Project Title: Doctoral Dissertation Improvement Grant: Taxonomic Efficacy of the Macaque Skeleton

Funding Program: National Science Foundation, Doctoral Dissertation Research Improvement Grant (DDRIG), Division of Behavioral and Cognitive Sciences, Biological Anthropology

Co-PI: Brittany Kenyon-Flatt, PhD Candidate, Department of Anthropology, University at Buffalo, SUNY, Buffalo, NY USA

Project Summary:

Project Overview. Here, we investigate the taxonomic efficacy of the macaque skeleton and examine the extent to which climate, geography, and/or behavior confounds taxonomic assessment of the genus *Macaca*. The cranium has long been thought to be particularly useful for taxonomic and phylogenetic assessment in non-human primates, however, researchers have recently begun testing similar questions using the post-cranium. While results from these studies suggest taxonomic differences among genera evident in post-cranial elements, there is a lack of research investigating intra-generic morphological differences in the skeleton. To date, the taxonomic valence of morphological features has largely been determined by tradition, and not by comparative evidence regarding intra- versus inter-taxon variation in extant species. Macaques are the best primate model for assessing intra-generic variation, given that they are specious, geographically widespread, and display variation in dietary and locomotor behaviors. The central question for this project is: **are different species of macaque taxonomically distinguishable based on their skeletal morphology?** Specifically, this project tests the following hypotheses: (**H1**) Taxonomy can be accurately predicted based on patterns of morphological variation in skeletal elements in geographically wide-spread macaque taxa, (**H2**) post-cranial bones, particularly the os coxa, scapula, and long bones, reflect sub-generic macaque taxonomy at the same level or better than the crania, and (**H3**) climate and geographic distance have a confounding effect on morphological distances among related macaque taxa. Data consists of a 3D geometric morphometric (GM) analysis of eight macaque species and three outgroups, each chosen for their phylogenetic relationship to macaques and for their diverse geographic locations and behavioral differences. Results from this project will show how skeletal variation informs sub-generic taxonomy, and can be applied to the fossil record to aid in identification of previously unknown taxa.

Broader Impacts. Results from this study will be presented to the general public in three main ways: developing a workshop for elementary and high school students belonging to underrepresented groups, presenting to the University at Buffalo groups WISE (Women in Science and Engineering) and the Asian Student Association, and by dissemination via blogs throughout the duration of the project. Results from this study promise to provide concrete evidence for the ways in which the skeletons of closely related individuals respond to different environments. Thus, the PI and Co-PI will develop two workshops to present to a K-6th grade and 9th-12th grade audience explaining how people and animals adapt to living in different environments. Given that understanding the human skeletal system is part of New York State's Common Core for third graders, this will be of particular interest to them. The Co-PI will use 3D models of macaques to teach students how similar animals have different skeletons depending on the environment they live in. A more advanced version of the workshop will be presented to high school students. Presentations will be given to the WISE group, and undergraduate students will have the opportunity to visit the lab and assist with the project. A presentation will also be given to the Asian Student Association, given several of the students who are members of the group come from locations where macaques live naturally. Throughout the project, blog posts focusing on novel results and data collection will be submitted to popular science outlets such as *Sapiens* and *Science Trends*.

Intellectual Merits. The comparative dataset developed from this project will have far-reaching intellectual value beyond the tenure of the project, as the first collated dataset of cranial and postcranial morphology for a large sample of Old World monkeys will be made available to future researchers. Moreover, the results of this project will be relevant to researchers wishing to assess levels of intra-specific or intra-generic variation in other primate species, including extinct hominins. These results will be widely disseminated as journal articles with targeted submissions planned to *The Anatomical Record, American Journal of Physical Anthropology, International Journal of Primatology* and *Journal of Human Evolution.* The pilot study was presented at the 2018 American Association of Physical Anthropologists' conference and an article detailing findings has been submitted for review to the *American Journal of Physical Anthropology.* Finally, the methods and findings employed here will be useful for museum curators looking to identify mislabeled or unknown specimens by providing extant models of macaque variation against which to compare their own data.

1. RESPOSE TO PREVIOUS COMMENTS

Previous reviewers commended the applicants for the organized research plan and overall project questions, noting that results issuing from this project would be of interest to multiple fields including biological anthropology, systematics, functional morphology, and paleobiology. Moreover, results have the potential to transform paleoanthropology's approach to defining hominin species. Reviewers felt that the use of *Macaca* as an analog for *Homo* was not entirely necessary, especially since morphological variation within genus *Macaca* is interesting by itself. On this basis, the panel suggested including a detailed discussion of the pilot study results, including clarifying that landmark selection was informed by overall bone shape and load-bearing (functional) morphology, and toning down the link between macaque biogeography and hominin systematics. Thus, we have removed the wider discussion of *Homo* fossil diversity to include more background on macaque phylogeny, included requested information on dietary and locomotor variation in macaques, and included a larger discussion of the pilot study.

2. INTRODUCTION

For this project, we investigate the taxonomic efficacy of eight skeletal elements to understand the extent and pattern of morphological variation within genus *Macaca*. Furthermore, we examine the extent to which climate, geography, and/or behavior confound or correlate with these taxonomic assessments. Results from this project will show how skeletal variation informs sub-generic taxonomy, and can be applied to the fossil record to aid in identification of previously unknown taxa. Data consists of a 3D geometric morphometric (GM) analysis of eight macaque species and three outgroups (**Figure 1**), each chosen for their phylogenetic relationship to macaques (**Figure 2**) (Perelman et al. 2011; Roos et al. 2019) and their diverse geographic locations and behavioral differences. As a result of the data collection necessary for this project, a unique digital dataset consisting of 3D scans of cranial, mandibular, and post-cranial bones will be available for future research projects.

It is well understood that the cranium is particularly useful for taxonomic and phylogenetic assessment in non-human primates (e.g., Fleagle et al. 2010; Frost et al. 2003; Groves 1967). As such, the cranium is often thought to be the most reliable skeletal region for taxonomic assessment and is frequently used to answer questions relating to systematics (von Cramon-Taubadel & Weaver 2009). More recently, researchers have begun testing the extent to which phylogeny and/or taxonomy can be reconstructed from post-cranial bones such as the os coxa (Lycett & von Cramon-Taubadel 2013), scapula (Green 2013; Young 2006), and long bones (Holliday & Friedl 2013; Holliday et al. 2010; Tallman 2012; Tallman 2016; Turley et al. 2011). Results from these studies suggest that there are taxonomic differences among genera evident in post-cranial elements, often due to differing locomotor preferences. However, there is a lack of research investigating intra-generic morphological differences evident in cranial and post-cranial bones.

The investigation of intra-generic morphology is particularly important to paleoanthropology, as there are a limited number of assigned hominin genera, each of which are considered relatively specious. Hence, species recognition is at the core of hominin systematics, given that taxonomic classification is essential to building our understanding of human evolution (White 2003). Accurate reconstructions and elucidations of the past, including the taxonomy and evolutionary adaptations of extinct hominins, require a thorough understanding of the biology of extant taxa (Smith & von Cramon-Taubadel 2015). In regards to hominins and non-human primate fossils, these interpretations are dependent on the development of accurate inference models of extant primate morphology (Smith & von Cramon-Taubadel 2015). Thus, a comparative dataset of extant primate morphological variation must form the basis for current and future systematic and evolutionary analyses.

To date, the taxonomic importance of morphological features has largely been determined by tradition, and not by comparative evidence regarding intra- versus inter-taxon variation in extant species (Zollikofer et al. 2014). Thus, the utilization of comparative datasets of extant primates offers anthropologists a means by which to determine intra- versus inter-taxon variation, and assess whether variation among fossil taxa is in line with that found in extant taxa or a byproduct of taphonomy (Zollikofer et al. 2014).

In order to address these debates, it is necessary to assess how much variation could, or should, exist among species within a single genus. These questions could be addressed in a variety of ways, yet macaques

are the best primate model available to test this. Macaques are specious and geographically widespread (e.g., Marchesi et al. 1995; Poulsen & Clark 2004; Russon et al. 2001; Tutin and Fernandez 1984) and, therefore, potentially adapted to a wide variety of ecological zones and climatic conditions. Furthermore, though macaques are a relatively well-known genus and the subject of numerous studies, their phylogenetic relationships and thus taxonomic nomenclature remains contentious. Due to discrepancies in genetic data (e.g., Deinard & Smith 2001; Fooden & Lanyon 1989; Morales & Melnick 1998; Tosi et al. 2000), which differ from morphological studies (e.g., Delson 1980), macaque phylogeny is complex. A new approach to analyzing macaque phylogeny, such as GM, has the potential to transform our understanding of levels of variation within a particular genus and species, while also elucidating the extent to which morphology tracks macaque phylogeny. Therefore, the central motivation for this project is to answer the question: **are different species of macaque taxonomically distinguishable based on their skeletal morphology?**

3. THEORETICAL BACKGROUND

Macaque monkeys (genus *Macaca*) are an ideal comparative dataset for the investigation of morphological variation within the order Primates because they have the widest geographic range of any primate genus aside from humans (Baab 2008; Terhune et al. 2007). However, unlike extant *Homo,* where a single species has a wide geographic range, genus *Macaca* is represented by 23 species (Deinard & Smith 2001). Macaques live within geopolitical boundaries ranging from Japan (*Macaca fuscata*) to Afghanistan (*Macaca mulatta*), to Morocco and Iberia (*Macaca sylvanus*) (**Figure 1**) (Weinstein 2011) and exploit a variety of ecological niches extending from lowland tropical forests to altitudes of over 2500 meters, living in highly seasonal and tropical rain forests, woodlands, swamps, mountains, grasslands, and human-made structures such as temples (Falk 2000). Consequently, *Macaca* represent the ideal comparative sample of extant primates, as their wide geographic range suggests that their skeletons might exhibit regional variants (Fooden 2006; Schillaci 2010).

For this project, we analyze the skeletons of eight macaque species, representing each of the seven major species-groups (Zinner et al. 2013) plus the cold-adapted *Macaca fuscata*, and three outgroup taxa closely related to macaques: *Cercopithecus ascanius*, *Trachypithecus cristatus,* and *Lophocebus albigena* (Perelman et al. 2011) (**Figure 2**). Data collection primarily consists of geometric morphometric (GM) methodologies (i.e., morphometric data collected from landmarked 3D scans), although also includes metric data for limb elements to calculate standard limb proportion indices.

Consistent with previous research, it is expected that geographic-specific adaptations will manifest on the skeleton (Schillaci 2010) and results will elucidate morphological similarities and differences among closely related taxa. Thereby, a comparative dataset will be created, and as such will offer insight into the cause of this variation. While this study focuses on *Macaca,* the overarching goal is to later apply the resultant findings to hominin and other mammal fossils to determine how much variation feasibly exists within a single genus, and within a single species.

This study focuses on *Macaca*, as they represent one of the most successful primate radiations (Delson 1980; Fooden 2006; Hoelzer & Melnick 1996; Li et al. 2009; Roos et al. 2019; Terhune et al. 2007; Ziegler et al. 2007), and exhibit the second largest geographic range after humans (Roos et al. 2019). There is wide behavioral contrast between different species of macaques, particularly as it relates to locomotion (e.g., Burr et al. 1989; Cant 1988; Dunbar & Badam 1999; Gebo 1995; Rodman 1979) and diet (e.g., Anton 1995; Borries et al. 2011; Hanya 2004; Krishnamani 1994; Menon & Poirier 1996; Thierry 2008; Sha & Hanya 2013; Su & Lee 2001; Yeager 1996; Zhou et al. 2011), which may manifest as distinct patterns of variation on the skeleton. The extent to which these behaviors correlate to morphology in macaques will, therefore, be tested by statistically comparing skeletal shape (quantified using GM data) against published behavioral and ecological data (**Table 3**).

We primarily seek to examine variation across diverse species in *Macaca,* thus revealing inter- and intra-generic morphological variation. Macaque taxonomy was initially assessed based on the penile morphology of males and pelage coloration, which resulted in the formation of four main species-groups: 1) *silenus-sylvanus*; 2) *fascicularis*; 3) *sinica*; and 4) *arctoides* (Fooden 1976; 2006). Delson (1980) reexamined macaque morphology based on evolutionary comparisons with fossil macaques and results

supported Fooden's (1976) initial classification with two exceptions: 1) *M. sylvanus* should have its own group based on biogeography and 2) *M. arctoides* should be included in the *sinica* group, suggesting that it represents an extreme in an otherwise clinal patterning (Delson 1980).

The monophyly of the genus *Macaca* is constantly questioned due to the lack of clear morphological distinctions that separate macaques from the rest of the cercopithecines (Groves 1989; Morales & Melnick 1998). Some argue that the placement of macaques in one genus is based on biogeography, and not the notion of shared morphological characteristics (Szalay & Delson 1979). Multiple genetic studies have sought to investigate the relationship of macaques to one another and the rest of the cercopithecines, with complex results.

Based on allozyme data, Fooden and Lanyon (1989) attempted to resolve the classification of *M. arctoides,* and found that the species represents a sister-group with the *sinica* species-group. Further analysis in the same study separated the Sulawesi macaques from all other macaque species, which had not been previously seen (Fooden & Lanyon 1989). Additional studies have attempted to use mitochondrial DNA (mtDNA) to resolve these complex phylogenetic relationships, though the results from the majority of these studies are too unexpected to be valid or otherwise unsupported by other data. For example, Morales and Melnick (1998) compared mtDNA among 19 species of extant macaques and one of their conclusions suggested that *M. fascicularis* was ancestral to all macaque clades except for *M. sylvanus* and Fooden's *silenus/*Sulawesi group (noted as Fooden's since other researchers consider these to be distinct groups) due to earlier divergence times. This would mean that, based on mtDNA analysis, *M. fascicularis* was the ancestor to the *fascicularis, mulatta, arctoides,* and *sinica* species-groups. These findings are generally unsupported by morphological data (Delson 1980), Y chromosome data (Tosi et al. 2000), and other mtDNA studies (Zhang & Shi 1993), which all report *fascicularis* only being ancestral to the *mulatta* species-group.

In a study based on determining phylogeny from the nuclear locus *NRAMP1*, the relationship of *M. arctoides* to the rest of the macaques remains unclear, suggesting it has an affinity to either *M. nemestrina* (from the *silenus*-group) or *M. fascicularis* (Deinard & Smith 2001). Almost all genetic studies support *M. sylvanus* as a monophyletic group. The *NRAMP1* data suggest that *M. fascicularis* haplotypes are derived with respect to *M. mulatta* haplotypes whereas most other mitochondrial and morphological comparisons suggest that *M. fascicularis* is more primitive than, and ancestral to, *M. mulatta* (Deinard & Smith 2001).

Though there is much discrepancy, genetic tests have concluded that insular populations are significantly less genetically variable than continental populations, and therefore conclusions about macaque phylogeny may be difficult to attain from allele frequency data (Fooden & Lanyon 1989). It is also the case that insular populations (i.e., *M. fuscata*) do not interbreed with other macaques, and therefore hybridization is not a factor for island populations. Hybridization could contribute to confusion among sub-generic taxonomy thereby allowing for continued speciation in continental populations such as *M. mulatta* and *M. fascicularis* (Hayasaka et al. 1988). It is clear that genus *Macaca* has likely undergone several population bottlenecks (Fooden & Lanyon 1989).

Although the intricacies of sub-generic taxonomy remain contentious, it is generally agreed upon that macaques represent a monophyletic grouping and results from multiple studies partly corroborate Fooden's (1976) species-groups. Based on these genetic and morphological analyses, Groves (2001) called for six, rather than four, species-groups: 1) *sylvanus;* 2) *silenus*; 3) Sulawesi; 4) *fascicularis*; 5) *mulatta*; and 6) *sinica*. The separation of *M. fascicularis* and *M. arctoides* into monophyletic groups based on morphology was recently proposed, thereby making seven recognized species-groups (Zinner et al. 2013).

To accurately assess morphological variation throughout *Macaca*, we will study one representative species from each species-group (following Zinner et al. 2013) (**Figure 2**), though we include two *mulatta*-group members: *M. mulatta* and the cold-adapted *M. fuscata*. The comprehensive approach taken in our study aims to add new insights into macaque taxonomy. Unlike pelage color and genital morphology, the focus on skeletal elements will also speak to the evolutionary history of macaque morphology, and integrating GM methodologies will update previous comprehensive morphological studies (e.g., Delson 1980). To our knowledge, no holistic study of the appendicular skeleton of macaques in regards to their sub-generic taxonomic classification has been undertaken, and based on previous work (e.g., Lycett & von

Cramon-Taubadel 2013; von Cramon-Taubadel & Lycett 2014) and our pilot study, results will undoubtedly contribute a more sophisticated understanding of the complex evolutionary relationships within genus *Macaca.*

The application of novel methods to the study of primate taxonomy is overdue. Though GM methods have been applied to phylogenetic and evolutionary studies since the 2000s (Baab 2008; Fleagle et al. 2010; Harvati 2003; Smith 2009; Terhune et al. 2007), these techniques have not yet been applied to an intra-generic dataset, despite the fact that primate taxonomy is one of the most important questions within bioanthropology. We adapted established GM methods from previous studies (e.g., Lycett & von Cramon-Taubadel 2013; Terhune et al. 2007; von Cramon-Taubadel & Smith 2012; Zichello et al. 2018) and will combine these techniques to create a comparative dataset. In turn, results will be applicable to extant specimens and the fossil record by statistically elucidating the amount of morphological variation that exists within a genus and within a species.

4. RESEARCH PLAN AND METHODOLOGY
4.1. Pilot Study
We conducted a pilot study to test the taxonomic efficacy of cranial versus pelvic morphology in *M. mulatta*, *M. fascicularis,* and an outgroup of *Chlorocebus pygerythrus*. 49 cranial (*n*=29) and os coxa (*n*=20) landmarks (Frost et al. 2003; Lycett & von Cramon-Taubadel 2013) from 20 males and 20 females from each species (*n*=120) were digitized with a MicroScribe G2X digitizer. Data were subjected to a Procrustes generalized fit, Principal Components Analysis (PCA), and Canonical Variates Analysis (CVA), performed in MorphoJ (Klingenberg 2011), and a Discriminant Function Analysis (DFA) was performed in SPSS based on PC scores (IBM Corp 2017).

Results from the pilot study suggested that the os coxa is also a reliable indicator of macaque taxonomy when compared with the cranium (Conaway et al. 2018; Kenyon et al. 2018). Results from the CVA showed clear separation of sexes and taxa for both the cranium and os coxa. For the os coxa, taxa are separated across CV1 by shape differences in the pubic symphysis and sex is distinguished on CV2 by shape differences in the ischial tuberosity and the pubic symphysis (**Figure 3**). In the cranium, genera are strongly distinguished across CV1 via changes in the cranial vault and sex is distinguished across CV2 via relative size differences in the eye orbit, consistent with previous findings (Fleagle et al. 2010) (**Figure 4**). DFAs were performed for both the crania and os coxa based on classifiers assigning individuals to 1) species and 2) combined species and sex, and cross-validated scores were obtained. When analyzing cranial shape within sexes, macaques are much more likely to be misclassified (~15%) as other macaques than as vervet monkeys (~2%). For the cranial DFA combining sex and species, male macaques had overall low correct classifications (~35%) and were quite likely to be misclassified as males of the other macaque species (~30-35%), while females had high correct classifications (80-85%). However, hardly any macaques were misclassified as ververts of either sex (<5%). Thus, we found that generic differences were clear in cranial shape but species-level differences (especially in males) were less obvious. For the os coxa, results from the DFA found that all taxa were correctly classified at higher rates than for the cranium (85-92%) and that macaques are more likely to be misclassified as another species of macaque than as a vervet monkey. The DFA testing the combined sex and species found higher rates of correct species classification for male (70-80%) and female (90%) macaques, with the highest rates of misclassification among male macaque species (15-25%). Hence, while generic-level taxonomic differences are still more prominent in the os coxa shape data than species-level differences, the os coxa shape was overall better at distinguishing macaque species (particularly male macaques) than the cranium.

The results were somewhat surprising, as conventionally the cranium is expected to offer the more reliable reflection of primate taxonomy. Although the cranium still served as a reliable indicator of generic differences, the os coxa provided a more accurate estimate of sub-generic differences than the cranium. These findings are in line with previous work by von Cramon-Taubadel and Lycett (2014), which found that pelvic shape was just as useful for recovering genetic relatedness of catarrhine taxa as cranial or mandibular shape. In our pilot study, females of each species tended to differentiate taxonomically more so than males, consistent with previous findings (e.g., Fleagle et al. 2010). *M. fascicularis* has a taller,

shallower eye orbit and a smaller cranial vault, which is in line with previous work suggesting a negative allometric relationship between orbit and body size (Ross 1995). DFA results suggest that closely related species are taxonomically distinguishable based on skeletal shape, and that it is more likely for species of the same genus to be misclassified as each other than it is for them to be misclassified as an individual from a closely related genus (i.e., *M. mulatta* are more likely to be misclassified as *M. fascicularis* than they are to be misclassified as *C. pygerythrus*). Hence, the results support the hypothesis that there are morphological differences in the macaque skeleton that contribute to sub-generic taxonomic assessment, which had not been tested previously. Therefore, further investigation is required to determine if other post-cranial bones, such as the scapula or long bones, exhibit similar levels of within- and among-species variation and if additional macaque species exhibit similar taxonomic signals on their skeletons. Results were presented in Spring 2018 (see Conaway et al. 2018; Kenyon et al. 2018) and submitted for publication in the *American Journal of Physical Anthropology* (currently under review).

4.2 Research Questions
This project is driven by the central question: **are species of macaques taxonomically distinguishable based on their skeletal morphology?** To test the main question, three hypotheses will be tested:

> **H1:** Taxonomy can be accurately predicted based on the patterns of morphological variation in skeletal elements in geographically wide-spread macaque taxa.
> **H2**: Post-cranial bones, particularly the os coxa, scapula, and long bones, reflect sub-generic macaque taxonomy at the same level or better than the cranium.
> **H3:** Climate and geographic distance have a confounding effect on morphological distances among related macaque taxa.

Previous research has demonstrated that distinct morphology is often driven by environmental adaptations (Fooden 2006) and that macaques often, though not always, follow Allen's and Bergmann's rules for climatic body size and shape variation (Schillaci 2010). Similar studies have demonstrated that *Homo sapiens* (Baab et al. 2010; Beall et al. 2012; Smithers & Smith 1997) and other hominins such as *Homo neandertalensis* (Holliday 1997) also follow Allen's and Bergmann's rules. Therefore, we know that environmentally-specific adaptations exist in widespread species and our pilot study and previous research suggests that taxonomy can be recovered from macaque skeletal elements (Fleagle et al. 2010). Thus, **H1** tests the notion that morphological variation among species is distinct enough to estimate taxonomy.

Historically, the cranium has been thought of as the best indicator for taxonomy (Fleagle et al. 2010; Frost et al. 2003; Groves 1967). However, the pilot study for this project suggested that the os coxa may be a better indicator of intra-generic taxonomic assessment than previously thought. This is in line with findings from studies on human (Betti et al. 2013; Betti et al. 2014) and catarrhine os coxae (von Cramon-Taubadel & Lycett 2014). Other recent studies have begun testing the taxonomic efficacy of the scapula, and have found morphological differences in blade shape and spine orientation (Green et al. 2016). It has been suggested that scapular morphology is most likely effected by either embryonic factors or epigenetic factors associated with muscle attachments, meaning taxonomic differences should be evident in scapular shape. However, there are also substantial differences in the quadruped versus non-quadruped scapula, likely a byproduct of stabilizing selection (Young 2006). Hence, it is currently not clear to what extent scapular morphology within a single genus, with largely similar locomotor patterns, will reflect sub-generic taxonomic similarities and differences. In other preliminary analyses for this project, we landmarked sample crania, os coxa, and scapula from one male and one female from 16 catarrhine species (*n*=32). Based on 2D multidimensional scaling, we found the scapula to be the best taxonomic indicator with clear distinctions between terrestrial and arboreal quadrupeds (see Kenyon & von Cramon-Taubadel 2019). Based on previous work and our preliminary findings, we will, therefore, test the null hypothesis (**H2**) that the post-cranial bones, particularly the os coxa and the scapula, are effective for assessing macaque taxonomy.

Previous studies show that the exploitation of new ecological niches is a driving factor for macaque diversification, which subsequently leads to morphological variation (Delson 1980). However, whether

these adaptations exist on a continuum is untested, as is the degree to which specific features, such as the nasal cavity, are adapted to climate as they are in Neandertals (Holliday 1997). Thus, the effect of climate on morphology will be examined, testing the null hypothesis (**H3**) that there is no effect on morphological distances as a result of climate or geography. It is expected that the null hypothesis will be rejected in favor of the notion that there are morphological adaptations as a result of climate, particularly in cranial morphology and limb bone dimensions. Therefore, these results will aid in elucidating whether or not climate adaptations could be a confounding factor on morphology.

4.2.1 Additional Considerations. There are obvious differences among macaque species, such as differing locomotor or dietary preferences which could have an effect on morphological variation. These confounding factors could alter patterns of variation in macaques or other primates. Consequently, two additional hypotheses relating to macaque morphology will be tested:

> **H4:** Locomotor behaviors do not affect post-cranial morphological variation at the species-level.
> **H5:** Dietary preferences do not affect craniomandibular variation at the species-level.

In addition to climate adaptations, the wide geographic range of macaques allows for extensive behavioral variation throughout the macaque genus (Thierry 2008). Since macaques are adapted to a wide range of environments, locomotor behaviors vary, with some species classified as arboreal quadrupeds and others as terrestrial quadrupeds (Burr et al. 1989; Cant 1988; Dunbar & Badam 1999; Rodman, 1979). Additionally, some species have other preferred methods of locomotion (e.g., swimming) which suggest anatomical adaptations in the post-cranium (Rodman 1979). The effect of locomotor preference on *Macaca* morphology will be examined in order to decipher how slight but noticeable differences in locomotion manifest on the skeleton (see **Table 3**). We will test the null hypothesis (**H4**) that there is no effect on the post-crania as a result of locomotor preferences. It is expected that this hypothesis will be rejected in favor of the idea that there are morphological differences resulting from locomotor preferences. The greatest differences are expected to be seen between *M. fuscata*, a more arboreal species, and *M. sylvanus*, a highly terrestrial species with some populations spending 100% of their time engaging in terrestrial quadrupedalism (Chatani 2003; Fooden 2007). Results that showcase differences in skeletal morphology due to differing locomotor preferences will be particularly useful when applying these questions to other primate taxa, both extant and extinct.

Given the broad range of environments occupied by macaques, there are also key differences in standard and/or preferred diet among species; some have a more frugivorous diet while others have a more folivorous diet (Thierry 2008). There are numerous studies that suggest that there are morphological correlates in the craniomandibular region as a result of biomechanical strain (Collard & Lycett 2009; Collard & Wood 2001; Collard & Wood 2007; von Cramon-Taubadel 2009; Wood & Lieberman 2001). Wood and Lieberman (2001) suggest that there are patterns of intraspecific variation in the craniodental region among extant anthropoid primates and these patterns are not related to within-species variability. It is not certain whether dietary differences aid or hinder taxonomic assessment. Thus, we will explore the extent to which diet confounds taxonomic assessment due to potential homoplasy (von Cramon-Taubadel 2009; Wood and Lieberman 2001) (**H5**). Given that the mandible is typically preserved in the fossil record (White 2003) and routinely used in taxonomic assessment, results should be particularly helpful when applied to fossil primate mandibulae and for identifying dietary behaviors in extant primates.

4.3 Samples
The project is comprised of a sample of eight macaque species and three closely related outgroups (Perelman et al. 2011) (**Figure 2**). The skulls and partial skeletons of each species will be 3D scanned with an HDI structured light scanner (using established lab protocol) and exported as ".ply" files for analysis. Long bones will be measured using traditional metric measurements (**Table 1**) (following Auerbach & Ruff 2004; 2006; Buikstra & Ubelaker 1994; Howells 1973).

All skeletons must be adult due to the fact that sub-adult skeletons are still developing and, therefore, are influenced by ontogeny and other external factors (Simons et al. 2018). Adult skeletons will be determined by eruption of third molars and fusion of long bone epiphyses. Additionally, all macaques must be wild-caught when possible and exhibit no obvious signs of trauma or pathology. The sample includes one representative species of each species-group: *M. fascicularis, M. mulatta, M. nemestrina, M. arctoides, M. nigra, M. radiata,* and *M. sylvanus*, plus *M. fuscata,* representing a cold climate (Fooden 1976; 2006) (**Figures 1 and 2**). Three outgroups will also be used for comparison: *Cercopithecus ascanius*, *Trachypithecus cristatus,* and *Lophocebus albigena* ensuring there is a representative from each major Catarrhine group (Perelman et al. 2011) (**Figure 2**). Comparisons with outgroups will help provide evidence as to which aspects of morphology demarcate sub-family or family-level variation and which are unique to macaques at the genus or species level.

Skeletal elements that correspond to behaviors tested in the hypotheses and those with previously demonstrated taxonomic efficacy will be studied including: cranium, mandible, scapula, os coxa, humerus, radius, femur, and tibia. Elements from the left side of the skeleton will be measured unless they are absent or otherwise unusable, in which case the right side will be used. Specimens must have all necessary skeletal elements to be included in the study. The target sample is 440 individuals: 20 males and 20 females from each species.

4.4 Methodology
This project will address the main question by using GM analysis coupled with metric analysis of long bones.

4.4.1 Geometric Morphometric Analysis. Landmarks (**Figures 5-12**) will be placed on each 3D surface scan using Landmark (Wiley et al. 2005), and coordinate data will be exported for statistical analysis. Landmarks were chosen to assess the overall bone shape, capture morphological aspects known to contribute to variation based on previous work (i.e., orbit shape), and allow for an assessment of functionally-important morphology (i.e., load-bearing regions or muscle attachment sites). All landmark configurations will be registered using Generalized Procrustes Analysis using MorphoJ (Klingenberg 2011) and resultant shape variables will be subject to a Principal Components Analyses (PCA) to investigate average shape variation among individuals (Zelditch et al. 2004) as well as how variation is partitioned within and among taxa (Harvati 2003). Resultant principal component (PC) scores generated in MorphoJ (Klingenberg 2011) will be exported into a Microsoft Excel spreadsheet for analysis. A multivariate analysis of variance (MANOVA) will be performed on the PC scores to determine which taxa (if any) show significant shape differences.

Thereafter, a Canonical Variate Analysis (CVA) will be employed to maximize inter-group differences (Zelditch et al. 2004). Once it is determined that the canonical variates (CVs) of shape variables are useful for distinguishing samples and assigning them to groups, it will be necessary to explore what shape differentiations the CVs represent. Thus, we will multiply the original shape variables by the coefficients of the CVs and sum them, which will result in a series of vectors of relative landmark displacements. In turn, this will show the shape differentiation represented by each CV, which is useful for trait discrimination against groups (Zelditch et al. 2004). Two versions of a jackknife procedure will be performed on the resulting canonical variate (CV) scores, which will resample the data leaving out a) one skeletal element and b) one species (Zelditch et al. 2004). The jackknife will be useful in this scenario since this is a large dataset with many parameters, meaning there is a high probability of overfitting the data. The jackknife will be used to estimate the predictive power of the models by predicting the dependent variables of each observation as if it were a new observation, thus creating an unbiased prediction model while minimizing the risk of over-fitting the data (Abdi and Williams 2010). Both the PCA and CVA will be performed in MorphoJ (Klingenberg 2011), MANOVA will be performed in PAST (Hammer et al. 2001) and jackknife procedures will be performed in *R* (R Core Team 2013).

A discriminant function analysis (DFA) based on the PC scores that explain 95% of overall shape information (Mitteroecker and Bookstein 2008) will be performed in SPSS (IBM Corp 2017) and will be

used to aid in predicting categorical memberships, thus determining the extent to which specimens are classified or misclassified. Preliminary results from the pilot study suggested that there are distinct aspects of morphology that aid in classifying specimens when using GM analysis, and therefore similar methods will be applied here (see Conaway et al. 2018; Kenyon et al. 2018). This portion of the analysis will be both integrative of all data and also include element-by-element analysis. The former is important since this project seeks to understand how specimens taxonomically classify based on their overall skeletal morphology. However, since complete fossils are rarely, if ever, found, it is equally important to understand how single elements differentiate taxa.

In order to test **H4** and **H5** it will be necessary to compare morphological data to geographic and behavioral data. This will be done by compiling published data on the percentage of time each species spends engaging in a particular locomotor behavior (**H4**) and the percentage of a specific food type included in each species diet (**H5**) (see **Table 3**). To compare these data with the morphological data, pairwise among-species matrices will be constructed based on the morphological and geographic or behavioral data. Mahalanobis distances (D^2) will be calculated among species based on morphological data (PC scores) (Zelditch et al. 2004). Geographic distances will be calculated among species as great circle distances in kilometers between average latitude and longitude coordinates for each taxon (Ramachandran et al. 2005), temperature distances will be calculated as average differences in mean annual temperature for each taxon location, while the locomotor and dietary data (**Table 3**) for each species will be coded and converted to Euclidean distance matrices (following von Cramon-Taubadel 2011). Mantel tests will be used to test for significant correlations between the morphological distance and distances representing geography, climate, locomotor behavior, and diet. Mantel tests will be calculated using PASSaGE software (Rosenberg & Anderson 2011).

4.4.2 Metric Analysis. In addition to the GM assessment of the 3D scans, standard metric measurements will be taken on all four limb bones using sliding calipers and an osteometric board (**Table 1**). Metric data from long bones will aid in the assessment of mean brachial, crural, and intermembral indices for each species, which will be used to infer locomotive pattern needed to test **H4**. A MANOVA test will be conducted for each element to assess species-level differences in element metrics. In cases where taxa are significantly different, an analysis of variance (ANOVA) and post-hoc Bonferroni adjusted *t*-tests will be employed to assess which traits best differentiate taxa.

4.5 Project Timeline
On average, five complete individuals can be scanned and measured in one eight-hour work day. This means, not including travel time, the entire dataset required for this project can be collected in 88 work days or 18 weeks. There must be added time for travel since no museum has all 440 specimens. About half of the data (*n*=250) were collected between summer 2018 and summer 2019. Additional time to process the scans and landmark scans is required, though some specimens are already landmarked and the remaining can be completed within the Fall 2019 semester. The entire project takes roughly three years to complete. **Table 6** lists the necessary components to complete the project, including the semesters they were/will be completed.

5. INTELLECTUAL MERITS
The central focus of this project is the answer the question: **are species of macaques taxonomically distinguishable based on their skeletal morphology?** We plan to test the taxonomic efficacy of eight skeletal elements to determine which element reflects sub-generic taxonomic relationships the best. Furthermore, we plan to determine whether or not there is an effect of climate and geographic distance on morphological distances, and if so, determine how this may impact *Macaca* morphology. Consequently, results will provide concrete evidence for the level of morphological variation within a particular genus and within species of the genus. Moreover, the results will shed light on longstanding debates about the relative efficacy of cranial versus post-cranial morphology in differentiating taxa at the species and genus level. As the pilot analyses results illustrated, it may be the case that certain aspects of post-cranial morphology are

more reliable for reconstructing taxonomic relationships than the cranium, which has important consequences for how we interpret taxonomic patterns in the fossil record based on different regions of skeleton.

The results of this study will also be important for formulating models of inter-specific and intra-generic patterns of variation, against which to test empirical data drawn from the hominin or mammalian fossil record. Future studies could employ the same landmarking protocols and analyses used here to compare patterns of morphology against those found as a result of this study.

Additional intellectual merits of the project include publication and presentation in academic settings, the applicability of these methods to museum curators, and the creation of a unique digital dataset. The methods, results, and conclusions of this project promise to contribute meaningfully to science, and as such will be published in scholarly journals and presented at conferences. The project is organized into three main hypotheses and two additional considerations. The first hypothesis concerns the taxonomic efficacy of various skeletal elements and the results obtained will have important implications for understanding morphological patterns throughout the skeleton. It will be submitted to *The Anatomical Record* for publication. Results from the test of the second hypothesis will elucidate which skeletal elements best reflect sub-generic phylogeny and will be submitted to the *American Journal of Physical Anthropology* as they are likely to be valuable for any number of phylogenetic studies of various species. The third major hypothesis concerns climate, and results obtained could have important ramifications for understanding climatic adaptations in extinct human species. Therefore, it will be submitted to the high-impact *Journal of Human Evolution.* The additional questions concern the impact of locomotion and diet on the skeletons of primates, and as such two additional journal articles will be submitted for publication. Results from the locomotion study will be submitted to the *International Journal of Primatology,* as findings have broad implications for primates more generally in determining how closely related animals move in differing environments. The results regarding the correlation of diet and morphology have important implications for identifying dietary behaviors in other living and extinct primates and thus the target journal for these results will be *Primates.* Conference presentations at the American Association of Anatomists, the American Association of Physical (Biological) Anthropology, and the American Society of Primatology will also occur at the completion of this project.

This study is also applicable to museums looking to re-curate their collection. Several skeletal collections have mislabeled, incorrectly labeled, or unidentified specimens. Many collections, for example, have online databases where specimens are labeled "*Macaca sp.*" or "unknown"; while sex and age are suggested based on skeletal features, species-level taxonomy cannot be accurately assessed to make these datasets more accurate. Mislabeled or unknown skeletons have little research value; hence, the results from this study could help curators in identification of specimens by targeting the analysis of particular skeletal regions, allowing for the better utility of these collections. Curators would be able to landmark unknown specimens, and compare resultant coordinates to the results from this project. Individuals from the same species should plot together, and therefore, the unknown specimen should plot in multivariate morphospace with the species to which it belongs.

Lastly, this project will result in a unique, digital dataset consisting of scans, and metric data for the cranium, mandible, and post-cranial bones for eleven primate species. As evidenced by the 2018 fire in Brazil's National Natural History Museum, digital datasets are of the utmost importance for the preservation of natural history collections. Our dataset is particularly unique in that it includes skulls and post-crania and these scans will then be useful for many future projects. Furthermore, the study of human remains is becoming increasingly difficult in many countries due to legal and ethical considerations. Macaque remains are not usually subject to ethical considerations in the same ways that human remains are. It is possible that access to human remains becomes more restricted in the future. Consequently, it is important that these non-human primate datasets exist and are easily accessible so that future researchers will always have access to closely related primate specimens to test taxonomic, geographic, or phylogenetic hypotheses, should samples of human remains become unavailable. As such, the PI and Co-PI will be sharing all scans, and metric limb data that result from this project. Museum curators will receive copies of specimens

scanned at their institutions for their databases. All data will be uploaded to MorphoSource, an NSF-funded database that allows researchers to store and distribute 3D data.

6. BROADER IMPACTS

This study seeks to determine the level of skeletal variation that exists within a single genus or species, and as such, particular aspects of this will be of interest to the general public. Results from this study will be presented not only to academic audiences, but to the general public in three main ways: developing a workshop for elementary and high school students belonging to underrepresented groups, presenting to the University at Buffalo group such as WISE (Women in Science and Engineering), and by dissemination via blogs throughout the duration of the project.

The skeleton provides important information about the human body, which is of interest to many students. Results from this study promise to provide concrete evidence for the ways in which the skeletons of closely related individuals respond to different environments. Using these results, the PI and Co-PI will develop two workshops to present to a K-6th grade and 9th-12th grade audience explaining how people and animals adapt to living in different environments. The Co-PI has over 15 years of experience working with public elementary students, and will therefore draw from her professional networks to develop these workshops. East High School (Rochester, NY) has a program for underrepresented high school students to enroll in particular courses based on their career goals; one program prepares students for a teaching career. The PIs will be presenting a workshop to students in this program explaining how anthropological research can inform our understanding of ourselves, with the hope that this information will be passed to future students.

Our data collection includes developing a digital set of macaque skeletons, and students will use these datasets to conduct their own experiment. The workshop will include a brief introduction to primate skeleton, followed by an explanation of results from the dissertation such as how and why the body responds to environmental differences. Students will then develop their own research question relating to the shape of the skeleton and the PIs will teach students how to extract data from the scans and conduct preliminary statistical analysis to answer their research questions. This will teach students how to engage in scientific research, equip them with research tools, and aid them in developing projects based in anthropology to teach to their future classes.

The PIs will also present a workshop to third graders at Theodore Roosevelt School #43 (Rochester, NY). The human body is a component of the third grade New York State Common Core and students spend about a month learning the basics of the human skeleton and body. At the conclusion of these lessons, the Co-PI will teach the students about human evolution by introducing primate skeletons. Students will then be shown models of a male and female macaque skeleton (3D printed from the collected scans and left in the classroom for future lessons) and asked to identify similarities and differences among them in order to understand how the skeleton differs based on where an individual lives, how they walk, or what they eat.

In addition to presenting workshops to local school-aged students, the PIs will be offering presentations on the University at Buffalo campus. The WISE student group is extremely active on campus, and offers lunchtime brown bag presentations for researchers to present their work to undergraduate women majoring in STEM disciplines. The PIs feel it is important to increase the presence of women in STEM, and therefore will present to this group at the conclusion of the project. The presentation will include how the PIs began their careers, explain the process of data collection, and the results of the dissertation. Following the presentation, students will be given the opportunity to visit the lab and complete their own research projects using the collected macaque scans.

Though most of the broader impacts include dissemination via presentation or workshop, results will also be disseminated by blog with planned submissions to *Sapiens, New Scientist, Scientific American,* and *Science Trends.* The Co-PI has experience with scientific blogging as she kept a blog during her field school in the Turkana Basin and contributed to the university's study abroad blog. All of the blogs mentioned above have open submissions to new, innovative work with the stipulation that it must be written for a general audience. These blog posts will be written and submitted throughout the project in order to engage the public through the duration of the project.
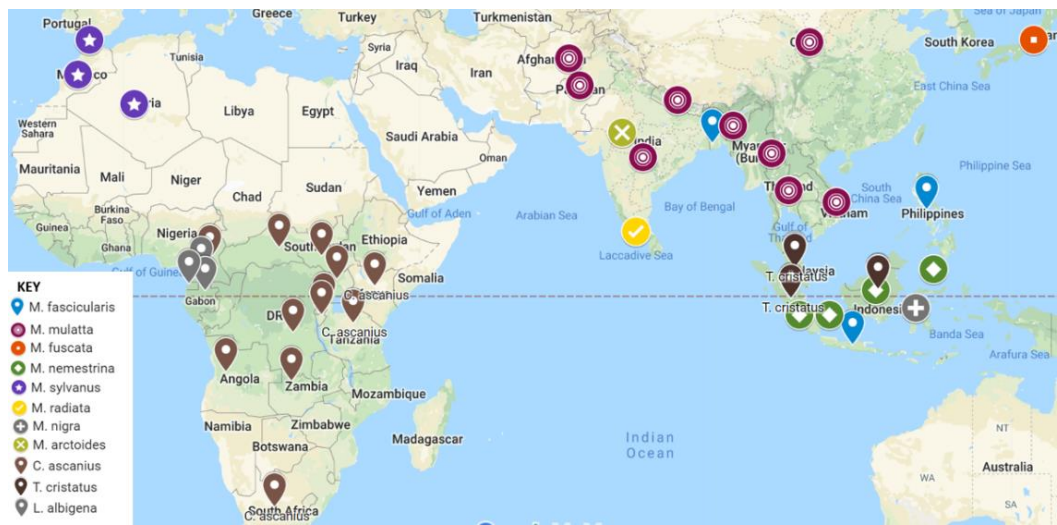
Figure 1. Macaque species and outgroup distribution by geopolitical boundaries.
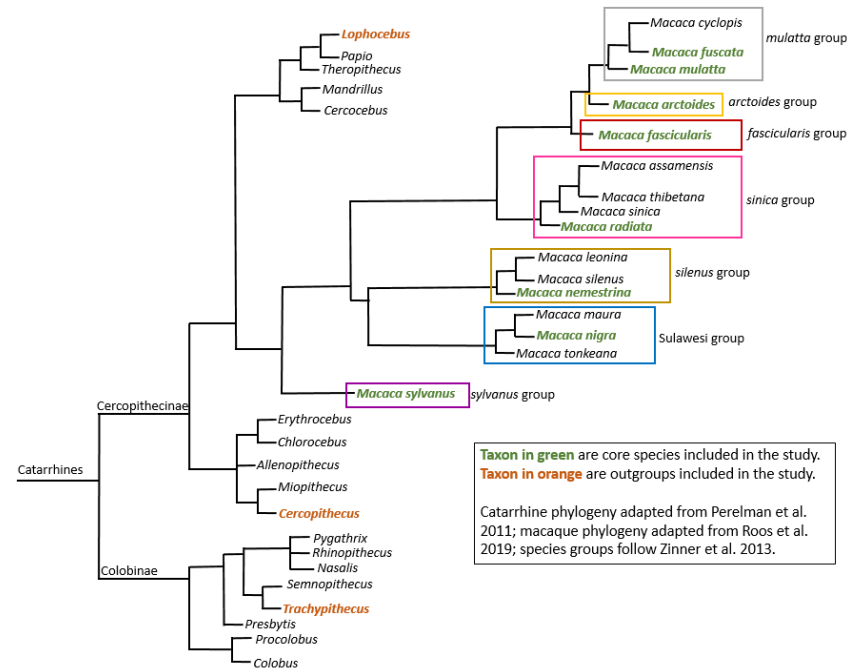

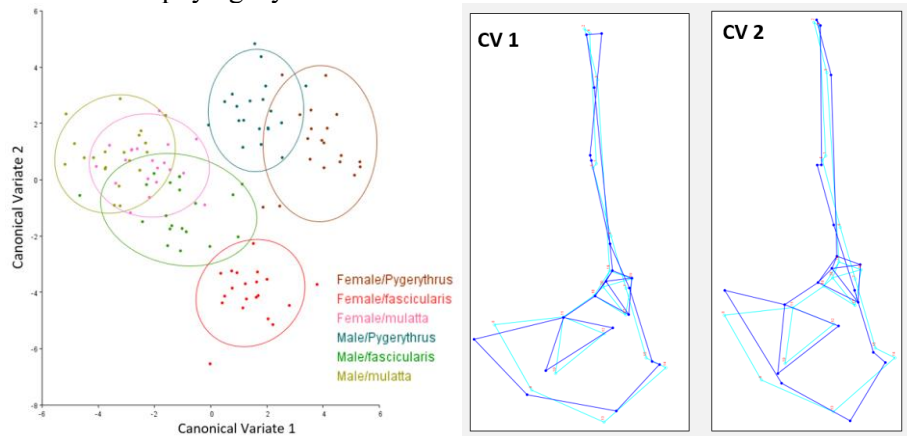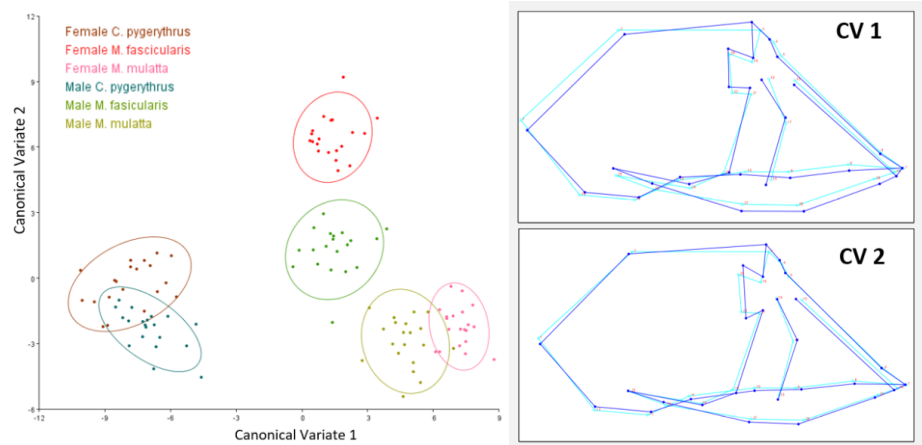**Figure 2.** Extant catarrhine phylogeny.

Figure 3. CVA results for the os coxa from the pilot study (Kenyon-Flatt et al., under review). Inserts show shape changes from negative (light blue) to positive (dark blue) ends of each CV.
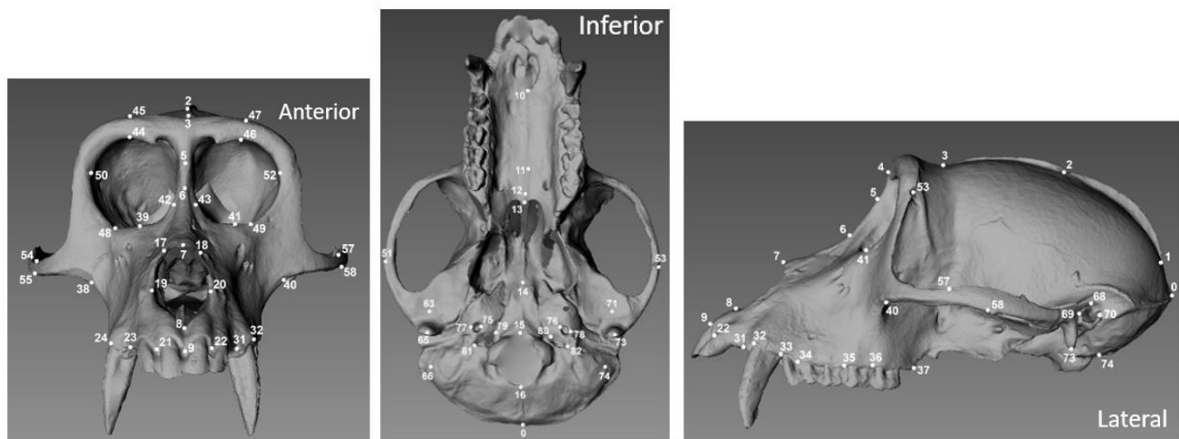
Figure 4. CVA results for the cranium from the pilot study (Kenyon-Flatt et al., under review). Inserts show shape changes from negative (light blue) to positive (dark blue) ends of each CV.

Figure 5. Cranial landmarks from Smith and von Cramon-Taubadel (2015), and Frost et al. (2003).
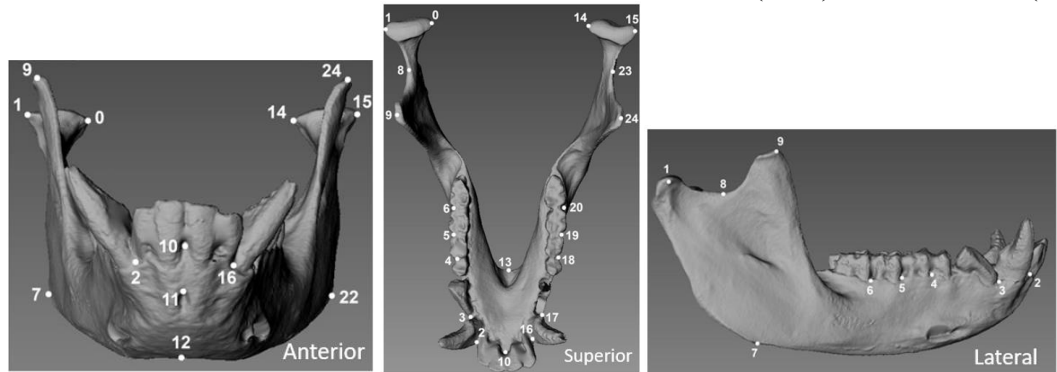

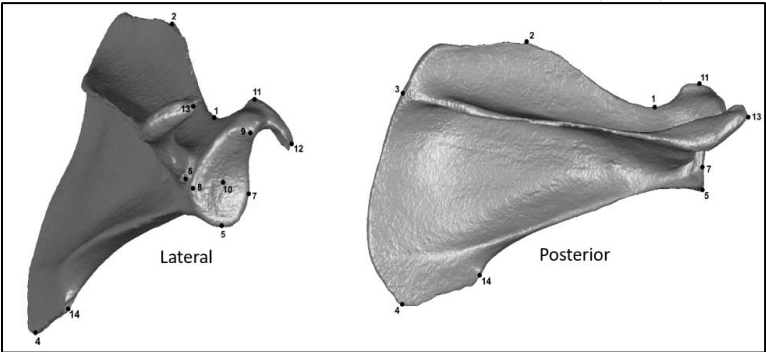**Figure 6.** Mandible landmarks from Smith and von Cramon-Taubadel (2015).

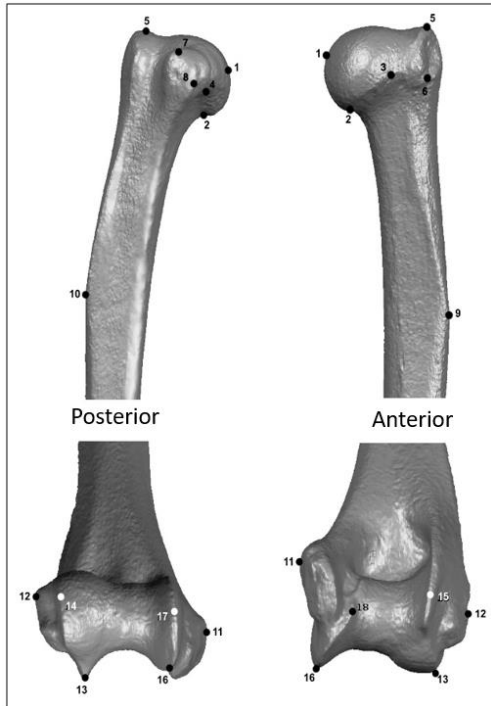**Figure 7.** Scapula landmarks from Young (2008).
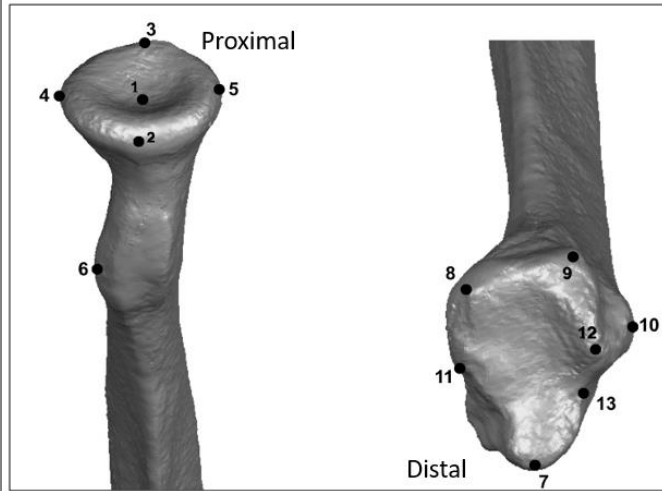

Figure 8. Humerus landmarks from Holliday & Friedl (2013).
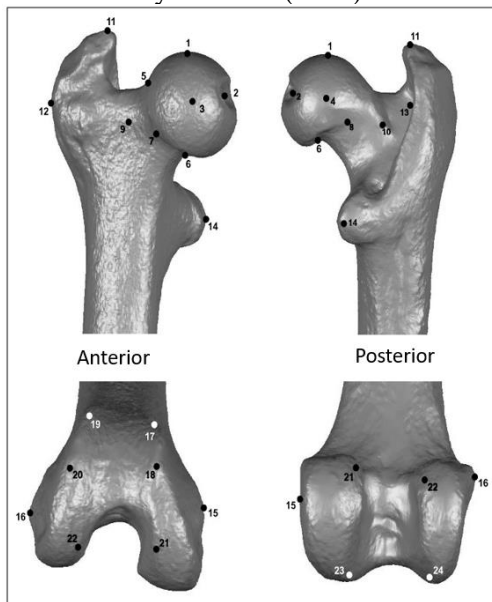

Figure 9. Radius landmarks from Tallman (2012).

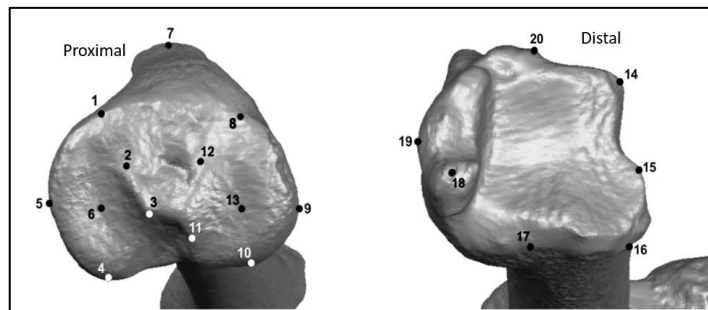**Figure 10.** Femur landmarks from Tallman (2016) and Holliday et al. (2010).
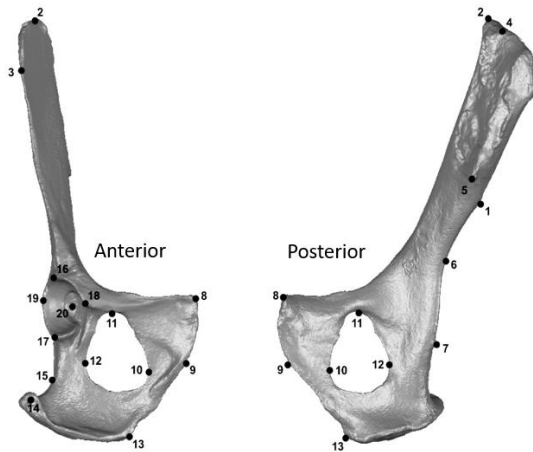

Figure 11. Tibia landmarks from Turley et al. (2011).

Table 1. Long Bone metric measurements, adapted from Auerbach & Ruff (2004; 2006). Each measurement will be performed on the humerus, radius, femur, and tibia as applicable.

Measurement	Description
XML	Maximum length
EB	Distal/epicondylar breadth
HD	Head/proximal diameter
MLD	Medial-lateral diameter
APD	Anterior-posterior diameter

Figure 12. Os coxa landmarks from Lycett & von Cramon-Taubadel (2013).

Table 2. Number of specimens per taxa located at each institution. Columns highlighted in green require NSF funding.

Species	Already collected	UCD	MNHN	BMNH	ZUM
M. arctoides	24	11	1	1	0
M. fascicularis	40	0	0	0	0
M. fuscata	40	0	0	0	0
M. mulatta	17	*	7	3	13+
M. nemestrina	34	*	4	6	0
M. nigra	18	0	0	2	3
M. radiata	21	7	0	1	9
M. sylvanus	6	0	3	4	3
C. ascanius	40	0	0	0	0
L. albigena	40	0	0	0	0
T. cristata	37	3	0	1	2
Total per institution	**317**	**21**	**15**	**17**	**30**

Collections visited previously: Museum of Comparative Zoology, Harvard University; Smithsonian Institution; Primate Research Institute, University of Kyoto; Museum of Vertebrate Zoology, University of California, Berkeley; Cleveland Museum of Natural History; Neil C. Tappen Collection, University of Minnesota; American Museum of Natural History; Field Museum of Natural History; Academy of Sciences, Philadelphia, PA. UCD = Primate Skeletal Collection, University of California, Davis; MNHN = Muséum National d'Histoire naturelle, Paris; BMNH = British Museum of Natural History; ZUM = Zurich University Museum.

* = UC Davis Primate collection contains several *M. nemestrina* and *M. mulatta* so the sample can be rounded out here if needed

Table 3. Dietary and locomotory data for comparison to morphological data. Note that not all percentages add up to 100 per reported data from original publication. D: dietary data. L: locomotory data

Species	DIET: % fruit	DIET: % leaves	DIET: % other	LOC.: % terrest.	LOC.: % arboreal	Citation
M. arctoides	37.5	58.4	0.3	14.6	3.9	Fooden et al. 1985 (D); O'Keefe & Lifshitz 1985 (L)
M. fascicularis	66.7	17.2	16.2	74	36	Yeager 1996; Sha & Hanya 2013 (D); Cant 1988 (L)
M. fuscata	13	41	38	54.5	46	Hanya 2004 (D); Chatani 2003 (L)
M. nemestrina	74.2	20	5	75	25	Caldecott 1986 (D); Rodman 1991 (L)
M. nigra	65	2.4	30	60	40	O'Brien et al. 1997 (D; L)
M. mulatta	8.5	84.4	42	70.85	40	Goldstein & Richards 1989 (D); Wells & Turnquist 2001 (L)
M. radiata	41.2	17.7	10.3	70	30	Krishnamani 1994 (D); Dunbar & Badam 1999 (L)
M. sylvanus	0.8	27.3	45.1	85	15	Fooden 2007 (D); Majolo 2013 (L)
C. ascanius	61.3	0.4	34.3	55	48	Cords 1986 (D); Gebo & Chapman 1995 (L)
L. albigena	40.6	6.6	49.9	62	44	Poulson et al. 2001 (D); Gebo & Chapman 1995 (L)
T. cristatus	30	71	15	50.6	49.4	Kool 1993 (D); Fleagle 1978 (L)

Table 4. Project timetable.

Stage	Year	Deliverables
1	Spring, Summer, Winter 2017	(1) Developed data collection protocol; (2) Established research objectives; (3) Collected and analyzed pilot study data
2	Spring 2018	(1) Present pilot study results at AAPA meeting
3	Summer 2018	(1) Data collection at Museum of Comparative Zoology, Harvard University and Smithsonian Institution
4	Summer/Fall 2018	(1) Specimen loans from Museum of Vertebrate Zoology, University of California, Berkeley and Academy of Natural Sciences, Philadelphia; (2) Data collection at Cleveland Museum of Natural History; (3) Submit pilot study for publication in American Journal of Physical Anthropology; (4) Landmark collected specimens and capture metrics
5	Winter 2018/2019	(1) Data collection at Primate Research Center in Japan
6	Spring/summer 2019	(1) Landmark PRI specimens and capture metrics; (2) Present preliminary results at AAPA meeting (3) Data collection at Neil C. Tappen Collection, Field Museum of Natural History, and American Museum of Natural History
7	Summer/fall 2019	(1) Data collection at University of California Davis, Muséum National d'Histoire Naturelle in Paris, British Museum of Natural History, and Zurich University Museum; (2) Landmark specimens and capture metrics
8	Winter/spring 2020	(1) Analyze data; (2) Dissertation write up and defense; (3) Publication write-up for academic and non-academic audiences