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Sexual Dimorphism of the Second Cervical Vertebra in Humans

by

Morgan Paskins

Under the Direction of Frank L'Engle Williams, Ph.D.

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Arts
in the College of Arts and Sciences
Georgia State University
2023

ABSTRACT

The pelvis and skull are frequently examined for the expression of sex-linked traits as are the humeral and femoral head dimensions. The second vertebra allows for rotation of the head, which is larger in males than in females. The axis is positioned close to other traits that have been shown to exhibit dimorphism, such as the mastoid process, gonial region, nuchal area, and the occipital protuberance. To explore which dimensions of the axis differ the most between females and males, and investigate its relationship to age, 149 individuals from the W.M. Bass Osteological Collection at the University of Tennessee, Knoxville were measured using 13 linear distances. The three age cohorts included 30-35, 50-55, and 70-75 years. The results indicate that all the traits show significant differences between the sexes. Using discriminant function analysis, predictive functions were created to estimate the sex of unknown individuals using 6 traits, 4 traits, 3 traits and 2 traits.

INDEX WORDS: Sexual dimorphism, Vertebrae, Axis, Biological profile, Anthropology, Statistical analysis

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Office of Graduate Services
College of Arts and Sciences
Georgia State University
May 2023

DEDICATION

I would like to dedicate this thesis to my parents who have supported me in every way - their sacrifices have not gone unnoticed. Thank you to my wonderful partner, Hanna, and our plethora of pets whose fur was usually used to wipe tears. To the Possum Posse/Chaos Incarnate and KOOKIES, thank you for keeping me smiling, keeping the wine flowing, and d20s rolling.

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LIST OF ABBREVIATIONS

AMA - Maximum height of axis

CMA - Most anterior part of the vertebra to the most posterior point

DSD - Dens sagittal diameter

DTD - Dens transverse diameter

DMFS - Distance between superior facets

CMFS - Max sagittal length of superior facet

LMFS - Max transverse width of superior facet

CMFV - Anterior to posterior measurement of foramen

DSMC - Sagittal diameter of vertebral body

LMFV - Max transverse diameter of foramen

AMD - Dens tooth length from the max height of dens to the superior articular facet

DTMC - Transverse diameter of vertebral body

LMA -Max vertebral width from the extreme side of the transverse process

1 INTRODUCTION

The purpose of this research is to explore a different approach to sex estimation. By utilizing the second cervical vertebrae as a means of sex estimation, there is the opportunity for it to serve as another means of developing a biological profile. The data collection uses many measurements deriving from past research, which are selected due to high accuracy rates (Gama et al. 2015; Wescott 2000; Medina 2011; Marlow and Pastor 2011).

A biological profile is a snapshot of traits a deceased individual could have possessed in life. This is used when a person is either fully skeletonized or partially skeletonized. A typical biological profile consists of sex estimation, age estimation, stature estimation, and, depending on the anthropologist, ancestry estimation (Bass 2006; Buikstra and Ubelaker 1994; Steele and Bramblett 1988; White and Folkens 2005; for critiques, see Williams et al., 2005). Skeletal trauma is also considered when constructing a case file (Bass 2006; Buikstra and Ubelaker 1994; Steele and Bramblett 1988; White and Folkens 2005). When constructing a biological profile, each portion of available information narrows down to the decedent's identity.

A correct sex estimation can halve the pool of potential decedents, increasing the likelihood of identification. It is important to note that sex traits can vary between humans, causing a biological male to exhibit feminine traits or vice versa. Due to this variation, it presents a challenge to studies that require two separate categories. While an individual may be considered male due to sex estimation methods, anthropologists should consider all context clues before deciding. Since gender and sexual identity vary, it is also important to note the usage of the term, ‘sex estimation.’ Unless the individual is alive and communicates their identity, anthropologists must estimate the decedent’s sex.

1.1 How Damage Can Affect Sex Estimation

Concerning the second cervical vertebrae, the fracturing of the dens accounts for 7-13% of all neck fractures (Jenkins et al. 2019). These fractures are broken into three types (Type I, Type II, and Type III), and all affect the morphology of the dens (Jenkins et al. 2019). While fractures such as Type I and Type III are considered stable and do not affect measurements, Type II is more violent and causes a separation of the dens from the vertebral body (Jenkins et al. 2019).

Another form of damage can occur during a sudden compression of the spine. If the decedent is involved in a high-speed motor accident, the atlas and axis can separate, driving the dens into the skull and severing the spinal cord. The severance is called an atlanto-occipital dissociation. As the dens passes into the skull and creates a ring fracture, damage can occur to the dens and the rest of the vertebrae, such as the transverse processes (Gaillard and Knipe 2022; Madadin et al. 2017). This thesis is interested in using sex estimation in a forensic context, so the possibility of damage to the vertebrae is of great concern.

1.2 Dimorphism of the Vertebral Column

Multiple studies have investigated the dimorphism surrounding the vertebral column (Allbright 2007; Amores et al. 2014; Banu et al. 2014; Bethard and Seet 2013; Ekizoglu et al. 2021; Gama et al. 2015; Kaeswaren and Hackman 2019; Marlow and Pastor 2014; Medina 2011; Rozendaal et al. 2019; Wescott 2000). The main goal of these studies is to examine the potential of using new and improved standards of measurement to estimate sex when traditional methods are unavailable or when the remains are comingled in the cases of multiple burials or mass graves. There is also interest in the cervical vertebrae. Since there is known sexual dimorphism of the skull, it is probable that the elements that bear the head's weight also present dimorphism (Bass 2006; Buikstra and Ubelaker 1994; Steele and Bramblett 1988; White and Folkens 2005).

1.3 Vertebral Column Anatomy

The vertebral column can be divided into four distinct regions. The superior of these is the cervical vertebrae which consist of seven vertebrae - all with unique morphologies. Beginning at the base of the skull, the atlas (C1) is a wide set bone lacking a true vertebral body and articulates with the axis (C2), the dens of which acts as the body of the C1. The dens or odontoid process on C2 is a large projection on the anterior portion of the bone that acts as a pivot which allows the head to swivel. Since C2 is tightly bound with ligaments, the axis is regarded as the strongest bone in the cervical column (Steele and Bramblett 1988). Inferior to the axis are C3 through C6 which share remarkably similar morphologies as they all contain attachment sites for the scalene muscles and approximately 12 other sets of muscles (Bridwell 2019). All remaining cervical vertebrae inferior to the axis exhibit transverse processes, a body, inferior and superior articular facets, spinous processes, transverse foramina, laminae, and pedicles (Bass 2006; Steele and Bramblett 1988; White and Folkens 2005).

However, C7 is unique among cervical elements in that it is a transitional bone that holds characteristics of the cervical and thoracic vertebrae. The C7 still has the transverse foramina, which allow blood flow and nerves to pass through, although, in size and shape, it reflects traits of the thoracic. The elongated spinous process of C7 can be easily palpated on the base of the neck. It signifies a change from lordosis to kyphosis in the spine, continuing the signature “S Curve” of the vertebral column.

The thoracic vertebrae consist of 12 individual bones with transverse processes, a body, pedicles, costal facets, demifacets, superior and inferior articular processes, and laminae (Bass 2006; Steele and Bramblett 1988; White and Folkens 2005). The most distinctive trait of the thoracic vertebrae is the costal facets. The main purpose of these facets is to articulate with and support the rib cage. Some variations within the thoracic vertebrae include small bones outside of the 12 which fuse to the existing vertebrae (Steele and Bramblett 1988). These false vertebrae can have costal facets and articular surfaces (Steele and Bramblett 1988). There is also a possibility for the T1 to fuse with the first rib (Steele and Bramblett 1988).

As the spine continues downward, the vertebrae become thicker and lose qualities such as the costal facets. These thickened bones of support are the lumbar vertebrae. There are five vertebrae in total, but due to human variation, there can be up to 6 or as few as 4. The further down the spine, the shallower the articular surfaces become, and the L5 widens to articulate with the sacrum (Steele and Bramblett 1988). L5 is the largest of all “true vertebrae” (Steele and Bramblett 1988). The term “true vertebrae” is used to describe the first three sections of the vertebral column, which are unfused from one another.

The sacrum is the widest portion of the spine consisting of multiple fused components, which Steele and Bramblett (1988) call the “false vertebrae.” The sacrum curves underneath the innominate bones creating support for the tissues which fill the pelvis and anchoring the posterior pelvic diaphragm. This spine portion is important in load bearing and is slightly changed in shape to assist reproductive purposes. This series of fused vertebrae harbors large foramina to pass nerves, arteries, and veins. The coccyx is underneath the true sacrum - a series of small bones lacking arches or any true vertebral shape. These bones are a remnant from an evolutionary ancestor and form a small internal tail (Steele and Bramblett 1988).

1.4 The Morphology of the Axis, Common Variations, and Age

The axis, or C2, is the second cervical vertebra and serves as a pivot for the lateral rotation of the head. There are attachment points on the axis for the “inferior oblique and rectus capitis posterior major, bulky portions of the semispinalis cervicis, spinalis cervicis, interspinalis and multifidus" (Sinha and Goyal 2015). These muscles mostly attach to the spinous process and help rotate the head from side to side and provide added stability (Sinha and Goyal 2015; Bridwell 2019). The intervertebral foramen of C1 and C2 gives passage for the C2 nerve, which innervates muscles surrounding the neck, allowing forward bending (Bridwell 2019; Dickerman 2019). It also allows for scalp and shoulder sensations (Dickerman 2019).

The axis is distinct from the other vertebrae by the presence of the dens which extends cranially to the atlas. There, the dens articulates with the atlantoaxial joint (Dickerman 2019). This joint can exhibit many variants and pathologies. The axis has the potential to ossify with the atlas, known as ankylosis, causing immobility of the neck. This can leave measurements incorrect or impossible to complete. The ligament that attaches to the apex of the dens can also ossify, causing a bony projection to emerge (Steele and Bramblett 1988). There is also the possibility for the dens to extend through the atlas to articulate with the inferior occipital bone (Steele and Bramblett 1988).

In addition to these morphological variants, another defect can occur in development where the dens remains unfused to the vertebral body and gains the name of *os odontoideum* (Steele and Bramblett 1988). There also is the anomaly of a missing dens or odontoid agenesis. "The true incidence of congenital absence of the odontoid process is impossible to determine, because most patients are thought to be asymptomatic and thus undiagnosed" (Tetradis and Kantor 2003). During the first few months of life, the base of C2 is fused, and by age 12, the dens is normally fused to the vertebral body (Tetradis and Kantor 2003).

The following chapter will discuss the evolution of sexual dimorphism, the cultural consequences of using sex as an identifier, and the usage of sexually dimorphic traits to produce a biological profile. In Chapter 3 the methods utilized in this study will be dissected as well as the basic statistical expectations of this dataset. Chapter 4 details the results of the study as well as providing formulas designed to assist forensic anthropologists in the creation of a sex estimation. Chapter 5 dives deeper into the successes, failures, consequences, and potential shortcomings of this study through discussion. Finally, the Conclusion brings together the results and provides inspiration for future study.

2 SEXUAL DIMORPHISM

There is reason to expect that sexual dimorphism is present in the axis. It has the potential to be used in tandem with other sex estimation methods to bolster an original conclusion. Sexual dimorphism of the axis could reflect stature distinctions and head size differences between the sexes. In addition, areas adjacent to the axis are among the most dimorphic in the skeleton, such as the cranium and mandible, where the sexes differ in both non-metric and metric characteristics (Best et al. 2018; Buikstra and Ubelaker 1994; Edwards et al. 2013; Franklin et al. 2013; Krishan et al. 2016; Musilova et al. 2016; Spradley and Jantz 2011; Walker 2008).

In Buikstra and Ubelaker (1994), much of the focus of cranial sex estimation is placed on the inferior and inferoposterior vault. Jaw and neck muscle attachments lie there, and there can be substantial heavy dimorphism reflecting the differential weights of the cranium. The external occipital protuberance, which lies on the occipital bone, can be quite prominent in males due to prominent nuchal muscle attachments. Also in this region, the mastoid process can be thick and extended in males due to the stronger sternocleidomastoid muscle attachment. These differences in muscularity, muscle attachment prominence, and mass imply the possibility of sexual dimorphism in the cervical vertebrae – most notably the axis.

2.1 Reproduction and the Origin of Sexual Dimorphism

The basis of all human existence surrounds reproduction. Evolutionarily, human ancestors varied in sexual dimorphism from major differences to very minimal differences. Plavcan (2012) argues that sexual dimorphism has changed quite fluidly throughout the *Homo* genus. Plavcan (2012) has hypothesized that sexual selection towards females is how these changes have come to be. He goes on to suggest that dimorphism could have been affected by the male-to-female ratio, mating rituals, and ecological systems (Plavcan 2012). While looking at our extinct *Homo* ancestors, it is worth noting that their sexual dimorphism is also being examined using modern human standards, yielding potential uncertainties (Plavcan 2012).

One theory suggests sexual dimorphism emerged in breeding groups of early humans. Plavcan (2012) cites Kappelman (1993) and Moore (1996) as the main proponents of this theory. While monogamous groups have limited dimorphism, breeding groups with a single male and multiple females tend to have substantial dimorphism - the males being the largest (Gray and Garcia 2013; Kappelman 1993; Moore 1996; Plavcan 2012). Human males have up to 65% more muscle mass than females, which suggests female accruement of fat has been selected to maximize neurodevelopmental resources during pregnancy. Dimorphism could have arisen from the need for males to perform specific tasks to gain attention or provide for a female. Gray and Garcia (2013) write that female reproductive choice relies more on having enough resources while male reproduction relies on access to a female. By providing the female with resources, the male gains a greater chance of mating (Gray and Garcia 2013; Dixson 2009). Unfortunately, the need to gather supplies comes with the cost of risky behavior and higher mortality. “Among Aka hunter-gatherer men in their young twenties, some of the elevated male mortality is due to risks in foraging activities, such as falling out of trees while collecting honey” (Gray and Garcia 2013). While Gray and Garcia (2013) hold an evolutionary psychology perspective, Kemper (2013) discusses the relaxed and cooperative communities of muriquis monkeys. These monkeys do not have harsh and combative tactics to gain the most females but form close brotherhoods and wait for female consent before approaching for mating (Kemper 2013). There is no competition to gain mates and males are often seen hugging and asking for comfort when stressful situations arise (Kemper 2013). Scenarios of early humans and their ancestors having a muriqui-type social organization or the one depicted by evolutionary psychologists are equally likely.

In terms of parturition, sexual dimorphism plays a key role in the ability of a female to give birth to her offspring. In the evolution of bipedalism, the pelvis has changed in shape to support the upright position. This has brought stress to the birthing process as the opening of the pelvis became smaller; therefore, to have offspring survive, fontanella evolved, and the pelvis became as wide and as deep as possible to support birth and bipedalism. When a female reaches sexual maturation, the pelvis is fully formed and exhibits an oval shape suitable for birth (Bass 1995; Buikstra and Ubelaker 1994; White and Folkens 2005; Iscan and Steyn 2013). Males on the other hand will have their pelvis fuse in a heart shape as viewed superiorly (Bass 1995; Buikstra and Ubelaker 1994; White and Folkens 2005; Iscan and Steyn 2013).

It is important that critiques be made due to the gendered nature of these aforementioned arguments. There is a heavy assumption that female and male roles within a society are divided into gendered work; that assumption can perpetuate the sexism often found in the field of anthropology and society as a whole. Conkey and Spector (1984) present information about the biases present in anthropology and how it has been assumed in the past, females had little to no involvement in hunting or manual labor in prehistoric society. This creates the illusion that women are incapable and upholds the western narrative of the division of work (Conkey and Spector 1984). There is a long history of putting the male narrative above the female one with the excuse that there is little to no evidence on female work life. In addition to this, the western ideas of sex equating gender erase the wide range of genders present in a multitude of cultures, including the United States’ LGBTQ+ community. Researchers must be aware that there is more to human life and culture than male and female reproduction.

2.2 Classification Variations

In the most accepted standards, there is a gradient scale to estimate sex. The Buikstra and Ubelaker (1994) standards scale range from 1-5 with 1 being definite female and 5 being definite male. In subpubic traits, 1-3 is used (Buikstra and Ubelaker 1994). While current cranial and postcranial methods rely on anthropologists to provide their expertise to rank order a continuous gradation of morphology, Gama et al. (2015) provide C2 measurements to present a more quantitively based assessment of sexual dimorphism of this element. An argument can be made that interobserver error is large for measurements, but the same could be applied to standard scoring systems. The collaboration between methods holds promise for developing a more accurate classification for sex. The C2 measurements featured in this study and the resulting discriminant function to classify the sexes could be a stand-alone methodology in modern forensic anthropology.

The Buikstra and Ubelaker (1994) book of standards promotes the Phenice method to examine the pelvis. This includes the scoring of the ventral arc, subpubic concavity, ischiopubic ramus ridge, greater sciatic notch, and preauricular sulcus (Buikstra and Ubelaker 1994). Each of these traits is evaluated using a rank ordered system that is interpreted by the observer. An anthropologist’s interpretation of the estimation of sex is often reliant on the individual’s previous experience in the field. Anthropologists must have an excellent grasp on the anatomy and orientation of the pelvis as these techniques often involve repositioning the *os coxae* to gain a better view. According to Ubelaker and Volk (2002), the Phenice method has an accuracy rate of 88.4%. The Phenice method was developed using the Terry collection housed at the Smithsonian; however, the developer of this method lacked any forensic training (Ubelaker and

Volk 2002). Ubelaker and Volk (2002) believe the previous investigator’s inexperience led to females being more accurately sorted than males.

3 METHODS

Data collection occurred on human remains of known age and sex curated at the William M. Bass Osteology Collection of the University of Tennessee, Knoxville. The samples chosen consisted of individuals who self-reported as “white” (the largest grouping by far) between the height of 5 feet 2 inches and 6 feet 2 inches, and between 100-210 pounds. The ages were divided into 5-year increments of 30-35, 50-55, and 70-75. The heights of 5 feet 2 inches to 6 feet 2 inches and weights of 100 to 210 pounds were chosen because this assisted in reducing the potential for outliers. With excessive height or diminutive stature, one runs the risk of including extreme individuals who may not be representative. The same rationale can be applied to the selection of weight at death. Also, splitting the individuals into 5–year increments allows for biological aging variation to be considered, while the 2–3 decade intervals between cohorts leaves the ability to compare the toll of aging between the sexes in C2. It was also requested that the occupations of the decedents lacked heavy lifting and muscle strain such as the motor skills used in construction work or body building.

This study group was chosen for the variety of ages available as well as their demographic information. While studies such as Wescott (2000) make the division between black and white individuals, since there are no anatomical differences between these races, there is no need to select one sample group over the other. In the case of this study, a self-identified white population is a strength due to the nature of donation. The bodies donated to the Bass Skeletal Collection are consensually donated, so there is a lesser chance of their bodies being the victim of exploitation, abuse, or cheap burials. Anthropology has a long history of utilizing vulnerable individuals and this includes the procurement of their remains for studies. Using this collection gives this research an ethically sound sample untainted by trauma.

3.1 Redundancy in Sample Sizes

The total number of individuals subjected to the study was 149. This consisted of 78 biological males and 71 biological females. All data surrounding sex, age, population affinity, occupation, weight, and height were provided by the University of Tennessee Knoxville where their sources were self-reported by the decedents. A total of 25-400 individuals is considered a reasonable statistical sample size according to Norman, Monteiro, and Salama (2012). These sample sizes are best used as a way to narrow down a large population to a more manageable level to work with statistically (Norman, Monteiro, and Salama 2012).

Some articles cite that in order to have a productive research population, you must have a sample following the formula of $N = (DF/k)+1$, with DF being the degrees of freedom and k being the number of groups (Serdar 2021). While authors such as Martinez-Mesa et al. (2014) use fewer specific metrics such as, "...when the target population size is sufficiently large, that is, surpasses an arbitrary value (for example, one million individuals), the resulting sample size tends to stabilize," (Martinez-Mesa et al. 2014). With this said, Martinez-Mesa et al. (2014) alludes to the idea that once a sample size of a population reaches a certain size, the data have become redundant, and it may be best to study the population rather than a small portion. On the other hand, if too small of a sample is taken, significant differences which are present may not be identified. With this in mind, a sample size of 149 will accurately reflect the population at large.

3.2 Testing for Dimorphism

To test for dimorphism, a set of 13 measurements was taken with a pair of digital calipers. They cover multiple angles of the C2 maximizing the chance of accurately capturing the morphology of this vertebra. These measurements include the dimensions of the dens, vertebral foramen, and other cervical vertebral traits (Table 1) and are derived from Gama et al. (2015).

Reference Figures 1 through 3 for illustrated versions. Gama et al. (2015) combined 8 measurements from Wescott (2000), 1 from Medina (2011), 1 from Marlow and Pastor (2011) and inserted 3 of their own variables. This study aims to validate the Gama et al. (2015) experiment and introduce age as a potential factor influencing axis dimorphism.

When using similar measurements, previous researchers have reached an accuracy rate as high as 92.9% (Torimitsu et al. 2016). The study conducted by Gama et al. (2015) reached an accuracy rate of 86.7% regarding their ability to assign sex to their individuals through statistical analysis correctly.

Gama et al. (2015) conducted their study at the Forensic Sciences Centre Portugal using a combination of previous researchers' measurements as well as their own to examine a Portuguese sample of 190 individuals. The study uses a smaller sample size to apply more complex statistical analysis to their results (Gama et al. 2015). Gama et al. (2015) only examined vertebrae without pathologies or trauma, and once measurements were complete, a two-sided t-test was conducted to compare symmetry.

Within their final analysis, Gama et al. (2015) found the maximum width of the axis (LMA) measurement showed an 11.18% difference between males and females, sagittal maximum body diameter (DSMC) had a 10.6% difference, and finally, length of the vertebral foramen (CMFV) had a 2.7% difference between males and females (Gama et al. 2015). Each trait showed males being larger than the females in the sample. These results suggest the human cervical vertebrae, specifically C2, exhibit an observable amount of sexual dimorphism. Gama et al. (2015) utilized logistic regression with a sample size of 190 individuals and a test sample of 47 to fully authenticate and apply their result as a generalization to the entire Portuguese population. In their conclusion, they suggest that this study be repeated with more variable age groups to receive a larger picture of this dimorphism.

Wescott (2000) pioneered the study of sexual dimorphism of C2 using 8 measurements of the axis and statistical tests to assign sex with an 83% accuracy in the population of 400 individuals (Wescott 2000). Marlow and Pastor (2011) built on the study conducted by Wescott (2000) in an effort to examine vertebrae where the preservation was poor. In addition, they helped corroborate the initial findings of Wescott (2000) and developed additional discriminant functions to classify the sexes using the axis. A total of 153 individuals aged 21-92 years were used in Marlow and Pastor's (2011) study. These individuals were of a known sex population, and a discriminant function analysis to exacerbate within-group differences was conducted to classify group membership. The results of this experiment reached a combined accuracy rate of 76.99%, and individual discriminant functions ranged from 70.91% to 78.9% (Marlow & Pastor 2011).

Gama et al. (2015) present one of their measurements as a reasonable addition to their own study: the maximum width of the vertebral foramen (LMFV). This measurement crosses the transverse plane of the vertebra covering only the open vertebral foramen where the spinal cord traverses. Previous studies have conducted measurements using the sagittal width of the vertebral foramen, but the transverse measurement was unique to this study when it was published (Wescott 2000; Marlow & Pastor 2011; Medina 2011).

In contrast to previous researchers, Torimitsu et al. (2016) uses fleshed cadavers of a Japanese population and postmortem computed tomography (PCT). A total of 224 cadavers were examined for this research, including 112 males and 112 females. The researchers received an accuracy rate of 92.9%, with significant size differences in males versus females (Torimitsu et al.

2016). Males were much larger than females in this study, and Torimitsu et al. (2016) suggest this is from sex-related occupation and hormone surges (Torimitsu et al. 2016).

3.3 Measurement Error Study

A pilot study was conducted involving a similar set of criteria to those outlined for C2. Ten cervical vertebrae were selected from the Georgia State University skeletal remains collection in the Department of Anthropology. Due to the university's limited vertebral collection, these ten vertebrae are different in location along the cervical spine. These ten cervical vertebrae were subjected to two trials of measurements against eleven criteria (Table 1). The eleven criteria are a combination of those developed for this study, as well as those from Medina (2011), Marlow and Pastor (2011), Wescott (2000), and Gama at al. (2015). The traits in Table 1 contain five of the measurements from Table 1 including DSMC, DTMC, CMFS, CMFV, and LMFV. The trials were completed to improve proficiency and precision in measurement. Each vertebra was measured once against these eleven criteria and once completed, a second trial was conducted to ensure the measurements were not influenced by an immediate remeasure. The time between trials was five minutes. The measuring itself was performed using a digital caliper calibrated in millimeters.

Table 1 A comprehensive list of the measurements in the measurement error study. These measurements are taken on the left side of the vertebra if applicable.

Measurement	**Description**
DSMC	Sagittal diameter of vertebral body
DTMC	Transverse diameter of vertebral body
LLT	Left lamina superior proximodistal to approximate midpoint excluding the spinous process

XCP	Length of costal process
CMFS	Maximum sagittal length of superior facet
SIF	Maximum sagittal length of inferior facet
XTP	Length of transverse process
XSP	Length of spinous process
PT	Midpoint of pedicle thickness
CMFV	Anterior to posterior measurement of foramen
LMFV	Maximum transverse diameter of foramen

The results of this measurement error study were examined using a t-test. The means were compared to see if there were any substantial differences between trial measurements. In Table 2, the results of the t-test show that there is no significant difference between trials suggesting there is repeatability in measurements. A Mann-Whitney u-test was also conducted to consider the probable nonnormal distribution of data resulting from small sample sizes. In Table 3, the results of the Mann-Whitney U test are presented. These results follow a similar pattern as the t-test in that there is no significant difference between trials. Again, this demonstrates precision in measurement.

Table 2 Results of the two-sided t-test conducted as a part of the measurement error study. These results suggest there is no significant difference between trials.

Trait	**2-Sided P Value**
DSMC	0.993
DTMC	0.989
LLT	0.991

XCP	1.000
SSF	0.931
SIF	0.914
XTP	0.900
XSP	0.982
PT	0.936
CMFV	0.886
LMFV	0.872

Table 3 Results of the Mann-Whitney u-test follow suit and show there is no significant difference between the two measurement trials.

Trait	**Asymp. Sig (2-tailed)**
DSMC	0.970
DTMC	0.970
LLT	0.970
XCP	0.859
SSF	0.820
SIF	0.793
XTP	0.874

XSP	1.000
PT	0.970
CMFV	1.000
LMFV	0.910

3.4 Statistical Methods and Expectations

After collecting these 13 measurements from 149 individuals, statistical methods must be applied to test their validity. To begin, a principal components analysis was run to obtain component loadings for the original 13 measurements. The variables with the highest correlation coefficient were selected to use in a discriminant function analysis from which beta weights and constants were obtained, as well as the percentage of correctly sorted individuals. The discriminant function separates individuals based on their similarities and the probability of them belonging to any group versus their own group. In this discriminant function, the known sex will be labeled to compare the proposed sex for females and males using classification rates. The beta weights and constant will be used to create formulas using the top 6, top 4, top 3 and top 2 variables with the highest correlation coefficients with the first PCA factor. These will be converted to vectors by using transform and compute option in SPSS whereby the beta weights will be multiplied to the top variables and added to the constant. The mean for females and males will be calculated from these vectors and the difference between these two are the breakpoints between the sexes. To examine the results visually, a graph will be produced with the known sex labels selected comparing the four prediction formulas with their respective discriminant scores and the breakpoint will be imposed on the scatter using adjusting the y-reference line in the graph editor to correspond to the calculated breakpoint between the sexes.

3.5 Description of variables

Starting with the maximum height of the axis (AMA), this measurement is of the anterior C2 covering the entire length of the dens and the vertebral body (Figure 1). The length of the vertebrae (CMA) stretches from the most anterior part of the vertebrae to the most posterior (Figure 2). This includes the length of the spinous process. The DSD measurement is taken sagittally at the superior tip of the dens. Also including the dens is the measurement of the transverse diameter (DTD) (Figure 2). Once more, this takes place on the superior tip of the dens yet from the maximum transverse plane.

Measurements are also taken of the facets (Table 4). All the sided measurements were taken from the left of the vertebrae except when the side was severely damaged or missing. Superiorly, the distance between the superior facets (DMFS) was taken (Figure 2). The distance between facets also includes the transverse measurement of the facet meaning the measurements were taken from the most transverse edge left to right across the vertebrae. The left facet was measured sagittally (CMFS) where measurements were taken from the maximum edges posteriorly to anteriorly (Figure 2). The maximum transverse diameter of the left superior facet (LMFS) extended left to right at the largest point near the middle of the surface (Figure 2).

The final measurements include examination of the vertebral body, foramen, maximum width, and the length of the dens tooth (Table 4). The vertebral body was measured both transversely and sagittally (DTMC, DSMC) (Figure 3). The foramen was measured the same (LMFV, CMFV) (Figure 2). AMD is the measurement of the dens tooth length from the max height of dens to the superior articular facet (Figure 1). This covers the length of the tooth without including the vertebral body in the measurement. Finally, the LMA is the maximum vertebral length (Figure 2). This includes both transverse processes in the measurement and is taken from the anterior side of the vertebrae.

Table 4 A comprehensive list of the measurements in this proposed study. If applicable, these measurements will be taken on the left side of the vertebra.

Measurement	**Description**
AMA	Maximum height of axis
CMA	Most anterior part of the vertebra to the most posterior point
DSD	Dens sagittal diameter
DTD	Dens transverse diameter
DMFS	Distance between superior facets
CMFS	Maximum sagittal length of superior facet
LMFS	Maximum transverse width of superior facet
CMFV	Anterior to posterior measurement of foramen
DSMC	Sagittal diameter of vertebral body
LMFV	Maximum transverse diameter of foramen
AMD	Dens tooth length from the maximum height of dens to the superior articular facet
DTMC	Transverse diameter of vertebral body
LMA	Maximum vertebral width from the extreme side of the transverse process

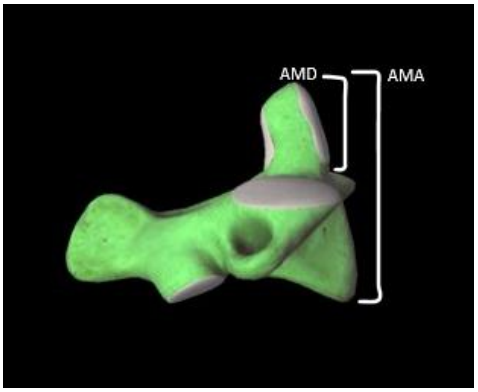


Figure 1 A lateral view of the measurements.

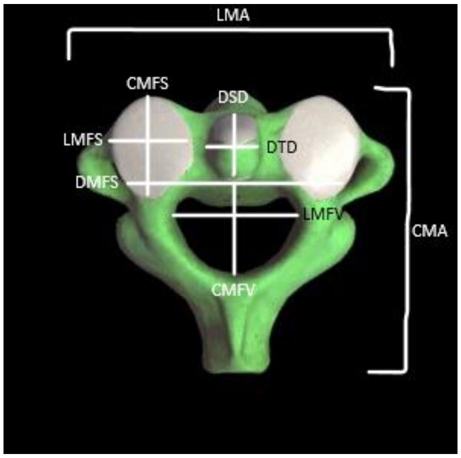


Figure 2 A superior view of the measurements.

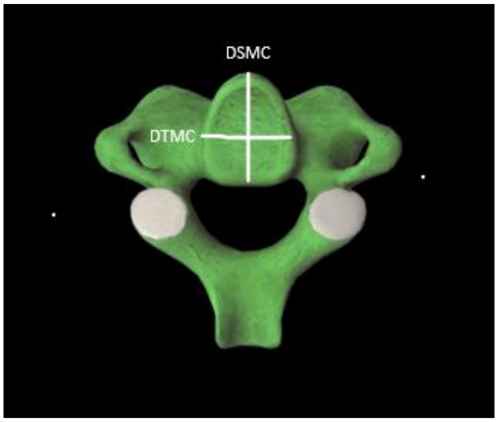


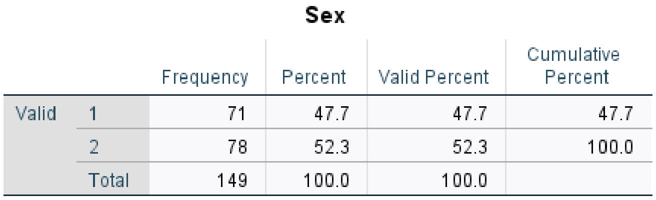
Figure 3 An inferior view of the measurements.

In addition to the discriminant function analysis, a t-test has been run. As stated before, the t-test serves as a way to discover the traits with p values indicative of significant difference between the means. These results can tell the researcher what traits differ the most between the sexes. This is an important test because it can assist in determining a potential formula to use the measurements of significant traits to estimate the sex of the individual. These tests and analyses were performed on the overall population to explore trends between females and males regardless of age.

4 RESULTS

The findings of this study give ample evidence to support the initial claims of there being significant sexual dimorphism in the second cervical vertebra. Tables 8, 9, and Figure 4 are the results of a principal component analysis run on the entire data set. This analysis is able to separate the data based on a multivariate comparison of all individuals regardless of sex. Table 8 concludes that all of the original traits have positive loadings to the first factor, CMA has the highest positive correlation. This means that the CMA has the greatest effect on the assignment of sex within Table 9.

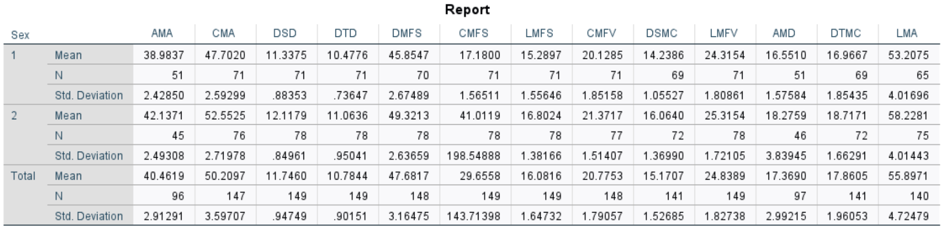
Table 4 Descriptive statistics of both sexes where 1 is all females and 2 is all males. N= 149



Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	71	47.7	47.7	47.7
	2	78	52.3	52.3	100.0
	Total	149	100.0	100.0	

Table 5 Mean and standard deviations of each trait compared to each sex.



Report

Sex		AMA	CMA	DSD	DTD	DMFS	CMFS	LMFS	CMFV	DSMC	LMFV	AMD	DTMC	LMA
1	Mean	38.9837	47.7020	11.3375	10.4776	45.8547	17.1800	15.2897	20.1285	14.2386	24.3154	16.5510	16.9667	53.2075
	N	51	71	71	71	70	71	71	71	69	71	51	69	65
	Std. Deviation	2.42850	2.59299	.88353	.73647	2.67489	1.56511	1.55646	1.85158	1.05527	1.80861	1.57584	1.85435	4.01696
2	Mean	42.1371	52.5525	12.1179	11.0636	49.3213	41.0119	16.8024	21.3717	16.0640	25.3154	18.2759	18.7171	58.2281
	N	45	76	78	78	78	78	78	77	72	78	46	72	75
	Std. Deviation	2.49308	2.71978	.84961	.95041	2.63659	198.54888	1.38166	1.51407	1.36990	1.72105	3.83945	1.66291	4.01443
Total	Mean	40.4619	50.2097	11.7460	10.7844	47.6817	29.6558	16.0816	20.7753	15.1707	24.8389	17.3690	17.8605	55.8971
	N	96	147	149	149	148	149	149	148	141	149	97	141	140
	Std. Deviation	2.91291	3.59707	.94749	.90151	3.16475	143.71398	1.64732	1.79057	1.52685	1.82738	2.99215	1.96053	4.72479

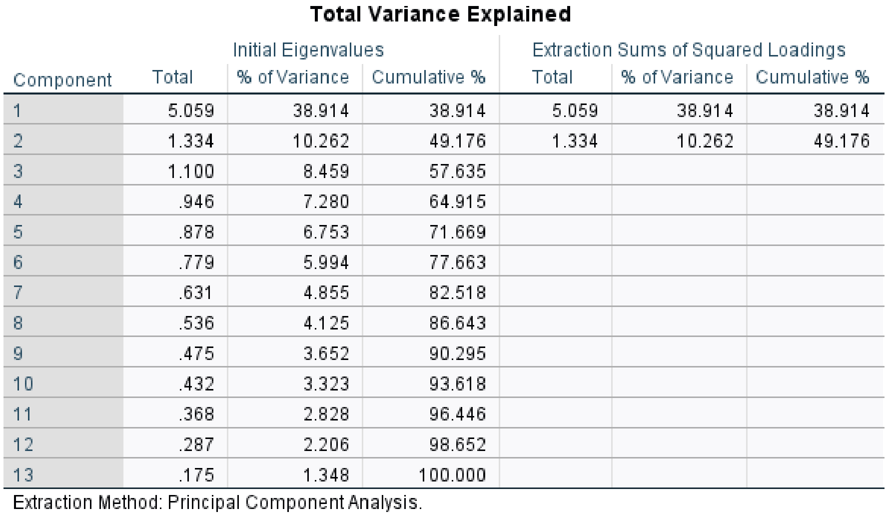
Table 6 Table depicting the t-test results for all variables.

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
AMA	136.099	95	<.000	40.46188	39.8717	41.0521
CMA	169.238	146	<.000	50.20973	49.6234	50.7961
DSD	151.324	148	<.000	11.74604	11.5927	11.8994
DTD	146.022	148	<.000	10.78436	10.6384	10.9303
DMFS	183.292	147	<.000	47.68169	47.1676	48.1958
CMFS	2.519	148	.013	29.65577	6.3899	52.9217
LMFS	119.164	148	<.000	16.08161	15.8149	16.3483
CMFV	141.152	147	<.000	20.77527	20.4844	21.0661
DSMC	117.983	140	<.000	15.17071	14.9165	15.4249
LMFV	165.919	148	<.000	24.83886	24.5430	25.1347
AMD	57.171	96	<.000	17.36897	16.7659	17.9720
DTMC	108.176	140	<.000	17.86050	17.5341	18.1869
LMA	139.982	139	<.000	55.89714	55.1076	56.6867

In the graph (Figure 4), we see numbered labels near points. These numbers are the known sex of the individual but during this Principal Components Analysis (PCA), sex was not stipulated. The x axis is labeled -3 to 3. Toward the negative terminus of the first factor are projected the largest number of females. Conversely, the positive extreme of factor 1 contains the larger individuals which included more males. These results reflect prior research indicating females are smaller than males. Since a divide is evident between the two groups, there must be traits that are dimorphic in some way. While 11 individuals are incorrectly assigned, most individuals have fallen into the correct sex grouping. Figure 5 is the PCA translated into a graphed format including a convex hull surrounding each sex. While observing this figure, there is a clear overlap where females are more likely to fall. This suggests that females are more likely to be incorrectly assigned based on the measurements and component loadings.

Table 7 Results of a PCA of the data set in full.

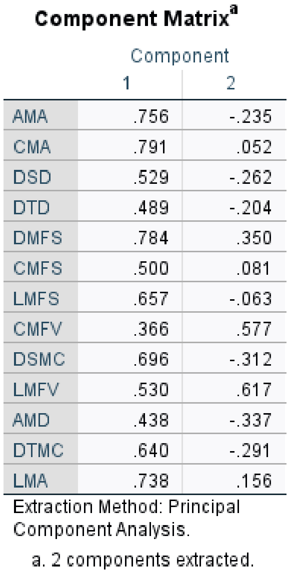


Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.059	38.914	38.914	5.059	38.914	38.914
2	1.334	10.262	49.176	1.334	10.262	49.176
3	1.100	8.459	57.635			
4	.946	7.280	64.915			
5	.878	6.753	71.669			
6	.779	5.994	77.663			
7	.631	4.855	82.518			
8	.536	4.125	86.643			
9	.475	3.652	90.295			
10	.432	3.323	93.618			
11	.368	2.828	96.446			
12	.287	2.206	98.652			
13	.175	1.348	100.000			

Extraction Method: Principal Component Analysis.

Table 8 Results of a PCA of the data set in full. The values with the highest number on component 1, have the greatest efficacy in separating the sexes.



Component Matrix[a]

	Component	
	1	2
AMA	.756	-.235
CMA	.791	.052
DSD	.529	-.262
DTD	.489	-.204
DMFS	.784	.350
CMFS	.500	.081
LMFS	.657	-.063
CMFV	.366	.577
DSMC	.696	-.312
LMFV	.530	.617
AMD	.438	-.337
DTMC	.640	-.291
LMA	.738	.156

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

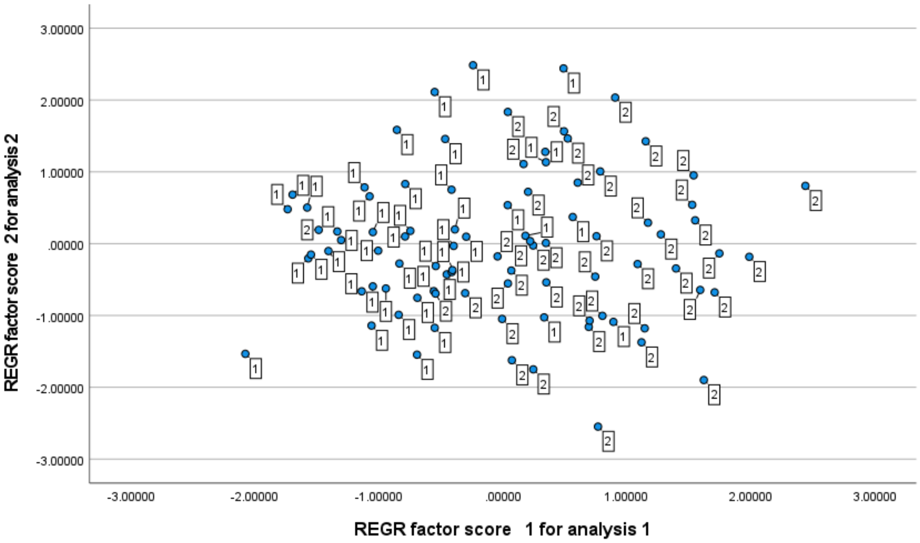


Figure 4 The first two factors of the PCA in a bivariate format. The number 1 is assigned to females while 2 is used to classify males.

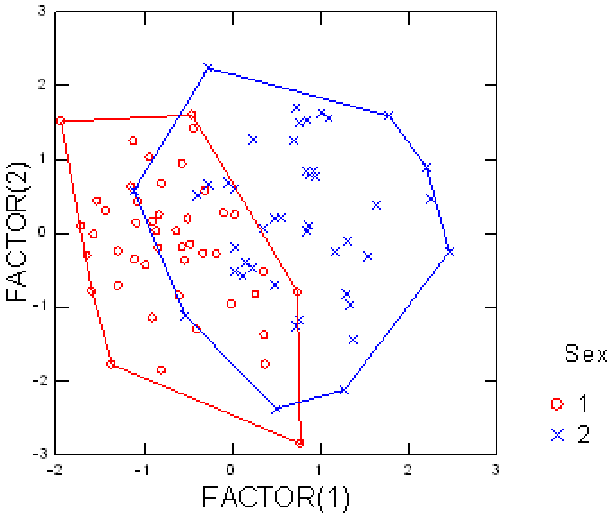


Figure 5 PCA of the 6 variables used in the first formula with a convex hull surrounding each sex.

After selecting the top 6 component loadings from Table 8 for a discriminant function analysis with sex as the grouping variable, the following beta weights and constant were calculated, and classifications estimated (Tables 9 and 10).

Table 9 Beta weights and constant for the top 6 variables.

Canonical Discriminant Function Coefficients

	Function
	1
AMA	.067
CMA	.186
DMFS	.051
LMFS	.084
DSMC	.390
LMA	.021
(Constant)	-22.904

Unstandardized coefficients

Table 10 Classification rates for females (1) and males (2) using 6 variables.

Classification Results[a,c]

			Predicted Group Membership		
		Sex	1	2	Total
Original	Count	1	41	5	46
		2	8	36	44
	%	1	89.1	10.9	100.0
		2	18.2	81.8	100.0
Cross-validated[b]	Count	1	40	6	46
		2	8	36	44
	%	1	87.0	13.0	100.0
		2	18.2	81.8	100.0

a. 85.6% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 84.4% of cross-validated grouped cases correctly classified.

The beta weights and constant were used to build the first function with 6 traits using the transform>compute function and the difference between the mean for females (-1.0248) and the mean for males (1.0949) was calculated as the breakpoint between the sexes for this function (0.3505). The classification rates for the top 6 traits feature 85.6% correctly classified individuals. For a visual representation of the above data (Table 10), see Figure 6. A darkened horizontal line has been placed at the breakpoint of 0.3505 to represent the aforementioned breaking point of the data.

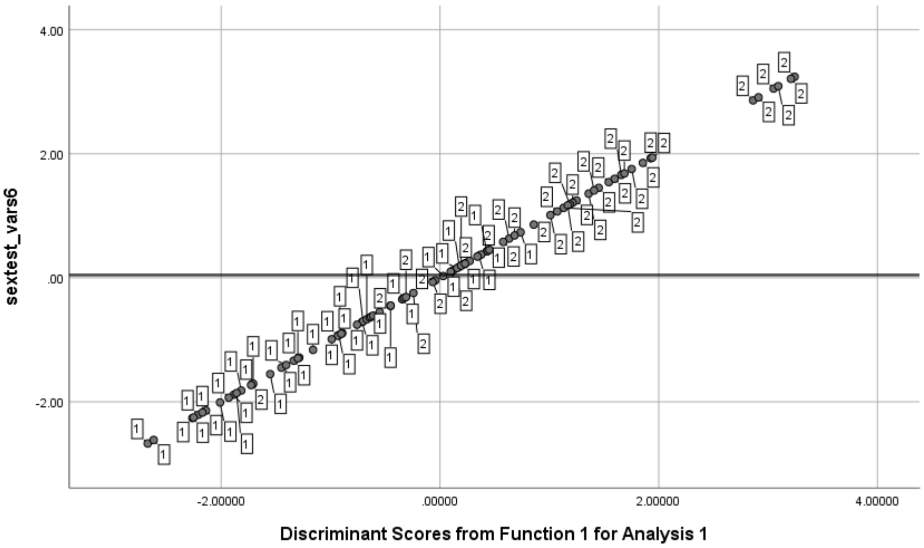


Figure 6 Comparison between predicted sex using 6 variables and discriminant scores for function 1.

After selecting the top 4 component loadings from Table 8 for a discriminant function analysis with sex as the grouping variable, the following beta weights and constant were calculated, and classifications estimated (Tables 11 and 12). According to the predictive formula, 82.2% of individuals were correctly classified (Table 12).

Table 11 Beta weights and constant for the top 4 variables.

Canonical Discriminant Function Coefficients

	Function
	1
AMA	.142
CMA	.264
DMFS	.026
LMA	.045
(Constant)	-22.673

Unstandardized coefficients

Table 12 Classification rates for females (1) and males (2) using 4 variables.

Classification Results[a,c]

			Predicted Group Membership		
		Sex	1	2	Total
Original	Count	1	38	8	46
		2	8	36	44
	%	1	82.6	17.4	100.0
		2	18.2	81.8	100.0
Cross-validated[b]	Count	1	38	8	46
		2	8	36	44
	%	1	82.6	17.4	100.0
		2	18.2	81.8	100.0

a. 82.2% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 82.2% of cross-validated grouped cases correctly classified.

The beta weights and constant were used to build the first function with 4 traits using the transform>compute function and the difference between the mean for females (-0.937) and the mean for males (1.047) was calculated as the breakpoint between the sexes for this function (0.055). Again, to assist in visualization, a darkened line has been placed at 0.055 to denote the cutoff point (Figure 7).

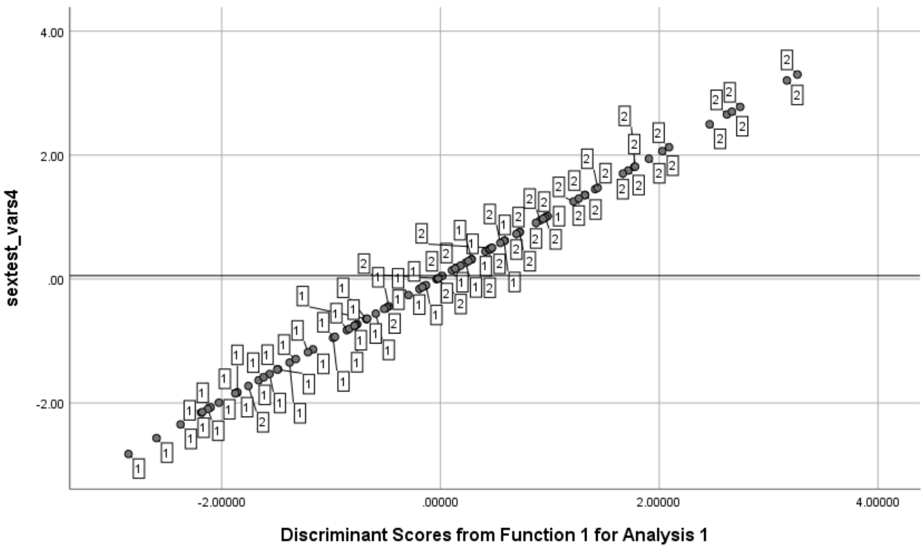


Figure 7 Comparison between predicted sex using 4 variables and discriminant scores for function 1.

After selecting the top 3 component loadings from Table 8 for a discriminant function analysis with sex as the grouping variable, the following beta weights and constant were calculated, and classifications estimated (Tables 13 and 14). While viewing the original classification rates, this predictive formula has correctly classified individuals at a rate of 85.3% (Table 14).

Table 13 Beta weights and constant for the top 3 variables.

Canonical Discriminant Function Coefficients

	Function 1
AMA	.151
CMA	.276
DMFS	.055

(Constant)	-22.431

Unstandardized coefficients

Table 14 Classification rates for females (1) and males (2) using 3 variables.

Classification Results[a,c]

			Predicted Group Membership		
		Sex	1	2	Total
Original	Count	1	42	8	50
		2	6	39	45
	%	1	84.0	16.0	100.0
		2	13.3	86.7	100.0
Cross-validated[b]	Count	1	42	8	50
		2	7	38	45
	%	1	84.0	16.0	100.0
		2	15.6	84.4	100.0

a. 85.3% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 84.2% of cross-validated grouped cases correctly classified.

The beta weights and constant were used to build the first function with 3 traits using the transform>compute function and the difference between the mean for females (-0.915) and the mean for males (1.103) was calculated as the breakpoint between the sexes for this function (0.094) as seen in Figure 8.

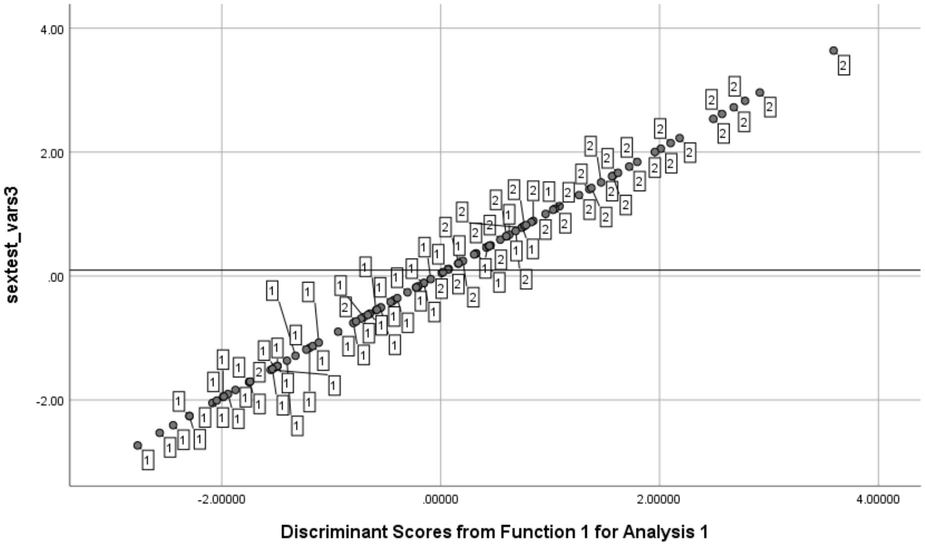


Figure 8 Comparison between predicted sex using 3 variables and discriminant scores for function 1.

After selecting the top 2 component loadings from Table 8 for a discriminant function analysis with sex as the grouping variable, the following beta weights and constant were calculated, and classifications estimated (Tables 15 and 16). Table 16 shows the classifications for this set of variables results in 83.6% of individuals being correctly assigned.

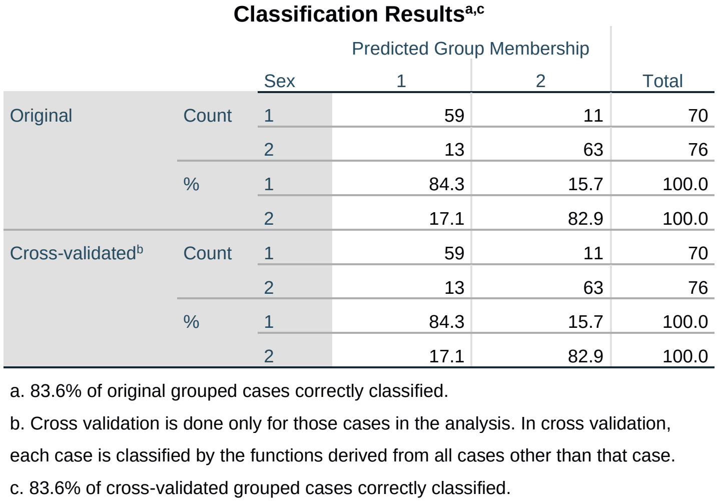
Table 15 Beta weights and constant for the top 2 variables.

Canonical Discriminant Function Coefficients

	Function 1
CMA	.298
DMFS	.144
(Constant)	-21.835

Unstandardized coefficients

Table 16 Classification rates for females (1) and males (2) using 2 variables.



Classification Results[a,c]

			Predicted Group Membership		
		Sex	1	2	Total
Original	Count	1	59	11	70
		2	13	63	76
	%	1	84.3	15.7	100.0
		2	17.1	82.9	100.0
Cross-validated[b]	Count	1	59	11	70
		2	13	63	76
	%	1	84.3	15.7	100.0
		2	17.1	82.9	100.0

a. 83.6% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 83.6% of cross-validated grouped cases correctly classified.

The beta weights and constant were used to build the first function with 2 traits using the transform>compute function and the difference between the mean for females (-1.017) and the mean for males (0.931) was calculated as the breakpoint between the sexes for this function (-0.0433). Figure 9 shows this data in a graphed format with a darkened line at -0.0433.

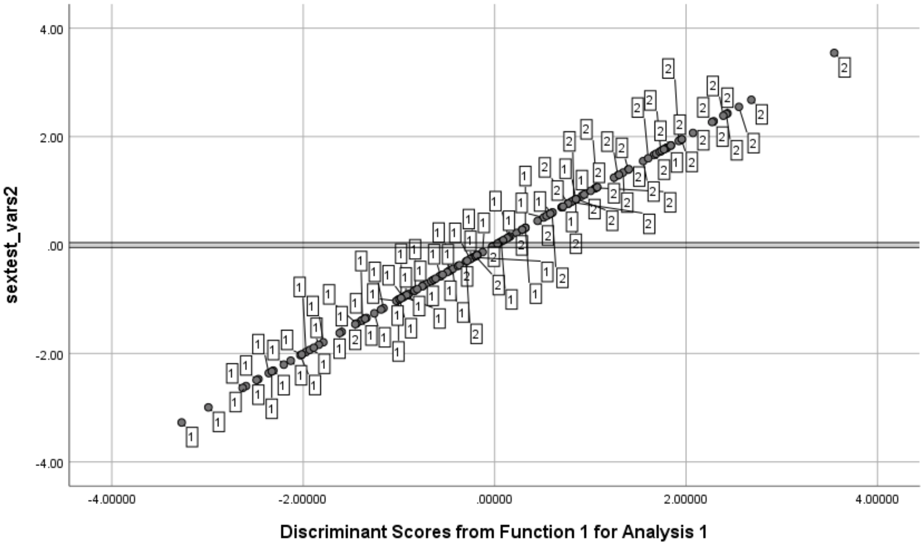


Figure 9 Comparison between predicted sex using 2 variables and discriminant scores for function 1.

5 DISCUSSION

Potential factors for these traits exhibiting the most dimorphism may lie in the muscle attachments. For example, the levator scapulae and splenius cervicis attach to the transverse processes of C2 (Perry and Salvador 2022). As more strain is applied to these muscles, the attachment becomes larger and can impact the LMA measurement in either sex. Due to the muscle attachments of the obliquus capitis inferior and rectus capitis posterior major on the spinous process, the same can be said about change in the CMA measurement (Perry and Salvador 2022). Finally, the DSMC and DTMC measurements have the potential to be influenced by the longus colli muscle which attaches directly to the vertebral body (Perry and Salvador 2022).

The rates of success reported in this thesis are comparable to those of other studies. Marlow and Pastor (2011) had the lowest rate of accuracy with their overall classification being 76.99%.

Wescott (2000) and his study produced formulas yielding 81.7 – 83.4% correct classification although his discriminant analysis provided 89% for one population and 81% for another. While this thesis includes a single population, it is important to note the difference in results Wescott obtained. Wescott (2000) also discovered the sagittal length of the vertebrae (CMA) yielded the highest correlation to sex-related size differences as seen in this study in the first factor of the PCA. While this study is a direct replication of Gama et al. (2015), they were able to achieve 89.7% accuracy on their sample population, while this study results in accuracies between 82.2 and 85.6%. The best results from this study involve the usage of the top 6 component loadings.

This thesis differs from Gama et al. (2015) in terms of which traits offer the most influential beta weights. Gama et al. (2015) marks the LMA measurement as the trait that is most effective in separating the sexes. This study marks CMA as the largest component loading while LMA is the fourth. Gama et al. (2015) also a fitted logistic regression to create their formula whereas this study produces a formula using beta weights derived from a principal components analysis. Another difference is that Gama et al. (2015) used a much older population to study. The study featured here, provides multiple age groups therefore testing the ability of the produced formulas to estimate sex accurately no matter the age.

As for cultural considerations, it is important to note that human bodies are complicated. Individuals who identify as male/female/neither/both may not have been genetically categorized as such. This information matters because "some 5.1% of adults younger than 30 are trans or nonbinary," (Pew Research Center 2022). While a typical genotype for male and female are xy and xx respectively, there is the possibility for mutations which result in genotypes outside of these. Intersex and transgender individuals may identify differently than their genetic makeup. As a society we know that gender identity and expression do not equal genetic sex. Gender is how a person feels and/or expresses themselves and are cultural and social categories. The LGBTQIA+ community continues to face alienation from family and friends which can lead to extensive violence. Black transgender women experience the highest rate of violent crime against them which, consequently, leads to an increase in the chance of them landing in a medicolegal investigation of their death (Transgender Europe 2022). There were 327 transgender individuals murdered in 2022 according to transrespect.org which is a website dedicated to tracking the violence against members of the trans community. It is crucial for forensic anthropologists to be aware of gender identities as it is integral to identification. While clothing found with the victim could represent one stereotypical gender, their body may not equate to the sex that gender is often correlated.

6 CONCLUSION

As methodology in forensic anthropology advances, new and improved tools emerge to improve the identification of unknown individuals in a medicolegal context. Methods to estimate sex are particularly relevant to constructing the biological profile. It is also important for bioarchaeologists to be able to identify the sex of individuals to reconstruct the demography, health, and life ways of prehistoric human populations. In this study, measurements of the axis from a known age and sex sample (n = 149) from the William M. Bass Osteological Collection of University of Tennessee Knoxville were investigated to determine if a formula could be created to identify the biological sex of unknown individuals relatively good accuracy. Four experiments were performed using 6, 4, 3 and 2 variables using the traits with the beta weights derived from a discriminant function analysis. These traits were selected from the component loadings of a principal components analysis conducted with all of the original 13 variables. Those with the highest correlation coefficients with the first axis were selected for the discriminant function analysis. Since the first factor of the PCA largely separated the sexes without the stipulation of known sex beforehand, the original traits with the highest correlations with this axis were considered particularly influential in separating size-based and, to a lesser extent, shape-based differences in C2 morphology between males and females. However, estimating the genetic sex as part of the biological profile in forensic anthropology is not enough to reconstruct identity in all cases. By keeping the identity of the whole human in mind while also providing inferred and estimated sex, forensic anthropologists can bridge the depersonalization gap often left in the medicolegal system.

The sample population selected for this study has provided crucial insight into the real-life application of the formulas created. Ranging from 82.2 to 85.6%, this sample population has brought to light the usefulness of C2 in creating a biological profile. Future studies of these data will compare the measurement values against height and weight across the age groups to estimate the extent to which the body mass index (BMI) influences distinctions between males and females in C2 morphology.

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