A Statistical Shape Analysis of the Neurocranium and Long Bones

by

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DECLARATION

I, ...*Petra Maass*..., hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Vir Oupa Willem

–Wat ek is, is net genade”

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ABBREVIATIONS

2D:	Two-dimensional
3D:	Three-dimensional
ANOVA:	Analysis of Variance
CT:	Computed Tomography
CV:	Canonical Variate
CVA:	Canonical Variates Analysis
DFA:	Discriminant Function Analysis
GPA:	Generalized Procrustes Analysis
HPCSA:	Health Professions Council of South Africa
MD:	Mahalanobis Distance
PC:	Principal Component
PCA:	Principal Component Analysis
QLFS:	Quarterly Labour Force Survey
SWGANTH:	Scientific Working Group for Forensic Anthropology
UCT:	University of Cape Town
WHO:	World Health Organization

ABSTRACT

Morphological variation of skeletal elements, and the potential use of such variation in distinguishing among demographic groups, is often investigated using traditional metric or non-metric assessments. Traditional approaches, however, often fail to sufficiently capture the "true" shape of features, thus also failing to identify potentially important feature characteristics. The development of geometric morphometrics has allowed more comprehensive and accurate three-dimensional data capture which maintains the geometric properties of an object while isolating the effect of size from the data.

The aim of this study was to employ the geometric morphometric approach to a 3D digitized sample of 1132 South African individuals from the skeletal collections of the Universities of Cape Town, Stellenbosch, Witwatersrand and Pretoria. Morphological variation among demographic groups was assessed using Generalized Procrustes Analyses applied to the individual bones of the neurocranium and the long bones of the limbs. The ability to distinguish groups based on the detected variation was assessed using Discriminant Function Analysis.

The results show that, when size is isolated from the data, only a few elements show sexual dimorphism, while all examined elements show high accuracy in distinguishing among ancestry groups (>74%). When variation is assessed using both parameters, classification accuracies of 70-83% are obtained. Comparison of the different elements shows that the best classification accuracies are based on the frontal bone (84% average) and the humerus (81% average).

This study shows that the morphologies of the neurocranium and long bones vary among sex and ancestry groups. This allows assessment of how the combination of variable intrinsic and extrinsic influences can manifest on different parts of the skeleton. In a population as genetically and historically complex as that of South Africa, understanding of the impact of such influences may inform forensic assessments of skeletal material, which is especially valuable considering the high rate of violent crimes and increasing number of unidentified remains being discovered in the country.

CHAPTER 1

INTRODUCTION

1.1. Traditional anthropological approaches

The shape of certain skeletal structures and the potential differences of these shapes among different human ancestral groups have given rise to many questions in the field of anthropology, for example ―Do the pelves of males differ in shape from that of females?" or ―Does the shape of the mandible change with age?". Traditionally, anthropologists have approached such questions using either visual assessment of non-metric features (e.g. absence or presence of specific features), or metric assessments of distances, angles or chords (Oettlé *et al.,* 2009). There are, however, advantages and disadvantages when using either of these methods. Non-metric features may provide a quick and easy preliminary assessment of a skeletal feature, but the assessment of such a feature may rely greatly on the experience of the observer, and thus involves a substantial amount of subjectivity (Walker, 2008; Bidmos *et al.,* 2010). Metric assessments are more objective, provide high levels of accuracy and repeatability, and can generally be performed by anthropologists with minimal training, but such often fail to capture the ―true" shape of the skeletal element being examined (Slice, 2007; Bidmos *et al.,* 2010). Increasing the number of measurements does not always provide a solution to this problem, as this causes an exponential increase in the number of potential variables (which may also be highly correlated to each other) to the point of impracticality (Franklin *et al.,* 2005; Slice, 2007).

Metric assessments also bear the caveat of often being very population-specific, with studies like those of Steyn & İşcan (1997), Asala *et al.* (2004) and Franklin *et al.* (2005) showing that application of metric standards of one population to an unknown individual of a different population may lead to misclassification of the unknown individual. This is most evident in studies of sexual dimorphism which show that the most sexually dimorphic feature in skeletal elements of one population may be only slightly dimorphic in another (Alunni-Perret *et al.,* 2003; Asala *et al.,* 2004; Macaluso, 2011; Vance *et al.,* 2011). Interpretation of morphological variation in a

population is complicated by the complex integration of intrinsic and extrinsic factors acting on that population. Even within a population, phenomena such as secular changes in morphology and influences of changing living conditions, health status and nutrition may cause a sample of a specific population to be very different from a sample of the same population taken from a different time period (Hamilton, 1982; Alunni-Perret *et al.,* 2008). In a historically and genetically complex population such as that of South Africa, the factors influencing morphological variation may thus be especially difficult to separate (Morris, 2010). A good example of this is the uniquely composed South African "Coloured" group. This group originally arose from admixture of several ancestral groups passing through the country throughout history, but has taken on more of a socio-cultural identity in more recent times (Adhikari, 2005; Patterson *et al.,* 2010; Petrus & Isaacs-Martin, 2012; Petersen *et al.,* 2013). As such, it is difficult to determine whether differences and similarities in morphology between this group and the other ancestral groups in the country are the result of shared genetic histories or of shared socio-economic conditions. Some previous studies suggest that the amount of morphological variation within the Coloured group is too large to allow useful differentiation of this group from the Black and White ancestral groups (Smay & Armelagos 2000; Armelagos & Goodman 1998). More recent studies, however, have shown that, by using newer and more sensitive techniques, this group can be differentiated from the contributing ancestral groups (Stull *et al.,* 2014). This allows for the comparison of the Coloured ancestry group to the Black and White ancestry groups which have genetically contributed to it, potentially providing some insight of how extrinsic factors (such as socio-cultural conditions) may act to modify the relationship between genotype and phenotype.

Another major challenge forensic anthropologists face (when using either metric or non-metric methods) is the fact that skeletal remains being analysed are often incomplete and/or damaged to some extent. This may prevent the anthropologist from investigating certain features and restrict them to make assessments based on a limited number of features or elements, thus potentially decreasing the accuracy of the assessment (Scheuer, 2002; Asala *et al.,* 2004; Macaluso, 2011; Spradley & Jantz, 2011). As such, it is necessary to develop techniques for skeletal analysis based on a wide range of isolated bony elements, and if possible, individual components of these elements which are likely to survive and be recovered in a forensic context (Edwards *et al.,* 2013).

1.2. Geometric morphometrics

Over the past few decades, anthropologists have started using more sophisticated statistical analyses to help overcome the limitations imposed by the traditionally employed techniques. Most popular of these is geometric morphometrics (Adams *et al.,* 2004; Slice, 2007). The main contribution of geometric morphometrics to the field of anthropology is its ability to isolate the influence of size from a data set while still retaining all the associated shape information encoded in that data set (Lockwood *et al.,* 2002; Slice, 2007). This is done by scaling all specimens in a sample to a common centroid size (the square root of the sum of squared distances), and aligning homologous landmarks of the different specimens by removing variation due to translation or rotation via a process called Procrustes superimposition. Once this has been done, the residual data set can be assessed for shape variations using multivariate statistical analyses such as principle components, canonical variables or discriminant functions. In essence, geometric morphometrics removes subjectivity from shape evaluation and creates the possibility of objective (and often more accurate) quantitative assessment of non-metric skeletal features (Franklin *et al.,* 2005; Pretorius *et al.,* 2006).

Geometric morphometrics has been applied to several anthropological questions, particularly the quantification of shape differences among species, populations or sexes (e.g. Lockwood *et al.,* 2002; Harvati, 2003a; Pretorius *et al.,* 2006; Yokley & Churchill, 2006; Kimmerle *et al.,* 2008). These studies have shown that geometric morphometric techniques provide improved detection of specific traits of dimorphism which are often difficult to detect or quantify using traditional visual or metric methods (Bidmos *et al.,* 2010; Bigoni *et al.,* 2010). This is especially useful in populations which express dimorphism to a lesser extent than others, and which often make simple assessments, such as sex estimation, difficult or even impossible (Hrdlička, 1939; Tobias, 1974; Alunni-Perret *et al.,* 2008). The application of geometric morphometric analyses to common anthropological questions creates a whole new field of potential study which would serve to both standardize and re-evaluate older techniques, and develop novel techniques of assessing demographic parameters such as sex, age and ancestry.

Since the application of geometric morphometrics in anthropology is relatively new, only a few select skeletal elements have been assessed using this approach, including the cranium or parts thereof (Ross *et al.,* 1999; Bulygina *et al.,* 2006; Kimmerle *et al.,* 2008), vertebrae (Albert *et al.,* 2003; Chatzigianni & Halazonetis, 2009), the pelvis (Pretorius *et al.,* 2006), and the scapula (Scholtz *et al.,* 2010). It is likely that future studies will continue to expand on the variety of elements assessed and extend this to investigating the differences in diverse populations, especially due to the increased access to the equipment and software required for such analyses and the attractive nature of the results produced (Slice, 2007; Webster & Sheets, 2010; McKeown & Schmidt, 2012).

1.3. Medico-legal considerations

In 1993, the United States Supreme Court passed a decision regarding the admissibility of expert witness testimony in court proceedings, the recommendations of which have since become known as the Daubert principles (Christensen & Crowder, 2009). The guidelines therein suggest that methods used in a scientific field should be repeatable, reliable and have a known or estimated error in order for such evidence to qualify as expert testimony in court (Christensen *et al.,* 2014a). Since this ruling, several anthropological studies have re-evaluated some of the most commonly used anthropological techniques. A few of these techniques have subsequently been shown to be less reliable than originally thought and fail to meet the requirements of the Daubert principles (Harrington *et al.,* 2003; Christensen, 2004; Steadman *et al.,* 2006).

Although expert witness testimony in South Africa (whether anthropological or otherwise) is not ruled by the Daubert principles, it is valuable for these principles to be considered and employed by South African anthropologists in order to ensure that the techniques used in the field are consistent and scientifically sound (Meintjes-Van der Walt, 2003; Gordon, 2011). It has been shown that the application of international standards to the complex South African population may result in poor classification accuracies which may not be usable in court (Steyn *et al.*, 1997; Vance *et al.*, 2011). Possible reasons for these differences may be either genetic or socio-cultural. Though it may be possible to trace the genetic history of many of the South African ancestral groups back to their origins in Europe or Africa, the local socio-economic conditions may have resulted in functional adaptation in the South African

groups, making them very different from their ancestral groups (Steyn & İşcan, 1998). It would be even more inappropriate to apply internationally-derived standards to the highly admixed Coloured ancestral group, which is unique to the country. It is thus imperative that reliable sex, age and ancestry estimation methods be developed in South Africa based on local populations, and that these methods are constantly re-evaluated and updated to remain relevant to the population to which they are applied. This is especially important considering the high incidence of violent crimes, increasing numbers of migrant workers and widespread disease in South Africa, all of which contribute to increasing numbers of forensic cases requiring analysis by well-trained anthropologists in an objective and reliable manner (Steyn *et al.,* 1997; Gordon, 2011; L'Abbé & Steyn, 2012).

In order to develop such methods, an extensive study of the shape variation of forensically informative skeletal elements of adult South African individuals must be done, focussing on elements which are likely to survive and be recovered in a forensic context. Compiling a database of morphological variation based on individuals of known sex and ancestry may provide a means of comparison for estimating such demographic parameters for unidentified skeletal remains in an objective, sensitive and reliable manner.

1.4. Aim and objectives

The aim of this study is to quantitatively describe morphological variation in the neurocranium and long bones of South African adult individuals, and the distribution of this variation within the three largest ancestry groups in the country – Black, White, and the uniquely South African Coloured (Mixed) group.

To achieve this, the objectives of this study are:

1. To assess possible associations between morphological variations of the neurocranium and long bones to known demographic information, namely sex and ancestry.
2. Evaluate the potential influences of temporal (time-related) changes and age on the morphology of these bones.
3. To assess which of these bones are most useful in estimation of the demographic parameters of sex, ancestry and sex-ancestry combined.

CHAPTER 2

BACKGROUND

2.1. Forensic anthropology

Forensic anthropologists are often called upon when unidentified skeletal remains are recovered, and are requested to assist in possible identification of the remains by constructing a biological profile of the individual through the application of the techniques of biological anthropology. Such a profile would often include estimations such as sex, age-at-death, living stature and ancestry. These estimations are used to narrow search parameters for the investigating law enforcement officials (Walsh-Haney *et al.,* 1999; Cattaneo, 2007; Spradley *et al.,* 2008). Several methods have been developed to aid the forensic anthropologist in the assessment of these demographic parameters, though two specific considerations are imperative to the accuracy of such estimations, namely the preservation of skeletal remains, and the applicability of a selected method on individuals of a specific population group (Bidmos & Dayal, 2004; White *et al.,* 2012; Bethard & Sheet, 2013; Jain *et al.,* 2013).

When considering which skeletal elements to use for developing techniques which will be applied to forensic cases, it is important to take the relative survival and recovery rates of the different skeletal elements into consideration (Bidmos *et al.,* 2010; Macaluso, 2011; Šlaus *et al.,* 2013). In the forensic context, bones may be damaged due to taphonomic processes such as erosion and animal scavenging, or due to intentional damage related to the events at death and/or deliberate attempts to prevent identification (Introna *et al.,* 1998; Konopka *et al.,* 2007). This may restrict forensic anthropologists from examining certain skeletal regions or features commonly used in demographic estimations, and force them to rely on assessments based on a limited number of features or ones which may be less diagnostic, thus reducing the accuracy of the final assessment (Scheuer, 2002; Bigoni *et al.,* 2010; Macaluso, 2011). It is thus vital to develop methods to analyse skeletal remains based on many different bones, especially the elements expected to have the best survival and recovery in a forensic context (Gapert *et al.,* 2009a).

In a comparison of studies on the survival of human bones, Stojanowski *et al.* (2009) showed that, despite the exact taphonomic conditions (for example temperature, humidity etc.), bone survival patterns are relatively constant across different recovery sites. Of the studies cited, common patterns of preservation include poor preservation rates for porous and less dense bones such as the ribs, sternum and vertebrae (Boddington *et al.,* 1987; Nawrocki, 1995), while dense areas such as shafts of long bones tend to survive better (Galloway *et al.,* 1997; Willey *et al.,* 1997). Spennemann (1992) reports 100% recovery of the cranium and 38 – 75% recovery of the various long bones, however, certain regions within a bone may also preserve better than others of that same bone. Waldron (1987) reports only 17% recovery of the whole cranium, but 40% for the frontal, 33% for the parietal, 40% for the occipital, and 47 – 67% for the various parts of the temporal bones of the same skeletal sample. Overall, the cranium (excluding the facial region) is often the best preserved unit, followed by the long bone shafts and the more dense epiphyses. The fragile scapula, vertebrae and sternum are usually not recovered or are very poorly preserved (Waldron, 1987; Stojanowski *et al.,* 2009). Based on this information, it would be more constructive to study some of the individual components of the cranium and the long bones when developing methods for application in forensic practice.

The next important consideration is that of the applicability of the designed method on individuals from the specific population group to which this method would be applied. A major criticism of the FORDISC® and 3D-ID software packages commonly used to estimate sex, ancestry and stature is that, while these programs perform well for Northern American individuals (from where many of the reference samples are derived), the application of these programs to individuals from other populations are less successful (Birkby *et al.,* 2008; Guyomarch & Bruzek, 2011; Wienker & Antúnez, 2012; L'Abbé *et al.,* 2013) and often produces ambiguous results due to the mixture of biological, ethnical and national groups of the samples and unknown individuals to which it is applied (Komar & Buikstra, 2008). This illustrates the need for consideration of population-specific approaches to analysing remains.

Populations may vary due to the specific balances reached between a large number of intrinsic factors such as genetic composition and health status, and extrinsic factors such as nutrition, access and quality of healthcare, physical activity patterns,

urbanization, increased population growth , etc (Stini, 1969; Ruff, 1987; Steyn & İşcan, 1999; Alunni-Perret *et al.,* 2008; Charisi *et al.,* 2011). Due to the fluctuating nature of these influences, populations may vary both geographically and temporally (Hamilton, 1982; Introna *et al.,* 1997; Jantz & Jantz, 1999; Alunni-Perret *et al.,* 2003 and 2008). It is thus prudent to develop analytical methods using samples which correspond as closely as reasonably possible to the contemporary population to which the methods may be applied.

2.2. Approaches to assessing skeletal variation

Traditionally, anthropologists approached assessments of skeletal variation using either visual evaluations of non-metric features (e.g. absence/presence, small/medium/large) or metric assessments of features such as measurements of distances, angles or chords (Oettlé *et al.,* 2009). Each of these approaches has its own advantages and disadvantages. Non-metric features may provide quick and easy preliminary assessment of a skeletal feature, but such assessments are prone to great subjectivity, and features are often hard to quantify and statistically analyze (Konigsberg & Hens, 1998; Williams & Rogers, 2006; Walker, 2008; Bidmos *et al.,* 2010). On the other hand, metric assessments are considerably more objective, providing high accuracy and repeatability, allowing relatively easy quantitative analysis (Kemkes-Grottenthaler *et al.,* 2002; Steyn *et al.,* 2004). However, the measurements usually require specialised equipment, and may sometimes be inadequate in describing complex features, and often require very sophisticated statistical analyses (Franklin *et al.,* 2005; Slice, 2007; Bidmos *et al.,* 2010).

Since the late 1980s, anthropologists began to employ more sophisticated approaches to capturing data and expanded statistical analysis through the use of various statistical software packages, helping to reduce the effect of some of the limitations of metric and non-metric approaches. The technique gaining the most momentum is geometric morphometrics (Rosas & Bastir, 2002; Adams *et al.,* 2004; Slice, 2007). This technique incorporates some of the advantages of both metric and non-metric approaches by allowing size to be isolated from the data, while still retaining all the geometric shape information encoded in the data set (Lockwood *et al.,* 2002; Slice, 2007; McKeown & Schmidt, 2012). This allows quantification of elements or regions in a more objective and robust manner, even when the regions being examined do not

have many anatomical landmarks (Pretorius *et al.,* 2006; Perez *et al.,* 2006; Franklin *et al.,* 2006 and 2007).

2.3. Geometric morphometrics

2.3.1. Brief overview

Geometric morphometrics requires complex statistical analyses which are based on sophisticated mathematical principles which are explained in detail in the literature (e.g. Bookstein, 1991; Marcus *et al.,* 1996; Dryden & Mardia, 1998; Slice, 2005). For the purposes of this study, a brief overview of these principles is given below. For a more detailed overview, see Slice (2007).

Geometric morphometrics is based on the capture of a set of Cartesian coordinate landmarks, which should be homologous among specimens (Bookstein, 1991), collectively forming a two- or three-dimensional configuration, representing each individual specimen that is digitized (Pavlinov, 2001; Slice, 2007). In morphological shape studies, size may be an informative parameter, as seen in the magnitude of osteometric studies in the literature. However, it may sometimes also be a hindrance in shape analyses, especially when absolute size differences among study groups are very large (e.g. comparison of species) or very small (e.g. comparison of sexes), or when allometry may play a role (e.g. when comparing juveniles to adults) (Rosas & Bastir, 2002; Schaefer *et al.,* 2004; McKeown & Schmidt, 2012).

Geometric morphometrics allows for the mathematical extraction of the influence of size on the data, allowing subsequent analyses to be performed in a ―size-free" shape space though still maintaining the integrity of the geometric information within the dataset (Rohlf, 1996). This extraction is most commonly performed through Generalized Procrustes Analysis (GPA) (Bookstein, 1991; McKeown & Schmidt, 2012). GPA uses a least-squares oriented approach to translate all landmark configurations (one per specimen) to a new common origin, scaling the configurations to a common centroid size (the square root of summed squared distances of all coordinates from their own centroid), and rotating all configurations around the origin until the sum of squared Euclidean distances (direct, two-dimensional distances) between the corresponding landmarks on the different specimens are minimized (Rohlf & Slice, 1990). The resulting superimposed

coordinates are called Procrustes shape coordinates and contain only shape information (Rohlf, 1996; Mitteroecker *et al.,* 2013), though size-related shape differences will remain in the presence of allometry (Green & Curnoe, 2009). The calculated centroid size provides a reasonable proxy for overall size of each specimen and can be used in exploration of size variation or allometric effects acting on a sample (Bookstein, 1991; Singleton, 2002; Mitteroecker *et al.,* 2013).

Procrustes shape coordinates, which lie in a hyper-spherical and non-Euclidean space called "Kendall's shape space" (Kendall, 1984), are then projected onto a Euclidean tangent space before further analyses can be performed (Dryden & Mardia, 1992; Slice, 2001). Since biological data tends to have relatively restricted variation, these projections usually do not significantly distort distances among specimens (Slice, 2001; Webster & Sheets, 2010). Multivariate statistical approaches such as Principal Component Analysis (PCA), canonical variate analysis (CVA) or discriminant function analysis (DFA) can now be used to assess shape variation among individuals or study groups (Green & Curnoe, 2009; Mitteroecker *et al.,* 2013).

One of the outstanding benefits of geometric morphometrics is the graphic output it produces, allowing visualization of shape differences in the form of deformation grids, vector diagrams, or wireframes (Adams *et al.,* 2004; Mitteroecker & Gunz, 2009; Webster & Sheets, 2010; McKeown & Schmidt, 2012). Such visualizations make interpretation of the shape variations more intuitive, and may allow identification of localized areas within a configuration where the most variation occurs (Pavlinov, 2001).

Geometric morphometrics has been widely used to address several questions in the field of anthropology (as will be discussed later in this chapter). These studies show that this new approach enables the detection of specific areas of variation between groups (e.g. sexes) which are often difficult to detect or describe using metric or non-metric methods, and it is also more objective, and occasionally also more accurate, than these methods (Franklin *et al.,* 2005; Pretorius *et al.,* 2006; Bidmos *et al.,* 2010; Bigoni *et al.,* 2010). This creates a whole new field of potential study which can serve to both standardize and evaluate existing techniques, and develop novel techniques of assessing demographic parameters such as age, sex and ancestry. These improvements in the reliability and accuracy of anthropological techniques would, in

turn, make them more suitable for medico-legal application, especially in the light of the recent shift in expert witness testimony from being based on ~~–e~~xpertise" to now being based more on reliable and tested principles and methodology, as suggested by the Daubert ruling by the US Supreme Court (Scheuer, 2002; Christensen & Crowder, 2009) and the subsequent Scientific Working Group for Forensic Anthropology (SWGANTH) guide to best practices.

2.3.2. Previous geometric morphometric applications in anthropology

Geometric morphometrics has been used to quantify morphology since the late 1980s, and the different components of the analysis were developed to address the specific interests and needs of each research group, which at this early stage was primarily aimed at improving taxonomic delineation (Kendall, 1981; Bookstein, 1989; Rohlf & Slice, 1990; Rohlf & Marcus, 1993; Slice, 1993; Rohlf, 1998). In the late 1990s, geometric morphometrics began to gain popularity in physical anthropology. Initial studies included systematic and taxonomic analyses (Delson *et al.,* 2001; Singleton, 2002; Guy *et al.,* 2003; Harvati, 2003a), morphological evolution and phylogenetic assessment (Lockwood *et al.,* 2002 and 2004; Bastir & Rosas, 2005; Nicholson & Harvati, 2006), and examination of morphological patterns of growth (O'Higgins & Jones, 1998; Viðarsdóttir *et al.,* 2002; Mitteroecker *et al.,* 2004). With the continued development and easier access to powerful computers, digitizers or scanners, and freely available and more user-friendly software such as MorphoJ®, Morpheus® and Morphologicka®, the use of geometric morphometrics has expanded exponentially within the anthropological research field (Richtsmeier *et al.,* 2002; Von Cramon-Taubadel *et al.,* 2007; Webster & Sheets, 2010; Hochstein, 2014). In fact, the three main parameters commonly assessed in an anthropological analysis of skeletal remains, namely sex, age and ancestry, have all been studied to some extent using this new approach.

2.3.2.1. Sex estimation

Sex estimation is one of the most important steps in the analysis of human skeletal remains, especially since techniques used for estimation of other parameters such as stature and age may vary depending on the sex of the individual being assessed (Krogman & İşcan, 1986; France, 1998; Bidmos *et al.,* 2010). The accuracy of sex estimation depends on the degree of sexual dimorphism present in the population, individual, and skeletal element being examined.

Sexual dimorphism manifests in two general forms (Scheuer, 2002). Firstly, males tend to have larger and more robust skeletal elements than females, both as a result of the higher levels of testosterone in males which result in greater muscle mass, as well as the longer growth period experienced by males towards the end of puberty (Bulygina *et al.,* 2006; Rösing *et al.,* 2007). Secondly, male pelves are adapted mainly for bipedal locomotion, with higher and narrower pelves to optimize the efficiency of weight transfer through the pelvic girdle (Bruzek & Murail, 2006; Kurki, 2007). Female pelves have the added requirements for obstetric adequacy acting on the girdle, making it wider and shallower (Scheuer, 2002; Bruzek & Murail, 2006). Based on this, it is logical that the most commonly assessed areas used for sex estimation are the pelvis and cranium, followed by the larger long bones such as the humerus and femur (DiBennardo & Taylor, 1979; Frutos, 2001; Bass, 1995; White *et al.,* 2012).

Non-metric methods of sex estimation have high levels of reported accuracy , for example 92 – 95% reported by Krogman & İşcan (1986), and 91 – 99% reported by Loth & Henneberg (1996). Unfortunately, subsequent validation tests of these methods seldom achieve the same level of accuracy as the original studies (Oettlé *et al.,* 2005; Spradley & Jantz, 2011). Some studies argue that metric assessments of bones are more reliable (with accuracies exceeding 80%), because they employ more sophisticated multivariate statistical analyses (Rightmire, 1971; Dayal *et al.,* 2008; Spradley & Jantz, 2011). The literature shows an extensive collection of metric studies performed using several diverse populations and assessing virtually all skeletal elements, including the bones of the upper limb (Berrizbeitia, 1989; İşcan *et al.,* 1998; Charisi *et al.,* 2011), lower limb (İşcan & Miller-Shaivitz, 1986; Tise *et al.,* 2013; Spradley *et al.,* 2015), pelvis (Patriquin *et al.,* 2005), vertebrae (Marino, 1995), ribs (İşcan & Loth, 1986), and the cranium or parts thereof (Suazo *et al.,* 2009a; Konigsberg *et al.,* 2009; Singh & Talwar, 2012).

Despite the fact that metric and non-metric methods of sex estimation produce high levels of reported accuracy, these approaches often fail to quantify the shape, rather than size differences of sexually dimorphic features. This becomes problematic when analysing skeletal material of mixed population origin, where females of one population may be larger or more robust than the males of another population (Bass, 1995), or when the degree of expression of sexual dimorphism within a population is

low (Hrdlička, 1939; Tobias, 1974; Alunni-Perret *et al.,* 2008). In such instances, size-independent shape analysis, such as geometric morphometrics, may provide useful insights.

As mentioned above, sex estimation is an important first step in the analysis of skeletal remains, and thus it is not surprising that it is the most common focus of geometric morphometric analyses in anthropology. Geometric morphometric studies of the mandible (Franklin *et al.,* 2007; Oettlé *et al.,* 2009), cranium (Green & Curnoe, 2009; Gonzalez *et al.,* 2011; Franklin *et al.,* 2012), humerus (Kranioti *et al.,* 2011; Vance & Steyn, 2013), tibia (Brzobohatá *et al.,* 2014 and 2016), femur (Purcell, 2013), pelvis (Steyn *et al.,* 2004; Pretorius *et al.,* 2006), and scapula (Scholtz *et al.,* 2010) have been performed. Some of these studies, such as those of Steyn *et al.* (2004) and Oettlé *et al.* (2009) yielded particularly interesting results, demonstrating that features (such as gonial eversion and the shape of the greater sciatic notch) which are widely used for sex estimation are not as reliable as previously reported. In a study of the greater sciatic notch, Pretorius *et al.* (2006) reported an average sex classification accuracy of 87% when using the geometric morphometric approach, compared to 75% using visual assessment of the same feature as reported by Patriquin *et al.* (2003). On the opposite end of the spectrum, studies like those of Pretorius *et al.* (2006) and Bigoni *et al.* (2010) have also shown that features, like the shape of the orbits, which have previously been overlooked or have low reported accuracy (48 – 66%) in metric sex assessments (Dayal *et al.,* 2008; Saini *et al.,* 2011), can yield high accuracies (>70%) when assessed using geometric morphometrics.

Many studies caution the application of sex estimation techniques to non-adult individuals, since secondary sexual development has not yet taken place and sexual dimorphism in such individuals may be difficult to assess (Mittler & Sheridan, 1992; Bogin, 1999). Again, geometric morphometric studies have shown high accuracy by assessing cranial dimorphism which could not be detected using metric or non-metric methods, with studies like those of Viðarsdóttir (1999) and Bulygina *et al.* (2006) showing dimorphism at the very early stages of ontogeny. The increased sensitivity provided by geometric morphometric analysis further makes it ideal for use in populations with low levels of sexual dimorphism, potentially as a result of reduced

sexual differences in labour and physical activity or nutritional stress (Ruff, 1987; Stini, 1975; Bogin, 1999; Charisi *et al.,* 2011).

Geometric morphometric assessment of sexual dimorphism of various skeletal elements has the potential to allow detection of new features for use in sex assessment. It is, however, important to remember that such assessments, like those of the traditional metric approaches, are still subject to population-specificity and potentially also temporal changes (Buretić-Tomljanović, 2006; Jonke *et al.,* 2007). It is thus vital that studies are performed to establish regional standards of assessment, and that these methods should continuously be updated to remain relevant to the population to which they are applied (Steyn & İşcan, 1997; Alunni-Perret *et al.,* 2008; Steadman, 2013).

2.3.2.2. Population structure and history

Many modern anthropologists consider racial classification as problematic in the study of human variation. This idea is supported by the lack of a genetic basis for defining ~~"r~~ace" (Royal & Dunston, 2005; Edgar & Hunley, 2009). However, geographic patterning due to gene flow among groups which live close to each other can be detected in skeletal remains in the frequency of occurrence of certain morphologies (Jorde & Wooding, 2004; Ousley *et al.,* 2009; Relethford, 2009). Knowledge of such geographic patterning in morphology may allow anthropologists to classify a skeleton according to broad ancestral groups (Ousley *et al.,* 2009; Relethford, 2009). This approach is most commonly applied in the forensic context, where ancestry estimations serve only to reduce the search parameters used in attempts to identify unknown individuals (White *et al.,* 2012; Christensen *et al.,* 2014b; King, 2015). It is important to remember that while ancestry estimation is based on biological differences among groups, morphology is greatly complicated by non-biological factors such as political and cultural influences on the ~~"m~~ixing" of groups, making ~~"a~~ncestry" more of a social than a biological construct (Sauer, 1992; Edgar & Hunley, 2009; Morris, 2010).

Metric and non-metric estimation of ancestry from skeletal material has favoured the skull, and especially the mid-facial region (White *et al.,* 2012; DiGangi & Hefner, 2012). The use of metric assessments of cranial dimensions is widespread, despite the debate surrounding the applicability of such methods (Howells, 1995; İşcan & Steyn,

1999; Buretić-Tomljanović *et al.,* 2006). The use of lists of non-metric features of the skull is also popular (e.g. Rhine, 1990; Hefner, 2002 and 2003; Byers, 2004), but is often criticized for supporting the idea of typology and ignoring the common occurrence of features in several population groups (Relethford, 2009; DiGangi & Hefner, 2012). Studies of the postcrania are less common, likely due to the expectation of less variation existing among populations and thus reduced accuracy of ancestry estimations based on these elements (Christensen *et al.,* 2014b). The few studies of postcranial elements for ancestry estimation include assessments of features such as femur curvature, size or sub -trochanteric shape (Stewart, 1962; St Hoyme & İşcan, 1989; Seidemann *et al.,* 1998; Wescott, 2005), the occurrence of bifid spines of cervical vertebrae (Duray *et al.,* 1999), and the dimensions of the innominate (Patriquin *et al.,* 2002). The results of these studies are highly variable, depending on the nature and quantity of features assessed, statistical approaches used, and heterogeneity of the populations or groups studied (Alunni-Perret *et al.,* 2008; Bidmos *et al.,* 2010; L'Abbé *et al.,* 2013). Even sophisticated programs such as FORDISC® or 3D-ID may produce ambiguous results when used for ancestry estimation, with the differences in definitions of measurements and/or mixture of biological, ethnic and national definitions of groups likely contributing to the issue (Ramsthaler *et al.,*2007; Komar & Buikstra, 2008; Ousley *et al.,* 2009).

Besides the difficulties associated with terminology, previous methods are also often unable to produce acceptable levels of accuracy when attempting to differentiate among groups with complex genetic histories (Ross *et al.,* 2004). With the dynamics of modern populations which are becoming more heterogeneous (Alunni-Perret *et al.,* 2008), and increased population growth of admixed populations such as Hispanic and Coloured individuals (Lisker *et al.,* 1996; Spradley & Jantz, 2005; Tishkoff *et al.,* 2009; De Wit *et al.,* 2012), this is becoming an increasingly important consideration in anthropological studies. An even more complex challenge when using existing metric and non-metric methods is that the standards produced are very population-specific and when standards developed based on one population are applied to an unknown individual of another population , it can lead to gross misclassification of the unknown individual (Steyn & İşcan, 1997; Asala *et al.,* 2004; Franklin *et al.,* 2005). Even within a population, phenomena such as secular changes in nutrition, living conditions and socio-economic conditions may cause a sample of a specific population to be vastly different from a sample of the same population taken from a

different time period (Hamilton, 1982; Jantz & Meadows-Jantz, 2000; Buretić-Tomljanović *et al.,* 2007), even when considering the dimensions of individual bones (Henneberg & Van den Berg, 1990; Jonke *et al.,* 2007).

Geometric morphometric analyses have been widely employed in studies of biological distance in terms of population structure and history (e.g. Hennessy & Stringer, 2002; Kuroe *et al.,* 2004; Bastir & Rosas, 2006; Chang *et al.,* 2014). Many studies were able to test the congruency of morphology and genetic information to answer questions related to the peopling of the Americas (Martinez- Abadías *et al.,* 2006; Perez *et al.,* 2009; Humphries *et al.,* 2015), the usefulness of certain cranial units in providing information about population history (Harvati & Weaver, 2006a; Smith, 2009; Von Cramon-Taubadel, 2011), and the influence of environmental conditions on morphology (Smith, 2009; Weisensee & Jantz, 2011; Brzobohatá *et al.,* 2014; Noback & Harvati, 2015).

Similar to its impact on sex estimation techniques, geometric morphometrics has also improved ancestry estimation techniques, primarily by improving the accuracy of distinguishing groups from each other, with several studies reporting classification accuracies exceeding 80% even when evaluating morphological variations of constrained anatomical areas among groups (Buck & Viðarsdóttir, 2004; Sholts *et al.,* 2011; King, 2015). Direct comparisons of classification accuracies using either traditional and geometric morphometric approaches applied to the same sample confirm this (Spradley & Jantz, 2016), with studies like that of Stull *et al.* (2014) reporting a 5% improvement when using the morphometric approach on a South African sample. Furthermore, because of its increased sensitivity to shape variation between groups, geometric morphometrics has enabled the detection of previously overlooked differences, and their use for accurate classification of groups which could not be distinguished before when using metric approaches. This is most evident in the work of Ross *et al.* (2004) and Duecker (2014) which demonstrated that the term ―Hispanic" is too broad to accurately capture the within group variation of Hispanic individuals, but that the different sub-groups of Hispanics could be differentiated from each other because of their unique population histories. Similar studies by Franklin *et al.* (2007), Badawi-Fayad & Cabanis (2007) and Ross *et al.* (2011) have shown that, despite a lack in significant size differences between groups such as the Khoi-khoi and San, or Europeans and European-Americans, these groups

can be reliably separated (with reported accuracies of up to 100%) according to their shape differences. While geometric morphometric analyses are unfortunately still susceptible to secular changes, such analyses can also be employed to detect small-scale differences between samples of the same population from different time periods and even allow assessment of the role of admixture on morphology as a consequence of colonization of a particular geographical region (Ross *et al.,* 2011; Weisensee & Jantz, 2011; Humpries *et al.,* 2013).

Lastly, the application of geometric morphometrics to the investigation of ancestry estimations have also allowed the re-evaluation of different areas of the skeleton, showing that some areas are more reliable than others (Steyn *et al.,* 2004; Smith, 2009; Sholts *et al.,* 2011; Spradley & Jantz, 2016), and that population differences can be detected even in the earliest stages of ontogeny (Viðarsdóttir *et al.,* 2002), which was previously thought to be "virtually impossible" (St Hoyme & İşcan, 1989; Scheuer & Black, 2000).

2.3.2.3. Growth and development

Studies of ontogeny and allometric trajectories are important not only for the information it provides about morphological variation during growth and development, but also the potential to identify forces responsible for morphological variations among individuals (McKeown & Schmidt, 2012). The main challenge facing such studies is the fact that differences in size among individuals of different ages often obscure differences in shape, which are often more informative (Richtsmeier *et al.,* 2002). Forensically, knowledge about the development and eventual deterioration of skeletal features can be employed to develop age estimation techniques (Buikstra & Ubelaker, 1994; Albert *et al.,* 2007). It is widely accepted that age estimation of non-adults is more accurate than that of adults, since the techniques used are mostly based on the highly regulated formation and fusion of different skeletal elements, as opposed to the highly variable deterioration of features (under even more varied environmental influences) classically used for adult age estimations (İşcan, 2001; Scheuer, 2002; Cattaneo, 2007). As a result, different parts of the skeleton can appear to age at different rates among individuals and even within the same individual (Franklin, 2010). To reduce the effect of this variation, anthropologists recommend using a multi-factorial approach when assessing age (Baccino *et al.,* 1999; Uhl, 2012). The efficiency of a multi-factorial approach is,

however, still debated, as there is no consensus regarding how to combine the estimates obtained using multiple techniques (Martrille *et al.,* 2007; Uhl, 2012) or account for the accumulation of estimation biases of the different techniques (Bocquet-Appel & Masset, 1982). Currently, the majority of adult age estimation techniques in use are based on ordinal classification of features such as the pubic symphysis, auricular surface, or sternal rib ends (Baccino *et al.,* 1999; İşcan, 2001; Uhl, 2012; White *et al.,* 2012). Unfortunately, the majority of these methods subsequently produce only wide estimated age ranges of ±10 years (Rösing *et al.,* 2007; Franklin, 2010).

The complicating factor of the influence of size on observations of the growth and development of skeletal features can be reduced using the geometric morphometric approach, which specifically involves scaling of all specimens to a common size (Viðarsdóttir *et al.,* 2002; Braga & Treil, 2007; Smith *et al.,* 2013). Geometric morphometrics has been employed to investigations of the patterns of human skeletal growth and development in comparison to that of other species (Mitteroecker *et al.,* 2004), and in relation to the development of differences between sexes or ancestral groups during ontogeny (Viðarsdóttir *et al.,* 2002; Bastir *et al.,* 2006; Gonzalez *et al.,* 2010; Pujol *et al.,* 2016). Very few studies, however, have explored the use of geometric morphometrics to assess age-related changes in the skeleton. Studies like those of Zollikofer & Ponce De Leon (2002) and Franklin *et al.* (2008) have explored several areas of the skeleton which could be used for age estimations, though the results were mixed, with one study reporting 1 – 74% accuracy in separating individuals based on the classifications of young/middle age/old using different cranial regions (Williams & Slice, 2010), and another reporting 60 – 88% based on the morphology of the femur and tibia (Stevens & Viðarsdóttir, 2008).

No geometric morphometric studies have yet been done on age-related changes in the adult skeleton. It is widely accepted that skeletal growth (and thus size increases of the elements) is completed in late adolescence or early adulthood (Buikstra & Ubelaker, 1994; Scheuer & Black, 2000), but remodelling of the elements have been shown to continue throughout an individual‘s lifespan (Hunter & Garn, 1972; Evans, 1976; Israel, 1977). It is thus possible that geometric morphometrics may allow the detection of previously overlooked age-related shape changes in the skeleton,

especially due to its increased sensitivity to small localized variations (Franklin *et al.,* 2006; Green & Curnoe, 2007).

2.3.2.4. Future geometric morphometric studies

The use of geometric morphometrics in the field of anthropology is still constantly evolving as new analyses and methodologies are added to its powerful toolkit (Adams *et al.,* 2004; Slice, 2007; Webster & Sheets, 2010). This also means that advancements on existing geometric morphometric studies can be made. Many studies have successfully used the technique on images of skeletal elements, either in the form of standardized photographs (Martinez- Abadías *et al.,* 2006; Vance & Steyn, 2013), radiographs (Lynch *et al.,* 1996; Perlaza, 2014), or CT scans (Franklin *et al.,* 2012; Morita *et al.,* 2012; Jantz *et al.,* 2013). This is mainly for practical purposes, but the increased availability of the equipment needed for capturing landmark data in three-dimensions, however, will allow future studies to capture data from the original specimen, giving more realistic representations than two-dimensional renderings are able to achieve (Jonke *et al.,* 2007; Adams *et al.,* 2013).

The increased use of geometric morphometrics will also allow the further study of more skeletal elements. Thus far, studies have mostly investigated the cranium as a whole (Ross *et al.,* 1999; Bastir *et al.,* 2006; Kimmerle *et al.,* 2008; Green & Curnoe, 2009), or constrained regions of it, such as the facial and temporal regions (Hennessy & Stringer, 2002; Harvati, 2003a and b; Bulygina *et al.,* 2006; Smith *et al.,* 2013). A few studies have also investigated the more morphologically complex postcranial elements such as the vertebrae (Albert *et al.,* 2003; Chatzigianni & Halazonetis, 2009), pelvis (Pretorius *et al.,* 2006; Gonzalez *et al.,* 2009), and scapula (Scholtz *et al.,* 2010). While these studies all contribute to the study of human skeletal variation, their applicability to forensic cases is limited due to the often poor recovery and preservation of these bones.

Lastly, despite all of the benefits geometric morphometric analysis provides, the standards obtained in the assessment of skeletal remains are still subject to population-specificity, as previously discussed. It is therefore necessary that extensive studies of the shape variation of several individual skeletal elements should be performed and assessed using forensically informative demographic parameters such as sex, age and ancestry. As recommended for metric data (Steyn & İşcan, 1997;

Mall *et al.,* 2000), the data obtained through geometric morphometrics should be gathered from a specified regional population or sample, and be constantly re-evaluated and updated in order to remain relevant to the population to which the developed estimations will be applied. The further application of geometric morphometric analyses to common anthropological questions creates a whole new field of potential study which would serve to both standardize and evaluate older techniques, and develop novel techniques of assessing demographic parameters from skeletal remains. These improvements would, in turn, make the techniques more suitable for application in medico-legal contexts, especially with the recent shift in forensic testimony from ―expertise" to more reliable and tested principles and methodologies (Scheuer, 2002; Christensen & Crowder, 2009).

2.4. The South African context

South Africa has a population of approximately 51.8 million individuals, of which the majority self-identify as Black (79.2%), Coloured (8.9%) or White (8.9%) (Statistics South Africa Census, 2011). Although ―race" is no longer a recorded category in the South African Population Register, many South Africans continue to identify each other along the racial classifications (using ―folk taxonomy") of the previous government system (Friedling & Morris, 2005). It is thus important for the forensic anthropologist to translate the observed biological information into the culturally constructed labelling system which was likely applied to the individual in question during their life (Sauer, 1992; Buck & Viðarsdóttir, 2004).

There is archaeological and genetic evidence that Black South Africans are the descendants of Bantu-speaking groups from West and East Africa which migrated to Southern Africa approximately 3000 years ago (Newman, 1995; Badenhorst, 2008; Henn *et al.,* 2008; Ribot *et al.,* 2010). Since then, many of these groups have mixed with each other and the indigenous Khoisan groups, as well as divided into even smaller ―tribal" groups, resulting in a vast number of subgroups which now live across Southern Africa (Newman, 1995; Hammond-Tooke, 2000). Many of these subgroups self-classify according to language and/or culture. However, the literature shows that these subgroup distinctions in South Africa are slowly disappearing due to Westernization and gene flow between groups, and that the different subgroups are very homogenous in assessments of both crania (De Villiers, 1968a and b; Franklin *et*

al., 2005 and 2007) and postcrania (Lundy, 1983). White South Africans are mainly of European descent, primarily from individuals arriving in the country from the Netherlands, England, France and Germany in the early years of colonization (Steyn & İşcan, 1998 and 1999).

In South Africa, the term "Coloured" is used to refer to a widely varied socio-cultural group of mixed ancestry. This group is the product of genetic admixture of the European settlers and the indigenous populations of the Cape, and later also the slaves which were imported from India and other parts of Africa (Adhikari, 2005; Petrus & Isaacs-Martin, 2012). Genetic studies have shown that this group has the "highest levels of intercontinental admixture of any global population group" (Tishkoff *et al.,* 2009; De Wit *et al.,* 2010). The shape variation within this group (irrespective of how large this variation is expected to be) and between this group and the other ancestry groups is worth investigating for potential forensic applications, especially since this group is unique to South Africa and constitutes over 50% of the Western Cape provincial population (De Wit *et al.,* 2010; Patterson *et al.,* 2010; Petersen *et al.,* 2013).

The need for anthropological studies in South Africa is greatly driven by the current socio-economic conditions in the country (Steyn *et al.,* 1997; L'Abbé & Steyn, 2012; Bernitz *et al.,* 2015). There has been a great increase in unnatural deaths in the country, which can be attributed to the interlinked factors of urbanization, unemployment, poor education, poverty, disease, past and present political conflict, as well as the influx of individuals (both legally and illegally) from other countries (Steyn *et al.,* 1997; Norman *et al.,* 2007). This has resulted in a large increase in the number of unidentified remains recovered and requiring anthropological analysis, and has sparked great interest in forensic anthropological research in the country (Steyn *et al.,* 1997; Gordon, 2011; L'Abbé & Steyn, 2012).

In South Africa, forensic pathologists and medically qualified forensic anthropologists are regulated by the Health Professions Council of South Africa (HPCSA), but there is no statutory organization governing the other disciplines within forensic anthropology (Bernitz *et al.*, 2015). As such, most of the research and practice of forensic anthropology in the country is primarily performed by laboratories associated with universities such as those of Cape Town and Pretoria

(Morris, 2010; Bernitz *et al.,* 2015). Due to the lack of agreed upon methodological approaches and the different social classifications of ancestry groups (i.e. two versus three ancestral group classifications) in the different areas of the country, the research focuses of these institutions vary greatly, especially when dealing with complex aspects of forensic anthropology such as ancestry estimation (Morris, 2010).

Aside from the socioeconomic motivations for studies of South African individuals, an even more important factor to consider is that of population-specificity. Several studies like that of L'Abbé *et al.* (2013) have shown that the application of anthropological techniques based on North American or European samples to South African individuals may lead to misclassifications of sex and ancestry. While many South African individuals are the descendants of European settlers, African migrants and indigenous populations, factors such as founder's effect and genetic admixture with indigenous and migrant slave groups have lead to these individuals having quite different skeletal morphology than their ancestors (Steyn & İşcan, 1998 and 1999). The unique Coloured population of South Africa also requires special consideration. As a result of their diverse genetic history and also the complicating influences of extrinsic factors such as socio-economic, health and nutritional conditions, this group cannot simply be treated as an intermediate (either in terms of genetics or morphology) to the Black and White South African groups. Previous South African anthropological studies have often chosen to exclude Coloured individuals from their samples either due to sample availability in certain parts of the country (L'Abbé & Steyn, 2012) or due to the belief that the amount of variation within the Coloured population is too large to be considered as a distinct ~~"b~~iological" group (Morris, 2010). It has, however, been shown that Coloured individuals are morphologically distinct from other South African ancestral groups despite the highly variable nature of their skeletal morphology and can be distinguished from the Black and White ancestral groups with high accuracy (Stull *et al.*, 2014). Further assessment of the morphological variation within this group, as well as comparisons to other contemporary South African groups is thus warranted.

Even within the South African population as a whole, phenomena such as secular changes in morphology and due to influences such as living conditions (which have changed significantly since 1994) may cause a contemporary sample of this

population to differ greatly from a historic sample of the same population, and thus needs to be investigated (Hamilton, 1982; Alunni-Perret *et al.,* 2008).

In the last few decades, there have been several metric and non-metric studies of South African individuals in the country‘s documented skeletal collections. These studies have examined a wide range of skeletal elements such as the skull and mandible (Rightmire, 1971; Kieser & Groeneveld, 1986; Franklin *et al.,* 2005), long bones of the upper and lower limbs (Kieser *et al.,* 1992; Steyn & İşcan, 1997, 1998 and 1999; Vance *et al.,* 2011; Siddiqi, 2013), and other postcranial elements such as the talus and calcaneus (Bidmos & Asala, 2003; Bidmos & Dayal, 2003). A few studies employing geometric morphometrics have also been published, focussing on the crania of indigenous groups (Franklin *et al.,* 2006), subadult mandibles (Franklin *et al.,* 2008), pelvis (Steyn *et al.,* 2004) and humerus (Vance & Steyn, 2013). Many of these studies confirm that South African individuals require unique standards when their skeletal remains are used to evaluate demographic parameters, especially when dealing with cases suspected to involve individuals of mixed ancestry, such as the unique Coloured population (Adhikari, 2005; Tishkoff *et al.,* 2009; De Wit *et al.,* 2012).

South Africa has become one of the leaders in anthropological research regarding human skeletal variation and forensic anthropology, driven mainly by the availability of large documented skeletal collections of diverse local population groups, as well as through international collaborations (Bernitz *et al.,* 2015). To maintain this status, it is vital that researchers continue to focus on the development of new analytical techniques and re-evaluation of old techniques, and the application of such techniques both in South Africa and internationally.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study sample

Only adult South African individuals, for whom sex, age and ancestry were recorded in the collection accession registers, were selected for the present study. The skeletal remains used in this study were obtained from the skeletal collections of the Universities of Cape Town (UCT), Stellenbosch, Pretoria and Witwatersrand. For all of these collections, the remains are those of donated individuals or unclaimed (but identified) individuals from public hospitals in the surrounding areas, as regulated by the *National Health Act* (Act No. 61 of 2003) and the *Human Tissue Act* (Act No. 65 of 1983).

The demographic information of each individual was obtained from the accession registers of the skeletal collections used and was originally obtained from the Medical Certificate of Cause of Death (*Births and Deaths Registration Act*; Act no. 51 of 1992). Only individuals for which sex, age, and ancestry were available were used in this study. Differences in the terminology used by different collection registers to classify the ―race", ―ethnicity" or ―ancestry" were problematic. Collections such as that of UCT use only the classifications of ―Black", ―Coloured" or ―White", while others such as the Raymond A. Dart collection of the University of the Witwatersrand specify tribal associations (e.g. Zulu, Xhosa, Sotho etc.) for some of the Black individuals in their collection. It has, however, been reported that some of these tribal specifications are only inferred from the individual's surname or from contextual information if tribal classification was not reported on the death certificate (Tal & Tau, 1983). Due to the potential subjectivity of these terms, only classifications of Black, Coloured (including Mixed South African) and White were used for the present study.

3.1.1. Skeletal collections used

3.1.1.1. University of Cape Town Human Skeletal Collection

The University of Cape Town Human Skeletal Collection is housed in the Department of Human Biology at the University of Cape Town. The cadaveric part of

the collection was established in the 1980s, with the accessioning of cadaveric remains after the bodies had been used for dissection in the medical training program (Ginter, 2005; Da Silva, 2006). The collection currently houses the remains of approximately 350 cadaveric individuals. The majority of these individuals are acquired from the Western Cape Province, and specifically the public hospitals and old age homes in the Cape Town metropolitan area (Da Silva, 2006; Robinson & Bidmos, 2009). The majority of individuals in the collection are of older White individuals which were donated as bequeathments, though the collection also contains the remains of several Black and Coloured individuals, of which the majority are of ―unclaimed" or ―pauper" donations to the university (Da Silva, 2006).

3.1.1.2. The Kirsten Skeletal Collection

The Kirsten Skeletal Collection is housed in the Department of Anatomy and Histology at the University of Stellenbosch, and contains the largest skeletal collection of ―Cape Coloured" individuals in the world (Alblas, 2016). The collection was established in 1945, and currently has the remains of approximately 670 complete individuals between the ages of 18 and 103 years (Robinson & Bidmos, 2009). As with the UCT collection, most of these remains are of ―unclaimed" individuals from teaching hospitals in the Cape Town metropolitan area and surrounding towns, though the collection also contains a few individuals from other parts of South Africa (Alblas, 2016). Only remains of individuals for which demographic information was available, i.e. those whose remains had previously been used in the medical dissection training program, were included in the present study.

3.1.1.3. The Pretoria Bone Collection

The Pretoria Bone Collection is housed in the Department of Anatomy of the University of Pretoria and was established in 1943. Most of the approximately 1000 individuals in the collection are acquired as ―unclaimed" individuals from public hospitals in the surrounding Tshwane metropolitan area and the wider Gauteng Province (L'Abbé *et al.,* 2005). The bodies of these individuals have all previously been used in the medical dissection training program at the university before the skeletal remains were accessioned into the collection. The majority of the individuals in the collection are Black males, and the age range of individuals in the collection extends from a few months after birth to 100 years (Robinson & Bidmos, 2009). The

selection criteria for skeletons which can be accessioned into the collection is primarily a full record of the demographic information of an individual as obtained from the death certificate of the individual (L'Abbé *et al.*, 2005).

3.1.1.4. The Raymond A. Dart Collection of Human Skeletons

Commonly referred to as "the Dart collection", this collection is the oldest and largest documented skeletal collection in South Africa, and is housed at the School of Anatomical Sciences at the University of the Witwatersrand in Johannesburg since 1923. This collection consists of approximately 2600 cadaveric individuals, the majority of which are Black South Africans, but the collection also has several individuals from other countries (Dayal *et al.,* 2009). Most of the individuals added to the collection from approximately 1960 to 1990 are those of individuals from the Johannesburg area received through bequests (Robinson & Bidmos, 2009). The more recent additions to the collection, however, are mostly of "unclaimed" individuals whose bodies have previously been used in for medical dissection training at the university and then accessioned into the skeletal collection (Dayal *et al.,* 2009). The majority of these individuals are likely to have been migrant workers from rural areas outside of the Johannesburg region (Dayal *et al.,* 2009).

3.1.2. Inclusion/Exclusion criteria

The sample was selected to include adult individuals with the majority of long bone epiphyses fused, thus a minimum age of 20 years was used. No maximum age was set, but individuals showing any form of traumatic or pathological change to the bones of interest were excluded from the sample. Further, any bones with post-mortem damage to the areas of interest were also excluded.

3.1.3. Sample summary

Studies like those of Komar & Buikstra (2008) and Komar & Grivas (2008) have shown that cadaveric collections may not be good representations of the larger populations from which they originate due to age, sex, ancestry and socio-economic biases introduced by the methods in which remains are acquired for skeletal collections (L'Abbé *et al*., 2005). However, using identified individuals from forensic cases as a study sample may also not provide a suitable sample size or reasonable representation of the larger population. Logistically, using a forensic sample would also be difficult in this context, as such remains are not covered by the *Human Tissues Act*, but by the *Inquests Act* (Act No. 58 of 1959) and would require

further application for permission of their use. It was thus decided to use only cadaveric individuals for this study and, to reduce sampling bias, the study sample was obtained from the different skeletal collections from different regions in South Africa, covering a wide range of age groups within each of the three largest ancestral groups according to the Statistics South Africa Census (2011).

A total of 1132 individuals were examined for the present study. The summary of the demographic composition of the total sample is given in Table 3.1. The sample included individuals with a recorded year-of-birth from 1887 to 1992, to allow a sufficiently large cohort to evaluate potential temporal trends present in the sample. The age-at-death of the individuals in the sample ranged from 20 to 100 years, with a mean age of 54 years. Preservation of the different skeletal elements differed among individuals due to damage to the bones as a result of the medical training dissection processes used by each university, and potential accidental damage or loss to the bones as a result of long term storage and handling. Even the different parts of the cranium were not equally preserved (Table 3.2). The process of medical dissection of the cranium involved the removal of the calvarium through sawing through it. The associated damage to the cranial bones, especially the frontal and occipital bones, often resulted in the loss of the areas where landmarks selected for this study are located, and could thus not be included in the sample. The landmarks on the temporal bones were less affected by this damage, thus it was possible to capture the landmark data of this element for more individuals. For bilateral elements, some individuals had the elements of only one side available for assessment, thus the sample of the two sides averaged is not simply half of the total sample for each of these elements. The elements of the lower limb were the most incomplete, with especially the fibula having a much smaller sample size than the other postcranial elements.

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

	Black	**Coloured**	**White**	**Total**
Female	187	144	170	501
Male	157	261	213	631
Total	344	405	383	**1132**

Table 3.2: Sample sizes of each skeletal element according to sex, ancestry and sex-ancestry groups.

	Sexes		Ancestry groups			Sex-ancestry groups						Total sample	Sides averaged
	Female	Male	Black	Coloured	White	Black females	Coloured females	White females	Black males	Coloured males	White males		
Cranial elements													
Whole	354	420	283	271	220	160	104	90	123	167	130	774	-
Frontal	356	421	287	270	220	162	103	91	125	167	129	777	-
Occipital	364	428	290	272	230	161	107	96	129	165	134	792	-
Parietal	367	434	290	278	233	162	107	98	128	171	135	1588	801
Temporal	375	449	300	287	237	165	108	102	135	179	135	1604	824
Upper limb													
Humerus	464	582	337	366	343	184	131	149	153	235	194	1971	1046
Radius	471	578	333	368	348	183	135	153	150	233	195	1935	1049
Ulna	465	578	334	361	348	183	131	151	151	230	197	1894	1043
Lower limb													
Femur	447	545	330	354	308	182	131	134	148	223	174	1843	992
Tibia	449	554	325	356	322	175	130	144	150	226	178	1835	1003
Fibula	412	507	309	323	287	170	122	120	139	201	167	1609	919

3.2. Data capturing

3.2.1. Landmark selection

Landmarks were chosen to be easily identifiable, repeatable, and to give a good representation of the shape of the skeletal element which they represent (Webster & Sheets, 2010). The cranial landmarks include some traditional metric landmarks and some used in previous geometric morphometric studies like those of Lockwood *et al.* (2002) and Franklin *et al.* (2006). Similar numbers of landmarks were chosen for all elements so that one element does not outperform another in the accuracy of classification of the demographic parameters simply because of that element having more landmarks, as suggested by Von Cramon-Taubadel (2009). A total of eleven to twelve landmarks were selected for each cranial element, and fourteen landmarks were chosen out of all cranial elements to represent the cranium as a whole for comparison purposes. The position and definitions of the selected cranial landmarks are shown in Figures 3.1. A – E, and are adapted from those defined by Martin & Saller (1957), Braüer (1988), Lahr (1992), Lieberman *et al.* (2000), Lockwood *et al.* (2002), Von Cramon-Taubadel (2009) and White *et al.* (2012).

A few studies, like those of Harmon (2007) and Kranioti *et al.* (2009) have suggested landmarks on postcranial elements, based on anatomical features they considered to best represent features expected to vary either between sexes or species. There are, however, no agreed upon defined landmarks for postcranial elements, as there are for the cranium. For the present study, it was decided to define landmarks on each postcranial element that are likely to differ between the sex and/or ancestry groups in the sample. For simplicity, landmarks were chosen to be easily identifiable without the use of traditional metric instruments, unlike those of Holliday & Friedl (2013). As suggested by Kranioti *et al.* (2009) and Brzobohatá *et al.* (2014 and 2016), the most identifiable landmarks, and often the most informative ones, are those which are located either at the ends of traditional measurements such as long bone lengths and widths, or at sites of muscle attachment. Considering all these suggestions, eight to nine landmarks were selected for each postcranial element, attempting to best capture the overall shape of the element. The position and definitions of the selected landmarks for each of the postcranial elements studied are shown in Figures 3.2. A – F.

3.2.2. Digitization of landmarks

The selected landmarks were marked on the bone before it was mounted in a stable position on a flat surface using modelling clay. In the case of the cranium, the cranium was positioned upside-down on a tripod stand and secured with modelling clay. A mirror was placed underneath the tripod, allowing capture of all cranial landmarks without having to reposition the cranium or requiring statistical –stitching" of landmarks. If the calvarium was cut for dissection purposes, the two parts were stuck together using masking tape.

Three-dimensional Cartesian coordinates of the selected landmarks were captured using a Microscribe® G2 3D digitizer (Immersion Corporation, San Jose, California, 2002). The configuration of landmarks of each skeletal element under study was digitized three times, and the Euclidean distance between these repeats was calculated. If this distance was larger than 1.0 mm, the entire set of landmarks was re-digitized until the distance was sufficiently small, as suggested by Terhune *et al.* (2007) and Smith *et al.* (2013).

3.2.3. Observer error assessment

To assess intra-observer error, 50 randomly selected specimens (with all elements of interest present) were re-digitized by the original observer three months after original digitization. For inter-observer error, 30 randomly selected specimens were re-digitized by an independent observer. The raw landmark coordinate sets were imported to Morphologika2® v2.5, and submitted to Generalized Procrustes Analysis (GPA) to superimpose all coordinate configurations to a common centroid. For each element studied, the between-group Procrustes chord distance between repeats of the same individual and among the different individuals was calculated and compared.

3.3. Data analysis

Raw landmark coordinates were entered into the program MorphoJ® (Klingenberg, 2011). Data were then submitted to GPA, which included reflection of sides, translation of landmark configurations to a common origin, scaling all coordinate configurations to unit centroid size (square root of sum of squared Euclidean distances between all landmarks and their centroid) in order to superimpose all specimen configurations to a common coordinate system, and rotation of

configurations to the optimal least-squares fit criterion (Nicholson & Harvati, 2006). For bilateral bones, Principal Components Analysis (PCA) was performed to assess whether there were significant differences between corresponding left and right elements of an individual. Unfortunately, information regarding handedness was not available for the sample, thus the influence of this could not be tested. If no significant differences were detected, data of the left and right sides were averaged and a new data set generated. PCA was also used to assess whether there were significant shape differences between the crania which had the calvarium cut during dissection and were taped together for this study, and those which were not cut for dissection. Unfortunately, it was not possible to test for the effect of cutting of the calvarium by digitizing the same sample of crania before and after cutting, due to the restrictions of the collections used. Lastly, PCA was used to assess potential shape differences the crania with teeth and those without teeth.

3.3.1. Sex

After GPA superimposition, GPA residuals were submitted to PCA to explore shape differences between sexes. PCA graphs were created to show the 90% probability ellipses of each sex, in accordance with the convention used by Smith *et al.* (2013), De Azevedo *et al.* (2015) and Rusk & Ousley (2015). The centroid sizes of males and females were compared to evaluate size differences between the means of the sexes. Data were then submitted to regression analysis to evaluate possible co-variation of (centroid) size, age and year-of-birth with the observed sample shape variation. All regression analyses were performed with pooled within-group variances to remove the potential effect of within-group variation before comparing groups. Each regression analysis was also performed with a permutation test of 10000 iterations to evaluate complete independence of variables.

The next step was to examine the separation of the *a priori* groups based on the observed shape variations, which was done using a Discriminant Function Analysis (DFA), which also calculated the Mahalanobis distance (MD) between sexes. MD is used as a measure of the distance between individuals from one group and the mean of another group, and is expressed in terms of the standard deviation of the latter group. It is important to note that the distance used in the present study (as calculated by the MorphoJ® software) is the Mahalanobis distance, and not the distance squared which is often reported. Using the MD allows the results to be interpreted as the

approximate distances scaled by the within-group standard deviations, giving an indication of the similarity or dissimilarity between groups (Klingenberg, 2011). The reliability of the separation of the sexes was assessed using a leave-one-out cross-validation test whereby each specimen is classified by the functions derived from the original sample excluding only that specimen. The results of the test represent the classification accuracy of the sex of each specimen when classified according to the functions derived from all other specimens in the sample. The cross-validation test was coupled with a permutation test of 10000 iterations to test for equal group means.

3.3.2. Ancestry

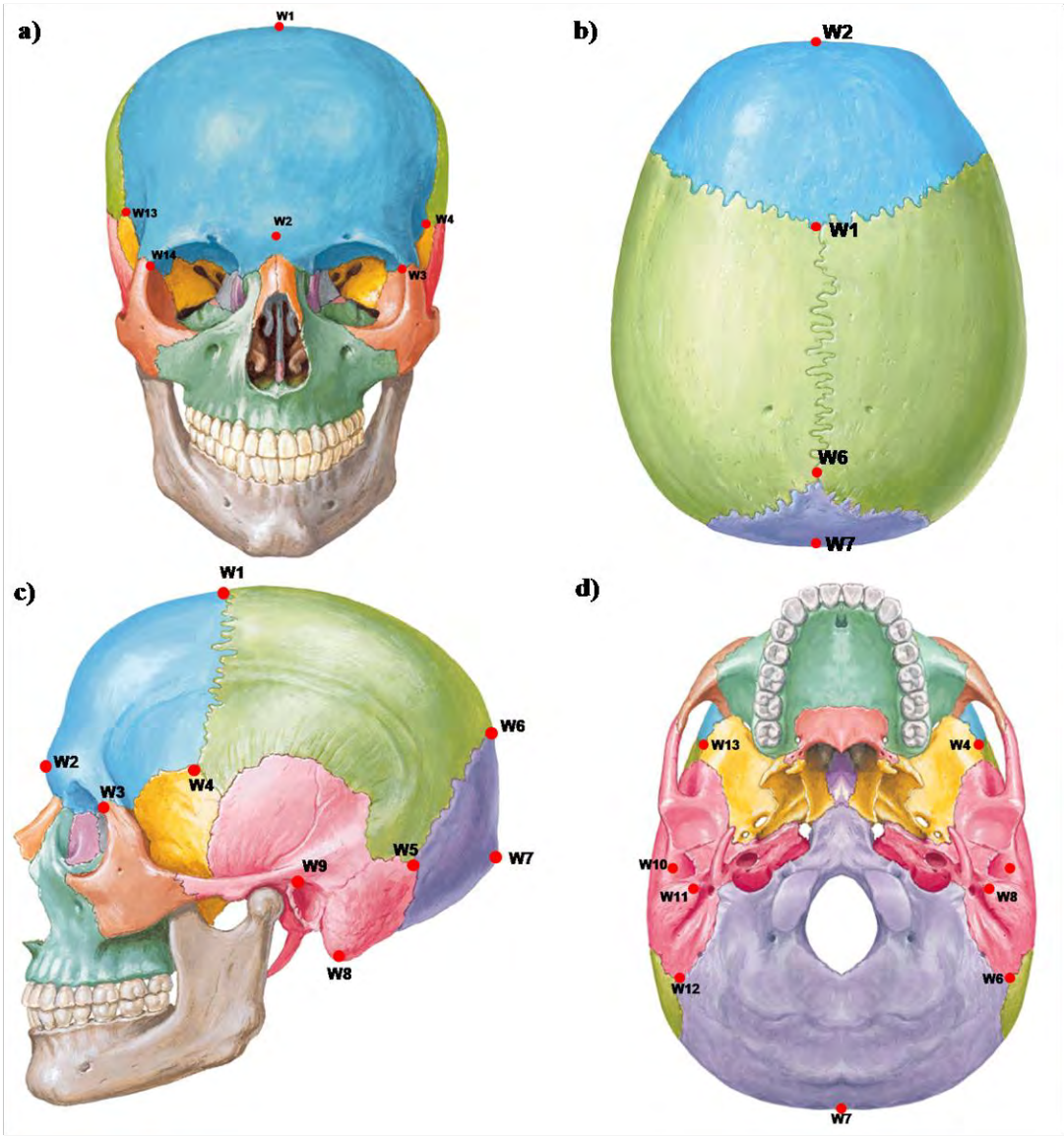
GPA residuals were submitted to a Canonical Variable Analysis (CVA) to explore which shape differences best distinguish ancestry groups from each other. The centroid sizes of the different groups were compared using Analysis of Variance (ANOVA) and Post-hoc Scheffé tests. Similar to the comparison of the sexes, pooled within-group regression analyses were performed to evaluate co-variation with size, age and year-of-birth, using a permutation test of 10000 iterations. A DFA was also performed for pair-wise comparisons of the three ancestry groups, and the associated Mahalanobis distance was used to assess group similarity. A leave-one-out cross-validation with a permutation of 10000 iterations was performed, allowing assessment of the classification accuracy of ancestry for each specimen based on functions derived from all other specimens in the sample.

3.3.3. Sex and Ancestry

To assess whether variation could be better described when ancestry and sex are considered together, the sample was divided in to six sex-ancestry groups (i.e. Black females and males, Coloured females and males, and White females and males). Analysis was done in the same manner as outlined for the ancestry groups above.

3.3.4. Accuracy assessment and comparison

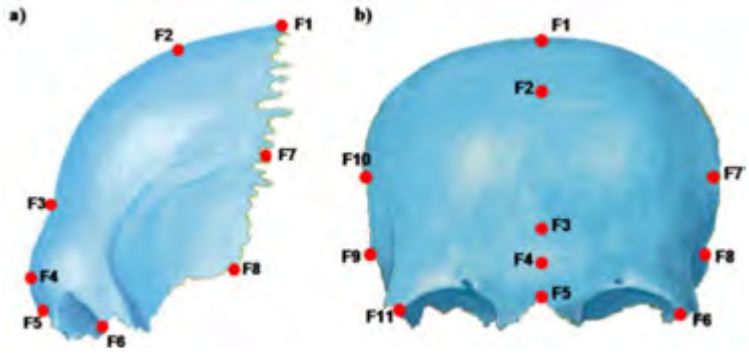
Differences in classification accuracy among the different bones examined were compared using Chi-squared tests using the leave-one-out cross-validated accuracies generated in the comparisons of the different sex, ancestry and sex-ancestry groups. Chi-squared tests were also used to assess whether there was significant association of the obtained accuracies with any of the classification groups used.



Name	Landmark	Landmark definition
Bregma	W1	Intersection of coronal and sagittal sutures
Glabella	W2	Most anterior point on the frontal bone on the supracilliary margin
Frontomalare orbitale	W3 (Left) W14 (Right)	Intersection of zygomatico-frontal suture and orbital margin
Sphenion	W4 (Left) W13 (Right)	Most anterior extent of the spheno-parietal suture
Asterion	W5 (Left) W12 (Right)	Junction of lambdoidal, parieto-mastoid and occipito-mastoid sutures
Lambda	W6	Intersection of sagittal and lambdoidal sutures
Opisthocranion	W7	Most posterior midline point, furthest from glabella
Mastoidale	W8 (Left) W11 (Right)	Most inferior and lateral point on the mastoid process
Porion	W9 (Left) W10 (Right)	Most superior point on the margin of the external auditory meatus

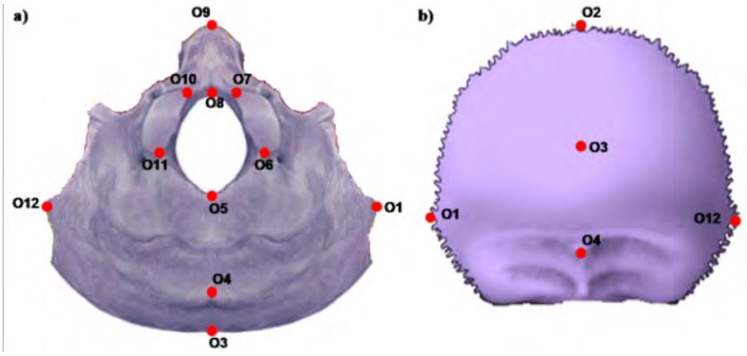
Figure 3.1A: Landmarks representing the whole cranium in a) anterior, b) superior, c) lateral, and d) inferior views; with definitions of landmarks.

[Images adapted from Hansen, 2014]



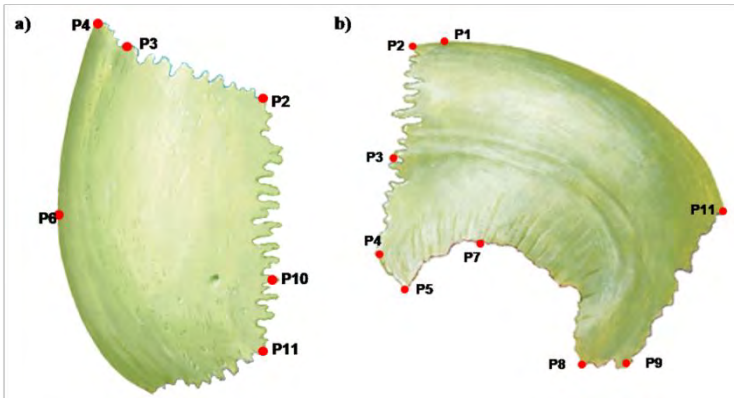
Name	Landmark	Landmark definition
Bregma	F1	Intersection of coronal and sagittal sutures
Midfrontal	F2	Approx. halfway between bregma and most superior point of frontal arch
Frontex	F3	Most inferior-posterior point on frontal bone (along midline) above glabella
Glabella	F4	Most anterior point on the frontal bone on the supracilliary margin
Nasion	F5	Intersection of naso-frontal suture and mid-sagittal plane
Frontomalare orbitale	F6 (Left); F11 (Right)	Intersection of zygomatico-frontal suture and orbital margin
Stephanion	F7 (Left); F10(Right)	Intersection of coronal suture and inferior temporal line
Sphenion	F8 (Left); F9 (Right)	Most anterior extent of the spheno-parietal suture

Figure 3.1B: Landmarks representing the frontal bone in a) lateral, and b) anterior views; with definitions. [Images adapted from Hansen, 2014]



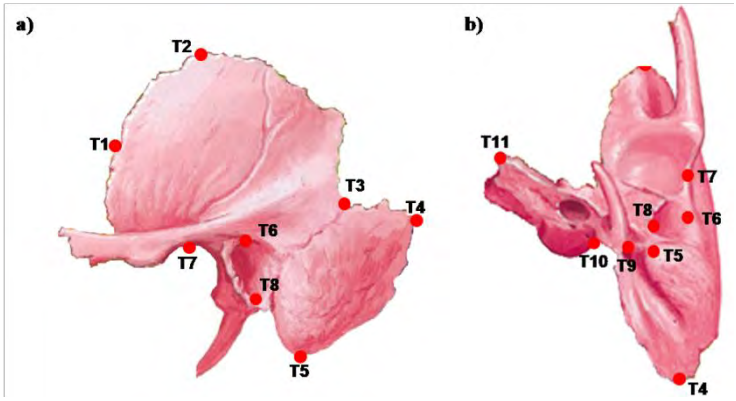
Name	Landmark	Landmark definition
Asterion	O1 (Left); O12 (Right)	Junction of lambdoidal, parieto-mastoid and occipito-mastoid sutures
Lambda	O2	Intersection of sagittal and lambdoidal sutures
Opisthocranion	O3	Most posterior midline point, furthest from glabella
Inion	O4	Point where superior nuchal lines merge in external occipital protuberance
Opisthion	O5	Intersection of posterior foramen magnum and mid-sagittal plane
Occipitocondyle (posterior)	O6 (Left); O11 (Right)	Most posterior point on the occipital condyle
Occipitocondyle (anterior)	O7 (Left); O10 (Right)	Most anterior point on the occipital condyle
Basion	O8	Intersection of anterior foramen magnum and mid-sagittal plane
Sphenobasion	O9	Midline point of the spheno-occipital suture

Figure 3.1C: Landmarks representing the occipital bone in a) inferior, and b) posterior views; with definitions. [Images adapted from Hansen, 2014]



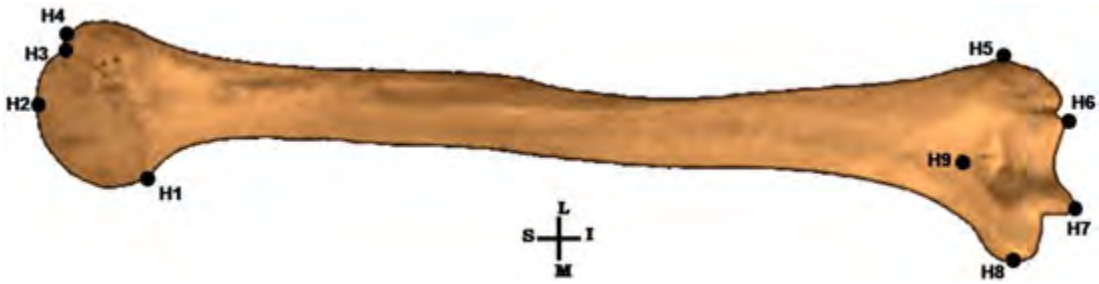
Name	Landmark	Landmark definition
Vertex	P1	Most superior point on sagittal suture
Bregma	P2	Intersection of coronal and sagittal sutures
Stephanion	P3	Intersection of coronal suture and inferior temporal line
Sphenion	P4	Most anterior extent of the spheno-parietal suture
Krotaphion	P5	Most posterior extent of the spheno-parietal suture
Euryon	P6	Ectocranial point of greatest cranial breadth
Spheno-squamosal apex	P7	Most superior point on squamosal suture (approx. directly above porion)
Entomion	P8	Junction of squamosal and parieto-mastoid sutures
Asterion	P9	Junction of lambdoidal, parieto-mastoid and occipito-mastoid sutures
Obelion	P10	Midline point between the parietal foramina
Lambda	P11	Intersection of sagittal and lambdoidal sutures

Figure 3.1D: Landmarks representing the parietal bone in a) superior, and b) lateral views; with definitions. [Images adapted from Hansen, 2014]



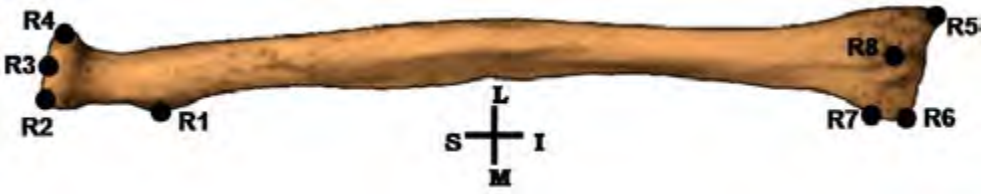
Name	Landmark	Landmark definition
Krotaphion	T1	Most posterior extent of the spheno-parietal suture
Spheno-squamosal apex	T2	Most superior point on squamosal suture
Entomion	T3	Junction of squamousal and parieto-mastoid sutures
Asterion	T4	Junction of lambdoidal, parieto-mastoid and occipito-mastoid sutures
Mastoidale	T5	Most inferior and lateral point on the mastoid process
Porion	T6	Most superior point on margin of the external auditory meatus
Articular eminence	T7	Midpoint of the tempero-mandibular articular surface lateral margin
External auditory meatus	T8	Most inferior point on margin of external auditory meatus
Styloid foramen (lateral)	T9	Most lateral point on styloid process opening
Jugular (lateral)	T10	Most lateral point on the jugular fossa
Medial spheno-squamosal	T11	Most medial point on spheno-squamousal suture

Figure 3.1E: Landmarks representing the temporal bone in a) lateral, and b) inferior views; with definitions. [Images adapted from Hansen, 2014]



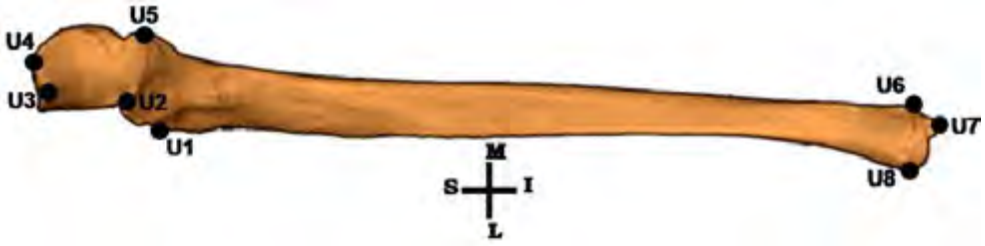
Landmark	Definition
H1	Most inferior point on anatomical neck
H2	Most superior point on head
H3	Most superior point on anatomical neck
H4	Most superior-lateral point on greater tubercle
H5	Most lateral point on lateral epicondyle
H6	Most inferior-lateral point on capitulum
H7	Most inferior-medial point on trochlea
H8	Most medial point on medial epicondyle
H9	Most superior point of olecranon fossa

Figure 3.2A: Position and definitions of landmarks on the humerus (posterior view) [Image adapted from White *et al*., 2012]



Landmark	Definition
R1	Most medial point on radial tuberosity
R2	Most superior-medial point on head
R3	Most posterior point on head (on articular surface margin)
R4	Most superior-lateral point on head
R5	Most inferior point of styloid process
R6	Most posterior-medial point on inferior articulating surface
R7	Most superior point on ulnar notch
R8	Most posterior point on dorsal tuberosity

Figure 3.2B: Position and definitions of landmarks on the radius (posterior view) [Image adapted from White *et al*., 2012]



Landmark	Definition
U1	Most lateral point on radial notch
U2	Most anterior point on coronoid process
U3	Most anterior point on olecranon process
U4	Most superior point on olecranon process
U5	Most medial point on trochlear notch
U6	Most medial point on head
U7	Most inferior point on styloid process
U8	Most lateral point on head

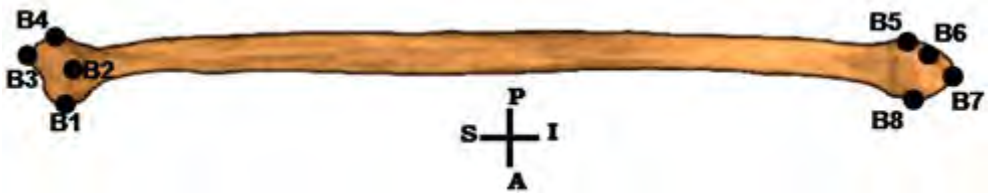
Figure 3.2C: Position and definitions of landmarks on the ulna (anterior view)
[Image adapted from White *et al*., 2012]

Landmark	Definition
F1	Most inferior-medial point on lesser trochanter
F2	Most inferior-medial point on border of head
F3	Most inferior point on fovea capitis
F4	Most superior-lateral point on border of head
F5	Most superior-lateral point on greater trochanter
F6	Most lateral point on lateral epicondyle
F7	Most inferior-lateral point on lateral condyle
F8	Most inferior-medial point on medial condyle
F9	Most medial point on medial epicondyle

Figure 3.2D: Position and definitions of landmarks on the femur (posterior view)
[Image adapted from White *et al*., 2012]

Landmark	Definition
T1	Most superior-anterior point on tibial tuberosity
T2	Most superior-lateral point on lateral condyle
T3	Most superior point on lateral intercondylar eminence
T4	Most anterior point on superior articulating surface
T5	Most superior point on medial intercondylar eminence
T6	Most superior-medial point on medial condyle
T7	Most inferior point on medial malleolus
T8	Most lateral point on inferior articulating surface

Figure 3.2E: Position and definitions of landmarks on the tibia (anterior view)
[Image adapted from White *et al*., 2012]



Landmark	Definition
B1	Most anterior point on head
B2	Most inferior point on superior articulation surface
B3	Most superior point on head
B4	Most posterior point on head
B5	Most posterior point on lateral malleolus
B6	Most superior point on malleolar fossa
B7	Most inferior point on lateral malleolus
B8	Most anterior point on inferior articulating surface

Figure 3.2F: Position and definitions of landmarks on the fibula (medial view)
[Image adapted from White *et al*., 2012]

CHAPTER 4

RESULTS

4.1. Observer error

The between-group Procrustes chord distances between repeats of the same specimen and between different specimens were calculated (example Figure 4.1; also Appendix A) and showed that, for both inter- and intra-observer repeats, distances between repeats of the same individual were all less than 5% of the mean distance between different individuals. Although no agreed-upon standards for acceptance of levels of observer error exist for morphometric data (Sholts *et al*., 2011), several studies like those of Braga & Treil (2007), Ross *et al*. (2011) and Holliday & Friedl (2013) have used this cut-off. These results indicated that observer error was small relative to sample variability and was thus unlikely to have influenced the results of this study.

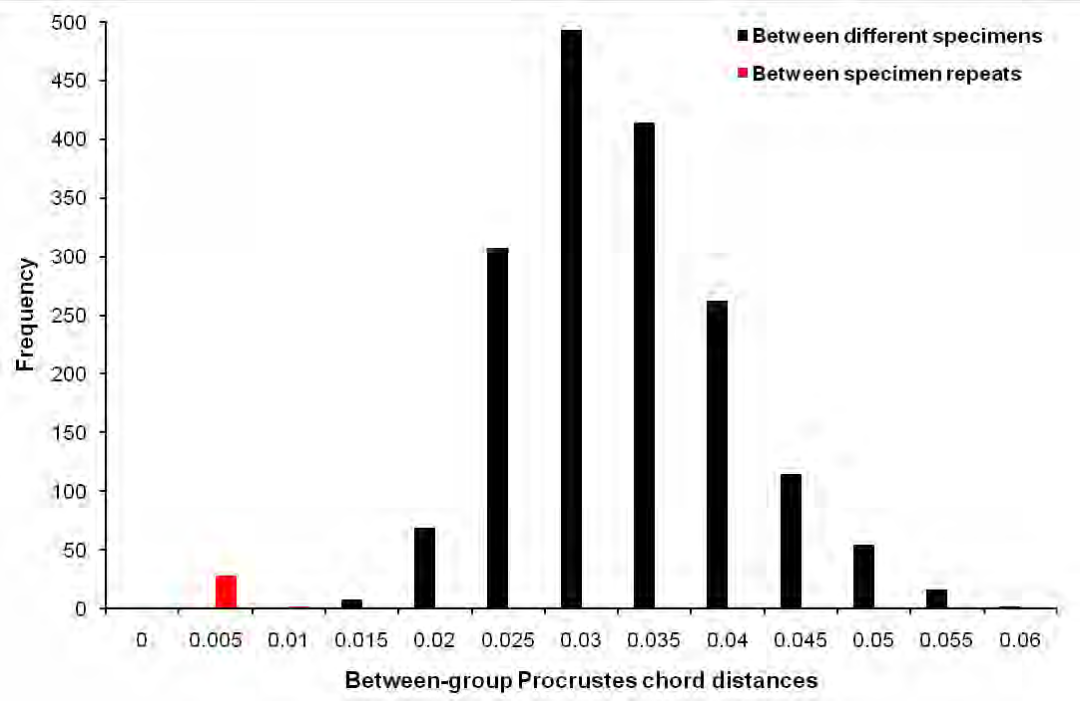


Figure 4.1: Example of the comparison of between-group Procrustes chord distances (for the right femur) between different specimens and between repeats of the same specimen.

4.2. Preliminary tests

4.2.1. Differences between left and right

PCA was performed on the Procrustes residuals of elements which occur bilaterally to assess whether significant differences existed between sides. None of the elements yielded significant separation of the sides on any of the principle components (PCs) generated, and DFA shows that the difference between bilateral elements was not significant ($p>0.07$). The shapes of the right and left sides were thus averaged and used for further analysis.

4.2.2. Effect of dissection of the crania

Procrustes residuals of crania which had the calvarium removed during dissection (n=788) and the whole and uncut crania (n=36) were compared using PCA. None of the cranial bones had separation of the groups on any of the principal components generated, which was supported by the DFA which showed that any separation between groups was not significant ($p>0.05$). The two groups were thus considered as a single group for further analyses.

4.2.3. Effect of edentualism

Procrustes residuals of all cranial bones were compared between individuals with teeth (n=534) and those without any teeth (n=290), using PCA. None of the cranial bones had separation of the two groups. This was supported by the DFA which shows that any separation between groups was not significant ($p>0.06$). The two groups were thus considered as a single group for further analyses.

4.3. Size variation

Comparison of mean centroid sizes showed that males were larger than females for all of the skeletal elements, with $p<0.001$ in all cases (Table 4.1)

Comparisons of mean centroid size among the three ancestry groups indicated that White individuals were larger than Black and Coloured individuals for all cranial elements, with $p\leq0.04$ in all cases (Table 4.2). The cranial elements of Black and Coloured individuals were not significantly different in size ($p>0.4$), except for the

occipital bone which was larger in Black individuals. Size differences among the ancestry groups were more varied for the postcranial elements. With the exception of the tibia and fibula, Black and White individuals differed significantly from each other, with Black individuals having the larger mean centroid size for the radius and ulna, while White individuals had larger mean centroid size for the humerus and femur. Coloured individuals tended to have the smallest mean centroid size for all postcranial elements, and were not significantly different from Black individuals for the mean centroid size of the humerus, and that of White individuals for the mean centroid size of the ulna.

Table 4.1: Comparison of mean centroid sizes between the sexes [All $p<0.001$].

Skeletal element	Females		Males	
	Mean centroid size (mm)	n	Mean centroid size (mm)	n
Cranium				
Whole	287.4 ± 8.3	354	300.3 ± 9.3	420
Frontal	180.7 ± 6.5	356	189.0 ± 7.0	421
Occipital	171.3 ± 6.4	364	177.2 ± 7.0	428
Parietal	198.0 ± 6.9	367	205.4 ± 7.5	434
Temporal	106.7 ± 4.4	375	112.3 ± 5.2	449
Upper limb				
Humerus	415.0 ± 24.3	464	452.0 ± 28.3	582
Radius	298.1 ± 19.1	471	328.1 ± 22.2	578
Ulna	308.4 ± 19.0	465	337.5 ± 21.8	578
Lower limb				
Femur	576.5 ± 30.7	447	617.1 ± 36.4	545
Tibia	426.4 ± 25.0	449	459.8 ± 30.1	554
Fibula	465.7 ± 27.7	412	500.2 ± 33.5	507

When centroid size was compared among the six sex-ancestry groups, the results were similar to those observed when comparing groups based on sex or ancestry independently (Table 4.3). The males of each ancestry group were found to have significantly larger mean centroid sizes than females of the same ancestry group, though there was still substantial overlap in the ranges of these groups. Coloured males and females tended to have smaller mean centroid size than the males and females of the other two groups, but the difference was not sufficient to distinguish the Coloured individuals from Black or White individuals of the same sex for any of

the elements. The difference in centroid size between Black and Coloured individuals was not significant for the frontal, parietal and temporal bones, or the humerus. White and Coloured individuals were not significantly different in the centroid size of the radius or ulna. White males and females tended to have larger cranial elements than the respective Black groups, but the difference between Black and White individuals was not significant for most of the postcranial elements ($p<0.05$).

Table 4.2: Comparison of mean centroid sizes among the ancestry groups.

Skeletal element	Black individuals		Coloured individuals		White individuals	
	Mean centroid size (mm)	n	Mean centroid size (mm)	n	Mean centroid size (mm)	n
Cranium						
Whole	293.1 ± 10.0	283	292.3 ± 10.8	271	298.7 ± 11.1	220
Frontal	183.9 ± 7.6	287	184.5 ± 7.9	270	187.6 ± 7.9	220
Occipital	174.1 ± 6.7	290	172.6 ± 7.2	272	177.2 ± 7.6	230
Parietal	200.6 ± 7.5	290	200.1 ± 8.0	278	205.8 ± 7.7	233
Temporal	109.5 ± 5.2	300	108.8 ± 5.8	287	111.1 ± 5.7	237
Upper limb						
Humerus	430.4 ± 30.2	337	428.4 ± 31.4	366	448.3 ± 31.6	343
Radius	321.8 ± 25.1	333	308.9 ± 25.2	368	313.8 ± 24.9	348
Ulna	331.9 ± 24.5	334	318.8 ± 25.1	361	323.3 ± 24.2	348
Lower limb						
Femur	597.7 ± 37.2	330	588.7 ± 38.6	354	611.6 ± 39.3	308
Tibia	449.9 ± 31.7	325	436.9 ± 32.0	356	448.5 ± 31.3	322
Fibula	491.2 ± 33.8	309	473.7 ± 34.8	323	490.2 ± 35.0	287

4.4. Shape variation

Shape variation was assessed according to sex, ancestry and sex-ancestry groups. There are several ways in which the shape differences between group means can be illustrated, for example using wireframes or vector diagrams. For the sake of clarity and conciseness, the detected differences were summarized using wireframe diagrams, though vector diagrams which aid in the interpretation of the results are given in Appendix B and will be referred to.

Table 4.3: Comparison of mean centroid sizes among the six sex-ancestry groups.

Skeletal element	Black females		Coloured females		White females		Black males		Coloured males		White males	
	Mean centroid size (mm)	n	Mean centroid size (mm)	n	Mean centroid size (mm)	n	Mean centroid size (mm)	n	Mean centroid size (mm)	n	Mean centroid size (mm)	n
Cranium												
Whole	287.4 ± 7.9	160	284.9 ± 8.7	104	290.5 ± 7.4	90	300.5 ± 7.2	123	296.9 ± 9.3	167	304.5 ± 9.5	130
Frontal	180.2 ± 6.4	162	179.3 ± 6.5	103	183.3 ± 6.1	91	188.8 ± 6.1	125	187.7 ± 6.9	167	190.7 ± 7.7	129
Occipital	171.6 ± 6.3	161	169.4 ± 6.2	107	172.8 ± 6.2	96	177.2 ± 5.8	129	174.7 ± 7.0	165	180.3 ± 7.0	134
Parietal	197.2 ± 6.6	162	195.7 ± 7.2	107	201.6 ± 5.6	98	205.0 ± 6.1	128	202.8 ± 7.3	171	208.9 ± 7.6	135
Temporal	106.7 ± 4.3	165	105.5 ± 4.5	108	108.0 ± 4.1	102	122.9 ± 4.0	135	110.9 ± 5.5	179	113.5 ± 5.6	135
Upper limb												
Humerus	412.8 ± 22.1	184	404.8 ± 24.3	131	426.7 ± 22.0	149	451.5 ± 24.5	153	441.6 ± 27.0	235	464.9 ± 27.5	194
Radius	306.8 ± 18.2	183	289.4 ± 16.9	135	295.4 ± 17.7	153	340.2 ± 19.7	150	320.1 ± 22.2	233	328.2 ± 19.7	195
Ulna	317.3 ± 17.7	183	299.5 ± 17.4	131	305.2 ± 17.3	151	349.5 ± 19.5	151	329.9 ± 21.9	230	337.1 ± 19.1	197
Lower limb												
Femur	578.7 ± 29.6	182	562.3 ± 28.8	131	587.6 ±28.7	134	621.1 ± 32.0	148	604.2 ± 35.2	223	630.2 ± 36.3	174
Tibia	432.6 ± 24.6	175	414.7 ± 24.1	130	429.3 ± 22.7	144	470.0 ± 26.8	150	449.6 ± 28.8	226	464.1 ± 30.6	178
Fibula	473.6 ± 27.1	170	450.9 ± 25.0	122	469.6 ± 25.5	120	512.7 ± 28.3	139	487.5 ± 32.6	201	504.9 ± 33.4	167

4.4.1. Sexual dimorphism

Cranial elements

4.4.1.1. Whole cranium

PCA of the Procrustes residuals produced 35 principal components (Figure 4.2), of which only PC3 showed separation between the 90% probability ellipses of the sexes, accounting for 7.8% of the observed sample variance.

The shape differences between the sexes on PC3 are shown in Figures 4.3a-c (Appendix B – Figure B1). Females tended to have a slightly larger medio-lateral dimension of the cranium (larger distance between landmarks 3 and 14, and between landmarks 4 and 13), but an antero-posteriorly longer occipital region (more anterior landmark 6 and 7), compared to males. This resulted in a more steeply sloped forehead but less steeply sloped occipital region in females. Males had more prominent glabellar regions (more anterior landmark 2) and larger mastoid processes than females (landmarks 8 and 11).

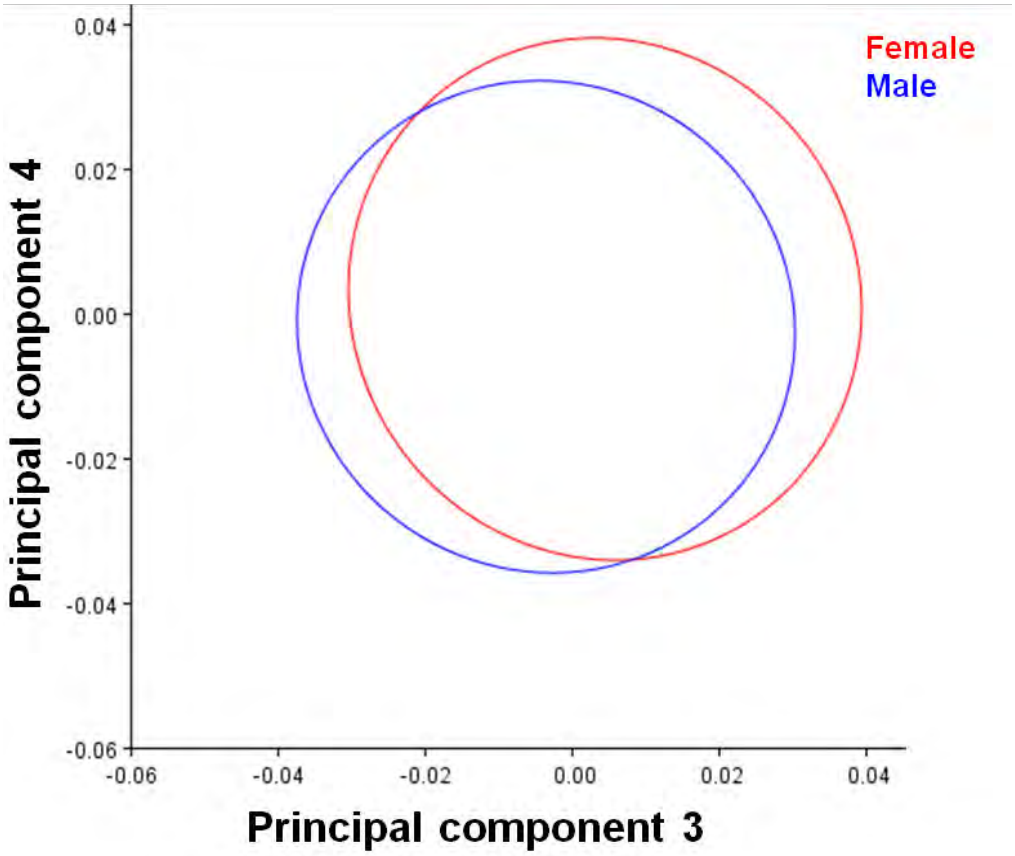


Figure 4.2: Whole cranial shape – plot of the third and fourth principal components, showing 90% probability ellipses of females and males. Sexes separate on PC3 only.

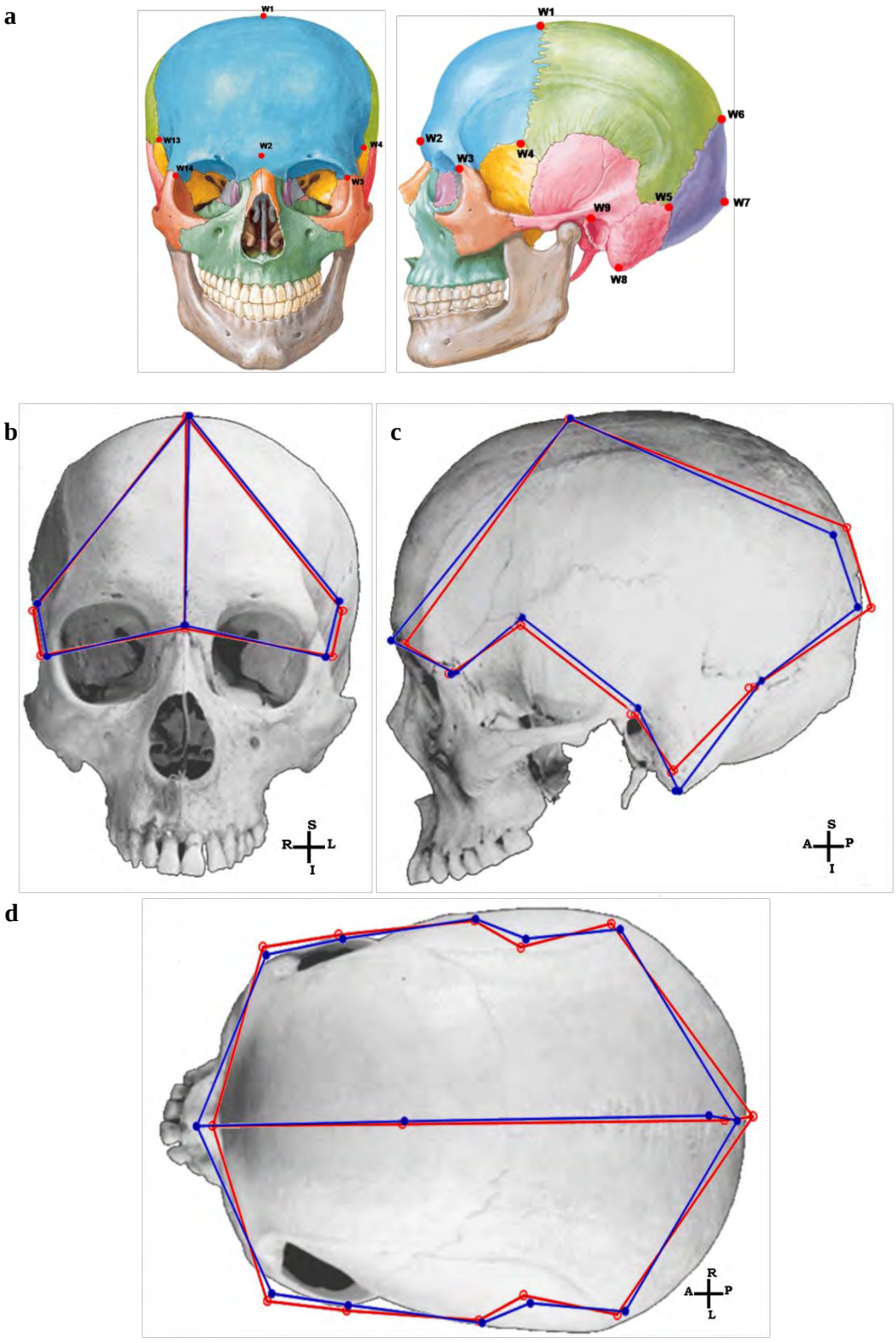


Figure 4.3: Whole cranial shape differences between the sexes - a) landmark locations, b) anterior view, c) lateral view, and d) superior view. Mean shape of females in red, mean shape of males in blue. [5X magnification of differences for visualization] [Images adapted from White et al. (2012) and Hansen (2014)]

4.4.1.2. Frontal bone

PCA of the Procrustes residuals produced 26 principal components, of which only PC2 showed separation between the sexes, accounting for 20.7% of the observed variance (Figure 4.4).

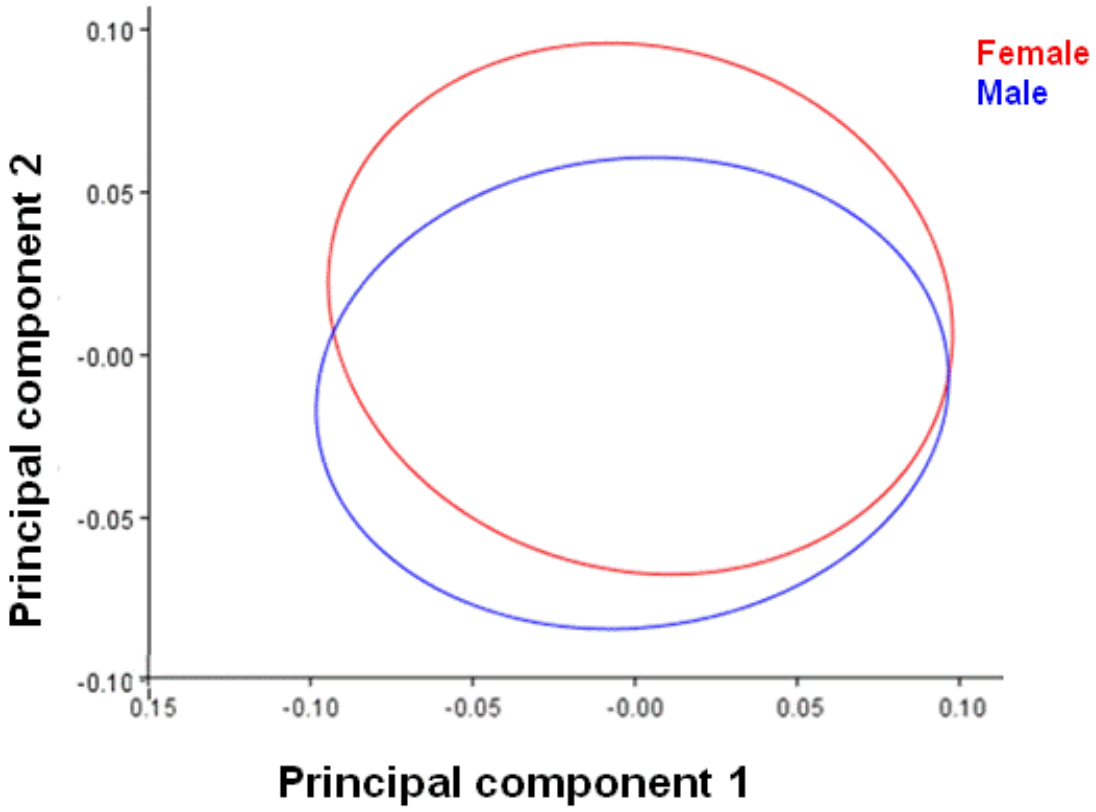


Figure 4.4: Frontal bone shape – plot of the first two principal components, showing 90% probability ellipses of females and males. Sexes separate on PC2 only.

The shape differences between the sexes on PC2 are shown in Figures 4.5a-b (Appendix B – Figure B2). Females tended to have relatively wider medio-lateral dimensions of the frontal bone (more lateral landmarks 6-11), and had a more superior bregma and glabella (landmarks 1 and 2), resulting in a steeper slope of the forehead, compared to males. Males had more prominent glabellar regions (more anterior landmark 4) with more inferior orbital regions (landmarks 6 and 11), and more superior and anteriorly positioned temporal lines (landmarks 7 and 10)

4.4.1.3. Occipital bone

PCA of the Procrustes residuals produced 29 principal components, though none of these yielded significant separation of the sexes and thus no further analyses of sexual dimorphism were performed.

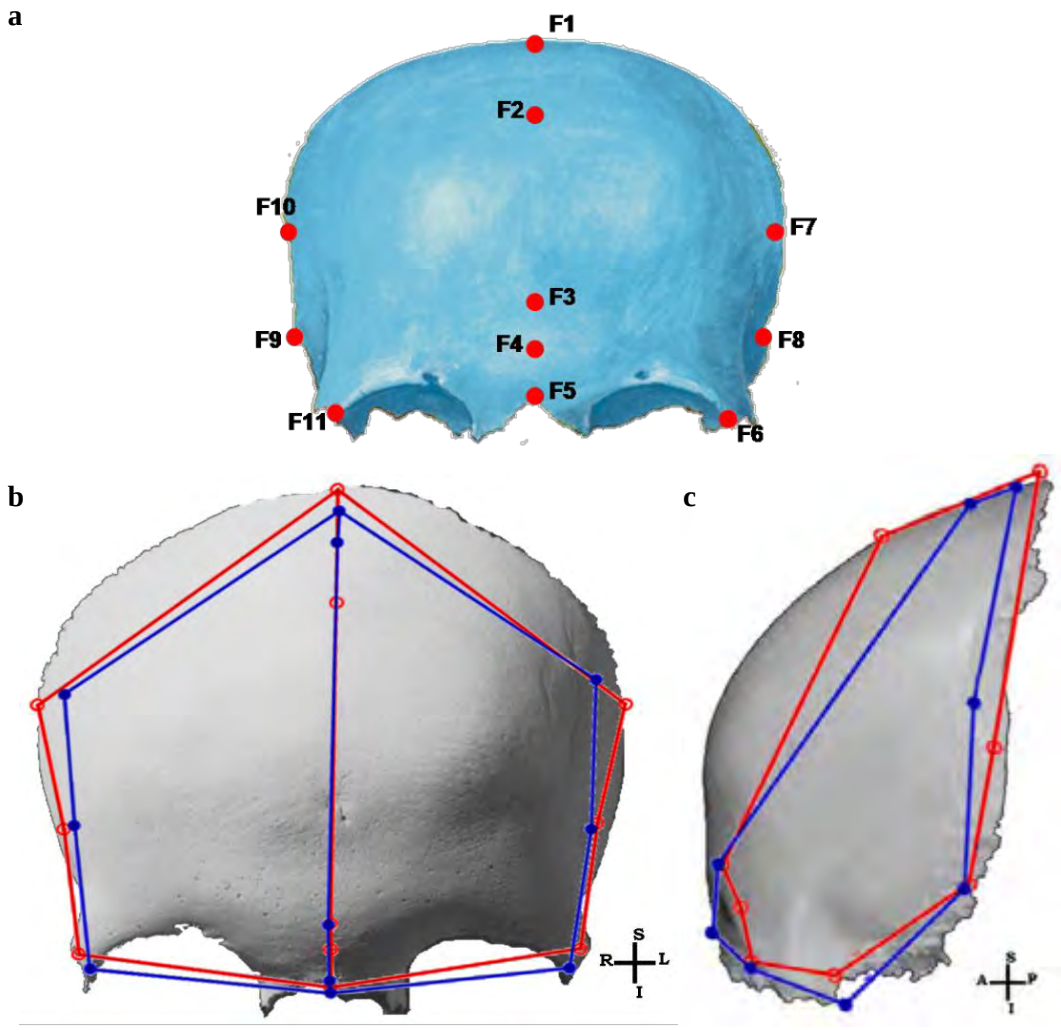


Figure 4.5: Frontal bone shape differences between the sexes – a) landmark locations, b) anterior view, and c) lateral view. Mean shape of females in red, mean shape of males in blue. [5X magnification of differences for visualization].
[Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.1.4. Parietal bone

PCA of the Procrustes residuals produced 26 principal components, though none of these yielded significant separation of the sexes and thus no further analyses of sexual dimorphism were performed.

4.4.1.5. Temporal bone

PCA of the Procrustes residuals produced 26 principal components, though none of these showed significant separation of the sexes and thus no further analyses of sexual dimorphism were performed.

Upper limb

4.4.1.6. Humerus

PCA of the Procrustes residuals produced 20 principal components, of which only PC2 yielded separation between the sexes, accounting for 14.8% of the observed variance (Figure 4.6).

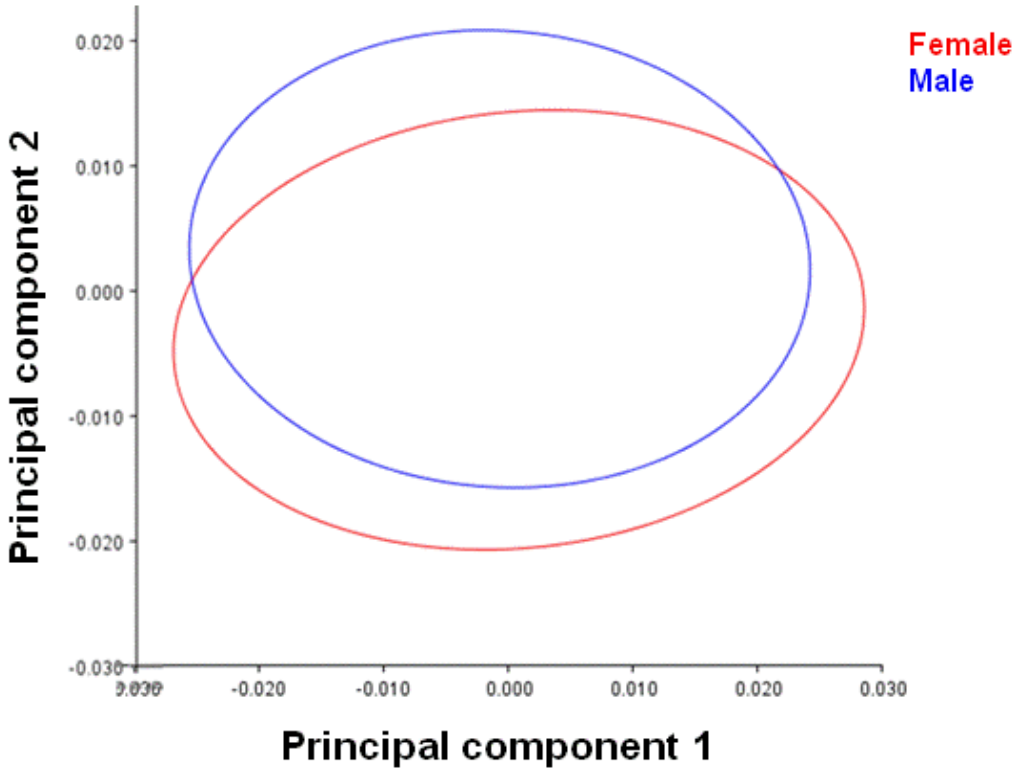


Figure 4.6: Humerus shape – plot of first two principal components, showing 90% probability ellipses of females and males. Sexes separate on PC2 only.

The shape differences between the sexes are shown in Figures 4.7a-c (Appendix B – Figure B3). Males tended to have medio-laterally wider proximal and distal epiphyses (larger distance between landmarks 1 and 4, landmarks 5 and 8, and landmarks 6 and 7), and supero-inferiorly larger humeral heads (more inferior landmark 1), though they did not differ significantly from females in overall supero-inferior length. Males also tended to have more anteriorly facing greater tuberosities (landmark 4), while that of females was less angled. It was also observed that males

had a counter-clockwise rotation of the distal epiphysis relative to the proximal epiphysis, giving a larger angle of humeral retroversion compared to that of females.

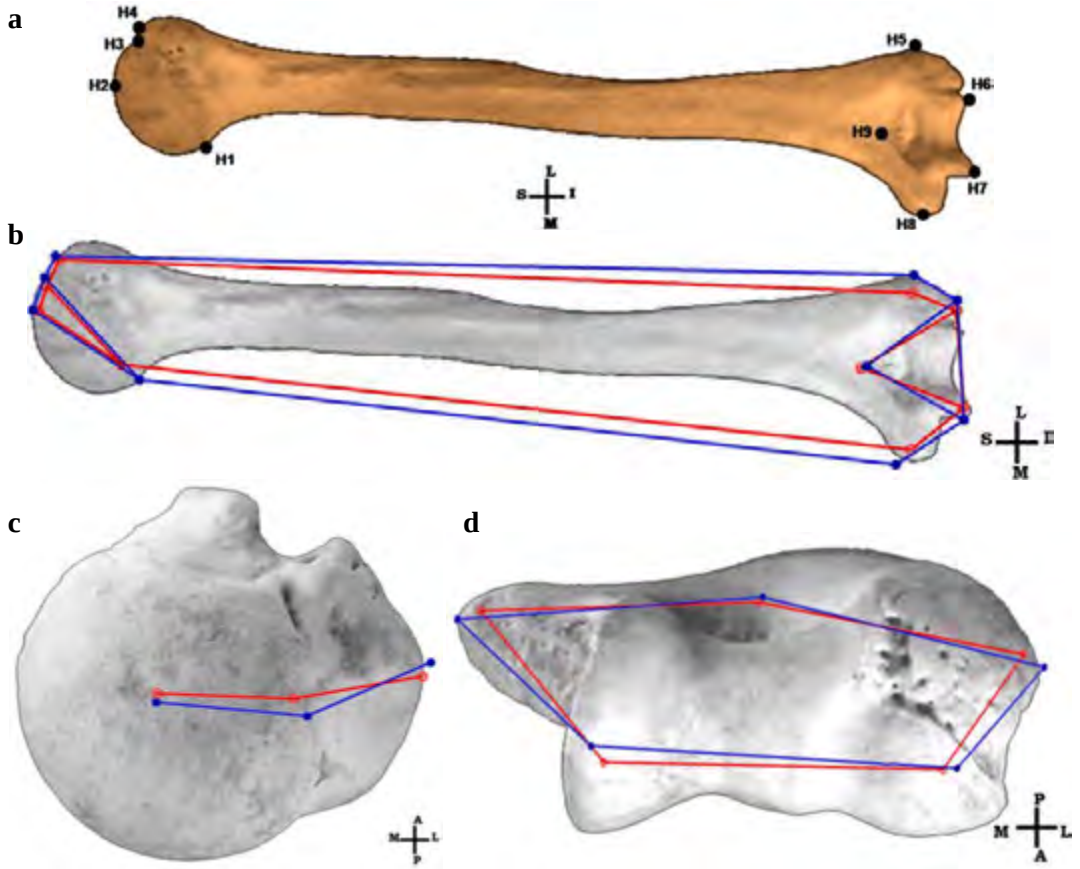


Figure 4.7: Humerus shape differences between the sexes – a) landmark locations, b) posterior view, c) humeral head, and d) distal epiphysis. Mean shape of females in red, mean shape of males in blue). [5X magnification of differences for visualization in b, 2X for c and d] [Images adapted from White *et al*. (2012)]

4.4.1.7. Radius

PCA of the Procrustes residuals produced 17 principal components, of which only PC1 showed some separation between the sexes, which accounted for 29.8% of the observed variance (Figure 4.8).

The shape differences between the sexes are shown in Figures 4.9a-c (Appendix B – Figure B4). Females had a medio-laterally narrower radial head (more lateral landmark 2), and a slightly more medial position of the dorsal tubercle (landmark 8), compared to males. The dorsal tubercle was also less posteriorly positioned in females, creating an antero-posteriorly flattened distal end. Females also had a slight clockwise rotation of the landmarks on the radial head (landmarks 2-4), but not the radial tuberosity (landmark 1), relative to that of males.

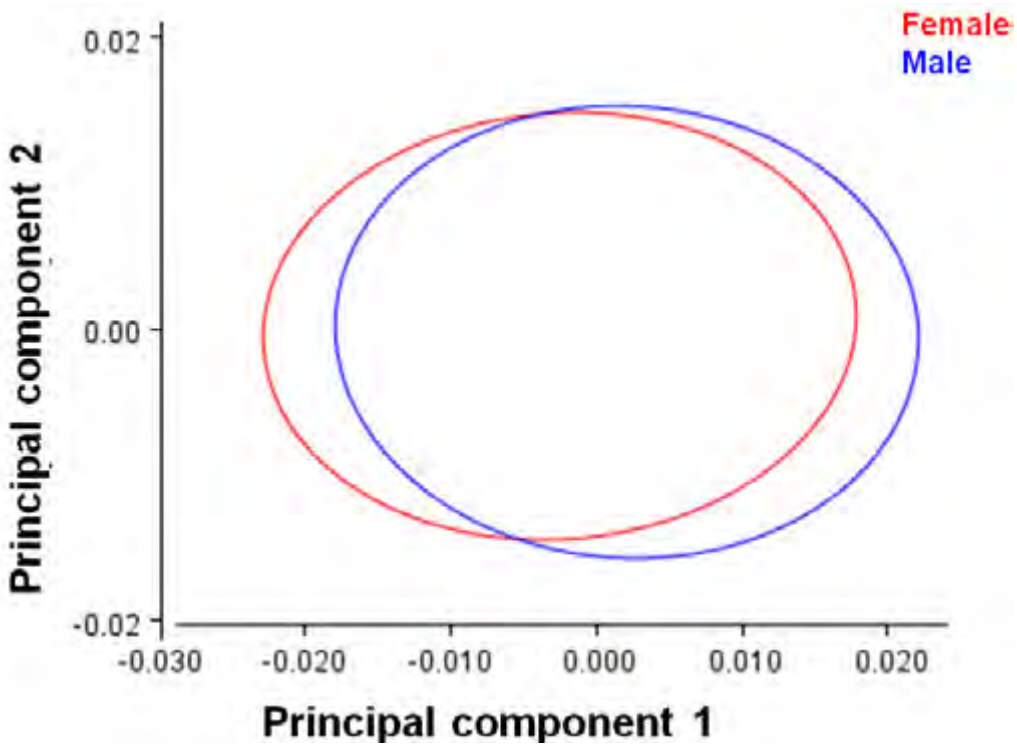


Figure 4.8: Radius shape – plot of first two principal components, showing 90% probability ellipses of females and males. Sexes separate on PC1 only.

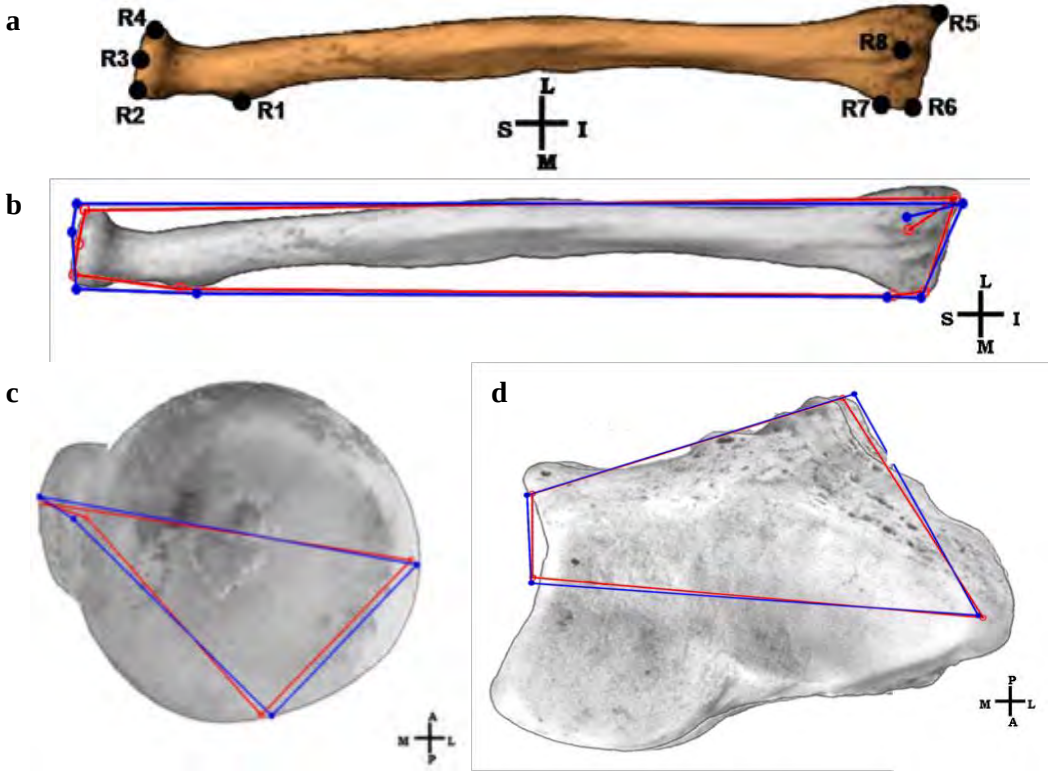


Figure 4.9: Radius shape differences between the sexes – a) landmark locations, b) posterior view, c) radial head, and d) distal epiphysis. Mean shape of females in red, mean shape of males in blue. [5X magnification of differences for visualization in b]
[Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.1.8. Ulna

PCA of the Procrustes residuals produced 17 principal components, of which only PC1 yielded separation between the sexes, which accounted for 26.1% of the observed variance (Figure 4.10).

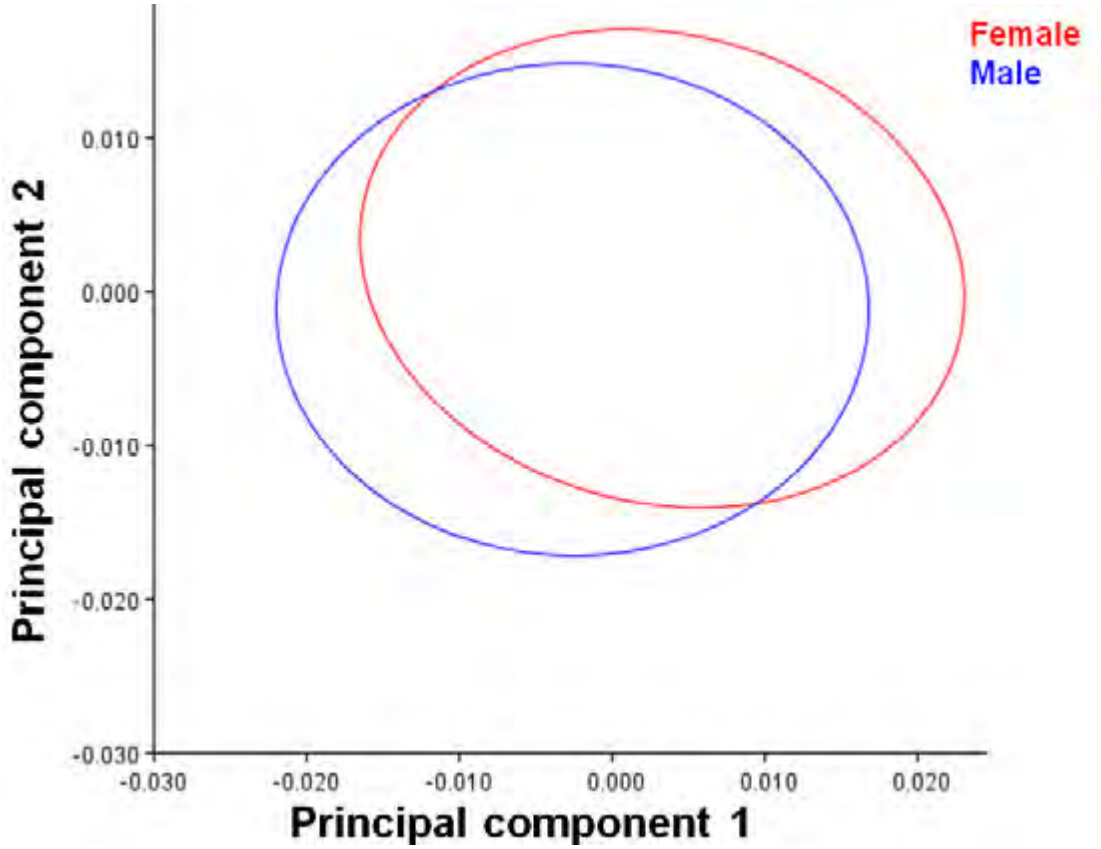


Figure 4.10: Ulna shape – plot of first two principal components, showing 90% probability ellipses of females and males. Sexes separate on PC1 only.

The shape differences between the sexes on PC1 are shown in Figures 4.11a-c (Appendix B – Figure B5). Males had medio-laterally wider proximal and distal epiphyses of the ulna (larger distance between landmarks 1 and 5, and between landmarks 6 and 8), and a more posteriorly positioned coronoid process (landmark 3), resulting in a more L-shaped trochlear notch, while females had a relatively C-shaped notch.

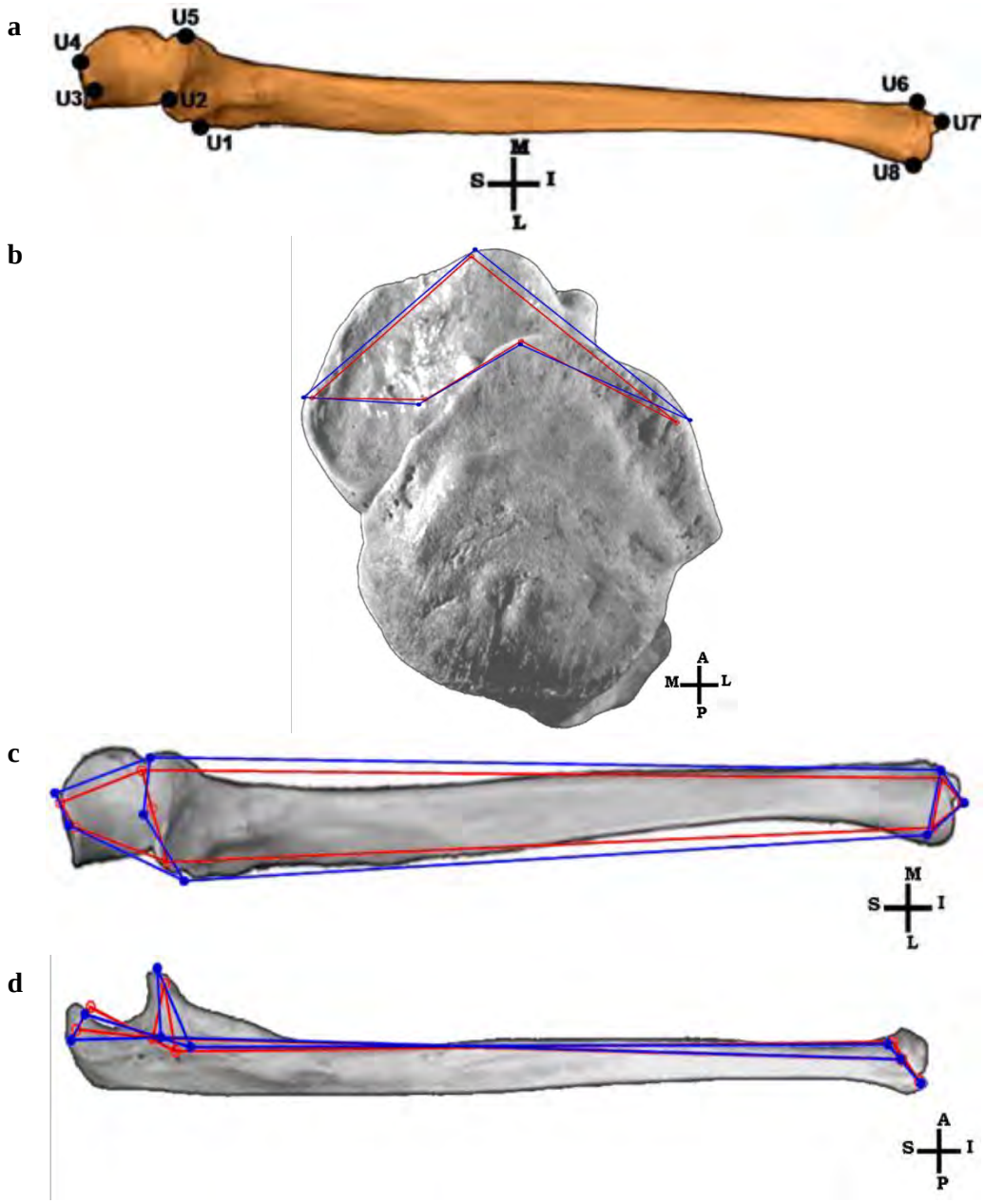


Figure 4.11: Ulna shape differences between the sexes – a) landmark locations, b) proximal epiphysis, c) anterior view, and d) lateral view. Mean shape of females in red, mean shape of males in blue. [5X magnification of differences for visualization in c and d] [Images adapted from White *et al*. (2012)]

Lower limb

4.4.1.9. Femur

PCA of the Procrustes residuals produced 20 principal components, of which only PC1 showed separation between the sexes, accounting for 22.2% of the observed variance (Figure 4.12).

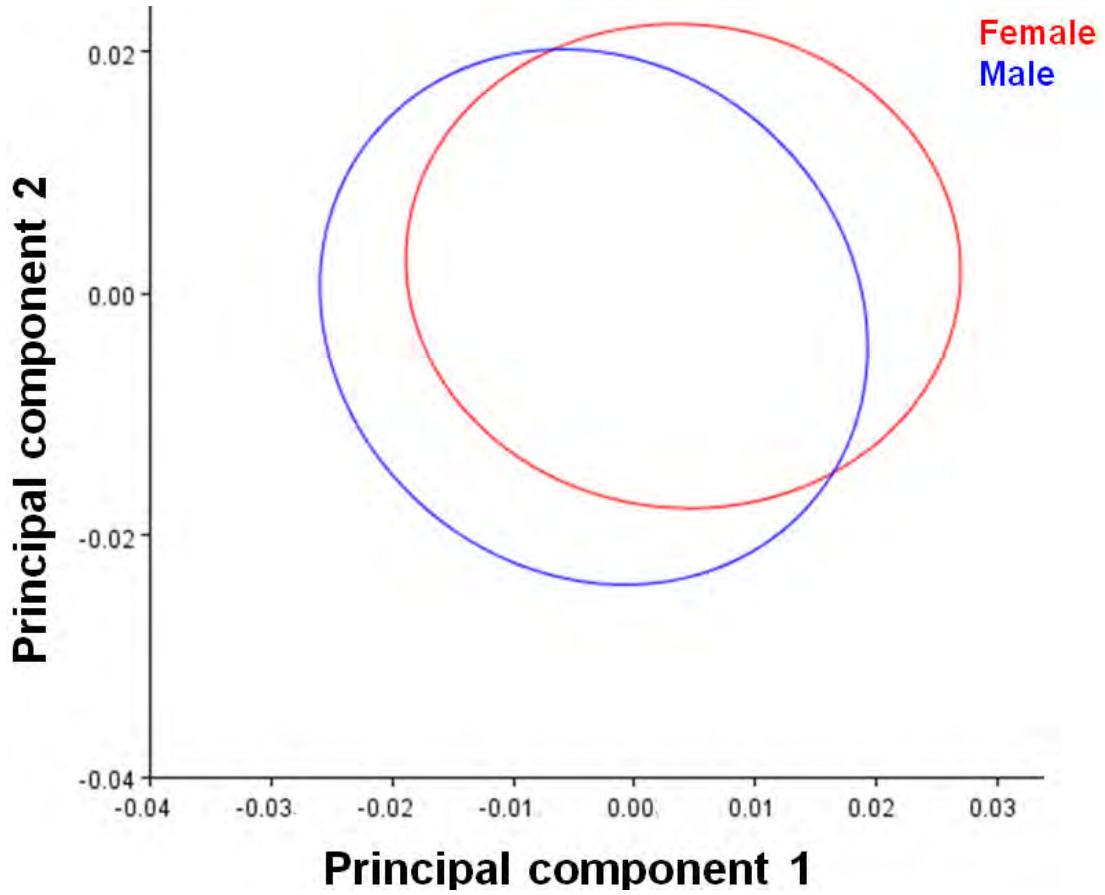


Figure 4.12: Femur shape – plot of first two principal components, showing 90% probability ellipses of females and males. Sexes separate on PC1 only.

The shape differences between the sexes on PC1 are shown in Figures 4.13a-c (Appendix B – Figure B6). Females had medio-laterally smaller proximal and distal epiphyses of the femur than males (smaller distance between landmarks 3 and 5, and between landmarks 7 and 8). Compared to males, females also had a slightly more superior lesser trochanter (landmark 1), a larger neck shaft angle (more superior and lateral landmarks 2 and 3), and supero-inferiorly smaller distal condyles (inferior landmarks 6 and 9). Males had a relative clockwise rotation of the fovea capitis and greater trochanter (landmarks 3 and 5), resulting in a smaller angle of anteversion of the femoral head, relative to that of females. The distal end of the femur also displays a relative counter-clockwise rotation, further contributing to the smaller anteversion angle in males.

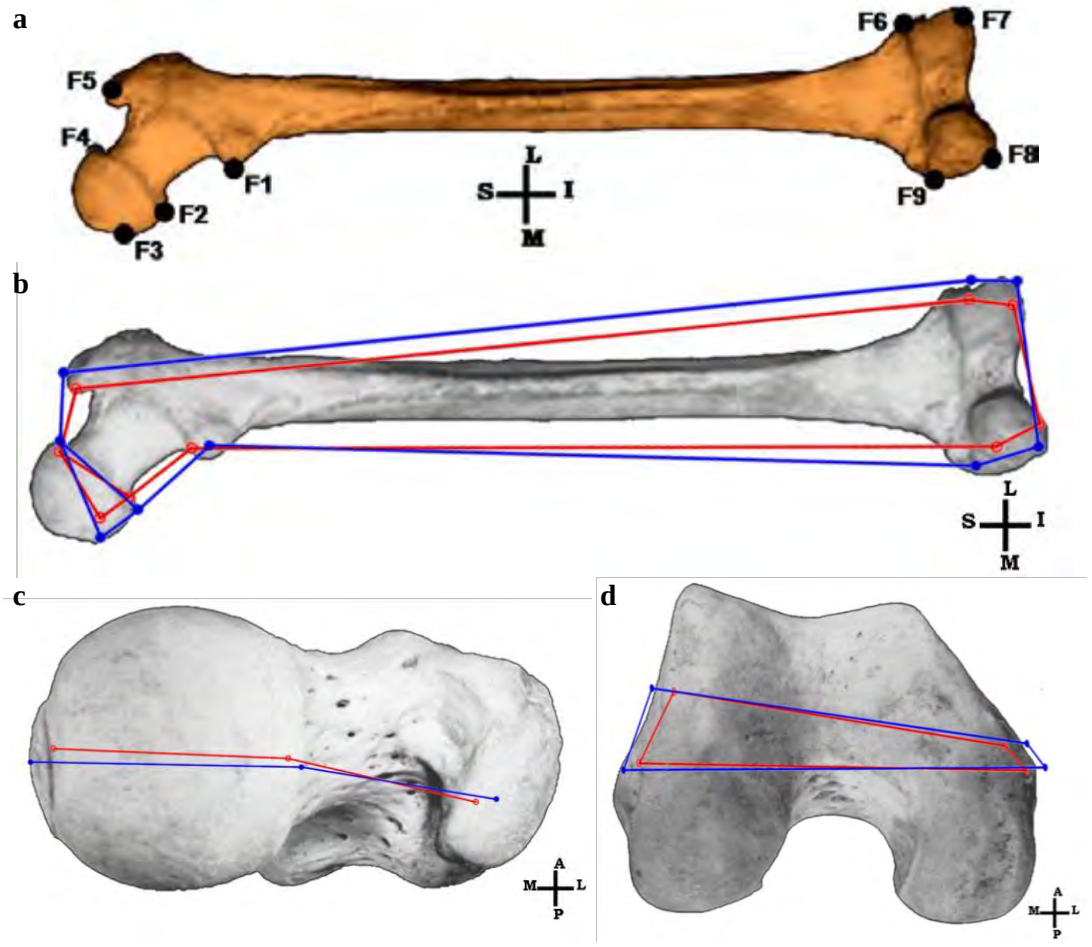
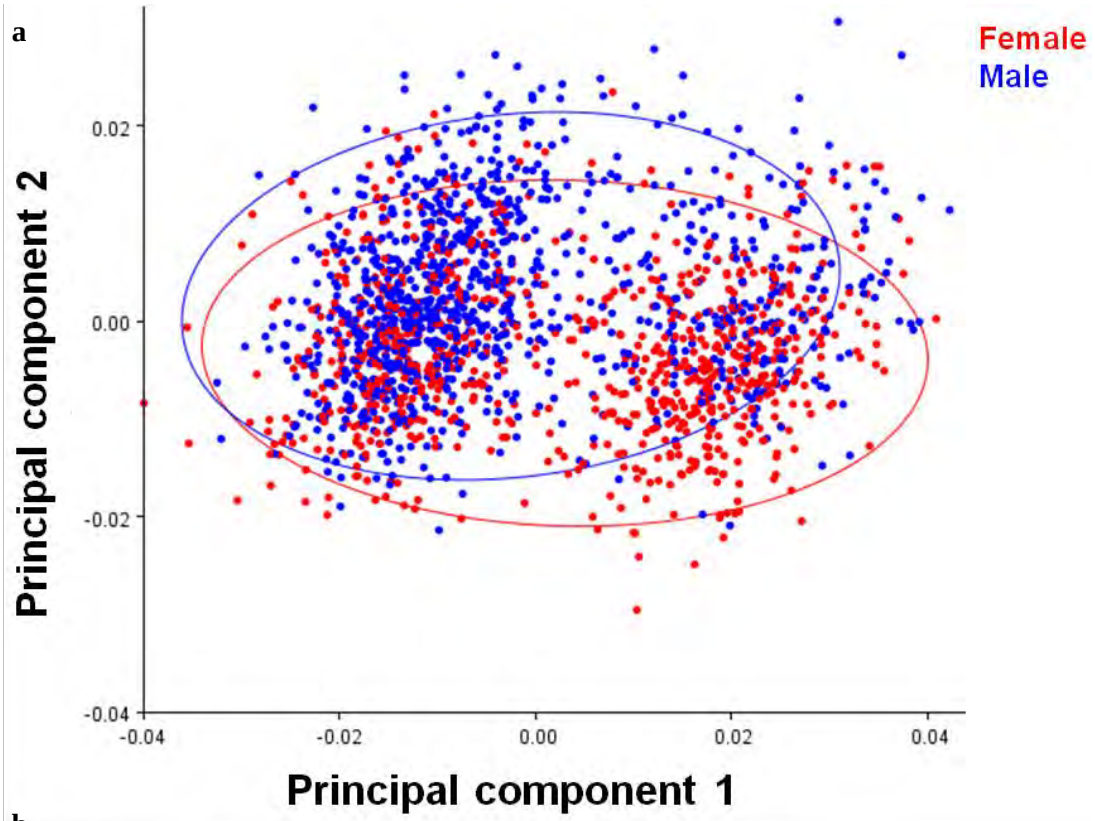
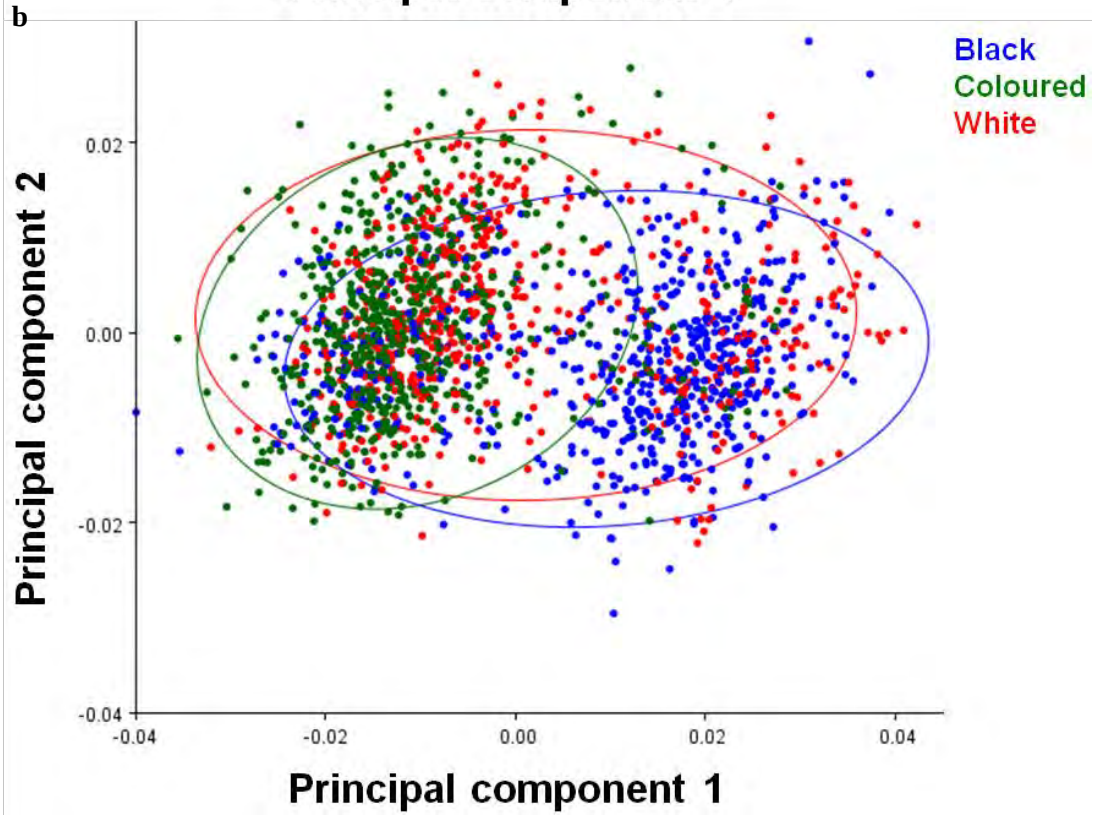


Figure 4.13: Femur shape differences between the sexes – a) landmark locations, b) posterior view, c) femur head, and d) distal epiphysis. Mean shape of females in red, mean shape of males in blue. [5X magnification of differences for visualization in b, 2X for c and d] [Images adapted from White *et al*. (2012)]

4.4.1.10. Tibia

Initial analysis of the tibia data showed two separate clusters of individuals across the first principal component. This clustering was observed within all sample groups when the variation was assessed according to sex, ancestry or sex-ancestry (Figures 4.14a-c). Investigation of the shape variation associated with this principal component indicated that the majority of the observed variation is related to variation in the position of the tibial tuberosity landmark. Due to the large amount of variation of this landmark alone, it is possible that the variation of the rest of the landmarks related to sex, ancestry and sex-ancestry may have been obscured. It was thus decided to perform two sets of analyses – one with the tibial tuberosity landmark included, and one with this landmark excluded from analysis.


a
Female
Male
Principal component 2
0.02
0.00
-0.02
-0.04
-0.04
-0.02
0.00
0.02
0.04
Principal component 1


b
Black
Coloured
White
Principal component 2
0.02
0.00
-0.02
-0.04
-0.04
-0.02
0.00
0.02
0.04
Principal component 1

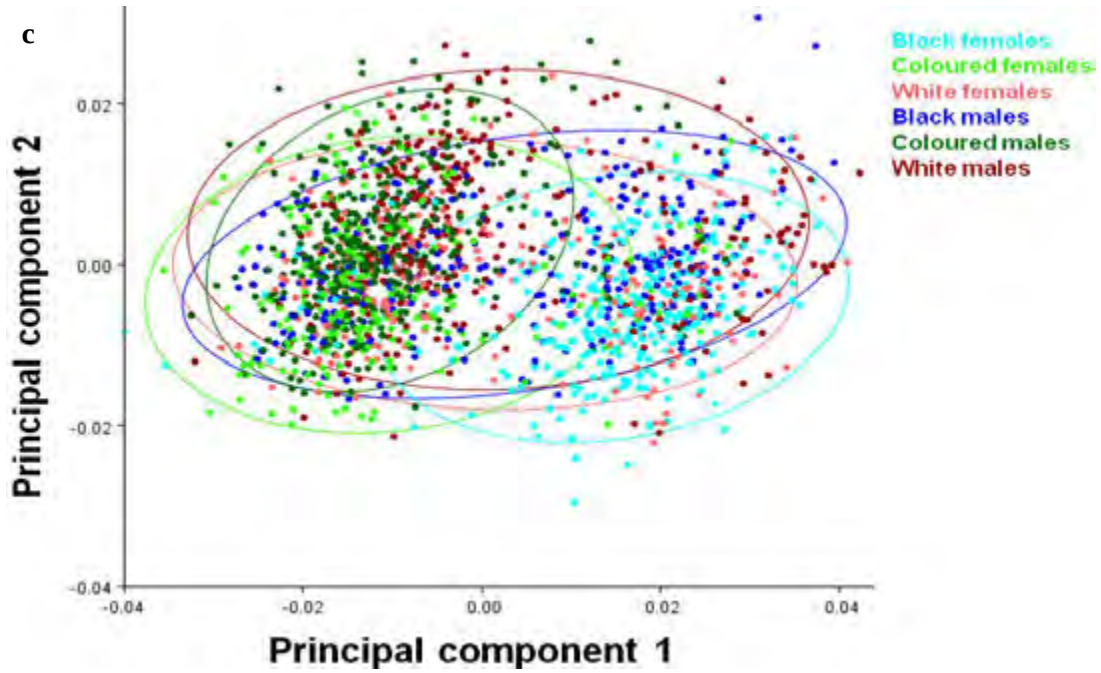


Figure 4.14: Tibia shape – plot of first two principal components, showing the distribution of individuals according to a) sex, b) ancestry, and c) sex-ancestry combined. Ellipses show 90% probability distribution of each study group. A separation of the data points is observed within each of the study groups.

When the tibial tuberosity landmark was excluded from the analysis of sexual dimorphism, PCA of the Procrustes residuals produced 14 principal components, though none of these yielded significant separation of the sexes. However, when this landmark was included, 17 principal components were produced, of which only PC2 showed some separation between the sexes (Figure 4.15). This principal component accounted for 14.0% of the observed sample variation.

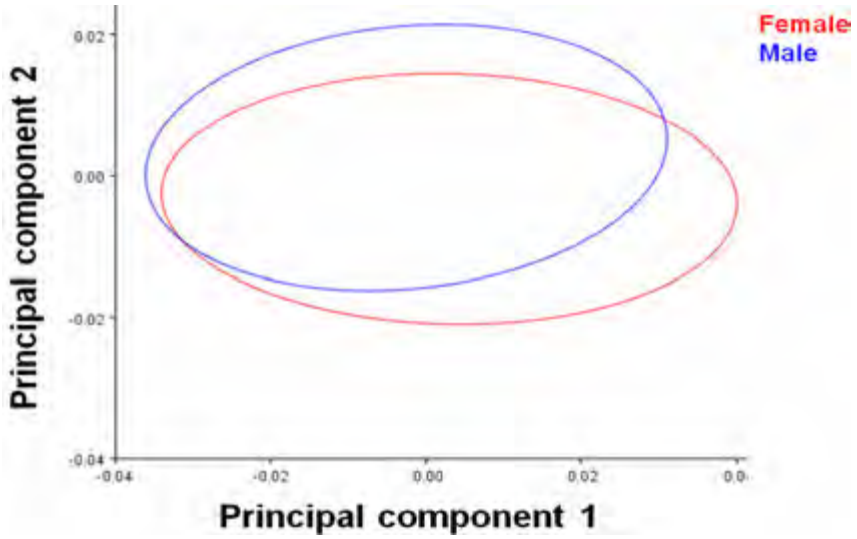


Figure 4.15: Tibia shape (including tuberosity) – plot of first two principal components, showing 90% probability ellipses of females and males. Sexes separate on PC2 only.

The shape differences between the sexes on PC2 are shown in Figures 4.16a-c (Appendix B – Figure B7), with the differences being a medio-laterally narrower proximal tibial epiphysis (smaller distance between landmarks 2 and 6), and a more infero-laterally positioned tibial tuberosity (landmark 1) in females compared to males.

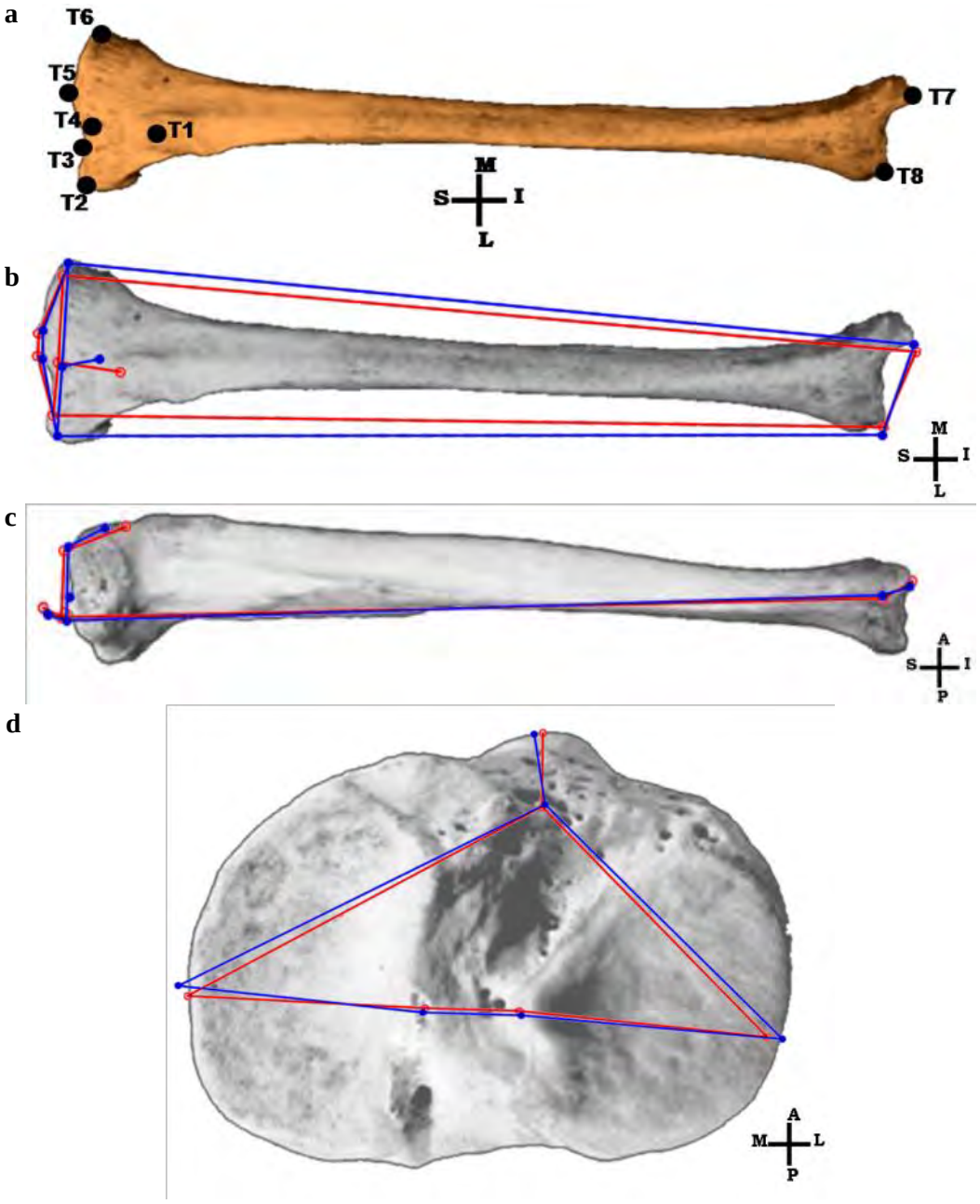


Figure 4.16: Tibia shape (including tuberosity) differences between the sexes – a) landmark locations, b) anterior view, c) lateral view, and d) superior view. Mean shape of females in red, mean shape of males in blue. [5X magnification of differences for visualization in b and c] [Images adapted from White *et al*. (2012)]

4.4.1.11. Fibula

PCA of the Procrustes residuals produced 17 principal components, but none of these principal components yielded significant separation between the sexes, thus no further analyses of sexual dimorphism were performed.

4.4.1.12. Relationships to other variables

For each skeletal element which showed significant separation of the sexes, regression analysis was performed to assess the potential relationship of the morphological differences between the sexes with centroid size, age and year-of-birth (Table 4.4). All elements, except the whole cranium, yielded significant association ($p<0.0001$) to centroid size. Some of these associations, however, were linked to less than 10% of the overall variation observed. The remaining elements with association of centroid size to the sexual differences were the bones of the upper limb, and the tibia. Regression analysis also showed significant association of age with the difference between the sexes ($p<0.0001$). Of the upper limb, only the radius and ulna had an association of more than 10% of the total observed variation, while the femur was the only element of the lower limb to show such association. None of the cranial elements yielded associations that were linked to more than 10% of the total variation. Lastly, significant association of year-of-birth with the difference between sexes was observed ($p\leq0.04$), but only those of the radius and femur exceeded 10% of the total observed variance.

Table 4.4: Relationship of sexual dimorphism in shape with centroid size, age and year-of-birth [All values shown have $p\leq0.04$].

Skeletal element	Coefficient of determination (R^2) (%)		
	Centroid size	Age	Year-of-birth
Cranium			
Whole	Not significant	1.5	1.6
Frontal bone	2.2	2.7	0.5
Upper limb			
Humerus	20.8	3.5	2.3
Radius	23.6	14.6	10.3
Ulna	11.8	12.0	8.9
Lower limb			
Femur	7.1	20.1	15.8
Tibia (with tuberosity)	25.8	4.0	1.3

*Note: No significant separation of the sexes was detected for the occipital, parietal and temporal bones, tibia (without tuberosity) and fibula.

4.4.1.13. Discriminant function analysis

The leave-one-out cross-validation test indicated that the shape differences between the sexes for the whole cranium, frontal bone, humerus, radius, ulna, femur and tibia were significant (all $p \leq 0.04$). The classification accuracies of each of these elements are shown in Table 4.5 (see Appendix C – Table C2 for sample sizes). Accuracies of the sex-pooled sample ranged from 64.0% for the tibia to 79.3% for the frontal bone. These elements also have the smallest and the largest Mahalanobis distances between the sexes (MD= 0.9 and 1.7, respectively; $p<0.0001$) (Appendix C – Table C1). The difference in classification accuracy between the sexes was not significant, except for the frontal bone and radius. For both of these elements, the classification accuracy of females was significantly higher than that of males ($\chi 2 = 7.84$ and 5.37, respectively; $p \leq 0.02$).

Table 4.5: Leave-one-out cross-validation classification accuracies of skeletal elements according to sex (Highest accuracies of cranial and postcranial elements indicated in red).

Skeletal element	Sex estimation accuracy (%)		
	Female	Male	Pooled sexes
Cranium			
Whole	70.9	69.5	**70.2**
Frontal	**83.7**	**75.5**	**79.3**
Upper limb			
Humerus	75.0	72.0	**73.3**
Radius	74.5	68.0	**70.9**
Ulna	70.5	67.5	**68.8**
Lower limb			
Femur	**75.6**	**73.2**	**74.3**
Tibia (with tuberosity)	64.4	63.7	**64.0**

*Note: No significant separation of the sexes was detected for the occipital, parietal and temporal bones, tibia (without tuberosity) and fibula.

4.4.2. Ancestry

Cranial elements

4.4.2.1. Whole cranium

CVA of the Procrustes residuals produced 2 canonical variates, both of which yielded significant separation of the three ancestry groups (Figure 4.17). The first canonical variate (CV1) accounted for 89.6% of the observed sample variance and separated Black individuals from White individuals, while Coloured individuals did not

separate from either of the other groups on this axis. The second canonical variate (CV2) accounted for 10.4% of the observed variance, and separated Coloured individuals from Black and White individuals.

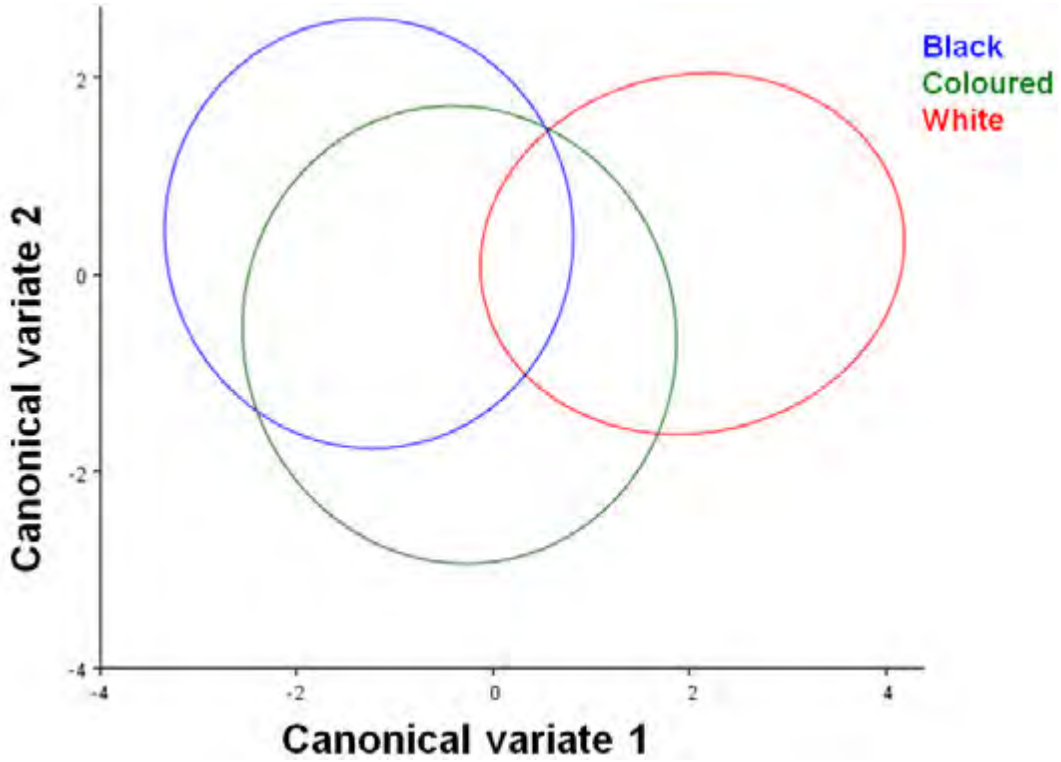


Figure 4.17: Whole cranial shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.18a-c (Appendix B – Figures B9 and B10). White individuals tended to have medio-laterally narrower orbital regions (more medial landmarks 3 and 14), but a medio-laterally wider cranial vault (more lateral landmarks 4 and 13) than Black or Coloured individuals. White individuals also had relatively more superior positioning of bregma and glabella (landmarks 1 and 2) and more anterior and superiorly positioned occipital landmarks 6 and 7, which resulted in more steeply sloped frontal and occipital regions, giving White individuals a more rounded cranium compared to individuals of the other groups. The crania of Black and Coloured individuals tended to be more antero-posteriorly elongated (more anterior landmarks 2 and 3, more superior landmark 1, more postero-inferior landmarks 6 and 7), with smaller mastoid processes (landmarks 8 and 11). Coloured individuals were similar to Black individuals, except for having a more prominent glabellar region (landmark 2) and more inferior opisthocranion (landmark 7) than Black individuals.

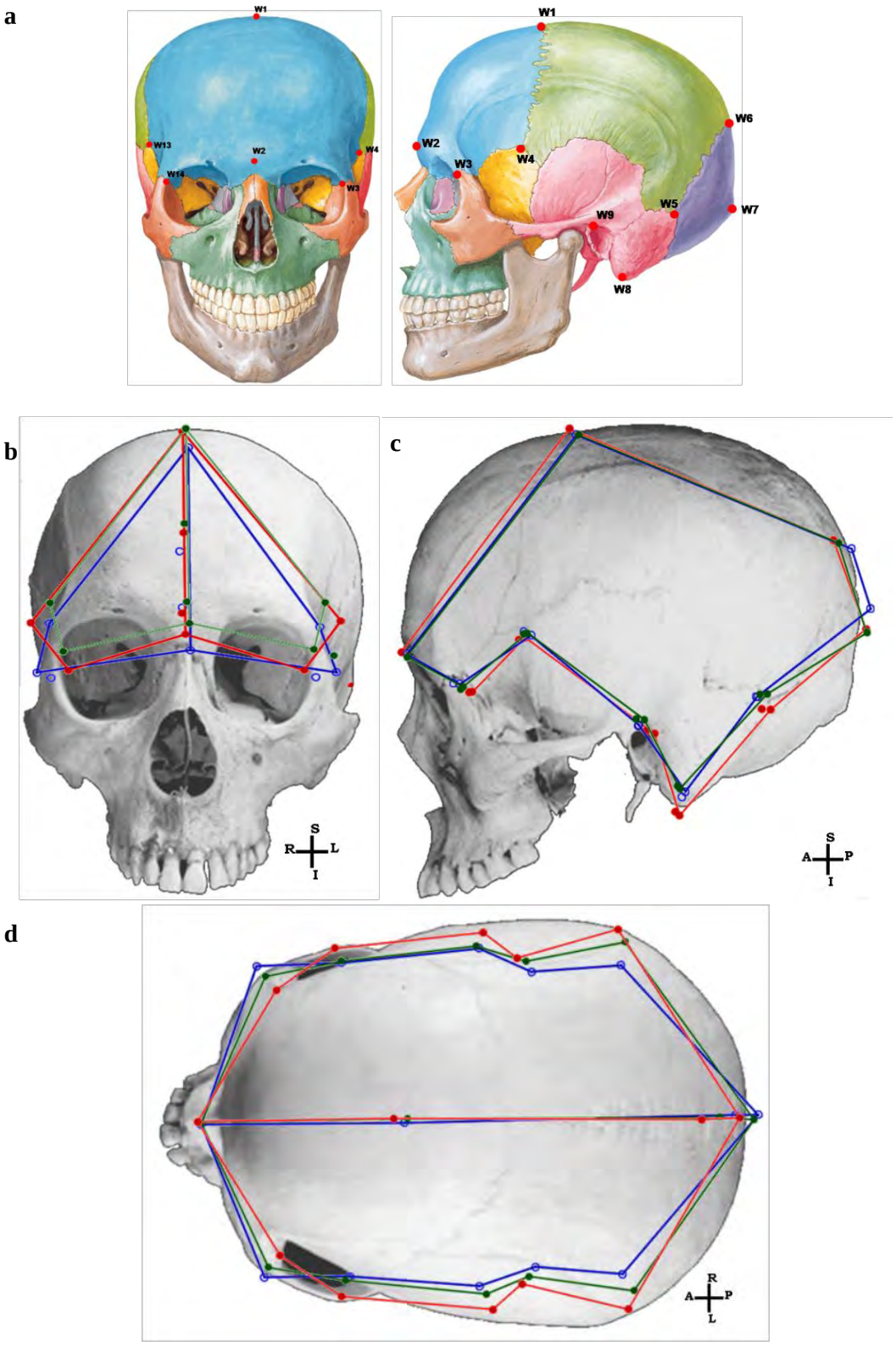


Figure 4.18: Whole cranial shape differences among ancestry groups – a) landmark locations, b) anterior view, c) lateral view, and d) superior view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization]

[Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.2.2. Frontal bone

CVA of the Procrustes residuals produced 2 canonical variates, both of which demonstrated significant separation of the three ancestry groups (Figure 4.19). CV1 accounted for 81.2% of the observed sample variance, separating Black individuals from White individuals, while Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 18.7% of the observed variance, separating Coloured individuals from Black and White individuals.

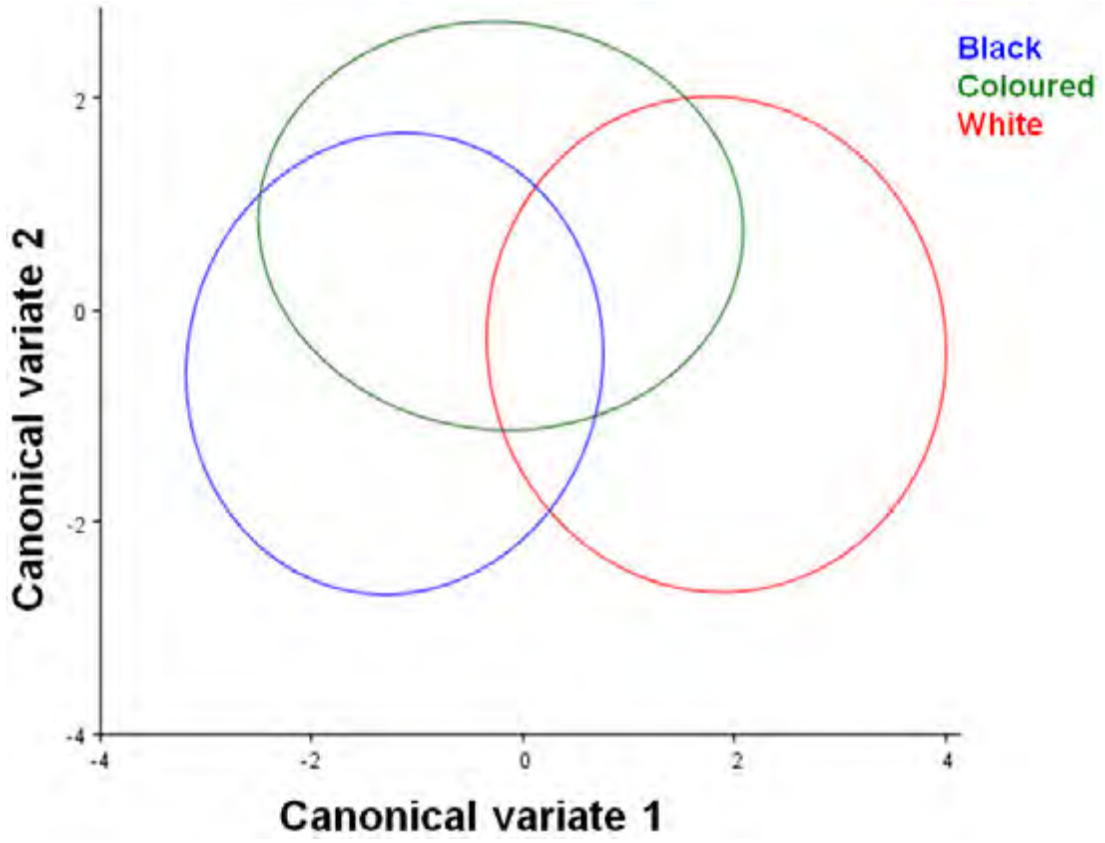


Figure 4.19: Frontal bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.20a-b (Appendix B – Figures B11 and B12). White individuals had a medio-laterally wider frontal bone (more lateral landmarks 7-10), but narrower orbital regions (landmarks 6 and 11), compared to individuals of the other two groups. Black and Coloured individuals were very similar, except for the more antero-inferior position of landmarks 2 and 3 of Black individuals, resulting in a more rounded forehead compared to both of the other groups.

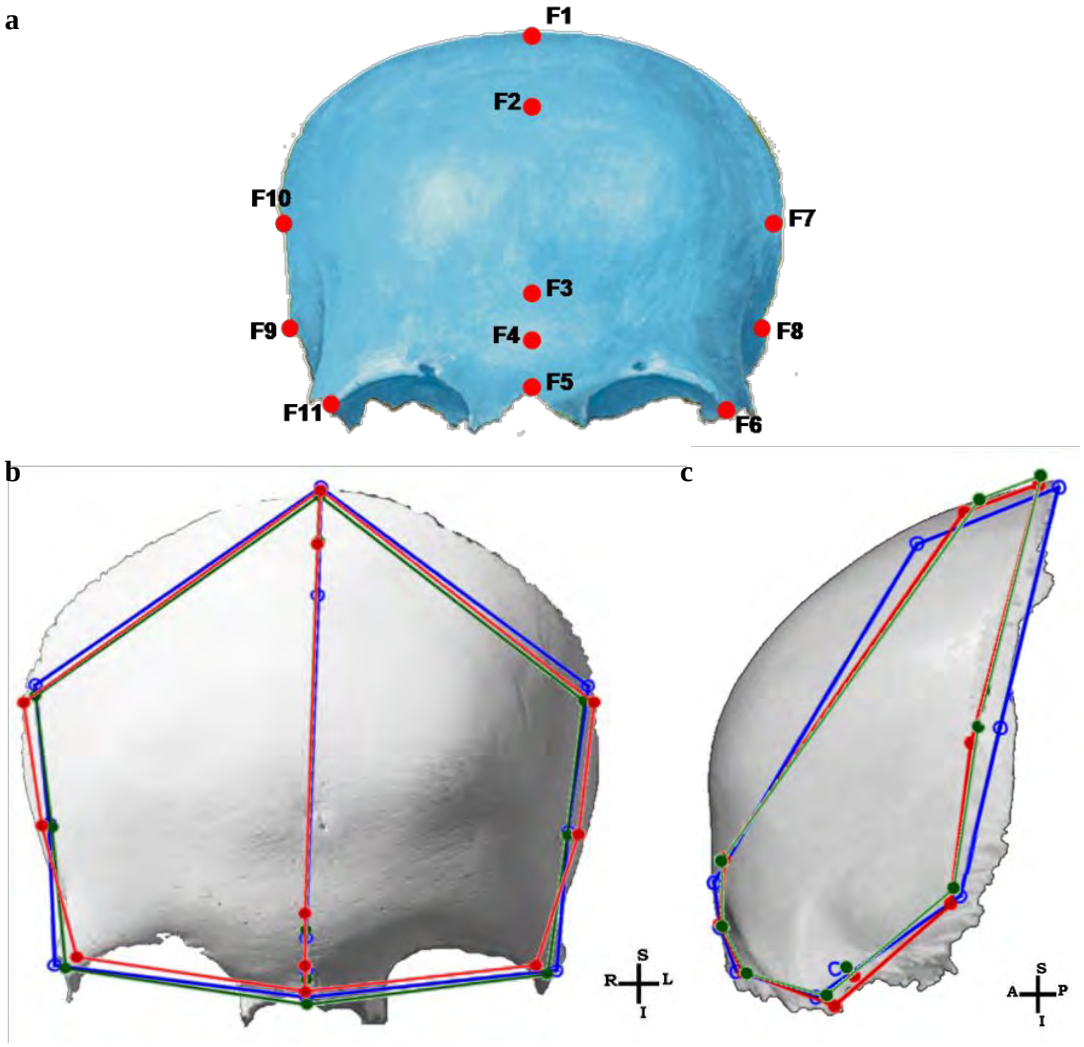


Figure 4.20: Frontal bone shape differences among ancestry groups – a) landmark locations, b) anterior view, and c) lateral view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [2X magnification of differences for visualization]

[Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.2.3. Occipital bone

CVA of the Procrustes residuals produced 2 canonical variates, both of which yielded significant separation of the ancestry groups (Figure 4.21). CV1 accounted for 84.9% of the observed sample variance, separating Black individuals from White individuals, while Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 15.1% of the observed variance, showing some separation of Coloured individuals from Black and White individuals.

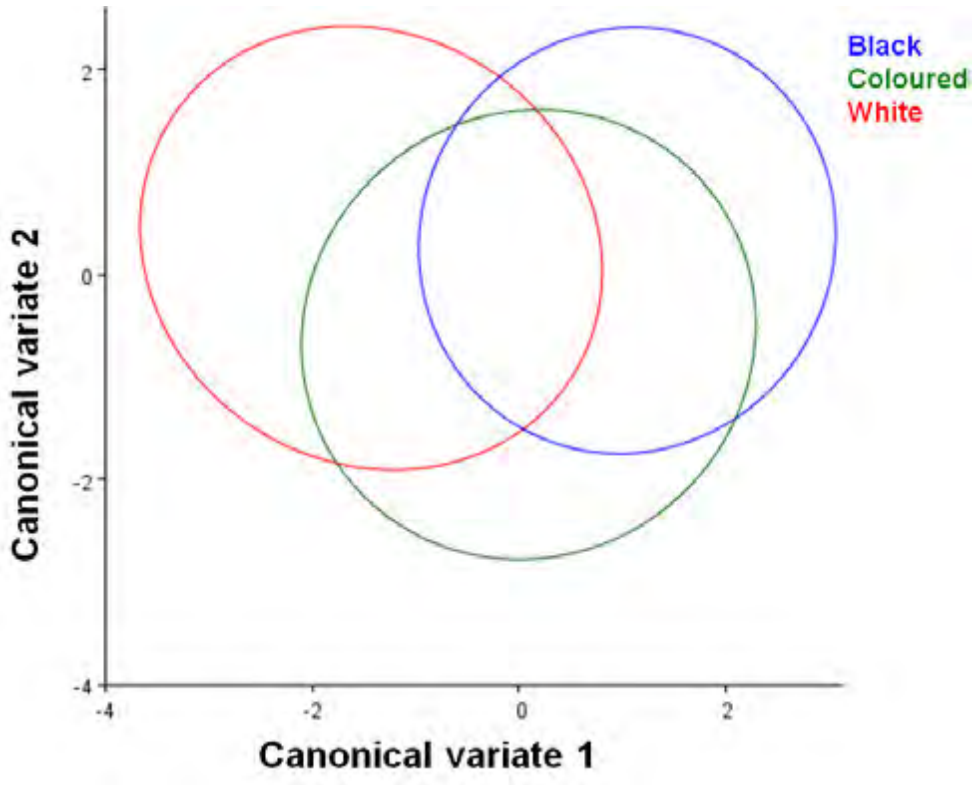


Figure 4.21: Occipital bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.22a-b (Appendix B – Figures B13 and B14). White individuals had antero-posteriorly longer occipital condyles (larger distance between landmarks 6 and 7, and between landmarks 10 and 11), but also had an antero-posteriorly shortened basilar portion (more posterior landmark 9) than Black and Coloured individuals. Coloured individuals differed from White individuals only in the more anterior projection of the basilar portion (landmark 9), and their shorter and more anterior occipital condyles compared to those of White individuals. Black individuals differed most from the other groups by having a much more anterior projecting basilar portion, but a relatively shorter bi-asterionic distance (more medial landmarks 1 and 12). The slope of the occipital bone was steeper in Black and Coloured individuals (more antero-superior landmarks 4 and 5), but Coloured individuals also had a more anteriorly positioned lambda and opisthocranion (landmark 2 and 3), giving the occiput a more elongated appearance than that of Black individuals. White individuals further differed from the other groups by having more inferiorly positioned landmarks at the superior part of the occiput (landmarks 2-5) and a more steeply sloped posterior region formed by the more posterior positions of landmarks

4-6 and 11. The angle formed between the basilar and squamous portions of the occipital bone also differed among ancestry groups, with this angle being largest in Black individuals and smallest in White individuals, with Coloured individuals being intermediate.

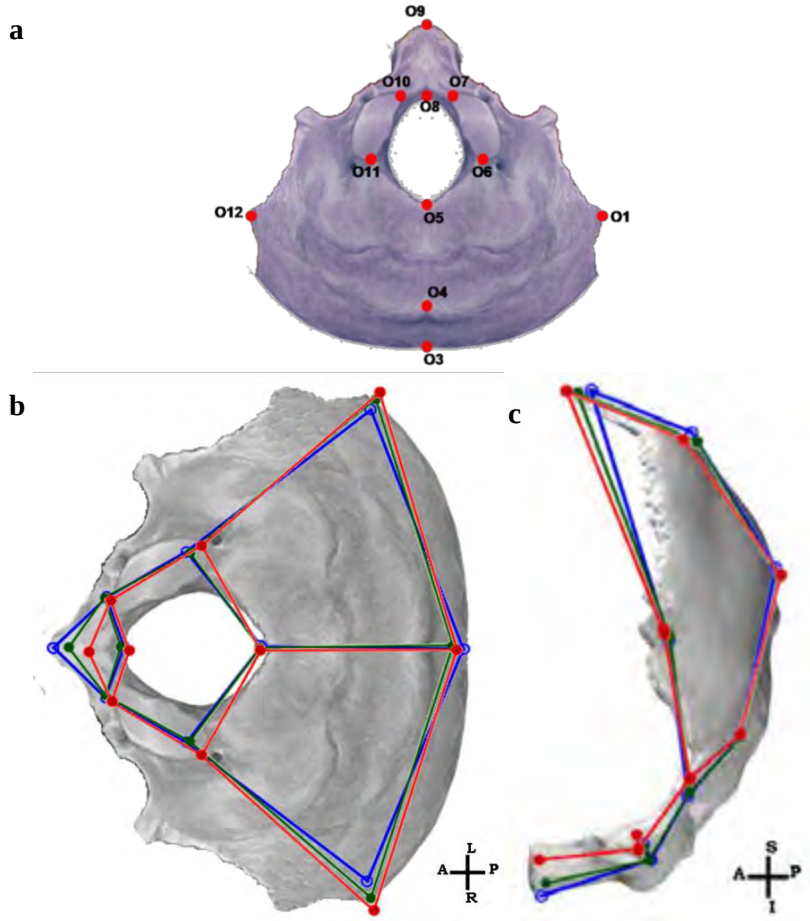


Figure 4.22: Occipital bone shape differences among ancestry groups – a) landmark locations, b) inferior view, and c) lateral view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization]

[Images adapted from White et al. (2012) and Hansen (2014)]

4.4.2.4. Parietal bone

CVA of the Procrustes residuals produced 2 canonical variates, both of which had significant separation of the ancestry groups (Figure 4.23). CV1 accounted for 81.7% of the observed sample variance, separating Black individuals from White individuals, while Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 18.3% of the observed variance, separating Coloured individuals from Black and White individuals.

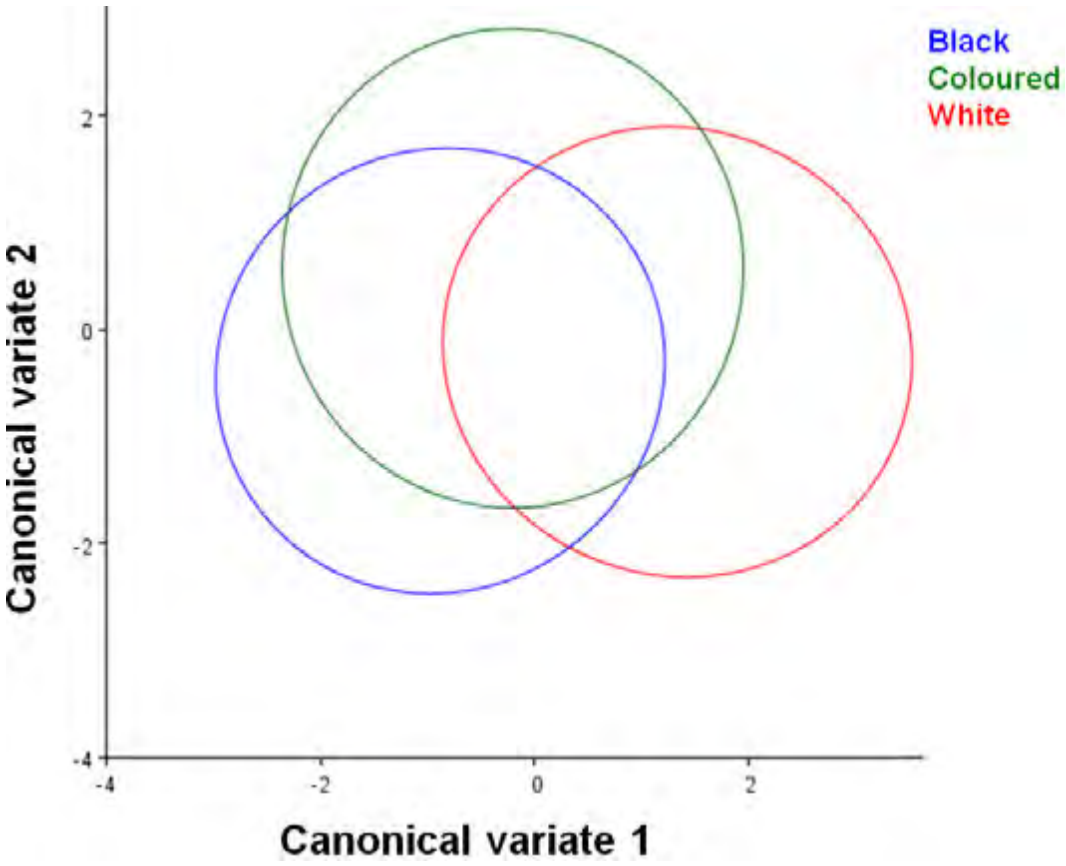


Figure 4.23: Parietal bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.24a-b (Appendix B – Figures B15 and B16). Black individuals had antero-posteriorly longer sagittal portions of the parietal bone (larger distance between landmarks 2 and 11), with a more anterior but less lateral euryon (landmark 6) than individuals of the other two groups. Coloured individuals had antero-posteriorly shorter sagittal portions, but a more postero-laterally positioned euryon. White individuals were intermediate to the other groups in the length of the sagittal portion, but had the most medially positioned euryon of the three groups. White individuals also had a more superiorly positioned vertex (landmark 1), more arched squamous border (landmarks 5, 7 and 8), and a more anteriorly positioned euryon and lambda (landmarks 6 and 11) compared to individual of the other ancestry groups. Black individuals had a more inferior sagittal portion (landmarks 1, 2, 10 and 11), with a more anterior vertex and more posterior obelion and lambda (landmarks 10 and 11) than White individuals. Coloured individuals were similar to Black individuals, except for having a more superior asterion (landmark 8) and more posterior euryon than the Black individuals.

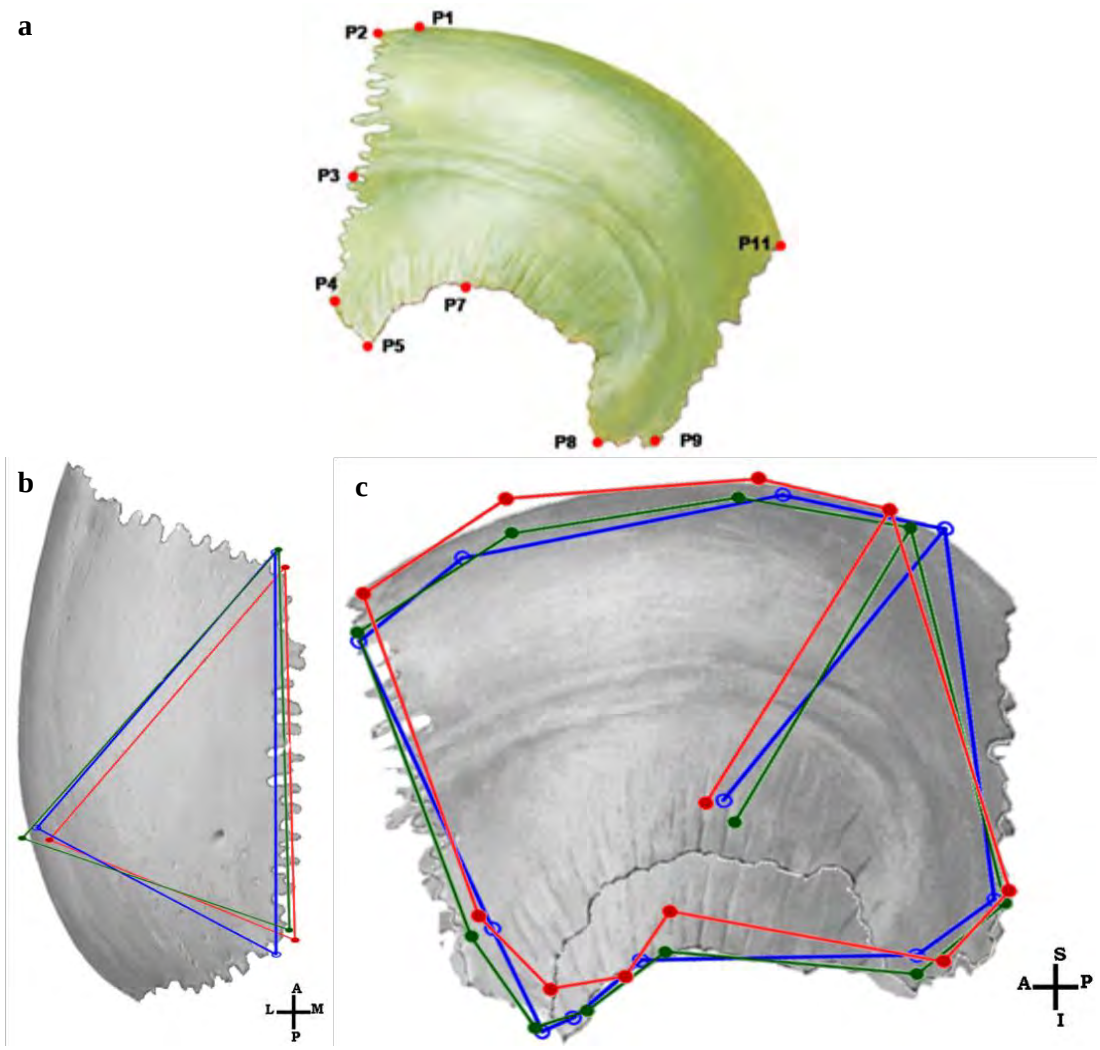


Figure 4.24: Parietal bone shape differences among ancestry groups – a) landmark locations, b) superior view, and c) lateral views, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization]

[Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.2.5. Temporal bone

CVA of the Procrustes residuals produced 2 canonical variates, both of which had significant separation of the ancestry groups (Figure 4.25). CV1 accounted for 85.0% of the observed sample variance, separating White individuals from Black and Coloured individuals. CV2 accounted for 15.0% of the observed variance, showing slight separation of Coloured individuals from Black and White individuals, though there was still considerable overlap among the groups.

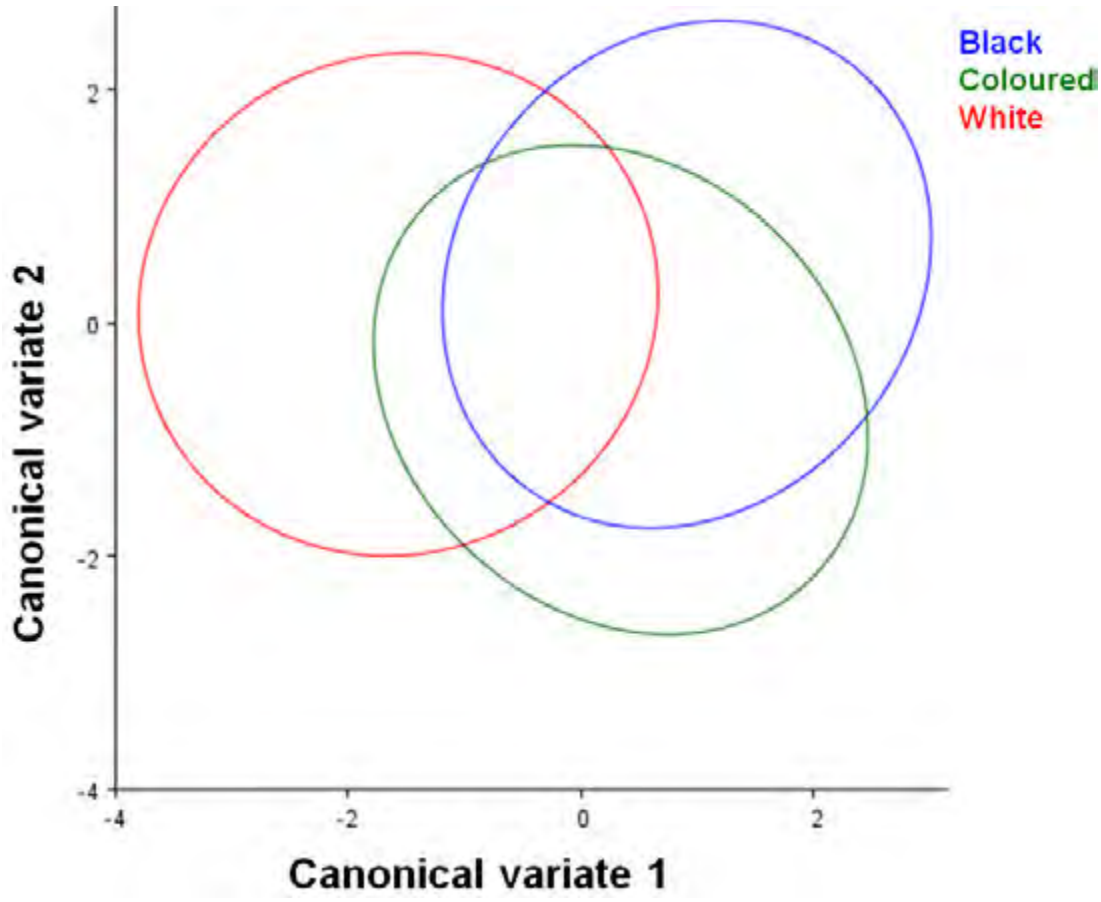


Figure 4.25: Temporal bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.26a-b (Appendix B – Figures B17 and B18). White individuals had an antero-posteriorly and medio-laterally smaller temporal bone (smaller distance between landmark 1 and 7, and between landmarks 1 and 11), more arched squamous border (landmarks 1-4), a larger mastoid process (landmark 5) and a more posteriorly positioned external auditory meatus and mandibular fossa (landmarks 6 and 9, landmark 7), compared to Black and Coloured individuals. Coloured individuals represented a combination of the features of to the other two groups, differing from these groups mainly in having a more posterior asterion (landmark 4) and a slightly more medial mandibular fossa (landmark 7).

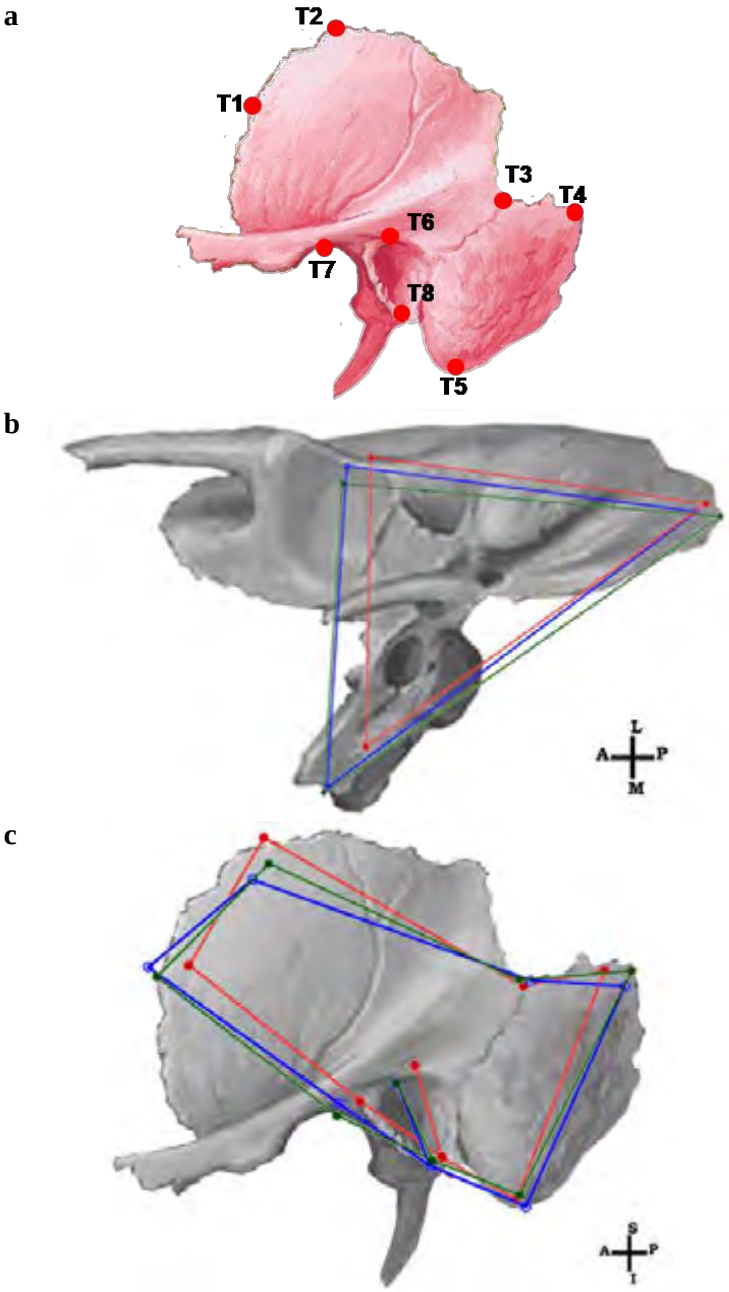


Figure 4.26: Temporal bone shape differences among ancestry groups – a) landmark locations, b) inferior view, and c) lateral views, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization]

[Images adapted from White *et al*. (2012) and Hansen (2014)]

Upper limb

4.4.2.6. Humerus

CVA of the Procrustes residuals produced 2 canonical variates, both of which had significant separation of the three ancestry groups (Figure 4.27). CV1 accounted for 83.3% of the observed sample variance, separating Black individuals from White

individuals, while Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 16.7% of the observed variance, separating Coloured individuals from both Black and White individuals.

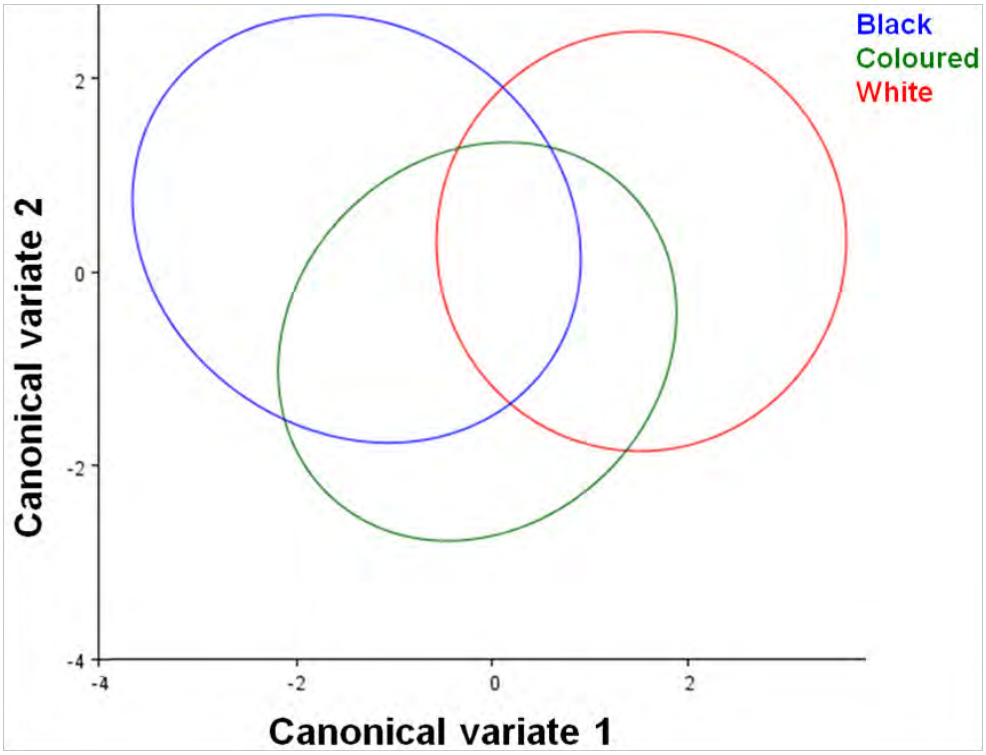


Figure 4.27: Humerus shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.28a-c (Appendix B – Figures B19 and B20). Black individuals tended to have a medio-laterally narrower proximal epiphysis (smaller distance between landmarks 1-4), resulting in a more vertically facing humeral head, compared to those of the individuals of the other ancestry groups. White individuals had a relatively more anterior projecting greater tuberosity, and the widest medio-lateral dimensions of the proximal humerus of all three groups. The more medial position of landmark 1 also resulted in a more horizontal facing head in White individuals, compared to the more vertical humeral head of Black individuals. Distally, the only differences between groups was in the slightly more medial position of the superior olecranon fossa (landmark 9) and the slightly more inferior projection of the trochlea (landmark 7) in White individuals. As with the comparison of the sexes, ancestry groups yielded no significant difference in relative supero-inferior length. It was further observed that the angle of retroversion was largest in Black individuals and smallest in White

individuals. Coloured individuals represented a combination of the features of the Black and White groups, but also had a more anteriorly positioned superior olecranon fossa (landmark 9), which suggested that the distal end of the humerus was antero-posteriorly flattened in Coloured individuals.

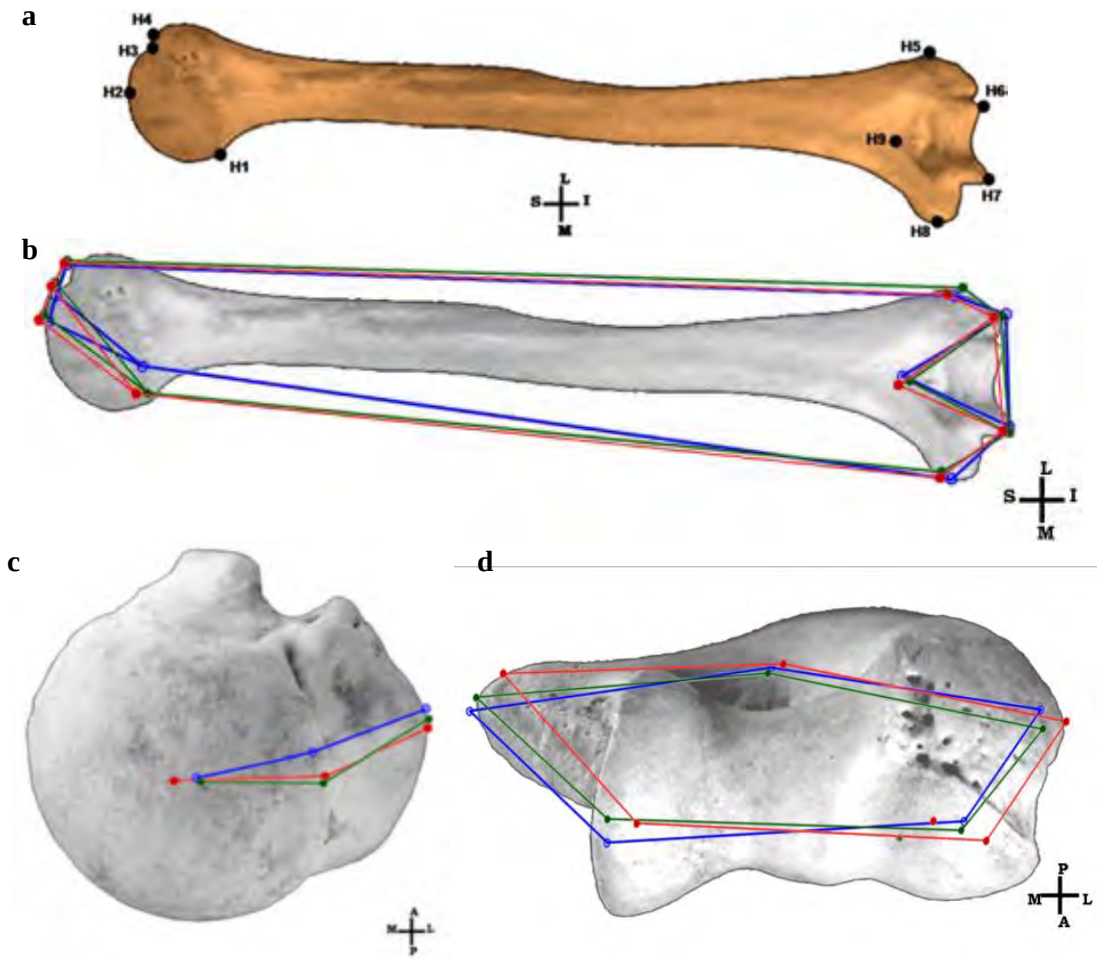


Figure 4.28: Humerus shape differences among ancestry groups – a) landmark locations, b) posterior view, c) humeral head, and d) distal epiphysis, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization in b]

[Images adapted from White *et al*. (2012)]

4.4.2.7. Radius

CVA of the Procrustes residuals produced 2 canonical variates, both of which showed significant separation of the ancestry groups (Figure 4.29). CV1 accounted for 66.5% of the observed sample variance, separating White individuals from Black individuals, while Coloured individuals did not separate from either group on this axis. CV2 accounted for 33.5% of the observed variance, separating Black and White individuals from Coloured individuals.

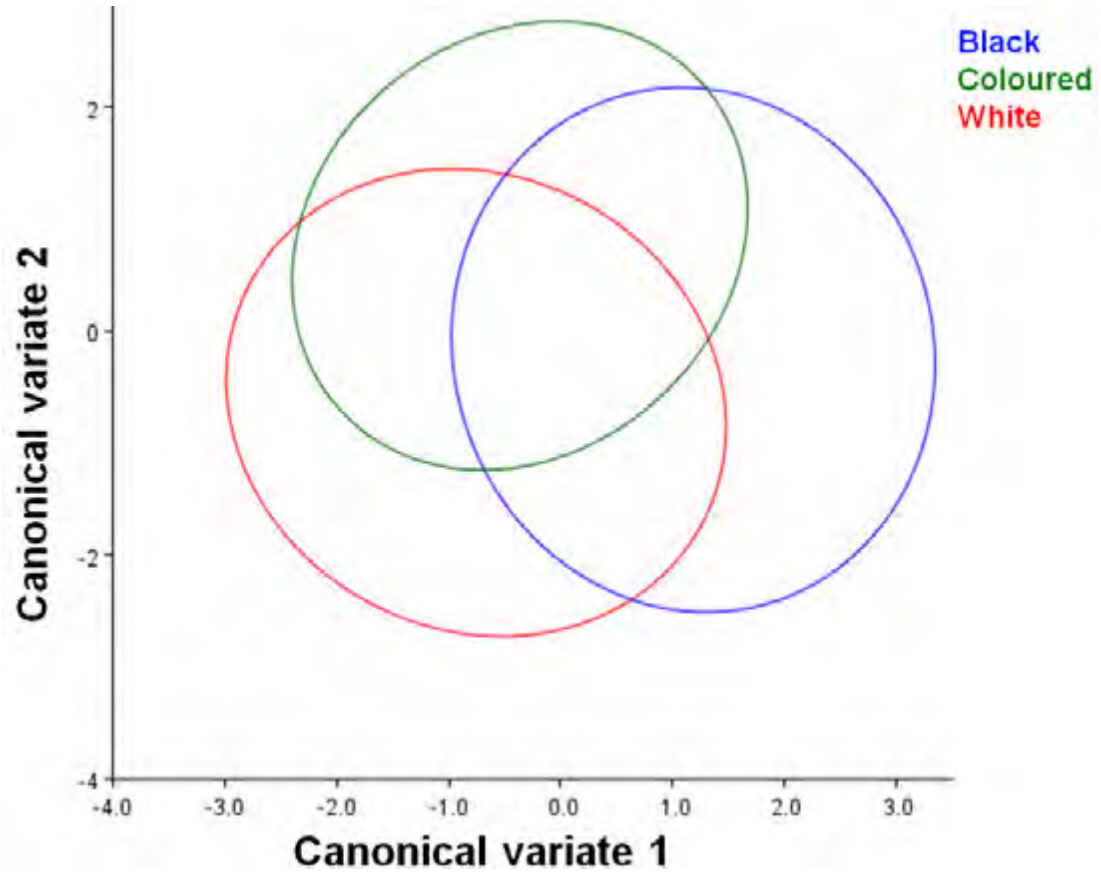


Figure 4.29: Radius shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.30a-c (Appendix B – Figures B21 and B22). Black individuals tended to have a medio-laterally narrower radius (smaller distance between landmarks 2 and 4, and between landmarks 5 and 6; more lateral landmark 1), a more medial dorsal tubercle (landmark 8), and a shorter supero-inferior length, compared to the individuals of the other groups. Black individuals also had a shorter styloid process (landmark 5) which resulted in a smaller angle of radial inclination compared to Coloured and White individuals. White individuals tended to have a medio-laterally wider radius, with a larger radial tuberosity (more medial landmark 1), and a more inferior projecting distal epiphysis, resulting in a larger radial inclination angle. Coloured individuals represented a combination of the features of the other two groups. Further, it was observed that there was a relative counter-clockwise rotation of the radial head in Black individuals, while that of White and Coloured individuals was less rotated.

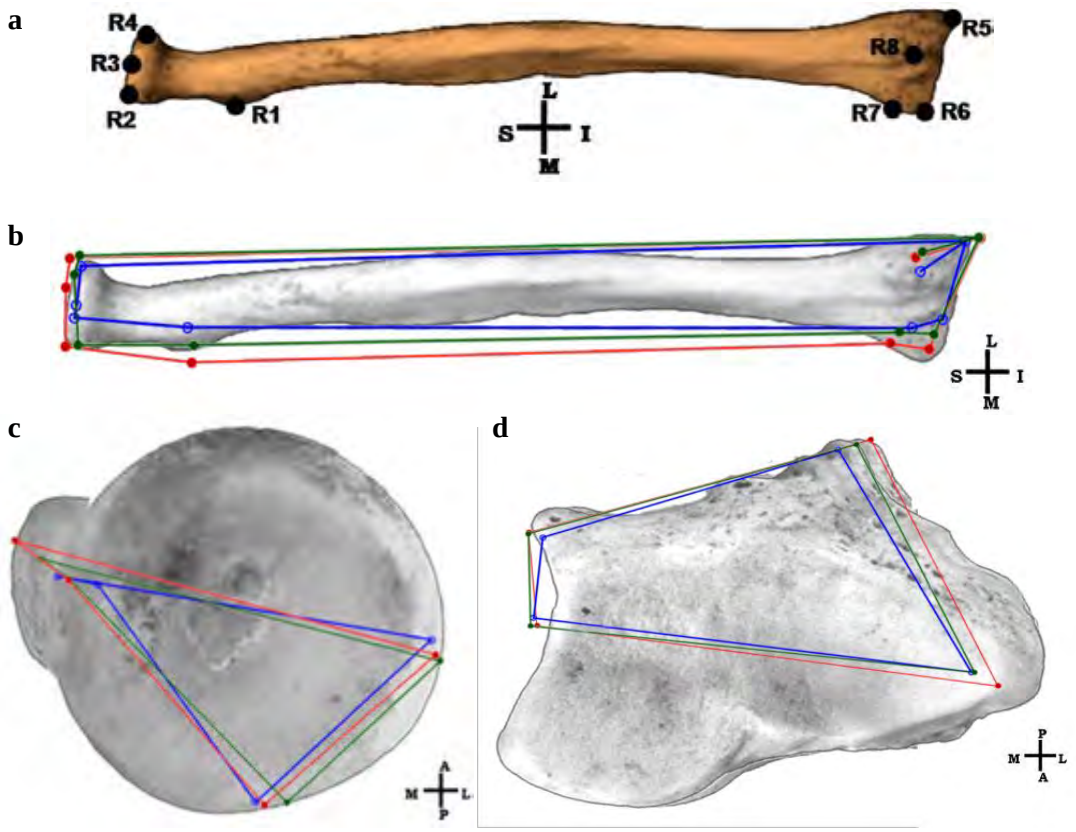


Figure 4.30: Radius shape differences among ancestry groups – a) landmark locations, b) posterior view, c) radial head, and d) distal epiphysis, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization in b]

[Images adapted from White *et al*. (2012)]

4.4.2.8. Ulna

CVA of the Procrustes residuals produced 2 canonical variates, both of which had significant separation of the three ancestry groups (Figure 4.31). CV1 accounted for 70.6% of the observed sample variance, separating Black individuals from White individuals, while Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 29.3% of the observed variance, separating Coloured individuals from Black and White individuals.

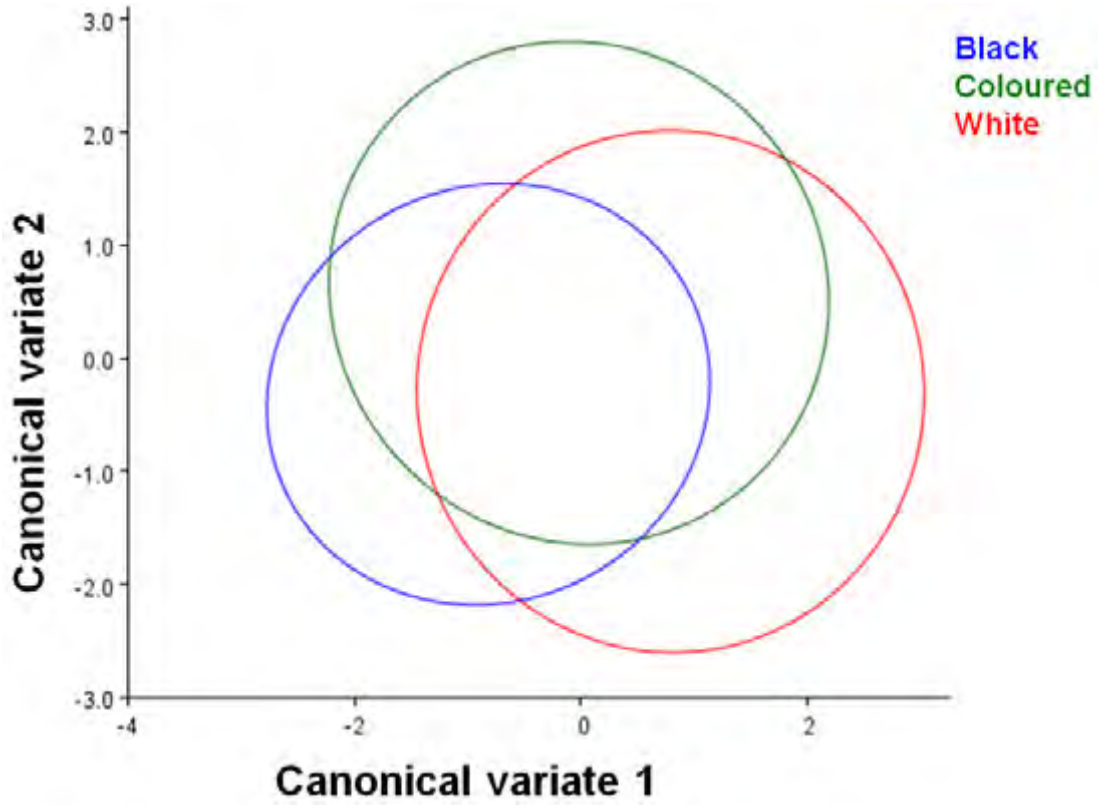


Figure 4.31: Ulna shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.32a-c (Appendix B – Figures B23 and B24). Black and Coloured individuals had a medio-laterally narrower proximal ulna than White individuals (smaller distance between landmarks 1 and 5; and between landmarks 6 and 8) which subsequently also had a more medially positioned coronoid process (landmark 2). Black individuals also had a shallower trochlear notch (more posterior landmark 2; more superior landmark 1), a smaller radial notch (more superior landmark 1), and a more angled and supero-inferiorly smaller ulnar head (more inferior landmark 6) than individuals of the other two groups. Coloured and White individuals differed only in Coloured individuals having a relatively supero-inferiorly shorter ulnar head (more superior landmarks 7 and 8) than White individuals.

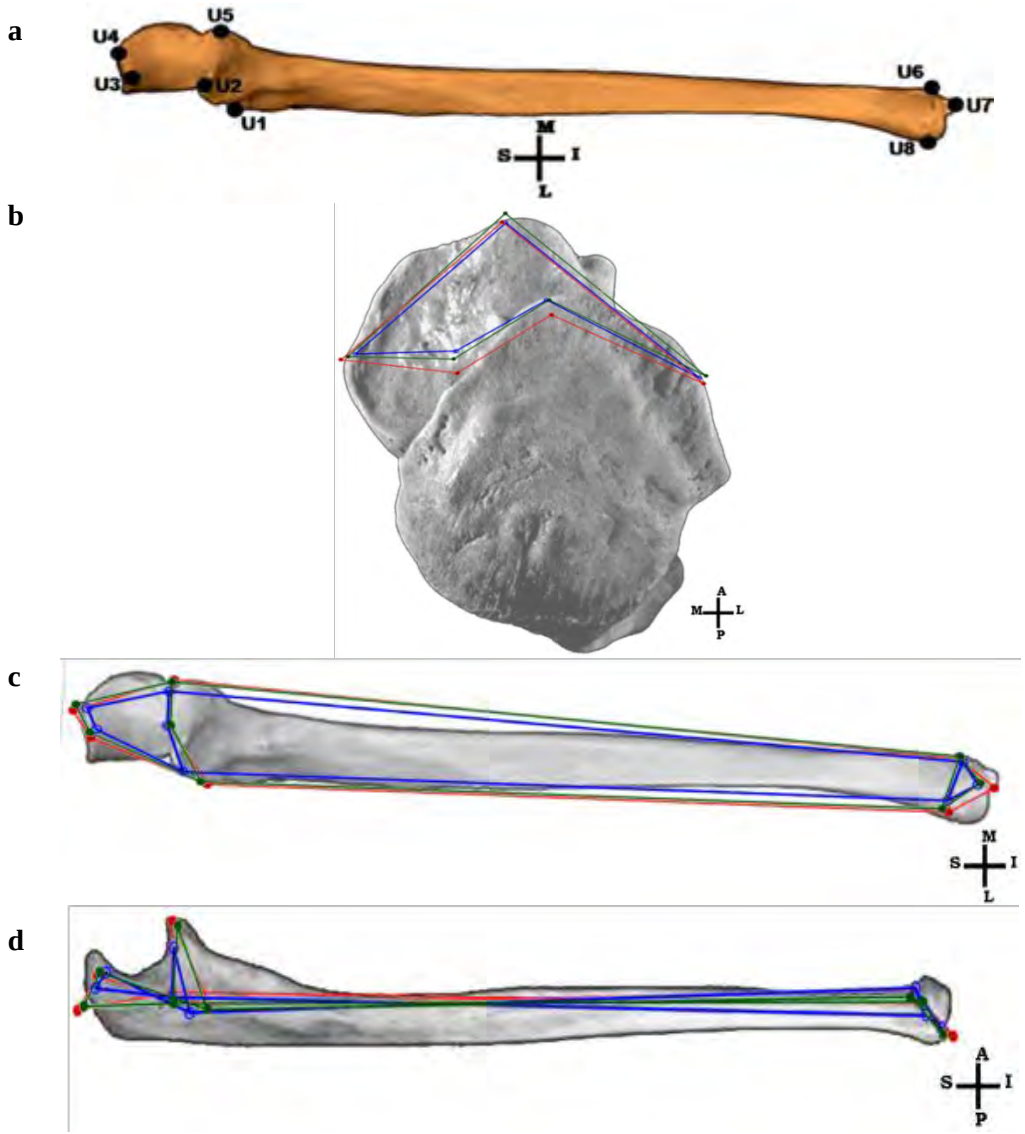


Figure 4.32: Ulna shape differences among ancestry groups – a) landmark locations, b) proximal epiphysis, c) anterior view, and d) lateral view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization in c and d]

[Images adapted from White *et al*. (2012)]

Lower limb

4.4.2.9. Femur

CVA of the Procrustes residuals produced 2 canonical variates, both of which had significant separation of the three ancestry groups (Figure 4.33). CV1 accounted for 67.6% of the overall sample variation, separating White individuals from Black individuals. Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 32.4% of the observed variance, separating Coloured individuals from Black and White individuals.

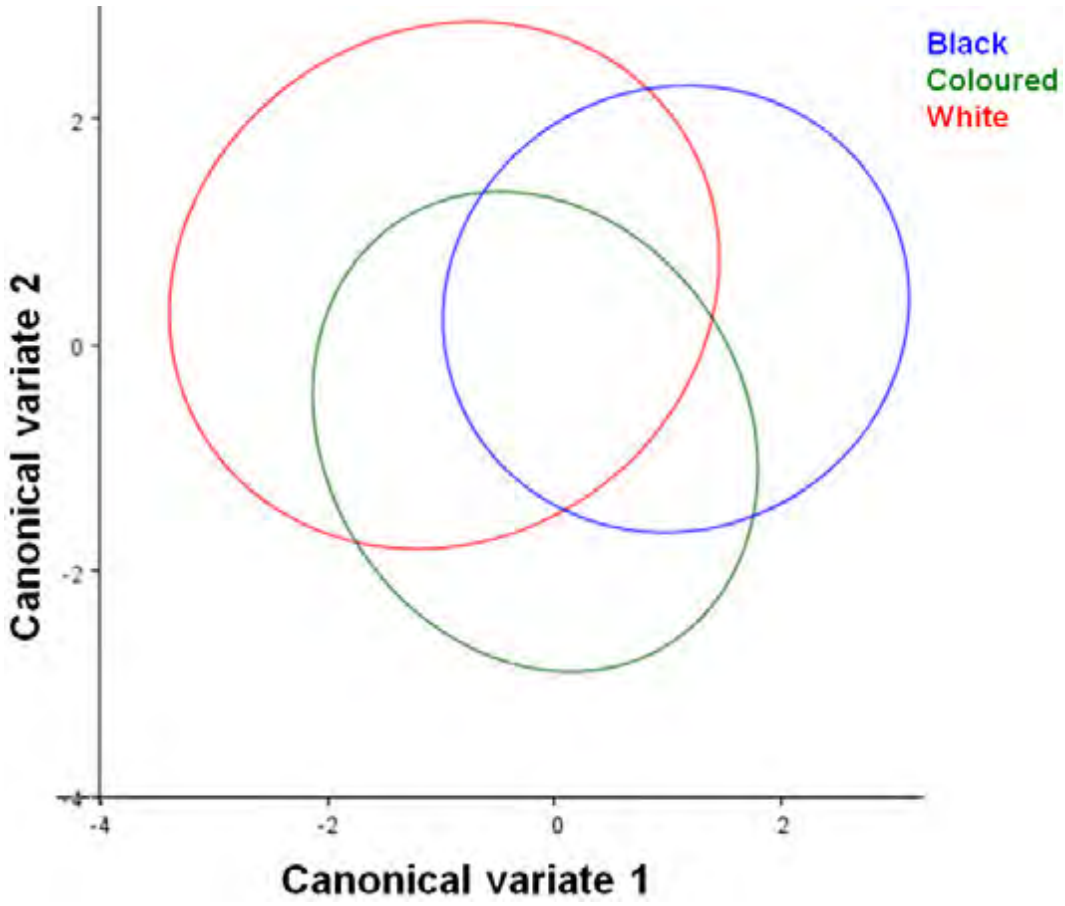


Figure 4.33: Femur shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.34a-c (Appendix B – Figures B25 and B26). Both White and Coloured individuals had supero-inferiorly larger medial condyles (more superior landmark 9) and medio-laterally wider proximal and distal epiphyses (larger distance between landmarks 3 and 5, and between landmarks 6 and 9) than Black individuals. White individuals also had supero-inferiorly larger femora (larger distance between landmarks 4 and 7) than both Black and Coloured individuals. Black individuals had a smaller lesser trochanter (more lateral landmark 1), larger neck-shaft angle (more superior landmark 3), and narrower femoral neck (smaller distance between landmarks 2 and 4) than the individuals of the other groups. Further, it was observed that Coloured individuals had the largest anteversion angle (clockwise rotation of landmarks 3 and 5, and of landmarks 6 and 7), followed by White individuals, while Black individuals had the smallest anteversion angle.

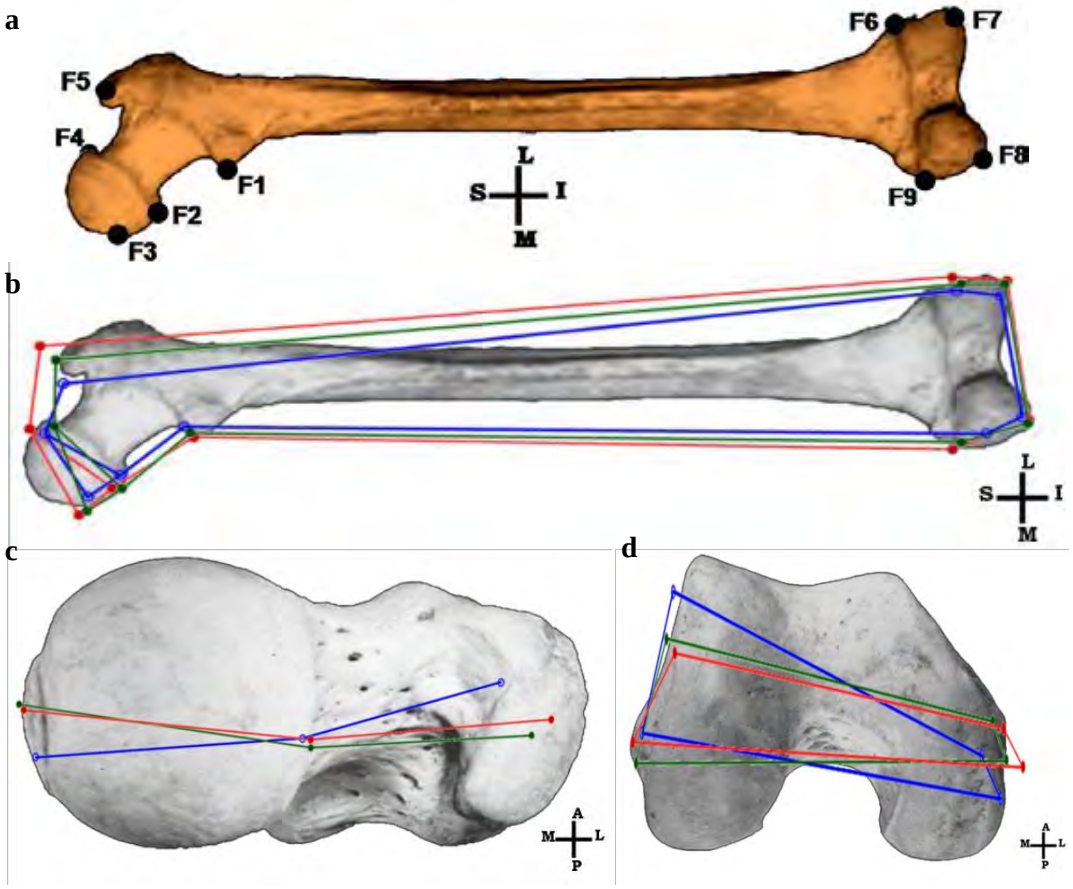


Figure 4.34: Femur shape differences among ancestry groups – a) landmark locations, b) posterior view, c) femoral head, and d) distal epiphysis, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization in b]

[Images adapted from White *et al*. (2012)]

4.4.2.10. Tibia

When the tibia data set was analysed with or without the tibial tuberosity landmark, significant separation of the ancestry groups was observed. The results of the two analyses are thus given separately.

Tibia with tuberosity

CVA of the Procrustes residuals produced 2 canonical variates, both of which yielded significant separation of the three ancestry groups (Figure 4.35). CV1 accounted for 58.4% of the overall sample variation, separating White individuals from Black individuals, while Coloured individuals did not separate from either of the other groups on this axis. CV2 accounted for 41.6% of the overall variation, separating Coloured individuals from Black and White individuals.

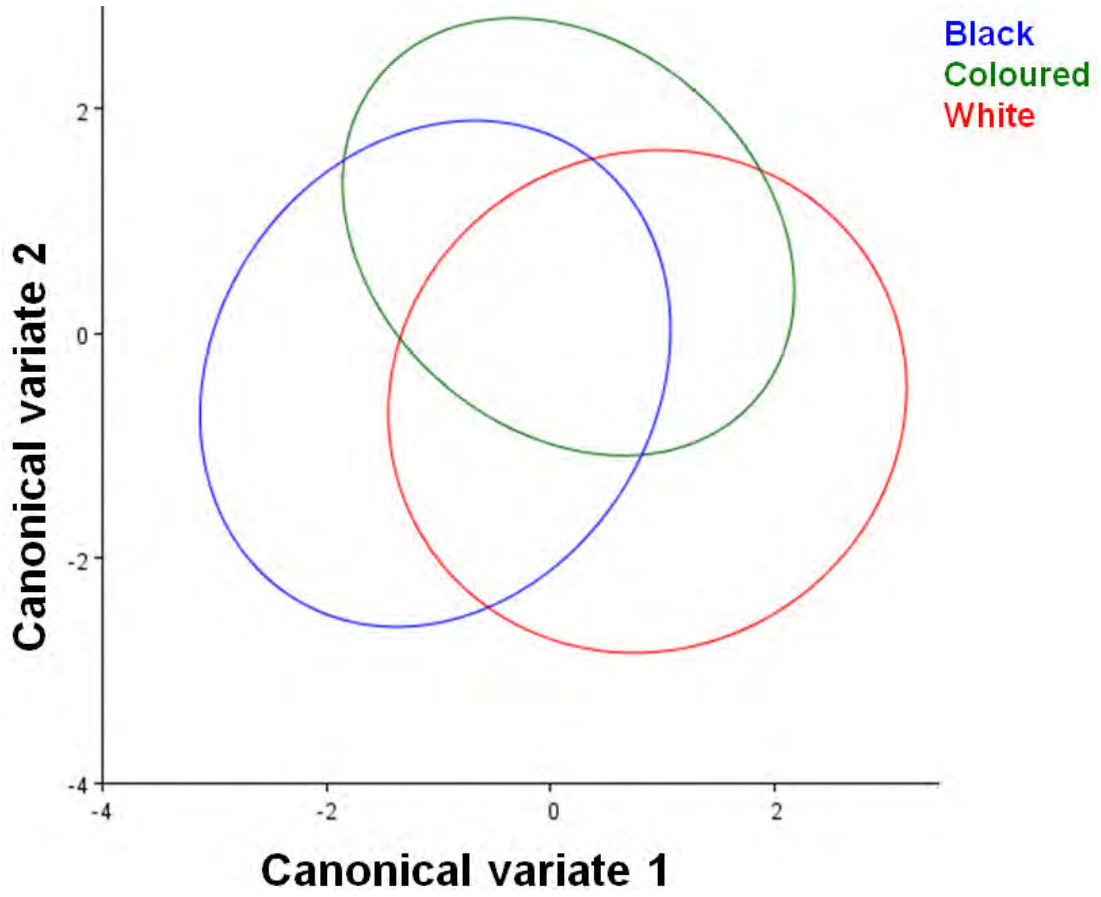


Figure 4.35: Tibia shape (including tuberosity) – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.36a-c (Appendix B – Figures B27 and B28). Black individuals had a medio-laterally narrower tibia both proximally and distally (smaller distance between landmarks 2 and 6, and between landmarks 7 and 8), a relatively more superior tibial plateau (landmarks 2, 3, 5 and 6), and a more infero-lateral tibial tuberosity (landmark 1), compared to individuals of the other two groups. Coloured individuals had smaller intercondylar eminences (more inferior landmarks 3 and 5), and a more supero-medial tibial tuberosity than Black or White individuals. The tibial tuberosity of White individuals was intermediate to that of the other two groups.

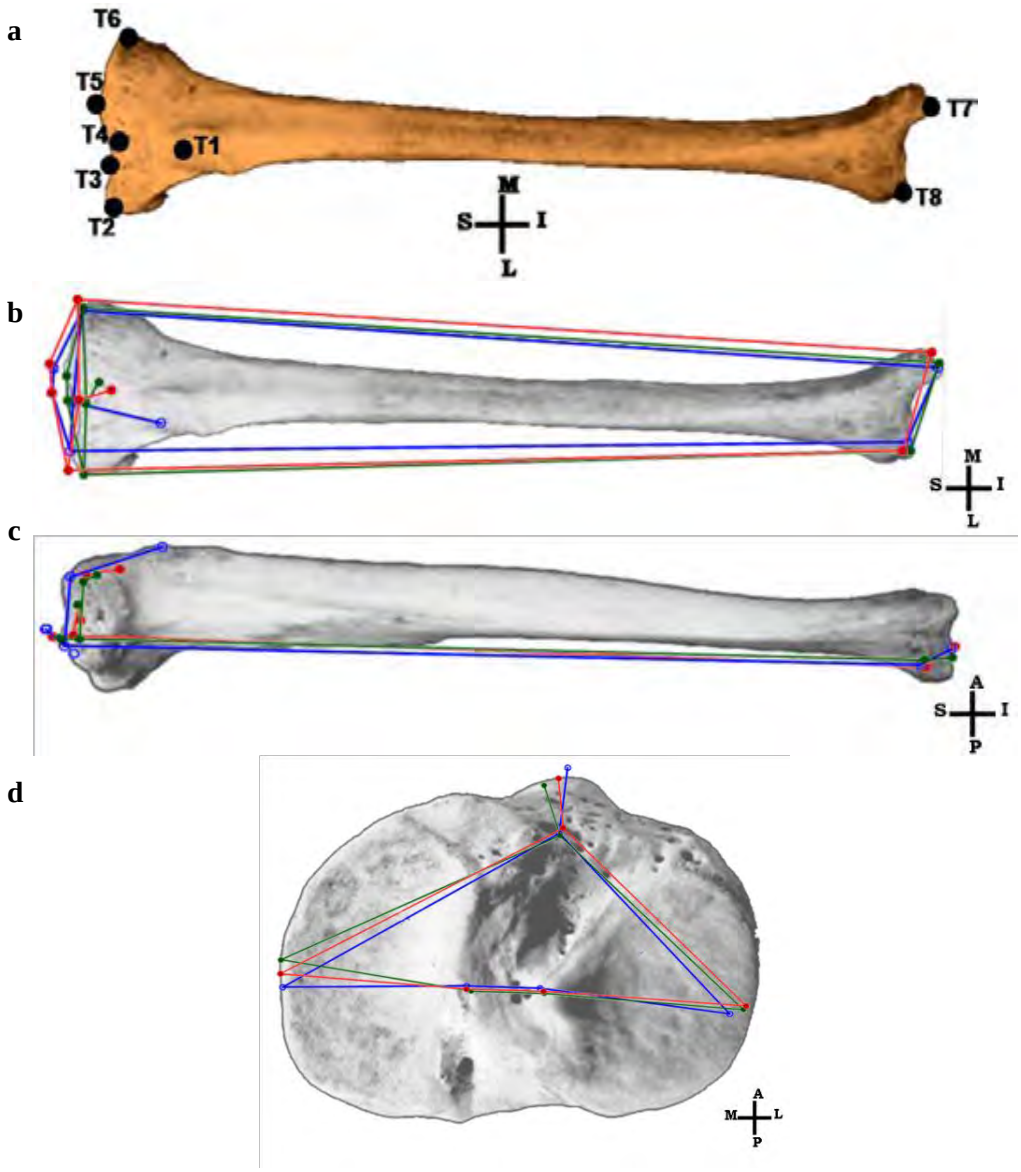


Figure 4.36: Tibia shape (including tuberosity) differences among ancestry groups – a) landmark locations, b) anterior view, c) lateral view, and d) superior view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization in b and c]

[Images adapted from White *et al*. (2012)]

Tibia without tuberosity

Once the tuberosity landmark was excluded from the analysis, CVA of the Procrustes residuals produced 2 canonical variates, both of which yielded significant separation of the ancestry groups (Figures 4.37). CV1 accounted for 57.6% of the overall sample variation, separating White individuals from Black and Coloured individuals. CV2 accounted for 42.4% of the overall variation, separating Coloured individuals from Black individuals. White individuals did not separate from the other groups on this axis.

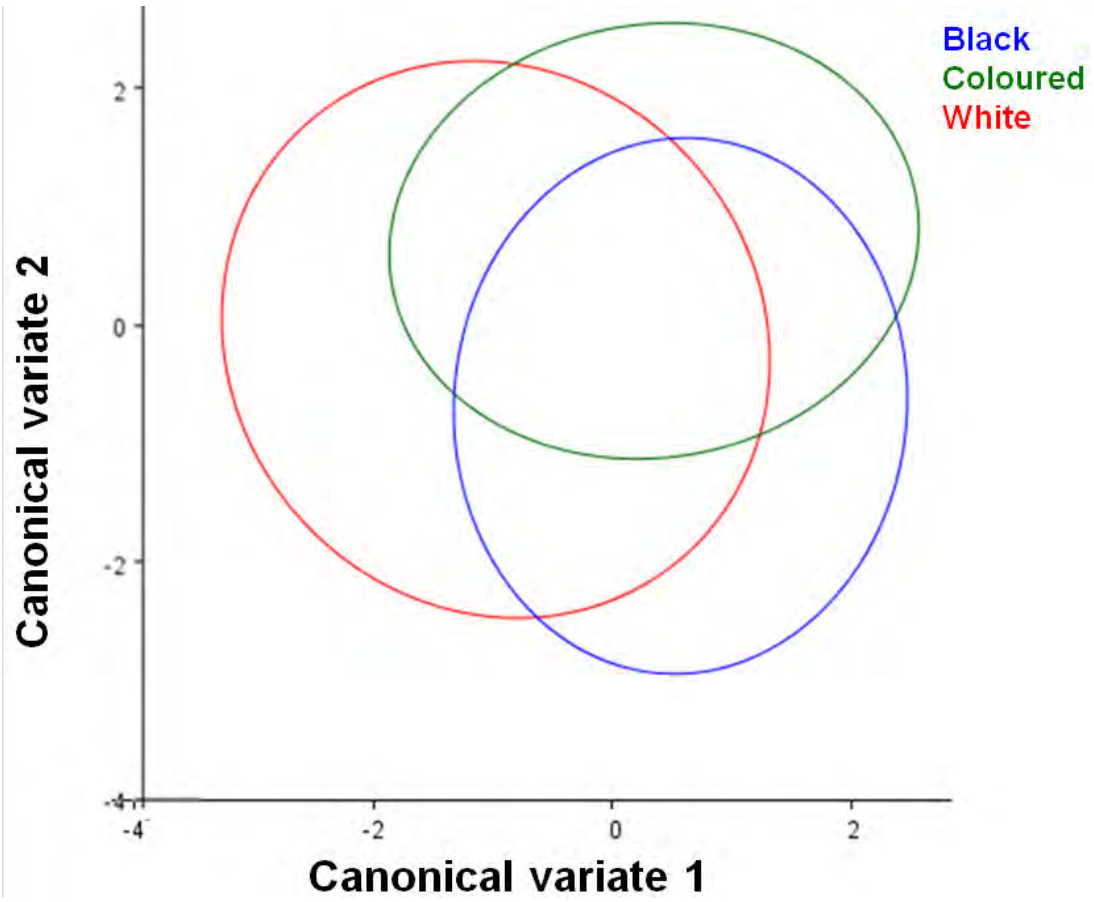


Figure 4.37: Tibia shape (excluding tuberosity) – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.38a-c (Appendix B – Figures B29 and B30). Black individuals still had a medio-laterally narrower tibia at both the proximal and distal ends (smaller distance between landmarks 2 and 6, and between landmarks 7 and 8) than Coloured and White individuals, but now also had more anteriorly positioned intercondylar eminences (landmarks 3 and 5). Coloured individuals were now observed to have a more anteriorly positioned fibular notch (landmark 8). As with the tuberosity included, White individuals represented a combination of the features of the other two groups.

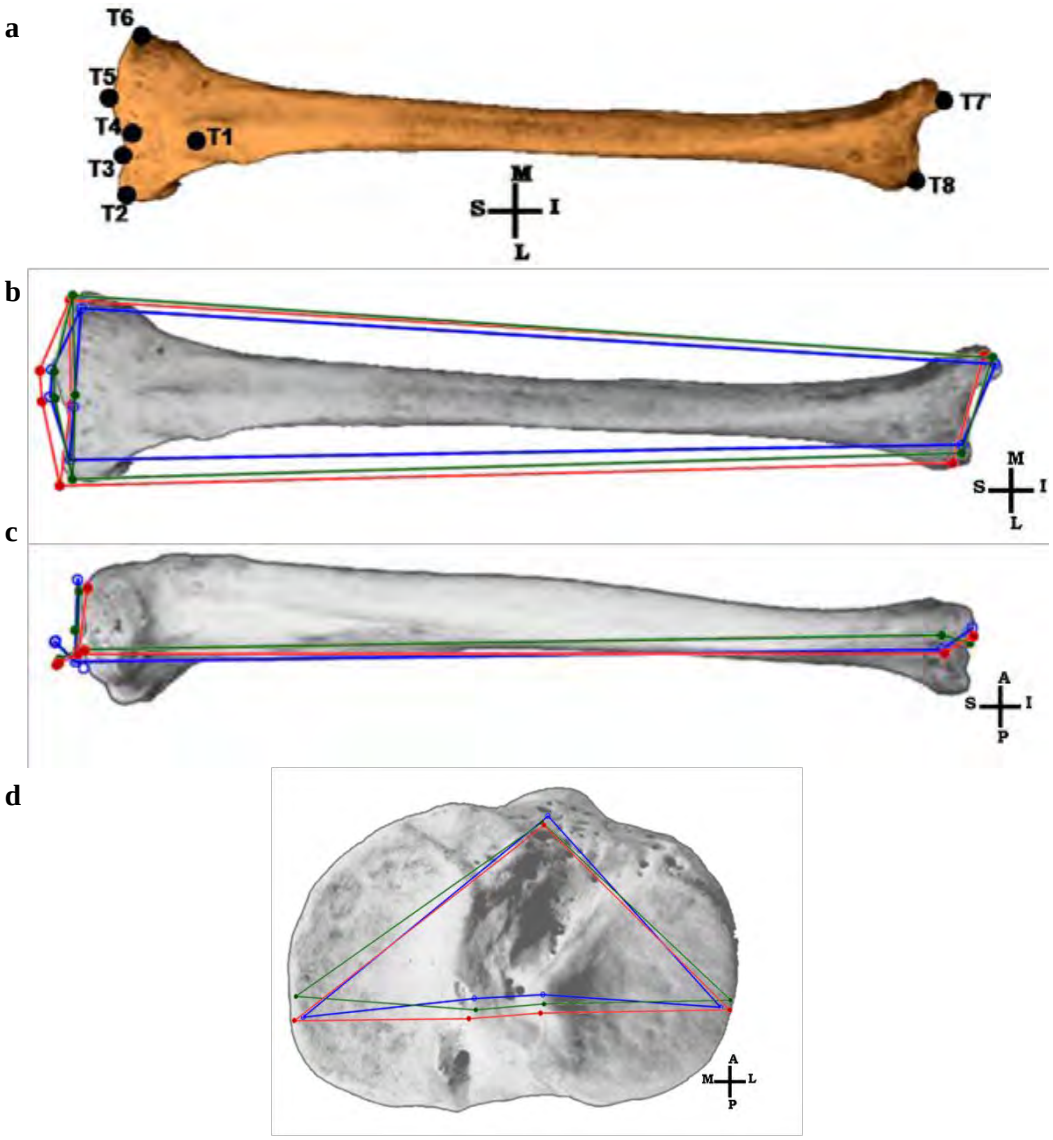


Figure 4.38: Tibia shape (excluding tuberosity) differences among ancestry groups – a) landmark locations, b) anterior view, c) lateral view, and d) superior view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization in b and c]

[Images adapted from White *et al*. (2012)]

4.4.2.11. Fibula

CVA of the Procrustes residuals produced 2 canonical variates, both of which presented significant separation of the three ancestry groups (Figure 4.39). CV1 accounted for 69.6% of the overall sample variation, separating White individuals from Black and Coloured individuals. CV2 accounted for 30.4% of the overall variation, separating Coloured individuals from Black and White individuals.

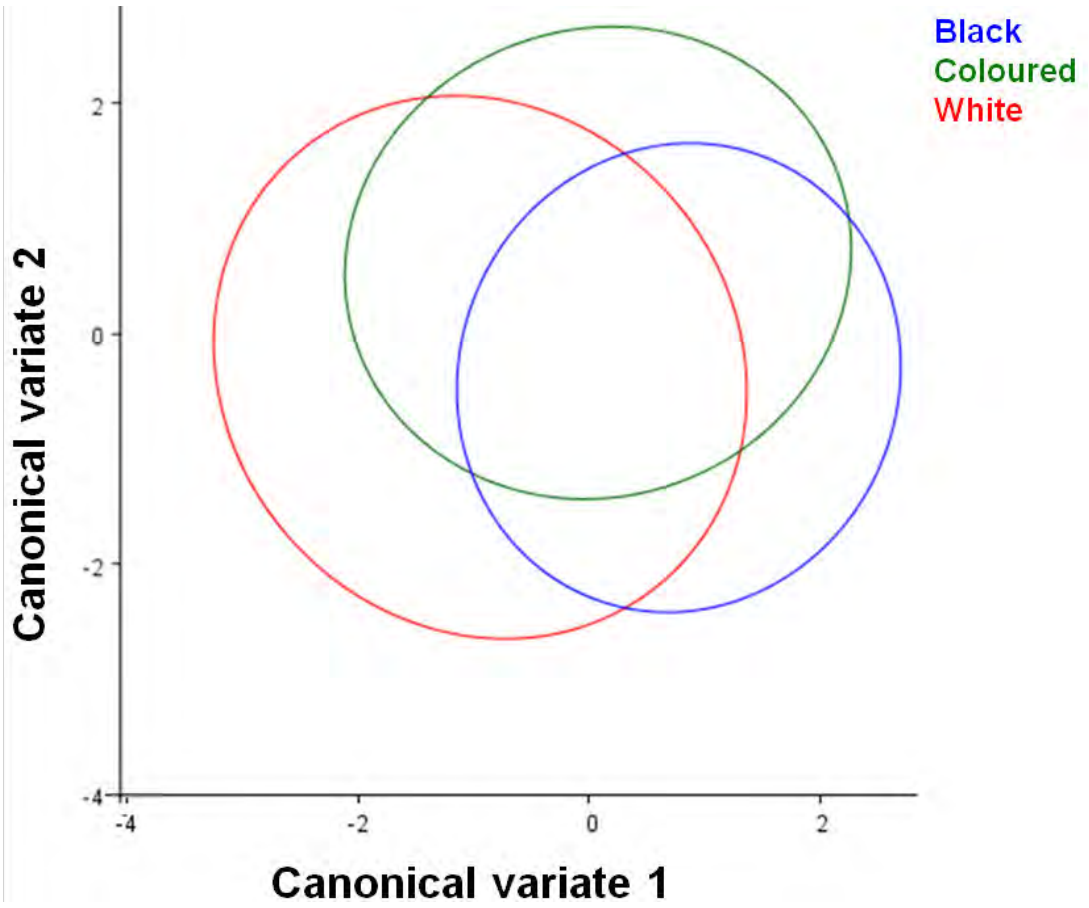


Figure 4.39: Fibula shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the three ancestry groups.

The shape variations associated with these canonical variates are represented in Figures 4.40a-b (Appendix B – Figures B31 and B32). Black individuals tended to have an antero-posteriorly and medio-laterally flattened fibula (smaller distance between landmarks 1 and 4, landmarks 5 and 8; landmarks 2 and 3, and between landmarks 7 and 8), as well as a supero-inferiorly shorter proximal epiphysis (smaller distance between landmarks 2 and 3) than Coloured or White individuals. White individuals had a slightly longer supero-inferior length, due mainly to the more superior position of landmark 3. Coloured individuals were similar to Black individuals at the proximal epiphysis, but more similar to White individuals at the distal epiphysis.

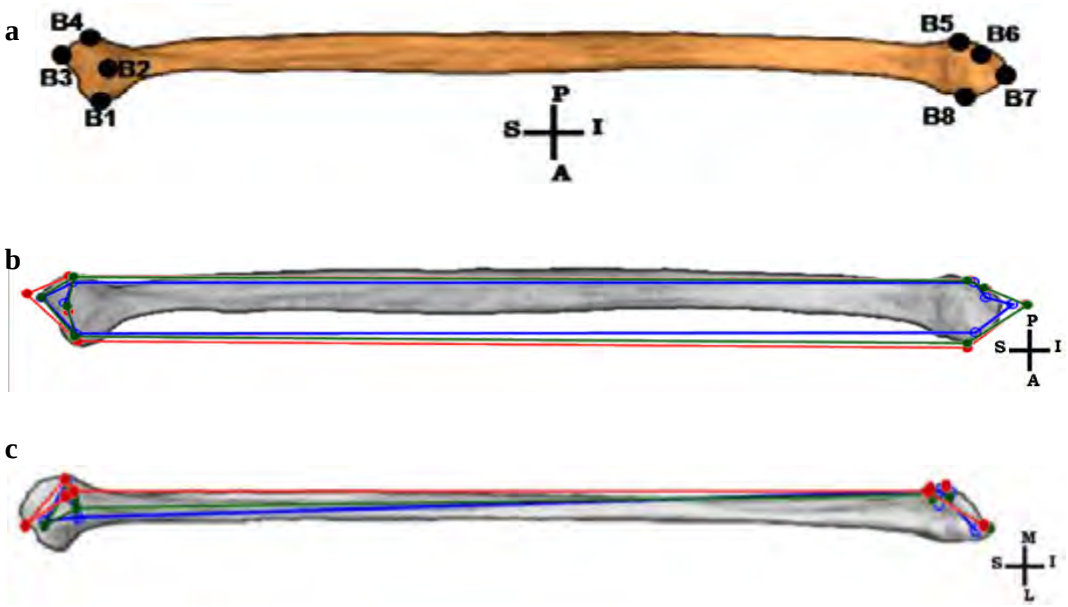


Figure 4.40: Fibula shape differences among ancestry groups – a) landmark locations, b) medial view, and c) anterior view, showing mean shapes of Black (blue), Coloured (green) and White (red) individuals. [5X magnification of differences for visualization] [Images adapted from White *et al*. (2012)]

4.4.2.12. Relationships to other variables

Regression analysis was performed to assess the potential relationship of the morphological differences among the three ancestry groups with centroid size, age and year-of-birth (Table 4.6). All elements showed a significant association of ancestry group differences to centroid size ($p \leq 0.03$), though only the associations of the frontal bone and radius were linked to more than 5% of the overall variation observed. All elements, except the whole cranium, occipital and parietal bones, had significant association of ancestry group differences with age ($p \leq 0.01$), but none of these associations exceeded 10% of the overall variation observed. Significant association of the differences among ancestry groups to year-of-birth were observed for all elements ($p \leq 0.02$), except for the whole cranium, occipital and temporal bones. However, only the associations of the radius and femur were linked to more than 5% of the overall observed variation.

Table 4.6: Relationship of differences in shape among ancestry groups with centroid size, age and year-of-birth [All values shown have p≤0.03].

Skeletal element	Coefficient of determination (R^2) (%)		
	Centroid size	Age	Year-of-birth
Cranium			
Whole	2.0	Not significant	Not significant
Frontal	10.1	4.0	0.9
Occipital	4.1	Not significant	Not significant
Parietal	4.1	Not significant	0.8
Temporal	2.1	2.4	Not significant
Upper limb			
Humerus	0.8	1.9	1.1
Radius	6.2	8.7	6.8
Ulna	1.8	6.3	2.8
Lower limb			
Femur	2.1	5.9	5.8
Tibia (with tuberosity)	2.9	5.4	3.2
Tibia (without tuberosity)	2.9	4.9	2.1
Fibula	1.2	4.7	3.6

4.4.2.13. Discriminant function analysis

Comparison of the Mahalanobis distances between groups demonstrated that Black and White individuals tended to be respectively more similar to Coloured individuals than to each other for most of the elements (Appendix C – Table C3). The distances between the three groups were, however, similar for the ulna and the tibia (with or without the tuberosity).

The leave-one-out cross-validation test indicated that the shape differences among the three ancestry groups were significant for all elements (all $p<0.0001$). The classification accuracies of the elements are shown in Table 4.7 (see Appendix C – Table C4 for sample sizes). Accuracies of the ancestry-pooled sample ranged from 73.6% for the ulna to 85.3% for the humerus. Classification accuracies between the ancestry groups did not significantly differ for the frontal bone, humerus, radius, tibia (without tuberosity) and the fibula. White individuals had significantly higher accuracies for the whole cranium, parietal and temporal bones than individuals of the other two groups (all $p\leq0.01$). Coloured individuals had significantly lower accuracies than those of the other groups for the occipital bone, ulna and tibia (with tuberosity), with all $p\leq0.03$. Lastly, Black individuals have significantly higher accuracies for the femur only ($p=0.005$).

Table 4.7: Leave-one-out cross-validation classification accuracies of the skeletal elements evaluated according to ancestry (Highest accuracies of cranial and postcranial elements indicated in red).

Skeletal element	Ancestry estimation accuracy (%)			
	Black	Coloured	White	Pooled ancestry
Cranium				
Whole	82.0	78.2	**90.2**	**83.0**
Frontal	**88.0**	**83.7**	88.0	**85.9**
Occipital	80.9	73.7	80.2	**78.2**
Parietal	77.9	74.8	82.6	**78.2**
Temporal	78.8	74.2	82.7	**78.3**
Upper limb				
Humerus	**84.7**	**84.0**	**87.2**	**85.3**
Radius	79.7	82.5	79.6	**80.6**
Ulna	76.9	70.6	73.2	**73.6**
Lower limb				
Femur	83.2	79.4	75.8	**79.5**
Tibia (with tuberosity)	78.6	83.4	80.0	**80.8**
Tibia (without tuberosity)	76.4	78.6	76.4	**77.2**
Fibula	77.2	72.4	75.1	**74.9**

4.4.3. Sex-ancestry

Cranial elements

4.4.3.1. Whole cranium

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two yielded significant separation of the sex-ancestry groups (Figure 4.41).

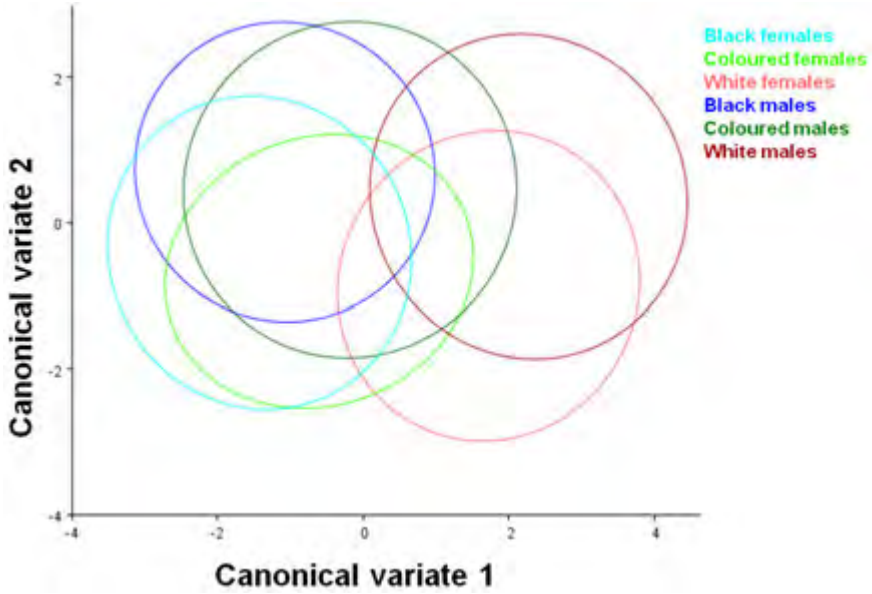


Figure 4.41: Whole cranial shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 73.6% of the total sample variation, and separated Black individuals from White individuals, while Coloured individuals did not separate from either group on this axis. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.18; also Appendix B – Figure B33). White individuals tended to have antero-posteriorly shorter, but medio-laterally wider crania, with more steeply sloped frontal and occipital regions and larger mastoids than Black and Coloured individuals.

CV2 accounted for 12.7% of the total sample variation, and produced only slight separation of females from males within each ancestry group. The shape variation associated with CV2 was similar to that seen when comparing the sexes independent of ancestry (Figure 4.3; also Appendix B – Figure B34). Females tended to have slightly wider medio-lateral dimensions, smaller mastoid processes and glabellar regions, with a more steeply sloped forehead, and a more posteriorly projecting occipital region resulting in a less steeply sloped occiput.

4.4.3.2. Frontal bone

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.42).

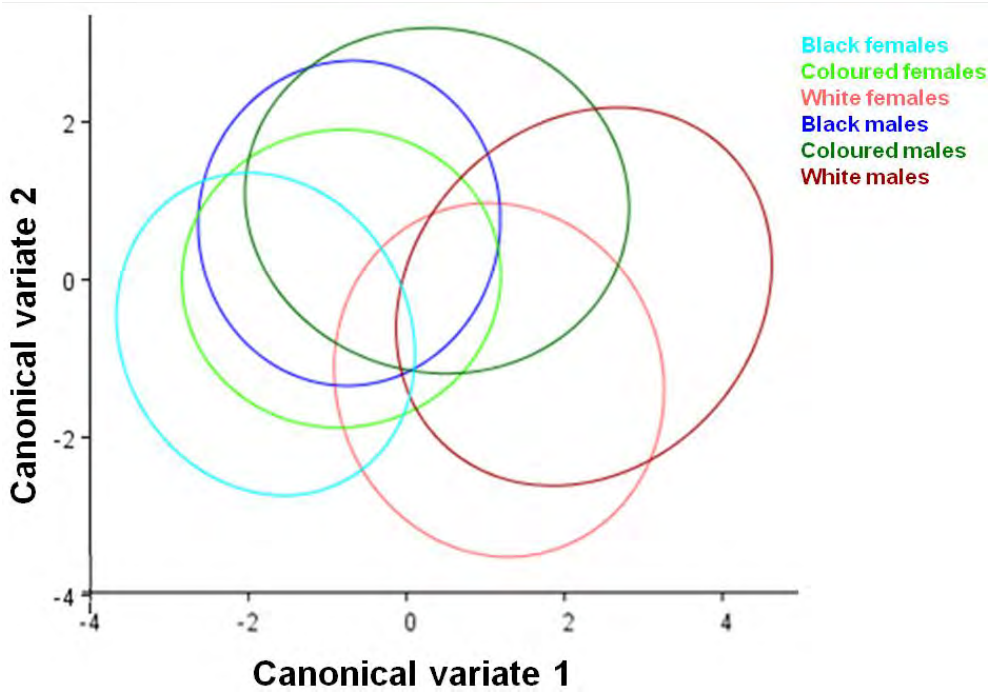


Figure 4.42: Frontal bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 64.4% of the observed sample variation, separating Black individuals from White individuals, while Coloured individuals did not separate from either group on this axis. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.20; also Appendix B – Figure B35). White individuals tended to have a medio-laterally wider frontal bone, but narrower orbital regions, while Black individuals had more rounded foreheads and antero-posteriorly elongated frontal bones. Coloured individuals represented a combination of the features of the other two groups.

CV2 accounted for 20.6% of the observed sample variation, separating females from males within each ancestry group. The shape variation associated with CV2 was similar to that seen when comparing the sexes independent of ancestry (Figure 4.5; also Appendix B – Figure B36). Females had a medio-laterally wider and more steeply sloped frontal bone, while males had more prominent glabellar regions, more inferior orbits, and more superiorly positioned temporal lines.

4.4.3.3. Occipital bone

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.43).

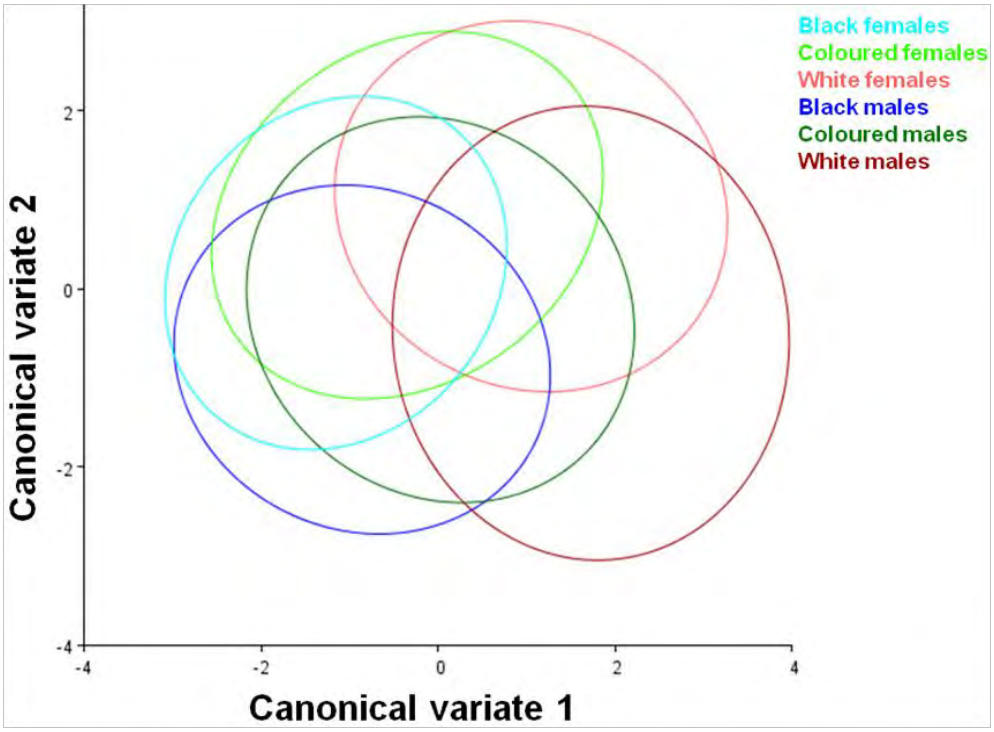


Figure 4.43: Occipital bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 62.7% of the total sample variation, separating Black individuals from White individuals, while Coloured individuals did not separate from either group on this axis. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.22; also Appendix B – Figure B37). Black individuals had an antero-posteriorly elongated but medio-laterally narrower occipital bone, with a larger angle between its basilar and squamous portions. White individuals had antero-posteriorly longer occipital condyles, but a shorter and less angled basilar portion which formed a less steep slope of the occiput. Coloured individuals represented a combination of the features of the other two groups.

CV2 accounted for 21.2% of the total sample variation, showing slight separation of the sexes within each ancestry group. This separation was not previously detected when sexes were examined independent of ancestry. An example of the sexual differences between the groups (using a comparison of Coloured females and males) is shown in Figures 4.44a-b (Appendix B – Figure B38). Females had shorter, more anterior and medially-inclined occipital condyles, and a more anteriorly positioned foramen magnum (more medial landmarks 6, 7, 10 and 11; more anterior landmarks 5, 6 and 11). Males tended to have a less steeply sloped occiput (more superior landmark 5), and a more horizontal angle between the basilar and squamous portions of the occipital bone (landmarks 7-11) than females.

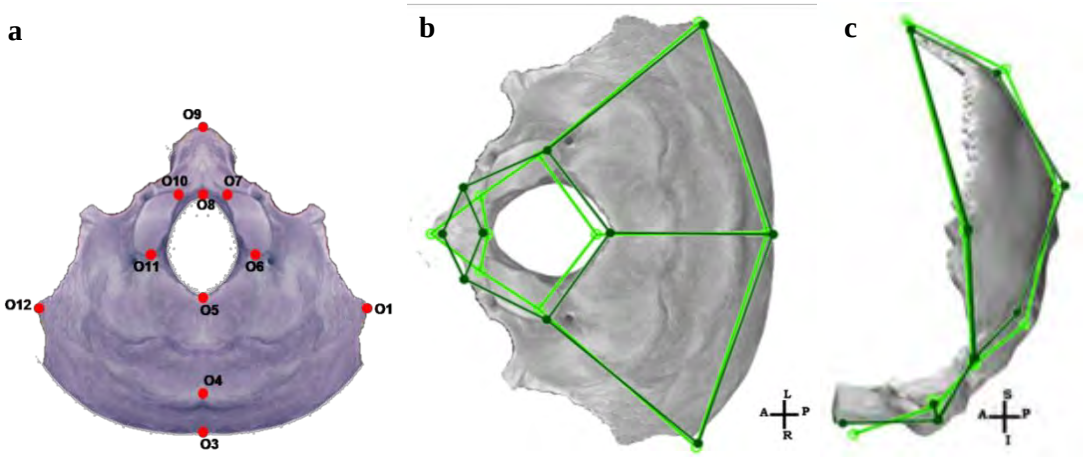


Figure 4.44: Occipital bone shape differences between the sexes as represented by Coloured females (light green) and Coloured males (dark green) – a) landmark locations, b) inferior view, and c) lateral view. [5X magnification of differences for visualization] [Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.3.4. Parietal bone

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.45).

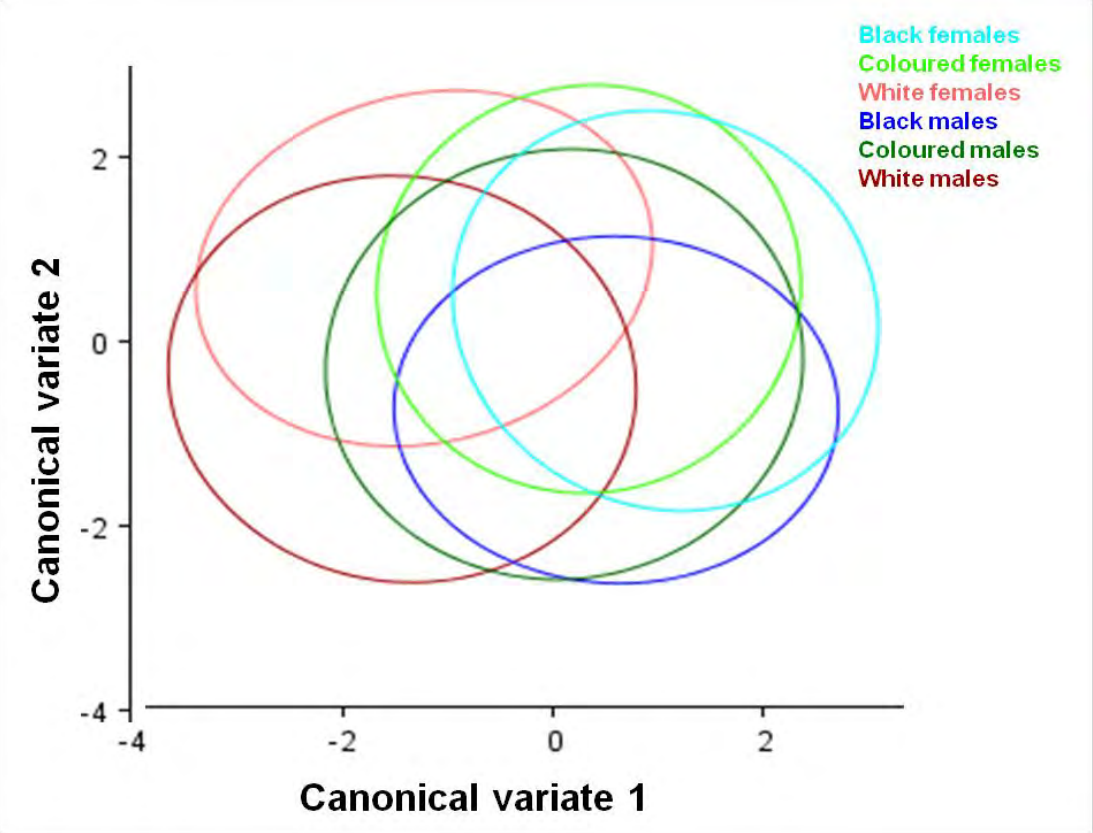


Figure 4.45: Parietal bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 59.2% of the total sample variation, separating Black individuals from White individuals, while Coloured individuals did not separate from either group on this axis. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.24; also Appendix B – Figure B39). Black individuals tended to have an antero-posteriorly shorter sagittal portion with a more antero-medially positioned euryon. Coloured individuals had a shorter sagittal portion with a more postero-lateral euryon. White individuals were intermediate to the other two groups, with the addition of a more arched squamous border.

CV2 accounted for 19.2% of the total sample variation, and indicated slight separation of the sexes within each ancestry group. This separation was not previously detected when sexes were examined independent of ancestry. An example of the sexual differences between the groups (using a comparison of Coloured females and males) is shown in Figures 4.46a-b (Appendix B – Figure B40). Males tended to have a more medially positioned euryon (landmark 6), more anteriorly positioned coronal border (more anterior landmarks 2-4), a more arched squamous border (landmarks 5, 7 and 8), and a slightly more posterior asterion (landmark 8), compared to females.

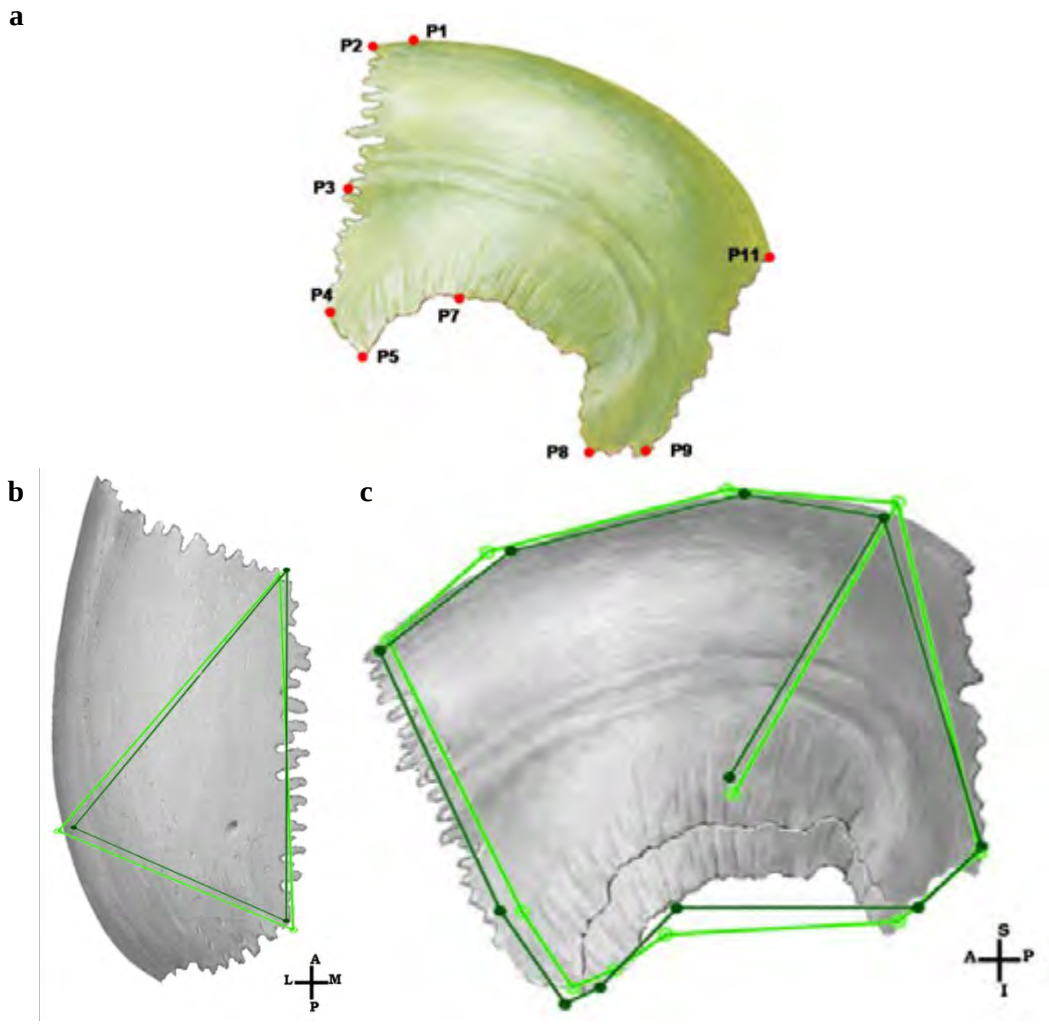


Figure 4.46: Parietal bone shape differences between the sexes as represented by Coloured females (light green) and Coloured males (dark green) – a) landmark locations, b) superior view, and c) lateral views. [5X magnification of differences for visualization] [Images adapted from White *et al*. (2012) and Hansen (2014)]

4.4.3.5. Temporal bone

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two yielded significant separation of the sex-ancestry groups (Figure 4.47).

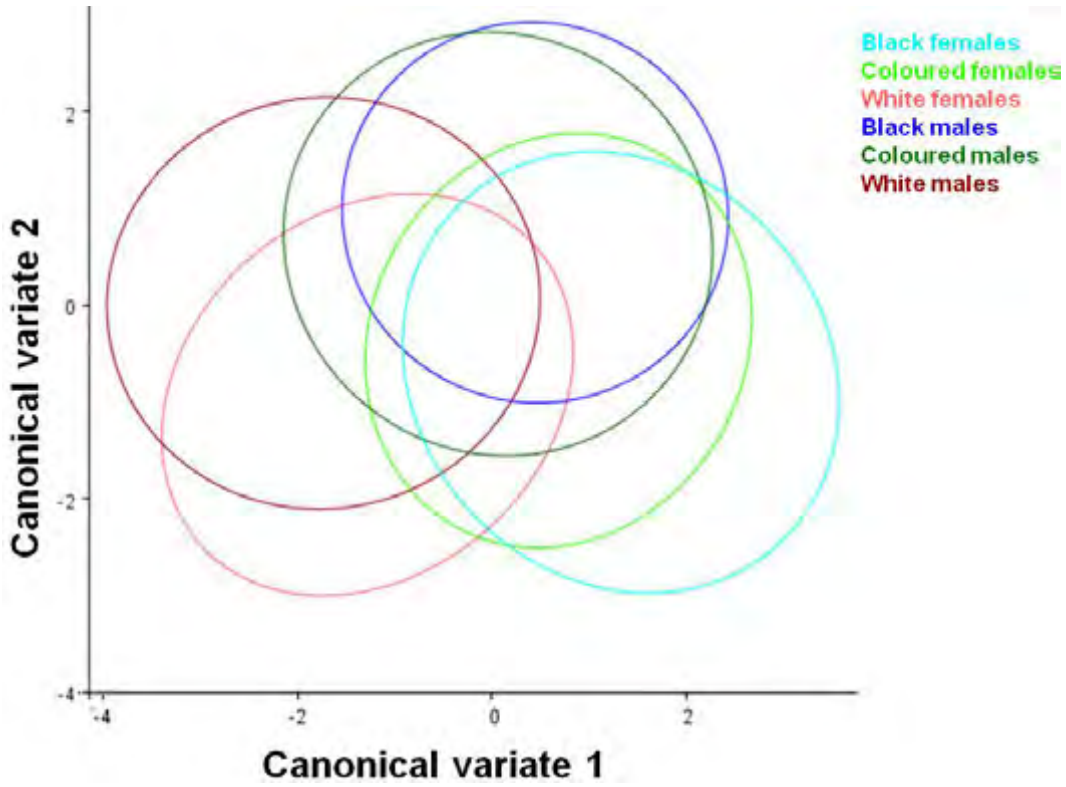


Figure 4.47: Temporal bone shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 58.0% of the total sample variation, separating Black individuals from White individuals, while Coloured individuals did not separate from either group on this axis. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.26; also Appendix B – Figure B41). White individuals tended to have an antero-posteriorly and medio-laterally smaller temporal bone, a more arched squamous border, and larger mastoid processes than individuals of the Black or Coloured groups. Coloured individuals differed from Black individuals only in having a slightly antero-posteriorly longer temporal bone, while both Black and Coloured individuals tended to have a more anteriorly positioned mandibular fossa and external auditory meatus than White individuals.

CV2 accounted for 23.1% of the total sample variation, separating the sexes within each ancestry group. This separation was not previously detected when sexes were examined independent of ancestry. An example of the sexual differences between the groups (using a comparison of Coloured females and males) is shown in Figures 4.48a-b (Appendix B – Figure B42). Females had an antero-posteriorly longer but

medio-laterally narrower temporal bone (more anterior landmarks 4 and 7; more lateral landmark 11), a shorter and less arched squamous border (landmarks 1-4), more posterior porion (landmark 6) and smaller mastoid processes (less inferior landmark 5), compared to males.

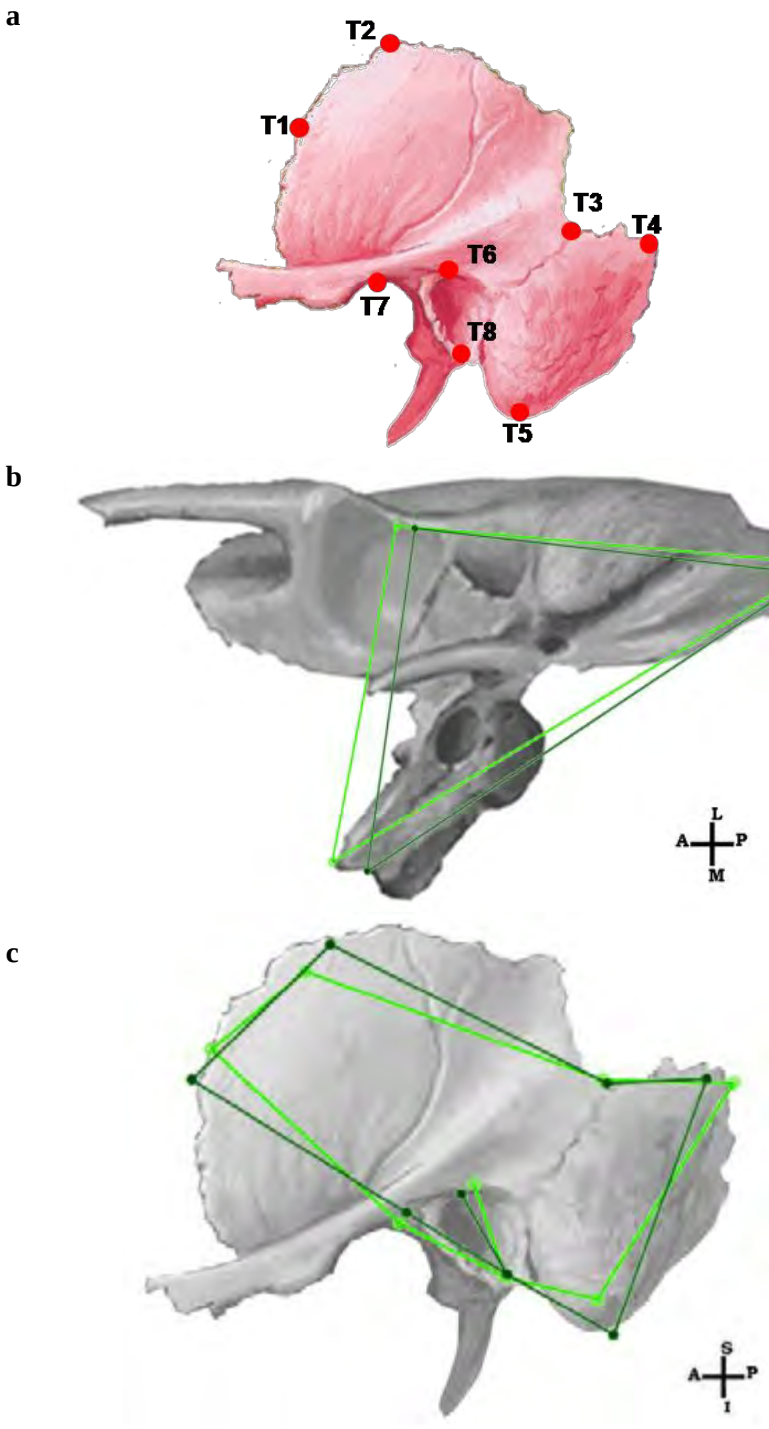


Figure 4.48: Temporal bone shape differences between the sexes as represented by Coloured females (light green) and Coloured males (dark green) – a) landmark locations, b) inferior view, and c) lateral views. [5X magnification of differences for visualization] [Images adapted from White *et al*. (2012) and Hansen (2014)]

Upper limb

4.4.3.6. Humerus

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.49).

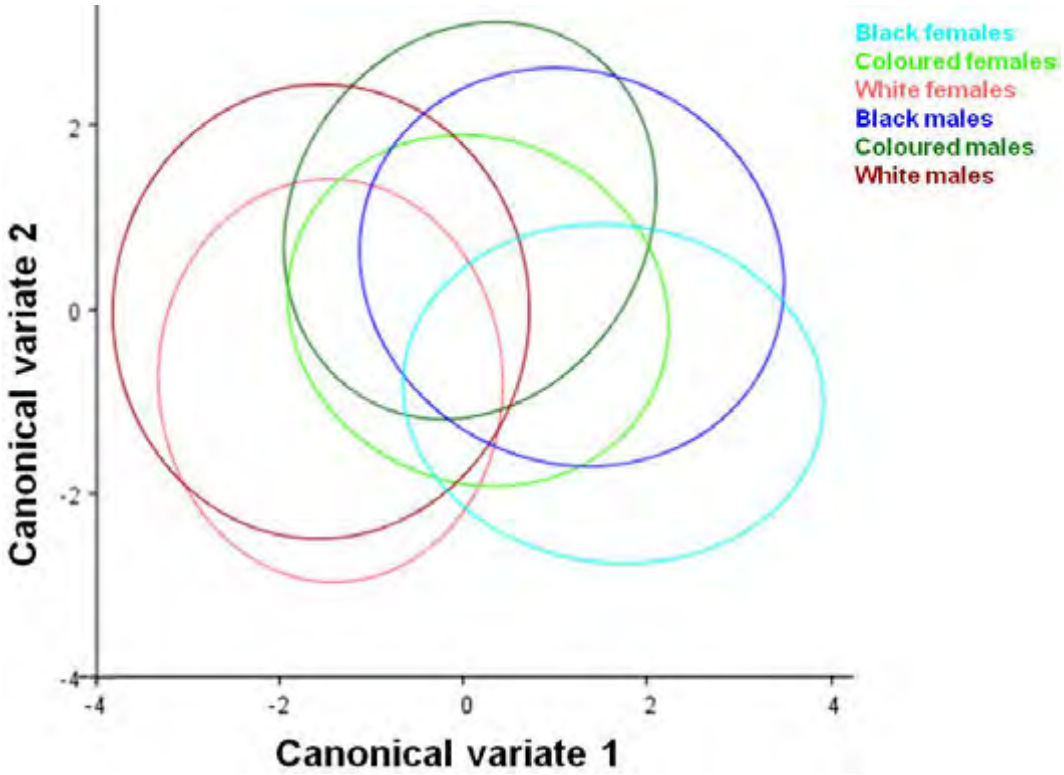


Figure 4.49: Humerus shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 64.7% of the observed sample variance, separating White individuals from Black individuals, while Coloured individuals did not separate from either of these groups on this axis. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.28; also Appendix B – Figure B43). Black individuals tended to have a medio-laterally narrower and more vertically facing humeral head, while White individuals tended to have a wider and more horizontal humeral head. White individuals also had a more anteriorly positioned greater tuberosity and the smallest angle of retroversion of the groups. Coloured individuals represented a combination of the features of the other groups.

CV2 accounted for 21.7% of the observed sample variance, separating the sexes within ancestry groups. The shape variation associated with CV2 was similar to that seen when comparing sexes independent of ancestry (Figure 4.7; also Appendix B – Figure B44), with males tending to have a supero-inferiorly larger humeral head, medio-laterally wider proximal and distal epiphyses, a more anteriorly positioned greater tuberosity, and a larger angle of retroversion, compared to females.

4.4.3.7. Radius

CVA of the Procrustes residuals produced 5 canonical variates, of which only CV2 yielded significant separation of the sex-ancestry groups (Figure 4.50). CV2 accounted for 28.9% of the observed variance, separating Black and White individuals from Coloured individuals.

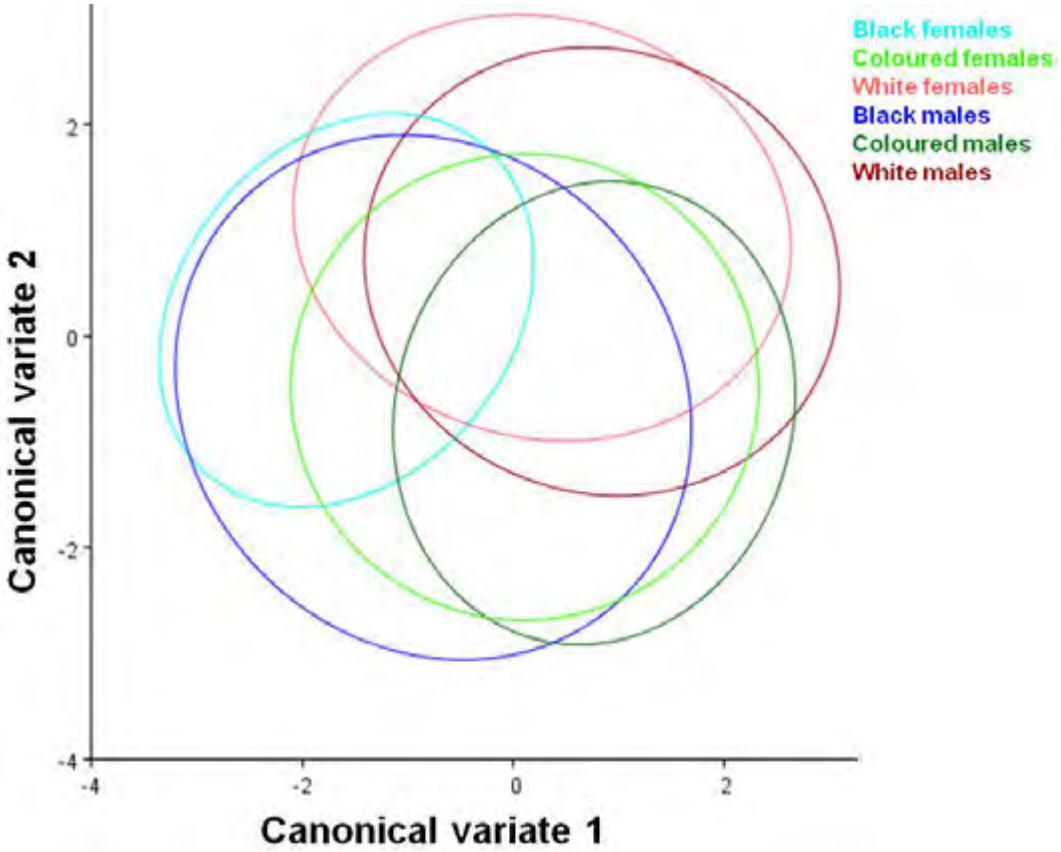


Figure 4.50: Radius shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

The shape variation associated with CV2 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.30; also Appendix B – Figure B45). Black individuals had a medio-laterally narrower, supero-inferiorly shorter radius, with a smaller radial inclination angle, and a relatively counter-clockwise rotation of

the landmarks of the radial head. White individuals had larger dimensions, a larger radial tuberosity and a larger radial inclination angle. Coloured individuals represented a combination of the features of the other two groups.

4.4.3.8. Ulna

CVA of the Procrustes residuals produced 5 canonical variates, of which only CV1 shows significant separation of the sex-ancestry groups (Figure 4.51). This canonical variate accounts for 56.8% of the observed variation, separating Black individuals from White individuals, as well as females from males within each ancestry group. Coloured individuals do not separate from the other ancestry groups, but also show the separation of females from males along this axis.

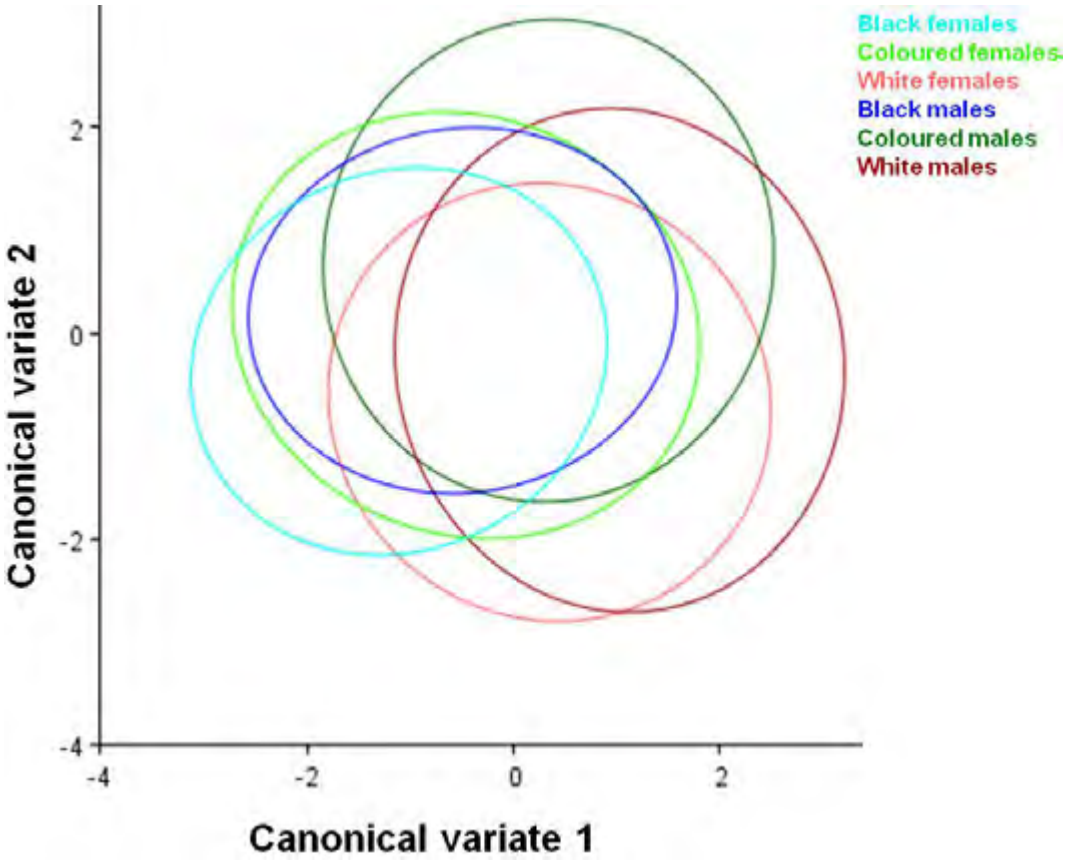


Figure 4.51: Ulna shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

The shape variation associated with CV1 was similar to that seen when comparing ancestry groups (Figure 4.32) and sexes independent of each other (Figure 4.11; also Appendix B – Figure B46). Black individuals had a medio-laterally narrower proximal epiphysis, smaller radial notch and a shallower trochlear notch. White and Coloured individuals were similar, except for a slightly more medial coronoid

process in White individuals. Males tended to have medio-laterally wider ulnae than the females within the same ancestry group, and had a more L-shaped trochlear notch while females had a more C-shaped notch.

Lower limb

4.4.3.9. Femur

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.52).

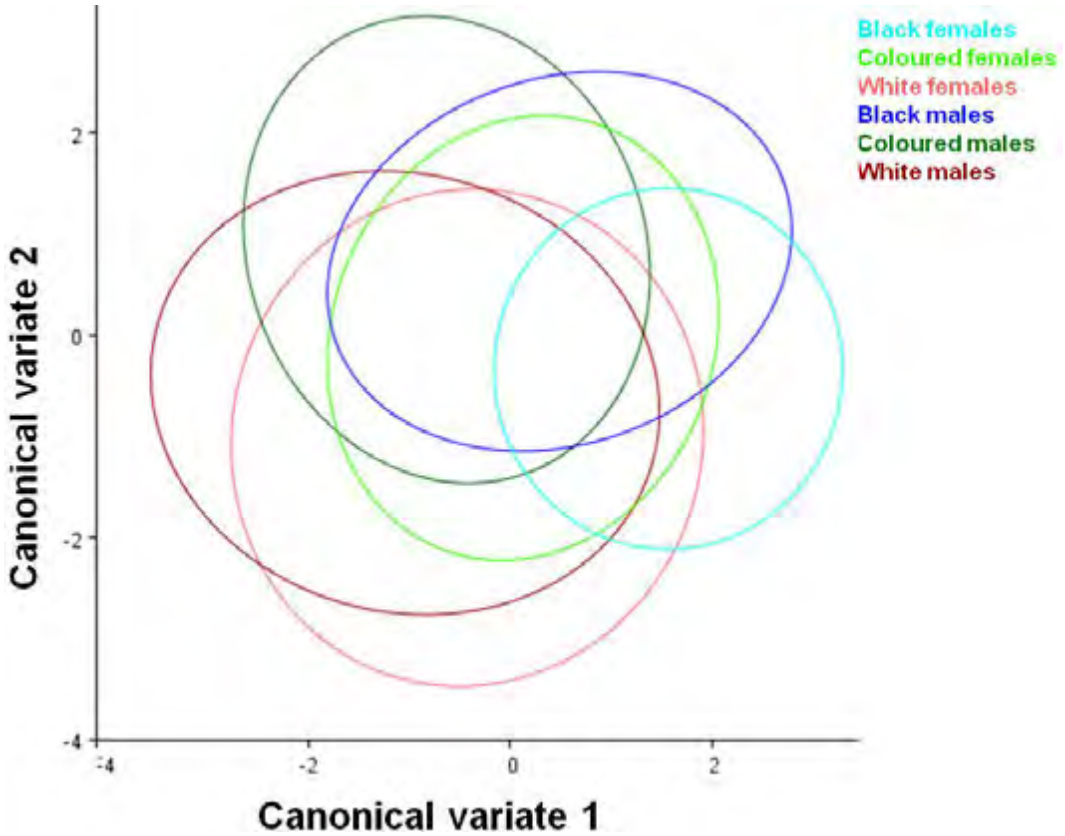


Figure 4.52: Femur shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 50.3% of the total sample variation, separating White individuals from Black individuals. The shape variation associated with CV1 was similar to that seen when comparing ancestry groups independent of sex (Figure 4.34; also Appendix B – Figure B47). Coloured and White individuals had medio-laterally wider femora with larger condyles, while Black individuals had a smaller lesser trochanter, a larger neck-shaft angle and a narrower femoral neck. Black individuals had the largest angle of anteversion, followed by White individuals, and then Coloured individuals.

CV2 accounted for 29.0% of the total sample variation, separating the sexes within each ancestry group. The shape variation associated with CV2 was similar to that seen when comparing sexes independent of ancestry (Figure 4.13; also Appendix B – Figure B48), with female femora being medio-laterally narrower both proximally and distally, with smaller condyles, a larger neck-shaft angle, and a larger angle of anteversion, compared to males.

4.4.3.10. Tibia

When the tibia data set was analysed with or without the tibial tuberosity landmark, significant separation of the sex-ancestry groups was observed. The results of the two analyses are thus given separately.

Tibia with tuberosity

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.53). There was no clear separation of the sexes on either CV.

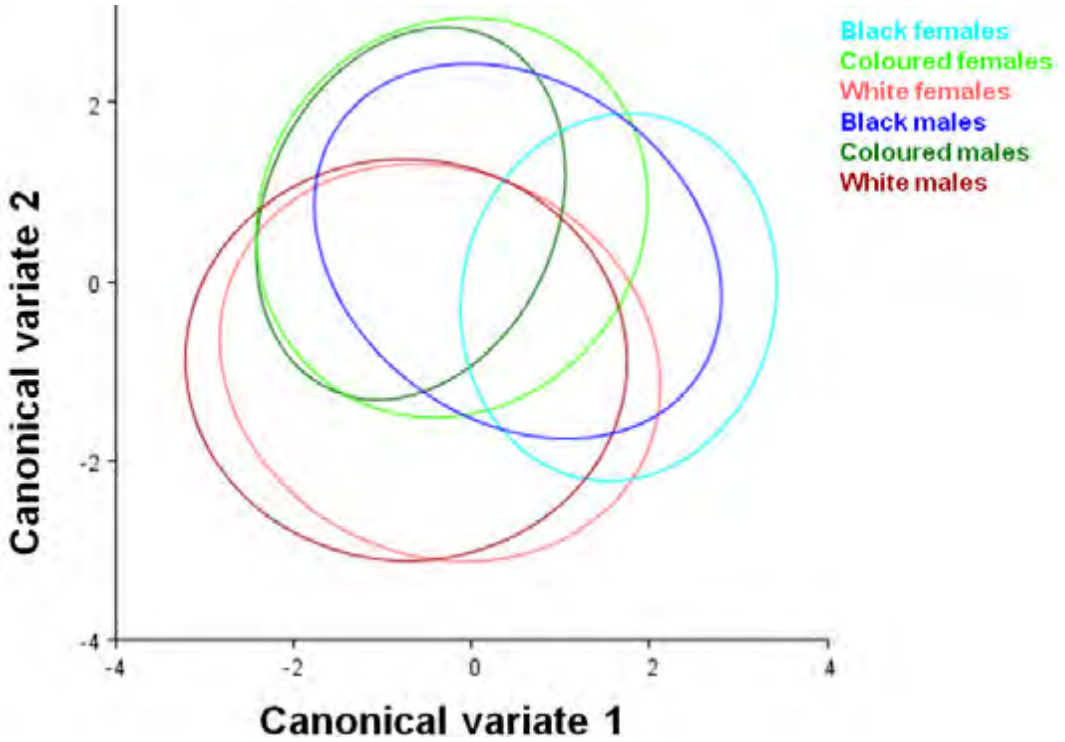


Figure 4.53: Tibia shape (including tuberosity) – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

CV1 accounted for 55.1% of the total sample variation, separating White individuals from Black individuals. CV2 accounted for 35.1% of the observed variation, separating Black and White individuals from Coloured individuals. The shape variations associated with these canonical variates were similar to that seen when comparing ancestry groups independent of sex (Figure 4.36; also Appendix B – Figures B49 and B50). Black individuals tended to have a medio-laterally narrower tibia both proximally and distally, a more superiorly positioned tibial plateau and an infero-lateral tibial tuberosity, compared to individuals of the other groups. Coloured individuals had smaller intercondylar eminences and a more supero-medial tibial tuberosity. White individuals represented a combination of the features of the other two groups.

Tibia without tuberosity

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two yielded significant separation of the sex-ancestry groups (Figure 4.54). CV1 accounted for 49.7% of the total sample variation, separating Black individuals from Coloured and White individuals. CV2 accounted for 40.4% of the observed variation, separating White individuals from Black and Coloured individuals. There was no clear separation between the sexes on either CV.

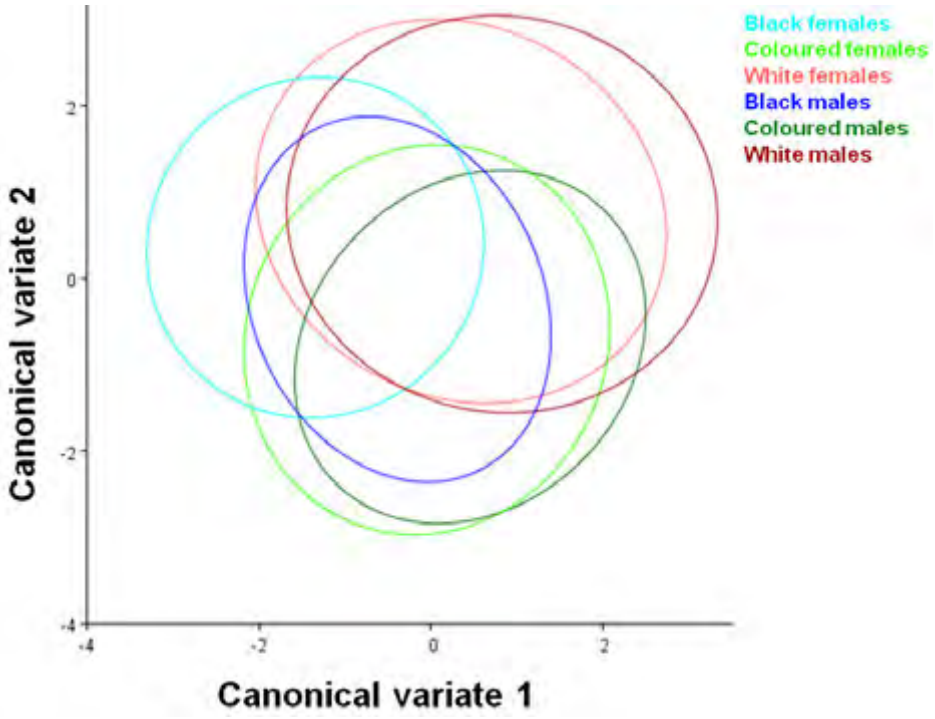


Figure 4.54: Tibia shape (excluding tuberosity) – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

The shape variations associated with these canonical variates were similar to those seen when comparing ancestry groups independent of sex (Figure 4.38; also Appendix B – Figures B51 and B52). Black individuals had a medio-laterally narrower tibia with more anteriorly positioned intercondylar eminences than individuals of the other groups, while Coloured individuals had a relatively more anterior fibular notch.

4.4.3.11. Fibula

CVA of the Procrustes residuals produced 5 canonical variates, of which only the first two had significant separation of the sex-ancestry groups (Figure 4.55). CV1 accounted for 60.0% of the total sample variation, separating Black individuals from Coloured and White individuals. CV2 accounted for 23.1% of the observed variation, separating Black and White individuals from Coloured individuals. There was no clear separation of the sexes on either CV.

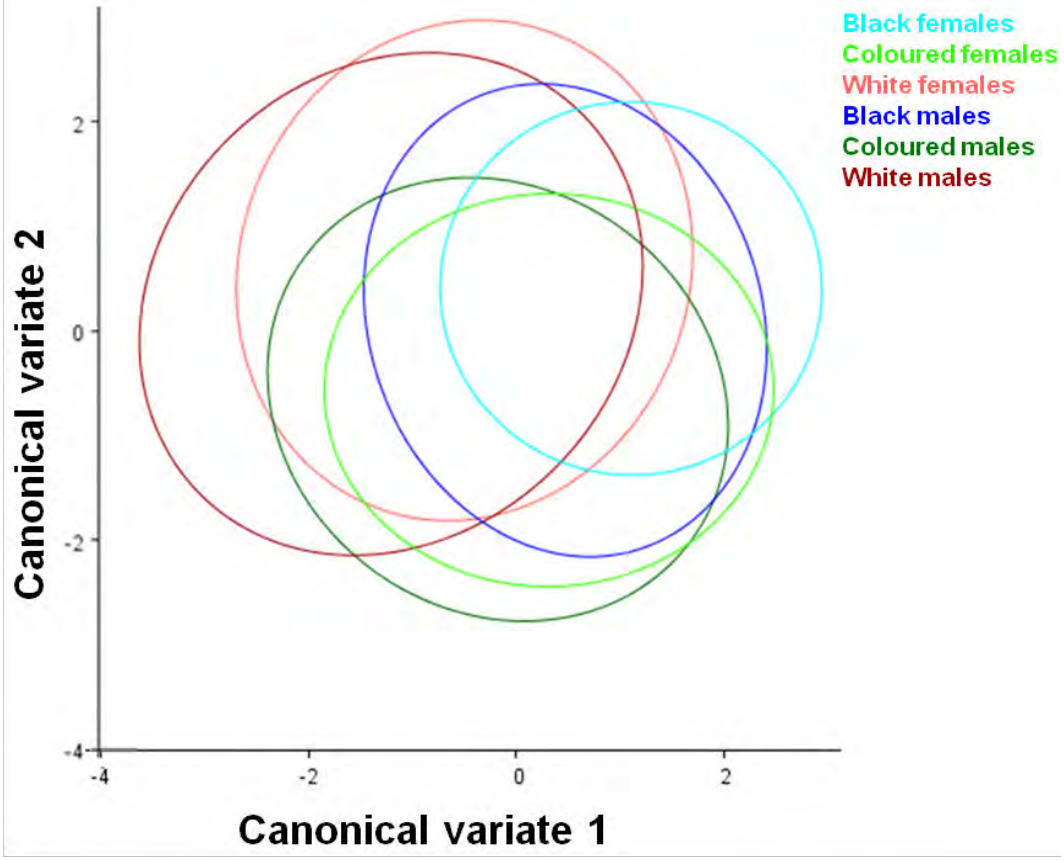


Figure 4.55: Fibula shape – canonical variate plot of the first two canonical variates, showing 90% probability ellipses for the six sex-ancestry groups.

The shape variations associated with these canonical variates were similar to those seen when comparing ancestry groups independent of sex (Figure 4.40; also Appendix B – Figures B53 and B54). Black individuals had an antero-posteriorly and medio-laterally narrower fibula, both proximally and distally, with a generally smaller proximal epiphysis, compared to the other groups, while White individuals had a more superior position of landmark 3. Coloured individuals represented a combination of the features of the other two groups.

4.4.3.12. Relationships to other variables

Regression analysis was performed to assess the potential relationship of the morphological differences among the six sex-ancestry groups with centroid size, age and year-of-birth (Table 4.8). All elements showed significant association of group differences to centroid size (all $p \leq 0.01$), except the parietal bone ($p=0.1$). All elements showed significant association of group differences to age (all $p \leq 0.02$), except the whole cranium, and the occipital and temporal bones ($p \geq 0.2$). Lastly, all elements showed significant association of group differences to year-of-birth (all $p \leq 0.007$), except the whole cranium, occipital and temporal bones, and the radius ($p \geq 0.1$). Despite the statistical significance of these associations, none of the detected associations were linked to more than 10% of the total observed variation.

Table 4.8: Relationship of differences in shape among sex-ancestry groups with centroid size, age and year-of-birth [All $p \leq 0.02$].

Skeletal element	Coefficient of determination (R^2) (%)		
	Centroid size	Age	Year-of-birth
Cranium			
Whole	2.8	Not significant	Not significant
Frontal	8.1	1.8	1.1
Occipital	1.9	Not significant	Not significant
Parietal	Not significant	0.9	1.9
Temporal	2.1	Not significant	Not significant
Upper limb			
Humerus	2.1	2.3	3.1
Radius	6.1	0.9	Not significant
Ulna	8.1	4.5	3.0
Lower limb			
Femur	8.4	9.0	8.7
Tibia (with tuberosity)	5.6	5.1	5.2
Tibia (without tuberosity)	7.4	4.2	4.0
Fibula	9.9	5.0	5.3

4.4.3.13. Discriminant function analysis

Comparison of the Mahalanobis distances (MD) between groups indicated that Black and White individuals tended to be respectively more similar to Coloured individuals than to each other for most of the elements (Appendix C – Table C5). The exceptions to this trend were the whole cranium, and the frontal and temporal bones, for which the MD was larger between the White female and male groups and the corresponding Black and Coloured female and male groups, while the MD between the Black groups and the corresponding Coloured and White groups was larger for the radius and the tibia (with tuberosity). The MD between the groups was similar for the tibia without the tuberosity.

The leave-one-out cross-validation test revealed that the shape differences among the six sex-ancestry groups were significant for all elements (all $p<0.0001$). The classification accuracies of the elements are shown in Table 4.9 (see Appendix C – Table C6 for sample sizes). Accuracies of the sex-ancestry pooled sample ranged from 70.6% for the tibia (without tuberosity) to 83.3% for the frontal bone. When comparing the sexes within ancestry groups, accuracies did not differ significantly for the whole cranium, parietal bone and the ulna (all $p\geq0.07$). Of the remaining skeletal elements, the classification accuracies for females tended to be higher than those of the male groups within the same ancestry. When comparing the accuracies within-sexes, the majority of the elements yielded higher accuracies for the Black males and females than for the corresponding Coloured and White female and male groups. The accuracies of the Coloured groups were also significantly lower than those of the Black and White groups, while the accuracy based on the whole cranium was the only one to be significantly larger in the White groups. The parietal bone and ulna again did not show significant differences in accuracy between groups ($p= 0.07$ and 0.11, respectively).

Table 4.9: Leave-one-out cross-validation classification accuracies of the skeletal elements evaluated according to sex and ancestry (Highest accuracies of cranial and postcranial elements indicated in red).

Skeletal element	Sex-ancestry estimation accuracy (%)						
	Black females	Coloured females	White females	Black males	Coloured males	White males	Pooled sex-ancestry
Cranium							
Whole cranium	73.5	74.4	80.0	75.1	75.8	**85.6**	**77.2**
Frontal	**86.8**	**83.8**	**85.0**	**83.7**	**76.6**	85.3	**83.3**
Occipital	78.9	69.5	75.7	73.6	71.3	79.1	**74.8**
Parietal	75.5	68.8	77.2	72.4	70.2	75.1	**73.1**
Temporal	83.3	75.6	83.3	78.3	68.5	79.3	**77.6**
Upper limb							
Humerus	85.5	**82.2**	**82.6**	**79.3**	**76.9**	**79.6**	**80.7**
Radius	85.8	76.5	78.0	72.4	75.7	74.2	**77.2**
Ulna	75.8	71.2	73.1	69.1	69.6	69.7	**71.3**
Lower limb							
Femur	**87.2**	75.1	72.4	78.4	76.2	76.4	**77.9**
Tibia (with tuberosity)	84.6	75.6	74.5	76.7	73.9	69.5	**75.5**
Tibia (without tuberosity)	81.0	51.3	72.2	73.3	71.3	70.2	**70.6**
Fibula	79.0	69.9	71.7	69.5	66.8	76.4	**72.3**

4.5. Comparison of classification accuracies between skeletal elements

4.5.1. Sex estimation

Comparison of the classification accuracies of each element indicated that the frontal bone was best at classifying each of the sexes, both individually and in a sex-pooled sample, with accuracies of 75.5–83.7% (Table 4.5; Figures 4.56a and b). When only the postcranial elements were considered, the femur had the best accuracy for each sex separately and for both sexes pooled (73.2–75.6%).

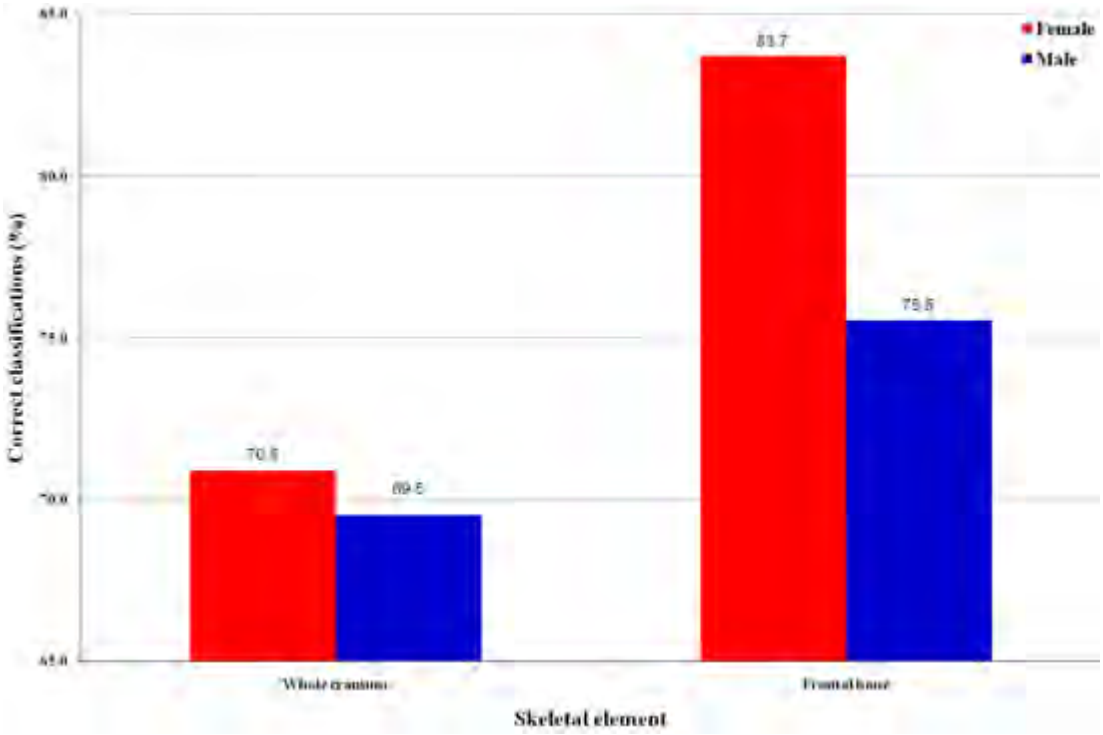


Figure 4.56a: Comparison of the leave-one-out cross-validated accuracies of sex estimation for the cranial skeletal elements. [Elements not shown had no significant separation of the sexes].

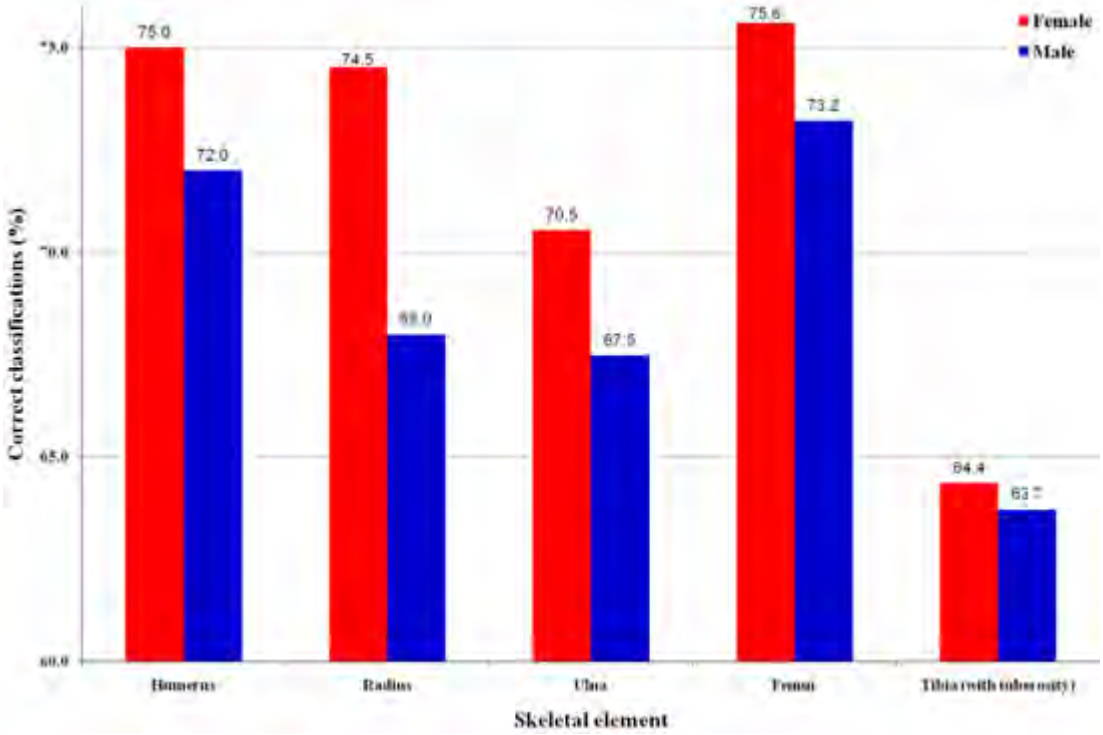


Figure 4.56b: Comparison of the leave-one-out cross-validated accuracies of sex estimation for the postcranial skeletal elements. [Elements not shown have no significant separation of the sexes].

4.5.2. Ancestry estimation

Comparison of the relative performances of the different skeletal elements in their ability to estimate ancestry yielded more varied results than when estimating sex (Table 4.7; Figures 4.57a and b). The frontal bone was the best element overall for correctly estimating the ancestry of Black individuals (88.0%), while the humerus was the best postcranial element for this group (84.7%). For Coloured individuals, the humerus was best overall with 84.0% accuracy, and the frontal bone being the best cranial element (83.7%). For White individuals, the whole cranium was the best overall (90.2%), and the humerus was the best postcranial element (87.2%). When all ancestry groups were pooled, the most accurate element for ancestry estimation was the frontal bone (85.9%), while the humerus was the best postcranial element (85.3%).

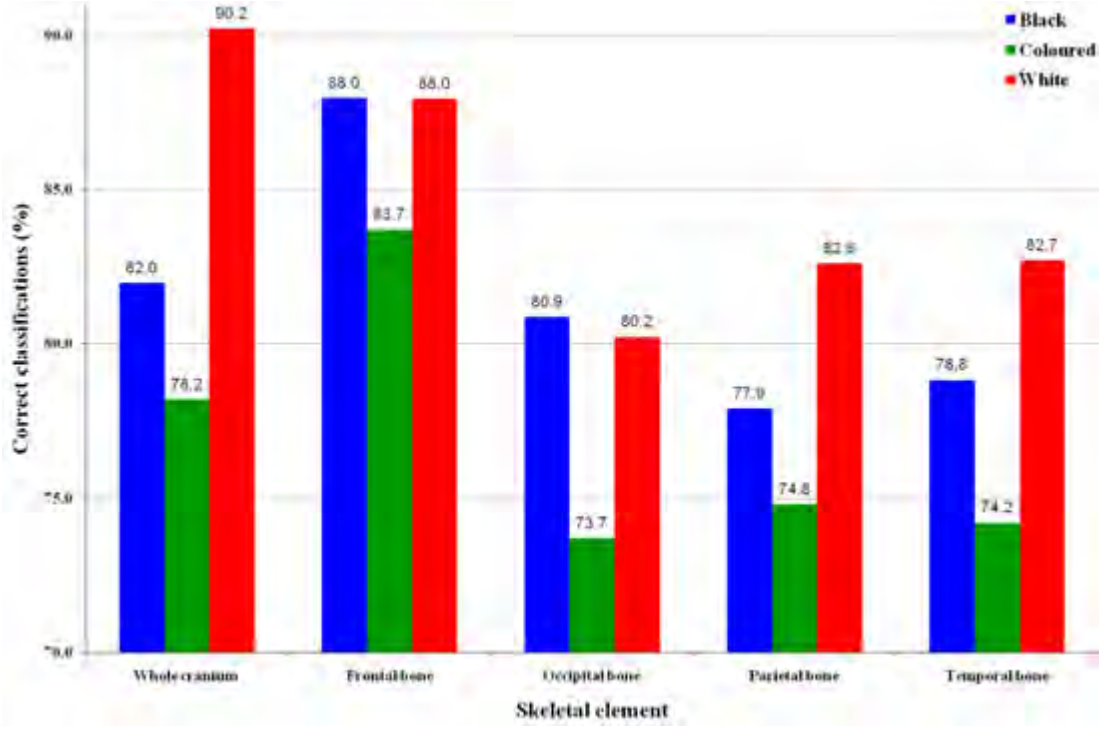


Figure 4.57a: Comparison of the leave-one-out cross-validated accuracies of ancestry estimation for the cranial elements.

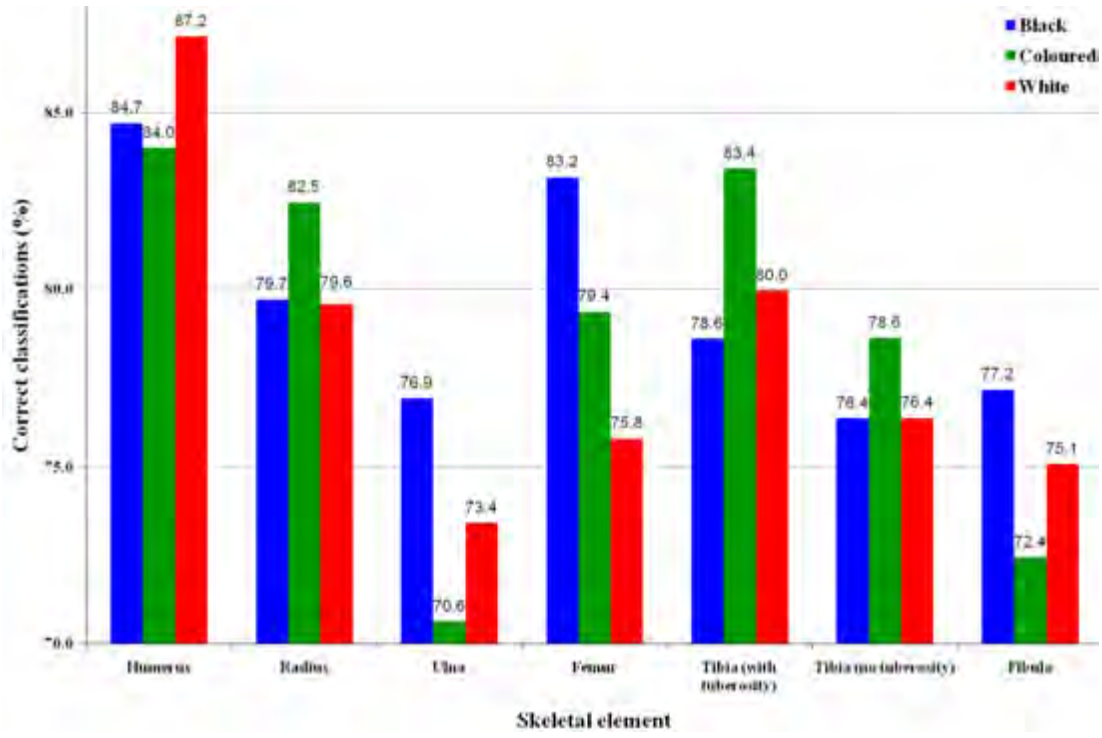


Figure 4.57b: Comparison of the leave-one-out cross-validated accuracies of ancestry estimation for the postcranial skeletal elements.

4.5.3. Sex-ancestry estimation

As with the estimation of sex and ancestry separately, the classifications based on the frontal bone and the humerus were most accurate for many of the sex-ancestry groups (Table 4.9; Figures 4.58a-c). The classification accuracy of the frontal bone was the best of the cranial elements for Black females and Coloured males, and best overall for Coloured females, White females, Black males and the ancestry-pooled sample (76.6–86.8%). The classification accuracy of the humerus was the best of the postcranial elements for Coloured and White females, Black and White males, and the ancestry-pooled sample, as well as the best overall for Coloured males (76.9–82.6%). The only exceptions to the trend were White males, for which the whole cranium had the best classification accuracy overall (85.6%), and Black females, for which the femur had the best classification accuracy overall (87.2%).

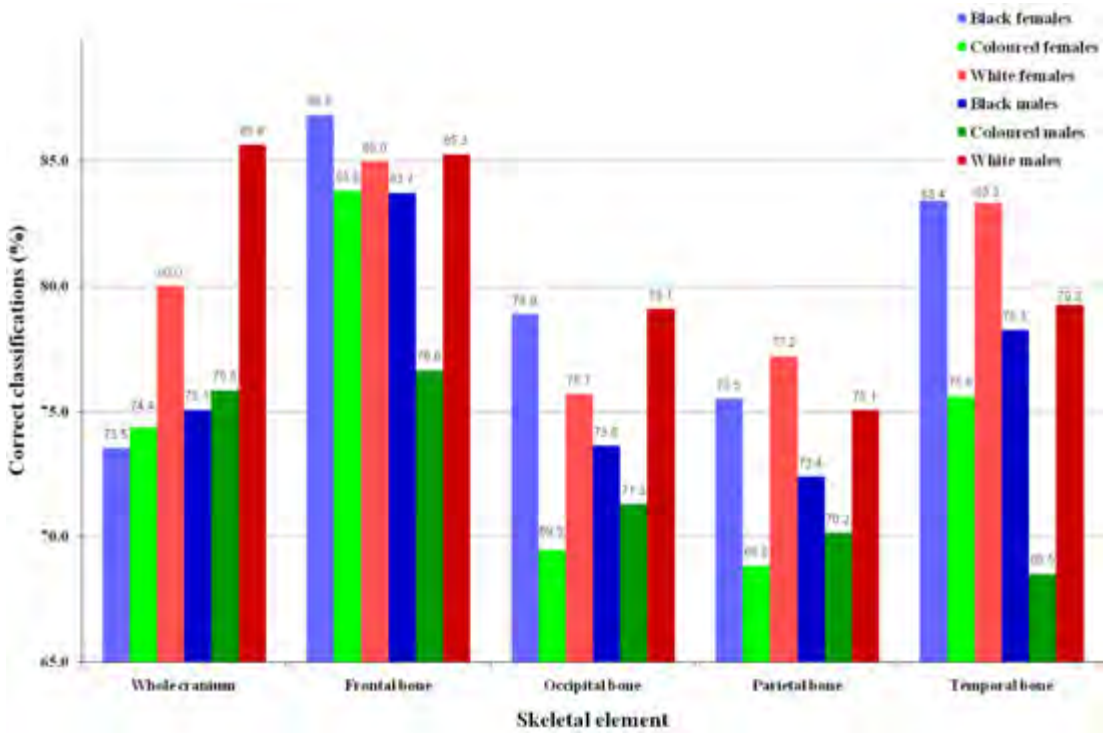


Figure 4.58a: Comparison of the leave-one-out cross-validated accuracies of sex-ancestry estimation for the cranial elements.

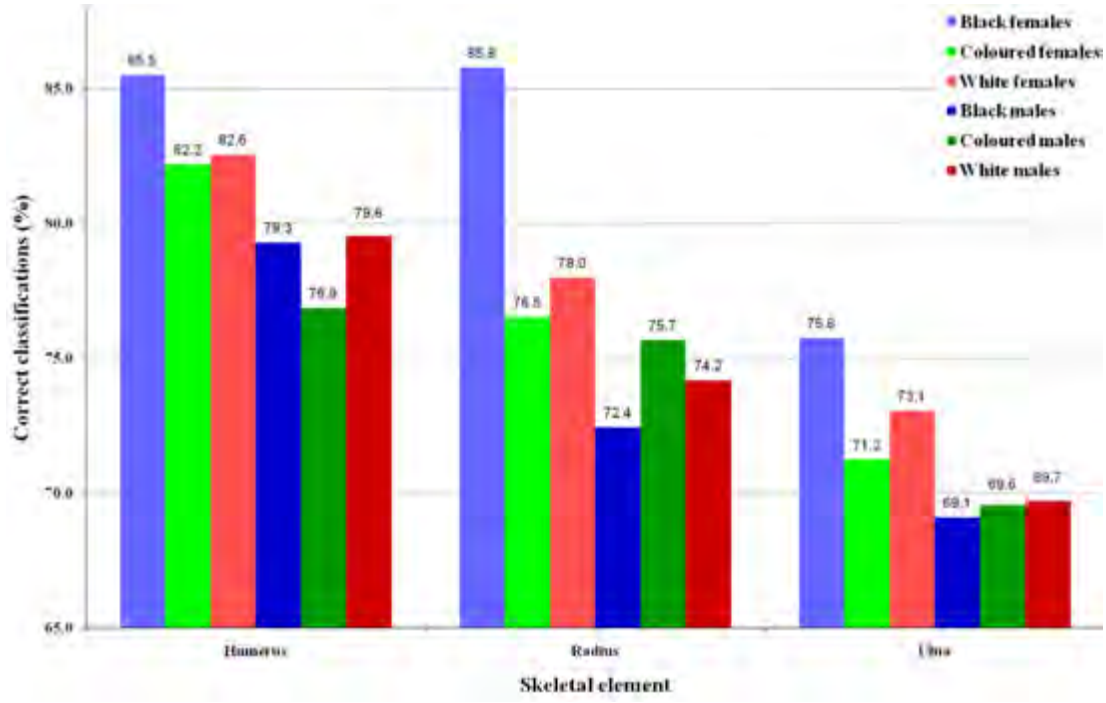


Figure 4.58b: Comparison of the leave-one-out cross-validated accuracies of sex-ancestry estimation for the skeletal elements of the upper limb.

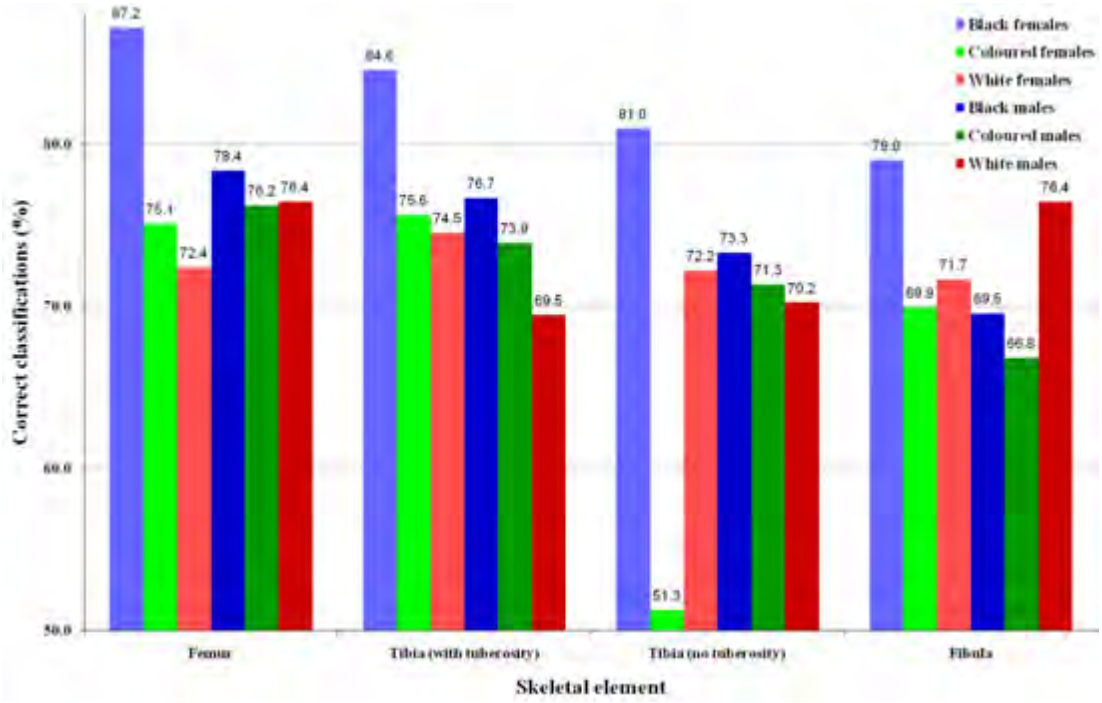


Figure 4.58c: Comparison of the leave-one-out cross-validated accuracies of sex-ancestry estimation for the skeletal elements of the lower limb.

4.5.4. Comparison between skeletal elements

Overall, for the classification of sex, ancestry and both variables combined, the frontal bone provided the best accuracy of estimation of all elements with an average of 83.5% (Figures 4.59a and b). The humerus was the best postcranial element with an average accuracy of 80.6%. The elements with the lowest average accuracy were the parietal bone (75.1%) for the cranial elements, and the ulna (71.5%) for the postcranial elements and for all elements collectively. Cranial elements generally yielded higher classification accuracies than the postcranial elements.

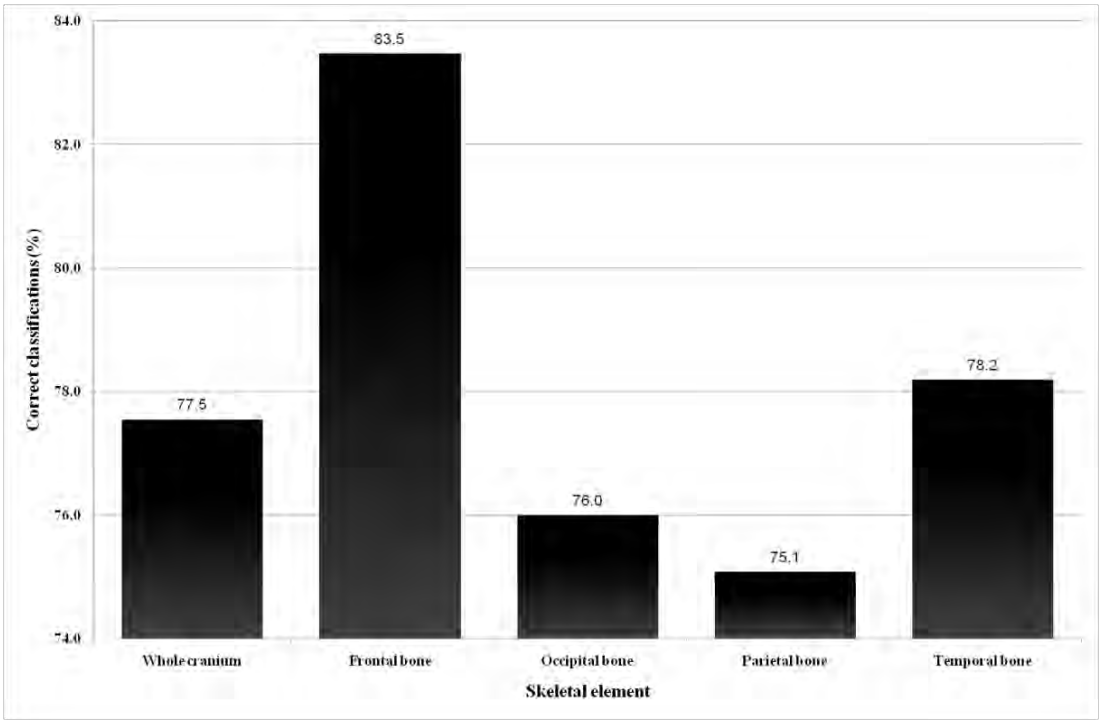


Figure 4.59a: Comparison of the average classification accuracies for the cranial skeletal elements.

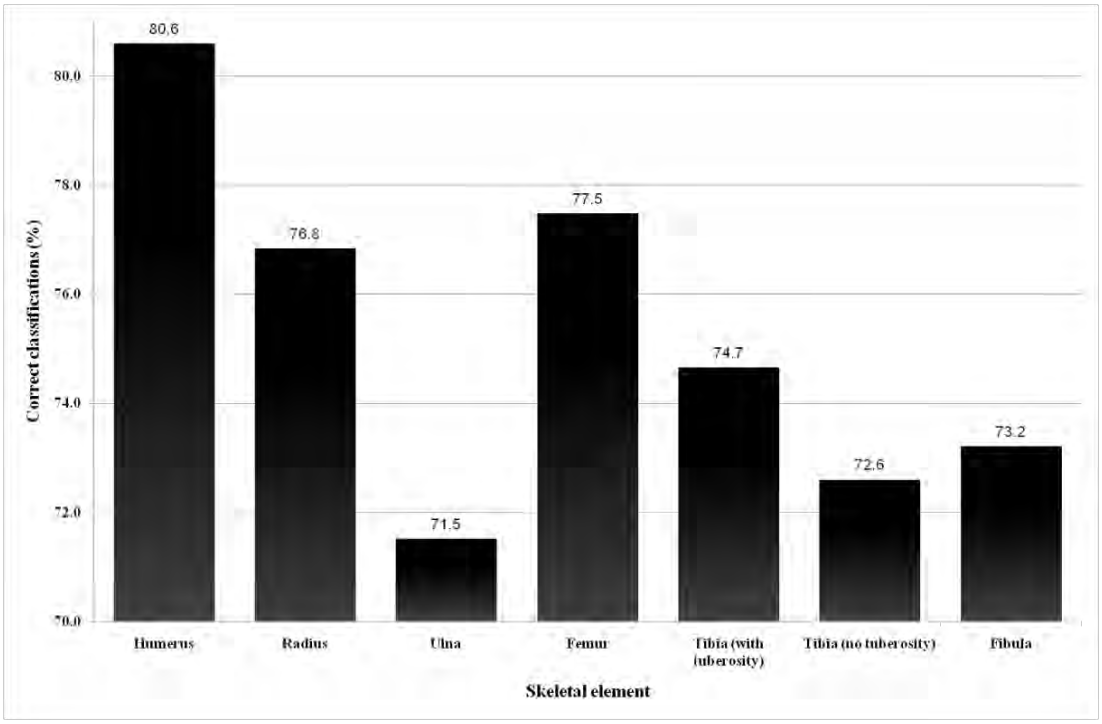


Figure 4.59b: Comparison of the average classification accuracies for the postcranial skeletal elements.

CHAPTER 5

DISCUSSION

The results presented in this study show that there is significant variation in the morphology of cranial and postcranial skeletal elements in terms of both size and shape. This variation can be statistically quantified and used to distinguish sex and/or ancestry groups from each other, even in a complex population like that of South Africa. As a result of its complex genetic and historical make-up, the South African population may enable elucidation of the interaction of environmental and social differences on the morphology of the different groups studied, while the Coloured group specifically allows insight into the interaction of genetic and historical backgrounds of these individuals.

5.1. Challenges experienced in this study

5.1.1. Study sample

The ideal for any anthropological study is the use of skeletal material from documented collections to develop methods and standards of analysis which could potentially be used to obtain information from the skeletal remains of individuals of the larger population represented in these collections (Dirkmaat *et al*., 2008). Unfortunately, the majority of individuals in many of the documented collections were acquired over 40 years ago, and often do not provide good representations of the population from which these individuals are taken from (Dirkmaat *et al*., 2008; Komar & Grivas, 2008). The main reason for this is the manner in which individuals are acquired for such collections, which already introduces bias to the collection in terms of the age, sex, ancestry and socioeconomic representations, causing the collection to be a poor reflection of the larger population (Dayal *et al*., 2009).

It has been noted that the public perception of donation and research, as well as cultural or religious beliefs influence which individuals tend to donate their bodies to science (Da Silva, 2006; Komar & Grivas, 2008). An example of this is the objection by the Muslim culture to the use of bodies for dissection, and the requirement that the body should be buried as soon as possible after death (Sarhill *et al*., 2001). Similarly,

L‘Abbé *et al.* (2005) reported that Black South Africans tend to not donate their bodies to science, likely as a result of the strong culture of ancestor reverence, which often specifically requires burial of remains near other family members. In a demographic analysis of the cadavers used at the University of Cape Town, Da Silva (2006) showed that only 0.61% of the Black individuals used by the university were received though bequeathments, in contrast to the 50% of White individuals. The remainder of the individuals were received by the university as ―unclaimed" individuals, and consist mainly of Black and Coloured individuals. L‘Abbé *et al*. (2005) suggests that the proportionately greater amount of Black individuals in South African collections may be due to socio-economic circumstances leading to their inclusion in these collections. Black individuals from rural areas often travel to urban areas to find work, forcing them to live far from their family members (Tal & Tau, 1983). When these individuals die in these urban areas, it may be difficult to locate their families to allow the return of their remains to their rural areas of origin, or the expense of doing so may be too great for the family (L‘Abbé *et al*., 2005). According to the *National Health Act* (Act No 61 of 2003) and the *Human Tissues Act* (Act 65 of 1983), the remains of such individuals who are not claimed by family members within 24 hours after death may be donated to institutions such as universities for medical education purposes.

As a result of the different acquisition practices of remains for medical training and eventually for skeletal collections, many of the South African skeletal collections are biased towards containing a disproportionate amount of remains of mostly old White individuals, and relatively younger Black and Coloured individuals, as seen in the reports of Dayal *et al*. (2009), L‘Abbé *et al*. (2005) and Da Silva (2006).

In order to reduce the possible influence of sample bias, the present study included as many Black, Coloured and White adults (over 20 years of age) as possible (c.f. Table 3.1). However, socio-economic data for these individuals were not available and could thus not be accounted for. While it may be possible, based on the general acquisition methods of the skeletal collections, that the majority of Black and Coloured individuals examined may be those of ―unclaimed" individuals and thus likely of lower socio-economic status, and that the majority of White individuals may be of higher socio-economic status (Da Silva, 2006), this could not be confirmed

from the accession records. If this was, however, the case, it is possible that the differences among these three ancestry samples may not be representative of inherent differences among the ancestry groups, but rather be more of a representation of the socio-economic differences in this potentially biased sample. It is thus important that this potential bias be considered when interpreting the observations made in the present study.

5.1.2. Landmark selection

Many geometric morphometric studies have used cranial landmarks which have previously been defined in metric studies (Lockwood *et al*., 2002; Franklin *et al*., 2006). Similar to the selection of metric measurements, one of the primary considerations in the selection of landmarks used to represent each cranial element should be how easily and reliably each landmark can be identified and digitized (Webster & Sheets, 2010). For many of the cranial elements in the present study, suitable and already defined landmarks were drawn from previous metric and geometric morphometric studies such as those of Lockwood *et al*. (2002) and Franklin *et al*. (2006). Many of the cranial landmarks were classified as Type I landmarks (Bookstein, 1991), based on their location at the point of intersection or juxtaposition of two or more distinct structures. As a result of their position, these landmarks were often easy to identify and digitize with little observer error (McKeown & Schmidt, 2012).

In contrast, postcranial elements have very few Type I landmarks, but instead have mostly Type II or III landmarks, which are located at points of maximal curvature or are defined based on the position of another landmark (Bookstein, 1991). Due to the nature of such landmarks on the long bones, clear definitions of landmarks similar to the existing landmarks defined for the cranium are difficult to standardize (McKeown & Schmidt, 2012). The existing studies of postcranial elements using landmark-based approaches tend to identify their own landmarks based on areas of potential interest (e.g. the distal humerus) and easily identifiable locations, for example the most distal point on the trochlea of the humerus. Some studies, like that of Holliday & Friedl (2013) employ osteometric instruments to assist in the identification of certain landmarks. However, this makes the process of landmark identification and digitization more time consuming and may introduce additional bias and error into

these observations (Smith & Boaks, 2014). The landmarks selected on the postcrania in the present study were thus chosen to not require equipment such as an osteometric board or callipers. The landmarks were thus mostly confined to the epiphyses and larger muscle attachments on the long bones, as these were easily identifiable and showed no significant observer error (c.f. Figure 4.1; also Appendix B – Table B1).

The other primary consideration in landmark selection was the number of landmarks. Similar to metric studies, the number of landmarks used should be determined by how many landmarks may be needed to give a good representation of the shape of the bone or feature of interest (Webster & Sheets, 2010). It is important to note that landmark-based analyses can only provide information about the variation at the location of the landmarks themselves, and any shape variation which is constrained to the areas between landmarks will not be detected (Richtsmeier *et al*., 2002; Webster & Sheets, 2010). Similar to the selection of metric measurements, the number of landmarks used could be increased without limit to give a more extensive representation of the object or feature of interest. However, the practical constraints of time and sample size should also be taken into account when deciding on the number of landmarks to use (Franklin *et al*., 2005; Slice, 2007; Webster & Sheets, 2010). For the present study, eight to nine landmarks were selected for each postcranial element, and eleven to twelve landmarks for each cranial element and fourteen landmarks for the whole cranial sample (c.f. Figures 3.1 and 3.2). Similar numbers of landmarks were purposefully chosen to allow comparison of the classification accuracy of the demographic parameters between all elements studied so that one element does not perform better than another by simply having more landmarks, as suggested by Von Cramon-Taubadel (2009).

Based on the above considerations in landmark selection for the present study, the chosen landmarks on the cranial elements were located mostly on suture lines (c.f. Figures 3.1A – E). Some of the cranial elements, such as the parietal bone, have very few identifiable landmarks on it, and the landmarks chosen for such elements were thus mostly located on the edges of these elements. This may have resulted in shape variations in the curvature of such elements to have remained undetected, thus reducing the ability to use such variation to distinguish between the study groups (Steyn *et al*., 2004; Webster & Sheets, 2010). In the present study, the shape of the

frontal bone appears to have been sufficiently captured, as great variation in this region was detected (c.f. Figures 4.5 and 4.20). The parietal bone, however, showed notably less variation (c.f. Figure 4.24), often not allowing for the separation of the study groups. While this lack of variation may be biologically determined, it is also possible that the landmarks used to capture the shape of the parietal bone failed to detect shape variations among groups which may have existed in the regions between landmarks. It was decided not to increase the number of landmarks on elements such as the parietal bone, as such landmarks would be more difficult to identify and reliably digitize. While semi-landmark (or sliding landmark) methods have been shown to allow the capture of the shape of a curve (e.g. Hochstein, 2014), the efficiency of semi-landmarks is constrained by the analyses which can be performed with such data (Sampson *et al*., 1996), and the application of this approach is limited due to the increased computational difficulty associated with such analyses (Slice, 2007).

Most of the landmarks chosen for the postcranial elements in this study were located on the epiphyses or at the attachment sites of muscles or ligaments (c.f. Figures 3.2A – F). These sites were chosen specifically because of the expectation that these areas would show the most variation between groups, as was shown in previous metric studies (Ruff, 1987; France, 1988; Purkait, 2005; Spradley & Jantz, 2011). The location of these landmarks, however, may have resulted in the failure to detect shape variations in the shafts of the long bones examined. As discussed above, landmarks on the long bones are difficult to standardize, and often involves the use of more equipment which may introduce additional bias or error into the observations. It was thus decided not to include any landmarks on the shafts besides those linked to muscle attachments, which are easier to reliably identify. The variation in these landmarks on muscle attachment sites also need to be considered with caution, as they can be affected by both genetic and environmental influences (Stinson, 2012; White *et al*., 2012).

5.2. Sexual dimorphism

Sexual dimorphism is the result of a complex interaction between intrinsic and extrinsic factors which affect human morphology (Stinson, 2012; White *et al*., 2012).

Intrinsic factors include genetic control and hormonal differences between the sexes, which mostly manifest as size differences after the start of puberty, leading to the development of larger and more robust bones in males, and smaller and more gracile bones in females (Humphrey, 1998; Scheuer & Black, 2000; Suazo *et al.,* 2008a). Despite the strong genetic control over this secondary sexual development, the degree of dimorphism between the sexes may vary greatly between populations as a result of the variable influences of extrinsic factors such as nutritional quality, health status and biomechanical behaviour (Frayer & Wolpoff, 1985). It was thus necessary to evaluate patterns of sexual dimorphism in the sample as a whole, as well as among ancestry groups to elucidate information regarding the interaction of intrinsic and extrinsic factors on the skeletal system, and how this manifests in the morphology of various skeletal elements.

5.2.1 Size variation

The females in the present study were found to have smaller skeletal elements (as reflected by mean centroid size) than males for all of the bones examined (c.f. Table 4.1). This was consistent with both previous metric (De Villiers, 1968b; Ahlström, 1996; İşcan *et al.,* 1998) and morphometric studies (Rosas & Bastir, 2002; Franklin *et al.,* 2006; Green & Curnoe, 2009) of different population groups, and was likely a reflection of the expected dimorphism in body size after the onset of puberty due to differential developmental patterns of secondary sexual dimorphism in size (Hamilton, 1982; Scheuer & Black, 2000; López-Costas *et al.,* 2012; White *et al.,* 2012).

5.2.2. Cranial elements

Sexual dimorphism in shape was detected for the whole cranial and frontal bone data sets but none of the other cranial elements in this study (c.f. Figures 4.4 and 4.6). The differences on the frontal bone were primarily in the slope of the forehead, prominence of the glabellar region, and in the position of the temporal lines (c.f. Figure 4.5). The dimorphism of the cranium as a whole also showed these features, as well as differences in the sloping of the occipital region and the prominence of the mastoid processes (c.f. Figure 4.3). All of these features occur in areas of muscle attachment, and thus the more robust appearance of these areas in males compared to those of females was most likely due to secondary sexual development (Hamilton,

1982; Çelbiş *et al.,* 2001; White *et al.,* 2012). The detection of dimorphism at these sites is widely reported, and the use of these features in sex estimation is widespread and often recommended in anthropology texts such as Buikstra & Ubelaker (1994), Bass (1995) and White *et al.* (2012). The present study did, however, show that the dimorphism in these cranial features manifested not only in size, but also in shape, an observation which may not be possible to make using metric assessments or may be difficult to quantify metrically (Bulygina *et al.,* 2006; Kimmerle *et al.,* 2008; Costello*,* 2016). Consequently, the sexes could potentially be distinguished from each other even when individuals are of similar size, for example, a female with a cranium of similar size to what is more commonly observed in males, could still be identified as female based on the shape of the features on the frontal bone and cranium as a whole.

The lack of sexual dimorphism detected in the occipital, parietal and temporal bones of the present sample was unexpected, especially given that each of these elements have features such as the nuchal crest, parietal eminences and mastoid processes which are widely accepted to show distinct sexual dimorphism (e.g. Buikstra & Ubelaker, 1994; Bass, 1995). Size dimorphism was detected for each of these elements individually (c.f. Tables 4.1 – 4.3), and shape dimorphism of areas located on these elements was detected as part of the whole cranial data set (c.f. Figure 4.3). Therefore it is possible that the degree of dimorphism of these elements may have been affected by factors other than differences in genetic control or hormones between the sexes (Frayer & Wolpoff, 1985; Stinson, 2012).

In order to evaluate whether extrinsic factors could have been responsible for the lack of detected dimorphism in these cranial elements, morphologies of the elements were compared between sexes within the ancestry groups, as variations in these factors often show population-specific variation (Bruns *et al*., 2002; Charisi *et al*., 2011). Once ancestry was considered, sexual dimorphism in the shape of these elements was observed in each of the three ancestry groups, showing similar differences between the sexes within each group (c.f. Figures 4.44, 4.46 and 4.48). This suggests that the intrinsic dimorphism of these regions, as a result of secondary sexual development (i.e. size), may have been augmented by the influence of extrinsic differences in, for example, nutrition, health and activity among ancestry groups (White *et al*., 2012;

Moore, 2012). It was thus important that the inherent differences in morphology among the ancestry groups be considered in order to elucidate how different selective forces influence the development of sexual dimorphism in the cranial elements (Bulygina *et al.,* 2006; Coquerelle *et al.,* 2011; Crespo *et al.,* 2015).

5.2.3. Postcranial elements

Sexual dimorphism in shape was detected for the majority of the postcranial elements in this study (c.f. Figures 4.6 – 4.13). Of these elements, all of the observed differences were related either to areas of muscle attachment, or to the relative proportions of the bone, with males being relatively larger and more robust than females. These observations show that dimorphism of the long bones manifest not only in size, but also in their shape. As with size dimorphism, these shape differences between the sexes were probably due to secondary sexual development after the onset of puberty (Hamilton, 1982; Scheuer & Black, 2000; López-Costas *et al.,* 2012; White *et al.,* 2012).

Aside from the general differences in size and shape observed for most postcranial elements, the variation of the morphology of the femur was of further interest in that it reflected the biomechanical adaptation of the lower limb to the broader pelvic girdle of females. A unique and important aspect of secondary sexual development in females is the functional adaptation of the pelvic girdle to facilitate its role in reproduction. In males, the primary function of the pelvis is to transmit the weight of the trunk to the lower limbs in order to facilitate efficient bipedal locomotion (Rosenberg, 1988; Bruzek & Murail, 2006; Kurki, 2007). In females, however, there is the additional requirement of obstetric adequacy which leads to widening of the pelvic girdle (Walrath, 1997; O'Connell, 2004; Bruzek & Murail, 2006; Costello, 2015). This widening reduces the efficiency and stability of the transfer of weight from the trunk to the lower limbs (Anderson & Trinkaus, 1998), and thus in order to compensate and ensure biomechanical stability, female femora develop larger anteversion and neck-shaft angles (allowing larger bicondylar angles distally) in order to reposition the centre of the body's gravity below the hip at the knee joint (Davivongs, 1963; Eckhoff *et al.,* 1994; Tohtz *et al.,* 2010; Bonneau *et al.,* 2012). The results of the present study and of previous metric studies (Aiello & Dean, 1990;

Purkait, 2003; Tardieu *et al.,* 2006) supported this, as females were shown to have both larger femoral neck-shaft and anteversion angles (c.f. Figure 4.13).

Unlike the femur, which experiences the combination of selective forces related to bipedal locomotion and obstetric adaptation of the pelvis, the tibia and fibula are primarily adapted for locomotion and weight-transfer through the lower limb and for muscle and ligament attachment (Sacragi & Ikeda, 1995). This is true for both sexes, though the magnitude of these forces acting on the bones may be greater in males than in females, resulting in similar secondary sexual dimorphism in size as was seen for the other skeletal elements examined (Holland, 1991). However, in order to maintain stability of the lower limb for it to be efficient in weight transfer, dimorphism in the shape of the tibia and fibula may be limited (France, 1988; Stevens & Viðarsdóttir, 2008). This was observed in the present study sample, with significant sexual dimorphism detected in tibial shape when the tibial tuberosity landmark, which is the site of insertion for the quadriceps muscles (Gilroy *et al.*, 2008; White *et al.*, 2012; Bhat *et al.*, 2016), was included in the analysis (c.f. Figure 4.15), but the lack of dimorphism observed when this landmark was excluded from the analysis. Even when morphological dimorphism was evaluated within ancestry groups, the tibia (excluding the tuberosity) and the fibula did not show dimorphism between the sexes. This suggested that extrinsic factors which may differ among ancestry groups were not responsible for the lack of sexual dimorphism in these elements and that the selection forces, in this case weight transfer and locomotion, were similar for these different groups, resulting in similar morphologies (White *et al.*, 2012).

5.3. Ancestry

The South African population has the potential to serve as a good example of the interaction between environmental and social influences on the morphological differences among ancestry groups. The three largest ancestral groups, according to the Statistics South Africa Census (2011), namely Black, Coloured and White, were expected to show inherent differences in skeletal morphology due to their unique genetic histories. Black South Africans were expected to have similar body sizes and shapes as their East and Central African ancestral groups, while White South

Africans were expected to be similar to their European ancestral groups (L'Abbé *et al*., 2013). However, modern Black and White South Africans are temporally far removed from their ancestral origins and are likely to have experienced some adaptations to their new environmental conditions as well as genetic admixture with indigenous and migrant groups (Stull *et al.,* 2014). It is thus expected that both groups are likely to differ from their ancestral origins to some extent in terms of their skeletal morphology (Steyn & İşcan, 1998 and 1999).

Coloured South Africans present an even more complex genetic history, with variable contributions not only from the Black and White populations, but also from the indigenous Khoisan groups, and migrant or enslaved individuals from Asia and other parts of Africa (Van der Ross, 2005; Petersen *et al.,* 2013). This has resulted in this group having the ~~h~~highest levels of intercontinental (genetic) admixture of any global population group" (Tishkoff *et al.,* 2009). It is thus likely that Coloured South Africans will have morphological commonalities with both Black and White individuals, but also with some of the other groups with which they share genetic history (e.g. the Khoisan), resulting in a unique, though highly varied, skeletal morphology (Adhikari, 2005; Petrus & Isaacs-Martin, 2012).

5.3.1. Size variation

The present study showed that, based on mean centroid size, White individuals tended to be larger than both Black and Coloured individuals for most of the skeletal elements examined (c.f. Table 4.2). This observation supported those of previous metric studies which have shown White South Africans to be larger than the Black South Africans for many skeletal elements (Steyn & İşcan, 1999; Liebenberg *et al.,* 2015a). It is widely accepted that an individual's growth potential is determined by genetic factors (Gray & Wolfe, 1980; Hall, 1982). However, the extent to which an individual is able to attain their growth potential is strongly influenced by extrinsic factors such as nutritional quality and biomechanical behaviours (Hiernaux, 1968; Stini, 1969; Tobias, 1972; Lazenby, 2001).

In the period from 1948 to 1994, the South African government passed legislature which enforced the separation of individuals based on social racial classification, a system commonly referred to as Apartheid (Christopher, 2002; Morris, 2010; Stull *et*

al., 2014). Even before this legislature, and as early as 1806, race-based separations had been enforced under British colonial rule (De Wit *et al.,* 2010). Under Apartheid, however, the socio-economic consequences of these racial classifications were amplified, as the legislature associated with this segregation included aspects such as criminalizing inter-racial marriage and prescribing distinct areas of residence for each ―race" group (De Wit *et al.,* 2010). As such, interaction and gene flow between groups was limited and over time led to an increase in the variation among them (Jacobson *et al.,* 2004; Stull *et al.,* 2014). Further, the designation of residential areas was followed by great disparities in socio-economic conditions, with non-White groups generally being provided with inadequate or inferior living and health conditions, which were further exacerbated by restrictions on education and employment (Price *et al.,* 1987; Henneberg & Van den Berg, 1990; Tobias, 1990; Thompson, 2001; L'Abbé *et al.,* 2013). As discussed for the manifestation of sexual dimorphism, these factors greatly influence the ability of individuals within a population to reach their full growth potential (Hiernaux, 1968; Lazenby, 2001).

Another factor which may influence an individual's ability to reach their growth potential is nutritional quality (Stini, 1969; Tobias, 1972). It has been shown that nutritional stress, especially in populations with very high or very low protein containing diets may result in a reduction of the mean adult body size within such populations (Gray & Wolfe, 1980; Hoppa, 1992; Kemkes-Grottenthaler, 2005). Puoane *et al*. (2002) reported that malnutrition is particularly prevalent in Black South African individuals, compared to individuals of other ancestry groups, and that this has increased over time due to the increased availability and low cost of unhealthy foods. Similar to poor living conditions, the poor nutritional status among Black individuals may thus also have reduced their ability to reach their full growth potential. Thus, while smaller size of the skeletal elements of the Black individuals in the present study may simply be due to historical genetic variation, extrinsic influences such as poor living conditions and/or nutrition may have amplified the extent to which this group differs in size from the White individuals in this study. This is especially likely considering the potential bias introduced into the sample as a result of acquisition bias of the skeletal collections used, and the lack of socio-economic information available for these individuals.

The Coloured individuals in the present study tended to have smaller skeletal elements than individuals of both of the other two ancestral groups (c.f. Table 4.2). This difference was observed even when the Coloured female and male groups were compared to individuals of the same sex in the other ancestry groups (c.f. Table 4.3). This supports arguments like those of Adhikari (2005) and Petrus & Isaacs-Martin (2012) that the Coloured group is not simply a morphological intermediate to the Black and White groups which have contributed to its genetic composition. This was further supported by the genetic results of Tishkoff *et al.* (2009) and De Wit *et al.* (2010) which show additional genetic contributions from Asian and indigenous Khoisan ancestral groups. Khoisan individuals are characterised by their small body size (Wilson & Lundy, 1994; Sealy & Pfeiffer, 2000; Kurki, 2007), and as such, it is possible that it was this genetic contribution to the Coloured group which was reflected in their smaller size in the present study. As with Black individuals, Coloured individuals were also subjected to poor living, health and nutritional conditions under Apartheid (Adhikari, 2005; Friedling, 2007), which may similarly have restricted growth in size in this population.

5.3.2. Cranial elements

Shape analysis of the cranial elements according to ancestry groups produced similar results as those of the metric studies of De Villiers (1968a and b) and Howells (1995), with Black individuals having more dolicocephalic crania (relatively antero-posteriorly longer than medio-laterally wide), and White individuals having more brachycephalic crania (more wide than long) (c.f. Figure 4.18). White individuals were also found to have generally more robust muscle attachment sites (e.g. the mastoid processes) than Black individuals, in agreement with the observations of Krüger *et al.* (2014).

Coloured individuals presented with features similar to both of the other groups, though they tended to be more similar to the Black individuals, as shown by the detection of smaller Mahalanobis distances between Black and Coloured individuals than between White and Coloured individuals (c.f. Appendix D – Table D3). This pattern of variation in the morphology of the skeletal elements of Coloured individuals was a better reflection of their genetic history than the observed pattern of size variation. Several studies have shown the cranium as a whole or parts thereof to

be a reliable indicator of genetic affinity (Harvati & Weaver, 2006a and b; Martinez-Abadías *et al.,* 2006; Von Cramon-Taubadel, 2011) and evaluation of cranial shape is often recommended in anthropology texts for the purpose of ancestry estimation (Buikstra & Ubelaker, 1994; Bass, 1995; White *et al.,* 2012). Genetic studies of South African populations have shown that there was little gene flow between Black and White ancestral groups over time (Jacobson *et al.,* 2004; Jorde & Wooding, 2004; Ousley *et al.,* 2009). Even after Apartheid legislature had been abolished in 1994, many groups still self-identify according to the Apartheid terminologies (Friedling & Morris, 2005; Stull *et al.,* 2014). Both Black and White ancestral groups have contributed to the genetic composition of the Coloured group, but according to the historical and genomic investigations of Adhikari (2005) and De Wit *et al.* (2010), the contribution from the Black group was more substantial. Jacobson *et al.* (2004) suggested that this could have been due to the less stringent enforcement of Apartheid laws regarding restriction of intermixture of Black and Coloured individuals than intermixture of White and non-White individuals. These genetic histories provide an explanation for the greater similarity between Black and Coloured individuals observed in the present study and those of Stull *et al.* (2014) and Liebenberg *et al.* (2015a). Since these two groups have more shared genetic history than either group has with the White group, it can be expected that their skeletal morphologies would be more similar to each other than to those of White individuals, as was observed in both the shape and size differences among the three groups in the present study.

5.3.3. Postcranial elements

The shape variation of the upper limb bones of the three ancestry groups in the present study was more varied than that of the cranial elements. In terms of the size of the bones, the humerus was larger in White individuals than for Black individuals (c.f. Table 4.2), fitting with the observed pattern of the cranial variation. In contrast, the radius and ulna were larger in Black individuals, deviating from this pattern. These bones were, however, more medio-laterally wider in White individuals, as seen in the shape analyses. A possible explanation for this may again be the different socio-economic conditions experienced by these groups. One component of the Apartheid legislation, besides the supply and quality of living conditions, were the limitations imposed on education and available work for each group (Stull *et al.,*

2014). As a result, Black individuals were often restricted to unskilled and often very physically demanding labour. As shown by the Quarterly Labour Force Survey (QLFS, 2016), this division of labour is still observed in the present-day South African population. Due to the increased physical activity required for many of these unskilled positions, it was expected that these individuals would have experienced increased muscle and bone strain, which, in turn, may have led to the development of more robust muscle attachment sites where this strain was applied (Ruff, 1992; Knüsel, 2000; Robling *et al.,* 2006; Micklesfield *et al.,* 2011). In the present study, this was observed in the bones of the forearm, suggesting that handling was more of a strenuous habitual task in Black individuals than in White individuals (Churchill & Morris, 1998; Stock & Pfeiffer, 2004). Despite the larger size of the radius and ulna of Black individuals, the relative shapes of these bones were more gracile in this group than in the White group (c.f. Figures 4.30 and 4.32). This suggests that the adaptation to this occupational stress may have manifested in the size but not the shape of these elements, perhaps as an indication of general strain to the forearm and not just localized strain to a few muscles as the result of specific muscle actions (Van Gerven, 1972). In terms of the upper limb bones, Coloured individuals shared size and shape similarities to both the Black and White groups, though they were often more similar to the Black group (c.f. Figure 4.28). As was the case for the cranial elements, this was likely a reflection of both their closer genetic relationship to the Black group than to the White group. However, this may also have been a reflection of their living conditions and the nature of the labour performed by Coloured individuals, which was more similar to that of the Black individuals than the White individuals (QLFS, 2016).

The bones of the lower limb were similar in size for Black and White individuals, which were both larger than Coloured individuals (c.f. Table 4.2). In terms of the shape of these elements, Coloured individuals were also found to be the most gracile in their overall morphology (c.f. Figures 4.34, 4.36, 4.38 and 4.40). This again supported the genetic and historical evidence which indicate that the Coloured group is not simply an intermediate to the Black and White groups, but that they may also display traits of the Asian and Khoisan contributions to their genetic history (Adhikari, 2005; Petrus & Isaacs-Martin, 2012), in this case expressed as their significantly smaller size. Despite the similarity in size to Black individuals, the

shape of the lower limb bones were comparatively more robust in shape in White individuals. Based on their involvement in more physically demanding labour such as agriculture and mining (QLFS, 2016), it would have been expected that Black individuals would have more robust bones than White individuals. The reduced robusticity in the Black individuals of this study may, however, have been a manifestation of the effects of poor living conditions and nutritional quality, which are more prevalent in Black communities than those of other ancestry groups, thus affecting the development of relatively larger body sizes within this group (Puoane *et al*., 2002; Hiernaux, 1968; Stini, 1969; Tobias, 1972; Lazenby, 2001). Due to the potential inherent socio-economic bias within the skeletal collections used for the present study, with White individuals most likely being of higher socio-economic status than the Black or Coloured individuals, it is possible that these differences which were detected as differences among the ancestry groups are, in reality, differences due to extrinsic (socio-economic) influences on skeletal morphology rather than intrinsic differences in genetic history among ancestry groups.

5.4. Sex-ancestry

Many of the extrinsic factors which have been reported to affect the manifestation of variation in skeletal morphology show population-specific patterning (Bruns *et al*., 2002; Charisi *et al*., 2011). It is thus important to assess features such as sexual dimorphism, which are greatly susceptible to extrinsic factors such as nutrition and biomechanical behaviour, within a population. Several metric and geometric morphometric studies such as those of Steyn & İşcan (1998 and 1999), Spradley *et al.* (2008) and Brzobohatá *et al.* (2014) have shown that variation in, for example, sexual dimorphism within a population may differ to such an extent from that of a different population that the applications of sex estimation standards of the one population to individuals of the other may lead to misclassification. Even when using sophisticated software programs such as FORDISC® or 3D-ID, population-specificity in parameters such as sex and stature has to be considered (Guyomarch & Bruzek, 2011).

The need for population-specific assessment was emphasized in the present study when sex and ancestry were considered together. Although the general size and shape

differences among individuals seen when assessing these parameters independently were still detected, some skeletal elements for which sexual dimorphism was not detected in the assessment of sex in a pooled ancestry sample were now also observed to show sexual dimorphism (c.f. Figures 4.44, 4.46 and 4.48). For all of the elements in the present study, the assessment according to sex-ancestry groups showed that ancestry explained a greater proportion of the observed sample variation than sex did, suggesting that the between-group variation was larger than the within-group variation for these three South African groups. It is important, however, to note that both ancestry and sex are expressed as continuous parameters in the skeleton, and because of the large overlaps among groups, females of one ancestry group may have very similar skeletal morphology (both in size and shape) to males of another ancestry group (White *et al.,* 2012). Lastly, both the intrinsic and extrinsic factors influencing skeletal morphology are dynamic and may cause groups to become more or less similar over time. Consequently, it was important to further explore the potential co-variation of parameters such as age and temporal (i.e. time-related) trends with the observed morphological variation (Sacragi & Ikeda, 1995; Walsh-Haney *et al.,* 1999; Rösing *et al.,* 2007; Kudaka *et al.,* 2013).

5.5. Relationships with other variables

5.5.1. Body size and allometry

The relationship of the morphological variation between the sexes and ancestry groups was further analysed to explore potential relationships of the variation between groups with three variables: centroid size, age and year-of-birth. The first of these, centroid size is isolated from the sample data in the Procrustes superimposition step of the statistical analysis, but represents a good indicator of overall body size which can be used in further analyses (Singleton, 2002). Most commonly, regression analysis is used to test for a co-variation of centroid size to the shape differences detected between groups as an evaluation of allometry, i.e. the influence of size on shape (e.g. Kimmerle *et al.,* 2008; Green & Curnoe, 2009). Allometric variation is likely to occur in complex structures such as the cranium, with adult morphology being influenced by factors such as morphological integration, developmental and functional constraints, and different levels of plasticity of the skeletal elements (Martinez-Abadías *et al.,* 2006). The ability to separate morphology into its size and

shape components through GPA makes the geometric morphometric approach ideally suited for tests of allometric effects on skeletal morphology, allowing the detection of subtle yet significant variations in very similar populations or detecting similarities in allometric trajectories among diverse population groups (Badawi-Fayad & Cabanis, 2007; Weisensee & Jantz, 2011; Mitteroecker *et al.,* 2013).

5.5.1.1. Sexual dimorphism

Cranial elements

Of all the cranial elements examined in the present study, only the whole cranium and frontal bone showed significant sexual dimorphism in morphology. When the relationship of these differences to (centroid) size was evaluated, only the frontal bones showed significant correlation ($p<0.0001$) of size and shape (c.f. Table 4.4). This lack of correlation for the whole cranium, and the very small proportion of variation linked to size in the frontal bone (only 2.2%) suggested that the shape of the cranial elements was influenced little, if at all, by variations in their size. In terms of differences between the sexes, this meant that, despite the difference in size between the sexes, the shape of the cranial elements of small and large females could be expected to differ approximately equally from that of small and large males. Previous studies have shown the neurocranial elements to reliably reflect population affinity, but that sexual dimorphism of shape in different population groups remains fairly uniform (Franklin *et al.,* 2004). The lack of allometric effect on the difference between sexes in the present study and that of Franklin *et al.* (2004) may be related to the development of the neurocranial elements, which reach their adult morphology early in ontogeny under the influence of their close relationship to the brain, which itself is highly constrained in morphology (Enlow, 1990). The neurocranial elements are thus less susceptible to the influence of environmental factors and have less plasticity in their morphological development than the elements of the facial skeleton (Viðarsdóttir *et al.,* 2002; Zollikofer & Ponce de León, 2002; Bastir *et al.,* 2006 and 2007; Harvati & Weaver, 2006a).

Postcranial elements

In the present study, the postcranial elements which were observed to be sexually dimorphic in shape showed stronger relationships to centroid size than the cranial elements (R^2 of up to 25.8%) (c.f. Table 4.4). These relationships show that as

centroid size increases, the bones tended to have morphologies which were similar to the mean female shape morphology, which was essentially a proportionately medio-laterally widened appearance of the long bones. A potential explanation for this observation is that a general increase in size can be expected to be associated with an increase in muscle size and subsequently an increase in the robusticity of the muscle attachment sites (Humphrey, 1998; Suazo *et al.,* 2008b). This would result in medio-lateral expansion of these attachment sites, making the bones appear proportionately larger, while the articular surfaces of the bone would be appear proportionately smaller. As a result, the long bones of larger sized individuals would tend to appear more rectangular in shape, similar to the proportions of these elements observed as the mean female shape.

5.5.1.2. Ancestry

Cranial elements

Centroid size was shown to be significantly correlated to the differences among ancestry groups for all skeletal elements in the present study, though the amount of variation linked to centroid size was low ($R^2 \leq 10.1\%$) (c.f. Table 4.6). The majority of the cranial elements tended to show an increase in relative size and robusticity with increasing centroid size, appearing more similar to the mean shapes of the White group. This was similar to the observations of Weisensee & Jantz (2011) that the crania from their Lisbon cemetery sample tended to be more robust for individuals with larger cranial sizes than those with relatively smaller crania. Exploring allometry of the face, Badawi-Fayad & Cabanis (2007) report that similar patterns of facial allometry was shared by their African, European, Asian and Native American samples, as well as a pooled sample of these groups, suggesting that the allometric effect was mainly due to morphological integration and functional constraints of the complex cranial structure, rather than to a plastic response to extrinsic factors (Martinez-Abadías *et al.,* 2006).

Postcranial elements

In contrast to the cranial elements which tended to become more robust (similar to White individuals) with increasing centroid size, the postcranial elements of the individuals in the present sample tended to become medio-laterally wider (similar to Black individuals). As observed in the assessment of differences between the sexes,

this may have been a reflection of larger muscle strain which would lead to increased size and robusticity of the muscle attachment sites, and which, in turn, makes these sites proportionally larger and the whole bone more rectangular in appearance.

5.5.1.3. Sex-ancestry

When the relationship of centroid size and shape was assessed according to sex and ancestry combined (c.f. Table 4.8), the nature of the detected correlation was similar, though the strength of the relationship was very weak ($R^2 \leq 9.9\%$), compared to the relationships seen when assessed according to sex or ancestry independent of each other. This suggested that the effect of changes in centroid size on the shape differences among the sex-ancestry groups was very small, likely due to the majority of the variation among the six groups already being incorporated into the consideration of sex and ancestry.

5.5.2. Age and remodelling

The next relationship that was assessed was that of age. While it is generally expected that little skeletal growth takes place after the completion of puberty, remodelling of bone has been shown to continue long after skeletal maturity (Hamilton, 1982; Lieberman, 1982; Scheuer & Black, 2000). Remodelling is greatly influenced by environmental factors such as physical activity and nutrition, and as such is highly variable, making its use in age estimations problematic (Franklin, 2010; White *et al.,* 2012; Steadman, 2013). Exploration of the effect of aging on skeletal morphology is, however, necessary, as it provides further information about the intrinsic and extrinsic influences which may alter the shape of skeletal elements and potentially lead to misclassifications of other variables such as sex and ancestry. An example of such change was noted by Walker (1995), which showed an increase in the robusticity of female crania, and the supra-orbital ridge in particular, in an English cemetery sample. Several studies have also shown an increase in osteometric dimensions of long bones with age through continued appositional growth (Smith & Walker, 1964; Pfeiffer, 1980; Ruff & Jones, 1981; Stevens & Viðarsdóttir, 2008; Vance *et al.,* 2010). These studies observed that such changes are more pronounced in post-menopausal females, leading to the conclusion that the reason for the observed changes may have been hormonal. Menopause is often associated with a loss of cortical bone thickness, and it is proposed that, as a method of compensation,

periosteal thickness of the long bones are stimulated to increase in order to maintain functional stability (Smith & Walker, 1964). This theory is supported by the fact that hormonal changes occur in both sexes, but to a greater extent in females (Khosla *et al.,* 2002; Seeman, 2002).

Regression analysis in the present study showed significant but weak correlation of age with several of the elements examined (R^2 <10% of sample variance). This suggests that age-related changes to these elements were small, having little, if any, effect on the overall morphology of the bone, and the subsequent differences between sex, ancestry or sex-ancestry groups. This is in agreement with previous observations by Smith & Walker (1964) and Stevens & Viðarsdóttir (2008) which showed these changes to be very subtle and to manifest as size rather than shape changes.

5.5.2.1. Sexual dimorphism

The femur, radius and ulna of the present sample showed stronger correlations to age than the other elements examined (R^2 = 12–20.1%), with these elements becoming increasingly more robust in shape with increasing age (c.f. Table 4.4). This is in agreement with the metric studies of Pfeiffer (1980) and Vance *et al.* (2010), which showed that the dimensions of the long bones in females approximate those of males in older individuals. The stronger relationship of age with these three elements suggested that the changes which occurred in these bones were more pronounced than those in the other bones. This may have been the result of differences in physical activity and nutrition between individuals, both of which may alter the magnitude of cortical bone changes in older age (Smith & Walker, 1964; Pfeiffer, 1980; Ruff & Jones, 1981). It is possible that increased levels of activity involving these areas may have led to increased bone density, thus reducing the magnitude of the impact of the hormonal changes to the bone in old age (Stevens & Viðarsdóttir, 2007). As both physical activity and nutrition often show population-specific variation, it was necessary to explore how the relationship of age to skeletal morphology may potentially vary among ancestry groups.

5.5.2.2. Ancestry

When the correlation of age with the differences among ancestry groups was assessed, it was observed that the majority of the elements studied became larger and

more robust with increasing age for most of the elements examined (c.f. Table 4.6). As shown for the differences between sexes, and previous metric and non-metric studies by Pfeiffer (1980) and Walker (1995), increased deposition of periosteal bone to compensate for the hormonally induced loss in cortical bone occurs later in life. Since White individuals already tended to be larger and more robust than the individuals of the other two ancestry groups, this might not have been as easy to detect in individuals of this group. Black and Coloured individuals, on the other hand, tended to be smaller and had more gracile morphologies, thus the increase in size and shape for these individuals may have been more evident, as they would become more similar to the larger, robust White individuals. A similar trend can be seen in the data of Vance *et al*. (2010) in their examination of age-related changes in the dimensions of long bones of Black and White South Africans. While their study did not discuss the potential overlap among ancestry groups, it did discuss how an increase in size in females with age, which causes them to appear more similar to their male counterparts, could lead to misclassification of sex. The data presented, however, showed that the older Black male sample (over 50 years of age) presented with measurement ranges more similar to White individuals than the younger Black individuals did. This was in agreement with the increased size and robusticity observed for Black (and Coloured) individuals in the present study.

As has been observed in the present study and several other sources in the literature (e.g. Bruns *et al*., 2002; Charisi *et al*., 2011), extrinsic influences on skeletal morphology are highly variable between populations and even between individuals, due to differences in diet, lifestyle (especially behaviours such as smoking and alcohol consumption), and health. It is also expected that differences between groups in terms of physically demanding activities would affect the deposition of bone with age, as individuals who perform more strenuous tasks, such as the habitual carrying of water on the head common among rural Black females (Lloyd *et al.,* 2010a and b), could compensate for this increased strain by having increased bone deposition at the sites on which this strain acts (Geere *et al.,* 2010; Vance *et al*., 2010; Micklesfield *et al.,* 2011). As a result of the highly variable nature of extrinsic influences on skeletal morphology, and thus also the differences among the different ancestry groups in the present study, it is possible that the morphologies of the three ancestry groups may become less distinct with age. This could potentially lead to a reduction in the

accuracy with which ancestry of an individual could be estimated, even when both size and shape of the skeletal elements are utilized (Vance *et al*., 2010). Such estimations should thus be interpreted with caution.

In contrast to the other elements in the present study, the whole cranium, occipital and parietal bones did not show significant correlation of ancestry differences with age (c.f. Table 4.6). This suggested that the variation in shape of these elements among the ancestry groups was not significantly influenced by age-related skeletal changes, i.e. the morphology of these elements were distinctive in both young and old individuals within each population group. This stability on the morphology of these elements may have been a reflection of their close relationship with the underlying neural structures, causing these skeletal elements to fuse relatively early in development and be less susceptible to extrinsic influences, such as those expected to vary among the ancestry groups of the present study (Scheuer & Black, 2000; Harvati & Weaver, 2006a and b; Gapert *et al*., 2009b; Smith, 2009).

5.5.2.3. Sex-ancestry

The relationship of age and shape variation according to sex and ancestry combined was similar to when they were assessed independently (c.f. Table 4.8), but the strength of these correlations was weaker ($R^2 \leq 9\%$). This suggests that sex and ancestry already accounted for most of the variation, while variation in age accounted for much less of the observed variation.

5.5.3. Temporal trends

Temporal trends are gradual changes in morphology which occur within a population over relatively short periods of time, e.g. one or two generations (Smith *et al.,* 1986; Cameron *et al.,* 1990). These changes can serve as indicators of the state of public health over time (Tanner, 1992), and are often influenced by socio-economic changes such as improvements in health care and nutrition, levels of physical activity and reproductive behaviours (Jantz & Jantz, 1999). As observed when sexual differences in morphology were analysed independent of ancestry, environmental conditions may have a great impact on the skeletal system of an individual, and since great socio-economic changes have occurred in South Africa over the past few decades, it is necessary to investigate the potential influences of temporal trends on the present

study sample. Year-of-birth was used as an indicator of different temporal groups. Previous studies often divide their samples into ~~–b~~irth cohorts" of e.g. 5 years (Price *et al.,* 1987), but due to the nature of the data in the present study, more information could potentially be gained using the year-of-birth data as a continuous variable (Tobias & Netscher, 1976 and 1977). The year-of-birth of individuals in the present study ranged from 1887 to 1992, and temporal changes have been observed in other study populations over similar timeframes (Price *et al.,* 1987; Henneberg & Van den Berg, 1990; Buretić-Tomljanović *et al.,* 2006; Jonke *et al.,* 2007; Hawley *et al.,* 2009). It was found that the morphological variation between sexes, ancestry groups and sex-ancestry groups showed significant correlation to year-of-birth, suggesting that temporal changes have indeed occurred in the present sample population.

5.5.3.1. Effect of temporal trends on sexual dimorphism

Cranial elements

The variation in the shape of the whole cranium and frontal bone between sexes of the present sample was found to be correlated to variation in year-of-birth (c.f. Table 4.4). It was observed that the morphology of the whole cranium tended to become more similar to the mean male cranial shape (i.e. medio-laterally narrower, with more robust mastoid processes and glabella) in the later part of the century. This was similar to the observations of Jantz & Meadows-Jantz (2000) and Weisensee & Jantz (2011) who noted a secular decrease in the width of the cranial vault but increased robusticity in the mastoid regions in their American skeletal sample. Secular trends in South African populations are poorly explored, with only Cameron *et al.* (1990) noting a decrease in the mean cranial height of South African Black individuals from 1880 to 1934. A similar trend was not observed in the present study, which may be due to the larger timeframe from which the individuals in this study were selected, or because the three ancestry groups were pooled for this initial analysis.

Postcranial elements

The present study showed that the morphology of the long bones tended to be more rectangular (similar to the mean female shape) in individuals born in the last few decades, while the bones of the individuals from the earlier part of the century had proportionately larger epiphyses (similar to the mean male shape). Most of the studies examining potential temporal trends in the South African populations have

only looked at the femur and tibia, or at living stature and weight (Tobias & Netscher, 1976 and 1977; Price *et al.,* 1987; Henneberg & Van den Berg, 1990; Hawley *et al.,* 2009). Many of these studies reported an absence of secular trend, but this may have been due to the short timeframes assessed in those studies, or the restriction of the samples to Black South African individuals (often only males). Others reported a slight secular decrease in the size and robusticity of their South African samples (Tobias & Netscher, 1976 and 1977; Price *et al.,* 1987), similar to the results of the present study. Over the past few decades, it has been reported that both males and females have become less physically active, both occupationally and recreationally, as a result of improvements in technology and increased mechanization of labour (Price *et al.,* 1987; Anderson & Trinkaus, 1998; Puoane *et al*., 2002; WHO, 2016). As a result, populations generally experience less extreme muscle development and bones appear less robust than those of individuals of the earlier part of the century performing more intensive physical labour (Ruff, 1987; Steyn & İşcan, 1999). This agreed with the more gracile skeletal morphology observed for more modern individuals in the present study. However, as was seen in the comparison of the ancestry groups, the socio-economic influences acting on skeletal morphology may vary greatly among the three largest ancestry groups in South Africa, largely due to the legislative separation of these groups during Apartheid. It was thus necessary to further explore the potential influence of temporal trends within each of these ancestral groups.

5.5.3.2. Effect of temporal trends on differences among ancestry groups

The majority of the skeletal elements examined showed correlation of year-of-birth with the variation among the ancestry groups (c.f. Table 4.6), with these bones tending to be more robust in shape for individuals born in the early part of the century, while those of the later part of the century were smaller and more gracile. After the abolishment of Apartheid, living conditions in South Africa have reportedly improved, especially for the previously oppressed Black and Coloured groups. These changes include improvements in health care and nutrition, and general access to services (Christopher, 2002; Hawley *et al.,* 2009; Statistics South Africa, Census, 2011).

In the present study, it was found that individuals of all three ancestry groups have become more robust in shape over time. This suggested that the influence of improved living and health conditions over time has had a similar effect in all three groups. It was, however also noted that Black and Coloured individuals tended to become more similar to the more robust White individuals over time. While this may have been a reflection of a greater magnitude of the effect of the socio-economic improvements in these groups, as suggested by Hawley *et al*. (2009), it may also relate to the biomechanical behaviours of these two groups. It has been noted that, despite the abolishment of legal barriers, there is still very little social and residential integration of the different groups in the country (Friedling & Morris, 2005; Stull *et al*., 2014). As a result, Black and Coloured individuals still constitute the majority of the unskilled labour force, often performing very physically demanding work in industries such as mining and agriculture (QLFS, 2016). It is thus expected that these individuals would consequently develop even more robust skeletal elements as a result of the increased strain being placed on their bodies by these activities, resulting in the increased robusticity observed in the present study.

5.5.3.3. Effect of temporal trends on differences among sex-ancestry groups

Analysis of groups according to sex and ancestry showed similar but weaker correlations to year-of-birth ($R^2 \leq 8.7\%$) (c.f. Table 4.8), compared to when these parameters were assessed independently. Similar to the observations of the relationships to centroid size and age, this suggested that sex and ancestry accounted for the majority of the variation in the sample, while the variation linked to year-of-birth accounted for a very small proportion of the observed variation.

5.6. Classification accuracy

5.6.1. Sex estimation

5.6.1.1. Cranial elements

The whole cranium yielded a classification accuracy of 70.2%, which was less than the approximately 80% accuracy normally reported for sex estimation using either metric or morphometric assessments of the cranium (e.g. Giles, 1970; Ross *et al.,* 1999; Spradley & Jantz, 2011; Best *et al.,* 2016). Sexual dimorphism in the size and robusticity of the cranium are widely reported in skeletal biology textbooks (e.g.

Buikstra & Ubelaker, 1994; Bass, 1995; White *et al.,* 2012). It is thus possible that the relatively lower accuracy obtained in the present study, which was based on shape dimorphism only, is an indication of the importance of size in distinguishing between the sexes (Best *et al.,* 2016). In the case of the frontal bone, an accuracy of 79.3% was achieved in the sex-pooled sample (c.f. Table 4.5; Figure 4.56a). This was similar to the results of Hochstein (2014), with reported accuracies of 63 – 73% using non-metric scoring of features of the frontal bone, and 70 – 88% when using DFA applied to 3D morphometric data of the same sample. It was also similar to the 79% accuracy reported by Garvin *et al.* (2014) for non-metric classification using only the appearance of the glabellar region. In these two studies and the present study, females could be classified with greater accuracy than males, likely because of the distinctive shape of the highly sloped and rounded female frontal bone. Nonetheless, the frontal bone was the element with the best sex classification accuracy of all the elements in this study, with accuracies of 75.5–83.7%. These high accuracies may have been due to the presence of highly dimorphic features such as the areas of muscle attachment at the supra-orbital ridge and the anterior part of the temporal line, and the close relationship of the frontal bone to the sinuses, which are known to be sexually dimorphic in size (Hylander *et al.,* 1991; Hsiao *et al.,* 1996; White *et al.,* 2012).

The lower accuracies achieved by the whole cranium compared to the high accuracies using the frontal bone alone may have been due to the inclusion of landmarks in the data set representing the whole cranium which are located on the parts of the cranium not showing significant dimorphism in the present study, i.e. the occipital, parietal and temporal bones. The present study found that these elements showed sexual dimorphism in size but not shape, similar to the metric results of King (1997). Green & Curnoe (2009) propose that this may have been due to population-specific responses to extrinsic influences, which tend to affect size to a greater extent than shape in certain populations. It may thus be better to use only the shape variation of the frontal bone, even when the whole cranium (or even the occipital, parietal or temporal bone) is available for analysis. These results supported the recommendation of Harvati & Weaver (2006a) and Bigoni *et al.* (2010) that the ~~"~~more is better" rule may not apply to analysis of the cranium, and that it may be better to exclude certain regions of the cranium from such analyses in order to achieve better classification accuracies. It is, however, important to note that the morphology of the cranium and

the sexual dimorphism thereof is often highly population-specific in nature (Cox & Mays, 2000; Viðarsdóttir *et al.,* 2002; Suazo *et al.,* 2008a). It is thus possible that sex classification accuracies may be improved when ancestry variation is considered in conjunction with the variation between the sexes.

5.6.1.2. Postcranial elements

The morphological variation of the postcranial elements produced sex classification accuracies of 64–74.3% (c.f. Table 4.5; Figure 4.56b). This was notably lower than the reported classification accuracies often exceeding 80% reported in metric studies based on both South African and international samples (e.g. Steyn & İşcan, 1997; İşcan *et al.,* 1998; Sakaue, 2004; Charisi *et al.,* 2011; Ahmed, 2013a and b; Tise *et al.,* 2013; Kranioti & Apostol, 2014). A possible reason for the lower accuracy observed in the present study may have been the population differences between international and South African samples, based on the observations of Steyn & İşcan (1999), Patriquin *et al.* (2003) and Barrier & L'Abbé (2008) that some South African groups have reduced sexual dimorphism of the postcranial elements, compared to their international counterparts. Similar to the lower classification accuracies observed using the cranial elements, the lower accuracies in the present sample compared to those of other South African samples evaluated using a metric approach may suggest that dimorphism of these individuals manifest in size rather than shape differences (Green & Curnoe, 2009). It is also possible that using a pooled sample consisting of individuals from three different ancestry groups may have reduced the ability to distinguish between sexes, since sexual dimorphism is highly population-specific (Spradley & Jantz, 2011; Macaluso, 2011). Sexual dimorphism in the Black and Coloured groups in the present study can be expected to be relatively less than those of the White group, due to the poor environmental conditions which may have influenced their morphologies, as seen when exploring the differences among the three ancestry groups. It was thus necessary to further explore sexual dimorphism of shape within specific ancestry groups.

The femur yielded the highest sex classification accuracy of all the postcranial elements, with an average accuracy of 74.3%. Since the femur is the primary weight-bearing bone of the lower limb (Williams & Warwick, 1980; Purkait & Chandra, 2004; Özer & Katayama, 2008), and since body size (as represented by centroid size

in this study) is generally larger in males than females, a high degree of sexual dimorphism in the size and shape of the femur can be expected (Plavcan, 2001; Byers, 2005; White *et al.,* 2012). Aside from these adaptations, the morphology of the femur is also tailored to compensate for the adaptations of the female pelvis to its reproductive function (Walrath, 1997; Bruzek & Murail, 2006; Nuger, 2008). It is thus the combination of the strong influences of weight-bearing and reproductive functional adaptations of the femur which leads to the distinctive morphologies of females and males, enabling more accurate differentiation between the sexes based on shape alone. The accuracy of the present study was less than the up to 92% accuracy reported by Costello (2015) for a sample of White American individuals when using either the proximal or distal ends of the femur. This lower accuracy in the present study may have been a reflection of lower levels of dimorphism in the postcrania of South African individuals compared to Costello‘s (2015) sample, as previously reported by Steyn & İşcan (1999), Patriquin *et al.* (2003) and Barrier & L‘Abbé (2008), thus resulting in less clear distinction between the sexes and lower classification accuracies, or the lower number of landmarks used in the present study. The tibia also showed significant differentiation between the sexes, but the detected differences were mainly constrained to the morphology and position of the tibial tuberosity, and thus yielded lower classification accuracies than the other long bones (64%). This result and the lack of sexual dimorphism in the shape of the fibula again suggest that the morphology of these elements may be constrained to ensure their function in locomotion and weight transfer.

5.6.1.3. Comparison of the classification accuracy between the sexes

Overall, the classification accuracies of females were higher than those of males (c.f. Table 4.5; Figure 4.56). This may have been due to the differences in susceptibility of males and females to environmental changes (Stini, 1969; Stinson, 1985; Ross *et al.,* 2003). It has been shown that under difficult conditions such as nutritional stress, the body size of males within a population is reduced, while females are more resistant to such stresses, likely as a mechanism to protect their ability to fulfil their reproductive roles (Stini, 1975; Wolanski & Kasprzak, 1976). As a result of the constrained variability in females, their skeletal morphologies may have been more distinctive and allowed more accurate classification than the more variable and less distinctive morphology of males. The classification accuracy for either sex is, however, similar

to the ranges reported for metric and nonmetric assessments of the different skeletal elements. This may suggest that the geometric morphometric assessment of these elements should be used as a supplement, rather than replacement, to the methods currently used by anthropologists (Bigoni *et al.*, 2010). In terms of forensic application, these methods would provide the objectivity and reliability that many of the other methods may not possess.

5.6.2. Ancestry estimation

The calculated Mahalanobis distances between ancestry groups supported the observation that Black and White individuals tended to be more similar in skeletal morphology to Coloured individuals than to each other, with Black individuals often being relatively more similar to Coloured individuals than White individuals are to Coloured individuals (c.f. Appendix D – Table D3). This again reflects that both of these groups contributed to the genetic history of the Coloured group, and the greater similarities in environmental conditions and shared genetic histories of the Black and coloured ancestry groups (Tishkoff *et al.,* 2009; De Wit *et al.,* 2010; L'Abbé *et al.,* 2013).

5.6.2.1. Cranial elements

The frontal bone was found to be the most accurate of all the skeletal elements for ancestry estimation in the present study, yielding accuracies of 83.7–88% (c.f. Table 4.7, Figure 4.57a). This was likely due to the distinguishing appearance of this bone in terms of its height, width and curvature, as suggested in several anthropology texts (Byers, 2005; White *et al.,* 2012; İşcan & Steyn, 2013). These texts also cite the shape of the cranium as a whole to be a good indicator of ancestry, which is in agreement with the whole cranium providing the second best classification accuracy in the present study. Besides a few of these texts mentioning the prominence of muscle markings on the individual bones of the cranium, very little attention is paid to ancestry estimation using these other cranial bones. One exception was the study by Von Cramon-Taubadel's (2009) which showed that the whole cranium, as well as the frontal and parietal bones show strong correlation to population affinity, while the occipital and temporal bones show weaker correlations, likely as a result of the confounding influence of large muscle attachments to the latter two elements. In the present study, the occipital, parietal and temporal bones yielded classification

accuracies of approximately 78% each, suggesting that there was indeed sufficient information available to estimate ancestry from any of these elements. The detection of shape differences of these bones among ancestry groups may have been overlooked in previous studies due to practical challenges in their assessment, such as lack of clear and quantifiable anatomical landmarks, the influence of size overshadowing shape differences, or because they have not been sufficiently investigated with a highly sensitive technique such as geometric morphometric analysis (Green & Curnoe, 2009; Bidmos *et al.,* 2010; Bigoni *et al.*, 2010).

5.6.2.2. Postcranial elements

For the postcranial elements, the pooled sample yielded ancestry classification accuracies of 73.6–85.3% (c.f. Table 4.7; Figure 4.57b). Despite the vast amount of studies examining variation of each of the long bones within specific population groups, few have assessed between-population differences in the morphology of these bones. Such studies might have been discouraged by the suggestion of researchers such as Brown *et al.* (2007) and Meeusen *et al.* (2015) that claim that the range of variation due to external influences like environmental conditions and physical activity is too large to allow confident ancestry estimations using the postcrania. The few studies which have examined population differences of the long bones report classification accuracies ranging from 70% to 90% (Saunders & Hoppa, 1997; Mall *et al.*, 2001; Sakaue, 2004; Tise *et al.*, 2013; Özer *et al.*, 2014), which was in agreement with the accuracies achieved in the present study.

The humerus yielded the highest ancestry classification accuracy of the postcranial elements, with accuracies of 84–87.2% (c.f. Table 4.7; Figure 4.57b). Several metric studies also report on ancestry-related differences in the size of the humerus and especially in its angle of retroversion (Jantz & Jantz, 1999; Shah *et al.,* 2006; Vance *et al.,* 2011). Overall, the postcranial elements performed well in ancestry estimation with each element having an average accuracy above 70%.

5.6.2.3. Comparison of the classification accuracy among the ancestry groups

Based on cranial elements, White individuals yielded better classification accuracies than Black and Coloured individuals (c.f. Table 4.7, Figure 4.57a). This was possibly due to the greater degree of similarity of Black and Coloured individuals seen for

many of the elements in this study (as reflected in the smaller Mahalanobis distances between these groups). This, in turn, is likely as a result of the greater genetic contribution and closer similarity of pre-democracy living conditions of Black groups to the highly heterogeneous Coloured groups, compared to the relative homogeneity of White South Africans (Jacobson *et al.,* 2004; Adhikari, 2005; Tishkoff *et al.,* 2009; De Wit *et al.,* 2012; L'Abbé *et al.,* 2013; Stull *et al.,* 2014; Liebenberg *et al.,* 2015b). Though less obvious, the opposite was true for the postcranial elements, where Black and Coloured individuals tended to yield higher classification accuracies than White individuals (c.f. Table 4.7, Figures 4.57b). This may suggest that the environmental differences in labour, health and nutrition may have played a bigger role in the shape of the postcrania (Brown *et al.,* 2007; Meeusen *et al.,* 2015). These influences may have a greater effect on Black and Coloured individuals than White individuals, making the shape of their long bones more distinguishable and producing higher classification accuracies (Jantz & Jantz, 1999; Jacobson *et al.,* 2004; Adhikari, 2005; Liebenberg *et al.,* 2015a).

It is also important to note since Coloured individuals can also be distinguished with relatively high accuracy from both Black and White groups, despite the great genetic admixture present in this group (Tishkoff *et al.,* 2009; De Wit *et al.,* 2010), they are not merely a mixture of the contributing Black and White groups. Instead, Coloured individuals can be seen as a unique group but with high within-group variation of cranial shape, as suggested by Petrus & Isaacs-Martin *et al.* (2012) and Stull *et al.* (2014). It is, however, important to acknowledge that the use of the three-group classifications used in the skeletal collections (and thus this study) may have to some extent obscured some potential variation between groups, as these classifications often do not take into account the considerable gene flow that may have taken place between groups (Morris, 2010). The classification accuracies achieved in the present study, even for the complex Coloured ancestry group, illustrate the sensitivity with which the geometric morphometric approach is capable of detecting differences between groups where previous metric and nonmetric approaches may have been unable to. This study also shows that there is considerable variation in the morphology of postcranial elements that can be used to objectively assess and reliably classify individuals into one of the three major ancestry groups in South Africa.

5.6.3. Sex-ancestry estimation

The calculated Mahalanobis distances between the sex-ancestry groups showed that individuals tended to be more similar to each other within ancestry groups, and that the differences between sexes were larger than those within. This was in agreement with the relationships observed between individuals when assessed according to ancestry independent of sex. Black and White individuals tended to be more similar in skeletal morphology to Coloured individuals than to each other. However, Black individuals often were relatively more similar to Coloured individuals than White individuals were to Coloured individuals. This again reflected the different contributions of these groups to the genetic history of the Coloured group (Jacobson *et al.,* 2004; Tishkoff *et al.,* 2009; De Wit *et al.,* 2012; Stull *et al.,* 2014).

DFA of the skeletal elements yielded classification accuracies of 70.6–83.3% for the pooled sample when estimating sex and ancestry combined (c.f. Table 4.9; Figures 4.58a - c). The general trends observed in these classification accuracies were that females tended to have slightly higher accuracies than the males within the same ancestry group, and Black females and males tended to have higher classification accuracies than the corresponding groups of White females and males, followed by Coloured females and males which often had lower accuracies than the other groups. The relatively higher accuracies of the Black and White groups were likely a reflection of the substantial differences in morphology among these groups, as determined by their different genetic and social histories (L'Abbé *et al.,* 2013), allowing individuals of these two groups to be classified with higher accuracy than the Coloured individuals. As seen when ancestry was observed independent of sex, the Coloured group has historical genetic contribution from both the Black and White ancestral groups, which explains the shared morphology of these individuals with those of the Black and White individuals in the present study. This, in turn, explains the relatively low classification accuracy achieved for Coloured individuals.

The difference in classification accuracy between the sexes within ancestry groups, similar to the differences observed when sexes were assessed independent of ancestry, suggested that females tended to have more distinctive skeletal morphologies, possibly due to the confounding influences of nutritional and activity-related differences, which are expected to affect males more than females, thus

making their morphologies more variable (Greulich, 1976; Wolanski & Kasprzak, 1976; Stinson, 1985). It is, however, important to note that, despite the good classification accuracies achieved for most of the skeletal elements in the present study, there was still substantial overlap in the morphologies of the sex-ancestry groups, especially that of the Black and White groups with the Coloured group. This may have resulted in some groups having significantly lower accuracies than the other sex-ancestry groups, as was seen for the classification based on the shape of the tibia (without the tuberosity) of Coloured females which was only 51.3% (c.f. Figure 4.58c). Despite these few exceptions, the overall classification accuracies achieved in the present study were comparable to those of previous studies across different populations under varied nutritional conditions (Franklin *et al.,* 2005; Suazo *et al.,* 2009; Devi *et al.,* 2013; Vance & Steyn, 2013; Garvin *et al.,* 2014).

The overall performance of the individual elements examined according to sex and ancestry were similar to those observed when either parameter was investigated independently. The frontal bone was found to be the best element overall at sex-ancestry classification, with accuracies of 76.6–86.8% (c.f. Table 4.9, Figure 4.58a). This again reflected the good representation of sex- and ancestry-related variation in the shape of the frontal bone. The other cranial elements all yielded accuracies over 70%, which indicated that each element could be used to obtain information when both sex and ancestry were considered together.

Of the postcranial elements, the humerus had the highest classification accuracy of sex and ancestry together, with accuracies of 76.9–85.5%, when compared to the other postcranial elements which, with the exception of a few groups, failed to yield accuracies higher than 80% (c.f. Table 4.9, Figures 4.58b and c). The femur was the second best postcranial element, with accuracies of 72.4–87.2%. These results were similar to those seen when observing the accuracy of these elements for sex and ancestry independently. When sex was considered alone, the classification accuracies achieved were lower than when ancestry was included for consideration. Some elements failed to separate sexes on their own, but once combined with ancestry, sufficient variation was detected to allow the distinction of groups. The opposite was true when ancestry was considered alone, as the classification accuracies per element were slightly reduced when ancestry was combined with sex. This suggested that

ancestry-related factors such as genetic composition and environmental differences among ancestral groups played a bigger role in the shape of the elements studied, while sex-related factors such as physical activity and differential development may have been more likely to affect size rather than shape (Van Gerven, 1972; Green & Curnoe, 2009). These results again highlighted the need for population-specific (both temporally and geographically) methods of sex and ancestry estimations, since the overlap between sexes of different ancestry groups may be considerably large and may lead to misclassification of unknown individuals.

Comparison of the classification accuracies of the six sex-ancestry groups showed that, despite considerable variation, Black females and White males tended to achieve the highest classification accuracies, while Coloured males and females tended to have the lowest. This again reiterates the large amount of overlap among sex-ancestry groups, showing that, for example, females of one ancestry group may have similar morphology to males of another ancestry group, thus reducing the accuracy of the classification of sex and ancestry. Since Black females and White males were most distinguishable from each other and the other groups, as seen in these groups having the greatest Mahalanobis distances for several of the elements examined in this study (Appendix D – Table D5a – l), they were able to achieve high accuracies, while the Coloured groups, which share great similarities in genetics and in influences such as living conditions with the other groups, were more difficult to distinguish. However, the high sensitivity of the geometric morphometric approach used in this study allowed the detection of significant variation between these closely-related groups so that they could be separated with a relatively good amount of confidence, which was not possible in many previous metric studies (Tobias, 1990; Thompson, 2001; Jones *et al.,* 2009; Hawley *et al.,* 2009). These results suggest that the geometric morphometric approach can be used to supplement current assessment methods and provide objective and reliable evaluations of skeletal elements to help estimate demographic parameters such as sex and ancestry.

CHAPTER 6

CONCLUSION

Skeletal morphological variation of South African individuals has been widely examined using metric and non-metric study approaches. These approaches are, however, often subject to high error rates or reduced sensitivity, resulting in poor accuracy in estimations of demographic parameters. This becomes especially problematic when only certain skeletal elements are available for analysis, as is often the case in forensic analyses.

The present study evaluated the morphology of the individual skeletal elements of the neurocranium and long bones of 1132 South African adults using a three-dimensional geometric morphometric approach. It was found that this approach is ideally suited to allow the exploration of variation between sexes and ancestry groups, as the manifestation of variation in terms of size and shape could be independently assessed. As a result of the isolation of size from the data, this approach was able to detect shape differences with more sensitivity than previous metric approaches.

This study has shown that the sexual dimorphism of the features of the examined skeletal elements manifest not only in size differences, but also in shape differences, reflecting the differences in secondary sexual development between the sexes. This suggests that even when individuals are of similar size, the differences in the morphology of these elements could still be used to distinguish between the sexes. This is especially true for elements such as the femur, which are greatly influenced by adaptations to their function in locomotion and reproduction, which often manifest more in shape than size differences between the sexes.

The three largest ancestry groups in the South African population, Black, White and Coloured, were also compared. Again, the geometric morphometric approach allowed evaluation of the variation within and between these groups, elucidating information regarding the complex interaction of the intrinsic (genetic) differences between these groups, and the strong influences of extrinsic factors (such as nutrition, health,

activity) which have historically been imposed on these groups as a result of the Apartheid legislation. It was shown that White individuals are larger and more robust than both Black and Coloured individuals. While this may be due to differences in genetic histories among these groups, it may also be a reflection of the better health, nutrition and living conditions of this group compared to those experienced by individuals of the other two groups. It was also noted that Coloured South Africans are not merely a mixture of the Black and White groups as commonly perceived, but that they have unique skeletal morphologies which allow them to be distinguished from both these groups. This highlights the fact that morphology remains only an approximation of ancestry, and due to the complexity of the relationship between genotype and phenotype, should still be interpreted with caution.

The potential co-variation of the observed morphology with factors such as size, age and temporal trends was found to be weak but significant in the present study sample. The results suggest that an increase in the size of the cranial elements shows little, if any, associated change in morphology. The postcranial elements tend to have relatively wider medio-lateral dimensions with increasing size, likely as a result of greater muscle strains acting on these elements in larger individuals. It was observed that the skeletal elements tended to become larger and more robust with increasing age. This is likely as a result of appositional bone growth under the influence of hormonal changes occurring later in life. Lastly, a weak increase in the size and robusticity of the skeletal elements was observed for individuals living in the later part of the century. This potentially serves as some indication of the improvements in living and health conditions in the country, and especially for the Black and Coloured groups after the abolishment of Apartheid. These results highlight the importance of the consideration of fluctuating extrinsic influences on the manifestation of skeletal morphology variation, even within the South African population, and can probably be expected to become an even more important consideration with the great changes which have begun to take place in South Africa over the last few years.

The last step of the present study was to evaluate the accuracy with which the demographic parameters sex and ancestry (or the combination of sex and ancestry) could be classified using the detected morphological variation in the sample. The results showed that all of the elements examined in this study yield average

classification accuracies of over 70%, with the frontal bone and humerus performing best overall with over 80% accuracies. This showed that potentially forensically useful information could be obtained from various skeletal elements when assessed using geometric morphometric analyses, even when using limited numbers of landmarks to represent the shape of the skeletal element in question. It is expected that future studies may be able to yield even higher classification accuracies when using more landmarks for this purpose. It would also be valuable to use this approach to further explore other skeletal elements which may potentially be used in a forensic context.

This study has provided a unique quantitative evaluation of the morphology of the neurocranium and long bones of South African adults and has shown how these morphologies may vary among demographic groups. The study also showed that these variations could be reliably used to assess certain demographic parameters of skeletal remains of individuals for whom sex and ancestry are unknown through comparison of the unknown individual to the known data of the present study. This approach would provide a sensitive, objective and reliable addition to the forensic anthropologist‘s construction of the biological profile of the unknown individual which is commonly based on more subjective metric or non-metric methodologies. The data gathered in this study may also be further explored by comparing different parts of a skeletal element (for example, proximal versus distal epiphyses). This would allow the application of this method to skeletal remains that may be damaged or incomplete, as is often the case in the forensic context.

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APPENDIX A

Table A1: Between-group Procrustes chord distances between intra- and inter-observer repeats.

		Intra-observer				Inter-observer			
		Max. difference between specimens	**Max. difference between repeats**	**Ave. difference between repeats**	**Error (%)**	**Max. difference between specimens**	**Max. difference between repeats**	**Ave. difference between repeats**	**Error (%)**
Cranium		0.151	0.020	0.006	4.10	0.137	0.020	0.007	4.88
Upper limb									
Humerus	Left	0.067	0.006	0.003	4.94	0.056	0.005	0.002	4.42
	Right	0.078	0.010	0.004	4.91	0.056	0.007	0.002	3.96
Radius	Left	0.074	0.005	0.004	4.89	0.053	0.004	0.002	4.64
	Right	0.073	0.005	0.004	4.85	0.040	0.003	0.002	4.75
Ulna	Left	0.095	0.006	0.004	3.74	0.048	0.005	0.002	4.89
	Right	0.089	0.006	0.003	3.82	0.043	0.004	0.002	4.89
Lower limb									
Femur	Left	0.064	0.014	0.003	4.61	0.057	0.010	0.002	3.77
	Right	0.069	0.006	0.003	4.40	0.056	0.005	0.002	3.58
Tibia	Left	0.080	0.012	0.004	4.72	0.055	0.007	0.002	3.94
	Right	0.081	0.011	0.003	4.32	0.044	0.004	0.002	4.46
Fibula	Left	0.050	0.006	0.002	4.76	0.042	0.005	0.002	4.89
	Right	0.050	0.007	0.002	4.86	0.039	0.005	0.002	4.67

APPENDIX B

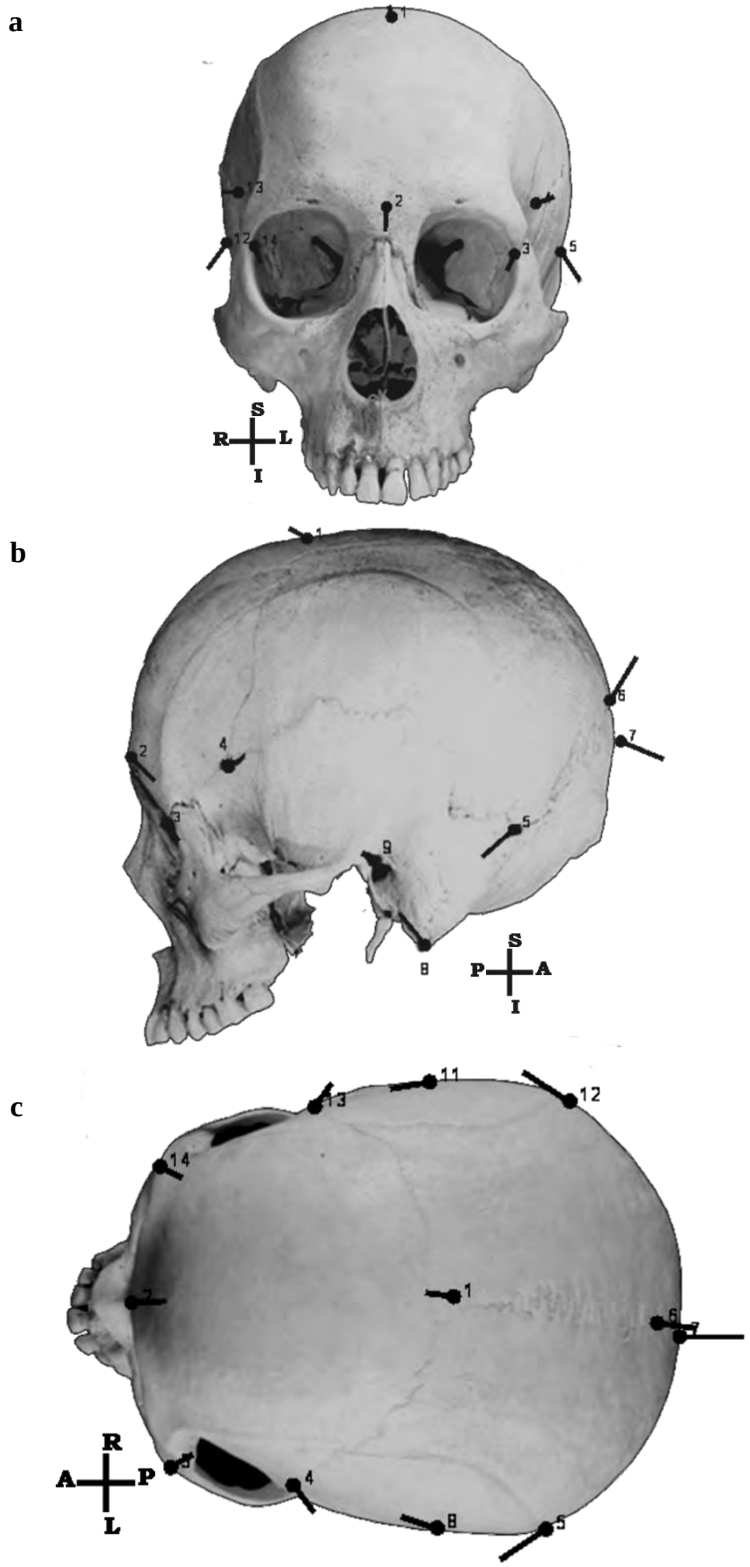

Figure B1: Whole cranial shape differences between the sexes (PC3) in a) anterior, b) lateral, and c) superior views. Vectors (―dllipops") represent the shape differences between mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.1A]

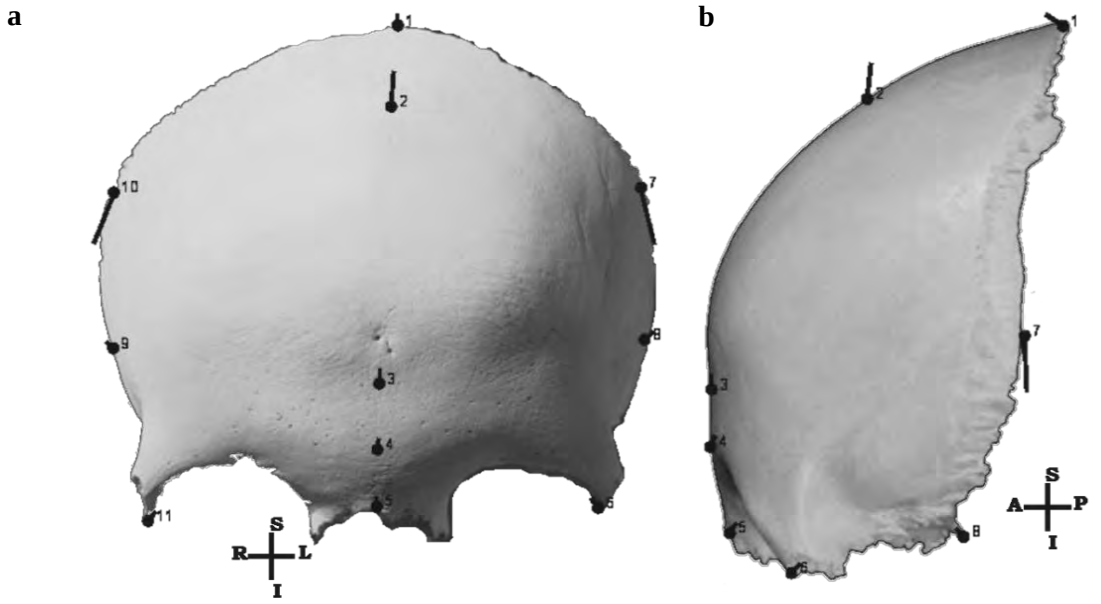

Figure B2: Frontal bone shape differences between the sexes (PC2) in a) anterior, and b) lateral views. Vectors (~~l~~ollipops") represent the shape differences between the mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.1B]

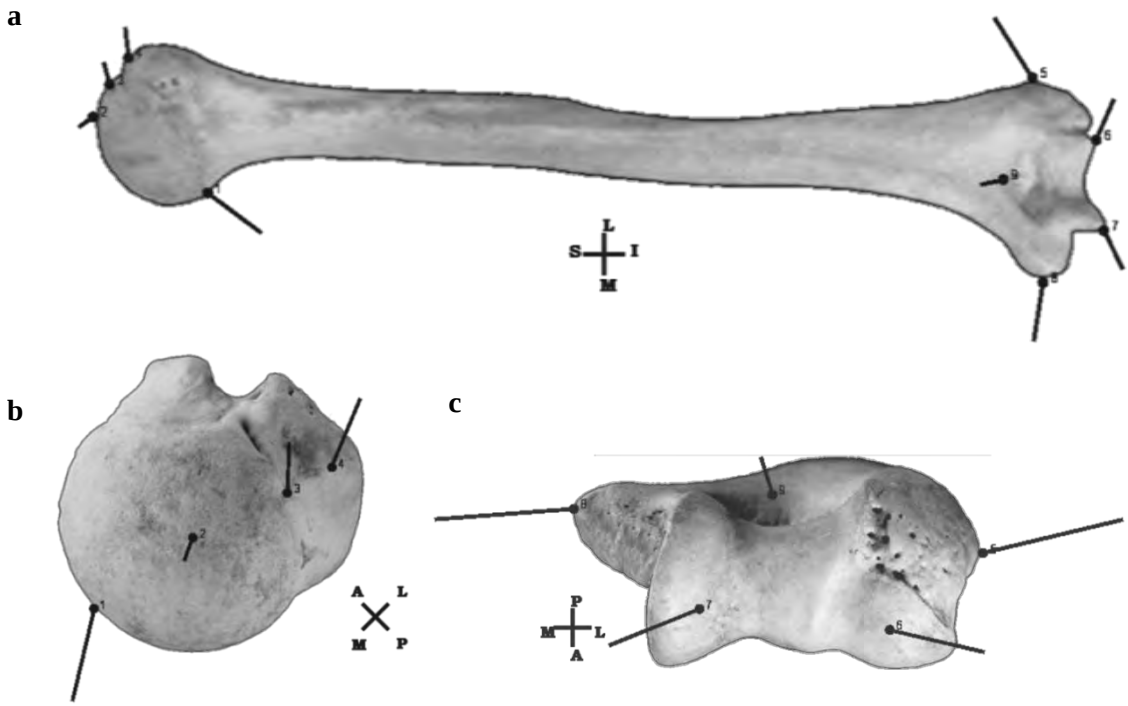

Figure B3: Humerus shape differences between the sexes (PC2) as viewed from the a) posterior, b) humeral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean female shape (dots) and mean male shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.2A]

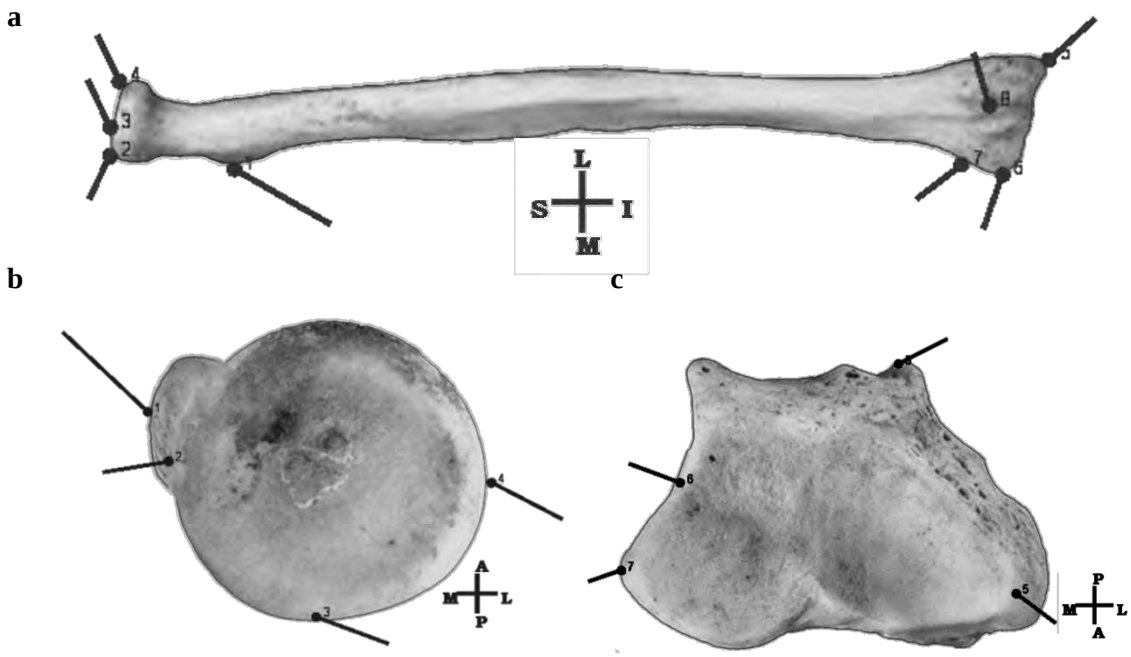

Figure B4: Radius shape differences between the sexes (PC1) as viewed from the a) posterior, b) radial head, and c) distal epiphysis. Vectors (―dllipops") represent the shape differences between the mean female shape (dots) and mean male shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.2B]

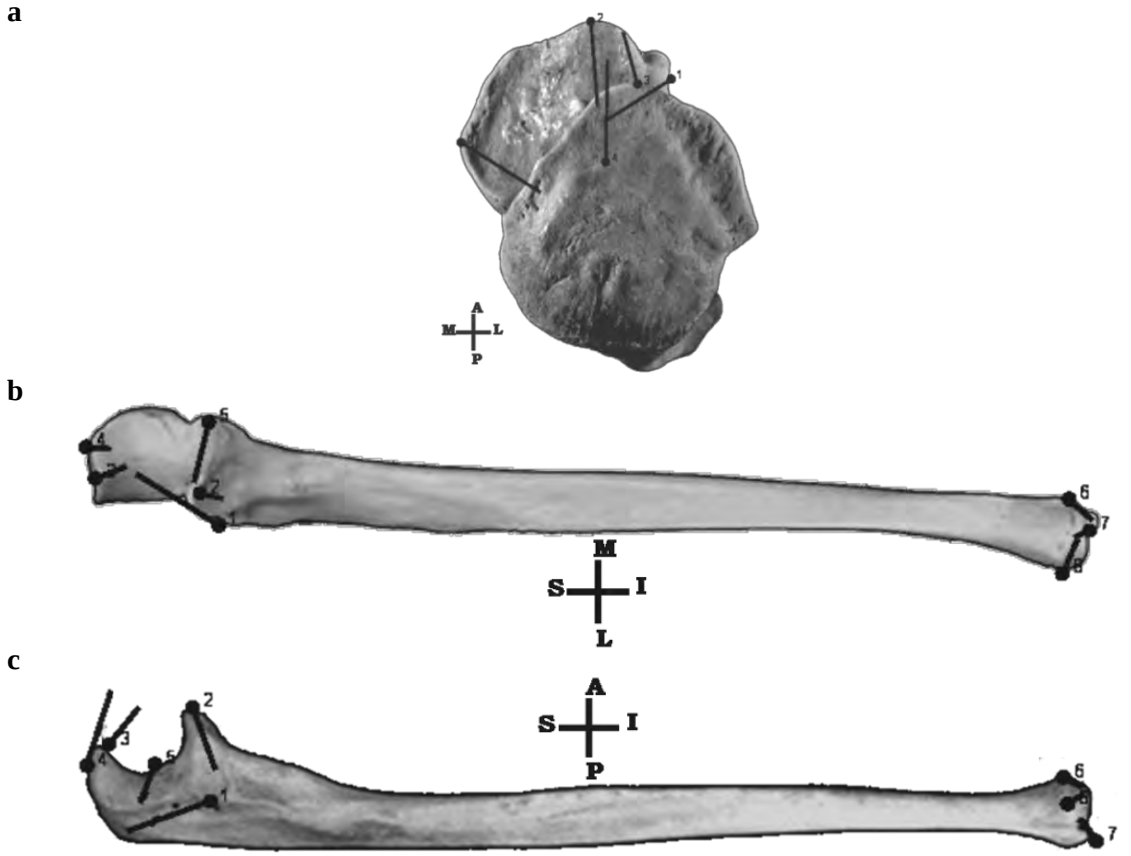

Figure B5: Ulna shape differences between the sexes (PC1) as viewed from the a) proximal epiphysis, b) anterior, and c) lateral sides. Vectors (―dllipops") represent the shape differences between the mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.2C]

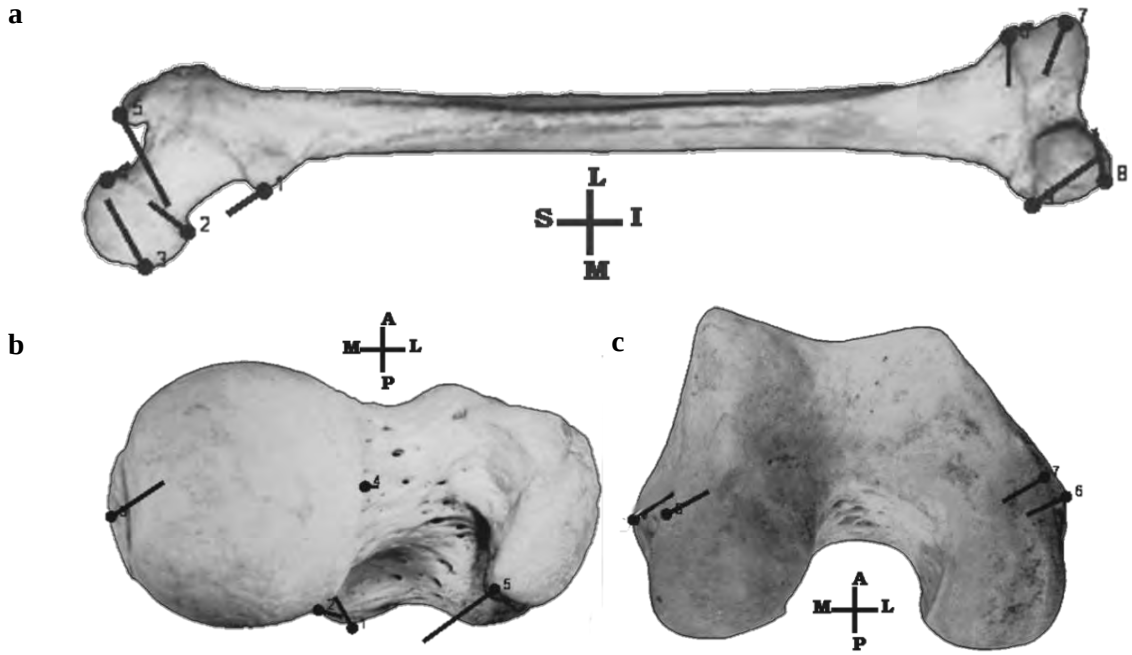

Figure B6: Femur shape differences between the sexes (PC1) as viewed from the a) posterior, b) femoral head, and c) distal epiphysis. Vectors (—dllipops") represent the shape differences between the mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.2D]

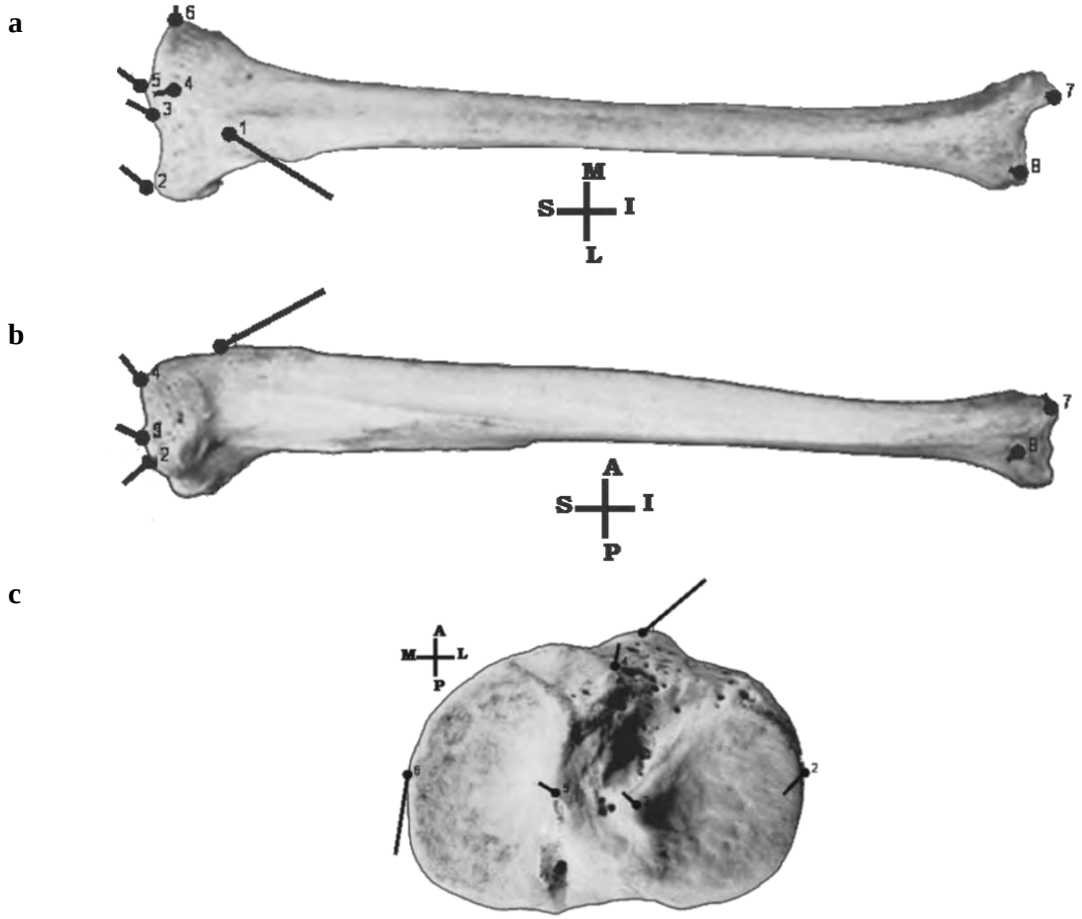

Figure B7: Tibia shape (including tuberosity) differences between the sexes (PC2) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (—llipops") represent the shape differences between the mean female shape (dots) and mean male shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

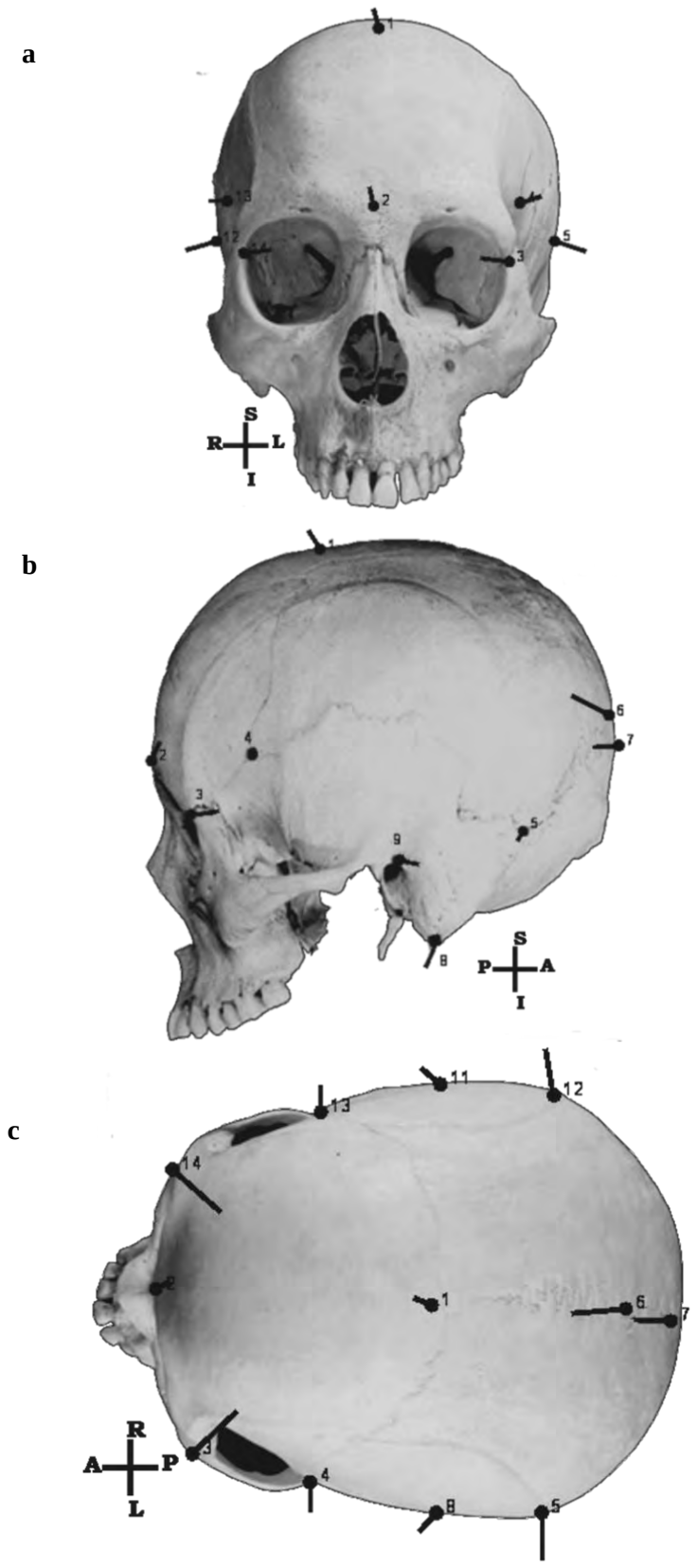

Figure B9: Whole cranial shape differences between ancestry groups (CV1) in a) anterior, b) lateral, and c) superior views. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1A]

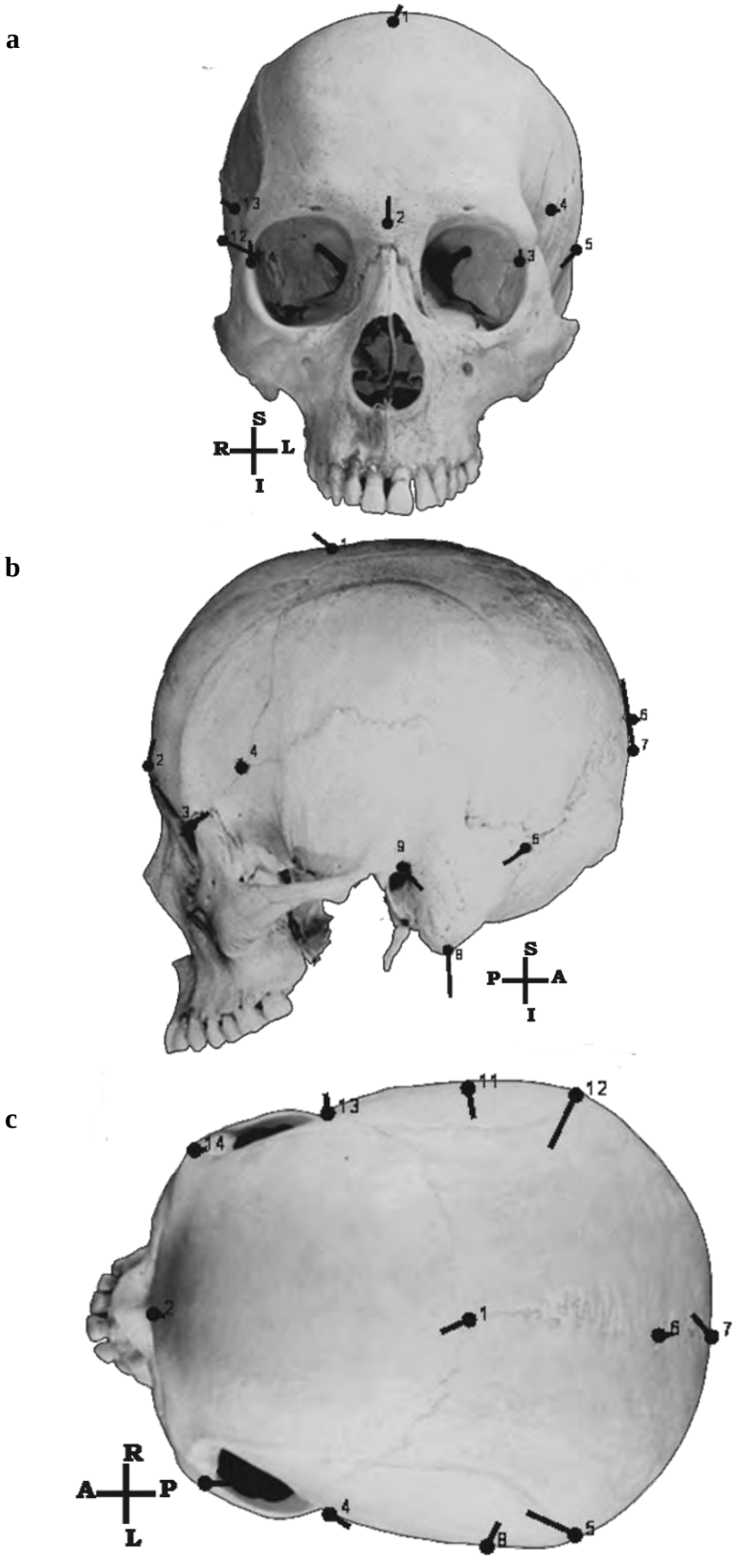

Figure B10: Whole cranial shape differences between ancestry groups (CV2) in a) anterior, b) lateral, and c) superior views. Vectors (―dllipops") represent the shape differences between the mean shape of Coloured individuals (dots) and mean shape of Black and White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1A]

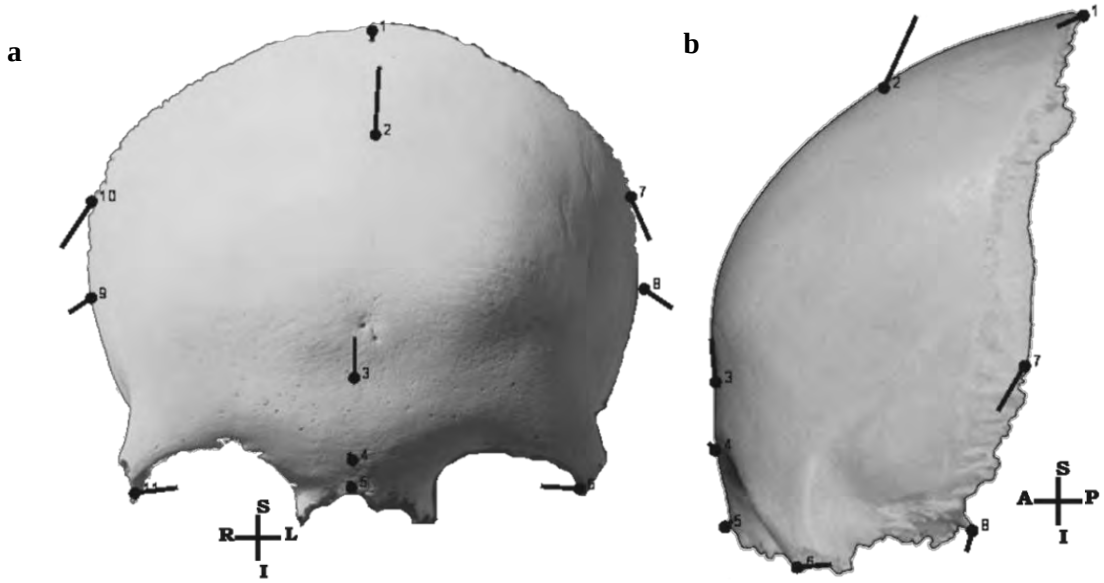

Figure B11: Frontal bone shape differences between ancestry groups (CV1) in a) anterior, and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1B]

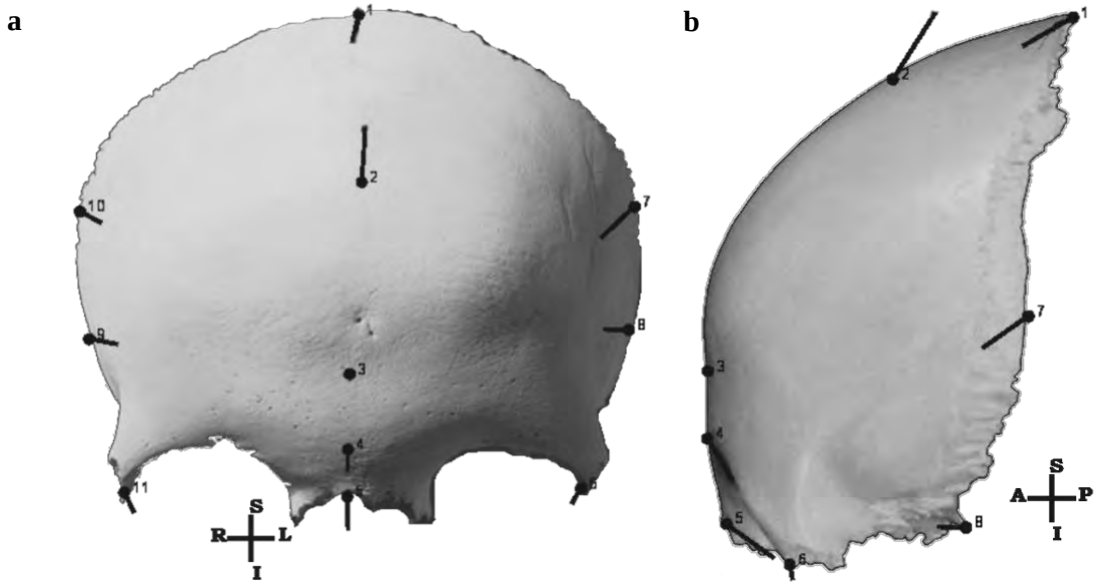

Figure B12: Frontal bone shape differences between ancestry groups (CV2) in a) anterior, and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of Black and White individuals (dots) and mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1B]

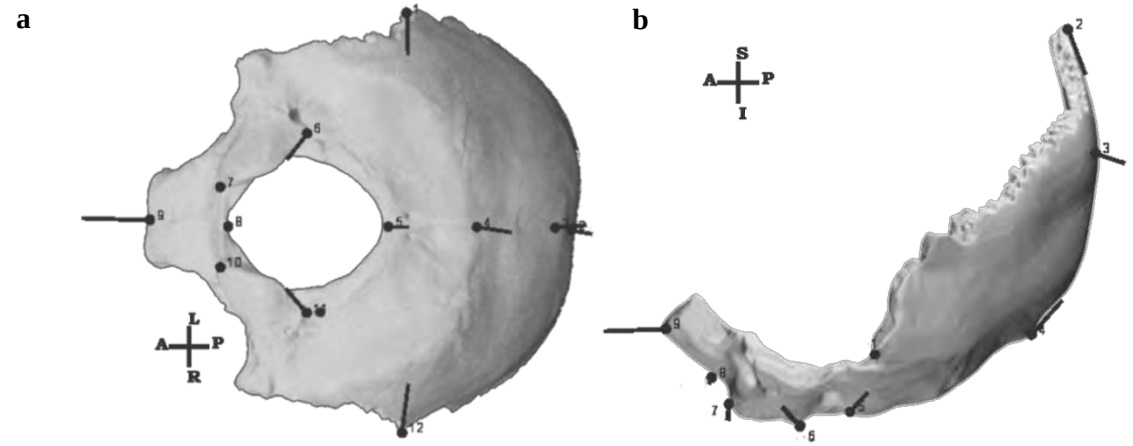

Figure B13: Occipital bone shape differences between ancestry groups (CV1) in a) inferior, and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1C]

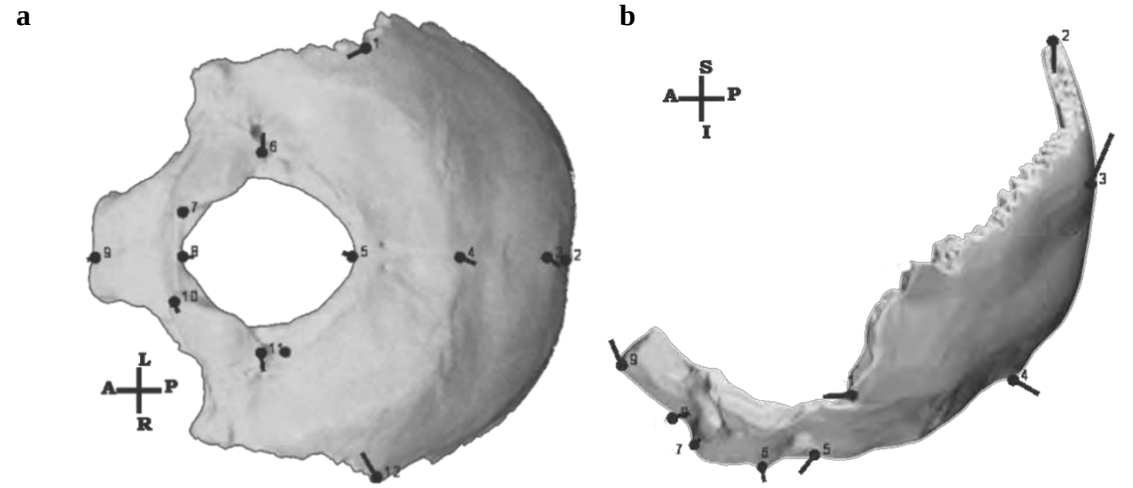

Figure B14: Occipital bone shape differences between ancestry groups (CV2) in a) inferior and b) lateral views. Vectors (―lollipops") represent the shape differences between the mean shape of Coloured individuals (dots) and mean shape of Black and White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1C]

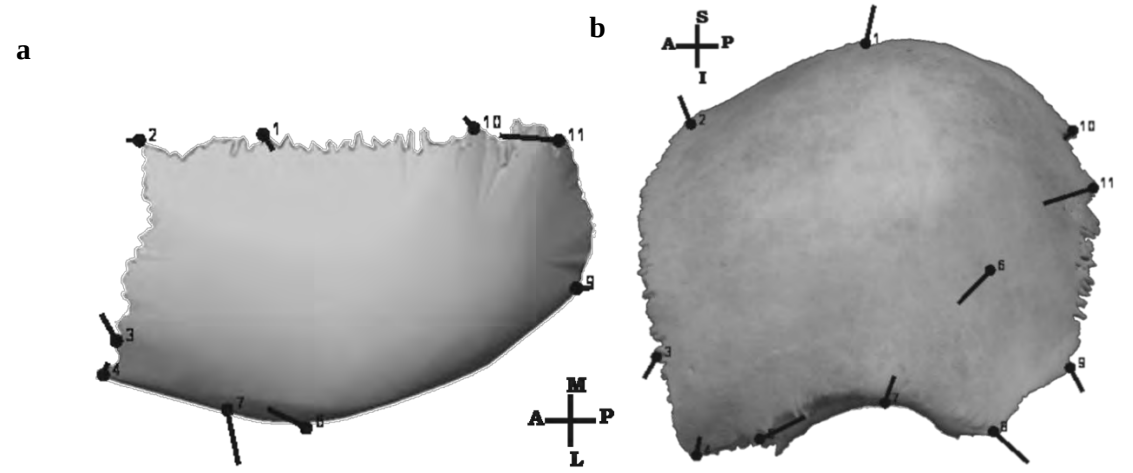

Figure B15: Parietal bone shape differences between ancestry groups (CV1) in a) superior and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1D]

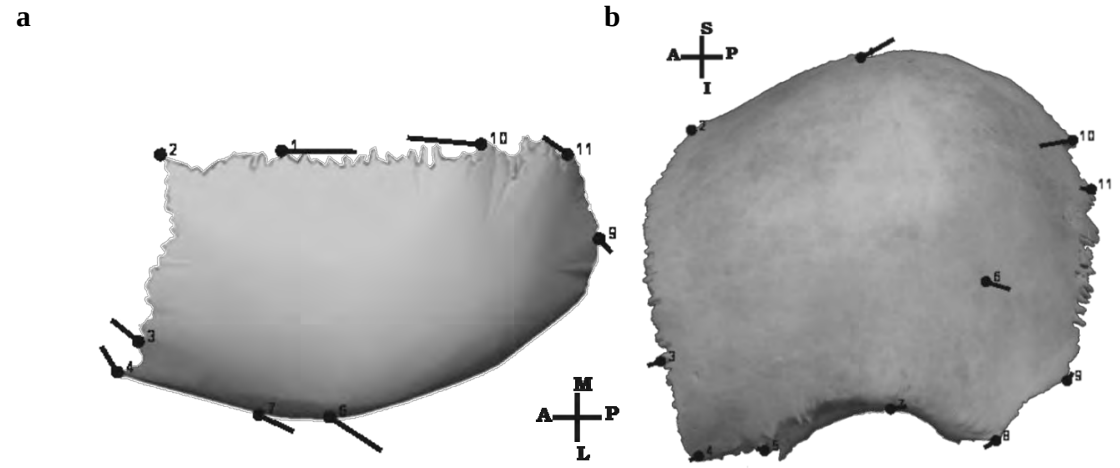

Figure B16: Parietal bone shape differences between ancestry groups (CV2) in a) superior and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of Black and White individuals (dots) and mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1D]

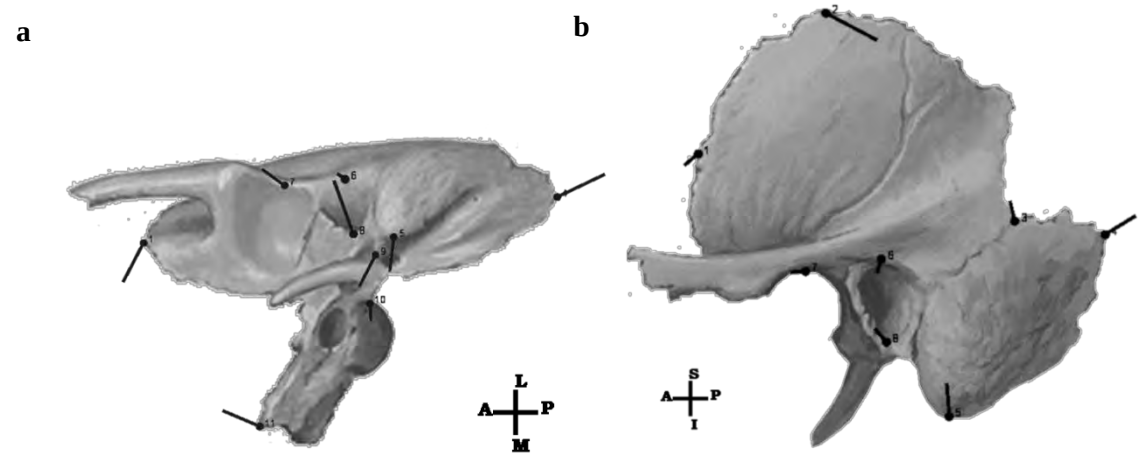

Figure B17: Temporal bone shape differences between ancestry groups (CV1) in a) inferior and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and mean shape of Black and Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1E]

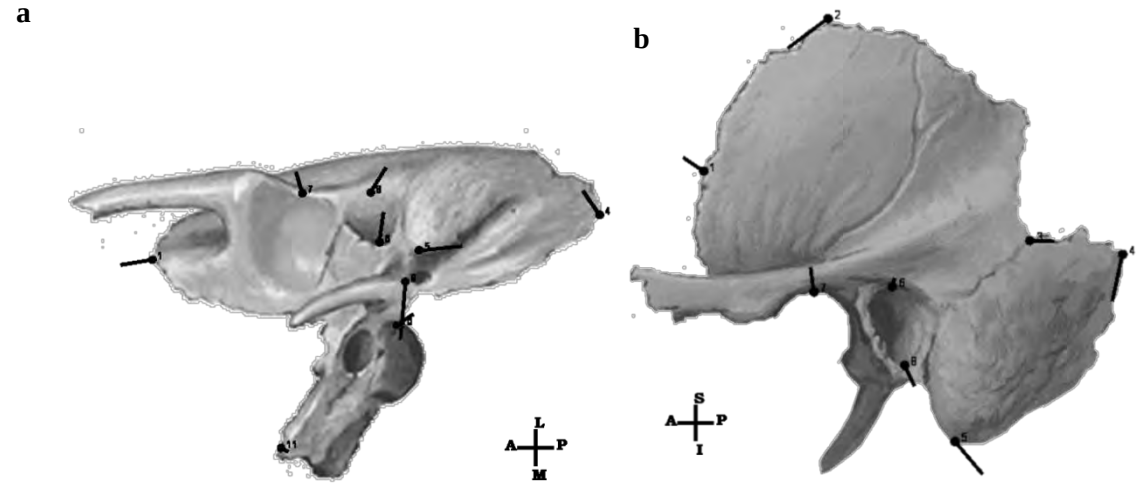

Figure B18: Temporal bone shape differences between ancestry groups (CV2) in a) lateral, and b) inferior views. Vectors (―lollipops") represent the shape differences between the mean shape of Coloured individuals (dots) and mean shape of Black and White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1E]

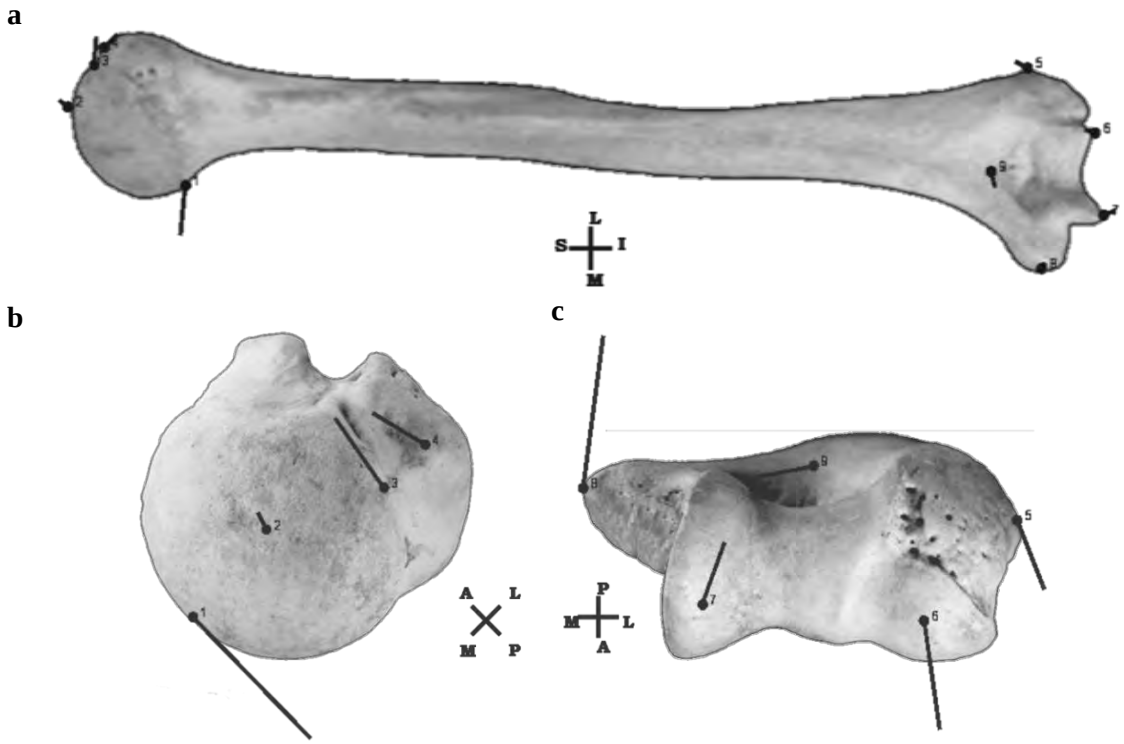

Figure B19: Humerus shape differences between ancestry groups (CV1) as viewed from the a) posterior, b) humeral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2A]

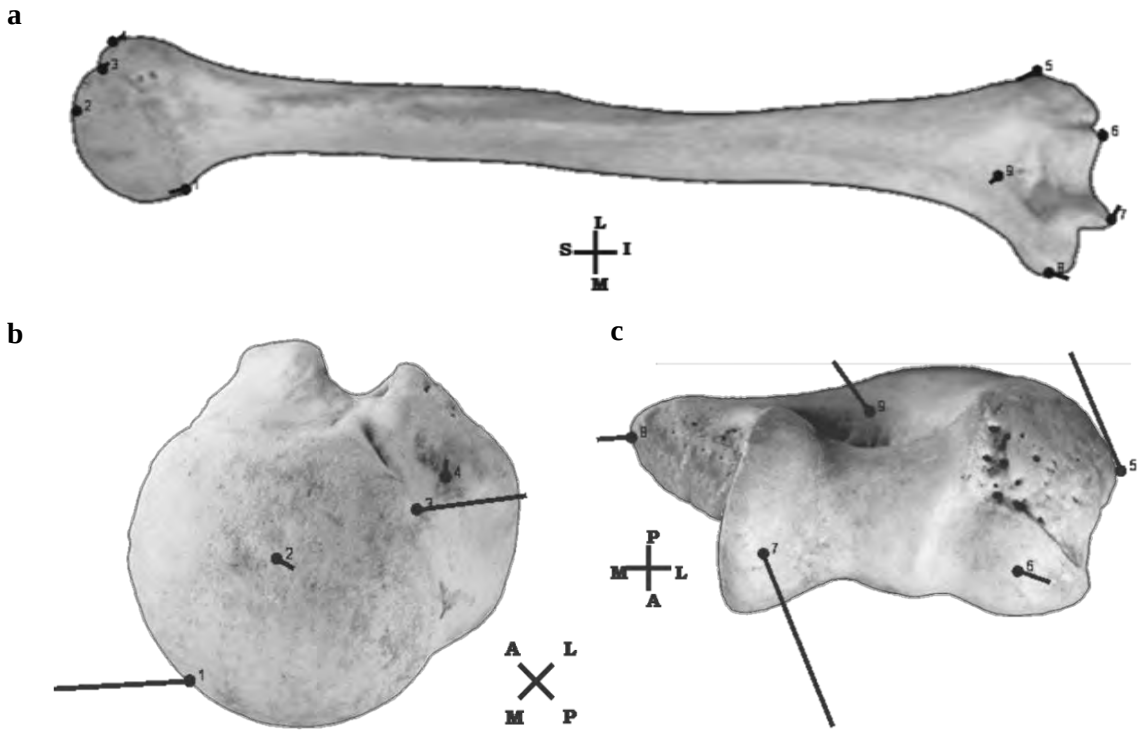

Figure B20: Humerus shape differences between ancestry groups (CV2) as viewed from the a) posterior, b) humeral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Coloured individuals (dots) and the mean shape of Black and White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2A]

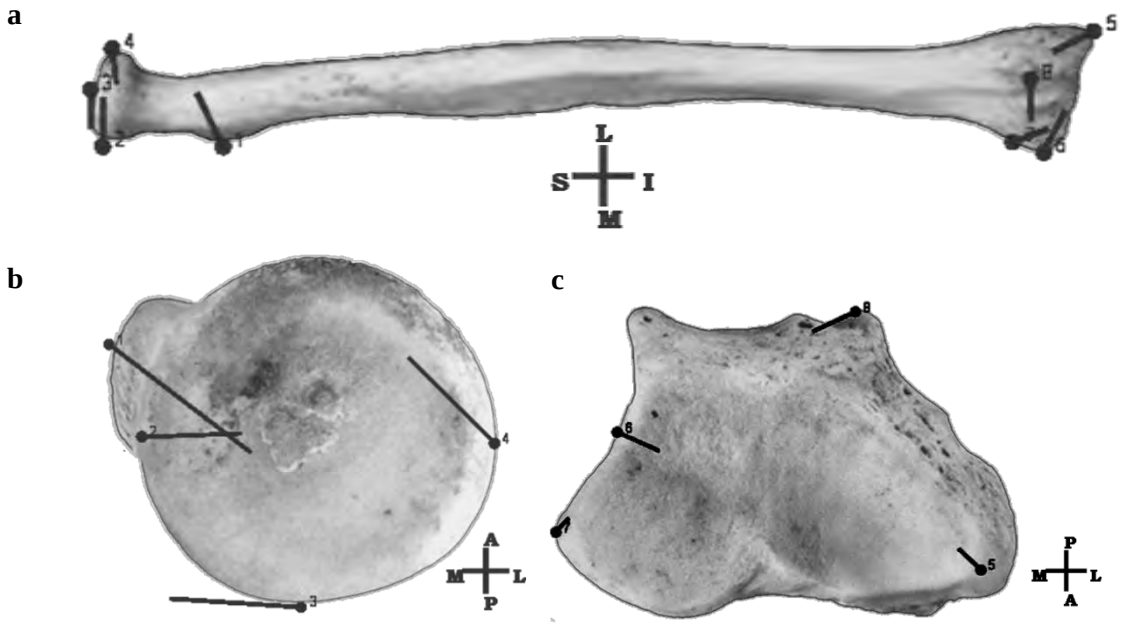

Figure B21: Radius shape differences between ancestry groups (CV1) as viewed from the a) posterior, b) radial head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2B]

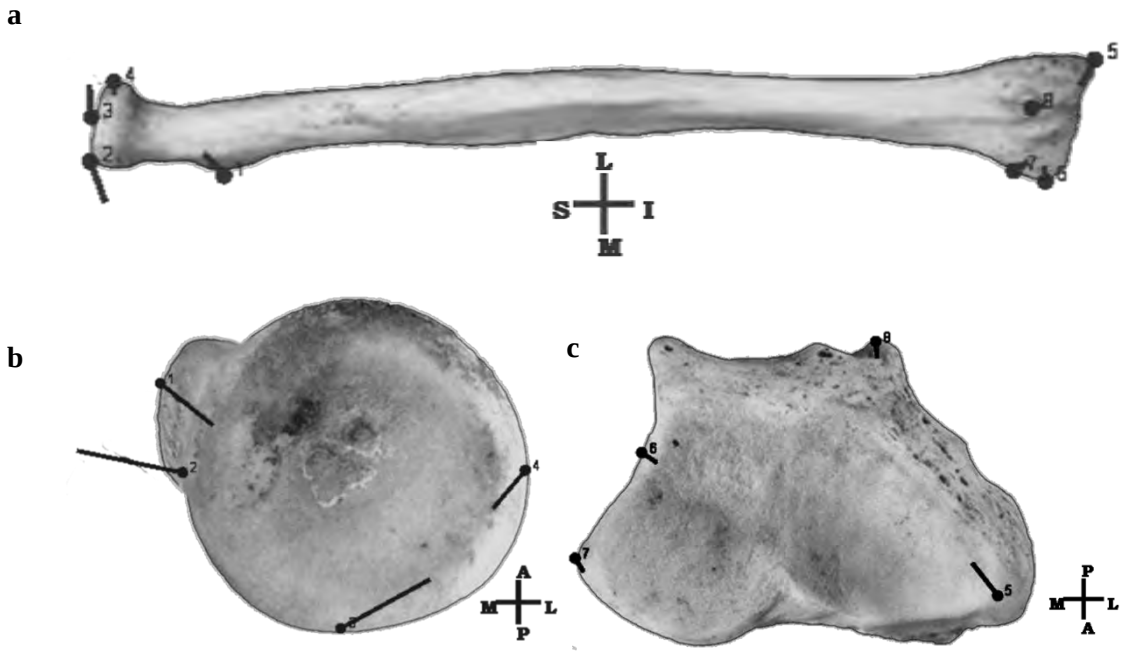

Figure B22: Radius shape differences between ancestry groups (CV2) as viewed from the a) posterior, b) radial head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black and White individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2B]

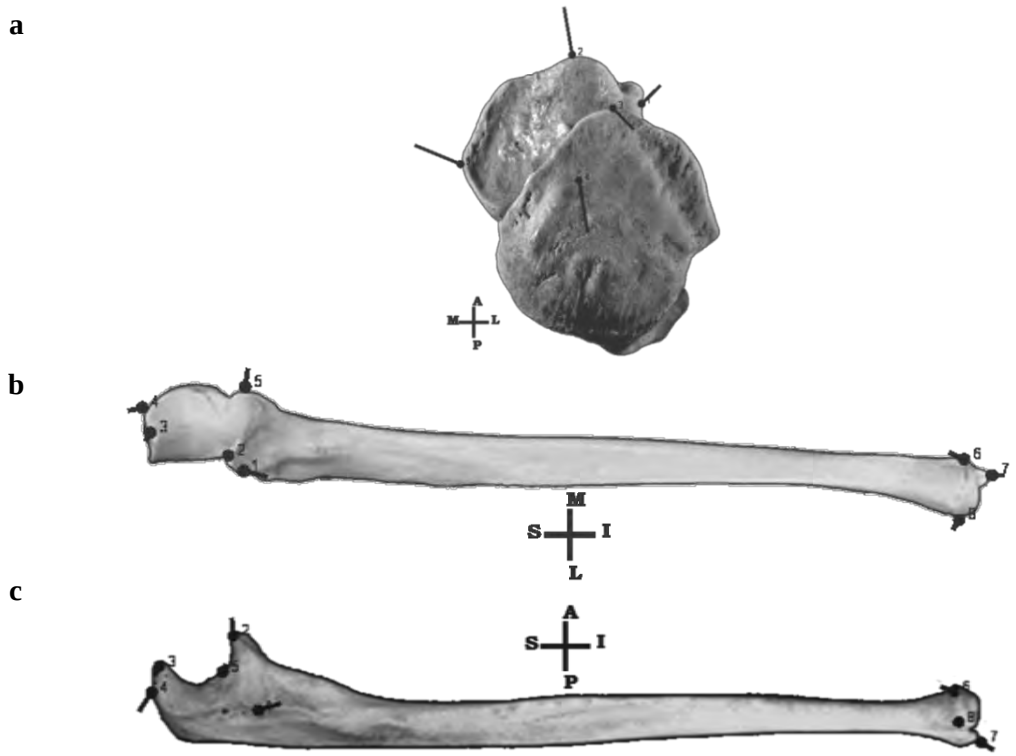

Figure B23: Ulna shape differences between ancestry groups (CV1) as viewed from the a) proximal epiphysis, b) anterior, and c) lateral sides. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2C]

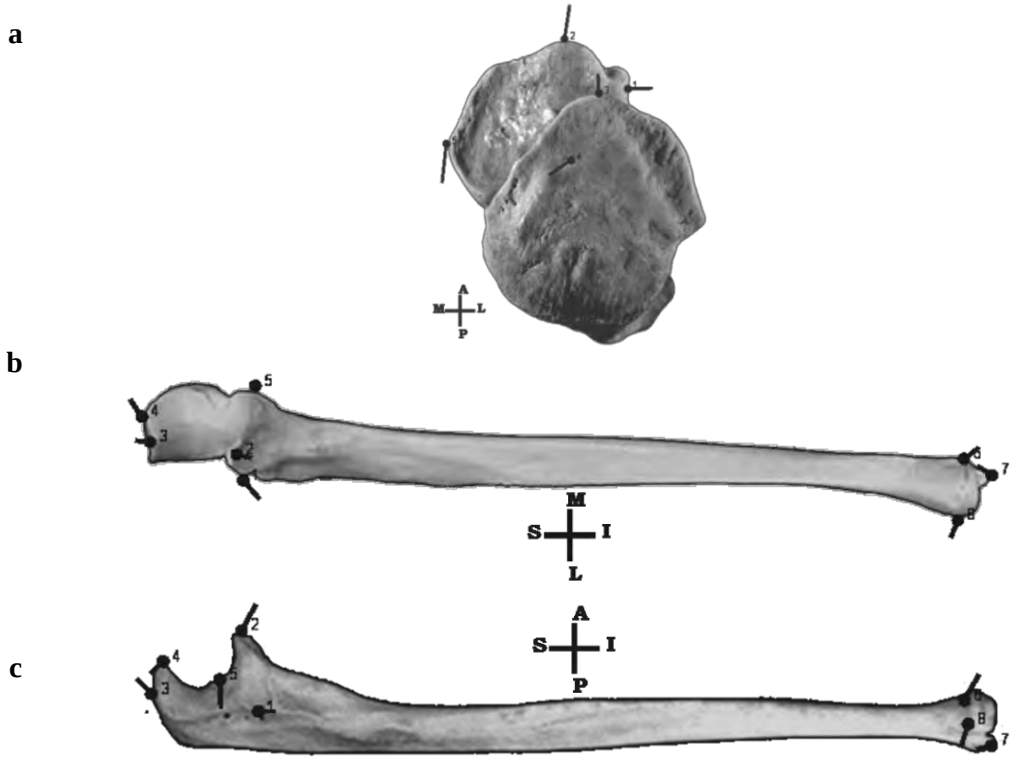

Figure B24: Ulna shape differences between ancestry groups (CV2) as viewed from the a) proximal epiphysis, b) anterior, and c) lateral sides. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black and White individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2C]

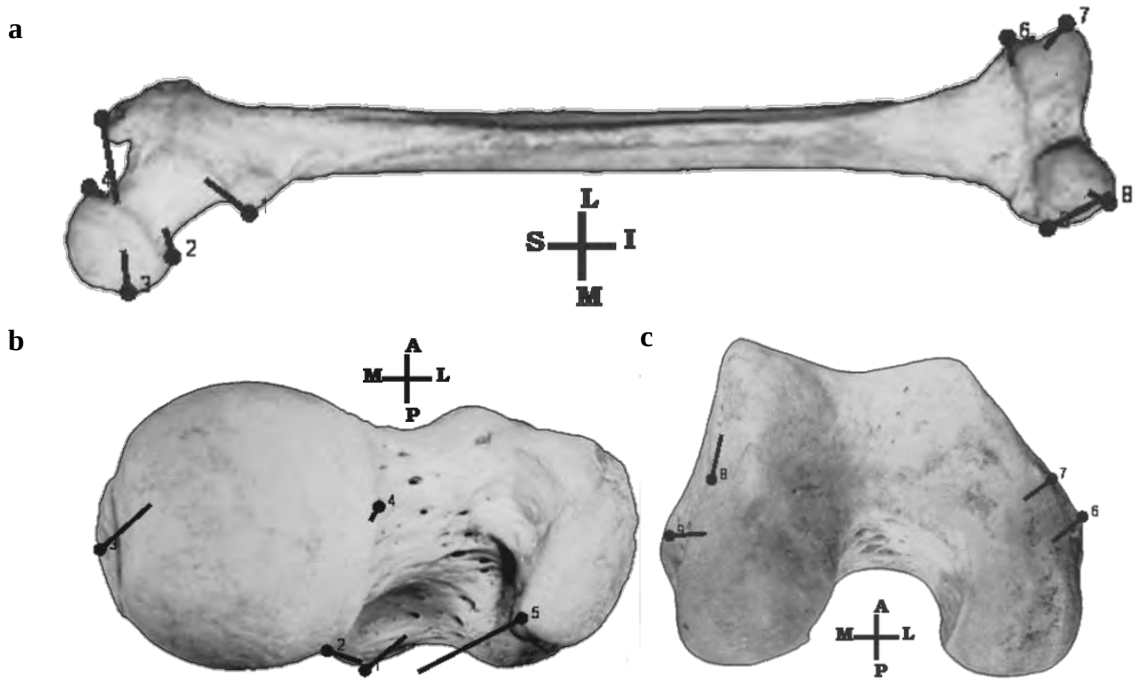

Figure B25: Femur shape differences between ancestry groups (CV1) as viewed from the a) posterior, b) femoral head, and c) distal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and the mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2D]

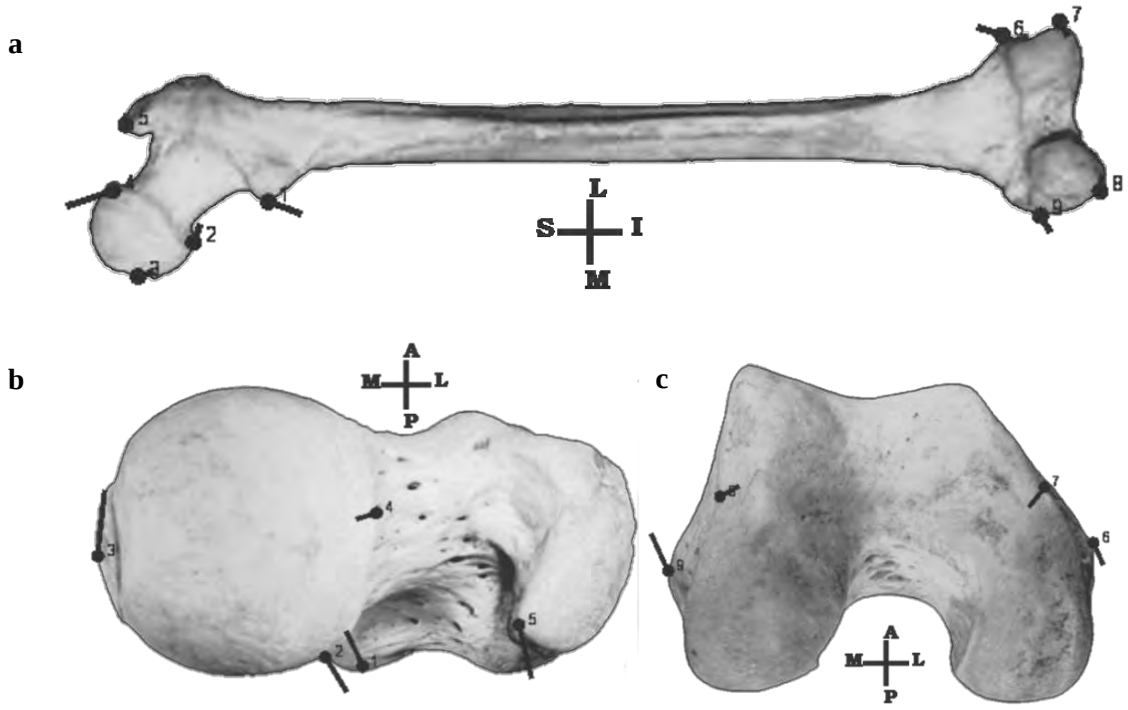

Figure B26: Femur shape differences between ancestry groups (CV2) as viewed from the a) posterior, b) femoral head, and c) distal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of Coloured individuals (dots) and the mean shape of Black and White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2D]

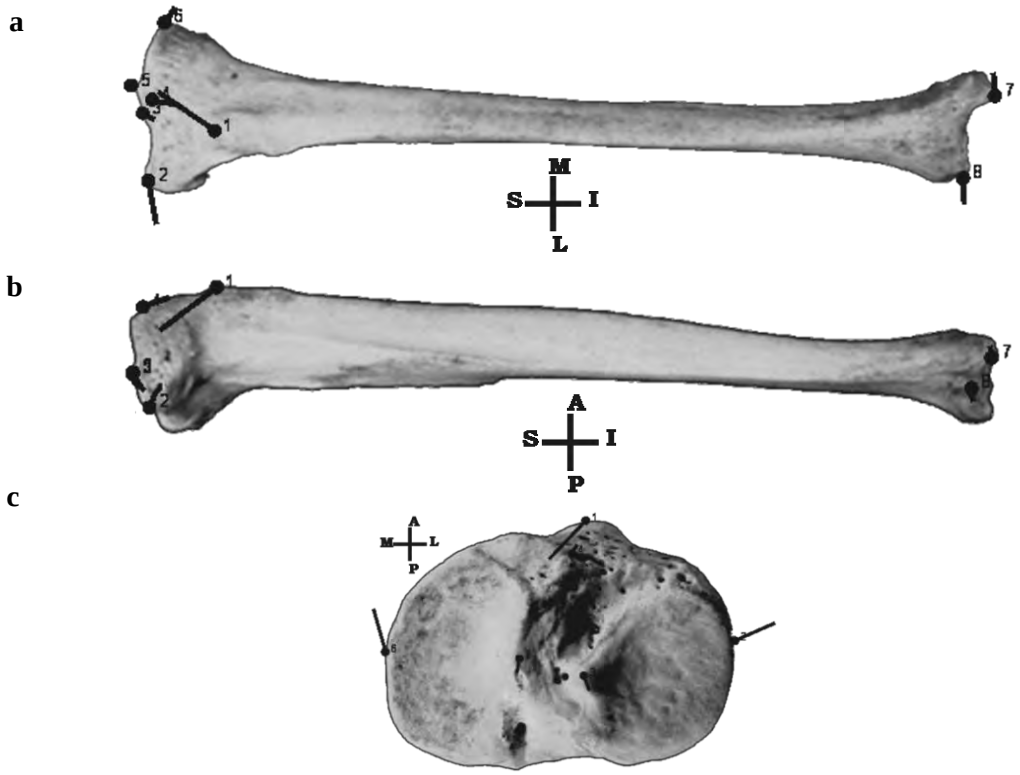

Figure B27: Tibia shape (including tuberosity) differences between ancestry groups (CV1) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

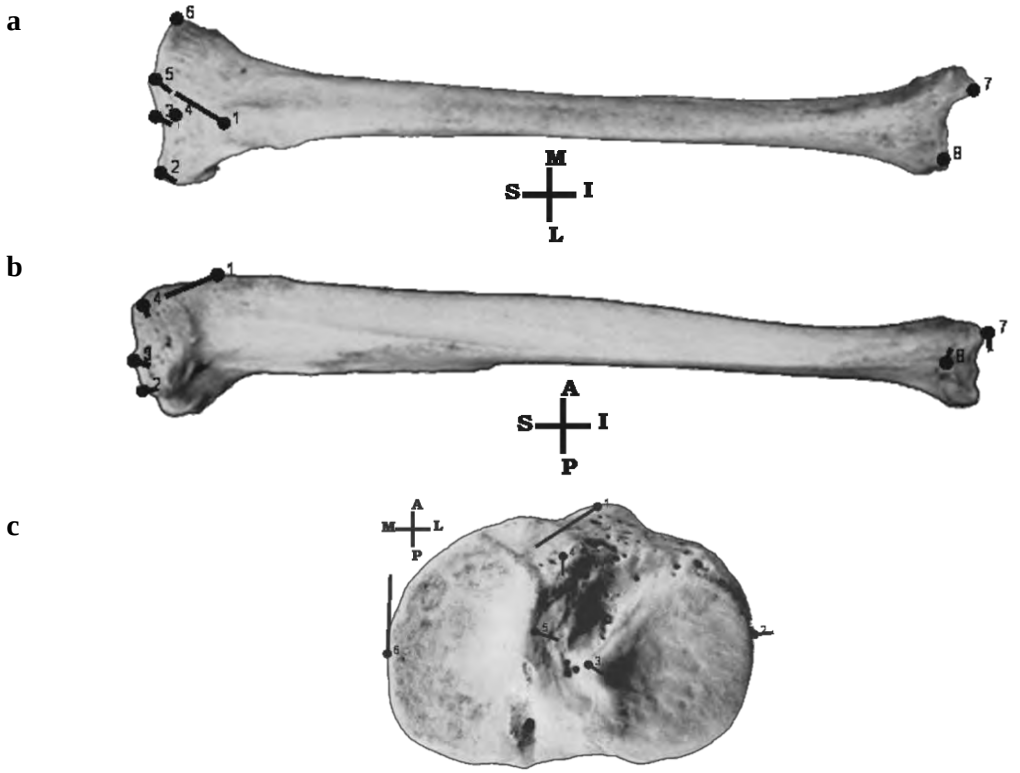

Figure B28: Tibia shape (including tuberosity) differences between ancestry groups (CV2) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

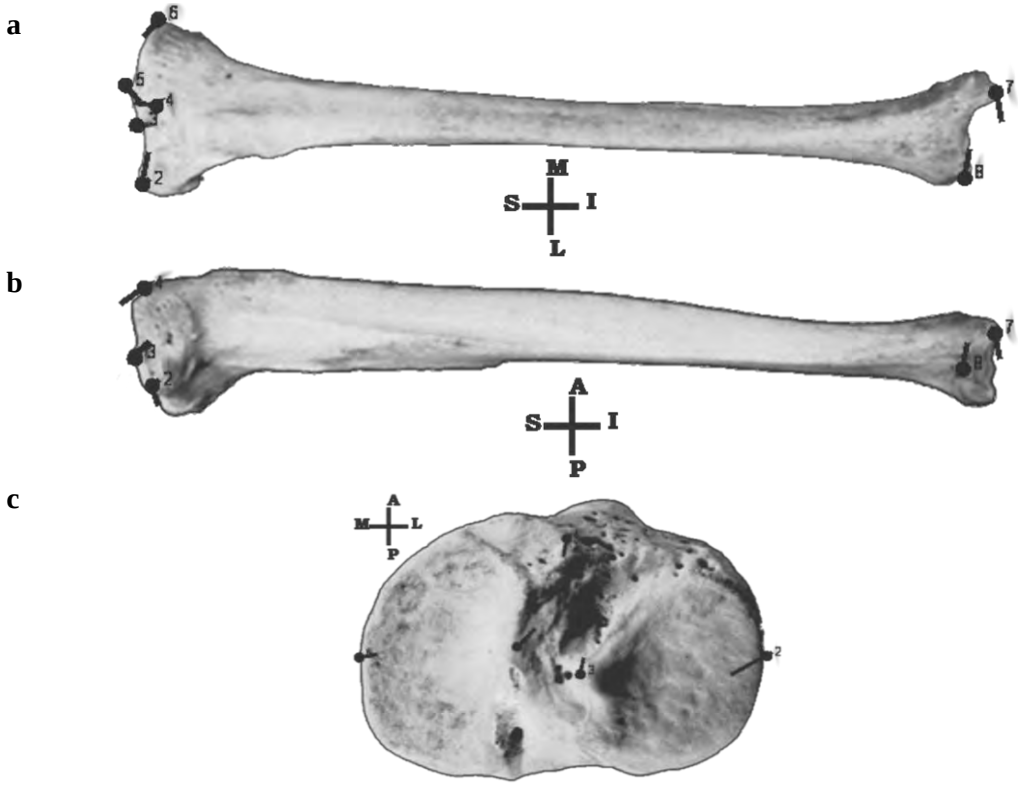

Figure B29: Tibia shape (excluding tuberosity) differences between ancestry groups (CV1) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and the mean shape of Black and Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

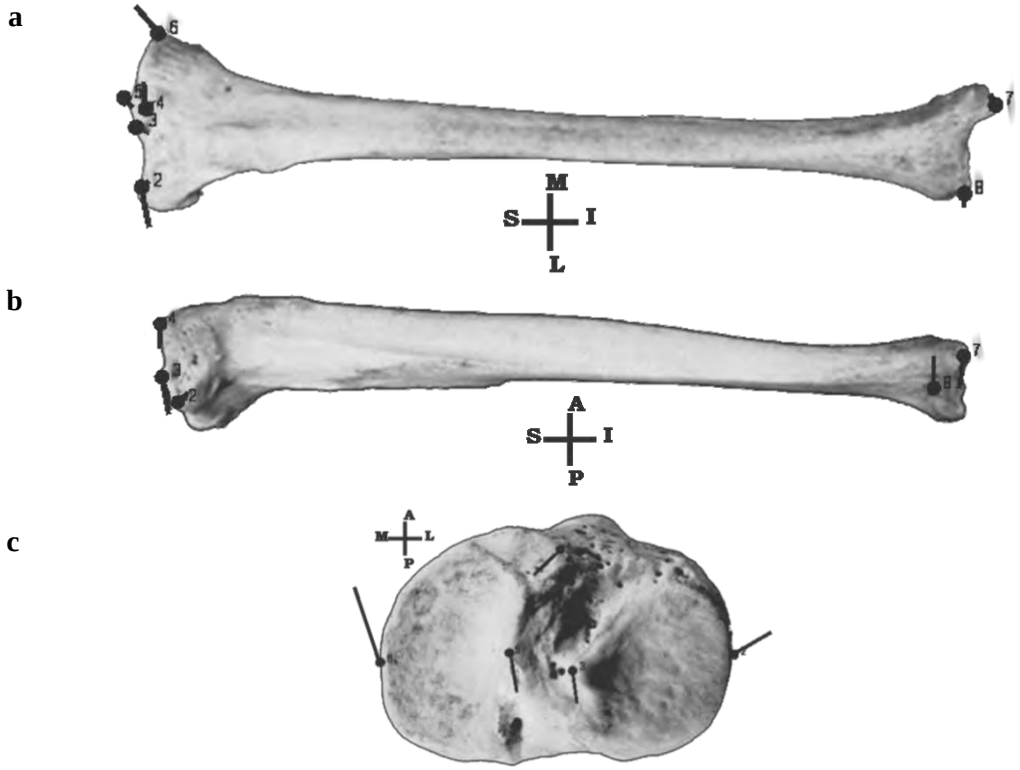

Figure B30: Tibia shape (excluding tuberosity) differences between ancestry groups (CV2) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

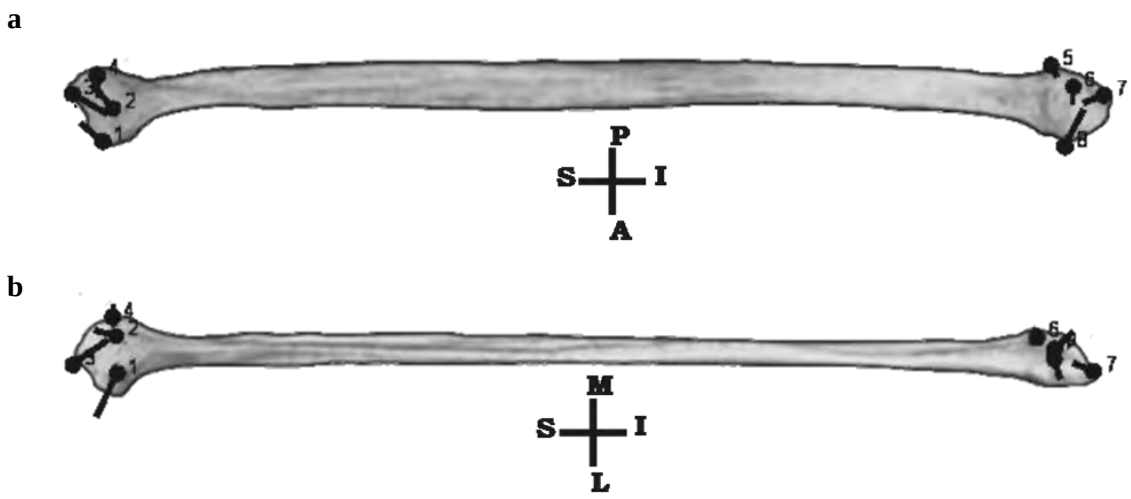

Figure B31: Fibula shape differences between ancestry groups (CV1) in a) medial and b) anterior views. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and the mean shape of Black and Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2F]

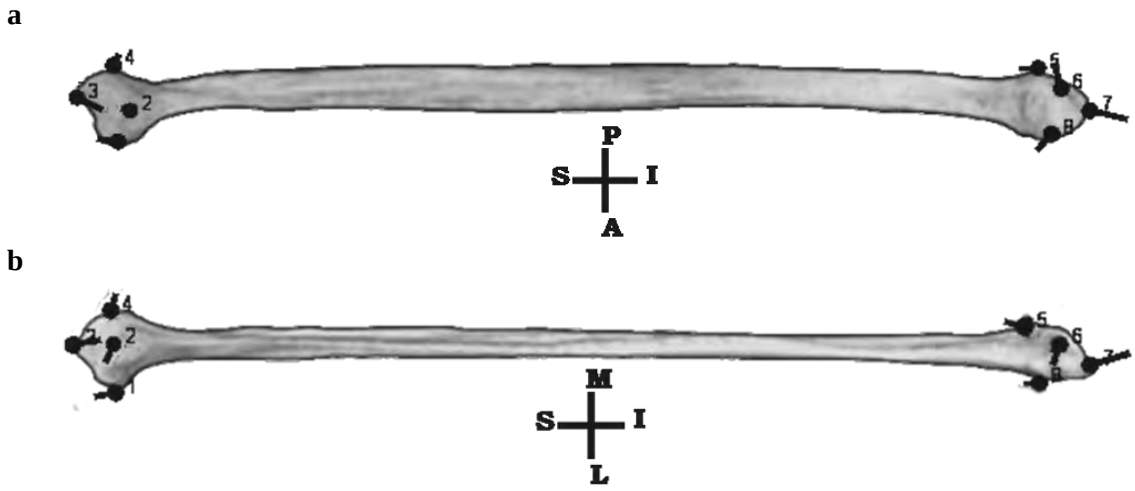

Figure B32: Fibula shape differences between ancestry groups (CV2) in a) medial and b) anterior views. Vectors (―dllipops") represent the shape differences between the mean shape of Black and White individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2F]

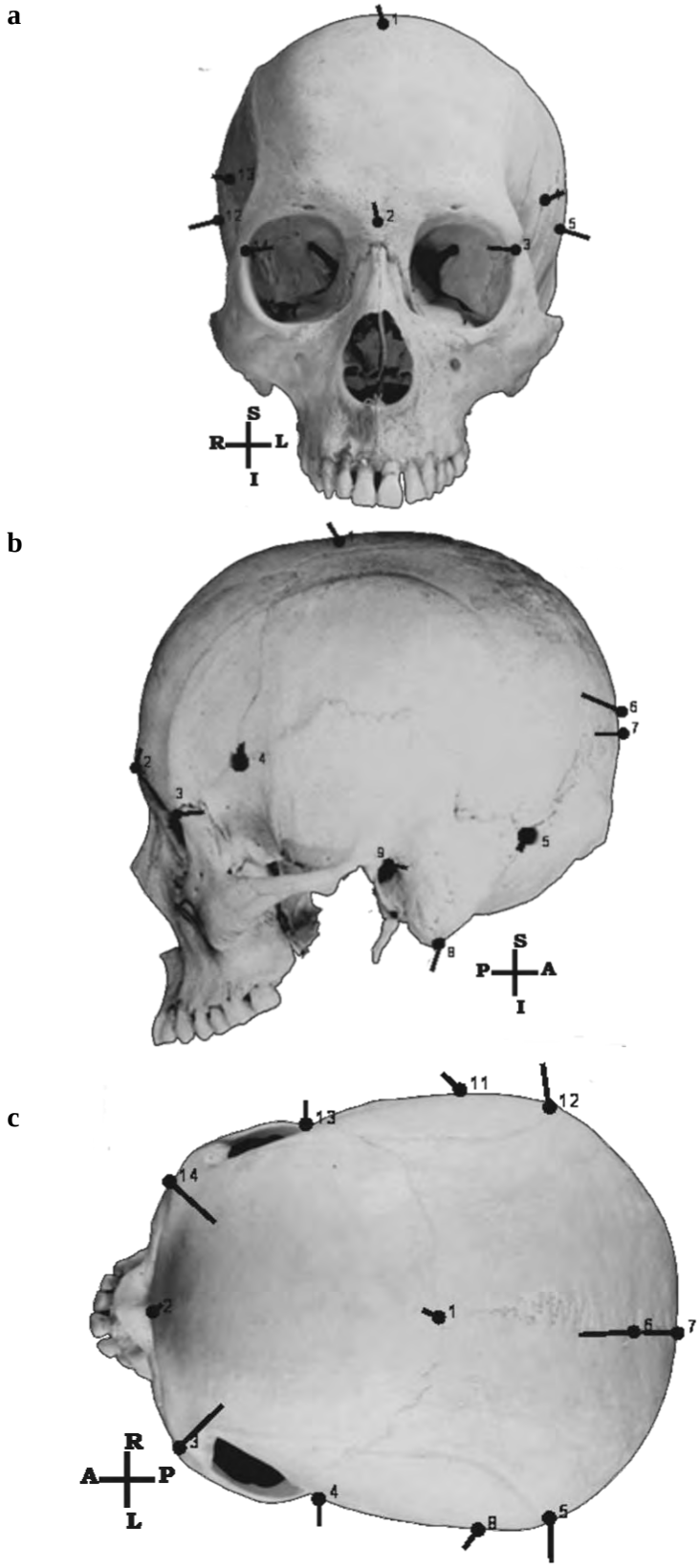

Figure B33: Whole cranial shape differences between sex-ancestry groups (CV1) in a) anterior, b) lateral, and c) superior views. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1A]

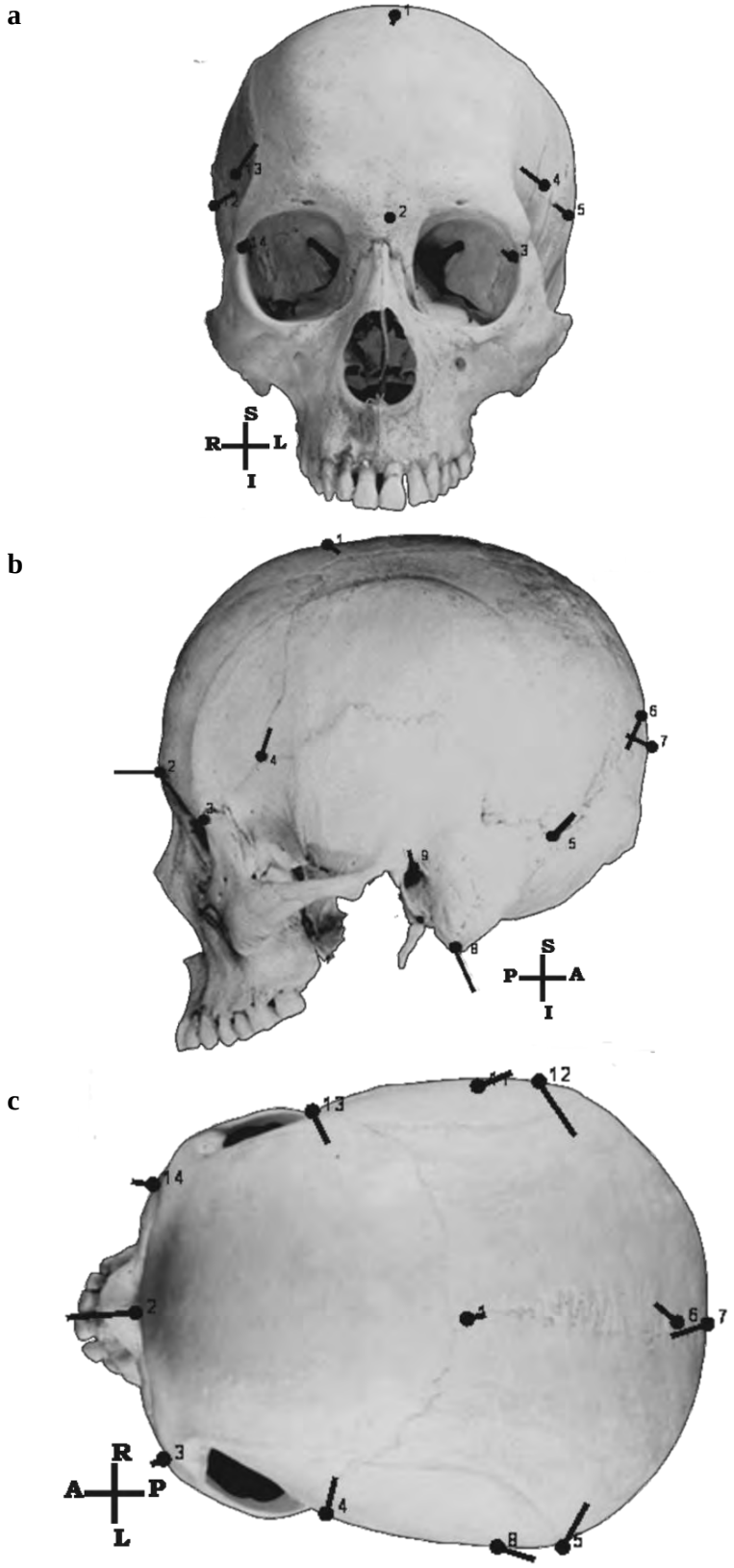

Figure B34: Whole cranial shape differences between sex-ancestry groups (CV2) in a) anterior, b) lateral, and c) superior views. Vectors (―dllipops") represent the shape differences between the mean female shape (dots) and mean male shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.1A]

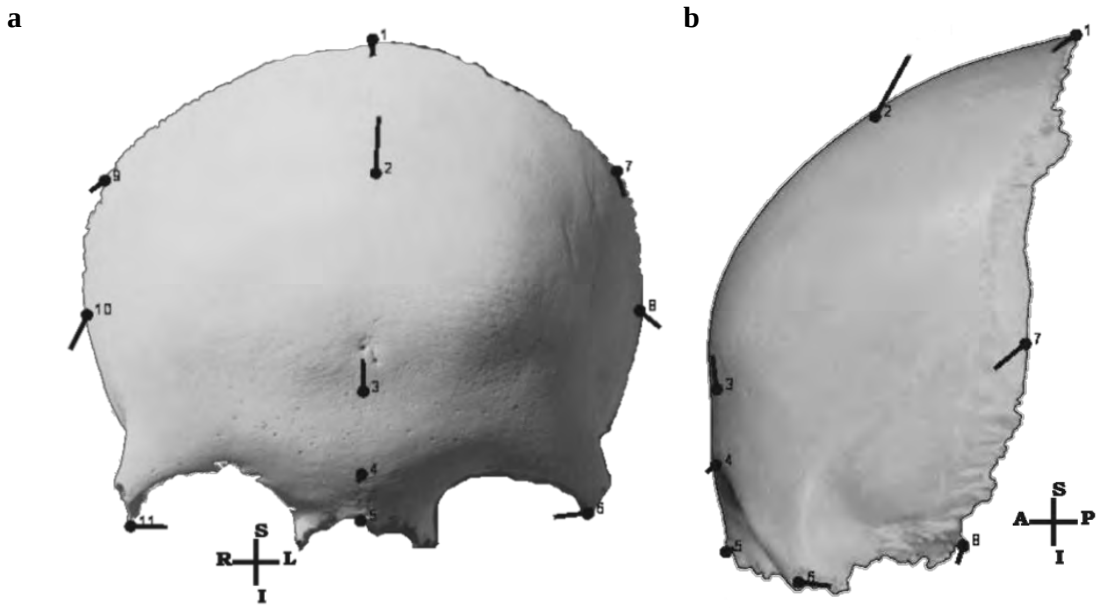

Figure B35: Frontal bone shape differences between sex-ancestry groups (CV1) in a) anterior, and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1B]

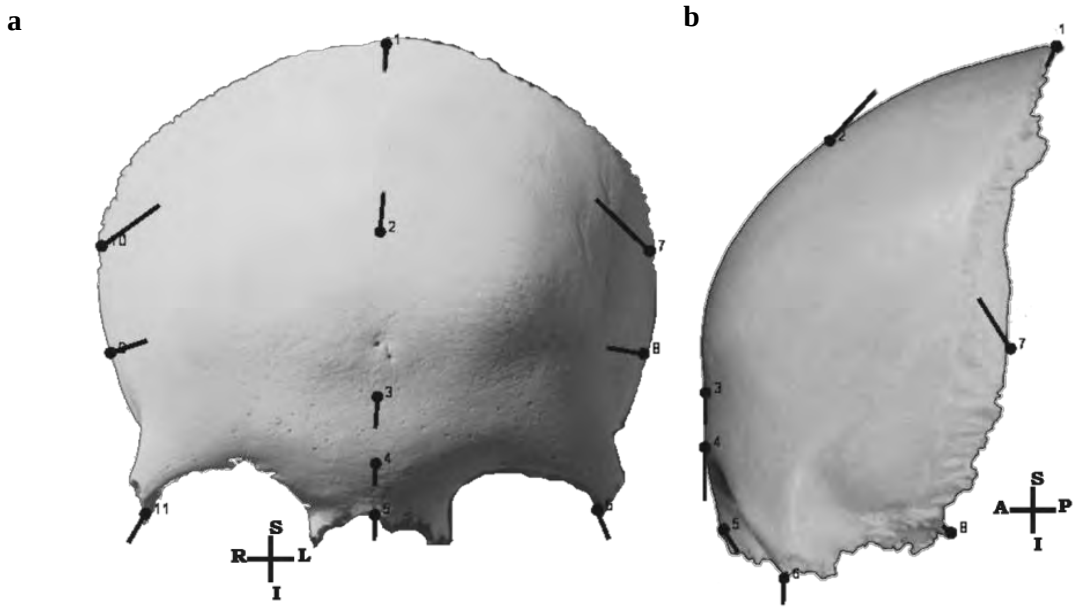

Figure B36: Frontal bone shape differences between sex-ancestry groups (CV2) in a) anterior, and b) lateral views. Vectors (―llipops") represent the shape differences between the mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Table 3.2]

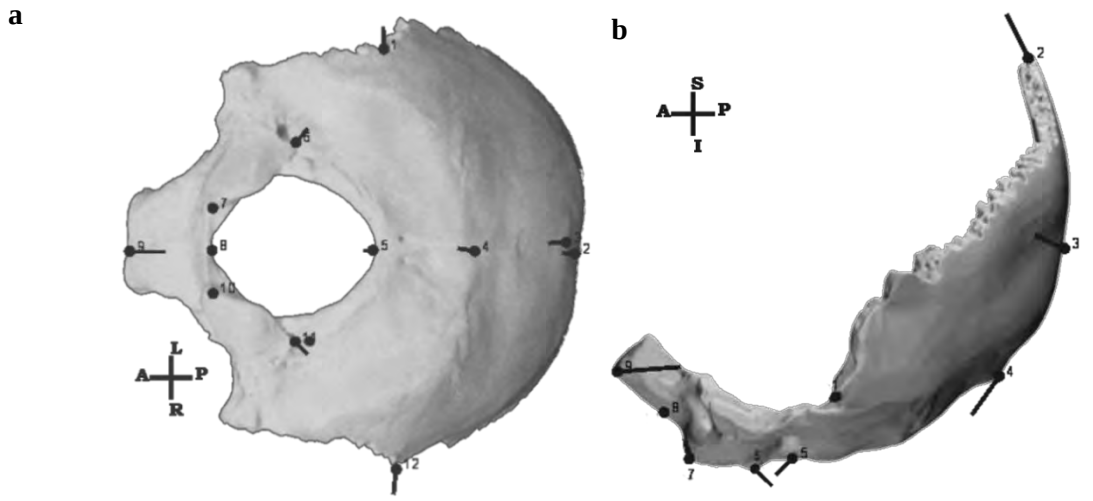

Figure B37: Occipital bone shape differences between sex-ancestry groups (CV1) in a) inferior, and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1C]

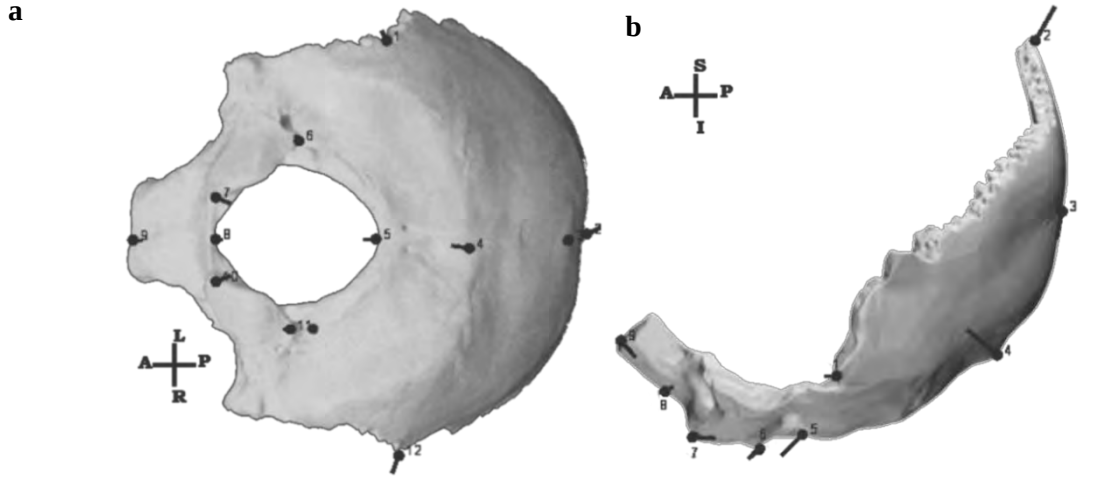

Figure B38: Occipital bone shape differences between sex-ancestry groups (CV2) in a) inferior, and b) lateral views. Vectors (―lollipops") represent the shape differences between the mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.1C]

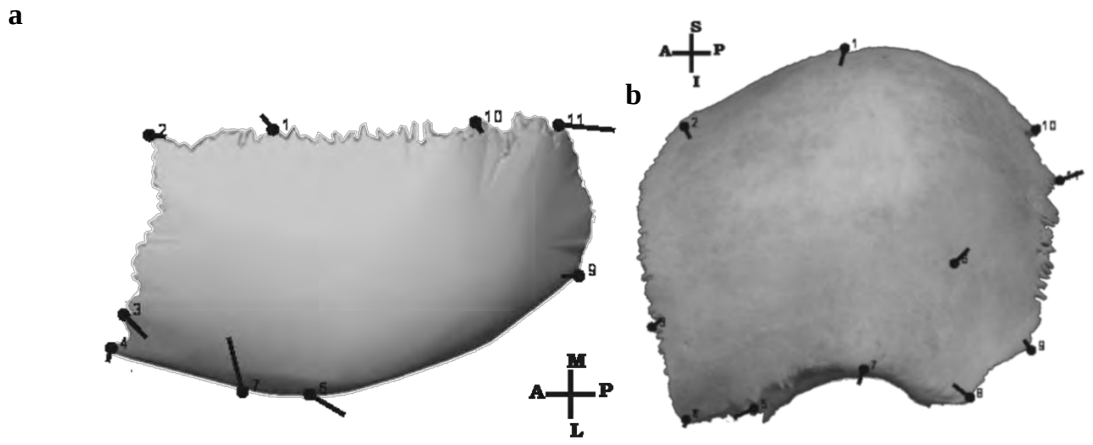

Figure B39: Parietal bone shape differences between sex-ancestry groups (CV1) in a) superior and b) lateral views. Vectors (―dllipops") represent the shape differences between the mean shape of White individuals (dots) and mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1D]

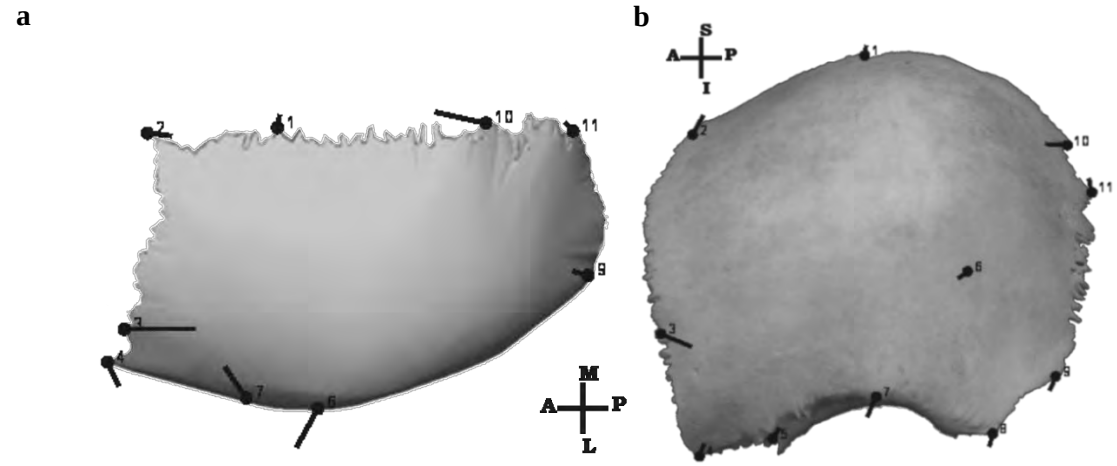

Figure B40: Parietal bone shape differences between sex-ancestry groups (CV2) in a) superior and b) lateral views. Vectors (“lollipops”) represent the shape differences between the mean male shape (dots) and mean female shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.1D]

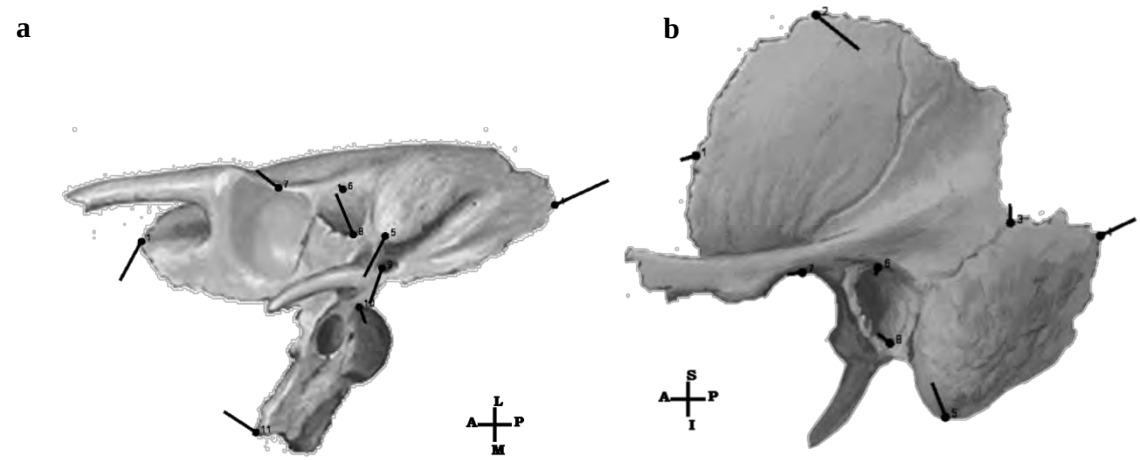

Figure B41: Temporal bone shape differences between sex-ancestry groups (CV1) in a) inferior and b) lateral views. Vectors (“dllipops”) represent the shape differences between the mean shape of White individuals (dots) and mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.1E]

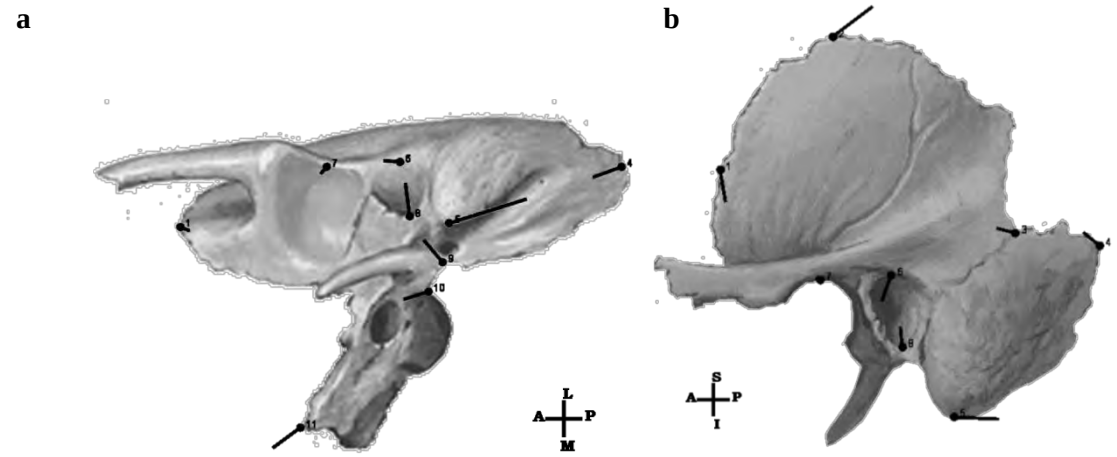

Figure B42: Temporal bone shape differences between sex-ancestry groups (CV2) in a) inferior, and b) lateral views. Vectors (“lollipops”) represent the shape differences between the mean female shape (dots) and mean male shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.1E]

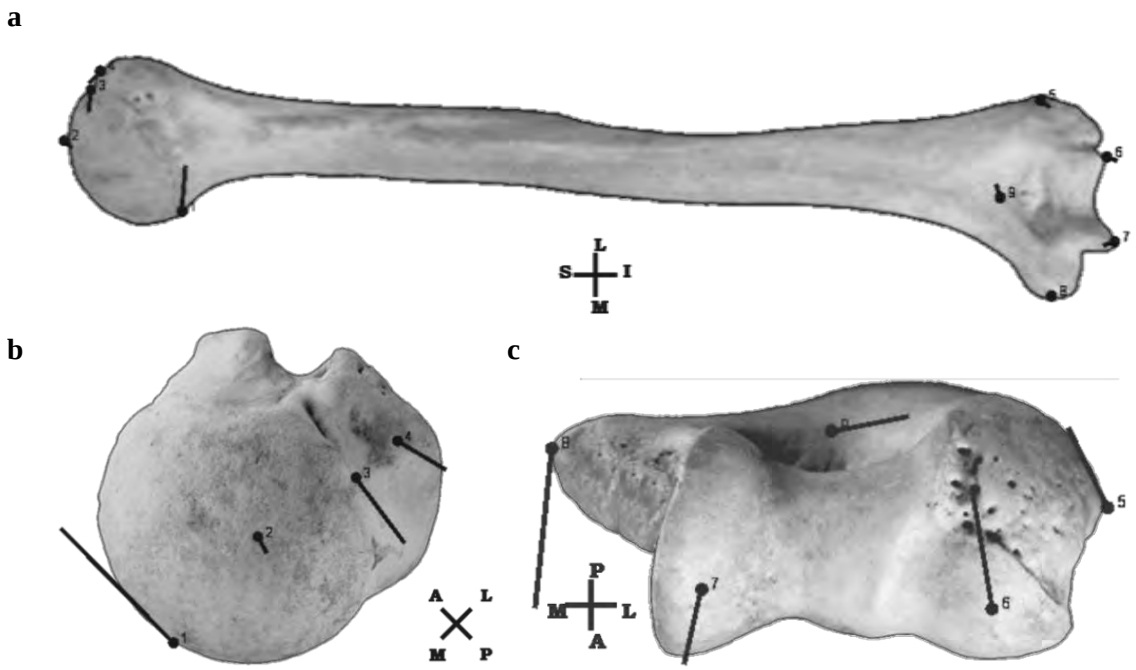

Figure B43: Humerus shape differences between sex-ancestry groups (CV1) as viewed from the a) posterior, b) humeral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of White individuals (dots) and the mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2A]

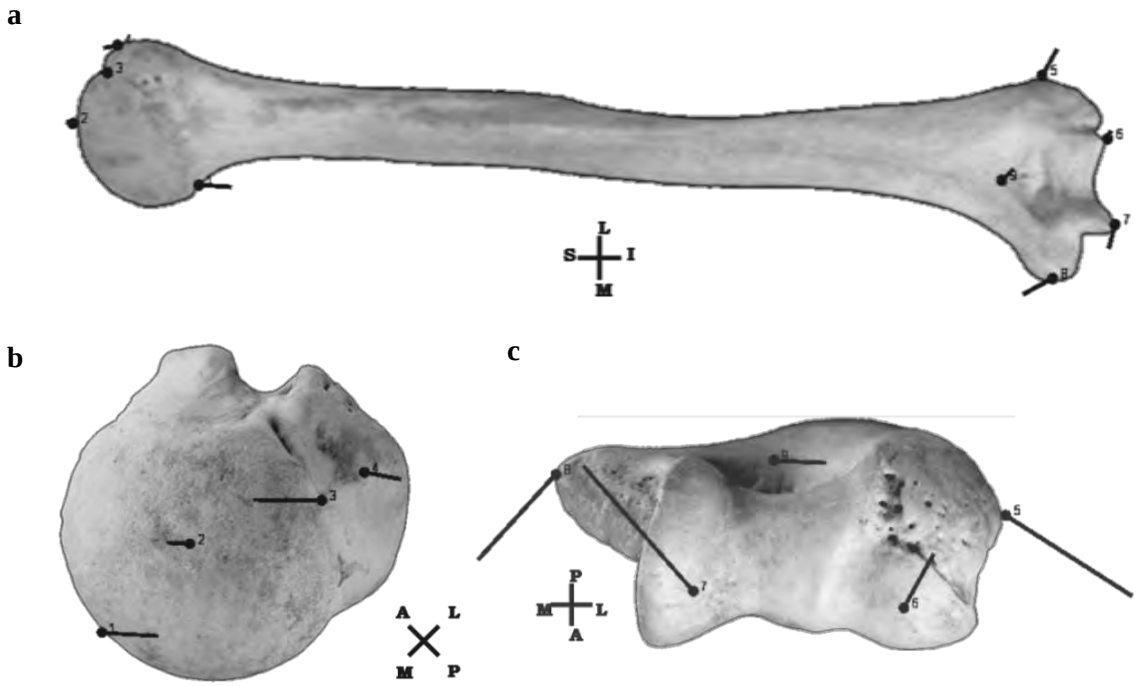

Figure B44: Humerus shape differences between sex-ancestry groups (CV2) as viewed from the a) posterior, b) humeral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean female shape (dots) and the mean male shape (end of stems). [Numbers correspond to landmark definitions in Figure 3.2A]

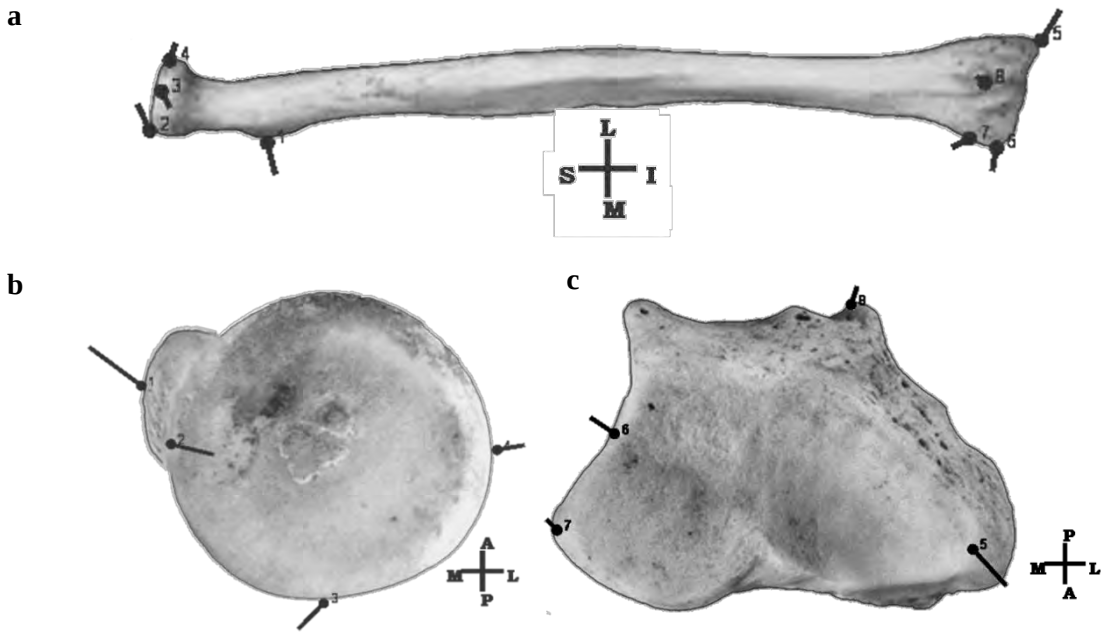

Figure B45: Radius shape differences between sex-ancestry groups (CV2) as viewed from the a) posterior, b) radial head, and c) distal epiphysis. Vectors (―dllipops") represent the shape differences between the mean shape of Black and White individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to radius landmark definitions in Figure 3.2B]

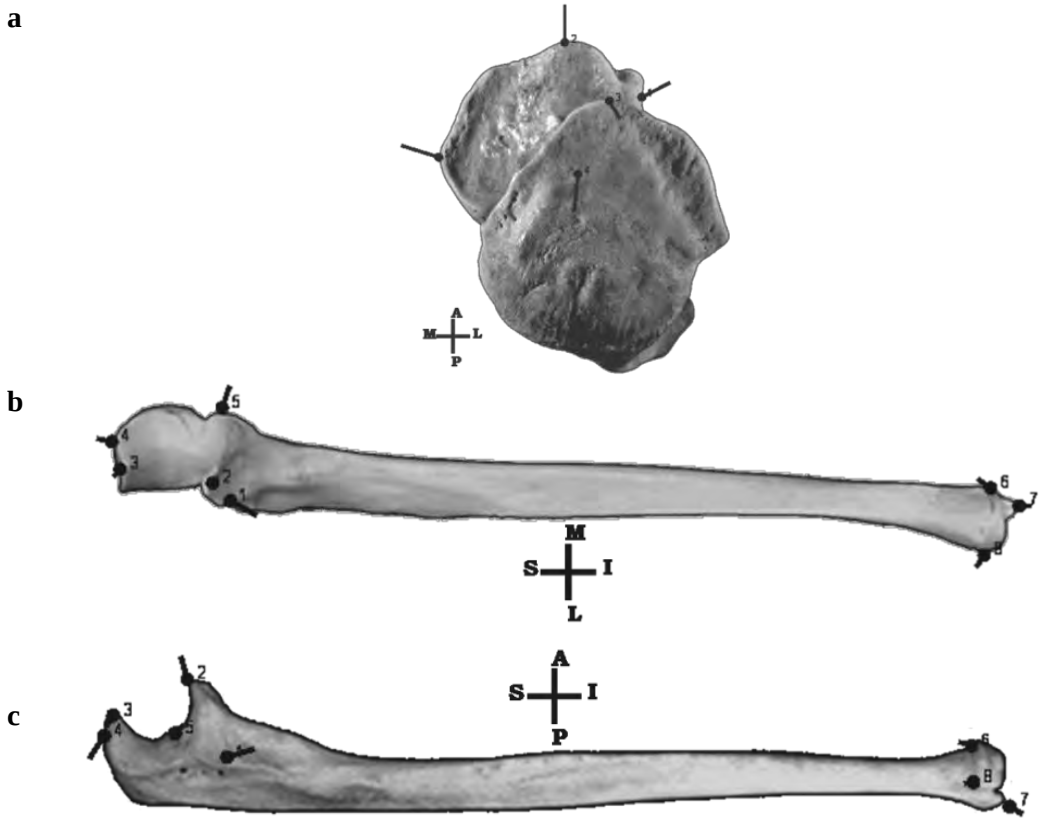

Figure B46: Ulna shape differences between sex-ancestry groups (CV1) as viewed from the a) proximal epiphysis, b) anterior, and c) lateral sides. Vectors (―dllipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2C]

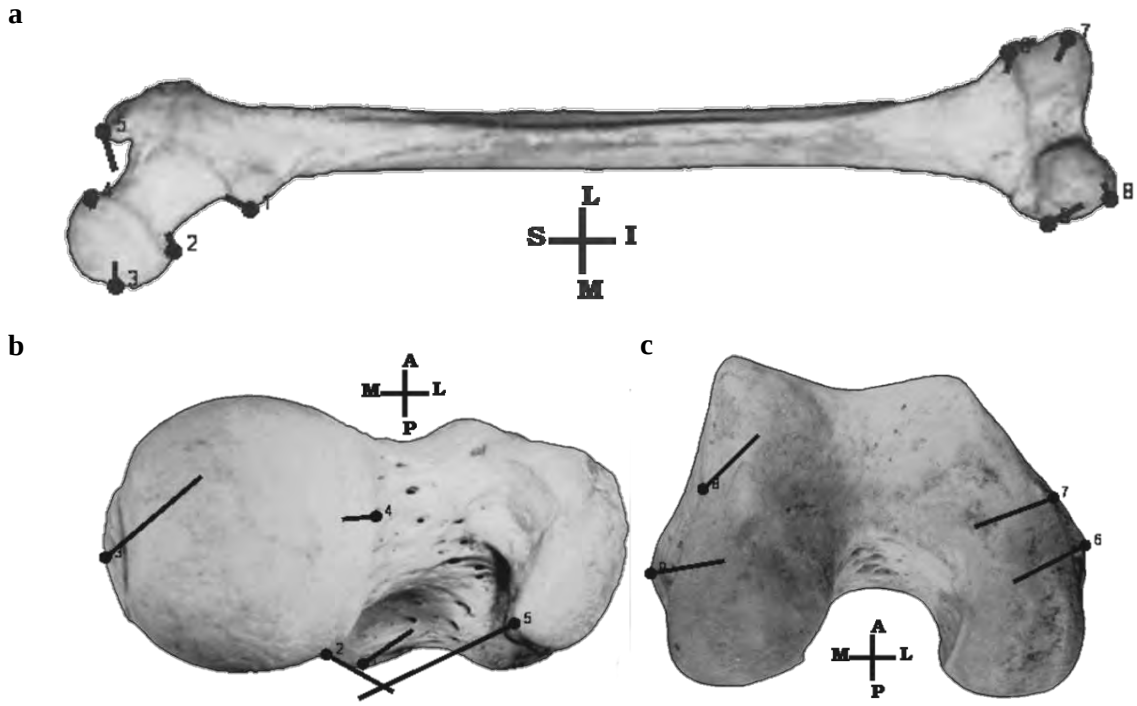

Figure B47: Femur shape differences between sex-ancestry groups (CV1) as viewed from the a) posterior, b) femoral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of White individuals (dots) and the mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2D]

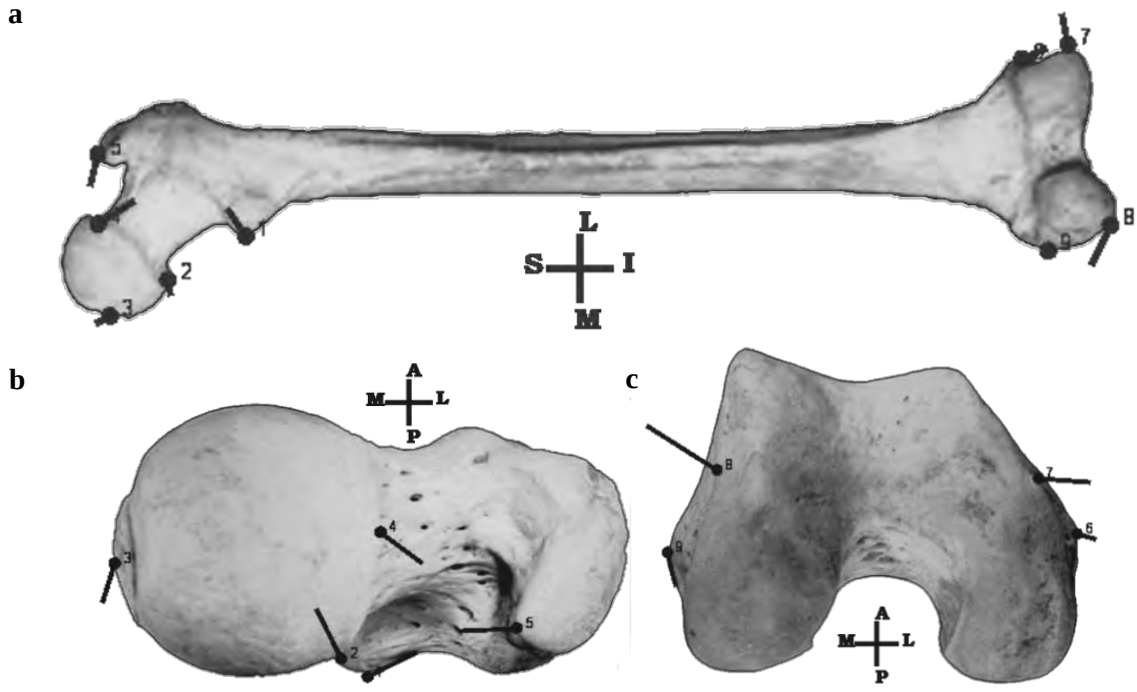

Figure B48: Femur shape differences between sex-ancestry groups (CV2) as viewed from the a) posterior, b) femoral head, and c) distal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of females (dots) and the mean shape of males (end of stems). [Numbers correspond to landmark definitions in Figure 3.2D]

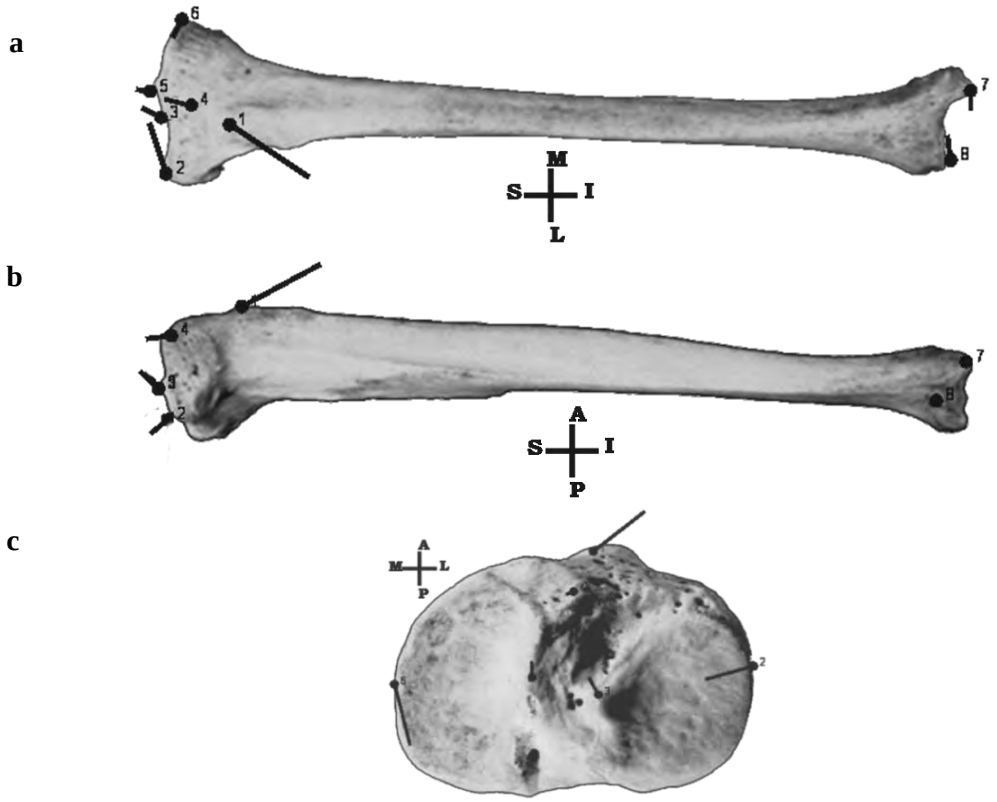

Figure B49: Tibia shape (including tuberosity) differences between sex-ancestry groups (CV1) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to tibia landmark definitions in Table 3.3]

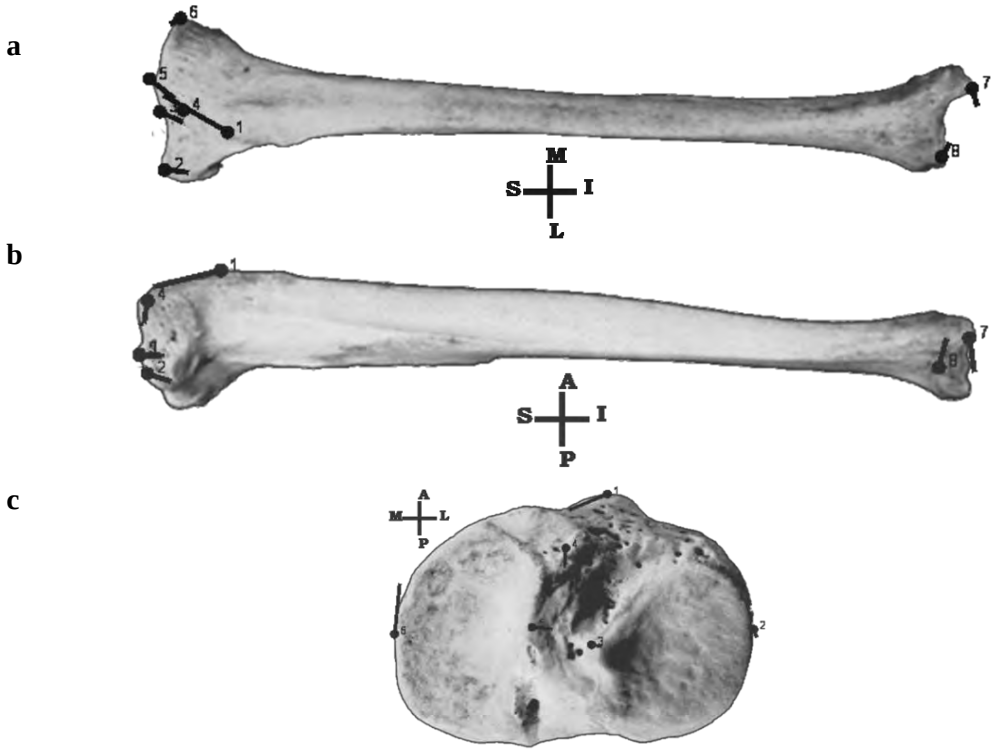

Figure B50: Tibia shape (including tuberosity) differences between sex-ancestry groups (CV2) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black and White individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

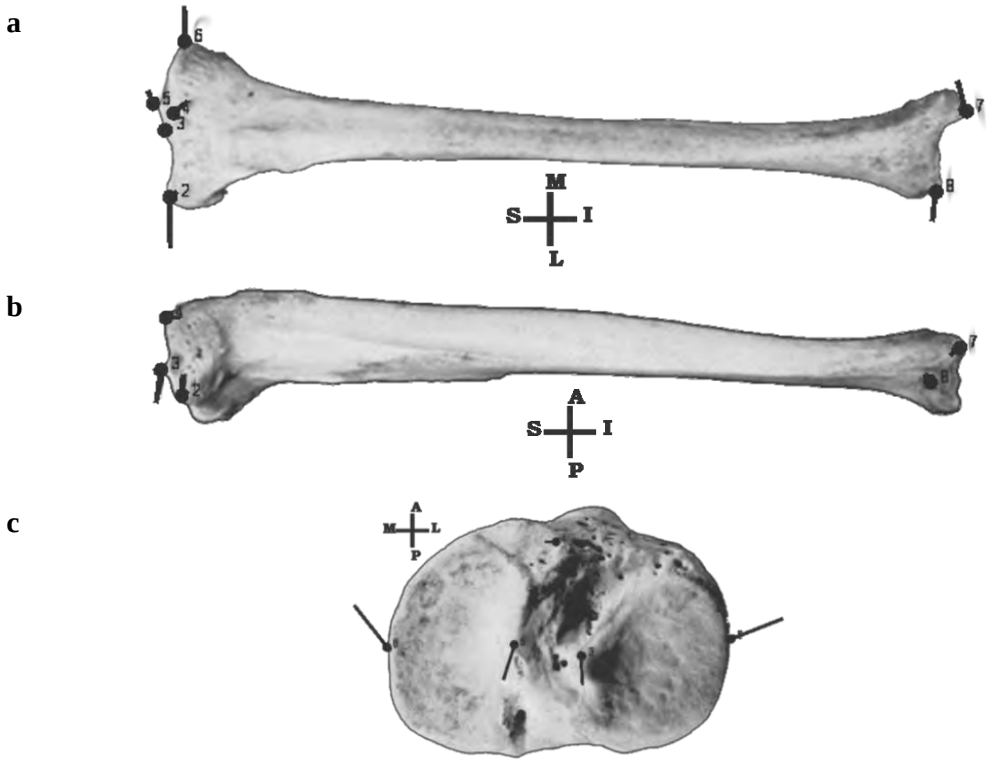

Figure B51: Tibia shape (excluding tuberosity) differences between sex-ancestry groups (CV1) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black and Coloured individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

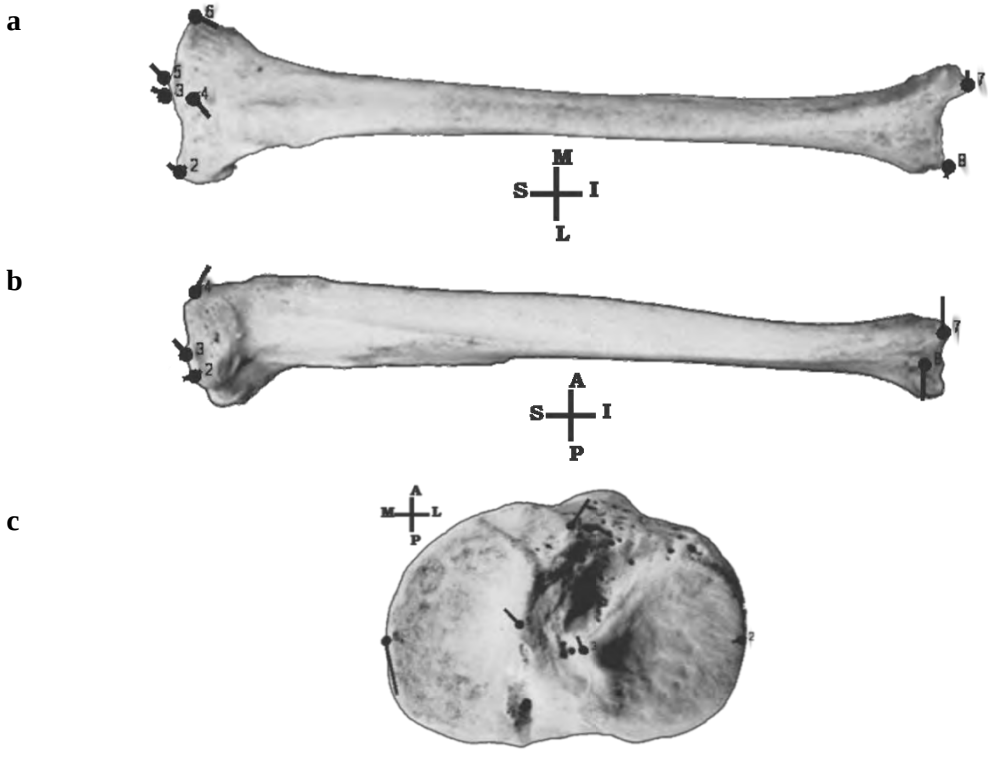

Figure B52: Tibia shape (excluding tuberosity) differences between sex-ancestry groups (CV2) as viewed from the a) anterior, b) lateral, and c) proximal epiphysis. Vectors (~~l~~ollipops") represent the shape differences between the mean shape of Black and Coloured individuals (dots) and the mean shape of White individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2E]

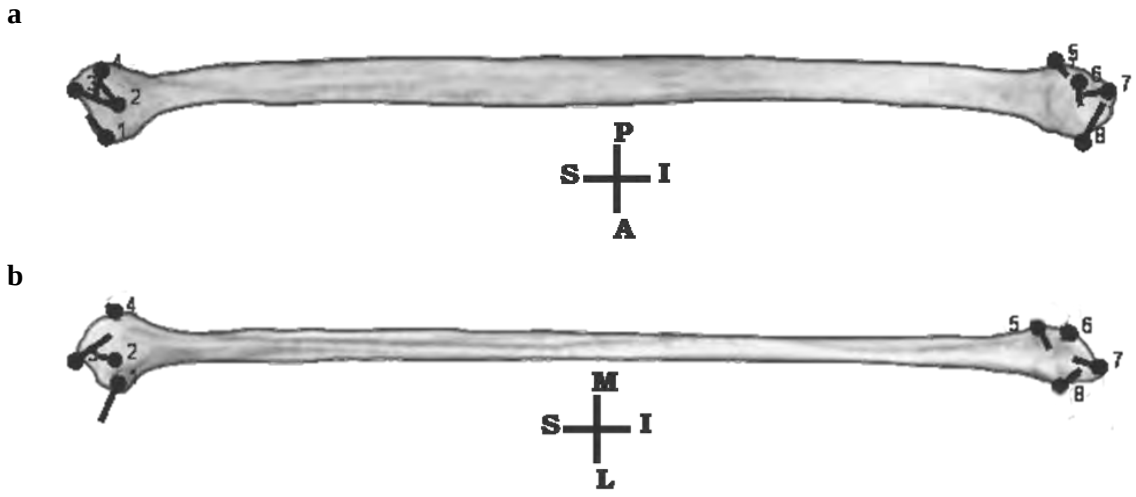

Figure B53: Fibula shape differences between sex-ancestry groups (CV1) in a) medial and b) anterior superior views. Vectors (―lollipops") represent the shape differences between the mean shape of White individuals (dots) and the mean shape of Black individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2F]

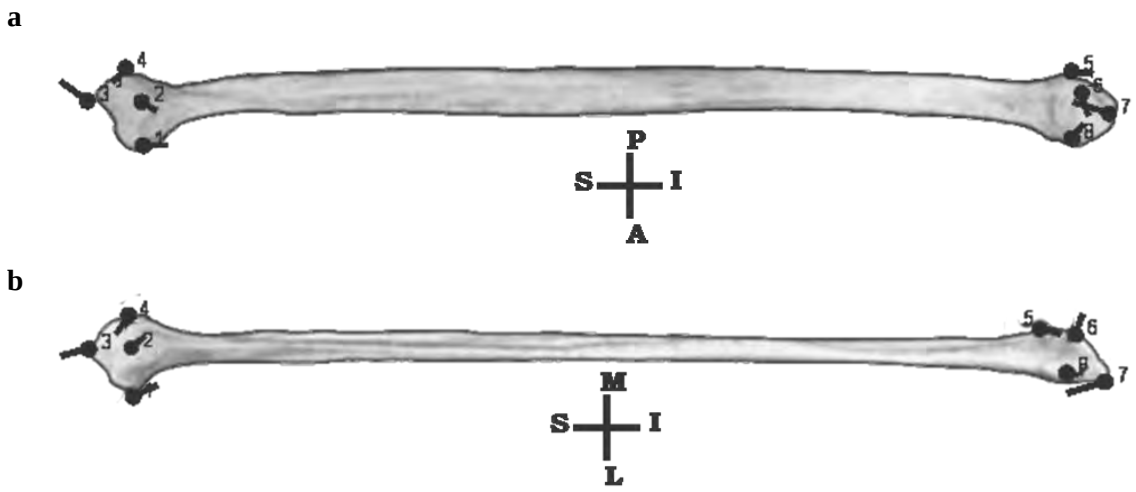

Figure B54: Fibula shape differences between sex-ancestry groups (CV2) in a) medial and b) anterior views. Vectors (―dllipops") represent the shape differences between the mean shape of Black and White individuals (dots) and the mean shape of Coloured individuals (end of stems). [Numbers correspond to landmark definitions in Figure 3.2F]

APPENDIX C

Table C1: Mahalanobis distance between sexes for skeletal element showing significant ($p<0.0001$) separation of the sexes.

Skeletal element	Mahalanobis distance (MD)
Cranium	
Whole cranium	1.2
Frontal	1.7
Upper limb	
Humerus	1.3
Radius	1.2
Ulna	1.0
Lower limb	
Femur	1.4
Tibia (with tuberosity)	0.9

Table C2: Raw output of the leave-one-out cross-validation classification accuracies of the skeletal elements evaluated according to sex (Highest accuracies of cranial and postcranial elements in red).

Skeletal element	Sex estimation (n correct/n total)		
	Female	Male	Pooled sexes
Cranium			
Whole cranium	251/354	292/420	**543/774**
Frontal	**298/356**	**318/421**	**616/777**
Upper limb			
Humerus	348/464	419/582	**767/1046**
Radius	351/471	393/578	**744/1049**
Ulna	328/465	390/578	**718/1043**
Lower limb			
Femur	**338/447**	**399/545**	**737/992**
Tibia (with tuberosity)	289/449	353/554	**642/1003**

*Note: accuracies for the occipital, parietal and temporal bones, tibia (without tuberosity) and fibula are not listed, as no significant separation of the sexes was detected.

Table C3: Mahalanobis distances between ancestry groups (all p<0.0001).

Skeletal element		Black	Coloured
Cranium			
Whole cranium	**Coloured**	1.4	
	White	3.3	2.5
Frontal	**Coloured**	1.6	
	White	3.0	2.3
Occipital	**Coloured**	1.3	
	White	2.5	1.7
Parietal	**Coloured**	1.1	
	White	2.2	1.7
Temporal	**Coloured**	1.1	
	White	2.5	2.0
Upper limb			
Humerus	**Coloured**	1.7	
	White	2.9	2.0
Radius	**Coloured**	1.8	
	White	2.0	1.5
Ulna	**Coloured**	1.2	
	White	1.6	1.2
Lower limb			
Femur	**Coloured**	1.6	
	White	2.1	1.5
Tibia (with tuberosity)	**Coloured**	1.7	
	White	1.9	1.6
Tibia (without tuberosity)	**Coloured**	1.4	
	White	1.6	1.6
Fibula	**Coloured**	1.2	
	White	1.7	1.4

Table C4: Raw output of the leave-one-out cross-validation of skeletal elements evaluated according to ancestry (Highest accuracies of cranial and postcranial elements indicated in red).

Skeletal element	Ancestry estimation (n correct/n total)			
	Black	**Coloured**	**White**	**Pooled ancestry**
Cranium				
Whole cranium	464/566	424/542	**397/440**	**1285/1548**
Frontal	**505/584**	**452/540**	387/440	**1344/1564**
Occipital	469/580	401/544	369/460	**1239/1584**
Parietal	452/580	416/556	385/466	**1253/1602**
Temporal	473/600	426/574	392/474	**1291/1648**
Upper limb				
Humerus	**571/674**	**615/732**	**598/686**	**1784/2092**
Radius	531/666	607/736	554/696	**1692/2098**
Ulna	514/668	510/722	511/696	**1535/2086**
Lower limb				
Femur	549/660	562/708	467/616	**1578/1984**
Tibia (with tuberosity)	511/650	594/712	515/644	**1620/2006**
Tibia (without tuberosity)	498/652	563/716	492/644	**1553/2012**
Fibula	477/618	468/646	431/574	**1376/1838**

Table C5a: Mahalanobis distances between sex-ancestry groups based on whole cranial shape variation.

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.41				
White females	3.28	2.61			
Black males	1.37	1.87	3.23		
Coloured males	1.84	1.34	2.52	1.53	
White males	3.79	3.17	1.64	3.45	2.64

Table C5b: Mahalanobis distances between sex-ancestry groups based on frontal bone shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.96				
White females	3.23	2.48			
Black males	1.88	1.72	2.98		
Coloured males	2.85	1.84	2.47	1.53	
White males	4.07	3.50	2.01	3.17	2.42

Table C5c: Mahalanobis distance between sex-ancestry groups based on occipital bone shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.49				
White females	2.42	1.76			
Black males	1.24	1.83	2.59		
Coloured males	1.71	1.27	1.81	1.29	
White males	2.98	2.58	1.72	2.67	1.98

Table C5d: Mahalanobis distance between sex-ancestry groups based on parietal bone shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.39				
White females	2.44	1.95			
Black males	1.35	1.66	2.41		
Coloured males	1.55	1.10	1.94	1.22	
White males	2.64	2.20	1.47	2.19	1.80

Table C5e: Mahalanobis distance between sex-ancestry groups based on temporal bone shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.76				
White females	2.85	2.28			
Black males	1.98	1.86	2.63		
Coloured males	2.03	1.46	2.16	0.89	
White males	3.17	2.86	1.60	2.48	2.08

Table C5f: Mahalanobis distance between sex-ancestry groups based on humeral shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	2.11				
White females	3.16	1.96			
Black males	1.55	1.78	2.99		
Coloured males	2.47	1.36	2.36	1.40	
White males	3.33	2.33	1.34	2.84	2.06

Table C5g: Mahalanobis distance between sex-ancestry groups based on radius shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	2.08				
White females	2.13	1.65			
Black males	1.32	1.57	2.10		
Coloured males	2.56	1.22	1.91	1.63	
White males	2.51	1.87	1.30	2.04	1.46

Table C5h: Mahalanobis distance between sex-ancestry groups based on ulna shape variation.

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.32				
White females	1.66	1.25			
Black males	0.94	1.23	1.47		
Coloured males	1.78	1.28	1.42	1.15	
White males	2.15	1.88	1.08	1.65	1.27

Table C5i: Mahalanobis distance between sex-ancestry groups based on femur shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.81				
White females	2.24	1.39			
Black males	1.72	1.80	2.17		
Coloured males	2.52	1.37	1.95	1.50	
White males	2.67	2.00	1.34	2.11	1.70

Table C5j: Mahalanobis distance between sex-ancestry groups based on tibia (including tuberosity) shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	2.15				
White females	2.21	1.69			
Black males	1.37	1.21	1.71		
Coloured males	2.53	0.85	1.79	1.36	
White males	2.51	1.85	0.89	1.80	1.67

Table C5k: Mahalanobis distance between sex-ancestry groups based on tibia (excluding tuberosity) shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.77				
White females	1.82	1.59			
Black males	1.22	0.98	1.42		
Coloured males	2.15	0.83	1.67	1.10	
White males	2.22	1.83	0.85	1.61	1.61

Table C5l: Mahalanobis distance between sex-ancestry groups based on fibula shape variation

	Black females	Coloured females	White females	Black males	Coloured males
Coloured females	1.43				
White females	1.78	1.46			
Black males	0.98	1.14	1.44		
Coloured males	1.68	0.89	1.45	1.13	
White males	2.33	1.89	1.18	1.76	1.41

Table C6: Raw output of the leave-one-out cross-validation classification accuracies of skeletal elements evaluated according to sex and ancestry (Highest accuracies of cranial and postcranial elements indicated in red).

Skeletal element	Sex-ancestry estimation (n correct/n total)						
	Black female	**Coloured female**	**White female**	**Black male**	**Coloured male**	**White male**	**Pooled sex-ancestry**
Cranium							
Whole cranium	353/480	232/312	216/270	277/369	380/501	**334/390**	**1792/2322**
Frontal	**422/486**	**259/309**	**232/273**	**314/375**	**384/501**	330/387	**1941/2331**
Occipital	381/483	223/321	218/288	285/387	353/495	318/402	**1778/2376**
Parietal	367/486	221/321	227/294	278/384	360/513	304/405	**1757/2403**
Temporal	413/495	245/324	255/306	317/405	368/537	312/405	**1919/2472**
Upper limb							
Humerus	472/552	**323/393**	**369/447**	**364/459**	**542/705**	**463/582**	**2533/3138**
Radius	471/ 549	310/393	358/453	326/453	529/690	434/591	**2428/3147**
Ulna	416/549	280/393	331/453	313/453	480/690	412/591	**2232/3129**
Lower limb							
Femur	**476/546**	295/393	291/402	348/444	510/669	399/522	**2319/2976**
Tibia (with tuberosity)	444/525	295/390	322/432	345/460	501/678	371/534	**2278/3019**
Tibia (without tuberosity)	425/525	200/390	312/432	332/453	488/684	375/534	**2132/3018**
Fibula	403/510	256/366	258/360	290/417	403/603	383/501	**1993/2757**