African	**45.8**	**43.5**	20.6	24.3	2.3	0
European	15.2	23.9	23.8	21.4	**95.4**	**91.7**
Asian	39	32.6	**25.4**	**31.4**	2.3	8.3
Zygomaxillary suture shape (ZS)						
African	11.9	13	30.2	12.9	37.2	**41.7**
European	13.6	34.8	8.7	14.3	16.3	6.3
Asian	**74.5**	**51.2**	**61.1**	**72.9**	**46.5**	52
Interorbital breadth (IOB)						
African	28.8	15.2	15.1	18.6	2.3	25
European	16.6	19.6	31	**41.1**	**76.7**	**75**
Asian	**57.6**	**65.2**	**54**	40	20.9	0
Orbital shape (OS)						
African	**45.8**	34.8	37.3	41.4	4.7	4.2
European	**47.4**	**54.3**	**48.4**	**45.7**	**83.7**	**91.7**
Asian	6.8	10.9	14.3	12.9	11.6	4.1
Alveolar prognathism (AP)						
African	**76.3**	**67.4**	**57.9**	42.1	18.6	16.7
European	23.7	32.6	44.3	**55.7**	**81.4**	**83.3**
Asian	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

- Bold values represent the likely ancestry estimations, based on the highest trait frequencies.
- NS: No standards
a) Nonmetric traits and ancestry estimation standards by Hefner (2003) (referencing Hooton (1946)) and Byers (2015).
b) Frequencies computed for ancestry groups in this study.

Table 11. Variables in the Final MLR model for African ancestry individuals. The final five variables chosen in the logistic equation, their coefficients (B values) and constants (A) for the equation are listed. Trait descriptions and illustrations are included in Appendix B.

	B	Std. Error	Wald	df	Sig.	Exp (B)
Intercept	-3.641	1.207	9.106	1	0.003	
Teeth	0.170	0.041	17.125	1	<0.0001	1.185
[NBC_OR=0]	2.563	0.839	9.332	1	0.002	12.976
[NBC_OR=1]	3.746	0.841	19.839	1	<0.0001	42.363
[NBC_OR=2]	0.430	0.870	0.244	1	0.621	1.537
[NBC_OR=3]	0.021	1.070	.000	1	0.984	1.021
[NBC_OR=4]	0	0	0	0	0	0
[NAW_OR=0]	-3.336	1.003	11.066	1	0.001	.036
[NAW_OR=1]	-1.921	0.547	12.319	1	<0.0001	.146
[NAW_OR=2]	0	0	0	0	0	0
[ANS_OR=0]	4.280	1.571	7.425	1	0.006	72.246
[ANS_OR=1]	1.667	0.779	4.580	1	0.03	5.294
[ANS_OR=2]	1.143	0.621	3.387	1	0.07	3.136
[ANS_OR=3]	0	0	0	0	0	0
[SS_OR=0]	1.755	1.522	1.329	1	0.25	5.782
[SS_OR=1]	-2.094	0.872	5.765	1	0.02	.123
[SS_OR=2]	-.714	0.528	1.828	1	0.18	.490
[SS_OR=3]	0	0	0	0	0	0
[OS_OR=0]	1.634	0.839	3.795	1	0.05	5.125
[OS_OR=1]	2.657	1.015	6.846	1	0.009	14.252
[OS_OR=2]	0	0	0	0	0	0

* The reference category is European ancestry

Table 12. Variables in the MLR model for Mixed ancestry individuals. The final five variables chosen in the logistic equation, their coefficients (B values) and constants(A) for the equation are listed. Trait descriptions and illustrations are included in Appendix B.

	B	Std. Error	Wald	df	Sig.	Exp(B)
Intercept	-1.345	1.002	1.804	1	0.18	
Teeth	0.044	.034	1.705	1	0.19	1.045
[NBC_OR=0]	2.835	.681	17.340	1	<0.0001	17.025
[NBC_OR=1]	3.440	.678	25.772	1	<0.0001	31.172
[NBC_OR=2]	0.406	.549	.547	1	0.46	1.501
[NBC_OR=3]	0.026	.591	.002	1	0.96	1.027
[NBC_OR=4]	0	0	0	0	0	0
[NAW_OR=0]	-1.737	.637	7.433	1	0.006	.176
[NAW_OR=1]	-1.108	.484	5.234	1	0.02	.330
[NAW_OR=2]	0	0	0	0	0	0
[ANS_OR=0]	3.136	1.501	4.363	1	0.04	23.014
[ANS_OR=1]	0.914	0.674	1.837	1	0.18	2.494
[ANS_OR=2]	0.378	0.474	0.634	1	0.43	1.459
[ANS_OR=3]	0	0	0	0	0	0
[SS_OR=0]	1.651	1.350	1.496	1	0.22	5.214
[SS_OR=1]	-0.617	0.635	0.944	1	0.33	.540
[SS_OR=2]	0.146	0.479	0.093	1	0.76	1.157
[SS_OR=3]	0	0	0	0	0	0
[OS_OR=0]	0.839	0.737	1.295	1	0.26	2.313
[OS_OR=1]	2.307	0.931	6.138	1	0.01	10.049
[OS_OR=2]	0	0	0	0	0	0

* The reference category is European ancestry

Table 13. Variables in the MLR model for African and Mixed ancestry males. The final five variables chosen in the logistic equation, their coefficients (B values) and constants(A) for the equation are listed. Trait descriptions and illustrations are included in Appendix B.

African	B	Std. Error	Wald	df	Sig.	Exp(B)
Intercept	-1.365	1.926	0.502	1	0.48	
Teeth	.218	0.058	14.251	1	<0.0001	1.244
[NBC_OR=0]	3.380	1.153	8.596	1	<0.003	29.381
[NBC_OR=1]	20.983	0.765	752.293	1	<0.0001	1296748886.
[NBC_OR=2]	0.444	1.090	0.166	1	0.68	1.559
[NBC_OR=3]	0.774	1.230	0.396	1	0.53	2.168
[NBC_OR=4]	0	0	0	0	0	0
[NAW_OR=0]	-2.697	1.194	5.102	1	.024	.067
[NAW_OR=1]	-1.893	0.780	5.884	1	.015	.151
[NAW_OR=2]	0	0	0	0	0	0
[ANS_OR=0]	-2.877	1.219	5.570	1	0.02	.056
[ANS_OR=1]	-2.088	1.158	3.250	1	0.07	.124
[ANS_OR=2]	0	0	0	0	0	0
[ANS_OR=3]	-.001	1.777	<0.0001	1	1.000	.999
[SS_OR=0]	-3.050	1.399	4.755	1	0.03	.047
[SS_OR=1]	-.782	0.869	0.810	1	0.37	.457
[SS_OR=2]	0	0	0	0	0	0
[SS_OR=3]	1.769	1.202	2.166	1	0.14	5.863
[OS_OR=0]	20.373	0.689	875.178	1	<0.0001	704315732.500
[OS_OR=1]	0	0	0	0	0	0
[OS_OR=2]	-1.365	1.926	.502	1	0.48	0
Mixed						
Intercept	0.384	1.002	1.804	1	0.18	
Teeth	0.040	0.034	1.705	1	0.19	1.045
[NBC_OR=0]	3.075	0.681	17.340	1	<0.0001	17.025
[NBC_OR=1]	20.694	0.678	25.772	1	<0.0001	31.172
[NBC_OR=2]	0.329	0.549	0.547	1	0.46	1.501
[NBC_OR=3]	0.361	0.591	0.002	1	0.96	1.027
[NBC_OR=4]	0	0	0	0	0	0
[NAW_OR=0]	-1.663	0.637	7.433	1	0.006	0.176
[NAW_OR=1]	-1.661	0.484	5.234	1	0.02	0.330
[NAW_OR=2]	0	0	0	0	0	0
[ANS_OR=0]	-0.779	1.501	4.363	1	0.04	23.014
[ANS_OR=1]	-0.689	0.674	1.837	1	0.18	2.494
[ANS_OR=2]	0.8690	0.474	0.634	1	0.43	1.459
[ANS_OR=3]	0	0	0	0	0	0
[SS_OR=0]	-0.355	1.350	1.496	1	0.22	5.214
[SS_OR=1]	0.308	0.635	0.944	1	0.33	.540
[SS_OR=2]	0.369	0.479	0.093	1	0.76	1.157
[SS_OR=3]	0	0	0	0	0	0
[OS_OR=0]	19.437	0.737	1.295	1	0.26	2.313
[OS_OR=1]	0.384	0.931	6.138	1	0.01	10.049
[OS_OR=2]	0	0	0	0	0	0

* The reference category is European ancestry

Table 14. Variables in the Final Logistic Regression model for African and Mixed ancestry females. The final five variables chosen in the logistic equation, their coefficients (B values) and constants(A) for the equation are listed. Trait descriptions and illustrations are included in Appendix B.

African	B	Std. Error	Wald	df	Sig.	Exp(B)
Intercept	-1.093	1.509	0.525	1	0.47	
Teeth	0.135	0.065	4.282	1	0.04	1.144
[NBC_OR=0]	4.316	1.383	9.738	1	0.002	74.865
[NBC_OR=1]	3.679	1.299	8.018	1	0.005	39.619
[NBC_OR=2]	0.190	1.636	0.013	1	0.91	1.209
[NBC_OR=3]	-18.594	<0.0001	0	1	0	8.411E-9
[NBC_OR=4]	0	0	0	0	0	0
[NAW_OR=0]	-20.655	7444.772	.000	1	1.00	1.071E-9
[NAW_OR=1]	-1.695	0.862	3.867	1	0.05	0.184
[NAW_OR=2]	0	0	0	0	0	0
[AP_OR=0]	-1.870	0.816	5.247	1	0.02	0.154
[AP_OR=1]	0	0	0	0	0	0
Mixed						
Intercept	-0.491	1.122	0.191	1	0.662	
Teeth	0.04	0.057	00.492	1	0.483	1.041
[NBC_OR=0]	4.476	0.984	20.683	1	<0.0001	87.922
[NBC_OR=1]	3.174	0.858	13.693	1	<0.0001	23.905
[NBC_OR=2]	-0.658	1.286	0.262	1	0.61	0.518
[NBC_OR=3]	-0.674	1.247	0.292	1	0.59	0.510
[NBC_OR=4]	0	0	0	0	0	0
[NAW_OR=0]	-1.552	1.070	2.104	1	0.15	0.212
[NAW_OR=1]	-0.483	0.800	0.364	1	0.55	0.617
[NAW_OR=2]	0	0	0	0	0	0
[AP_OR=0]	-1.137	0.744	2.335	1	0.13	0.321
[AP_OR=1]	0	0	0	0	0	0

* The reference category is European ancestry

Appendix J: Lollipop diagrams depicting craniofacial shape variation

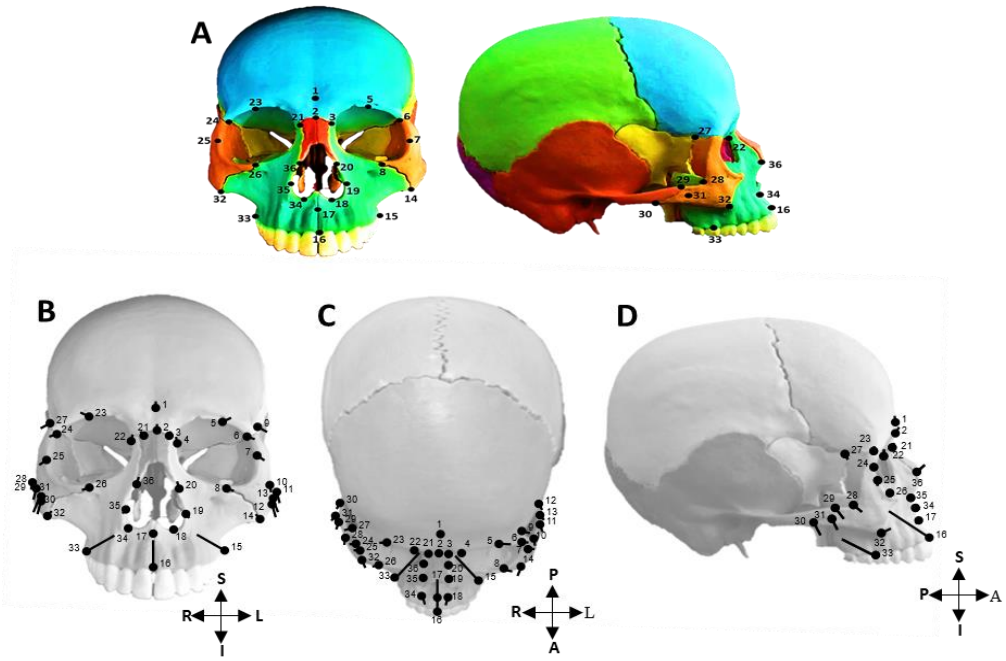

Figure 1. The relationship between total craniofacial shape variation and tooth loss. A. Illustrations of landmark locations correspond to definitions in Table 3.3. Views of shape change from regression analyses include: B. Anterior; C. Superior and D. Lateral. Dots represent the positive regression scores (more teeth present) and stems represent the magnitude and direction of variation in negative regression scores (fewer teeth present). [40X magnification of differences for visualisation] [Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

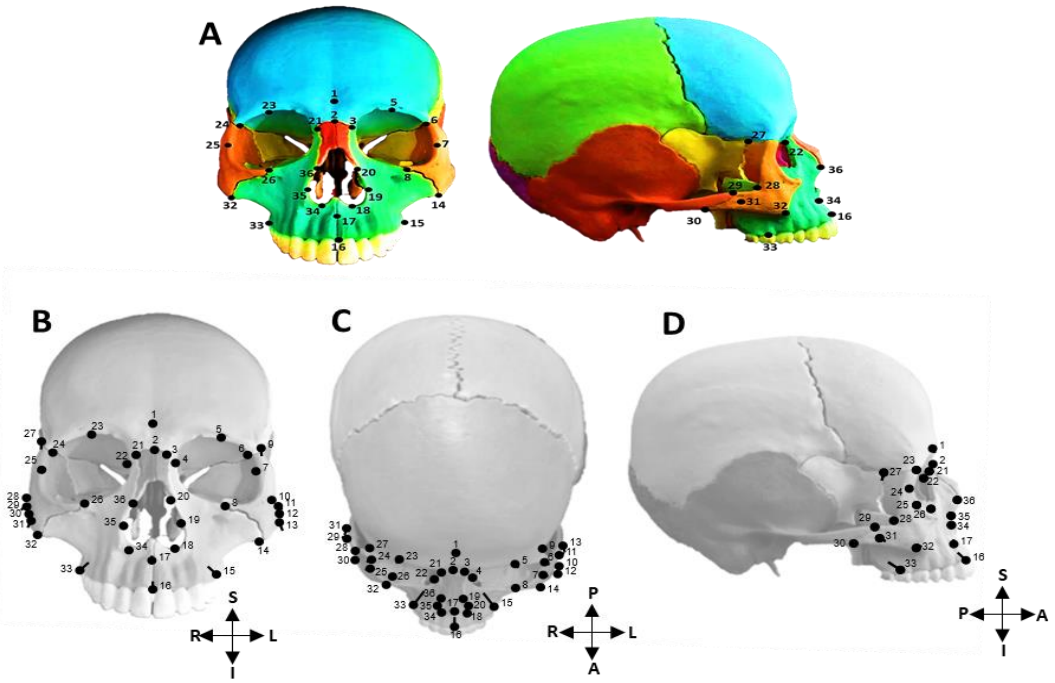

Figure 2. The relationship between total craniofacial shape and age. A. Illustrations of landmark locations correspond to definitions in Table 3.3. Views of shape change from regression analyses include: B. Anterior; C. Superior and D. Lateral. Dots represent the negative regression scores (lower ages at death) and stems represent the magnitude and direction of variation in positive regression scores (higher ages at death). [40X magnification of differences for visualisation] [Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

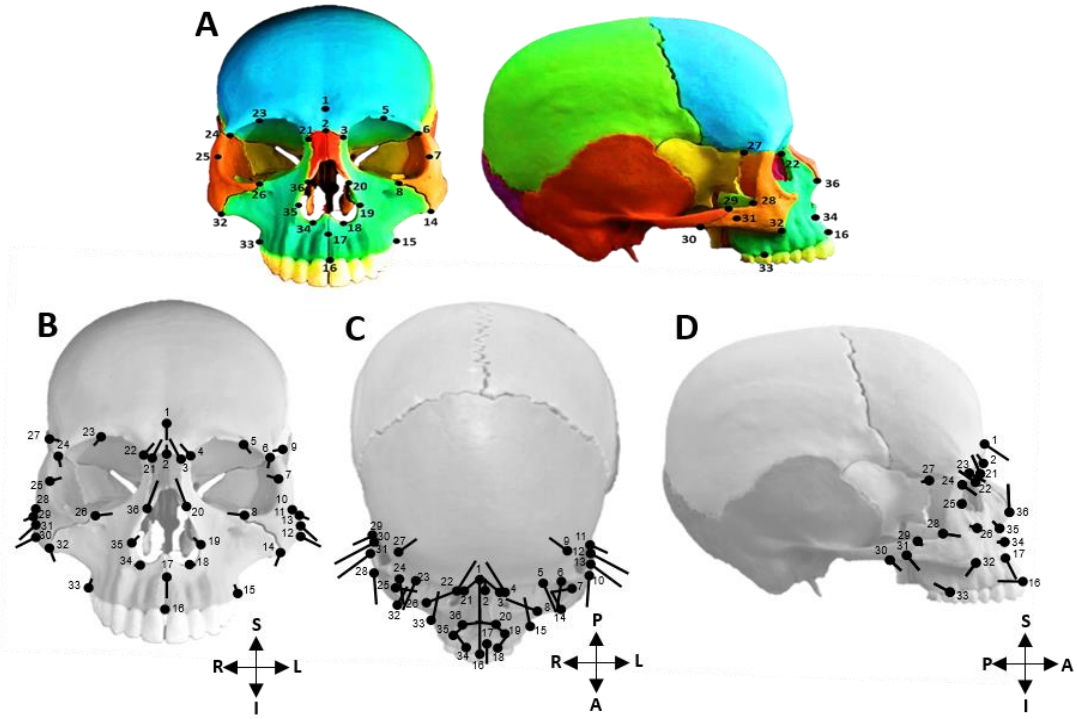

Figure 3. Total craniofacial shape variation between males and females. A. Illustrations of landmark locations correspond to definitions in Table 3.3. Views of shape change from PCA include: B. Anterior; C. Superior and D. Lateral. Dots represent the average shape in females and stems represent the magnitude and direction of variation in males. *[*5X magnification of differences for visualisation] [Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

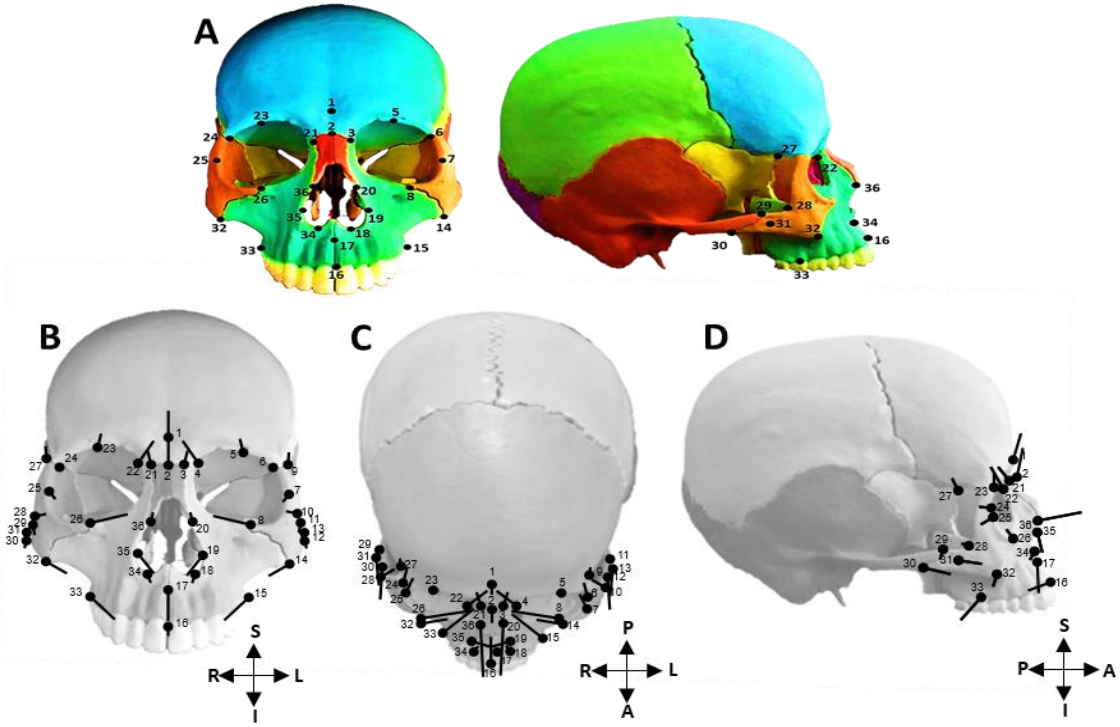

Figure 4. Total craniofacial shape variation between EA individuals and AA and MA individuals (CV 1). A. Illustrations of landmark locations correspond to definitions in Table 3.3. Views of shape change from CVA include: B. Anterior; C. Superior and D. Lateral. Dots represent the average shape in AA and MA individuals and stems represent the magnitude and direction of variation in the EA group.

[5X magnification of differences for visualisation]
[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

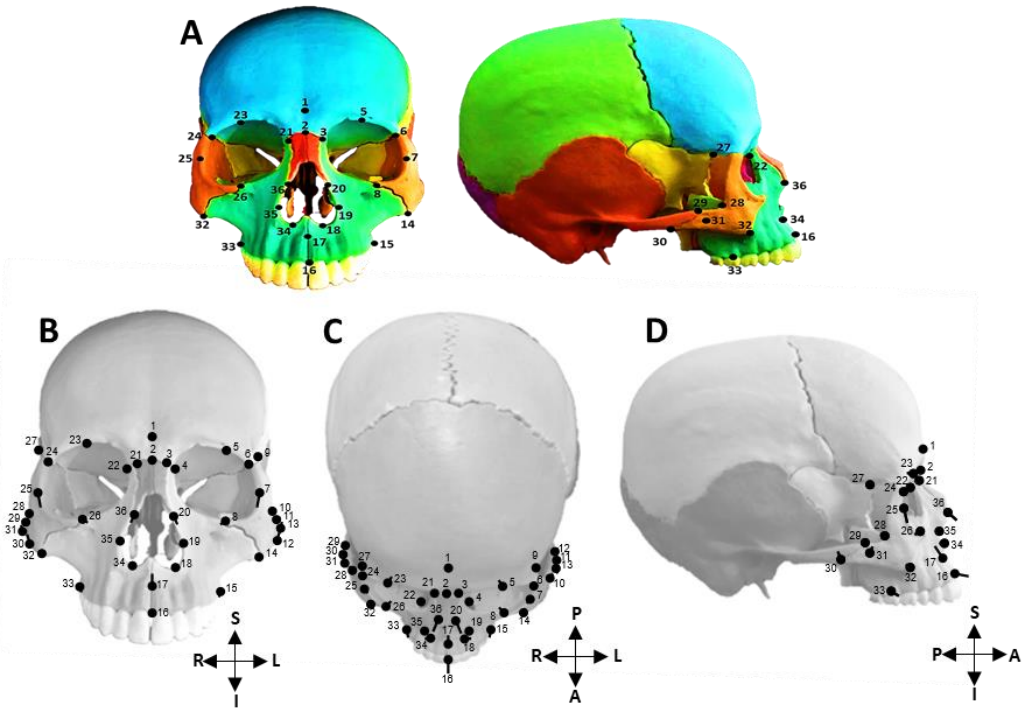

Figure 5. Total craniofacial shape variation between AA individuals and EA and MA individuals (CV 2). A. Illustrations of landmark locations correspond to definitions in Table 3.3. Views of shape change from CVA include: B. Anterior; C. Superior and D. Lateral. Dots represent the average shape in EA and MA individuals and stems represent the magnitude and direction of variation in the AA individuals. [5X magnification of differences for visualisation]
[Photograph of osteopathic teaching model (manufactured by Erler Zimmer 2016)]

Appendix K: Mahalanobis distances and ancestry estimation accuracies between ancestry and sex-ancestry groups

Table 1. Leave-one-out cross validation (LOOCV) ancestry estimation accuracies from pairwise comparisons of craniofacial regions.

Groups compared		Percentage accuracies per craniofacial region				
		Total	**Orbital**	**Nasal**	**Maxilla**	**Zygomatic**
AA—EA	AA	92.3	88.6	88.6	93.3	80
	EA	97.8	89.0	80.2	91.2	76.9
EA-MA	EA	91.2	82.4	74.7	92.3	80.2
	MA	91.3	86.2	82.7	82.3	83.7
AA-MA	AA	71.4	57.1	52.4	65.7	71.4
	MA	80.1	58.2	58.2	71.9	70.4

- Highest accuracies for African ancestry highlighted in red, European ancestry in blue and Mixed ancestry in green.

Table 2. Mahalanobis distances between ancestry groups ($p<0.01$).

		Ancestry groups	
Craniofacial region		**AA**	**EA**
Total	EA	3.8	
	MA	2.2	3.3
Orbital	EA	2.5	
	MA	0.8	2.1
Nasal	EA	2.0	
	MA	0.4	1.7
Maxilla	EA	3.3	
	MA	1.4	2.9
Zygomatic	EA	2.1	
	MA	1.3	2.0

Table 3. Leave-one-out cross validation (LOOCV) ancestry estimation accuracies from pairwise comparisons of craniofacial regions in males.

Groups compared		Percentage accuracies per craniofacial region				
		Total	**Orbital**	**Nasal**	**Maxilla**	**Zygomatic**
AA—EA	AA	78.6	76.8	80.4	87.5	80.4
	EA	72.7	84.1	75.0	86.4	75.0
EA-MA	EA	75.0	75.0	72.7	84.1	81.8
	MA	87.3	89.0	78.6	84.9	78.6
AA-MA	AA	60.7	46.0	60.7	64.3	58.9
	MA	69.0	56.3	55.5	68.3	61.9

- Highest accuracies for African ancestry highlighted in red, European ancestry in blue and Mixed ancestry in green.

Table 4. Leave-one-out cross validation (LOOCV) ancestry estimation accuracies from pairwise comparisons of craniofacial regions in females.

Groups compared		Percentage accuracies per craniofacial region				
		Total	Orbital	Nasal	Maxilla	Zygomatic
AA—EA	AA	81.6	85.7	87.8	93.9	77.6
	EA	91.5	87.2	78.7	97.9	68.1
EA-MA	EA	93.6	89.4	78.7	95.7	74.5
	MA	94.3	88.6	87.1	94.3	85.7
AA-MA	AA	65.3	57.1	55.1	69.4	67.3
	MA	77.1	61.4	45.7	70	74.3

- Highest accuracies for African ancestry highlighted in red, European ancestry in blue and Mixed ancestry in green.

Table 6. Mahalanobis distances for total craniofacial shape between sex-ancestry groups.

Sex-ancestry groups	AF	AM	EF	EM	MF
AM	1.9				
EF	4.2	3.7			
EM	4.6	3.9	1.7		
MF	2.8	2.3	3.7	3.9	
MM	2.9	2.2	3.4	3.4	1.5

Table 7. Leave-one-out cross validation (LOOCV) ancestry estimation accuracies from pairwise comparisons of craniofacial regions after correcting for the effect of tooth loss.

Groups compared		Percentage accuracies per craniofacial region				
		Total	Orbital	Nasal	Maxilla	Zygomatic
AA—EA	AA	91.4	88.6	***89.5***	91.4	79.0
	EA	93.4	85.7	79.1	***93.2***	*78.0*
EA-MA	EA	92.3	80.2	72.5	92.3	80.2
	MA	89.3	85.7	82.1	***89.3***	83.2
AA-MA	AA	63.8	***59.0***	***53.3***	63.8	67.6
	MA	67.3	53.1	***60.2***	67.3	70.4

- Highest accuracies for African ancestry highlighted in red, European ancestry in blue and Mixed ancestry in green.
- Bold and italicised values represent improvements in estimation accuracies after correcting for tooth loss.

Table 8. Mahalanobis distances between ancestry groups (p<0.03), after correcting for tooth loss.

Craniofacial region		Ancestry groups	
		AA	EA
Total	EA	3.1	
	MA	1.8	0.04
Orbital	EA	2.3	
	MA	0.7	2.0
Nasal	EA	1.9	
	MA	0.3	1.7
Maxilla	EA	3.1	
	MA	1.3	2.9
Zygomatic	EA	2.1	
	MA	1.3	2.0