Assessing the accuracy of the zygoma for estimating ancestry using geometric morphometrics in a South African sample

by

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ABSTRACT

The large number of unidentified, decomposed and skeletonised remains found in South Africa (SA) necessitates relevant and reliable methods to assist in victim identification. Ancestry estimation from unknown skeletal remains is essential when reconstructing a demographic profile of a missing person. In the SA population, estimating ancestry is problematic as standards developed internationally rarely apply to the local, biologically heterogenous population. Craniofacial morphology is known to be ancestrally distinct and studies are yet to explore shape and size variation in the zygomatic bone of the SA population. The aim of this study was to assess ancestral variation in zygomatic shape and size in a SA population using three-dimensional geometric morphometric analyses. A sample of 158 individuals were analysed from Bantu-speaking (BA), European (EA) and Mixed Ancestral (MA) South African groups. Males were larger in size than females, but no size differences were observed between ancestral groups. Significant shape differences were observed between ancestral groups, while none were observed between males and females. BA and MA individuals had narrower, shorter and more anteriorly projecting zygomas than EA individuals. The zygoma was shown to accurately distinguish EA (84%) from BA (81%), and MA (80%) from EA (68%) individuals, but unreliably distinguished BA (60%) from MA (66%) individuals. This is likely correlated to the historical peopling of SA and historical forced racial classification. Age-related changes and antemortem tooth loss did not confound the ancestral variation in size, despite minor changes in zygomatic shape being associated with these two factors. These confounders did not impact ancestry estimation accuracies, further suggesting a minor impact on overall zygomatic shape. Furthermore, the patterning of ancestral variation in the zygoma revealed the need for further research to distinguish between the biologically heterogenous ancestral groups in SA.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
2D	Two-dimensional
3D	Three-dimensional
AD	Anno Domini
ANOVA	Analysis of variance
BA	Bantu-speaking ancestry
CVA	Canonical variate analysis
DFA	Discriminant function analysis
EA	European ancestry
GM	Geometric morphometrics
GPA	Generalised Procrustes analysis
LOOCV	Leave-one-out-cross-validation
MA	Mixed ancestry
PCA	Principle component analysis
SA	South Africa
UCT	University of Cape Town

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1. INTRODUCTION

Throughout the world, interpersonal violence has been associated with unnatural deaths and unidentified individuals. This has given rise to the critical need for forensic anthropologists to provide probable identification to victims. They apply knowledge and techniques in the analysis of human skeletal remains (Cattneo *et al.*, 2006; Byers, 2011). Forensic anthropologists provide an estimated demographic profile to the police and this is matched against the decedents profile reported by the family, thereby, assisting in victim identification and provide the victims' families with closure, social and criminal justice.

The crime rate in South Africa (SA) increases annually due to crimes against humanity, poverty and gangsterism (Steyn *et al.*, 2016); with the current murder rate of 35.8 per 100 000 population for 2017/18 (Africa Check, 2018). The precincts that report the highest murder rates are found in Cape Town (Nyanga, Delft, Khayelitsha, Harare, Phillipi East, Gugulethu and Kraaifontein) and Kwazulu Natal (Umlazi, Inanda and Plessislaer) (CrimeStatisticsSA, 2015). These high levels of murder rates usually correspond to missing persons, decomposed or skeletonised individuals, and are represented as the proportion of documented cases to the exclusion of unaccounted or undocumented murders. In the absence of an investigative lead, skeletal analysis can provide information to construct the biological profile of the deceased (Ferguson *et al.*, 2011) and provide a probable identity.

Forensic anthropologists are requested to construct a demographic profile of a decedent (Steyn *et al.*, 1997; Byers, 2011), by assessing the biological parameters inclusive of sex (Steyn *et al.*, 1997), ancestry (Hefner, 2009; L'Abbé *et al.*, 2011), age (Franklin, 2010) and stature (Dayal *et al.*, 2008a; Mummert, 2011). Estimating ancestry is arguably the most important aspect in victim identification (Byers, 2011), as research has shown that standards for sex, stature and age estimation are sensitive to ancestry. Biological sex estimation is possible because of sexual dimorphism, which refers to the differences between males and females as a result of variation between the sexes (Black & Ferguson, 2010). Sexual dimorphism of the cranium is closely associated with ancestry; therefore, ancestral origin must be considered when assessing sex (Loth, 2000; Barrier & L'Abbé, 2008; Macaluso Jr, 2010; Krishan *et al.*, 2016).

1.1 Ancestry

Ancestry refers to a scientifically derived descriptor of biological variation that forensic anthropologists estimate (Konigsberg *et al.*, 2009; Ferguson *et al.*, 2011; Stull *et al.*, 2014); or the affinity to a population group in terms of skeletal shape and size, often expressed in geographical terms (Chrysostomou & Thompson, 2015). This is because some populations have higher frequencies of certain traits that are adaptations to a geographical region of ancestral origin (Patterson *et al.*, 2009; Byers, 2011; Montinaro *et al.*, 2017). For example, thermoregulatory adaptations occur in the nasal region of a populations adapted to colder climates (narrow nasal aperture) and hotter climates (wider aperture). Therefore, ancestry is also an indication of one's genetic and cultural heritage, including environmental adaptations (Nawrocki *et al.*, 2018). These factors impact bone morphology, which may be assessed using scientific methods and permit the use of skeletal analyses to assess ancestry.

Worldwide, the greatest challenge forensic anthropologists face is the expectation to estimate ancestry, which is often erroneously mistaken with race. In SA, this is very problematic as forensic anthropologists are expected to provide a socially translatable profile of a deceased individual. The use of past typological approaches inclined to racial terminology and classification have been present in forensic research. Forensic anthropological researchers opt to use scientifically derived descriptors rather than racial identification. Therefore, the social implications of terminologies used in forensic research remain an important consideration as forensic anthropological researchers strive to distance themselves from past typological approaches of racial classification and identification that suggest greater biological grouping than is scientifically detectible (Hunt & Albanese, 2005; Saunders & Rainey, 2008). *Race* is a social construct that does not exist biologically. Social notions of race exist wherein individuals identify with social and bureaucratic identities. Due to prejudice and political associations of *race,* this has been evident in past research within the South African populations that used the racial categories in ancestral studies. Some studies in SA have used racial terminology for research, whilst others prefer ancestral terms (McDowell *et al.*, 2012; Maass, 2016). Understanding the historical use of race as a category of identification in SA is crucial when investigating ancestral variation because of the expectation in providing a socially translatable profile to the police.

1.1.1 Racial Identities in South Africa

Racial identities and ancestral variation in SA have been shaped by colonial and apartheid policies and ideologies. The complex genomic admixture in SA is evidence of the intra-and-

inter-continental contribution from different ancestral backgrounds (African, Asian, European, Indian and Mixed Ancestry) (Patterson *et al.*, 2009; Petersen *et al.*, 2013; Montinaro *et al.*, 2017). The Cape of Good Hope (present day, Cape Town) was established in 1652 as a refreshment and fuelling station by the Dutch East India Company (VOC) (De Wit *et al.*, 2010). This led to the arrival and settling of different European colonists (*i.e.* Dutch, German, Portuguese, Spanish and French Huguenot) with the British settling much later during the mid-1800's (Inwood & Masakure, 2013). The presence and continued construction at the refreshment station increased the demand for labour, but due to a lack of work force; political exiles and slaves were brought to the Cape by the Dutch (and later British) from East India and Malaysia (De Wit *et al.*, 2010; Patterson *et al.*, 2010). The expansion of the Cape of Good Hope resulted in further displacement of the indigenous Khoesan population and agro-pastoral Bantu language-speakers, who were settled in the Cape area; and their subsequent assimilation into the Cape colony economy for labour (De Wit *et al.*, 2010; Patterson *et al.*, 2010; Petersen *et al.*, 2013). The abolition of slavery in 1834 resulted in assimilation of different ancestral groups in the Cape as populations mixed and migrated freely (South African History Online, 2018). However, this was short lived as the beginning of apartheid in 1940s resulted in the gradual implementation of segregationist policies, the legacy of which shaped ancestral diversity in South African and the Western Cape.

Under the apartheid government in SA, the Population Registration Act of 1950 imposed segregation of racial categories based on skin colour ('black', 'white' and 'coloured') and the Group Areas Act of 1950 for ethnic segregation (Petrus & Isaacs-Martin, 2012). Despite this, marriages were common place between ancestral groups especially between European and freed slaves (primarily from Malay, India) (Petersen *et al.*, 2013). Adhikari (1992) suggests that marriage between these specific groups could be due to shared similarities in cultural practices and living within close proximity with each other (Adhikari, 1992). However, marriage infrequently occurred between European and African individuals because of cultural differences (Adhikari, 1992; Inwood & Masakure, 2013) and imposed segregation laws of that time. These marriages led to biological admixture (Patterson *et al.*, 2009), shared culture with exchanges in socially transmitted ideas, values and perceptions (Petrus & Isaacs-Martin, 2012).

In the present day, SA included, social racial categories remain a reality where individuals attribute themselves to different identities based on social or legal terms

(population centred identities) (Stull *et al.*, 2014). Certain morphological features are affiliated with ancestral estimation of individuals found on European, Asian or African continents ("old world continents"); thus, classified into three broad ancestral groups namely: African, European and Asian (Buikstra & Ubelaker, 1994; Hefner, 2009; Byers, 2011). Research has shown that ancestral estimation methods initially developed for European and North American populations (Buikstra & Ubelaker, 1994; Byers, 2011) cannot be successfully applied to the South African population (L'Abbé *et al.,* 2011; McDowell *et al.,* 2012; Stull *et al.,* 2014; Liebenberg *et al.,* 2015; Small *et al.,* 2016). This is due to the unique ancestral composition of the South African population that complicates establishing ancestry in skeletal analysis for a population with unique admixture *e.g.* Cape Town (Buikstra & Ubelaker, 1994; Hefner, 2009; Byers, 2011; Stull *et al.,* 2014).

1.1.2 Current South African Population Groups

Generally, in SA people still affiliate with social categories ('black', 'coloured', 'white') defined by the government of the time, whilst others affiliate with population affinity (*e.g.* African, European, Asian) (Byers, 2011; İşcan & Steyn, 2013a) People may often use these terms interchangeably. However, having standardising terminology of biological descriptors such as ancestry ensures relatability to the population of interest. To understand the context of the terminology used in the skeletal collections within South Africa, one must convert socially defined or legislative terminology to biological identities.

South Africans of Bantu-speaking ancestry (BA) legislatively identify as 'black' and comprise 79% of the SA population (Statistics South Africa, 2013); 39% of the population in Cape Town (City of Cape Town, 2012). These individuals include descendants of the Bantu-speaking agro-pastoralist migration from West and Central Africa, who migrated throughout sub-Saharan Africa in 1700AD (Murdock, 1959; Liebenberg *et al.,* 2015), and are non-Khoesan descendants *i.e.* non-KhoeKhoe or non-San descendants (Petersen *et al.,* 2013). Language origin cannot be used in other parts of the world to track gene flow, but genetic studies within sub-Saharan Africa link directly to ancestry because of the Bantu agro- pastoralist migration (Veeramah *et al.,* 2012; Montinaro *et al.,* 2017). Linguists have also been able to track and link Bantu heritage through language and cultural practices and this is evident in linguistic diversity in present day Sotho-Tswana and Nguni languages (Murdock, 1959). Evidence of click containing languages spoken throughout Southern Africa amongst herder and hunter gatherers suggest possible contribution in genetic admixture and interaction between the Bantu agro-pastoralists and Khoesan (Murdock, 1959; Montinaro *et al.,* 2017).

South Africans of European ancestry (EA) bureaucratically identify as ‘white’ and comprise 9% of the SA population, (Statistics South Africa, 2013) and 16% of the population in Cape Town (City of Cape Town, 2012). They are descendants of colonial migrants who were British, Dutch, Portuguese, French Huguenot, Italian and Greek (Stull *et al.*, 2014; Krüger *et al.*, 2018), and considered a heterogenous group. The segregation of racial groups led to restricted genetic flow between individuals, as years progressed, they had limited genetic admixture from the parent populations. This led to the those present in SA to be isolated over generations, creating a founder group and subsequently led to the conservation of variation in this group; therefore, South Africans of European ancestry are considered genetically distinct from European individuals (L’Abbé *et al.*, 2011).

South Africans of Mixed Ancestry (MA) bureaucratically identify as ‘coloured’ and make up 9% of the SA population, (Statistics South Africa, 2013) and comprise 42% of the population in Cape Town (City of Cape Town, 2012), whereby the largest proportion of these individuals, including the Khoesan, are found to constitute 50% of the Western Cape population (De Wit *et al.*, 2010; Patterson *et al.*, 2010; Petersen *et al.*, 2013). The ‘coloured’ South Africans are from diverse origins including individuals who are descendant from indigenous Khoesan (Griquas, Namas and Basters) (Adhikari, 2005), Europe, Bantu speaking ancestry (West Africa), Asia (Malaysia, Indonesia and East India) and Madagascar (Adhikari, 2005; De Wit *et al.*, 2010). The genetic contributions in this group are as follows: 32–43% Khoesan, 20- 36% Bantu-speaking Africans, 21–28% European and 9–11% Asian (De Wit *et al.*, 2010). As marriage became common among people from various ancestral origins, especially between Europeans and freed slaves (primarily from Bengal, Southern India, Sri Lanka, Madagascar, Indonesia) (South African History Online, 2017), Bantu-speakers, Khoesan, Madagascan Cape Slaves and Asians, this resulted in diverse genetic admixture. Miscegenation occurred more frequently between Khoesan, Madagascan Cape Slaves and Bantu-speaking individuals (Adhikari, 1992, 2005), because of shared similarities in cultural practices and living within close proximity with each other (Adhikari, 1992, 2004, 2009). Therefore, the complex admixture is a legacy of historical peopling that contributes to the biologically heterogenous group of South Africans of MA (Liebenberg *et al.*, 2015).

The Khoesan are individuals who are unique and indigenous to SA, with homogeneous genetic input, shared culture and languages from Khoe and San descent (Schelebusch *et al.*, 2012; South African History Online, 2012; Thompson, 2018). The term ‘The Khoe’, refers to individuals who were skilled in the practice of nomadic pastoral

agriculture and maintained large herds, while 'The San', were skilled nomadic hunter-gatherers. Both these groups migrated throughout the Southern African region (Thompson, 2018). The influx of colonialists and the continued expansion at the Cape of Good Hope, led to some Khoesan individuals being assimilated as workers (Adhikari, 1992), while the remainder migrated further from the Cape (South African History Online, 2012). Under the apartheid regime, Khoesan people were forced to self-classify with the bureaucratic group 'coloured' and many still use that classification today (Adhikari, 1992).

In this study, the researcher acknowledges that individuals of BA origin, as well as Khoesan individuals are of African origin, which may not be reflected in the skeletal collections used in the study. Skeletal collections used may have accessioned individuals who may socially identify and socially classify as 'coloured' but are considered African *e.g.* Khoesan. These individuals will be misclassified as 'coloured', when they are not of MA origin. This further resonates the complexity among the individuals who socially classify in this category. Therefore, the Mixed Ancestral group encompasses individuals of MA origins and possibly Khoesan individuals. While BA and Khoesan individuals are considered African, the skeletal collections may classify individuals who socially classify as 'black' as BA individuals and not of Khoesan descent.

1.2 Ancestral Estimation Methods

Metric and non-metric methods have been used to characterise and interpret human biological variation by assessing ancestral differences in shape that exists on the skeletal components (Patriquin *et al*., 2002; Hefner, 2009; Mitteroecker & Gunz, 2009; Ferguson *et al.*, 2011; Stull *et al.*, 2014). Ancestral estimation using these methods attributes specific skeletal variation to geographic origin (*e.g.* Africa, Europe or Asia) (Hunt & Albanese, 2005). Traditionally, craniofacial and postcranial regions have been used for morphological and metric analysis to assess ancestry (Hefner, 2009; Byers, 2011; Xing *et al.,* 2013). To do this accurately, ancestry estimation methods need to conform to *Daubert* expert testimony standards (Daubert, 1993). These standards highlight the four principles that must be addressed when estimating ancestry, which include: (i) empirical support, (ii) estimated error rates, (iii) method standardisation and (iv) method validation via peer review (Sauer & Wankmiller, 2009). Therefore, it is of paramount importance that forensic anthropologists standardise and ensure the test methods used for all aspects of skeletal analysis are population specific and reliable (İşcan & Steyn, 1999; Hefner, 2009; Sauer & Wankmiller, 2009; Byers, 2011; L'Abbé *et al.,* 2011; Hefner & Ousley, 2014; Stull *et al.*, 2014; Liebenberg *et al.,* 2015).

Anthroposcopic traits are used to assess ancestry by visually analysing features of shape on bone (Chrysostomou & Thompson, 2015), *e.g.* height of the nose bridge, width of nasal aperture (Saunders & Rainey, 2008), nose structure (Hefner, 2009; DiGangi & Hefner, 2013) and suture shape (Hefner, 2009). According to Patriquin and associates (2002) and Relethford (2009), the cranium is widely used to estimate ancestry (Giles & Elliot, 1962a; Krogman, 1962; Brues, 1990; Rhine, 1990; İşcan & Steyn, 1999; Patriquin *et al.*,, 2002; Hefner, 2009; Relethford, 2009; L'Abbé *et al.*, 2011) and sexual dimorphism (Giles & Elliot, 1962b; Giles, 1970; Buikstra & Ubelaker, 1994; İşcan & Steyn, 1999; Franklin *et al.*, 2005; Dayal *et al.*, 2008a; Spradley & Jantz, 2011). Research conducted on the mid- craniofacial region particularly the orbital (Xing *et al.*, 2013) and nasal regions (Byers, 2011; Stull *et al.*, 2014) exhibit significant variation in the ability to distinguish between ancestral groups. Morphological features of the mid-craniofacial region, including zygomatic features, are suggested to have the ability to distinguish between ancestral groups (J.A.S., 1963; Hefner, 2009; Schlager & Rüdell, 2013; Kamal & Rathee, 2015). However, inconsistencies in scoring these qualitative traits varies across individuals (Byers, 2011), as it may be influenced by an individuals' level of experience and exposure. According to the *Daubert* standards (Daubert, 1993), issues were raised concerning the subjective nature and repeatability of non-metric methods. This led to metric methods being often favoured over non-metric methods. Furthermore, combining the methods is informative in establishing demographic profile.

Quantitative analysis using metric methods allows objective inference of the variation under investigation by providing discrete numerical measurements Estimation accuracy using discriminant function analyses (DFA) within the SA population for ancestry and sex has been previously reported. For ancestry, the estimation accuracy was above 95% (cranium) (İşcan & Steyn, 1999; İşcan & Steyn, 2013a), and sex was 80-95% (cranium and pelvis) (İşcan & Steyn, 1999; Franklin *et al.*, 2005; Dayal *et al.*, 2008b; DiGangi & Hefner, 2013; İşcan & Steyn, 2013b; Spradley & Stull, 2018). However, an objects' three-dimensional (3D) information is often lost when using traditional metric methods. Therefore, an informative method such as geometric morphometrics (GM), is favoured over traditional metric and non-metric methods (Kimmerle *et al.*, 2008; Stull *et al.*, 2014).

1.3 Geometric Morphometrics

GM has emerged as a paradigm of biological shape analysis, allowing variation to be visualised and quantified in two and three dimensions (Adams *et al.*, 2004; Mitteroecker & Gunz, 2009; McKeown & Schmidt, 2013). Modern computational and technological advances have allowed for the acquisition, processing, and analysis of shape variables that retain *all* geometric information contained within biological data (McKeown & Schmidt, 2013; Maass, 2016). Shape analysis is one of the approaches used to understand morphological variation in biological studies (Bookstein, 1997; Zelditch *et al.*, 2004).

These processes are influenced by genetic or environmental conditions to bring about effects in development (shape change with age and growth), growth (size change with age) and allometry (shape change with size) (Viðarsdóttir *et al.*, 2002).

GM has developed largely within the field of biological anthropology to enable quantification of morphology (McKeown & Schmidt, 2013). Different research investigations into shape analysis alluded to the difficulty of generating or capturing geometric relationships from linear measurements gained from object analysis, as some shape aspects were lost (Gelsvartas, 2001; Adams *et al.*, 2004; Zelditch *et al.*, 2004). Advancements in the field of morphometrics to address the loss of shape gave rise to the GM analyses and its use in assessing sex (Gonzalez *et al.*, 2011) and ancestry (Xing *et al.*, 2013; Stull *et al.*, 2014) due to the sensitivity of the methods in shape analysis.

There are two types of sub-classifications within GM techniques; namely, outline and landmark-based methods. The outline method involves the digitising of points along the perimeter or curvature of an object and fitting the points with mathematical functions (*e.g.* Fourier analysis) (Adams *et al.*, 2004; Webster & Sheets, 2010). The landmark-based approach involves the summary of 2D and 3D Cartesian coordinate configurations of biologically homologous landmarks (Adams *et al.*, 2004; Webster & Sheets, 2010). The digitised landmark coordinates contain configurations (shape and size) in terms of location and information in multiple planes (McKeown & Schmidt, 2013), which allows placement of landmarks in anterior-posterior, superior-inferior and medial-lateral planes (McKeown & Schmidt, 2013).

Landmarks

GM analysis involves the capturing of *landmarks*, which are defined as precise locations on biological individuals that hold some functional, structural, developmental, or evolutionary significance (Bookstein, 1991; Maass, 2016). In biological anthropology, anatomical landmarks (including standard craniometric or linear distances on the skeleton) are used to acquire landmark coordinate data (Bookstein, 1991; Gelsvartas, 2001; Zelditch *et al.*, 2004; Ferguson *et al.*, 2011; McKeown & Schmidt, 2013).

Landmarks may be classified under three Types (I, II, and III) based on whether they are a specific point, a relative or midway location or outline points along a curvature (Viðarsdóttir *et al.*, 2002; Webster & Sheets, 2010). In earlier studies, it was noted that not all landmarks were defined the same, thus, Bookstein (1991) addressed this inconsistency by defining three types of landmarks: Type I, Type II and Type III. Landmarks defined by a specific location or a point of intersection between sutures are Type I landmarks (McKeown & Schmidt, 2013). Type II landmarks are points that are located at the sharpest curvature along boundaries or are defined as the most inferior or superior point along a margin or feature of interest being studied (Bookstein, 1991). Type III landmarks are relational points whose placement is

dependent on the location of another landmark (*e.g.* maximum bizygomatic breadth) (Bookstein, 1991). The challenge of using Type III landmarks is the lack repeatability due to the variability in landmark location from individual to individual (McKeown & Schmidt, 2013).

Landmark coordinates for an object under study are typically transformed into points in the Kendall shape space (Kendall, 1977) via scaling and alignment procedures in Generalised Procrustes Superimposition (Slice, 2007). Generalised Procrustes Superimposition brings the landmark configurations of all the individuals into a common coordinate system by translating, scaling to unit centroid size and then rotating every landmark (Figure 1.1), until the sum of the squared Procrustes distances between all configurations is minimised (Slice, 2001, 2007). Thus, differences due to location, orientation and scaling multiple coordinate-based configurations are removed; until the least-squares fit all landmarks to a reference individual (Rohlf & Slice, 1990). The Procrustes coordinates may be used to investigate the Procrustes distance between corresponding landmarks on each configuration (Slice, 2007; McKeown & Schmidt, 2013). The resulting landmarks can be used to assess Procrustes distance between landmark coordinates.

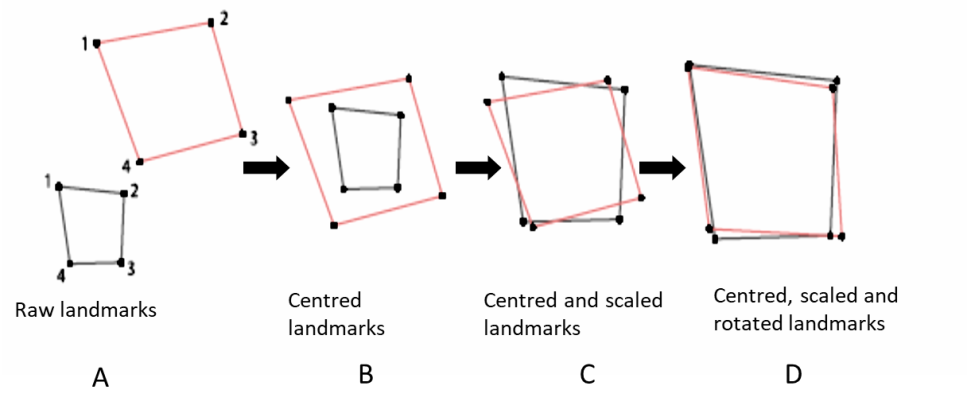

Figure 1.1: Three steps of Generalised Procrustes Superimposition to Procrustes coordinates starting at the raw landmarks (A) and resultant Procrustes shape coordinates (D).
[Image adapted from Figure 3, (Mitteroecker & Gunz, 2009: 239)

The superimposed landmark coordinates are usable as shape variables and can be subjected to multivariate statistical methods such as Principal Component Analysis (PCA); Canonical Variate Analysis (CVA), DFA, and multivariate regression analysis of variance or covariance (McKeown & Schmidt, 2013). These tests are used to quantify and identify covariance structure, group differences, and functional relationships (Slice, 2007). One of the disadvantages of the Generalised Procrustes Superimposition method lies in that the superimposed landmarks are susceptible to the 'Pinocchio Effect' (Webster & Sheets, 2010). The Pinocchio Effect occurs when large variance differences at one or two landmarks are distributed over many landmarks by least-squares rotation, thus, providing misrepresentations of landmark variation (Webster & Sheets, 2010). Despite this, Generalised Procrustes Superimposition is considered a

statistically robust method to process 3D coordinate data (Adams *et al.*, 2004; Slice, 2007).

PCA is used in anthropological morphometrics to investigate size and shape variation and interrelationships that may be present in a dataset (McKeown & Schmidt, 2013) by reducing the dimensions of variation (Saunders & Rainey, 2008). CVA is similar to PCA, however, it is used for grouped data. In CVA the axes or canonical variates maximise between-group differences rather than entire sample variation as in PCA (Katzenberg & Saunders, 2007). CVA generates Mahalanobis distances, a distance measure to gauge similarity of an unknown set of measurements to a known reference sample, between groups based on sample centroids (McKeown & Schmidt, 2013). DFA is a multivariate statistical analysis designed to calculate the distance from an unknown individual to the centroids for reference groups for classification purposes (*e.g.* sex or ancestry estimation) (Marcus, 1990). The smallest distance is indicative of the greatest similarity to the group mean (McKeown & Schmidt, 2013).

GM techniques require rigid and robust structures for analyses and are well suited for characterising variation in bones (Adams *et al.*, 2004; Slice, 2007). Several studies have used GM to analyse the morphology of past and present humans; to provide unique insight into the extent of ancestral variation and the possible forces shaping this variation (Bookstein, 1991; Viðarsdóttir *et al.*, 2002; Adams *et al.*, 2004; Kimmerle *et al.*, 2008; Mitteroecker & Gunz, 2009; Webster & Sheets, 2010; Stull *et al.*, 2014; Freidline *et al.*, 2015; Small *et al.*, 2016).

GM in ancestral studies

Research in ancestral shape variation using GM has been conducted in North American populations (Kimmerle *et al.*, 2008), Portuguese (Weisensee & Jantz, 2011), Italian (Dedouit *et al.*, 2017) as well as SA populations (L'Abbé *et al.*, 2011; McDowell *et al.*, 2012; Stull *et al.*, 2014; Franklin *et al.*, 2006; Xing *et al.*, 2013; Small *et al.*, 2016). However, none of these studies have explored the variation in the zygomatic bone in isolation for ancestral estimation within a SA population (L'Abbé *et al.*, 2011; Stull *et al.*, 2014; Liebenberg *et al.*, 2015; Small *et al.*, 2016). A study investigating the nasal apertures of 'black' and 'white' South Africans found significant differences between the groups with approximately 94% cross-validated accuracy (McDowell *et al.*, 2012). Another study investigating craniofacial features, found the significant differences between the cranial shapes of 'black', 'coloured' and 'white' South Africans, with approximately 79% cross-validated accuracy (Stull *et al.*, 2014). However, the landmarks and regions of analysis used differed between the studies (McDowell *et al.*, 2012; Xing *et al.*, 2013; Stull *et al.*, 2014). A study examining the orbital region for possible ancestral differences found good discriminatory ability in this region for distinguishing between people of different ancestries (Xing *et al.*, 2013). The study concentrated on the internal and lateral aspects of the upper margin formed by the frontal bone, and the internal aspects of the lower margins formed by the

zygoma and maxilla (Xing *et al.*, 2013). The study by Xing and associates (2013) showed that comparisons between the superior orbital contour and inferior orbital contour, that comprise the zygomatic bone, was more easily discerned when GM analysis was employed (Xing *et al.*, 2013). Therefore, there is need for investigation of the zygomatic region, using an informative method such as GM

Before GM analysis can be performed, an understanding of the anatomical structure of the zygomatic bone is required to better comprehend how differences in morphology may arise. Knowing the anatomy of the zygomatic region will improve understanding of the mechanisms contributing to variation in the mid-craniofacial region.

1.3.1 Zygoma

The zygoma, also known as the malar bone has a rough quadrangular shape with two processes: the frontal and maxillary processes (Figure 1.2) (Standring, 2008). It bridges the facial skeleton to the cranial bones by connecting the maxilla of the facial skeleton to the temporal and frontal bones (Oettlé *et al.*, 2017). The quadrangular shaped zygoma is anchored by a zygomatic arch to the temporal bone. The zygomatic arch comprises the zygomatic process of the temporal bone posteriorly, and the temporal process of the zygomatic bone, which articulates anteriorly at the zygomaticotemporal suture (Oettlé *et al.*, 2017). The zygomatic arch correlates to the widest part of the face, forming the cheek prominence (Oettlé *et al.*, 2017). Sutures present around the zygomatic area include the zygomaticomaxillary, temporozygomatic and frontozygomatic (Schwartz, 1995). The frontozygomatic suture is between the zygomatic bone and frontal bone of the facial region, and the zygomaticomaxillary suture is between the zygomatic bone linking to the maxillary bone (Schwartz, 1995) (Figure 1.2). The temporozygomatic suture lies between the zygomatic and temporal bone and the internal sphenozygomatic suture is found between the zygomatic bone and sphenoid bone (Schwartz, 1995) (Figure 1.2).

Zygoma ontogeny

Modularity refers to the variation in a system, module or anatomical regions which are dependent on the variation in structural and functional relationship of other components (Bruner, 2007; Klingenberg, 2008). Integration proposes the high level of covariation within the whole structure, that arises from the interaction and cohesion of biological processes (Bruner, 2007; Klingenberg, 2016). Some researchers have suggested that the whole cranium functions as an integrated unit, and variation in shape and size is influenced by the close integration of the nasal bone, maxillae and zygomatic bones (Hylander *et al.*, 1991). To understand facial changes, the knowledge of how facial growth occurs is required. Two models have been suggested to impact growth (Bastir *et al.*, 2006). The functional model explores the biomechanical relations of the facial skeleton, and functional integration among the regions; the structural approach focuses on the developmental and growth changes in different parts affects each other (Bastir *et*

al., 2006). Research in exploring the possibility of how skeletal components change with respect to shape and size are influenced by modularity and integration (Bastir, 2008).

The zygomatic arch grows laterally and inferiorly along with all the other cranial structures (Dechow & Wang, 2017; Oettlé *et al.*, 2017). This is due to bone deposition on the zygomatic arch while the projecting zygoma area remodels posteriorly, with continued deposition of new bone on its posterior side and resorption from its anterior side (Dechow & Wang, 2017; Oettlé *et al.*, 2017). "Deposition and resorption processes require adjustment within the sutural connective tissue development; thus, as deposition exceeds resorption, the whole zygomatic protuberance and zygomatic arch relocate posteriorly as it enlarges vertically. By the increase in size of the arches, the growing muscles attached to them are accommodated (masseter and temporalis); displacement is driven by the functional relationships established by the soft tissues that surround and interact with a given bone. Although, these bony features are said to be inherited separately, they do have an interaction with the growth of each other. Further displaced zygomatic arches, for instance, suggest a more developed and bulkier masseter" (Oettlé *et al.*, 2017). Freidline and associates (2015) suggest that subtle differences in facial shape are due to the prolonged midfacial development when compared with other cranial components and are often subject to external influences (masticatory stress) (Freidline *et al.*, 2015). External stresses often exert longer effects on facial morphology when compared with other cranial components (Freidline *et al.*, 2015). Thus, the increase in facial size in relation to total size and modification in shape of craniofacial structures is related to mastication (Paschetta *et al.*, 2010).

Although by age ten, the cranial and orbital cavities have reached adult dimensions, orbital margins and the zygomatic bone continue to grow due to bone deposition in these areas (Freidline *et al.*, 2015). Other researchers' findings suggested that before puberty, males and females are more similar in facial growth (Bulygina *et al.*, 2006). At puberty, usually 12 years-of-age, females experience rapid growth influenced by hormonal changes with growth ceasing by age 14 or 15; whilst the males have a broader and continued growth spurt until 16 or 17 years-of-age (Bulygina *et al.*, 2006). The growth patterns enable an understanding of the variation amongst ancestral groups; and the important correlations of morphology of other features of the face such as the orbit, nasal cavity and the mandible (Oettlé *et al.*, 2017). Oettlé and associates (2017) stated that shape and size features of cranial robusticity are correlated; this may provide information regarding the degree of development of the zygoma, among both broad ancestral groups and more narrowly defined population groups.

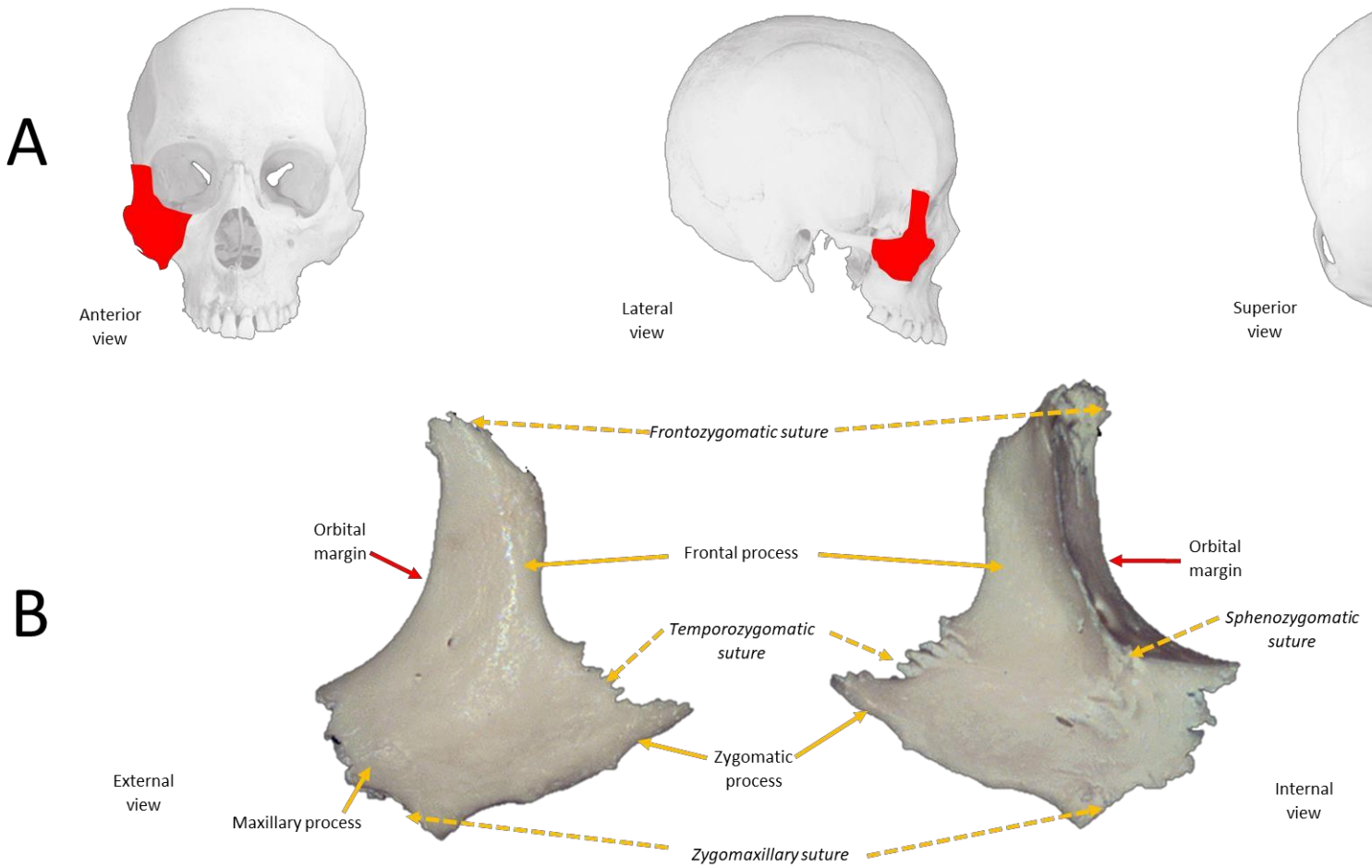

Figure 1.2: Anatomy of the zygomatic bone. A: The craniofacial region showing the zygomatic bone in anterior, lateral and superior views. B: Left zygomatic bone with the external view on the left and internal view on the right. Processes are indicated by solid orange arrows, the orbital margin by solid red arrows, and associated sutures by broken orange arrows.

[Images adapted from Eskeletons, 2018)].

Musculoskeletal function

Masticatory stress is driven by the adaptation to process mechanically resistant food further influencing muscle size and attachment sites on the zygoma (Paschetta *et al.,* 2010; Dechow & Wang, 2016; Oettlé *et al.,* 2017). This includes overall enlarged and anteriorly positioned temporalis and masseter muscles; enlarged attachment sites of the masseter muscle on the zygomatic arch and of the temporalis muscle on the lateral side of the cranium, and a larger cross-section of the infratemporal fossa for the temporalis muscle (Oettlé *et al.,* 2017). Additionally, the posteroinferior border of the zygomatic bone is roughened for masseter muscle attachment (Standring, 2008).

The masseter muscle is a quadrangular shaped muscle anchored to the zygomatic arch and to most of the lateral surface of the ramus of the mandible (Drake *et al.,* 2015). The superficial part of the masseter originates from the maxillary process of the zygomatic bone and the anterior two-thirds of the zygomatic process of the maxilla (Drake *et al.,* 2015). Augmented masticatory forces generally lead to growth and overall robustness of the cranium (Drake *et al.,* 2015). The temporalis muscle is a large, fan-shaped muscle that fills much of the temporal fossa and originates from the bony surfaces of the fossa superiorly to the inferior temporal line and is attached laterally to the surface of the temporal fascia. The orientation of the fibres differs with the more anterior fibres being vertical, while the more posterior fibres are horizontal. The fibres converge inferiorly to form a tendon, which passes between the zygomatic arch and the infratemporal crest of the greater wing of the sphenoid to insert on the coronoid process of the mandible. The temporalis muscle attaches down the anterior surface of the coronoid process and along the related margin of the ramus of the mandible, almost to the last molar tooth. The temporalis is a powerful elevator of the mandible and this movement involves posterior translocation of the head of the mandible from the articular tubercle of the temporal bone and back into the mandibular fossa, the temporalis also retracts the mandible or pulls it posteriorly. In addition, the temporalis participates in side-to-side movements of the mandible (Drake *et al.*, 2015).

The muscles around the zygoma consist of the upper group of oral muscles such as zygomaticus major and zygomaticus minor, and contributary muscles such as *levator labii superioris, levator labii superioris alaeque nasi, risorius,* and *levator anguli oris* (Drake *et al.*, 2015) (Figure 1.3). These contributory muscles are responsible for upward or downward movement of the lips. The zygomaticus major is formed from the posterior part of the lateral surface of the zygomatic bone (Drake *et al.,* 2015). It draws the corner of the mouth upward

and laterally and is used when smiling (Drake *et al.*, 2015). *Zygomaticus major* is a superficial muscle deep to *orbicularis oculi* along the posterior part of the lateral surface of the zygomatic bone. It passes downward and forward, blending with *orbicularis oris* and inserts under the skin at the corner of the mouth (Drake *et al.*, 2015). *Zygomaticus minor* is situated on the lateral surface of the zygomatic bone and functions in moving the lip upwards (Drake *et al.*, 2015). It originates from the anterior side to the origin of the *zygomaticus major*, runs parallel to the *zygomaticus major* path, and inserts into the upper lip medial to the corner of the mouth (Drake *et al.*, 2015). Both muscles are responsible for moving the corners of the mouth laterally (Drake *et al.*, 2015).

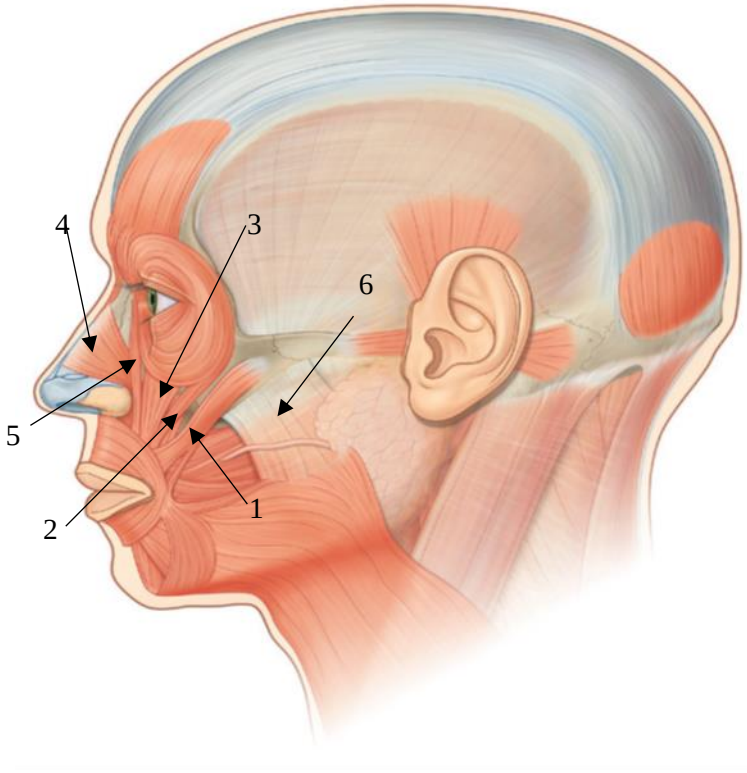

Figure 1.3: Facial muscles around the zygomatic bone. 1. Zygomaticus major, 2. Zygomaticus minor, 3. Levator labii superioris, 4. Nasalis, 5. Levator labii superioris alaeque nasi and 6. Masseter muscle.
[Image adapted from Figure 8.53 (Drake *et al.*, 2015: 904)].

Zygoma in ancestral studies

Oettlé and associates (2017) stated that extensive research in ancestral variation was thought to reflect adaptations to climatic conditions due to isolation, and diet; which could be expressed in zygomatic shape and size variation. In a study by Freidline and associates (2015), the Inuit population was suggested to have laterally projecting zygomatic bone whilst the Khoesan had anterolateral projecting zygomas (Freidline *et al.*, 2015). The explanation for this difference

was that the Iniut population had pronounced development in nasal aperture when compared with the Khoesan; possibly related to adaptation to a colder climate (Freidline *et al.*, 2015). Furthermore, comparing the Inuit and Khoesan population groups; the zygomatic bone morphology was very different possibly due to dietary differences between the climatic and geographic regions (Freidline *et al.*, 2015). In this study review, they that stated the following; "Ancestral variation in the zygoma reflects genetic variation because of selective pressures exerted by genetic drift, natural selection and epigenetic changes to adapt to diet and climate variation with possible intensification by isolation" (Oettlé *et al.*, 2017).

GM has been used to assess variation of different cranial regions; however, no research has examined the zygomatic bone independently. Previous research for estimating ancestry within South Africa has mainly focused on samples derived from the Gauteng Province (L'Abbé *et al.*, 2011; McDowell *et al.*, 2012; Stull *et al.*, 2014; Small *et al.*, 2016). The composition of the population in Gauteng Province contrasts with that of the Western Cape. This has posed a challenge, as well as an opportunity to validate ancestral estimation methods which are applicable to populations found in and around Cape Town. This study therefore aims to investigate whether the zygomatic bone, can be used to accurately distinguish between individuals from different ancestral origins.

1.4 Aim

To use GM analysis to assess shape and size variation of the zygomatic bone in three-dimensions (3D) and its potential use for estimating ancestry in a South African population; taking into consideration the effect of presence of maxillary teeth, ageing, year-of-birth and sex.

Hypothesis: The zygoma can be used to accurately distinguish between individuals of different ancestries in a South African population sample.

Objective 1: Investigate whether the shape of the zygoma is influenced by size.

Question: Is the shape of the zygoma influenced by its size?

Objective 2: Characterise shape and size difference between South African ancestral groups

Question: Can the zygoma be used to estimate ancestry?

Determine whether differences between ancestral groups are evident in zygomatic shape and size.

Question: Is there a difference based on zygomatic shape and size for individuals of

different ancestries?

Question: Can we estimate ancestry from zygomatic shape and size?

Objective 3: Assess the effect of tooth presence, ageing and year-of-birth on zygomatic shape and size.

Question: Does the presence of teeth impact zygomatic size and shape?
Follow through Question: Does tooth loss influence our ability to accurately estimate ancestry?

Objective 4: Investigate whether the shape of the zygoma is influenced by sex

Question: Is the shape of the zygoma influenced by its sex?

2. MATERIALS and METHODS

2.1 Materials

Skeletal collections are frequently used for research and teaching purposes because they comprise individuals that have known ancestry, sex, age-at-death, and year-of-birth. There is great value obtained from the use of the skeletal collections, which have aided in advancing knowledge in forensic anthropology (L'Abbé *et al.*, 2005; Alblas *et al.*, 2018). Unfortunately, skeletal collections are affected by bias due to various acquisition practices and may not be representative of the population from which they are derived (L'Abbé *et al.*, 2005; Komar & Grivas, 2008). It is common for documented human skeletal collections to include a mix of unclaimed and bequeathed remains (L'Abbé *et al.*, 2005; Komar & Grivas, 2008). This affects the sample distributions of individuals from different population groups. Despite the biases and challenges in cadaveric collections, the research for application in a forensic context is vital. While including individuals from forensic cases was considered in this study, legal restrictions of the National Health Act (Act No. 61 of 2003) (Government Gazette, 2004), prevent this and forensic collections have limited sample sizes which may also introduce biases (Maass, 2016).

For this study, crania were sampled from established skeletal collections commonly used for research studies within the Western Cape, which are the University of Cape Town (UCT) Human Skeletal Collection and the Kirsten Collection (Stellenbosch University). These Collections comprise individuals from different social, economic and health statuses; and represent different ancestral groups encountered within the Cape Town population *i.e.* BA, MA and EA (Statistics South Africa, 2013). However, both collections have a lower representation of female individuals (38%) when compared with males (Alblas *et al.*, 2018; Gibbon, 2018).

2.1.1 University of Cape Town (UCT) Human Skeletal Collection

Unless otherwise stated, the information about the UCT Collection was sourced from the manuscript by Gibbon and Morris (*in press*). The UCT Human Skeletal Collection was started in 1911 by Robert Black Thompson and was officially accessioned by Matthew Robertson Drennan in 1925. It is housed and curated under the Division of Clinical Anatomy and Biological Anthropology in the Department of Human Biology, Faculty of Health Science, UCT.

Acquisition of the individuals is achieved through two sources: Bequest and State donation. Individuals may be fully/partially bequeathed (by the donors themselves or their families) to the Department for use in scientific (academic teaching) or medical research. Cadaver records indicate that the UCT Skeletal Collection represents an adult population from the early to mid-20th century. The majority of those bequeathed are often older individuals of EA (above 50 years) and are from a higher socio-economic status. The younger cohort of individuals (less than 50 years) mostly consists of State donated BA and MA individuals; often associated with lower socio-economic status. The State donated individuals are unidentified or unclaimed individuals whose relatives could not be located, or the family could not afford the burial costs. These include donated individuals from state hospitals, prisons, palliative care hospices and retirement centres.

2.1.2 Kirsten Collection

Unless otherwise stated, information about the Kirsten Collection was sourced from Alblas and colleagues (2018). The Kirsten Collection is housed and curated under the Division of Anatomy and Histology, Faculty of Medicine and Health Sciences, Stellenbosch University. It was started in the 1950's by J. F. van E. Kirsten, who was tasked to collect skeletal material for anatomical study. Prior to 1960, remains used for teaching and research were received from the Universities of Pretoria and the Witwatersrand.

Unlike UCT, most individuals accessioned in this collection are from State donations and are of BA and MA. Cadaver records indicate that the Kirsten Skeletal Collection represents an adult population from the mid to late-20th century. Furthermore, approximately 87% of the individuals represented in the Kirsten Collection are associated with lower socioeconomic status, implying marginal to poor employment, housing and health care (Pfeiffer *et al.*, 2016). To date, this collection is the largest cadaver-derived 'Cape coloureds' (or MA) skeletal collection in SA.

2.1.3 Study Sample

Consent for this study was obtained from the UCT Faculty of Health Science, Human Research Ethics Committee (HREC Ref# 843/2017) (Appendix A), and curators of both skeletal collections formally approved the study.

The demographic information pertaining to sex, ancestry, age-at-death and year-of-birth for each individual in the sample were obtained from the accession registers at each collection. This information was obtained from the Medical Certificate of Cause of Death

(Births and Deaths Registration Act; Act 51 of 1992) for each donor. The exclusion criteria were:

i. Individuals with trauma, pathology or deformity of the zygoma. Where unilateral zygomatic deviations occurred, only the unaffected side was digitised.

ii. Individuals under 18 or over 75 years of age at time-of-death.

iii. Individuals born prior to the 1900s.

Due to these exclusion criteria and previously-discussed biases in these skeletal collections, a total sample of 158 individuals were selected. This sample comprised individuals from BA, EA and MA. 3D shape and size variation using geometric morphometrics has been recommended that sample sizes exceed 15 for adequate statistical power (Cardini *et al.*, 2015). Therefore, the sample size selected for this study is enough to explore variation with sufficient statistical power, the above-mentioned biases will remain a consideration when interpreting results.

Table 2.1: Sample demographics according to sex and ancestry

Ancestral group	**Male**	**Female**	**Total**
Bantu-speaking Ancestry	30	17	47 (30%)
Mixed Ancestry	34	27	61 (39%)
European Ancestry	32	18	50 (31%)
Total	96 (61%)	62 (39%)	158 (100%)
Total column percentages were calculated out of the 158 individuals for the sex and ancestral groups.			

2.2 Cranial Landmarks

Eight zygomatic landmarks defined by Howells (1973) and Buikstra and Ubelaker (1994) were selected to provide information regarding the superior, inferior and lateral dimensions of the zygoma (Figure 2.1, Table 2.1). Type I and Type II landmarks are more repeatable than Type III; and of the eight landmarks chosen for this study; six were Type II and two were Type I.

In cases where there was evidence of unilateral zygomatic trauma and deformation, only the unaffected zygoma was digitised (either left or right side). For crania without trauma or deformation both zygomas (left and right) were digitised separately. Shape and size difference between left and right zygoma were evaluated using a Procrustes Analysis of variance (ANOVA), and if no side-related differences were detected, coordinates were averaged for further analyses.

Table 2.2: Landmarks name, descriptions and types analysed in this study.

Name	Description	Type
Frontomalare temporalis	Posterior point of intersection between frontozygomatic, sphenozygomatic and sphenofrontal sutures	I
Jugale	Deepest incurvature along the posterior edge of the zygomatic bone between the frontal and temporal processes; or the midpoint in the notch between temporal and frontal process of the zygomatic bone	II
Zygotemporale superior	Most superior point on the temporozygomatic suture	II
Zygotemporale inferior	Most inferior point on the temporozygomatic suture	II
Zygomaxillare	Most inferior, anterior point on the frontomaxillary suture	II
Zygoorbitale	The intersection between the zygomaticomaxillary suture and inferior orbital margin	I
Ectoconchion	The intersection at the most anterior surface of the lateral border of the orbit and a line bisecting the orbit into two equal portions	II
Frontomalare orbitale	Point where the zygomaticofrontal suture crosses the orbital margin at the most anteriorly positioned point on the frontomalar suture.	I
Descriptions of landmarks (Martin & Saller, 1957; Howells, 1973; Buikstra & Ubelaker, 1994)		

2.2.1 Data Capture

A Microscribe G2® digitiser (Immersion Corp, San Jose, California, 2002) was used to digitise landmarks in 3D. All raw landmark coordinates were captured onto a Microsoft Excel spreadsheet. The configurations of landmarks were digitised thrice, and the digitisation error was evaluated by calculating the mean Euclidean distances (straight line distance between two points), between consecutive repeats. If an error greater than 1.0mm was found; the configurations of landmarks were re-digitised until distances between successive digitisations were less than 1.0mm (Von Cramon-Taubadel *et al.*, 2007; Robinson & Terhune, 2017).

Crania marked with landmarks shown in Figure 2.1 and Table 2.1. During the digitisation process, the crania were anchored with modelling clay (one by the maxilla and the other by foramen magnum) onto a wooden structure/rig, which was fixed on a clipboard using Prestik adhesive (Bostik South Africa, Cape Town).

Landmark name	
1 - Frontomalare temporalis	5 - Zygomaxillare
2 - Jugale	6 - Zygoorbitale
3 - Zygotemporale superior	7 - Ectoconchion
4 - Zygotemporale inferior	8 - Frontomalare orbitale

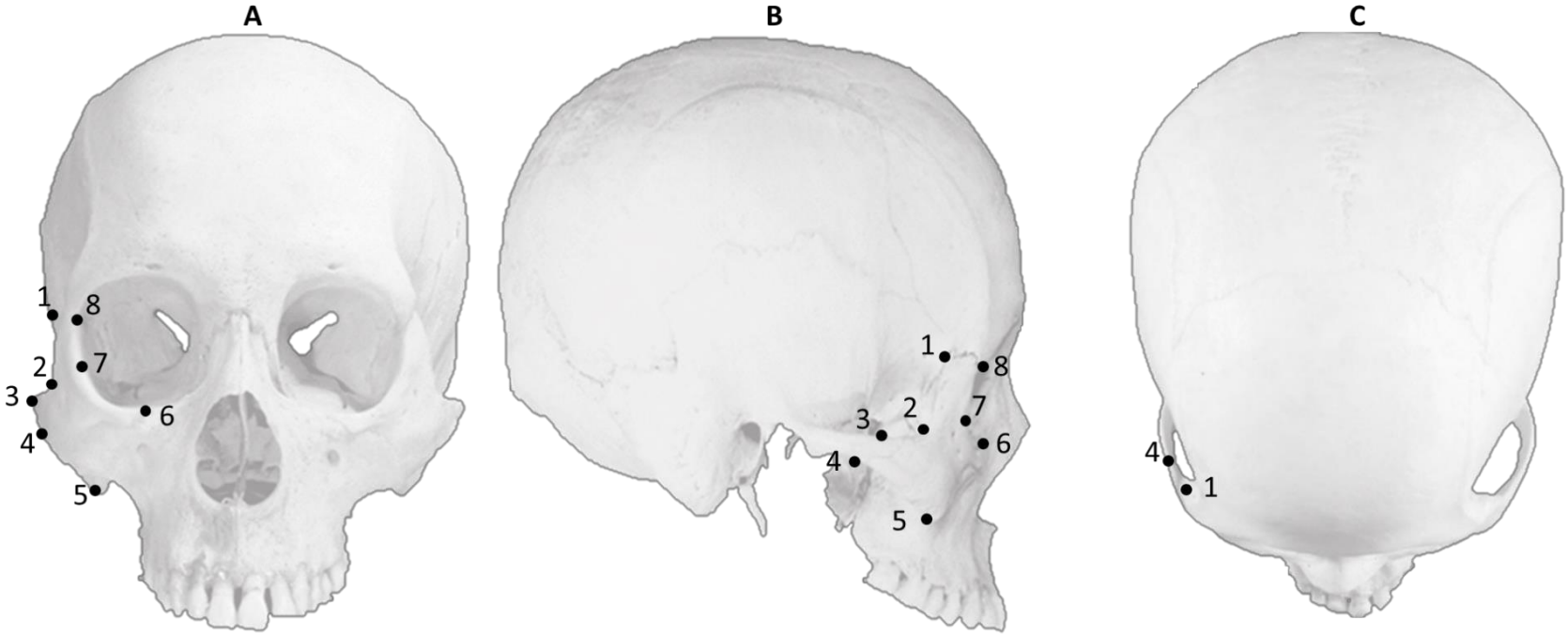

Figure 2.1: Digitised landmarks on the zygomatic bone in three views: anterior (A), lateral (B) and superior (C). Landmark names in the insert table above correspond to Table 2.1, and the features are described in Table 2.1.

[Image adopted from (eSkeletons, 2005)].

2.2.2 Data Analyses

Unless otherwise stated, sample distributions were computed in International Business Machine (IBM®) Statistical Package for the Social Sciences (SPSS®) program, version 25.0.0 (IBM Corporation, New York). GM analyses were analysed in MorphoJ (Klingenberg, 2011). A significance level (*p* value) of less than or equal to 0.05 was considered significant for all analyses.

2.2.2.1 Method Repeatability

Data were collected and assessed for inter-and intra-observer error using Procrustes ANOVA in MorphoJ version 1.06d (Klingenberg, 2011). Thirty crania were analysed by the researcher in the initial data collection phase and re-analysed two months later to test repeatability. A PhD student, proficient in the digitising technique, from the Division of Clinical Anatomy and Biological Anthropology was given the same 30 crania to assess for inter-observer error.

In GM, there are no recognised standards for defining acceptable error (Sholts *et al.*, 2011). Observer agreement was deemed unacceptable when 'error between repeats' was more than 5% of the variation, based on the percentage contribution of each factor to the total variation (Al Shahrani, 2012; Muñoz-muñoz *et al.*, 2018).

2.2.2.2 Sample Demographics

Descriptive statistics were computed for sex, ancestry and the impact of sex on ancestral groups, and a Shapiro-Wilk test for normality was used to test the distribution of continuous variables. Covariates such as age-at-death, year-of-birth and presence of maxillary teeth were investigated using two-way ANOVA tests to find any association of covariates with sex, ancestry and impact of sex on ancestral groups.

2.2.2.3 Analysis of Size and Shape

Data were examined for outliers before any analyses were performed. The data were converted to allow 3D viewing with no object symmetry required as unaffected left and right sides were digitised separately. Shape and size variation were visualised and interpreted using lollipop diagrams and wireframes. The lollipop 'circle' represents the mean shape and the stems represent the magnitude and direction of variation. The scaling factor for visualisations were noted, and caution was exercised to avoid exaggerating variation.

Generalised Procrustes Analysis (GPA) was used to remove differences due to location, orientation and scaling multiple coordinate-based configurations (Rohlf & Slice, 1990; Slice, 2007; McKeown & Schmidt, 2013) by placement onto a common coordinate system.

Centroid size is a mathematical measure independent of shape; calculated as the square root of the sum of squared distances of every landmark to their centroid (Kimmerle *et al.*, 2008). If the range of size measure is large, logarithm- transformed centroid size (log centroid size) is used (Klingenberg, 2016). Log centroid size is the independent variable in the multivariate regression (Zelditch *et al.*, 2004; Mitteroecker *et al.*, 2013). The effect of the log-transformation can stretch the scale of size for small values and shrink it for large values (Klingenberg, 2016). Since the allometric change is usually concentrated among the smaller sizes, the use of log centroid size often results in a better fit to a straight-line relationship (Klingenberg, 2016).

The effect of centroid size on shape (allometry) was then explored using a multivariate statistical test and corrected for using the residuals from multivariate regression, so that shape and size could be extracted as two separate variables for analyses.

Allometric variation

According to Klingenberg (2016), allometry (size-related variation) remains in the shape data even after Procrustes superimposition. As scaling accounts for only the isometric component of size and influence the separation of groups in multivariate analyses such as PCA and CVA (Gonzalez *et al.*, 2011; Klingenberg, 2013). Therefore, corrections were done using a multivariate regression of the Procrustes coordinates on log centroid size allowing the Procrustes residual data (size-corrected) to be retained for shape analysis (Klingenberg, 2016). To eliminate the possible effect of within-group variation; pooled within-group variances were used in the regression analysis before comparing the groups. The use of permutation tests for all the analyses were used to evaluate the significance of the regression results.

Size variation

Furthermore, the effect of log centroid size variation was assessed against the covariates age-at-death, year-of-birth and the presence of maxillary teeth, using multivariate regression analyses when pooled according to ancestry. With regards to sex and ancestral group analyses, mean and standard deviation of log centroid size were calculated.

Shape variation

Multivariate analyses such as PCA and CVA were performed for sex and ancestral groups respectively, and DFA used to investigate the accuracy of group classifications. Furthermore, multivariate regression analyses were performed for allometry regression residuals of shape against confounders (maxillary teeth present, age-at-death and year-of-birth); and the shape variation was assessed when individuals were pooled according to ancestral group.

The data set was split according to sex to explore shape variation between males and females. CVA using Procrustes residuals (size-corrected) was performed by minimising the squared distances between sexes. Each dataset (male or female) was analysed according to the variation within the three ancestral groups by investigating between-group differences relative to within-group variation from generated Mahalanobis distances. Mahalanobis distances represent the distance between individuals from one group when compared against the mean of another group. It is expressed as the standard deviation of the latter group (Klingenberg, 2011). Visualisation of variation in shape was achieved by using generated data scatterplots and wireframes. CVA was used to assess variation when there were more than two groups under investigation, whilst PCA assesses variation by maximising differences between two groups. Although CVA is like PCA, the difference lies in the axes or canonical variates maximised between-group variation rather than entire sample variation (Katzenberg & Saunders, 2007).

2.2.2.4 Ancestral Estimation

CVA using size-corrected Procrustes residuals was used to explore shape differences between ancestral groups. CVA graphs were generated with 90% confidence ellipses to assess variation based on ancestral groups and the impact of sex on ancestral groups. The resultant shapes were plotted based on the CVA graph showing the differences between the groups. A series of pairwise comparisons were conducted for the three ancestral groups and their associated Mahalanobis distances were computed to assess group similarity or dissimilarity.

Furthermore, a pairwise-DFA was used to investigate similarities between group means for classification purposes based on ancestry. These estimation accuracies were calculated from the leave-one-out-cross-validation (LOOCV) percentages. LOOCV involves the removal of one individual from the sample and recalculating the percentage accuracy using all the remaining individuals when the removed individual is treated as an unknown (Ousley *et al.*, 2009; Gillick, 2012). LOOCV provides a more realistic predicted probability estimate of the discriminant function by combatting optimistic bias and overfitting of the data (Ousley *et al.*, 2009; Krüger, L'Abbé & Stull, 2017).

2.2.2.5 Sex and Ancestry-Linked Estimation

Regression analyses were performed for allometry; to investigate the effect of log centroid size on shape when individuals were pooled according to sex. PCA were done with size- corrected Procrustes residuals to explore shape variation. PCA graphs generated had 90% confidence ellipses for variation based on sex (Paschetta *et al.*, 2010; Maass, 2016). The resultant shape

based on the allometry regression residuals were plotted to show the differences between the male and female groups with the principle component of interest highlighted.

A CVA using size-corrected Procrustes residuals, was used to explore shape differences occurring due to the impact of sex on ancestral groups (*i.e.* BA females and males, MA females and males). The DFA using LOOCV percentage estimation accuracy were used to investigate similarities between sex and impact of sex on ancestral group means for classification purposes. The highest percentage estimation accuracies of the ancestral group classification were recorded from the pairwise comparisons in the DFA analysis.

3. RESULTS

Ancestral variation in shape and size of the zygomatic bone was assessed in 3D using GM. Presence of antemortem maxillary teeth, age-at-death and year-of-birth were assessed as confounders of the effects of ancestral variation on zygomatic morphology. Shape and size were assessed separately after correcting for the effect of size on shape. Differences in zygomatic morphology between ancestral groups were investigated first, after which the impact of sex on ancestry was investigated.

In cases where there was evidence of unilateral zygomatic trauma and deformation, only the unaffected zygoma was digitised. For crania without trauma or deformation both zygomas (left and right) were digitised separately, and both sides were averaged for further analyses due to the absence of side-related shape and size differences ($p \geq 0.09$).

3.1 Method Repeatability

Repeated digitisations of zygomatic landmarks by two different observers (inter-observer and intra-observer error) produced error that accounted for less than 5% of the variation in the sample (Appendix C: Table C.1). Shape analysis in inter-observer error showed more variation possibly due to digitisation technscaklinique. Despite the variability, the technique produced good repeatability in both centroid size and Procrustes shape, therefore, these two components were analysed further.

3.2 Sample Demographics

A total of 158 individuals were sampled, with a greater number being male (61%) when compared with female (39%). Ancestral group distribution was as follows: MA (39%), EA (31%) and BA (30%).

3.2.1 Confounders

Fewer maxillary teeth were present in individuals of EA and MA when compared with BA individuals ($p \leq 0.0001$) (Table 3.1). Age-at-death was higher for those of EA ($p=0.02$) when compared with BA and MA (Table 3.1). BA and MA groups in this sample were born significantly later (mid- late 1900's) when compared with those of EA (early-mid 1900's) ($p=0.001$) (Table 3.1). Due to the disparity in year-of-birth and the small sample size in this study, the presence of a secular trend could not be reliably evaluated.

Table 3.1: Summary of median and (interquartile range) of confounders for the three ancestral groups.

	Ancestry		
Variable	**Bantu-speaking** ***n=47***	**Mixed** ***n=61***	**European** ***n= 50***
Age-at-death [Range]	40 (18) † [20-66]	45 (24) † [18-73]	62 (14) §* [28-75]
Antemortem maxillary teeth present [Range]	15 (4) *† [0-16]	6 (13) § [0-16]	0 (16) § [0-16]
Year-of-birth [Range]	1960 (37) † [1918-1991]	1951 (30) † [1914-1988]	1928 (14) §* [1911-1962]
Units for age-at-death and year-of-birth: years. *Statistically different results to the p≤0.05 level of significance with Bantu-speaking ancestry are indicated by § and those from Mixed ancestry by * and those from European ancestry indicated by †.*			

A similar trend in the distribution of confounders was noted for sex and impact of sex on ancestral groups. Fewer maxillary teeth were present in males of EA and MA when compared with BA males ($p \leq 0.0001$); similarly, EA and MA females had fewer maxillary teeth when compared to BA females (Table 3.2). Age-at-death was higher for the males (BA, MA, EA) when compared females across the ancestral groups. The youngest individual sampled in the study was 18- years-old, whilst the oldest individuals were 75-years-old (Table 3.2). The BA females were born much later than all the other groups with a year-of-birth range of 1935-1991 (Table 3.2).

Table 3.2: Summary of median and interquartile range of confounders for ancestry and sex demographics.

	Ancestry					
	Bantu-speaking ***n=47***		**Mixed** ***n=61***		**European** ***n= 50***	
Variable	**Female** n = 17	**Male** n = 30	**Female** n = 27	**Male** n =34	**Female** (n =18)	**Male** n =32
Age-at-death (years) [Range]	39 (16) † [22-50]	40 (24) † [20-66]	51 (23) [18-72]	39 (27) † [20-73]	60 (18) § [28-75]	64 (13) §* [45-75]
Antemortem maxillary teeth present [Range]	15 (4) †* [8-16]	12 (4) †* [0-16]	1 (13) § [0-16]	7 (12) § [0-16]	0 (5) § [0-6]	0 (9) § [0-12]
YOB (years) [Range]	1962 (26) †* [1935-1991]	1959 (41) † [1918-1988]	1945 (17) †§ [1914-1986]	1954 (38) † [1919-1988]	1931 (17) §* [1917-1976]	1927 (13) §* [1911-1949]
Units for age-at-death and year-of-birth: years *Statistically different results to the p≤0.05 level of significance with Bantu-speaking ancestry are indicated by § and those from Mixed ancestry by * and those from European ancestry indicated by †.* *Pairwise comparisons are computed within same sex only (i.e. in female group, compare confounders across ancestral groups). [Males are shown with green symbols, and females are shown with purple symbols]*						

3.3 Allometric Variation

A multivariate linear regression analysis of the effect of log centroid size on Procrustes shape showed a significant relationship between size and shape ($p \leq 0.0001$), which accounted for 4.8% of the shape variation in the zygoma. Therefore, as the size increased, it resulted in the following shape changes: an anteroposterior elongation of the zygoma, with mediolateral migration of landmarks around the cheek bone, and more anteriorly projecting orbital margins (Figure 3.1 and Figure 3.2).

3.4 Size Variation

Due to the limitations of log centroid size as a three-dimensional size measurement, ancestry could not be estimated from size.

3.4.1 Confounding Factors

No relationship between log centroid size and the presence of antemortem maxillary teeth (p=0.15), age-at-death (p=0.40), or year-of-birth (p=0.36) were observed. These confounders accounted for less than 2% of the variation in zygomatic size.

3.4.2 Ancestry Variation

Individuals of BA had the largest mean centroid sizes (60.1 ± 3.4mm) followed by those of EA (58.5 ± 3.8mm) and those of MA (58.1 ± 3.3mm) (Appendix C: Table C.2). However, significant size differences were only detected between MA and BA groups (p=0.01), suggesting that individuals of EA are more similar in size to people of both MA (p=1) and BA (p=0.09) (Figure 3.3).

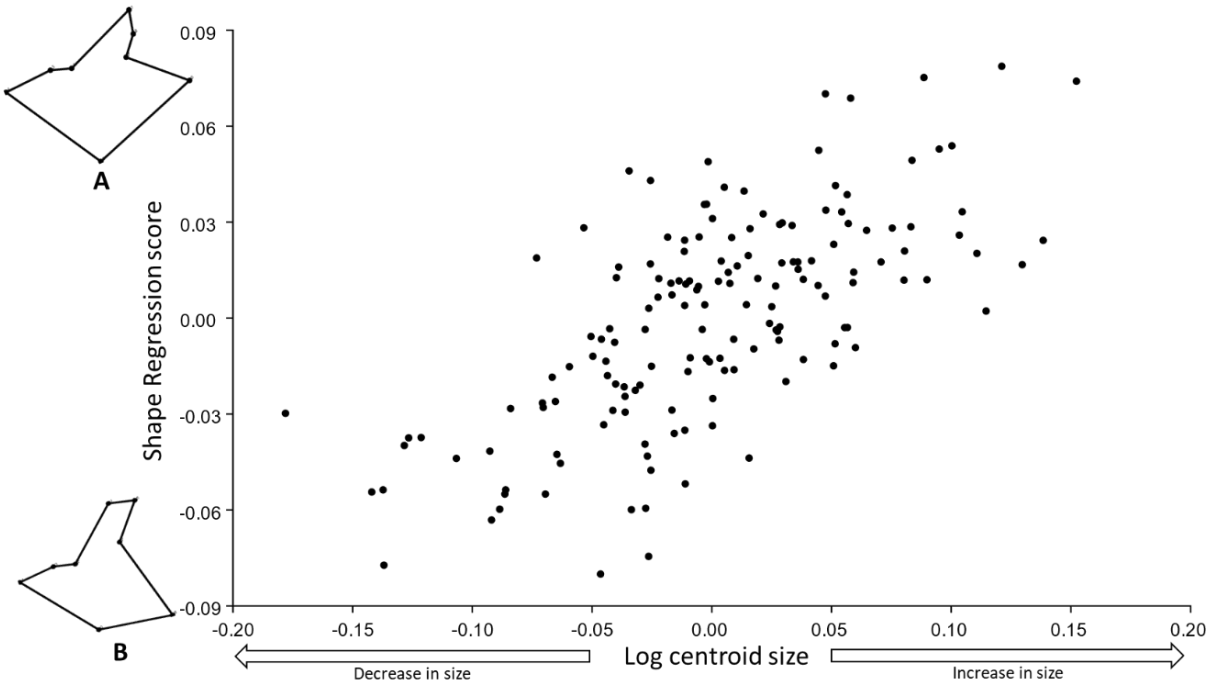

Figure 3.1: Multivariate linear regression analyses of the relationship between Procrustes shape and log centroid size. Wireframes A and B show shape changes associated with positive and negative regression scores, respectively.

[Scale factor: 0.5].
[Scaling is for visualisation and may result in unnatural distortion].

3.4.3 Sex and Ancestry-Linked Variation

Males had larger zygomatic sizes (60.3 ± 3.1mm) than females (56.5 ± 2.9mm) (p<0.0001) (Figure 3.3, and Appendix C: Table C.2). When assessed relative to ancestral groups, no size differences were observed between the males (p=1) across ancestral groups and females (p>0.09) from different ancestral groups. This suggests that the size differences observed were primarily due to sexual dimorphism and not ancestral variation (Figure 3.4).

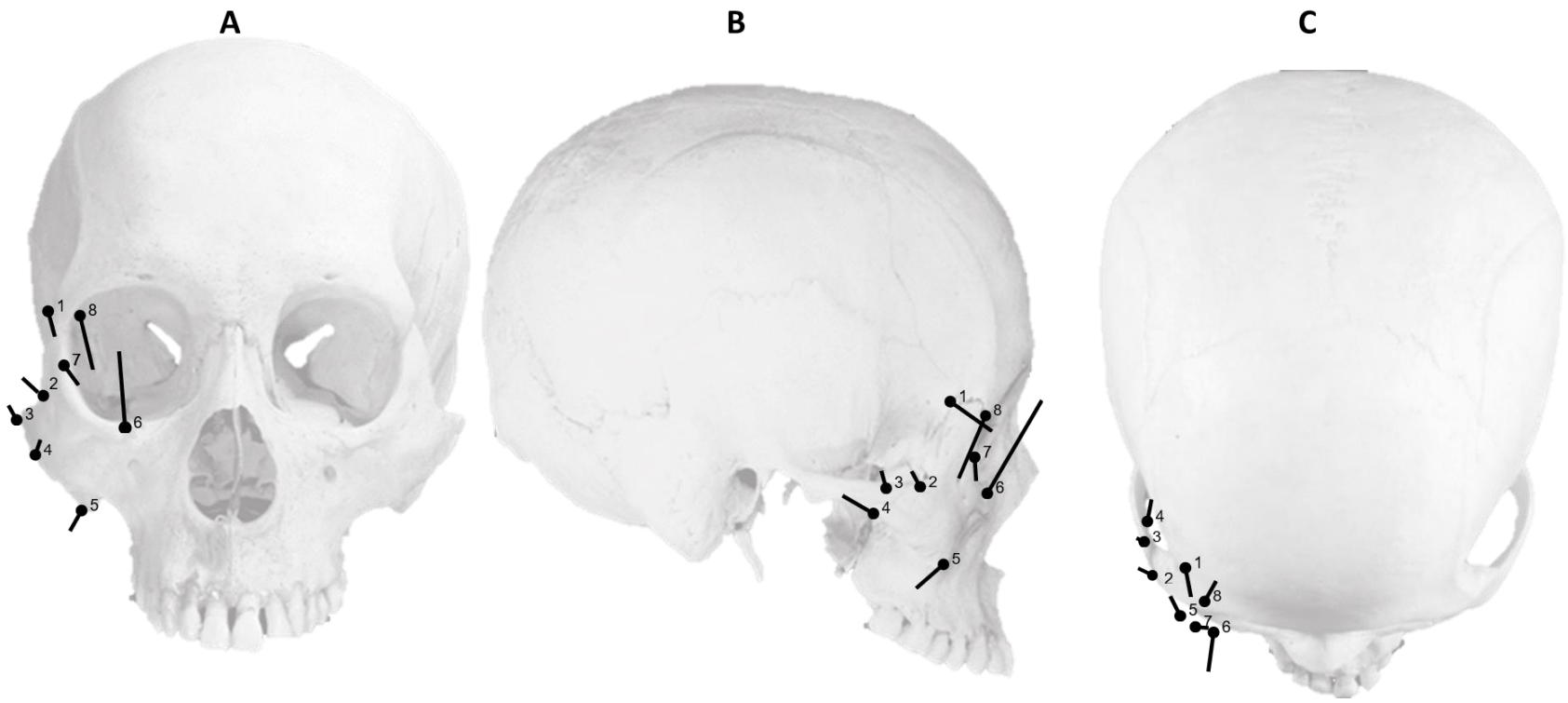

Figure 3.2: Shape variation associated with log centroid size. Dots represent the average shape and stems represent the magnitude and direction of shape variation when size increases. Views of shape change include: A. Anterior; B. Lateral and C. Superior.

[Scale factor: 0.15].
[Scaling is for visualisation and may result in unnatural distortion].
[Image of cranium adapted (eSkeletons, 2005)].

3.5 Shape Variation

To mitigate the effect of log centroid size on shape, size-corrected Procrustes-residuals from the multivariate linear regression analysis of shape and size were used in analyses of shape variation.

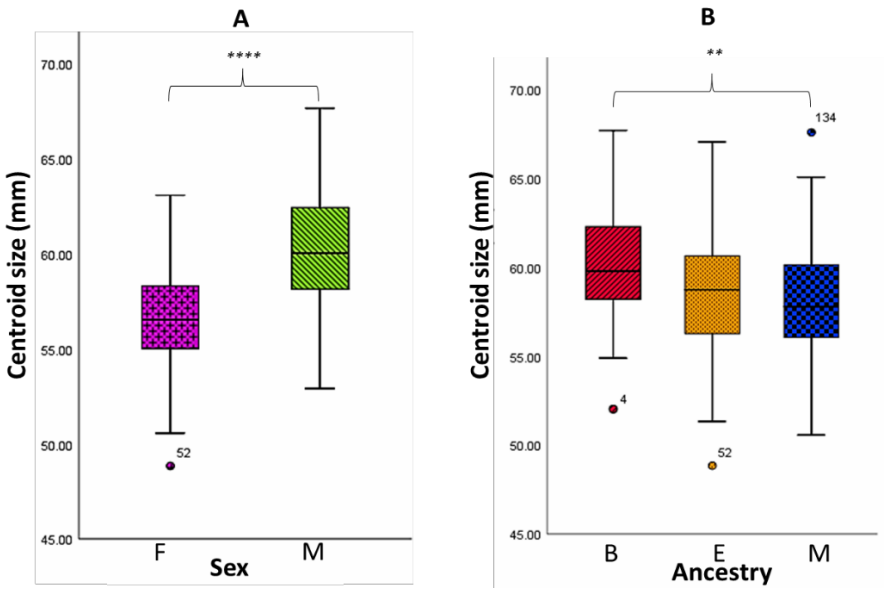

Figure 3.3: Box plots of centroid size mean and standard deviation of the zygomatic bone for sex (A) and ancestry (B). All measurements were taken in mm. Samples were annotated accordingly: F= female, M= male; B=Bantu-speaking Ancestry, E= European Ancestry and M= Mixed Ancestry. Significant values are indicated in asterisks (*): *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

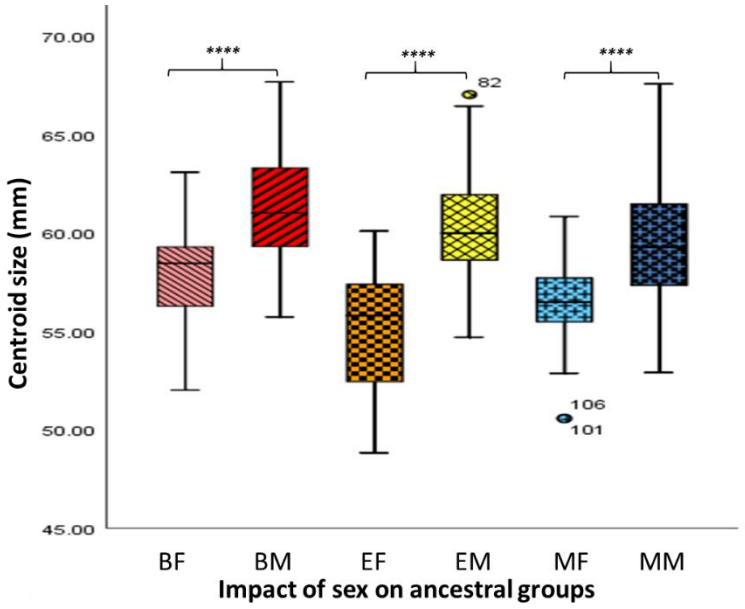

Figure 3.4: Box plots of centroid size mean and standard deviation of zygomatic bone for the impact of sex on ancestral groups. All measurements were taken in mm. Samples were annotated accordingly F= female, M= male; B=Bantu-speaking Ancestry, E= European Ancestry and M= Mixed Ancestry. Significance bars show differences between males and females within ancestral groups (BA, EA and MA). Significant values are indicated in asterisks (*): *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

3.5.1 Confounding Factors

Presence of maxillary teeth

A significant relationship was detected between zygomatic shape and the presence of maxillary teeth (p=0.004). This relationship accounted for 1.68% of the shape variation (Figure 3.5). Individuals with fewer antemortem maxillary teeth present had slightly more negative regression scores with posteriorly located orbital regions of the zygoma (landmarks 7 and 8), narrower zygomatic arches (superior-medial migration of landmarks 4 and 6) and more compressed, shortened zygomatic bones (superior and inferior migration of landmarks 1 and 5) (Figure 3.6). Notably, more individuals of EA and MA had negative regression scores, and it is possible that shape variation associated with tooth loss may be influenced by ancestral variation in these groups. Additionally, differences in tooth loss may also impact assessments of ancestral variation and this will remain a consideration when interpreting variation in zygomatic shape.

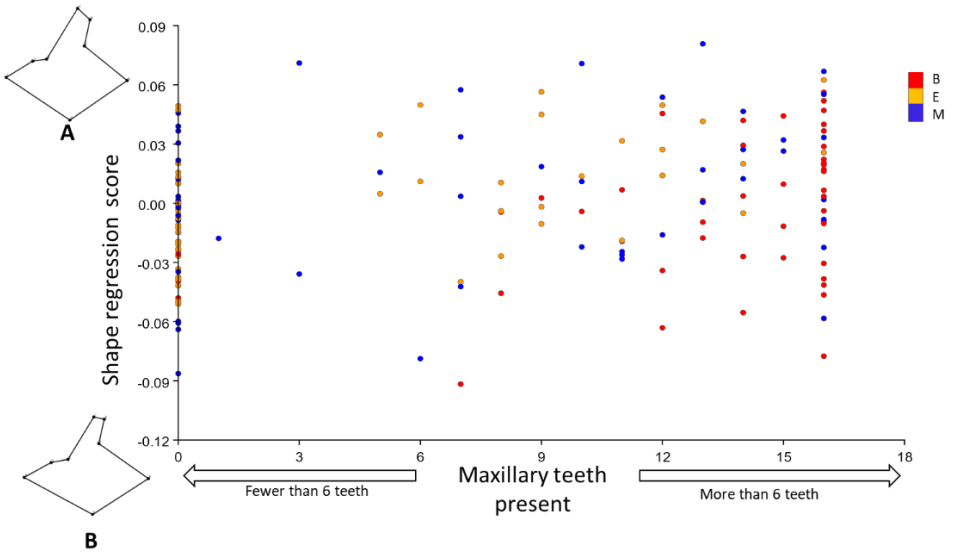

Figure 3.5: Multivariate regression analysis to assess the relationship between antemortem maxillary teeth present and zygomatic shape, with variances pooled by ancestry. Wireframe A shows the average shape when more maxillary teeth are present, and B shows average shape when fewer teeth are present. Positive maxillary teeth regression scores (associated with more teeth present) and negative regression scores (associated with fewer teeth present). Colours represent BA (red), EA (orange) and MA (blue).

[Scale factor: 40].

[Scaling is for visualisation and may result in unnatural distortion].

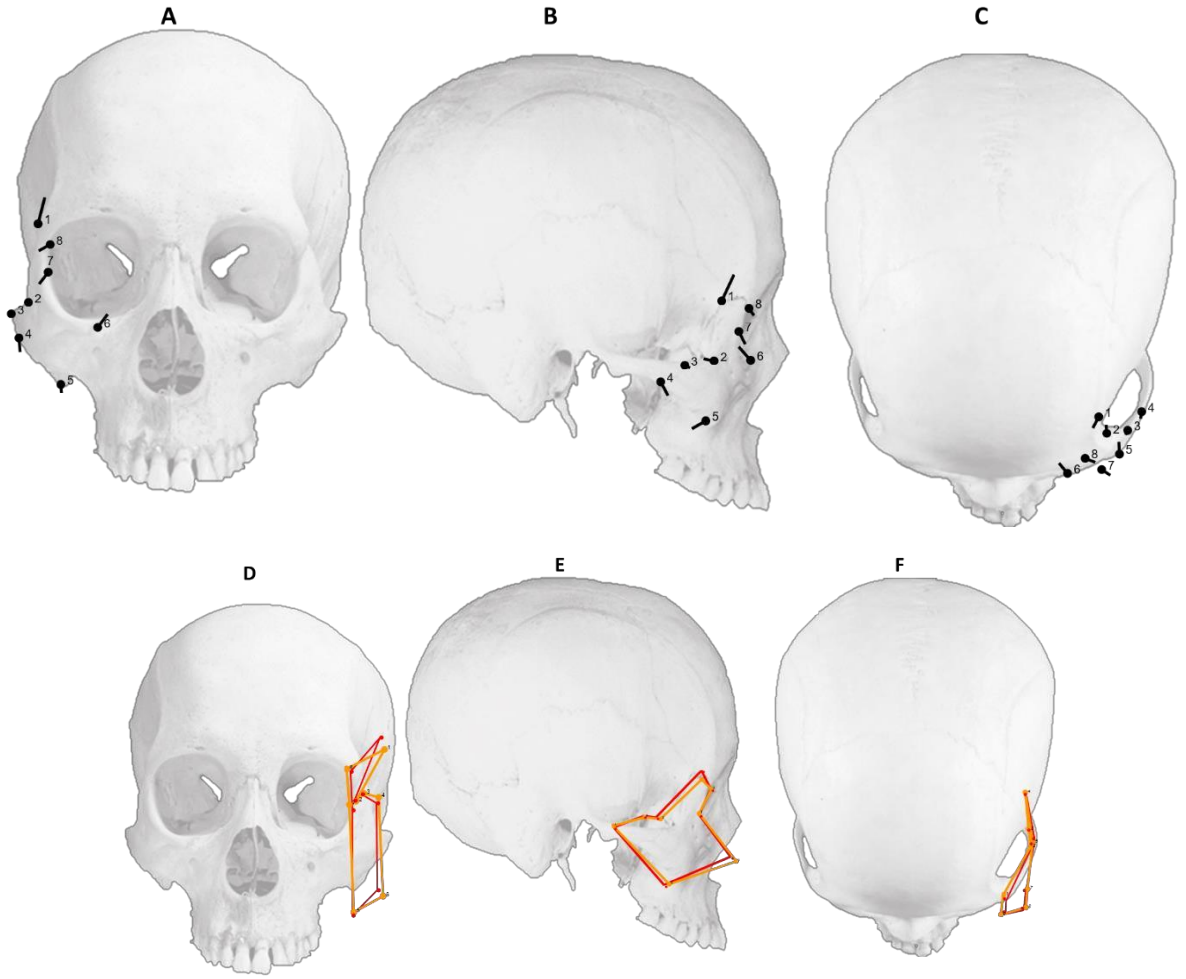

Figure 3.6: Zygomatic shape variation associated with antemortem maxillary teeth present, with variances pooled by ancestry. A-C: Dots represent the average shape when fewer maxillary teeth are present and stems represent the magnitude and direction of variation when there are more maxillary teeth present. Wireframes D-F: The red shape is associated with more teeth present in Bantu-speaking individuals and the orange wireframe shows when fewer maxillary teeth are present in European individuals. Views of shape change from include: A, D. Anterior; B, E. Lateral and C, F. Superior.

[Scale factor: 40].
[Scaling is for visualisation and may result in unnatural distortion].
[Image adopted from (eSkeletons, 2005)].

Age-at-death

A significant relationship was detected between shape and age-at-death (p=0.001) (Figure 3.7), and age-at-death accounted for 2% of the shape variation. The effect of ageing on zygomatic shape was the same for all ancestral groups. Older individuals had more positive regression scores, associated with receding lateral and inferior orbital margins (posterior and inferior landmarks 7 and 8), slightly narrower zygomatic arches (medial migration of landmarks 4 and 6) and more shortened and compressed zygomatic heights (inferior and superior migration of landmark 1 and 5, respectively) (Figure 3.8). Although, it is unlikely that the effect of age differs among the populations, this was not tested statistically

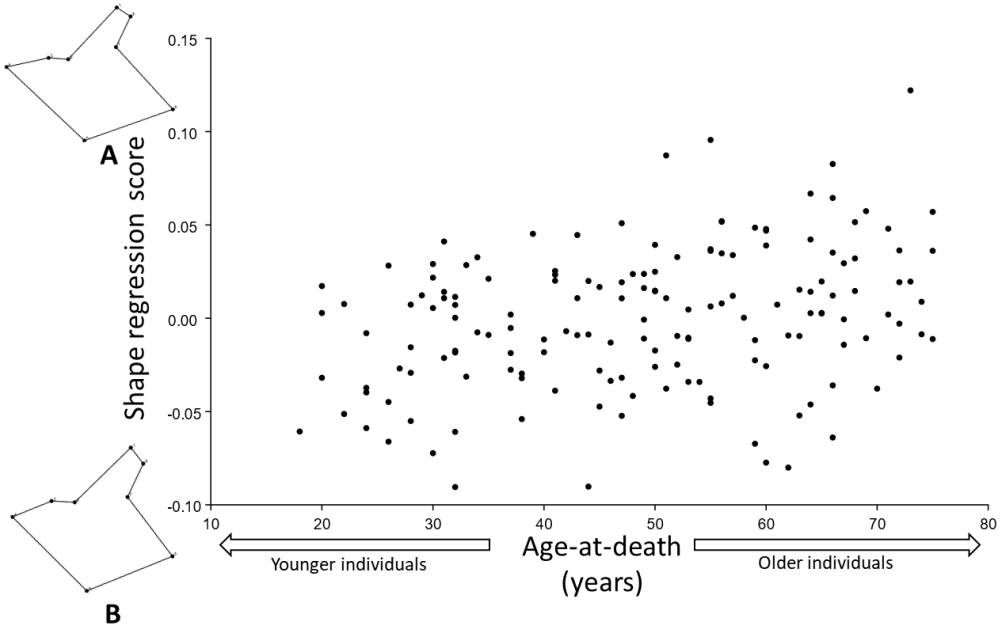

Figure 3.7: Multivariate regression analysis to assess the relationship between age-at-death and zygomatic shape, with variances pooled by ancestry. Positive age-at-death regression scores (associated with older individuals) and negative regression scores (associated with younger individuals). Wireframes A and B show the average shape changes associated with positive and negative regression scores, respectively.

[Scale factor: 40].

[Scaling is for visualisation and may result in unnatural distortion].

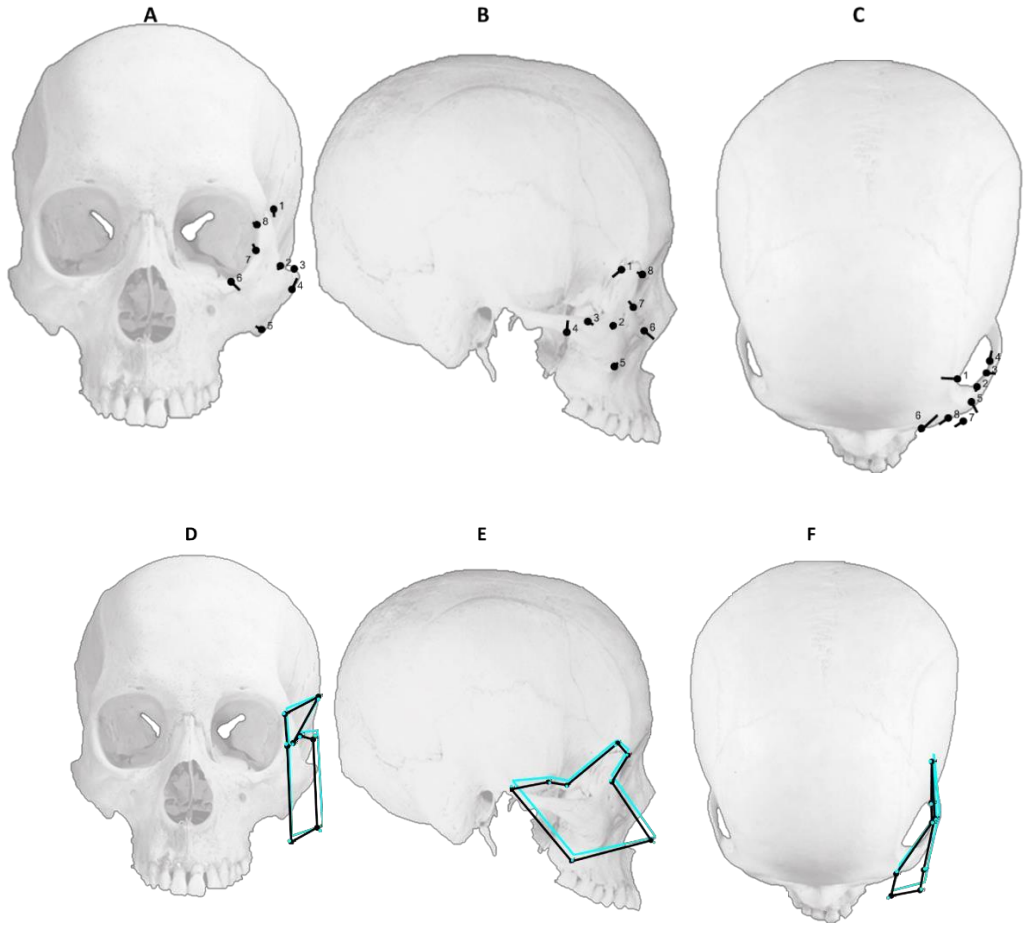

Figure 3.8: Zygomatic shape variation associated with age-at-death, with variances pooled by ancestry. A-C: Dots represent the average shape for younger individuals and stems represent the magnitude and direction of variation for older individuals. Wireframe D-F: The cyan blue shape is associated with older individuals and the black wireframe shows the zygomatic shape for younger individuals. Views of shape change include: A, D. Anterior; B, E. Lateral and C, F. Superior.

[Scale factor: 40].

[Scaling is for visualisation and may result in unnatural distortion].

[Image adopted from (eSkeletons, 2005)].

3.5.2 Ancestry Variation

Variation in shape between ancestral groups was analysed using CVA. CV1 and CV2 accounted for 80% and 20% of the observed variance between ancestral groups. On CV1 individuals of EA separated from both MA and BA groups who largely clustered together. On CV2, slight separation was noted between BA and MA groups. Significant shape differences were observed between the BA and EA groups (Mahalanobis distance: 2.30, p<0.0001); BA and MA groups (Mahalanobis distance: 2.02, p<0.0001) and EA and MA groups (Mahalanobis distance: 1.21, p=0.001). Regardless, large overlap in confidence ellipses between ancestral groups suggests the zygoma may yield low ancestry estimation accuracies.

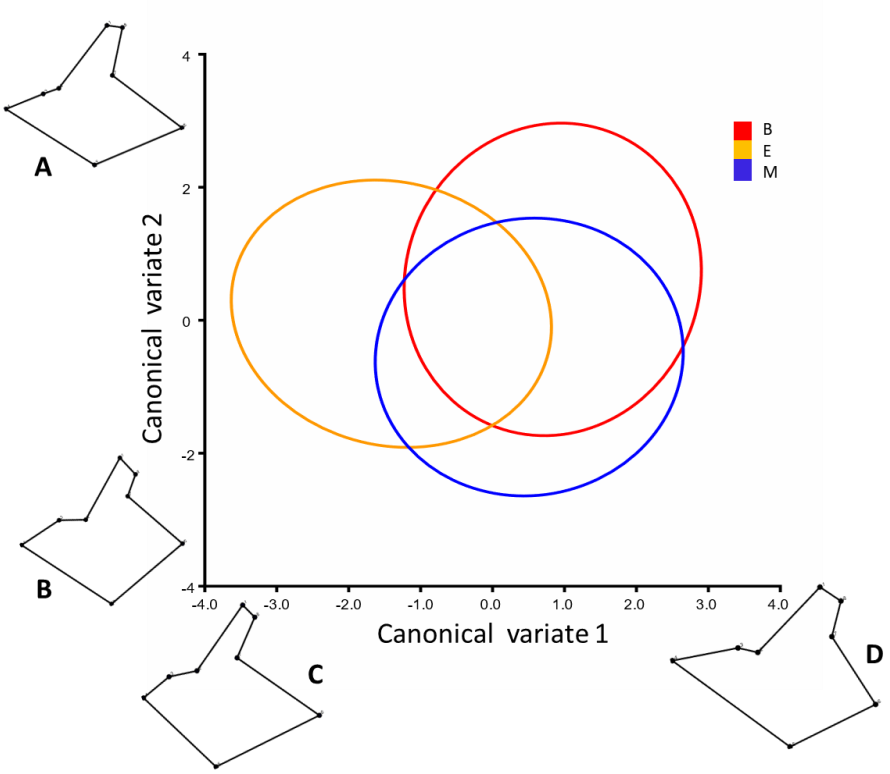

Figure 3.9: Scatter plot for variation on canonical variate 1 and 2 showing separation between ancestral groups, depicted using 95% confidence ellipses. Wireframes A-D show the average shape changes associated with positive and negative canonical variate scores. Ancestral groups include**:** BA (red), EA (orange), and MA (blue).
[Scale factor: 5].
[Scaling is for visualisation and may result in unnatural distortion].

Observed shape differences

On CV1, individuals of EA separated from those of BA and MA (Figure 3.9). The BA and MA groups exhibited anterior and inferior projecting orbital regions (landmarks 7 and 8), slightly narrower zygomatic arches (landmark 4), superior and inferior reduction in height of the zygomas (landmark 1 and 5) (Figure 3.9). Therefore, BA and MA groups had anteriorly projecting and shorter zygomatic shape. Contrary to this, the EA group exhibited anteromedial

and inferior orbital components of the zygoma, slightly wider zygomatic arches (landmark 4) and elongated zygomatic height (landmark 1 and 5) (Figure 3.9). Thus, the EA group had a receded orbital region with wider and vertically elongated zygoma.

On CV2, individuals of MA separated from BA (Figure 3.9). The BA group exhibited anterior projecting orbital component of the zygoma (landmarks 6 and 8), slight narrowing due to inferior and lateral migration of landmark 6, superior and inferior reduction in height of the zygomas (landmark 1 and 5) (Figure 3.11). Contrary to this, the MA group had posterior and superior orbital components of the zygoma (landmarks 6 and 8), superior and inferior elongation (landmarks 1 and 5) and wider zygomatic arches (landmarks 4 and 6) (Figure 3.11). Therefore, the MA group mirrored the EA group, whilst the BA group had more anteriorly projecting orbital region with a narrower and shorter zygoma (Figure 3.11).

3.5.3 Sex and Ancestry-Linked Variation

Variation in shape between sex and the interaction of sex and ancestry were analysed by PCA and CVA of size-corrected Procrustes residuals, respectively. This PCA produced 17 principal components, with the first 13 components accounting for 97% of the observed variation. There appeared to be a separation of males and females detected only on principal component 10 (Figure 3.12) and principal component 13 (Figure 3.14). DFA results showed that females were accurately sexed 50% of the time, and males were accurately sexed 60% of the time

The same shape changes observed when assessing for ancestral differences, were also observed when the ancestral groups were split according to sex (Appendix C, Figure C.2). The BA and MA groups had anteriorly projecting and shorter zygomatic shape whilst the EA group had a receded orbital region with wider and vertically elongated zygoma.

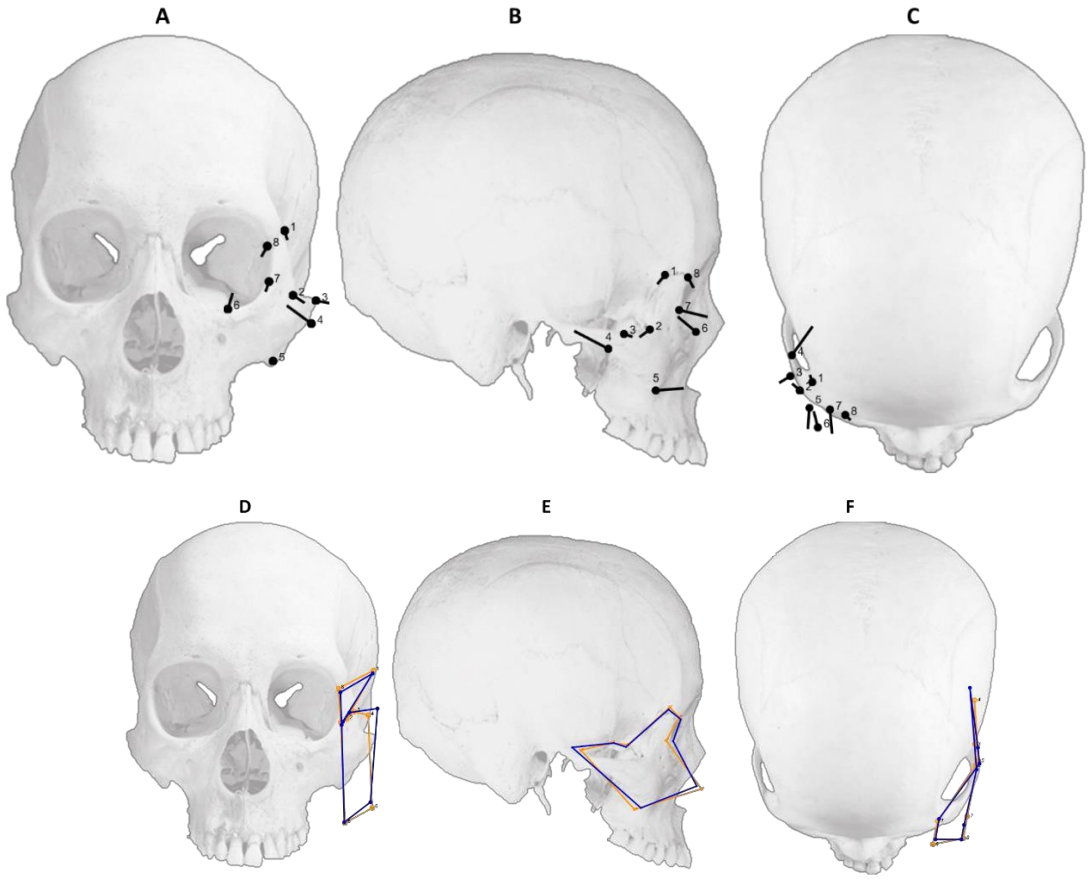

Figure 3.10: Ancestral shape variation on canonical variate 1. A-C: Dots represent the average shape for EA and stems represent the magnitude and direction of variation for BA and MA individuals. Wireframes D-F: The blue wireframe shows the average shape for MA and BA individuals and the orange wireframe shows the average shape for EA individuals. Views of shape change from CVA include: A. D Anterior; B. E Lateral and C. F Superior.

[Scale factor: 5].
[Scaling is for visualisation and may result in unnatural distortion].

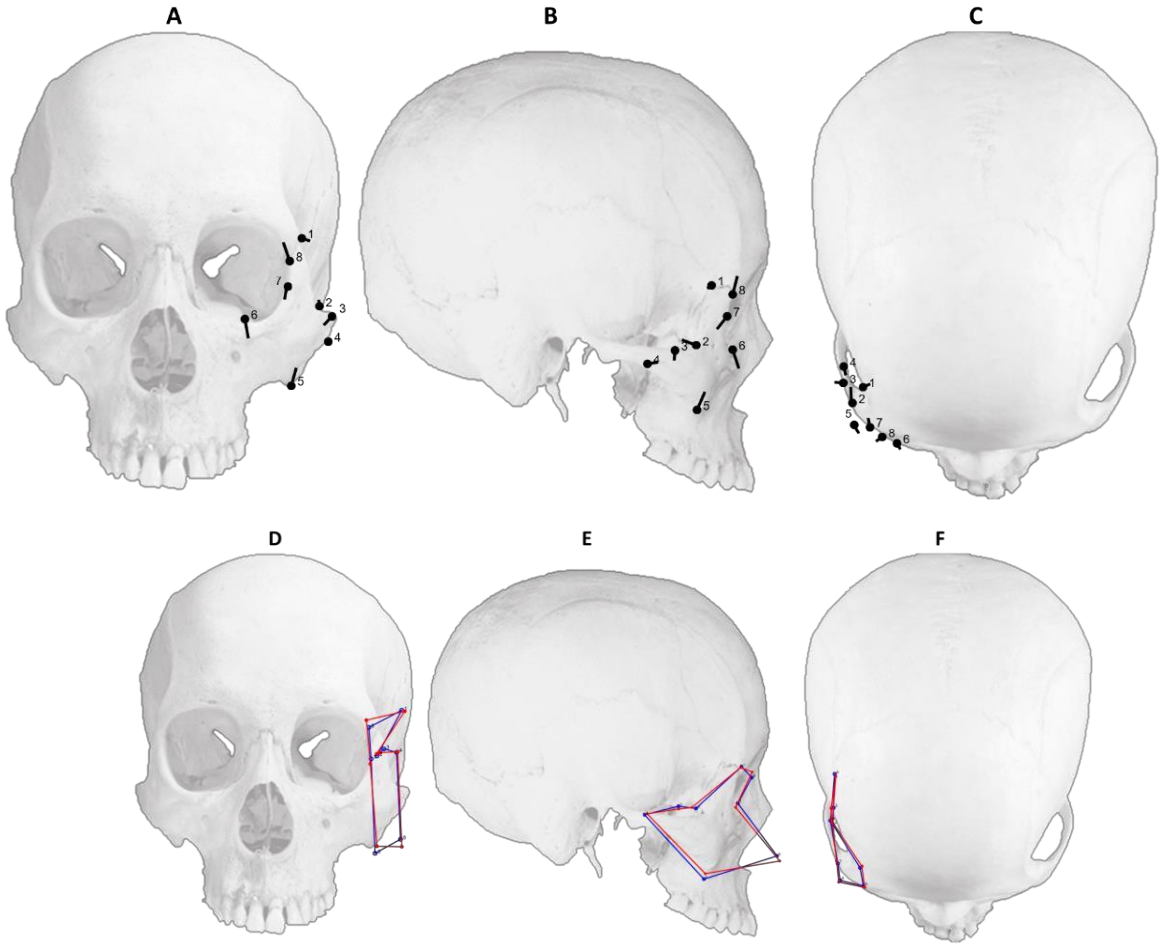

Figure 3.11: Ancestral shape variation on canonical variate 2, when pooled according to ancestry. A-C: Dots represent the average shape in MA individuals and stems represent the magnitude and direction of variation in BA. Wireframe D-F: The blue wireframe shows the average shape for MA individuals and the red wireframe shows the average shape for BA individuals. Views of shape change analysis include: A. D Anterior; B. E Lateral and C. F Superior.

[Scale factor : 5].

[Scaling is for visualisation and may result in unnatural distortion].

On principal component 10, males exhibited more positive principal component scores than females (Figure 3.12), while on principal component 13 females exhibited more positive scores (Figure 3.14). Regardless, similar shape differences were observed in both principal components. Males exhibited more compressed zygomatic heights (landmarks 1 and 5), less flared and narrow zygotemporale (medial migration of landmarks 4 and 6) and more anteriorly projecting orbital region (landmarks 6 and 8) and antero-posteriorly elongated zygomas (landmarks 4 and 6) (Figure 3.13). Contrary to this, females exhibited wider and more flared zygomas (lateral migration of landmark 3), with slight steeply-angled zygomatic arch landmarks (3 and 4) (Appendix C, Figure C.1). Overall, the zygomatic bone appeared slightly shorter and narrower in males than in females, who exhibited wider and more elongated zygomatic bones. However, the overlapping regions in the confidence ellipses between males and females (Figure 4.12, Figure 4.14) indicated low levels of sexual dimorphism in zygomatic shape.

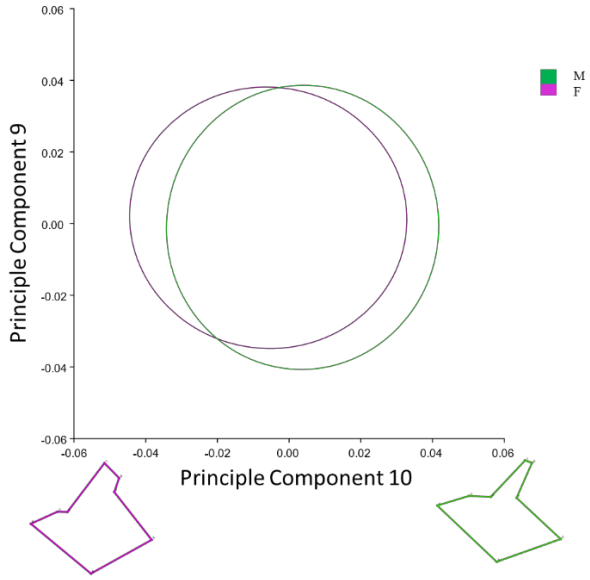

Figure 3.12: Scatter plot for principle component 10 showing slight separation between males (green) and female (purple), depicted using 95% confidence ellipses. Shape changes towards the positive principle component are inclined towards the male shape, whilst, the negative principle component scores are inclined towards the female shape.

[Scaling may result in unnatural distortion]

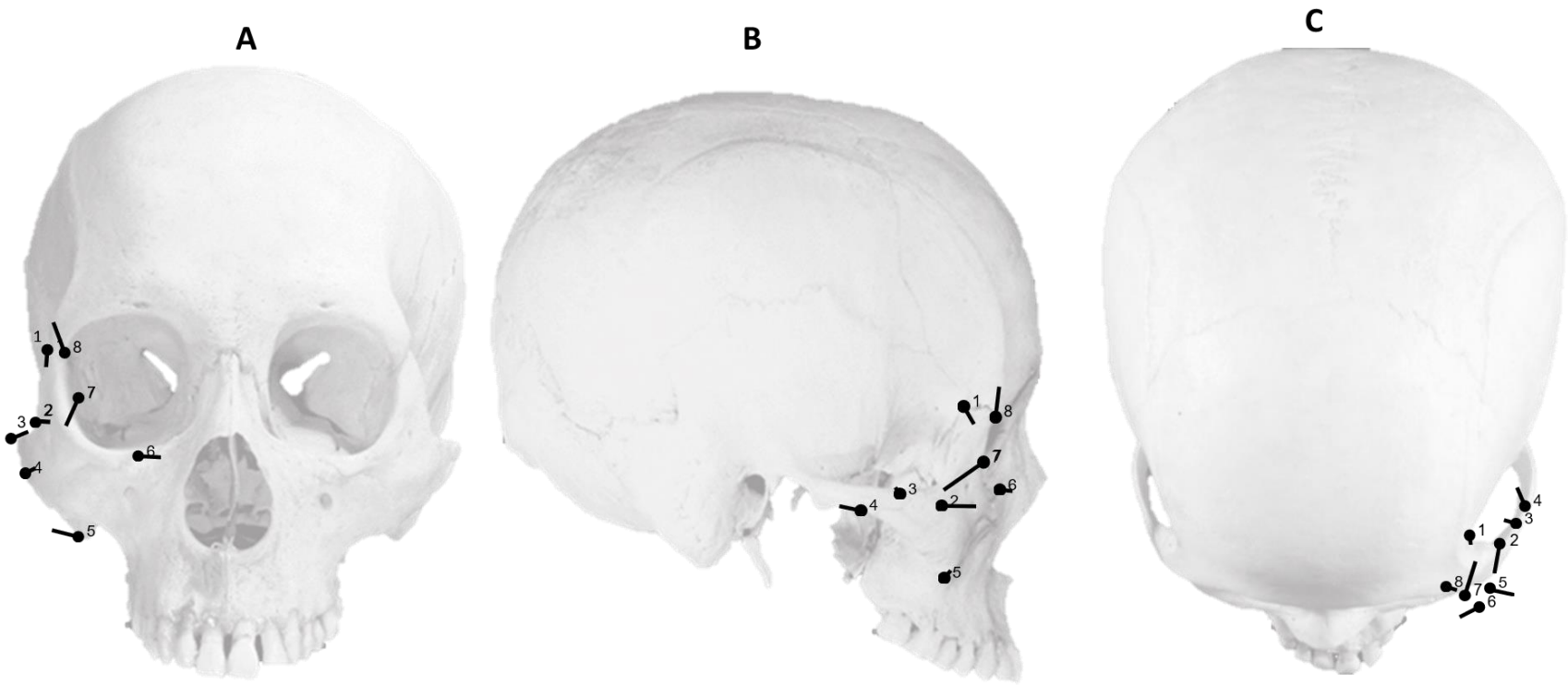

Figure 3.13: Shape variation on principle component 10 for male and female individuals, when pooled according to sex. Dots represent the average shape in females and stems represent the magnitude and direction of variation in males. Views of shape change from include: A. Anterior; B. Lateral and C. Superior.

[Scale factor: 0.1].

[Scaling is for visualisation and may result in unnatural distortion].

[Image adopted from (eSkeletons, 2005)].

The overall shape in males is similar in both principal components, 10 and 13 for males (Appendix C, Figure C.1), except for the following: wider flaring (posterior and lateral migration of landmarks 3 and 5), and posterior and medial migration of the orbital region (landmarks 7 and 8). Thus, males have a shorter zygoma, with compressed orbital region and wide flare in the zygomatic arch.

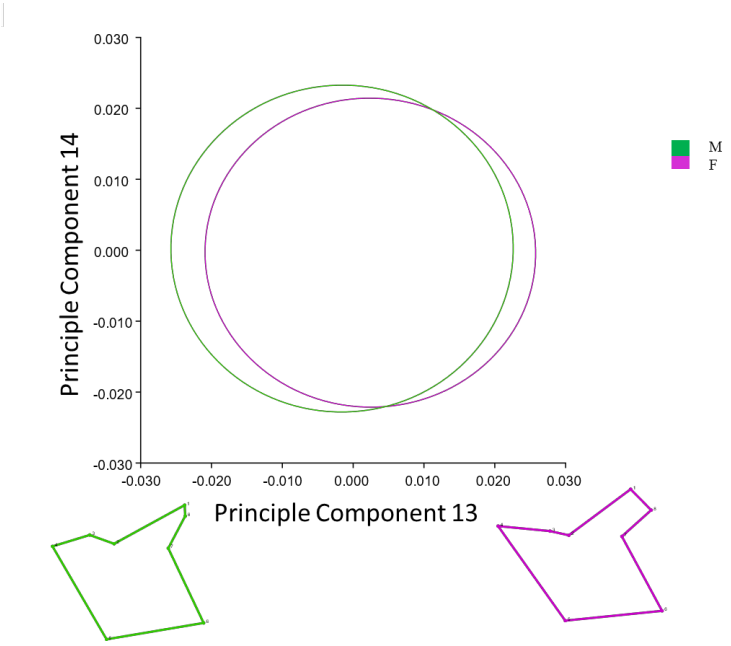

Figure 3.14: Scatter plot for principle component 13 showing slight separation between males (green) and females (purple), depicted using 95% confidence ellipses. Shape changes towards the positive principle component are inclined towards the female shape, whilst, the negative principle component score was inclined towards the male shape.

[Scaling is for visualisation and may result in unnatural distortion].

3.6 Ancestry Estimation Accuracies

Ancestry estimation

A pairwise DFA was used to estimate ancestry from zygomatic shape. The greatest classification accuracy was between the BA (81%) and EA (84%) individuals. Ancestry estimation accuracy between the other group comparisons were; EA (68%) and MA (80%) individuals, and BA (60%) and MA (66%). According to Mahalanobis distances, individuals of EA had the most distinct zygomatic shape when compared with BA, whilst MA was more distinguishable from EA individuals (Table 3.3). Additionally, the BA and MA groups were highly similar in shape, as indicated by the poorer ancestry estimation accuracies produced when comparing these two groups (Table 3.3).

Table 3.3 Summary of the highest ancestry estimation accuracies from pairwise comparisons using leave-one-out cross validations of classification accuracies.

Group 1	**Group 2**	**Group 1 Classified correctly (%)**	**Group 2 Classified correctly (%)**	**Mean Mahalanobis distance**	***p value***
Bantu-speaking Ancestry	European Ancestry	**81**	**84**	2.42	*(p≤0.0001)*
Bantu-speaking Ancestry	Mixed Ancestry	60	66	1.19	*(p≤0.05)*
European Ancestry	Mixed Ancestry	68	**80**	2.01	*(p≤0.0001)*

Bold values *represent the highest ancestry estimation ancestries for each ancestry.*
Mahalanobis distance represent the magnitude of variation between individuals from one group when compared against the mean of another group.

Sex and ancestry-linked estimation accuracies

Impact of sex on ancestral groups was investigated using CVA residuals from Procrustes shape to investigate how knowing sex prior to assessing ancestry, may impact ancestry estimation. Statistically significant shape differences in females between ancestry was represented by 59% of the variation (p<0.0001). In males, 84% of shape variation, with statistically significant differences occurring between EA and both MA and BA individuals (p<0.0001, Appendix C, Table C.3). No significant shape differences were observed between BA and MA males (p>0.1, Appendix C, Table C.3).

When accounting for sex of the individuals, males showed better ancestral estimation accuracy when compared with females. Ancestral estimation accuracy for females produced the greatest accuracy between BA (76%) and EA (72%), BA (71%) and MA (59%) and EA (72%) and MA (63%) (Appendix C, Table C.4). In males, ancestral estimation accuracies produced the greatest accuracy between BA (77%) and EA (81%), EA (72%) and MA (82%), and BA (53%) and MA (59%) (Appendix C, Table C.4). Lower ancestral estimation accuracy was obtained when classifying the BA and MA individuals for either sex, and this suggests possible similarities in the shape of the zygoma in these groups. While the zygomatic bone has shown less sexual dimorphism in terms of zygomatic shape, it appears that ancestry can be estimated more accurately when sex is known prior to the DFA (Appendix C, Table C.4).

3.7 Summary

Confounders impacted zygomatic shape and not size. The low variation associated with tooth loss and the similar effect of ageing across the ancestral groups, suggests that the associated shape changes were likely due to ancestral variation. Zygomatic bone size was larger in males than females, therefore, the differences in size were largely due to sexual dimorphism and not ancestral variation. There was a significant size difference between the BA and EA groups. BA and MA individuals had more shared similarities in shape of the zygoma (anteriorly projecting orbital region with a narrower and shorter zygomatic bone) when compared with the differences shared between individuals of EA (receding orbital margins, vertically elongated zygomas with wider zygomatic arches). The MA group displayed similarities with features expressed by both the EA and BA individuals. Low levels of shape differences between males and females showed that the zygomatic shape has low sexual dimorphism. Therefore, zygomatic bone size may be better for sex estimation, whilst zygomatic bone shape is more accurate in ancestral estimation. Furthermore, ancestry estimation is more accurate when sex is known prior to the ancestry estimation.

4. DISCUSSION

The present study sought to assess shape and size variation in the zygomatic bone using the GM method. Findings suggested that zygomatic shape differences were most evident between ancestral groups, whilst zygomatic size differences were exclusively observed between males and females. The most notable zygomatic shape differences were observed in EA individuals, who separated from both BA and MA individuals. Males had significantly larger zygomatic size when compared with females regardless of ancestry. Considering the genetic and historical composition of SA population groups, the zygomatic bone was evaluated for accuracy in ancestry estimation. The results from discriminant functions agreed with the distribution of shape (in PCA/CVA plots) as EA individuals produced higher ancestry estimation accuracy when compared with BA individuals. Ancestry estimation was less accurate when BA individuals were compared with MA individuals. This study also examined confounding factors that may impact zygomatic morphology and showed that they had a minor impact on zygomatic shape variation and did not affect zygomatic size. Possible explanations for the zygomatic shape and size variation are discussed below.

4.1 Allometric Variation

In biological studies, it is often a challenge to state whether observed variation is entirely size-related, shape-related, or if there is an influence of size on shape, termed allometry (Slice, 2007; Mitteroecker & Gunz, 2009; Klingenberg, 2016; Dedouit *et al.*, 2017). It is important when extracting shape information that allometry is corrected for as not doing so may lead to erroneous deductions about shape variation. Fortunately, the effect of allometry can be corrected for using established statistical GM methods, which allow for independent assessment of size and shape (Mitteroecker *et al.*, 2013; Klingenberg, 2016).

In the current study, a significant linear relationship between size and shape was observed (*i.e.* as zygomatic size increased, zygomatic shape changed in various dimensions). An increase in zygomatic size resulted in the following zygomatic shape changes: anteroposterior elongation, mediolateral migration of landmarks and anteriorly projecting zygomas. However, the observed shape changes in the zygomatic bone were neither uniform nor consistent for every landmark. There was variable magnitude of change in the landmarks, with greater landmark changes observed in zygoorbitale, frontomalare orbitale, frontomalare temporalis and zygomaxilla (Slice, 2007; Mitteroecker & Gunz, 2009; Klingenberg, 2016). This shows that if size is not corrected for, the variation in those landmarks would have been

exaggerated. The ability to attain this depth in the analyses is only achievable when one employs the GM technique.

4.2 Zygomatic variation and its impact on ancestry

4.2.1 Zygomatic shape variation

Statistically significant differences between ancestral groups were observed in zygomatic shape and not in size. Zygomatic shape differences may be the result of intrinsic factors (genetic difference) between ancestral groups. Individuals of EA separated from both BA and MA individuals. Ancestral variation in the shape of the zygomatic bone produced ancestry estimation accuracies between the groups as follows: EA (84%) and BA (81%), MA (80%) and EA (68%) individuals. It was however, unreliable to distinguish between BA (60%) and MA (66%) individuals. The most accurate estimations were between individuals of EA and BA, based on landmark differences. This correlated to a study by Gillick (2012), who found differences between sub-Saharan African or 'black American' origin or European or 'white American' individuals. Similarly, GM was used to assess variation across cranial landmarks with 3D imaging and findings proposed that European individuals could be distinguished from sub-Saharan individuals (Gillick, 2012; Stull *et al.*, 2014).

BA individuals had more anteriorly projecting orbital margins on the zygoma, with narrower zygomatic arches and smaller zygomatic heights. This was also noted by Xing and colleagues (2013), who found BA individuals had a shorter orbital height and anteriorly projecting zygomas. Conversely, the isolated zygomatic bone shape similarity between MA and EA showed vertical elongation of the zygoma and correlated to findings by Stull and associates (2011), though the similarities observed were assessed on the entire craniofacial region. Therefore, this present study showed the potential in distinguishing ability of the isolated zygomatic bone in a uniquely admixed population. The observed variation in zygomatic bone shape between MA, BA and EA individuals showed evidence of the intra- and-inter-continental contribution from different ancestral backgrounds within the SA context (African, Asian and European) (Patterson *et al.,* 2009; Petersen *et al.,* 2013; Montinaro *et al.,* 2017).

In the present study, people of EA had receded orbital regions and vertically elongated zygoma, suggesting they originated from a colder climate in agreement to Maddux and Butaric (2017). Climatic adaptation has been suggested to explain why certain shape changes occur in the zygomatic region (Bernal *et al.,* 2006; Maddux & Butaric, 2017; Oettlé *et al.*, 2017). Considering the attachment of the masseter muscle on the inferior part of the zygomatic arch

and zygomatic bone, it has been suggested that the zygomatic bone is likely strongly influenced by the strain of the masseter muscle and stresses resulting from mastication (Witzel and Preuschoft, 2002). As the temporalis muscle partly attaches to the zygomatic arch, the temporalis muscle runs deep to the arch, the size of the temporalis muscle may be responsible for the shape variation in the zygomatic bone (Oettlé *et al.*, 2017). Maddux and Butaric (2017) suggested that individuals from colder climatic regions have a larger maxillary sinus, reduced nasal size, and an increased zygomatic height and maxillary height as thermoregulatory adaptations (Maddux & Butaric, 2017). Considering the colonial history of SA, EA individuals are descendants of British, Dutch, Portuguese, French Huguenot, Italian and Greek colonial migrants (Stull *et al.*, 2014; Krüger *et al.*, 2018). Thus, it is expected that South Africans of EA inherited features adapted for the cold climates from parent populations. Under the apartheid regime in SA, the mixing of races, particularly in the form of relationships or marriage, was considered illegal. Thus, variation was conserved, particularly within EA individuals. (L'Abbé *et al.,* 2011). The current study, anteriorly projecting zygoma were observed in both BA and MA people, this may suggest shared African influence and climatic adaptations in both groups (Patterson *et al.,* 2009; De Wit *et al.,* 2010; South African History Online, 2012; Petersen *et al.,* 2013; Busby *et al.,* 2016). Thermoregulatory features adapted to a hot climate, such as higher and wider cheek bones and anterio-posteriorly elongated zygomatic bones were expressed in BA and MA groups. A greater surface area on the zygomatic bone may be a result for thermoregulation requirement in the sub-Saharan climate in which features close to Bantu-speakers and Khoesan may dominate. Although the proof of this theory remains uncertain to some researchers, this accentuates the existing complexity in distinguishing BA and MA individuals (Patterson *et al.,* 2009; De Wit *et al.,* 2010; Petersen *et al.,* 2013; Busby *et al.,* 2016).

The challenge to distinguish between BA and MA individuals in SA has been widely reported (Stull *et al.*, 2014; Liebenberg *et al.,* 2015; Small *et al.*, 2016). De Wit and colleagues (2010) carried out a genetic study and found that South Africans of MA have genetic contributions from the following people; Khoesan (32-34%), Bantu-speakers (20- 36%), Europeans (21-28%) and Asians (9-11%). These various genetic contributions influence the expressed features present in MA individuals with variable degree with some having features closely aligned to Khoesan or Bantu-speakers or both. The degree of expressed genetic influence is unpredictable as MA individuals express variable admixture.

Although genetic influence is considered the drive to retain ancestral characteristics (Maddux & Butaric, 2017), mediation by hormonal changes have been suggested to influence

facial robusticity (Bernal *et al.*, 2006). Therefore, this presents a challenge on how one may distinguish the effects attributed to independent or aggregated genetic or environmental influence. Thus, a holistic approach is needed to understand these different effects and their impact on zygomatic shape changes. Unfortunately, investigation of these possible effects was beyond the scope of this study and would be considered in future research.

4.2.2 Zygomatic size variation

Significant zygomatic size differences were only detected between BA (60.1 ± 3.4mm) and MA (58.1 ± 3.3mm) individuals. The zygomatic size showed large overlap between all ancestral groups. Individuals of EA were similar in zygomatic size to those of both MA and BA and thus, zygomatic size showed minimal ancestral distinguishing ability between ancestral groups. There is limited research on the isolated zygomatic centroid size for comparison, with most studies analysing bizygomatic breadth. This is a relative inter- landmark distance measured from zygion to zygion on the either side of the zygomatic bone (Oettlé *et al.*, 2017). Zygion, a Type III landmark, is defined as the widest point on the zygomatic bone (Bookstein, 1991), although sometimes it may not correspond to the most prominent area on the zygoma (Oettlé *et al.*, 2017). Some landmarks, particularly in the internal surface of the orbit that bridges with the zygoma were considered, they were classified as Type III landmarks, which were excluded due to a lack of repeatability (Von Cramon-Taubadel *et al.*, 2007; Sholts *et al.*, 2011). Bizygomatic breadth size was not assessed in the current study as several individuals only had one side digitised due to trauma or damage to the zygomatic bone. Although, there were some differences in zygomatic size between ancestral groups, this was considered negligible due to the high degree of overlap and poor discrimination. Therefore, future research would allow for the validation of the zygomatic size measurements for comparative analyses across other population groups in SA.

4.3 Zygomatic variation and its impact on sex

4.3.1 Zygomatic shape variation

There were minor shape differences observed between males and females in this study. Sexual dimorphism of the cranial skeleton comes about through two distinct processes: i) differences in the shape of the elements as a direct result of biological differences between males and females (*i.e.* allometry), and ii) differences due to the indirect effect of (adult) hormones on muscle mass and thus the size and rugosity of the bony elements involved in muscle attachment or resisting the strains of mastication or holding of the head. Since the effect of allometry on shape was negated, the minor effect remaining is likely due to hormone-mediated muscle-size differences (temporalis and masseter muscle size differences), rather than males possessing

relatively larger viscerocrania from their prolonged growth (allometric). The findings from the present study showed low sex estimation accuracies using DFA, and that zygomatic bone shape was not sexually dimorphic. Previous research on sex estimation accuracy using DFA on the whole crania within the SA population have reported 80% to be the sex estimation benchmark (İşcan & Steyn, 1999; Franklin *et al.*, 2005; Dayal *et al.*, 2008b; DiGangi & Hefner, 2013; İşcan & Steyn, 2013b; Spradley & Stull, 2018). In this present study, females were accurately sexed, only 50% and males 60% of the time using zygomatic shape only. Although the sex estimation accuracies may not be ideal, zygomatic bone size would be informative for sex in a situation where fragmented remains are encountered. Sex is considered a confounder in ancestry estimation studies, and thus, better ancestry estimation accuracies were obtained when sex was considered in conjunction with ancestry. Male individuals produced better accuracies than females across the ancestral groups suggesting sex estimation before ancestry will improve accuracy and this will therefore, be discussed in more detail below.

When comparing males only across ancestral groups, the highest ancestry estimation accuracy was accredited to MA males and the least being ascribed to MA females. Lower ancestry estimation accuracies were obtained when classifying BA and MA individuals for either sex, which suggested possible similarities in the shape of the zygoma in these groups. While the zygomatic bone demonstrated minimal sexual dimorphism in terms of zygomatic shape, it appears that ancestry can be estimated more accurately when sex is known. Rosas and Bastir (2002) investigated sexual dimorphism on a Portuguese population using 2D GM and found that size and sex had a significant influence on shape of the craniofacial region. This current study found that size affected zygomatic shape but not sex. The sex estimation accuracies observed in this current study correlated to those of 60% for males and females from the Coimbra Collection (Gonzalez *et al.*, 2011). The possible reason for this disparity may be due to the present study assessing variation of an individual bone and not the whole cranium and mandible like in the study conducted by Rosas and Bastir (2002). Additionally, the present study explored variation across three ancestral groups, whilst Rosas and Bastir (2002) explored variation in 2D and did not specify if all the individuals were from different ancestries or if this was negated in their study. Moreover, the current study lacks the uniform age-at-death distribution in the individuals across ancestral and sex groups. Therefore, in future studies, there is a need to address this and explore if the same results are observed with a larger sample size and more uniformly distributed age-at-death.

In the present study, minimal sexual dimorphism was observed in zygomatic shape. Using size-independent shape analysis, such as GM provides useful insights when analysing

skeletal material of different origins where: (i) females of one population may possibly be larger or more robust than the males of another population (Maass, 2016); or (ii) when the degree of expression of sexual dimorphism within a population is low (Franklin *et al.,* 2006; Gonzalez *et al.,* 2011; King, 2015). The low level of sexual dimorphism observed in the present study correlated to findings by Gonzalez and colleagues (2011), who showed males and females differed more in size than in shape. This present study showed males with more compressed zygomatic heights, anteriorly projecting orbital margins and anteroposterior elongated zygomas, while females had wider and more flared zygomas with slight steeply-angled zygomatic arch landmarks. Generally, the zygomatic bones appeared slightly shorter and narrower in males than in females who exhibited a wider and more vertically elongated zygomatic bone. However, while significant zygomatic size differences were observed, zygomatic shape differences showed low levels of sexual dimorphism. Gonzalez and colleagues (2011), investigated sexual dimorphism in the crania of individuals from the Portuguese population using GM and found the zygomatic bone to be the least sexually dimorphic facial bone (Gonzalez *et al.*, 2011).

4.3.2 Zygomatic size variation

Males had larger zygomatic size than females, 60.3 ± 3.1mm and 56.5 ± 2.9mm respectively. The existence of zygomatic size differences between males and females was also observed by Franklin and colleagues (2006). Size differences in the mid-facial region (nasal, cheek bone and maxilla) between adults and sub-adults are thought to be due to hormonal differences (Ferrario *et al.*, 1998; Bastir *et al.*, 2006; Bulygina *et al.*, 2006; Windhager *et al.,* 2011). Before puberty, males and females experience similar facial growth rates (Bulygina *et al.*, 2006). However, females go through puberty earlier than males (approximately 12 years-of- age), due to increased levels of oestrogen and progesterone. These hormones result in rapid facial growth that ceases by 14-15 years-of-age in females. Males experience puberty slightly later (approximately 13-15 years-of-age), and the testosterone hormone results in continued facial growth until 16 or 17 years-of-age (Ramachandran *et al.,* 2005; Rogers, 2005; Bulygina *et al.*, 2006; Micklesfield *et al.*, 2011; Windhager *et al.*, 2011; Freidline *et al.*, 2015). In males, testosterone is known to increase muscle mass and bone density and since the cranium is made up of different integrated components, the impact of testosterone will affect growth in some facial bone components. Increased muscle mass in males would also increase the zygomatic size to accommodate larger muscle attachment sites (Drake *et al.,* 2015). This is a possible reason as to why males in the current study had a greater size in the zygomatic bone when compared with females.

The zygomatic bone provides anchoring support for the muscles of mastication, which would directly impact the zygomatic morphology. Another influence in zygomatic morphology may stem from the masticatory-function hypothesis (Larsen, 2013), which showed facial bones are dynamic and respond to demands from chewing muscles (temporalis and masseter). Thus, the consumption of either 'hard-textured or soft-textured foods' affects masticatory stress *e.g.* hard-textured foods (grains and nuts) may require stronger masticatory force as opposed to the force required to consume soft foods (cooked, processed or pre- cooked products). Therefore, consuming predominantly hard foods requires stronger, more robust masticatory muscles when compared with gracile muscles needed to break down softer foods. Craniofacial alterations due to changes in diet were associated with shorter and rounder cranial vaults, smaller and posteriorly placed faces and reduced robusticity of faces and jaws (Larsen, 1995, 2006, 2013). Since the masticatory muscles *e.g.* masseter and temporalis muscles attach to the zygomatic bone, the impact the zygomatic morphology may reflect geographic and cultural adaptations that relate to diet. Although one cannot assume the dietary requirements in BA, MA, and EA individuals, the dynamic change in bone in response to masticatory stress is probable.

4.4 Confounding factors

The potential disparities in socio-economic status remain an important consideration when evaluating confounding factors such as presence of maxillary teeth, age-at-death and year-of-birth. Although the effect of year-of-birth is considered when assessing secular trends, it was not assessed in the present study due to the limited sample size and year-of-birth distributions in the sample showed that individuals of different ancestries were born in different time periods. The absence of maxillary teeth and the effect of ageing did not influence zygomatic size but had a minor impact on the zygomatic shape, which accounted for less than 5% of the observed variation. To observe the associated zygomatic shape changes as a result of confounding factors, scaling was exaggerated when compared with the magnification of view used to observe shape changes due to ancestry or sex. It was observed that the most variant landmarks (*e.g.* zygomaxilla, zygoorbitale, frontomalare orbitale) were those closely integrated with neighbouring regions not assessed in the study
e.g. nasal or maxillary bone (McDowell *et al.*, 2012; Richard *et al.*, 2009; Williams & Slice, 2010; Mendelson & Wong, 2012; Small *et al.*, 2016). However, failure to assess and correct for confounding factors may led to erroneous deductions.

4.4.1 Presence of maxillary teeth

In the current study, presence of maxillary teeth did not impact zygomatic size but rather its shape. Alveolar bone provides structural support for teeth, and when tooth loss occurs, there is

irreversible and non-uniform alveolar bone resorption (Bodic *et al.,* 2005). Thus, individuals with alveolar bone resorption were taken to be indicative of individuals with antemortem tooth loss. Factors that influence tooth loss may be ascribed to disease (Bodic *et al.,* 2005), diet (Brennan *et al.*, 2008; Zhu & Hollis, 2014), age-related atrophy (Bodic *et al.,* 2005) and cultural practices (Friedling & Morris, 2007). Additionally, decreased masticatory stress has been suggested to influence tooth loss on the mandible and maxilla (Bodic *et al.,* 2005), and may occur in conjunction with age-related atrophy. Thus, there is the possibility of combined effects of the presence of maxillary teeth and ageing.

In this study, younger individuals had more maxillary teeth present when compared with older individuals. The former cohort was represented by most BA individuals, and the latter were represented by the MA and EA individuals. The older, edentulous MA and EA individuals had narrow, compressed and shortened zygomatic bones. There is a possibility that tooth loss may also be culturally driven especially amongst the MA males. Friedling and Morris (2007) stated that individuals of MA in the Western Cape, commonly practiced tooth extraction, particularly younger males (below 50 years). This may impact the presence of maxillary teeth in MA males, as antemortem tooth loss reflects ancestral variation. Therefore, understanding dental health practice and cultural practice is critical in the interpretation of tooth loss.

4.4.2 Effect of ageing

No significant relationships were observed between the presence of maxillary teeth and effect of ageing based on size. However, the effect of ageing showed similar shape changes between the ancestral groups. Older individuals had receded orbital margins, with narrower and shortened zygomatic bones. It has been suggested that the maxilla is more susceptible to age-related bone loss than the zygoma (Mendelson & Wong, 2012; Dinkele, 2018), because mid-craniofacial resorptions are site specific and uneven due to non-uniform bone resorption. Small and colleagues (2016) used GM to investigate tooth loss in the crania of individuals in the European individuals. They did not find any significant effects on the zygomatic bone due to tooth loss (Small *et al.*, 2016). Another study by Richard and colleagues (2009), who examined the effects of ageing on the craniofacial skeleton, observed a decrease in the anterior projection of the zygoma. This may support the idea that the bone is likely to experience bone atrophy. However, they found that the cortical bone mass remained rather stable irrespective of tooth loss (Richard *et al.,* 2009). Although the current study assessed the zygomatic bone in isolation to the whole cranium, having no observed size differences irrespective of the effect of ageing or the presence of maxillary teeth may suggest stability of zygomatic morphology during

alveolar resorption.

4.5 Forensic application and future work

The crime rate in SA has been attributed to various factors, *e.g.* gangsterism, unemployment, poverty, disease, interpersonal violence and substance abuse (Steyn *et al.*, 1997; Norman, Bradshaw, *et al.,* 2007; Norman, Matzopoulos, *et al.,* 2007; Norman *et al.,* 2010). Consequently, the annual increase in crime rate (Africa Check, 2018) exacerbates the number of unidentified individuals, which necessitates collaborative effort between different stakeholders (*e.g.* forensic anthropologists, pathologists and South African Police Service) to assist in identification. The pending formalisation of forensic anthropology in SA does not negate the expertise forensic anthropologists provide in analysing skeletonised, burnt and fragmented remains. Therefore, forensic anthropologists need to use relevant and reliable methods to assist with identification. Additionally, the improvements in peer-reviewed forensic anthropological methods continues the advancement of forensic anthropology in SA (L'Abbé *et al.*, 2011; Liebenberg *et al.*, 2015; Steyn *et al.*, 2016).

Considering the population of Cape Town comprises MA (49%), BA (39%) and EA (16%) (Census2011.adrianfrith.com, n.d.; De Wit *et al.,* 2010; Patterson *et al.,* 2010; Petersen *et al.,* 2013), the precincts that report the highest murder rates in South Africa are found in Cape Town (Nyanga, Delft, Khayelitsha, Harare, Phillipi East, Gugulethu and Kraaifontein) and Kwazulu Natal (Umlazi, Inanda and Plessislaer (CrimeStatisticsSA, 2015). The present study has demonstrated the capacity of zygomatic shape as an alternative method to distinguish individuals of EA from non-EA. Thus, the subsequent validation of this method would lead to establishing improved ancestral variation standards, assisting in the identification of unidentified remains.

In this present study, the zygomatic bone was shown to assist in ancestry and sex estimation. In most forensic cases, perpetrators often attempt to conceal or destroy the body to ensure the murdered individual is not found (Spradley & Stull, 2018). In some scenarios, the perpetrator may opt to dismember the body parts to deter possible identification. If few complete fragments (inclusive of the zygomatic bone) are recovered, they may be used to estimate sex or ancestral group affinity (Spradley & Stull, 2018). This potential further emphasises the need for continued research to develop standards that apply to SA among MA and BA individuals, ensuring the methods and techniques are validated to assess ancestry and sex variation. This would allow for crucial information on sex and ancestry affinity to be gained from the zygomatic bone and assist in victim identification. This current study also allows the

dissemination of information to other complementary fields such as forensic facial reconstruction, wherein forensic artists conduct facial reconstruction for victim identification or in reburial. These facial reconstructions serve to trigger possible recognition from the public and further assist in victim identification.

Exploring variation in the zygomatic bone, when integrated in the cranium, is often challenging as the bone is not easily accessible, traditional measurements using callipers are not suitable for irregular shaped bones like the zygoma. GM becomes advantageous because one can use the stylus to obtain the correct variation previously negated. Moreover, the advantage of using GM allows for convenient use of standardised instrumentation to capture data and software to perform complex analyses. This alleviates some of the shortcomings and biases associated with traditional metric and non-metric methods in multivariate statistical methods. Additionally, GM has the potential for the development of automated scanning due to technological advancement and allow for estimating elements of the demographic elements as well as assist in future collaborative research. This would combine GM and 3D scanners with validated pre-set algorithms to estimate demographic profile of (*e.g.* zygomatic bone and size variation for ancestry and sex). Future developments may allow the system to be highly accurate and rapidly produce output data, which is easily understandable and gives a high turnaround rate for identification.

4.6 Study limitations

The use of skeletal collections in forensic research remains controversial in the literature. Skeletal collections provide large samples for obtaining information about the population from which they are derived (Usher, 2002; Dayal *et al.,* 2009). However, others argue that their use introduces bias into research as acquisition practices at different skeletal collections, have introduced age, sex and socio-economic biases into collections (L'Abbé *et al.,* 2005; Komar & Grivas, 2008). A study conducted by Komar and Grivas (2008) on sample from the New Mexican population showed that older individuals are more likely to donate their bodies prior to death, whereas, younger individuals are less likely to donate their remains. Thus, the presence of younger individuals in skeletal collections is more likely because they are of lower socio-economic status and were donated by a medicolegal authority after their death. This is also known to occur in SA and may have impacted the skeletal collections used in this study. In this present study, BA and MA individuals in both collections used (UCT Human Skeletal Collection and Kirsten Collection) more likely came from State donations whilst the EA individuals were more likely bequeathed by the individuals themselves or by family members (Alblas *et al.*, 2018; Gibbon & Morris, *in press*). While no information on the socio-economic

status of the people in this study was available, if this assumption holds true, individuals of BA or MA are more likely to be of lower socio-economic status while those of EA may be of higher economic status (da Silva, 2006). Therefore, while it seems unlikely, there is a possibility that the results may reflect socio-economic disparities and not necessarily ascribed to ancestral variation.

Limited representation of individuals from BA and MA may be attributed to cultural and religious reasons (Adhikari, 1992; Maass, 2016). According to Adhikari (2005), many individuals of MA residing in Cape Town are Islamic (Adhikari, 2005). Therefore, acuity to donations is restricted due to the requirement for immediate burial after death (Adhikari, 2004; Maass, 2016). Similarly, the perception to donation is minimal in BA individuals due to cultural beliefs and ancestor reverence that advocates for individuals to be buried near other family members (L'Abbé *et al.*, 2005). Thus, limited sample sizes remain a consideration in skeletal collections, particularly for these population groups.

Due to the disparity in year-of-birth and the small sample size in this study, the presence of a secular trend could not be reliably evaluated. Tobias (1985) explored the secular trend in different populations and defined the positive, negative and absence of a secular trend (Tobias, 1985). Positive secular trend is associated with the direct greater, upward changes in growth over time (Tobias, 1985). "The difference between negative and absent secular trend is that, in the former, demonstrable changes have occurred in a population over a secular span of time but these have been in a slower, downward or negative direction; whereas absent secular trend implies that no changes have taken place in rate of growth or adult size, in a particular population over a span of time" (Tobias, 1985: 352). Whilst an interesting concept, the analysis of this was however, beyond the scope of the study.

Individuals in the study were born either in the early to mid-19th century or mid to late-19th century. One is unable to account for changes that may have occurred due to environmental changes (climatic changes, availability of food and resource, disease, wars *etc.*), technological advancement in medical intervention (decreased mortality) or urbanisation trends that may have introduced changes in cranial morphology. Weisensee and Jantz (2011) found no craniofacial variation due to allometry when they investigated secular trend patterns impacting cranial morphology in the New Lisbon collection (year-of births from 1806 to 1945). They assessed numerous cranial landmarks using GM, including the ones utilised in this study, but they were interpreted as overall changes with reference to the whole crania (Weisensee & Jantz, 2011). Individuals used were from a considerable homogeneous population and in this

current study, the limited sample size were from three different ancestral groups. Furthermore, variation assessments in the present study were restricted to the zygomatic bone, wherein variation was interpreted independent of the whole crania. Further research is required to investigate secular trends in the zygoma using a larger sample representative of the SA population groups.

Unfortunately, the practical application of GM in a forensic context may be problematic. One major limitation of allometric variation is that size influence on shape is negated, which would not be practical in a forensic context as size influences shape. However, in this study, shape and size were done separately to inform methods, which analyse shape and size variation exclusively to estimate ancestry (metric and non-metric methods). Another limitation of DFA in GM studies is with the use of 3D data, one cannot attain a discriminant function equation. The Microscribe equipment is expensive and requires a trained individual to operate. Despite these limitations, the information gained from assessing the zygomatic bone variation has shown great potential. Further research would improve reliability of ancestry estimation standards relevant for a South African population.

5. CONCLUSION

Estimating ancestry in a forensic context is crucial to establish the demographic profile of the deceased and to assist with victim identification. Due to SA's heterogenous population, there exists the pertinent need for reliable and relevant methods that accurately estimate ancestry within the admixed South African population. Ancestral differences have been attributed to mid-craniofacial variation, however, no study has explored the variation in the zygomatic bone exclusively. The purpose of the study was to use GM to explore zygomatic shape and size variation in 3D for ancestral estimation within SA. Applied to a sample of 158 individuals, representative of the three largest population groups in SA, the majority were males, who were concomitantly older. Cranial landmarks selected encompassed the zygomatic bone morphology were deemed repeatable, thereby, allowing shape and size variation to be assessed accurately.

With regards to zygomatic shape, variation was informative for differences in ancestral groups (BA, EA and MA individuals), although zygomatic bone shape had minimal sexual dimorphism. There was observed similarity between BA and MA individuals, who had narrower, shorter and more anteriorly projecting zygomas than EA individuals. This is likely correlated to genetic admixture due to the historical peopling of SA and historical forced racial classification. The zygoma was shown to accurately distinguish between EA (84%) and BA (81%), MA (80%) and EA (68%) individuals; and unreliably distinguish between BA (60%) and MA (66%) individuals. Thus, the resultant difficulty in distinguishing between MA and BA individuals in the SA context highlights the need for further research in ancestry estimation.

Zygomatic size differences were ascribed to sexual dimorphism, but MA were significantly smaller than EA. Males had considerably larger zygomatic bone size when compared with females. This is indicative of size differences being hormonally or environmentally controlled. Zygomatic size differences between the ancestral groups produced great similarities between EA individuals with MA and BA individuals. Although estimation accuracy was not considered ideal when assessing sexual dimorphism, females were accurately distinguished 50% and males 60%. The zygomatic bone has shown great potential in providing sex and ancestry demographic profiles in cases where fragmented remains are encountered. Furthermore, ancestry estimation accuracy improved when ancestry was aggregated with sex. Furthermore, the presence of maxillary teeth and effect of ageing was found to have a minor impact on zygomatic shape and did not affect size. Variation in the zygomatic shape and size

has demonstrated the possibility of an alternative method to aid in establishing the demographic profile in a biologically heterogenous population.

Future endeavours propose an expansion in sample size will improve statistical robusticity. Targeting a more balanced sample in terms of age-at-death and sex distributions will allow informative deductions. Moreover, future studies can assess variation using GM for aggregated facial bones *e.g.* zygoma and nasal, zygoma and orbit, zygoma and maxilla. Technological advancements in GM may involve a more portable Microscribe and user-friendly software to generate faster results for assessing elements of the demographic profile. Additionally, social classifications do not represent biology, thus, there is need for biologically relevant categories that reflect biological descriptors, thus, alleviating the complexity in ancestral estimation. Furthermore, improvements in method validation and comparative studies propagates the development of standardised, reliable and relevant ancestry estimation methods within SA, that are pivotal in the forensic anthropology fields and criminal justice system in SA.

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7. APPENDICES

Appendix A Ethics Approval Letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

28 February 2018

HREC REF: 843/2017

Dr V Gibbon
Division of Clinical Anatomy & Biological Anthropology
Human Biology
Level 5, Room 5.14
Falmouth Building

Dear Dr Gibbon

PROJECT TITLE: GEOMETRIC MORPHOMETRIC ANALYSES TO ASSESS THE ACCURACY OF THE ZYGOMA FOR ESTIMATING ANCESTRY IN A SOUTH AFRICAN POPULATION (MASTERS CANDIDATE - MS T TAWHA)

Thank you for submitting your response to the Faculty of Health Sciences Human Research Ethics Committee received on 8 February 2018.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that student Tafadzwa Tawha will also be involved in this study.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS/HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

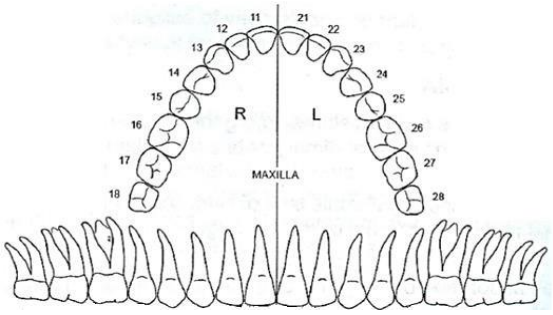
HREC 843/2017

Appendix B Diagram for Teeth Presence

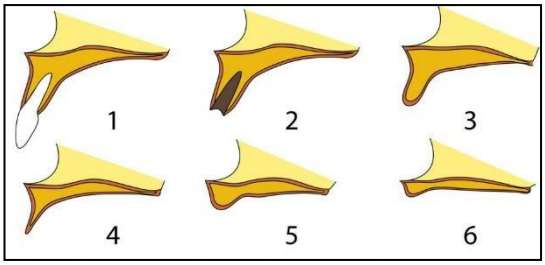
Accession number:

Date:

STATUS KEY	
A	Antemortem Loss
Pe	Perimortem Loss
Po	Postmortem Loss

	STATUS	Resorption	Comment		STATUS	Resorption
11				21		
12				22		
13				23		
14				24		
15				25		
16				26		
17				27		
18				28		

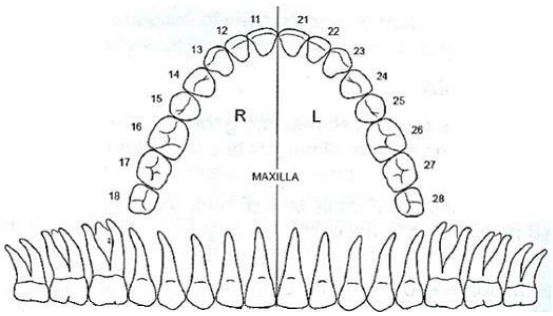
Resorption scale Maxilla
(Reichs *et al.*, 2011)



Accession number:

Date:

STATUS KEY	
A	Antemortem Loss
Pe	Perimortem Loss
Po	Postmortem Loss

	STATUS	Resorption	Comment		STATUS	Resorption
11				21		
12				22		
13				23		
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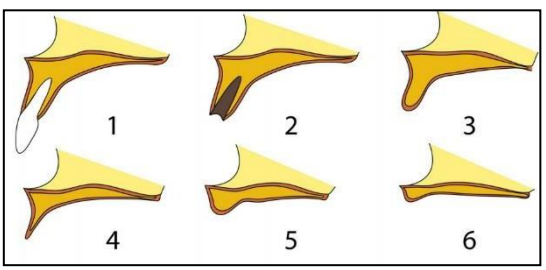
15				25		
16				26		
17				27		
18				28		

Resorption scale Maxilla
(Reichs *et al.*, 2011)



Appendix C Supporting Data

Table C.1: Inter and intra reliability tests for averaged left and right sides. Values in the brackets are attributed to the error component.

Effect	**% Contribution**	**Sum of squares**	**Mean sum of squares**	**Degrees of freedom**	**F* statistic**	***p value***
Intra-observer: Centroid size						
Individual	**99.9**	1123.7	35.7	32	845.2	****
Error	**0.1**	1.6	0.1	33		
Procrustes Shape ANOVA						
Individual	**99.2**	1.0	1.5×10^{-3}	630	153	****
Error	**0.8**	8.0×10^{-3}	1.2×10^{-5}	650		
Intra-observer: Centroid size						
Individual	**96.9**	2011.0	52.9	38	64.4	****
Error	**1.5**	32.1	0.9	39	1.24	*0.23*
Residual	**1.6**	33.0	0.7	50		
Procrustes Shape ANOVA						
Individual	**88.2**	1.5	2.3×10^{-3}	646	24.9	****
Error	**3.6**	6.0×10^{-2}	9.1×10^{-5}	663	0.6	*0.09*
Residual	**8.2**	1.4×10^{-1}	1.6×10^{-4}	850	2.7	*****
*Significant p values are represented in asterisks (*) as follows *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001*						

Table C.2: Summary of sex, ancestry, ancestry-linked categories for centroid size (mm)**.** Values shown are represented as Mean ± standard deviation.

Variable	**Female**	**Male**	**T stat.**	***p value***
Centroid Size (mm)	56.46 ± 2.9	60.31 ± 3.1	60.1	****

	Ancestry				
Variable	**Bantu-speaking**	**Mixed**	**European**	**F stat.**	***p value***
Centroid Size (mm)	60.05 ± 3.4	58.10 ± 3.3	58.48 ± 3.8	4.44	*

	Ancestry							
Variable	**Bantu-speaking**		**Mixed**		**European**		**F stat.**	***p value***
	Female	**Male**	**Female**	**Male**	**Female**	**Male**		
Centroid Size (mm)	58.03 ± 2.8	61.19 ± 3.2	56.28 ± 2.4	59.54 ± 3.3	55.25 ± 3.1	60.29 ± 2.8	15.27	*<0.0001*

*Significant p values are represented in asterisks (*) as follows *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001*

Table C.3: Summary of Mahalanobis distances for pairwise ancestry-linked group comparisons from canonical variate analyses. Variances pooled by ancestry-sex classifier.

	Bantu-speaking female	**Bantu-speaking male**	**European female**	**European male**	**Mixed female**
Bantu-speaking male	1.70*				
European female	2.35****	2.25****			
European male	2.70 ****	2.67 ****	1.47*		
Mixed ancestry female	1.99***	1.46 *	1.96 ***	2.36 ****	
Mixed ancestry male	1.88 ****	1.25 *(p=0.11)*	2.11 ****	2.30 ****	1.22 *(p=0.22)*
*Significant p values are represented in asterisks (*****) as follows *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.*					

Table C.4: Summary of Mahalanobis distances for pairwise ancestry-linked group comparisons and estimation accuracies from leave-one-out cross validations (LOOCV) of classification accuracies.

Ancestral Group 1	**Ancestral Group 2**	**Ancestral Group 1 Classified correctly (%)**	**Ancestral Group 2 Classified correctly (%)**	**Mahalanobis distance**	***p value***
Bantu-speaking female	European female	**76**	**72**	3.95	*
Bantu-speaking female	Mixed female	71	59	2.14	*0.10*
European female	Mixed female	**72**	**63**	2.24	*0.05*
Bantu-speaking male	European male	**77**	**81**	2.71	****
Bantu-speaking male	Mixed male	53	59	1.34	*0.29*
European male	Mixed male	72	**82**	2.54	****

Bold values *represent the highest ancestry estimation ancestries for the impact of sex on ancestry group. The colours correspond to the ancestral groups: Bantu-speaking (red), European (orange) and Mixed Ancestry (blue)*
Significant differences with the following ancestry groups: Bantu-speaking females with both European females; Bantu-speaking males with both European males and Mixed ancestry males ($p \leq 0.0001$).
*Significant p values are represented in asterisks (*) as follows *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$*

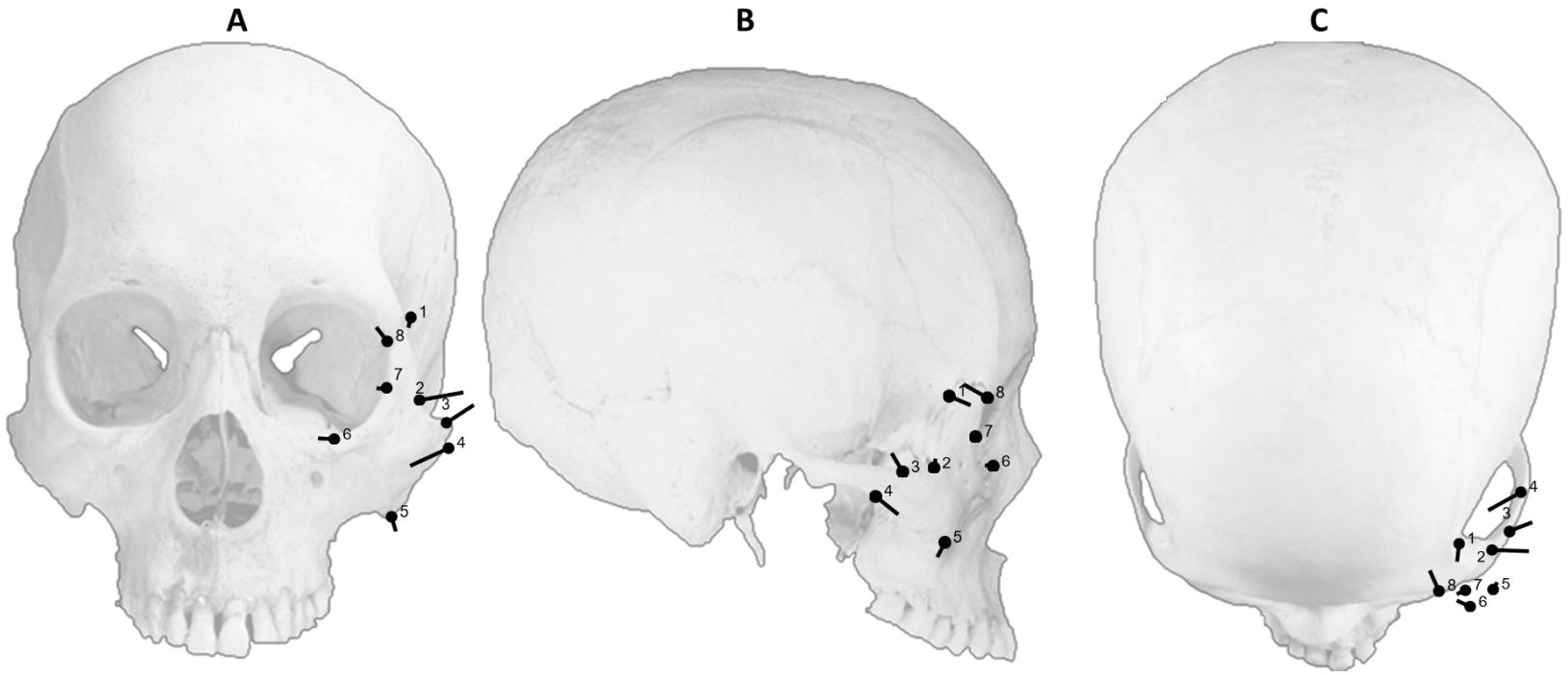


Figure C.1: Shape variation on principle component 13 for males and females, when pooled according to sex. Dots represent the average shape in females and stems represent the magnitude and direction of variation in males. Views of shape change from Principal Component Analyses include: A. Anterior; B. Lateral and C. Superior.
[Scale factor: 0.1].
[Scaling is for visualisation and may result in unnatural distortion].
[Image adopted from (eSkeletons, 2005)].

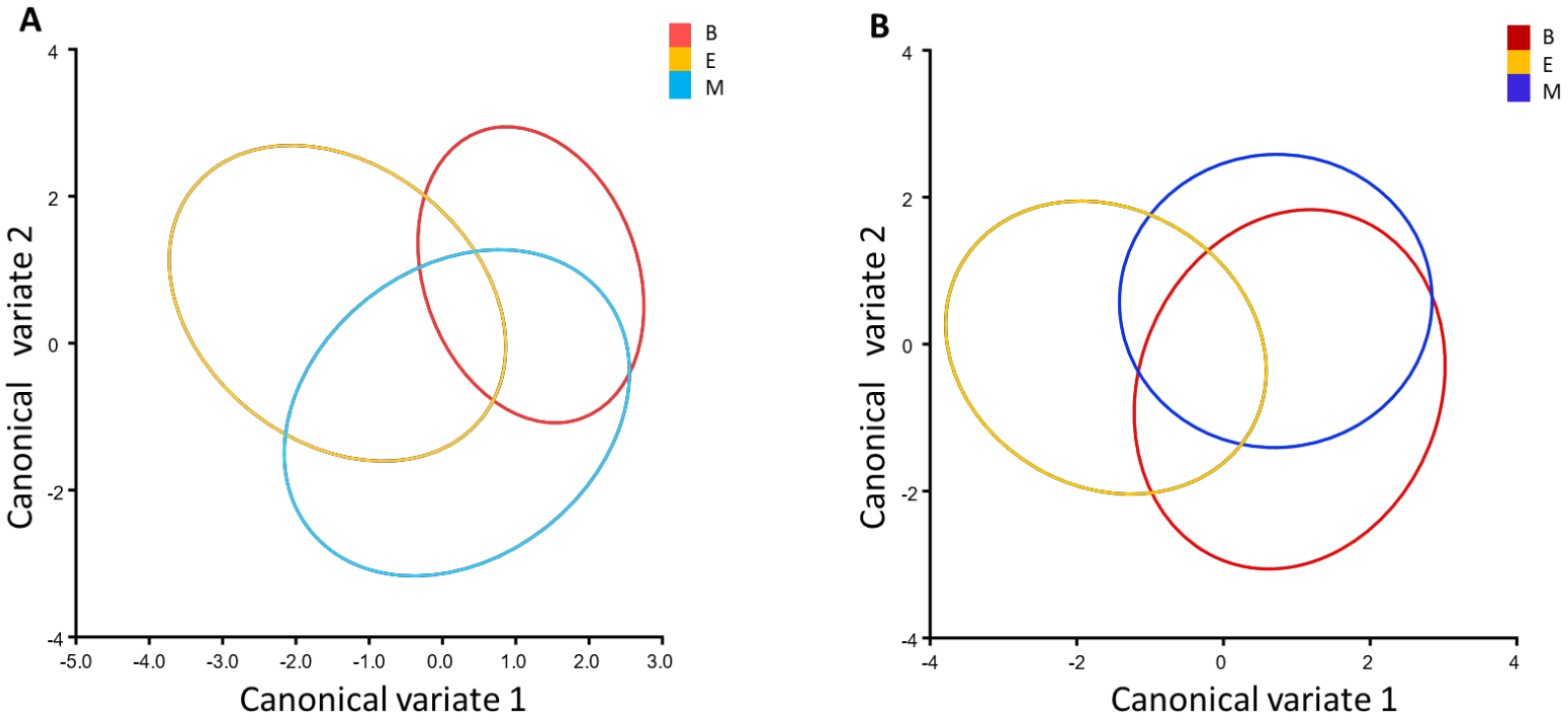


Figure C.2: Scatter plot for zygomatic shape variation on canonical variate 1 and 2 for ancestry-linked groups, A represents ancestral variation in females and B represents ancestral variation in males, depicted using 95%confidence ellipses. Ancestral groups include: BA (red), EA (orange) and MA (blue).

[Scaling may result in unnatural distortion]