Assessing the sexual dimorphism of the upper limb in a Free State skeletal population using univariate measurements.

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Abstract

Increasing numbers of incomplete unidentified skeletal remains requiring anthropological analysis have created the need for studies of bones other than the pelvis and cranium for sex estimation purposes. This study evaluated the use of 20 measurements of the radius, ulna, humerus, scapula and clavicle of 30 males and 30 females from a Free State skeletal collection. Measurements were assessed for side differences and sexual dimorphism. Sectioning points were calculated for each measurement and used to classify the sex of each individual with the accuracies of classifications also being calculated and compared to those obtained using existing classification standards.

All measurements showed a significant positive correlation to each other, ranging from moderate (R=0.6) to very strong (R=0.99), reflecting the proportional biological relationship between body dimensions. Males were larger than females for all measurements ($p<0.001$), despite large overlaps in the ranges, reflecting the genetic and adaptive differences between the sexes. The largest proportional difference between the means of the males and females was found in the sagittal diameter at the midshaft and the acromial epiphyseal width of the clavicle, as well as in the anterior-posterior diameter at the midshaft of the radius, while the smallest was found in the maximum lengths of the radius, ulna, humerus and clavicle. Classification of sex based on the Free State derived sectioning points of each measurement was most accurate for measurements taken at joints or muscle attachment sites, such as the vertical head diameter of the humerus and the sagittal diameter at the midshaft of the clavicle with accuracies of more than 90%. The least accurate measurement for classification of sex was the acromial epiphyseal width (71.67%), which may have been as a result of the different type and rates of ossification of the clavicle affecting the variability of its morphology beyond sexual differences. For most measurements, the Free State-derived sectioning points performed better than those of the existing sectioning points and the existing classification standards, suggesting that regionally specific standards for classification may be useful.

These results indicate that there is sufficient dimorphism in the upper limb bones to allow for accurate classification of sex in this Free State skeletal sample, but that differences in expression of dimorphism may exist between regional South African populations and may need to be considered when deciding which measurements to use for sex estimation. Further

studies on the need for such regionally specific standards would be useful in the South African context.

Keywords: skeletal; sex estimation; sexual dimorphism; univariate measurements; upper limb bones; Free State, South Africa

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Chapter 1: Introduction

In South Africa, as in many other parts of the world, there has been a marked increase in the levels of violent crimes and in the resultant number of unidentified remains discovered that require forensic analysis (Steyn *et al.*, 1997; Steyn & İşcan, 1999). This increase can most likely be ascribed to socio-economic factors such as amplified urbanisation, poverty, poor education, diseases, excessive drug or alcohol use, unemployment, past and present political conflicts, as well as an influx of people (immigrants) from other countries, both legally and illegally (Norman *et al.*, 2007; L'Abbé & Steyn, 2012; Steyn *et al.*, 2016). Forensic pathologists who have to examine these unidentified remains often encounter circumstances in which the standard avenues for identification (e.g. fingerprints, DNA, and even antemortem dental records) are of little or no use, due to poor recovery or damage to the bones and/or due to the unavailability of antemortem records (Barrier & L'Abbé, 2008; L'Abbé & Steyn, 2012; Kranioti, 2019). Furthermore, DNA in the bones undergo degradation due to exposure, even if the DNA was intact with unknown individuals there may not be any familial comparative samples available (Barrier & L'Abbé, 2008; L'Abbé & Steyn, 2012; Kranioti, 2019).

In cases where the remains are badly decomposed or already skeletonised, the task then falls to forensic anthropologists to reconstruct a biological profile of the remains (Kranioti, 2019). A great deal of information can be deduced from the skeletal remains both at the population and at the individual level (White & Folkens, 2005; Moore, 2012). At the population level, bones can provide information on the geographic or historic origins and the biological diversity of the group to which the individual belonged (White & Folkens, 2005; Ruff *et al.*, 2006; Okai, 2010; Moore, 2012). At the individual level, the bones reflect the basic biological characteristics of the individual such as age, sex, stature and ancestry (White & Folkens, 2005; Ruff *et al.*, 2006; Moore, 2012). It also documents how the individual may have fitted into their society or have experienced life, via social status, occupation, diet, disease, past injuries and the like (Okai, 2010; Moore, 2012; Manifold, 2015). It is thus important for the anthropologist working with the remains to have access to methods to construct an accurate biological profile, consisting of the estimated sex, age and ancestry of the individual being examined (Pietrusewsky, 2008; Bidmos *et al.*, 2010), and which can subsequently be used by law enforcement agencies to compare to missing person profiles in their attempts to identify the skeletal remains (Scheuer, 2002; Peckmann *et al.*, 2017).

Of the key elements in the biological profile, sex estimation is usually performed first, as it reduces the number of possible matches to missing persons, and because other estimations such as age, stature and ancestry are strongly influenced by whether the individual is male or female (Robinson & Bidmos, 2009; Bidmos *et al.*, 2010; Dabbs & Moore-Jansen, 2010). The accuracy and reliability of the estimation of sex are dependent on the type, nature and maturity of the skeletal elements available, as well as the technique used for the assessment (Buikstra & Ubelaker, 1994). As such it is of utmost importance to select skeletal elements for study that can reasonably be expected to show morphological dimorphism between sexes, but also have sufficient sexual dimorphism to be easily detected and that can provide accurate classification rates (Maass & Friedling, 2019a).

Based on these considerations, the most commonly assessed skeletal elements for the purpose of sex estimation are the pelvis and cranium, which are considered to be the most accurate (Vance *et al.*, 2011; Albanese, 2013). However, these bones are not always available for analysis due to low preservation and/or recovery rates in a forensic or archaeological context (Vance *et al.*, 2011; Spradley *et al.*, 2015). This is mainly because the preservation of the bones may be affected by their density (Waldron, 1987; Spennemann, 1992; Stojanowski *et al.*, 2002). Less dense bones, like the sternum, fibula and clavicle have low preservation rates of only 11–30%, while the radius, ulna and femur have a high density and also higher preservation rates of 58–84% (Waldron, 1987; Spennemann, 1992; Stojanowski *et al.*, 2002). Bones such as the cranium and pelvis have low bone density and as a whole have preservation rates as low as 17% or 39%, while some isolated parts of these bones can have preservation rates of 33% and 27%, respectively (Waldron, 1987; Spennemann, 1992; Stojanowski *et al.*, 2002). Other factors that may affect bone preservation are the size and shape of the bone, as well as external environmental (e.g. burial/disposal) conditions such as the soil type and the presence of water in the direct vicinity of the remains (Spennemann, 1992; Katzenberg & Saunders, 2008). The recovery of the bones may be further affected by damage to the bones due to exposure to the elements or scavenging animals over an extended period, or due to deliberate attempts to prevent identification through, for example, dismemberment or burning (Dabbs & Moore-Jansen, 2010; Spradley & Jantz, 2011). Consequently, the recovery of the smaller bones of the hand and foot is less likely, as they are easily lost and damaged, while larger bones of the arms and legs are more likely to be recovered because of their larger size and density (Waldron, 1987 Spennemann, 1992; Stojanowski *et al.*, 2002).

Due to differing preservation and/or recovery rates of bones, the anthropologist may be restricted to the use of a limited set of measurements or observations of these skeletal elements, or the assessment of other skeletal elements that may be less sexually dimorphic and thus possibly less accurate (Scheuer, 2002; Macaluso, 2011). It is thus necessary for anthropologists to develop methods of sex estimation that could be used on skeletal elements other than the pelvis and cranium, such as the long bones, which may also yield high classification accuracies, but are also more likely to be recovered undamaged in a forensic or archaeological context (Waldron, 1987; Spennemann, 1992; Stojanowski *et al.*, 2002).

Multiple studies have been conducted on the radius, ulna, humerus, scapula, clavicle, or a combination of two or three of these bones (e.g. Spradley & Jantz, 2011; Papaioannou *et al.*, 2012; Devi *et al.*, 2013). These studies found the bones of the upper limb to be highly sexually dimorphic, with classification accuracies of 77–98% when used for sex estimation. These bones also have moderate preservation rates of 30–58%, and while the scapula as a whole has a low preservation rate of only 22%, its glenoid cavity has a high preservation rate of 64% (Waldron, 1987; Stojanowski *et al.*, 2002). This suggests that, in a forensic context, it may be more useful to examine isolated regions of specific bones, as some may have a higher preservation rate than the entire bone and are more likely to be available for analysis (Boldsen *et al.*, 2015; Maass & Friedling, 2018). It is thus necessary that multiple univariate measurements of several bones be examined to determine their usefulness in sex estimation.

1.1. Aim of the study

The aim of the present study is to assess the sexual dimorphism of the bones of the upper limb in a Free State skeletal sample and compare the use of univariate sex estimation methods based on this sample with those of previous South African studies.

The objectives of the study are:

- To perform univariate measurements of the radius, ulna, humerus, clavicle and scapula on cadaveric individuals from a Free State skeletal collection.
- To evaluate the possible correlation between these measurements for the purpose of exploring potential relationships or proportionality of the different aspects of the bones.

- To evaluate the sexual dimorphism of these measurements by comparing the data of males with that of females.
- To use the data of the Free State sample to develop and evaluate the accuracy of regionally specific ranges for the estimation of sex.
- To evaluate the application of existing sex estimation ranges, developed elsewhere in South Africa (Gauteng and Western Cape), on a Free State skeletal sample by comparing classification accuracies of this Free State sample with those reported by the original studies.

Chapter 2: Background

2.1. Sexual dimorphism

Sexual dimorphism refers to the differences in size and shape between males and females of a species and includes differences in the rate and/or timing of development and behaviour, including anatomical, functional and psychological differences (Vance *et al.*, 2011; Moore *et al.*, 2016; Sehrawat, 2018). Sexual dimorphism mostly becomes evident after the onset of puberty, due to an increase in the production of sex hormones that control and regulate the development of secondary sexual characteristics (Scheuer & Black, 2000; White & Folkens, 2005; Vance *et al.*, 2011; Moore *et al.*, 2016). Not only does this affect the development of soft tissue but also the development of hard tissue in a systematic and indirect manner (Moore *et al.*, 2010; Berg, 2012; Cabo *et al.*, 2012). Sexual dimorphism of the bones can range from extremely marked to only subtle differences, thus making them potentially useful in the estimation of sex in forensic anthropology (White & Folkens, 2005; Özer *et al.*, 2006; Sehrawat, 2018; Mokoena *et al.*, 2019).

Forensic anthropological studies on the sexual dimorphism of the bones take either a morphological/qualitative or a metric/quantitative approach (Ahmed, 2013). The morphological approach is a quick method that does not require a great deal of expensive equipment and is based on visual assessment of the relative shape of bony features, for example, the olecranon fossa which is triangular in males and oval in females (Rogers, 2009; Vance *et al.*, 2011). This is combined with the relative size and robustness of the bones, with males expected to be larger and females expected to be smaller (Kranioti & Michalodimitrakis, 2009; Scholtz *et al.*, 2010). This approach, however, is a subjective method, and may be susceptible to both inter- and intra-observer errors, resulting in challenges in standardisation and statistical analysis (Frutos, 2002; Christensen & Crowder, 2009). Furthermore, morphological parameters may vary between and within populations and may be influenced by factors such as age, biological affinity and musculature (Spradley & Jantz, 2011; Steyn & İşcan, 2013; Sehrawat, 2018). Thus, differences in the range of variation may also affect standardisation and the statistical analysis of this methodology (Frutos, 2002; Krüger *et al.*, 2017). Recently more statistically advanced techniques such as geometric morphometrics and Fourier analysis allowed for the analysis of shape variance in quantitative form. These methods allowed shape and size to be analysed separately, and thus

morphological differences between the sexes that were previously too difficult or complex to quantify could now be described both reliability and objectively (Maass & Friedling, 2019a). However, in spite of the great potential of these new methods and the growth of the studies in the literature making use thereof, there is still a lack of standardisation procedures for these methods when applied to postcrania (Dayal et al, 2008; Maass & Friedling, 2018). Thus, traditional methods are still mostly preferred due to the costs of the specialised equipment needed and the complexity of the statistical analyses used for the more advanced analyses (Dayal *et al*, 2008; Maass & Friedling, 2018).

The metric approach, unlike the morphological approach, is often more time-consuming and may require expensive equipment. The metric approach uses precise measurements of the bones for sex estimation, with males usually being absolutely larger than females within a population (Scheuer, 2002; Scholtz *et al.*, 2010; Hudson *et al.*, 2016). Such measurements allow the description of bones or bony features to be more objective, with good repeatability and often also higher levels of accuracy (Bidmos *et al.,* 2010). This approach is thus considered more appropriate for forensic anthropological analysis, as the levels of accepted accuracy in a forensic context is expected to be higher than those deemed acceptable in general biological anthropological contexts (Scheuer, 2002; Peckmann *et al.*, 2016 & 2017; Maass & Friedling, 2018). Lastly, with this approach, a combination of measurements can be selected to maximise accuracy through multivariate analysis, which allows robust statistical evaluations of within- and between-group differences (Coward & McConathy, 1996). However, if multivariate analysis is not possible, univariate analysis is still useful as it can be used on fragmented or damaged elements and often has a reasonable accuracy (Albanese *et al.*, 2005; Milner & Boldsen, 2012; Boldsen *et al.*, 2015).

The degree of expression of sexual dimorphism in bones may be influenced by genetic (intrinsic) factors such as genetics and hormones, as well as epigenetic (extrinsic) factors such as nutrition, health status, physical activity, socioeconomic status and the environment (Lesk, 2012; Torimitsu *et al.*, 2016; Scott *et al.*, 2018). Genetic (intrinsic) factors determine the growth potential of an individual, with males expected to be generally larger, heavier and more robust than females within a population due to the longer adolescent growth period of males (Purkait & Chandra, 2004; White & Folkens, 2005). Additionally, the growth potential of the individual may be positively or negatively affected by the amount of sex-specific

hormones, testosterone and oestrogen, which primarily affect the critical growth phase of individuals within a population (Tanner, 1978; McGraw *et al.*, 2009; Moore *et al.*, 2016).

While genetic (intrinsic) factors determine the potential of growth and expression of sex-specific skeletal features of an individual, the extent of the potential growth achieved is influenced by the mediating role of the epigenetic (extrinsic) factors (Hawley *et al.*, 2009). Socio-economic status may influence the degree of expression of sexual dimorphism as it impacts on the availability and quality of nutrition, and access to basic health care available to individuals (Steyn & İşcan, 1999; Puoane *et al.*, 2002; Maass & Friedling, 2018). In terms of nutrition, if food is of poor quality or inadequate, it often leads to individuals within a population being smaller and/or less sexually dimorphic (Steyn & İşcan, 1999; Puoane *et al.*, 2002; Spradley *et al.*, 2015). This more commonly affects males, resulting in a decrease in their robusticity, while females are better protected against such stress, likely because of their role in procreation, and generally do not experience such significant changes in robusticity or gracility (Stini, 1969; Jantz & Jantz, 1999). Thus, with males becoming less robust and females not showing significant changes, the population as a whole will experience a decrease in dimorphism. The health of the individual and access to good health care during early development may also affect linear growth (Puoane *et al.*, 2002). This is because linear growth may be negatively affected by low levels of important growth factors and poor responsiveness of growth plates, as a result of exposure to repeated or prolonged illness or disease (Jantz & Jantz, 1999; Scott *et al.*, 2018). Even once growth has been completed, sexual dimorphism of the long bones may be further affected by other epigenetic (extrinsic) factors such as individual occupation, physical activity and, biomechanical stress which all lead to variation in robusticity of the skeletal elements (Ahmed, 2013; Scott *et al.*, 2018; Sehrawat, 2018). This variation is the result of continual differential cortical remodelling in response to different functional demands on the muscles acting on the bone (Ahmed, 2013; Scott *et al.*, 2018; Sehrawat, 2018). Therefore, even though males are usually larger, heavier and more robust than females, there are females who can also be larger, heavier and more robust than expected due to their high activity levels, causing their remains to appear more like those of males.

The balance between genetic (intrinsic) and epigenetic (extrinsic) factors differs both between and within populations, leading to population-specific degrees of expression of sexual dimorphism (Jantz & Jantz, 1999; Charisi *et al.*, 2011; Sehrawat, 2018). It is thus

important to account for such population differences when developing or using metric methods for sex estimation. Scott *et al.* (2018) illustrated this by applying sectioning points developed using measurements of the clavicles of Black American, White American and Greek samples to those of a modern South African Coloured sample. The study reported classification accuracies of below 76% for the South Africans, which is less than the 80% accuracy considered forensically acceptable and may result in a decrease in the accuracy of the biological profile as a whole (Peckmann *et al.*, 2016 & 2017). Spradley & Jantz (2011) and Spradley *et al.* (2015) produced similar results, finding that the accuracy of sex estimation using the humerus was higher in Black Americans than in White Americans and Hispanics. The lower accuracy in the latter two groups may indicate population-specific variation in the genetics, socioeconomic status, access to good nutrition and health care, as well as their geographical locations, climate and migration patterns (Hawley *et al.*, 2009; Sehrawat, 2018). This is because individuals adapt to their climate and geography, with those in colder climates being smaller in stature to preserve heat and those in warmer climates being larger and taller as heat preservation is not as important (Ruff, 1994; White & Folkens, 2005; Wells, 2012). As a result of all these influences, the most accurate bone or measurement for one group may not be as useful for another group. It is thus important in forensic studies that the reference population used to develop standards for sex estimation must resemble, as closely as possible, that of the population to which the method will be applied and that the variation within many different groups or populations be evaluated (Steyn *et al.*, 1997; Steyn & İşcan, 1999).

2.2. Bones of the upper limb

In a forensic context, the most accurate bones for estimating aspects of the biological profile are not always available for analysis, especially in cases where the remains are commingled, damaged or absent. To combat this, anthropological methods and standards of sex estimation using less sexually dimorphic bones, such as the fibula, calcaneus and ulna, must be developed (Spradley *et al.*, 2015). These methods must be population-specific, as well as time- and geographically specific, given the differences within and between populations (Mall *et al.*, 2001; Spradley & Jantz, 2011; Spradley *et al.*, 2015). Preference should be given to bones that are frequently recovered in a forensic context such as the humerus, femur and tibia, but also other bones such as the scapula and patella, in the event that they are the only

bones recovered (Stojanowski *et al.*, 2002; Papaioannou *et al.*, 2012; Spradley *et al.*, 2015). Univariate (single) measurements may be better than multivariate (multiple) measurements in this context, as such measurements can be collected quickly and have been shown to be reasonably accurate indicators of sex (Milner & Boldsen, 2012; Boldsen *et al.*, 2015). This is especially important in cases where the skeletal elements are fragmented and/or incomplete, thus reducing the number of measurable sex-informative features available (Milner & Boldsen, 2012).

Previous metric studies on sex estimation typically use the pelvis and the cranium, as these elements have been shown to be highly sexually dimorphic and are considered the most accurate and reliable (Frutos, 2005; Vance *et al.*, 2011; Albanese, 2013; Spradley *et al.*, 2015). However, postcranial bones such as the femur, tibia, humerus, radius, ulna, clavicle and calcaneal bones can also be used, as it has been shown that these elements may sometimes be more accurate than the cranium (Robinson & Bidmos, 2009; Spradley *et al.*, 2015; Kranioti, 2019). The high level of dimorphism, and the associated estimation accuracy of these bones, has been reported for various global populations (Spradley & Jantz, 2011; Lee *et al.*, 2014; Moore *et al.*, 2016; Tomczyk *et al.*, 2017). The recovery and recovery rates of the bones of the upper limb are further motivation for their study. Bones such as the humerus and radius have relatively high recovery rates of 47–58% and are thus likely to be recovered in a forensic context (Waldron, 1987; Stojanowski *et al.*, 2002). The remaining bones of the upper limb have moderate to high recovery rates ranging from 30–55%, and could still be useful in the forensic context should they be the only bones recovered or undamaged (Waldron, 1987; Stojanowski *et al.*, 2002). Individual parts of bones such as the glenoid cavity of the scapula, which has a high preservation rate of 64%, must also be evaluated as this could be useful for fragile bones - such as the scapula - which have low recovery rates (22%) as a whole (Waldron, 1987; Stojanowski *et al.*, 2002).

2.2.1. Radius

The radius is the shorter of the two forearm bones and is located laterally in anatomical position (Moore *et al.*, 2010). It articulates with the capitulum of the humerus superio-laterally as part of the elbow joint, and with the scaphoid and lunate bones of the hand inferiorly to form the wrist joint (Moore *et al.*, 2010). Superiorly, the head of the radius

articulates with the radial notch of the ulna to form the proximal (superior) radio-ulnar joint, whereas inferiorly the ulna articulates with the ulnar notch of the radius to form the distal (inferior) radio-ulnar joint (Moore *et al.*, 2010). All of these joints are strengthened and supported by several multidirectional ligaments (Moore *et al.*, 2010).

Earlier studies of the radius have shown the maximum length of the radius to be the most accurate univariate measurements for sex estimation in German, Greek, American, Korean, Colombian and Polish populations, with sex estimation accuracies ranging between 80–90% (Mall *et al.*, 2001; Charisi *et al.*, 2011; Spradley & Jantz, 2011; Lee *et al.*, 2014; Moore *et al.*, 2016; Tomczyk *et al.*, 2017; Kranioti, 2019). This high accuracy may be the result of the general difference in body size between the sexes due to the longer growth period of males during puberty, which allows for the development of larger skeletal elements than females (Lesk, 2012; Sadler, 2015). In Spanish and Japanese populations, the radial tuberosity circumference (93% accuracy) and the maximum distal breadth (92% accuracy) were the most accurate univariate measurements, respectively (Safont *et al.*, 2000; Sakaue, 2004). A possible reason for the high accuracy of these measurements in these two populations may be that the articular surfaces (proximally and/or distally) experience a substantial portion of the applied force from the muscles acting across the joint, with this force being greater on average in males, resulting in the joint being larger than those of females (France, 1988; White & Folkens, 2005). It is evident that the functional differences in the anatomy of the radius between the sexes act more strongly on different regions of the bone in the different population groups. This emphasises the need to consider population specificity in the selection of measurements for sex estimation within different populations and later onset of hormonal upregulation (Spradley & Jantz, 2011; Spradley *et al.*, 2015).

For the studies that indicated the least accurate univariate measurement for the populations, the distal width, the sagittal diameter, the transverse diameter and/or the medio-lateral diameter measurements were identified, all with accuracies below 79% (Mall *et al.*, 2001; Spradley & Jantz, 2011; Tomczyk *et al.*, 2017). This may be because areas of muscle insertion are subjected to a greater muscle strain force than the areas of muscle origin, and thus to counteract this force more bone will be added via appositional growth to the affected area (Purkait & Chandra, 2004; Ross & Pawlina, 2011). Males are expected to have larger muscles and larger muscle insertion areas; however, the appositional growth is not always

clearly distinguishable between males and females which in turn make them less accurate in osteometric sex estimation (White & Folkens, 2005; Cabo *et al.*, 2012).

These studies show that, although many of the radial measurements can be accurately used in various groups, the most accurate and least accurate measurements for estimation still vary between groups. This indicates that caution should still be exercised when using measurements from one group to estimate the sex of an individual of another group.

2.2.2. Ulna

The ulna is the medially located and longer of the two forearm bones (Moore *et al.*, 2010). It articulates superio-medially with the trochlea of the humerus to form part of the elbow joint and it articulates inferiorly with an articular disc, as such it does not participate in the formation of the wrist joint (Moore *et al.*, 2010). It also articulates proximally and distally with the radius, as described previously, to form the radio-ulnar joints.

The ulna has been studied in several global populations (e.g. Mall *et al.* 2001, Cowal & Pastor, 2008; Srivastava *et al.*, 2013; Tomczyk *et al.*, 2017). Unlike the radius, however, there is more variation in the most accurate sexually dimorphic measurements for different groups. While accuracies of more than 80% have been reported for the maximum and physiological lengths, and the minimum circumference (Safont *et al.*, 2000; Spradley & Jantz, 2011), other measurements such as the midshaft area, the olecranon width, the radial notch width and the maximum proximal width also produce relatively high accuracies in some populations (Sakaue, 2004; Charisi *et al.*, 2011).

Notably, while maximum length produced higher sex estimation accuracies for the German, American, Indian, Korean and Polish populations, it had an accuracy of only 79% for the Colombian population. Similarly, the minimum circumference, distal articular breadth, proximal width and radial notch width produced lower accuracies of 63–79% for German, Japanese, Indian and Korean populations (Mall *et al.*, 2001; Sakaue, 2004; Srivastava *et al.*, 2013; Lee *et al.*, 2014). This again emphasises the need for the use of population-specific equations for metric sex estimation. The least accurate measurements all showed accuracies below 79% and included measurements such as the distal width, the radial notch height, the

coronoid and olecranon height, the transverse (medio-lateral) and dorso-volar (antero-posterior) diameter (Cowal & Pastor, 2008; Spradley & Jantz, 2011; Tomczyk *et al.*, 2017).

2.2.3. Humerus

The humerus is the largest bone of the upper limb. Its head articulates superiorly with the scapula at the glenohumeral joint, and inferiorly its capitulum and trochlea articulate with the radius and ulna at the elbow joint (Moore *et al.*, 2010). A prominent feature located on the lateral surface of the shaft is the deltoid tuberosity for attachment of the deltoid muscle (Moore *et al.*, 2010).

İşcan *et al.* (1998) studied the humerus of individuals from three Asian populations and found that the most accurate univariate measurements for sex estimation in the Chinese population was the vertical head diameter (81%), while for the Japanese and Thai populations it was the epicondylar breadth (90–93%). In other studies, the vertical head diameter was also found to be the best univariate measurement for German, Greek, Indian, Korean and Colombian populations, with accuracies ranging from 86–92% (Mall *et al.*, 2001; Kranioti & Michalodimitrakis, 2009; Charisi *et al.*, 2011; Devi *et al.*, 2013; Lee *et al.*, 2014; Moore *et al.*, 2016). The maximum head diameter (the diameter between the most superior and inferior point of the articular surface), which is not always defined the same as the vertical head diameter (the diameter of the head in the vertical/ coronal plane), was found to be the best univariate measurement for Guatemalan (Frutos, 2005), Hispanic (Tise *et al.*, 2013) and Mexican populations (Spradley *et al.*, 2015), with accuracies of 96%, 86% and 88%, respectively. Similar to the Japanese and Thai samples of the İşcan *et al.* (1998) study, Spradley & Jantz (2011) found that the epicondylar breadth of the humerus was the most accurate measurement for both White and Black American populations with accuracies of 86% and 87%, respectively. Other studies have shown the minimum circumference of the humerus to be the most accurate for Spanish (93%), the distal articular width for Japanese (95%), the humeral head circumference for Bulgarian (90%), and the transverse head diameter for Polish (85%) population groups (Safont *et al.*, 2000; Sakaue, 2004; Fasova & Timonov, 2017; Tomczyk *et al.*, 2017). Additionally, the maximum length also had a high accuracy rate for the German, Greek, American and Hispanic populations, with accuracies

ranging from 81–85% (Mall *et al.*, 2001; Charisi *et al.*, 2011; Spradley & Jantz, 2011; Tise *et al.*, 2013)

In the İşcan *et al.* (1998) study, the least accurate univariate measurement of the humerus in all three Asian populations was the midshaft circumference with accuracies ranging from 77–88%. Sakaue (2004), however, found that the trochlear width, maximum and proximal lengths were the least accurate for the Japanese population, with accuracies as low as 70%. The maximum midshaft diameter was found to be the least accurate for the Guatemalan, Greek and American populations with accuracies below 80% (Frutos, 2005; Kranioti & Michalodimitrakis, 2009; Spradley & Jantz, 2011). In contrast to some of the earlier studies, Lee *et al.* (2014) found that the epicondylar breadth was the least accurate for a Korean population (75%), Moore *et al.* (2016) found that the maximum length was the least accurate for a Colombian population (77%), while Tomczyk *et al.* (2017) found that the vertical head diameter was the least accurate for a Polish population (79%). Possible reasons for variation within and between populations are the degree of expression of sexual dimorphism, which may be influenced by the variations in both the genetic (intrinsic) and epigenetic (extrinsic) factors of the populations (Lesk, 2012; Torimitsu *et al.*, 2016; Scott *et al.*, 2018).

2.2.4. Clavicle

The clavicle connects the upper limb to the trunk, transferring the weight of the upper limb across the glenohumeral joint to the thorax (Moore *et al.*, 2010; Alcina *et al.*, 2015). The clavicular shaft has a double curvature in the transverse (horizontal) plane, with its medial half being convex anteriorly and its lateral half being concave anteriorly (Moore *et al.*, 2010). The medial end of the clavicle articulates with the manubrium of the sternum to form the sternoclavicular joint, while the lateral end articulates with the acromion process of the scapula to form the acromioclavicular joint (Moore *et al.*, 2010). Both of these joints are strengthened and supported by several multidirectional ligaments (Moore *et al.*, 2010).

The maximum length of the clavicle was the most accurate univariate measurement for sex estimation in various global populations with accuracies of more than 80% (Papaioannou *et al.*, 2012; Lee *et al.*, 2014; Moore *et al.*, 2016, Sehrawat, 2018). Despite also being the most accurate measurement in a Korean population, its accuracy did not exceed 75% (Lee *et al.*, 2014). Tomczyk *et al.* (2017) found that the most accurate measurement in a Polish

population was the antero-posterior diameter of the clavicle, though also yielding an accuracy of only 74%.

There was more variation reported for the least accurate measurements between these populations, with accuracies below 77% for measurements such as the sagittal and vertical diameter, the midshaft circumference, the mid-articular distance and the maximum breadth of the acromial and sternal ends (Spradley & Jantz, 2011; Kralik *et al.*, 2014; Lee *et al.*, 2014; Moore *et al.*, 2016; Kaewma *et al.*, 2017; Tomczyk *et al.*, 2017; Sehrawat, 2018).

2.2.5. Scapula

The scapula is a flat, triangular bone that overlies the second to seventh ribs on the postero-lateral aspect of the thorax (Moore *et al.*, 2010). The spine of the scapula, which is a thick, projecting ridge of bone, unevenly divides the posterior surface of the scapula and continues laterally as the flat, expanded acromion process which articulates with the clavicle to form the acromioclavicular joint (Moore *et al.*, 2010). The glenoid cavity is located superolaterally on the lateral surface of the scapula and articulates with the head of the humerus to form the glenohumeral joint (Moore *et al.*, 2010).

Sexual dimorphism of the scapula has also been studied in various global populations, however, most of these studies only focused on two to four measurements of the scapula, namely the scapular height and breadth and/or the glenoid cavity height and breadth (Frutos, 2002; Özer *et al.*, 2006; Spradley & Jantz, 2011; Peckmann *et al.*, 2017). Only the studies by Dabbs (2010) and Papaioannou *et al.* (2012) included various other scapular measurements, such as the maximum length of the spine.

In Guatemalan, Mexican, Chilean and Thai populations, the glenoid cavity breadth was found to be the most accurate measurement with sex estimation accuracies of more than 80% (Frutos, 2002; Hudson *et al.*, 2016; Peckmann *et al.*, 2016 & 2017). The glenoid cavity height was found to be the most accurate for Turkish, Egyptian and Greek populations, with accuracies of more than 80% (Dabbs, 2010; Papaioannou *et al.*, 2012). In contrast to the studies mentioned above, Spradley *et al.* (2015) found that the scapular height was the most accurate measurement for a Mexican population (92% accuracy). This is similar to the findings by Spradley & Jantz (2011), Papaioannou *et al.* (2012), Tise *et al.* (2013) and Moore

et al. (2016) for American, Greek, Hispanic and Colombian populations, with accuracies of higher than 80%. The scapular breadth was found to be the most accurate measurements for a Turkish population, with accuracies above 90% (Özer *et al.*, 2006).

The high classification accuracies obtained using the glenoid cavity and/or the scapular measurements may be the result of this articular surface reacting in some degree to the applied force of the muscles over the glenohumeral joint. The larger the muscle, the greater the force acting across the joint will be, thus the articular surface needed will also be larger, making the joint size distinctive between the sexes and more accurate in sex estimation (France, 1988; Purkait & Chandra, 2004; Charisi *et al.*, 2011). These studies show that parts of a bone can have similar accuracies than the whole bone, thus it may not be necessary to measure the bone in its entirety which is especially useful in forensic cases where the bones are often damaged (Milner & Boldsen, 2012).

Taking the existing differences in the accuracies of measurements into consideration, it is clear that the measurements and standards of one population cannot simply be applied to another population due to differences in their genetic (intrinsic) and epigenetic (extrinsic) factors, and may lead to the misclassification of individuals. It is thus important to test various measurements in different populations and, once the most appropriate measurements have been chosen, to set standards for sex estimation that resemble, as closely as possible, the population from which the unidentified individual is expected to have come from.

2.3. The South African context

In South Africa, the estimated population size is 55.7 million individuals, consisting of individuals of various and diverse ancestral and cultural origins and practices (Statistics South Africa, 2018). It is expected that the skeletal morphology of these contemporary South Africans will differ from their ancestors from elsewhere in Africa and Europe, due to adaptations to their specific environment and admixture with other local groups in this environment (Steyn & İşcan, 1999; Micklesfield *et al.*, 2011; Mokoena *et al.*, 2019). These morphological differences between ancestral groups have been historically broadened through disparities in the socio-economic living conditions, including the physical activity, access to good nutrition and health care, of these groups (Puoane *et al.*, 2002; Stull *et al.*, 2014). Additionally, these groups were both geographically and socially segregated by South

Africa's Apartheid legislation, which was enforced from 1948 to 1994, and served to maintain such distances between groups and amplify morphological variation between these groups (Harris *et al.*, 2011; Liebenberg *et al.*, 2015; Maass & Friedling, 2018 & 2019a). Since 1994 and the end of the Apartheid era, however, there has been some change in these socio-economic living conditions, such as an increase in the quality of and access to nutrition and good health care, allowing previously disadvantaged groups to more readily reach their growth potential (Steyn *et al.*, 1997; Cole 2003; Maass & Friedling, 2018). This can be seen in the skeletal morphology of these groups as secular trends such as increased lengths of the long bones of Black individuals and a subsequent increase in average stature has been reported in recent years (Hawley *et al.*, 2009). However, some variation between the groups is caused by positive assortative mating, geographical distance and social factors that still act as barriers between population groups, and are still maintained to a lesser extent due to persisting social and cultural attitudes (Stull *et al.*, 2014). Though some differences between groups are still expected and many have been indicated in previous studies, it is important to remember that skeletal morphology is a product of both internal (genetic) and external factors (socio-economic, nutrition, activity) and that this balance is constantly shifting (Hawley *et al.*, 2009). It is nonetheless difficult to determine the extent of the effect these differences within and between the population groups may have and how they may manifest on the skeleton (Sealy & Pfeiffer, 2000; Maass & Friedling, 2019a). However, in order to account for the fact that skeletal morphology is constantly changing within populations, the application of observations of morphology (e.g. measurements) for a biological profile estimation must be continuously re-evaluated and updated if necessary (Steyn *et al.*, 1997; Mall *et al.*, 2000; Hawley *et al.*, 2009).

Ongoing changes in the South African population structure have two main consequences relating to forensic anthropology. Firstly, due to the increasing population size, there is a corresponding increase in the need for housing and employment, which resulted in an increase in urbanisation (L'Abbé & Steyn, 2012). This sometimes results in the accidental discovery of unidentified skeletal remains, which require forensic anthropological analysis in order to determine whether they are of archaeological, historical or forensic interest (L'Abbé & Steyn, 2012). Secondly, there has been an increase in the number of unnatural deaths reported in the country and in some cases, where the remains are in advanced stages of soft tissue decomposition or even completely skeletonised, the identification is made difficult by the fact that standard identification procedures such as fingerprinting or DNA comparisons

may not be possible (Steyn *et al.*, 1997; Norman *et al.*, 2007; L'Abbé & Steyn, 2012; Krüger *et al.*, 2017). In these cases, the anthropologist may assist in the identification using appropriate osteological standards that can be applied to a contemporary South African population in order to assist with potential identification of the unidentified remains.

Previous studies on sex estimation using the upper limb bones in South Africa were based on samples consisting mostly of Black and/or White individuals from the Raymond A. Dart Collection and the Pretoria Bone Collection, both based in Gauteng, and occasionally some Coloured individuals from the Kirsten Skeletal Collection based in the Western Cape (Steyn & İşcan, 1999; Barrier & L'Abbé, 2008; Macaluso, 2011; Krüger *et al.*, 2017; Scott *et al.*, 2018). This, along with the fact that most of these studies were performed over 20 years ago, may suggest that they are no longer good representations of the current South African population, due to changing living conditions and secular trends, as mentioned above (Washburn, 1949; De Villiers, 1968; Steyn *et al.*, 1997). These South African studies, however, show similar results as the global studies, with high sex estimation accuracies of approximately 80% when using univariate measurements of the bones of the upper limb, though the exact measurements yielding the highest accuracies vary between studies. A summary of some South African studies of the bones of the upper limb is presented in Table 2.1.

Table 2.1.: Summary of the results of South African studies on sexual dimorphism of the upper limb bones.

Author	Sample size	Bone(s)	Most accurate univariate	Least accurate univariate
Barrier & L'Abbé (2008)	400	Radius	Distal breadth (80–83%) Maximum head diameter (80–82%) Minimum midshaft diameter (82–86%)	Not indicated
		Ulna	Maximum olecranon breadth (76–83%)	Not indicated
Steyn & İşcan (1999)	192	Humerus	White = epicondylar breadth (90%) Black = head diameter (91%)	White = deltoid circumference (80%) Black = deltoid circumference (82%)
Ogedengde *et al.* (2017)	211	Humerus	Upper epiphyseal breadth (84%) Epicondylar breadth (83%) Vertical head diameter (83%)	Midshaft transverse diameter (55%)
Scott *et al.* (2018)	198	Clavicle	Maximum length (71%)	Vertical diameter (67%) Sagittal diameter (63%)
Macaluso (2011)	120	Scapula	Glenoid cavity height (87%) Glenoid cavity breadth (86%)	Not indicated
Krüger *et al.* (2017)	360	Radius	Anterior-posterior diameter (89%) Maximum length (81%)	Transverse diameter (74%)
		Ulna	Transverse diameter (85%) Physiological length (81%) Maximum length (80%)	Dorso-volar diameter (79%)
		Humerus	Epicondylar breadth (85%) Minimum midshaft diameter (83%)	Maximum midshaft diameter (79%) Vertical head diameter (78%) Maximum length (75%)
		Clavicle	Anterior-posterior diameter (81%),	Maximum length (78%) Vertical diameter (75%)
		Scapula	Height (82%) Breadth (81%)	Not indicated

It is unclear whether the application of sex estimation ranges based on samples derived from Gauteng or Western Cape skeletal collections will be able to produce accurate results when applied to skeletal remains found in other local populations or other provinces such as the Free State. There is reason to expect that the Free State population differs significantly from both these populations in terms of demographic composition, socio-economic conditions, and environmental influences – all of which may influence the expression of sexual dimorphism (Steyn & İşcan, 1999; L'Abbé & Steyn, 2012; Scott *et al.*, 2018). The Free State population has the largest provincial Sotho/Southern Sotho constituency in South Africa, whereas the Gauteng population is composed of the largest Zulu constituency and the Western Cape population is composed of the largest provincial Coloured constituency (Statistics South Africa Census, 2011). The socio-economic conditions of the Free State also differ from that of Gauteng and the Western Cape, with relatively high levels of unskilled labour and unemployment, especially among the Black population (Quarterly Labour Force Survey, 2019). Further, environmental influences such as the rainy summers and frosty winters may also result in regional specific adaptations of individuals living in the Free State province, as opposed to those living in the Western Cape which experiences hot and dry summers and rainy winters (Statistics South Africa Census, 2011). Considering these differences between regional populations, it is thus necessary to evaluate the applicability and accuracy of the use of sex estimation ranges developed elsewhere in the country on a Free State skeletal population.

In a pilot study on the osteometric assessment of the sexual dimorphism of the glenohumeral joint using a Free State sample, it was found that the scapula and humerus show sufficient sexual dimorphism to allow for accurate sex estimation (Smal, 2018). Similar to previous studies (İşcan *et al.*, 1998; Steyn & İşcan, 1999; Mall *et al.*, 2001; Devi *et al.*, 2013; Fasova & Timonov, 2017; Ogedengde *et al.*, 2017), the most accurate measurements of the humerus were that of the vertical head diameter and epicondylar breadth. This pilot study also found that the glenoid cavity height was the most accurate measurement of the scapula, with findings similar to those of Frutos (2002), Dabbs (2010) and Macaluso (2011). It is thus necessary to evaluate such potential differences, and it would be further beneficial to evaluate whether other bones like the radius, ulna and clavicle also differ between these regional groups and may be used for accurate univariate sex estimation in a Free State sample.

2.4. Problem statement

Based on the issues discussed, there is a clear interest to assess the need for population-specific ranges for sex estimation in South Africa using bones such as the radius, ulna, humerus, clavicle and scapula. It would also be beneficial to assess which bones or regions thereof are best for use in sex estimation in this population, especially for cases where only one or parts of these bones are recovered in a forensic context. While some studies addressing these subjects have been performed in South Africa, there is a need to re-assess the accuracy of the existing South African ranges when applied to regional samples other than those on which they were originally based.

Chapter 3: Materials & Methodology

3.1. Ethical approval

The present study forms part of a larger ongoing study *Anthropological research on the skeletal collection of the Department of Basic Medical Sciences, University of the Free State* [Ethical clearance number UFS-HSD2017/1129]. Ethical approval for the present study was applied for and obtained from the Head of the Department, the Head of Sciences, Health Sciences Research Ethics Committee (HSREC) and appointed evaluation committee. Ethical clearance number UFS-HSD2019/1509/2708 was assigned to the present study.

3.2. Sample

The present study sample consisted of 60 individuals of known sex and age, selected from the human skeletal collection housed in the Department of Basic Medical Sciences at the University of the Free State. The skeletal collection consists of the remains of individuals who were obtained either as donations or as unclaimed individuals after the year 2000 from public hospitals in the Free State, in accordance with the *National Health Act* (Act No. 61 of 2003) of South Africa. The collection is used as a representative Free State sample, not in the sense of individuals who were born and lived in the Free State, but rather individuals whose skeletal remains may likely be found in the province in a forensic context. This would likely include individuals who have migrated to the area in the period shortly before their death.

Individuals were selected on the basis of having all left and right upper limb bones present (excluding the hand). Furthermore, individuals were excluded if there were any trauma and/or pathologies present. As prescribed by the existing protocols within the Department, the age range of individuals in the skeletal collection is 20 to 50 years at the time of death. The age range of the individuals selected for the present study was 22 to 50 years. Selection of individuals was performed without consideration of ancestry, as many collections no longer record this information (Tal & Tau, 1983). In a forensic context, ancestry would also have to be estimated independently as part of the compilation of the biological profile. It is thus better to evaluate sex estimation methods as "generic" for a regional population rather than according to a specific ancestral group to avoid confounding errors in the profile (Feldesman & Fountain, 1996; Albanese, 2013).

3.3. Measurements and equipment used

Twenty standard univariate measurements were performed on the radius, ulna, humerus, clavicle and scapula (four measurements each) of each individual. The measurements were taken using an osteometric board or digital Vernier calliper, as appropriate. The definitions of the measurements of the bones are given in Tables 3.1 to 3.5 and illustrated in Figures 3.1 to 3.5.

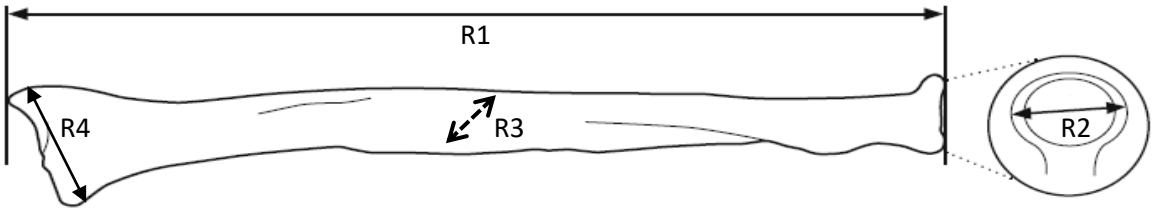

Figure 3.1: Univariate measurements of the radius (anterior view of right radius) [as defined in Table 3.1, adapted from Moore-Jansen, *et al*. 1994.].

Table 3.1: Definitions of univariate measurements of the radius [adapted from Buikstra & Ubelaker (1994) and Bass (2005)].

Measurements		Definitions
R1	Maximum length.	The distance from the most proximally positioned point of the head of the radius to the most distal end of the styloid process.
R2	Maximum head diameter.	The diameter of the radial head between the most lateral and medial points.
R3	Anterior-posterior diameter at midshaft.	The maximum diameter of the radial shaft at the midpoint of the diaphysis (anterior-posterior plane).
R4	Distal breadth.	The distance from the most laterally protruding point of the lateral side to the corresponding projection on the medial side.

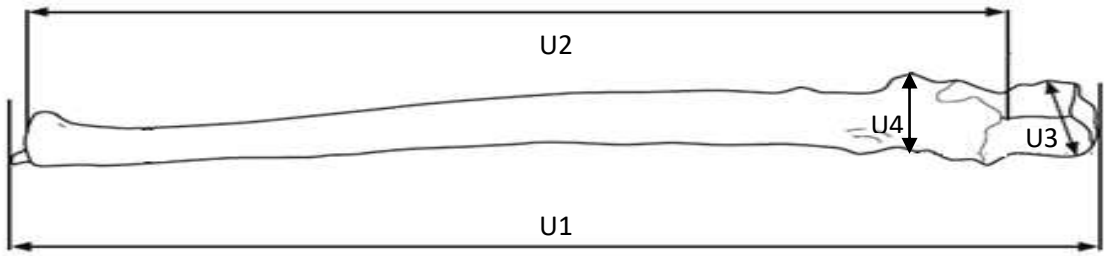

Figure 3.2: Univariate measurements of the ulna (anterior view of right ulna) [as defined in Table 3.2, adapted from Moore-Jansen, *et al*. 1994.].

Table 3.2: Definitions of univariate measurements of the ulna [adapted from Buikstra & Ubelaker (1994) and Bass (2005)].

Measurements		Definitions
U1	Maximum length.	The distance between the most superior/proximal point on the olecranon and the most inferior/distal point on the styloid process.
U2	Physiological length.	The distance between the deepest point on the articular surface of the coronoid process on the guiding ridge and the most inferior point on the inferior/distal articular surface of the ulna.
U3	Olecranon breadth.	The maximum breadth of the olecranon process, taken perpendicular to the longitudinal axis of the semilunar notch.
U4	Transverse diameter.	The proximal width of the ulna inferior to the trochlear notch.

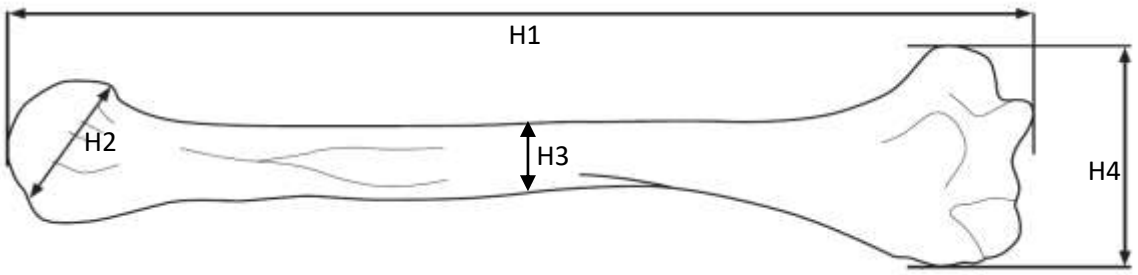

Figure 3.3: Univariate measurements of the humerus (anterior view of the right humerus) [as defined in Table 3.3, adapted from Moore-Jansen, *et al*. 1994.].

Table 3.3: Definitions of univariate measurements of the humerus [adapted from Buikstra & Ubelaker (1994)].

Measurements		Definitions
H1	Maximum length.	The distance from the most superior point of the head of the humerus to the most inferior point on the trochlea.
H2	Maximum vertical head diameter.	The distance between the most superior and inferior points on the border of the articular surface.
H3	Maximum midshaft diameter.	The maximum diameter of the humeral shaft at the midpoint of the diaphysis (medial-lateral plane).
H4	Epicondylar breadth.	The distance from the most laterally protruding point of the lateral epicondyle to the corresponding projection on the medial epicondyle.

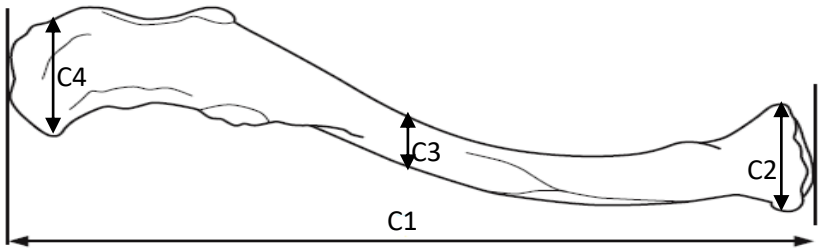

Figure 3.4: Univariate measurements of the clavicle (superior view of right clavicle) [as defined in Table 3.4, adapted from Moore-Jansen, *et al*. 1994.].

Table 3.4: Definitions of univariate measurements of the clavicle [adapted from Moore-Jansen *et al.* (1994) and Bass (2005)].

Measurements		Definitions
C1	Maximum length.	The maximum distance between the most medial and lateral ends of the clavicle.
C2	Sternal epiphyseal width.	The anterior-posterior distance of the epiphyseal end of the sternal part of the clavicle.
C3	Sagittal diameter at midshaft	The anterior-posterior distance (maximum width) of the clavicle at the midpoint of the diaphysis.
C4	Acromial epiphyseal width.	The anterior-posterior distance of the epiphyseal end of the acromial part of the clavicle.

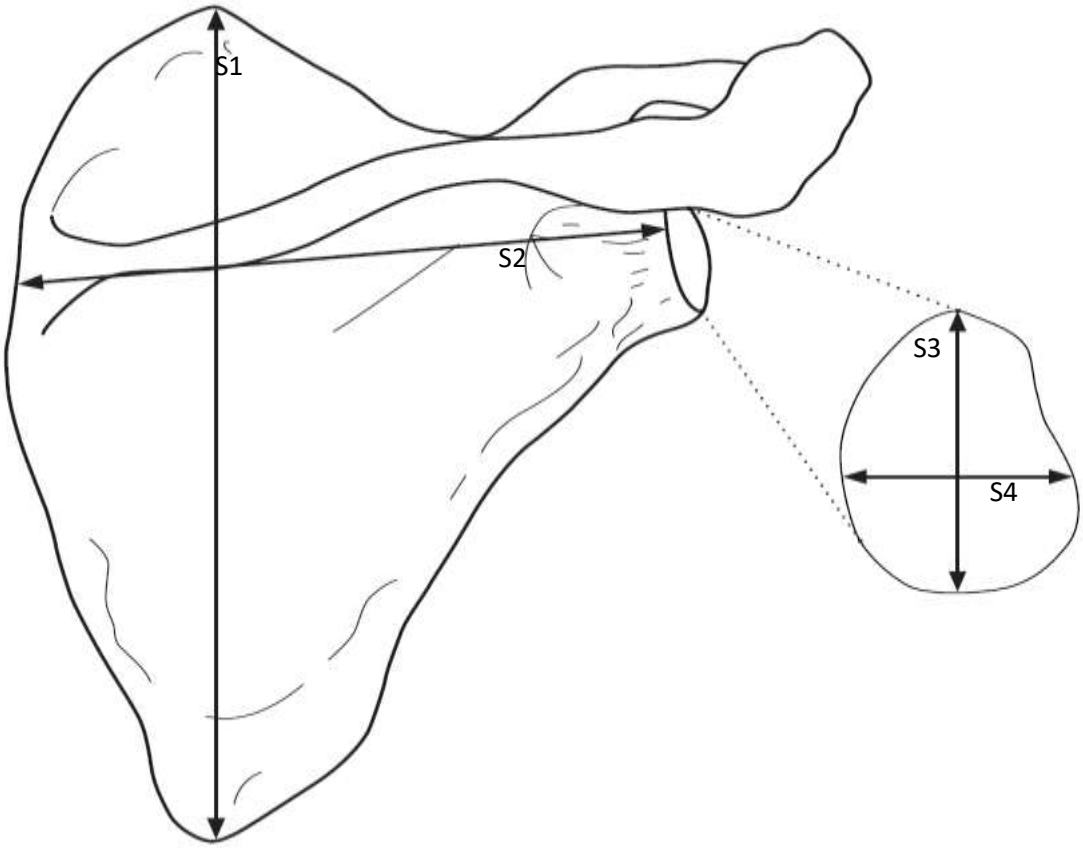

Figure 3.5: Univariate measurements of the scapula (posterior and lateral views of right scapula) [as defined in Table 3.5].

Table 3.5: Definitions of the univariate measurements of the scapula [adapted from Buikstra & Ubelaker (1994)].

Measurements		Definitions
S1	Scapular height.	The distance from the most superior point of the superior angle to the most interior point on the inferior angle.
S2	Scapular breadth.	The distance from the midpoint on the dorsal border of the glenoid fossa to midway between the two ridges of the scapular spine on the vertebral border.
S3	Glenoid cavity height.	The distance from the most superior located point on the margin of the glenoid cavity to the most inferior located point on the margin, taken perpendicular to the glenoid cavity breadth.
S4	Glenoid cavity breadth.	Maximum distance from the ventral to dorsal margins of the glenoid cavity, taken perpendicular to glenoid cavity height.

3.4. Errors in methodology and measurements

Intra- and inter-observer error rates were calculated using repeated measurements of a randomly selected sub-sample of 30 skeletal individuals. The intra-observer error was

statistically assessed by comparing repeated measurements of the principal researcher, taken a few days apart. The inter-observer error was assessed by comparing the measurements taken by the principal observer and the independent observer.

Comparisons of repeated measurements followed the guidelines of Ulijaszek & Kerr (1999). For each variable, the technical error of measurement (TEM) was calculated using the formula:

$$TEM = \sqrt{(\Sigma D^2/2N)}$$

Where D is the difference between measurements and N is the sample size. Since TEM is an estimated standard deviation of the difference between repeated measurements, it serves as an evaluation of measurement precision (Scott *et al.*, 2018).

Relative TEM was calculated using the formula:

$$rTEM = (TEM/mean)\ X100$$

The coefficient of reliability (R) was calculated using the following formula:

$$R = 1- [(total\ TEM)^2/ SD^2]$$

Where SD is the total standard deviation for all measurements used when evaluating TEM.

International standards for acceptable observer error as reflected in these measurements are rTEM of greater than 5% and an R of less than 0.90 (Ulijaszek & Kerr, 1999), though R-values as low as 0.75 have also been considered acceptable (Weinberg *et al.*, 2005). In cases where the observer error rates of the present study did not meet these criteria, both observers repeated the measurements and whereupon the error was reassessed. If, however, the criteria were met, the repeated measurements were averaged and added to the larger data pool to be used for further analysis.

3.5. Data analysis

Statistical analysis of the collected data was performed using the Data Analysis package of Microsoft Excel 2010. Descriptive statistics for each variable such as means and standard deviations were calculated. Shapiro-Wilk testing was performed to determine if the data were normally distributed (Appendix A, Tables A.1 & A.2). For analysis, a significance level of $p<0.05$ was used. The potential differences between the left and right sides were assessed by applying paired T-tests to the normally distributed data, or Wilcoxon matched tests to the

non-normally distributed data. While differences between sides were assessed, previous studies have shown that measurements of the left and right side are strongly correlated in most cases (Mahakkanukrauh *et al.*, 2011), and it would also be impractical in the forensic context to develop and use separate measurements for the left and right side. Thus the data for the left and right sides of the measurements were pooled and further analysed as a combined sample. A similar method was used to determine if there was a difference between the sexes by applying the unpaired T-test to the normally distributed data, or Mann-Whitney tests to the non-normally distributed data. Lastly, the data was submitted to Spearman Rank testing to evaluate the possible association between variables.

3.5.1. Classification accuracy

The Free State data collected in the present study was used to calculate identification and sectioning points for each measurement. The sectioning point values were subsequently used to classify the sex of each individual, with those higher than the sectioning point being males and those lower that the sectioning point being females (Jit & Singh, 1966). Accuracies of these classifications were then calculated to assess which individual measurement is best for use in sex estimation in this sample. Next the obtained accuracies were compared to accuracies achieved using the existing South African criteria.

The data of the Free State sample was further used to evaluate the existing South African criteria for sex estimation based on univariate measurements of the radius and ulna (Barrier & L'Abbé, 2008), the humerus (Ogedengbe *et al.*, 2017), the clavicle (Scott *et al.*, 2018), and the scapula (Macaluso, 2011) as shown in Appendix A, Tables A.3 and A.4. In the case of measurements for which there are no South African criteria available, the international criteria for these measurements were tested: the radius (Mall *et al.*, 2001; Spradley & Jantz, 2011), the ulna (Mall *et al.*, 2001; Charisi *et al.*, 2011; Spradley & Jantz, 2011), the humerus (Sakaue, 2004; Frutos, 2005; Charisi *et al.*, 2011), clavicle (Spradley & Jantz, 2011; Tise *et al.*, 2013) and scapula (Papaioannou *et al.*, 2012; Moore *et al.*, 2016) as shown in Appendix A, Tables A.3 and A.4.

Chapter 4: Results

4.1. Observer error

The TEM, rTEM and R-values were calculated for both the inter- and intra-observer errors as shown in Table 4.1. For all comparisons, TEM was less than 1.68, and rTEM was less than 4.83%, whereas the coefficient of reliability (R) was greater than 0.85 for both the inter- and intra-observer comparisons for all the measurements evaluated. These results met the criteria of Ulijaszek & Kerr (1999) and indicated that the intra- and inter-observer errors were minor relative to the sample variability, and thus unlikely to have a significant effect on the results of the present study.

Table 4.1: Observer error test statistics.

Measurements	Inter-observer			Intra-observer		
	TEM	rTEM (%)	R	TEM	rTEM (%)	R
R1	0.13	0.05	1.00	0.90	0.38	1.00
R2	0.13	0.63	1.00	0.18	0.84	0.99
R3	0.57	4.83	0.86	0.12	0.99	0.99
R4	0.04	0.13	1.00	0.69	2.13	0.88
U1	1.29	0.51	0.99	0.65	0.25	1.00
U2	0.52	0.23	1.00	0.52	0.23	1.00
U3	0.62	2.58	0.94	0.43	1.77	0.97
U4	0.58	2.76	0.97	0.13	0.60	1.00
H1	1.55	0.51	0.99	0.90	0.29	1.00
H2	0.61	1.49	0.98	0.75	1.82	0.96
H3	0.34	1.79	0.98	0.45	2.36	0.96
H4	1.68	2.94	0.85	0.07	0.13	1.00
C1	0.13	0.09	1.00	0.39	0.26	1.00
C2	0.33	1.63	0.99	0.52	2.56	0.97
C3	0.44	3.63	0.91	0.49	4.07	0.87
C4	0.69	2.82	0.98	1.16	4.78	0.93
S1	1.03	0.72	0.99	0.00	0.00	1.00
S2	0.26	0.27	1.00	0.41	0.43	1.00
S3	1.16	3.30	0.86	0.46	1.35	0.98
S4	0.10	0.41	1.00	0.40	1.58	0.97

TEM = Technical error of measurement
rTEM = Relative technical error of measurement
R = Coefficient of reliability

4.2. Differences between left and right side

A summary of the descriptive statistics of each of the measurements evaluated is shown in Table 4.2. As seen in the table, there is a noticeable overlap between the standard deviations and ranges for all measurements.

The initial Shapiro-Wilks analysis showed that the variables R4, U2, U3, U4, H3, C1, C2, C4 and S1 were normally distributed for both sides, while all other variables showed non-normal distributions for one or both of the sides (as shown in Appendix A, Table A.1). A significant difference between sides was found for all measurements (as shown in Table 4.3), except for the transverse diameter of the ulna (U4), the acromial epiphyseal width of the clavicle (C4) and both the scapular height (S1) and breadth (S2), with all $p>0.06$. For most of the measurements, the left side was smaller than the right side, except for the olecranon breadth (U3), the maximum clavicular length (C1) and the scapular breadth (S2) where the left was larger than the right side.

Table 4.2: Descriptive statistics of univariate measurements used in the present study (all measurements in mm).

Variables	Left			Right			% Difference between sides	Pooled		
	Mean	Standard Deviation	Actual Range	Mean	Standard Deviation	Actual Range		Mean	Standard Deviation	Actual Range
R1	240	16.91	208-275	242	17.50	212-280	1.09	241	17.19	208-280
R2	21.11	2.00	17.03-25.09	21.13	2.38	10.83-25.00	0.13	21.12	2.19	10.83-25.09
R3	11.69	1.34	9.13-14.56	11.86	1.40	9.10-14.39	1.43	11.77	1.37	9.10-14.56
R4	32.65	2.98	23.17-40.27	32.99	3.09	23.70-39.76	1.02	32.82	3.03	23.17-40.27
U1	256	17.12	225-291	259	17.71	226-295	1.18	258	17.41	225-295
U2	229	15.73	198-260	231	15.92	198-263	1.12	230	15.81	198-263
U3	24.87	2.68	19.69-30.94	24.62	2.70	19.79-31.77	-1.02	24.75	2.68	19.69-31.77
U4	20.80	2.90	14.92-27.71	20.83	2.70	16.06-26.84	0.16	20.81	2.79	14.92-27.71
H1	311	21.90	273-372	313	21.66	276-375	056	312	21.71	273-375
H2	41.48	3.85	33.12-49.49	41.96	3.92	33.93-49.67	1.14	41.72	3.88	33.12-49.67
H3	18.72	2.09	14.78-23.67	19.22	2.09	15.40-24.40	2.60	18.97	2.10	14.78-24.40
H4	58.34	5.47	48.91-76.25	58.79	5.17	49.43-68.86	0.75	58.56	5.31	48.91-76.25
C1	149	11.39	120-179	147	12.01	116-172	-1.84	148	11.73	116-179
C2	20.79	2.81	13.36-26.69	21.43	2.89	13.56-27.79	2.98	21.11	2.85	13.36-27.79
C3	11.78	1.72	9.11-15.67	12.18	1.69	9.55-16.38	3.30	11.98	1.71	9.11-16.38
C4	25.21	4.37	15.32-35.40	25.88	4.67	15.50-34.37	2.59	25.54	4.52	15.32-35.40
S1	145	12.36	119-178	145	12.64	121-182	0.33	145	12.45	119-182
S2	99.45	6.93	87.34-115.23	99.19	6.94	86.76-118.87	-0.27	99.32	6.91	86.76-118.87
S3	34.79	2.89	29.09-40.92	35.55	2.95	28.58-41.01	2.14	35.17	2.93	28.58-41.01
S4	24.76	2.35	20.34-28.89	25.73	2.49	21.34-30.78	3.77	25.24	2.46	20.34-60.78

Table 4.3: Comparison of the left and right side variables.

Measurement	Test statistic	p-value
R1	W = 79.0	p<0.001
R2	W = 638.5	p=0.04
R3	W = 520.5	p<0.01
R4	T = -4.1	p<0.01
U1	W = 158.5	p<0.001
U2	T = -7.2	p<0.001
U3	T = 2.9	p<0.01
U4*	T = -0.2	p=0.85
H1	W = 312.5	p<0.001
H2	W = 353.0	p<0.001
H3	T = -3.8	p<0.001
H4	W = 404.5	p<0.001
C1	T = 6.4	p<0.001
C2	T = -4.0	p<0.001
C3	W = 354.5	p<0.001
C4*	T = -2.0	p=0.06
S1*	T = -1.3	p=0.20
S2*	W = 802.5	p=0.41
S3	W = 281.0	p<0.001
S4	W = 65.0	p<0.001

* = no significant difference
T = Paired T-test statistic
W = Wilcoxon matched test statistic

4.3. Correlation of variables

The correlation coefficients (R) of the pair-wise correlations are presented in Table 4.4. All the measurements show a positive correlation to each other, with correlation strength ranging from weak (R=0.36) to very strong (R=0.99).

The strongest correlation for the radius among its own measurements was between the maximum head diameter and the anterior-posterior diameter at the midshaft, with R=0.83. For the ulna, its strongest correlation was between its maximum length and its physiological length (R=0.99), while for the humerus it was between its maximum vertical head diameter and its epicondylar breadth (R=0.86). The clavicle's strongest correlation was between its maximum length and its sagittal diameter at the midshaft, with R=0.64. Lastly, for the scapula, the strongest correlation was between its glenoid cavity height and glenoid cavity breadth, with R=0.81.

The variables of the radius had moderate to strong correlations with those of the ulna, especially between the maximum length of the radius and the ulnar maximum and physiological lengths (R=0.99 and R=0.98, respectively). This was also true for the correlations with the maximum lengths of the humerus, clavicle and the scapula (R=0.80–0.85). Additionally, the correlations of the radius with the humerus and scapula were moderate to strong (R=0.62–0.85), while correlations with the clavicle were relatively weaker, with the correlation between the maximum head diameter of the radius and the sternal epiphyseal width of the clavicle having a correlation coefficient of only R=0.52.

For the ulna, the best correlations were with the humerus and the scapula. The strongest correlations with the humerus were between the ulnar olecranon breadth and both the maximum vertical head diameter and epicondylar breadth of the humerus, with R=0.87 for both. With the scapula, the strongest correlation was between the maximum ulnar length and the glenoid cavity breadth (R=0.81). The correlations with the clavicle were again relatively weaker, with the correlation between the transverse diameter of the ulna and the sternal epiphyseal width of the clavicle being only R=0.43.

The variables of the humerus had moderate to strong correlations with those of the scapula, with the strongest being between the maximum humeral vertical head diameter and the glenoid cavity height of the scapula (R=0.88), and the glenoid cavity breadth of the scapula (R=0.83). However, the humeral correlations with the clavicle were weaker (R=0.36–0.75).

Lastly, the variables of the clavicle had weak to moderate correlations with those of the scapula, the correlation between the sternal epiphyseal width of the clavicle and the scapular breadth being weakest at only R=0.48.

Table 4.4: Correlation between univariate measurements of the bones of the upper limb.

	R1	R2	R3	R4	U1	U2	U3	U4	H1	H2	H3	H4	C1	C2	C3	C4	S1	S2	S3	S4
R1																				
R2	0.80																			
R3	0.77	0.83																		
R4	0.78	0.78	0.77																	
U1	0.99	0.80	0.77	0.77																
U2	0.98	0.77	0.74	0.74	0.99															
U3	0.77	0.79	0.78	0.82	0.76	0.72														
U4	0.56	0.66	0.68	0.69	0.55	0.52	0.65													
H1	0.84	0.70	0.64	0.62	0.83	0.82	0.65	0.42												
H2	0.81	0.84	0.83	0.83	0.81	0.78	0.87	0.67	0.71											
H3	0.62	0.70	0.76	0.69	0.62	0.60	0.62	0.72	0.42	0.68										
H4	0.82	0.78	0.81	0.85	0.82	0.79	0.87	0.59	0.71	0.86	0.67									
C1	0.83	0.76	0.73	0.70	0.81	0.80	0.73	0.48	0.72	0.73	0.51	0.75								
C2	0.55	0.52	0.59	0.54	0.57	0.54	0.50	0.43	0.36	0.57	0.50	0.55	0.53							
C3	0.71	0.69	0.74	0.67	0.71	0.70	0.66	0.58	0.52	0.69	0.72	0.70	0.64	0.53						
C4	0.60	0.61	0.58	0.64	0.62	0.59	0.57	0.49	0.50	0.59	0.53	0.68	0.51	0.51	0.50					
S1	0.80	0.76	0.79	0.66	0.79	0.76	0.72	0.56	0.77	0.77	0.63	0.75	0.76	0.49	0.73	0.49				
S2	0.75	0.70	0.70	0.68	0.77	0.72	0.70	0.52	0.67	0.72	0.51	0.73	0.69	0.48	0.54	0.55	0.67			
S3	0.78	0.76	0.75	0.78	0.77	0.75	0.75	0.61	0.64	0.88	0.70	0.83	0.68	0.51	0.72	0.54	0.75	0.69		
S4	0.80	0.79	0.85	0.79	0.81	0.77	0.72	0.68	0.67	0.83	0.75	0.82	0.63	0.56	0.69	0.61	0.73	0.73	0.81	

Red = stronger correlation

Blue = weaker correlation

4.4. Sexual dimorphism

A summary of the descriptive statistics of each of the measurements evaluated is shown in Table 4.4. As can be seen in the table, females have lower absolute values compared to males for all of the measurements. Additionally, there was a noticeable overlap between the ranges of the sexes, especially at the maximum diameter of the humerus at midshaft (H3), with nearly 100% overlap.

Shapiro-Wilks analysis was performed and showed that the variables U2, U3, U4, H3, C1, C2, C4 and S1 were normally distributed for both sexes, while all other variables showed non-normal distribution (as shown in Appendix A, Table A.2). To determine if there was a difference between the two sexes, comparisons of the males and females were then made for each measurement, using unpaired T-tests if the data for both sides were normally distributed, or Mann-Whitney U-tests if either was not normally distributed. It was found that males were significantly larger than females for all measurements with $p<0.001$ for all comparisons (Table 4.5). The largest percentage difference between the sexes was found in measurements R3, C3 and C4, with percentages of more than 16%. The measurements H1 (7.48%) and S2 (7.70%) had the smallest percentage difference between sexes, with the measurements R1, U1, U2 and C1 also having less than 10% difference between the means of the sexes.

Table 4.5: Descriptive statistics of univariate measurements used in the present study (all measurements in mm).

Variables	Female			Male			% Difference between sexes	Pooled		
	Mean	Standard Deviation	Actual Range	Mean	Standard Deviation	Actual Range		Mean	Standard Deviation	Actual Range
R1	229	10.82	208-256	254	12.97	226-280	9.75	241	17.19	208-280
R2	19.46	1.60	10.83-22.60	22.77	1.23	20.32-25.09	14.53	21.12	2.19	10.83-25.09
R3	10.71	0.69	9.10-12.70	12.84	0.99	10.09-14.56	16.59	11.77	1.37	9.10-14.56
R4	30.62	2.34	23.17-36.71	35.01	1.80	31.29-40.27	12.54	32.82	3.03	23.17-40.27
U1	246	11.77	225-275	270	12.90	246-295	9.09	258	17.41	225-295
U2	219	10.71	198-247	241	12.07	218-263	9.09	230	15.81	198-263
U3	22.81	1.67	19.69-26.49	26.68	2.02	22.43-31.77	14.50	24.75	2.68	19.69-31.77
U4	19.17	2.17	14.92-23.64	22.46	2.35	17.45-27.71	14.64	20.81	2.79	14.92-27.71
H1	300	13.74	273-333	325	21.07	285-375	7.70	312	21.71	273-375
H2	38.65	2.25	33.12-45.06	44.79	2.47	39.49-49.67	13.70	41.72	3.88	33.12-49.67
H3	17.51	1.30	14.78-20.65	20.43	1.69	15.12-24.40	14.27	18.97	2.10	14.78-24.40
H4	54.79	3.52	48.91-65.91	62.34	3.92	55.40-76.25	12.12	58.56	5.31	48.91-76.25
C1	140	8.21	116-159	156	9.44	136-179	9.93	148	11.73	116-179
C2	19.58	2.61	13.36-25.40	22.64	2.20	16.64-27.79	13.51	21.11	2.85	13.36-27.79
C3	10.66	0.90	9.11-13.47	13.31	1.22	10.45-16.38	19.93	11.98	1.71	9.11-16.38
C4	23.05	3.38	15.32-31.26	28.04	4.12	20.47-35.40	17.82	25.54	4.52	15.32-35.40
S1	136	8.10	119-157	154	9.62	132-182	11.35	145	12.45	119-182
S2	95.27	5.30	86.76-106.65	103.37	5.89	94.33-118.87	7.84	99.32	6.91	86.76-118.87
S3	32.88	2.02	28.58-37.87	37.46	1.62	34.42-41.01	12.22	35.17	2.93	28.58-41.01
S4	23.45	1.90	20.34-29.49	27.03	1.43	24.11-30.78	13.26	25.24	2.46	20.34-30.78

Table 4.6: Comparisons of the female and male variables.

Measurements	Test statistic	P-value
R1	U= 237.5	p<0.001
R2	U= 106.0	p<0.001
R3	U= 162.0	p<0.001
R4	U= 193.5	p<0.001
U1	U= 266.5	p<0.001
U2	T= -10.5	p<0.001
U3	T= -5.7	p<0.001
U4	T= -5.1	p<0.001
H1	U= 634.0	p<0.001
H2	U= 184.5	p<0.001
H3	T= -5.1	p<0.001
H4	U= 253.5	p<0.001
C1	T= -9.5	p<0.001
C2	T= -6.9	p<0.001
C3	U= 156.5	p<0.001
C4	T= -7.3	p<0.001
S1	T= -10.7	p<0.001
S2	U= 528.0	p<0.001
S3	U= 150.0	p<0.001
S4	U= 264.5	p<0.001

T = Unpaired T-test statistic
U = Mann-Whitney U-test statistic

4.5. Classification Accuracy

The accuracies of these classifications were then calculated for the female, male and pooled samples, and are shown in Table 4.8.

The most accurate upper limb measurements for females were the maximum head diameter of the radius (R2), the anterior-posterior diameter at the midshaft of the radius (R3), the vertical head diameter of the humerus (H2), the sagittal diameter at the midshaft of the clavicle (C3) and the glenoid cavity height of the scapula (S3) – all with accuracies of 90.00% or more. The least accurate measurements for females were the transverse diameter of the ulna (U4), and the acromial epiphyseal width of the clavicle (C4), both with accuracies of 73.33%. For the males, the most accurate measurements were the distal radial breadth (R4), the sagittal diameter at the midshaft of the clavicle (C3), and the glenoid cavity height of the scapula (S3), all with accuracies of 90.00%. The maximum length of the humerus (H1), the acromial epiphyseal width of the clavicle (C4), and the scapula breadth (S2) were

the least accurate measurements for males, with accuracies of 70.00% or less. In general, the accuracies of the females were higher than those of the males with the exception of the distal radial breadth (R4), the transverse diameter of the ulna (U4) and the glenoid cavity breadth of the scapula (S4).

A comparison of the overall classification accuracies of the different bones and their measurements was made using the pooled (combined male and female) sample, as shown in Figure 4.1. The vertical head diameter of the humerus (H2) and the sagittal diameter at the midshaft of the clavicle (C3) were the most accurate, both with 90.83% accuracy. This was followed by the maximum head diameter (R2) and the anterior-posterior diameter at the midshaft (R3) of the radius, and the glenoid cavity height of the scapula (S3), all with 90.00% accuracy. In contrast to the majority of the measurements that had 80% to 90% accuracies, there were four measurements that had lower accuracies of 70% to 75%. These were the transverse diameter of the ulna (U4; 75.00%), the maximum length of the humerus (H1; 73.33%), the acromial epiphyseal width of the clavicle (C4; 71.67%), and the scapular breadth (S2; 74.17%).

Table 4.7: The calculated identification and sectioning points for the Free State sample (measurements in mm).

	Female identification point	Sectioning point	Male identification point
R1	226	241	256
R2	20.32	21.12	22.60
R3	10.09	11.77	12.70
R4	31.29	32.82	36.71
U1	246	258	275
U2	218	230	247
U3	22.43	24.75	26.49
U4	17.45	20.81	23.64
H1	285	312	333
H2	39.46	41.72	45.06
H3	15.12	18.97	20.65
H4	55.40	58.56	65.91
C1	136	148	159
C2	16.64	21.11	25.40
C3	10.45	11.98	13.47
C4	20.47	25.54	31.26
S1	132	145	157
S2	94.33	99.32	106.65
S3	34.42	35.17	37.87
S4	24.11	25.24	29.49

Table 4.8: Classification accuracy of measurements for the estimation of sex.

Variables	Female				Male				Pooled			
	Correct		Incorrect		Correct		Incorrect		Correct		Incorrect	
	n	%	n	%	n	%	n	%	n	%	n	%
R1	53	88.33	7	11.67	52	86.67	8	13.33	105	87.50	15	12.50
R2	55	**91.67**	5	8.33	53	88.33	7	11.67	108	**90.00**	12	10.00
R3	55	**91.67**	5	8.33	53	88.33	7	11.67	108	**90.00**	12	10.00
R4	51	85.00	9	15.00	54	**90.00**	6	10.00	105	87.50	15	12.50
U1	52	86.67	8	13.33	51	85.00	9	15.00	103	85.83	17	14.17
U2	52	86.67	8	13.33	49	81.67	11	18.33	101	84.17	19	15.83
U3	52	86.67	8	13.33	51	85.00	9	15.00	103	85.83	17	14.17
U4	44	73.33	16	26.67	46	76.67	14	23.33	90	75.00	30	25.00
H1	48	80.00	12	20.00	40	66.67	20	33.33	88	73.33	32	26.67
H2	56	**93.33**	4	6.67	53	88.33	7	11.67	109	**90.83**	11	9.17
H3	53	88.33	7	11.67	50	83.33	10	16.67	103	85.83	17	14.17
H4	53	88.33	7	11.67	47	78.33	13	21.67	100	83.33	20	16.67
C1	50	83.33	10	16.67	46	76.67	14	23.33	96	80.00	24	20.00
C2	48	80.00	12	20.00	48	80.00	12	20.00	96	80.00	24	20.00
C3	55	**91.67**	5	8.33	54	**90.00**	6	10.00	109	**90.83**	11	9.17
C4	44	73.33	16	26.67	42	70.00	18	30.00	86	71.67	34	28.33
S1	52	86.67	8	13.33	50	83.33	10	16.67	102	85.00	18	15.00
S2	48	80.00	12	20.00	41	68.33	19	31.67	89	74.17	31	25.83
S3	54	**90.00**	6	10.00	54	**90.00**	6	10.00	108	**90.00**	12	10.00
S4	50	83.33	10	16.67	52	86.67	8	13.33	102	85.00	18	15.00

Highest percentages per group are indicated in bold

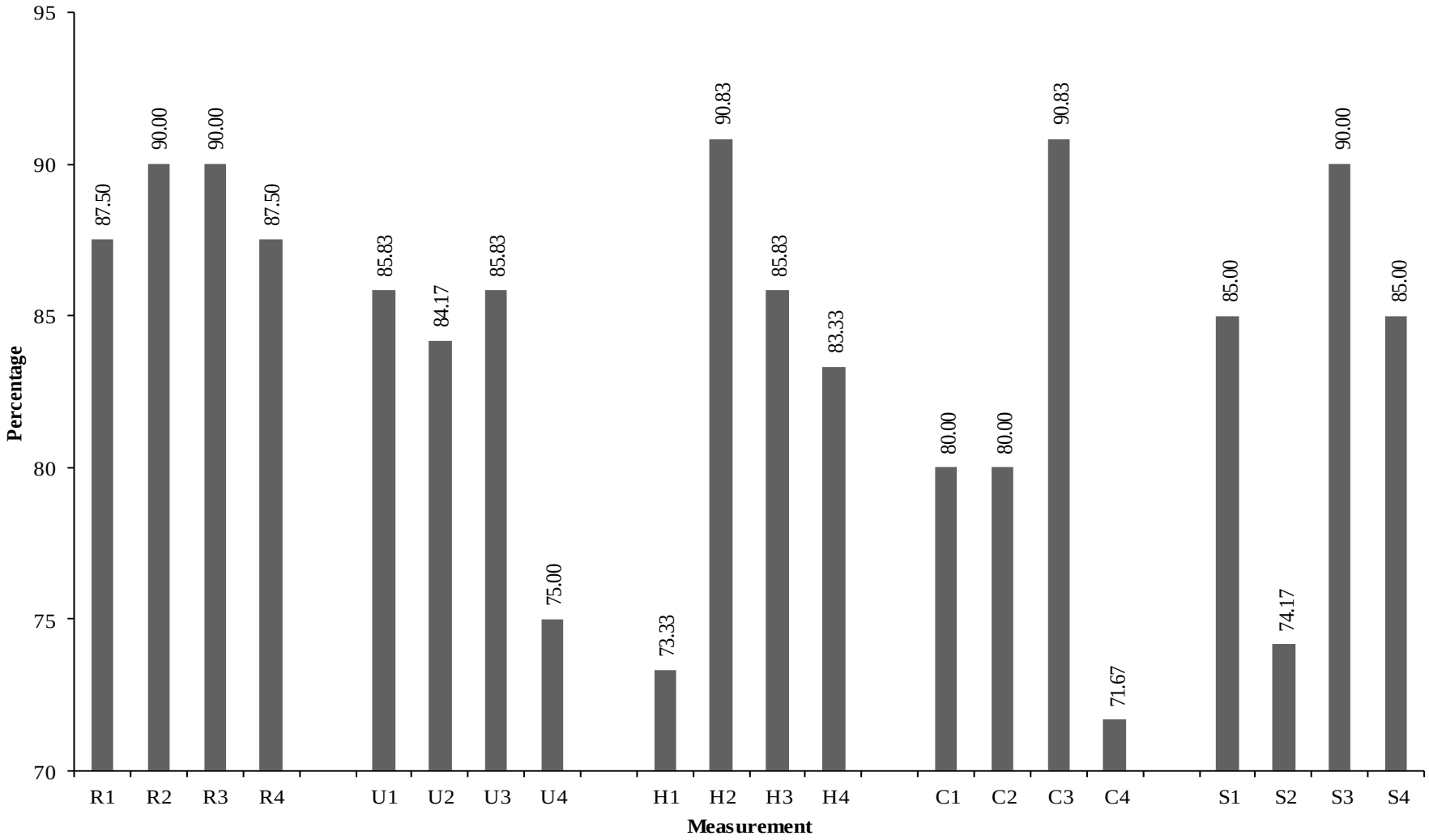

Figure 4.1: Comparison of classification accuracy of measurements in the pooled sample.

4.6. Accuracy of existing univariate methods on the Free State sample

Existing published sectioning points and/or equations (based on other skeletal samples) were applied to the collected Free State data to allow comparison of classification accuracies to those derived from the present study sample. Where available, South African standards were used, but if no such standards were available, standards from other populations were applied (references as listed in Appendix A: Table A.3 and Table A.4). The resulting classification accuracies (as seen in Appendix A: Table A.5 and Table A.6) were then compared to the Free State derived standards, as shown in Figures 4.2–4.6.

In general, as can be seen in Figure 4.2, the accuracies obtained when using radial measurements were equal to or higher when using the Free State sample sectioning points than when using the published sectioning points. The only comparison for which the accuracy based on the Free State sample sectioning points was lower than that of the published sectioning points was the R4 measurement, though the difference was only 1.67%. The difference in the accuracies of the present study and the published regression equations, however, was greater, with the Free State-derived sectioning points giving accuracies of up to 40% higher, as seen for the R3 measurement. Classification accuracies obtained using the regression equations were all less than 77%.

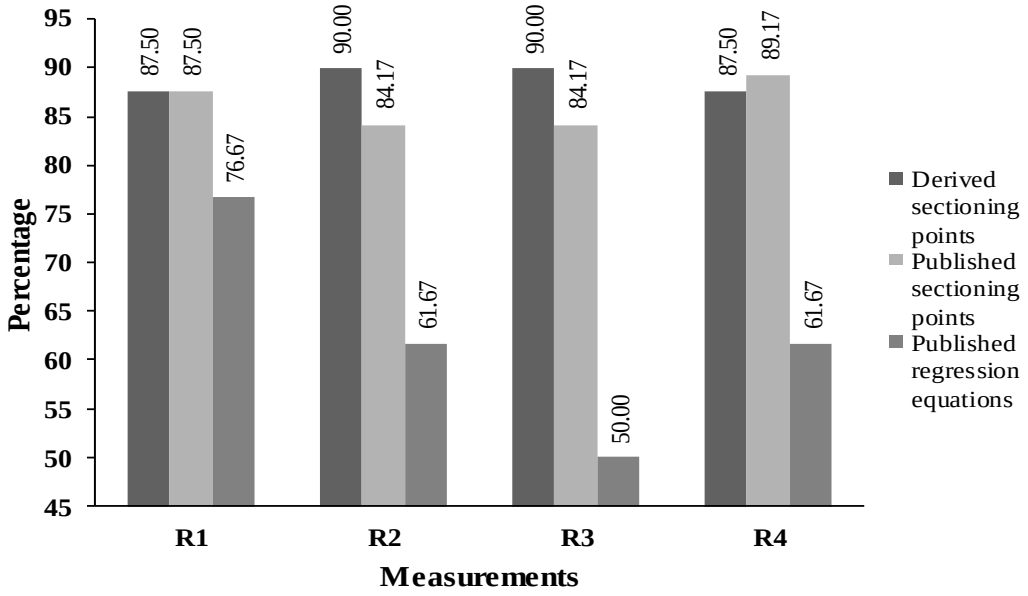

Figure 4.2: Comparison of classification accuracies for the radius using the different standards for classification.

The accuracies obtained when using the Free State-derived ulnar sectioning points for measurements U1 and U2 were equal to those obtained when using the published sectioning points, as seen in Figure 4.3. For the U3 measurement, the accuracy was higher than those of the published sectioning points, though the difference was less than 5%. Since no published sectioning points for the measurement U4 were available in the literature, its accuracy in the present study could only be compared with those obtained when using the published regression equations. Similarly, no published regression equations were available for the U2 measurement, and thus it was excluded from the comparison. Similar to the radius, the classification accuracies of the regression equations were lower for all three univariate measurements (U1, U3, U4), with a difference of up to 25% observed for the U3 measurement.

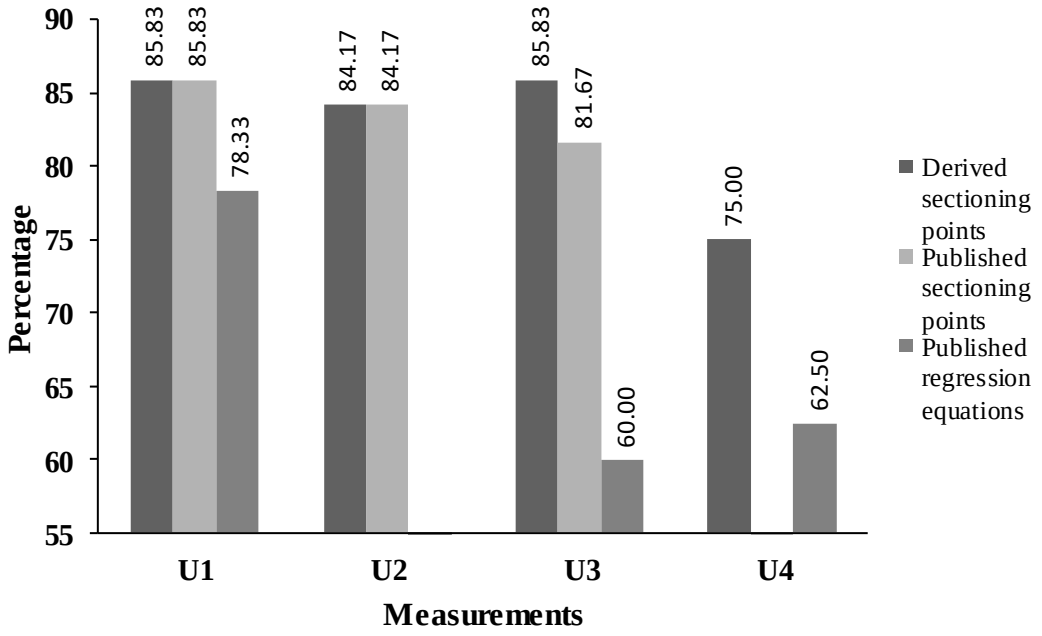

Figure 4.3: Comparison of classification accuracies for the ulna using the different standards for classification.

As can be seen in Figure 4.4, the accuracies obtained when using the humeral measurement sectioning points of the present study were similar to those obtained when using the published sectioning points, with less than 4% difference for all four measurements. In contrast to the previous findings for the radius and ulna, the accuracies obtained when using the published regression equations and those obtained when using the Free State sample sectioning points did not differ as substantially. The classification accuracies of the present

study and the published regression equations for measurements H1, H2, and H4 were similar, while the accuracy of the regression equations was 5.83% lower for the H3 measurement.

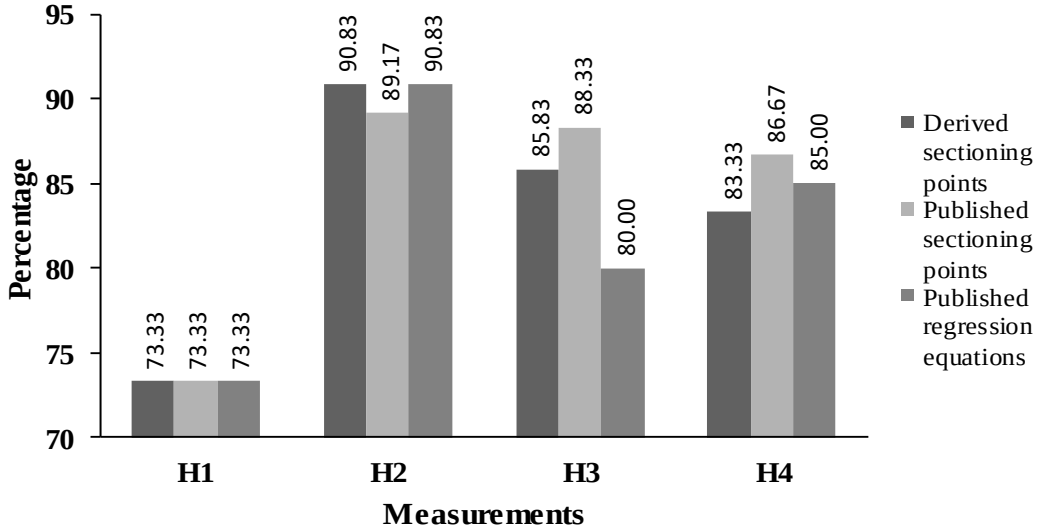

Figure 4.4.: Comparison of classification accuracies for the humerus using the different standards for classification.

For the clavicle, published standards existed only for measurements C1 and C3, thus only these could be compared (Figure 4.5). The accuracy obtained using the Free State-derived sectioning point of C3 was equal to that obtained when using the existing sectioning points, and only 2.50% higher than that of the regression equations. For measurement C1, the accuracies were similar for the Free State and published sectioning points, though the regression equation accuracy was again much lower, with a difference of 17.50% noted.

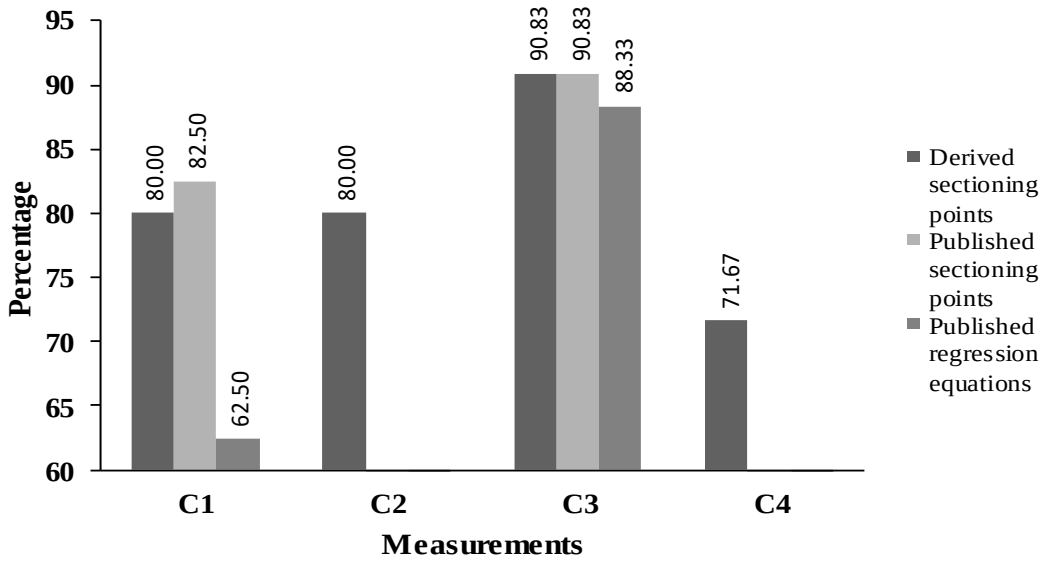

Figure 4.5: Comparison of classification accuracies for the clavicle using the different standards for classification.

Overall, as seen in Figure 4.6, the accuracies obtained when using the Free State-derived scapular sectioning points were higher than those obtained using the published sectioning points. The only exception was for the S2 measurement, for which the accuracy of the Free State sectioning points was only 2.50% lower. Published regression equations were found only for measurements S3 and S4, thus only these could be compared. For both measurements, the regression equations were outperformed by the derived sectioning points of the present study, with a difference of 30.00% noted for the S3 measurement, and 16.67% for the S4 measurement.

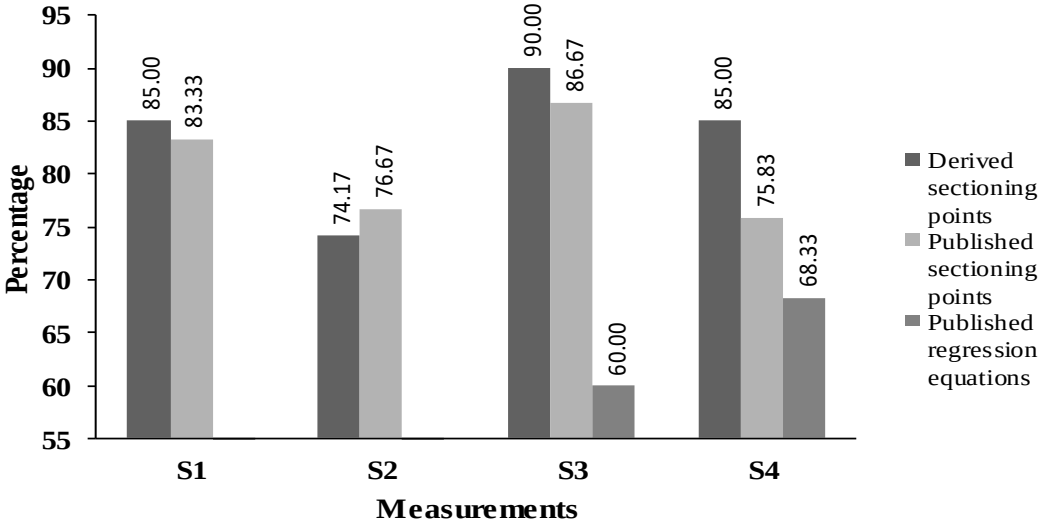

Figure 4.6: Comparison of classification accuracies for the scapula using the different standards for classification.

Chapter 5: Discussion

The results show significant differences between sexes for all of the bones examined, thus the variation can be expected to be useful in classifying sex with reasonable accuracy. The obtained accuracies were compared to those recorded in the existing literature. In comparison to the published standards, some of the accuracies obtained with the Free State derived standards were higher, suggesting that regionally-specific standards should be considered more appropriate for forensically use. However, some classification accuracies were lower than those of the published standards, suggesting that highly regionally-specific standards may be misleading. These results indicate that the applicability of the existing South African standards on groups other than the ones they have been derived from must be assessed and adapted if necessary to optimise the accuracy with which they can be used to estimate parameters such as biological sex.

5.1. Differences between Left and Right

The present study found that there was a significant difference between the left and right sides for most of the upper limb bone measurements ($p \leq 0.04$; Table 4.3). In most of these cases, the mean values of the right side were larger than those of the left side. A significant difference between the sides has been noted in many other studies (Selvaraj *et al.*, 1998; Sharma *et al.*, 2013; Sparacello, *et al.*, 2017). These studies have found the muscles and bones of the right side to be larger and heavier than those of the left, which is likely associated with the development of side dominance (Dogra & Singh, 1970; Chhibber & Singh, 1972; Blackburn, 2011; Bongiovanni & LeGarde, 2018). The development of left-side/right-side dominance has been shown to be the result of asymmetry based on genetic, physiological, and biomechanical influences, such as handedness (Ruff & Jones, 1981; Lazenby, 2002; Charisi *et al.*, 2011; Moore *et al.*, 2016). Furthermore, directional asymmetry is the result of innate factors, such as left-/right-side difference in blood oxygen levels that can potentially lead to unequal bone growth and side partiality in the skeleton (Lazenby, 2002; Mays, 2002; Blackburn, 2011). Additionally, there may be an increase in appositional growth due to potential occupation-related adaptations on the side of greater mechanical stress because of its increased use, whether it is the dominant or non-dominant side (Charisi *et al.*, 2011; Ahmed, 2013). It is also of interest to note that right-handed individuals exhibit a

greater degree of asymmetry, followed by bimanual individuals, while the side differences in left-handed individuals were not statically significant (Charisi *et al.*, 2011).

In the present study, the measurements of the left side were larger than the right side for only the olecranon breadth (U3), the maximum clavicular length (C1), and the scapular breadth (S2) measurements (Table 4.2). This contralateral pattern may be a function of the general asymmetry of the thorax transferred to the limb, as well as part of the biomechanical function of the upper limb (Auerbach & Ruff, 2006; Auerbach & Raxter, 2008). The olecranon breadth (U3) is measured at a region of the ulna that plays a part in the formation of the carrying angle of the upper limb at the elbow, with the left side carrying angle previously reported to be larger than the right in both males and females (Paraskevas *et al.*, 2004; Fasova & Timonov, 2017). This difference in carrying angle may be the result of asymmetrical bone growth, as joints are modelled through loading, thus areas with greater mechanical stresses on the cartilaginous epiphysis grow slower than areas with less strain (Paraskevas *et al.*, 2004; Plochocki, 2004; Drapeau, 2008; Niinimäki *et al.*, 2013). It is thus possible that the larger U3 measurement observed in the present study is a reflection of some of these mechanical stresses acting at the elbow joint. Similar to the larger dimension of the clavicular length observed in the present study, Auerbach & Raxter (2008), Sharma *et al.* (2013) and Waidhofer & Kirchengast (2015) reported that the left clavicle of right-handed individuals was longer, while the curve of the right clavicle became greater and led to a shortened bone on the right during the development of right-left handedness. It is thus possible that the observed larger C1 measurement in the present sample is reflective of similar clavicular development since it can reasonably be expected that the majority of the sample were right-handed (Blackburn, 2011; Charisi *et al.*, 2011; Bongiovanni & LeGarde, 2018). Lastly, the greater scapular breadth (S2) of the left side in the present study may be due to variable ossification of the medial borders of the scapula, due to the appearance and fusion of the elongated plates during adolescence (Hrdlička, 1942; White & Folkens, 2005; White *et al.*, 2012). Furthermore, it may be affected by the balancing force of the superficial (appendicular) muscle group that inserts onto it, to ensure an upright posture (Drake *et al.*, 2015; Osborn & Homberger, 2015; Kim, 2017).

Only four measurements in the present study showed no significant difference between sides (Table 4.3), namely the transverse diameter of the ulna (U4), the acromial epiphyseal width of the clavicle (C4), and the scapular height (S1) and breadth (S2). A possible explanation for

the lack of difference between sides in these measurements may be that the areas incorporated by these measurements may have been affected by muscle activity that is expected to act similarly on both sides of the body (Scheuer, 2002; White & Folkens, 2005). The transverse diameter of the ulna (U4) may have been enlarged during development so as to withstand the opposing forces of the muscles that originate, insert and act across this area to support and stabilise the joints during transmission of weight and simple movements, such as carrying, swinging or throwing (Drapeau, 2008; Niinimäki *et al.*, 2013; Osborn & Homberger, 2015; Kazi *et al.*, 2017). It is thus possible that the observed lack of difference in these measurements is the result of all these muscle actions, which are likely to act on both sides of the body, and thus would result in little, if any, differences between the sides.

5.2. Correlation of variables

All the measurements taken of the upper limb bones showed a significant positive correlation to each other (Table 4.4), with correlation strength ranging from weak (R=0.36) to very strong (R=0.99). Previous studies have found that there is a definite and proportional biological relationship between the body parameters, such as the head, face, trunk and limbs and the body size or height of an individual (Mansur *et al.*, 2014; Mumtaz & Sharman, 2015; Lukpata *et al.*, 2016). Thus, the larger an individual, the larger all parts of their skeleton and the more robust the areas of articulation and muscle insertion on the bone are expected to be (Charisi *et al.*, 2011; Cabo *et al.*, 2012). The pattern of correlation found in this study is similar to those of previous studies, where bones that are closest to each other, such as the radius and ulna or the humerus and scapula, have strong correlations with each other, due to their close anatomical position and their relative proportions to each other and the overall body size (Sehrawat & Pathak, 2016; Anzellin & Toyne, 2019). In contrast, the clavicle, which is the only horizontally oriented bone of the limb, has a greater variation in both size and shape and thus has weaker correlations with other elements (Auerbach & Sylvester, 2011). Though the general pattern of correlation found in this study and the literature show similarities between different population groups, it is important to note that the proportionality of the different body parts can be affected by internal factors such as genetics or external factors such as environmental conditions, resulting in exact proportions differing between populations, individuals, and even between pairs of bones within the same individual

(Ahmed, 2013; Howley *et al.*, 2018; Maass & Friedling, 2018). Hence it is important to evaluate these correlations in different samples.

Within each bone, measurements were all positively correlated to each other. This is likely because the long bone dimensions such as the articular breadths, maximum lengths and midshaft diameters are generally in proportion with each other and with body size, and will thus show covariance (Sehrawat & Pathak, 2016; Anzellin & Toyne, 2019). The lengths of the upper limb bones also had strong correlations (R=0.72–0.99; Table 4.4) with each other. This is likely because of the linear relationship of the upper limb bones with each other, and body proportions of an individual, which are genetically determined and tend to show covariance (Turan *et al.*, 2005; Celbis & Agritmis, 2006; Howley *et al.*, 2018; Gamble, 2020). Furthermore, the upper limb bone lengths have been found to be less susceptible to environmental stresses during growth and development than the lower limb bones (Holliday & Ruff, 2001; Weinberg *et al.*, 2017; Anzellin & Toyne, 2019). For this reason, a general proportionality of the upper limb dimensions can be expected (Jantz & Jantz, 1999; Cabo *et al.*, 2012), with larger individuals having larger measurements and smaller individuals having smaller measurements, as seen in the present study.

The areas of joint articulation also had strong correlations to each other (R=0.81–0.88; Table 4.4). These correlations can be divided into two groups. The first group consisted of correlations between different joints of the same bone, for example the maximum vertical head diameter (H2) and the epicondylar breadth (H4) of the humerus. This correlation could be the result of general proportionality with each other and body size (Sehrawat & Pathak, 2016; Anzellin & Toyne, 2019). The second group of correlations consisted of corresponding parts of the same joint but on different paired bones, for example as seen between the vertical head diameter (H2) and the glenoid cavity height of the scapula (S3) and/or the glenoid cavity breadth (S4), which are all measurements of components of the glenohumeral joint. A strong correlation was expected, as it reflects the response of the bones to forces from the muscles that act across the joint areas, and which transfer weight from the appendicular to the axial skeleton (France, 1988; Purkait & Chandra, 2004; Cabo *et al.*, 2012; Horbaly *et al.*, 2019).

The clavicle generally produced the weakest correlations (R=0.36–0.68; Table 4.4) with measurements of the other elements. This may be the result of it being the only horizontally orientated bone of the upper limb, and it having more variation in its size and shape as the

result of muscle activity from muscles that originate, insert and act across its various parts (Tanner, 1978; Drapeau, 2008; Niinimäki *et al.*, 2013; Osborn & Homberger, 2015). Furthermore, the clavicle may also be affected by the different types and rates of ossification of its different parts. The clavicle begins the process of ossification during embryological development, but only completes the process between 20 to 30 years of age (White & Folkens, 2005; Cunningham *et al.*, 2016; Hyland *et al.*, 2020). The lateral end of the clavicle forms via intramembranous ossification between 18 to 20 years of age, while the medial end undergoes endochondral ossification between 16 to 30 years of age (Prescher, 2000; Cunningham *et al.*, 2016; Hyland *et al.*, 2020). It is consequently one of the late-maturing bones of the body, and it is more susceptible to environmental stresses during its prolonged growth and development period (White & Folkens, 2005; Murphy, 2002; Kralik *et al.*, 2014; Cunningham *et al.*, 2016). A result of the different ossification processes of the parts of the clavicle and the expected variability of environmental influences on its later maturing part is that its dimensions are likely to show more variation than bones that mature earlier, and thus it is not surprising that the correlations of the sternal epiphyseal width (C2; R=0.36–0.59) and the acromial epiphyseal width (C4; R=0.48–0.68) show weaker correlations to other measurements of the upper limb bones.

5.3. Sexual dimorphism

All of the measurements taken of the upper limb bones differed significantly between the sexes, with all $p<0.001$ (Table 4.6). For each of the upper limb measurements, males were found to have larger means than females, though there was a noticeable overlap between the ranges of the sexes for most of the measurements (Table 4.5). This corresponds to the documented global trend of males being larger than females for many skeletal elements, including those of the upper limb, as indicated for German, Japanese, Greek, American and Colombian samples (Mall *et al.*, 2001; Sakaue, 2004; Charisi *et al.*, 2011; Spradley & Jantz, 2011; Moore *et al.*, 2016). Generally, the difference in size and shape between males and females within the same population is attributed to differences in genetics, as well as differences in hormone levels and growth rates from puberty onwards (Purkait & Chandra, 2004; White & Folkens, 2005). The degree of sexual dimorphism between populations, however, may differ due to the genetic variation as a result of gene mutation, genetic drift and/or environmental influences (White & Folkens, 2005; Charisi *et al.*, 2011). The degree of

sexual dimorphism in the skeletal remains of South African individuals has been shown to be less than that of other global populations, which may be due to external influences such as climate, altitude, availability of and access to nutrition, health care and physical activity (Steyn & İşcan, 1998 & 1999; White & Folkens, 2005; Maass & Friedling, 2018).

In the present study, the largest proportional difference between the means of the males and females was found in the sagittal diameter at the midshaft (C3) and the acromial epiphyseal width (C4) of the clavicle, as well as in the anterior-posterior diameter at the midshaft of the radius (R3), with a difference of more than 15% (Table 4.5). Compared to the lower degree of dimorphism observed for the other measurements, this larger degree of sexual dimorphism was expected. The midshaft diameters and the epicondylar width measurement areas are greatly affected by mechanical stresses, as well as by the genetic and physiological factors that affect the growth and development in both sexes (Akhlaghi *et al.*, 2012; Charisi et al., 2011; Anzellin & Toyne, 2019). Thus, the larger an individual is the larger and more robust the area of articulation and insertion will be, resulting in males with larger muscles, and having noticeably larger muscle attachment sites than females for these measurements (France, 1988; Cabo *et al.*, 2012). Some females may, however, be larger and more robust, and thus have larger bones with larger muscle attachment sites (Akhlaghi *et al.*, 2012; Charisi et al., 2011; Anzellin & Toyne, 2019).

The smallest differences between the sexes were in the maximum lengths of the radius (R1), ulna (U1), humerus (H1) and clavicle (C1), and the physiological length of the ulna (U2) – all with less than 10% difference between the means of males and females (Table 4.5). This smaller degree of dimorphism may be because the lengths of these bones are determined genetically, and are minimally affected by environmental stresses or other factors that may result in differences in morphology between the sexes (Anzellin & Toyne, 2019; Gamble, 2020). Once the epiphyseal plates of these bones have fused, the bones can no longer grow in length, but can only grow appositionally (in thickness) to accompany the larger muscle attachments (France, 1988; White & Folkens, 2005; Ross & Pawlina, 2011). The difference between the sexes was also small for the scapular breadth (S2). This may again be because the size of the scapula is genetically determined, as well as because the muscles that originate on the scapula function in movement or posture and not in the transfer of shock or weight from the upper limb to the axial skeleton (Drake *et al.*, 2015; Osborn & Homberger, 2015).

Thus the force applied does not affect its growth as much as at the other areas, resulting in less distinction between the sexes (Hrdlička, 1942; Osborn & Homberger, 2015).

5.4. Classification Accuracy

The accuracy of the estimation of sex was calculated for all the measurements. For most measurements, the accuracies did not differ substantially (≤5%) between the sexes (Table 4.8). However, the measurements of the maximum length of the humerus (H1), the epicondylar breadth of the humerus (H4), the maximum length of the clavicle (C1) and the scapular breadth (S2), were up to 13.33% higher in females than in males. It was expected that these measurements would be less accurate when used to identify males, as the percentage difference between the sexes were as low as 7.70% (Table 4.5). This may be because the females are more distinctive than males, while males who have larger ranges for the measurements are less distinctive. The accuracies of the females may also be higher than that of the males in this study, as the males in this population may be smaller and more gracile as they may be affected to a greater extent by environmental stresses during their growth and development period (Steyn & İşcan, 1998; İşcan & Steyn, 1999; Jantz & Jantz, 1999; Charisi *et al.*, 2011). This may be a reflection of the impact of socio-economic conditions on the skeletal morphology of Black South African individuals, which constitute the sample of this study. Before 1994, under the Apartheid government, South African "race" groups were segregated both geographically and socially by the country's Apartheid legislation (Harris *et al.*, 2011; Liebenberg *et al.*, 2015; Maass & Friedling, 2018 & 2019a). This mostly resulted in poor socio-economic conditions in the areas allocated to Black and Coloured groups, where individuals experienced crowded living conditions, poor housing and sanitation and with limited access to quality nutrition and health care (Stull *et al.*, 2014; Maass & Friedling, 2019a). Many of these conditions have been shown to have an impact on the general growth of a population, and thus it is likely to result in a decrease in the degree of sexual dimorphism within and between the population groups (Steyn & İşcan, 1999; Puoane *et al.*, 2002; Liebenberg *et al.*, 2015; Maass & Friedling, 2018). Since the growth of males is expected to be more sensitive to such environmental stressors than that of females, who appear to be protected due to their vital role in procreation, the decrease in the degree of sexual dimorphism within a population is mostly due to males developing with more gracile morphologies (Stini, 1969; Tobias, 1972; Hall, 1978; Jantz & Jantz, 1999). After 1994,

Apartheid was abolished, leading to significant changes in socio-economic conditions such as an increase in access to and quality of health care, wellness and safety of Black and Coloured individuals (Steyn *et al.*, 1997; Cole 2003; Maass & Friedling, 2018). It is expected that in the years to come, a positive secular trend may be detected in the skeletal morphology and expression of sexual dimorphism in these groups as a result of these improvements (Puoane *et al.*, 2002; Hawley *et al.*, 2009). Thus, the population-specific standards for this, as well as other local and global populations, must be re-evaluated at a later time to determine if any secular trends arise or change between generations (Charisi *et al.*, 2011).

In the pooled sample, the vertical head diameter of the humerus (H2), the sagittal diameter at the midshaft of the clavicle (C3), the maximum head diameter (R2), the anterior-posterior diameter at the midshaft of the radius (R3), and the glenoid cavity height of the scapula (S3) were the most accurate measurements for sex estimation, with accuracies of approximately 90%. Based on the earlier observation that the means of these measurements differed significantly with up to 19% between the sexes (Table 4.5), it was expected that the sexes would be more distinctive and would allow higher classification accuracies than other measurements with smaller differences between male and female means. The high classification accuracies of the radial head diameter (R2), the vertical head diameter of the humerus (H2), and the glenoid cavity height of the scapula (S3) may be because these areas were affected by the force of the muscles that act across the relevant joints that transfer weight and shock from the appendicular skeleton to the axial skeleton (France, 1988; Purkait & Chandra, 2004; Cabo *et al.*, 2012). Thus, males were expected to have larger and more robust areas of articulation and insertion than females, due to differences in internal factors such as genetics and longer adolescent growth periods and external factors such as sex-specific occupations (Purkait & Chandra, 2004; White & Folkens, 2005; Lesk, 2012; Sadler, 2015). Additionally, females have a larger range of motion than males at the glenohumeral joint, due to having smaller muscles and less robust articulation areas and muscle insertion points (Prescher, 2000; Vance *et al.*, 2011; Moore *et al.*, 2016), thus the measurements of both the humeral head and glenoid of the scapula are expected to be sufficiently smaller in females than in males. As a result, measurements of both these surfaces are expected to produce high classification accuracies, as seen in the vertical head diameter (H2) and the glenoid cavity height (S3) measurement of the present study. For the anterior-posterior diameter at the midshaft of the radius (R3) and the sagittal diameter at the midshaft of the clavicle (C3), the high accuracies of sex estimation observed in the present study may be

because of an increase in the diameter of the bones through appositional growth (Vance *et al.*, 2011; Lesk, 2012; Moore *et al.*, 2016). This may be the result of the higher torques and mechanical stress being placed on these skeletal components due to sex-specific division of labour, with males expected to be larger than females (Prescher, 2000; Vance *et al.*, 2011; Cabo *et al.*, 2012; Lesk, 2012).

For the pooled sample, the measurements with the weakest sex classification accuracies were the transverse diameter of the ulna (U4), the maximum length of the humerus (H1), the acromial epiphyseal width of the clavicle (C4), and the scapular breadth (S2). The maximum length of the humerus (H1) and the scapular breadth (S2) had accuracies of 73.33% and 74.17%, respectively. These relatively low accuracies may be because the percentage differences between the means of the sexes were low (<8%) for these measurements (Table 4.5), as well as the largest ranges observed for males which suggest their morphologies to be less distinctive than the females, as previously discussed. The transverse diameter of the ulna (U4) and the acromial epiphyseal width (C4) also had relatively low accuracies of 75.00% and 71.67%, respectively. These were lower than expected, as these measurements had a high degree of sexual dimorphism (14% and 17%, respectively; Table 4.5), likely due to sexual differences in the forces acting across the humero-ulnar and acromioclavicular joints, respectively. The lower accuracies, however, may be due to internal factors such as genetics and hormone levels and/or external factors such as malnutrition or inactivity which resulted in smaller and more gracile males, as discussed above (Tobias, 1972; Hall, 1978; Ahmed, 2013; Maass & Friedling, 2018).

5.5. Accuracy of existing univariate methods

The accuracies obtained when using the sectioning points derived from the Free State sample were generally equal to or higher than when applying either the published sectioning points or regression equations which are based on South African and other samples.

For most measurements, the accuracies did not differ substantially (≤5%) between the derived and published sectioning points or between the derived sectioning points and the published regression equations for the H1, H2, H4 and C3 measurements (Figures 4.2 to 4.6). This may be because the expression of sexual dimorphism in the present study sample is similar to that of the South African and global samples on which the published classification

standards were based (Safont, 2000; Charisi *et al.*, 2011). This similarity in the expression of sexual dimorphism may suggest that the internal and external factors that may affect the expression of sexual dimorphism in different groups may not have had a substantial effect on these specific bone dimensions due to common ancestry and/or geographic proximity (Albanese *et al.*, 2005; Charisi *et al.*, 2011). The fact that the difference in accuracy between the populations is low may suggest that there is no practical reason to develop region-specific sectioning points; however, using sectioning points that are based on samples as similar as possible to the current sample should still be considered best practice in a forensic context till further studies can determine how specific the regional standards must be (Albanese *et al.*, 2005).

There were, however, some exceptions such as the glenoid cavity breadth of the scapula (S4), the maximum head diameter (R2) and the anterior-posterior diameter at the midshaft of the radius (R3) for which the differences in the accuracies between the Free State-derived and the published sectioning points were up to 9.17% (Figures 4.2 and 4.6). This large difference in the accuracies between the derived and published sectioning points may be because the level of sexual dimorphism in some skeletal elements of the Free State sample appears to be lower than that of the rest of the South African and global populations (Steyn & İşcan, 1998; Steyn *et al.*, 1997; Barrier & L'Abbe, 2008). The Free State population is expected to differ significantly from other South African and global populations in terms of its demographic composition, socio-economic conditions, and environmental influences – all of which may influence the expression of sexual dimorphism (Steyn & İşcan, 1999; L'Abbé & Steyn, 2012; Scott *et al.*, 2018). The Free State population consists of the largest provincial Sotho/Southern Sotho constituency in South Africa, with relatively high levels of unskilled labour and unemployment, especially among the Black population (Quarterly Labour Force Survey, 2019). In contrast, the demographic composition of the Pretoria and Dart collections, on which most South African classification standards are based, consists mostly of Black Zulu individuals, while the Stellenbosch collection consists mostly of Coloured individuals, and the University of Cape Town collection consists mostly of White individuals (L'Abbé *et al.*, 2005; Dayal *et al.*, 2009; Albas *et al.*, 2018; Maass & Friedling, 2019b). Furthermore, environmental influences such as the rainy summers and frosty winters may also result in regional specific adaptations of the individuals living in the Free State province in accordance with Bergmann's rule to decrease heat loss in colder climates (Ruff, 2002; White & Folkens, 2005; Wells, 2012). Thus it may be more appropriate to use Free State-derived sectioning

points for these measurements, as the available published South African and global population sectioning points may not be reliable for sex estimation in this population if the expression of sexual dimorphism in skeletal morphology differs from those of the populations on which the published sectioning points were based (Charisi *et al.*, 2011; Peckmann *et al.*, 2016 & 2017; Scott *et al.*, 2018).

The present study also allowed the calculation of sectioning points for the sternal epiphyseal width (C2) and the acromial epiphyseal width (C4) of the clavicle where it was previously not available. Though the sex classification accuracies of these measurements were lower than 75%, which is lower than forensically acceptable, these measurements may still be used to allow for some estimation of sex in cases where other measurements are not possible (Steyn & İşcan, 1998; Steyn & İşcan, 1999). As these sectioning points were developed on a Free State population, it is unknown if they are appropriate to the rest of the South African or other global samples, and should thus be re-evaluated in future studies.

Overall, the sex classification accuracies obtained when using the existing regression equations for the upper limb bones were significantly lower than those obtained using the Free State-derived section points, with a difference of between 10–40% (Figures 4.2 to 4.6). This was expected as the regression equations are considered to be population- and region-specific, and the populations are expected to differ due to internal factors such as genetics and external factors such as altitude, climate, as well as socio-economic factors, as discussed above (Frutos, 2005; Duyar & Pelin, 2010; Charisi *et al.*, 2011). Furthermore, the degree of sexual dimorphism may also differ between populations even if they have geographic proximity, due to localised genetic variations and/or living conditions which may affect one, but not another close-by group (İşcan *et al.*, 1998; Barrier & L'Abbé, 2008; Charisi *et al.*, 2011). Thus the standards developed on one population group should not be applied to others if there is reason to suspect that the groups are likely to differ in expression of dimorphism or until such time as can be assessed (İşcan *et al.*, 1998; Barrier & L'Abbé, 2008; Charisi *et al.*, 2011). It was also noted that the accuracies obtained when using the regression equations were as low as 50% for the Free State sample (Figure 4.2), which is much lower than the 80% accuracy considered forensically acceptable (Peckmann *et al.*, 2016 & 2017). Thus the published regression equations are not considered applicable in a forensic context for a Free State sample, as it may result in a decrease in the accuracy of classification of sex and the biological profile as a whole (Charisi *et al.*, 2011; Peckmann *et al.*, 2016 & 2017; Scott *et al.*,

2018). Instead, it would be more appropriate to use the Free State-derived sectioning points or even the published sectioning points than the regression equations, as their accuracies were higher. Overall, the Free State-derived sectioning points performed better than both the published sectioning points and the regression equations. Though the published sectioning points can be used in the Free State sample with reasonable accuracy that may be forensically acceptable, the regression equations cannot, due to the accuracies being lower than the 80% accuracy considered forensically acceptable (Peckmann *et al.*, 2016 & 2017). Furthermore, some of the sectioning points and regression equations have been shown to be population- and region-specific, and should thus be applied with caution on this Free State population if no other classification standards are available. Though many of the differences in classification accuracy are small, it should still be considered best practice to use standards that are as close to the sample as possible to reduce the potential effect that the population-level differences in skeletal morphology may have. Thus it may be appropriate to use South African or even global standards for sex estimation using some measurements, while others might benefit from using more regionally specific standards as this may lead to higher classification accuracies, as seen in the present study of a Free State skeletal sample. The extent to which regional-specific standards may be necessary still requires further investigation. Further study may therefore be needed using a new Free State sample and other South African samples as they are made available to determine if regional-specific standards are necessary and/or if population-specific standards will be sufficient.

Chapter 6: Conclusion

Along with the increase in both legal and illegal immigrants, there has been an increase in the rate of crime and urbanisation in South Africa and other parts of the world. This has, in turn, led to an increase in the number of incomplete unidentified remains discovered which require anthropological analysis. The forensic anthropologist assists in the compilation and assessment of a biological profile that can aid in the identification of such remains. The components of the profile are, however, only estimates and can vary between and within populations. Sex is usually the first estimate that is made, as the estimation of other components of the profile may be dependent on it. The sex of the individual can be estimated with either a morphological or metric approach, by preferably using bones that are highly sexually dimorphic and thus most likely to yield accurate classifications. The accuracy of the estimation is, however, dependent on which bones are available. The pelvis and cranium, which are considered the most suitable for this purpose, may not always be present due to taphonomic processes. Thus it is important to also study bones that may be less sexually dimorphic such as the upper limb bones or even bones with a low recovery rate so that estimations can be made should it so happen that these are the only bones available for analysis. Previous studies show high accuracies when using the bones of the limb, separately or in conjunction with other bones for sex estimation, because of the variation in size and shape between sexes. However, populations can differ in expression of dimorphism, thus it is important that standards for classification must also be developed and evaluated for applicability in different population groups.

The purpose of this study was to assess the sexual dimorphism of the bones of the upper limb in a Free State skeletal population using univariate measurements. Four measurements of each bone were used with univariate analysis. Similar to other studies, it was found that there was some difference between the left and right sides, with the right side being larger, which emulated the global trend and may be the result of the development of handedness. However, it would be impractical to develop and use separate measurements for both the left and right side in a forensic context; thus the sample was further analysed as one pooled group. The study found that there was a significant positive correlation of all the univariate measurements with each other, which was expected as a result of the body's proportions. This may be because of greater variation in the size and shape of the clavicle itself due to the different types and rates of ossification of its different parts.

All measurements differed significantly between the sexes, with the males being significantly larger than females. This is consistent with the globally documented trend of males being larger and more robust than females for many skeletal elements. The percentage difference in dimorphism between the sexes was largest for the anterior-posterior diameter of the radius, the midshaft diameter of the clavicle and the acromial epiphyseal width of the clavicle. This is considered to be a reflection of general intrinsic and extrinsic size differences between the sexes for the former and lower susceptibility to external influences in the latter. The sex estimation accuracy of the measurements for the upper limb bones for the pooled sample was high, ranging from 71% to 90%. Though some of the accuracies obtained in this study were lower than the forensically acceptable 80%, these univariate measurements of the upper limb bones may still be used in a forensic context if the upper limbs or even segments thereof are the only bones recovered and available for analysis. Comparisons of the accuracies obtained using Free State-derived sectioning points and published sectioning points and regression equations were made. The published regression equations produced accuracies that were lower than expected, and should not be applied to this Free State sample if more appropriate classification standards, like the ones derived in this study, are available. The accuracies produced by the Free State sectioning points and the published sectioning points' accuracies were similar for many measurements, suggesting regionally specific standards may not be needed for these, though the Free State-derived sectioning points did yield higher accuracies for the other measurements, suggesting that regionally specific standards may require further investigation.

The present study has shown that many upper limb measurements are highly sexually dimorphic and can produce forensically acceptable levels of accuracy when used for sex estimation in this Free State skeletal population. Future studies may also be able to incorporate more bones or even a larger sample which includes populations from other areas of South Africa to determine the need for the expansion of population-specific standards of development of more regionally specific standards.

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Appendix A

Table A.1: Normality test for left and right sides.

Variables	p-value (L)	p-value (R)	p-value (Pooled)
R1	p>0.05	p>0.05	p<0.05
R2	p>0.05	p<0.05	p<0.05
R3	p<0.05	p<0.05	p<0.05
R4	p>0.05	p>0.05	p>0.05
U1	p>0.05	p>0.05	p<0.05
U2	p>0.05	p>0.05	p>0.05
U3	p>0.05	p>0.05	p>0.05
U4	p>0.05	p>0.05	p>0.05
H1	p>0.05	p<0.05	p<0.05
H2	p>0.05	p<0.05	p<0.05
H3	p>0.05	p>0.05	p>0.05
H4	p>0.05	p>0.05	p<0.05
C1	p>0.05	p>0.05	p>0.05
C2	p>0.05	p>0.05	p>0.05
C3	p<0.05	p<0.05	p<0.05
C4	p>0.05	p>0.05	p>0.05
S1	p>0.05	p>0.05	p>0.05
S2	p>0.05	p>0.05	p<0.05
S3	p>0.05	p>0.05	p<0.05
S4	p>0.05	p<0.05	p<0.05

Table A.2: Normality test for males and females.

Variables	p-value (F)	p-value (M)	p-value (Pooled)
R1	p>0.05	p<0.05	p<0.05
R2	p<0.05	p>0.05	p<0.05
R3	p>0.05	p<0.05	p<0.05
R4	p<0.05	p>0.05	p>0.05
U1	p>0.05	p>0.05	p<0.05
U2	p>0.05	p>0.05	p>0.05
U3	p>0.05	p>0.05	p>0.05
U4	p>0.05	p>0.05	p>0.05
H1	p>0.05	p>0.05	p<0.05
H2	p>0.05	p<0.05	p<0.05
H3	p>0.05	p>0.05	p>0.05
H4	p>0.05	p<0.05	p<0.05
C1	p>0.05	p>0.05	p>0.05
C2	p>0.05	p>0.05	p>0.05
C3	p<0.05	p>0.05	p<0.05
C4	p>0.05	p>0.05	p>0.05
S1	p>0.05	p>0.05	p>0.05
S2	p<0.05	p>0.05	p<0.05
S3	p>0.05	p>0.05	p<0.05
S4	p<0.05	p>0.05	p<0.05

Table A.3: Sectioning points used.

Variables	Author	Population	Criteria
R1	Spradley & Jantz (2011)	Black American	SP=241
R2	Barrier & L'Abbé (2008)	Black South African	SP=22
R3	Barrier & L'Abbé (2008)	Black South African	SP=11
R4	Barrier & L'Abbé (2008)	Black South African	SP=33
U1	Spradley & Jantz (2011)	Black American	SP=258
U2	Spradley & Jantz (2011)	Black American	SP=229
U3	Barrier & L'Abbé (2008)	South African	SP=24
U4		No Data	
H1	Ogedengbe *et al.* (2017)	Black South African	SP=307.72
H2	Ogedengbe *et al.* (2017)	Black South African	SP=40.73
H3	Ogedengbe *et al.* (2017)	Black South African	SP=19.43
H4	Ogedengbe *et al.* (2017)	Black South African	SP=57.88
C1	Tise *et al.* (2013)	Hispanic	SP=147
C2		No Data	
C3	Spradley & Jantz (2011)	Black American	SP=12
C4		No Data	
S1	Papaioannou *et al.* (2012)	Greek	SP=147
S2	Moore *et al.* (2016)	Colombian	SP=98.15
S3	Papaioannou *et al.* (2012)	Greek	SP=35.81
S4	Papaioannou *et al.* (2012)	Greek	SP26.82

The definitions of the abbreviations of the measurements of the bones as in Tables 3.1 to 3.5 in Materials and Methodology (Chapter 3).

Table A.4: Equations used.

Variables	Author	Population	Equation	Criteria
R1	Mall *et al.* (2001)	German	(R1x0.878)-20.340	SP=0; M>0; F<0
R2	Barrier & L'Abbé (2008)	Black South African	(R2x0.64)-14.02	MIP=0.83; FIP= -0.83
R3	Barrier & L'Abbé (2008)	Black South African	(R3x0.98)-10.88	MIP=0.78; FIP= -0.78
R4	Barrier & L'Abbé (2008)	Black South African	(R4x0.48)-15.71	MIP=0.83; FIP= -0.83
U1	Mall *et al.* (2001)	German	(U1x0.767)-19.188	SP=0; M>0; F<0
U2			No Data	
U3	Barrier & L'Abbé (2008)	Black South African	(U3x0.51)-12.43	MIP=0.78; FIP= -0.78
U4	Charisi *et al.* (2011)	Greek (Right side)	(U4x1.52259)-35.9847	SP=0; M>0; F<0
H1	Charisi *et al.* (2011)	Greek (Left side)	(H1x0.12076)-37.1895	SP=0; M>0; F<0
H2	Sakaue (2004)	Japanese	(H2x0.514)-21.320	SP=0; M>0; F<0
H3	Frutos (2005)	Guatemalan (max)	(H3x5.828)-11.826	SP=-0.1135
H4	Charisi *et al.* (2011)	Greek (Right side)	(H4x0.80501)-46.0255	SP=0; M>0; F<0
C1	Scott *et al.* (2018)	Black South African	(C1x0.093)-13.63	SP=0; M>0; F<0
C2			No Data	
C3	Scott *et al.* (2018)	Black South African	(C3x0.661)-8.21	SP=0; M>0; F<0
C4			No Data	
S1			No Data	
S2			No Data	
S3	Macaluso (2011)	Black South African	(S3x0.179)-6.419	SP=0.5; M>0.5; F<0.5
S4	Macaluso (2011)	Black South African	(S4x0.215)-5.393	SP=0.5; M>0.5; F<0.5

The definitions of the abbreviations of the measurements of the bones as in Tables 3.1 to 3.5 in Materials and Methodology (Chapter 3).

Table A.5: Classification accuracies of the Free State sample obtained when using published sex estimation univariate demarcation points.

Variables	Female correct	%	Male correct	%	Pooled correct	%
R1	53	88.33	52	86.67	105	87.50
R2	58	96.67	43	71.67	101	84.17
R3	43	71.67	58	96.67	101	84.17
R4	53	88.33	54	90.00	107	89.17
U1	52	86.67	51	85.00	103	85.83
U2	51	85.00	50	83.33	101	84.17
U3	46	76.67	52	86.67	98	81.67
U4			No Data			
H1	43	71.67	45	75.00	88	73.33
H2	52	86.67	55	91.67	107	89.17
H3	58	96.67	48	80.00	106	88.33
H4	52	86.67	52	86.67	104	86.67
C1	50	83.33	49	81.67	99	82.50
C2			No Data			
C3	55	91.67	54	90.00	109	90.83
C4			No Data			
S1	57	95.00	43	71.67	100	83.33
S2	46	76.67	46	76.67	92	76.67
S3	55	91.67	49	81.67	104	86.67
S4	58	96.67	33	55.00	91	75.83

Table A.6: Classification accuracies of the Free State sample obtained when using published sex estimation univariate regression equations.

Variables	Female correct	%	Male correct	%	Pooled correct	%
R1	34	56.67	58	96.67	92	76.67
R2	50	83.33	24	40.00	74	61.67
R3	12	20.00	48	80.00	60	50.00
R4	36	60.00	38	63.33	74	61.67
U1	37	61.67	57	95.00	94	78.33
U3	33	55.00	39	65.00	72	60.00
U4	59	98.33	16	26.67	75	62.50
H1	43	71.67	45	75.00	88	73.33
H2	54	90.00	55	91.67	109	90.83
H3	59	98.33	37	61.67	96	80.00
H4	47	78.33	55	91.67	102	85.00
C1	37	61.67	38	63.33	75	62.50
C3	58	96.67	48	80.00	106	88.33
S3	60	100.00	12	20.00	72	60.00
S4	58	96.67	24	40.00	82	68.33

U2, C2, C4, S1 and S2 do not have any published equations.