

A geometric morphometric analysis of the primate clavicle

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Table of Contents

Abstract.....	3
Acknowledgements.....	5
Chapter 1: Introduction.....	6
Chapter 2: Materials & Methods.....	14
Chapter 3: Results.....	24
Chapter 4: Discussion.....	31
Bibliography.....	39

Abstract

The clavicle has been an historically understudied bone when compared with other elements of the primate pectoral girdle, even though it likely contains important information about locomotion due to its role in upper arm stabilization. Because of this, little is known about levels of morphological variation both within and among species. To address this issue, this study presents a geometric morphometric analysis of clavicular variation in multiple catarrhine species including *Homo sapiens*.

Using an established protocol, seven three-dimensional landmarks were collected on 215 right clavicles from *Trachypithecus cristatus* (n = 36), *Pan troglodytes* (n = 12), *Gorilla gorilla* (n = 27) *Homo sapiens* (n = 46), *Hylobates lar* (n = 36), and *Macaca mulatta* (n = 58). These landmarks were then superimposed using a generalized Procrustes analysis. Comparative clavicular morphology was visualized via a principal component's analysis, and was quantified using Procrustes ANOVA with permutation. Within species morphological disparity was also analyzed using Procrustes variance, calculated using species means.

The Procrustes ANOVA results showed significant shape differences among species in general ($F_{(5,209)} = 39.905$, p-value < 0.001), which was largely related to acromial end curvature, and to the position of the conoid tubercle relative to the acromial end. The results of the morphological disparity analysis found that *Gorilla gorilla* has significantly more shape variation than the other species. The greatest contributing factor associated with this variation was the relative distance of the conoid tubercle from the acromial end. Interestingly, results from the

Procrustes ANOVA analysis also found sexual dimorphism in shape in *Trachypithecus cristatus*, but not in the remaining species.

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I would also like to express my thankfulness to the curators of the Museum of Comparative Zoology collection at Harvard University, the Cleveland Museum of Natural History Hamann-Todd Human and Non-Human Primate Osteological collection, and the Buffalo Human Evolutionary Morphology Laboratory collection, that all housed the specimens that were examined during this research project.

Chapter One: Introduction

The clavicle, along with the scapula and the humerus, are the three bones that make up the primate pectoral girdle. However, there have been many more studies conducted involving the scapula and humerus, and there is much more information available that has been derived from those studies (Arias-Martorell et al., 2015; Larson and Stern, 2007; Schmidt and Krause, 2011; Young, 2006). There have been very few studies conducted involving the morphological variation of primate clavicles, even though this bone likely contains important information about locomotion due to its role in upper arm stabilization. It is possible that the primate clavicle is overlooked due to its relatively limited shape complexity compared to other bones that have attracted more attention. It is also possible that the primate clavicle is overlooked because of its relatively small size and potential to be broken, and because of these factors it is not as well represented in museum collections as other bones are.

The primate clavicle has utility as a strut that facilitates connectivity between the sternum and the scapula (Squyres and DeLeon, 2015). A non-anatomical analogous comparison can be seen in the jib of a mechanical crane where the jib facilitates appropriate distancing from the crane's mast so that the trolley can move. The clavicle's inclusion in the pectoral girdle likely plays a significant functional role regarding the way in which primates are able to locomote and may be a significant factor associated with certain behaviors. Some reasons for this may be because the shape of the clavicle can affect the facilitation of arboreal or terrestrial habitation or the shape may also play a contributing role in other

diverse primate behaviors like quadrupedal travel or brachiation. For example, *Hylobates lar*'s brachiation locomotor functions utilize muscles in the shoulder called flexor muscles. These shoulder flexor muscles, like the pectoralis major, as well as the anterior fibers of the deltoid are connected to the clavicle (Michilsens et al., 2009). Thus, *Hylobates lar*'s clavicle shape, combined with its attached flexor muscles, likely plays a role in their brachiating locomotor abilities.

Even though there has been very limited research regarding primate clavicles in general, there has been research conducted in a medical context in humans (e.g., Barberini, 2014; Makandar et al., 2011), that provides important anatomical information. For example, Makandar et al. (2011) found that left human clavicles have increased length compared to right human clavicles in both males and females. Right now, the reason for this is unclear. However, another study found that even though the left clavicle has a tendency to be longer than the right clavicle in humans, they did not find this to be statistically significant (Andermahr et al., 2007). There may be benefit in acquiring handedness information associated with human clavicles to examine possible reasons for left-side and right-side clavicular size disparities. Barberini (2014) describes the progression of clavicular skeletogenesis in human embryos, and notes that human clavicles are well developed by the seventh week of embryotic development. There has also been research conducted on mammalian and bird clavicles (Hall, 2001), which provide information that can certainly be useful when looking at clavicle shape from an evolutionary perspective. For instance, clavicles are evolutionary “leftovers” of skeletal armor that covered a lot of early vertebrates. Hall (2001) explains that

clavicles are considered to be dermal bones and that dermal bones are left over from dermal exoskeletons. In fact, clavicles are the only relics leftover from the dermal skeletons of mammals.

Another category of primate clavicle research can be seen in paleoanthropology (Boaz, 1980; Roach and Richmond, 2015; Sankhyan 1997; Voisin, 2006; Voisin 2008) which is also very beneficial for the purpose of examining primate clavicle shape from an evolutionary perspective. Even though these paleoanthropological clavicular studies usually have very small sample sizes, they provide interesting information regarding extinct hominin clavicular profiles. For example, Voisin (2008) studied 3D shape variations within genus *Homo*, and found that two different clavicle morphological groups exist. His study found that there is one clavicular morphology group that is associated with four different species of genus *Homo*, *Homo habilis*, *Homo ergaster*, *Homo antecessor*, and *Homo neanderthalensis*, and another clavicular morphology group that is associated with modern *Homo sapiens*. Voisin (2008) suggested that the reason for the difference in the clavicular morphology of modern *Homo sapiens* could potentially be resultant from genetic drift due to small population sizes. Roach and Richmond (2015), using claviculohumeral ratios and claviculocostal ratios to calculate clavicle length, found that *Homo erectus* clavicle lengths are within the same variation range as modern human clavicles and that clavicle length does not have a significant relationship with throwing abilities.

Though not many, there have also been studies that involve clavicular morphology research that focus on clavicular structure as well as functionality and

its relation to variation in primate locomotion (Voisin, 2006; Preuschoft et al., 2010; Melillo et al, 2018, Squyres and DeLeon, 2015). For example, Preuschoft et al. (2010) conducted a functional analysis of the primate shoulder girdle and found that the primate clavicle had utility for keeping the shoulder harness away from the rib cage during quadrupedal walking and during suspension. In another study, Squyres and DeLeon (2015) used 3D geometric morphometrics to examine clavicle curvature in multiple primate species and found that greater degrees of ventral curvature are associated with more suspensory locomotion behaviors.

There is still a lot of information about primate clavicles that is unknown. For example, there have not been any studies with a sample representing all major primate families. There could be potential benefit in being able to examine variation among both catarrhine and platyrrhine parvorders and it may be even more desirable to examine comparative primate clavicular morphology among both suborders to have an opportunity to examine variation between strepsirrhines and haplorrhines. There is also potentially important information that could be gained by analyzing primate clavicles at different stages of ontogeny or conducting research based on primate clavicular injuries. There have not been any comparative studies that were able to include handedness which could be beneficial for understanding differences in shape between left-side and right-side clavicles in humans. Although Squyres and DeLeon (2015) examined clavicle curvature in catarrhines, further studies are still needed to examine overall clavicle shape in this group. Another example of where there is an absence of clavicular morphology

knowledge is that there haven't been any previous studies that examine potential sexual dimorphism in clavicle shape among primates.

This study addresses some of the gaps in our knowledge regarding primate clavicle variation by examining shape variation in a broad sample of catarrhine taxa. It also examines sexual dimorphism, which has not previously been considered in non-human primate clavicular morphology studies. There were several motivations for this investigation. First, the information derived from this study could potentially be useful for understanding relationships between clavicle shape and the utility of the primate shoulder harness. For example, there may be differences in the shape of primate clavicles among species that travel quadrupedally versus bipedally. Conversely, there may be differences in clavicle shape between primate species that live a mostly terrestrial lifestyle versus species that primarily have an arboreal lifestyle. Second, this study aimed to examine if there were any differences in clavicle shape that may be associated with behavioral differences among the sexes. In addition to these gaps in anthropological knowledge, this research also set out to determine levels of clavicle morphological disparity among different primate species which could be beneficial for understanding if ranges of variation in clavicular morphology is related to locomotion. Information derived from this study could potentially assist researchers when determining the species and sex of unknown osteological remains.

Clavicles from six catarrhine species were sampled for this research, representing a range of behavioral and locomotor differences. Even though all of the species included in this study are from the same parvorder, two of them are old-

world monkeys while the others are apes. The included species spanned those that are primarily arboreal, species that are primarily terrestrial, and species that spend time in both the trees and on the ground. This was significant because different primate behaviors and associated locomotion could possibly affect or be affected by pectoral girdle functionality and therefore possibly clavicular morphological variation. Most of the catarrhines observed in this study have different methods of locomotion when compared to one another. Of the species studied, *Trachypithecus cristatus* is a mostly arboreal quadruped who is also a semi-brachiator (Harding, 2010). *Hylobates lar* travels mostly arboreally and is an excellent brachiator (Strier, 2015). *Gorilla gorilla* is a mostly terrestrial primate who travels as a quadrupedal knuckle-walker, similarly to *Pan troglodytes* (Strier, 2015). *Homo sapiens*, of course, have the most unique locomotion due to being obligative terrestrial bipeds.

It is important to acknowledge that there are similarities and differences between the locomotion capabilities of each of the species involved in this study due to the presumption that clavicular utility and function may have had significant importance concerning clavicular morphology among the species. It may be important to take into consideration that there are behavioral differences between the males and females of different primate species which could potentially be a cause for clavicular sexual dimorphism disparities between the different taxa included in this study. Hence, studying patterns of sexual dimorphism can help us further understand sources of morphological variation in primate clavicles.

This study will test several hypotheses regarding clavicular morphological variation. First, this investigation will determine if there are quantifiable differences

in clavicular shape among species. The null hypothesis in this regard is that clavicles do not differ in shape among taxa. The alternative hypothesis is that clavicle shape differs among species. The reason for these hypotheses is because the clavicle has a functional role in locomotion (Voisin, 2006; Preuschoft et al., 2010; Melillo et al, 2018, Squyres and DeLeon, 2015) and it might be expected that clavicle shape is determined by use in locomotion, rather than by taxonomic affiliation. Next, this study will determine if there are quantifiable differences in clavicular shape within species, that is, if there is sexual dimorphism in clavicle shape. The null hypothesis in this regard is that there is no clavicular sexual dimorphism within species. The alternative hypothesis is that there is clavicular sexual dimorphism within species. The reason for these hypotheses is because there is some level of general sexual dimorphism in all of the species included in this research with the exception of *Hylobates lar*, which exhibits minimal sexual dimorphism (Strier, 2015). It is worth reiterating that there have not been any studies that examined clavicular sexual dimorphism before, so it is currently unknown if sexes differ in clavicle shape. However, because sexes often locomote in similar ways, it is possible that there will be no detectable sexual dimorphism in shape. Also, this study will examine how much variation there is in clavicular morphology within species. The null hypothesis in this regard is that there is no difference among species regarding clavicular morphological disparity. The alternative hypothesis is that species have different amounts of clavicular morphological disparity. The reason for these hypotheses is because, even among the different species of genus *Homo*, there are different amounts of clavicular

morphological disparity (Voisin, 2006). This is suggestive of the possibility that there will be different levels of clavicle shape disparity among the different species included in this study, who all belong to different genera.

Chapter Two: Materials and Methods

Materials

Sample

The specimens used for this research consisted of 3D surface scans of 214 right clavicles from six catarrhine species (one cercopithecine, one colobine, and four ape species (see Table 2.1)), representing the broad range of geographic and behavioral variation in this parvorder. Data were collected from the Museum of Comparative Zoology collection at Harvard University, the Cleveland Museum of Natural History Hamann-Todd Human and Non-Human Primate Osteological collection, and the Buffalo Human Evolutionary Morphology Laboratory collection. Both male and female specimens were collected, with sex assessed using collection tags and visual inspection of canines. Specimen breakdown by sex is provided in Table 2.1. The raw scans were collected by multiple researchers, as part of a larger project examining integration in the primate skeleton.

Methods

Data Collection

Scanning/Aligning procedures

Each clavicle was digitally scanned with an HDI Advance high-definition 3D scanner (LMI Technologies Inc., Vancouver, Canada). Putty was used to hold each clavicle in place for imaging. The 3D digital scanner was placed approximately six inches away from each clavicle that was being held by the putty. After scanning each clavicle, it would then be flipped to its opposite side in order

to capture the clavicle image from the alternate side. This process is necessary in order to form a complete 3D image. The images were captured using FlexScan3D software (Polyga, 2021). After all necessary 3D images were captured, each 3D clavicle image was uploaded to a laboratory computer for subsequent processing procedures. Each individual clavicle was also aligned using FlexScan3D software in order to form a complete digitalized 3D image that could be saved in a .ply file for landmarking (see Figure 2.1).

Table 2.1. Study sample.

<i>Species</i>	<i>Male</i>	<i>Female</i>
<i>Trachypithecus cristatus</i>	18	18
<i>Pan troglodytes</i>	7	5
<i>Gorilla gorilla</i>	15	12
<i>Homo sapiens</i>	23	23
<i>Hylobates lar</i>	16	19
<i>Macaca mulatta</i>	29	29
Total	108	106

Landmarking Procedure

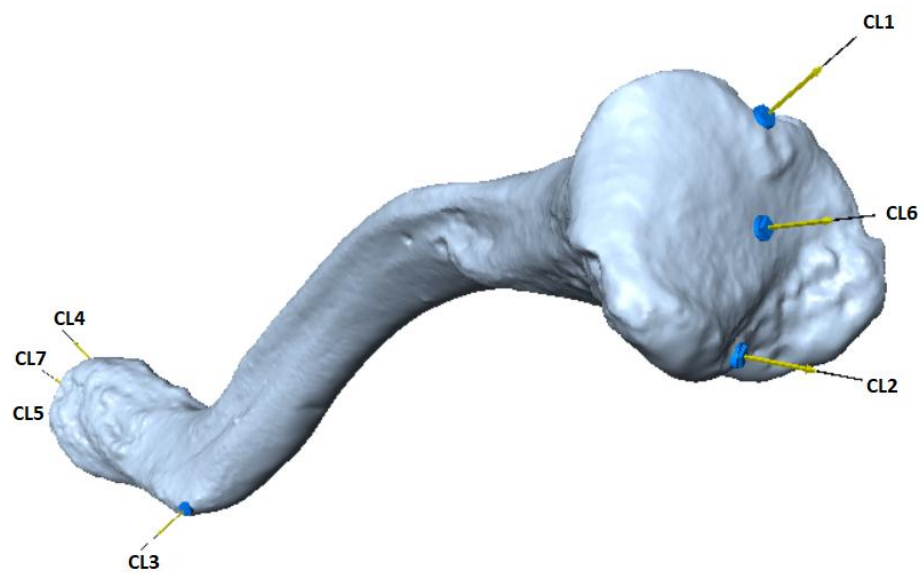
After all specimens had been 3D digitally scanned and saved for storage, they were then each individually landmarked in order to conduct geometric morphometric analyses. Seven landmarks were placed on each clavicle by a single

observer (Kevin Klier) utilizing IDAV Visualization and Graphics Research Group's Landmark 3.6 software (Wiley, 2006). Each of the seven landmark placements were replicated from a previously established clavicle landmarking protocol developed by Melillo et al. (2019). The established protocol was used because it has been previously shown to adequately capture primate clavicular shape information (Melillo et al., 2019). Landmarks collected from the sternal end are illustrated in Figure 2.1A and landmarks from the acromial end are illustrated in Figure 2.1B.

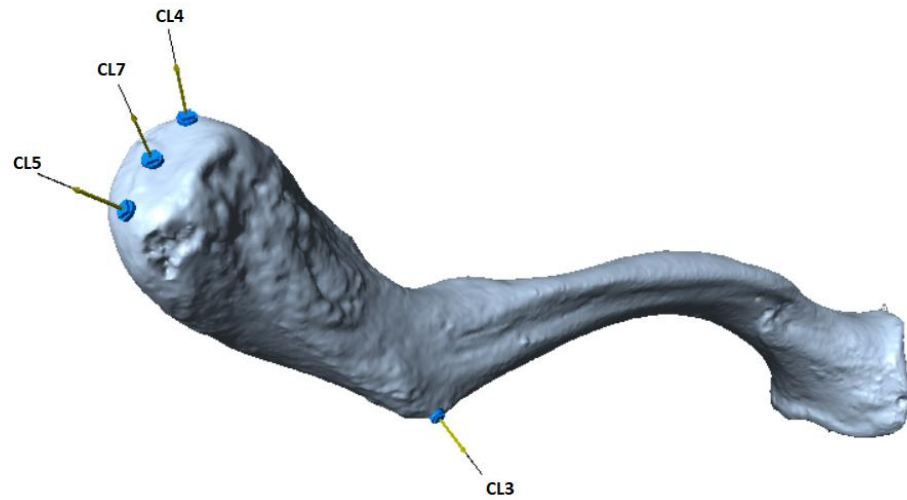
The landmarks were labeled CL1 through CL7 and corresponded to different locations on each clavicle. CL1 was placed on the anterior extent of the sternal articulation. CL2 was placed on the posterior extent of the sternal articulation. CL3 was placed on the inferior-most projection of the conoid tubercle. CL4 was placed on the anterior extent of the acromial articulation. CL5 was placed on the posterior extent of the acromial articulation. CL6 was placed at the intersection of the midline curve and the external surface of the sternal end, and CL7 was placed at the intersection of the midline curve and the external surface of the acromial end (see Figure 2.1 and Melillo et al, 2019). The three-dimensional landmark-coordinates were then saved and stored on the laboratory computer for further geometric morphometric analysis and intra/inter-specific comparison.

All landmark files were then visually examined individually for any discrepancies or noticeable errors that may have occurred during the landmarking or file storage processes (e.g., if there were only six landmarks collected rather than the full set of seven on a particular clavicle). If discrepancies or errors were detected

then files were corrected and reprocessed prior to analytical data inclusion (e.g., if missing, a landmark was added and then the complete specimen was included in the dataset). All seven landmarks were able to be collected on all specimens (i.e., none were damaged such that one of the seven landmarks were not able to be collected).



A



B

Figure 2.1. Clavicle landmarks viewed from the sternal end (A) and acromial end (B).

Analytical Methods

The analyses for this research were performed in R (R Core Team, 2021) and all the figures were created utilizing the geomorph package (Adams et al., 2021) and ggplot2 (Wickham, 2016). R is a free open-source software program that provides an environment for conducting statistical analysis. It uses a unique computer programming language which has been developed and maintained by both statisticians and computer programmers. The R program is easily accessible and is commonly used globally in research for data analysis.

Generalized Procrustes Analysis

To attain a meaningful interpretation of the data collected from the seven superimposed clavicle landmarks, the first analytical step was to perform a Procrustes superimposition via a generalized Procrustes analysis (GPA) by running the “gpagen” command in the geomorph package. This technique is commonly used in geometric morphometrics to attain shape information from landmark placements. It superimposes the specimens’ structures based on their geometric centers (centroids) and then scales them based on the size of their centroids. Then the GPA rotates the specimens’ structures until comparable landmarks are as close to each other as possible (Baab et al. 2012). After the GPA is done, superimposed specimen structures can be visualized as a single point in a high-dimensional shape space. As shape is defined as the properties of an object that are invariant to its location, orientation, and scale, by removing these variables, a GPA allows the shapes of objects to be compared. The amount of difference in shape corresponds to the difference in distance in the high-dimensional shape space. Procrustes distance is the square root of the sum of all the squared distances between corresponding landmarks in the two superimposed objects, and is the standard measure of shape difference in Procrustes-based geometric morphometrics (Baab et al., 2012).

Data Visualization

A Principal Component Analysis (PCA) plot was produced by running the “plotTangentSpace” command and by using the plotting functions in ggplot2, which plotted out information based on species and sex for an increased

visualization capacity for observation purposes. A PCA rotates the data space to a set of orthogonal (i.e., perpendicular) axes that describe the most variation in the dataset, and allows for 2D viewing of this variation, which is useful in the present analysis as the clavicle landmark data are in 21-dimensional space. So, the first principal component (PC1) explains the largest proportion of the overall shape variation, PC2 explains the next biggest proportion of the overall variation, and so forth. It is important to keep in mind that PCAs are not a statistical test and do not show complete representations of all data but preserve enough important information via linear algebraic algorithms to be useful for viewing the major patterns of variation in the data.

Shape Differences Among Taxa

Using the geomorph package in the R program, a Procrustes Analysis of Variance (Procrustes ANOVA) was used to find out if any of the species significantly differed in shape from each other. Instead of using conventionally derived continuous variables to calculate the group means to assess differences, the Procrustes ANOVA uses the mean Procrustes distances between groups (here, species). This was performed by running the “advanced.procD.lm” command in geomorph. A significant result from the Procrustes ANOVA would indicate that at least one of the species differed in clavicle shape from the other species. Results of pairwise comparisons (that is, all pairs of species compared with each other) of differences in clavicle shape (which is automatically performed in the “advanced.procD.lm” function) allowed for a more precise determination of which species differed from each other. This was used to determine if, for example, one

species was very different from the rest, but the remaining species were relatively similar to each other, or if all of the species differed in shape from each other. To determine if the differences in shape reached statistical significance, a permutation test was performed using 10,000 replicates, which is also automatically included as part of the “advanced.procD.1m” function. This permutation procedure shuffles all of the specimens so that they are no longer categorized into specific groups (here, species) and then recalculates the difference in means of the Procrustes distances to check if the results are the same or not. This is useful because it reveals information on whether differences in shape reach statistical significance. That is, the permutation test assesses how likely it is that observed group differences are due to chance. A significant result from the permutation tests would indicate that differences due to chance would be highly unlikely. However, because there were multiple comparisons in the pairwise tests, a strict Bonferroni correction was also applied to facilitate an increasingly scrutinized comparative observation and reduce the likelihood of finding (false) differences among species simply due to making repeated observations.

Sexual Dimorphism

A separate Procrustes ANOVA analysis was performed within each species to test for differences in clavicle shape between the sexes. Rather than calculating the Procrustes ANOVA based on species groups, the “advanced.procD.1m” function was performed on species and sex. As a significant result in this analysis would indicate that at least one of the species was sexually dimorphic, pairwise comparisons (within each species) were also examined (as described above).

Significance was again assessed using permutation with 10,000 replicates. This method allows for a determination of whether sexual dimorphism in clavicle shape exists, and if so, in which species. Then, the “trajectory.analysis” command was run in geomorph. This command uses all of the specimen’s clavicle shape sequences (here, the sequence from the average male shape to the average female shape) in tangent space and uses it to make a quantifiable shape change trajectory for comparison. After “trajectory.analysis” has been performed, it creates a 2D plot of the trajectories for visualization purposes. All collected and processed specimen data files were then checked for sexual dimorphism using observed path distances between the males and females. Stated another way, “trajectory.analysis” was used to measure the Procrustes distance between males and females (i.e., how much they differ in shape), and if these values differ among taxa. As with the “advanced.procD.lm” function, statistical significance was assessed using permutation with 10,000 replicates. Thus, this test provides another assessment of the degree of sexual dimorphism present within each taxon.

Morphological Disparity

The “morphol.disparity” command was run in order to quantify levels of morphological variation within species. Morphological disparity is the quantification of levels of difference in shape for an observed group (here, species) that can be compared with the levels of difference in shape with another group. This shows the comparative statistical significance of these species’ clavicle shape differences. This was achieved by quantifying how much Procrustes variance there was in clavicle shapes within species. Then, “morphol.disparity” used the

quantified data acquired from the within species morphological variation estimates and compared the calculated differences to the calculated differences in each of the other species on a pairwise basis with permutation.

Although a broader sample of catarrhines would be desirable, there were obstacles that prevented a more species diverse sample size while conducting this research. Restrictions due to the COVID-19 pandemic limited the ability to travel to alternate collection sites or to gain access to laboratories for the 3D imaging of more species' clavicles. Fortunately, there were enough species diverse clavicles available that could be used already on hand that we were able to include in our sample for 3D imaging, landmarking, and analysis. Also, as previously mentioned, it was not always possible to redo 3D images of clavicle that were not suitably captured during the scanning process if those clavicles came from an offsite collection pre-pandemic.

Chapter Three: Results

Data Visualization

A plot of the first two axes from the principal components analysis is presented in Figure 3.1. Also presented in this figure are wireframes (i.e., schematics where landmarks are linked to display shape differences) depicting clavicular shape associated with each end of the PCA axes. The first principal component accounted for 41.8% of shape variation, and the second principal component accounted for 25.2% of shape variation, together accounting for 67% of the total variation. Examination of the PC loadings indicated that PC1 variation is mostly related to the position of the conoid tubercle relative to the acromial end and positive PC2 values are associated with increased acromial end curvature which mostly separates *Hylobates lar* from the other species (see also wireframes in Figure 3.1). Also, after examining the PCA plot, it was clear that *Gorilla gorilla* had a lot more clavicular morphological variation than that observed in any of the other species, spanning almost the entirety of PC1 (see also Morphological Disparity analysis, below). Additionally, PC1 does not appear to be directly related to size, as *Gorilla gorilla* have the largest clavicles in the sample, but also span the entirety of the axis.

There is some overlap among species' clavicular shape variation that is apparent in Figure 3.1. However, there is also a lot of shape variation between species that can be easily identified from the PCA. For example, almost all of the *Hylobates lar* specimens were morphologically different than the rest of the species, with the exception of some overlap with *Trachypithecus cristatus*. The

PCA plot does not appear to reflect any taxonomic divergence between monkeys and apes, at least not on the first two axes of variation. There does not appear to be any obvious group separation between any of the species' males or females that would suggest any significant sexual dimorphism with the possible exception of *Trachypithecus cristatus* on PC2. *Pan troglodytes* appears to have the smallest amount of shape variation, but this is most likely due to their having a smaller sample size than the other species.

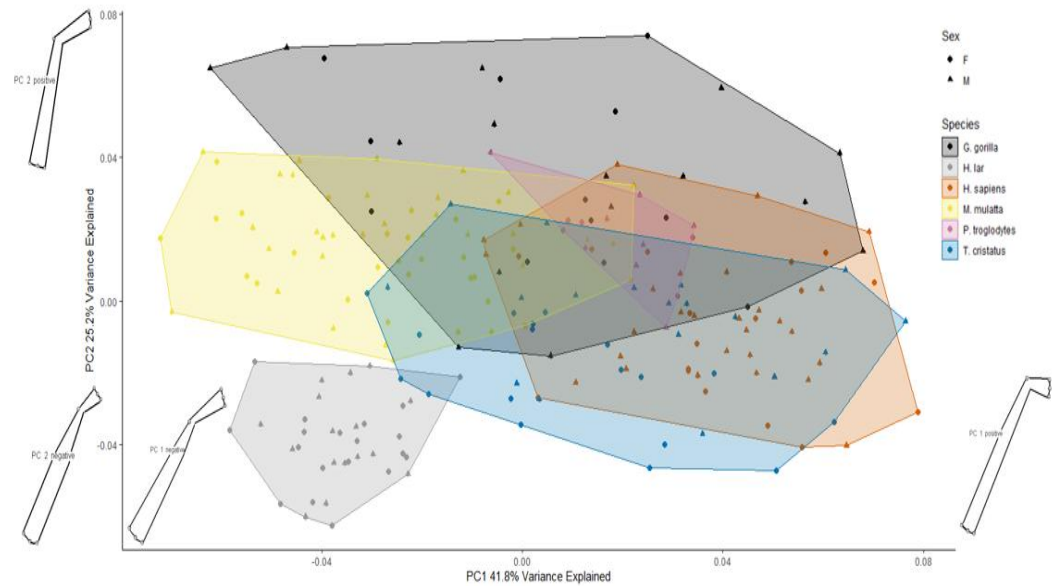


Figure 3.1. Plot of the first two principal components representing 67% of total shape variation.

Shape Differences Among Taxa

The results of the among species Procrustes ANOVA indicated that there were significant differences in clavicle shape among species ($F_{(214,209)} = 11.384$, $p\text{-value} = 0.0001$). Therefore, the null hypothesis that clavicles do not differ in shape among taxa is rejected: at least one species differs from the other species in

clavicular shape. Furthermore, the results of the pairwise analysis (to evaluate which species differed in shape from each other and if this difference was most likely due to chance) showed that all species significantly differed in shape from each other (Table 3.1). However, after correcting for multiple comparisons (Bonferroni adjusted alpha = 0.0033), the amount of shape difference between *Gorilla gorilla* and *Pan troglodytes* was no longer significant (Table 3.1). Thus, the null hypothesis was rejected in almost all cases.

Table 3.1. Between species Procrustes ANOVA results. Above diagonal: Procrustes distance among species means; Below diagonal: p-value from pairwise comparisons (Permutation procedure). Bold = significant differences after Bonferroni correction.

	<i>G. gorilla</i>	<i>H. lar</i>	<i>H. sapiens</i>	<i>M. mulatta</i>	<i>P. troglodytes</i>	<i>T. cristatus</i>
<i>G. gorilla</i>	—	0.08558	0.0527	0.0461	0.0305	0.0517
<i>H. lar</i>	0.0001	—	0.0815	0.0597	0.0813	0.0641
<i>H. sapiens</i>	0.0001	0.0001	—	0.0702	0.0341	0.0363
<i>M. mulatta</i>	0.0001	0.0001	0.0001	—	0.0501	0.0618
<i>P. troglodytes</i>	0.0336	0.0001	0.003	0.0001	—	0.0454
<i>T. cristatus</i>	0.0001	0.0001	0.0001	0.0001	0.0002	—

Sexual Dimorphism

The results from the within species sexual dimorphism Procrustes ANOVA indicated that there is some sexual dimorphism in clavicle shape ($F_{(208,203)} = 1.219$, p-value = 0.004). Therefore, the null hypothesis that males and females do not differ in clavicular shape is rejected. A pairwise analysis was conducted to see which

species showed significant sexual dimorphism, and *Trachypithecus cristatus* was the only species that had significant sexual dimorphism in clavicular morphology (see Table 3.2). Thus, although the null hypothesis was rejected, this result was being driven by the large differences between male and female *Trachypithecus cristatus* specimens.

Table 3.2. Procrustes ANOVA results for sexual dimorphism in clavicle shape. Bold = significantly different in shape.

	<i>Procrustes distance between sexes</i>	<i>p-value</i>
<i>G. gorilla</i>	0.00921	0.9694
<i>H. lar</i>	0.00594	0.9977
<i>H. sapiens</i>	0.01315	0.5782
<i>M. mulatta</i>	0.01341	0.4193
<i>P. troglodytes</i>	0.01734	0.7937
<i>T. cristatus</i>	0.02844	0.0047

A trajectory analysis was also performed to provide an independent test for the presence of sexual dimorphism, and to improve visual representation of the inter-specific sexual dimorphism analysis. The results of this analysis corroborated the results of the Procrustes ANOVA test for sexual dimorphism above. Out of the six catarrhine species observed in this study, *Trachypithecus cristatus* had the highest level of clavicular shape sexual dimorphism and *Hylobates lar* had the least, as measured by the path distance between males and females (see Table 3.3 and Figure 3.2). The pairwise absolute differences between path distances with associated p-values results are presented in Table 3.4. The results in Table 3.4 show that *Trachypithecus cristatus* has a significantly longer path distance (which means a greater shape difference) between males and females than the other species,

though after Bonferroni correction this is only true for the comparison of *Trachypithecus cristatus* and *Hylobates lar*. Thus, the results of the trajectory analysis confirm that the largest differences between male and female clavicular shape are found in *Trachypithecus cristatus*. It should be noted again that *Pan troglodytes* had the smallest sample size which could be influencing the results, as it is possible that there are not enough specimens to capture actual levels of sexual dimorphism. Thus, the results for *Pan troglodytes* should be considered preliminary.

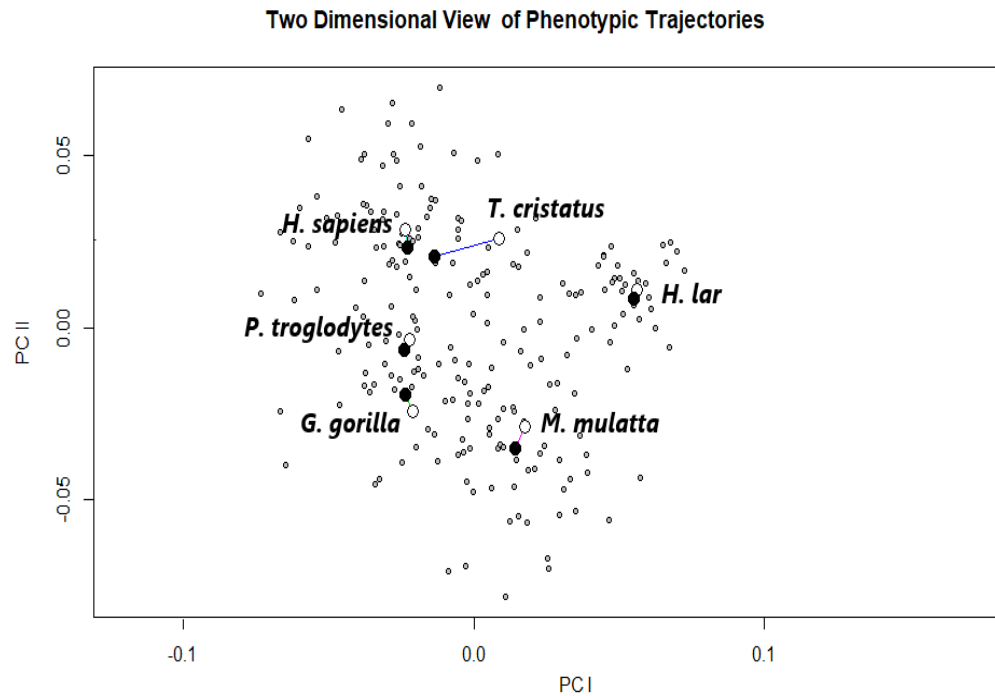


Figure 3.2. 2D plot of trajectory analysis. Open circles: females. Closed circles: males. Lines between sexes illustrate levels of sexual dimorphism and roughly correspond to the path distances in Table 3.3 (longer lines = greater shape differences between males and females).

Table 3.3. Phenotypic sexual dimorphism observed path distances.

<i>G. gorilla</i>	<i>H. lar</i>	<i>H. sapiens</i>	<i>M. mulatta</i>	<i>P. troglodytes</i>	<i>T. cristatus</i>
0.0092	0.0059	0.0132	0.0134	0.0173	0.0284

Table 3.4. Between species sexual dimorphism Procrustes ANOVA results. Above diagonal: Pairwise absolute differences between path distances; Below diagonal: p-value from pairwise comparisons (Permutation procedure). Bold = significant differences after Bonferroni correction.

	<i>G. gorilla</i>	<i>H. lar</i>	<i>H. sapiens</i>	<i>M. mulatta</i>	<i>P. troglodytes</i>	<i>T. cristatus</i>
<i>G. gorilla</i>	—	0.0033	0.0039	0.0042	0.0081	0.0192
<i>H. lar</i>	0.6047	—	0.0072	0.0075	0.0114	0.0225
<i>H. sapiens</i>	0.5371	0.2167	—	0.0003	0.0042	0.0153
<i>M. mulatta</i>	0.5421	0.2021	0.9586	—	0.0039	0.0150
<i>P. troglodytes</i>	0.4257	0.3132	0.7519	0.8115	—	0.0111
<i>T. cristatus</i>	0.0054	0.0008	0.0090	0.0123	0.3282	—

Morphological Disparity

The null hypothesis that each of the species in this study have similar amounts of morphological variation in clavicular shape was falsified. Out of the six catarrhine species observed in this study, the morphological disparity analysis results suggested that *Gorilla gorilla* had the most clavicular morphology variation and that *Hylobates lar* had the least (see Table 3.5). This is also apparent in the PCA plot above (Figure 3.1). The pairwise absolute differences between variances with associated p-values results can be seen in Table 3.6. After a Bonferroni correction there were significant pairwise absolute differences between variances

observed between *Gorilla gorilla* and all the other species, and between *Hylobates lar*/*Homo sapiens*, *Hylobates lar*/*Macaca mulatta*, and *Hylobates lar*/*Trachypithecus cristatus*. Again, the results for *Pan troglodytes* in the morphological disparity analysis should be considered preliminary, as it is possible that there are too few specimens in this study to obtain an accurate picture of morphological disparity in this species.

Table 3.5. Procrustes variances for defined groups.

<i>G. gorilla</i>	<i>H. lar</i>	<i>H. sapiens</i>	<i>M. mulatta</i>	<i>P. troglodytes</i>	<i>T. cristatus</i>
0.0029	0.0006	0.0015	0.0015	0.0014	0.0018

Table 3.6. Within species morphological disparity Procrustes variance results. Above diagonal: Pairwise absolute differences between variances; Below diagonal: p-value from pairwise comparisons (Permutation procedure). Bold = significant differences after Bonferroni correction.

	<i>G. gorilla</i>	<i>H. lar</i>	<i>H. sapiens</i>	<i>M. mulatta</i>	<i>P. troglodytes</i>	<i>T. cristatus</i>
<i>G. gorilla</i>	—	0.0023	0.0014	0.0014	0.0015	0.0011
<i>H. lar</i>	0.0001	—	0.0009	0.0008	0.0007	0.0012
<i>H. sapiens</i>	0.0002	0.0017	—	0.0001	0.0001	0.0003
<i>M. mulatta</i>	0.0002	0.0026	0.8242	—	0.0001	0.0003
<i>P. troglodytes</i>	0.0008	0.0571	0.7318	0.8391	—	0.0004
<i>T. cristatus</i>	0.0005	0.0001	0.3035	0.1908	0.2915	—

Chapter Four: Discussion

The results of this investigation show that the primate clavicle provides some taxonomic information, even for species that have broadly similar locomotion patterns. For example, there was clavicular morphological variation found even among suspensory apes. The results from the among-species Procrustes ANOVA revealed that there were significant shape differences among the different species' clavicles and the PCA showed that this is mostly because of acromial end curvature, and due to the position of the conoid tubercle relative to the acromial end. These results meant that the null hypothesis, that clavicles do not differ in shape among taxa, was rejected. These results also matched with results from other clavicular morphology variation studies (e.g., Voisin 2006).

Even though all of the primate species included in this study were from the catarrhine parvorder, the biggest taxonomic difference between species in this study was that two of the species were old world monkeys and the other four were apes. After examining the results, there did not appear to be any one factor associated with clavicular morphology that would distinguish these two phylogenetic categories from one another. However, even though some of the species have similar locomotion patterns, all of the species in this study have some differences in their modes of locomotion, with the most noticeably different species being *Homo sapiens* who are obligative bipeds. It appears, therefore, that variations in clavicle shape may be associated with both taxonomy and functionality.

Even though some overlap can be seen on the PCA plot, all the species in this study do show significantly different clavicular morphological variation from

each other. This information can be useful for several reasons. For example, this information can be used as a reference for researchers who may be examining undetermined osteological or fossil primate remains. The data derived from this study could potentially be used to assist in the exclusion of unknown taxonomic candidates based on clavicular morphology measurements that exclude certain primate species. For example, *Hylobates lar* clavicles appear to be mostly morphologically separated from the other species in this study. Therefore, it is possible that, along with a comparative sample, clavicle shape as measured using these landmarks could help place specimens of unknown taxonomic affiliation with their conspecifics. However, caution should be used with this technique, as there is a large amount of overlap in morphology among taxa. Another potential use of the information revealed in this study could be to assist with theoretical concepts concerning taxonomic and behavioral categories that are associated with primate clavicle utility during locomotion and how that may relate to evolution. For example, this study showed that after correcting for multiple comparisons (Bonferroni adjusted $\alpha = 0.0033$), there was no statistical significance regarding clavicle shape differences between *Gorilla gorilla* and *Pan troglodytes*. This is interesting because these two African apes are closely related and are both knuckle-walkers. However, it should be noted that the sample size for *Pan troglodytes* was much smaller than the other species and that this factor could have potentially affected the results.

The results from the within species clavicular sexual dimorphism comparisons found that significant differences between males and females were

only found in *Trachypithecus cristatus*, but not in any of the other remaining species. These results are interesting because even though *Trachypithecus cristatus* is a generally sexually dimorphic species, it is a far less noticeably sexually dimorphic species when compared to some of the other species included in the sample. For example, *Gorilla gorilla* has a high degree of size difference between males and females (Taylor, 1997) compared to *Trachypithecus cristatus* where the males only weigh about 11% more than the females (Harding, 2010). Other noticeable differences between the *Trachypithecus cristatus* sexes are that there is a presence of irregular white patches located on the female's groin area and that the males have considerably larger canine-sectoral teeth (Harding, 2010). One might expect to see a bigger difference among the sexes in the clavicular morphology of *Gorilla gorilla* due to having extreme sexual dimorphism for traits such as body mass and canines when compared to some of the other species in this study (Plavcan, 2001).

The only major differences that separates *Trachypithecus cristatus* from almost all of the other species is that it is a primarily arboreal species (Harding, 2010). The only other species in this study that belong in this category is *Hylobates lar*. However, *Hylobates lar* and *Trachypithecus cristatus* travel through the trees in two totally different ways. Also, *Hylobates lar* shows very little sexual dimorphism in general (Strier, 2015) when compared to any of the other species included in this study. Another thought that was considered prior to the Procrustes ANOVA for within species sexual dimorphism results was that if there were taxonomic differences associated with sexually dimorphic clavicular morphology

that it may be between the old-world monkeys and the apes due to broad differences in locomotion. Ape locomotion broadly differs from monkeys because they travel by true brachiation, knuckle walking, or bipedally in the case of *Homo sapiens* (Strier, 2015). However, the results revealed that there was no clavicular sexual dimorphism observed in *Macaca mulatta*, the other old-world monkey included in this study.

None of the within-species clavicular sexual dimorphism results fit in with any of the literature that was known at the time of this study. We now know that there is no clavicular sexual (shape) dimorphism observed in five of the six catarrhine species that were included in this study. However, once again, the smaller sample size of *Pan troglodytes* should be taken into consideration. The data derived from this study could potentially be used to assist in the sexing of undetermined *Trachypithecus cristatus* clavicle remains if desired. Unfortunately, observations regarding the sexual dimorphism of clavicles within catarrhines is limited to these six species. Further research involving a larger sample sizes of more diverse primate taxa is desirable. It would be especially beneficial to examine the clavicles of more colobine monkeys to see if there is any pattern that can be observed there. There are very few colobine behavioral or locomotor differences that would be suggestive of a reason for bone shape differences. However, since *Trachypithecus cristatus* is the only species in this study that has clavicular sexual dimorphism and belongs to the colobine family, there could be benefit in pursuing more investigations within that family. There may also be potential in examining the clavicles of other arboreal primate species as well as examining the clavicles of

more monkeys that travel through the trees in a quadrupedal manner similar to the way that *Trachypithecus cristatus* does.

The results of the trajectory analysis, which was performed to provide another test to see if there were any patterns of sexual dimorphism among species, and to provide an improved visual representation of the inter-specific sexual dimorphism analysis, were consistent with the results derived from the Procrustes ANOVA that showed limited evidence of sexual dimorphism within all of the species with the exception of *Trachypithecus cristatus*. After examining the Procrustes distances between males and females, *Hylobates lar* had the least amount of clavicular sexual dimorphism which was not surprising, given that *Hylobates lar* is not a very sexually dimorphic species in general (Strier, 2015).

The information regarding the relative absence of sexually dimorphic *Gorilla gorilla* clavicles is significant because it shows that primate clavicle shape differences are not consistent with the levels of general species sexual size dimorphism. This information implies that the reason for clavicular sexual shape dimorphism found in *Trachypithecus cristatus* is possibly occurring due to differences in locomotor functionality between the sexes. That particular locomotor functionality is possibly resultant from behavioral differences between the males and females of *Trachypithecus cristatus*. Both *Trachypithecus cristatus* (Harding, 2010) and *Gorilla gorilla* (Strier, 2015) live in single male multi female groups, often referred to as harem-based groups, so there must be something else about the relationship between the males and females of these two species that is driving the patterns of clavicular sexual dimorphism observed. It is possible that the compound

factors of being quadrupedal, arboreal, and living in harem-based groups (Harding, 2010) are causing clavicular sexual shape dimorphism or there are other behaviors that differentiates *Trachypithecus cristatus* from the other species that has been overlooked. Further research regarding the sexual dimorphism prevalence in other lutungs could be beneficial as well as further research into what differences in locomotion behaviors between the lutungs' sexes could possibly be driving these clavicular morphology variations. Also of potential benefit would be future research on levels of sexual dimorphism of lutung scapulae and humeri which might help explain why there is sexual dimorphism in *Trachypithecus cristatus* clavicles but not in the other five species.

All six primate species included in this study were examined for within species clavicular morphology disparity by comparing Procrustes variances. The results showed that the null hypothesis that there would be equal amounts of clavicular morphological disparity within species was rejected, and instead different amounts of clavicular morphological disparity was found in all of the species examined. *Gorilla gorilla* clavicles were found to have the most morphological disparity among the sample species and *Hylobates lar* clavicles were found to have the least amount of morphological disparity among the sample species. This result was also visually apparent in the PCA plot (Figure 3.1). The information found during this analysis is significant because it can potentially be used as reference for researchers analyzing undetermined primate clavicle remains to determine whether or not a clavicle is within the morphological disparity range of a particular species. For example, a researcher could measure the unknown

remains of a clavicle and be able to decide if it could possibly belong to a member of one of the six species included in this study by observing if the clavicle falls into known clavicle morphology disparity range. However, this is complicated by the observation that there is a lot of overlap in the clavicular shape variation of these species.

After all the species were examined pairwise for clavicular morphology disparity, it was apparent that there were statistically significant differences in clavicular morphology disparity levels between *Gorilla gorilla* and all the other species. We can also see that most of the species, with the exception of *Pan troglodytes*, have different levels of clavicular morphology disparity than *Hylobates lar*. This information is important simply because it opens the door for more questions to be asked. There must be some reason that some of these species have more clavicular morphological disparity than other species do. The reason that *Hylobates lar* has such a low level of morphological disparity when compared to *Gorilla gorilla* could potentially have evolutionary divergence implications. For example, bottleneck effects could be the reason for one species to have higher inter-specific variation than other species do. In this sense, it is possible that the current population of *Hylobates lar* are descended from a small population of ancestors that were a remainder of a larger population that underwent a reduction, resulting in the current population having less variation than other species that had not gone through a bottleneck event. Another possibility that warrants further exploration is that stabilizing selection has been important in shaping the morphology of the gibbon shoulder in relation to brachiation, in such a way as to reduce levels of

within-species shape variation in the clavicle. Further research involving the non-clavicular osteological morphology variances of these six species combined with this research could potentially provide further insight as to why these different levels of variation exist. For example, there may be benefit in understanding if there are any non-clavicular osteological morphology disparity patterns that are consistent with clavicular morphology disparity patterns.

With unlimited funding and time, there are several desirable avenues of future research that would increase the depth of knowledge associated with the geometric morphometric analysis of the primate clavicle presented here. It would be very desirable to maximize the sample sizes of each of the six catarrhine species included in this study, which would increase the precision of the overall analysis. It would also be very desirable to maximize the number of different species that could be included. There should be increased benefit from including every single species from the primate order so, at minimum, representations of all major taxonomic groups. Including examples with different forms of locomotion, different diets, differing social group structures, and different levels of general sexual dimorphism would widen our scope of current knowledge and would increase our ability to gain insight from inter-specific comparisons. All of these inclusions would decrease the possibilities of confounding variables that might affect analytical results. There would also be added benefit to having handedness information associated with human clavicles in order to investigate the possibility that previously observed length variation between left and right-side human clavicles is related to hand lateralization (Makandar, 2011).

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