ASSESSMENT OF SEXUAL DIMORPHISM ON THE FIRST AND SECOND RIBS: EXPLORING GEOMETRIC MORPHOMETRICS

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Clifford T. Brown, Department of Anthropology, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Dorothy F. Schmidt College of Arts and Letters and was accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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ABSTRACT

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Estimating the sex of unknown human skeletal remains is important to the fields of forensic anthropology, bioarchaeology, and other specialties. I studied sexual dimorphism on the first and second ribs to estimate sex from skeletal remains. I employed two approaches. I used geometric morphometrics to analyze landmark and semilandmark coordinate points to examine the overall shape of the ribs. I also examined the sternal end of the ribs for size using the superior-inferior height (SIH) and anterior-posterior breadth (APB) in a binary logistic regression (BLR) model. Differences in male and female first and second ribs are undetectable when landmark coordinate points are used to capture shape variability, but significant differences in the shape of the ribs, however, are detected through the use of semilandmark coordinate points.

Using semilandmark points to estimate sex presented an accuracy rate of 80.7% from the first rib, and 72.9% from the second rib. The use of the sternal end presents consistent results in its ability to estimate sex with an accuracy rate of 84.2%. The BLR model reveals significant differences between males and females than the geometric morphometric approach; it is more applicable for discerning sexual dimorphism of unknown individuals. This study reveals that while geometric morphometrics provides a powerful approach to assessing morphological differences, it is not always better than simpler methods, in this case, simple measurements analyzed through BLR.

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TABLES

FIGURES

I. INTRODUCTION

The analysis of sexual dimorphism is a major component of understanding human variation. Sexual dimorphism is the morphological, genetic, and hormonal differences that allow for distinction between the male and female sexes (McPherson & Chenoweth, 2012). The degree to which these traits manifest varies between and within population groups. Biological anthropologists rely upon methodologies using various aspects of the human skeleton to estimate the sex of unidentified remains. The most popular sex estimation methodologies use the crania, os coxae, and long bones; however, little research has been conducted to explore the use of the skeletal thorax. An exploration of the morphological variations observed on the thorax is needed to understand sexually dimorphic properties of the entire skeleton because of the systemic and mechanical links among all skeletal elements.

This thesis explores the morphological differences between males and females through an analysis of the right first and second ribs. Observations of variation in human morphology were used to answer two questions: (1) Are there statistically significant differences in the morphology of the first and second ribs in male and females? and (2) What dimension of the rib reveals the greatest amount of variability with regards to sex?

Two approaches were used to assess sexual dimorphism. The first method utilized the sternal dimensions of the ribs applying a binary logistic regression model; the second method looked at the curve of the rib using geometric morphometrics. The binary logistic regression (BLR) model that was applied to the sternal end of the rib was created during preliminary research leading up to this thesis. Data from Gavit's (2009) Master's thesis were reanalyzed to produce the BLR model used in this thesis. Geometric morphometrics is a form of statistical shape analysis that removes the variable of size to look at the differences in shapes. Landmark and semilandmark coordinate points were used to uncover the meaningfulness of the rib's curvature for the purpose of estimating sex. The second aspect of this research compared the degree of sexual dimorphism on the rib's curvature to its sternal ends. This was done to understand which aspect of the rib presents the greatest amount of sexual dimorphism. These approaches expand the application of geometric morphometric techniques and contribute towards methodologies that aid in understanding human morphology.

In the following pages, I discuss human sexual dimorphism and established methodologies within the field of anthropology. Then I explain the research design and methodology used in this research. Finally, the resulting data as well as their outcomes and implications of this research to the field of anthropology are presented.

Human Sexual Variation

The analysis of sex plays a pivotal role in understanding other dimensions of the individual biological profile. The presentation of diagnostic skeletal features is influenced by culture, development and ancestry. The osteological perception of sexual dimorphism stems from the understanding that there are general morphological trends observed between males and females. Understanding these trends has led to the formulation of the methodologies that are used today to estimate sex. It is necessary to examine the theoretical basis upon which prior methodologies were developed in order to understand the biological factors associated with sexual characteristics and their presentation. Those theoretical approaches were used in the construction of this research and aided in the analysis of its results.

The theoretical approach of biological anthropologists is described as one that does not rely upon having a single overarching explanatory statement (Boyd and Boyd 2011). The use of a wide array of theoretical approaches is deemed necessary due to anthropologists' need to explore all aspects of the human environment in order to properly gain insight into the individual. To properly conduct an analysis, a statistical approach is required, as well as taphonomic, pathogenic, genetic, psychological, and evolutionary approaches (Boyd et al., 2011).

The methodologies developed by biological anthropologists enable them to assess elements of an individual's biological profile using skeletal material. The data collected allows them to infer an individual's behavior, age, ancestry, sex; all of these observations are made with a specified degree of confidence. The reliability of the methodological approaches continues to be examined, leading researchers to call for new approaches or refinement (Boyd et al., 2011). It is understood that the biological anthropologist can only function within a margin of certainty when exclusively analyzing skeletal remains. Though these limits are understood and recognized, the data acquired from skeletal analysis are major contributors toward understanding individuals and their lifestyles. Anthropological perspectives deem it irresponsible to construct definitive biological profiles for the individuals whose pasts are not clearly noted.

The male-female dichotomy is a product of sociocultural norms and thus varies across space and time. Sex in the general modern western society is perceived as being a scientific, unbiased biological trait that is universal; this perception is incorrect (Jones, 2014). Both sex and gender are culturally constructed; they are designed based on regional cultural variation. Sex and gender assignments to skeletal individuals may not be equivalent across cultural groups (Jones, 2014; Wesp 2017). Methodologies for sex estimation utilize both metric and non-metric characteristics. These estimations still require the inclusion of social context, including gender roles (Burris & Harris, 1998; Bass 2005; Byers 2011; Christensen et al., 2014; Edgar 2013; Hefner 2007; Hefner 2009; Hefner et al., 2012). A single binary system that is applied to every culture ignores the variation that occurs across space and time and within the varied cultural perceptions of sex and gender (Wesp, 2017). To apply the same sex estimation methodologies all skeletal remains without a consideration of cultural, temporal, or regional variations, creates a false lens. Sex cannot be determined, it can only be estimated within a range of certainty.

The categorization of sexual difference refers to an analyst's degree of certainty with respect to categorization, rather than the presence of sexual variability or ambiguity (Geller 2005). The analyst's ability to estimate sex of unknown individuals is influenced by their prior experiences and the amount of human variation they have encountered. This influences their recognition and categorization of distinguishing traits and is aided by the use of material culture. Biological anthropologists attempt to maintain a broader perspective of the individual to avoid the definitive reconstruction of a false history. Though it may seem subjective to do so, a misrepresentation of individuals would be an unjust interpretation of human past. Understanding the subjectivity of sex and gender is crucial to gauging the magnitude to which sexual dimorphism influences humans, including the aspects of dimorphism evaluated in this thesis. Sex within the human species is not dichotomous and presents itself in a wide range of variation. This variation contributes to the errors and difficulties biological anthropologists experience when using skeletal material alone to estimate sex. A similar range of variation between males and females from the sample population used is expected. This will influence the reliability of the proposed methodologies.

Within the field of biology and medicine, sex takes on a different meaning. The complex biological systems that are associated with the regulation and presentation of the individual's genotype are diverse. An individual's biological components are understood through their genetics; males are known to have an X and Y chromosome, whereas females are known to have two X chromosomes. The specific sex chromosomes are responsible for the expression of morphological characteristics that produce the dichotomy. However, there is variation in these genetic combinations that produce morphological variability. Even though biological sex is known not to occur in a dichotomous manner, biomedical studies continue to present them as such. The concept of maleness and femaleness is dictated by the fulfillment of biological roles in reproduction; the ability for an individual to carry a child to term and give birth makes her a female. Though biomedicine does not attempt to propose that sex is exclusively bimodal it analyzes the human body, both physically and genetically, into two categories.

Those in biomedicine are not the only ones to ignore the outliers that do not fit into these categories; anthropologists are guilty of the same practices. All individuals or aspects that do not meet this biological "norm" are considered to be unique or abnormal; they are omitted from the researcher's sample population and analysis (Geller 2012). The issue of ignoring individuals who express a degree of ambiguity is that a large number of population data is excluded, and the true nature of the population's culture becomes obscured. Whereas the social context may have been ignored or unintentionally omitted in osteological analysis, and the cultural biology of the individual may have been skewed, these errors do not necessarily warrant the dismissal of osteological methods (Holliman 2011; Geller 2005).

Categorizing individuals into bimodal sex categories does not provide a complete depiction of the population, biologically or culturally, however there are clear morphological differences between *typical males* and *typical females*. The development and use of sex estimation methodologies continue to have practical applications. However, it should be noted that they can only estimate aspects of an individual with a degree of uncertainty. The data utilized in this research are from a collection where the biological sex of each individual is known and presented dichotomously. Based upon the data provided regarding the sample population, this research was limited to examining sex dichotomously. Nevertheless, the presentation of morphological ambiguity observed throughout the human body indicates that no sex estimation methodology is capable of producing perfect results because sex is not a truly dichotomous variable.

Sexual Dimorphism

Human sexual dimorphism is represented in varying degrees across the world. There are notable trends that can be used to distinguish between biological males and females. Overall, females of a population group appear more gracile (Braz 2016). Male bones are longer, thicker, and have greater muscle mass, which requires greater surface area for attachment to bone, making them appear more robust (Braz 2016; Christensen et al., 2014). The degree of definition observed at muscle origin and insertion sites is limited by muscle size and the amount of force acting on the bone (Christensen et al., 2014). The amount of force acting on the bone influences its overall structure and how robust or gracile it appears (Christensen et al., 2014; Fernandez et al., 2014).

Compared to other organisms in our ancestral lineage, modern humans have a significantly longer childhood. This trait is beneficial for cognitive development, and also impacts sexual maturity; delayed reproductive maturity catalyzes morphological changes as the individual grows (Gage 2003). Individuals undergo a wide series of transformations that influence muscle definition and bone morphology, including stature, shape of the pelvis, muscle insertion or origin sites, and bone density. The presentation of sexually dimorphic characteristics on the human skeleton is contingent upon when the individual reaches sexual maturity: puberty (Bogin 1994; Gluckman & Hason 2006). Due to these changes, diagnostic traits may not be fully expressed on a subadult skeleton, thus rendering current methodologies inapplicable (Lewis 2007; Sutter 2003; Weaver 1980; Schutkowski 1993). Due to the uncertainty associated when dealing with non-adult remains, they were not included in the sample population used in this study.

Beyond childhood and puberty, humans continue to undergo fluctuations in hormone levels within the body. This results in the continued morphological changes and presentation of diagnostic features present throughout the skeleton. Females tend to be more neotenous or pedomorphic, retaining juvenile traits such as a higher voice, larger eyes, and a more gracile skeleton, however with age skeletal remodeling results in the masculinization of the skeleton (Christensen et al., 2014). During middle adult stages of life, between ages 46 and 54, females undergo menopause; this stage is characterized by a decrease in estrogen production and an increase in testosterone and loss of reproductive capabilities (Campbell & Reece 2005). Males retain their reproductive capabilities throughout life, though it is decreased. Post-menopausal women may display a more robust, or masculinized skeleton, ultimately increasing potential for error in estimating their sex (Campbell & Reece 2005; Christensen et al., 2014). Due to the morphological transformations associated with growth and development of humans, it is necessary to account for age when estimating an individual's sex. In addition, sex estimation is most accurate after the individual reaches maturity (White & Folkens, 2005, p. 385). The age of the individuals sampled in this research was taken into account.

Morphological variation can also be attributed to ancestry of the population. "Human populations differ in various ways as a function of evolutionary processes such as natural selection, genetic drift, mutation, and gene flow, which collectively shape phenotypic variation, including variation in the skeleton," (Christensen et al., 2014). Ancestral variation impacts the degree to which skeletal traits are displayed and therefore the estimation of individual's sex (L'Abbe et al., 2013). The degree to which robustness is expressed varies within and between ancestral populations: what may appear robust in one population may be considered gracile in another. Due to the strong relationship between ancestry and sexual dimorphism, this study takes into account the ancestry of individuals within the sample population.

Established Methodologies

A variety of methods are currently used for estimating the sex of skeletons. The methods naturally focus on skeletal elements that are sexually dimorphic. The crania and os coxae are often perceived as the most sexually dimorphic skeletal elements. Due to the wide variation that is observed across the human population, sex estimations use a scale to indicate how male-like or female-like the features appear. Craniofacial features are scored on a scale of 0 to 5: undetermined (0), female (1), probable female (2), ambiguous sex (3), probable male (4), and male (5) (Bukistra & Ubelaker, 1994, p. 21). When assessing pelvic shape, the Phenice method scores each feature as: unobservable (0), female (1), ambiguous (2), or male (3) (Bukistra & Ubelaker, 1994). Global studies have also observed the presence of sexually dimorphic properties on long bones including the femur, tibia, humerus, and ulna (Cavaignac et al., 2016; Srivastava et al., 2012; Srivastava et al., 2013; & Steyn & Isçan 1999; Tommasini et al., 2007). All of these approaches follow the general rule of gracility and robustness, in which prominent craniofacial features are considered to be more male-like and delicate features are considered more female-like (Bukistra & Ubelaker, 1994; Gonzales et al., 2009).

While these methodologies are useful, they do provide challenges. Several of these methodologies rely on the use of non-metric traits; the successful application of their use is contingent upon the knowledge and experience of the individual. Non-metric methodologies are prone to observational errors (Gonzales et al., 2009). Bone is also vulnerable to taphonomic influences such as scavenging, fragmentation, and warping. This increases the level of difficulty and expertise needed to gauge non-metric traits. Postmortem damage to the crania and os coxae can also obscure the dimorphic traits that sex estimation methodologies rely upon, thus contributing additional complications of these methodologies. The issue associated with using the methodologies provides one of the motivations behind this research. First and second ribs can be easily identified amongst commingled remains and are flat, compact bones, which increases the probability of preservation and reduces the likelihood of taphonomic damage. In addition, the methodologies examined in this research focuses on metric analyses alone. Metric approaches to sex estimation are viewed positively; for example, the geometric morphometric technique is "minimally influenced by the observer" (Gonzales et al., 2009).

Sexual Dimorphism in Ribs

Methodologies utilizing ribs for sex estimation are not commonly implemented. These methodologies rely upon various ribs and different dimensional features to identify sexually dimorphic characteristics. Like other methodologies assessing sex, those using ribs present merits and faults. One issue associated with using ribs is that it is difficult to accurately distinguish between ribs of the middle and lower thorax in disarticulated and commingled remains. Unfortunately, most of the current approaches rely upon those lower ribs. Ribs from the midsection of the body are also more susceptible to taphonomic influences and scavenger damage; this can further impede the anthropologist's ability to identify those (Kubicka & Piontek, 2015). General assessments report that females tend to have relatively longer ribs than males, and their morphology is, to some degree, influenced by age, height and weight (Bellamare et al., 2006). As this research attempts to expand upon sex estimation using the first and second ribs, it is necessary to explore this literature.

Rib morphology

The average human thorax is composed of twelve pairs of ribs. The structure of the rib can be divided into three parts: the head, which articulates with the body of the thoracic vertebrae; the neck, articulated with the transverse processes of the thoracic vertebrae; and the shaft, encapsulating the thoracic organs. Their unique morphology and placement along the thorax further divides the ribs into three groupings: true ribs, false ribs, and floating ribs. Ribs 1 through 7 are classified as true ribs; they are characterized by their individual articulation to the sternum via costal cartilage. Ribs 8, 9, and 10 are classified as false ribs because of their shared cartilaginous attachment to the sternum. Ribs 11 and 12 are floating ribs; they lack any articulation to the sternal body.

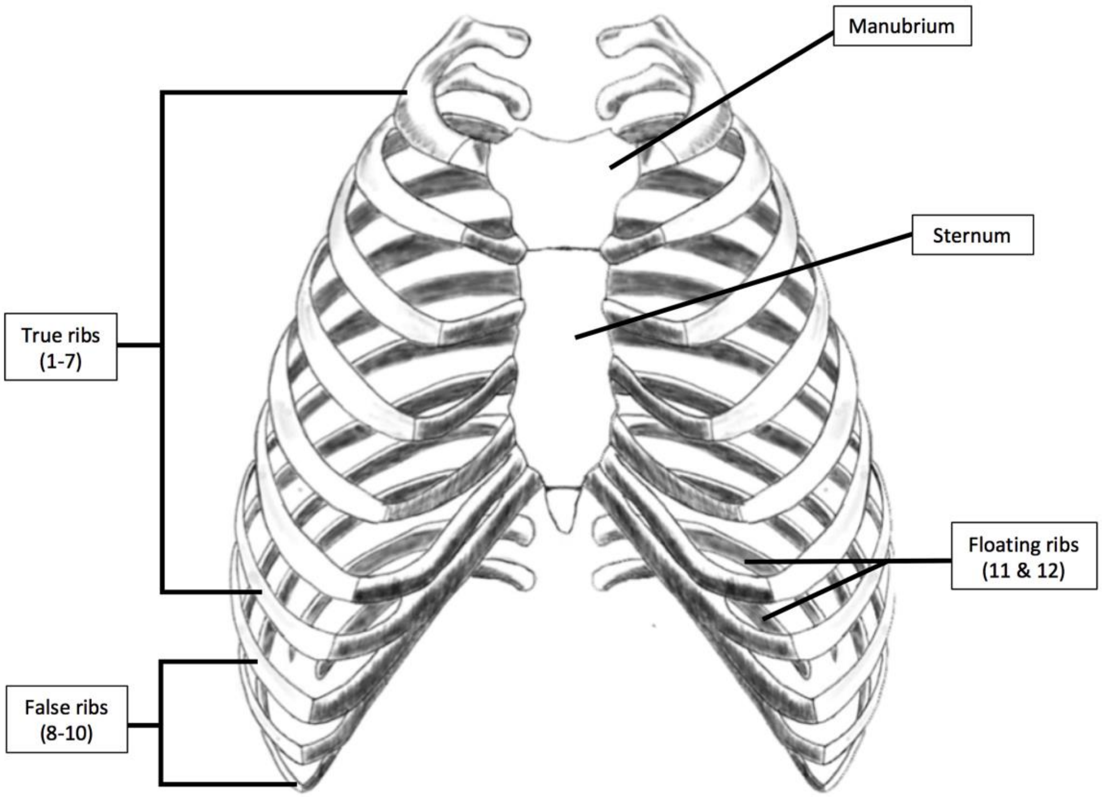

Figure 1. Skeletal anatomy of the human thorax

The construction of the rib cage enables it to serve several functions, thus its overall morphology is influenced by those functions. Ribs possess the mechanical role of protecting and supporting internal organs and stabilizing the trunk during respiration and spinal flexion (Dansereau & Stokes 1988). An assessment of the ribcage conducted by

Dansereau & Stokes (1988), and later by Bertrand et al. (2005), used stereoradiography to create a 3-dimensional model of the human ribcage in an effort to understand morphological variability. Intrinsic measurements focused on the shape of the rib cage and included the arc length and the distance between the costovertebral and costalchondral joints. Extrinsic measurements focused on the orientation of the rib through assessing the angle made by the rib. Assessments revealed that there is no significant asymmetry in the thoracic cage (Bertrand et al., 2005; Dansereau & Stokes 1988). As attributes of the left and right ribs are the same, my research progressed focusing on the right first and second ribs, as is consistent with other sex estimation methodologies that utilize ribs. The first and second ribs possess unique morphology due to their structural position in forming the thoracic cage. A complex nexus of nerves and vessels lay over the ribs; there are several muscles that also insert on the bone to allow for movement of the neck. These ribs are easily identifiable within a commingled assemblage and possess many notable features.

Additional modeling of the ribcage has been conducted in order to understand the morphological changes that are observed as a product of age and biological adaptations. These factors are known to influence the skeletal morphology of the cranium, pelvis, and long bones; therefore, it is necessary to understand their influence on the ribs. An assessment of age-related changes to morphology of the thoracic cage revealed the consistent changes undergone as a child grows. The rib cage, accessory muscles, diaphragm, and abdominal muscles are thought to be less efficient in the very young child than in mature adults because of the instability of the thoracic cage and lower efficiency of the diaphragm (Openshaw et al., 1984). When compared to adults, a child's rib cage is more pliable and the ribs are arranged with a more horizontal orientation. Openshaw et al. (1984) observed that the greatest amount of change in rib arrangement occurred from birth to 2 years of age. Another trend this study revealed was a change in the roundedness of the thoracic index: by age 3, the cage transformed to a more ovoid shape as the child matured. The changes in thoracic shape occurred primarily during early childhood. Openshaw et al. (1984) attributed these changes to the upright posture developed in the child, while the thorax was also being pressured by the weight of developed lungs and other internal organs.

Though a great amount of change in the ribs morphology and orientation occurs during the early years of a child's development, as the individual progresses into their later years, changes in rib morphology persist. In an attempt to quantify these changes throughout the thorax, Gayzik et al. (2008) assessed the morphology through measuring changes in the position of the ribs' head, angle, sternal end, and position of sternal facets. This study sample specifically focused on the thorax of 63 adult males ranging from 20 to 80 years of age (Gayzik et al., 2008). Overall, there was an observed change in the general morphology of the rib across the age spectrum (Gayzik et al., 2008). The entire thoracic cage became laterally contracted and longer anterior-posteriorly; the rib angle increased as the rib's roundedness decreased (Gayzik et al., 2008). These changes may be associated with the changes that are observed with aging including decrease in respiratory muscle function, stiffening of the chest wall, and the development of kyphosis due to osteoporosis (Gayzik et al., 2008). Coupled together, the many aspects of aging observed in adults can be noticeably correlated with changes in rib shape and thoracic morphology. Understanding the age implications involved with age morphology was another factor that motivated me to account for age variability within my sample population. Most individuals in the sample collection are over the age of 50, many of their ribs present the morphological changes caused by aging.

Overall chest morphology can also be attributed to environmental factors that influence the individual's respiration and associated mechanisms. When comparing the shape of the chest across populations it is apparent that there is variability in the overall size and capacity of the chest. The chest shape and size of the individuals living at a higher elevation has been observed to be greater in dimensions than someone living at or below sea level (Heath 1984). The enlargement of the chest cavity is a byproduct of respiratory demands and enlargement of the lungs. Comparisons of child-growth surveys of individuals living in the highlands of Peru revealed that the patterns of this growth and development are population specific and acquired during a child's growth (Frisancho 1975; Heath 1984). These physical adaptations arise in an effort to counter the negative effects of living at a high altitude, such as hypoxia, and allowing for survival in these conditions.

Another adaptation observed in the shape of the thorax is associated with reproductive capabilities. The requirements entailed by the need to carry a child during pregnancy are suspected to have influenced the rib cage to ensure it provides adequate space and strength. "Horizontal orientation of the spinous processes in females could allow for a greater thoraco-lumbar lordosis during pregnancy," (Bastir et al., 2014). The orientation of the vertebral spinous processes results in an alteration in the transverse processes with which the rib articulates. Ultimately the changes would produce a bilateral divergence in the position of the ribs and a wide, short thoracic cage in females, when compared to males (Bellamare et al., 2005; Bastir et al., 2014). This produces a recognizable amount of sexual dimorphism between the shape of male and female lower ribs (Bastir et al., 2014; Bellemare et al., 2006).

Sexual dimorphism using the 4th rib

One of the earliest studies of sexual dimorphism on the rib, conducted by Isçan (1985), proposed a method for estimating sex using the right fourth rib. His methodology relied upon sternal end dimensions: the superior-inferior height (SIH), anterior-posterior breadth (APB), and depth of the articular surface on the sternal end of the rib. Isçan (1985) used these measurements to produce a stepwise discriminant function analysis testing the precision of the approach. He also accounted for age by analyzing his sample population in three groups, young, old, and a combined young and old group. According to his results, the SIH and APB were the most diagnostic of the measurements and were the best dimensions to use for sex estimations (Isçan, 1985; Koçak et al., 2003). Isçan (1985) notes, "Sexual dimorphism in a single sternal rib can be assessable with some reliability" and the approach is applicable to individuals of a broad age range.

Further studies of Isçan's methodology have applied his approach to other ancestral populations revealing varying degrees of success. One study conducted by Koçak et al. (2003) applied Isçan's approach to a Turkish population using the same specified age groups. Results of this study presented a higher degree of accuracy in estimating young females. Success of this approach ranged from approximately 85% to 91% (Koçak et al., 2003). As with other examinations of Isçan's method, Koçak et al. (2003) provided additional confirmation that ribs can serve as a useful resource for sex estimation.

Geometric morphometric analysis of the curvature of lower ribs

Utilizing geometric morphometrics, Curran and Griffith (2012) assessed evidence of sexual dimorphism as it is presented on ribs 6 through 9. Their research tested the morphology of the ribs in two aspects: the overall curvature and the angle of the tubercle. Their methodology relied upon the use of the Microscribe G2 to gather semilandmark coordinate points needed. Data points were input into Rhinoceros 3D, a graphics program; this step was necessary for recreating a 3-dimensional copy of the rib's original curvature (Curran & Griffith, 2012). The program then chose 20 semilandmark points that corresponded to the same proportion of each rib (Curran & Griffith, 2012). These points were exported from Rhinoceros 3D and were analyzed in Morphologika to complete a generalized Procrustes and further statistical analyses (Curran & Griffith, 2012). Variation in the position of each corresponding point was then statistically assessed using a canonical variate analysis (CVA); cross-validation and resubstitution analyses were used to assess the effectiveness of this approach. Testing the effectiveness of their proposed methodology produced correct reclassification rates ranging from 74% to 84% (Curran & Griffith, 2012).

To test the angle of the tubercle in relation to sexual dimorphism, three points on each rib were taken using the Microscribe G2: the vertebral head of the rib, the position of the tubercle, and the sternal end of the rib (Curran & Griffith, 2012). The coordinate points were then input into Rhinoceros 3D and the angle of the tubercle was measured. Testing of the effectiveness for this approach yielded correct reclassification rates ranging from approximately 95 % to 81%. Though the methodology proposed shows much promise, as with the 4th rib, it is difficult to identify ribs 6 through 9 in disarticulated remains. Also, further testing of this methodology using a larger sample group would help validate this experimental approach and provide a better understanding of its reliability

Sexual dimorphism of the 1st and 2nd ribs

In her research, Gavit (2009) perceived the first and second ribs to be more appropriate for methodologies due to the factors influencing their identification and recovery. Like Isçan (1985), she relied upon the SIH and APB taken on the sternal ends of the first and second ribs. Her study sampled 323 skeletal remains from the William M. Bass and Maxwell Museum osteological collections 236 male and 87 female. It included specimens with anomalous rib morphologies. The left rib served as a substitute whenever a right rib was not present for the individual. She used a discriminant function analysis to develop a model that could be applied to other populations. Her analysis reported a pooled accuracy of 84%, and found greater success with correctly classifying females.

I reviewed Gavit's (2009) proposed methodology for sex estimation intended to evaluate the reliability of the approach for making sex estimations. This research was prompted by the assessment of normality of Gavit's (2009) published data; her data were found to be non-normally distributed. It has been observed that when parametric tests, such as discriminant function analysis, are applied to non-normal data, overall error may increase (SAS Institute Inc., 2010). My preliminary research tested the accuracy of the discriminant function formula created by Gavit (2009) and then compared it to an alternative statistical perspective, a stepwise binary logistic regression (BLR) model. The methodology and results of that examination are discussed further in the Methodology section.

II. METHODOLOGY

The analysis of skeletal remains is not purely dependent upon the researcher's observations. It is also dependent upon the statistical analysis of the data obtained, whether metric or nonmetric. Many statistical approaches for assessing variation in shape are housed in the anthropologist's toolbox, and each serves to answer a specific kind of question through utilizing a unique set of calculations. This study uses metric approaches to gather information on the shape and size of the first and second ribs with relation to sex.

Examining Gavit (2009)

To carry out this analysis I chose individuals from Gavit's (2009) primary sample, excluding those with rib anomalies and left rib substitutions, in order to perform stepwise BLR. My goal was to evaluate whether this non-parametric approach, seemingly more appropriate than linear discriminant analysis, reinforced or undermined her original conclusions. I excluded the left rib substitutions and anomalies because I thought it would be more useful to test first whether the method worked with a high-quality data set, to prove or disprove the concept, rather than evaluating a perhaps more realistic situation in which there were anomalies and substitutions because, obviously, the substitutions and anomalies add additional uncontrolled variables to the study and thereby make the results more difficult to interpret. I used the Minitab statistical program to carry out my analysis. The modified sample consisted of 237 individual cases, 163 males and 74 females. The modified dataset was analyzed using a stepwise binary logistic regression. The statistical model indicated that a cutoff of 0.56 presented the greatest amount of separation between the male and female samples. Thus, values below 0.56 were classified as female while values 0.56 or above were classified as male.

BLR model:

$Y = -17.59 + (0.3081)\textit{First rib SIH} + (0.298)\textit{Second rib SIH} + (0.893)\textit{Second rib APB}$

$Y' = e^{Y}$

$P = Y'/(1+Y')$

Female: $P < 0.56$

Male: $P \geq 0.56$

A second sample of specimens was measured with permission from Florida Atlantic University's College of Medicine. The right first and second ribs were dissected from eight human cadavers, four known male and four known females. Measurements as specified by Gavit (2009) were taken of the first and second ribs using a digital sliding caliper. The accuracy of the BLR formula and Gavit's (2009) discriminant function formula were tested. Both formulas were applied to measurements obtained from Gavit's sample and the new sample.

Output from the stepwise BLR, using the modified data set, indicates that the most diagnostic dimensions collected were the first rib's SIH and the second rib's SIH and APB. Using those three dimensions, the BLR formula accurately estimated 84.8% of males and females within the modified data set. Applying measurements of the secondary sample to the BLR equation resulted in the correct estimation of 7 cadavers;

only one cadaver was incorrectly estimated (Table 1). When the BLR model was applied to the primary sample obtained from Gavit (2009), there was an observed pooled accuracy of 85.8% (Table 2). Gavit's (2009) discriminant function formula relied upon all four dimensions: the first rib SIH and APB, and the second rib SIH and APB. Based on her model's parameters, values above 0 indicate female, values below 0 indicate male. Applying her discriminant function formula to the primary sample set produced inconclusive results (Table 3). When her discriminant function formula was applied to the secondary data set, the results were also inconclusive (Table 4).

Cadaver	Known Sex	Calculated P	Estimated Sex
1	Female	0.01091	Female
2	Female	0.02275	Female
3	Female	0.18929	Female
4	Female	0.09731	Female
5	Male	0.69338	Male
6	Male	0.99625	Male
7	Male	0.68753	Male
8	Male	0.26688	Female

Table 1. Cadaver rib dimensions applied to the BLR equation.

	Known Male	Known Female	Total
Estimated Male	**210**	20	230
Estimated Female	26	**67**	93
Total	236	87	323
Accuracy	0.8898	0.7701	0.857585

Table 2. Cross validation of the BLR equation.

	Known Male	Known Female	Total
Estimated Male	**236**	87	323
Estimated Female	0	**0**	0
Total	236	87	323
Accuracy	1.00	0.00	0.73065

Table 3. Cross validation of the discriminant function formula proposed by Gavit (2009).

Cadaver	Known Sex	Calculated Value	Estimated Sex
1	Female	-27.8655883	Male
2	Female	-27.8498029	Male
3	Female	-30.0371871	Male
4	Female	-30.3467035	Male
5	Male	-32.3470294	Male
6	Male	-36.6584723	Male
7	Male	-31.9722443	Male
8	Male	-30.3460192	Male

Table 4. Cadaver rib dimensions applied to Gavit's (2009) discriminant function equation.

This preliminary study was intended to compare an alternative approach to analyzing rib dimensions to Gavit's (2009) proposed method. Unfortunately, my inability to effectively use her method to distinguish between male and female individuals prevented the successful comparison of either model. Gavit (2009) used a linear discriminant analysis to examine her data, which is a parametric statistical test preferred for normal data. Inability to apply her formula may be a result of the nature of her data. The descriptive statistics of her original data revealed that it was non-normal. Gavit (2009) used a linear discriminant analysis to examine her data, which is a parametric statistical test utilizing normally distributed data. Additionally, when using non-normal data, a nonparametric statistical analysis, such as the stepwise binary logistic regression conducted, is preferred. Though it is possible to successfully apply a parametric statistical test, like discriminant function analysis, to non-normal data, the resulting error rate may be biased (SAS Institute Inc., 2010).

This study utilizes the BLR model to estimate sex using the sternal end of the first and second ribs. It compares the accuracy of using the sternal end to using the overall shape of the rib. Sexual dimorphism presented on the shape of the rib will be assessed using geometric morphometric techniques.

Geometric Morphometrics

Geometric morphometrics is a suite of multivariate statistical analysis methodologies that assess biological shape and its variation within the sample population. The approach utilizes landmark and semilandmark coordinate points to gather information; these points are then manipulated to isolate shape by removing size. Shape is considered invariant to location, scale, and orientation. Separating shape from size allows for the mathematical evaluation of shape characteristics and the analysis of shape changes in morphology. Calculating the distance between points and position changes from one individual to the next accounts for the amount of variation that is observable within the sample population group (Slice, 2002). This approach evaluates variables such as ancestry, age, or sex and their influence on morphological similarities or differences between and within populations. In doing so, it is possible to shed light on the influence of these variables on skeletal anatomy through mapping population variation; this will also aid in the development of a biological profile.

Landmarks and Semilandmarks

Assessment of morphological structure relies upon coordinate points, which are analogous to landmark or semilandmark points. Landmark points are positions on the subject that correspond to biologically or geometrically significant features (Polly, 2012). The points may correspond to a muscle insertion or origin site, the position of a foramen, or the intersection of two sutures (Polly, 2012). Semilandmark points are taken at positions that are arbitrarily chosen to capture topographic variation between landmark points such as curvature or surface contours (Polly, 2012). Together, landmark and semilandmark points capture shape variations on the outline and surface of the object. To capture these landmark points, it is necessary to use instruments such as a Microscribe or a NextEngine laser scanner to log the corresponding x, y, and z coordinates of the position of specific landmarks.

Statistical analysis

Geometric morphometrics relies upon the analysis of shape variation. In order to efficiently apply the statistical analysis, it is necessary to first isolate size through methods of standardization. The first step in standardization requires Procrustes superimposition of the points; this process is effective in revealing small-scale variation in the position of biological structures. Isolating shape from size and orientation using the Procrustes approach cleans the data and retains information that is pertinent to the object's shape.

Once size and orientation variables are removed from the data, it is necessary to create shape variables that quantify the position of the landmark points. One of the most effective ways to do this is through calculating the Principal Component Analysis (PCA) scores. PCA is a statistical approach that teases out the underlying principal components of the data set while maintaining the measure of shape variation shown in the data (Webster & Sheets, 2010). This is determined by calculating a vector direction that measures the greatest amount of variance in the data points. The distance between the points and the vector line is measured. The final output of the PCA provides two outputs: eigenvector (direction of the line) and eigenvalue (the amount of variance), the highest of which is the first principal component (Webster & Sheets, 2010). Using this form of analysis allows for the identification and simplification of the data set, thus removing non-pertinent information and allowing for analysis of variance within the data set.

Through processing data in this way, the meaningfulness of the variance within the sample set can be assessed. Two of the most common forms of analysis are regression analysis for continuous variables, and analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA) for categorical variables. The use of ANOVA/MANOVA tests is most pertinent to this project; this statistical approach assesses the relationship between the delineating variable and the associated geometric shape. It emphasizes the statistical differences in shape that can be used to differentiate between two categories, as well as the amount of variation observed between the groups.

Research Design

My research was divided into three phases. The first phase, data acquisition, consisted of collecting data on the first and second ribs from a blind sample of 140 skeletal remains of known sex and age. The sample was acquired from the William M. Bass Donated Skeletal Collection, located at the University of Tennessee. The skeletal collection consists of over 1,800 donated human remains, with 89% of the collection being of European ancestry (Forensic Anthropology Center, UTK). 65% of the population is male, and while the age range of this population is from 16 to 100 years old, a majority of them are between 40 and 80 years old (Forensic Anthropology Center, UTK). The first and second right ribs of 70 adult males and 70 adult females of European ancestry were measured.

The experimental methodology was first be applied to the right first and second ribs; this assessed the curvature of the rib using landmark and semilandmark points. A linear regression to reveal the effects of sexual dimorphism in the sample population was conducted. Next, the dimensions of the sternal end of the first and second ribs were measured. The dimensions included the superior-inferior height (SIH) and anterior-posterior breadth (APB).

The second phase of this research was statistical analysis of all data acquired from phase 1 and the estimations of sex. The analysis of the rib's curvature using both landmark and semilandmark coordinate points required a geometric morphometric approach. The analysis of the sternal end dimensions utilized the binary logistic regression model. The final phase of this project compared the variance observed between using the rib's shape and its sternal dimensions. Ultimately, the goal of this final phase was to discern which method captures the greatest amount of variation with regards to sexual dimorphism.

At the University of Tennessee, landmark coordinates corresponding to muscle insertions sites and vessel grooves were identified on the first and second ribs. The anatomical landmark points were selected for clarity and ease of identification, and the significance of the vessels and musculature of the region. Though the degree to which the landmarks are expressed varied, they were all easily identified through macroscopic evaluation of the superior surface of the ribs. The chosen landmark points in Figures 2 and 3 were used to assess rib morphology based upon form and function. Because landmark coordinates are focused on particular features on the rib, they cannot be expected to capture all variation seen on the ribs. Sampling of the entire curve of the rib was done using semilandmark points. A total of 20 semilandmark coordinate points were positioned on each first and second rib. 10 semilandmark points were obtained on the rib's lateral border of the ribs, and 10 were taken on the medial border.

Landmarks points on the first rib (Figure 2):

On lateral border:

a1. Most posterior-medial point of the vertebral head

b1. Medial border of costotranverse ligament insertion site

c1. Most inferior-medial edge of tubercle

d1. Most superior-lateral edge of tubercle

e1. Posterior border of scalenus medius insertion site

f1. Most anterior-medial points of the sternal end

g1. Anterior border of subclavius origin site

On medial border:

h1. Most posterior-medial point of sternal end

i1. Anterior border of groove for subclavian vein

j1. Anterior border of scalene tubercle

k1. Posterior border of scalene tubercle

l1. Posterior border of groove for subclavian artery

m1. Anterior-medial point of vertebral body

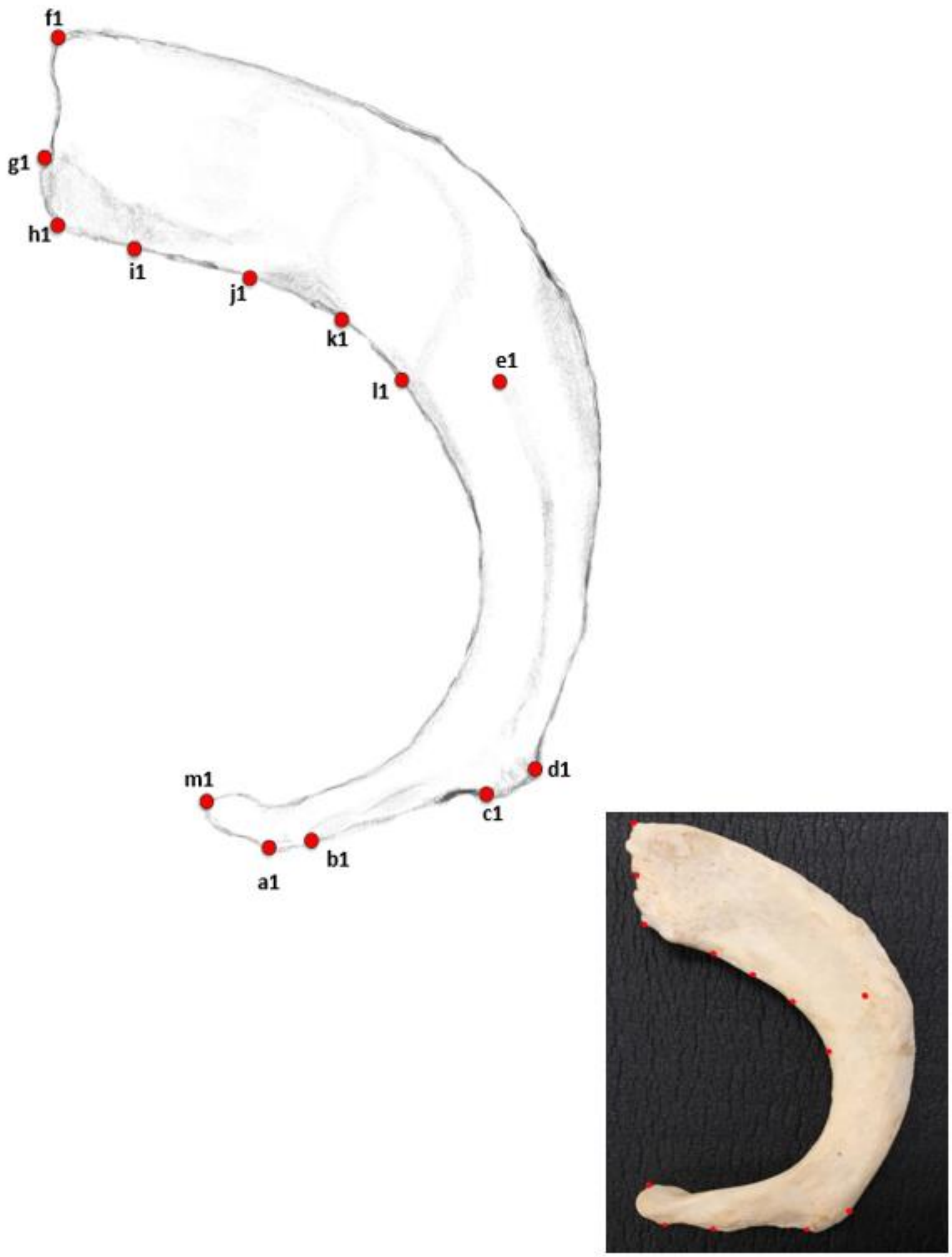

Figure 2. First rib landmark points.

Landmark points on the second rib (Figure 3):

<u>*On lateral border:*</u>

a2. Crest of neck

b2. Medial border of articular facet of tubercle

c2. Lateral border of articular facet of tubercle

d2. Lateral border of tubercle

e2. Costal angle

f2. Posterior-lateral border of scalenus posterior insertion site

g2. Anterior-lateral border of scalenus posterior origin site

h2. Posterior-lateral border of tuberosity

i2. Anterior-lateral border of tuberosity

j2. Most inferior-medial point of sternal end

<u>*On medial border:*</u>

k2. Most superior-medial point of sternal end

l2. Medial border of rib parallel to crest of neck

m2. Most anterior medial point on vertebral head

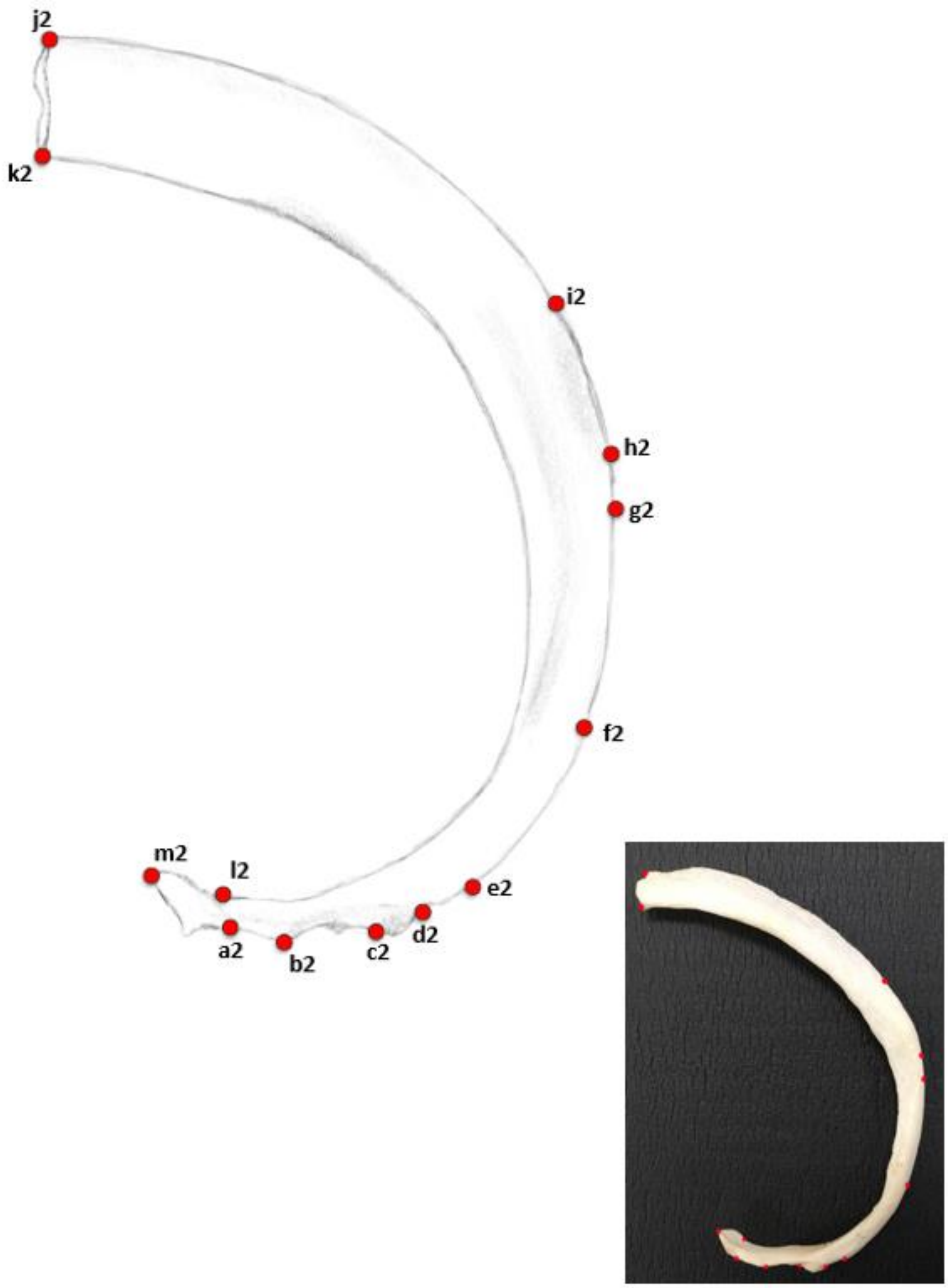

Figure 3. Second rib landmark points.

Methodology

All data was acquired digitally through the use of a Next Engine 3D Laser Scanner. The scans were then cleaned using the integrated *ScanStudio HD* software to remove any captured elements of the modeling clay used to orient the rib for scanning. Each rib was individually scanned, and then the digital model was input into software to capture the coordinate points and measurements. The landmark coordinate points and measurements needed for the BLR model were obtained through *Landmark*. *Landmark* is a software program created and published by the Institute for Data Analysis and Visualization (IDAV), within the University of California, Davis. The program allowed points to be placed as desired on the surface of the scan. *Landmark* also allowed for the distance between points to be measured. Points were placed corresponding to the rib's SIH and APB; the measurement of the distance between the points was noted and used in the BLR model. The semilandmark points were acquired using *tpsDIG*, which allowed the borders of the scanned ribs to be digitized. The digitized curve of the lateral and medial borders of the rib was then resampled in order to obtain the semi-landmark coordinate points.

Once all coordinate data was obtained and organized, geometric morphometric analysis began. All statistical analysis was conducted using the *Geomorph R package.* Statistical analysis of landmark and semilandmark data was conducted separately. The first and second ribs were analyzed separately. For the landmark data, a Procrustes analysis was used to remove size and orientation variables, allowing the data that communicates shape to stand alone. Procrustes analysis is the size measure that is used to scale a configuration of landmarks; the amount of size variation can be determined by understanding the degree to which the element was scaled (Slice 2007). In order to incorporate the variable of size, the centroid size (Csize) of each first rib was used to determine the significance of size differences with respect to sex. The normality of the centroid size data was assessed using a Shapiro-Wilks normality test. Upon determining the nature of the data a T-test or Mann-Whitney U test was used to measure the variance between the means and determine the presence of significant differences between male and female size adjustments

The coordinate points produced from the Procrustes were then used to conduct a Principal Components Analysis (PCA). To produce a better depiction of the position of males and females within the PCA plot females were color coded black, while the males were coded red. The cumulative proportions of the PCA scores were assessed. PCA scores that accounted for ~80% of the population variance, as indicated by the cumulative proportions, were used to conduct a MANOVA. The Wilks' Lambda statistic was used to measure the significance of the variation between males and females.

Human variation is not only a product of sex, there are also genetic and environmental factors that influence the morphology of the body. In order to detect the degree to which this study was able to capture morphological variation with respect to sex, a stepwise multiple linear regression was performed. The PC scores of both landmark and semilandmark data of ribs 1 and 2 were analyzed to determine the amount of variation of the variable was captured within each individual component. R^2 values were also accounted for; however, in a data set such as this, where there is potential for noise, the R^2 value cannot always be trusted. Thus it was necessary to conduct a cross-validation analysis to determine the predictive accuracy of the regression model and its potential to be used to estimate the sex of unknown individuals.

To measure the presentation of sexual dimorphism on the sternal end of the rib, the SIH and APB measurements of the dimensions were analyzed using the BLR sternal end methodology. This was necessary to determine the accuracy of the method when applied to the data set. A MANOVA was conducted on the sternal end dimensions to identify the amount of variation seen between females and males. The amount of variation detected from the ribs' shape was then compared to the amount of variation detected using the sternal end BLR model. The accuracy of the predictive models created was also compared.

III. RESULTS

Analysis of the statistical data obtained from the rib occurred in many stages. Due to the exploratory nature of this study, results from each stage guided me in determining how to proceed. Doing so allowed me to optimize the data used in each step in order to better identify variability between the male and female groups. Results from the principal component analysis (PCA) were especially important in that it provided multidimensional scaling that is used to portray the greatest amount of information about the sample. The principal component (PC) represents the various principal coordinate planes that present some degree of the population variance. PC scores indicate the position of an individual within the PC plane; these are used to create a plot that visually represents the population variance observed on each PC plane.

The use of the proportion variance and cumulative proportion of those principal components were especially important. Proportional variance depicts the amount of variability that is identified within that particular principal component. PC1 always has the greatest variance, whereas PC2 through *n* have progressively less variance; the amount of variance decreases as one moves to higher principal components. Cumulative proportion indicates the amount of variance that is accounted for within a given set of principal components. The cumulative proportion statistic was used to determine how many principal components would be needed to measure at least 80% of the variability observed between males and females.

The centroid size (Csize) adjustment obtained from the Procrustes analysis was assessed for normality using the Shapiro-Wilks Normality test. Determining the distribution of the data is important for identifying the best statistical analysis that can be used to measure the variance between male and female Csize. The data were tested at a 95% confidence interval and yielded a p-value of 1.715e-11. From this output, p-value is less than 0.05, indicating that the Csize data demonstrate a normal distribution; thus normality was assumed.

Statistic	Value	
T	-1.3996	
Df	133.24	
p-value	0.164	
	Female	Male
95% confidence	-498.55	85.36
Sample means	2822.31	3028.913

Table 5. T-test of the centroid size adjustments obtained from the Procrustes analysis.

Using the results of the Shapiro-Wilks test a T-test was deemed appropriate for measuring the significance of variance between the male and female groups (Table 5). The sample mean of the female Csize is 2822.31, whereas male Csize sample mean is 3028.91. A test of the significance of the variance between the means at a 95% confidence interval produced a p-value of 0.164. Based on this statistic, male and female Csize adjustments do not differ significantly.

	Standard Deviation	Proportion of Variance	Cumulative Proportion
PC1	0.3597	0.2204	0.2204
PC2	0.3104	0.1641	0.3846
PC3	0.2781	0.1318	0.5163
PC4	0.2531	0.1091	0.6254
PC5	0.19381	0.06398	0.68938
PC6	0.16614	0.04701	0.73639
PC7	0.15547	0.04117	0.77756
PC8	0.14407	0.03535	0.81291
PC9	0.133	0.03013	0.84304
PC10	0.12513	0.02667	0.86971
PC11	0.1126	0.0216	0.8913
PC12	0.10384	0.01837	0.90967
PC13	0.08973	0.01371	0.92339
PC14	0.08623	0.01266	0.93605
PC15	0.07624	0.0099	0.94595
PC16	0.06952	0.00823	0.95418
PC17	0.06177	0.0065	0.96068
PC18	0.05998	0.00613	0.96681
PC19	0.05731	0.00559	0.9724
PC20	0.05239	0.00467	0.97707
PC21	0.04985	0.00423	0.98131
PC22	0.04711	0.00378	0.98509
PC23	0.04118	0.00289	0.98798
PC24	0.03907	0.0026	0.99058
PC25	0.03727	0.00237	0.99294
PC26	0.035	0.00209	0.99503
PC27	0.03251	0.0018	0.99683
PC28	0.03106	0.00164	0.99847
PC29	0.02994	0.00153	1
PC30	1.80E-16	0.00E+00	1.00E+00
PC31	1.44E-16	0.00E+00	1.00E+00
PC32	1.27E-16	0.00E+00	1.00E+00
PC33	1.14E-16	0.00E+00	1.00E+00
PC34	1.07E-16	0.00E+00	1.00E+00
PC35	8.80E-17	0.00E+00	1.00E+00
PC36	7.19E-17	0.00E+00	1.00E+00

Table 6. Principal components of landmark coordinate points on Rib 1

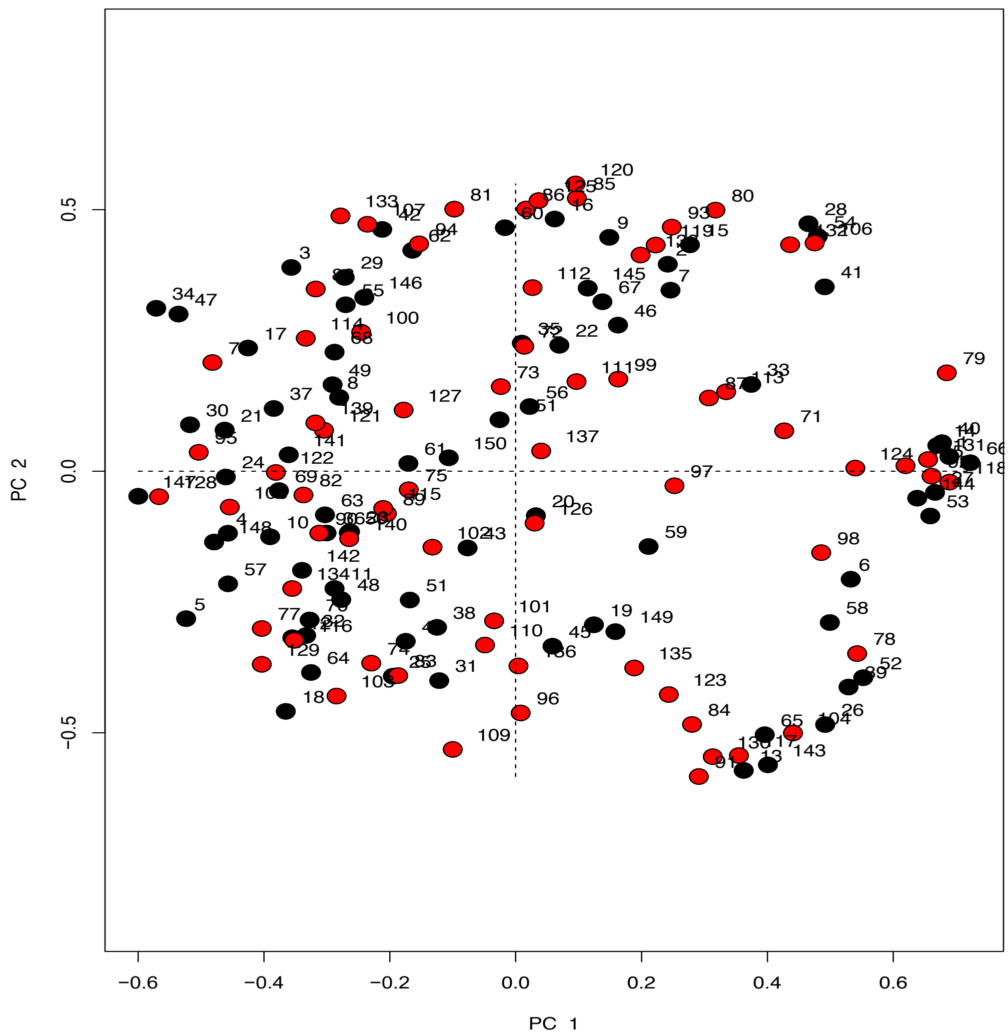

Figure 4. Plot of PC1 scores x PC2 scores from PCA of Rib 1 with landmark coordinate points.

Analysis of landmark coordinate points on the first rib depicts a low proportion of variance between the first two principal components. Within the first two principal components only 0.3846 has been accounted for (Table 6). At least 0.80 of variance is accounted for within the first eight principal components; the cumulative proportion of PC8 is 0.81291. A plot of PC1 and PC2 (Figure 4) reveals that there is no clear trend in the organization of individuals.

	Standard deviation	Proportion of Variance	Cumulative Proportion
PC1	0.2577	0.1511	0.1511
PC2	0.232	0.1225	0.2736
PC3	0.20419	0.09486	0.36845
PC4	0.19427	0.08587	0.45432
PC5	0.17841	0.07242	0.52674
PC6	0.17488	0.06959	0.59632
PC7	0.16921	0.06515	0.66147
PC8	0.15207	0.05261	0.71408
PC9	0.1487	0.0503	0.7644
PC10	0.13467	0.04127	0.80565
PC11	0.1234	0.03464	0.8403
PC12	0.116	0.03062	0.87091
PC13	0.10972	0.02739	0.8983
PC14	0.104	0.0246	0.9229
PC15	0.0866	0.01706	0.93997
PC16	0.08406	0.01608	0.95605
PC17	0.07056	0.01133	0.96737
PC18	0.06087	0.00843	0.9758
PC19	0.05873	0.00785	0.98365
PC20	0.04986	0.00566	0.98931
PC21	0.04456	0.00452	0.99382
PC22	0.03599	0.00295	0.99677
PC23	0.02494	0.00141	0.99819
PC24	0.01976	0.00089	0.99907
PC25	0.01661	0.00063	0.9997
PC26	0.01146	0.0003	1
PC27	4.46E-16	0.00E+00	1.00E+00
PC28	2.44E-16	0.00E+00	1.00E+00
PC29	1.37E-16	0.00E+00	1.00E+00
PC30	1.25E-16	0.00E+00	1.00E+00
PC31	9.02E-17	0.00E+00	1.00E+00
PC32	8.42E-17	0.00E+00	1.00E+00
PC33	7.18E-17	0.00E+00	1.00E+00

Table 7. Principal components of landmark coordinate points on Rib 2.

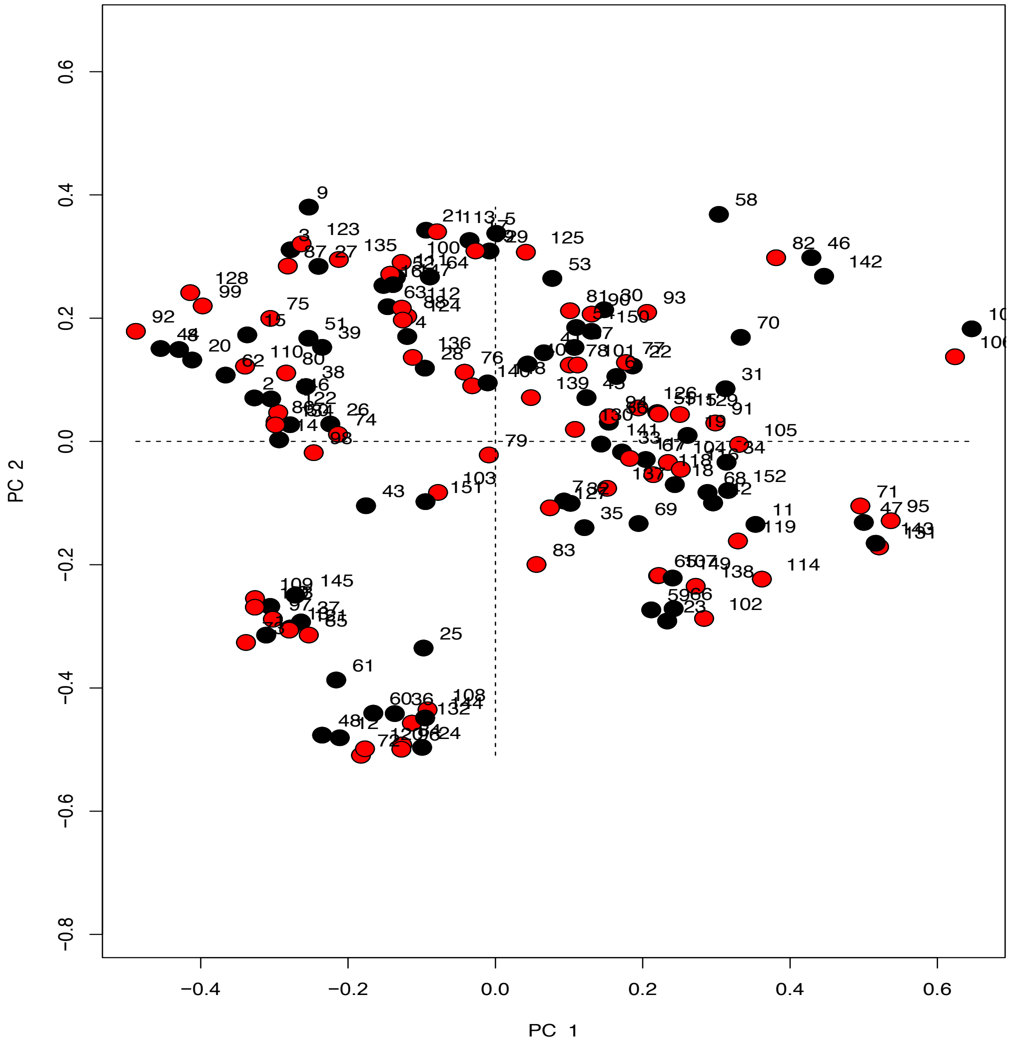

Figure 5. Plot of PC1 scores x PC2 scores from PCA of Rib 2 with landmark coordinate points.

An analysis of landmark coordinate points of the second rib present lower proportions of variance than the first rib. PC1 and PC2 accounts for 0.2736 of the sample's variance (Table 7). Approximately 0.8056 of variance is accounted for within the first ten principal components. A plot of PC1 and PC2 (Figure 5) shows individuals are broadly mixed with respect to sex. Like the first rib's landmark analysis (Figure 5), they appear widely scattered between the first and second principal component axes.

	Standard deviation	Proportion of Variance	Cumulative Proportion
PC1	0.1338	0.4837	0.4837
PC2	0.1052	0.2992	0.7829
PC3	0.05557	0.08351	0.86641
PC4	0.04203	0.04776	0.91417
PC5	0.03466	0.03248	0.94665
PC6	0.02627	0.01867	0.96532
PC7	0.01724	0.00804	0.97336
PC8	0.01544	0.00645	0.97981
PC9	0.01384	0.00518	0.98498
PC10	0.009742	0.00257	0.98755
PC11	0.009263	0.00232	0.98987
PC12	0.007872	0.00168	0.99154
PC13	0.007314	0.00145	0.99299
PC14	0.006656	0.0012	0.99419
PC15	0.006497	0.00114	0.99533
PC16	0.006175	0.00103	0.99636
PC17	0.005267	0.00075	0.99711
PC18	0.004481	0.00054	0.99765
PC19	0.003928	0.00042	0.99807
PC20	0.003709	0.00037	0.99844
PC21	0.003321	0.0003	0.99874
PC22	0.003008	0.00024	0.99899
PC23	0.002777	0.00021	0.99919
PC24	0.002699	0.0002	0.99939
PC25	0.002448	0.00016	0.99955
PC26	0.002398	0.00016	0.99971
PC27	0.001707	0.00008	0.99979
PC28	0.001591	0.00007	0.99986
PC29	0.001255	0.00004	0.9999
PC30	0.0009919	0.00003	0.99993
PC31	0.0008188	0.00002	0.99994
PC32	0.0007334	0.00001	0.99996
PC33	0.0007116	0.00001	0.99997
PC34	0.0006199	0.00001	0.99998
PC35	0.0005774	0.00001	0.99999
PC36	0.000564	0.00001	1
PC37	1.40E-16	0.00E+00	1.00E+00
PC38	6.54E-17	0.00E+00	1.00E+00
PC39	4.36E-17	0.00E+00	1.00E+00
PC40	3.05E-17	0.00E+00	1.00E+00

Table 8. Principal components of semilandmark coordinate points on Rib 1.

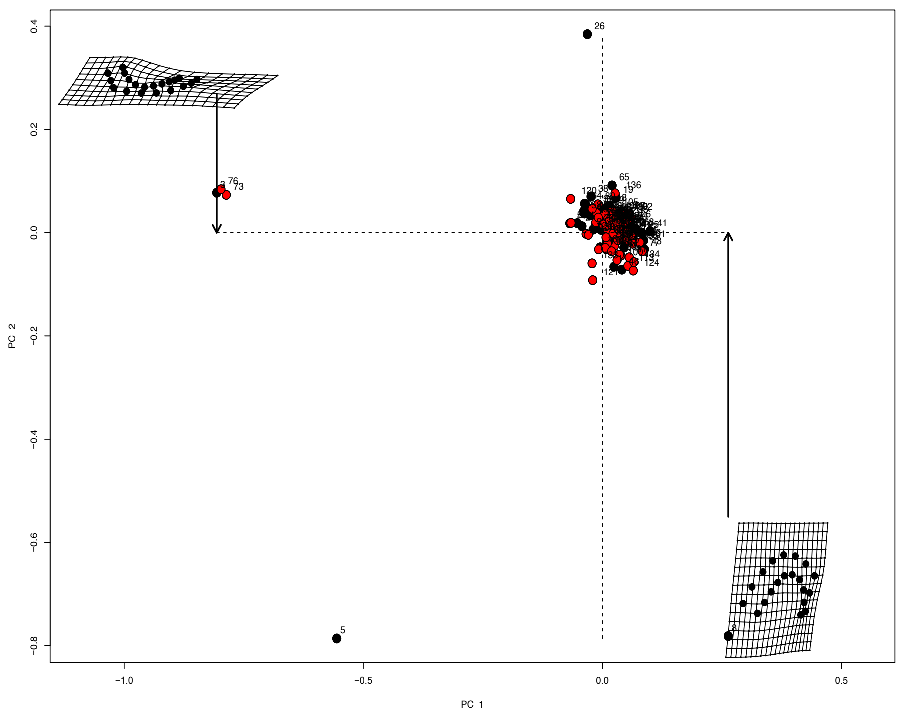

Figure 6. Plot of PC1 scores x PC2 scores from PCA of Rib 1 with semilandmark coordinate points. Outliers are present in the sample.

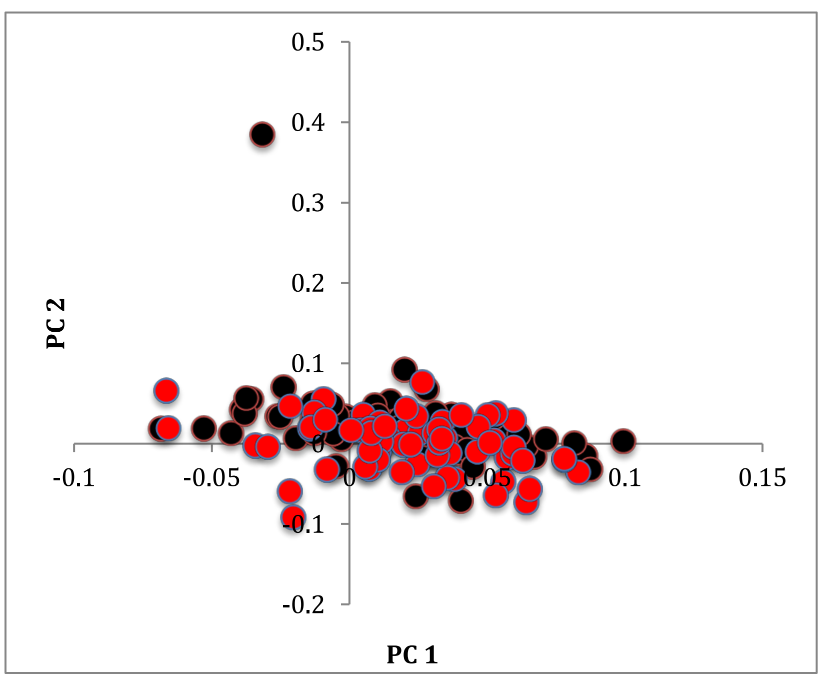

Figure 7. Plot of Rib 1 Semilandmark PC1 x PC2 plot with outliers removed.

Compared to landmark points, principal component analysis of semilandmark coordinate points reveal better proportions of variance. Within PC1 and PC2 a 0.7829 cumulative proportion of variance is observed between individuals (Table 8). Approximately 0.8664 of variance is accounted for within the first three principal components. A plot of PC1 and PC2 (Figure 6) portrays a clearer organization of individuals on the principal component axes. The plot also reveals few individuals who appear as outliers who are a mixture of both male and female individuals. Further inspection of these outliers did not reveal any strange or anomalous shape of those ribs. It is unclear why these specific individuals were so far from the rest of the cluster (Figure 7).

	Standard deviation	Proportion of Variance	Cumulative Proportion
PC1	0.1646	0.6176	0.6176
PC2	0.1119	0.2853	0.9029
PC3	0.03869	0.03411	0.93702
PC4	0.02967	0.02005	0.95707
PC5	0.0236	0.01269	0.96976
PC6	0.02011	0.00921	0.97897
PC7	0.01904	0.00826	0.98723
PC8	0.01271	0.00368	0.99091
PC9	0.009293	0.00197	0.99288
PC10	0.008332	0.00158	0.99446
PC11	0.007509	0.00128	0.99574
PC12	0.006355	0.00092	0.99666
PC13	0.005978	0.00081	0.99748
PC14	0.004417	0.00044	0.99792
PC15	0.004294	0.00042	0.99834
PC16	0.003526	0.00028	0.99863
PC17	0.003291	0.00025	0.99887
PC18	0.003122	0.00022	0.99909
PC19	0.002724	0.00017	0.99926
PC20	0.002583	0.00015	0.99942
PC21	0.002325	0.00012	0.99954
PC22	0.002184	0.00011	0.99965
PC23	0.001859	0.00008	0.99973
PC24	0.001771	0.00007	0.9998
PC25	0.001649	0.00006	0.99986
PC26	0.001497	0.00005	0.99991
PC27	0.001187	0.00003	0.99994
PC28	0.0009275	0.00002	0.99996
PC29	0.0007091	0.00001	0.99997
PC30	0.0005838	0.00001	0.99998
PC31	0.0004513	0	0.99999
PC32	0.0004484	0	0.99999
PC33	0.0003886	0	0.99999
PC34	0.0003457	0	1
PC35	0.0002587	0	1
PC36	0.0002226	0	1
PC37	1.01E-16	0.00E+00	1.00E+00
PC38	5.07E-17	0.00E+00	1.00E+00
PC39	4.58E-17	0.00E+00	1.00E+00
PC40	4.33E-17	0.00E+00	1.00E+00

Table 9. Principal components of landmark coordinate points on Rib 2.

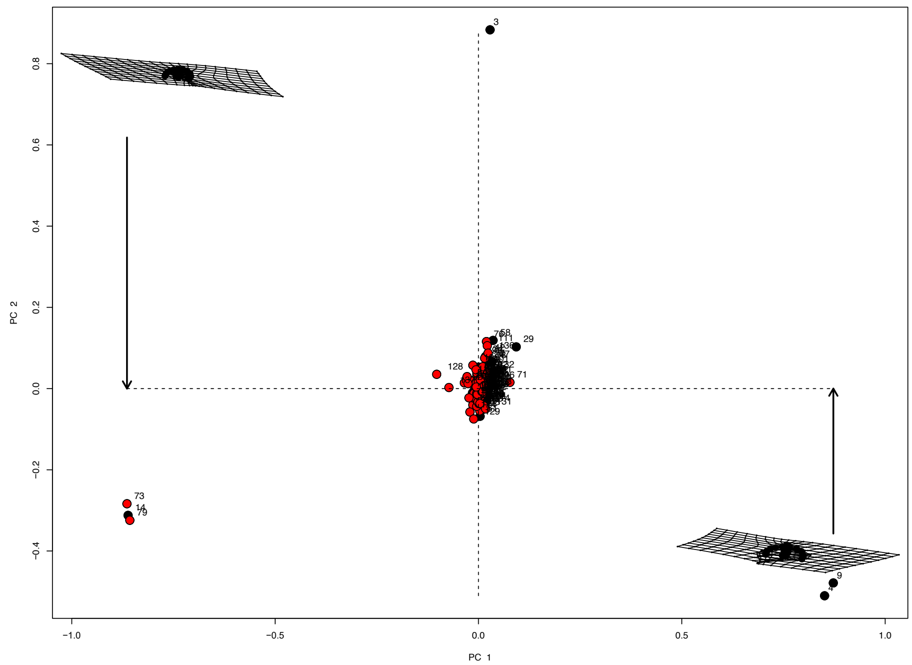

Figure 8. Plot of PC1 scores x PC2 scores from PCA of Rib 2 with semilandmark coordinate points. Outliers are present in the plot.

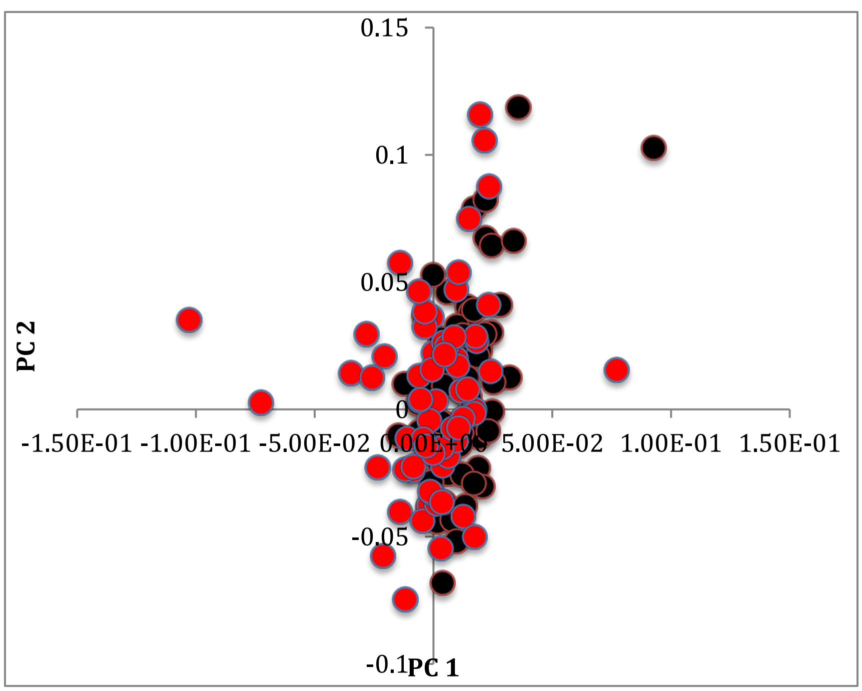

Figure 9. Primary cluster of Rib 2 Semilandmark PC1 x PC2 plot with outliers removed.

Analysis of semilandmark coordinate points on the second rib present the highest proportions of variance amongst PC1 and PC2. Together, PC1 and PC2 account for 0.9029 cumulative proportion of variance between individuals; PC1 alone measures 0.6176 of variance (Table 9). A plot of PC1 and PC2 (Figure 8) reveals the greatest organization of individuals across the principal component axes. As with the semilandmark points of the first rib, the plot also reveals outliers that are a mixture of both male and female individuals. It is unclear why these individuals deviate from the rest of the cluster, as their overall shapes do not appear anomalous in any way (Figure 9).

The completed principal component analysis of this study's data provided over 30 principal component planes for each data set. The second rib landmark PCA possessed the lowest rates of proportion variance and cumulative proportion; at least 0.80 of cumulative proportion was observed until PC10 (Table 7). In contrast, the second rib semilandmark PCA had the highest rates of proportion variance and cumulative variance, with approximately 0.90 cumulative proportion observed at PC2 (Table 9). To maintain consistency for each rib analyzed and method of assessing sexual dimorphism, PC scores from PC1 to PC10 were used in each analysis of variance.

	Df	Wilks	Approx. F	num Df	Den Df	p-value
as.factor (Sex)	1	0.92148	0.71364	16	134	0.7763
Residuals	149					

Table 10. Results from MANOVA on PC scores of landmark coordinate points on Rib 1.

	Df	Wilks	Approx. F	num Df	Den Df	p-value
as.factor (Sex)	1	0.95132	0.43177	16	135	0.9717
Residuals	150					

Table 11. Results from MANOVA on PC scores of landmark coordinate points on Rib 2.

Multivariate analysis of variance (MANOVA) of principal component scores of each individual from PC1 through PC10 is used to measure the Wilks' Lambda test statistic. For landmark coordinate points on the first rib $p = 0.7763$; there are no significant differences between male and female first ribs using landmark coordinate points (Table 10). On the second rib's landmark coordinates, $p = 0.9717$ (Table 11). As with the first rib, the coordinate points do not present any significant differences between male and females. These analyses suggest that the use of landmark points is ineffective for identifying sexual dimorphism on the first and second ribs.

	Df	Wilks	Approx. F	num Df	Den Df	p-value
as.factor (Sex)	1	0.88212	1.7239	10	129	0.08193
Residuals	138					

Table 12. Results from MANOVA on PC scores from semilandmark coordinate points on Rib 1.

	Df	Wilks	Approx. F	num Df	Den Df	p-value
as.factor (Sex)	1	0.78188	3.5988	10	129	0.000306
Residuals	138					

Table 13. Results from MANOVA on PC scores from semilandmark coordinate points on Rib 2.

MANOVA of semilandmark coordinate points reveal significant differences between male and female ribs. For semilandmark coordinate points on the first rib, the p-value is approximately 0.082 suggesting that there is some difference between male and female first ribs using semilandmark coordinate points; these are not significant differences as they do not satisfy the alpha level of 0.05 (Table 12). On the second rib's semilandmark coordinates, the p-value is approximately 0.00031, indicating that there are strong significant differences between male and female ribs (Table 13). The analysis of semilandmark coordinate points indicates that there are significant differences between male and female first and second ribs. However, these differences were not detectable using landmark coordinate points. This suggests that the use of semilandmark coordinate points is most appropriate for capturing variability on the first and second ribs.

	PC score	Coefficients	Std. Error	t-value	p-value
Landmark 1st Rib y-intercept = 0.4643	PC5	0.373426	0.2119	1.781	0.0771
	PC8	-0.493179	0.2842	-1.736	0.0848
	PC9	0.471659	0.3004	1.570	0.1186
	PC22	-1.19632	0.8559	-1.398	0.1642
	PC24	1.460478	1.023	1.428	0.1555
	PC36	-7.3739e+14	4.084e+14	-1.805	0.0731
Landmark 2nd Rib y-intercept = 0.4655	PC10	-0.492551	0.2995	-1.645	0.1022
	PC20	-1.259556	0.8027	-1.569	0.1188
	PC25	-5.655612	2.375	-2.381	0.0185
	PC27	-1.8351e+14	9.623e+13	-1.906	0.0586
	PC33	-5.9255e+14	4.138e+14	-1.432	0.1542
Semilandmark 1st Rib y-intercept = 0.5109	PC1	-0.5325651	0.3594	-1.482	0.140889
	PC6	4.582353	1.277	3.588	0.000479
	PC7	3.616818	1.967	1.839	0.068368
	PC10	9.385628	3.445	2.725	0.007366
	PC11	7.091056	3.628	1.955	0.052881
	PC13	-16.94633	4.587	-3.695	0.003850
	PC15	15.23214	5.171	2.946	0.003850
	PC17	20.55082	6.368	3.227	0.001600
	PC18	-18.87029	7.48	-2.522	0.012948
	PC19	-17.29265	8.539	-2.025	0.044996
	PC24	40.50556	12.54	3.229	0.001590
	PC33	106.1973	47.26	2.247	0.026398
	PC36	124.0379	59.60	2.081	0.039478
	PC37	-7.342426e+14	2.510e+14	-2.925	0.004095
	PC40	1.844290e+15	9.864e+14	1.870	0.063878

Semilandmark 2nd Rib y-intercept = 0.4929	PC1	0.4549723	0.2243	2.028	0.044627
	PC4	2.2696791	1.2450	1.823	0.070645
	PC5	2.3030489	1.5652	1.471	0.143642
	PC6	4.4015133	1.8354	2.398	0.017934
	PC8	-11.0451	2.9043	-3.803	0.000221
	PC9	-11.00824	4.242	-2.549	0.012348
	PC13	18.193408	6.1786	2.945	0.003847
	PC17	-18.35177	11.2280	-1.634	0.104637
	PC18	17.81134	11.8245	1.506	0.134471
	PC25	-30.35032	22.3996	-1.355	0.177840
	PC29	70.54579	52.0864	1.354	0.178014
	PC34	-178.03104	106.8454	-1.666	0.09812

Table 14. Stepwise multiple linear regression of PC scores.

A stepwise multiple linear regression of each data set's PC scores revealed only a few components to use to capture sexual variations on the first and second ribs. PC scores from the landmark points on the first rib produced an R^2 value of 0.0441, suggesting there is low fitness of the data and poor predictive abilities of the model. PC scores of the landmark points on the second rib produced an R^2 value of 0.0628, revealing that it is also a poor predictive model. Regression of PC scores using semilandmark points produced an R^2 value of 0.3793 for the first rib, and 0.2468 for the second rib. This indicates that semilandmark coordinate points have better a predictive accuracy than the landmark coordinate points, though still poor.

Regression Model of First Rib Landmark PC scores:

$$X = 0.4643 + (0.373426*PC5) + (-0.493179*PC8) + (0.471659*PC9) + (-1.19632*PC22) + (1.460478*PC24) + ((-7.3739E+14)*PC36)$$

	Known Male	Known Female	Total
Estimated Male	**45**	34	79
Estimated Female	25	**36**	61
Total	70	70	140
Accuracy	0.643	0.514	0.579

Table 15. Cross validation of regression model.

Regression Model of Second Rib Landmark PC scores:

$$X = 0.4655 + (-0.4926*PC10) + (-1.26*PC20) + (-5.656*PC25) + ((-1.834e+14)*PC27) + ((-5.925e+14)*PC33)$$

	Known Male	Known Female	Total
Estimated Male	**46**	32	78
Estimated Female	24	**38**	62
Total	70	70	140
Accuracy	0.657	0.543	0.60

Table 16. Cross validation of regression model.

Regression Model of First Rib Semilandmark PC scores:

X = 0.5109 + (-0.5326*PC1) + (4.5824*PC6) + (3.6168*PC7) + (9.3856*PC10) + (7.0911*PC11) + (-16.9463*PC13) + (15.2321*PC15) + (20.5508*PC17) + (-18.8702*PC18) + (-17.2927*PC19) + (40.5056*PC24) + (106.1973*PC33) + (124.0379*PC36) + ((-7.3424E+14)*PC37) + ((1.8443E+15)*PC40)

	Known Male	Known Female	Total
Estimated Male	**57**	14	71
Estimated Female	13	**56**	69
Total	70	70	140
Accuracy	0.814	0.80	0.807

Table 17. Cross Validation of regression model.

Regression Model of Second Rib Semilandmark PC scores:

X = 0.4929 + (0.4549723*PC1) + (2.2696791*PC4) + (2.3030489*PC5) + (4.4015133*PC6) + (-11.0451*PC8) + (-11.00824*PC9) + (18.193408*PC13) + (-18.35177*PC17) + (17.81134*PC18) + (-30.35032*PC25) + (70.54579*PC29 + (-178.03104*PC34)

	Known Male	Known Female	Total
Estimated Male	**53**	19	72
Estimated Female	17	**51**	68
Total	70	70	140
Accuracy	0.757	0.729	0.729

Table 18. Cross validation of regression model.

To thoroughly assess the predictive accuracy of the PC scores using the linear regression models, a cross-validation analysis was completed. Females in this study were coded *1* and males were coded *0;* $X \leq 0.5$ is classified as male, $X > 0.5$ is classified as female. The regression models of semilandmark PC scores were better predictive models in this study; the first rib had an accuracy rate of 80.7% (Table 17), the second rib had an accuracy of 72.9% (Table 18). The regression models of landmark PC scores were not very effective in estimating sex; the first rib's accuracy was 57.9% (Table 15), while the second rib had an accuracy of 60% (Table 16). All models showed slightly better performance in correctly estimating male individuals than female individuals.

	Known Male	Known Female	Total
Estimated Male	**28**	4	32
Estimated Female	8	**36**	44
Total	36	40	76
Accuracy	0.777	0.9	**0.842**

Table 19. BLR model applied to the sample of ribs.

	Df	Wilks	Approx. F	num Df	Den Df	p-value
as.factor (Sex)	1	0.53471	15.446	4	71	3.904e-09
Residuals	74					

Table 20. Results from MANOVA on the measurements taken for the BLR model.

Applying the binary logistic regression model to the individuals within the sample used a subset of 76 individuals, 40 females and 36 males. Within this subsample, 28 of the 36 males sampled were correctly identified and 36 of the 40 females sampled were correctly identified (Table 19). An 84.2% overall accuracy was observed within this population. A MANOVA was then performed on the measurements taken, and produced a p-value of 3.09e-09, indicating that there are significant differences between males and females (Table 20).

V. DISCUSSION AND CONCLUSION

The human rib presents an interesting depiction of the morphological variation between males and females; however, size also plays a role. Within the human species, size variation between males and females is one of the clearest markers of sexual dimorphism. There is overlap in size amongst various populations though males tend to be distinctly larger and more robust. Geometric morphometric approaches function through focusing on the position of the points, and the proportional variation between and within the groups. From the t-test that was conducted (Table 5) it is evident that some degree of sexual dimorphism present on the ribs was lost due to the removal of size.

Comparing success of both landmark and semilandmark coordinate points presents insight into geometric morphometric methodologies and their application. The landmark points used in this study indicate that there are no significant differences in the shape of male and female first and second ribs. In contrast, the use of semilandmark points indicates that there are significant differences between the rib's shapes. The difference in results seen between the landmark and semilandmark approaches are engrained in the function of geometric morphometrics.

The landmark points chosen were associated with anatomical structures that interacted with the bone, including muscle attachment sites and grooves produced from the vessels that lay atop the bone. The positions of these landmarks are proportional to the size of the individual. Regardless of the individual, the expressions of those features are generally unified across the population. When size variation is removed in the Procrustes analysis, the new coordinate points represent the proportions of the landmark's positions; because the proportions between male and female musculature is equivalent, there are no significant differences. Thus, the PCA and MANOVA are unable to detect significant variation between males and females of the sample population. Though this does not reveal any significant differences in the male and female ribs through the landmark coordinates, it does confirm proportional anatomical development between males and females. These results suggest that sexual dimorphism of the thorax may not be attributed to the interaction of the muscle or mechanical demands; further research is needed to thoroughly examine this.

The semilandmark points applied to the first and second ribs were able to reveal significant differences between male and female rib morphology. The semilandmark points captured the overall curvature of the lateral and medial borders of the rib. While this does not exclude the influence that size has on the rib, shape variation at each semilandmark point is identifiable after the removal of the size component. Landmark morphometrics is applicable for identifying shape variations in humans; however, it is not best suited for distinguishing sexual dimorphism on the first and second ribs. Semilandmark points are better suited for capturing the significant shape differences of the rib. This is further supported by the stepwise multiple linear regressions performed on each principal component data set. Regression models created from the semilandmark points PC scores showed to possess a better predictive accuracy, ranging from 80.71% to 72.86%.

The female reproductive requirements may be one source of the variability observed on the first and second ribs through the use of semilandmark coordinate points. The need to accommodate a fetus impacts the structure of the pelvis as well as the thorax (Bastir et al., 2014; Garcia-Martinez et al., 2016). Examining the orientation of the rib cage revealed that the female thorax declined more than the male's; thus, the rib curves further inferiorly in females than in males (Bellamare, 2003; Bastir et al., 2014; Garcia-Martinez et al., 2016). In-depth exploration of the elements influencing the morphology of the thorax is needed to properly understand the sources of variation.

The age range of the individuals within this sample was fairly limited. All individuals within the population were adults, a majority of them being over the age of 50. Age is known to play a role in the shape of other skeletal elements due to hormone fluctuations, however this factor does not appear to significantly obscure sexual dimorphism on the ribs. One issue that was caused by the older age of the individuals in the sample is calcification of the costal cartilage. The calcification observed occurs locally and is common in aging. The costal cartilage bridging the sternum and rib of several individuals was calcified or in the process of becoming calcified. In several individuals the sternal end of the rib developed additional bone deposits, causing it to become enlarged and flared; in extreme cases the rib became completely fused to the sternal bodies.

The calcification did not negatively impact the morphometric coordinate points taken, but it did affect the ability to properly measure the sternal end dimensions for the BLR model. It was necessary to exclude any individuals who presented any anomalous bone on the sternal end of either the first and second ribs to prevent the additional calcification from skewing results. Eliminating those individuals reduced the sample size to 76 individuals. Within that subsample the BLR model was able to accurately estimate the sex of 84.2% of the group. The MANOVA performed on the sternal end measurements of the first and second ribs produced a p-value significantly lower than the p-value of any morphometric analysis of the rib's shape. While there is a significant difference in the shape of the ribs, the use of sternal end dimensions may be better suited for distinguishing sexual dimorphism. This success may be because the size of the rib is the component being assessed in the model.

The goal of this study was to examine variability in the shape of the first and second rib with respect to sex. Through measuring the shape of the rib using both landmark and semilandmark coordinate points it is clear that morphological variability exists between males and females on the upper thorax. A regression model to estimate sex using the PC scores of unknown individuals does prove to be an effective methodology, though less accurate than the BLR model. Geometric morphometric models have been applied to create programs such 3D-ID and BioImage XD, which are used to estimate elements of the biological profile. Unfortunately, designing a similar program for estimating sex using morphological variation of the first and second rib was beyond the scope of this project. The simplified binary logistic model refined from Gavit's (2009) research currently shows greater promise as a tool for biological anthropologists. The model can be easily applied to measurements taken on the sternal end of the ribs and has been applied to several sample sets with relatively consistent success.

This research expands upon the discussion of human morphological variation observed on the thorax. Through examining ribs, it lends insight to an often-overlooked element of the skeletal anatomy. While the tested approach to examine sexual dimorphism through overall shape of the rib may be complex, the outcome of this study identifies ribs as a skeletal element that should not be overlooked in sex estimation. While it may take several studies to properly understand the factors influencing the shape and dimensions of the ribs and to validate its use, the use of the BLR methodology does have real applications for biological anthropologists. Continued research on variability observed on the thorax as a whole contributes to the understanding of factors influencing of human evolution, sexual dimorphism, adaptations, age, ancestry and other factors influencing morphological variation.

Geometric Morphometrics has been used globally to model and analyze biological systems. While the use of landmark coordinates may appear well suited because it focuses on established features, its use is not always appropriate. In this case, removal of the size variability actual appears to render it unfit for this purpose. A simpler approach that shows consistency and lends good insight into the variation has greater viability and chance of application. Due to the complexity of geometric morphometric techniques, it does require further refinement to become an effective and applicable model that can be tested and applied in forensic anthropology and bioarchaeology.

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