An evaluation of the cranial variation of Indian South Africans in comparison to other modern South Africans

A Computed Tomography Approach

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Submitted in fulfilment of the requirements for the degree
Doctor of Philosophy
PhD (Anatomy)

In the Department of Anatomy
Faculty of Health Sciences
School of Medicine
University of Pretoria

2024

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DECLARATION

I, Gabriele Christa Krüger, declare that the thesis, which I hereby submit for the degree Doctor of Philosophy (Anatomy) to the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

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25 October 2023

Date

ETHICAL APPROVAL

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Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

16 February 2023

Approval Certificate
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Ethics Reference No.: 771/2018 – Line 4
Title: An evaluation of the cranial variation of Indian South Africans in comparison to other modern South Africans: A computed tomography approach

The **Annual Renewal** as supported by documents received between 2023-01-18 and 2023-02-15 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-02-15 as resolved by its quorate meeting.

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Yours sincerely

On behalf of the FHS REC, Dr R Sommers
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ACKNOWLEDGEMENTS

First and foremost, I extend my deepest gratitude to my supervisors, Prof Ericka N. L'Abbé and Prof Richard L. Jantz, for their invaluable guidance, support, and expert insights throughout every stage of this research journey. Your mentorship has been instrumental in shaping both my academic and personal growth.

I want to express my heartfelt gratitude to my esteemed colleagues and peers. Our lively discussions, collaborative energy, and constant encouragement have been the driving force behind me actually finishing this research with some sanity intact.

To my beloved partner, Es, your unwavering belief in me, your patience, and your understanding have been my lifeline throughout this academic journey. Your love and encouragement have been a constant source of strength, providing me with stability amidst the chaos of this degree.

To my parents, from the earliest days of my education to the conclusion of this PhD journey, your support has been endless. Your belief in my abilities, even when I doubted myself, has been a source of immeasurable strength. Thank you for instilling in me the values of perseverance, resilience, and hard work.

Last, but no least, to Mila, Delilah, Sammy and Archie, your cuddles, tail wags, and purrs have kept me sane when the research world got too crazy.

SUMMARY/ABSTRACT

Constant re-evaluation of standards used in forensic anthropological analyses are necessary, particularly as new methods are explored or populations change. Even though Indian South Africans are not a new addition to the South African population, the lack of skeletal material available for analysis has resulted in a lack of information on the variation present in the crania of this group. Furthermore, although black, white and coloured South African crania have been previously researched and standards created, by similarly making use of three-dimensional (3D) models created from computed tomography (CT) scans, the data are comparable to the Indian South African data collected and all four groups could be explored simultaneously.

The aim of this project was to use 3D CT models to explore cranial variation in Indian South Africans when compared to current black, coloured and white South Africans to distinguish among the groups when estimating sex and population affinity from the cranium.

3D cranial models were created from 409 head CT scans of black, coloured, white and Indian South Africans (equal sex and population distribution). A total of 42 landmarks were recorded on each skull. The coordinates were used to assess shape differences using geometric morphometrics, generalized Procrustes analysis and principal component analysis. Standard and non-standard interlandmark distances (ILD) were also created from the landmark coordinate data and assessed using analysis of variance for significant sex and population differences, and symmetric percent differences for comparisons of the degree of sexual dimorphism among the different population groups. Furthermore, linear discriminant analysis (LDA) was used to assess the classification potential of the various ILDs to estimate sex and population affinity. Four morphoscopic traits were also scored on each cranium according to the methodology by Walker (2008). The scores were then assessed for their ability to separate

between the sexes in each of the population groups using ordinal logistic regression and random forest modelling.

Indian South Africans obtained the highest correct classification rates for sex using morphoscopic traits (95.7%) and demonstrated substantial differences between Indian South African males and females for the ILDs. Similarly, the remaining three population groups had excellent correct classification rates for the morphoscopic traits (88.0% - 91.5%) and sex could be estimated with high rates using ILDs (90.7%). Furthermore, acceptable classification rates were obtained when estimating population affinity for the four South African populations when the ILDs (up to 62.2%) and 3D coordinates (up to 63.8%) were assessed, indicating cranial differences among the four groups. Even though population affinity could be estimated, substantial overlap between coloured, Indian and white South Africans was noted, most likely due to the similarities in genetic influences that have contributed to the various populations.

The assessment of current Indian South Africans as well as the exploration of the cranial variation present in the other three larger current South African populations, was only possible through the use of 3D cranial models created from head CT scans, and was able to provide novel information that can be applied in both biological and forensic anthropology.

Keywords: computed tomography, sex estimation, population affinity, Indian South Africans, cranial variation, morphoscopic, random forest modelling, geometric morphometrics, linear discriminant analysis, interlandmark distances

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LIST OF ABBREVIATIONS

2D – two-dimensional
3D – three-dimensional
ANOVA – analysis of variance
CNRS – Centre national de la recherche scientifique
CS – centroid size
CT – computed tomography
CVA – canonical variate analysis
FD3 – Fordisc 3 (various versions)
GLS – generalised least squares
GM – geometric morphometrics
GPA – generalised Procrustes analysis
GS Imp% – relative variable importance
HMH – half-maximum height
HSD – honestly significant distance
ILD – interlandmark distances
LDA – linear discriminant analysis
LOOCV – leave-one-out cross-validation
MANOVA – multivariate analysis of variance
mm – millimetres
OLR – ordinal logistic regression
OLS – ordinary least squares
PC – principal component
PCA – principal component analysis
PopSex – combined population and sex groups
ProCoords – Procrustes coordinates
RF – Random Forest modelling
s% – sympercents
SD – standard deviation
TEM – absolute technical error of measurement
%TEM – relative technical error of measurement
TIVMI – Treatment and Increased Vision for Medical Imaging
USA – United States of America

CHAPTER 1: INTRODUCTION

In forensic anthropology, great emphasis is placed on the validity of methodologies and their ability to provide the best results using the most accurate methods. The *Daubert* criteria were implemented in the United States of America (USA) following a court ruling in 1993 as a means to supervise the admissibility of expert testimony and to improve the methods and standards for forensic analyses. The criteria require methods to have been tested, be repeatable, have known error rates, be peer-reviewed and be generally accepted by the scientific community (Daubert v. Merrell Dow Pharmaceuticals, Inc. 1993). While the *Daubert* criteria are not currently required in the South African court system, the inclusion of these criteria when creating new methods simply underlines that the methods are scientifically valid and meet the standards required for expert testimony. Researchers in forensic anthropology are continuously validating existing methodology and exploring new methods for their potential in assessing skeletal variation. The variation inherent in the skeleton of different populations, different sexes, as well as different age cohorts can be quantified and used to create biological profiles to aid in providing a presumptive identification for unknown skeletal remains. While multiple methods have been explored to assist in creating a biological profile, current methods typically make use either of a scoring system (morphoscopic) or of measurements (osteometric) to quantify skeletal features that may be useful in distinguishing different populations as well as between the sexes.

While the use of morphological cranial variation to estimate sex and population affinity has been described for many years, the lack of robust statistics and decreased repeatability has made the method less applicable to forensic cases. In South Africa, the Walker (2008) method is typically applied to estimate sex from the skull. The method is based on the assessment of five features on the skull (glabella, mastoid process, mental eminence, nuchal crest and supra-orbital margin) that have been shown to depict sexual dimorphism. The features were described

as early as 1875 (Walker 2008) and over the years have been amended by adding different scoring systems and complex diagrams (-2 to +2; Açsádi & Nemeskéri 1970; Buikstra *et al.* 1994; Walker 2008). Only in 1994 were a five-point ordinal scale and simplified line diagrams added to better describe the range of variation that existed in these traits and to improve repeatability (Buikstra *et al.* 1994). However, even with the improvements, the lack of robust statistics meant the method was not compliant with the *Daubert* criteria. Walker (2008) added linear discriminant functions to the scores and added probabilities and correct classification rates. The combination of line diagrams, scoring procedures, robust statistics, known error rates and peer-review of the published material meant the method could be accepted in the USA court system.

Although the method was sound and shown to work when applied to a sample similar to the reference data, when the method was applied to a different group, such as the South African population, the resulting correct classification rates were extremely low (31% correct classification; Krüger *et al.* 2015). As a means to improve on the existing methodology, the original formulae were re-calibrated to create not only South African-specific standards, but also formulae specific for the estimation of sex from the skull in black and white South Africans, separately.

Similarly, 13 morphological qualitative features in the cranium have been assessed for significant relationships with population affinity in black, white and coloured South Africans (L'Abbé *et al.* 2011; Hefner 2009). While the majority of variables assessed showed significant relationships with population variation, the scoring of the features showed poor scoring consistency (low inter-observer repeatability) and without improvements to the trait definitions, the method may not be very useful at present in estimating population affinity (L'Abbé *et al.* 2011; Hefner 2009). Therefore, to estimate sex and population affinity, a

combination of osteometric and morphoscopic results may provide the best possible option for obtaining accurate estimates.

For osteometric methods, discriminant functions can be used to assess the data and provide estimates of both population affinity and sex. Fordisc 3.0 (FD3) was created in 2005 as a software program that runs linear discriminant functions (LDA) and can make use of an automatic stepwise variable selection process to choose only the most discriminatory variables on a case-by-case basis (Jantz & Ousley 2005; Ousley & Jantz 2012). The FD3 program has steadily become the more preferred method to use in many forensic labs throughout the world to estimate the population affinity and sex of an unknown individual (Krüger *et al.* 2017; Manthey *et al.* 2018). Reference databases, to which unknown individuals can be compared, are typically made up of measurements obtained from measured and digitised skeletal material. However, while three-dimensional (3D) coordinates may be available for the individuals that were digitised and can thus be assessed for both size and shape variation, the data obtained from linear measurements is limited in the shape differences that can be observed.

Geometric morphometrics (GM) makes use of 3D coordinate data to compare the size and shape of different objects or groups and is not limited to two dimensions as are traditional measures and interlandmark distances (ILDs) (Klingenberg 2011). GM has been used in multiple fields for many years including biological anthropology, where it involves the assessment of cranial and postcranial variation in human skeletal remains (Kimmerle *et al.* 2008; Stull *et al.* 2014a; Spradley & Jantz 2016; Maass & Friedling 2018; Small *et al.* 2018; Krenn *et al.* 2022). Previous studies using both linear measurements and GM to assess population affinity from the crania of modern black, coloured and white South Africans have shown the potential of using GM over linear measurements, where correct classification rates were higher for the former method (Stull *et al.* 2014a). Furthermore, a study comparing the use of GM, non-standard ILDs and standard linear measurements noted that non-standard ILDs

outperformed the other two methods when estimating population affinity for the current North American population structure. The results further suggested that the non-standard cranial ILDs derived from landmark data collection might be able to improve classifications in diverse populations (Spradley & Jantz 2016).

However, while digitising physical skulls is possible to be able to make use of GM to compare an unknown to a reference sample, digitising is also more difficult as the skull needs to remain in the same position when locating each landmark so that the relationships between the coordinates is consistent with the original size and shape of the object. However, some landmarks are located on the inferior surface of the skull and are hard to reach, making this method less user-friendly. Furthermore, the digitising equipment required to collect the coordinate data is expensive and alternative methods need to be available for an instance when the equipment is not available at all. Therefore, taking linear measurements using calipers is not only less time-consuming than digitizing remains, it is an affordable method of exploring population and sex variation from skeletal remains and is most often used when assessing skeletal remains in forensic casework.

While digitising skeletal material provides new avenues for applying advanced methods as a means to assess skeletal variation, the variation observed may be limited to the skeletal material that is available in a collection. Skeletal collections are not only limited by the number of individuals that donate themselves for teaching and/or research, but also in their inability to change as continuously as the contemporary population does. The Raymond A. Dart Collection was started in the early 1920s, whereas the Pretoria Bone Collection and Kirsten Collection were initiated in the 1940s and 1950s, respectively (Dayal *et al.* 2009; Alblas *et al.* 2018; L'Abbé *et al.* 2021). While some individuals in the collections are representative of the current South African population, many individuals are most likely not due to changes in the

population variation and secular trends that may have created differences in the skeletal variation present in the collections and in the current South African population.

Skeletal remains of black, coloured and white South Africans are available in multiple University skeletal collections throughout the country and are typically used to create reference databases for use in South Africa. While these databases include measurements of black, coloured and white South Africans that were considered modern at the time, populations are constantly changing, and reference data that was once an appropriate representation of the South African population may no longer be as effective as reference samples when estimating the parameters of the biological profile. Furthermore, multiple other population groups (minority groups in South Africa and immigrants from other countries) reside in South Africa that are not represented in these medical school skeletal collections. For example, Indian South Africans are typically not available in any skeletal collection, mainly due to issues with regard to body donation and their religious beliefs and traditions (Mehta *et al.* 2015). The majority of Indian South Africans are of Hindu faith. In Hinduism, deceased individuals need to be cremated without unnecessary delay (Burton & Underwood 2007). Hindus only make up approximately 41% of the Indian South African population; however, another 25% of Indian South Africans are Muslim, which similarly requires deceased individuals to be buried without delay (Burton & Underwood 2007). While the practices differ between the two religions, the traditions indicate that donations to medical schools are not common practice, which in turn creates a lack of skeletal remains available for research. While Indian South Africans only make up a small proportion of the country's population when compared to black, coloured and white South Africans, the numbers are far larger in selected areas, particularly certain suburbs, throughout the country. The suburbs mainly populated by Indian South Africans include Chatsworth (KwaZulu-Natal), Lenasia (Gauteng) and Erasmia (Gauteng), where 55-77% of the individuals living in those areas are of Indian descent (Statistics South Africa 2011). In

addition, storage of skeletal remains is an issue in many Universities, as space is limited and often finite, which in turn limits the sample sizes available for study and limits the addition of new individuals. Therefore, in recent years, attempts have been made to find alternative sources of skeletal samples.

The use of computed tomography (CT) scans of living individuals is a common practice in biological anthropological research, as the use of living individuals has great advantages, particularly with regard to collection and use. Issues associated with digitising and landmark placement are not relevant, as the skull models can be rotated in the virtual space without affecting the relationship between coordinates. Additionally, the 3D nature of reconstructed scans allows the skulls to be assessed using multiple techniques, namely traditional linear measurements, non-standard ILDs and GM, as well as scoring morphological traits according to the expressions of the traits (morphoscopic analysis; Dereli *et al.* 2018). Traditional linear measurements are the direct distances between specific landmarks on a bone. ILDs are similar to traditional linear measurements in that they are the direct distances between two landmarks; however, this method also makes use of other distances that are not typically assessed using traditional linear measurements.

Previously, issues were raised as to whether measurements or landmarks taken from CT scans were comparable to measurements taken from bone as well as whether the placement of landmarks or taking of measurements was repeatable on scans. Both issues have been addressed in recent studies and have shown that not only are CT scans comparable to bone, but also the landmark placement is repeatable with standard descriptions (Cavalcanti *et al.* 2004; Stull *et al.* 2014b; Colman *et al.* 2017). Additional advantages of using CT scans include that religious beliefs/death rituals are not factors that need to be considered, as the individuals are still living and could provide consent. Furthermore, by making use of living individuals, the research samples are contemporary and more representative of the current population.

While multiple standards are available to which an unknown individual can be compared, most standards are population-specific, necessitating the re-evaluation of existing methods, the addition of novel techniques and the exploration of additional modern comparative samples (Krüger *et al.* 2015; Krüger *et al.* 2018; Manthey *et al.* 2018). Although 3D reconstructed models (from CT scans) have been used in anthropological research (Decker *et al.* 2011; Franklin *et al.* 2012, 2014; Mehta *et al.* 2014, 2015) and dry skulls have been assessed using GM (Stull *et al.* 2014a; Small *et al.* 2018), the combination of using GM to analyse 3D coordinates taken from reconstructed CT scans has not been used to evaluate modern cranial variation in South Africa.

The purpose of this research was to use 3D CT models to explore the cranial variation present in current Indian South Africans. Additionally, cranial sexual dimorphism and size and shape variation were explored and contrasted among the various current South African populations to establish whether the data could be used to confidently distinguish among the groups when estimating sex and population affinity from the cranium. This includes assessing sexual dimorphism of four morphoscopic Walker (2008) traits and creating new population-specific standards for estimating sex using those traits. Furthermore, assessing 3D landmarks and ILDs to assess any size and shape differences that can be used to estimate sex and population affinity for current black, coloured, Indian and white South Africans, and resulting in a new reference craniometric database for use with FD3.

CHAPTER 2: LITERATURE REVIEW

2.1 The race concept in biological anthropology

The history of the biological concept of race in biological anthropology has undergone significant shifts over time, reflecting changes in scientific understanding and socio-political contexts (Littlefield *et al.* 1982; Dubow 1995; Caspari 2003; Edgar & Hunley 2009). Early anthropologists attempted to classify humans into discrete racial "types" based on physical traits. However, this typological approach, prevalent in the 18th and 19th centuries, supported theories of racial hierarchy and scientific racism, perpetuating discrimination and inequality (Caspari 2003; Dubow 1995; Littlefield *et al.* 1982).

However, it became increasingly clear that human populations cannot be neatly categorized into distinct biological groups, as human diversity is shaped by a complex interplay of biological, cultural, and environmental factors (Edgar & Hunley 2009; L'Abbé *et al.* 2013a). Human populations are not simply biological subgroups. Culture, religion, language and geography can all be used to define significant groupings of people and are factors that contribute to human variation (Edgar & Hunley 2009; L'Abbé *et al.* 2013a).

Therefore, rather than a biological concept, race is recognised as a social concept that can be defined as the categorisations people use to identify themselves and the people around them (Edgar 2009). A person's social race, whether it is a self-identified label or one imposed by society, remains an important part of their identity. The social construct does not fade away when the individual passes away; it persists throughout the identification process, making its mark on records such as death certificates. As noted by Cunha and Ubelaker (2020), forensic anthropologists often use social race terminology when describing missing persons, thereby necessitating its usage in their own work. However, there is a growing recognition within the

field that the traditional race concept is overly simplistic when it comes to explaining human biological diversity.

Therefore, most recent studies have moved away from the historical approach of estimating defined racial “types” to estimating ancestry, which comprises assessing inherent variation between populations based on population origins (Ousley *et al.* 2009). Yet, the landscape of forensic anthropology is evolving. Researchers, such as Spradley and Jantz (2021) and Ross and Pilloud (2021) are advocating for a fundamental shift away from the terms "ancestry" and "race”. Instead, they propose embracing the concept of "population affinity". This approach recognizes that human biological variation is shaped by a complex interplay of microevolutionary forces, temporal shifts and historical events like colonization and the various slave trades, and in South Africa, the forced segregation of populations during Apartheid. The term "population affinity" is grounded in statistical methodologies, aligning with the rigorous approaches used in population structure analysis and indicates similarity to a population rather than a forced racial categorization (Ross & Pilloud 2021; Spradley & Jantz 2021). It emphasizes populations as groups defined by shared characteristics without the limitations and biases inherent in racial typology (Spradley & Jantz 2021). Regardless of the terminology used, research into the accuracy of population affinity estimations by forensic anthropologists has reported commendable success rates, reaching as high as 90.9% (Stull *et al.* 2021). The estimation of population affinity is possible because a relationship exists between skeletal dimensions and self-designated or peer-reported social groupings, which is as a result of the different origins and population histories of each group (Jantz & Ousley 2005; Ousley *et al.* 2009).

Past segregation laws and social barriers also played a role in the morphological variation present in a population. Institutional racism associated with *Apartheid* supported endogamy, which in turn limited gene flow and resulted in distinct skeletal differences among the social

groups in South Africa (L'Abbé *et al.* 2013a). While the segregation laws implemented during *Apartheid* were abolished, social behaviour continued to limit gene flow among groups. Therefore, while ancestry or population affinity is not a vindication of the race concept or the notion of biological "types", the biological consequences of racism can be used to help explain the range of variation currently observed in the South African population and can aid in estimating the population affinity of an unknown individual (Sauer 1992).

In the scope of this dissertation, "ancestry" denotes discernible skeletal differences evident within and among global populations. "Population" refers to a collective assembly of individuals residing in a defined geographic area. For example, when examining the South African population, the term population encompasses individuals who identify as South African. Additionally, the term "population group" denotes specific subdivisions within the South African population, characterized by the prevailing social categorization system in the nation. These subdivisions include individuals of diverse racial backgrounds, including black, coloured, Indian and white South Africans.

2.2 Population histories

The 1950 Population Registration Act established the designations of black, white, coloured and Indian South Africans for the purpose of physically separating groups based on skin colour (Erasmus 2012). However, while the Population Registration Act was no longer used after 1991, the social classifications used in South Africa were retained for the purpose of transformation and redress (Posel 2001).

As of 2022 the South African population was estimated to consist of 60.6 million people of which the largest proportion is black (81.0%), followed by coloureds (8.8%), whites (7.7%) and Indians/Asians (2.6%) (Statistics South Africa 2022).

2.2.1 Black South Africans

The current black South African population arose mainly from Bantu-speaking groups, originally from Nigeria/Cameroon, that migrated into South Africa within the past 5000 to 3000 years (Beck 2000; Tishkoff & Williams 2002; Tishkoff *et al.* 2009; May *et al.* 2013). While gene flow was present between the historical Bantu-speaking and indigenous Khoesan groups early in history, these groups are still genetically and morphologically different (Herbert 1990; Stynder 2009; Liebenberg *et al.* 2015a). In historical literature, Bantu-speaking males were described as having "imposing height" and the females were "no less well-built" (Thompson 2001:20). However, over time Bantu-speakers in southern Africa differentiated from their ancestors in Central Africa due to changes in the environment as well as genetic influences from the indigenous Khoesan, with substantial Khoesan genetic contribution, (20-23%), identified in the various South African ethnic groups (Petersen *et al.* 2013; Gurdasani *et al.* 2015; Uren *et al.* 2016). In this instance, "ethnic groups" refers to linguistic and cultural groups categorised within the black South African population group and include the Pedi, Sotho, Venda, Swazi, Xhosa, Ndebele, Tsonga and Zulu people.

2.2.2 Coloured South Africans

South African coloureds are a highly varied population that has been shown to possess the highest levels of intercontinental genetic diversity of any global population. Similar parental contributions from Khoesan and Bantu-speaking groups, with slightly lower contributions from both Indian and European ancestry were noted in the genetic makeup (Tishkoff 2009; de Wit *et al.* 2010; Patterson *et al.* 2010; Quintana-Murci *et al.* 2010). According to historical records, the first Indians were brought to the then Dutch Cape Colony in the Cape in 1653 and were subsequently sold to early Dutch settlers as slaves. Most of the Indian slaves were shipped from Bengal (Northeast India) or the Coromandal coast (Southeast India) but were not allowed to

preserve their distinct "Indian" identity in the Cape. It was common for the slaves to marry other East Asian or African slaves, or even the indigenous Khoesan inhabitants. Their progeny subsequently became known as "Malays". In later years, the term "Malays" was used to refer to all the Muslims in the Cape, irrespective of their geographic origin. Later on, however, during *Apartheid* they came under the designation of "Coloured" (Indian Council of World Affairs 2001).

Unbalanced sex-specific contributions of the various population genetic components have been shown in the South African coloured group, the most pronounced being the massive maternal contribution of Khoesan ancestry (more than 60%) and the almost negligible maternal contribution of European ancestry with respect to their paternal counterparts (Quintana-Murci *et al.* 2010). The paternal contribution for coloured South Africans included a substantial proportion of European genes and an almost equal proportion of African genes (Quintana-Murci *et al.* 2010). The sex-biased admixture indicates that the modern South African coloured population results mainly from the early encounters of European and African males with Khoesan and Indian females (Quintana-Murci *et al.* 2010; Chimusa *et al.* 2013). The Khoesan ancestors were small, delicately built people, particularly in stature, which can be noted in the current coloured population (Thompson 2001).

2.2.3 White South Africans

White South Africans are descended largely from colonial immigrants including Dutch, French, British and German groups (Steyn & İşcan 1998; L'Abbé *et al.* 2011), with recent research suggesting almost equal contributions from all European groups (Greeff 2007). Genetic evidence also suggests genetic contributions from European males and their slaves from southern Africa, most likely the Khoesan and Indian females (Hollfelder *et al.* 2020). Low frequencies of alleles typical to the Khoesan and Bantu-speaking people are found in

white South Africans, which is most likely as a result of admixture between the European males and their slaves from southern Africa, most likely the Khoesan. Slightly higher frequencies of Indian genetic contribution have been noted in white South Africans (Hollfelder *et al.* 2020). As the emigrants were predominantly male, a female bias of non-European influence was noted (Steyn & İşcan 1998; Greeff 2007).

2.2.4 Indian South Africans

After 1652, the next time Indians were brought to South Africa was only in 1860, more than 200 years after the first group. The latter group of Indians came mainly from the southern and eastern parts of India, most escaping extreme poverty conditions (Bhat & Narayan 2010). Among the first groups 75% were male, and 25% were female. No children were among the passengers. In later trips, more females (up to 41%) were brought to SA (Indian Council of World Affairs 2001). However, other groups of Indians also came over to SA on their own, most from the Gujarat region in north-west India (Bhat & Narayan 2010) and some from Mauritius and East Africa (Indian Council of World Affairs 2001). With the different origins came different languages, cultures and religions. While a few of the individuals were of Christian faith, and a small number were Muslim, the majority were Hindus of different castes (Indian Council of World Affairs 2001). Most of the free or passenger Indians were Muslim and came from northern and western India to trade among the indentured Indians and later expanded inland as shopkeepers that supplied all populations. Indians were only considered permanent residents of South Africa in 1961 (Indian Council of World Affairs 2001; Bhat 2010). Genetic evidence in the Indian South African population indicates potential limited maternal gene flow from indigenous Khoesan, while the remaining genetic contributions demonstrate very little variation from the genes of Indians (in India) (Soodyall & Jenkins 1992). Strict endogamy and religious customs are most likely responsible for the limited

genetic heterogeneity of the Indian South Africans when compared to the other South African populations.

A combination of linguistic and genetic evidence indicates that Indians (in India) have multiple language influences that have shaped their languages for over thousands of years. The linguistic evidence suggests that Indians in the north-western states of India tend more towards languages with Indo-European influence, whereas Indians in the south-eastern states tend more toward languages with Dravidian influence (Reich *et al.* 2009; Moorjani *et al.* 2013). The attached map is a rough indicator of the spread of different influences on the languages spoken throughout India (Figure 2.1).

However, the language influence is also supported by genetic evidence, which suggests that Ancestral North Indians have larger genetic influences from West Eurasians, whereas Ancestral South Indians have a larger genetic influence from indigenous Andaman Islanders. Ancestral North Indian ancestry ranges from 39-71% in India and is higher in the traditionally upper castes and in Indo-European speakers (Reich *et al.* 2009). Some historians have argued that the caste system in modern India was initially implemented by colonialists as it became more rigid under colonial rule; however, a study by Reich *et al.* (2009) suggested that many current distinctions among groups are ancient and that strong endogamy must have shaped marriage patterns in India for thousands of years. Allele frequency differences between groups in India are larger than in Europe, reflecting strong founder effects whose signatures have been maintained for thousands of years due to endogamy (Reich *et al.* 2009).

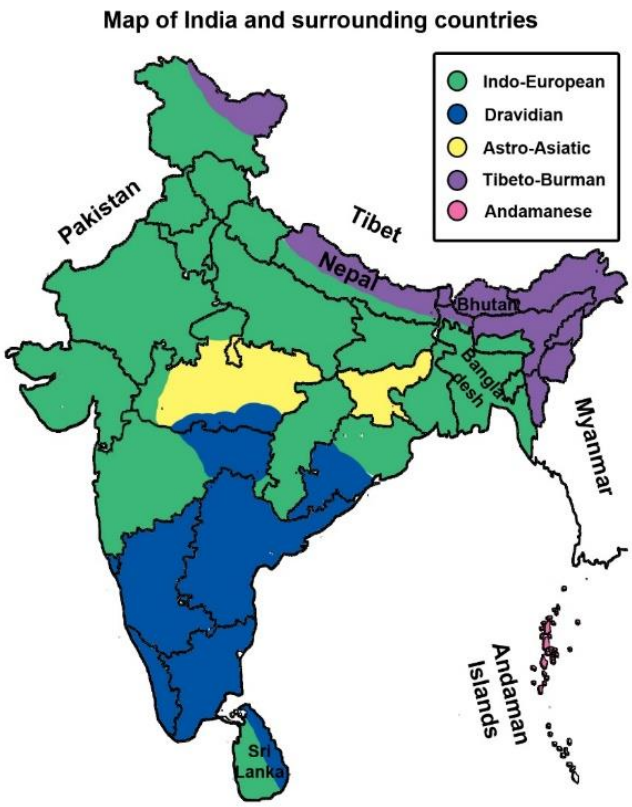

Figure 2.1. Map depicting the linguistic origins in the modern languages spoken in India.

2.3 The effects of diasporas and secular trends on skeletal morphology

The term diaspora, or the spread of a population away from the homeland, is often used to define large-scale migration events, including forced migrations, such as the slave trade between West Africa and the Americas and between India and South Africa, among others. Because migrations often result in adaptation to new environments and the restructuring of gene flow, they produce biological consequences in the human genotype and phenotype (developmental plasticity) (Spradley 2006). When dramatic shifts in living standards or exposure to a new environment occur, physical changes may take place within the given population (Relethford 2004; Spradley 2006). These types of changes over time are known as secular change and are thought to be the result of an improvement or a decline in environmental conditions (Cameron *et al.* 1990; Spradley 2006; Langley & Jantz 2020).

Significant craniofacial secular changes have been documented in American Whites and Blacks over the past 150 years (Jantz & Meadows Jantz 2000; Jantz 2001; Wescott & Jantz 2005; Langley & Jantz 2020). Other studies have focused exclusively on the craniofacial morphological changes taking place since the arrival of Africans in the Americas, some 350 years ago, and have assessed whether these changes result from selection, environment, plasticity, or gene flow (admixture) (Spradley 2006). Results suggest that significant craniofacial secular changes have taken place from 1700 to 1975 and that a genetic association with craniofacial morphology is apparent (Spradley 2006).

Further studies have also suggested that cranial morphology retains population history and that different aspects of cranial morphology can preserve different kinds of information. Both the temporal bone and neurocranium shape have shown the ability to retain neutral genetics well, even though, past research has noted that the neurocranium is generally too developmentally plastic and heavily influenced by environmental factors to retain any information with regard to population history (Harvati & Weaver 2006). While developmental plasticity and climatic adaptation can have an impact on cranial variation, these factors generally do not obscure or erase the underlying patterns of population structure and history (Relethford 2004).

Living conditions not only have an effect on population variation, but also on the degree of sexual dimorphism observed in a population (Harris 1997; Spradley *et al.* 2016). Enhanced environments and dietary conditions can cause an increase in the degree of sexual dimorphism, whereas unfavourable conditions can cause a decrease (Tobias 1971; Gray & Wolfe 1980; Henneberg & Van den Berg 1990; Barrier 2007). The complex interaction between genetics and environmental effects influence bone shape and size; therefore, factors such as diet, activity and disease have the potential to alter bone morphology (Klepinger 2001; Ruff *et al.* 2006).

A study on secular changes in the basicranium of North Indians revealed that significant differences were present between contemporary and sub-recent museum specimens (added to the collection approximately 50 years prior to analysis) from Northern India. The degree of sexual dimorphism present in the basicranium decreased over time, even though the dimensions of both contemporary males and females increased in size (Saini *et al.* 2014). For the most part males tend to be more robust than their female counterparts (Rogers & Mukherjee 1992; Loth & İşcan 2000; Gustafsson & Lindenfors 2004). However, the degree of sexual dimorphism within a group can be affected by genetics, social status, environmental and nutritional factors (Cameron 2002; Barrier 2007). As age, environment, nutrition and socio-economic standings vary within and among populations, understanding these factors may help in understanding skeletal variation observed in different geographic populations.

2.4 Population affinity estimation

While some anthropologists argue that population affinity estimates are generally more difficult, less precise and overall less reliable than the estimates of other biological parameters, many advances have been made in recent years (White & Folkens 2005; Liebenberg *et al.* 2015). With the development of new methods and the application of more advanced statistical techniques, the estimation of population affinity is becoming much more reliable and valid (Gill 1998a,b; Gill 2009).

In past research, the major social racial groups (European, African, and East Asian) have been shown to exhibit some general differences in facial features. Many European skulls exhibit a somewhat flat face with zygomatic bones that retreat or slant back and nasal openings that tend to be longer and narrower than in other groups (Stavrianos *et al.* 2012). The African skulls often exhibit alveolar prognathism, which is expressed as a projection of the lower face, as well as nasal openings that tend to be wider and shorter than in European and East Asian

individuals with a bridge that is broader and flatter (Stavrianos *et al.* 2012). The East Asian category also takes Native Americans, American Eskimos and various Asian groups into account. The faces tend to be flattened with a short cranial vault (distance from glabella [front] to opisthocranion [back]) and a cheek area that is usually quite wide with projecting zygomatic bones. The width of the nasal aperture is also somewhere between European and African groups (Stavrianos *et al.* 2012). Although the midface traits show general differences between groups, without a quantification of the traits, the morphology alone would not be useful in forensic contexts.

The cranium is generally used to facilitate the estimation of population affinity and in an attempt to quantify the variations observed among European, African and East Asian groups, morphoscopic methods have been applied to multiple cranial features, with mid-facial features, such as the nose, often producing the best results (Gill 1998a,b; Hefner *et al.* 2009). While morphoscopic studies have assessed trait frequencies in different population groups in North America, research is somewhat limited. A validation study on thirteen morphoscopic traits developed in North America address the application of the traits to the estimation of population affinity in three South African groups, namely white, black and coloured South Africans (Hefner *et al.* 2009; L'Abbé *et al.* 2011). The frequency distributions were tested and revealed that frequency distributions were highly variable among the groups, which limits the applicability of the original method to estimating population affinity in South Africa (L'Abbé *et al.* 2011). Furthermore, the study noted poor repeatability in most scores, which may be due to unclear definitions or due to the character states not adequately describing the observable morphology in the population. The validation study revealed the need for a re-evaluation and re-calibration of the traits to make the methodology more applicable to the South African population and to create standards for possible use in forensic anthropology casework (L'Abbé *et al.* 2011).

While an improvement in the scoring methodology and the addition of population-specific standard may make the method more useful, geometric morphometric studies have been used to observe some of the shape difference in the cranium that are also noted in the morphoscopic traits. The use of GM to assess population variation has shown great potential (correct classification rates of 89% in black, coloured and white South Africans) and also proves more versatile as the methodology is not limited to using either size or shape, but can make use of both variables (form) to assess variation (Stull *et al.* 2014a). Furthermore, observer error rates tend to be lower for osteometric and geometric morphometric methods than for morphoscopic techniques, which are still somewhat subjective.

While the postcranial skeleton has been assessed for multiple populations and has shown great promise in estimating population affinity with correct classification rates of 84% and above (Liebenberg 2015, Liebenberg *et al.* 2015b), the cranium still holds valuable information and may sometimes be the only skeletal element available for analysis. Some studies that have proven to be proficient in discriminating among South Africans, yielded accuracies between 73% and 98%. However, the total number of groups compared differed among studies with some studies only comparing black and white South Africans (İşcan & Steyn 1999; McDowell *et al.* 2012; L'Abbé *et al.* 2013a; Stull *et al.* 2014a).

2.5 Sexual dimorphism and sex estimation

2.5.1 Genetic expressions of sexual dimorphism

The interaction of hormonal environments and genes on the X-chromosome lead to distinct variations in the expressions of different quantitative traits in males and females (Cameron 2002; Weiss *et al.* 2006). Sexual dimorphism is population-specific due to the differences in geographical location as well as in the evolutionary influences and experiences of these groups (Garvin *et al.* 2014); therefore, the degree of sexual dimorphism as well as the features that are

more or less dimorphic may vary between and among populations. Considerable differences in sexual dimorphism between different populations invalidate sex estimation techniques when data sources are based on populations with different geographical and ancestral origins. Therefore, methods based on one population need to be validated and possibly re-calibrated to improve standards in all populations and their sub-groups.

Diversity in size and shape of the facial skeleton arises through ontogenesis, environmental and epigenetic influences, and masticatory function (Kemkes & Göbel 2006). Sexual dimorphism in facial size has been noted around 14 years of age and develops with the onset of puberty in association with the skeletal adolescent growth spurt. While the age at onset and duration differs between males and females, the process is mainly genetic and under hormonal control, which produces extreme differences in later growing regions (mandible, maxilla, upper face, cranial head height and parts of the cranial base) (Humphrey 1998; Rogers 2005; Saini *et al.* 2014).

During growth, parts of the upper face, particularly the orbits, are said to be less dimorphic as they gain their final size before the later growing regions such as the maxilla and nasal regions, which continue to grow for longer and therefore have greater opportunity to develop sexual dimorphism (Enlow 1990; Ferrario *et al.* 1993; Saini *et al.* 2011). While orbits generally display limited sexual dimorphism in the overall shape, the supraorbital margin thickness has been shown to be useful in estimating sex in a South African sample (Walker 2008; Krüger *et al.* 2015).

2.5.2 Sex estimation methods from the skull

Accurate sex estimation is based on the interpretation and quantification of the expression of sexual dimorphism and on the degree of sexual dimorphism present in a particular population (Loth & İşcan 2000; Gapert *et al.* 2009; McDowell *et al.* 2012). Craniometric

studies on South African groups noted considerably lower levels of sexual dimorphism than in North American groups (Ousley & L'Abbé 2010). Overall, South African black males are relatively smaller and South African black females are relatively larger when compared to their North American counterparts (Ousley & L'Abbé 2010; L'Abbé *et al.* 2013a). Greater size differences exist between the crania of South African white males and females compared to South African black males and females, which also suggests a greater degree of sexual dimorphism in white than in black South Africans.

Similar findings were obtained in a morphoscopic study that estimated sex in a South African sample (Krüger *et al.* 2015). Five morphoscopic traits (glabella, mastoid process, mental eminence, nuchal crest and supraorbital margin) were assessed for their use in estimating sex in white and black South Africans. The modified formulae were created for ease of application in forensic cases specific to South Africa and achieved accuracy rates that ranged between 84% and 93% (Tables 8-10). White South African males obtained overall higher scores, whereas black South African males obtained more intermediate results, suggesting that white males were more robust. White South African females similarly had slightly higher scores than black South African females, which suggested that black South African females were overall the most gracile of the four groups. The overall larger difference in scores between white South African males and females when compared to the difference in scores between black South Africans males and females suggests a greater degree of sexual dimorphism in white compared to black South Africans (Krüger *et al.* 2015).

Craniometric studies estimating sex from the skulls of South Africans obtained classification accuracies of 86% (white South Africans only) (Steyn & İşcan 1998) and of approximately 76% (black and white South Africans; L'Abbé *et al.* 2013a). Although the classification accuracies of the studies are at least 50% better than chance, other non-standard measurements may be able to explore more sexually dimorphic regions or features in the skull

and thereby obtain higher classification accuracies that are comparative to results obtained from postcranial remains (classification accuracies up to 98%; Krüger *et al.* 2017). Morphoscopic analyses in South Africa have obtained higher correct classification rates than cranial linear measurements, which suggests that sexual dimorphism is present in the cranium, but alternative methods need to be utilized, such as shape analysis or non-standard ILDs, to be able to fully explore the sexual dimorphism present in the cranium.

2.6 Anthropology research on Indian populations

Most studies in India revolve around sexual dimorphism and the estimation of sex and are often limited to individual postcranial bones, such as the femur (Purkait 2002, 2005; Purkait & Chandra 2004; Srivastava *et al.* 2013), the sternum (Chandrakanth *et al.* 2014), the ulna (Srivastava *et al.* 2013), and the sacrum (Arora *et al.* 2010), or even anthropometric comparisons (hand, foot or finger lengths or sizes – Kanchan & Rastogi 2009; Kanchan & Krishan 2011; Krishan *et al.* 2011; Krishan *et al.* 2013). However, many have also assessed the cranium, mandible and/or dentition for differences between males and females (Deshmukh & Devershi 2006; Saini *et al.* 2011, 2014; Raghavendra Babu *et al.* 2012; Kanchan *et al.* 2013; Raj & Ramesh 2013; Mehta *et al.* 2014, 2015; Singh *et al.* 2015; Ramamoorthy *et al.* 2016). While some cranial studies have made use of dissected skulls, the more current studies have made use of CT scans to assess the skeleton. While different modes of assessment are used, the main consensus is that the skull is sexually dimorphic and, according to most studies, can be used to estimate sex in an Indian population. Accuracies range from 66.7% to 89% (most studies obtain accuracies between 85-88%), with some regions of the skull showing more potential than others (Deshmukh & Devershi 2006; Saini *et al.* 2011, 2014; Raghavendra Babu *et al.* 2012; Kanchan *et al.* 2013; Raj & Ramesh 2013; Mehta *et al.* 2014, 2015; Singh 2015; Ramamoorthy 2016). Nasal height, nasal breadth, mastoid process length and the foramen magnum length and breadth have shown to be sexually dimorphic in a South Indian population

(Vidya *et al.* 2012). However, even though the skull has been shown to be sexually dimorphic, most studies do not make full use of all landmarks or measurements that can be taken from the skull and as such may not be exploring the full potential of the skull for estimating sex. Furthermore, most studies also make use of indices or individual non-standard measurements to estimate sex but multivariate approaches far outperform univariate analyses (Deshmukh & Devershi 2006; Ousley & Jantz 2012; Ousley & Jantz 2013). Therefore, while many researchers have explored different aspects of bones, very few have made use of multivariate techniques or GM and no research was encountered on the use of morphoscopic assessments, such as the Walker (2008) method of estimating sex from the skull, in Indian populations.

In most sex estimation studies in India, males and females of either North Indian or South Indian samples are compared. The lack of population affinity estimation studies is understandable due to limited number of non-Indian population groups or admixture with other populations that occurred in India. Some studies, however, have attempted to explore the possibility of differences among North Indian and South Indian individuals. In her doctoral thesis, Rogers (1999) compared North and South Indians and noted no significant differences between the two groups (Rogers 1999). However, a study by Bhasin *et al.* (1994) had shown that North Indians tended to cluster together and separate from other Indian regional groups (Bhasin *et al.* 1994). Other studies have also noted some differences among Indians from different regions in India (Mehta *et al.* 2014). The differences may be due to different geographical regions, environments, climates and cultural effects on the skeleton (Mehta *et al.* 2014). Kanchan *et al.* (2014) also noted different frequencies of specific cranial shapes/types in different regions throughout India (Kanchan *et al.* 2014). While variation within the Indian population has been assessed in multiple studies, very little research is available that compared Indian and non-Indian groups. Rogers (1999) also compared the skeletal remains of Indians with those of European ancestry (white Canadians and North Americans). The major findings

of the research suggested that the two population groups were significantly different. The major distinction was noted in the robusticity, whereas other differences seemed more subtle. Indians tend to be more gracile and smaller than the Europeans in the study. Some areas of the midface morphology showed similarities; however, other regions showed significant differences, particularly in the inter-orbital region and the degree of prognathism (Rogers 1999). While this research seems promising in revealing variation between Indians and individuals of European descent, multiple points of sample bias were noted that might affect the validity of the results. Samples used in Rogers' (1999) study were collected from various sources (cadaver and skeletal material obtained through a biological supply company) and although the population affinities were known, the ages and sexes had to be estimated prior to analysis. Furthermore, the sample was skewed with more estimated males available for analysis than females (Rogers 1999). Another study that explored differences between Indians and other groups noted some difference to other populations outside of India (Mehta *et al.* 2014). However, no statistical test was used to examine the significance of the differences. Furthermore, the differences were based on craniofacial indices, which are extensively limited in the amount of human variation that can be captured and should not be used in forensic anthropological analyses (Kanchan *et al.* 2014; Liebenberg *et al.* 2015a).

2.7 Computed tomography in anthropology

Computed tomography (previously referred to as computed axial tomography, or CAT scan) makes use of radiation (X-Rays) to produce an image of a subject that contains volumetric data (Allard 2006; Garcia de Leon Valenzuela 2014). The resulting data can then be saved in a variety of formats, both two-dimensional (2D) and 3D.

Similar to many European countries, India is extremely limited in terms of skeletal availability, which has resulted in a shift towards using both 2D CT scans and 3D models

created from the CT scans as alternatives to using bones. Studies have assessed some postcranial bones as well as the skull and have noted the usefulness of using this format for anthropological research (Decker *et al.* 2011; Franklin *et al.* 2012, 2014; Mehta *et al.* 2014, 2015). While CT scans are mostly used to estimate sex or regional variation from the skull, the researchers rarely made use of all the standard landmarks available in the cranium and rather focused on finding new methods or skeletal areas to assess (Kanchan *et al.* 2014; Mehta *et al.* 2014, 2015). One problem noted in the Indian CT studies is the use of large slice thicknesses up to 5mm, which may cause inconsistencies in measurements (Mehta *et al.* 2014). In anthropological analyses, measurements discrepancies of 2mm between observers are generally considered acceptable (Stull *et al.* 2014b; Langley *et al.* 2016; Colman *et al.* 2017; Mbonani *et al.* 2023). In a study to compare the geometrical precision of virtual bone models when varying imaging conditions, such as scanner type and standard patient scanning protocol, slice thickness and exposure level were tested. The results indicated segmentation and imaging conditions rarely exceeded the generally accepted linear error of 2 mm (Stull *et al.* 2014b; Colman *et al.* 2017). However, the study compared slice thickness of 0.9 mm and 3.0 mm, which are still smaller than the 5mm used in some studies. Furthermore, most studies use only linear measurements taken directly on the skull, rather than making full use of the 3D nature of the scans (Mehta *et al.* 2014, 2015).

The use of CT scans for anthropological research is not limited to India. Many anthropologists all over the world have made use of CT scans in recent years; however, the focus is usually on discovering new features or structures to assess, or to examine a combination of soft tissues and bone to improve facial reconstruction methods (Turner *et al.* 2005; Tilotta *et al.* 2009; Guyomarc'h *et al.* 2012a; Guyomarc'h *et al.* 2013, 2014; Ridel *et al.* 2018). Research using CT scans has often focused more on assessing inner cranial structures that are hard to explore on physical skulls, such as the sinus shapes or the cochlea, than on

measurements of the cranium (Rodt *et al.* 2002; Fatterpekar *et al.* 2006; Vidya *et al.* 2013; Belaldavar *et al.* 2014). While this research is valuable when CT scanners or X-ray machines are available during forensic analyses, some features, such as frontal sinus shape, cannot be assessed in dry bones.

In addition to the limitations in the availability of skeletal collections in some countries, the stagnant nature of most skeletal collections suggests that anthropological studies on modern populations may benefit from the incorporation of CT scans from living individuals that are representative of the current populations. The use of CT scans has the potential to make continuously updating reference databases possible, so that the data are always applicable to modern populations. Furthermore, while robust statistical analyses are necessary in anthropological research, the large sample sizes required by the methods often necessitate the use of multiple skeletal collections. Therefore, studies that require large sample sizes may also benefit from the near unlimited numbers of CT scans available.

CHAPTER 3: MATERIALS AND METHODS

3.1. Sample, scanning procedures and data acquisition

A total of 408 head CT scans was assessed for the current study with equal sample sizes for males and females of black, coloured, white and Indian South Africans (51 per group; Table 3.1). All scans were obtained from the Steve Biko Academic Hospital (Pretoria, Gauteng), the Groote Schuur Hospital (Cape Town, Western Cape) and the Inkosi Albert Luthuli Central Hospital (Durban, KwaZulu-Natal). As certain populations are more populous in certain areas, the scans were obtained from hospitals in areas where the groups are most prevalent. For example, as coloured South Africans reside mainly in the Western Cape, the scans for the population were obtained from the Groote Schuur Hospital. Furthermore, as Indian South Africans have a larger presence in KwaZulu-Natal, the scans for Indian South Africans were collected from the Inkosi Albert Luthuli Central Hospital. CT scans for black and white South Africans were widely available and thus collected from all three hospitals.

Table 3.1. Distribution and sources of the sample

Population-sex group	Abbreviation	*n*	Source
Black South African males	BM	34	Groote Schuur Hospital
		17	Inkosi Albert Luthuli Central Hospital
Black South African females	BF	22	Groote Schuur Hospital
		29	Inkosi Albert Luthuli Central Hospital
Population group total		**102**	
White South African males	WM	16	Steve Biko Academic Hospital
		18	Groote Schuur Hospital
		17	Inkosi Albert Luthuli Central Hospital
White South African females	WF	28	Steve Biko Academic Hospital
		4	Groote Schuur Hospital
		19	Inkosi Albert Luthuli Central Hospital
Population group total		**102**	
Coloured South African males	CM	51	Groote Schuur Hospital
Coloured South African females	CF	51	Groote Schuur Hospital
Population group total		**102**	
Indian South African males	IM	51	Inkosi Albert Luthuli Central Hospital
Indian South African females	IF	51	Inkosi Albert Luthuli Central Hospital
Population group total		**102**	
Total sample		***408***	

CT settings can vary in slice thickness (e.g., 0.625 mm, 1.25 mm, etc.), often depending on the anatomical area of study. In order for the crania to be comparable across the various individuals, only individuals with slice thicknesses less than 0.625mm were used in the current study. Furthermore, isotropy and thin slices were necessary for the reconstructed 3D model to retain resolution quality in visualisations and to be the most accurate representation of the actual object (cranium). Isotropy refers to an equal number of slices obtained in all three dimensions in order for each image data element, or voxel, to be of equal dimensions in all spatial axes (Figure 3.1; Kalender 1995; Levy 1995; Dalrymple *et al.* 2007). Therefore, in order for all scans to be comparable and isotropic, the scans were re-sliced and saved as isotropic versions at a slice thickness in each dimension of 0.60mm.

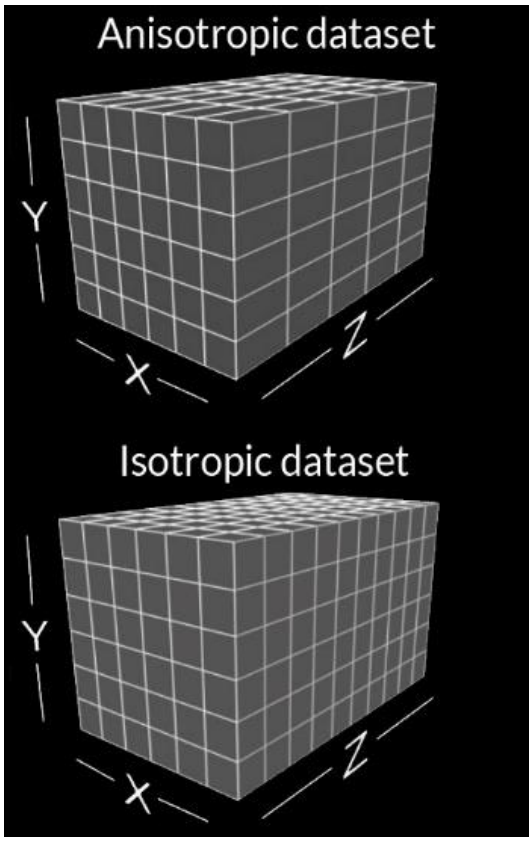

Figure 3.1. Illustration of an example of anisotropic and isotropic datasets. In anisotropic data, the voxels do not have the same dimensions in all three planes (top), whereas in isotropic data, the voxels are cube-shaped with equal dimensions in all three planes (bottom; taken from Dalrymple et al. 2007:50).

As this was a retrospective study, the scans for this research were only collected once patients had been scanned for reasons not related to the current project (i.e. for suspected trauma or pathology). Only scans where the necessary structures were clearly identifiable and with no pathological lesions or injuries disrupting any of the landmarks were included. As pathological conditions and traumatic injuries can disrupt the underlying bone, all scans were assessed for the potential presence and extent of change to the bone prior to inclusion in the sample. Furthermore, no scans with any metal objects causing artefacts in imaging acquisitions were retained for further study. All scans were anonymised and no information that could be used to identify any individuals was retained. Only general demographic information, such as population affinity (self-assigned), sex and age were stored for the individuals.

3.2. Image processing, scoring and landmarks

To prepare the scans for creating the 3D models, the first step, alignment, involved rotating the CT scans to orientate the skull following the Frankfurt horizontal plane. The Frankfurt horizontal plane is a standard anatomical position in which the inferior borders of the orbits are in line with the upper margin of the external auditory meatus (porion) (Figure 3.2; Oh *et al.* 2013). Thermo Scientific™ Avizo™ software 9 (license: AVIZO.59228) was used for alignment and four landmarks, namely left and right porion and left and right inferior orbital margin were placed on each cranium. The slices were then aligned according to the landmarks. All cranial scans were stored in their aligned state for the next steps.

The Treatment and Increased Vision for Medical Imaging (TIVMI) program, a software designed to assist anthropology researchers in obtaining precise and reproducible measurements in 3D and medical imaging was used to segment and volume-render the CT scans to create 3D models of the skulls (bone only) on which landmarks were placed (Dutailly *et al.* 2009; Dutailly 2016). TIVMI was developed by Bruno Dutailly at the PACEA laboratory,

at the French National Center for Scientific Research (CNRS), University of Bordeaux, France (Dutailly 2009).

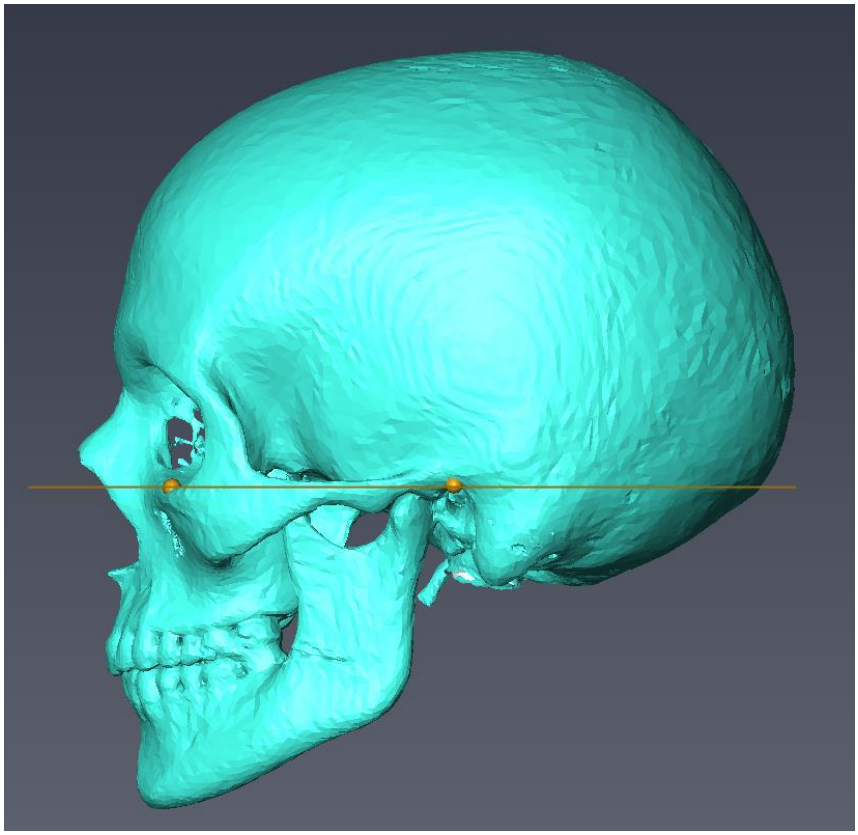

Figure 3.2. Skull placed in the Frankfort horizontal plane.

The second step, segmentation, involved finding a particular grey value threshold that was able to distinguish the bone from all surrounding structures, such as the air, soft tissues, blood vessels, etc. For this step, TIVMI was employed, as the segmentation process in TIVMI was very user-friendly and has been shown to result in lower error rates when compared to other imaging software (Guyomarc'h *et al.* 2012b). Half-maximum height (HMH) thresholding, a common method to use for segmentation, was used and involved calculating the average of the greyscale values along the boundary transition, which is a row of voxels that illustrate the transition between two materials (e.g. between different tissue types or between the tissue and the air surrounding it; Coleman & Colbert 2007; Dutailly 2009). More recently, an improved version of the HMH protocol became the recommended thresholding method to improve the

distinction between structures when identifying the threshold value. The modified HMH protocol only differed in that it required the repetition of the process on 10 randomly selected slices and then the mean value of the slices was taken as the threshold for the entire stack (Fajardo *et al.* 2002; Coleman & Colbert 2007).

Finally, the third and last step involved the volume-rendering of the segmented materials (i.e., bone) into the 3D model of a cranium. While thinner slices generally result in smoother model surfaces, the smoother surface may also occasionally make locating sutures more difficult. By either adjusting the threshold or by including orthoslices (individual aligned 2D slices/cross-sections incorporated into the 3D model) visualisation of small structures was improved for more accurate landmark placement. Once the 3D models were created, morphological features were scored and landmarks were placed on each 3D-rendered cranium.

3.2.1. Morphoscopic traits to estimate sex

Four morphoscopic traits, namely the glabella, mastoid process, nuchal crest and supra-orbital margin were scored on each of the 3D skull models. Each trait was scored according to a scale of 1-5 using score descriptions and depending on the expression of the trait when compared to line diagrams published by Walker (2008) (Figures 3.3 and 3.4; Walker 2008). The cranial models were assessed using the Thermo Scientific™ Avizo™ software 9 (license: AVIZO.59228). Using Avizo, the cranial models could be rotated similar to how a physical cranium could be adjusted to score the various morphoscopic features. The bilateral features were only scored on the left, as testing asymmetry was not within the scope of the current study.

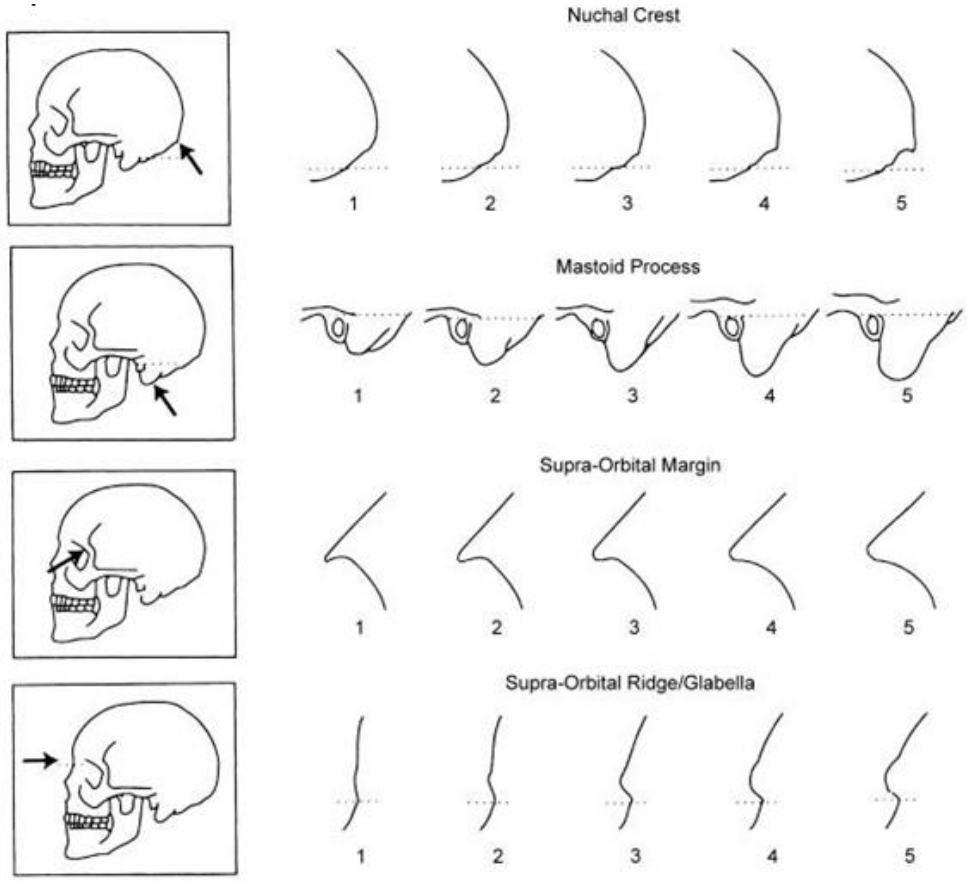

Figure 3.3. Line diagrams depicting each of the trait expressions for five traits on the skull for the estimation of sex (taken from Walker 2008).

Skull scoring procedures

Hold the skull at arms length a few inches from the diagram. Orient it so that its features can be directly compared with those illustrated. Move the skull from diagram to diagram until the closest match is obtained. Score each trait independently, ignoring the other features.

The following are descriptions of the procedures to use in scoring cranial traits. These are followed by descriptions of minimal (score = 1) and maximal (score = 5) expressions of the traits.

Nuchal crest

View the lateral profile of the occipital and compare it with the diagrams. Feel the surface of the occipital with your hand and note any rugosities on its surface. The important feature to consider in scoring this trait is the development of bone on the external surface of the occipital associated with the attachment of the nuchal muscles. Ignore the contour of the underlying bone (e.g., the presence or absence of an occipital bun in scoring this trait.

a. Minimal expression (score = 1)

The external surface of the occipital is smooth with no bony projections visible from when the lateral profile of the occipital is viewed.

b. Maximal expression (score = 5)

A massive nuchal crest that projects considerable distance from the bone and forms a well defined ledge or hook of bone.

Mastoid process

Score this feature by comparing its size with that of surrounding structures such as the external auditory meatus and zygomatic process of the temporal bone. Mastoid processes vary considerably in their proportions. The most important variable to consider in scoring this trait is the volume of the mastoid not its length.

a. Minimal expression (score = 1)

A very small mastoid process that projects only a small distance below the inferior margins of the external auditory meatus and the digastric groove.

b. Maximal expression (score = 5)

A massive mastoid process with lengths and widths several times that of the external auditory meatus

Orbital margin

Hold your finger against the margin of the orbit in the area lateral to the supra-orbital foramen. Look at each of the diagrams to determine which diagrams it feels like it matches most closely.

a. Minimal expression (score = 1)

Extremely sharp, border feels like the edge of a dull knife

b. Maximal expression (score = 5)

A thick rounded margin with a curvature that approximates that of a pencil

Glabella-supra-orbital ridge

View the cranium from its lateral side and compare the profile of the glabella/supra-orbital area with the profiles in the diagrams.

a. Minimal expression (score = 1)

The contour of the frontal is smooth with little or no projection in the glabellar area.

b. Maximal expression (score = 5)

The glabella and/or supra-orbital ridge are massive and from a rounded loaf shaped projection

Mental eminence

Hold the mandible between your thumbs and your index fingers with your thumbs on either side of the mental eminence. Move your thumbs medially so that they delimit the lateral borders of the mental eminence.

a. Minimal expression (score = 1)

Area of the mental eminence is smooth. There is little or no projection of the mental eminence above the surrounding bone.

b. Maximal expression (score = 5)

A massive mental eminence that occupies most of the anterior portion of the mandible

Figure 3.4. Scoring procedure for the five morphoscopic traits described by Walker (2008) (taken from Walker 2008).

3.2.2. Landmarks for linear measurements and geometric morphometrics

A total of 40 landmarks described by Ousley and McKeown (2001) (Figures 3.5 and 3.6; Table 3.2) as well as an additional bilateral mid-zygomaticomaxillary suture landmark not previously described (total of 42 landmarks per cranium) were recorded on each 3D cranial model using the landmarking tool in Avizo (Ousley & McKeown 2001). The additional mid-zygomaticomaxillary suture landmark was added to incorporate a landmark that could be used to assess the distance from porion, asterion, and ectoconchion to the additional landmark, respectively, to potentially comment on projection of the zygomaticomaxillary area. The landmark coordinates were extracted and used first to calculate distances between landmarks

to explore the size variation present in the crania among the various South African populations (Table A1.1). Two types of distances were assessed: the standard and non-standard ILDs. Standard ILDs are used to measure the direct distances between predefined landmarks and refer to 25 linear measurements typically used to measure crania in anthropology (Moore-Jansen & Jantz 1994). Non-standard ILDs are also linear measurements; however, these can be the direct distances between any two landmarks and can be used to further explore cranial variation (Spradley & Jantz 2016).

Furthermore, the raw landmark coordinates data were imported into MorphoJ, an integrated freeware software package for assessing shape variation in the crania among the populations, and were assessed using GM (Klingenberg 2011). Once the landmark coordinates were exported from the imaging software, the coordinate data was assessed through multiple statistical tests. All descriptive statistics were run using the stats package in R (R Core Team 2023).

Table 3.2. List of cranial landmarks from Ousley and McKeown (2001)

#	Landmark	Side	Abbreviation	#	Landmark	Side	Abbreviation
1	subspinale		ssp	22	nasion		nas
2	most inferior nasal border	L	nlhil	23	glabella		glb
3	most inferior nasal border	R	nlhir	24	bregma		brg
4	alare	L	alarl	25	lambda		lam
5	alare	R	alarr	26	opisthion		ops
6	zygoorbitale	L	zygool	27	basion		bas
7	zygoorbitale	R	zygoor	28	FOB point	L	fobl
8	lower orbital border	L	obhil	29	FOB point	R	fobr
9	upper orbital border	L	obhsl	30	radiculare	L	aubl
10	dacryon	L	dacl	31	radiculare	R	aubr
11	ectoconchion	L	ectl	32	asterion	L	astl
12	dacryon	R	dacr	33	asterion	R	astr
13	ectoconchion	R	ectr	34	mid-malar projection point*	L	mlxppl
14	lower orbital border	R	obhir	35	porion	L	porl
15	upper orbital border	R	obhsr	36	mastoidale	L	mastl
16	zygion	L	zygl	37	mid-malar projection point*	R	mlxppr
17	zygion	R	zygr	38	porion	R	porr
18	frontomalare temporale	L	fmtl	39	mastoidale	R	mastr
19	frontotemporale	L	ftl	40	eurion	L	eurl
20	frontotemporale	R	ftr	41	eurion	R	eurr
21	frontomalare temporale	R	fmtr	42	opisthocranion		opg

**The mid-malar projection point is the midpoint (superior-inferior) on the zygomaticomaxillary suture when observed from the lateral view and is not considered a standard landmark.*

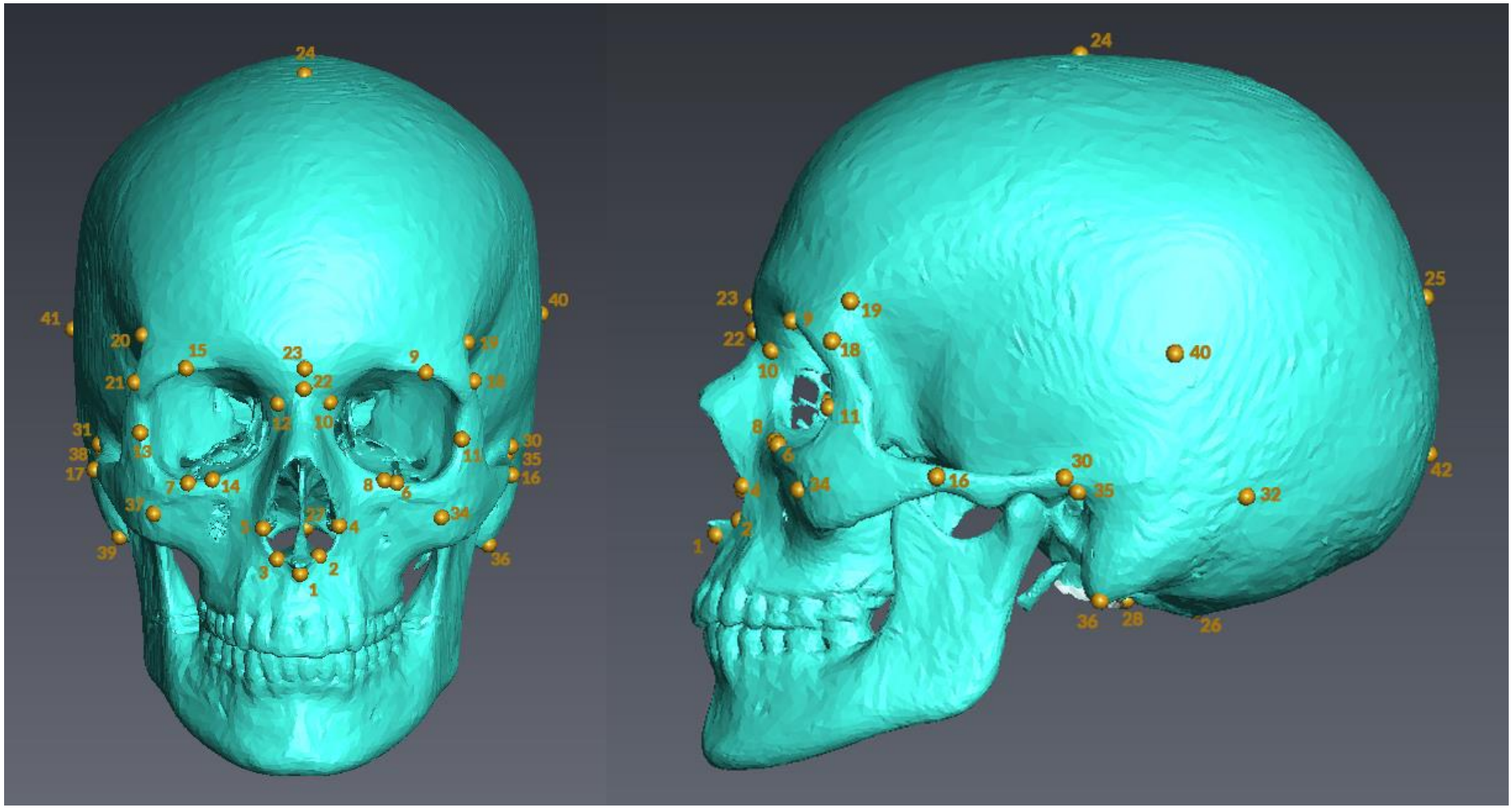

Figure 3.5. Landmarks on anterior and lateral view of skull.

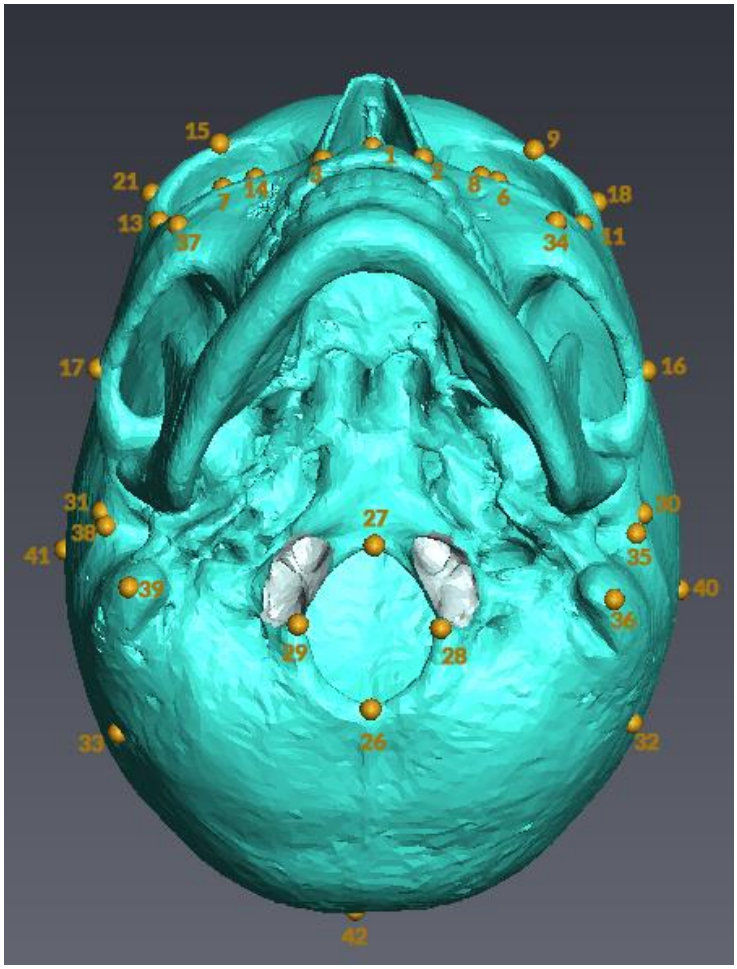

Figure 3.6. Landmarks on the inferior view of the skull.

For some landmarks, namely the left and right eurion and the opisthocranion landmarks, the most lateral or most posterior point of the skull needed to be located. In order to find those points, the most lateral slices (perpendicular to the Frankfort horizontal plane) were isolated and a midpoint placed. In order to locate eurion on the opposite side and in the same planes as the first side, orthoslices (a feature available in Avizo) were used to mark the location of the first eurion landmark and so the same point could be located on the second side on the most lateral slice. Similarly, for opisthocranion, the most posterior slice was isolated and the midpoint was marked (Figure 3.7).

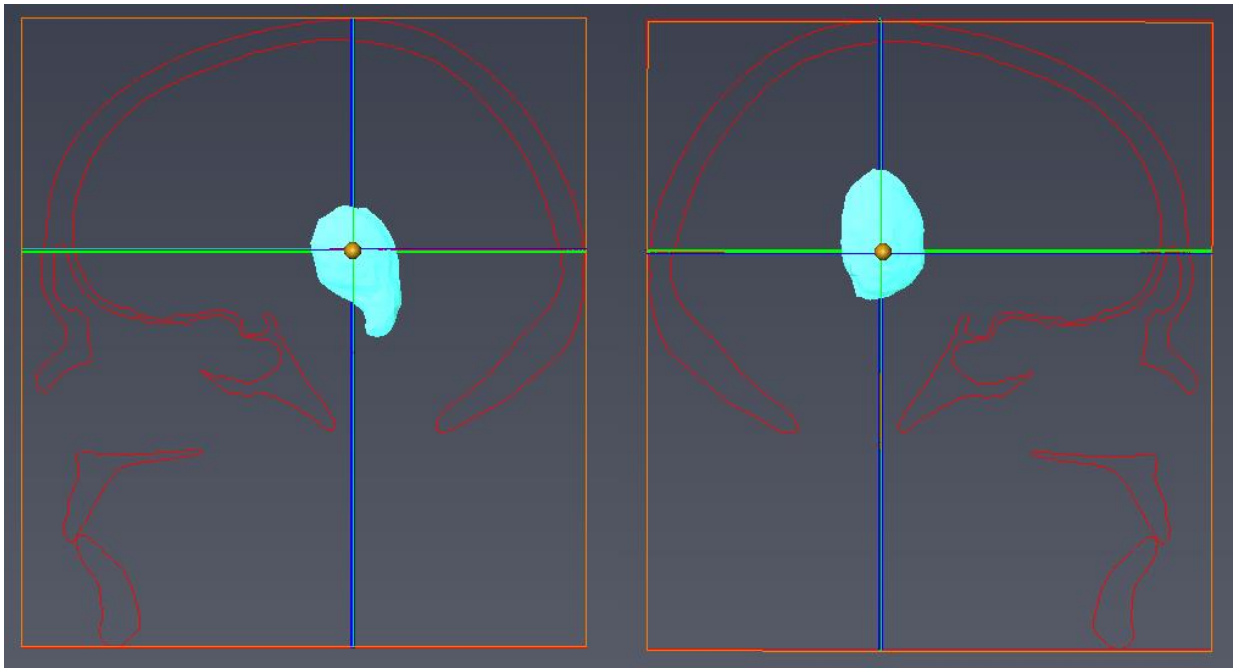

Figure 3.7. Isolation of the most lateral and most posterior slices on the cranium to obtain the eurion and opisthocranion landmarks. Horizontal and vertical lines indicate the orthoslices.

3.3. Statistical Analyses

3.3.1. Morphoscopic data

3.3.1.1. Repeatability

The intra- and inter-observer agreement for the scores of the four Walker (2008) traits were assessed using weighted Cohen's kappa (κ), in order to evaluate consensus within (intra-observer) and between raters (inter-observer) (Fleiss & Cohen 1973). If raters are in complete agreement, then $\kappa = 1$. If no consistency is found among raters, then $\kappa = 0$. Values less than 0.40 represent poor to fair agreement, values between 0.40 and 0.75 indicate medium or good agreement, and values greater than 0.75 may be taken as excellent agreement between observers (Landis & Koch 1977). Cohen's kappa was assessed using the *irr* package in R (R Core Team 2023).

3.3.1.2. Descriptive statistics

Frequency distributions of the scores were used to identify general distributions of the scores across the different sex and populations. Furthermore, the frequencies were used to observe the pattern of sexual dimorphism within black, coloured, white and Indian South Africans. Large differences in the prevalent scores between males and females within a population indicate a greater degree of sexual dimorphism, whereas overlap in the scores indicates limited sexual dimorphism in the group. More scores of 5 also indicate increased robustness, whereas more scores of 1 indicate extreme gracility (Krüger *et al.* 2015). Means and SD were calculated for all scores to assist in interpretation of sex and population differences.

3.3.1.3. Exploratory statistics

Chi-squared tests of independence were also used to test for significant differences in the score frequencies among the populations and between the sexes in each population. The test is appropriate for ordinal data and non-parametric and thus robust and does not require normality of the data (Kruskal & Wallis 1952; Grissom 2000; McHugh 2011). The Chi-squared tests of independence were run using the *stats* package in R (R Core Team 2023).

3.3.1.4. Classification

The score frequencies for black and white South Africans have been tested previously and were noted to have significant differences, which warranted the creation of population-specific logistic regression equations for black and white South Africans, separately (Krüger *et al.* 2015). Even though recently recalibrated logistic regression equations exist for black and white South Africans, multiple factors could have a potential influence on the comparability of the data, such as the temporality of the samples, the differences in modalities assessed, and the

geographical origins of the samples. Therefore, in order to create the most direct comparison between population groups, new equations were created for all four populations in this study.

Ordinal logistic regression (OLR) was used to predict the probability of the occurrence of an event fitting the data and was used to identify relationships between the independent variable (sex) and the ordinal dependent variables (scores of the four morphoscopic traits; Dawson-Saunders & Trapp 2004). OLR adds weights to the scores of the non-metric traits based on the significance of the variable when estimating the sex of an individual (Kachigan 1991; Anderson & Trinkaus 1998). Logistic regression assumes an equal relationship between each pair of outcome groups, referred to as the proportional odds. OLR was run using the *MASS* package in R (R Core Team 2023).

Recent research has also shown the potential of using RF for sex estimation using morphoscopic traits (Klales 2020). The statistical program, Morphopasse, was recently created and includes both OLRs and RF to estimate sex using cranial morphoscopic traits (Klales 2020). Decision trees and random forest models have predominantly been applied to continuous data for sex estimation. Nevertheless, these statistical techniques have demonstrated significant potential for handling morphological traits (binary, discrete, and ordinal data) as well as combined morphological and metric ancestry estimation (Hefner & Ousley 2014; Hefner *et al.* 2014). RF is a flexible machine learning algorithm that operates by constructing a multitude of decision trees through bootstrap aggregating of random training subsets. Overall, model predictions are made by calculating the prediction for each decision tree, then taking the most popular result from all the trees and combining their predictions to determine the best classification rules (Klales 2020). RF was run using the *randomForest* package in R (R Core Team 2023).

3.3.2. Interlandmark distances

3.3.2.1. Repeatability

In order to test the repeatability of not only landmark placement, but also the repeatability of the resulting linear measurements, absolute and relative technical error of measurement (TEM and %TEM, respectively) were used to calculate inter- and intra-observer agreement. TEM reflects the typical measurement error size and examines the SDs of the repeated measurement sets (Knapp 1992; Stull 2014). In order to remove the size component, the %TEM is calculated by converting the absolute TEM into a percentage and dividing by the mean of each of the measurement pairs (Knapp 1992; Perini *et al.* 2005; Jamaiyah *et al.* 2008; Stull 2014; Krüger 2015). A measurement error (%TEM) of less than 3.5% was deemed acceptable, although measurement size differences (TEM) were also considered and limited to less than 2mm.

The inter- and intra-observer error rates associated with each of the measurements were visualised using Bland-Altman plots. The plots show trends in the differences between the measurements. The repeated measurements are plotted along the x-axis and the differences between the measurement pairs on the y-axis (Rothwell 2000; Harris & Smith 2009; Krüger 2015). A 95% confidence interval is illustrated by dashed lines and is based on the SD. The larger the difference between measurement pairs, the wider the spread of the data away from the mean of zero, whereas smaller differences group closer to the mean (Bland & Altman 1996; Stull 2014; Krüger 2015). Bland Altman plots were created using the *ggplot2* package in R (R Core Team 2023).

3.3.2.2. Descriptive statistics

The direct distances between the landmark coordinates, the standard and non-standard ILDs, were subjected to a variety of exploratory statistics.

Basic descriptive statistics, including measurement mean, SD and variance were calculated. A further analysis included the Analysis of Variance (ANOVA) to test for significant differences between the sexes, among the four populations and to test for any interaction of sex and population affinity in the cranial measurements. ANOVA is useful in testing for significant differences in means between two or more groups; however, additional *post hoc* testing using Tukey's Honestly Significant Difference (HSD) test is necessary to show where (i.e. between which groups) the significant differences are (Fenech 1979). ANOVA assumes normality and homoscedasticity of the data and may not produce reliable results if the assumptions are not met. While the data should follow a normal distribution, if large enough sample sizes (>30 or 40) are available, the sampling distribution tends to be normal, even if the shape of the data is not (Elliott & Woodward 2007; Field 2013). ANOVA was run using the *stats* package in R (R Core Team 2023).

3.3.2.3. Symmetric percent differences to assess sexual dimorphism

Sympercents (s%), or symmetric percentage differences, were used to assess the level of sexual dimorphism within the South African population. The males and females of each population were compared to assess the pattern and degree of sexual dimorphism in the cranial measurements of black, coloured, Indian and white South Africans. Because the sizes of the cranial dimensions (i.e. nasal breadth versus maximum cranial length) affect the mean difference between males and females, the unit of measure is removed when calculating sympercent; instead the log of a ratio are presented in terms of s% and is calculated as follows (Cole 2000):

$$\mathbf{s\% = (100 \log_e x_2) - (100 \log_e x_1)}$$

The s% of black, coloured, Indian and white South African males and females were used to assess the differences in the degree of sexual dimorphism among the populations (Cole 2000).

3.3.2.4. Classification statistics

Once the presence of significant differences had been explored, LDA was used to test the classification potential of different combinations of measurements (multivariate classification). Leave-one-out cross-validation (LOOCV) was then used to validate the LDA results.

LDA is a linear combination of weighted, independent variables (measurements) that, in combination, have the ability to maximise the differences between two or more dependent variables (sex, population affinity (pop), or PopSex; Dawson-Saunders 2004; Pietrusewsky 2008; Ousley & Jantz 2012). The results of a LDA are accompanied by a posterior probability that the unknown belongs to one of the groups in the reference sample. Assumptions associated with LDA include adequate sample sizes of at least one greater than the number of variables used in each of the models, normality, homogeneity of variance and no remaining outliers (Ousley & Jantz 2012, Jantz & Ousley 2023). LDA was run using FD3 that included options for removing outliers, running forward Wilks variable selection and made use of LOOCV (Ousley & Jantz 2012).

In order to maintain large enough sample sizes, a variable selection process needed to be incorporated to reduce the extremely large number of measurements obtained in the current study, so only the most discriminatory variables were used in model creation. One useful variable selection process is forward stepwise selection, which sifts through, tests each measurement at a time, and only adds that measurement to the model if the improvement in correct classification is greater than 5%. If a variable had little to no impact when separating groups, the variable was not included in model creation.

Once a model was created, LOOCV was used to authenticate the results by classifying each individual (one at a time) using the created model and the remaining individuals in the reference data. The resulting correct classification rate was thus a more effective indication of the ability

of the model to classify an individual into one of the reference groups (dependent variables) (Cheng *et al.* 2017).

3.3.3. Geometric morphometrics

3.3.3.1. Repeatability

To test for repeatability of landmark placement, the mean Euclidean distance, or dispersion was calculated for each landmark when the principal investigator (intra-observer error) and a second observer (inter-observer error) repeated the landmarking process on 15 randomly selected 3D cranial models. Dispersion analysis calculates the mean Euclidean distance between the repeated landmarks and the mean of the landmark (Guyomarc'h *et al.* 2014). The mean Euclidean distance (Δ_{ij}) of the sample landmark $\boldsymbol{p}_{ijk}$ to the mean $\bar{\boldsymbol{p}}_{ij}$ of the (x, y, z)-coordinates of landmark *i* over all observations *k* for subject *j* was calculated using the formula:

$$\Delta_{ij} = \sum_{k=1}^{K} \|\boldsymbol{p}_{ijk} - \bar{\boldsymbol{p}}_{ij}\|/K, \text{ with } \bar{\boldsymbol{p}}_{ij} = \sum_{k=1}^{K} \boldsymbol{p}_{ijk}/K$$

The acceptable error level for the intra- and the inter-observer dispersion error was set at 2 mm, following the recommendations from literature (Bräuer & Knussmann 1988, Ridel *et al.* 2018, Braun *et al.* 2022).

3.3.3.2. Generalised Procrustes Analysis, Principal Component Analysis, and lollipop graphs

The landmark coordinates underwent further exploration through GM, which included removing nuisance parameters such as initial differences in centroid size (CS), position, and orientation of the specimens through the use of generalised Procrustes analysis (GPA). The raw 3D coordinate data were uploaded into MorphoJ (Klingenberg 2011) and R using the RStudio environment for further analysis, including GPA and Canonical Variate Analysis (CVA). GPA

in R was run using the *geomorph* package (R Core Team 2023). MorphoJ makes use of generalised least-squares (GLS) Procrustes superimposition of the 3D landmark coordinates and once size, orientation, and position are removed, defines the shape of each specimen in terms of Procrustes residuals or coordinates (ProCoords; Rohlf & Slice 1990; Nicholson & Harvati 2006; Maass 2016). The ProCoords were used to assess the shape differences among the populations, between the sexes, and among the population-sex (PopSex) groups (black females, coloured males, Indian females etc.).

Landmark displacements or differences in the ProCoords among groups were also assessed through principal component analysis (PCA) and visualised using lollipop graphs. PCA is useful in removing unnecessary variables (reducing dimensionality) within models and subsets (Jolliffe 2002; Ringnér 2008; Krüger 2015). The principal component (PC) scores, or linear combinations of the original variables, were ordered with the first few components extracting the most variance present in all the original variables of a subset, with the last few PC scores retaining the least variance (Jolliffe 2002; Tabachnick & Fidell 2007; Krüger 2015). Only the PC scores that extracted at least 1% of the total variance were used to replace the raw measurements in model creation in CVA. PCA was run using the *geomorph* package in R (R Core Team 2023).

Procrustes ANOVA using the GPA coordinates was used to assess the presence of significant differences both between the sexes and among the four populations. Procrustes ANOVA evaluates whether the variability observed within and across these groups exceeds any inherent measurement error present in the collected data (Daboul *et al.* 2018). Multivariate ANOVA (MANOVA), an extension of the univariate ANOVA, was used to assess multiple dependent variables simultaneously. The created combinations of variables were then tested to determine if significant group differences were present. 50-50 MANOVA is a modified version of MANOVA that can take into account the potential collinearity of the response variables by

making use of the PC scores (Lansgrud & Mevrik 2023). 50-50 MANOVA was run using the *ffmanova* package in R (R Core Team 2023).

Furthermore, to explore the statistical significance of the findings, permutation testing was used and involved the iterative random reordering of data to recalibrate the *p*-value associated with the significance test. In this context, the objective was to determine the likelihood of obtaining a *p*-value equal to or greater than the originally observed *p*-value under the null hypothesis, i.e. to investigate whether notable differences exist within and among the diverse groups being assessed (Anderson 2001). The permutation tests compared distances between two group means when random individuals were assigned to the groups. In addition to the distances between groups, *p*-values were used to show whether the distances between groups were significant. The permutation test and distances were calculated using the *permudist* function in the *Morpho* package in R (R Core Team 2023).

Lollipop graphs were created in MorphoJ to assist in presenting the shape and size differences among different groups by indicating the landmark starting position with a dot and the shift of the landmark as a line (Klingenberg 2013). The resulting lollipop graphs were superimposed on an image of a 3D cranial model to improve interpretation of the graphs (Figure 3.8).

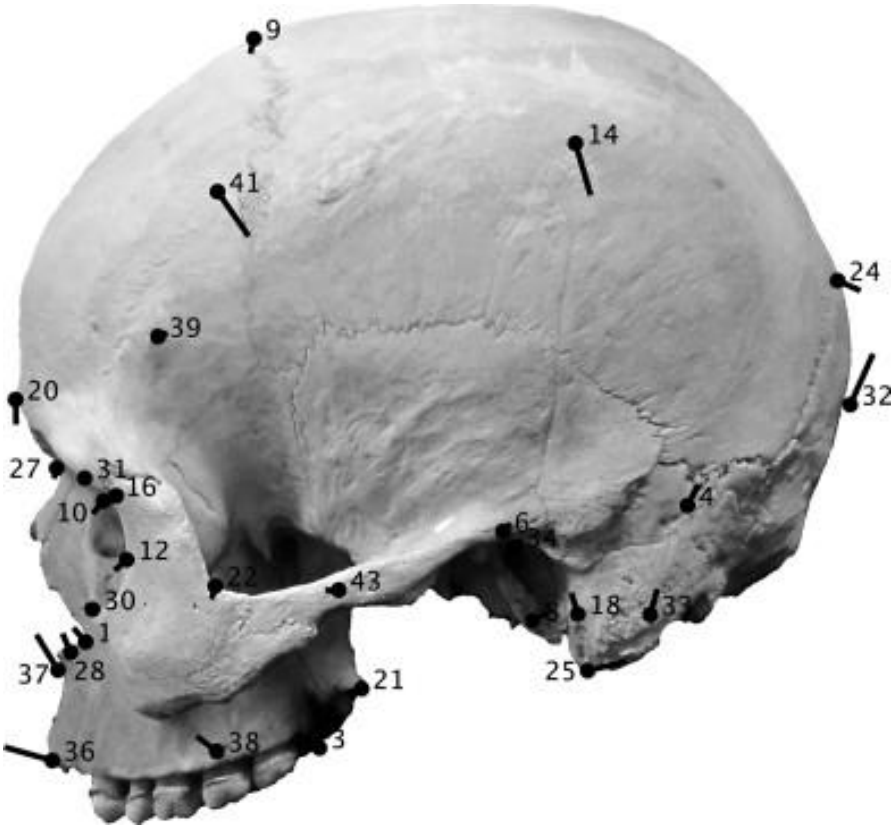

Figure 3.8. Example of a lollipop graph superimposed on the lateral view of a skull (taken from Stull et al. 2014).

3.3.3.3. Canonical Variate Analysis

Various CVA functions were run to show the potential of shape variables in classifying according to population group, sex, and PopSex groups using the cranium. CVA was run using the *Morpho* package in R (R Core Team 2023).

CVA is a multivariate technique for data reduction similar to discriminant function analysis. In CVA, variance parameters are used to maximize the differences among various groups. Each canonical variate (CV) represents a linear combination of variables, typically shape coordinates, with specific weights added to capture distinct shape variations. The primary objective is to extract shape variable combinations that offer the most effective differentiation among the groups present within the dataset (Weinberg *et al.* 2009).

CHAPTER 4: RESULTS

4.1. Morphoscopic variation

4.1.1. Repeatability

To test the repeatability of scoring the four morphoscopic traits, 15 3D cranial models were selected randomly and scored twice by the principal investigator (intra-observer agreement) and by a second observer (inter-observer agreement). The results showed medium to excellent intra-observer agreement, with the lowest agreement obtained for the mastoid process (κ = 0.848) and the highest agreement obtained for the glabella (κ = 0.970; Table 4.1). A similar level of agreement was obtained between the principal investigator and the second observer, where the agreement ranged from medium to excellent. However, for the inter-observer agreement, repeatability was lowest for the nuchal crest (κ = 0.719) and highest for the mastoid process (κ = 0.963; Table 4.1).

Table 4.1. Intra- and inter-observer agreement for the four morphoscopic traits.

	Intra-observer agreement			Inter-observer agreement		
	Cohen's κ	*p*-value	Landis & Koch (1977) scale	Cohen's κ	*p*-value	Landis & Koch (1977) scale
glabella	0.970	<0.01	Excellent agreement	0.811	<0.01	Excellent agreement
mastoid	0.848	<0.1	Excellent agreement	0.963	<0.01	Excellent agreement
nuchal	0921	<0.01	Excellent agreement	0.719	<0.01	Medium or good agreement
orbit	0.896	<0.01	Excellent agreement	0.857	<0.01	Excellent agreement

4.1.2. Descriptive statistics

The trait score frequencies, means and SD were tabulated for each trait for both sexes (Table 4.2) and each population and sex group (Table 4.3). When the means and SDs were explored with only the sexes separated but all the populations pooled, males presented with overall higher scores (3.44 to 3.66) than the females did (1.39 to 2.81). The SD also indicated higher levels of variation in the males for all four traits when compared to the females (Table 4.2). While score differences were present, a certain amount of overlap in scores was still evident between males and females, as is illustrated in the radar graphs (Figure 4.1), with greater overlap present for the nuchal crest and supra-orbital margin scores, while the glabella presented with the least amount of overlap between the sexes.

Table 4.2. Frequencies, means, and SD of the scores for males and females for each of the four morphoscopic traits.

	Glabella						
	1	2	3	4	5	mean	SD
M	18	56	77	81	72	3.44	1.20
F	206	82	12	3	1	1.39	0.65
	Mastoid						
	1	2	3	4	5	mean	SD
M	3	32	95	109	65	3.66	0.96
F	38	106	121	30	9	2.56	0.94
	Nuchal						
	1	2	3	4	5	mean	SD
M	10	53	86	90	65	3.48	1.11
F	56	112	91	37	8	2.44	1.01
	Orbit						
	1	2	3	4	5	mean	SD
M	4	41	88	96	75	3.65	1.04
F	31	81	121	56	15	2.81	1.01

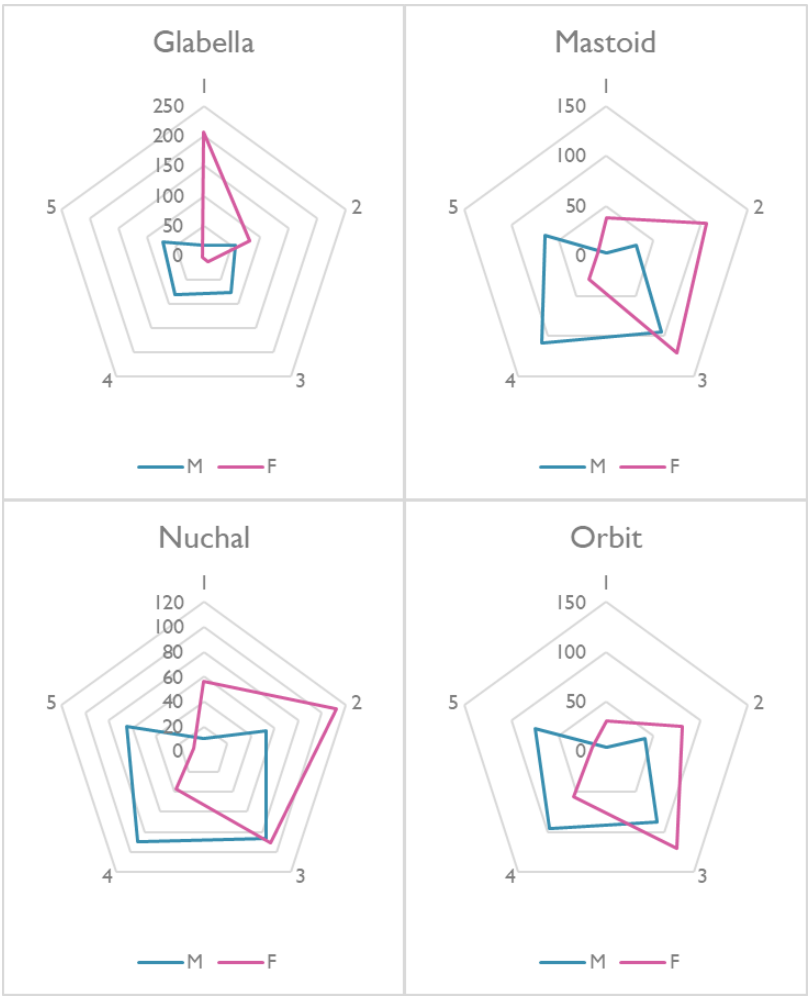

Figure 4.1. Graphs depicting the score frequencies for the males and females for each of the four morphoscopic traits.

To further assess the levels of sexual dimorphism within the populations, variation between the population and sex groups were compared to one another to explore the differences among the females and among the males of the various South African populations as well as between the males and females of each population (Table 4.3).

The score frequencies revealed that of the females, the black South African females were the most gracile for the glabella and nuchal crest, while the Indian South African females were most gracile for the mastoid process and supra-orbital margin. White South African females were the most robust for the glabella, while the coloured South African females were the most robust for the supra-orbital margin and nuchal crest (similar with Indian females; Table 4.3).

Of the males, the black South African males were the most gracile for the glabella and mastoid process, while the Indian South African males were the most gracile for the mastoid process and supra-orbital margin. White South African males were the most robust for the glabella, mastoid process and nuchal crest, while coloured South African males were the most robust for the supra-orbital margin (Table 4.3).

When exploring the sexual dimorphism within the Indian South African population, Indian South African males were more robust than their female counterparts. The mean scores for males ranged from 3.16 (supra-orbital margin) to 3.93 (nuchal crest), whereas the female mean scores ranged from 1.49 (glabella) to 2.68 (nuchal crest). The males were more variable in their scores, with the SDs from 0.93 (mastoid process) to 1.13 (glabella), while the females had SDs from 0.55 (glabella) to 0.94 (nuchal crest; Table 4.3).

Similar to the Indian South Africans, the coloured South African males were also more robust than their female counterparts, with score means that ranged from 3.22 (nuchal crest) to 4.03 (supra-orbital margin), while the female score means ranged from 1.38 (glabella) to 3.21 (supra-orbital margin). For three of the four traits, the males were more variable than the females, with the females more variable for the supra-orbital margin (SD = 1.09; Table 4.3).

As with the previous two populations, white South African males were more robust than their female counterparts, with score means that ranged from 3.82 (mastoid process) to 3.95 (nuchal crest), while the female score means ranged from 1.5 (glabella) to 2.86 (mastoid process). White South African males were more variable than females for the glabella and mastoid process, while white South African females were more variable than their male counterparts for the nuchal crest and supra-orbital margin (Table 4.3).

Black South African males were more robust for all four traits when compared to their female counterparts, with score means from 2.83 (nuchal crest) to 3.61 (mastoid process), while

the females had score means that ranged from 1.2 (glabella) to 2.8 (supra-orbital margin). The black South African males were more variable for the glabella and supra-orbital margin, while the females were more variable for the mastoid process and nuchal crest (Table 4.3).

Table 4.3. Frequencies, means, and SDs of scores for each population-sex group for each of the four morphoscopic traits. Abbreviations are defined in Table 3.1.

	Glabella						
	1	2	3	4	5	mean	SD
BM	8	23	20	18	7	2.91	1.16
BF	65	7	4	0	0	1.20	0.52
CM	6	15	13	28	14	3.38	1.22
CF	55	15	4	2	0	1.38	0.71
IM	2	10	26	15	23	3.62	1.13
IF	41	33	2	0	0	1.49	0.55
WM	2	8	18	20	28	3.84	1.12
WF	45	27	2	1	1	1.50	0.74
	Mastoid						
	1	2	3	4	5	mean	SD
BM	0	9	23	33	11	3.61	0.88
BF	14	25	26	9	2	2.47	1.01
CM	1	7	23	24	21	3.75	1.01
CF	8	22	35	9	2	2.67	0.91
IM	1	10	27	28	10	3.47	0.93
IF	12	38	22	4	0	2.24	0.78
WM	1	6	22	24	23	3.82	1.00
WF	4	21	38	8	5	2.86	0.92
	Nuchal						
	1	2	3	4	5	mean	SD
BM	4	24	32	13	3	2.83	0.91
BF	31	25	16	3	1	1.92	0.95
CM	5	17	20	24	10	3.22	1.14
CF	6	29	27	11	3	2.68	0.96
IM	0	7	19	22	28	3.93	1.00
IF	5	31	26	11	3	2.68	0.94
WM	1	5	15	31	24	3.95	0.95
WF	14	27	22	12	1	2.46	1.01
	Orbit						
	1	2	3	4	5	mean	SD
BM	0	12	26	27	11	3.49	0.93
BF	6	19	37	12	2	2.80	0.89
CM	0	4	21	20	31	4.03	0.95
CF	6	11	29	21	9	3.21	1.09
IM	3	18	27	20	8	3.16	1.03
IF	11	31	26	8	0	2.41	0.87
WM	1	7	14	29	25	3.92	1.00
WF	8	20	29	15	4	2.83	1.04

Figure 4.2 illustrates the score differences and overlap present among the scores of the eight population-sex groups. For both the glabella and mastoid process, the females tend to group together with similar score frequencies, whereas the males, while still grouped together to a certain extent, are more variable in their score frequencies than the females. For the supra-orbital margin, more overlap is apparent with almost no separation of the males and females. For the nuchal crest, three of the four male groups overlap and appear more robust in their scores than black South African males, who overlap considerably with the female groups (Figure 4.2).

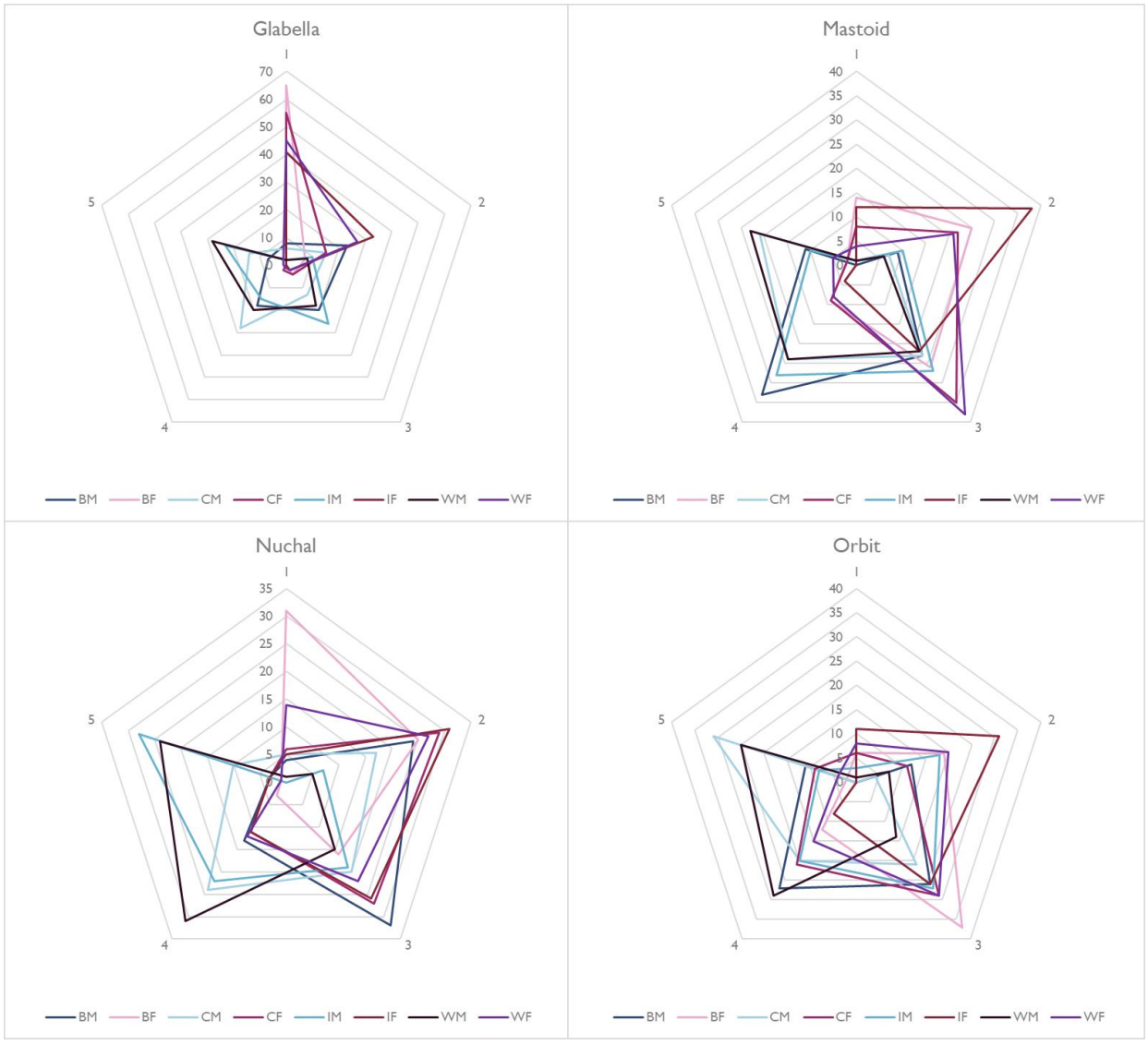

Figure 4.2. Graphs depicting the score frequencies for the males and females of the various population groups for each of the four morphoscopic traits.

4.1.3. Exploratory statistics

Pairwise Chi-squared tests were run to test for significant sex differences between male and female scores obtained for each population. The results show that the black, Indian and white South African males and their female counterparts are significantly different for all four traits, whereas coloured South African males and coloured females are significantly different from one another for all traits, except for the nuchal crest (p = 0.336; Table 4.4).

Table 4.4. Pairwise Chi-squared tests of nominal independence to assess for significant differences in the score frequencies between the males and females of each population group for the four morphoscopic traits. Bold indicates significant differences.

	p-values*			
Group comparisons	glabella	mastoid	nuchal	orbit
BM-BF	**<0.001**	**<0.001**	**<0.001**	**<0.01**
CM-CF	**<0.001**	**<0.001**	0.336	**<0.01**
IM-IF	**<0.001**	**<0.001**	**<0.001**	**<0.01**
WM-WF	**<0.001**	**<0.001**	**<0.001**	**<0.001**

* p-value was calculated using a Bonferroni correction factor

For the glabella, significant differences (p < 0.05) were noted between black and Indian South Africans, between black and white South Africans and between coloured and Indian South Africans. No significant differences were noted between black and coloured South Africans (p = 0.642), between coloured and white South Africans (p = 0.329) and between Indian and white South Africans (p = 1.0; Table 4.5).

For the mastoid process, significant population differences were only noted between Indian and white South Africans (p < 0.05), whereas all other groups were not significantly different for the mastoid process scores (Table 4.5).

For the nuchal crest, significant population differences were noted between black and coloured South Africans, between black and Indian South Africans and between black and white South Africans. No significant differences were noted between coloured and Indian

South Africans (p = 0.199), between coloured and white South Africans (p = 0.377) and between Indian and white South Africans (p = 0.496; Table 4.5).

For the supra-orbital margin, significant differences were noted between black and coloured South Africans, between coloured and Indian South Africans and between Indian and white South Africans. No significant differences were noted between black and Indian South Africans (p = 0.152), between black and white South Africans (p = 0.157) and between coloured and white South Africans (p = 1.0; Table 4.5)

Table 4.5. Pairwise Chi-squared tests of nominal independence to assess for significant group differences in score frequencies for the four morphoscopic traits. Bold indicates significant differences between population groups.

	p-value*			
Group comparisons	glabella	mastoid	nuchal	orbit
B-C	0.642	1.00	**<0.001**	**<0.01**
B-I	**<0.01**	1.00	**<0.001**	0.152
B-W	**<0.01**	0.0672	**<0.001**	0.157
C-I	**<0.05**	0.14	0.199	**<0.001**
C-W	0.329	1.00	0.377	1.00
I-W	1.00	**<0.01**	0.496	**<0.001**

* p-value was calculated using a Bonferroni correction factor

4.1.4. Classification statistics

As significant differences were noted between most populations for at least some of the traits using the Chi-squared tests, specific OLR formulae were created for each population. Therefore, in order to obtain the best possible outcome in classification, the population affinity needs to be estimated prior to estimating sex using the equations below (Tables 4.6 to 4.9).

Using all four morphoscopic traits, sex could be estimated for black South Africans with a 90.8% correct classification rate and no sex bias. The glabella contributed the most to the equation, while the nuchal crest contributed the least (Table 4.6).

Table 4.6. OLR coefficients, constant, cross-validated correct classification rate, and classification matrix for the estimation of sex in black South Africans using all four morphoscopic traits.

Black South Africans			
	coefficient	constant	% correct*
glabella	1.8972	-9.4953	90.8%
mastoid	0.7621		
nuchal	0.6756		
orbit	0.6778		

Classification matrix			
	M	F	% correct
M	69	7	90.8%
F	7	69	90.8%

*K-fold cross-validated percent correct

Using only two of the four variables, sex could be estimated for coloured South Africans with an 88.8% correct classification rate and only a slight female sex bias. Once again, similar to the equation for black South Africans, the glabella trait contributed the most to the equation; however, the glabella only contributed slightly more than the mastoid process trait (Table 4.7).

Table 4.7. OLR coefficients, constant, cross-validated correct classification rate, and classification matrix for the estimation of sex in coloured South Africans using two morphoscopic traits.

Coloured South Africans			
	coefficient	constant	% correct*
glabella	1.674	-7.305	88.8%
mastoid	1.1397		

Classification matrix			
	M	F	% correct
M	67	9	88.2%
F	8	68	89.5%

*K-fold cross-validated percent correct

The ordinal logistic equation for the Indian South Africans was able to estimate sex with a correct classification rate of 94.1%, which is the highest of the four South African population groups assessed. Similar to coloured South Africans, a slight female sex bias was noted for Indian South Africans. Furthermore, the glabella once again contributed the most to the equation, while the supra-orbital margin contributed the least (Table 4.8).

Table 4.8. OLR coefficients, constant, cross-validated correct classification rate, and classification matrix for the estimation of sex in Indian South Africans using all four morphoscopic traits.

Indian South Africans			
	coefficient	constant	% correct*
glabella	2.5998	-17,3249	94.1%
mastoid	1.4532		
nuchal	1.3475		
orbit	1.0820		

Classification matrix			
	M	F	% correct
M	70	6	92.1%
F	3	73	96.1%

*K-fold cross-validated percent correct

Similar to the equation for coloured South Africans, the OLR equation for white South Africans also only made use of the glabella and mastoid process scores; however, in the equation for white South Africans, the glabella contributed considerably more to the equation than the mastoid process. Furthermore, the equation for white South Africans was able to estimate sex with a 91.5% correct classification rate, which is higher than the correct classification rate obtained for coloured South Africans. A slight female sex bias was noted (Table 4.9).

Table 4.9. OLR coefficients, constant, cross-validated correct classification rate and classification matrix for the estimation of sex in white South Africans using two morphoscopic traits.

White South Africans			
	coefficient	constant	% correct*
glabella	1.8784	-7.2109	91.5%
mastoid	0.8104		

Classification matrix			
	M	F	% correct
M	68	8	89.5%
F	5	71	93.4%

*K-fold cross-validated percent correct

While high accuracies were obtained using the OLR equations, recent research has highlighted the potential of using RF for estimating sex when using morphoscopic traits (Klales 2020). Therefore, sex was also estimated using RF and the classification accuracies ranged from 88.0% to 95.7%, with coloured South Africans obtaining the lowest and Indian South Africans obtaining the highest classification accuracies (Table 4.10). A female sex bias was noted for all populations, except for Indian South Africans where both males and females classified equally well. The correct classifications rates were similar between the methods for all groups, except for the Indian South Africans for which the accuracy increased slightly with RF (Table 4.10).

Table 10. Classification using RF. The predictive values and accuracies represent the classification of a separate test samples into male and female groups.

	Female predictive value	Male predictive value	*p*-value	κ	Accuracy
Black SA	91.7%	88.9%	<0.001	0.8056	90.5%
Coloured SA	89.7%	85.7%	<0.001	0.7537	88.0%
Indian SA	95.7%	95.7%	<0.001	0.9130	95.7%
White SA	91.7%	90.9%	<0.001	0.8258	91.3%

*All population groups combined

4.2. Interlandmark distances

4.2.1. Repeatability

TEM and %TEM were conducted for each measurement and showed overall low intra-observer and inter-observer error rates. The mean intra-observer TEM and %TEM are fairly small at 0.48 mm (ranging from 0.11 mm to 1.26 mm) and 1.02% (ranging from 0.1% to 5.49%), respectively. The mean TEM and %TEM for the inter-observer error were slightly higher at 0.73 mm and 1.45%, and the ranges notably wider at 0.17 mm to 2.11 mm and 0.12% to 5.26%, respectively (Table 4.11). The mid-orbital width (MOW) and the left and right distances between the mid-malar projection points and ectoconchions (MAP2L and MAP2R, respectively) were associated with the greatest repeatability errors for both observers. As the

TEM and %TEM were higher for the three distances than the generally acceptable limits in anthropological research, the ILDs were removed from further analyses.

The Bland-Altman plot of the intra-observer error revealed overall high agreement with very few ILD differences exceeding 2mm or systematic bias (Figure 4.3). Inter-observer plots showed similar results, despite a few more ILD differences greater than 2mm (Figure 4.4). While the means and range of differences does not vary considerably between the intra- and inter-observer errors, the spread of the differences is larger for the inter-observer error than for the intra-observer error.

Table 4.11. Absolute (TEM) and relative technical error of measurement (%TEM) for the 35 interlandmark distances (ILD). Bold indicates values greater than the generally acceptable standards for intra- and inter-observer error for ILDs in anthropological analyses.

ILD	Intra-observer error		Inter-observer error	
	TEM (in mm)	%TEM (in %)	TEM (in mm)	%TEM (in %)
GOL	0.18	0.10	0.36	0.19
XCB	0.39	0.28	0.18	0.13
ZYB	0.18	0.13	0.48	0.37
BBH	0.16	0.11	0.17	0.12
BNL	0.33	0.32	0.85	0.82
ASB	0.46	0.41	0.50	0.45
WFB	0.11	0.11	0.30	0.31
UFBR	0.17	0.16	0.46	0.43
NLHL	0.35	0.68	0.44	0.86
NLHR	0.37	0.71	0.62	1.21
NLH	0.34	0.66	0.49	0.94
NLB	0.20	0.73	0.45	1.64
OBBL	0.78	1.78	0.86	1.93
OBBR	0.40	0.91	0.67	1.51
OBHL	0.21	0.58	0.60	1.63
OBHR	0.37	1.00	0.58	1.57
EKB	0.62	0.63	1.70	1.71
DKB	0.65	3.09	0.68	3.29
FRC	0.30	0.26	0.42	0.37
PAC	0.62	0.52	0.71	0.60
OCC	0.30	0.30	0.47	0.47
FOL	0.29	0.74	1.13	2.81
FOB	0.45	1.48	0.95	3.09
MDHL	0.55	1.67	0.70	2.14
MDHR	0.31	0.93	0.74	2.26
AUB	0.25	0.21	0.42	0.34
NOL	0.22	0.12	0.49	0.26
MOW	1.21	2.22	**2.11**	**3.88**
NLH2	0.42	0.78	0.78	1.44
MAPL	0.83	1.09	1.12	1.48
MAPR	0.87	1.11	0.89	1.14
MAP2L	1.05	**4.95**	1.01	**4.59**
MAP2R	1.26	**5.49**	1.20	**5.26**
MAP3L	0.90	0.71	1.01	0.80
MAP3R	0.76	0.60	1.02	0.81

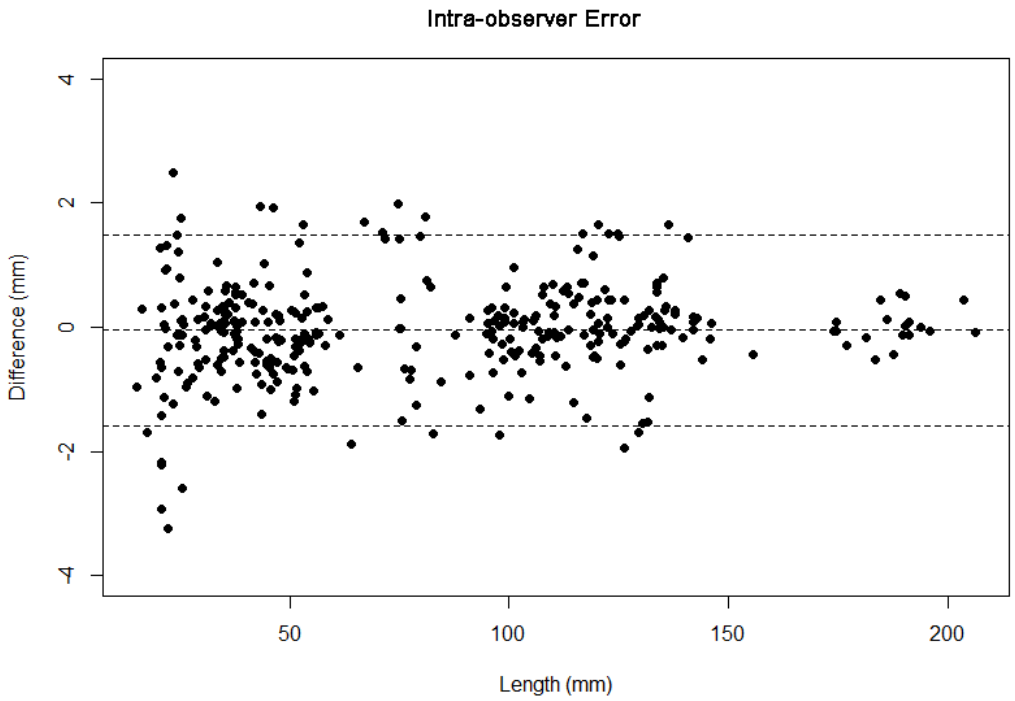

Figure 4.3. Bland-Altman plot illustrating the intra-observer agreement when the principle investigator repeated the landmark placement on 15 randomly selected 3D cranial models. The majority of the resulting ILDs fall within the upper and lower agreement levels (dashed lines), which are based on the SD.

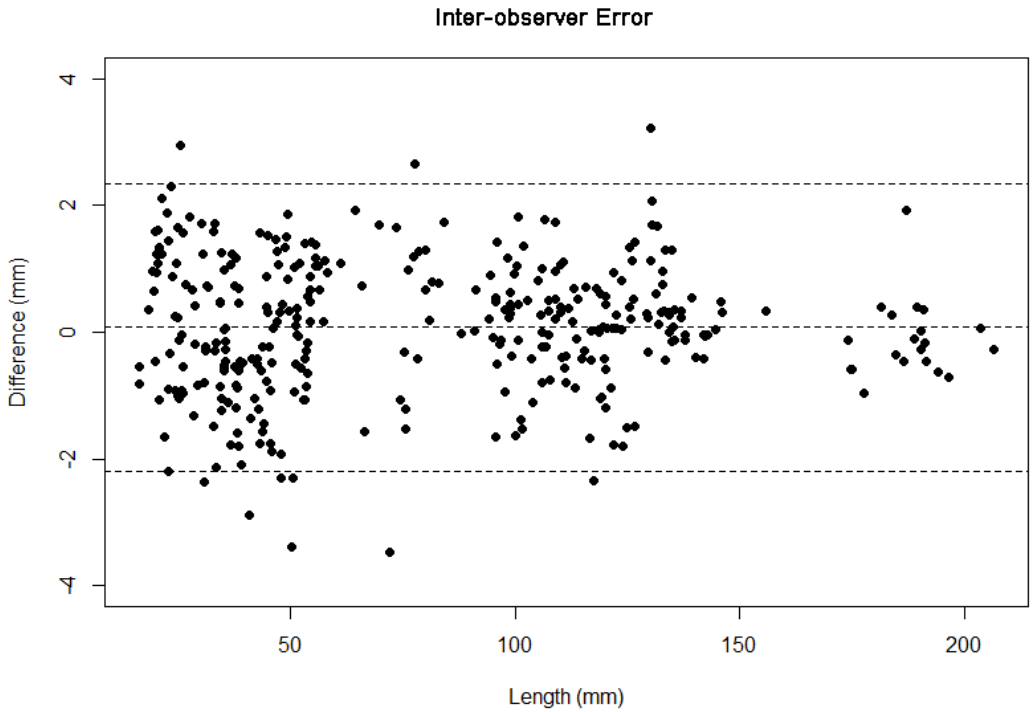

Figure 4.4. Bland-Altman plot illustrating the inter-observer agreement when the principle investigator repeated the landmark placement on 15 randomly selected 3D cranial models. The majority of the resulting ILDs fall within the upper and lower agreement levels (dashed lines), which are based on the SD.

4.2.2. Descriptive statistics

4.2.2.1. Means and standard deviations

Means and SDs were calculated for all ILDs for black, coloured, Indian and white South Africans as well as for the males and females of each population.

When the group means were compared among the four populations, white South Africans had the largest means for most of the cranial ILDs (15 of 32), followed closely by black South Africans with the largest means for 13 of the 32 ILDs (Figure A2.1). Black South Africans were larger for the midface breadths (NLB, EKB, DKB, WFB, UFBR), as well as both anterior and lateral the cheek projections (ZYB, MAPL, MAPR, MAP3L, MAP3R) and the cranial lengths (NOL and GOL). White South Africans, on the other hand, had larger measurement means for the cranial vault and base ILDs (XCB, ASB, AUB), as well as larger distances for the vertical measurements (NLHL, NLHR, NLH2, MDHL, MDHR, BBH, BNL). In contrast, Indian South Africans had the smallest means for 24 of the 32 measurements. The only measurements for which Indian South Africans did not have the smallest means included the nasal height (NLHL, NLHR, NLH, NLH2), and orbital breadth distances (OBBL, OBBR), as well as FOB. Coloured South Africans had intermediate means for all distances except the orbital heights (OBHL, OBHR) and OBBR, for which they were the largest of the four populations (Table 4.12; Figure A2.1).

Of the four populations, black South Africans had the greatest SD for most of the ILDs (13 of 32), whereas Indian South Africans had the smallest SD for the most ILDs (13 of 32). Coloured South Africans rarely had the greatest SD, but had the smallest SD for 8 of the 32 ILDs.

Table 4.12. Black, coloured, Indian and white South African group means and SD for the various ILDs. Bold indicates the largest mean for each ILD.

ILD	black South Africans			coloured South Africans			Indian South Africans			white South Africans		
	n	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*	mean	SD
GOL	101	**185.1**	7.5	101	182.0	7.5	102	180.0	8.0	102	184.9	8.2
XCB	98	136.6	4.7	101	136.0	5.4	102	134.1	5.5	102	**140.5**	5.4
ZYB	101	**127.2**	5.9	101	123.9	5.0	101	123.1	4.9	101	126.0	5.6
BBH	101	134.2	5.6	100	134.0	5.6	100	131.3	5.9	102	**138.0**	6.0
BNL	102	101.3	5.1	100	101.4	5.0	102	101.3	5.6	102	**104.5**	5.5
ASB	102	109.4	5.4	100	108.9	4.6	101	106.8	5.8	102	**113.3**	5.5
WFB	102	**95.6**	5.2	102	94.0	4.3	102	92.9	4.4	101	95.0	4.5
UFBR	102	**105.4**	4.9	101	102.5	4.0	101	101.3	4.1	97	102.4	4.4
NLHL	101	49.5	3.0	101	51.3	3.4	102	50.8	4.1	102	**53.2**	3.4
NLHR	102	49.6	3.0	102	51.4	3.5	102	50.8	4.0	101	**53.1**	3.2
NLH	100	49.5	2.8	102	51.4	3.5	102	50.8	4.0	101	**53.2**	3.2
NLB	99	**27.9**	2.0	98	25.3	1.5	100	24.7	1.7	102	25.1	2.0
OBBL	102	44.2	2.5	99	44.4	2.1	96	**44.7**	1.8	100	44.1	2.1
OBBR	99	44.5	2.3	100	44.1	2.1	99	**44.7**	1.8	102	44.3	2.2
OBHL	101	35.4	1.9	101	**35.9**	2.0	101	35.2	1.8	101	35.8	2.0
OBHR	100	35.7	1.9	102	**35.7**	2.1	101	35.1	1.9	101	35.5	2.0
EKB	100	**99.5**	4.0	102	96.7	4.0	98	95.9	3.4	98	96.9	3.4
DKB	100	**19.5**	2.4	99	17.1	2.0	101	16.4	1.9	102	17.7	1.7
FRC	100	112.4	5.5	101	111.5	4.6	101	110.5	5.4	102	**114.0**	5.4
PAC	101	**116.5**	6.9	102	114.2	7.3	100	112.1	6.1	102	115.1	7.4
OCC	101	98.2	5.2	102	95.1	6.1	102	94.5	5.1	102	**100.2**	5.3
FOL	101	37.6	2.5	102	38.0	2.6	99	36.5	2.4	101	**38.1**	2.7
FOB	102	30.4	2.4	101	31.6	2.2	101	30.6	2.2	98	**32.4**	2.3
MDHL	102	31.8	3.8	102	30.7	3.6	99	29.9	3.1	99	**31.9**	3.2
MDHR	102	32.0	3.9	102	31.1	3.4	99	30.8	3.5	102	**32.7**	3.4
AUB	100	118.3	4.9	100	118.2	5.0	98	116.5	4.2	102	**121.5**	5.3
NOL	101	**183.2**	7.3	100	179.7	7.2	102	178.0	7.7	100	182.3	7.1
MAPL	97	**74.8**	3.8	102	72.9	3.9	101	71.2	4.3	99	73.2	4.1
MAPR	101	**75.4**	4.3	102	73.1	4.1	101	71.5	3.9	102	73.7	4.1
MAP3L	101	**123.4**	6.1	100	120.2	5.4	100	118.1	6.3	101	121.9	7.0
MAP3R	100	**124.5**	6.0	99	121.5	5.5	100	118.2	6.8	101	122.2	7.2
NLH2	102	52.9	3.3	102	55.4	3.8	101	54.7	4.0	99	**57.1**	3.2

For most of the IDLs (25 of 32), Indian South African females had the smallest means when compared to the females of the other populations. The only distances for which Indian females did not have the smallest means included the nasal height, orbital breadth and FOB distances. Both black and white South African females had the same number of distances for which they

had the largest means (14 of 32). White females had the larger means for the vertical (midline) heights (BBH, BNL, NLHL, NLHR, NLH, NLH2), as well as the cranial breadths (XCB, ASB, AUB), FRC, OCC, MDHL, MDHR and FOB. Black South African females had the largest means for the cranial lengths (GOL, NOL, MAPL, MAPR, MAP3L MAP3R), as well as the facial breadths (ZYB, WFB, UFBR, NLB, EKB, DKB, OBHR) and PAC. Indian and coloured South African females were only the largest for the OBBL and OBBR and for the FOL and OBHL distances, respectively (Table 4.13).

Similar to the females, white South African males had overall the largest means for the most ILDs (22 of 32). For the majority of the remaining ILDs, black South African males had the largest means, except for OBHL, for which coloured South African males had the largest means. The black South African males also had the smallest means for all nasal height distances (NLHL, NLHR, NLH, NLH2), OBHL and FOB. Coloured South African males had the smallest means for 9 of the 32 distances, while Indian South African males had the smallest means for the majority of the distances (20 of 32). Unlike the females, white South African males had larger means for the cranial length distances (GOL and NOL), as well as OBHR, PAC, MAP3L and MAP3R when compared to the black South African males (Table 4.14).

Black South African males were most variable for 12 of the 32 ILDs, whereas coloured South African males were most variable for 11 of 32 ILDs. Indian and white South African males were only most variable for six and three of the 32 ILDs, respectively. Indian South African males were the least variable for the most ILDs (10 of 32), followed by white South African males that were least variable for eight of the 32 variables. Overall, when comparing the SD for the males and females, the males were more variable than the females (Table 4.13 and Table 4.14).

Table 4.13. Comparison of ILD means and SD for the females of the four populations. Bold indicates the largest mean for each ILD.

ILD	black SA females			coloured SA females			Indian SA females			white SA females		
	n	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*	mean	SD
GOL	50	**181.3**	6.0	50	178.3	5.9	51	174.7	5.7	51	179.5	5.5
XCB	49	134.8	4.0	51	133.5	4.6	51	132.1	4.6	51	**137.5**	4.4
ZYB	51	**124.0**	4.5	51	121.1	4.2	50	120.3	3.8	51	121.8	3.3
BBH	51	131.8	4.9	51	131.3	4.9	50	127.5	4.0	51	**134.1**	4.4
BNL	51	98.2	4.1	51	99.0	4.2	51	97.5	3.4	51	**100.9**	3.9
ASB	51	108.3	5.4	51	107.6	3.8	51	105.4	4.8	51	**110.9**	4.0
WFB	51	**94.3**	4.6	51	92.9	3.4	51	90.7	3.6	51	93.3	4.0
UFBR	51	**103.6**	4.3	51	101.7	3.4	51	99.1	3.4	49	99.9	3.0
NLHL	51	48.0	2.7	51	49.9	2.7	51	48.2	2.9	51	**51.8**	2.9
NLHR	51	48.1	2.7	51	49.8	2.6	51	48.2	3.3	50	**51.9**	2.7
NLH	51	48.0	2.7	51	49.8	2.6	51	48.2	3.0	50	**51.9**	2.7
NLB	49	**27.9**	2.0	50	25.3	1.4	51	24.4	1.5	51	24.8	1.9
OBBL	51	43.1	2.0	50	43.8	1.9	48	**44.1**	1.7	51	42.7	1.6
OBBR	49	43.6	1.7	50	43.7	1.9	51	**44.2**	1.6	51	42.9	1.9
OBHL	51	35.3	1.9	51	**35.7**	2.0	50	34.8	1.7	50	35.5	2.1
OBHR	49	**35.6**	1.8	51	35.4	2.0	51	34.5	1.9	51	35.3	1.9
EKB	50	**98.0**	3.5	51	95.6	3.4	51	94.3	2.9	51	95.1	2.7
DKB	49	**19.8**	2.4	49	17.0	2.0	51	15.9	2.2	51	17.7	1.3
FRC	51	110.5	5.2	51	109.8	3.9	51	107.5	4.3	51	**110.8**	4.8
PAC	50	**115.0**	7.0	51	111.4	6.1	49	109.1	5.4	51	112.2	7.3
OCC	50	97.9	5.1	51	94.0	5.6	51	93.1	4.9	51	**98.9**	5.5
FOL	51	36.6	2.0	51	**37.0**	2.4	51	35.6	2.4	51	36.8	2.3
FOB	51	29.4	2.3	50	30.9	2.1	51	29.8	1.8	50	**31.2**	2.0
MDHL	51	29.7	3.2	51	29.1	2.5	51	28.0	2.2	50	**30.1**	2.7
MDHR	51	29.9	3.2	51	29.5	2.6	51	28.6	2.4	51	**31.0**	2.8
AUB	51	116.6	4.8	50	115.6	4.3	51	114.3	3.4	51	**118.2**	3.8
NOL	50	**179.7**	5.9	50	176.5	6.2	51	173.0	5.5	51	178.0	5.5
MAPL	49	**73.1**	3.5	51	71.4	3.8	51	68.9	3.2	51	71.3	3.3
MAPR	51	**74.0**	4.0	51	71.6	3.9	51	69.8	2.9	51	71.7	2.8
MAP3L	50	**120.9**	5.6	50	117.7	4.5	51	114.6	4.1	51	117.2	4.5
MAP3R	49	**122.0**	5.4	49	119.0	4.4	51	114.3	4.8	51	117.2	4.4
NLH2	51	51.1	2.7	51	53.6	2.8	51	52.0	2.8	50	**55.9**	2.4

Table 4.14. Comparison of IDLs means and SD for the males of the four populations. Bold indicates the largest mean for each ILD.

ILD	black SA males			coloured SA males			Indian SA males			white SA males		
	n	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*	mean	SD
GOL	51	188.8	7.0	51	185.5	7.2	51	185.3	6.4	51	**190.3**	6.8
XCB	49	138.4	4.7	50	138.6	5.1	51	136.2	5.6	51	**143.5**	4.5
ZYB	50	**130.5**	5.3	50	126.8	3.9	51	125.9	4.2	50	130.3	3.9
BBH	50	136.6	5.3	49	136.8	4.9	50	135.2	4.8	51	**141.9**	4.8
BNL	51	104.4	4.1	49	103.9	4.5	51	105.2	4.7	51	**108.1**	4.4
ASB	51	110.5	5.3	49	110.3	5.0	50	108.4	6.4	51	**115.6**	5.9
WFB	51	**96.8**	5.6	51	95.2	4.8	51	95.0	4.0	50	96.7	4.4
UFBR	51	**107.3**	4.7	50	103.2	4.5	50	103.5	3.6	48	105.0	4.1
NLHL	50	51.1	2.4	50	52.8	3.5	51	53.5	3.2	51	**54.5**	3.4
NLHR	51	51.2	2.5	51	52.9	3.6	51	53.5	2.9	51	**54.3**	3.1
NLH	49	50.9	2.2	51	52.9	3.6	51	53.5	3.0	51	**54.4**	3.2
NLB	50	**28.0**	2.0	48	25.4	1.7	49	25.0	1.7	51	25.4	2.0
OBBL	51	45.3	2.6	49	45.0	2.1	48	45.3	1.7	49	**45.5**	1.6
OBBR	50	45.3	2.5	50	44.6	2.1	48	45.1	1.8	51	**45.7**	1.6
OBHL	50	35.6	2.0	50	**36.0**	2.1	51	35.7	1.9	51	**36.0**	2.0
OBHR	51	35.9	2.1	51	**36.0**	2.2	50	35.7	1.8	50	35.7	2.1
EKB	50	**101.0**	4.0	51	97.8	4.2	47	97.7	3.1	47	98.9	3.0
DKB	51	**19.2**	2.4	50	17.1	2.1	50	16.8	1.5	51	17.8	2.0
FRC	49	114.3	5.1	50	113.3	4.6	50	113.6	4.6	51	**117.2**	3.9
PAC	51	117.9	6.5	51	117.1	7.3	51	115.0	5.3	51	**118.0**	6.3
OCC	51	98.6	5.3	51	96.3	6.3	51	95.8	4.9	51	**101.5**	4.9
FOL	50	38.6	2.6	51	38.9	2.5	48	37.6	1.9	50	**39.4**	2.4
FOB	51	31.4	2.0	51	32.4	2.1	50	31.5	2.2	48	**33.6**	2.0
MDHL	51	**33.9**	3.2	51	32.3	3.8	48	31.9	2.7	49	33.7	2.6
MDHR	51	34.0	3.4	51	32.7	3.4	48	33.2	2.8	51	**34.3**	3.1
AUB	49	120.1	4.5	50	120.8	4.4	47	118.8	3.8	51	**124.7**	4.5
NOL	51	186.7	6.9	50	182.9	6.6	51	182.9	6.4	49	**186.8**	5.8
MAPL	48	**76.5**	3.2	51	74.4	3.4	50	73.5	4.0	48	75.3	4.0
MAPR	50	**76.9**	4.1	51	74.7	3.6	50	73.3	4.0	51	75.7	4.2
MAP3L	51	125.8	5.7	50	122.7	5.0	49	121.7	6.2	50	**126.8**	5.6
MAP3R	51	127.0	5.5	50	124.0	5.4	49	122.3	6.2	50	**127.3**	5.8
NLH2	51	54.7	2.7	51	57.1	3.9	50	57.5	3.1	49	**58.3**	3.4

The ILD means for black South African males were overall larger than the means of their female counterparts for all distances except DKB, for which the females were larger than the males. Black South African males were more variable than their female counterparts for 18 of the 32 ILDs, whereas black South African females were more variable for nine of the 32 ILDs when compared to their male counterparts. For BNL, NLB, DKB, MDHL and NLH2, the SD were the same for both males and females (Table 4.15).

Table 4.15. Black South African male and female means and SD for the various ILDs. Bold indicates the larger mean for each ILD.

ILD	black SA females			black SA males			ILD	black SA females			black SA males		
	n	mean	SD	*n*	mean	SD		*n*	mean	SD	*n*	mean	SD
GOL	50	181.3	6.0	51	**188.8**	7.0	EKB	50	98.0	3.5	50	**101.0**	4.0
XCB	49	134.8	4.0	49	**138.4**	4.7	DKB	49	**19.8**	2.4	51	19.2	2.4
ZYB	51	124.0	4.5	50	**130.5**	5.3	FRC	51	110.5	5.2	49	**114.3**	5.1
BBH	51	131.8	4.9	50	**136.6**	5.3	PAC	50	115.0	7.0	51	**117.9**	6.5
BNL	51	98.2	4.1	51	**104.4**	4.1	OCC	50	97.9	5.1	51	**98.6**	5.3
ASB	51	108.3	5.4	51	**110.5**	5.3	FOL	51	36.6	2.0	50	**38.6**	2.6
WFB	51	94.3	4.6	51	**96.8**	5.6	FOB	51	29.4	2.3	51	**31.4**	2.0
UFBR	51	103.6	4.3	51	**107.3**	4.7	MDHL	51	29.7	3.2	51	**33.9**	3.2
NLHL	51	48.0	2.7	50	**51.1**	2.4	MDHR	51	29.9	3.2	51	**34.0**	3.4
NLHR	51	48.1	2.7	51	**51.2**	2.5	AUB	51	116.6	4.8	49	**120.1**	4.5
NLH	51	48.0	2.7	49	**50.9**	2.2	NOL	50	179.7	5.9	51	**186.7**	6.9
NLB	49	27.9	2.0	50	**28.0**	2.0	MAPL	49	73.1	3.5	48	**76.5**	3.2
OBBL	51	43.1	2.0	51	**45.3**	2.6	MAPR	51	74.0	4.0	50	**76.9**	4.1
OBBR	49	43.6	1.7	50	**45.3**	2.5	MAP3L	50	120.9	5.6	51	**125.8**	5.7
OBHL	51	35.3	1.9	50	**35.6**	2.0	MAP3R	49	122.0	5.4	51	**127.0**	5.5
OBHR	49	35.6	1.8	51	**35.9**	2.1	NLH2	51	51.1	2.7	51	**54.7**	2.7

ILD means for coloured South Africans showed a similar pattern to black South Africans with males generally having larger means than females. However, unlike the black South Africans, coloured South African females were smaller than their male counterparts for all measurements. Coloured South African males were overall also more variable than their female counterparts, with the SD for males larger for 29 of the 32 measurements than for the females. Females only had the larger SD for ZYB, MAPL and MAPR and were equally variable for BBH and FOB when compared to the males (Table 4.16).

Table 4.16. Coloured South African male and female means and SD for the various ILDs. Bold indicates the larger mean for each ILD.

ILD	coloured SA females			coloured SA males			ILD	coloured SA females			coloured SA males		
	n	mean	SD	*n*	mean	SD		*n*	mean	SD	*n*	mean	SD
GOL	50	178.3	5.9	51	**185.5**	7.2	EKB	51	95.6	3.4	51	**97.8**	4.2
XCB	51	133.5	4.6	50	**138.6**	5.1	DKB	49	17.0	2.0	50	**17.1**	2.1
ZYB	51	121.1	4.2	50	**126.8**	3.9	FRC	51	109.8	3.9	50	**113.3**	4.6
BBH	51	131.3	4.9	49	**136.8**	4.9	PAC	51	111.4	6.1	51	**117.1**	7.3
BNL	51	99.0	4.2	49	**103.9**	4.5	OCC	51	94.0	5.6	51	**96.3**	6.3
ASB	51	107.6	3.8	49	**110.3**	5.0	FOL	51	37.0	2.4	51	**38.9**	2.5
WFB	51	92.9	3.4	51	**95.2**	4.8	FOB	50	30.9	2.1	51	**32.4**	2.1
UFBR	51	101.7	3.4	50	**103.2**	4.5	MDHL	51	29.1	2.5	51	**32.3**	3.8
NLHL	51	49.9	2.7	50	**52.8**	3.5	MDHR	51	29.5	2.6	51	**32.7**	3.4
NLHR	51	49.8	2.6	51	**52.9**	3.6	AUB	50	115.6	4.3	50	**120.8**	4.4
NLH	51	49.8	2.6	51	**52.9**	3.6	NOL	50	176.5	6.2	50	**182.9**	6.6
NLB	50	25.3	1.4	48	**25.4**	1.7	MAPL	51	71.4	3.8	51	**74.4**	3.4
OBBL	50	43.8	1.9	49	**45.0**	2.1	MAPR	51	71.6	3.9	51	**74.7**	3.6
OBBR	50	43.7	1.9	50	**44.6**	2.1	MAP3L	50	117.7	4.5	50	**122.7**	5.0
OBHL	51	35.7	2.0	50	**36.0**	2.1	MAP3R	49	119.0	4.4	50	**124.0**	5.4
OBHR	51	35.4	2.0	51	**36.0**	2.2	NLH2	51	53.6	2.8	51	**57.1**	3.9

Similar to coloured South Africans, Indian South African males had larger means for all ILD when compared to those of their female counterparts (Table 4.17). Indian South African males were more variable for 24 of the 32 ILDs, with SDs larger than those of females. Indian South African females were more variable than their male counterparts were for NLHR, OBHR, DKB, PAC, and FOL (Table 4.17).

Likewise, white South African males were also larger for all ILD means when compared to their female counterparts and were also more variable for 24 of the 32 ILDs (Table 4.18). White South African females were more variable than the white South African males for OBBR, OBHL, FRC, PAC, OCC, MDHL (Table 4.18).

Table 4.17. Indian South African male and female means and SD for the various ILDs. Bold indicates the larger mean for each ILD.

ILD	Indian SA females			Indian SA males			ILD	Indian SA females			Indian SA males		
	n	mean	SD	*n*	mean	SD		*n*	mean	SD	*n*	mean	SD
GOL	51	174.7	5.7	51	**185.3**	6.4	EKB	51	94.3	2.9	47	**97.7**	3.1
XCB	51	132.1	4.6	51	**136.2**	5.6	DKB	51	15.9	2.2	50	**16.8**	1.5
ZYB	50	120.3	3.8	51	**125.9**	4.2	FRC	51	107.5	4.3	50	**113.6**	4.6
BBH	50	127.5	4.0	50	**135.2**	4.8	PAC	49	109.1	5.4	51	**115.0**	5.3
BNL	51	97.5	3.4	51	**105.2**	4.7	OCC	51	93.1	4.9	51	**95.8**	4.9
ASB	51	105.4	4.8	50	**108.4**	6.4	FOL	51	35.6	2.4	48	**37.6**	1.9
WFB	51	90.7	3.6	51	**95.0**	4.0	FOB	51	29.8	1.8	50	**31.5**	2.2
UFBR	51	99.1	3.4	50	**103.5**	3.6	MDHL	51	28.0	2.2	48	**31.9**	2.7
NLHL	51	48.2	2.9	51	**53.5**	3.2	MDHR	51	28.6	2.4	48	**33.2**	2.8
NLHR	51	48.2	3.3	51	**53.5**	2.9	AUB	51	114.3	3.4	47	**118.8**	3.8
NLH	51	48.2	3.0	51	**53.5**	3.0	NOL	51	173.0	5.5	51	**182.9**	6.4
NLB	51	24.4	1.5	49	**25.0**	1.7	MAPL	51	68.9	3.2	50	**73.5**	4.0
OBBL	48	44.1	1.7	48	**45.3**	1.7	MAPR	51	69.8	2.9	50	**73.3**	4.0
OBBR	51	44.2	1.6	48	**45.1**	1.8	MAP3L	51	114.6	4.1	49	**121.7**	6.2
OBHL	50	34.8	1.7	51	**35.7**	1.9	MAP3R	51	114.3	4.8	49	**122.3**	6.2
OBHR	51	34.5	1.9	50	**35.7**	1.8	NLH2	51	52.0	2.8	50	**57.5**	3.1

Table 4.18. White South African male and female means and SD for the various ILDs. Bold indicates the larger mean for each ILD.

ILD	white SA females			white SA males			ILD	white SA females			white SA males		
	n	mean	SD	*n*	mean	SD		*n*	mean	SD	*n*	mean	SD
GOL	51	179.5	5.5	51	**190.3**	6.8	EKB	51	95.1	2.7	47	**98.9**	3.0
XCB	51	137.5	4.4	51	**143.5**	4.5	DKB	51	17.7	1.3	51	**17.8**	2.0
ZYB	51	121.8	3.3	50	**130.3**	3.9	FRC	51	110.8	4.8	51	**117.2**	3.9
BBH	51	134.1	4.4	51	**141.9**	4.8	PAC	51	112.2	7.3	51	**118.0**	6.3
BNL	51	100.9	3.9	51	**108.1**	4.4	OCC	51	98.9	5.5	51	**101.5**	4.9
ASB	51	110.9	4.0	51	**115.6**	5.9	FOL	51	36.8	2.3	50	**39.4**	2.4
WFB	51	93.3	4.0	50	**96.7**	4.4	FOB	50	31.2	2.0	48	**33.6**	2.0
UFBR	49	99.9	3.0	48	**105.0**	4.1	MDHL	50	30.1	2.7	49	**33.7**	2.6
NLHL	51	51.8	2.9	51	**54.5**	3.4	MDHR	51	31.0	2.8	51	**34.3**	3.1
NLHR	50	51.9	2.7	51	**54.3**	3.1	AUB	51	118.2	3.8	51	**124.7**	4.5
NLH	50	51.9	2.7	51	**54.4**	3.2	NOL	51	178.0	5.5	49	**186.8**	5.8
NLB	51	24.8	1.9	51	**25.4**	2.0	MAPL	51	71.3	3.3	48	**75.3**	4.0
OBBL	51	42.7	1.6	49	**45.5**	1.6	MAPR	51	71.7	2.8	51	**75.7**	4.2
OBBR	51	42.9	1.9	51	**45.7**	1.6	MAP3L	51	117.2	4.5	50	**126.8**	5.6
OBHL	50	35.5	2.1	51	**36.0**	2.0	MAP3R	51	117.2	4.4	50	**127.3**	5.8
OBHR	51	35.3	1.9	50	**35.7**	2.1	NLH2	50	55.9	2.4	49	**58.3**	3.4

4.2.3. Significance testing

4.2.3.1. ANOVA and Tukey's HSD

Analysis of variance (ANOVA) was used to test for significant differences for the means of both sex and populations and to assess for any interaction between the two variables. Significant differences between males and females were observed for all ILDs, except NLB (p = 0.0929) and DKB (p = 0.598). Only OBBL (p = 0.191), OBBR (p = 0.307), OBHL (p = 0.0839), and OBHR (p = 0.0932) where not significantly different when explored by population group. Significant interactions between sex and population were present for 11 of the 32 ILDs (Table 4.19).

The *post hoc* Tukey's HSD tests among which populations overlap was present. At least two populations overlapped for all ILDs. The orbital height and breadth ILDs showed group overlap among all population groups. Indian and coloured South Africans overlapped for most (25 of 32) ILDs, except for BBH, ASB, FOL, FOB, MAPL, MAPR, and MAP3R (Table 4.19). Indian and white South Africans overlapped for the fewest ILDs, with overlap only present for UFBR, NLB and EKB. Coloured and black South Africans overlapped for 11 of the 32 ILDs, which was fewer than white and black South Africans (12 of 32 ILDs) or white and coloured South Africans (14 of 32 ILDs) overlapped (Figures A2.1-2.4).

Table 4.19. ANOVA to assess the ILD for significant differences between the sexes and populations, as well as for any significant interactions between sex and population affinity. A *post hoc* Tukey's HSD test was used to explore which populations overlapped for the various ILDs. Bold *p*-values indicate non-significance.

ILD	ANOVA						Tukey's HSD
	Sex		Population Affinity		Pop*Sex interaction		Overlap between groups
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	
GOL	182.9	<0.001	10.1	<0.001	2.3	**0.076**	W-B; I-C
XCB	81.2	<0.001	26.1	<0.001	1.3	**0.263**	C-B; I-C
ZYB	218.6	<0.001	12.5	<0.001	2.7	<0.05	W-B; I-C
BBH	147.2	<0.001	22.5	<0.001	2.6	**0.050**	C-B
BNL	224.5	<0.001	8.9	<0.001	2.3	**0.074**	C-B; I-B; I-C
ASB	31.5	<0.001	25.3	<0.001	1.1	**0.345**	C-B
WFB	49.3	<0.001	6.7	<0.001	1.1	**0.332**	C-B; W-B; I-C; W-C
UFBR	75.4	<0.001	16.7	<0.001	3.9	<0.01	I-C; W-C; W-I
NLHL	118.0	<0.001	18.8	<0.001	4.5	<0.01	I-C
NLHR	118.7	<0.001	17.5	<0.001	4.3	<0.01	I-C; I-B
NLH	118.8	<0.001	20.3	<0.001	4.8	<0.01	I-C
NLB	2.8	**0.0929**	66.6	<0.001	0.6	**0.619**	I-C; W-C; W-I
OBBL	88.6	<0.001	1.6	**0.191**	3.9	<0.01	All groups
OBBR	66.8	<0.001	1.2	**0.307**	5.6	<0.001	All groups
OBHL	7.2	<0.01	2.2	**0.0839**	0.6	**0.637**	All groups
OBHR	10.6	<0.01	2.2	**0.0932**	1.2	**0.304**	All groups
EKB	73.1	<0.001	17.4	<0.001	1.0	**0.399**	I-C; W-C; W-I
DKB	0.3	**0.598**	44.6	<0.001	2.5	**0.062**	I-C; W-C
FRC	111.4	<0.001	8.0	<0.001	2.9	<0.05	W-B; I-C; C-B; I-B
PAC	60.0	<0.001	7.0	<0.001	1.2	**0.300**	C-B; W-B; I-C; W-C
OCC	12.8	<0.001	24.8	<0.001	0.8	**0.508**	I-C; W-B
FOL	82.3	<0.001	7.5	<0.001	0.6	**0.604**	C-B; W-B; W-C
FOB	73.9	<0.001	15.5	<0.001	0.9	**0.435**	I-B; W-C
MDHL	154.2	<0.001	7.7	<0.001	0.7	**0.576**	C-B; W-B; I-C; W-C
MDHR	155.6	<0.001	5.6	<0.001	1.1	**0.341**	C-B; I-B; W-B; I-C
AUB	117.8	<0.001	18.1	<0.001	2.2	**0.084**	C-B; I-C
NOL	154.7	<0.001	10.9	<0.001	1.7	**0.163**	W-B; I-C; W-C
MAPL	97.6	<0.001	13.6	<0.001	1.0	**0.389**	W-C
MAPR	75.0	<0.001	15.7	<0.001	0.6	**0.651**	W-C
MAP3L	14.0	<0.001	13.5	<0.001	4.5	<0.01	W-B; I-C; W-C
MAP3R	151.8	<0.001	16.5	<0.001	5.8	<0.001	W-C; W-B
NLH2	124.9	<0.001	23.3	<0.001	4.3	<0.01	I-C

4.2.4. Symmetric percent differences to assess sexual dimorphism

Sympercents (s%) were used to compare sexual dimorphism between males and females of each population. Differences between males and females were observed in the s% calculated for all ILDs for all four groups (Table 4.20).

Overall, males presented with larger mean values and produced positive s% for all ILDs except for the DKB for black South Africans, where the females were larger than their male counterparts. Indian South African males and females had the highest degree of sexual dimorphism for the most measurements (16 of 32), followed closely by white South Africans (15 of 32). Coloured South Africans were the least sexually dimorphic, with an average s% of 1.74% (Table 4.20). The various nasal height ILDs (NLHL, NLHR, NLH, NLH2) were also highly sexually dimorphic for black, coloured and Indian South Africans, whereas the cheek projections (MAP3L and MAP3R) were more dimorphic for white South Africans. The mastoid processes had the greatest s% for all four populations, while NLB had the smallest s% for black South Africans, DKB had the smallest s% for coloured and white South Africans and OBBR had the smallest s% for Indian South Africans (Table 4.20).

The graph in Figure 4.5 demonstrates the differences in sexual dimorphism among the various populations for the various ILDs. Indian and white South Africans lines are consistently on top, indicating greater degrees of sexual dimorphism for the majority of the ILDs (Figure 4.5).

Table 4.20. Comparison of s% between black, coloured, Indian and white South African populations. A negative s% indicates females are larger than males and bold indicates the group with the largest s% for each ILD.

ILD	B	C	I	W
GOL	1.75	1.72	**2.55**	2.53
XCB	1.14	1.61	1.32	**1.87**
ZYB	2.24	2.02	1.98	**2.96**
BBH	1.54	1.79	**2.54**	2.46
BNL	2.66	2.09	**3.33**	3.01
ASB	0.84	1.07	1.23	**1.77**
WFB	1.12	1.06	**2.01**	1.53
UFBR	1.53	0.63	1.89	**2.14**
NLHL	2.66	2.46	**4.61**	2.26
NLHR	2.77	2.64	**4.47**	1.95
NLH	2.55	2.62	**4.54**	2.04
NLB	0.18	0.15	0.99	**1.03**
OBBL	2.18	1.15	1.18	**2.68**
OBBR	1.65	0.90	0.91	**2.79**
OBHL	0.35	0.44	**1.15**	0.59
OBHR	0.35	0.80	**1.53**	0.43
EKB	1.28	1.00	1.53	**1.70**
DKB	-1.44	0.14	**2.37**	0.23
FRC	1.49	1.35	2.42	**2.46**
PAC	1.11	2.17	**2.28**	2.21
OCC	0.30	1.01	**1.23**	1.14
FOL	2.24	2.25	2.33	**3.06**
FOB	2.89	2.04	2.39	**3.20**
MDHL	**5.84**	4.46	5.58	4.87
MDHR	5.54	4.53	**6.42**	4.41
AUB	1.27	1.92	1.69	**2.30**
NOL	1.65	1.56	**2.41**	2.09
NLH2	3.01	2.78	**4.32**	1.84
MAPL	1.97	1.78	**2.83**	2.33
MAPR	1.64	1.83	2.15	**2.41**
MAP3L	1.71	1.81	2.61	**3.41**
MAP3R	1.75	1.78	2.94	**3.62**
Average	1.81	1.74	**2.55**	2.29
Smallest s%	0.18	0.14	0.91	0.23
Largest s%	5.84	4.53	6.42	4.87

Figure 4.5. Chart illustrating the differences in sexual dimorphism among the various population groups represented by the sympercents (s%).

4.2.5. Classification statistics

The software FD3 was used to estimate population affinity, sex, and sex and population affinity, simultaneously, by combining the most discriminatory variables to produce the best possible group separation and thus the best possible classification accuracy. To give a more realistic correct classification rate, LOOCV was also applied.

For each analysis, the ILDs selected for model creation, as well as the relative importance (GS Imp %) of each distance were recorded to show the contribution of each ILD to the separation of the reference groups (Table A4.1).

When estimating population affinity, 24 variables were selected using Forward Wilks stepwise selection. The overall cross-validated correct classification rate was 62.2%; however, the various populations had individual correct classification rates from 41.6% to 81.8%. Black South Africans had the least misclassifications while coloured South Africans had the most misclassifications. Most of the coloured South Africans misclassified as Indian or white South Africans, while the majority of the Indian South Africans misclassified as coloured or white South Africans. Similarly, most white South Africans misclassified as either coloured or black South Africans (Table 4.21).

The graph below also depicts the group classifications with the ellipses indicating a 95% confidence interval. A large amount of overlap is apparent for coloured, Indian, and white South Africans, with black South Africans more separated from the other three groups when visualised in a graph comparing the first two canonical variables (Figure 4.6).

Table 4.21. Classification matrix and cross-validated (CV) correct classification rates when estimating population affinity using 24 Forward Wilks selected cranial ILDs.

From group	Into group				Total	Correct classification
	B	**C**	**I**	**W**		
B	63	10	0	4	77	81.8%
C	9	32	20	16	77	41.6%
I	5	15	44	12	76	57.9%
W	3	14	10	55	82	67.1%

Total correct = 194 of 312
CV correct classification = 62.2%
Mean Cohen's kappa = 0.496
Mean Balanced Accuracy = 0.747

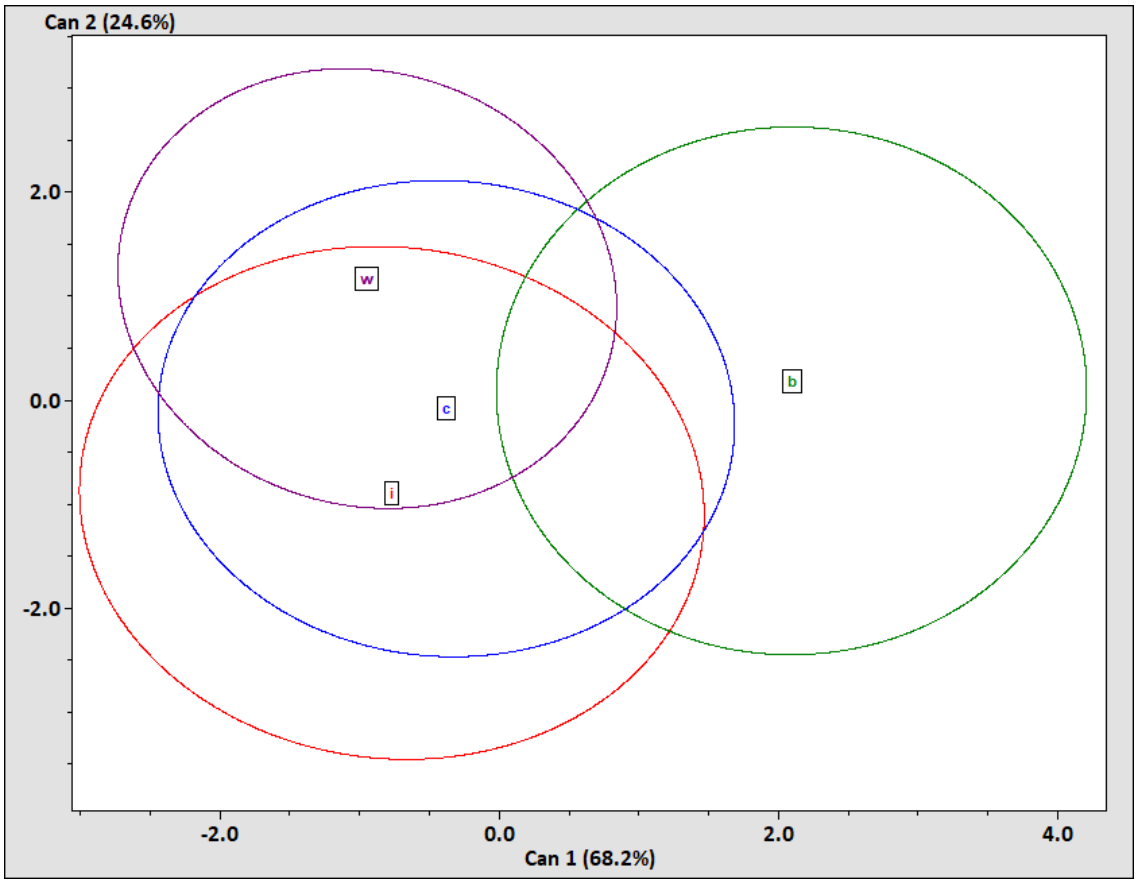

Figure 4.6. Graph illustrating the classification of the four population groups. The letters depict the group centroids. Note the extensive group overlap among coloured, Indian and white South Africans.

As significant sex differences were noted with the ANOVA test, LDAs were also run with the sexes separated. When only the females of the various populations were assessed using FD3, Forward Wilks variable selection identified 22 variables that would maximise group differences (Table 4.22). Correct classifications rates were overall higher than when the males

and females were combined, with the rates ranging from 56.1% to 81.6%. Black South African females still had the lowest number of misclassifications and coloured South African females the highest number. Coloured South African females misclassified mostly as Indian or white South African females, while the Indian females misclassified mostly as coloured or white South Africans. White South African females misclassified mostly as coloured females.

Table 4.22. Classification matrix and cross-validated (CV) correct classification rates when estimating population affinity among the females of the various population groups using 22 Forward Wilks selected cranial ILDs.

From group	Into group				Total	Correct classification
	BF	**CF**	**IF**	**WF**		
BF	31	4	0	3	38	81.6%
CF	5	23	5	8	41	56.1%
IF	2	4	32	4	44	77.3%
WF	2	6	2	36	46	78.3%

Total correct = 124 of 169
CV correct classification = 73.4%
Mean Cohen's kappa = 0.645
Mean Balanced Accuracy = 0.822

Similar to when the sexes were combined, the black South African females were more distinct from the other three populations. However, the coloured, Indian and white South African females overlapped a little less when compared to the graph of the LDA where the sexes were pooled (Figure 4.7).

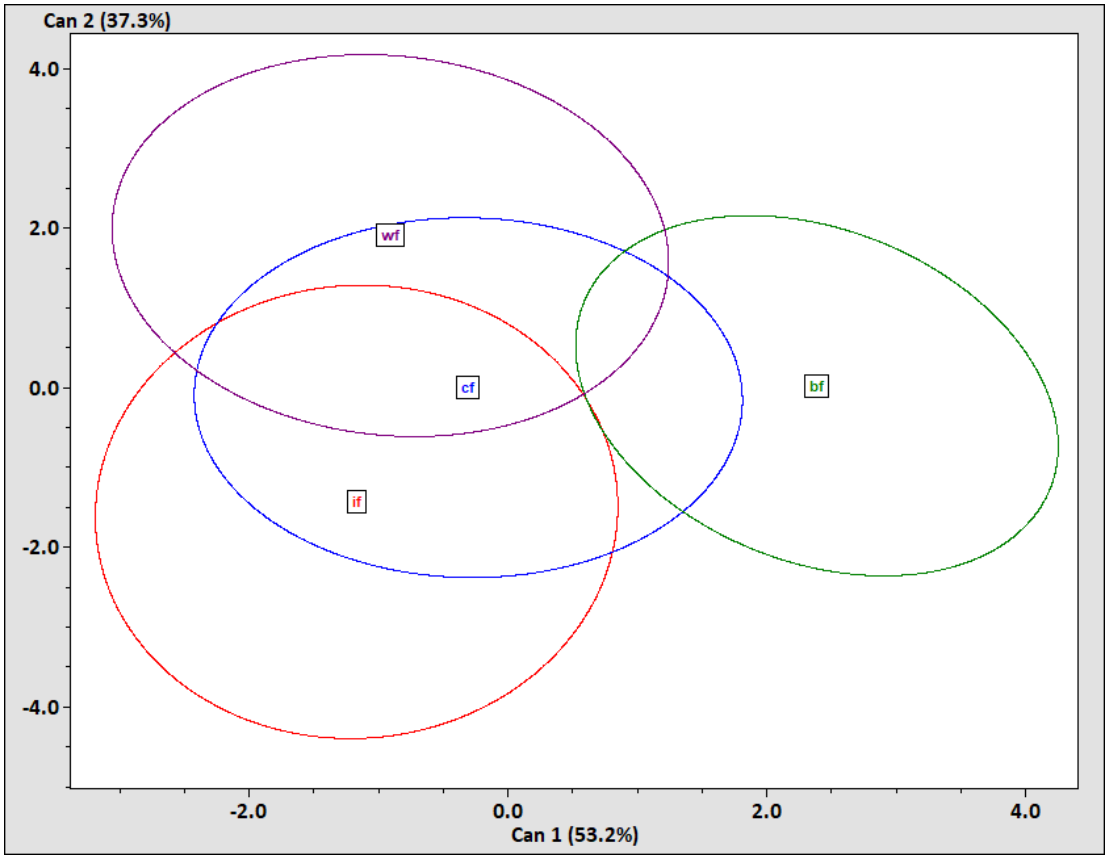

Figure 4.7. Graph illustrating the classification of the females of the four population groups. The letters depict the group centroids.

When only the males of the four populations were combined, the overall correct classification rate was substantially lower than for the females. For the male analysis, 26 variables were selected and combined to maximise group differences. The correct classification rates ranged from 40.5% to 70.0% with a similar pattern to the females, with black South African males obtaining the highest and coloured South African males the lowest correct classification rates. A large number of coloured males (9 of 36) misclassified as Indian South African. Similarly, a large number of Indian South African males misclassified as coloured South African males. Both black and white South African males misclassified mostly as coloured South African males (Table 4.23).

In the graph all groups appear to overlap considerably; however, unlike in the female analysis, the overlap between coloured and Indian South African males is more substantial (Figure 4.8).

Table 4.23. Classification matrix and cross-validated (CV) correct classification rates when estimating population affinity among the males of the various population groups using 26 Forward Wilks selected cranial ILDs.

From group	Into group				Total	Correct classification
	BM	**CM**	**IM**	**WM**		
BM	25	9	2	2	38	65.8%
CM	4	15	11	7	37	40.5%
IM	1	13	15	5	34	44.1%
WM	1	6	5	28	40	70.0%

Total correct = 83 of 149
CV correct classification = 55.7%
Mean Cohen's kappa = 0.411
Mean Balanced Accuracy = 0.702

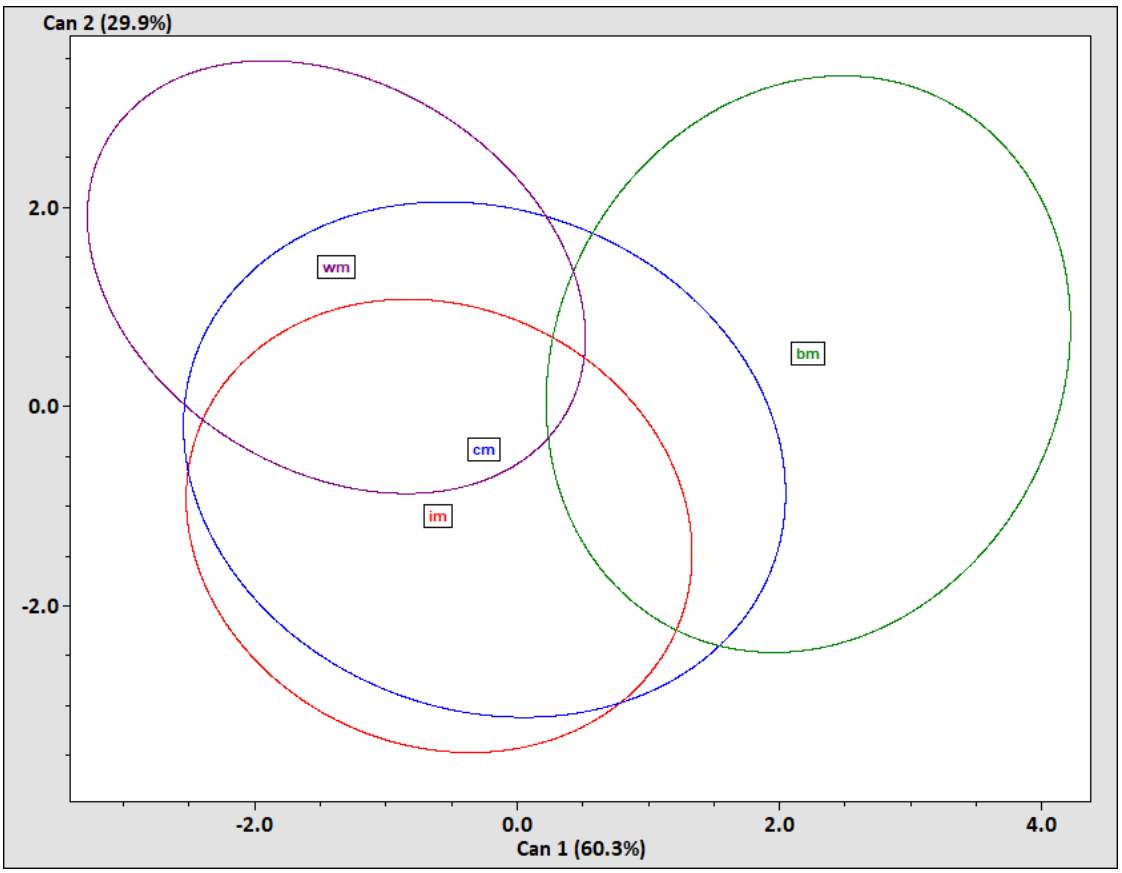

Figure 4.8. Graph illustrating the classification of the males of the four population groups. The letters depict the group centroids.

To explore the classification potential of the cranial ILDs to separate into both sex and populations, simultaneously, a further LDA was run using 22 Forward Wilks stepwise selected variables. Furthermore, simultaneous sex and population affinity estimation was performed to assess whether individual groups misclassified more as a result of sex or as population affinity. The classification rates ranged from 24.3% to 73.7%, where coloured South African males obtained the lowest correct classification rate and black South African females and white South African males obtained the highest rates. The coloured South African males misclassified mostly as Indian or white South African males, although some misclassified as coloured or white South African females. Coloured South African females, with the seconds lowest correct classification rate, misclassified mainly as Indian or white South African females; however, some misclassified as coloured or Indian South African males (Table 4.24). For most groups, misclassifications were more prevalent according to population affinity than according to sex, indicating that differences between males and females were present even when all population groups were assessed simultaneously.

While extensive overlap is present in the graph illustrated in Figure 4.9, clear separation between the males and females can be observed along the x-axis (representing the first canonical variate score), while separation between black South Africans (males and females) and the other population-sex groups can be observed along the y-axis (second canonical variate score).

Table 4.24. Classification matrix and cross-validated (CV) correct classification rates when estimating population affinity and sex simultaneously using 22 Forward Wilks selected cranial ILDs.

From group	Into group								Total	Correct classification
	BF	**BM**	**CF**	**CM**	**IF**	**IM**	**WF**	**WM**		
BF	28	3	3	1	2	0	1	0	38	73.7%
BM	1	28	0	5	0	3	1	2	40	70.0%
CF	4	1	18	3	7	4	5	0	42	42.9%
CM	0	4	4	9	0	10	3	7	37	24.3%
IF	3	0	5	2	33	1	2	0	46	71.7%
IM	1	3	2	11	3	9	4	2	35	25.7%
WF	1	1	7	2	0	5	30	0	46	65.2%
WM	0	0	0	6	0	3	1	28	38	73.7%

Total correct = 183 of 322
CV correct classification = 56.8%
Mean Cohen's kappa = 0.507
Mean Balanced Accuracy = 0.749

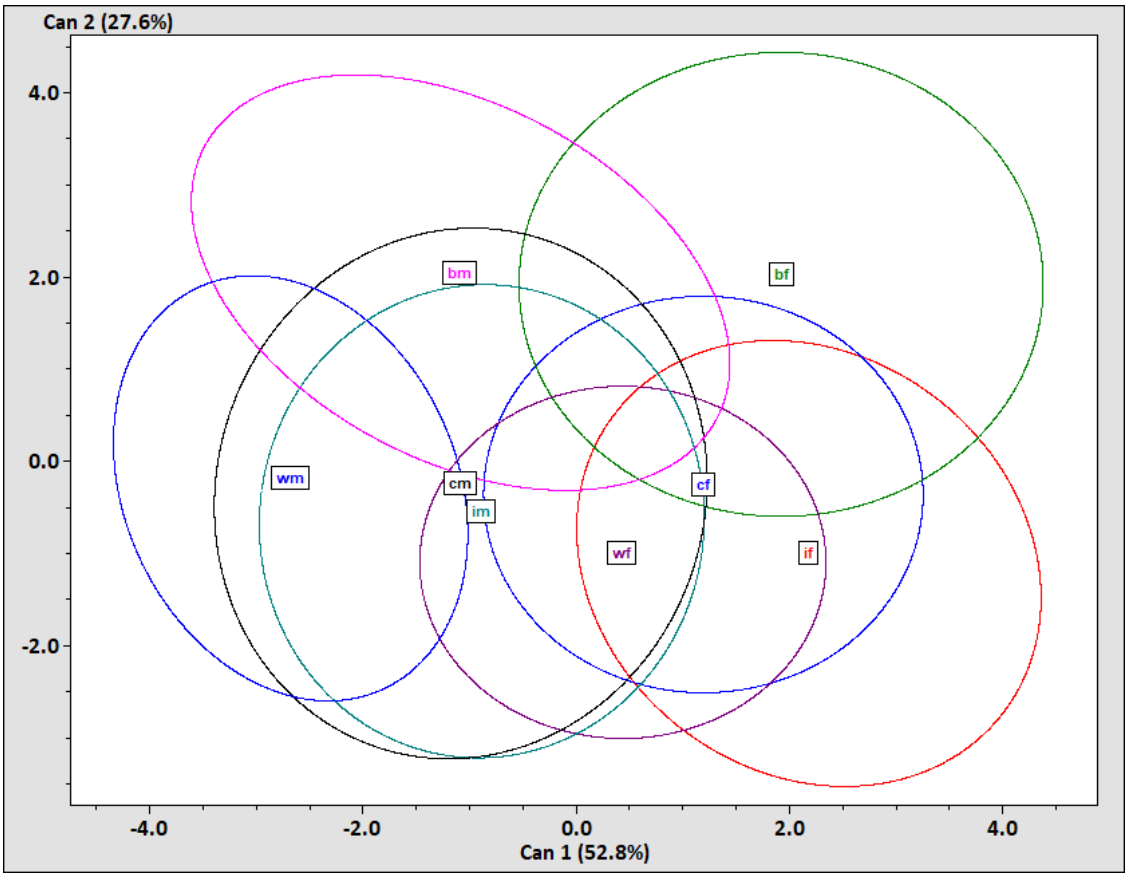

Figure 4.9. Graph illustrating the classification of the sample according to both sex and population affinity simultaneously. The letters depict the group centroids.

To explore the cranial ILDs for their potential in estimating sex, 21 stepwise selected variables were combined. The correct classification rate was substantially higher than for estimating population affinity and only a slight female sex bias was noted for the cross-validated correct classification rates (Table 4.25).

Table 4.25. Classification matrix and cross-validated (CV) correct classification rates when estimating sex using 21 Forward Wilks selected cranial ILDs.

From group	Into group		Total	Correct classification
	F	**M**		
F	160	15	175	91.4%
M	15	132	158	89.8%
Total correct = 292 of 322				
CV correct classification = 90.7%				
Mean Cohen's kappa = 0.812				
Mean Balanced Accuracy = 0.906				

4.3. Geometric Morphometrics

4.3.1. Repeatability

Mean Euclidean distance was calculated for each landmark for the repeated landmark placement for both observers to assess the repeatability of the landmarking process. For most landmarks, except the right alare, the left asterion, and the right asterion, the mean Euclidean distance was lower for the intra-observer error than for the inter-observer error (Figure 4.10; Table A5.1). However, for the right alare, the mean Euclidean distance was equal for both observers, and for the left and right asterion landmarks, the inter-observer error was only slightly lower than the intra-observer error by 0.1mm and 0.08mm, respectively (Table A5.1). All intra-observer mean Euclidean distances were less than 2mm and only the left ectoconchion, left lower orbital border, and the right lower orbital border differed by more than 2mm between observers. Although the three landmarks had mean Euclidean distances greater than 2mm, the extent beyond the 2mm was at most 0.13mm (Table A5.1).

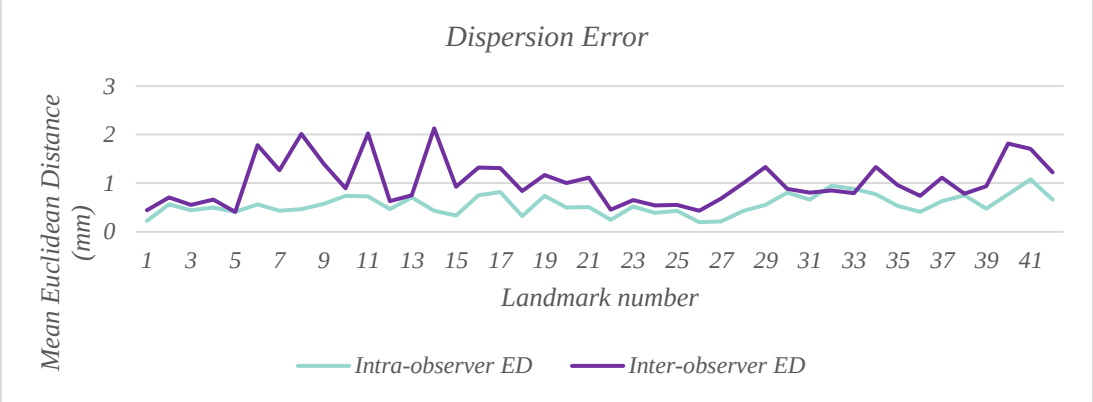

Figure 4.10. Comparison of the dispersion error (mean Euclidean distances) obtained for both the intra- and inter-observer error for the various landmarks.

4.3.2. Generalised Procrustes analysis

Once the landmark coordinates had undergone GPA and the coordinates had been translated, rotated, and rescaled, a mean shape was created (Figure 4.11). A larger amount of variation was noted around the cranial vault landmarks, particularly eurion, lambda, opisthocranion, and asterion.

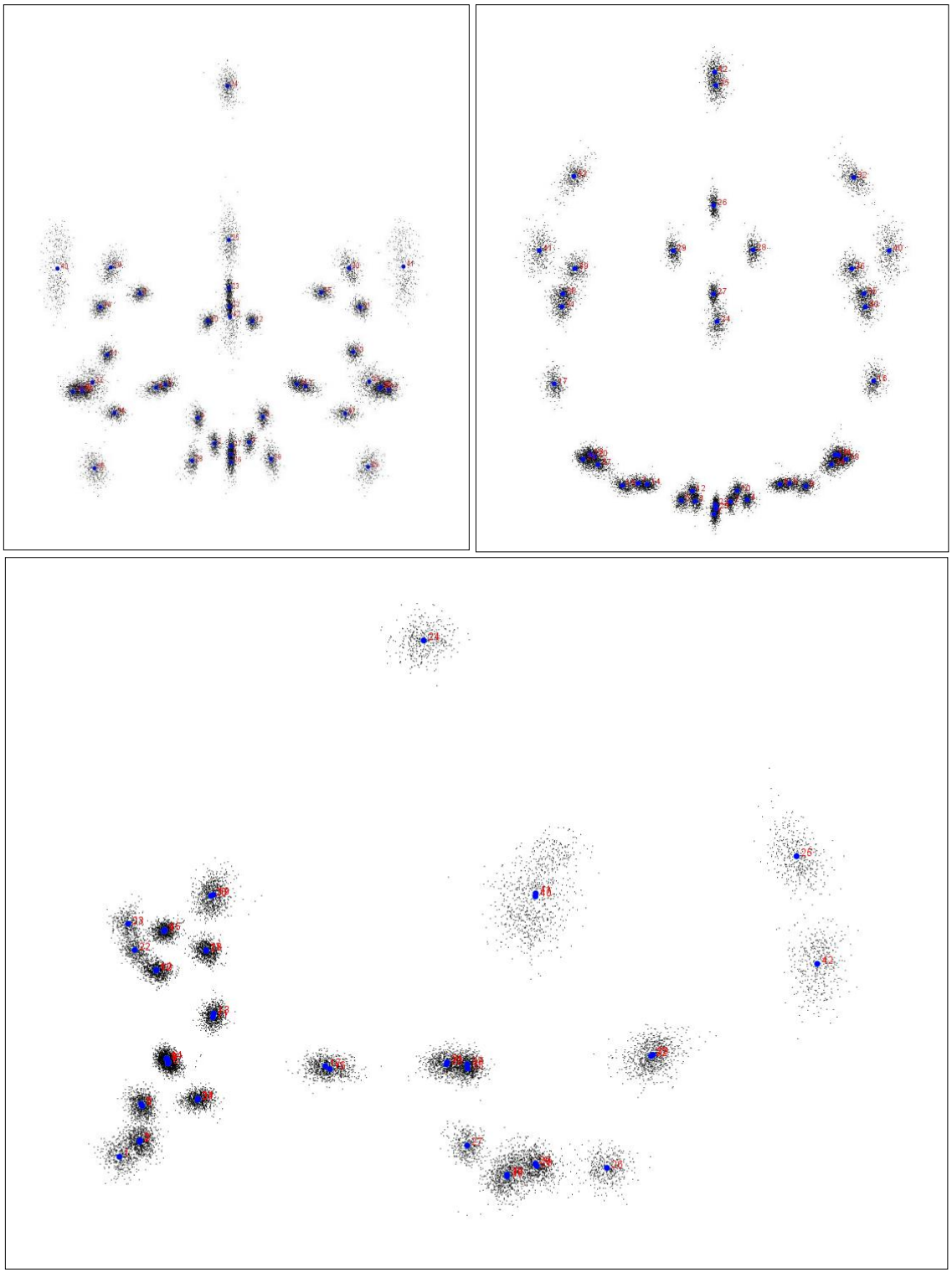

Figure 4.11. Anterior (top left), inferior (top right), and lateral (bottom) views of the mean landmarks (blue dot) and the migration of the individually scored cranial landmarks (black dots) in relation to the mean shape.

4.3.3. Principal Component Analysis and lollipop graphs

PCA was used to reduce the dimensionality of the 3D coordinate data. In order to decrease the number of variables, only PC scores that contributed 1% or more to the total variation were included in further analyses (Figure A6.1). Of the 119 PC scores, only 22 contributed enough variation to be included in further analyses and accounted for 80.56% of the total variation in the sample (Figure 4.12; Figure A6.1).

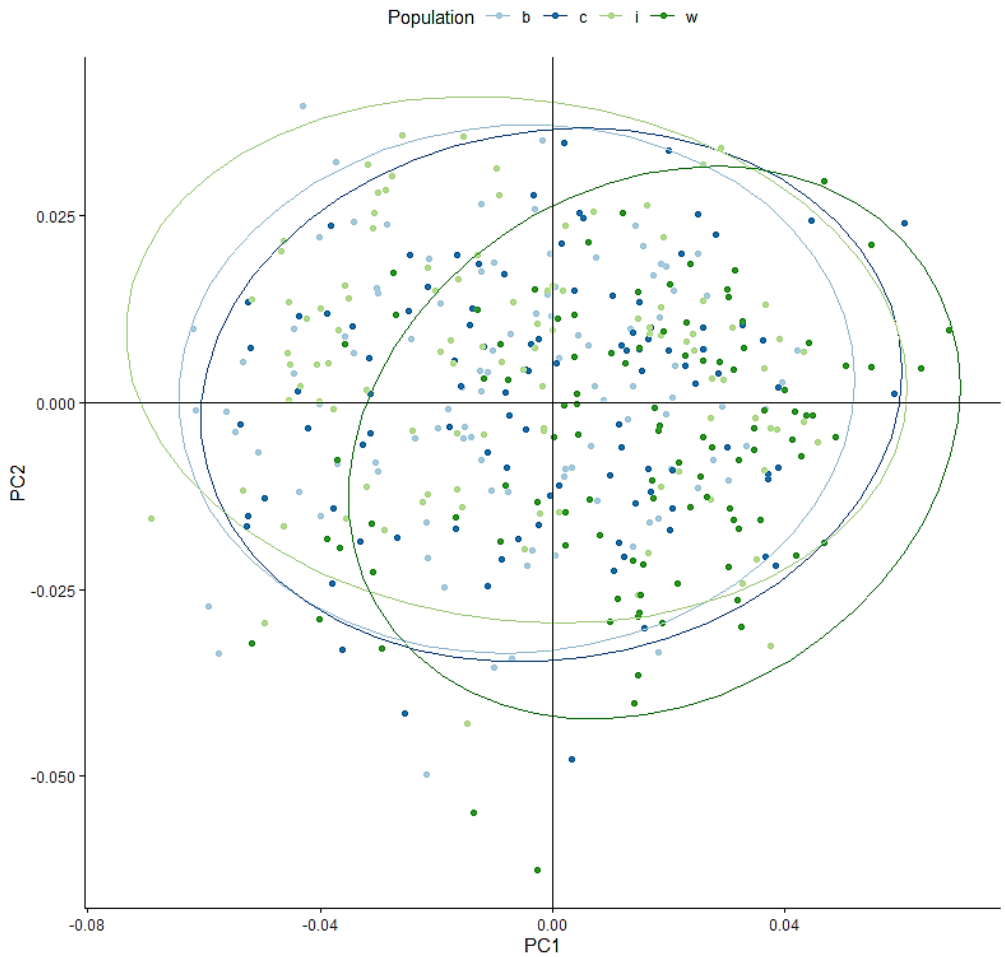

Figure 4.12. Plot of PC1 (20.1%) versus PC2 (7.3%) when assessing population affinity among black, coloured, Indian and white South Africans.

Principal component 1 (PC1) accounted for 20.1% of the total shape variation, while PC2 accounted for 7.3%. The major movement in PC1 occurred in the cranial vault, with minor movement in the nasal and midsagittal landmarks. The left and right eurion landmarks (landmarks 40 and 41, respectively) showed a tendency toward a slight anterior, but mostly

inferior, migration, whereas both lambda (landmark 25) and opisthocranion (landmark 42) showed a tendency toward a superior and slightly anterior migration. Similarly, both nasion (landmark 22) and glabella (landmark 23) showed a slight tendency toward an anterior and superior migration; however, to a lesser extent than the posterior landmarks. Both left and right asterions (landmarks 32 and 33, respectively) had a tendency toward a posterior and superior migration, to a similar extent than the lambda (landmark 25) and opisthocranion (landmark 42) landmarks (Figure 4.13).

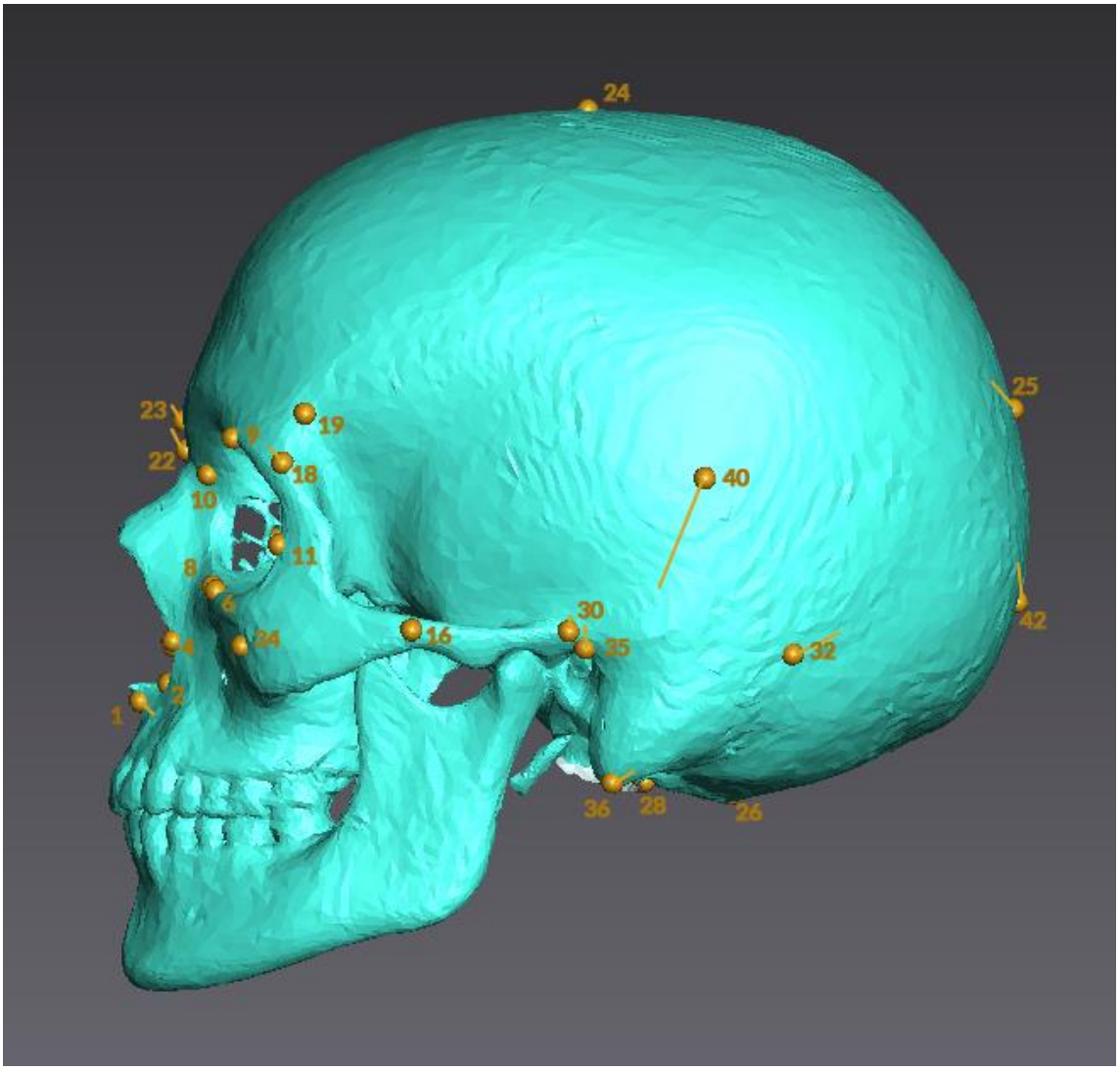

Figure 4.13. Lollipop graph superimposed on lateral view of the cranium showing the shape changes associated with PC1. Only the left landmarks visible for ease of viewing. Landmark names available in Table 3.2.

The major movements in PC2 occurred in the posterior and superior cranium, with some minor movements in the base and facial landmarks. Bregma (landmark 24) tended to migrate more superior and slightly posterior, which is similar to opisthocranion (landmark 42), while lambda (landmark 25) tended to migrate more inferiorly and posteriorly. Most cranial base landmarks tended to migrate more superiorly and anteriorly, while most facial landmarks tended to migrate more inferiorly and posteriorly (Figure 4.14).

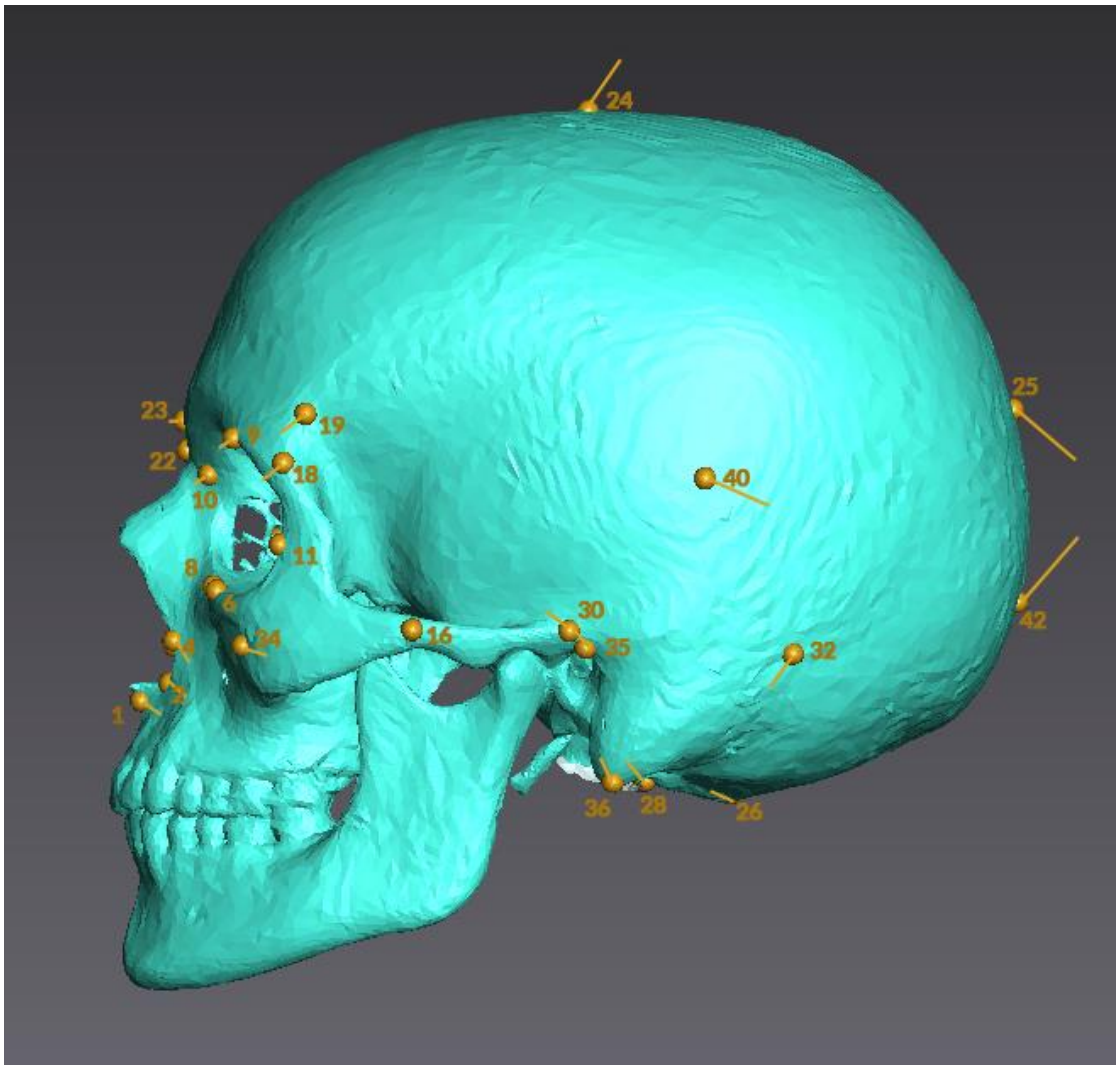

Figure 4.14. Lollipop graph superimposed on lateral view of the cranium showing the shape changes associated with PC2. Only the left landmarks visible for ease of viewing. Landmark names available in Table 3.2.

4.3.4. ANOVA, 50-50 MANOVA, and two-way population differences

The first 22 PC scores were also assessed using ANOVA with 1000 permutations and ordinary least squares. The results showed that significant population differences were present in the data ($p < 0.01$) with F-value = 10.64. An additional 50-50 MANOVA with a Holm's adjustment for multiple comparisons was run to explore the relationships among the various populations within the sample. All populations showed significant differences from one another (Table 4.26).

Table 4.26. 50-50 MANOVA with distances and *p*-values with Holm's adjustment for multiple comparisons.

Distances between group means with *p*-values			
	B	C	I
C	0.0214 ($p < 0.01$)		
I	0.0244 ($p < 0.01$)	0.0156 ($p < 0.05$)	
W	0.0361 ($p < 0.01$)	0.0230 ($p < 0.01$)	0.0333 ($p < 0.01$)
Overall 50-50 MANOVA *p*-value < 0.01			

ANOVA with 1000 permutations and ordinary least squares was again used to assess the first 22 PC scores, this time to test for differences between the sexes. The results indicated significant differences between males and females ($p < 0.001$) with F-value = 15.95. A 50-50 MANOVA with a Holm's adjustment revealed a significant distance of 0.026 ($p < 0.001$) between males and females.

Similarly, ANOVA with 1000 permutations was also used to assess for any significant differences between the various PopSex (population and sex combined) groups. The results indicated significant PopSex differences in the data ($p < 0.01$) with F-value = 8.07. The additional 50-50 MANOVA with a Holm's adjustment for multiple comparisons demonstrated

that while certain PopSex groups were significantly different from one another, multiple groups overlapped considerably (Table 4.27).

Table 4.27. 50-50 MANOVA with distances and *p*-values with Holm's adjustment for multiple comparisons when assessing PopSex differences. Bold indicates non-significance.

	Distances between group means with *p*-values						
	BF	BM	CF	CM	IF	IM	WF
BM	0.028 ($p < 0.05$)						
CF	**0.023 (*p* = 0.119)**	0.030 ($p < 0.05$)					
CM	0.033 ($p < 0.05$)	**0.020 (*p* = 0.228)**	**0.0230 (*p* = 0.119)**				
IF	0.027 ($p < 0.05$)	0.038 ($p < 0.05$)	**0.020 (*p* = 0.228)**	0.034 ($p < 0.05$)			
IM	0.036 ($p < 0.05$)	**0.260 (*p* = 0.081)**	**0.024 (*p* = 0.119)**	**0.015 (*p* = 0.382)**	0.030 ($p < 0.05$)		
WF	0.04 ($p < 0.05$)	0.034 ($p < 0.05$)	0.031 ($p < 0.05$)	**0.025 (*p* = 0.096)**	0.044 ($p < 0.05$)	0.033 ($p < 0.05$)	
WM	0.047 ($p < 0.05$)	0.036 ($p < 0.05$)	0.036 ($p < 0.05$)	**0.025 (*p* = 0.080)**	0.050 ($p < 0.05$)	0.031 ($p < 0.05$)	**0.021 (*p* = 0.228)**
Overall 50-50 MANOVA *p*-value < 0.001							

Pairwise comparisons were made between two of the populations at a time using discriminant function analysis in MorphoJ. The analyses were not used for classification purposes as all four groups should be compared simultaneously in order to observe the most realistic potential of the data in classifying the sample according to population affinity.

Lollipop graphs were assessed to explore the shape changes observed between the groups, with the first group represented by the point and the second by the line (lollipop stick). When black and coloured South African ProCoords were compared, the major group differences were noted in the nasal height-related landmarks (nasion, subspinale, and inferior nasal border), as well as in the cranial base and posterior vault landmarks (Figure A7.1). Subspinale and inferior nasal borders were located more superiorly and anteriorly, while the nasion and glabella were

located more inferiorly and posteriorly for black South Africans when compared to coloured South Africans. The opisthocranion and lambda landmarks were located more superiorly and posteriorly, while the foramen magnum breadth landmarks were located more superiorly and medially for black South Africans when compared to coloured South Africans. Mastoidale was also located more medially and inferiorly for black South Africans (Figure A7.1).

Similar difference could be seen between black and Indian and between black and white South Africans. Glabella and nasion landmarks were located more superiorly and anteriorly for both Indian and white South Africans when compared to black South Africans. Furthermore, the mid-malar projection point was located more posteriorly, the inferior nasal border and sub-spinale landmarks were located more inferiorly and posteriorly, and the alare landmarks were located slightly more medially for both Indian and white South Africans when compared to black South Africans (Figures A7.2 to A7.4). The eurion landmarks for white South Africans were also located more inferiorly and laterally than for both black South Africans and for Indian South Africans (Figure A7.2), although the landmark was located slightly more anteriorly for Indian South Africans when compared to black South Africans. Similarly, the asterion landmark was located more laterally for white South Africans than for both Indian and black South Africans. Opisthocranion was also located more superiorly for white South Africans when compared to both black and Indian South Africans; however, the landmark was slightly more inferior for Indian South Africans than for black South Africans.

Coloured and Indian South Africans were very similar for most landmarks. A very slight anterior shift was noted of the glabella and nasion landmarks of Indian South Africans, as well as a slight superior and anterior shift of the eurion landmark, when compared to coloured South Africans (Figure A7.5). Coloured and white South Africans were also very similar with a slight anterior and superior shift of nasion and glabella as well as bregma and lambda, with a slight superior-only shift of opisthocranion for white South Africans. A slight inferior and lateral shift

was noted for eurion and mastoidale, as well as an inferior shift of opisthion for white South Africans when compared to coloured South Africans (Figure A7.6).

4.3.5. Classification statistics

The CVA assessing the first 22 ProCoords, resulted in a 63.8% cross-validated classification accuracy for the four populations. Coloured South Africans had the lowest correct classification rate, while black South Africans had the highest. Most overlap was noted between coloured and Indian South Africans, with both groups also misclassifying as white South Africans and vice versa (Table 4.28). However, both black and white South Africans rarely misclassified as one another and both were more distinct from coloured and Indian South Africans (Table 4.28, Figure 4.15). Figure 4.15 depicts the first two canonical variate scores, which when combined express 85.4% of the variation.

Table 4.28. Classification matrix and correct classification rate for CVA when classifying the sample according to population using ProCoords (size-free shape variables).

From group	Into group				Correct classification
	B	**C**	**I**	**W**	
B	77	13	11	1	75.5%
C	12	47	23	20	46.1%
I	5	24	62	12	60.2%
W	1	17	9	75	73.5%
Total correct: 261 of 408 (63.8% cross-validated)					

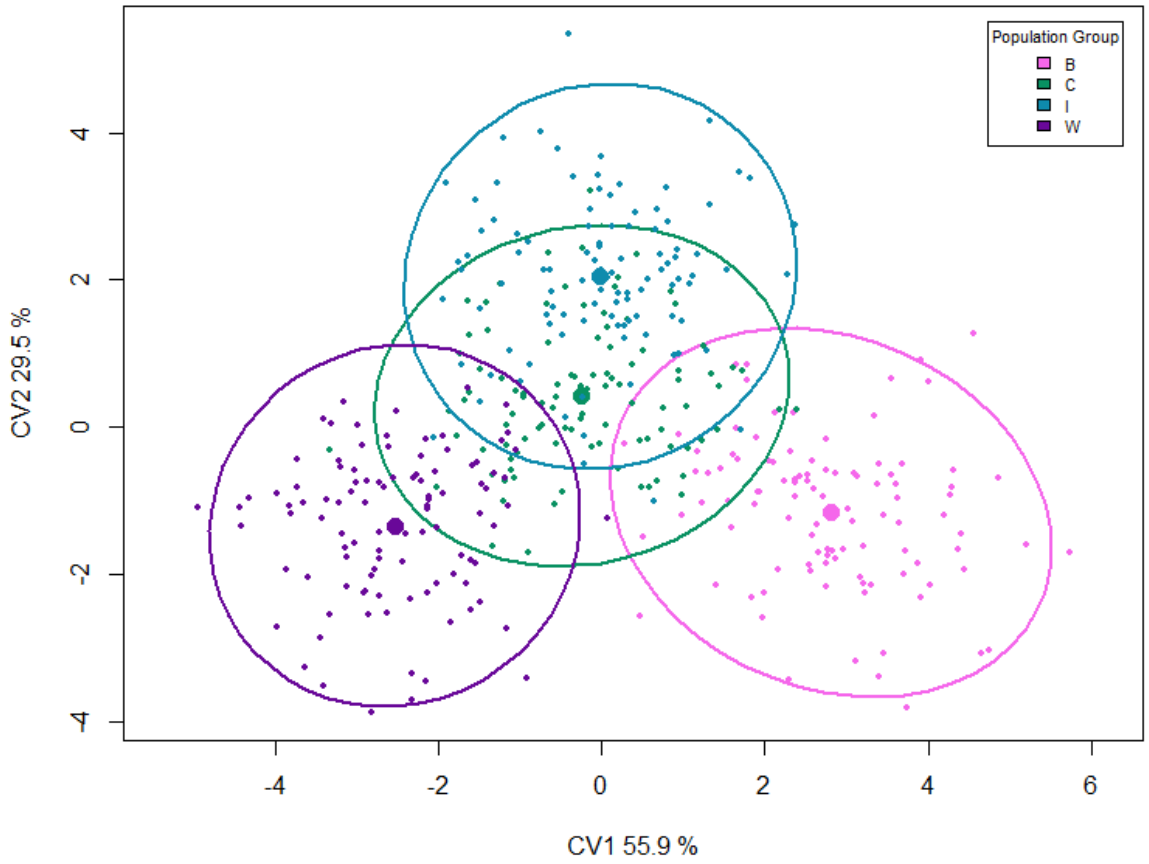

Figure 4.15. Plot of canonical variate group means and 95% confidence intervals for the CVA using ProCoords. The overall correct classification rate was 63.8%.

When the PC scores of the ProCoords were assessed and used to classify the sample, the cross-validated correct classification decreased slightly to 62.6%. However, similar to when the ProCoords themselves were assessed, most misclassifications occurred between coloured and Indian South Africans with black South Africans once again classified best (Table 4.29). The graph in Figure 4.16 illustrates a similar graph to the CV analysis of the ProCoords, with black and white South Africans showing more separation from the other two groups when assessing the first two PC scores that express 88% of the total variation (Figure 4.16).

Table 4.29. Classification matrix and correct classification rate for CVA when classifying the sample according to population using ProCoord PCs.

From group	Into group				
	B	**C**	**I**	**W**	Correct classification
B	79	15	4	4	77.5%
C	15	39	28	20	38.2%
I	5	23	61	14	59.2%
W	1	14	10	77	75.5%
Total correct: 255 of 408 (62.6% cross-validated)					

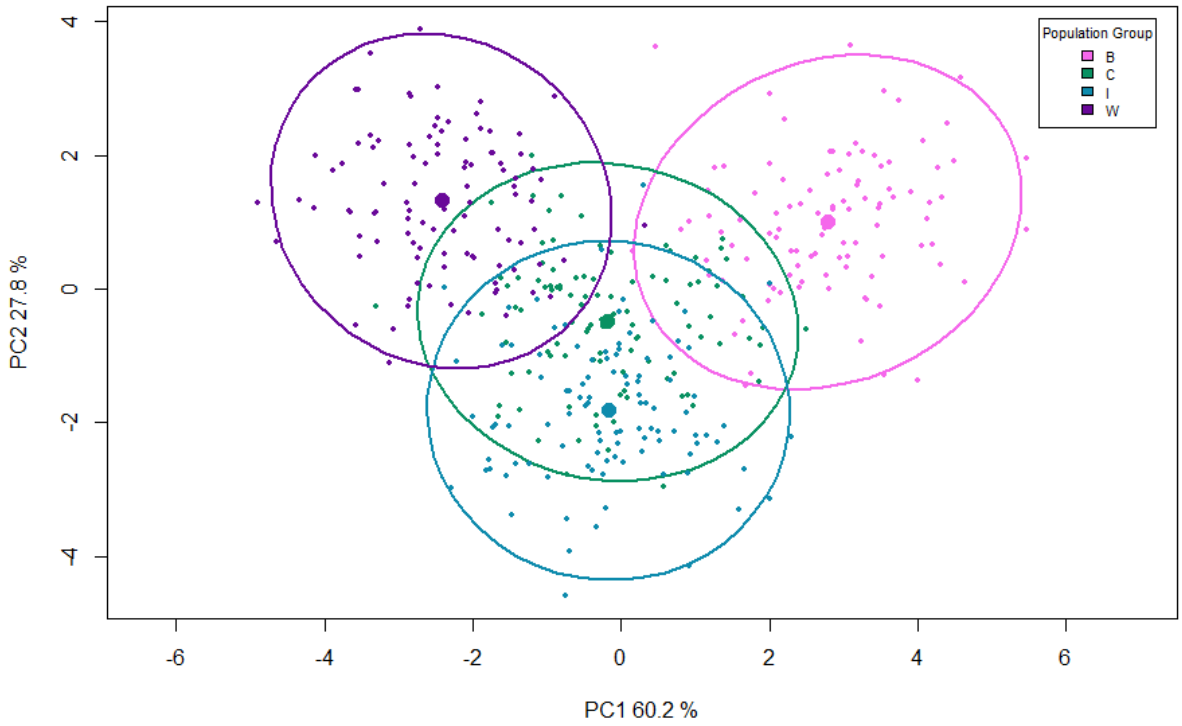

Figure 4.16. Plot of canonical variate group means and 95% confidence intervals for the CVA using ProCoord PCs. The overall classification rate was 62.6%.

When the ProCoords of the sexes were assessed using CVA, a correct classification of 93.2% was obtained with a slight male sex bias. Similarly, when the PC scores of the ProCoords were assessed, a slightly lower correct classification rate of 81.9% was obtained, again with a male sex bias (Table 4.30).

Table 4.30. Classification matrix and correct classification rate for CVA when classifying the sample according to sex using ProCoords and ProCoord PCs.

ProCoords				ProCoord PCs			
From group	Into group		Correct classification	From group	Into group		Correct classification
	F	**M**			**F**	**M**	
F	189	16	92.2%	**F**	162	43	79.0%
M	12	192	94.1%	**M**	31	173	84.8%
Total correct: 380 of 408 (93.2% cross-validated)				Total correct: 334 of 408 (81.9% cross-validated)			

When the ProCoords of both sex and population were assessed simultaneously using CVA, overall correct classification decreased, but only slightly when compared to the analysis when the population were compared with sexes pooled (Table 4.31, Figure 4.17). Black South African females obtained the highest correct classification rate while coloured South African males obtained the lowest (Table 4.31). The females overall classified better than their male counterparts, and the graph depicts much greater overlap and variation in the male sample when compared to the females (Figure 4.17).

Table 4.31. Classification matrix and correct classification rate for CVA when classifying the sample according to population and sex simultaneously using ProCoords.

From group	Into group								Correct classification
	BF	**BM**	**CF**	**CM**	**IF**	**IM**	**WF**	**WM**	
BF	43	2	2	0	3	0	1	0	84.3%
BM	2	33	0	7	3	5	0	1	64.7%
CF	2	0	32	1	8	2	5	1	62.7%
CM	0	7	0	23	0	9	0	12	45.1%
IF	4	1	10	1	32	1	2	1	61.5%
IM	0	1	2	10	1	30	4	3	58.8%
WF	0	1	5	2	3	0	34	6	66.7%
WM	0	0	0	8	1	7	4	31	60.8%
Total correct: 258 of 408 (63.1% cross-validated)									

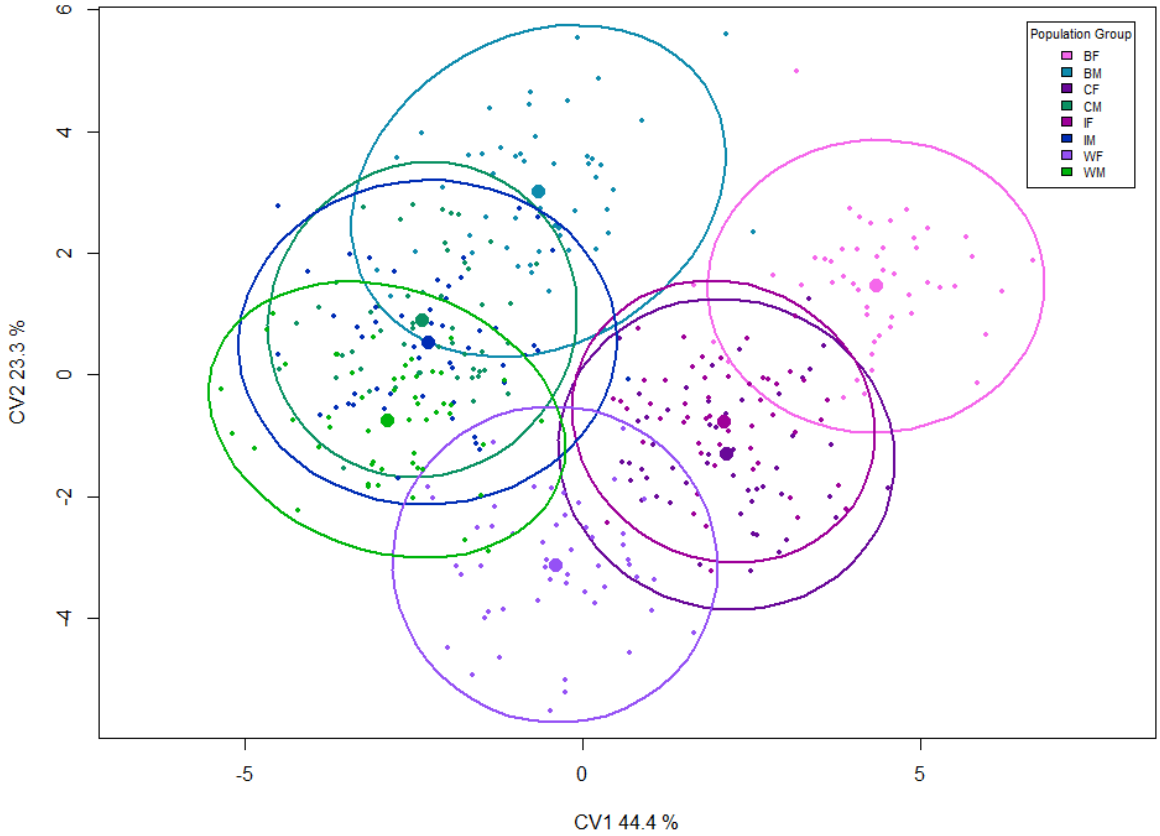

Figure 4.17. Plot of canonical variate group means and 95% confidence intervals for the CVA using ProCoords to estimate PopSex simultaneously. The overall correct classification rate was 63.1%.

Similar to the previous ProCoord PC CVAs, the classification accuracy decreased slightly, although the patterns of misclassification remained unchanged (Table 4.32, Figure 4.18).

Table 4.32. Classification matrix and correct classification rate for CVA when classifying the sample according to population and sex simultaneously using ProCoords PCs.

From group	Into group								Correct classification
	BF	**BM**	**CF**	**CM**	**IF**	**IM**	**WF**	**WM**	
BF	37	8	3	0	1	1	1	0	72.6%
BM	5	30	1	7	2	3	3	0	58.8%
CF	4	0	22	2	15	4	4	0	43.1%
CM	0	7	1	15	1	12	7	8	29.4%
IF	2	2	12	1	32	2	1	0	61.5%
IM	0	0	2	8	6	25	2	8	49.0%
WF	0	0	3	2	3	1	33	9	64.7%
WM	0	2	0	7	0	4	9	29	56.9%
Total correct: 222 of 408 (54.5% cross-validated)									

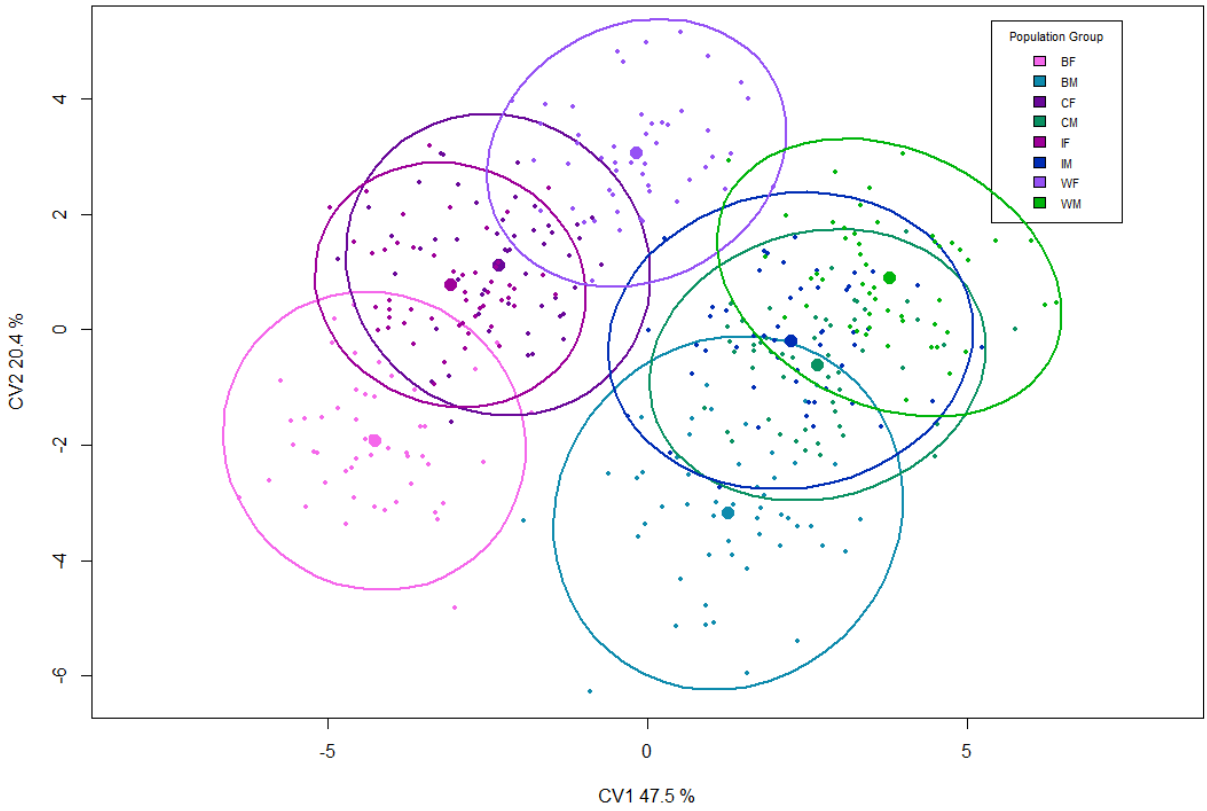

Figure 4.18. Plot of canonical variate group means and 95% confidence intervals for the CVA using ProCoord PCs when estimating PopSex *simultaneously. The overall classification rate was 54.5%.*

CHAPTER 5: DISCUSSION

Constant re-evaluation of standards used in forensic anthropological analyses are necessary, particularly as new methods are explored and populations change. Even though Indian South Africans are not a new addition to the South African population, the lack of skeletal material available for analysis has resulted in a lack of information on the variation present in the crania of this group. Furthermore, although black, white and coloured South African crania have been previously researched and standards created, by similarly making use of 3D models, the data are comparable to the Indian South African data collected and all four groups could be explored simultaneously. The results provide a comprehensive description of the patterns of sexual dimorphism and the cranial variation present in the current South African population.

5.1. Patterns of cranial sexual dimorphism

Sexual dimorphism is highly population-specific and making use of standards based on a reference population unrelated to the individual being classified leads to decreased accuracies and probabilities. Therefore, in order to obtain the best possible outcome, the sexual dimorphism present in a population needs to be quantified and population-specific standards created (Loth & İşcan 2000; Gapert *et al.* 2009; McDowell *et al.* 2012).

The results of this study indicate that the various South African populations are all highly sexually dimorphic and able to classify according to sex with high correct classification rates for morphoscopic and craniometric methods. The accuracies for both methods were overall higher than previous research estimating sex from the crania of South African black, coloured and white South Africans (L'Abbé *et al.* 2013a, b; Krüger *et al.* 2015). The new standards for sex estimation were able to produce correct classifications rates up to 94.1% when morphoscopic traits were assessed, while correct classification rates up to 90.7% were

produced when ILDs were assessed with LDA, and up to 93.2% when shape variables were assessed using CVA.

While black, coloured and white South Africans are all sexually dimorphic and sex can be estimated with high accuracies, the greatest degree of sexual dimorphism and the highest correct classification rates were obtained for Indian South Africans. Similar, although slightly lower, accuracies were obtained in studies from India (Deshmukh & Devershi 2006; Saini *et al.* 2011, 2014; Raghavendra Babu 2012; Kanchan *et al.* 2013; Raj & Ramesh 2013; Mehta *et al.* 2014, 2015; Singh 2015; Ramamoorthy 2016). Enhanced environments and dietary conditions can cause an increase in the degree of sexual dimorphism within a population, whereas unfavourable conditions can cause a decrease (Tobias 1971; Gray & Wolfe 1980; Henneberg & Van den Berg 1990; Barrier 2007). Indian South Africans, while segregated during *Apartheid*, were considered semi-privileged or as a part of the trader class, indicating that living conditions were most likely not completely unfavourable for them (Johnson 2022). Furthermore, many contract workers that came to South Africa from India, when given the option to stay after their contract expired, chose to remain in South Africa. Their choice was most likely heavily influenced by the possibility of even poorer living conditions back in India when compared to the conditions in South Africa (Bhat & Narayan 2010). Therefore, potentially better living conditions in South Africa could explain the difference in sexual dimorphism between the South African Indian and the Indian population in India. Furthermore, Indian groups (in India) have been shown to be highly variable both within and among the various Indian populations (Arya *et al.* 2002; Saini *et al.* 2017). Because South African Indians are descendants of Indians from limited geographic origins (Bhat & Narayan 2010), the amount of variation within the population is most likely more limited than the Indian population (in India). Therefore, the South African Indian population may be more sexually dimorphic than the Indians in India due to both genetic drift (Founder's effect) and improved living conditions.

While environment, dietary conditions, and limited genetic heterogeneity may have played a role in the degree of sexual dimorphism present in the Indian South Africans and the higher classification rates compared to previous studies in India, another reason for lower correct classification rates in the Indian studies may be the variables selected for analysis. Certain cranial features are more sexually dimorphic than others and when these are not included in the analyses, lower correct classification rates may be produced. Sex estimation using the four Walker traits resulted in excellent correct classification rates for Indian South Africans. A recent South African study testing correlations between the Walker (2008) cranial morphoscopic traits and standard craniometrics showed moderate to strong correlations between the traits and the standard cranial measurements, particularly with the mastoid height, cranial base length, and cranial height measurements (Krüger *et al.* 2015). In the current study, Indian South Africans had the highest degree of sexual dimorphism for those measurements, all three of which were included in model creation for LDA as well. Therefore, previous Indian studies may have benefited from including these measurements in their studies when estimating sex (Raghavendra Babu *et al.* 2012; Jain *et al.* 2016; Ramamoorthy *et al.* 2016).

The classification rates for the other South African populations, although similar, were slightly lower than for Indian South Africans. Coloured South Africans obtained the lowest correct classification rates (88.8%) for morphoscopic sex estimation. Similarly, when the ILD s% differences were assessed, coloured South Africans presented with the overall smallest differences between the sexes (lowest degree of sexual dimorphism). Previous research assessing the cranial variation among black, coloured and white South Africans also noted lower sexual dimorphism for coloured South Africans (L'Abbé *et al.* 2013b). For the most part, males tend to be more robust than their female counterparts (Rogers & Mukherjee 1992; Loth & İşcan 2000; Gustafsson & Lindenfors 2004), which is also true for the current research. However, the degree of sexual dimorphism within a group is affected by genetics and socio-

economic status among other factors (Cameron 2002; Barrier 2007). Due to the extensive heterogeneity of the coloured South African population (L'Abbé *et al.* 2013b), as well as a most likely low socio-economic status (Adedeji *et al.* 2022), overlap between the males and females is present in both size and shape and, therefore, most likely resulted in the decreased correct classification rates.

The current OLR correct classification rates for the morphoscopic traits in black (90.8%) and white (91.5%) South Africans were higher or similar to those obtained previously (Krüger *et al.* 2015). The correct classification for black South Africans increased by 5.8% from the 2015 study; however, only glabella and mastoid scores were included in the previous equation, whereas glabella, mastoid, nuchal and orbit were included in the current analysis. The additional traits most likely added more information that was able to better separate the males and females during classification. Even though black South Africans are not the most sexually dimorphic population when compared to the other groups assessed in this study, the differences between black South African males and females appear greater when compared to the study by Krüger *et al.* (2015). The 2015 study made use of samples that are temporally older than the current sample (Krüger *et al.* 2015; L'Abbé *et al.* 2021). While the effect of secular trends was not tested in the current study, secular trends are often the cause of a shift in the degree of sexual dimorphism in a population; however, the shift is typically towards a decrease in sexual dimorphism (Saini *et al.* 2014). Another potential reason for the improved sexual dimorphism noted in the black South African sample in this study is the origin of the data collected. Previous studies on black South African cranial variation (sexual dimorphism and population variation) assessed crania from the Pretoria Bone Collection. The collection houses the skeletal remains of individuals from mostly low socio-economic status (L'Abbé *et al.* 2021). A large proportion of the CT scans used in the current study were collected from the Inkosi Albert Luthuli Central Hospital, which is located in an urban, high- to middle-income area (Seahloli *et al.* 2015). The

potentially higher socio-economic standing of patients scanned at the hospital may have influenced the level of sexual dimorphism present in the black South African sample, and may possibly be a more accurate reflection of sexual dimorphism within this group.

In contrast to the classification rates for black South Africans, the correct classification rate for white South Africans decreased by 1.5% from the previous research, even though both analyses used only the glabella and nuchal scores (Krüger *et al.* 2015). The white South African females obtained slightly higher trait scores (i.e. were slightly more robust) for the glabella and nuchal traits in the current study, resulting in a marginal increase in misclassifications from the previous equations. Scoring variation between bone and CT scans may have affected the score frequencies. Previous research testing the repeatability of scoring on bone and on 3D models noted slightly higher repeatability between observers on the 3D models than on the dry bone (Braun *et al.* 2022). While the scoring procedure could have influenced the sexual dimorphism present in the white South African sample, another potential cause for the disparity is an actual decrease in the degree of sexual dimorphism between white South African males and females. Again, secular trends may have had an effect on the differences noted between the males and females. As the samples were collected from different regions (less than half from Gauteng) when compared to the Krüger *et al.* (2015) study (all from Gauteng), regional variation could also potentially have caused the slight difference in correct classification rate for white South Africans. However, a change in socioeconomic status is less likely responsible for the differences, as the samples for both the Krüger *et al.* (2015) study and the current research consisted of individuals with most likely low socio-economic statuses.

5.2. Cranial variation among black, coloured Indian and white South Africans

In addition to differences in sexual dimorphism, size and shape differences were also noted among black, coloured, Indian and white South Africans. The LDA of ILDs (size differences) were able to estimate population affinity with correct classification rate up to 62.2%, while the

CVA of ProCoords (shape differences) were able to estimate population affinity with correct rates up to 63.8%. Similar to the study by Stull *et al.* (2014a), the ProCoords performed better than simple craniometrics and better than ProCoord PCs. While shape variables were able to maximise group separation better, the improvement over linear measurements were not as much as expected, indicating that linear measurements are able to extract a similar amount of information to ProCoords, and can classify South African black, coloured, Indian and white South Africans with accuracies greater than chance.

In numerous laboratories across the world, linear measurements are assessed using FD3. The user-friendly nature of the software, as well as the simplicity with which population-specific reference samples can be uploaded, make the program very valuable in forensic anthropological casework. Therefore, the similar correct classification rates obtained for both ILDs and shape analyses indicate that LDA of craniometrics (whether manual or using a program like FD3) can be used to produce group separation with high probabilities and acceptable correct classification rates.

While the overall correct classification rate is lower than previous research comparing black, coloured and white South African crania (L'Abbé *et al.* 2013b, Stull *et al.* 2014), the current study is exploring four populations instead of three groups. For methods to be acceptable in forensic anthropological casework, they typically should be 50% better than chance, which for the current group comparisons would be 37.5%. However, at least 50% or greater is generally preferred. Therefore, even though the accuracies appear lower than previous studies, they are still able to provide good probabilities of group membership for black, coloured, Indian and white South Africans.

The highest correct classification rate was obtained for black South Africans. When the various populations were compared for size and shape, the most notable differences between

black South Africans and coloured, Indian, and white South Africans included the presence of a more prognathic facial profile and a narrower and longer cranial shape. The diversity in craniofacial features among populations and throughout the fossil record has been attributed to factors such as geographical location, adaptations to the environments and climate, gene flow, mechanical factors (i.e. adaptation to differences in diets), population histories, and cultural and religious traditions (Klepinger 2001; Viðarsdóttir *et al.* 2002; Jantz & Ousley 2005; Ruff *et al.* 2006; Ousley *et al.* 2009). Migrations and the necessary resulting adaptation to new environments and the restructuring of gene flow can affect the genotype and phenotype (Spradley 2006). However, while developmental plasticity and climatic adaptation have been shown to impact cranial variation, population differences in craniofacial morphology most likely emerge from pre-existing facial shape templates present at birth that are further emphasized during the growth and developmental phases (Viðarsdóttir *et al.* 2002; Relethford 2004).

Prognathism is characterized by the degree of projection of the mid-face in relation to the remaining cranium (De Villiers 1968). In contrast, orthognathism is defined by a profile in which there is a vertical appearance, with both the maxilla and mandible positioned under the orbits with no anterior projection. Although a more orthognathic facial profile has been recognized as a contemporary craniofacial trait compared to early *Homo*, various degrees of prognathism and orthognathism have been recognized as distinctive traits among geographically distinct human populations (Krogman & İşcan 1989; İşcan & Steyn 1999; İşcan *et al.* 2000; Brown & Maeda 2004; Gonzalez-Jose *et al.* 2007; Patriquin 2013).

The main landmark movements noted in the shape analyses demonstrated an inferior and anterior shift in the landmarks of the upper face (glabella, nasion, dacryon, upper orbital border, frontotemporale and frontomalare temporale) with a posterior and inferior shift in the inferior nasal landmarks (inferior nasal border, subspinale, alare, and mid-malar projection point). The

shift resulted in a change from a more prognathic or slanted facial profile in black South Africans to a more orthognathic or vertical facial profile in coloured, Indian and white South Africans. Overall, lower projecting faces, wide nasal apertures, wide interorbital regions with longer and narrower neurocrania have been associated with African populations in the literature (Jantz & Owsley 2001; Neves *et al.* 2007).

While black South Africans have genetic contributions from both Bantu-speakers and Khoesan ancestry, limited gene flow was noted between the genetic ancestors of black South Africans and European settlers or Indian/Asian slaves, potentially resulting in the distinction of black South Africans from the other three groups. Furthermore, even though the literature has noted that Khoesan crania displayed a reduced projection of the sub-nasal region and overall smaller crania, Bantu-speakers had more prognathic facial profiles and larger, longer, and narrower crania in comparison (Rightmire 1970; Howells 1973; Franklin *et al.* 2006). The craniofacial features noted in black South Africans in this research appear more consistent with the descriptions of the Bantu-speaking ancestors, which is different from the features noted for coloured, Indian, and white South Africans. Even though overlap is observed in both shape and size between coloured and black South Africans, the amount is somewhat smaller than in previous studies that compared black, coloured and white South Africans (Stull *et al.* 2014). A potential reason for the decrease in overlap may be the increased overlap between coloured and Indian South Africans, with these groups showing greater size and shape similarities than coloured and black South Africans.

Coloured South Africans received genetic contributions from Bantu-speakers, Europeans, Indians, and Khoesan groups; however, the levels of contribution from each parent group differ considerably for coloured males and females. A much stronger Khoesan contribution was noted for coloured females while a stronger European contribution was noted for coloured males (Destro-Bisol *et al.* 1999; Tishkoff *et al.* 2009). Due to the sex-specific contributions of the

Khoesan, greater similarities occur between modern coloured females and Khoesan ancestors than between coloured males and the Khoesan groups (Thompson 2001, Tishkoff *et al.* 2009; de Wit *et al.* 2010; Quintana-Murci *et al.* 2010). Khoesan were described as delicate and small people, which could explain why coloured females and Indian females misclassified as one another (Thompson 2001). In this study, the most notable cranial feature for Indian South Africans was the smaller overall size, followed closely by the overall size of the coloured South Africans when compared to the other two groups. Previous research has linked the overall smaller cranial size noted in ancestral Indians to the adaptation to warmer climates, which is typical of the Indian subcontinent (Matsumura *et al.* 2022). Even though Indian South Africans have lived in South Africa for many generations, very strict endogamy and religious customs have most likely limited the amount of gene flow present in the Indian South Africans. Limited variability has also been shown between South African Indians and Indians still living in India (Soodyall & Jenkins 1992). Soodyall and Jenkins (1992) also noted some limited Khoesan genetic contributions to the Indian South Africans. This could further explain the large amount of overlap noted between coloured and Indian South Africans. Similar group relationships to the current study were noted between anatomical specimens (obtained before 1985), believed to be of Indian origin, when compared to various other world populations (Hefner *et al.* 2016). During classification using craniometric variables (size), the anatomical specimens also occupied the middle of the space defined by the other populations demonstrating extensive overlap between the anatomical specimens and the other groups (Hefner *et al.* 2016).

In this study, extensive overlap was noted between coloured, Indian, and white South Africans. Genetic contributions from similar parental lineages most likely played a large role in the similarities noted in both the size (ILDs) and the shape analyses (GM) among the three groups. In the period of the Dutch colonization of the Cape, the early Dutch settlers most likely admixed with their Khoesan and Indian slaves, resulting in white South Africans with both

Khoesan (up to 1.3%) and Indian heritage, similar to some of the parental genetic contributions noted in coloured South Africans (Chimusa *et al.* 2013; Hollfelder *et al.* 2020).

However, while white South Africans have genetic contributions from Khoesan and Indian ancestral groups, similar to coloured South Africans, the contributions are smaller than those present in coloured South Africans. Genetic research has demonstrated that ancestral Indian genetic contributions are present not only in Indian South Africans, but also in smaller frequencies in white (up to 2.6%) and in coloured South Africans (up to 12 %) (Chimusa *et al.* 2013; Hollfelder *et al.* 2020). More than 90% of the genetic contributions in white South Africans are from European parent groups, when compared to the approximately 16% contribution in coloured South Africans. Therefore, the much larger contribution present in white South Africans had a greater influence on the cranial shape when compared to coloured South Africans.

White South Africans presented with overall larger crania that were more rounded (shorter but wider), similar to the cranial shape noted in Europeans, which is often related to the adaptation to colder environments, such as the European climate. Even though similarities and overlap are present between coloured, Indian and white South Africans, the Indian and coloured groups misclassify more as one another than as white South Africans. The larger Indian genetic contributions to the coloured South African population when compared to the Indian genetic contributions to white South Africans are most likely responsible for the slightly greater amount of overlap between Indian and coloured South Africans. White South Africans misclassified more as coloured South African than as Indian South African, which is most likely as a result of the European ancestry noted in both coloured and white South Africans, but not in Indian South Africans. Greater overlap between coloured and white South Africans was noted for size than for shape. Coloured South Africans presented with mostly intermediate sizes when compared to Indian and white South Africans, where white South Africans typically

had the largest ILDs and Indian South Africans the smallest ILDs. The cranial shape differences were smaller for coloured and Indian South Africans, creating extensive overlap between the two groups and less (although still present) overlap with white South Africans. Variable genetic contributions most likely accounted for the similarities and differences among coloured, Indian, and white South Africans.

While this study compared the size and shape variation present among current black, coloured, Indian, and white South Africans and proposed potential reasons for the differences and similarities noted, certain additional aspects (such as variations among the various ethnic groups or regional variation) could have influenced the variation in the South African population and may require further investigation.

5.3. Limitations and future recommendations

Some of the differences noted in this research when compared to previous studies could be explained by secular trends or variations in levels of genetic heterogeneity in the samples. However, regional variation may be a further aspect that could have affected the variation expressed in the current data, and should be explored in future research.

Furthermore, even though slice thickness was standardized in the processing phase of this research, the original scan parameters were not monitored, as the CT scans were obtained retrospectively. Additionally, as the scans were obtained from multiple different hospitals with multiple different CT scanners, any effect due to the scanning procedure or the scanner itself that may have influenced the data cannot be accounted for. Future studies should compare different CT scanners and various scanning protocols and their effect on 3D rendered models.

Furthermore, future research will need to validate the data collected for the current study on an osteological sample to confirm that the data is in fact applicable for use in dry bone samples. Additionally, by making use of retrospective data, patient information obtained is limited,

particularly with regard to information on population affinity and, potentially, on ethnic affiliations, which may explain the variation observed in the current study. If prospective data can be collected, more detailed information may be available.

CHAPTER 6: CONCLUSION

The ability of a forensic anthropologist to produce an accurate biological profile that may assist in providing a presumptive identification for an unknown individual relies heavily on the methods employed, the statistical analyses incorporated and, of course, the reference data used. Creating reference databases is influenced by the available reference samples, and in the case of Indian South Africans, skeletal material is severely limited, mainly due to conflicts with regard to body donation and the religious beliefs and traditions of the Indian South African population (Mehta *et al.* 2015). Until now, no biological anthropological research existed on the cranial variation in the modern Indian South African population and as such, no reference data was available for estimating the parameters of the biological profile for this population.

Furthermore, even though multiple studies have previously assessed the skeletal variation present in black, coloured and white South Africans, the reference data was based on sample populations from the previous century that may not be completely representative of the current population variation. This research, therefore, is not only the first to have explored the cranial variation of modern Indian South Africans when compared to current black, coloured and white South Africans, but also provided new information on the current variation present among black, coloured and white South Africans. As a result of this research, new standards were created to estimate sex and population affinity through morphoscopic analysis, standard craniometric analysis and shape analysis. The assessment of current Indian South Africans as well as the exploration of the cranial variation present in the other three larger current South African populations, was only possible through the use of 3D cranial models created from head CT scans, and was able to provide novel information that can be applied in both biological and forensic anthropology.

Even though this research has added new information to the understanding of variation within the modern South African population, continued re-assessment is necessary as populations change over time. With the creation of new methods and the addition of more robust statistical analyses, even more information may become available and could further improve the understanding of the complex interactions of microevolution, population history, endogamy, and environmental effect and their effect on skeletal variation.

In previous research, secular trends have shown to affect cranial dimensions and, therefore, by using individuals from the same period, the temporality of the skeletal material is of no consequence when comparing the populations. Furthermore, any standards created based on the current data that may be potentially applied to current forensic casework will be more appropriate reference material, which will perhaps in turn result in improved identifications.

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APPENDIX I

Table A1.1. Interlandmark distance abbreviations and landmarks involved.

Abbreviation	Landmark 1	Landmark number	Landmark 2	Landmark number
GOL	glabella	23	opisthocranion	42
XCB	left eurion	40	right eurion	41
ZYB	left zygion	16	right zygion	17
BBH	basion	27	bregma	24
BNL	basion	27	nasion	22
ASB	left asterion	32	right asterion	33
WFB	left frontotemporale	19	right frontotemporale	20
UFBR	left frontomalare temporale	18	right frontomalare temporale	21
NLHL	nasion	22	left most inferior nasal border	2
NLHR	nasion	22	right most inferior nasal border	3
NLH	average of NLHL and NLHR			
NLB	left alare	4	right alare	5
OBBL	left dacryon	10	left ectoconchion	11
OBBR	right dacryon	12	right ectoconchion	13
OBHL	left lower orbital border	8	left upper orbital border	9
OBHR	right lower orbital border	14	right upper orbital border	15
EKB	left ectoconchion	11	right ectoconchion	13
DKB	left dacryon	10	right dacryon	12
FRC	nasion	22	bregma	24
PAC	bregma	24	lambda	25
OCC	lambda	25	opisthion	26
FOL	opisthion	26	basion	27
FOB	left border of foramen magnum (FOB point)	28	right border of foramen magnum (FOB point)	29
MDHL	left porion	35	left mastoidale	36
MDHR	right porion	38	right mastoidale	39
AUB	left radiculare	30	right radiculare	31
NOL	nasion	22	opisthocranion	42
MOW	left zygoorbitale	6	right zygoorbitale	7
NLH2	nasion	22	supspinale	1
MAPL	left mid-malar projection point	34	left porion	35
MAPR	right mid-malar projection point	37	right porion	38
MAP2L	left mid-malar projection point	34	left ectoconchion	11
MAP2R	right mid-malar projection point	37	right ectoconchion	13
MAP3L	left mid-malar projection point	34	left asterion	32
MAP3R	right mid-malar projection point	37	right asterion	33

APPENDIX II

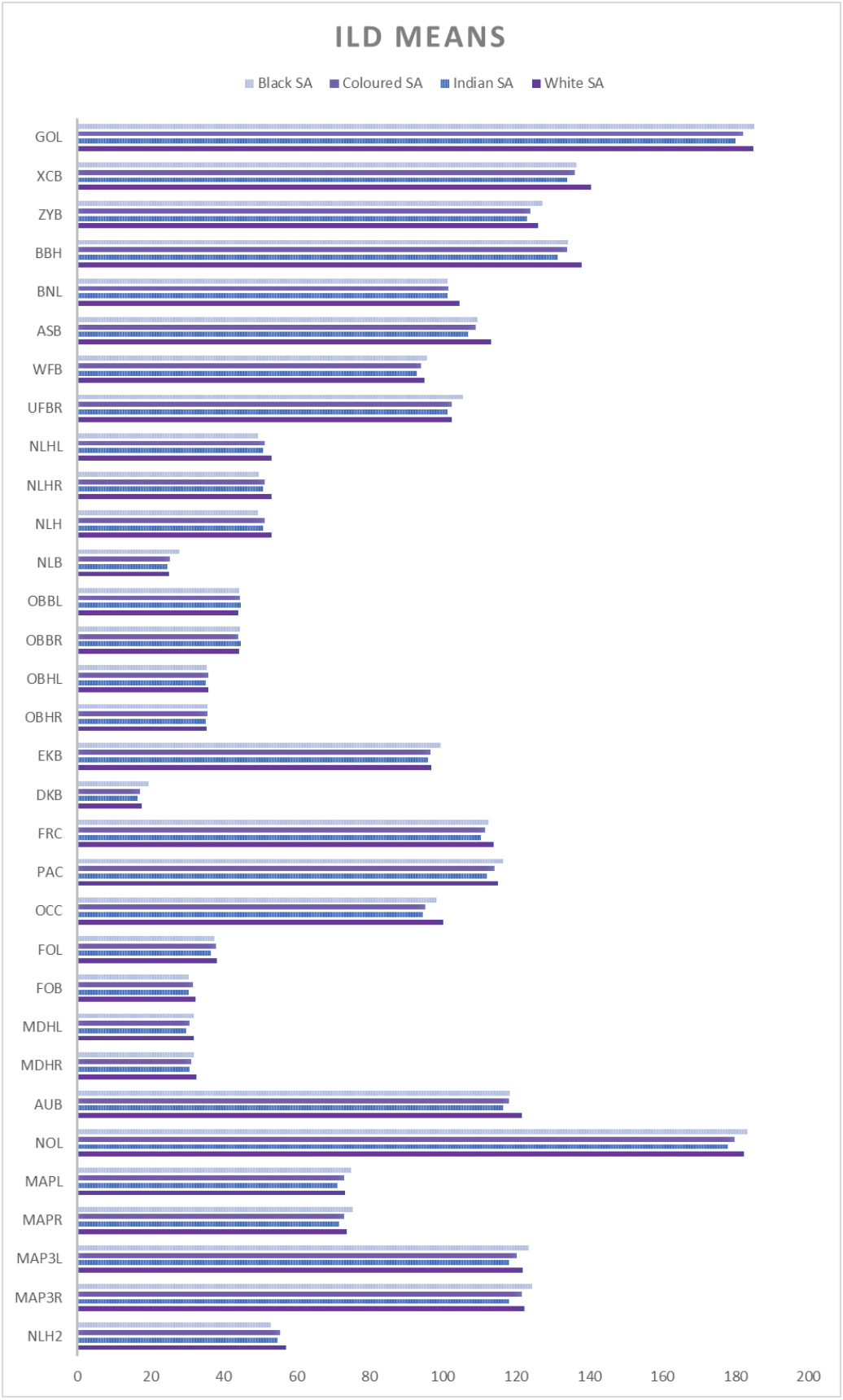

Figure A2.1. Bar chart depicting the means for black, coloured, Indian and white South Africans for each of the ILDs.

APPENDIX III

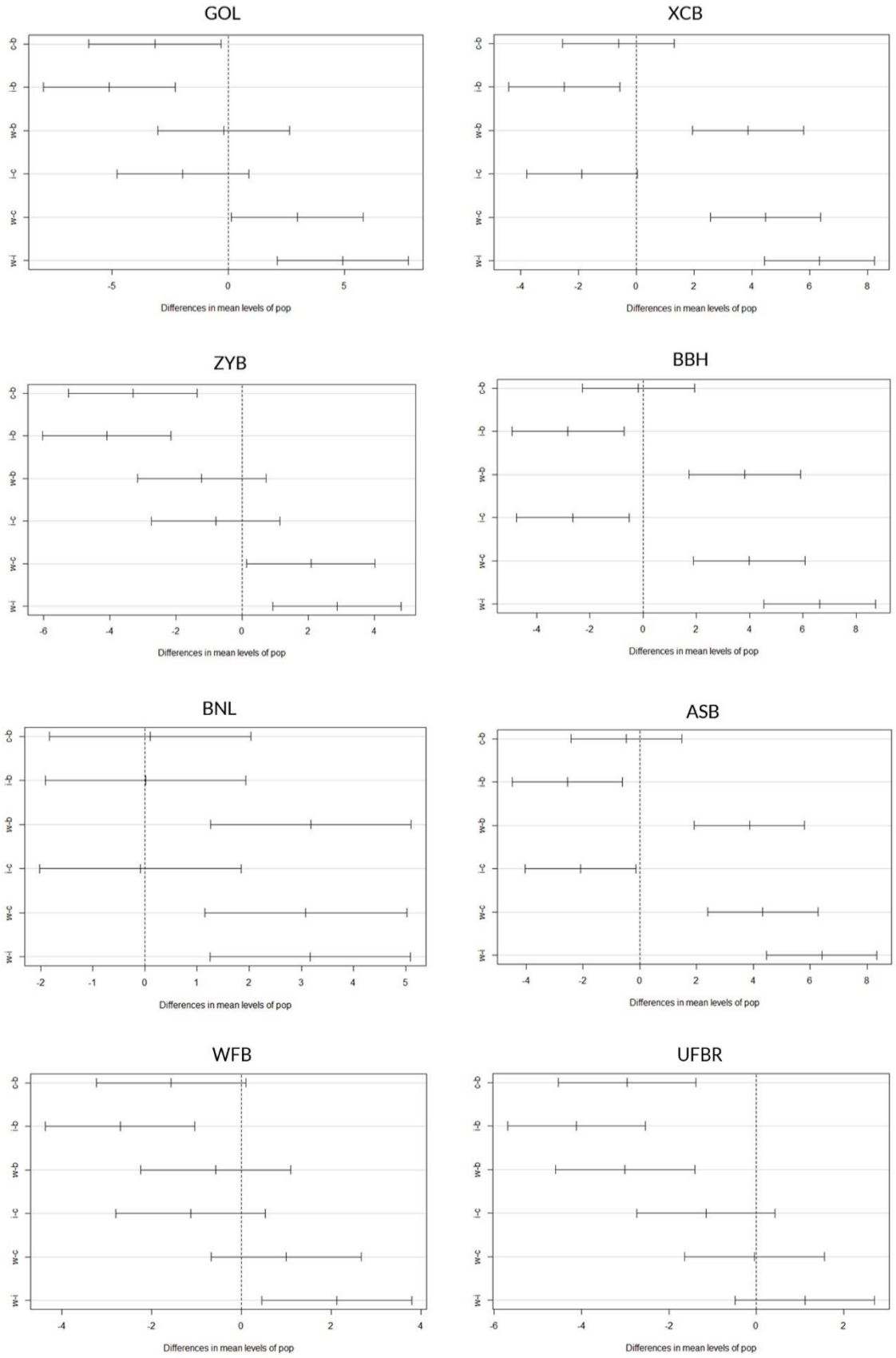

Figure A3.1. Tukey's HSD plots for GOL, XCB, ZYB, BBH, BNL, ASB, WFB, and UFBR depicting significant group differences or overlap.

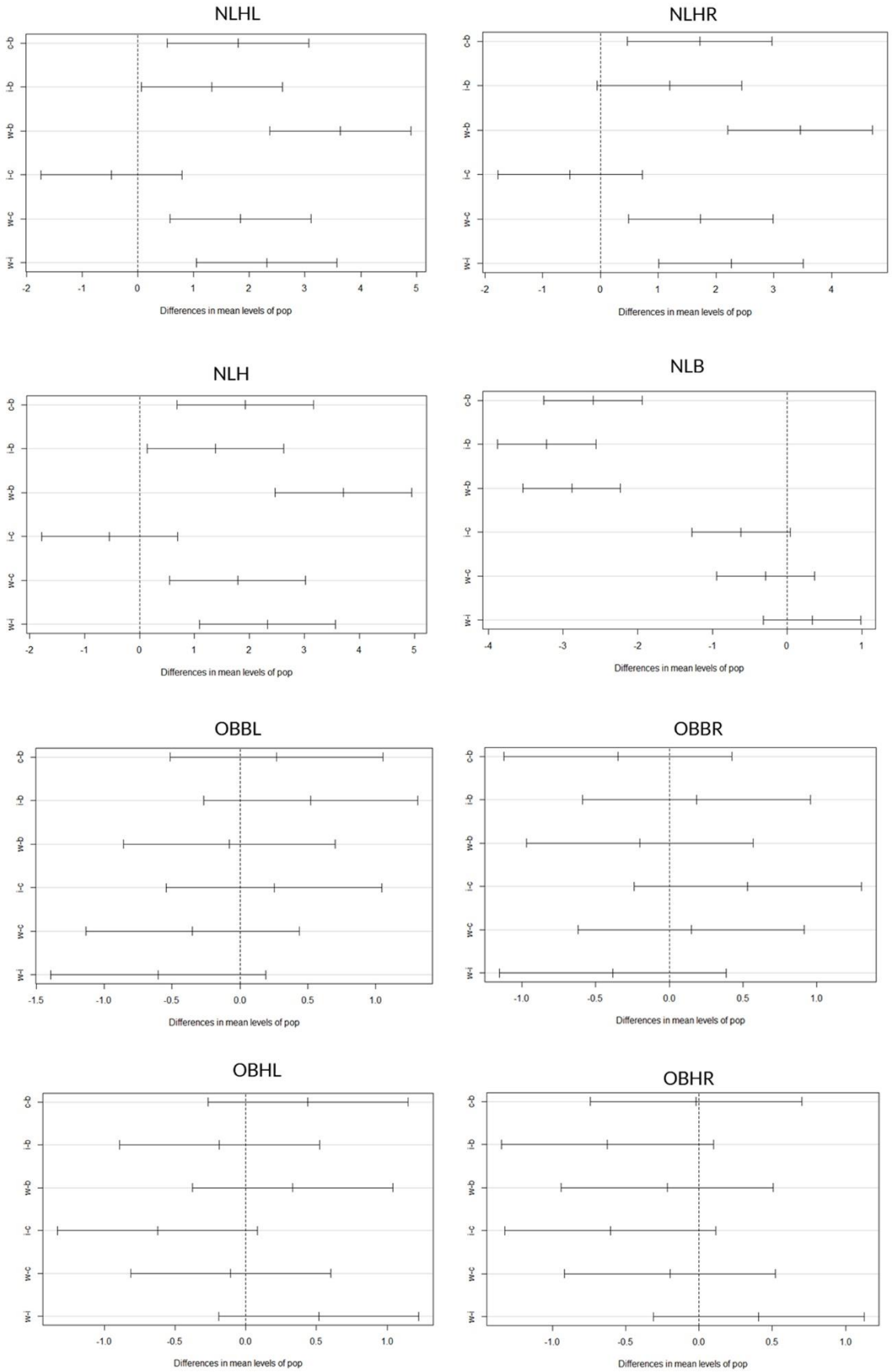

Figure A3.2. Tukey's HSD plots for NLHL, NLHR, NLH, NLB, OBBL, OBBR, OBHL and OBHR depicting significant group differences or overlap.

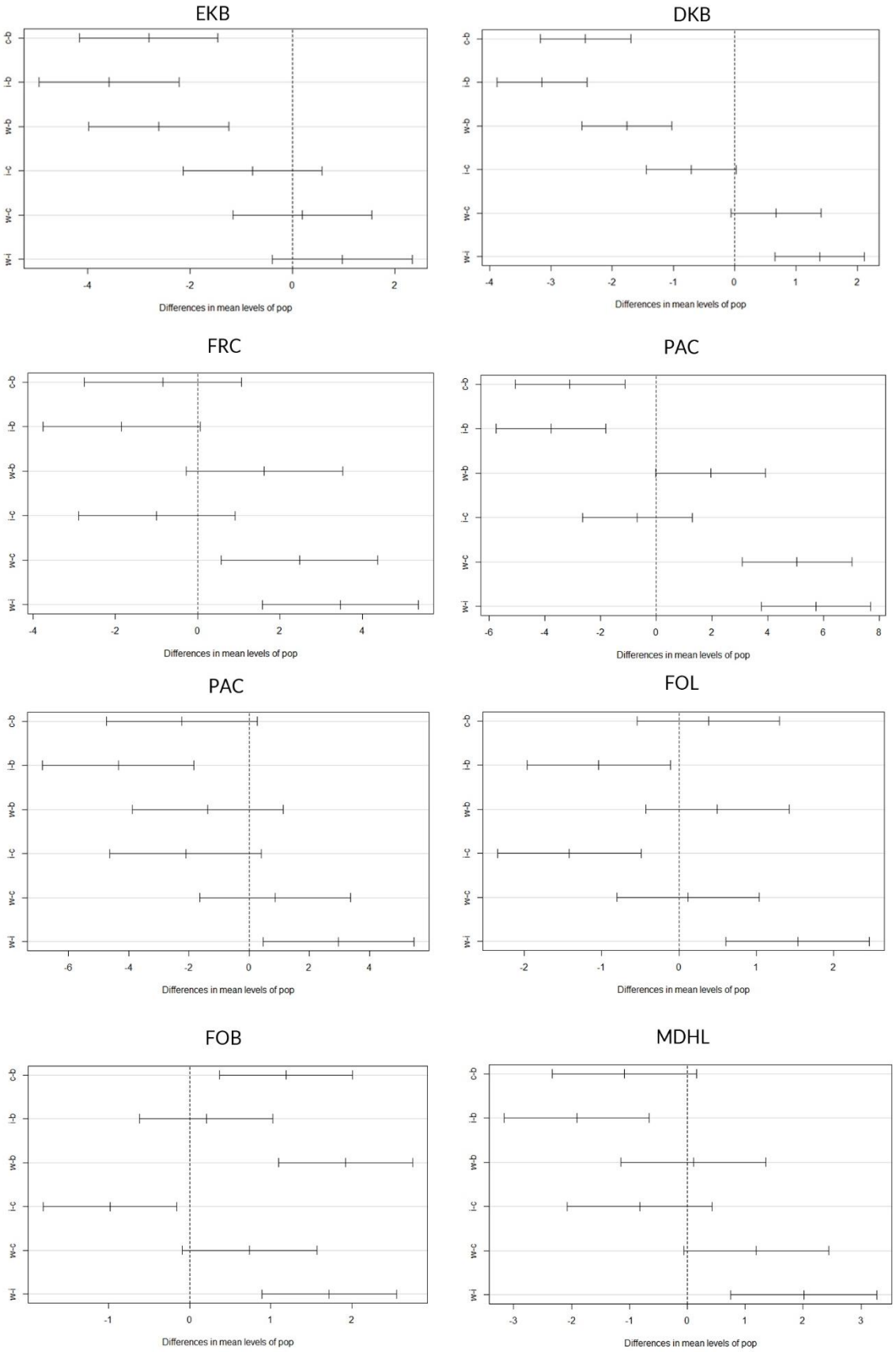

Figure A3.3. Tukey's HSD plots for DKB, EKB, FRC, OCC, PAC, FOL, FOB, and MDHL depicting significant group differences or overlap.

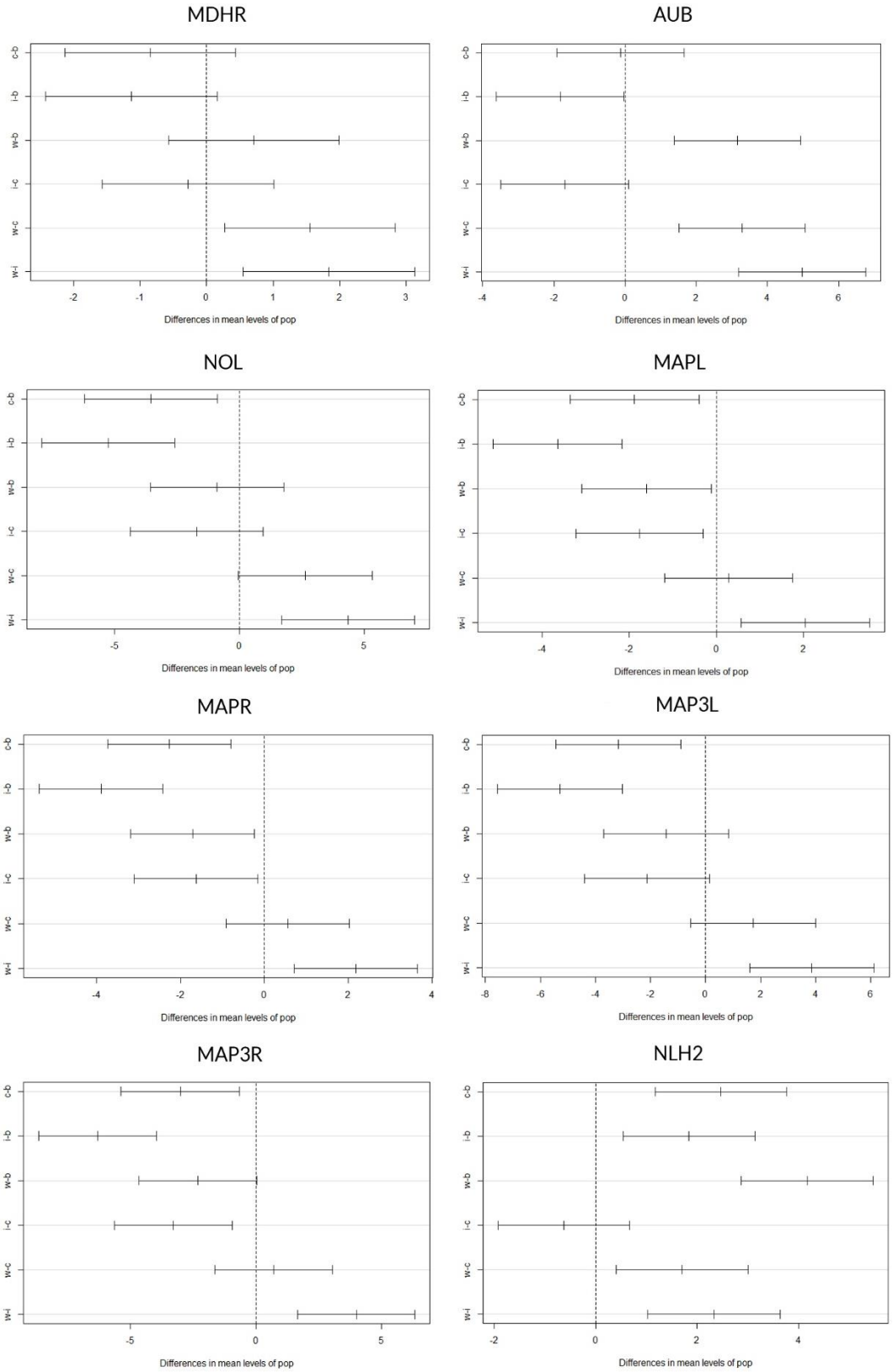

Figure A3.4. Tukey's HSD plots for MDHR, AUB, NOL, MAPL, MAPR, MAP3L, MAP3R, AND NLH2 depicting significant group differences or overlap.

APPENDIX IV

Table A4.1. Relative variable importance (GS Imp %) and ILDs selected for model creation in FD3 for the estimation of population affinity (sexes pooled and separate), PopSex, and sex. Variables are sorted from largest to smallest GS Imp%.

Population Affinity (pooled sexes)		Population Affinity (females only)		Population Affinity (males only)		PopSex		Sex	
ILDs	GS Imp %	ILD	GS Imp %	ILD	GS Imp %	ILD	GS Imp %	ILD	GS Imp %
NLB	15.5	NLB	10.9	NLB	10.9	ZYB	7.7	GOL	26.0
DKB	11.5	DKB	9.7	DKB	7.4	BNL	7.4	NOL	25.6
UFBR	7.1	MAP3R	8.5	BBH	6.8	BBH	7.0	ZYB	9.4
MAP3R	6.6	NLH2	7.9	ZYB	5.7	MAP3R	6.8	BNL	6.3
NLH2	5.5	MAPR	6.0	MAP3L	5.4	MAP3L	6.5	AUB	5.1
ZYB	5.5	UFBR	5.7	XCB	5.1	NLH2	6.3	MAP3R	3.5
XCB	4.8	EKB	5.5	ASB	4.8	MDH	5.7	MDH	3.3
ASB	4.8	BBH	5.1	UFBR	4.8	AUB	5.6	BBH	2.8
MAPL	4.7	MAPL	5.0	EKB	4.6	XCB	5.0	FRC	2.7
BBH	3.9	NOL	4.5	FOB	4.6	FRC	4.6	MAP3L	2.4
NOL	3.8	GOL	4.3	AUB	4.2	FOB	4.5	NLH2	2.2
OCC	3.8	OCC	4.0	MAP3R	4.2	MAPL	4.3	MAPR	1.7
AUB	3.6	ASB	3.8	NLHL	4.1	UFBR	4.2	FOL	1.6
FOB	3.5	XCB	3.7	NLH	3.9	EKB	4.1	PAC	1.6
GOL	3.4	PAC	2.8	BNL	3.5	NLB	4.0	UFBR	1.6
MDH	2.7	BNL	2.7	FRC	3.4	MAPR	3.8	MAPL	1.6
PAC	2.6	FOB	2.7	GOL	3.2	ASB	3.4	XCB	1.2
MDHR	1.8	OBB	2.4	NOL	3.1	DKB	3.1	FOB	1.1
BNL	1.7	OBBR	1.8	OCC	2.7	OBB	2.1	ASB	0.4
FRC	1.4	FRC	1.7	MAPL	2.4	OCC	1.9	NLB	0.2
OBHR	0.6	OBHR	1.0	MDHR	1.7	OBBR	1.6	OCC	0.1
OBB	0.5	OBHL	0.5	FOL	1.3	OBHR	0.5		
OBHL	0.4			PAC	1.2				
OBBR	0.2			OBBR	0.9				
				OBHL	0.3				
				OBHR	0.1				

APPENDIX V

Table A5.1. Intra- and inter-observer error represented by Euclidean distances to show the dispersion of repeated landmark placements.

	Intra-observer Error		Inter-observer Error	
Landmark number and name	Euclidean distance (mm)	SD	Euclidean distance (mm)	SD
1. subspinale	0.23	0.10	0.45	0.20
2. most inferior nasal border (L)	0.57	0.30	0.71	0.38
3. most inferior nasal border (R)	0.45	0.31	0.56	0.29
4. alare (L)	0.50	0.31	0.66	0.40
5. alare (R)	0.41	0.25	0.41	0.21
6. zygoorbitale (L)	0.57	0.86	1.78	1.42
7. zygoorbitale (R)	0.43	0.32	1.27	0.85
8. lower orbital border (L)	0.47	0.40	2.02	1.04
9. upper orbital border (L)	0.58	0.49	1.40	0.84
10. dacryon (L)	0.74	0.44	0.90	0.53
11. ectoconchion (L)	0.73	0.65	2.03	4.60
12. dacryon (R)	0.47	0.36	0.63	0.51
13. ectoconchion (R)	0.71	0.50	0.75	0.53
14. lower orbital border (R)	0.43	0.30	2.13	1.28
15. upper orbital border (R)	0.34	0.27	0.93	0.58
16. zygion (L)	0.75	0.39	1.32	0.71
17. zygion (R)	0.82	0.60	1.31	0.82
18. frontomalare temporale (L)	0.33	0.19	0.84	0.34
19. frontotemporale (L)	0.74	0.37	1.17	0.73
20. frontotemporale (R)	0.50	0.48	1.00	0.84
21. frontomalare temporale (R)	0.51	0.16	1.12	0.66
22. nasion	0.25	0.10	0.46	0.41
23. glabella	0.52	0.33	0.65	0.26
24. bregma	0.39	0.25	0.54	0.47
25. lambda	0.43	0.17	0.55	0.37
26. opisthion	0.20	0.12	0.43	0.25
27. basion	0.21	0.15	0.69	0.37
28. FOB point (L)	0.44	0.31	1.00	1.04
29. FOB point (R)	0.55	0.35	1.33	0.89
30. radiculare (L)	0.81	0.46	0.88	0.65
31. radiculare (R)	0.66	0.48	0.81	0.60
32. asterion (L)	0.95	0.67	0.85	0.67
33. asterion (R)	0.88	0.60	0.80	0.51
34. mid malar projection point (L)	0.77	0.35	1.34	0.81
35. porion (L)	0.53	0.50	0.96	0.66
36. mastoidale (L)	0.41	0.26	0.74	0.59
37. mid malar projection point (R)	0.63	0.23	1.12	0.69
38. porion (R)	0.75	0.62	0.79	0.48
39. mastoidale (R)	0.48	0.25	0.94	0.84
40. eurion (L)	0.77	0.56	1.82	1.12
41. eurion (R)	1.08	0.41	1.71	0.92
42. opisthocranion	0.67	0.33	1.23	0.55
Mean	**0.56**	**0.37**	**1.02**	**0.74**

APPENDIX VI

Table A6.1. PC scores. Eigenvalues. % variance and the cumulative variance expressed by the PC scores.

PC score	Eigenvalues	% Variance	Cumulative %
PC1	0.00077990	20.112	20.112
PC2	0.00028424	7.330	27.441
PC3	0.00024504	6.319	33.760
PC4	0.00021742	5.607	39.367
PC5	0.00019371	4.995	44.362
PC6	0.00017094	4.408	48.770
PC7	0.00014137	3.646	52.416
PC8	0.00011840	3.053	55.469
PC9	0.00011262	2.904	58.373
PC10	0.00010491	2.705	61.078
PC11	0.00010140	2.615	63.693
PC12	0.00008714	2.247	65.941
PC13	0.00007775	2.005	67.946
PC14	0.00007053	1.819	69.764
PC15	0.00006434	1.659	71.424
PC16	0.00006393	1.649	73.072
PC17	0.00006196	1.598	74.670
PC18	0.00005154	1.329	75.999
PC19	0.00005014	1.293	77.292
PC20	0.00004366	1.126	78.418
PC21	0.00004260	1.099	79.517
PC22	0.00004058	1.047	80.563

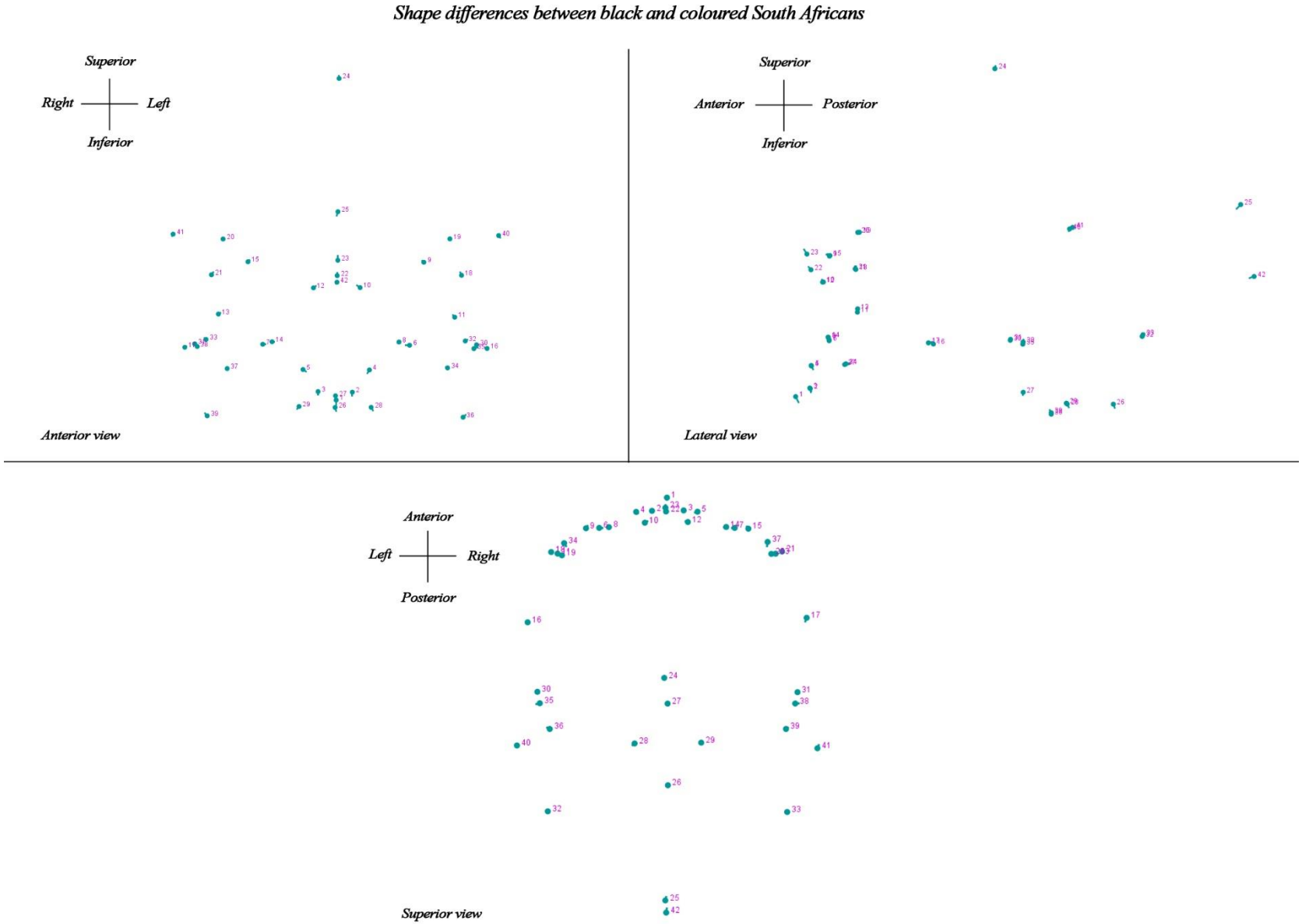

Figure A7.1. Lollipop graphs illustrating the shape differences between black and coloured South Africans in anterior (top left), lateral (top right), and superior (bottom) view. The dot indicates the black mean shape, while the line (lollipop stick) indicates the coloured mean shape.

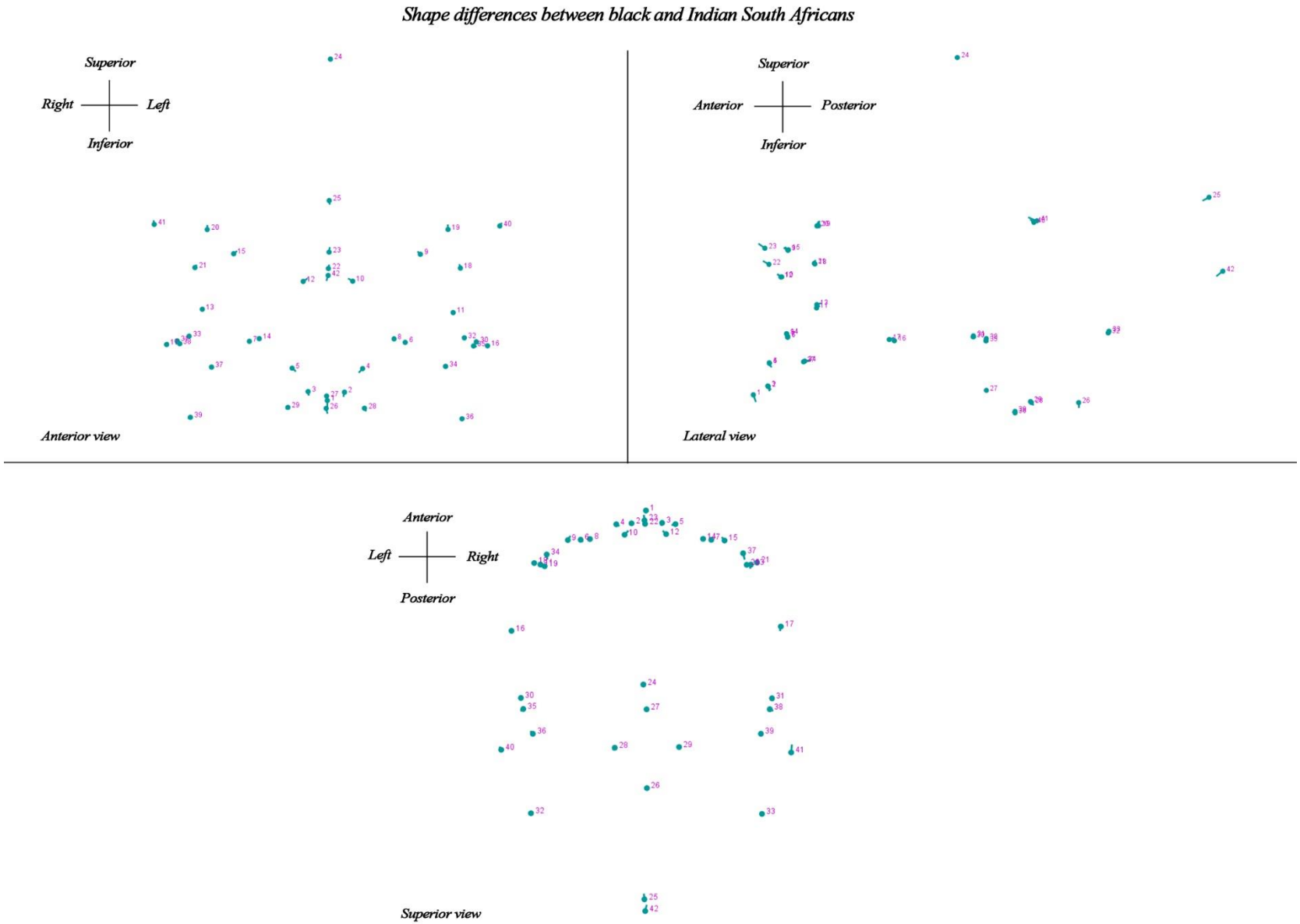

Figure A7.2. Lollipop graphs illustrating the shape differences between black and Indian South Africans in anterior (top left), lateral (top right), and superior (bottom) view. The dot indicates the black mean shape, while the line (lollipop stick) indicates the Indian mean shape.

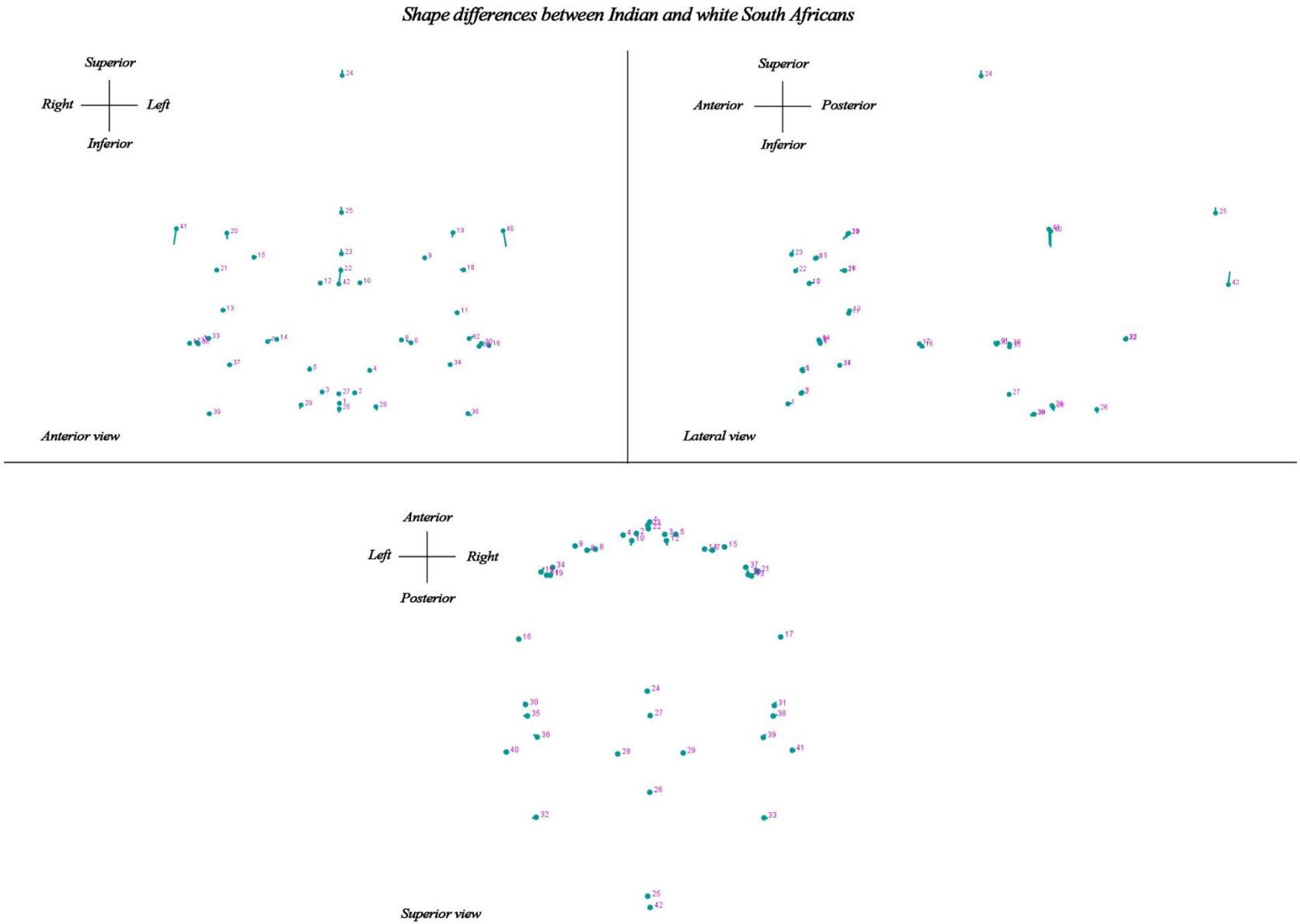

Figure A7.3. Lollipop graphs illustrating the shape differences between Indian and white South Africans in anterior (top left), lateral (top right), and superior (bottom) view. The dot indicates the Indian mean shape, while the line (lollipop stick) indicates the white mean shape.

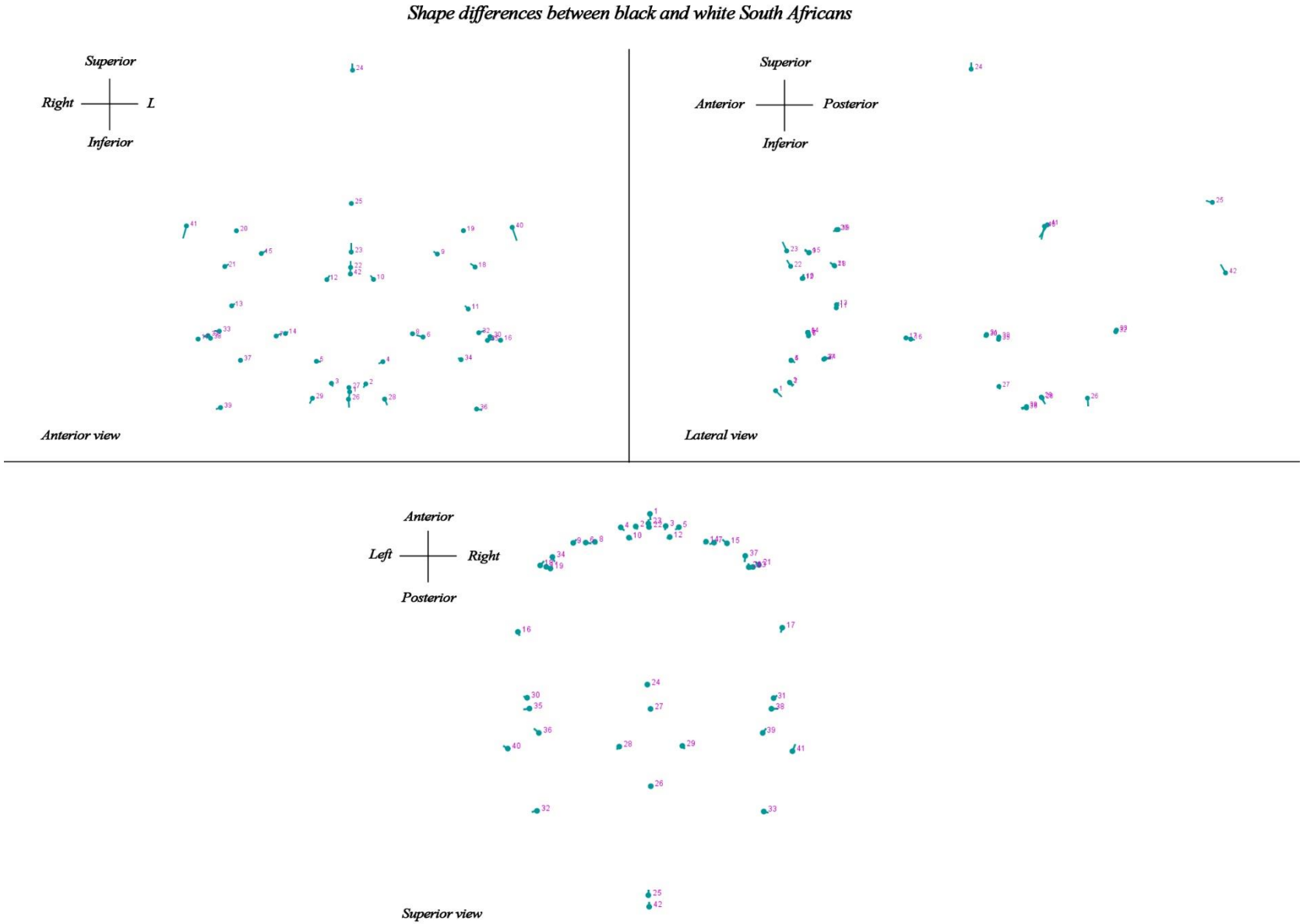

Figure A7.4. Lollipop graphs illustrating the shape differences between black and white South Africans in anterior (top left), lateral (top right), and superior (bottom) view. The dot indicates the black mean shape, while the line (lollipop stick) indicates the white mean shape.

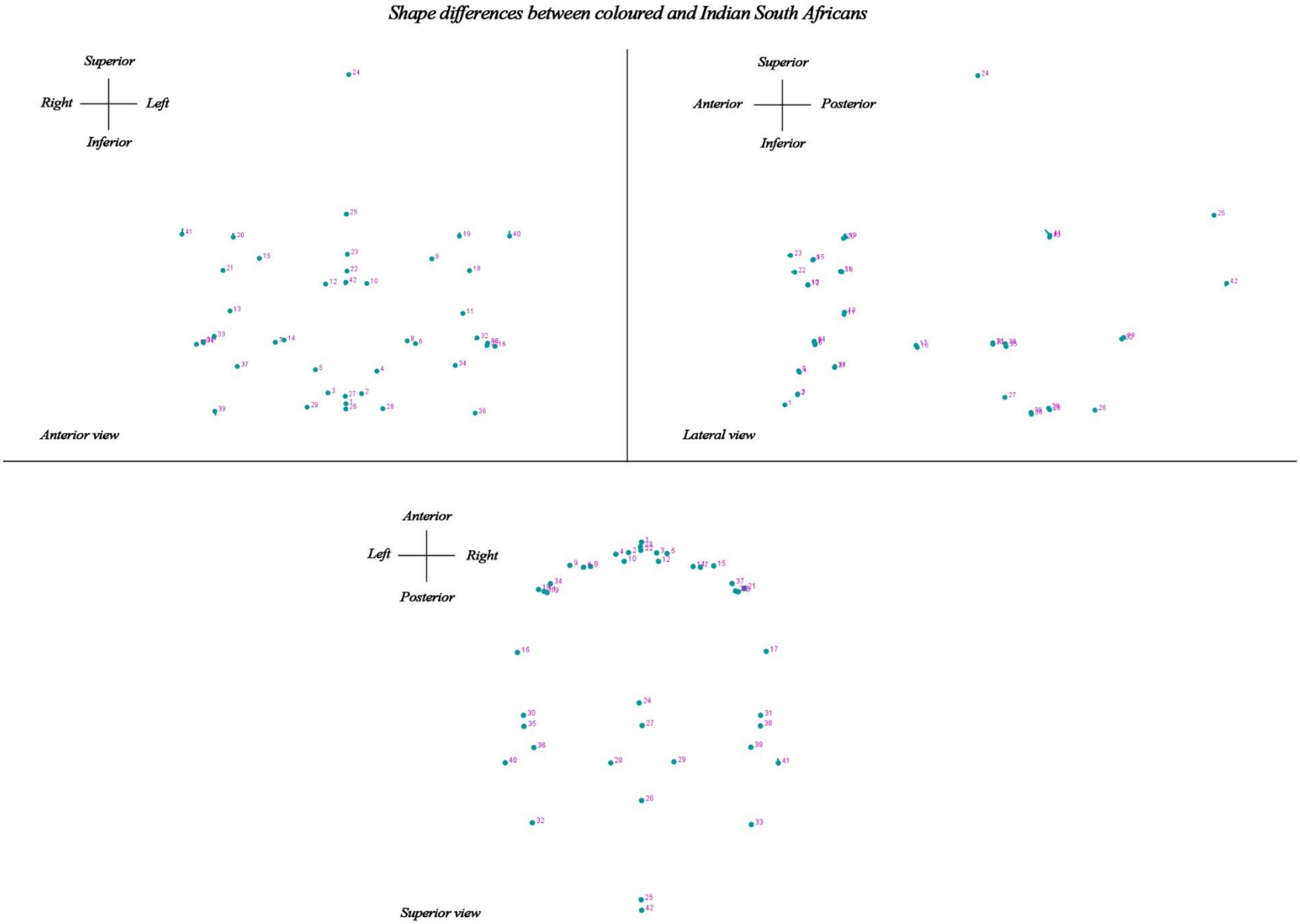

Figure A7.5. Lollipop graphs illustrating the shape differences between coloured and Indian South Africans in anterior (top left), lateral (top right), and superior (bottom) view. The dot indicates the coloured mean shape, while the line (lollipop stick) indicates the Indian mean shape.

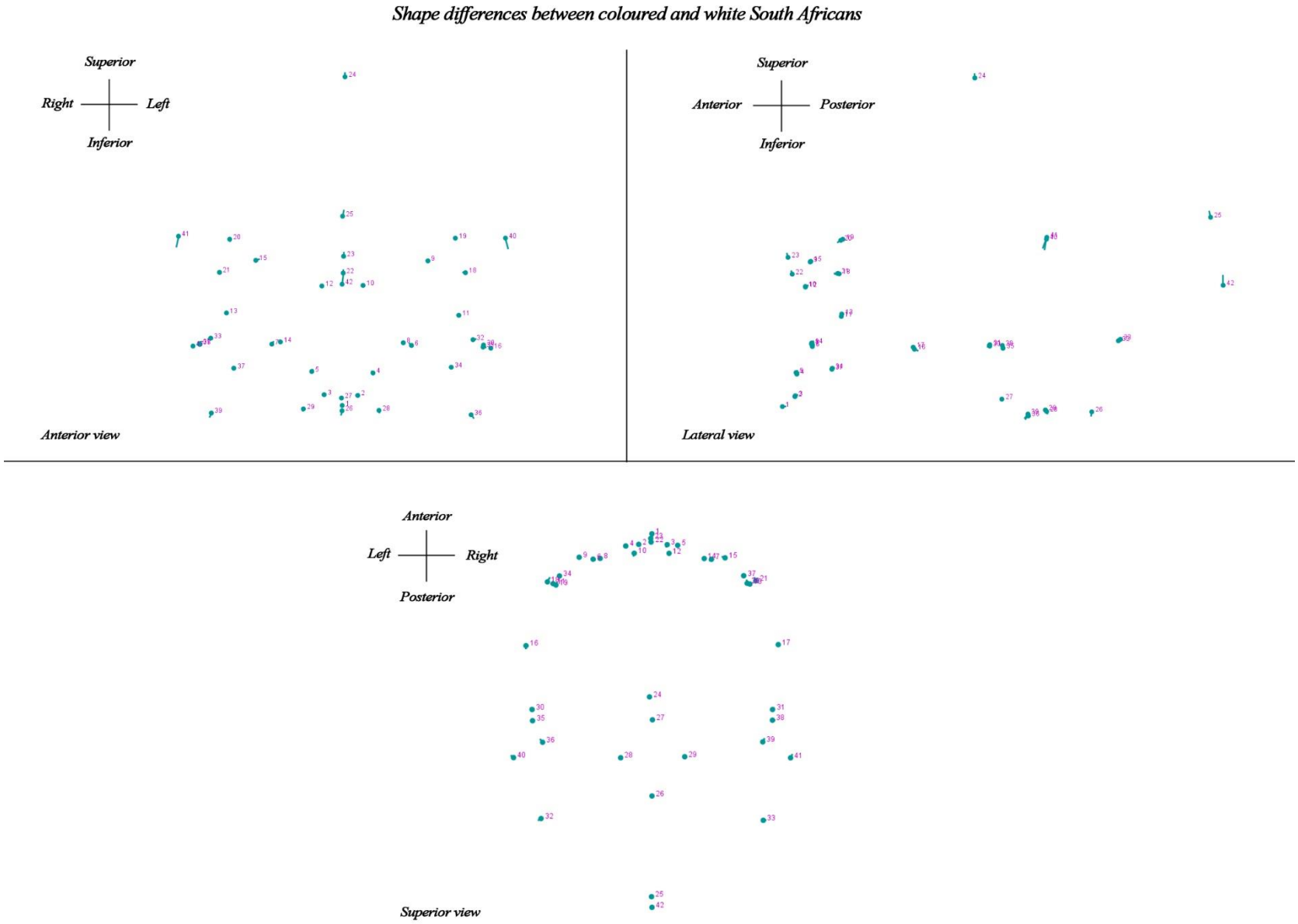

Figure A7.6. Lollipop graphs illustrating the shape differences between coloured and white South Africans in anterior (top left), lateral (top right), and superior (bottom) view. The dot indicates the coloured mean shape, while the line (lollipop stick) indicates the white mean shape.