Estimating population affinity using dental morphoscopic traits in a South African sample

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DECLARATION

I, Cassidy Brooks, declare that this dissertation is my own work. It is being submitted for the degree of MSc in Anatomy at the University of Pretoria. It has not been submitted before for any other degree or examination at this or any other institution.

Sign: ________________________________

23rd of October 2024

ABSTRACT

Multiple methods exist to estimate population affinity, with country-specific standards available to effectively assess the variation of South Africans. However, analyses are also case-specific, so the overall presence and condition of certain skeletal elements will dictate which methods can possibly be used. Dental characteristics are of importance in biological anthropology, as teeth demonstrate population variation, and are typically well-preserved and intact when recovered in most contexts. However, the application of dentition in South African forensic casework has been fairly limited. The purpose of this study was to explore dental morphological variation in the South African population in order to provide an additional method for the estimation of population affinity for application in forensic cases. The objectives of the study included to test the observer repeatability of identifying and scoring 15 dental morphological traits described in the Arizona State University Dental Anthropology System (ASUDAS) guidelines; to assess the prevalence of each trait among black and white South African males and females; and to test the accuracy with which the traits can be used to estimate population affinity. The overall study sample consisted of 191 individuals, with varying sample sizes per tooth. Any skulls or traits with post-mortem damage, deciduous dentition, pathology and/or excessive tooth loss and dental work that prevents the accurate scoring of the traits were excluded from the study. A series of 15 dental traits were visually assessed on the relevant incisors, premolars and molars of each individual in the sample and scored according to the Arizona State University Dental Anthropology System (ASUDAS). While the traits were considered repeatable based on the intra-observer agreement, the inter-observer agreement was overall poor. This suggests the need for experience with both teeth and the scoring method itself to apply the method accurately. Frequency distributions revealed a substantial amount of overlap in trait prevalence between black and white South Africans. Ultimately, the results of the Kruskal-Wallis tests revealed that only three traits demonstrated statistically significant differences for population affinity (pegged/reduced/missing third molar, four cusped lower second molar, and the seventh cusp on the lower first molar) and only two traits demonstrated differences for sex (the interruption groove on the upper lateral incisors, and the protostylid). Random forest modelling was used to assess the classification accuracy of the traits. Univariate models assessing each trait individually yielded accuracies ranging from 28% to 70%, with the number of cusps of the lower second molar (LM2-4C) and the presence of a metaconulid (LM1-C7) on the lower first molar performing the best. The multivariate model including all traits yielded a classification accuracy of 78%.

Although the classification accuracy is comparable to other frequently employed methods (such as metric and morphoscopic methods applied to the cranium), several considerations must be accounted for when attempting to use this method. Firstly, the sample was influenced by patterns of antemortem and postmortem tooth loss as well as dental wear, which was primarily a problem caused by the advanced age of many individuals in the skeletal collection. Thus, traits were often not available to score or may have been obscured or altered. Secondly, many traits or specific trait states were noted to be exceedingly rare in the sample. Rare traits and variants may not only affect a user's ability to correctly identify and score a trait (as is evidenced by the poor inter-observer repeatability), but also offers limited information in terms of classification models and the estimation of population affinity.

The current study failed to observe the promising results in the South African population reported in other global studies. Thus, based on the results the dental traits should be further explored on a more extensive sample. But in its current state the morphoscopic method should not be used on its own in skeletal analyses in South Africa.

Keywords: Forensic anthropology, Ancestry, rASUDAS, Teeth, Classification

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CHAPTER 1: INTRODUCTION

The South African population consists of nearly 62 million individuals (Statistics South-Africa, 2022). According to the rapid mortality surveillance reports by the South African Medical Research Council (saMRC), approximately 46 471 unnatural deaths occurred in the year 2020 (Dorrington *et al*., 2021). Additionally, 1173 unidentified bodies were recorded as unnatural deaths in 2020 by the Gauteng Health Department since the individuals remained in a state mortuary (Molelekwa, 2021). When bodies are unidentified or unclaimed, forensic anthropologists are tasked to analyse the unknown individuals to assist in identification by using the skeletal remains. In most cases any information or context is absent or incomplete, except for the police district where the remains were discovered (Krüger *et al*., 2018). In South Africa, the field of forensic anthropology is continuously advancing as forensic practitioners strive to research the variation among individuals and develop improved methods for identification. This has resulted in a greater number of unknown individuals being identified.

Population affinity (previously referred to as ancestry) is one of the four parameters of the biological profile (Spradley and Jantz, 2022). The term population affinity refers to skeletally quantifiable traits attributable to a specific group resulting from a combination of genetics and population history (Spradley and Weisensee, 2012; Dunn *et al*., 2020). South Africa has a very diverse population consisting of four major population groups (black, white, coloured and Indian/Asian South Africans). Each group is considered to originate from different geographical locations and have unique histories which has led to skeletal differences, although a considerable amount of group overlap has also been observed (Krüger *et al*., 2018). With the biological profile, estimates of population affinity are very valuable as it can assist in narrowing down the number of possible matches in forensic investigations (L'Abbé *et al*., 2011).

Multiple methods exist to estimate population affinity, with country-specific standards created to effectively assess the variation of South Africans (Stull *et al*., 2014; Liebenberg *et al*., 2015; Shakoane *et al.*, 2021; Bothma *et al.*, 2024; Liebenberg *et al.,* 2024). However, analyses are case-specific, where the condition and presence/absence of skeletal elements can be affected by factors such as taphonomy, pathology and trauma, and will thus dictate which methods are available to use for the most accurate results (L'Abbé *et al.*, 2013). For many cases the use of existing methods will either not be possible, or an estimation can be made but the accuracy will be compromised and become questionable.

The use of dental characteristics offers a lot of potential as teeth preserve well and are usually intact when recovered in most forensic contexts. While the dentition is frequently assessed in biological anthropology or bioarchaeology, its application in forensic anthropology has been more limited (Scott *et al.,* 2013; Maier, 2017). Dentition can be useful in cases with limited intact skeletal elements but can also be used to validate or support other methods of population affinity estimation, especially when supplementation is needed for the confident exclusion of a population group or when there is a large amount of overlap, and a sound estimation cannot be made. Research examining dental morphological traits for population classification globally has reported classification accuracies ranging from 70% to 72% (Maier, 2017; Scott *et al*., 2018; Rathmann and Reyes-Centeno, 2020). But to date there are no studies that have compared these traits among the South African population groups.

The aim of this study was to explore dental morphological variation in a South African sample using morphoscopic traits as defined in the Arizona State University Dental Anthropology System (ASUDAS) and assess their performance to estimate population affinity. Specifically, this study seeks to answer the question: *How effective and accurate are dental morphological traits, as defined by ASUDAS, in estimating population affinity within a South African sample?* By addressing this question, the study aspires to provide an additional method for estimating population affinity for forensic applications. The main objectives of the study included to test the observer repeatability of identifying and scoring 15 dental morphological traits described in the Arizona State University Dental Anthropology System (ASUDAS) guidelines; to assess the prevalence of each trait among black and white South African males and females; and to test the accuracy with which the traits can be used to estimate population affinity.

CHAPTER 2: LITERATURE REVIEW

2.1. Population affinity and sex in forensic anthropology

Forensic anthropologists are often asked to estimate the biological profile from human skeletal remains. Along with population affinity and sex, forensic anthropologists also estimate stature and age at death, which altogether make up the four parameters of the biological profile (Dunn *et al.,* 2020).

The term "population affinity" describes skeletally quantifiable morphological variation among humans and classifies humans into groups typically associated with their social race (Ousley *et al.,* 2009; Spradley and Jantz, 2022). The relationship between these two factors is the combined result of genetics, positive assortative mating practices and population history (Ousley *et al.,* 2009). The South African population is largely comprised of four major population groups. Black South Africans are considered descendants from central and western African populations (Liebenberg *et al*., 2015) and makes up 81.4% of the population. Coloured South Africans make up 8.2% of the population. Coloured individuals are considered the modern descendants of indigenous southern Africans but is an extremely heterogenous group with additional genotypic contributions from Africa, Europe, and Asia (Stull *et al*., 2014). White South Africans are decedents from European settlers and make up 7.3% of the population (L'Abbé *et al*., 2011; Statistics South-Africa, 2022). Finally, Indian/Asians are descendants from Eastern and Southern Asian populations that were initially transported to South Africa as indentured labourers and make up 2.5% of the population (Christopher, 1990).

Additional to knowing what group an individual is likely from, population estimation also contributes to the accuracy of other biological estimations, such as sex and stature. This is because different populations show variable expressions of sexual dimorphism (Krüger *et al*. 2015) as well as different expressions in proportions, sizes, or shapes of skeletal elements (Relethford, 2004). Although it was not within the scope of the current study to estimate sex, the integrated nature of sex and population affinity as biological variables should always be acknowledged and explored together. Biological sex is determined by the XX (female) and XY (male) chromosomes (Gilbert, 2000). When sex is estimated from skeletal remains, it is based on the premise that differences exist between males and females regarding the shape and size of skeletal elements, both within and among populations (Klales, 2020). The reason differences are observed between males and females are because of the influence of sex hormones during development of the bones. Oestrogen, the female sex hormone, promotes growth hormone secretion at low concentrations, and is also responsible for the formation of trabecular bone. Inversely, when there are high concentrations of oestrogen, linear growth is halted (Juul, 2001). Oestrogen will also limit periosteal expansion of bones (Callewaert *et al.*, 2010). Testosterone, which is the male sex hormone, is responsible for periosteal bone apposition, which contributes to thickness of bones. This is why males usually have bones that are wider, or have a larger diameter, and is thus typically stronger than the bones of females (Callewaert *et al.*, 2010). Sex estimation is population-specific, with the magnitude of size and shape differences between males and females varying among different populations (L'Abbé and Steyn, 2012).

Two broad approaches exist to quantify skeletal variation; these are (1) osteometric, and (2) morphological/morphoscopic. The osteometric method makes use of standard definitions of landmarks, which are taken with precise instruments to yield accurate and repeatable measurements (Hefner *et al.,* 2012). Craniometry is typically preferred for population estimations as methods from the skull are more accurate than methods using postcrania (Liebenberg *et al*., 2015). However, when there is no skull recovered, postcranial remains can also provide population affinity estimates with high accuracy (Liebenberg *et al*., 2015). The morphological approach is also known as the non-metric or morphoscopic method. This approach makes use of the visual assessment of morphological variation of discrete or ordinal traits among groups. It entails assessing the shape of a skeletal element, the presence or absence of a feature, or the degree of expression or development of a feature for analysis (Hefner, 2009). Using non-metric methodology has some difficulties, such as inconsistency due to subjectivity and observer error, but this can be mitigated with attempts to standardise the criteria used for morphological analysis (Hefner *et al.*, 2012). These standardisations usually include comparative diagrams or illustrations that is paired with a scoring system, in which case the method is referred to as morphoscopic rather than simply morphological (Hefner *et al.,* 2012). To date South African forensic anthropologists frequently employ morphoscopic methods for the estimation of sex using the cranium and pelvis (Krüger *et al*., 2015; Kenyhercz *et al*., 2017). More recent studies have also created population-specific standards for morphoscopic traits to estimate population affinity using the cranium and postcranial skeleton (Bothma *et al*., 2024; Liebenberg *et al*., 2024). However, the application of dental variation (both metric and non-metric) among South Africans have not been sufficiently explored.

2.2. Dentition and dental morphology

2.2.1. Dental anatomy and development

Teeth consist of four tissue types, namely: enamel, dentine, cementum, and pulp. Enamel and the cementum form the visible parts of teeth. The enamel covers the anatomic crown and is made up of calcium hydroxyapatite (the inorganic component of a tooth), which is tremendously hard and durable (Schreid and Weiss, 2017). Enamel is the hardest tissue in the human body and preserves itself in most conditions – such as pH, moisture, salinity, and high temperatures, protecting teeth from most taphonomic influences that usually affect other skeletal elements (Ash and Nelson, 2009; Scheid and Weiss, 2017). The cementum covers the anatomic root of the tooth. The periodontium can also be found in relation to the roots of teeth; it consists of the cementum, alveolar bone, gingival tissue (gums), and the periodontal ligament. The periodontium makes it possible for the tooth to attach to bone (Torabi and Soni, 2023).

Regarding the embryonic development of teeth, an epithelial-mesenchymal interaction occurs between the underlying mesenchyme (derived from the neural crest cells of the ectoderm) and the overlying oral epithelium. The dental lamina is formed when the basal layer of the epithelial lining of the oral cavity moves into a c-shape. During the bud stage, ten dental buds arise from this lamina and are present in each jaw (Teaford *et al.,* 2000). These buds form the primordia of the ectodermal components of the teeth (Sperber *et al.,* 2010; Sadler, 2019). The cap stage of tooth development occurs soon after, where the deep surface of the bud invaginates (Teaford *et al.,* 2000). The cap is made up of an inner and outer layer, also referred to as the inner and outer enamel epithelium, respectively (Nanci, 2017). In-between these layers a central core called the stellate reticulum can be found, which is made up of loose woven tissue. The dental papilla is formed from the mesenchyme that originates in the neural crest. The dental cap grows, and the tooth starts to look like a bell (bell stage) as it continues forming a deeper indentation (Teaford *et al.*, 2000). The cells of the papilla that are adjacent to the inner dental epithelium then differentiate into odontoblasts which produce dentine. As the dentine layer thickens, the odontoblasts move into the dental papilla, forming a thin cytoplasmic layer in the dentine. The remaining cells of the dental papilla become the pulp of the tooth (Sperber *et al.,* 2010; Sadler, 2019).

Cells of the inner dental epithelium concurrently differentiate into ameloblasts which form the enamel that is deposited over the dentine. An enamel knot forms from a cluster of these cells which is responsible for regulation of early tooth development. After the enamel has thickened, the ameloblasts move into the stellate reticulum where they regress and leave a thin membrane on the enamel called the dental cuticle which gradually sloughs off after eruption of the tooth into the oral cavity. Root formation is initiated when the dental epithelial layers move into the underlying mesenchyme forming the epithelial root sheath. The cells in the dental papilla form a layer of dentine that is continuous with the dentine of the crown. Continuous dentine deposition causes the pulp chamber to narrow and forms a canal for the blood vessels and nerves of the tooth (Sperber *et al.,* 2010; Sadler, 2019).

On the external surface of the tooth, mesenchymal cells that are in contact with the dentine are differentiated into cementoblasts, which are responsible for producing the cementum. Outside of this layer, mesenchyme forms the periodontal ligament that holds the tooth in position and absorbs shock. As the root increases in length, the crown is pushed through the overlying tissue until it erupts into the oral cavity. Deciduous teeth erupt from 6 to 24 months after birth. The buds for the permanent dentition form during the third month of development on the lingual aspect of the deciduous teeth. The buds of the permanent teeth are dormant until an individual reaches the age of 6 years, after which the buds begin to grow. The permanent teeth then push against the deciduous teeth which results in shedding. As the permanent teeth grow, the root of the overlying deciduous tooth is resorbed by osteoclasts, and after shedding the permanent teeth will erupt as well (Sperber *et al.,* 2010; Sadler, 2019). The permanent dentition consists of four incisors, two canines, four premolars and six molars in each jaw (Scott and Turner, 1997).

Each tooth develops in such a way that the morphology suits the function that it needs to perform. The crowns for instance, possess cusps which are different for each tooth type and among individuals. Cusps consist of occlusal and marginal ridges and have grooves and fissures that vary in depth which divides the tooth into cusp and ridge components. Incisor crowns are usually spatulate; canine crowns are conical and have one cusp; premolars are bicuspid; and molars are multi-cusped (Scott and Turner, 1997). In the same way that crowns differ between type of tooth and between individuals, the roots differ as well. Incisors, canines and lower premolars usually have one root, whereas upper molars have three roots, and lower molars have two roots (Scott and Turner, 1997).

2.2.2. Dental variation

While all human dentition is fundamentally similar, slight differences in the number or size of certain dental traits can occur, reflecting an individual's genetic constitution. The size and morphological structure of a tooth is formed completely histo-embryologically, and unlike bone is not changed or remodelled after development has ended. The only changes that occur are due to dental wear, attrition, and secondary dentine formation (Ash and Nelson, 2009; Zinni and Crowley, 2012). Different forms of dental wear can also influence dental morphology and account for variation observed, such as attrition and abrasion. Attrition results from occlusal contact between adjacent teeth. It can be functional or parafunctional (bruxism). Functional attrition refers to activities of the jaw that are due to controlled muscle activities such as chewing and speaking and occurs when normal functions are performed with minimal damage. Parafunctional attrition or bruxism refers to muscle hyperactivity such as clenching or grinding (Reddy *et al*., 2014). It manifests as well defined, flat-planed opposing facets with small striations (Scott and Irish, 2013). Abrasion results from friction between teeth and other objects introduced into the mouth. This includes wear from food causing wear during mastication, but also non-dietary causes such as use of dental hygiene items such as toothbrushes and toothpicks, as well as other task-related behaviours (Scott and Irish, 2013).

Potential reasons for variation observed in dental morphology apart from genetics include geographical separation, environmental factors such as orthodontic appliances, frequency of tooth brushing, dental hygiene and water fluoride levels which will directly impact occlusal surfaces through wear over time or increase/decrease the risk of dental disease such as caries, hormone activity, mechanical stress, positive assortative mating, dietary preferences/habits, and socio-economic status (Ash and Nelson, 2009; Wang *et al*., 2012; Shakoane, *et al*., 2021).

According to Scott and Turner (1997), two types of morphological variants can be observed in human dentition. The first type of variant has to do with major deviations from the most common and basic dental blueprint such as fused, twinned, or supernumerary teeth, as well as hyperdontia, agenesis and hypodontia. This type also includes crown differences such as conical lateral incisors, 3-cusped upper premolars, 'mulberry' molars and other anomalies. The second type of variant has to do with subtle differences that mainly entail minor variation in characteristics of the tooth such as secondary cusps, fissure patterns, marginal ridges, supernumerary roots, etc. These minor differences have been noted to vary between different populations and have a larger significance in an evolutionary sense than rare anomalies that is found in the first type of variation, which is usually more linked to environmental factors present at the time of development (Scott and Turner, 1997).

Patterned geographic variation exists among human populations when looking at morphology of the tooth, as well as the size of the crowns (Scott and Turner, 1997). For example, the root number of upper premolars have been noted to depend on the geographic area, where upper premolars from individuals located in Greenland have one root, but the same teeth more frequently have two roots in Nairobi. In a South African population, it has been observed that the distributions of the root numbers of maxillary first premolars were 44% for one root, 54.1% for two roots and 1.9% for three roots (Buchanan *et al.,* 2020). Scott and Irish (2013) also described dental patterns and traits present in high frequencies in specific populations; these have been named the Afridont, Eurodont, Sinodont (East Asia) and Sundadont (Southeast Asia and Pacific regions) dental patterns. The Afridont dental pattern is typically characterised by the "*Bushman*" (sic) canine, two-rooted upper first premolars, Carabelli's trait (UM1-Car), three-rooted upper second molars, a Y-Groove on the lower second molars, presence of cusp 7 on the lower first molars (LM1-C7), Tomes root on the lower first premolars, two-rooted lower second molars and presence of the upper third molars. The "Bushman" canine and the seventh cusp on the lower first molars are considered the “most African” traits based on how rarely they occur outside of the African continent. The characteristics of a typical Eurodont dental pattern include Carabelli’s trait (UM1-Car), spatulate and featureless upper incisors (no shovelling or ridges), two rooted lower canines, five-cusped lower first molars and four cusped lower second molars. Traits that are more frequently observed in Sinodonts are shovelling of the upper central incisors (UI1-S), double shovelling of the upper central incisors, one-rooted upper first premolars, enamel extensions on the upper first molar, a third molar that is pegged, reduced or missing, a deflecting wrinkle on the lower first molars and three-rooted lower first molars. The Sinodont dentition is characterised by intensification (features appear more pronounced/displays higher degrees of expression) of crown and root features and is very common in Eastern Asian populations. In contrast, Sundadonts are characterised by retained/simplified traits (features appear less pronounced/displays lower degrees of expression) and a less complex dentition (Scott and Irish, 2013; Scott *et al.*, 2018). Despite being geographically close, the typical Sinodont traits are present in substantially lower frequencies among Sundadont dental complexes, which have a higher frequency of a four cusped lower second molars (Scott *et al.,* 2018). Scott and Irish (2013) reports that Sub-Saharan African samples demonstrate the largest within group variation, followed by Southeast Asian and West Asian samples, whereas European and East/Northern Asian samples demonstrate relatively low intraregional variation.

Size differences have been shown to exist in crown dimensions, where males typically possess larger teeth than females. But sexual dimorphism has also been observed in dental morphology among populations (Schwartz and Dean, 2005; Yuwanati *et al.,* 2012; Shakoane *et al.*, 2021). For example, Chowdhry *et al.* (2023) noted statistically differences between Indian males and females for the shovelling (UI1-S), tuberculum dentale, hypocone absence (UM2-H) and protostylid traits (LM1-P). The apparent sexual dimorphism observed in tooth crowns have been ascribed to different proportions of enamel or dentine, of which dentine is present in greater amounts in males (Schwartz and Dean, 2005).

2.3. Dentition in forensic anthropology

In the South African forensic context, skeletal remains are typically discovered in outdoor environments. In these instances, cranial and postcranial elements are commonly absent or damaged (L'Abbé and Steyn, 2012; Shakoane, *et al.,* 2021). Teeth are of importance in forensic anthropology as they are usually well preserved in most forensic cases. Scott *et al.* (2018) argues that incorporating dental morphology into estimations of the biological profile are extremely advantageous as they circumvent the limitations and shortcomings that are often associated with other skeletal traits (such as the craniofacial region or the pubic bone) that are currently used in skeletal analyses. In addition to its durability, the heritability involved in dental morphology has been extensively researched and are deemed a good indicator of population variation. More specifically, the genes and biological processes responsible for the development of teeth are concordant with the processes that influence other skeletal elements commonly used to estimate population affinity, such as the cranium (Scott *et al.,* 2018). Thus, teeth provide similar biological information as the cranium (which is considered a suitable proxy for genetic information) and should theoretically be useful in attempts to classify unknown individuals based on population affinity (Irish *et al*., 2020).

A number of studies have compared dental measurements among populations for classification purposes (e.g., Pilloud *et al.,* 2014; Maier, 2017; Yang *et al*., 2023). Pilloud *et al.* (2014) used buccolingual and mesiodistal measurements of tooth crowns and identified a general pattern: Africans tend to have the largest teeth, Asians have more intermediate sized teeth, and Europeans tend to have the smallest teeth. Discriminant function analysis of crown measurements of all teeth except the third molar classified individuals correctly with an accuracy of 71.3% with unknown sex parameters, and with increased accuracy when sex was known (88.1% in females and 71.9% in males). Yang *et al.* (2023) measured dental size variation in a heterogeneous sample of Latin American descent. The authors found that the heterogeneous group had substantial dental size diversity, and that the dental variation demonstrated overlap with the specific parental populations of specific groups. These studies help us understand the influence that knowledge of dental information of population groups can contribute to the forensic context; however, limited information is available for dental metric variation among South African groups. Shakoane *et al*. (2021) explored sex differences in dental measurements among black, white and coloured South Africans. Overall, sex could be estimated with 86% accuracy. In terms of population affinity, Shakoane *et al.* (2021) demonstrated significant size differences between black, white, and coloured South Africans, where black South Africans were observed to have significantly larger dentition for all tooth types. However, no classification standards were created to assess the use of dental measurements to estimate population affinity in South Africa.

Additional to metric methods, morphological methods are also very commonly used in estimations of population affinity. Morphoscopic traits refer to observable physical characteristics used to assess population affinity or other biological parameters. Morphoscopic traits are visible features of a bone or tooth, where macromorphoscopic (MMS) traits include more defined, specific, measurable details of shape and morphology. MMS traits are considered more precise for population affinity estimations as it involves standardized criteria (in this case a defined scoring system) making it less subjective than morphoscopic traits alone (Hefner, 2008; Plemons and Hefner (2016). In terms of dental morphology, Scott and Turner (1997) and Scott *et al.* (2018) have assessed secondary structural variants of tooth crowns and roots that manifest either as binary characters (i.e., the trait is present or not), such as supernumerary cusps and roots, or accessory ridges and furrow patterns, or as differences in ordinal form (i.e., when present, the trait can vary in size) such as variations present in curves and angles. The authors focused on the prevalence of traits within dentition of an individual as well as variations in the degree of expression within a population noted as slight, moderate, or pronounced when the specific trait was present (Scott *et al.,* 2018). Additionally, they observed large differences in trait frequency and expression among populations and emphasised its significance in the field of anthropology. Although more than 100 such dental crown and root traits are discussed in anthropological literature, only 21 features are commonly used in research pertaining to population differences and classification. These traits have been studied and catalogued as the Arizona State University Dental Anthropology system (ASUDAS). The ASUDAS system makes use of dental plaques (casts) demonstrating ranges of variation for a series of traits located on the incisors and molars of both the maxillary and mandibular dentition (Ash and Nelson, 2009). Recently, a web application (rASUDAS) that employs the traits was developed for use in the statistical software R (R Core Team, 2020). The rASUDAS application makes use of crown and root frequencies for the 21 variables collected from over 30 000 individuals encompassing 21 populations spanning 7 geographical regions (Scott *et* *al*., 2018). Predictive models can be created using the rASUDAS database to estimate which population an unknown individual most likely belongs to, with reported accuracies ranging between 51.8% and 72.7% (Scott *et al.,* 2018). Sub-Saharan Africans have been included in rASUDAS, with data even collected in South Africa, but the particular population affinity is not further specified. Thus, it is not currently possible to use rASUDAS to confidently estimate population affinity in South Africa beyond identifying an individual as originating from sub-Saharan Africa.

In terms of predictive performance, Rathmann and Reyes-Centeno (2020) obtained accuracies of 51.8% to 72.2% with rASUDAS when assessing twenty modern human populations. The authors notably contend that a suite of useful dental traits was omitted from rASUDAS and that the application leaves room for improvement. In a comparison among three North American groups (black, white and Hispanic), Maier (2017) achieved 64.7% to 70.1% classification accuracy. The results indicated that the cranial morphology and dental morphology provide slightly different information about population affinity, and the use of the dental traits yielded improvement in estimating the population affinity of Hispanic individuals (Maier, 2017). The ability of the dental traits to more accurately classify highly heterogeneous groups, such as Hispanics in Maier (2017), demonstrates significant potential that may yield promising results in the similarly diverse South African population.

CHAPTER 3: MATERIALS AND METHODS

3.1. Sample

The sample consisted of the dentition of 191 skulls (cranium and mandible) of black and white South African males and females. The sample distribution is presented in Table 3.1.1. The original sample as in Table 3.1.1 consisted of 191 individuals; however, since each trait was assessed in isolation, the sample sizes for individual traits varied. This variation occurred because each tooth was typically subject to exclusion criteria (taphonomy, damage, dental wear, ante- and postmortem tooth loss) as not all teeth were affected by these factors equally which is why in the same individual, some teeth could be scored, and others not. The skulls were obtained from the Pretoria Bone Collection (PBC), housed at the University of Pretoria. The PBC is a contemporary collection comprised of individuals donated to the Medical School (L'Abbé, *et al.,* 2021). The Black South Africans in the population are descendants of the Bantu-speaking groups that migrated throughout sub-Saharan Africa from western-ventral Africa, and has since further divided into numerous subgroups which exist in Southern Africa today, namely Nguni, Sotho, Venda and Shangaan-Tsonga (Stull *et al.,* 2016). During the 17th century, the Cape was colonized by European settlers, mainly of Dutch origin with contributions from French Huguenots and Germans that arrived in the late 18th century, which has formed the population of White South Africans (Liebenberg, *et al,* 2015). The ages of the individuals in the sample ranged from 16 to 89 years, with an average of 55 years (Table 3.1.2). Overall, the mean age of the white South Africans in the sample is considerably older than the black South Africans. Any skulls or traits with post-mortem damage, deciduous dentition, pathology and/or excessive tooth loss and dental work that prevents the accurate scoring of the traits were excluded from the study. As such, the sample size varies for each trait.

Table 3.1.1 - Sample distribution.

	Black SA	White SA	Total
Male	50	47	97
Female	50	44	94
Total	100	91	191

Table 3.1.2 – Age distribution (years) of the sample.

	Black			White		
	Males	Females	Pooled	Males	Females	Pooled
Mean	43	44	43.5	65	69	67
Range	16 - 70	21 - 80	16 - 80	36 - 82	21 - 89	21 - 89

3.2. Procedure

A series of 15 dental traits were visually assessed on the relevant incisors, premolars and molars (as indicated in Table 3.2) of each individual in the sample and scored according to the Arizona State University Dental Anthropology System (ASUDAS). Refer to Appendix A for more detail regarding the trait descriptions, scoring systems, methods employed and refer to Appendix C for images of the traits. Although the ASUDAS system consists of 21 traits, six of these pertain to the morphology of the tooth root. All teeth were scored *in situ* and were not removed from the alveolar sockets of the maxilla and mandible; thus, any trait pertaining to the root could not be observed and was omitted from the current study. This is in concordance with Maier (2017) who also excluded root traits and discourages removal of teeth from their sockets due to a risk of damaging alveolar bone. Only the teeth on the left side were scored. In instances where the scores could not be obtained for the left side, the right side was used.

Table 3.2 - rASUDAS traits (taken from Scott, *et al*., 2018).

Trait	Trait abbreviation	Trait location
Winging	UI1-W	First upper incisor
Shovelling	UI1-S	First upper incisor
Interruption grooves	UI2-IG	Second upper incisor
Hypocone	UM2-H	Second upper molar
Carabelli's trait	UM1-Car	First upper molar
Cusp 5 (Metaconule)	UM1-C5	First upper molar
Enamel extensions	UM1-EE	First upper molar
Multiple lingual cusps	LP2-MC	Second lower premolar
Groove pattern	LM2-GP	Second lower molar
4-cusped (Cusp number)	LM2-4C	Second lower molar
Cusp 6 (Entoconulid)	LM1-C6	First lower molar
Cusp 7 (Metaconulid)	LM1-C7	First lower molar
Protostylid	LM1-P	First lower molar
Deflecting wrinkle	LM1-DW	First lower molar
Pegged-reduced-missing (PRM)	PRM	Third upper molar

3.3. Statistical analyses:

A variety of statistical analyses were employed to best interpret the data. This included tests to measure observer agreement, exploratory analyses, and classification models. All statistical analyses were completed using the software R version 4.0.2 (R Core Team, 2020).

3.3.1. Observer agreement

A total of 12 skulls were randomly selected from the sample and re-scored by the principal investigator as well as an additional observer. The inter- and intra-observer agreement was then tested with Cohen's kappa. The kappa coefficient measures the agreement between observers in assigning categorical variables adjusted by the standard measure of reliability that could be expected due to chance (Walrath *et al*., 2004; Ferrante and Cameriere, 2009). The calculated kappa values may range from -1 to 1, where a negative value indicates agreement due to chance (Walrath *et al*., 2004). To be consistent when describing the strength of agreement associated with kappa statistics, the parameters proposed by Landis and Koch (1977) were used. The parameters are outlined in Table 3.3 as follows:

Table 3.3. - Kappa values (taken from Landis and Koch, 1997).

Kappa value	**Level of agreement**
< 0.00	Poor
0.00 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.00	Almost perfect

Different weights can be assigned to categorical variables depending on the data structure of the trait (i.e., binary, nominal or ranked ordinal) and how harshly disagreement in a score should be assessed (Sim and Wright, 2005; Tran *et al*., 2018). While an unweighted kappa is suitable for binary and nominal structured traits (where any score disagreement is equally penalised), a weighted kappa should be considered for ordinal traits that have a specific rank or order to the scores (Sim and Wright, 2005). Based on the scoring structure UI2-IG, LP2-MC, LM1-P, LM1-DW and PRM were unweighted. The remaining traits were assessed using a quadratic-weighted Cohen's kappa.

3.3.2. Exploratory analyses

Frequency distributions were created to assess the occurrence of each trait per population group. Kruskal-Wallis tests were used to identify any significant differences in the prevalence of each trait between the two population groups and between the two sexes. The Kruskal-Wallis test is a non-parametric statistic that is robust to variables that may violate the assumptions of comparable parametric tests, such as a non-normal distribution or small sample sizes.

Polychoric correlations were conducted to assess the relationships among the different dental traits using the *polycor* package in R (Fox and Dusa, 2022). Correlation coefficients may indicate either a positive or negative relationship, where greater coefficients suggest higher degrees of covariance between variables (Taylor, 1990; Krüger *et al*., 2015). Multicollinearity occurs when two variables demonstrate increased correlation coefficients, which can inflate standard errors, create bias within inference statistics, and can yield unstable parameter estimates, ultimately affecting the interpretation of results (Dormann *et al*., 2012). The presence of multicollinearity in data should be acknowledged and is suggested to occur with correlations greater than 0.9 (Tabachnick and Fidell, 2007).

3.3.3. Classification models

Random forest models (RFM) were employed to classify the dentition according to population affinity and sex and has been used in many studies to estimate population affinity (Hefner and Ousley, 2014; Maier, 2017; Bothma *et al*., 2024; Liebenberg *et al*., 2024). RFM is a non-parametric machine learning method that was introduced as an improvement upon decision trees (Breiman, 2001; Klales and Kenyhercz, 2015). Decision trees are a type of classification model that uses sequential splitting values (such as dental traits) to predict the probability of an unknown belonging to a certain class (i.e., population and sex) to separate a dataset into groups (Hastie *et al*., 2009). It is commonly used in forensic anthropology especially because it works well with missing data (Deng *et al.*, 2011). A total of 2500 classification trees were used for each model with four variables at each split. The RFM then ranks the importance of each variable included in the classification ensemble, giving an indication of which variables are most discriminatory in the model and which variables do not contribute to the classification. The variable importance was assessed by calculating the mean decrease in the Gini index; the Gini index measures how much each predictor variable contributes to the overall reduction in node impurity achieved by splitting the data on each variable across all trees in the forest. The mean decrease is calculated for each variable by averaging the reduction in the Gini index across all nodes where that specific variable is used for splitting. Finally, out-of-bag observations can be used to gauge the external prediction accuracy of the tree (comparable to leave-one-out cross-validation commonly used with discriminant analysis). Thus, the classification accuracy for the training model as well as the testing model (comprised of the out-of-bag sample) was calculated. Both univariate (assessing each trait individually) and multivariate (assessing all traits combined together) models were created to most effectively assess the positive predictive performance of the traits in classifying population affinity. The *randomForest* package was used to create the RFM classifications (Liaw and Wiener, 2002).

CHAPTER 4: RESULTS

4.1. Observer agreement

The results of observer agreement tests can be seen in Table 4.1. The intra-observer agreement was assessed using Cohen's kappa. The intra-observer repeatability was mostly high, ranging from 0.127 (slight) to 0.957 (almost perfect), demonstrating substantial consistency in repeated scoring by the same observer (principal investigator). Only one trait, multiple lingual cusps (LP2-MC), demonstrated poor repeatability (0.127). The trait was not removed from subsequent analyses, but its poor repeatability should be considered when interpreting results. The inter-observer repeatability was considerably lower than the intra-observer repeatability for every single trait, ranging between -0.187 (poor) and 0.731 (substantial). More than half of the traits had very low values with only one trait scoring more than 0.4 (moderate level of agreement) which was LM1-C6, and two traits scoring more than 0.6 (substantial level of agreement) which were UI2-IG and LM2-4C. Despite the poor inter-observer agreement, all traits were retained for further analysis as all the data were collected by the principal investigator.

Table 4.1: Observer agreement

	Intra-observer	**Inter-observer**
Winging (UI1-W)	0.793	0.204
Shovelling (UI2-S)	0.906	-0.187
Interruption groove (UI2-IG)	0.957	0.731
Hypocone (UM2-H)	0.695	0.174
Carabelli (UM1-Car)	0.843	0.156
Cusp 5 (UM1-C5)	0.763	0.035
Enamel extensions (UM1-EE)	0.856	0.310
Multiple lingual cusps (LP2-MC)	0.127	0.120
Groove pattern (LM2-GP)	0.832	0.087
4-cusped (LM2-4C)	0.934	0.701
Cusp 6 (LM1-C6)	0.856	0.443
Cusp 7 (LM1-C7)	0.880	0.298
Protostylid (LM1-P)	0.884	0.000
Deflecting Wrinkle (LM1-DW)	0.774	0.343
Pegged-reduced missing third molar (PRM)	0.838	0.167

4.2. Exploratory analysis

Frequency distributions were created to assess the prevalence of each trait and its different trait states when comparing black and white South Africans as in Table 4.2. The sample size varies for each trait; some samples were very small due to absent or unscorable teeth. Traits where more than half of the original sample could not be scored include UI2-W, UI2-S and PRM (i.e., traits located on the incisors and third molars). Thus, the small sample size should be considered when interpreting results. Many traits demonstrated near equal distribution between black and white South Africans, such as UI2-W, UM1-Car, LP2-MC, LM2-GP and LM1-DW. Thus, no significant differences were seen between populations with these traits. Many traits were noted to be exceedingly rare in the sample (i.e. predominantly receiving a score of 0 – absent); this includes LM1-C6, PRM, LM1-EE, and UI2-W. Finally, certain trait states, were not observed in the current sample. Some traits have scores that can range from 0 to 7 and is typically related to the size of a feature, where the highest scores indicate larger or more pronounced features. However, numerous traits did not present with the higher trait scores in the current sample. This was most notable for the LM1-P, where there was only one specimen that was assigned a score between 4 and 7. Other traits that also had traits states that were not represented in the sample include LM1-DW, LM1-C6, UI2-S, PRM, UM1-EE and UI2-W. This might be the result of patterns of population-specific variation in the current sample but may also suggest that the codification system for these traits is too complex.

Table 4.2 – Trait frequencies for the population groups. (n = number of teeth available for scoring per population affinity in the trait specific sample)

	Population group			
	Black		White	
Trait scores	**n**	**%**	**n**	**%**
Winging (UI1-W)	(n = 35)		(n = 15)	
0	30	85.7%	13	86.7%
1	5	14.3%	2	13.3%
2	0	0.0%	0	0.0%
3	0	0.0%	0	0.0%
Shovel (UI1-S)	(n = 18)		(n = 7)	
0	3	16.7%	2	28.6%
1	4	22.2%	1	14.3%
2	3	16.7%	1	14.3%
3	5	27.8%	1	14.3%
4	2	11.1%	1	14.3%
5	0	0.0%	1	14.3%
6	1	5.6%	0	0.0%
7	0	0.0%	0	0.0%
Interruption groove (UI2-IG)	(n = 63)		(n = 32)	
0	23	36.5%	11	34.4%
1	10	15.9%	8	25.0%
2	12	19.0%	6	18.8%
3	5	7.9%	3	9.4%
4	13	20.6%	4	12.5%
Hypocone (UM2-H)	(n = 87)		(n = 42)	
0	3	3.4%	9	21.4%
1	7	8.0%	2	4.8%
2	7	8.0%	2	4.8%
3	18	20.7%	6	14.3%
4	24	27.6%	10	23.8%
5	18	20.7%	10	23.8%
6	10	11.5%	3	7.1%

Table 4.2 (continued) – Trait frequencies for the population groups

Carabelli cusp (UM1-Car)	(n = 94)		(n = 46)	
0	32	34.0%	15	32.6%
1	13	13.8%	4	8.7%
2	21	22.3%	7	15.2%
3	12	12.8%	9	19.6%
4	9	9.6%	7	15.2%
5	5	5.3%	2	4.3%
6	1	1.1%	1	2.2%
7	1	1.1%	1	2.2%
Cusp 5 (UM1-C5)	(n = 91)		(n = 31)	
0	56	61.5%	23	74.2%
1	17	18.7%	2	6.5%
2	8	8.8%	2	6.5%
3	4	4.4%	1	3.2%
4	5	5.5%	1	3.2%
5	1	1.1%	2	6.5%
Enamel extensions (UM1-EE)	(n = 94)		(n = 49)	
0	67	71.3%	40	81.6%
1	20	21.3%	7	14.3%
2	6	6.4%	2	4.1%
3	1	1.1%	0	0.0%
4	0	0.0%	0	0.0%
Pegged/ Reduced/ missing third molar (PRM)	(n = 77)		(n = 15)	
0	68	88.3%	10	66.7%
1	9	11.7%	5	33.3%
2	0	0.0%	0	0.0%
3	0	0.0%	0	0.0%

Table 4.2 (continued) – Trait frequencies for the population groups

Multiple lingual cusps (LP2-MC)	(n = 91)		(n = 46)	
0	0	0.0%	0	0.0%
1	30	33.0%	14	30.4%
2	45	49.5%	27	58.7%
3	16	17.6%	5	10.9%
Groove pattern (LM2-GP)	(n = 83)		(n = 14)	
0	0	0.0%	0	0.0%
1	48	57.8%	8	57.1%
2	19	22.9%	3	21.4%
3	16	19.3%	3	21.4%
4 Cusps (LM2-4C)	(n = 86)		(n = 34)	
0	17	19.8%	16	47.1%
1	10	11.6%	2	5.9%
2	16	18.6%	6	17.6%
3	20	23.3%	3	8.8%
4	17	19.8%	3	8.8%
5	6	7.0%	4	11.8%
Cusp 6 (LM1-C6)	(n = 83)		(n = 21)	
0	67	80.7%	18	85.7%
1	12	14.5%	1	4.8%
2	0	0.0%	0	0.0%
3	3	3.6%	1	4.8%
4	1	1.2%	1	4.8%
5	0	0.0%	0	0.0%

Table 4.2 (continued) – Trait frequencies for the population groups				
Cusp 7 (LM1-C7)	(n = 90)		(n = 27)	
0	32	35.6%	15	55.6%
1	30	33.3%	9	33.3%
2	18	20.0%	0	0.0%
3	5	5.6%	1	3.7%
4	5	5.6%	2	7.4%
Protostylid (LM1-P)	(n = 93)		(n = 28)	
0	26	28.0%	14	50.0%
1	64	68.8%	10	35.7%
2	3	3.2%	2	7.1%
3	0	0.0%	1	3.6%
4	0	0.0%	0	0.0%
5	0	0.0%	0	0.0%
6	0	0.0%	0	0.0%
7	0	0.0%	0	0.0%
Deflecting wrinkle (LM1-DW)	(n = 75)		(n = 11)	
0	44	58.7%	6	54.5%
1	26	34.7%	4	36.4%
2	4	5.3%	0	0.0%
3	1	1.3%	1	9.1%
4	0	0.0%	0	0.0%

4.3. Kruskal-Wallis tests

Kruskal-Wallis tests were conducted to identify any traits that demonstrate a significant association with population affinity and would thus potentially be useful in classification models (Table 4.3). Of the 15 traits that were scored, only three traits show statistically significant differences between black and white South Africans ($p<0.05$); these traits are PRM, LM2-4C and LM1-C7 (the pegged/reduced/missing third molar, number of cusps on the lower second molar, and cusp 7 on the lower first molar). However, it should be acknowledged that the sample size for the PRM was quite small, especially among white South Africans. Although the Kruskal-Wallis test is robust to smaller samples, this should still be taken into consideration as a potential reason for the significant result. None of the other traits were noted to be significantly different, suggesting a lack of population differences. Two of the 15 traits showed statistically significant differences between males and females ($p<0.05$); these traits are the interruption groove on the upper second incisor, and the protostylid on the lower first molar (UI2-IG and LM1-P). The rest of the traits were not observed to be statistically different for sex.

Table 4.3. - Results of the Kruskal-Wallis test comparing trait score frequencies between the populations and the sexes. Bold indicates significant differences.

Trait	Population	Sex
Winging (UI1-W)	0.93	0.52
Shovel (UI1-S)	0.95	0.89
Interruption groove (UI2-IG)	0.65	**<0.01**
Hypocone (UM2-H)	0.26	0.11
Carabelli cusp (UM1-Car)	0.34	0.46
Cusp 5 (UM1-C5)	0.36	0.96
Enamel extensions (UM1-EE)	0.17	0.75
Reduced/missing third molar (PRM)	**0.03**	0.46
Multiple lingual cusps (LP2-MC)	0.8	0.67
Groove pattern (LM2-GP)	0.92	0.45
4 Cusps (LM2-4C)	**0.03**	0.69
Cusp 6 (LM1-C6)	0.7	0.45
Cusp 7 (LM1-C7)	**0.04**	0.31
Protostylid (LM1-P)	0.11	**0.02**
Deflecting wrinkle (LM1_DW)	0.74	0.1

4.4. Correlations

Polychoric correlations were used to analyse the relationship between the dental traits, with correlation coefficients ranging between -0.99 and 0.9 (Table 4.4). All the notable correlations, whether positive or negative, were related to the winging of the upper first incisors (UI1-W). More specifically, a strong positive correlation was observed between winging (UI1-W) and shovelling (UI1-S) of the upper first incisors (r=0.99). This is likely because both traits are located on the central upper incisors. Additionally, strong negative correlation correlations were noted between UI1-W and UM1-C5 (r =-0.99), UI1-W and UM1-EE (-0.98), UI1-W and PRM (-0.98), as well as UI1-W and LM1-C6 (-0.98). These negative correlations could be because of the differences in the crown anatomy of the incisors (UI1-W) and the molars (UM1-C5, UM1-EE, PRM, LM1-C6). The only characteristic among those listed above that shows statistically significant population differences and is highly correlated with another trait is PRM. This suggests that the probability of the correlation being driven by population differences is low. It is also important to note that the sample for which UI1-W could be scored was very small and this could also affect the results seen. The high correlation coefficients obtained with these traits are indicative of multicollinearity, which should be taken into consideration with the creation of classification models. A moderate positive correlation was also observed between UI1-W and UI2-IG- (r=0.54), while UI1-S and UM1-EE (r=-0.55), and UI1-S and LM1-C6 (r=-0.57) yielded a moderate negative correlation.

Table 4.4 – Polychoric correlations demonstrating the relationship between traits.

	Winging	Shovelling	Interruption groove	Hypocone	Carabelli cusp	Cusp 5	Enamel extension	PR M third molar	Lingual cusps	Groove pattern	4 Cusps	Cusp 6	Cusp 7	Protostylid	Deflecting wrinkle
Winging	-														
Shovelling	0.9	-													
Interruption groove	0.54	-0.05	-												
Hypocone	-0.04	-0.21	0.17	-											
Carabelli cusp	0.01	-0.18	0.02	0.26	-										
Cusp 5	-0.99	-0.38	0.2	0.19	-0.01	-									
Enamel extension	-0.98	-0.55	0.16	-0.14	-0.21	-0.07	-								
PRM third molar	-0.98	0.09	-0.32	-0.09	-0.12	-0.2	-0.36	-							
Lingual cusps	-0.36	-0.44	-0.12	0.22	0.15	-0.06	-0.09	-0.01	-						
Groove pattern	-0.16	-0.09	-0.19	-0.2	0.18	0.07	0.16	-0.12	-0.05	-					
4 Cusps	-0.31	-0.33	-0.2	0.21	0.13	-0.23	0.03	-0.01	-0.14	-0.14	-				
Cusp 6	-0.98	-0.57	0.32	-0.05	-0.09	0.35	0.05	0.21	-0.44	0.09	0.09	-			
Cusp 7	0.21	0.38	0.02	-0.01	0.11	0.05	-0.03	-0.13	0.1	0.09	-0.17	-0.17	-		
Protostylid	-0.23	0.28	-0.2	0.07	0.15	0.16	0.1	0.11	-0.1	0.02	-0.15	0.23	0.22	-	
Deflecting wrinkle	-0.02	-0.29	-0.2	-0.14	0.07	0.2	-0.01	0.15	0.03	-0.04	-0.1	0.16	0.16	0.04	-

4.5. Random Forest Models:

Univariate random forest models were first created to explore the predictive ability to estimate population affinity of each dental trait on its own prior to building a multivariate model. Training accuracies for the univariate models ranged from 28.17% to 69.72% (Table 4.5.1). Amongst the traits scored, UI1-S performed the worst, which may be a result of the small sample size. The traits LM2-4C and LM1-C7 performed the best, which is expected since both of these traits demonstrated significant differences with the Kruskal-Wallis test. Inversely, PRM which is the only other trait that showed significance for population differences (p=0.03), is the trait with the third lowest accuracy (52.82%). Once again, this may be the result of a reduced sample size for this trait. Notably, the traits of the upper and lower molars mostly resulted in higher accuracies compared to the other teeth (incisors and premolars).

Table 4.5.1.– Univariate classification accuracy (%) of each MMS trait using RFM for population affinity.

Trait	Training accuracy
4 Cusps (LM2-4C)	69.72
Cusp 7 (LM1-C7)	69.72
Hypocone (UM2-H)	69.01
Carabelli cusp (UM1-Car)	69.01
Interruption groove (UI2-IG)	64.08
Cusp 5 (UM1-C5)	64.08
Groove pattern (LM2-GP)	61.97
Multiple lingual cusps (LP2-MC)	61.27
Deflecting wrinkle (LM1-DW)	60.56
Enamel extensions (UM1-EE)	58.45
Cusp 6 (LM1-C6)	55.63
Protostylid (LM1-P)	54.93
Reduced/missing third molar (PRM)	52.85
Winging (UI1-W)	52.82
Shovelling (UI1-S)	28.17

All traits were combined into a multivariate model. Table 4.5.2 presents the training accuracies, with a breakdown of the predictive performance of each population group and group overlap. The training model correctly classified 76.0% of black individuals and 79.1% of white individuals, for an overall accuracy of 77.46%. The testing accuracy for the model, which made use of the holdout sample for validation purposes was 76.6%. Thus, the model performs similarly on training and testing datasets which means that it has captured the underlying patterns in the data effectively without overfitting the data which means that the model is generalising the data quite well.

Table 4.5.2 – Confusion matrix showing patterns of overlap and misclassification among the groups for the training model employing the macromorphoscopic (MMS) traits ordinally/categorically.

		Classifies into:		**% Correct**
		Black	White	
Group:	Black	57	18	76.0
	White	14	53	79.1
				77.46

The variable importance was calculated to assess how much discriminatory power each trait contributes to the model, with the variable importance ranging from 0.84 to 9.93 (Table 4.5.3). Figure 4.5.1 graphically demonstrates the contribution of each trait to the model. The highest ranked traits include LM2-4C (9.93), UM1-C5 (6.84), LM1-C7 (6,.57) and UM2-H (6.48). The lowest ranked traits include UI1-W (0.84), PRM (0.93), UI1-S (1.56) and LM1-C6 (1.65).

Table 4.5.3 – RFM variable importance for MMS traits.

Trait	Variable importance
4 Cusps (LM2-4C)	9.93
Cusp 5 (UM1-C5)	6.84
Cusp 7 (LM1-C7)	6.57
Hypocone (UM2-H)	6.48
Carabelli cusp (UM1-Car)	5.55
Interruption groove (UI2-IG)	4.47
Groove pattern (LM2-GP)	4.13
Multiple lingual cusps (LP2-MC)	3.98
Deflecting wrinkle (LM1-DW)	3.70
Enamel extensions (UM1-EE)	3.07
Protostylid (LM1-P)	2.63
Cusp 6 (LM1-C6)	1.65
Shovel (UI1-S)	1.56
Reduced/missing third molar (PRM)	0.93
Winging (UI1-W)	0.84

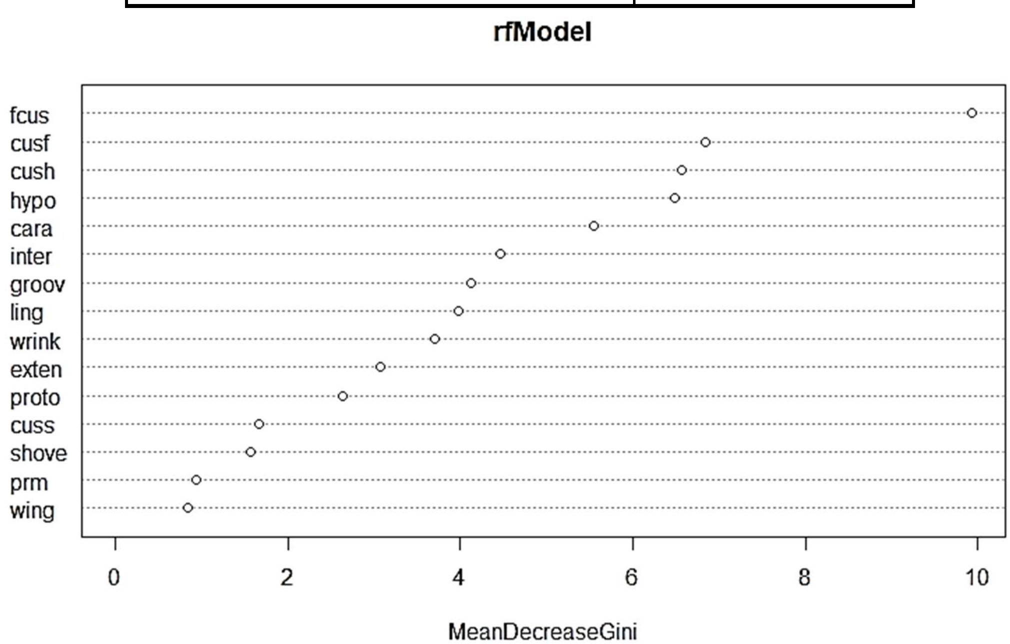

Figure 4.5.1. Variable importance for each trait in the sample based on the GINI index.

Given sample constraints and the apparent rarity of some traits in the sample, some additional models were created to further explore the accuracy of the traits. A first additional model was created to determine the effect of the scoring system on the results. The traits were dichotomised (codification of the traits was changed from ordinal/categorical to binary) so that each trait is only scored as absent (0) or present (1 – visible regardless of size or shape). This was done for all traits except for traits that do not have "absent" as one of the trait states (e.g. LM2-GP). Table 4.5.4 presents the training accuracies with a breakdown of the predictive performance of each population group and group overlap. The overlap seems to be more pronounced when ordinal scoring is eliminated as the classification accuracy for the white South Africans decreased with more than 10% (from 79.1% to 68.66%); however, the classification accuracy for the black South Africans increased slightly (from 76.1% to 78.67%), for an overall training accuracy of 73.94%. With the dichotomisation, the testing accuracy decreased to 65.96%, which is considerably lower than when ordinal scoring was used (76.6%). Table 4.5.5 and Figure 4.5.2 presents the variable importance with the contribution of each trait to the model. When comparing this to the original ordinal model, it is interesting to note that LM2-C, which demonstrated the highest variable importance, is now amongst the lowest, while UI1-S and UI1-W remain the two lowest in both models. The variable importance for LM2-GP was now much higher in the dichotomised model compared to the ordinal model. The reason for this is because this trait is scored as an X, Y or + and there is no score for absent (i.e. more trait states).

Table 4.5.4 – Confusion matrix showing patterns of overlap and misclassification among the groups for the training model employing the MMS traits as absent or present (dichotomised).

		Classifies into:		**% Correct**
		Black	White	
Group:	Black	59	16	78.67
	White	21	46	68.66
				73.94

Table 4.5.5 – RFM variable importance for MMS traits using the dichotomised scoring system.

Trait	**Variable importance**
Groove pattern (LM2-GP)	8.46
Deflecting wrinkle (LM1-DW)	5.68
Multiple lingual cusps (LP2-MC)	4.68
Cusp 7 (LM1-C7)	3.57
Cusp 5 (UM1-C5)	3.48
Carabelli cusp (UM1-Car)	3.47
Interruption groove (UI2-IG)	2.73
Cusp 6 (LM1-C6)	2.29
Enamel extensions (UM1-EE)	2.16
Protostylid (LM1-P)	2.14
Hypocone (UM2-H)	1.58
Reduced/missing third molar (PRM)	1.50
4 Cusps (LM2-4C)	1.49
Shovelling (UI1-S)	0.46
Winging (UI1-W)	0.41

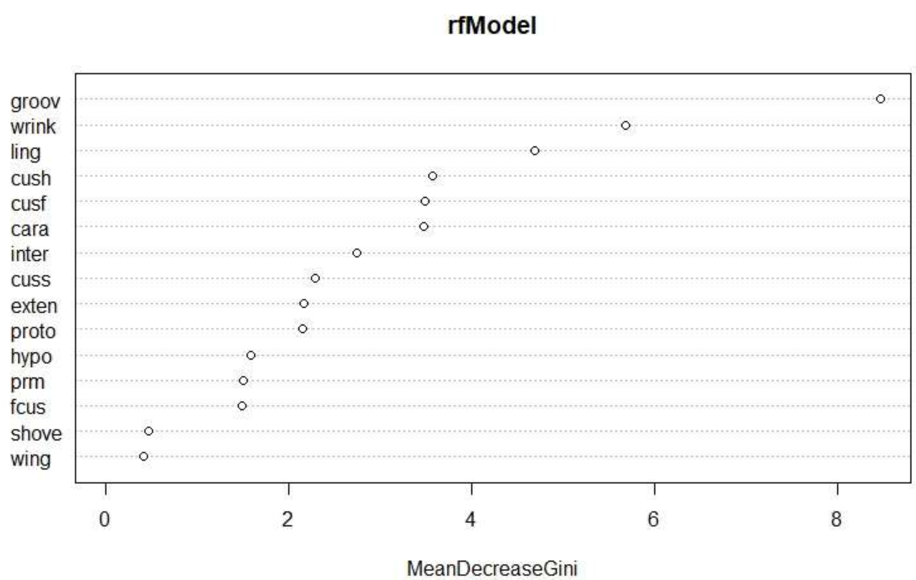

Figure 4.5.2. Variable importance for each trait in the sample using the dichotomised scoring system based on the GINI index.

A second additional model was created where the number of traits employed was reduced (Table 4.5.6). More specifically, the variables with very small samples were omitted; this includes UI1-W, UI1-S, PRM, and LM2-GP. The model yielded slightly increased training accuracies compared to the model that includes all traits (78.17% vs 77.46%), but a decreased testing accuracy (70.21% vs 76.6%). In this model, the difference between the training accuracy and testing accuracy is much larger than the model that included all traits (7.96% vs 0.6%). This suggests that all variables provide some useful information to the model, and their omission affects the generalisability of the model on an independent sample. The variable importance for this model is shown in Table 4.5.7 and Figure 4.5.3.

Table 4.5.6 – Confusion matrix showing patterns of overlap and misclassification among the groups for the training model employing the MMS traits with smaller samples dropped.

		Classifies into:		**% Correct**
		Black	White	
Group:	Black	57	18	76.0
	White	13	54	81
				78.17

Table 4.5.7 – RFM variable importance for MMS traits with the reduced variables.

Trait	Variable importance
Interruption groove (UI2-IG)	5.56
Hypocone (UM2-H)	7.51
Carabelli cusp (UM1-Car)	6.33
Cusp 5 (UM1-C5)	8.03
Enamel extensions (UM1-EE)	3.52
Multiple lingual cusps (LP2-MC)	4.93
4 Cusps (LM2-4C)	11.29
Cusp 6 (LM1-C6)	1.53
Cusp 7 (LM1-C7)	7.80
Protostylid (LM1-P)	3.23
Deflecting wrinkle (LM1-DW)	4.52

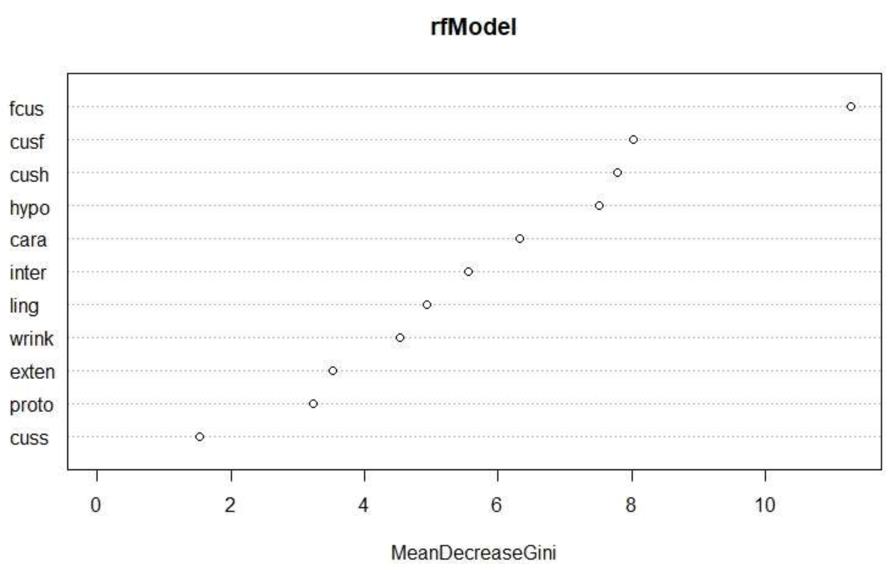

Figure 4.5.3. Variable importance for each trait in the sample based on the GINI index with the reduced variables.

CHAPTER 5: DISCUSSION AND CONCLUSION

Several studies have assessed the morphology of dentition, particularly focusing on variation among geographic populations (Scott *et al*., 2018; Irish *et al*., 2020; Rathmann and Reyes-Centeno, 2020). This is the first study to assess differences in dental morphology among population affinities within a South African population using the ASUDAS traits with the aim to create population-specific classification standards for use in forensic skeletal analyses. Overall, the study observed few significant differences in trait frequencies which culminated in moderate classification accuracies. Some factors that potentially play a role include trait repeatability, complexity of the scoring system, and difficulties pertaining to the sample.

5.1. Scoring methodology and trait repeatability

Assessing trait repeatability and observer agreement is an essential step in the creation of standards, especially when working with non-metric traits. Before data collection commenced, the principal investigator studied and practiced the scoring procedure. This is considered good practice for morphoscopic studies as greater repeatability is typically noted when observers are more experienced with the traits (Klales and Kenyhercz, 2015; Hay *et al.,* 2019). Since teeth are small, the traits themselves, and variations between the trait states were minute and often difficult to discern with the naked eye. For example, with winging of the incisors (UI1-W), a score of 0 refers to incisors positioned at an angle greater than 180°, while a score of 1 refers to incisors angled between 160° to 180° (Scott and Irish, 2017). Similarly, a 5th cusp on the lower second molar is described as being "very small, small, medium-sized, large, or very large" (Scott and Irish, 2017). Even with larger traits, such as those on the cranium, it can be difficult to objectively distinguish whether a variant is small rather than medium without a particular frame of reference. Although comparative reference guides are available, such as the manual published by Edgar (2017), the traits remain difficult to score based on visual assessment. In their descriptions for macromorphoscopic (MMS) traits of the cranium, Plemons and Hefner (2016) recommend the use of additional tools to assist with the scoring procedure. For instance, the use of a clear ruler is recommended to assist with an assessment of the size of the malar tubercle and posterior zygomatic tubercle (Plemons and Hefner, 2016). The same approach was adopted in the current study, where a clear ruler was used to gauge the size of cusps (such as the 5th cusp on the lower second molar and the 7th cusp on the lower first molar), and a clear protractor was used to assess the degree of winging of the incisors (UI1-W). Refer to the descriptions in Appendix A for more information on the additional measures used to improve repeatability of the traits. These measures ultimately helped to increase the intra-observer repeatability, apart from multiple lingual cusps on the lower 2nd premolar (LP2-MC). With this trait even the presence of extremely small cusps is considered as an additional cusp, and they are sometimes overlooked, leading to low repeatability. It is possible that magnification might be beneficial when scoring these traits. Maier (2017) reported higher values for intra-observer agreement for all traits compared to the current study (ranging between 0.61 and 1.00), but similarly observed lower agreement for LP2-MC (0.61).

As for inter-observer agreement, the repeatability was much lower, as the second observer had no prior experience with the traits and only had the descriptions (as presented in Appendix A) to guide them. This affects future research in this field as it may reduce reliability of findings, limit comparability between studies, affect accuracies of population affinity estimations and create challenges in standardization of the method. The lower inter-observer agreement suggests that the method is difficult to employ when the observer is unfamiliar with the traits and the variations between traits. This confirms results presented by Hay *et al.* (2019), who concluded that greater experience is required for greater agreement. Additionally, the number of lingual cusps on the 2nd premolar (LP2-MC) was once again identified as the least repeatable trait (Hay *et al*., 2019) just as in this study. As previously mentioned, trait states tend to have very slight differences from one score to the next. However, numerous traits are scored on a scale varying from 0 to 6 or 7, such as UI1-S, UM2-H, UM1-Car, and LM1-P; this is a very large number of trait states for such apparently small differences among the states. As a result of the large number of states per trait, several trait states were not observed, or not represented in the sample. Thus, the low frequency of these states in the sample begs to know if such a complex array of states is truly required, as the complexity may influence the repeatability of the method. It may be argued that the low frequency of certain traits is due to population-specific variation among modern South Africans not observed in other populations. However, numerous authors have opted to dichotomise the traits in recent studies (Rathmann and Reyes-Centeno, 2020; Corron *et al*., 2022; Chowdhry *et al.,* 2023), and cited difficulties with the scoring procedure as the reason. While a dichotomised scoring system would certainly make data collection easier, the classification models based on dichotomised scores did yield lower accuracies. This is most likely as some variation between the groups is lost when the scores are simplified. Further research is required is to reach a balanced compromise between good classification accuracy and ease of use of the method.

5.2. Factors influencing teeth

Although teeth are lauded for their exceptional postmortem preservation, many antemortem factors can influence their morphology and subsequent use in anthropological analyses. These factors include advanced age of the person at the time of death, antemortem tooth loss, damage (attrition, wear etc.), and dental practices (Scott and Irish, 2013). Abrasion and attrition interfered with the scoring of various molar traits; this is because constant friction between the molars have worn away characteristics on the crown that is scorable, such as the peaks of cusps. In some specimens the grooves in between cusps were abraded to the point that it was not possible to distinguish between cusps and therefore judge cusp size (affecting scoring of traits such as UM2-H, UM1-Car, UM1-C5, LM2-4C, LM1-C6, and LM1-C7) as well as groove patterns (affecting scoring of LM2-GP). Shovelling on the incisors (UI1-S) was also frequently obscured due to dental wear.

This would not be the first case where dental wear has interfered with research due to loss of biological information (Hrdlicka, 1920, Maier, 2017, Scott *et al.,* 2018; Fidalgo *et al.*, 2021). Maier (2017) also mentions that dental wear plays a role in intra-observer agreement of dental morphology, because sometimes the researcher needs to decide whether the trait should be recorded or excluded, which results in a dilemma between maximising sample size and accurately recording the traits observed. Fidalgo *et al.* (2021) concluded that as occlusal wear increases, crown traits become less distinct, resulting in lower trait scores by observers. Traits that should have been marked as unobservable are still scored, likely due to an unconscious bias to achieve a sufficient sample size. In numerous cases where teeth exhibit excessive wear, researchers are compelled to exclude these teeth from analyses and only score those without wear, acknowledging the limitation of a reduced sample size.

The effects of dental wear and poor dental care increases with advancing age. Kimmie-Dhansay *et al*. (2021) reports that in the South-African population the prevalence of edentulism and tooth loss increases after the age of 45 years. This was confirmed and observed in the current study sample as the white South Africans in the sample, who were on average much older compared to the black South Africans (67 years versus 43 years), presented with more frequent and extensive tooth loss and wear in all teeth, but especially molars. Thus, the white South African sample had less scorable traits and subsequently smaller trait samples. The possibility that the age of the sample has had an impact on the results of this study cannot be excluded.

Another issue faced when scoring teeth, especially molar crowns, was dental caries and cavities, as these lesions either destroy the enamel or leads to antemortem tooth loss. A dental carious lesion refers to the localised destruction of susceptible dental hard tissues and can lead to cavitations, and in extreme cases results in tooth loss (Rathee and Sapra, 2023). Since teeth are unable to remodel, the number of caries typically increase with age, especially among individuals that lack access to proper dental care. In this sample, caries has been noted to lead to the absence or damage of a whole cusp, which results in the cusp not being scored, or for example, obscuring the junction of the groove pattern making scoring of the trait as either X-, Y-, or +-shaped not possible. When a carious lesion becomes large enough to extend into the root, it is likely that the tooth will be removed. Other risk factors for tooth loss include sociodemographic factors such as age and education level, chronic conditions such as asthma and arthritis, health risk behaviour such as smoking, and low fruit/vegetable intake (Peltzer *et al.,* 2014). Sometimes patients will get crowns, dental implants and in cases of extreme tooth loss, dentures. While these dental treatments would be developed to fit its recipient in terms of size, it will not contain any individualising features such as cusp variations.

Secondary to dental wear and tooth loss, there is also the impact that braces, cultural modification, or other aesthetic modification of teeth has on scoring. Braces are apparatus made of metal bands, square metal brackets and metal wire that are glued to the outside of the teeth. Braces aim to change and improve the position of teeth, as well as the position of the mandible and maxilla due to malocclusion and misalignments (Hung *et al.*, 2023; Institute for Quality and Efficiency in Health Care, 2006). Braces for example, will rectify winging (UI1-W) so that this trait will not be present when scoring, which will not be an accurate representation of that individual’s biological information. This can lead to, for example that an individual that would have scored for the presence of a trait to a varying degree is then scored as a 0. In this sample, majority of the scores for UI1-W (86%) was a 0, and there is a possibility that some of these individuals would have scored higher if orthodontic practices were not applied. However, there is no way of knowing if any of the individuals in the PBC indeed had braces. Hung *et al.* (2023) evaluated the demographic differences of orthodontic patients in a North American population (those who typically get braces, retainers, etc.) and found statistically significant results that usage of orthodontics was more common in females, in the white population, in individuals under the age of 18 years old and a family total income of $100 000 or more. However, since the majority of the individuals in the sample used in the current study are likely of lower socio-economic status, it is not expected that many of them had access to orthodontic treatment (Branson *et al.,* 2024).

Similar to the incisors, the third molars (which are scored as pegged, reduced or missing/absent) may also potentially be affected by dental practices. Molars can be highly variable, with up to 50% of third molars presenting with some form of anomaly (Sujon *et al*., 2016). If simply considering the presence of the tooth in the oral cavity, third molars frequently do not erupt, erupt partially (and may become impacted), or are congenitally absent. Among many individuals the eruption of the third molar leads to overcrowding in the mouth, which may lead to discomfort (especially in the case of impaction). Thus, it is not uncommon for the third molars to be extracted or surgically removed. Kennesey *et al.* (2023) noted in a multi-population study (which included a South African cohort), that dental crowding was the most prevalent among white and coloured South Africans, and least prevalent among black South Africans. Thus, it could be argued that the third molar was more commonly extracted in the white individuals in the sample, which is why it was scored as absent more frequently. However, this assumption would most likely be completely biased. When there is no third molar present in the oral cavity of a dry bone specimen, it is not always possible to determine whether it is congenitally absent, not yet erupted, or surgically removed (Maier, 2017). Maier (2017) had completely excluded this trait from the study for the exact same reason – stating that it is impossible to distinguish congenital absence from surgical removal in modern skeletons without taking dental radiographs.

5.3. Teeth as variables for classification

The univariate models yielded moderate accuracies; except for UI1-S, all traits had a training accuracy between 50% and 70%.The multivariate model performed better than the univariate model, which is consistent with the literature that states using multiple traits will produce higher accuracies and are less prone to false positive predictions resulting from chance than using single traits (Ousley and Jantz, 2012; Liebenberg *et al.,* 2024). The multivariate model yielded a moderate accuracy (76.6%) in classifying unknown individuals as either black or white South Africans, which was fairly surprising given the lack of significant differences that were observed. In comparison to other, similar methods, dental traits appear to have performed better than the macromorphoscopic traits of the postcrania (54.6% to 62.1%) (Bothma *et al.,* 2024), and similar to the macromorphoscopic traits on the cranium (79%) (Liebenberg *et al.,* 2024). However, it should be acknowledged that both the aforementioned studies assessed three groups (black, white and coloured South Africans), whereas the current study only compared two. As such, the classification accuracy for the dental models will most likely be much lower if additional groups are added.

In comparison to results from the United States, many traits that did not show any statistically significant differences in the South African sample were noted to be different among black, white and Hispanic North Americans, such as UM1-EE, UI1-S, LM2-GP, UM2-H, UI1-W, UM1-C5, LM1-DW, among others that were not scored in this study (Maier, 2017). Conversely, the traits noted in this study as significantly different among South Africans (PRM, LM1-C7 and LM2-4C) were not significantly different for North Americans. The differences in trait prevalence and significance may be due to population variation, which distinguishes South Africans from North Americans; but observer agreement cannot confidently be excluded as an impacting factor. Although the current study, achieved high intra-observer agreement, it simply indicates consistency, but does not guarantee that the traits were scored reliably (or the same as other researchers). Ultimately, Maier (2017) achieved accuracies ranging from 64.7 to 70.1%, and concluded that dental morphology can be used to accurately estimate individual population affinity and that the high rate of accurate classification the research yielded supports further inclusion of dental morphology in forensic estimations of population affinity. It should also be mentioned that Maier (2017) advocates for the use of mixed models combining the dental traits with cranial traits for optimal results.

On a more global scale, Scott and colleagues (2018) has applied the rASUDAS system to a total of seven geographic populations; these were then reduced to smaller comparisons by decreasing the number of populations to a four-group and then a three-group model, with accuracies ranging from 65.96% to 76.6%. Compared to other studies that evaluated dentition for differences in population affinity (Maier, 2017; Scott *et al.*, 2018; Shakoane *et al.,* 2018), this study simply did not observe the same level of significant differences between the South African groups.

When looking at African populations specifically, Irish (1997) proposed that sub-Saharan Africans (which included the black south Africans as well as Khoesan, which more closely represents today's coloured population in South Africa) are best differentiated from other worldwide samples by high frequencies of the following traits: The "*Bushman*" (sic) canine, a two rooted upper first premolar, Carabelli's trait (UM1-Car), a three-rooted upper second molar, a y-groove pattern (LM2-GP), Cusp 7 (LM1-C7), tomes root on the lower first premolar, presence of the upper third molar (PRM). Additionally, low frequency traits in this population are double shovelling of the central incisors, and enamel extensions on the upper first molar (UM1-EE). Irish (1997) termed this the sub-Saharan African dental complex. The lack of major significant differences in the current study is most likely the result of micro-evolutionary factors, such as genetic drift and gene flow.

5.4. Limitations and recommendations

Several limitations were encountered throughout the study that should be considered and addressed in future research. Firstly, the sample was greatly affected by tooth loss and dental wear. Many specimens were excluded from the sample, so that the individuals that were included represented the best possible, most scorable sets of dentition. Yet, there were still many missing traits, and damaged or absent teeth which made scoring of certain traits impossible or very difficult. As previously discussed, the mean age of the sample certainly contributed to this problem, which could be mitigated by the inclusion of younger individuals. However, the advanced age of individuals housed in skeletal collections are a universal problem (Dayal *et al*., 2009; Alblas *et al*., 2018; Campanacho *et al*., 2021; L'Abbé *et al*., 2021).

In addition to age, the PBC is also limited in its representation of the South African population. More specifically, the PBC primarily houses skeletal remains of black and white South Africans, with a very small sample of coloured South Africans (L'Abbé *et al*., 2021). There are also currently no skeletal collections in South Africa with modern Indian or Asian South Africans. As such, the current study only assessed the variation of black and white South Africans, which provides an incomplete picture of the population's variation. For the method to truly be useful in forensic casework, more population groups need to be assessed to better understand the within- and among-group variation and overlap, and to more realistically gauge the predictive performance of the traits in South Africa.

Finally, all of the traits pertaining to the tooth roots had to be omitted from the current study because they were obscured by alveolar bone. Teeth that were already loosened from the alveolar socket could be scored, but this was rarely the case. Several root traits have previously been found to be indicative of population affinity, and the information that those traits provide is not included in many current models because they cannot be scored on dry bone (Maier, 2017).

One potential solution that can address all the above limitations is the use of 3D imaging technology. Three-dimensional scans can provide a means to document skeletal features, such as trauma, traits and anomalies. It can also be used to create replicas that can be used for archival and illustrative purposes (Garvin and Stock, 2016). Additionally, with access to various collections of 3D scans, researchers have access to a wider range of populations to include in research on population affinity and other biological parameters. Making use of three-dimensional scans do not only enhance specimen preservation but can be a useful tool for virtual human studies (Johnson and Pandey, 2019). Scans of specimens as well as living individuals can be used to build databases for forensic anthropologists to use for research and casework purposes. With regard to this specific study, having micro-CT as well as cone beam computed tomography (CBCT) scans available would have enabled the observation and scoring of root traits. Micro-CT could assist in studying root morphology of teeth in specimens where roots are not visible, where CBCT could provide high image quality at low cost in living individuals as well, with visibility of root morphology (Liang *et al,* 2017). These scans could have provided a younger sample where the dentition was less subject to tooth wear and loss, and it could possibly have alleviated the limitations of small sample sizes.

Although the extent of variation expected based on other dental macromorphoscopic studies was not observed, three traits showed statistically significant differences between the black population and the white population. When compared to other methods in terms of accuracy in a South African population, the dental traits performed better than postcrania, but not as well as crania.

Scott et al. (2018) mentions that using dental morphology in estimations of the biological profile circumvent the limitations and shortcomings that other skeletal traits face in forensic casework, however, this study sample has shown that teeth did not solve as many problems as we had hoped – as they are just as subject to loss, wear, damage and disease as the rest of the skeleton. This is, however, strongly linked to the poor condition of the teeth of the White South African population in skeletal collections, and may not be the case in all other study samples where the populations in the collections are possibly younger, and thus have better and more scorable teeth that have less changes to the dentition, as the White South African population in this sample had a mean age of 67 years.

This method may not be suitable to incorporate into South-African forensic casework, but with refinement of methods, improving expertise on scoring of traits by employing method-specific training, and possibly a more representative sample (younger) with less tooth loss, damage and changes, this method has promising potential for future use as a standalone or as a mixed model such as in Maier (2017) where cranial morphoscopic traits was combined with dental morphoscopic and yielded better results than dental traits alone.

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APPENDIX A: TRAIT DESCRIPTIONS

<table>
<tr><td colspan="5">Table A1. Table of trait definitions, descriptions, methods employed and examples.</td></tr>
<tr><th>Characteristic</th><th colspan="2">Tooth</th><th>Trait type</th><th>Grade/Description</th></tr>
<tr><td>Winging (UI1-W)</td><td>UI1</td><td>Central Upper Incisor</td><td>Crown</td><td>Rotated maxillary central incisors.
0) If the line is parallel to the labial surfaces or if the distal margins fall below the line, winging is absent. Angle >= 180 degrees.
1) Mesial margins of the upper incisors fall slightly below the line. Angle 160-180 degrees.
2) Mesial margins are more removed from the line. Angle 135-150 degrees.
3) There is a distinct distance between the line and the mesial margins. Angle < 135 degrees.
Following Scott and Irish (2017)
Note: Grade of 1 or greater counts as presence in the rASUDAS application.</td></tr>
<tr><td colspan="5">The rASUDAS criteria uses angles such as >180, 160-180, 135-150 and <135. When scoring winging both incisors needed to be present. In certain instances where it was not clear whether the score is a 1 or a 2, a protractor was used to measure the angle. Figure 5.1.1 (Scott and Irish, 2017) shows different degrees of winging of the central upper incisors.</td></tr>
</table>

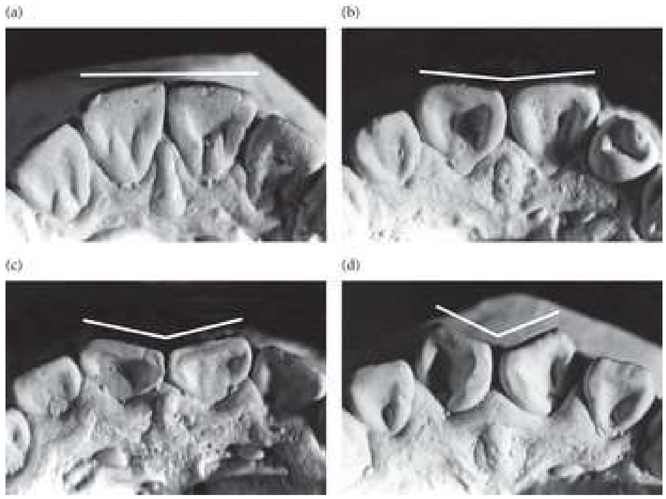


Figure A1: UM1-W - Degrees of winging (Scott and Irish, 2017)

Shovelling (UI1-S)	UI1	Central Upper Incisor	Crown	The presence of lingual marginal ridges. 0) None. Lingual surface is essentially flat. Rare to find a complete lack of marginal ridges. 1) Trace. Marginal ridges can be discerned, but expression is slight, with mesial marginal ridge not extending to the basal eminence. 2) Low moderate. Ridges more pronounced, with mesial marginal ridge extending further down on basal eminence. 3) High moderate. Ridges more pronounced, almost coalescing at basal eminence. 4) Low pronounced. Well-developed ridges that converge at the basal eminence. 5) Medium pronounced. More

<table>
<tr><td></td><td></td><td></td><td></td><td>pronounced ridges that meeting at basal eminence.
6) High pronounced. Pronounced ridges that meet a basal eminence, almost folding around on themselves.
7) Extreme pronounced. Any expression that exceeds grade 6 can be placed in grade 7. It is a rare expression. This grade would involve marginal ridges that folded around on themselves, similar to grade 6 on the UI2 shovelling plaque.</td></tr>
<tr><td colspan="5">To assist with consistent scoring figure 5.1.2 (Burnett et al., 2013) was used. Additionally, how far the marginal ridges on both sides posterior side of the crown are formed in relation to the crown length.
1 – ridges are small and close to the occlusal ridge
2 – the ridges do not meet the middle of the crown yet
3 – the ridges end between the middle and ¾ toward the base.
4 – the ridges very close to the base
5 – the ridges are at the base but have not come together or folds around on each other yet
6 – the ridges are touching the base and they tough or fold around on each other.


Figure A2: UI1-S - Degrees of shovelling (Burnett et al., 2013).</td></tr>
</table>

Figure A3: Attrition on upper incisors (Vailati and Belser, 2011)

Figure A4: Dental wear on incisors (Valilati and Belser, 2016)

Interruption grooves (UI2-IG)	UI2	Upper Lateral Incisor	Crown	Distinct grooves that interrupt the normal course of the mesial or distal marginal ridges or even the basal cingulum. 0) Absence of grooves on lingual marginal ridges and basal cingula. 1) [M] Groove on mesiolingual marginal ridge. 2) [D] Groove on distolingual marginal ridge. 3) [MD] Grooves on both mesiolingual and distolingual marginal ridges. 4) [med] Groove on medial aspect of basal cingulum, sometimes extending onto root.

<table>
<tr><td></td><td></td><td></td><td></td><td>*Note: Any presence is counted in rASUDAS – regardless of location. *</td></tr>
<tr><td colspan="5">Figure A5 (Scott and Irish, 2013) serves as an example of what interruption grooves look like. In scoring interruption grooves the rASUDAS criteria was sufficient, clear and made it easy to score consistently.

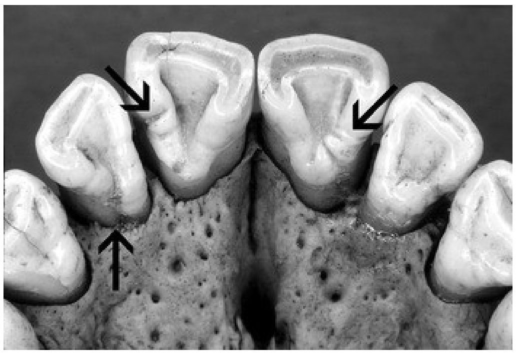



Figure A5: UI2-IG - Interruption grooves (Scott and Irish, 2017).</td></tr>
<tr><td>Hypocone (UM2-H)</td><td>UM2</td><td>Upper Second Molar</td><td>Crown</td><td>The distolingual cusp (cusp 4) on maxillary molars. Plaque #10.
0) No hypocone.
1) Faint ridging present at site.
2) Faint cuspule present.
3) Small cusp present.
4) Moderate-sized cusp present (not shown on plaque - interpolation necessary).
5) Large cusp present.
6) Very large cusp present.</td></tr>
<tr><td colspan="5">The rASUDAS criteria ranges from a score of 0 which is not present, after which subjective descriptions such as small, moderate, large and very large are used. To limit the subjectivity and to stay consistent, the hypocone was scored in relation to the metacone.
It was considered small (3) if the hypocone was less than or equal to half the size of the metacone, moderate (4) if the hypocone was larger than half of the metacone,</td></tr>
</table>

large (5) if it was similar in size to the metacone and very large (6) if the hypocone was larger than the metacone. Figure 5.1.4. (Louail, 2018) was used as a guide.

Figure A6: UM2-H - Degrees of the hypocone (Louail, 2018)

Carabelli's trait (UM1-Car)	UM1	Upper First Molar	Crown	A Cingular derivative expressed on the lingual surface of the protocone (mesiolingual cusp of upper molars). 0) Mesiolingual cusp does not exhibit any grooves or pits on the lingual surface. 1) A vertical groove separates the protocone from the mesial marginal ridge complex; grade 1 expression occurs when there is a slight eminence that deflects distally from this groove. 2) When expression goes beyond a slight groove or eminence and takes the form of a pit. 3) Expression is still slight but takes on a more distinct form than shown by grades 1 and 2. 4) The most pronounced Carabelli's trait that does not involve a tubercle with a free apex; grade 4 takes the classic

				bird-wing form. 5) Small tubercle with a free apex. 6) Moderate tubercle with a free apex. 7) Pronounced tubercle with a free apex.

Figure A7(Sujitha *et al.*, 2011) was used to assist with scoring of Carabelli's trait, in addition to the rASUDAS criteria which was very specific.

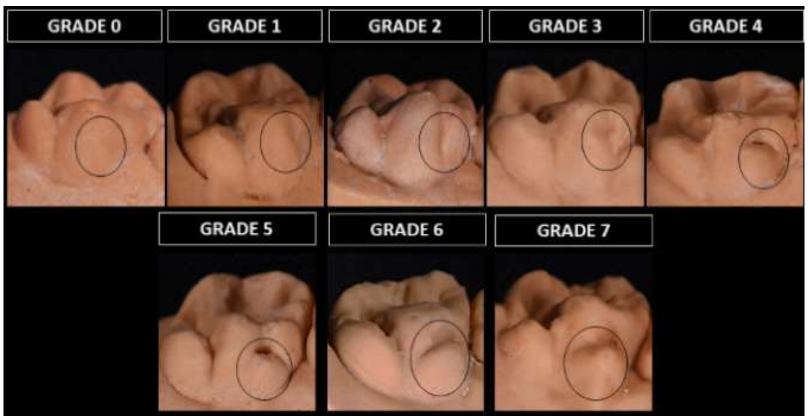


Figure A7: UM1-Car - Degrees of Carabelli's cusp (Suijtha et al., 2021)

Cusp 5 (Metaconule) (UM1-C5)	UM1	Upper First Molar	Crown	Also called the **Metaconule. A fifth cusp that occasionally may be present in the distal fovea of the upper molars between the metacone and hypocone (distobuccal and distolingual cusps). This cuspule is much smaller than the other cusps of the upper molars. 0) Site of cusp 5 is smooth, there being only a single distal groove present separating cusps 3 and 4. 1) Faint cuspule is present. 2) Trace cuspule is present. 3) Small cuspule present.

<table>
<tr><td></td><td></td><td></td><td></td><td>4) Small cusp present.
5) Medium-sized cusp present.</td></tr>
<tr><td colspan="5">To assist with consistency during scoring, some extra methods were employed: To distinguish between a 1 and 2 was to identify if there was a separating groove or a little cusp that could be felt with the fingernail. To distinguish between 3-5 which is described as small cuspule, small cusp and medium, not only was the height or point of the cusp considered but the proportion of the tooth surface it took up, as if considering a “slice of a pie”. E.g. a very sharp angled triangle was considered a 3, an angle that is not sharp, and not square but in between was considered a 4, and the cusps that almost resembled a right angle (square angle) was considered a 5. Figures A8 was used to assist with scoring of Cusp 5.


Figure A8: UM1-C5 - Degrees of cusp 5 (Scott and Irish, 2017)</td></tr>
<tr><td>Enamel extensions (UM1-EE)</td><td>UM1</td><td>Upper First Molar</td><td>Crown</td><td>Enamel extends beyond the cementum-enamel junction towards the apex of the roots – typically in the bifurcation of the two buccal roots of either the upper or lower molars.

0) Cervical enamel is straight.
1) Enamel line extends ~1 mm toward root apex.
2) Enamel line extends ~2 mm toward root apex.
3) Enamel line extends 4 mm or more toward root apex.</td></tr>
<tr><td colspan="5">Example:</td></tr>
</table>

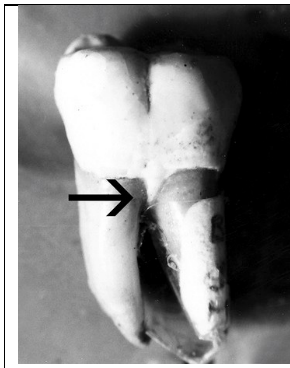


Figure A9: Example of enamel extensions (Scott and Irish, 2017)

Multiple lingual cusps (LP2-MC)	LP2	Lower Second Premolar	Crown	Lingual cusp variation of the lower premolars. 0) Lingual cusp has no free apex. 1) Single lingual cusp (Grades 0 - 1). 2) Two lingual cusps (Grades 2 - 7). 3) Three lingual cusps (Grades 8 - 9). Following Scott and Irish (2017), simplification of Turner *et al*. (1991)
Figure A10 (Scott *et al*., 2018) was used to assist with scoring.				

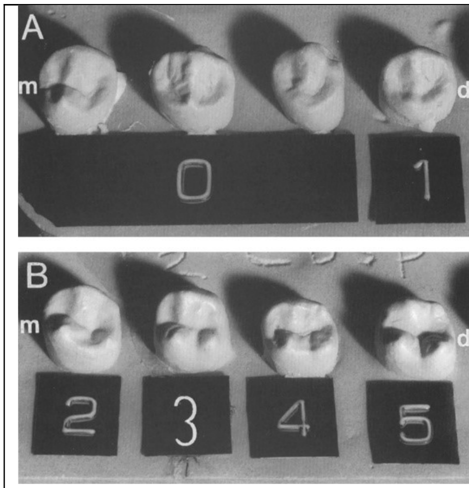
<table>
<tr><td colspan="5">

Figure A10: LP2-MC - Degrees of multiple lingual cusps (Scott et al., 2018)</td></tr>
<tr><td>Groove pattern (LM2-GP)</td><td>LM2</td><td>Lower Second Molar</td><td>Crown</td><td>Pattern of contact of major cusps of lower molars.

1) [X pattern] Contact between cusps 2 and 3 (mesiolingual and distobuccal).
2) [Y pattern] Contact between cusps 1 and 4 (mesiobuccal and distolingual).
3) [+ pattern] Contact between cusps 1, 2, 3, and 4 at central sulcus (all four cusps touch).</td></tr>
<tr><td colspan="5">A score of 0 was not possible with the groove pattern trait. Figure A11 (Scott and Irish, 2017) depicts examples of the various patterns; however, this trait was only scored on the lower second molar.</td></tr>
</table>

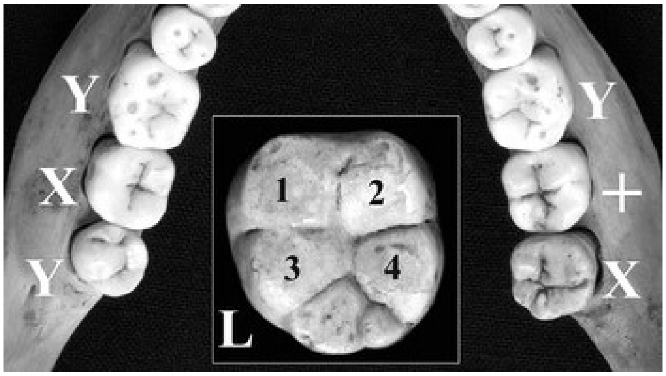


Figure A11: Examples of X, Y and + groove patterns on the molars (Scott and Irish, 2017).

4-cusped (Cusp number) (LM2-4C)	LM2	Lower Second Molar	Crown	This is also the scoring of Cusp 5 or Hypoconulid. Lower first molars generally have five cusps (though not always), there is much more geographic variation in this trait on the lower second and third molars. Both teeth are Graded the same way. Sometimes a molar can have 6 cusps – two in the area of cusp 5. If you see this, then you definitely have a cusp 5, the second, lingual cusp, is a cusp 6. Grade the cusp 5 according to the table below, still looking at its size relative to the other cusps. 0) No occurrence of cusp 5. The molar has only 4 cusps. 1) Cusp 5 is present and very small. 2) Cusp 5 is small. 3) Cusp 5 is medium-sized.

				4) Cusp 5 is large. 5) Cusp 5 is very large. **Note: For rASUDAS any presence of cusp five should be marked as a five-cusped molar. Absence of cusp 5 is a four-cusped molar.**

. Once again it was in the interest of consistency to look at its size relative to the other cusps. Figure A12. (Davies, *et al*., 2021) illustrates where one will find cusp 5 and cusp 6. Since the scoring for this trait used descriptions “very small” to “very large”, the following guidelines were used to improve consistent and accurate scoring:

Very small: - about 1mm (visible from mandibular ramus) – sometimes measured with a small ruler to distinguish between 1 and 2.

Small – about 1-2mm.

Medium – relatively smaller than the entoconid and hypoconid (about ½).

Large – almost the size of the entoconid and hypoconid (more than ½).

Very large – the same size as the entoconid and hypoconid.

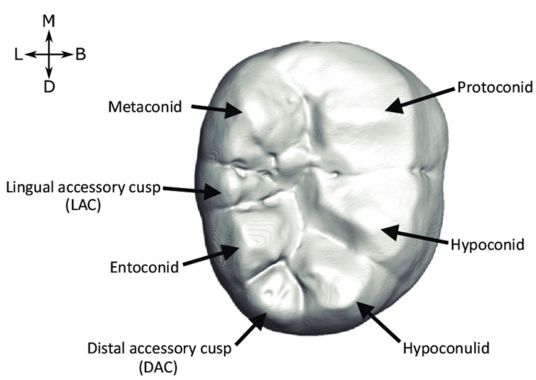


Figure A12.: position of cusps on a molar (Davies, et al., 2021)

Cusp 6 (Entoconulid) (LM1-C6)	LM1	Lower First Molar	Crown	Cusp 6 or **tuberculum sextum* is only present if there is also a cusp 5. It is expressed on the distal portion of the lower molars – cusp 6 is distal to cusp

				5 (it is more associated with the entoconid (distolingual cusp). * 0) Absence of cusp 6. 1) Cusp 5 is more than twice the size of cusp 6. 2) Cusp 5 is about twice as large as cusp 6. 3) Cusps 5 and 6 are about equal in size. 4) Cusp 6 is slightly larger than cusp 5. 5) Cusp 6 is markedly larger than cusp 5.
Cusp 6 is depicted in Figure A12. as the distal accessory cusp (DAC For scoring of cusp 6 the rASUDAS criteria was simple to use and sufficient on its own. It contained descriptions that scored cusp 6 relative to cusp 5. Similar to the approach taken with cusp 5 on the first upper molar, the height of the cusp was looked at, but in cases where there was not a distinctive tip the proportion that the cusp had taken up has been used. (pie slice trick).				
Cusp 7 (Metaconulid) (LM1-C7)	LM1	Lower First Molar	Crown	Cusp 7 occurs in the lingual groove between cusps 2 and 4 (the 2 lingual cusps). It has no relation to cusps 5 or 6. 0) No occurrence of cusp 7. 1) Faint cusp is present. 1A) Faint tipless cusp occurs displaced as a bulge on the lingual surface of cusp 2 (the mesiolingual cusp). 2) Cusp 7 is small. 3) Cusp 7 is medium sized. 4) Cusp 7 is large.
When scoring this trait, the rASUDAS traits used descriptions small to large for scored 2-4. In addition to using figure 5.1.12. (Scott and Irish, 2017) as a guideline,				

a small modification has been made to increase repeatability. The cusp width (mesial to distal) was measured from the lingual surface with a small ruler, the following criteria were implemented:

2 – small (less than 2mm)

3 – medium (about 2mm)

4 – Large (more than 2mm)

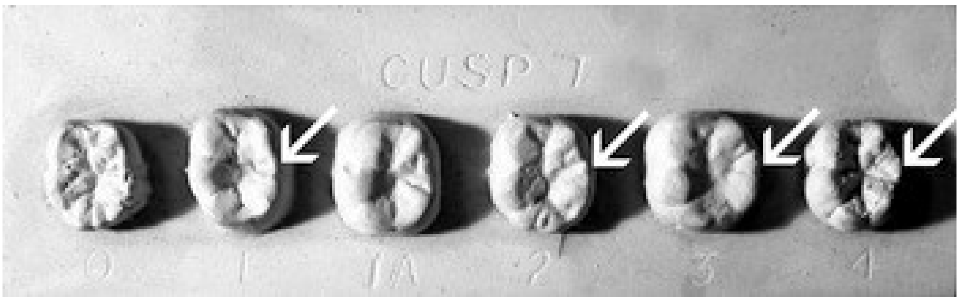
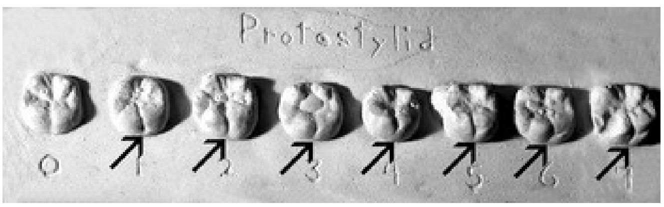


Figure A13: LM1-C7 - Degrees of cusp 7 (Scott and Irish, 2017).

Protostylid (LM1-P)	LM1	Lower First Molar	Crown	An extra cusp found on the buccal surface of cusp 1. It is normally associated with the buccal groove separating cusps 1 and 3 (the two buccal cusps). 0) No expression of any sort. Buccal surface is smooth. 1) A pit occurs in the buccal groove. 2) Buccal groove is curved distally. 3) A faint secondary groove extends mesially from the buccal groove. 4) Secondary groove is slightly more pronounced. 5) Secondary groove is stronger and can be easily seen.

<table>
<tr><td></td><td></td><td></td><td></td><td>6) Secondary groove extends across most of the buccal surface of cusp 1. This is considered a weak or small cusp.
7) A cusp with a free apex occurs.</td></tr>
<tr><td colspan="5">The method used to distinguish between 0-2 was that 0 was a groove with the absence of a pit, a one was a groove with the presence of a pit, which in many cases was a small little hole, then a score of 2 was given when the groove started curving distally. Figure 5.1.13. (Scott and Irish, 2017) was used as a guide for scoring.



FigureA14: LM1-P - Degrees of the protostylid (Scott and Irish, 2017).</td></tr>
<tr><td>Deflecting wrinkle (LM1-DW)</td><td>LM1</td><td>Lower First Molar</td><td>Crown</td><td>This trait occurs on the median occlusal ridge of the mesiolingual cusp (cusp 2). Cusp 4 is the distolingual cusp. No dentine involvement in trait, difficult to Grade on teeth with wear.

0) Deflecting wrinkle is absent – essential ridge of metaconid runs a straight course from cusp tip to central occlusal fossa.
1) Cusp 2 essential ridge is straight but shows a midpoint constriction.</td></tr>
</table>

				2) Essential ridge deflects at halfway point toward central occlusal fossa but does not contact hypoconid (cusp 4). 3) Essential ridge shows strong deflection at midpoint and does contact hypoconid (cusp 4).

Some confusion is also present when cusp 7 is present on the lower first molar, this is because its grooves separating it from the other cusps can be confused with the wrinkle. Thus, when scoring the deflecting wrinkle, one must be certain that cusp 7 is not involved. Figure 5.1.14. (Haddow, 2012) was used as a guide during scoring of this trait.

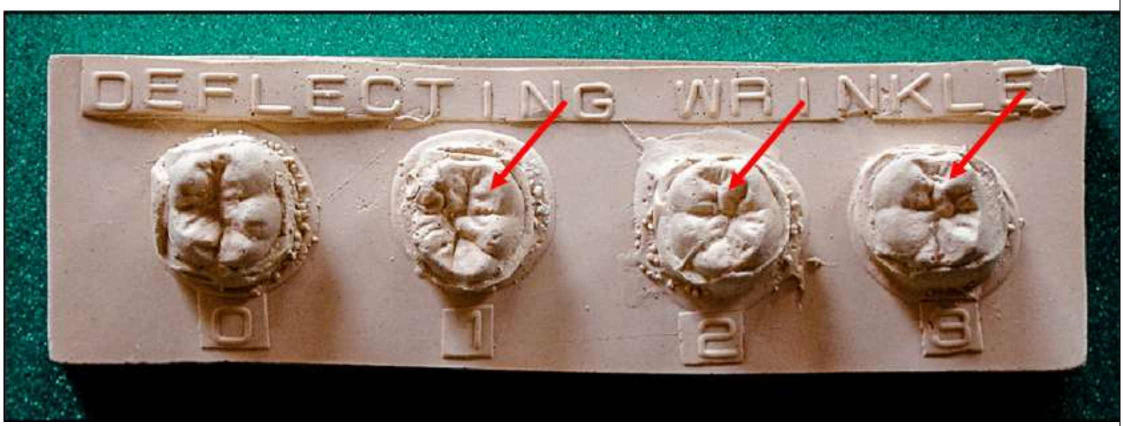


Figure A15.: LM1-DW - Degrees of the deflecting wrinkle (Haddow, 2012)

Pegged-reduced-missing (PRM)	UM3	Upper Third Molar	Root	A reduced in size to missing third molar. 0) Third molar present and normal. 1) Third molar significantly reduced in size (½ normal size with two or more cusps). 2) Third molar peg-shaped (only a single cusp evident). 3) Third molar congenitally absent.

A reduced in size to missing third molar. Figures A16, A17 and A18 (Scott and Irish, 2017) show examples of what this looks like for each.

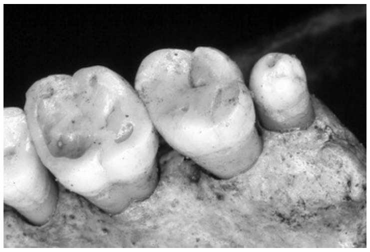


Figure A16: Pegged molar (Scott and Irish, 2017)

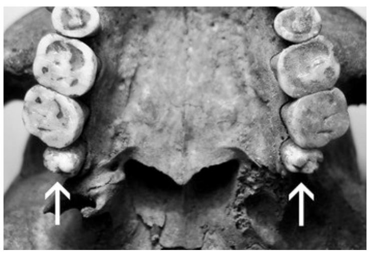


Figure A17: Reduced molar (Scott and Irish, 2017)

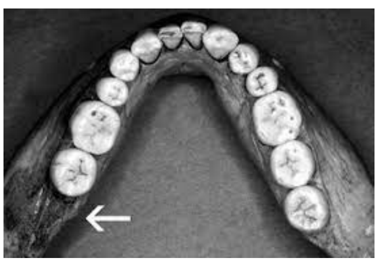


Figure A18. Missing molar (Scott and Irish, 2017)

APPENDIX B: ETHICS

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

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. 0990-0279 Approved for use through June 30, 2025 and Expires 07/28/2026.

Faculty of Health Sciences **Research Ethics Committee**

13 September 2023

Approval Certificate
New Application

Dear Miss C Brooks

Ethics Reference No.: 502/2023
Title: Estimating population affinity using dental morphoscopic traits in a South African sample

The **New Application** as supported by documents received between 2023-08-28 and 2023-09-13 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-09-13 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-09-13.
- Please remember to use your protocol number (502/2023) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
***Deputy Chairperson** of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Figure B1. Ethical Approval Certificate 1

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

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.
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Faculty of Health Sciences **Research Ethics Committee**

14 August 2024

Approval Certificate
Annual Renewal

Dear Miss C Brooks,

Ethics Reference No.: 502/2023 – Line 1
Title: Estimating population affinity using dental morphoscopic traits in a South African sample

The **Annual Renewal** as supported by documents received between 2024-07-03 and 2024-08-13 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2024-08-13 as resolved by its quorate meeting.

Please note the following about your ethics approval:
- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2025-08-14.
- The Research Ethics Committee (REC) must monitor your research continuously. To this end, you must submit as may be applicable for your kind of research:
  a) annual reports;
  b) reports requested *ad hoc* by the REC;
  c) all visitation and audit reports by a regulatory body (e.g. the HPCSA, FDA, SAHPRA) within 10 days of receiving one;
  d) all routine monitoring reports compiled by the Clinical Research Associate or Site Manager within 10 days of receiving one.
- The REC may select your research study for an audit or a site visitation by the REC.
- The REC may require that you make amendments and take corrective actions.
- The REC may suspend or withdraw approval.
- Please remember to use your protocol number (502/2023) on any documents or correspondence with the Research Ethics Committee regarding your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Figure B2. Ethical Approval Certificate Annual Renewal

APPENDIX C: PHOTOS FROM SAMPLE

Examples of traits seen in the sample.

Winging (UI1-W):

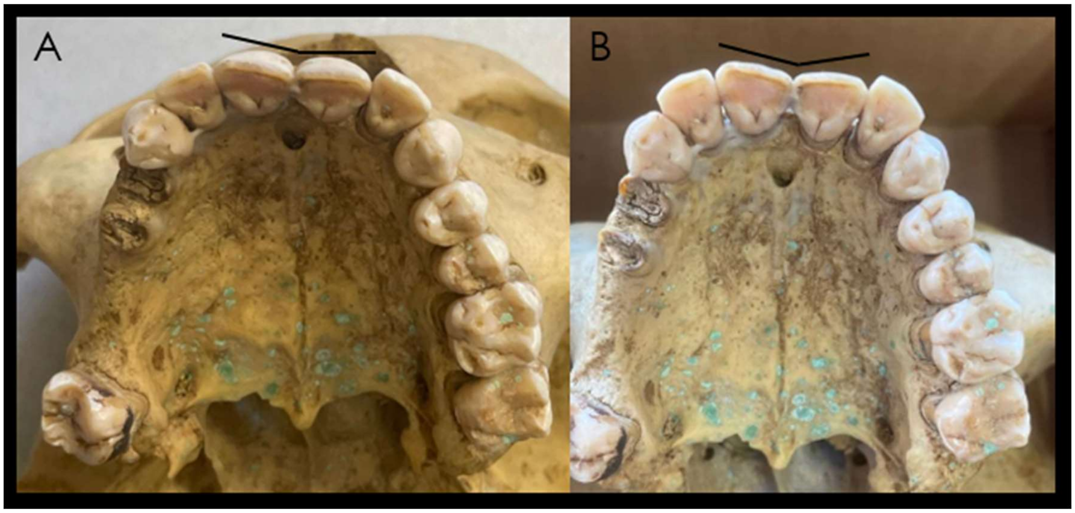

Figure C1. Winging of the central upper incisors. (A) Score 1: Mesial margins of the upper incisors fall slightly below the line. Angle 160-180 degrees. (B) Score 2: Mesial margins are more removed from the line. Angle 135-150 degrees.

Carabell's trait (UM1-Car):

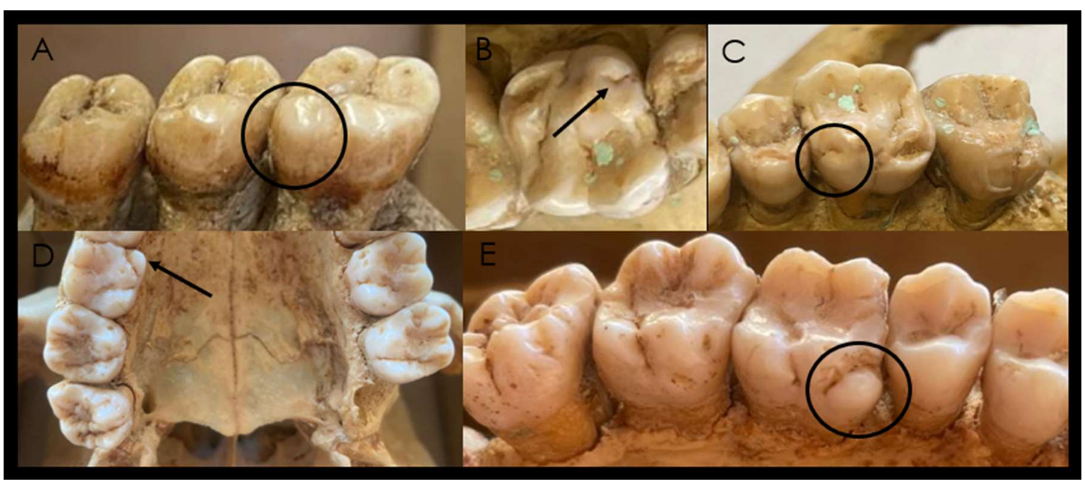

Figure C2. Carabelli's trait on the upper first molar. (A) Score 0: Mesiolingual cusp does not exhibit any grooves or pits on the lingual surface. (B and C) Same individual – Score 4: The most pronounced Carabelli's trait that does not involve a tubercle with a free apex; grade 4 takes the classic bird-wing form. (D and E) Same individual – Score 7: Pronounced tubercle with a free apex. Variation in trait seen between left and right.

Cusp 5 (UM1-C5):

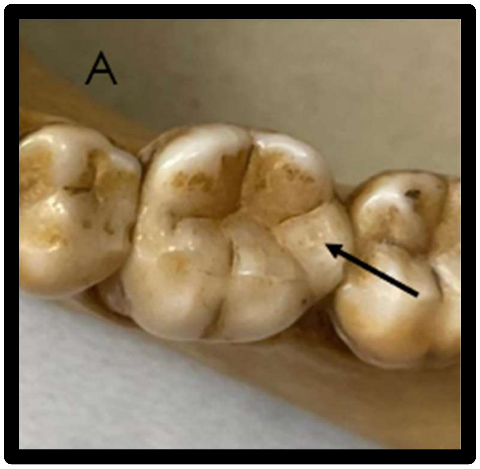

Figure C3. Cusp 5 on the upper first molar (A) Highest expression of the fifth cusp on the upper first molar. Score 5: Medium-sized cusp present. (Arrow indicates fifth cusp)

Cusp 7 (LM1-C7):

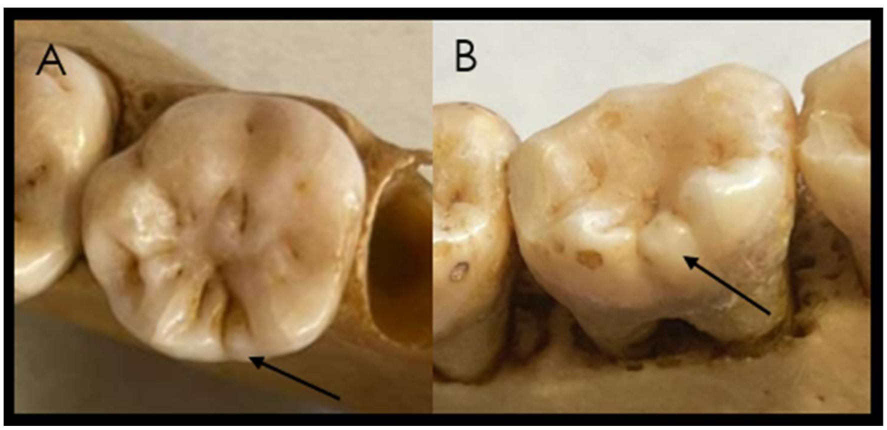

Figure C4. Cusp 7 on the lower first molar. (A) Score 2: Cusp 7 is small (Arrow indicates cusp 7). (B) Score 4: Cusp 7 is large (Arrow indicates cusp 7).

Enamel extensions (UM1-EE):

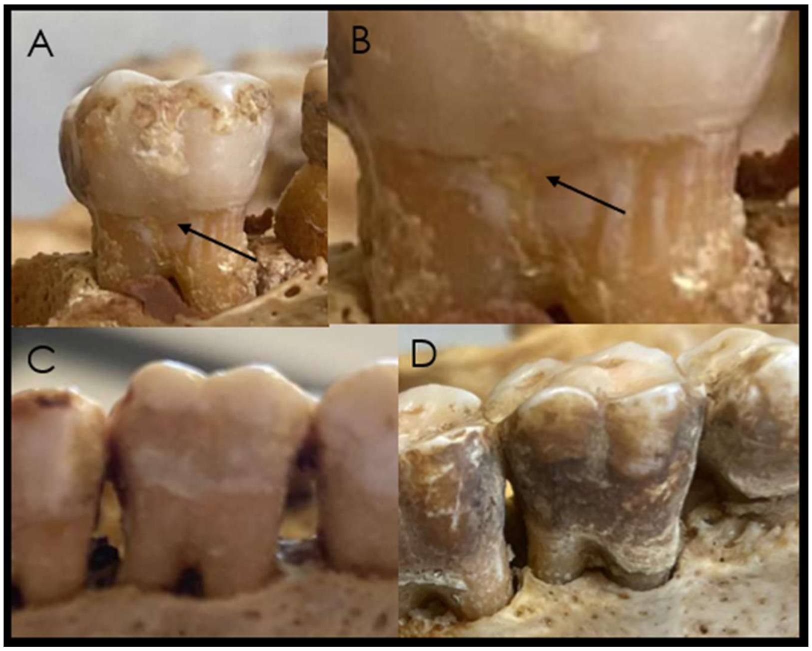

Figure C5. Enamel extensions on the upper first molar. (A and B) Same individual – Score 1: Enamel line extends ~1 mm toward root apex (Line indicates enamel line). (C) Score 0: Cervical enamel is straight. (D) Enamel unobservable.

Hypocone (UM2-H):

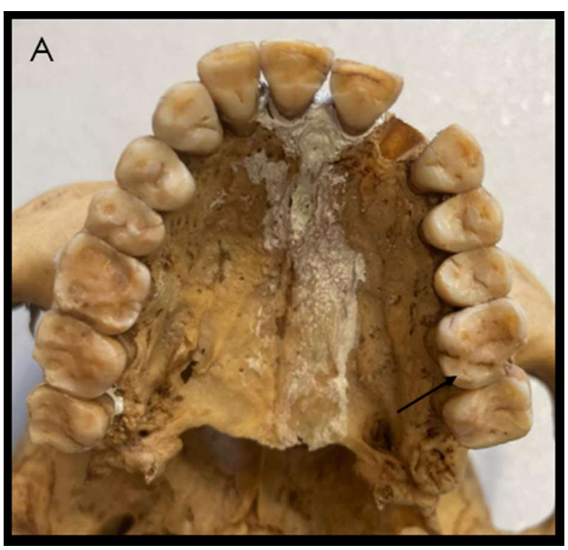

Figure C6. Hypocone on the upper first molar. (A) Score 6: Very large cusp present. (Arrow indicates hypocone/distolingual cusp).

Protostylid (UM1-P):

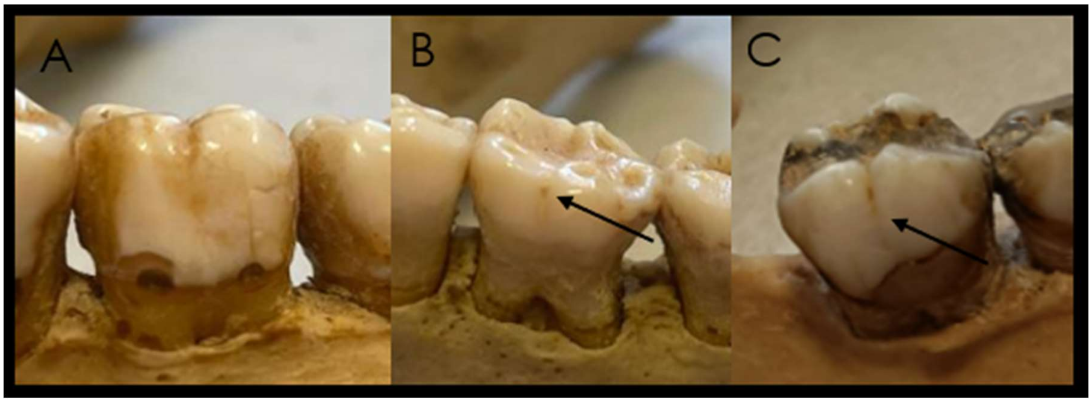

Figure C7. Protostylid on the upper first molar. (A) Score 0: No expression of any sort. Buccal surface is smooth. (B) Score 1: A pit occurs in the buccal groove (arrow indicates pit). (C) Score 3: A faint secondary groove extends mesially from the buccal groove (arrow indicates pit and faint groove).

Deflecting wrinkle (LM1-DW):

Figure C8. Deflecting wrinkle on the lower first molar. (A) Score 0: Deflecting wrinkle is absent – essential ridge of metaconid runs a straight course from cusp tip to central occlusal fossa. (B) Score 1: Cusp 2 essential ridge is straight but shows a midpoint constriction. (C) Score 2: Essential ridge deflects at halfway point toward central occlusal fossa but does not contact hypoconid (cusp 4). (D) Score 3: Essential ridge shows strong deflection at midpoint and does contact hypoconid (cusp 4).

4 cusped (LM2-4C):

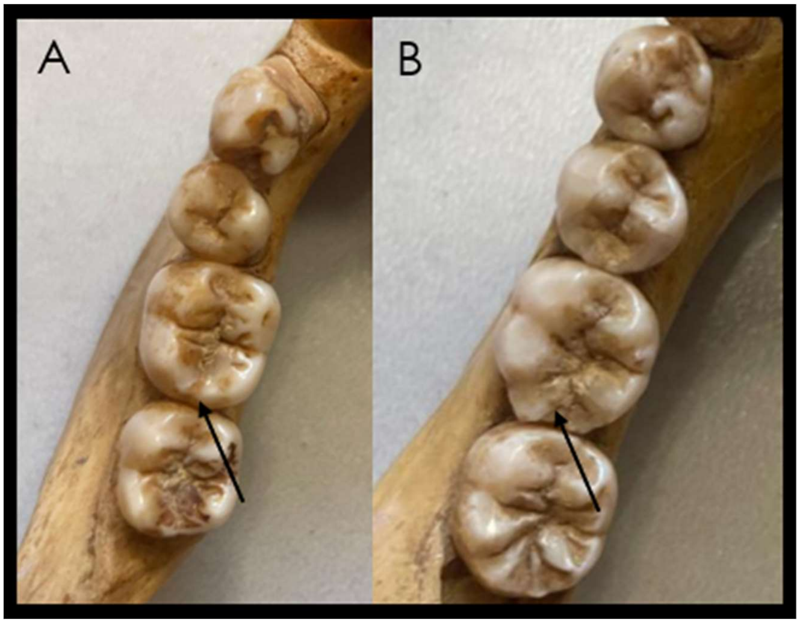

Figure C9. 4-cusped lower second molar (Cusp number). (A) Score 0: No occurrence of cusp 5. The molar only has 4 cusps. Score 3: Cusp 5 is medium-sized – highest expression.

Multiple lingual cusps (LP2-MC):

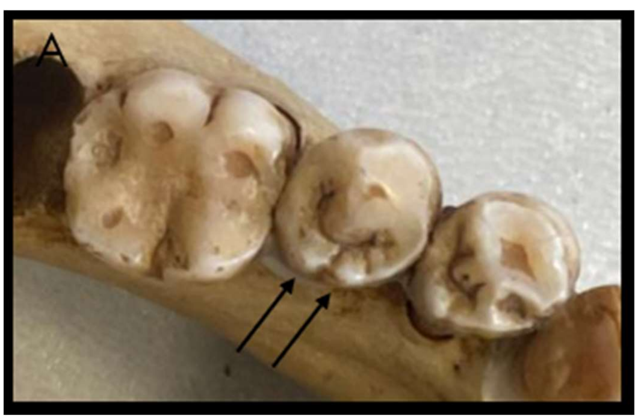

Figure C10. Multiple lingual cusps on the lower second premolar. (A) Score 2: Two lingual cusps (arrows indicate individual cusps).

Third molar:

Figure C11. Lower third molar unerupted but visible. Not scored.

Groove pattern (LM2-GP):

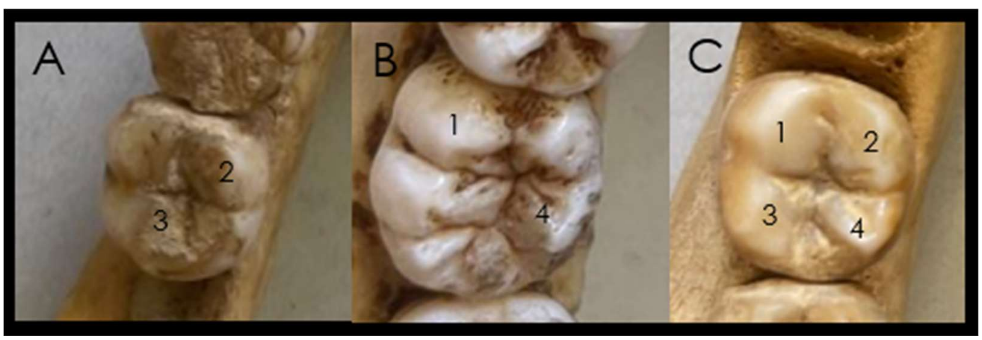

Figure C12. Groove pattern on the lower second molar. (A) Score 1: [X pattern] Contact between cusps 2 and 3 (mesiolingual and distobuccal). (B) Score 2: [Y pattern] Contact between cusps 1 and 4 (mesiobuccal and distolingual). (C) Score 3: [+ pattern] Contact between cusps 1, 2, 3, and 4 at central sulcus (all four cusps touch).

Cusp 6 (LM1-C6):

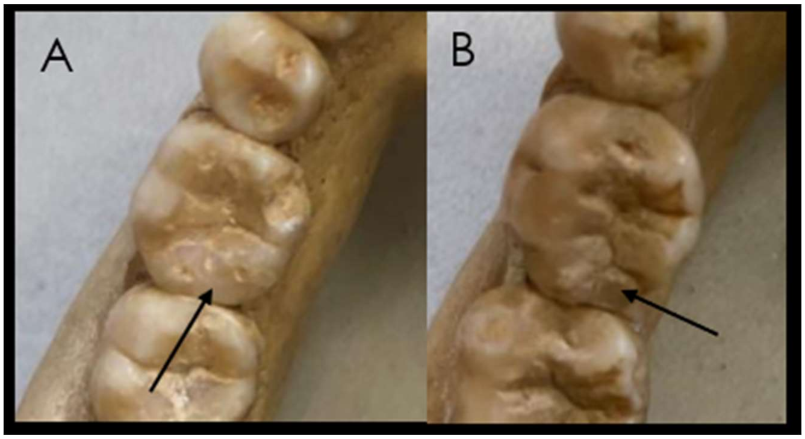

Figure C13: Cusp 6 on the lower first molar. (A) Score 0: Absence of cusp 6. (B) Score 1: Cusp 5 is more than twice the size of cusp 6.