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Geometric Morphometric Analysis of the Humerus in New and Old World Vultures

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By

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Table of Contents

Signature Page.....	ii
Acknowledgments.....	iii
List of Tables.....	v
List of Figures.....	vi
Abstract.....	vii
Introduction.....	1
Materials and Methods.....	4
Results.....	7
Discussion.....	11
Conclusion.....	17
Literature Cited.....	18
Appendix A.....	24
Appendix B.....	40

List of Tables

Table 1.....	24
Table 2.....	25
Table 3.....	26
Table 4.....	26
Table 5.....	26
Table 6.....	26
Table 7.....	27
Table 8.....	27
Table 9.....	40
Table 10.....	43

List of Figures

Figure 1 A – D.....	28
Figure 2 A – D.....	29
Figure 3 A – D.....	30
Figure 4 A – D.....	31
Figure 5 1A – 1D, 2A – 2D.....	32
Figure 6 A – D.....	33
Figure 7 A – D.....	34
Figure 8 1A – 1D, 2A – 2D.....	35
Figure 9 A and B.....	36
Figure 10 A and B.....	36
Figure 11 1A – 1B, 2A – 2B.....	37
Figure 12 A and B.....	38
Figure 13 A and B.....	38
Figure 14 A and B.....	39
Figure 15A – D.....	40
Figure 16A – D.....	43

Abstract

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The vulture guild is comprised of two distinct groups, Old and New World, that provide a unique insight into how morphology varies among convergent species. All vultures are considered to be large birds of prey that utilize a style of flight called thermal soaring to search and feed primarily on already dead animal flesh. Even though this flight style is exhibited among all 23 species, slight variations in their skeletal morphology may relate to their differences in ecology. I hypothesized that vulture humeral morphology varies in relation to these organisms' habitat, average body mass, courtship displays, and migration capabilities.

To address this hypothesis, I used three-dimensional geometric morphometrics to measure the overall shape differences of vulture humeri. Computer models of each bone were created to observe the crucial skeletal features that relate to muscular functions and were analyzed using discriminant function analyses. Humeral morphology was found to vary most by habitat preference and body mass. Vultures that inhabit forested areas have humeri that exhibit features that suggest increased flapping flight compared to those in open and mountainous regions. The results for the heaviest species allude to these birds enhancing their wingbeats in ways other than humeral morphology

Introduction

The forelimbs of flying organisms are the leading factors for aerial locomotor ecology (Norberg 1981) and research should combine a variety of tools, methods, and strategies to fully understand this biological relationship. Ecomorphology is the study that bridges the gap between an organism's ecological role and its morphology. Birds are an excellent class of organisms to observe this connection due to the diversity of ecological adaptations within the group (James 1982). Past studies exploring the ecomorphology of birds have included topics relating to foraging (Hertel 1994, 1995, Sustaita and Rubega 2014), locomotion (Dial 2003, Kaboli et al. 2007, Corbin et al. 2013, Degrange 2017), habitat (Gamauf et. al 1998, Kaboli et al. 2007), and migration (Marchetti et al. 1995, Calmaestra and Moreno 2000, Voelker 2001, Provinciato et al. 2018).

Ecomorphological understanding is crucial in two facets of biological science: convergent evolution and paleobiology (Hertel 1995, Plummer et al. 2008, Degrange 2017, Serrano et al. 2017). Old and New World vultures (Accipitridae and Cathartidae, respectively) provide a strong example of the convergence of form and function between two taxonomically distinct groups of organisms. Phylogenetically, Old World vultures are related to other raptors, such as hawks and eagles whereas the New World Vultures have historically been placed with the ciconiid storks (Ligon 1967, Sibley and Ahlquist 1990). Recent DNA analyses have shown that New World vultures are closely related to birds of prey (raptors), excluding owls and those in Falconidae (Jarvis et al. 2014). Even though all species are members of the scavenger guild, ecomorphological studies have shown intra and interspecific differences among these two families by observing feeding and locomotor behaviors in relation to various cranial structures (Hertel 1995), myology of the wings and hindlimbs (Hertel et al. 2015), and behavior (Houston 1985, Hertel 1995).

Vultures, as a guild, share a similar flying technique known as thermal soaring in which the flying organism uses convection currents to remain aloft. Even though this ability is shared among these birds, previous studies have shown how the wing morphology of raptors relates to various flight styles, habitats, and migration patterns (Gamauf et. al 1998, Sievwright and Macleod 2012). Wing elements such as size, shape, and feathers have been shown to relate to species' ecology and behavior (Brewer and Hertel 2007, Nudds et al. 2007, Wang et al. 2011,

Hieronymus 2015, Wang and Clarke 2015, Sausner et al. 2016, Serrano et al. 2017, van Oordt et al. 2018), but relatively less work has been performed on the bones of the forelimb. The primary muscles responsible for avian flight are the *M. pectoralis* muscles that power the downstroke and the *M. supracoracoideus* that elevates the wing during the upstroke. The *M. pectoralis* of vultures is divided into a superficial and deep section with the latter containing slow muscle fibers that are associated with soaring and gliding (Meyers and McFarland 2017). These muscles originate from the coracoid, scapula, and sternum and insert on various skeletal features of the humerus. The combination of key muscle attachments and skeletal articulation points makes the avian humerus a logical target for an ecomorphological study of understanding flight in soaring birds.

Differences among vulture behavior and ecology translate to variability in flight characteristics. These include differences in habitat preference, general body mass, courtship performances, and migration abilities. The habitat a species occupies is a critical aspect of an organism's ecology and relates to flight capabilities especially in terms of foraging and maneuverability (Marchetti et al. 1995, Gamauf et al. 1998, Sievwright and Macleod 2012, Corbin et al. 2013). Species such as the Griffon Vulture (*Gyps fulvus*) and many other Old World vultures inhabit open spaces such as the savannahs of Africa whereas others like the New World King Vulture (*Sarcoramphus papa*), occupy the forested Amazonian Basin (del Hoyo et al. 1994). These terrains provide certain challenges that may be overcome through different soaring and flying techniques such as adjustments in the ratio between flapping and soaring flight.

Body mass has been shown to be a predictor of flight performance (Hertel and Ballance 1999, Dial 2003, Tobalske et al. 2003, Corvidae et al. 2006, Sievwright and Macleod 2012) because of its relationship to mechanical power output. Vulture body masses range from the small Lesser Yellow-headed Vulture (*Cathartes burrovianus*), with a mean body mass of 1.25 kg, to the massive 12 kg Andean Condor (*Vultur gryphus*), one of the heaviest flying avian species (del Hoyo et al. 1994).

As a group, raptors are known to perform elaborate aerial courtship maneuvers in which both mating pairs participate in clutching of talons, aerial flips, and high speed dives (del Hoyo et al. 1994). Only a few species of vultures are known to perform these mating displays and that ability may relate to wing morphology. Wing morphology varies between migratory and sedentary species because migration is an energetically costly behavior and a strong selective

pressure for various flight elements (Marchetti et al. 1995, Calmaestra and Moreno 2000, Voelker 2001, Kaboli et al. 2007, Provinciato et al. 2018). Even though the home ranges of many vultures are generally large, only a handful of species actually migrate, which may be reflected in their morphology.

Geometric morphometrics (GM) is a technique that uses morphological landmarks to describe biological shape and shape variation among species. Previous studies utilized traditional morphometrics to procure measurements and data to understand ecomorphological relationships. These classic methods proved to be adequate when describing basic avian features such as wingspan, body mass, or bone lengths but more precise techniques, such as GM, are crucial in comparing the subtle differences in the entire wing form. Geometric morphometrics has been shown to provide greater insight into the relationship between flight performance and various morphological features such as wing shape (Tobalske et al. 2003, Wang and Clarke 2015, Provinciato et al. 2018) and skeletal elements (Hui 2002, Sievwright and Macleod 2012, Corbin et al. 2013).

In this study, I used three-dimensional geometric morphometrics to perform a qualitative examination of the vulture humerus as it relates to ecological factors pertaining to flight. I predicted that vulture humeri will exhibit osteological variation that relates to avian flight in the areas of habitat preference, body mass, courtship performance, and migratory abilities. This exploration will provide a framework for understanding how skeletal features on the humerus differ among similar flight style in avian species.

Materials and Methods

Specimens

A total of 97 right wing humeri from 23 species of Cathartidae and Accipitridae were utilized from various museum collections across the United States (Table 1). Specimens studied were housed at: the American Museum of Natural History, New York (AMNH); the Natural History Museum of Los Angeles County (LACM); the Smithsonian National Museum of Natural History, Washington DC (USNM); the University of California, Berkeley, Museum of Vertebrate Zoology (UCMVZ); University of Kansas Museum of Natural History (KUNHM); Louisiana State University Museum of Natural Science (LSUMZ); and the University of California, Los Angeles, Bird and Mammal collection (UCLA).

To examine ecomorphological correlations, I assigned each species to various ecological categories based on the literature. Information was gathered from: *Handbook to the Birds of the World Volume 2* (del Hoyo et al. 1994), *Eagles, Hawks, and Falcons of the World* (Brown and Amadon 1968), *The Vultures of Africa* (Mundy et al. 1992), and *Birds of Prey of the World* (Grossman and Hamlet 1964). The categories include habitat preference (open, forested, or mountain), species weight class (light, medium, or heavy), aerial courtship abilities (perform or no performance), and migration capabilities (migratory or sedentary). Assignments for each species are outlined in Table 1.

Imaging and 3D model creation

Capturing images of each humerus was done using a Nikon D3200 with an 18 – 55mm lens. Each bone was held in place, using clay, in the center of a round swivel turntable with the proximal end facing up. Turning the swivel, photos were taken every 10° until the whole bone was captured. This was done at three various angled heights: 0° (straight on), 30°, and 60°. The process was repeated with the distal end facing up. In total, each bone had a set of 216 photos, 108 for the proximal and distal ends, respectively. 3D-surface models were created using the AgiSoft PhotoScan Professional software from the photos for each specimen.

Geometric Morphometrics

Landmark-based geometric morphometrics has been shown to be an effective method to observe three-dimensional morphological differences (Zelditch et al. 2012). A total of 32 landmarks (Table 2) were chosen based on important avian muscular and osteological features (Howard 1929, George and Berger 1966, Gilbert et al. 1985, Baumel and Witmer 1993). Completed surface models for each humerus were imported into the Landmark Editor Version 3.0 software (Wiley 2006) for landmark application. The raw landmark coordinates were imported into the MorphoJ Version 1.06d software (Klingenberg 2011) that performs a full Procrustes fit, which ordinales the landmarks in both space and size creating Procrustes coordinates. Each landmark produces three different Procrustes coordinates corresponding to the x, y, and z planes.

Multivariate Analyses

A canonical variates analysis (CVA) is an ordination method used to determine which variables discriminate between two or more assigned groups (Zelditch et al. 2012). This technique was performed in MorphoJ for each of the ecological categories along with discriminant function analyses (DFA) that examine group assignments. Both techniques were applied due to how MorphoJ accepts groups for an analysis. The CVA allowed for inspection of all pairings whereas the DFA only considered two groups at a time and provided cross-validated correct classification rates of cases to their respective groupings.

Phylogenetic Consideration

To test the effects of phylogeny, MorphoJ performs permutation tests to determine if a phylogenetic signal, the degree to which morphology can be ascribed to phylogenetic relations, is present within the data (Klingenberg and Gidaszewski 2010). Following the methods of Figueirido et al. (2016), a phylogenetic MANOVA was performed on the canonical variates of each category using the Geiger library (Harmon et al. 2008) in R (R Development Core Team).

Quantifying Effects of Allometry

Avian forelimb morphology has been shown to relate in some way to organism size (Kilbourne 2013). Wing mass, center of mass, and total length scale with positive allometry relative to body mass (Kirkpatrick 1990, Van den Berg and Rayner 1995). The effects of allometry on humerus shape were tested for each category by regression analysis of shape (canonical variates of independent contrasts) on size (average body mass and log centroid Size). Phylogenetically independent contrasts were used to control for phylogeny (Martín-Serra et al. 2014).

Results

A phylogenetic signal was revealed in the landmark dataset ($P < 0.0001$) but the groups for each category were still found to be significantly different using the PhyMANOVA ($P < 0.0001$).

CVA Results

The CVA on habitat preference provided two eigenvectors that explain 100% (89.43% for CV1 and 10.57% for CV2) of total variation among species. Permutation tests on both Procrustes (PD) and Mahalanobis (MD) distances showed significant difference between each of the habitat pairs (Table 3). The plot for CV1 vs. CV2 (Fig. 1A) showed differentiation among the three habitat groups: forested, mountainous, and open. All groups were separated along the CV1 axis. Mountainous species fell on the negative side, the open group showed intermediate values closer to 0, and forested species are found on the positive end. CV2 only separated open from mountainous and forested which grouped around a similar negative value.

The CVA performed on the various weight classes provided two eigenvectors that explained 100% (83.97% for CV1 and 16.03% for CV2) of total variance. Permutation tests on PD and MD showed significant differences among the weight class pairs (Table 4). The plot for CV1 vs. CV2 (Fig. 1B) showed variation in the weight classes: light, medium, and heavy. CV1 separated each group with medium falling on the negative end, light at the positive end, and heavy in between. The CV2 axis separated the light and medium classes from the heavy class.

The CVAs performed on both courtship abilities and migratory behavior provided one eigenvector each that explains 100% of total variation for each category. These permutation tests also showed significant differences (Table 5 and 6). The plot for the CV1 axes (Fig. 1C and 1D) separated the respective categories. For aerial courtship abilities, those species that perform fell on the negative side whereas those that do not, group in the positive (Fig. 1C). Migratory behavior was divided with sedentary species falling on the negative side of the axis and migratory species on the positive (Fig. 1D).

DFA Results

The accuracies of the discriminant function analyses were assessed by observing the classification of vulture species into their respective groups within each ecological category. The DFA classified all species correctly but leave-one-out cross-validations showed that the post-hoc probabilities did decrease for each category (Table 7).

Shape Changes

Relevant humerus skeletal features are outlined in Figure 2. Shape differences can be described from the CVA by plotting the shape transformations between the various groups (Fig. 3 – 14). The DFA provided shape change plots between group pairings within ecological categories that best separate morphological features.

Habitat

The shape transformation plots that describe the morphological differences among vulture species that inhabit forested, mountainous, and open areas are presented in Figures 3 – 5. The deltopectoral crest of mountain dwelling species are relatively longer and more curved than the other groups (Fig. 3B – C and 4B – C). This is in contrast to forested individuals that exhibit a relatively short and flat crest (Fig. 3C – D and 4C – D). The bicipital crests of forested and mountainous species (Fig. 3C) are similar but this feature is relatively less robust and more curved among those found in open habitats (Fig. 3B, D). Forested species display a comparatively wide and robust head that projects laterally toward the deltopectoral crest (Fig. 3C, D and 5-1C, D). These species also exhibit relatively pronounced external tuberosities whereas those of open areas were reduced and displaced medially toward the head (Fig. 3C, D and 5-1C, D). Both the proximal and distal ends of the forested humeri are relatively wider (medio-laterally) than the other two groups due to various features (Fig. 3C, D). On the distal end, the external condyle is more pronounced in the forested group (Fig. 5-2C, D).

Weight Class

The shape transformation plots that describe the morphological differences among light, medium, and heavy vulture species are presented in Figures 6 – 8. Species that are classified as light-weight have a relatively shorter deltopectoral crest with a prominent peak that is

transitioned distally (Fig. 6, 7, and 8-1C, D). This crest for mid-weight vultures is comparatively wider, laterally, with a more distal terminus thus making the feature relatively longer than the other two groups (Fig. 6B, C and 8-1B, C). The heaviest species have a deltopectoral peak that is most proximal, displaying a relatively short distance from the head (Fig. 8B, D). For medium and heavy species, the bicipital crests are similar, but those in the light-weight class have a relatively less robust crest (Fig. 6B, C, D). This crest is also comparatively flatter, and positioned more posteriorly, in the light-weight species (Fig. 8-1C, D). The humeral heads of medium and heavy species are less robust than those of lighter species and also angled toward the deltopectoral crest (Fig. 8-1B, C, D). The external tuberosities of each group are shown to have different morphological characteristics. This feature is relatively more pronounced in lighter species, diminutive in mid-weight species, and intermediate among heavier individuals (Fig. 8-1B, C, D). Internal tuberosities for light-weight species are angled relatively more proximally and the other two groups are similar (Fig. 7B, C, D). The external condyles of mid-weight vultures are comparatively the smallest of the three (Fig. 8-2B, C). Those in the heavy-weight class possess more robust internal condyles (Fig. 8-2B, D). The olecranon fossa of this category is also relatively the deepest (Fig. 8-2B, D).

Courtship

The shape transformation plots that describe the morphological differences between species that perform aerial courtship and those that do not are presented in Figures 9 – 11. The proximal end for performing species is relatively wider compared to the other group, but this is due to the exaggerated width and size of the humeral head (Fig. 9B and 11-1B). The shapes of both the deltopectoral and bicipital crests are similar except for the aforementioned width and the prominence of the deltopectoral peak (Fig. 9, 10, and 11-1B). Performers also possess a comparatively more robust external condyle (Fig. 11-2B).

Migration

The shape transformation plots that describe the morphological differences between migratory and sedentary species are presented in Figures 12 – 14. The peak of the deltopectoral crests of migratory species is flared laterally, providing a relatively wider shape compared to sedentary individuals (Fig. 12B and 14-1B). The external tuberosity for species that partake in migration appears to be relatively shorter, but this is caused by the shift in angle of the humeral

head toward the deltopectoral crest (Fig. 14-1B). The remaining morphological features for these categories are comparatively similar.

Effects of Phylogeny and Allometry

A phylogenetic signal was revealed in the landmark dataset ($P < 0.0001$) but the groups for each category were found to be significantly different using the PhyMANOVA ($P < 0.0001$). Regression analyses of shape on body mass were significant for both habitat and weight when looking at shapes along the first canonical axes ($P = 0.031$, $R^2 = 0.18$, and $P < 0.0001$, $R^2 = 0.73$, respectively; Appendix B). All analyses of shape on log centroid size were found to be non-significant (Appendix B).

Discussion

Overview

Results suggest that there are differences among vulture humeral morphologies related to functional variability of ecological factors. Skeletal shape differences were described for vultures in each category, but the factors of habitat and weight were better at discriminating group association compared to courtship or migration when looking at their higher percentage of correct, leave-one-out cross-validated, classifications (Table 7). This outcome was possibly due to the lack of courtship displaying species and that many vultures have incredibly large home ranges without actively partaking in migration.

The results from the regression analyses suggest that the shape differences among species in different habitats may be slightly attributed to the effects of allometry. About 18% of the variance in shape found in this category can be explained by the increasing body mass of the organisms in this study. Important avian linear morphological measurements, such as wing length, have been shown to scale with positive allometry relating to inertial properties required to fly (Van den Berg and Rayner 1995). Results for the regression in the weight category, in which body mass explained 73% of the variance in shape, coincide with previous notions of scaling that larger birds require different skeletal morphology to resist the demands for flight as body mass increases (see Weight discussion).

The humeral features that displayed the most variation were the deltopectoral crest, bicipital crest, humeral head, external and internal tuberosities, external and internal condyles, olecranon fossa, supracondylaris dorsale, and entepicondyle (Fig. 2). Previous studies have provided evidence for how bone shape and structure relate to muscle anatomy in a variety of organisms (Hui 2002, Sievwright and Macleod 2012, Martín-Serra et al. 2014, MacLaren and Nauwelaerts 2016, Mayr 2017, Dickson and Pierce 2019, Martin et al. 2019). These musculoskeletal correlations may not just indicate the size and shape of muscles, but also how the levers of the avian wing differ in mechanical advantages (Hildebrand and Goslow 2001). The variations found in this study relate to musculoskeletal anatomies that suit a vulture's ecology by modifying the wing's biomechanical properties. Relevant functions of these muscles and features are detailed in Table 8.

Habitat

Humeral shape was shown to differ the most among the three habitat categories, the two extremes being forest and mountain as noted along CV1 of Figure 1A. The deltopectoral crest provides the insertion surface for both the *M. pectoralis* and the *M. deltoideus major*. Both New and Old World vultures are known to possess similar flight muscle plans in terms of cross-sectional area which is a valid indicator of muscle force-generating capacity (Hertel et al. 2015). The relatively short crest of forested species implies that these muscles have their insertions closer to their origins, shortening the overall length. This suggests that forest species have wings that operate with increased velocity, therefore possibly flapping more compared to mountainous and open inhabitants. Another key feature driving shape difference was the external tuberosity, the point of insertion for the *M. supracoracoideus* tendon. This tuberosity was relatively longer and more exaggerated in forested individuals creating a more distal attachment, which increases the mechanical advantage for the upstroke muscle. Articulation points for both the proximal site (humeral head) and one of the distal sites (external condyle) were relatively more pronounced and rounded among forested species, creating a potentially greater range of motion for the wing at the glenohumeral joint and radius, respectively. The last feature separating this group from the others is the relatively wider distal end of the humerus caused by the protrusion of the supracondylaris dorsale (dorsal) and the entepicondyle (ventral). Muscles of the antebrachium, *M. extensor carpi radialis* and *M. pronator profundus*, respectively, originate from these tubercles and provide the wing with more refined movements. Relative widening of the distal humerus attachment points potentially create more distal insertions for muscles, thereby potentially increasing mechanical advantage.

Mountain and open groups had relatively similar humeral shapes except for some proximal features. The deltopectoral crests were comparatively the same length which may enable the wings of these groups to act with more mechanical advantage, more forceful wing beats, in contrast to the forest group. The main difference with open species is the shape of the bicapital crest that segregates this group along the CV2 axis in Figure 1A. This feature is relatively the least robust of the three, shortening the distance between the muscle attachments and the glenohumeral joint, potentially increasing the velocity advantage for the recovery stroke.

Both the external and internal tuberosities are displaced medially toward the humeral head, which might also contribute to the velocity advantage of the open habitat wings.

Examining these features simultaneously, I can conclude that there is some skeletal variation among vultures occupying different habitats. Variation in wing morphology has been shown to have a strong correlation with foraging style for a variety of avian species (Marchetti et al. 1995, Corvidae et al. 2006, Sievwright and Macleod 2012, Corbin et al. 2013), whereas foraging behavior has been associated with habitat (Gamauf et al. 1998). Because all vultures have a very similar foraging style (Hertel et al. 2015), using habitat to infer the subtle differences in wing morphology is an appropriate substitute. The species found in mountain and open habitats have the characteristically long deltopectoral crest of soaring birds (Tobalske et al. 2011) that provide forceful downstrokes and upstrokes due to the *M. pectoralis* and *M. deltoideus* major. Due to the accessible thermals and updrafts, vultures inhabiting these areas only require small adjustments in wing orientation to stay aloft. The bicipital crest, external tuberosity, and internal tuberosity are the features that might enable open species' wings to act at a velocity advantage compared to the other categories. The relevant muscles of the bicipital crest and external tuberosity, including the vital *M. supracoracoideus*, are responsible for upstroke movements including flexing, stabilizing, and retracting the wing, which affects overall maneuverability during various modes of flight (Dial 1992a, Warrick and Dial 1998, Corvidae et al. 2006). Along with the muscles that insert on the internal tuberosity that assist the *M. pectoralis* on the downstroke, the short moment arm of the wings might affect the speed at which these species achieve takeoff, which is critical in environments that expose individuals to predation such as accessible grasslands or savannas.

The dense foliage coverage of woodland and forested habitats provides unique challenges to static soaring birds such as navigation and locating prey items. In this way, forest vultures were hypothesized to possess wing morphologies similar to species that partake in more flapping flight. This flight style is represented in humeral structure with shorter deltopectoral crests (Serrano and Chiappe 2017) and increased area for muscle attachment sites that equate to relatively larger muscles (Dial et al. 1991, Corvidae et al. 2006). Our results for forest vultures comply with the former description of flapping flight indicating the muscles of the deltopectoral crest work at a velocity advantage, but other features were found to have the opposite effect. The

external tuberosity, supracondylaris dorsale, entepicondyle, ectepicondyle, and flexorius process might increase the mechanical advantage for the wing at different stages of the wingbeat. The *M. supracoracoideus* is considered the primary upstroke muscle in birds but previous studies have concluded that it may be recruited at different points during the wingbeat cycle such as takeoff (Corvidae et al. 2006, Bribiesca-Contreras et al. 2019), low-speed soaring (Warrick and Dial 1998), or at the transition between the downstroke and upstroke (Dial et al. 1991, Dial 1992a, Dial 1992b, Tobalske et al. 2011). Because forest species flap at a higher frequency, I suggest that this muscle acts more to decelerate the wing at the termination of the downstroke. These species may counteract the high inertia of quick downstrokes by utilizing more forceful upstrokes accomplished by the *M. supracoracoideus*. This action may be facilitated by the *M. flexor* and *extensor carpi ulnaris* that both flex the wrist as the wing is beginning its accession (Dial 1992a, Vazquez 1994). The *M. extensor carpi radialis* is also active during the transition period but at the upstroke – downstroke conversion, the opposite time compared to the previous muscles (Dial 1992a). This muscle, along with the *M. pronator profundus*, are parts of the wing responsible for altering its shape and position (Biewener 2011) and are subjected to the greatest aerodynamic forces during flapping flight (Dial 1992a). The relatively higher mechanical advantage for these muscles may allow forest species to compensate for the quick change of wing shape and position during flight.

Weight

Among the four ecological categories, weight was only slightly worse at discriminating group association compared to habitat (Table 7). Medium and light species were found to have the greatest difference in humeral shape along CV1 (Fig. 1B). One would suspect that heavy and light species would be the two extremes, but heavier vultures were closer to small vultures along CV1 and separated from the other groups along CV2 (Fig. 1B). Skeletal shape variation is known to be influenced by allometry (Bright et al. 2016), but our results suggest feeding ecology of birds might be constrained by body size (Bright et al. 2016); our results also suggest that humeral shape may not be related to allometric scaling.

Body mass is one of the largest constraints on a flying organism's aerial performance especially in energetically costly takeoff flight (Marden 1994, Dial 2003, Tobalske et al. 2011). In general, larger birds exert more energy achieving vertical takeoff and other flight modes

because the power from muscles to attain this form of locomotion increases slower than what is required to fly (Tobalske et al. 2011). I hypothesized that the heavy group would have the most force behind the downstroke that would be represented by the deltopectoral crest. There are many ways heavier birds alleviate this issue such as altered muscle physiology and wing shape and size. The results in this category indicate that the deltopectoral crest for heavy vulture species causes the wings to work at a greater mechanical disadvantage than those with a medium weight. When compared to the light-weight group, heavy vultures still had a lower mechanical advantage due to the peak of the deltopectoral crest positioned relatively closer to the humeral head. This result leads us to believe that heavy vultures enhance the downstroke in other ways besides the shape of the deltopectoral crest. These species might have larger cross-sectional area for the pectoralis relating to potential force production (Hertel et al. 2015), overall lower wing loading and aspect ratio for more lift surface area, different feather proportions (Norberg 1979), or different recruitment of downstroke muscle fibers (Rosser and George 1986, Corvidae et al. 2006, Peters and Dobbins 2012).

Heavy vultures were also found to have less potential range of motion at various articulation sites. The shape of the humeral head and internal condyle were found to be the most restrictive of the three weight classes. However, the olecranon fossa was relatively the deepest in comparison. The range of motion for limb joints decreases with increasing size (Biewener 1991) and the combination of these features may allow large vultures to resist twisting of the humerus during flight, increasing the critical torsional strength of the bone (Biewener and Dial 1995). This is also seen in the medium weight species but to a lesser extent.

Vultures considered medium weight were found to have the relatively greatest mechanical advantage on the downstroke due to the shape of the deltopectoral crest (Serrano and Chiappe 2017). This feature, similar to the habitat results, is what drives the position of the groups on the CV1 axis in Figure 1B. The deltopectoral crest is an insertion site for the *M. deltoideus major* that assists the *M. supracoracoideus* in elevation of the wing near the downstroke – upstroke transition (Dial 1992a). Medium weight species' wings receive a velocity advantage on the upstroke because of the relatively less robust external tuberosity. Examining both upstroke muscles simultaneously reveals that these birds may have a more balanced flight cycle.

Features of light-weight vultures were found to be remarkably similar to those of forest species, given that two of three forest vultures are categorized as light-weight (Table 1, Fig. 3 – 7). Such similarities include a relative velocity advantage of the deltopectoral crest, mechanical advantage of the external tuberosity, and a relatively greater potential range of motion at the glenohumeral joint. However, light-weight species exhibited a relatively less robust bicipital crest indicating a wing that has more velocity advantage on both flight strokes. In general, smaller vultures would be able to flap their wings at a higher frequency and the similarities between light and forest species might also be a result of the low number of vultures found in this habitat type.

Courtship/Migration

The discriminant function analysis was able to correctly classify the species into the correct courtship and migration categories but after a leave-one-out cross-validation these groupings were less predictive (Table 7). In the shape change plots (Fig. 8 – 13), species that perform aerial courtship displays and those that migrate tend to have more robust proximal features. Both ecological classifications have been shown to be valid predictors of wing shape, size, and skeletal morphology in a variety of other avian species (Marchetti et al. 1995, Calmaestra and Moreno 2000, Voelker 2001, Sievwright and Macleod 2012, Provinciano et al. 2018). Only a handful of vulture species partake in aerial courtship and, being scavengers, usually have incredibly large home ranges in which they may search hundreds of miles for a chance to acquire a meal. For these reasons, courtship abilities or migration patterns may not be reliable predictors of humeral morphology in this avian group.

Conclusions

Vultures provide unique insight to how avian skeletal morphology relates to the locomotor form of flight. This group of birds is considered to have the same style of flight, thermal or static soaring, and are usually clustered together for ecomorphological studies. Even though phylogeny had a significant role in the separation of some features, that does not discredit the humerus as a functional trait through evolutionary history. I presented a novel approach of applying 3D geometric morphometrics to analyze two convergent families of birds, which allowed us to examine slight differences in their generally similar skeletal morphology. Most previous morphological studies implemented linear measurements to understand wing shape and function. Traditional morphometrics fail to capture the complete variation in shape, which is easily summarized using the various methods of visualization provided by geometric morphometrics. Using the methods outlined here may provide a more detailed understanding of the subtle shape differences that affect an organism's ecology.

Our research provides a general framework for future introductory 3D geometric morphometric studies on the avian humerus. The morphometric results presented here can be improved with more precise imaging tools, advancements in software, or larger datasets that include other species or structures. Continuing to develop ecomorphological techniques will increase our understanding of the linkage between morphology and ecology on both extinct and extant organisms.

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Appendix A

Table 1. General information for vulture species including ecological groupings.

Species	Family	Habitat	Weight Class	Courtship	Migration	N
<i>Aegypius monachus</i> (Cinereous Vulture)	Accipitridae	Mountain	Heavy	No	Migratory	5
<i>Gypaetus barbatus</i> (Bearded Vulture)	Accipitridae	Mountain	Medium	Yes	Sedentary	8
<i>Gyophierax angolensis</i> (Palm-nut Vulture)	Accipitridae	Open	Light	Yes	Sedentary	5
<i>Gyps africanus</i> (White-backed Vulture)	Accipitridae	Open	Medium	No	Migratory	8
<i>G. bengalensis</i> (White-rumped Vulture)	Accipitridae	Open	Medium	No	Sedentary	1
<i>G. coprotheres</i> (Cape Vulture)	Accipitridae	Mountain	Heavy	No	Migratory	1
<i>G. fulvus</i> (Griffon Vulture)	Accipitridae	Open	Heavy	No	Migratory	3
<i>G. indicus</i> (Indian Vulture)	Accipitridae	Open	Medium	No	Sedentary	1
<i>G. rueppelli</i> (Rüppell's Vulture)	Accipitridae	Open	Heavy	No	Sedentary	2
<i>Necrosyrtes monachus</i> (Hooded Vulture)	Accipitridae	Open	Light	No	Sedentary	6
<i>Neophron percnopterus</i> (Egyptian Vulture)	Accipitridae	Mountain	Light	No	Migratory	5
<i>Sarcogyps calvus</i> (Red-headed Vulture)	Accipitridae	Forest	Medium	Yes	Sedentary	3
<i>Trigonoceps occipitalis</i> (White-headed Vulture)	Accipitridae	Open	Light	No	Sedentary	3
<i>Torgos tracheliotos</i> (Lappet-faced Vulture)	Accipitridae	Open	Heavy	No	Sedentary	5
<i>Coragyps atratus</i> (Black Vulture)	Cathartidae	Open	Light	Yes	Sedentary	4
<i>Cathartes aura</i> (Turkey Vulture)	Cathartidae	Open	Light	No	Migratory	4
<i>C. burrovianus</i> (Lesser Yellow-headed Vulture)	Cathartidae	Open	Light	No	Sedentary	7
<i>C. melambrotus</i> (Greater Yellow-headed Vulture)	Cathartidae	Forest	Light	No	Sedentary	4
<i>Gymnogyps californianus</i> (California Condor)	Cathartidae	Mountain	Heavy	No	Sedentary	4
<i>Sarcoramphus papa</i> (King Vulture)	Cathartidae	Forest	Light	Yes	Sedentary	11
<i>Vultur gryphus</i>	Cathartidae	Mountain	Heavy	No	Sedentary	4

(Andean Condor)						
<i>Aquila chrysaetos</i> * (Golden Eagle)	Accipitridae	Open	Medium	Yes	Migratory	2
<i>Haliaeetus leucogaster</i> * (White-bellied Sea Eagle)	Accipitridae	Open	Light	Yes	Sedentary	1

* Species used as phylogenetic controls.

Table 2. Humerus landmarks

1. Anterior-most point of the humeral head
2. Superior-most point of the humeral head
3. Posterior-most point of the humeral head
4. Inferior-most point of the humeral head
5. Center of the humeral head
6. Apex of external tuberosity
7. Proximal origin of deltopectoral crest
8. Apex of deltopectoral crest
9. Distal terminus of deltopectoral crest
10. Inferior margin of deltopectoral crest
11. Posterior-most point of internal tuberosity
12. Proximal origin of bicipital crest
13. Apex of bicipital crest
14. Distal terminus of bicipital crest
15. Proximal margin of ligamental furrow
16. Distal margin of ligamental furrow
17. Apex supracondylaris dorsale
18. Proximal-most point of external condyle
19. Center of the external condyle
20. Superior-most point of external condyle
21. Apex of ectepicondyle prominence
22. Distal-most point of intercondylar groove
23. Superior-most point of internal condyle
24. Proximal-most point of internal condyle
25. Inferior-most point of internal condyle
26. Distal-most point of internal condyle
27. Center of internal condyle (between 23 and 25)
28. Apex of entepicondylar prominence
29. Superior point of olecranon fossa
30. Distal point of flexorius process
31. Distal point of ectepicondyle
32. Anterio-distal point of humeral head

Table 3. Results of the CVA performed from the Procrustes coordinates describing humeral shape to separate among forested, mountainous, and open habitat groups. Numbers in bold type indicate Procrustes distances (PDs) among pairs of groups and normal-type numbers indicate Mahalanobis distances (MDs).

	Forested	Mountainous
Mountainous	< 0.0001 < 0.0001	
Open	< 0.0001 < 0.0001	0.0002 < 0.0001

Table 4. Results of the CVA performed from the Procrustes coordinates describing humerus shape to separate among light, medium, and heavy groups. Numbers in bold indicate PDs among pairs of groups and normal-type numbers indicate MDs.

	Heavy	Light
Light	< 0.0001 < 0.0001	
Medium	0.0001 < 0.0001	< 0.0001 < 0.0001

Table 5. Results of the CVA performed from the Procrustes coordinates describing humerus shape to separate among the groups that perform or not perform aerial courtship. Numbers in bold indicate PDs among pairs of groups and normal-type numbers indicate MDs.

	Courtship
No Courtship	0.0005 < 0.0001

Table 6. Results of the CVA performed from the Procrustes coordinates describing humerus shape to separate among the groups that migrate or those that are sedentary. Numbers in bold indicate PDs among pairs of groups and normal-type numbers indicate MDs.

	Migrate
Sedentary	0.0003 < 0.0001

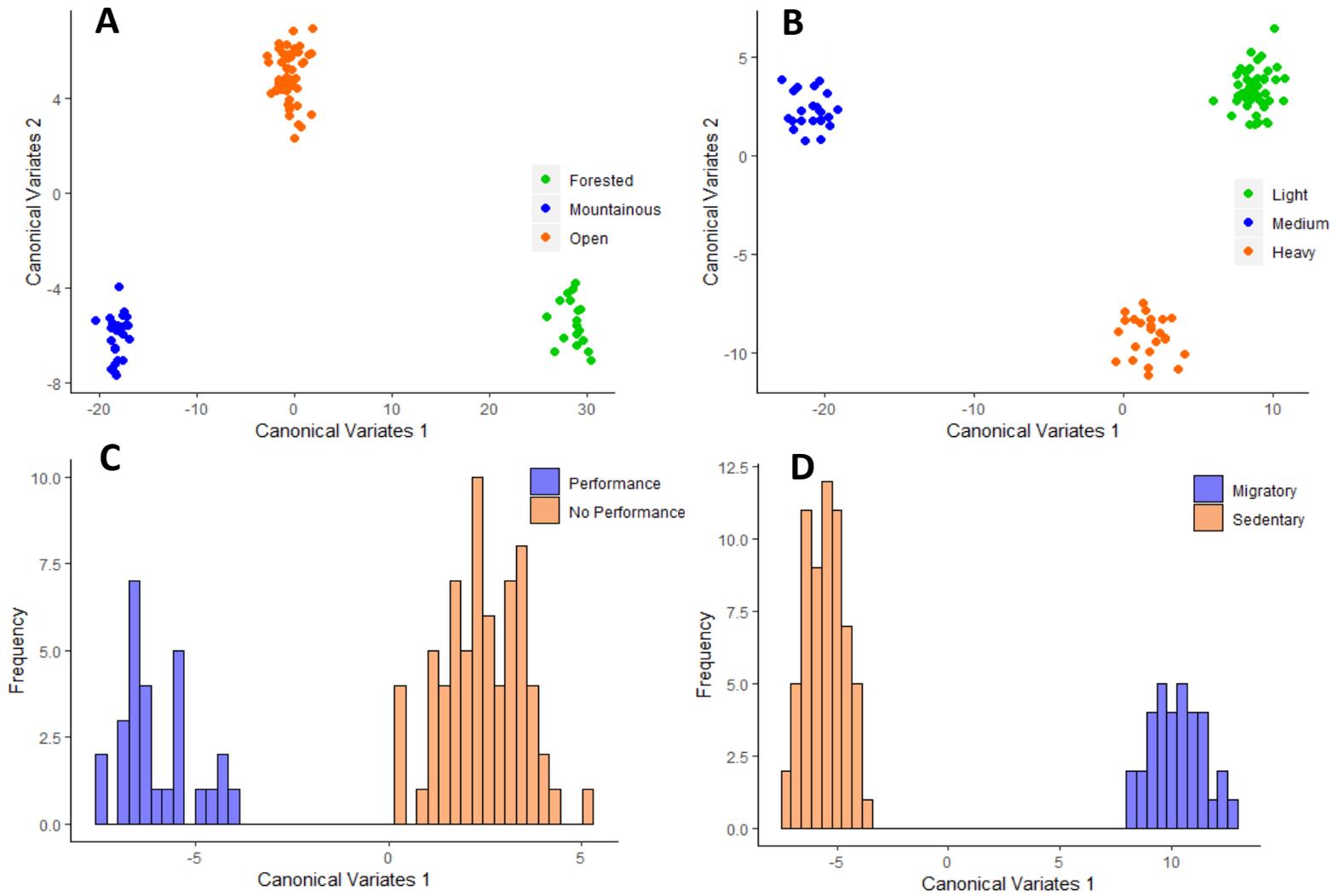
Table 7. Overall percentages of post-hoc correct classifications of species to their respective groupings from the discriminant functions (DF) and after leave-one-out cross-validation (LCV).

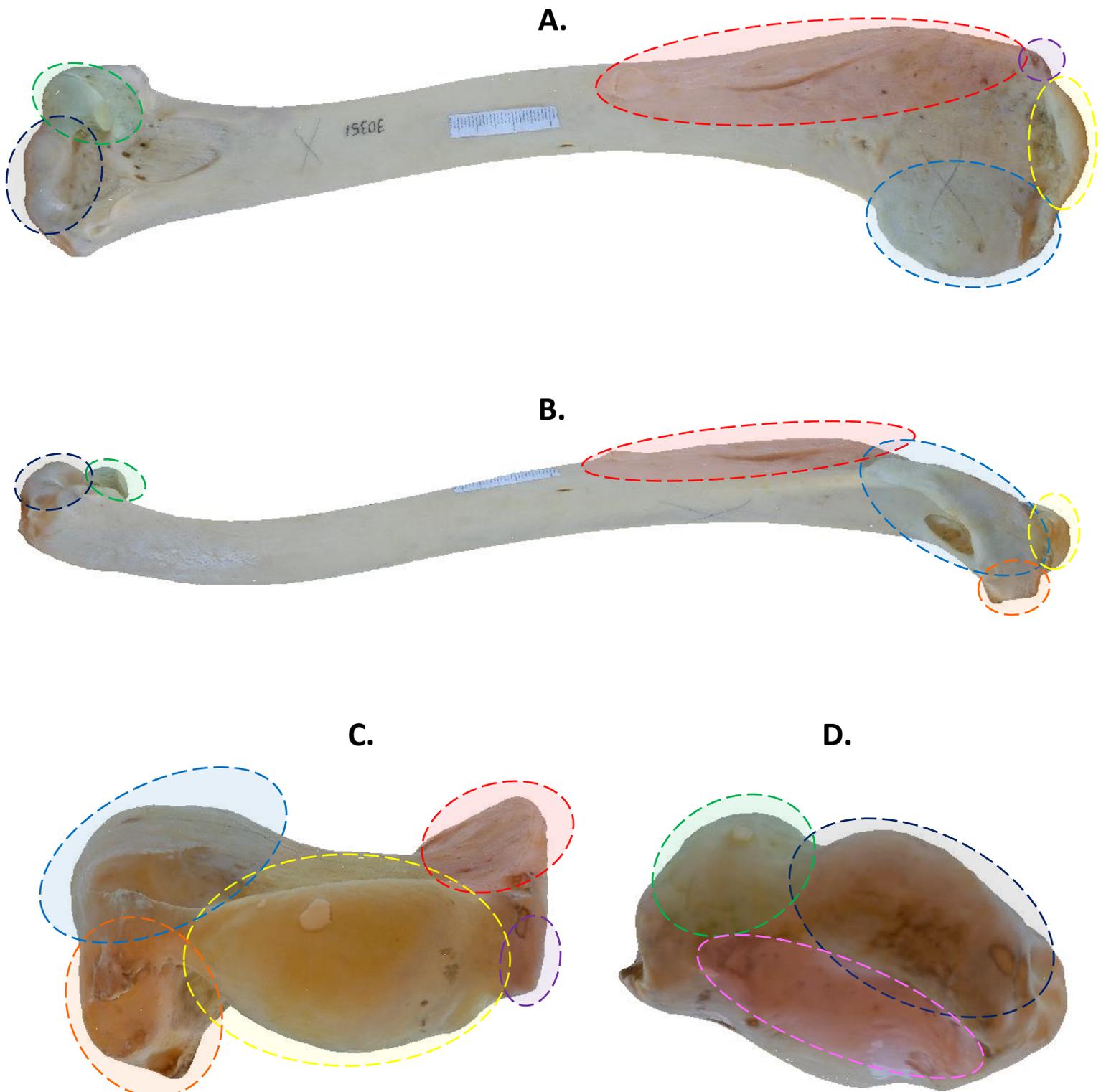
Analysis	Groups	Percentages (DF, LCV)
Habitat	Forested/Mountainous/Open	100, 82
Weight	Light/Medium/Heavy	100, 75
Aerial Courtship	Perform/No Performance	100, 60
Migration	Migratory/Sedentary	100, 57

Table 8. Humerus features and related muscles and functions.

Skeletal Feature	Associated Muscle(s)	Function
Deltopectoral Crest	<ol style="list-style-type: none"> 1. Insertion of M. pectoralis (anterior surface) 2. Insertion of M. deltoideus major (posterior surface) 	<ol style="list-style-type: none"> 1. Prime depressor of wing during downstroke 2. Assists M. supracoracoideus during upstroke
Bicipital Crest	<ol style="list-style-type: none"> 1. Insertion of Scapulohumeralis caudalis 2. Origin of M. biceps brachii 	<ol style="list-style-type: none"> 1. Rotates wing and retracts humerus during upstroke 2. Flexor of the antebrachium and stabilizes elbow during downstroke
Humeral Head	N/A	Articulation site with scapula and coracoid
External Tuberosity	Insertion of M. supracoracoideus	Prime elevator of wing during upstroke
Internal Tuberosity	<ol style="list-style-type: none"> 1. Insertion of M. coracobrachialis caudalis 2. Insertion of M. subcoracoideus 3. Insertion of M. subscapularis 	<ol style="list-style-type: none"> 1. Depressor of wing 2. Depressor of wing 3. Depressor of wing
External Condyle	N/A	Articulation site with radius
Internal Condyle	N/A	Articulation site with ulna fossa
Olecranon Fossa	N/A	Articulation site with olecranon of ulna
Supracondylaris Dorsale	Origin of extensor carpi radialis	Extension of manus
Entepicondyle	Origin of M. pronator profundus	Pronates antebrachium
Ectepicondyle	Origin of M. extensor carpi ulnaris	Flexion of manus
Flexorius Process	Origin of M. flexor carpi ulnaris	Flexion of manus

Figure 1. Canonical variates plots for humerus shape differences among vultures. Ordination of the species coded by habitat (A), weight class (B), migration (C), and courtship (D) groupings.





- Deltopectoral Crest
- Bicipital Crest
- Humeral Head
- External Tuberosity
- Internal Tuberosity
- External Condyle
- Internal Condyle
- Olecranon Fossa

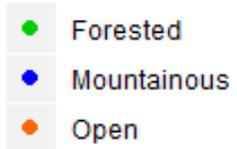
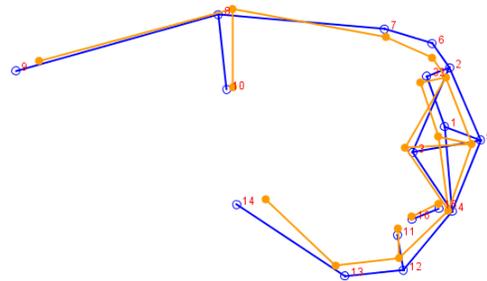
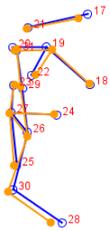
Figure 2. Various views of the right humerus of *Vultur gryphus*. A. Anterior; B. Dorsal; C. Proximal; D. Distal.

Figure 3. Shape changes between group centroids from the CVA on habitat preference. The superimposition views correspond to the orientation of the right humerus (A) in the x vs y dimension as shown. Comparisons shown correspond to the shape changes between: (B) mountainous (blue) and open (orange) inhabitants, (C) mountainous and forested (green), and (D) forested and open.

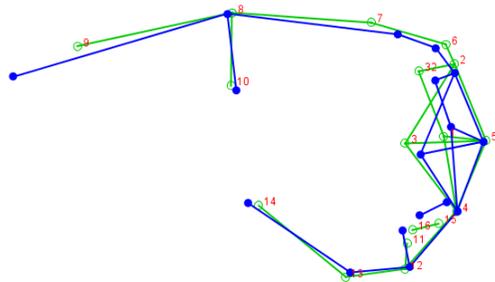
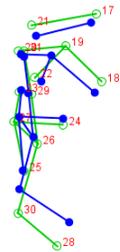
A



B



C



D

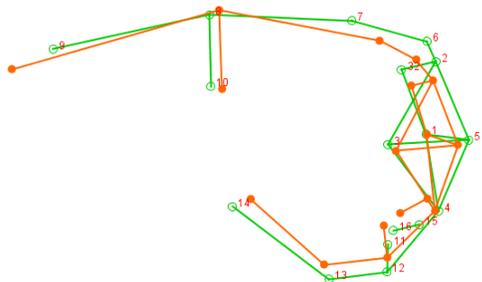
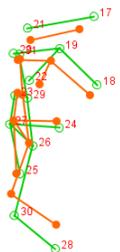
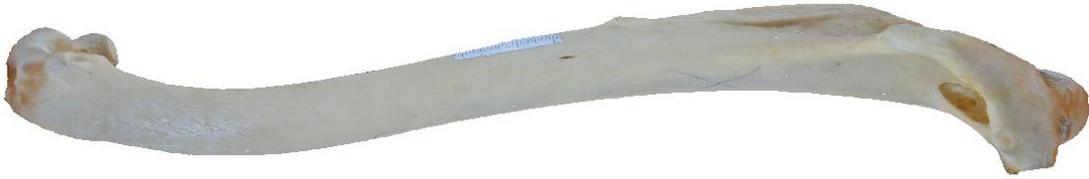


Figure 4. Shape changes between group centroids from the CVA on habitat preference. The superimposition views correspond to the orientation of the right humerus (A) in the x vs z dimension as shown. Comparisons shown correspond to the shape changes between: (B) mountainous (blue) and open (orange) inhabitants, (C) mountainous and forested (green), and (D) forested and open.

A



B



C



D

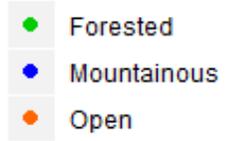


Figure 5. Shape changes between group centroids from the CVA on habitat preference. The superimposition views correspond to the orientation of the right humerus (A) in the y vs z dimension as shown. (1) The proximal view and (2) is the distal view. Comparisons shown correspond to the shape changes between: (B) mountainous (blue) and open (orange) inhabitants, (C) mountainous and forested (green), and (D) forested and open.

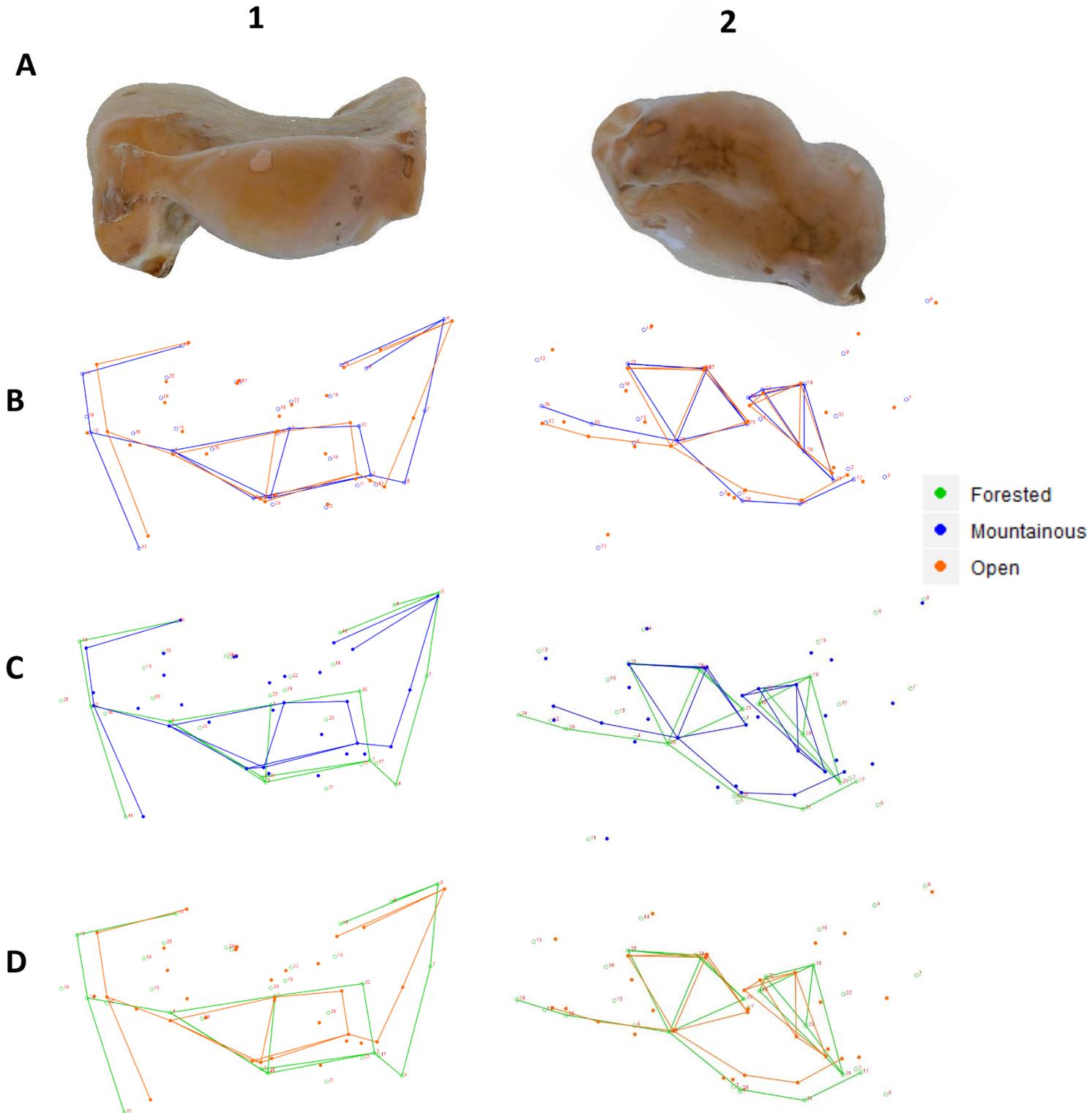


Figure 6. Shape changes between group centroids from the CVA on weight classes. The superimposition views correspond to the orientation of the right humerus (A) in the x vs y dimension as shown. Comparisons shown correspond to the shape changes between: (B) medium (blue) and heavy (orange) classes, (C) medium and light (green), and (D) light and heavy.

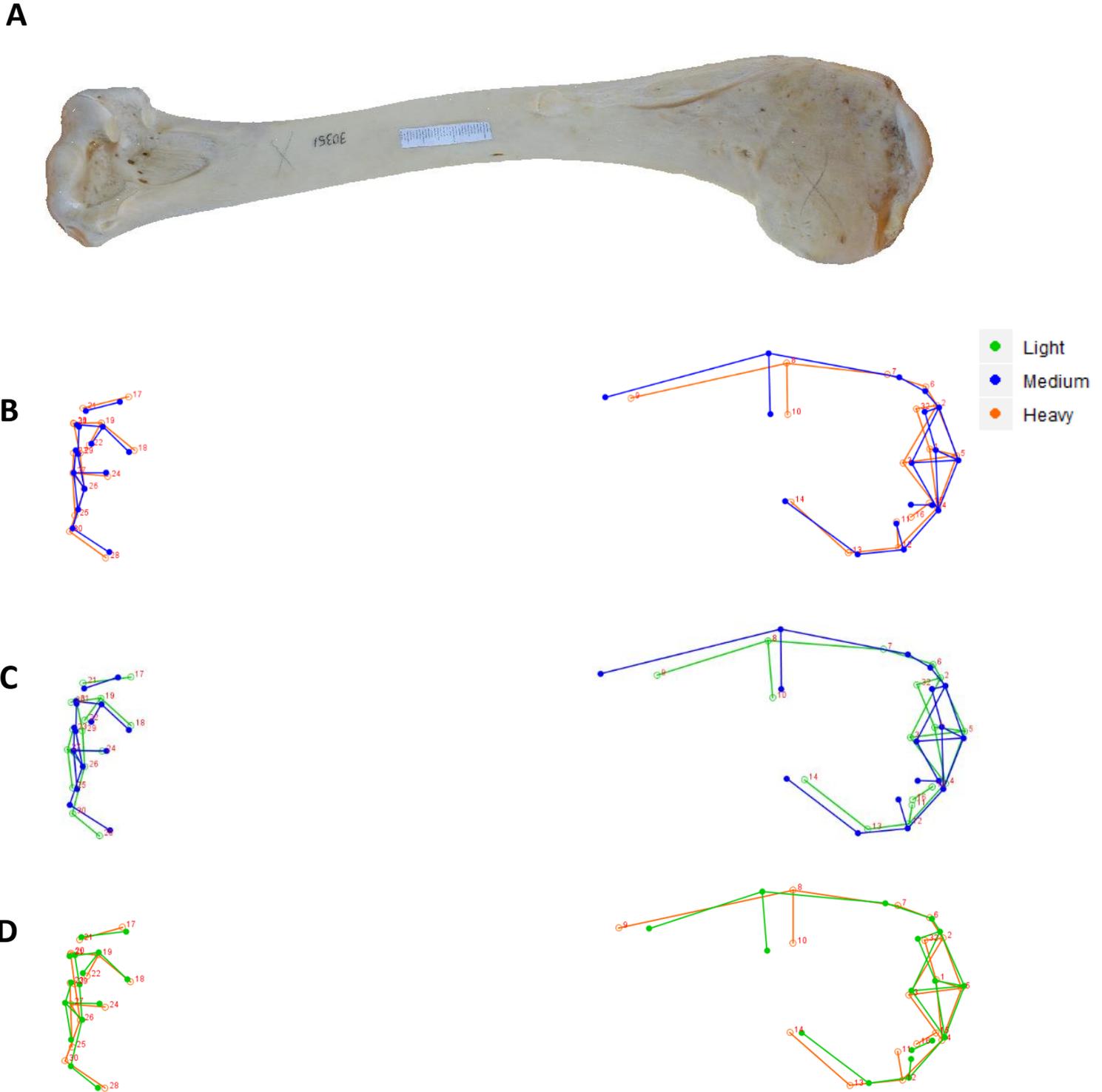


Figure 7. Shape changes between group centroids from the CVA on weight classes. The superimposition views correspond to the orientation of the right humerus (A) in the x vs z dimension as shown. Comparisons shown correspond to the shape changes between: (B) medium (blue) and heavy (orange) classes, (C) medium and light (green), and (D) light and heavy.

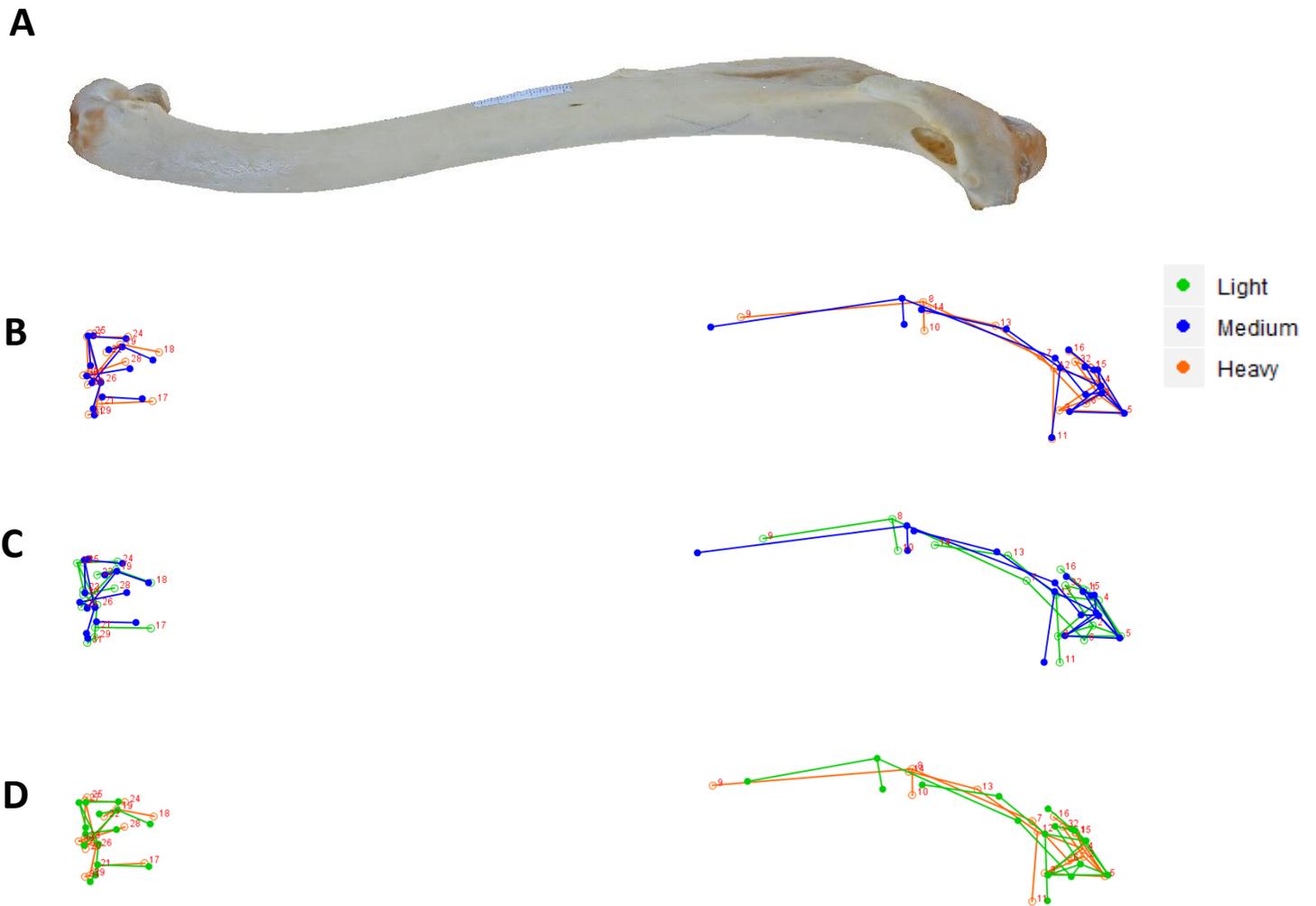


Figure 8. Shape changes between group centroids from the CVA on weight classes. The superimposition views correspond to the orientation of the right humerus (A) in the y vs z dimension as shown. (1) The proximal view and (2) is the distal view. Comparisons shown correspond to the shape changes between: (B) medium (blue) and heavy (orange) classes, (C) medium and light (green), and (D) light and heavy.

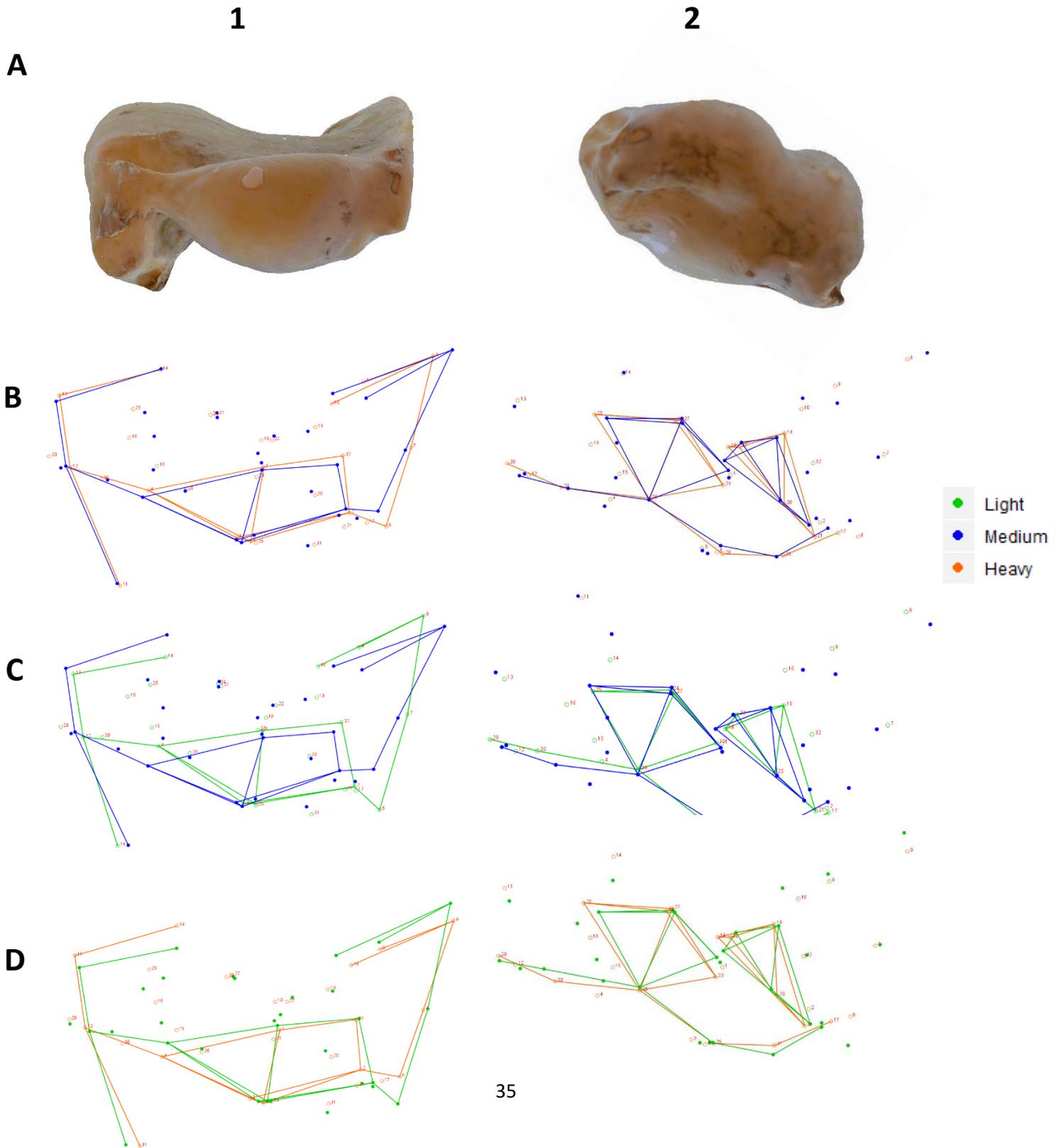


Figure 9. Shape changes between group centroids from the CVA on aerial courtship abilities. The superimposition views correspond to the orientation of the right humerus (A) in the x vs y dimension as shown. Comparisons shown correspond to the shape changes between (B) those that perform aerial courtship (blue) and species that do not (orange).

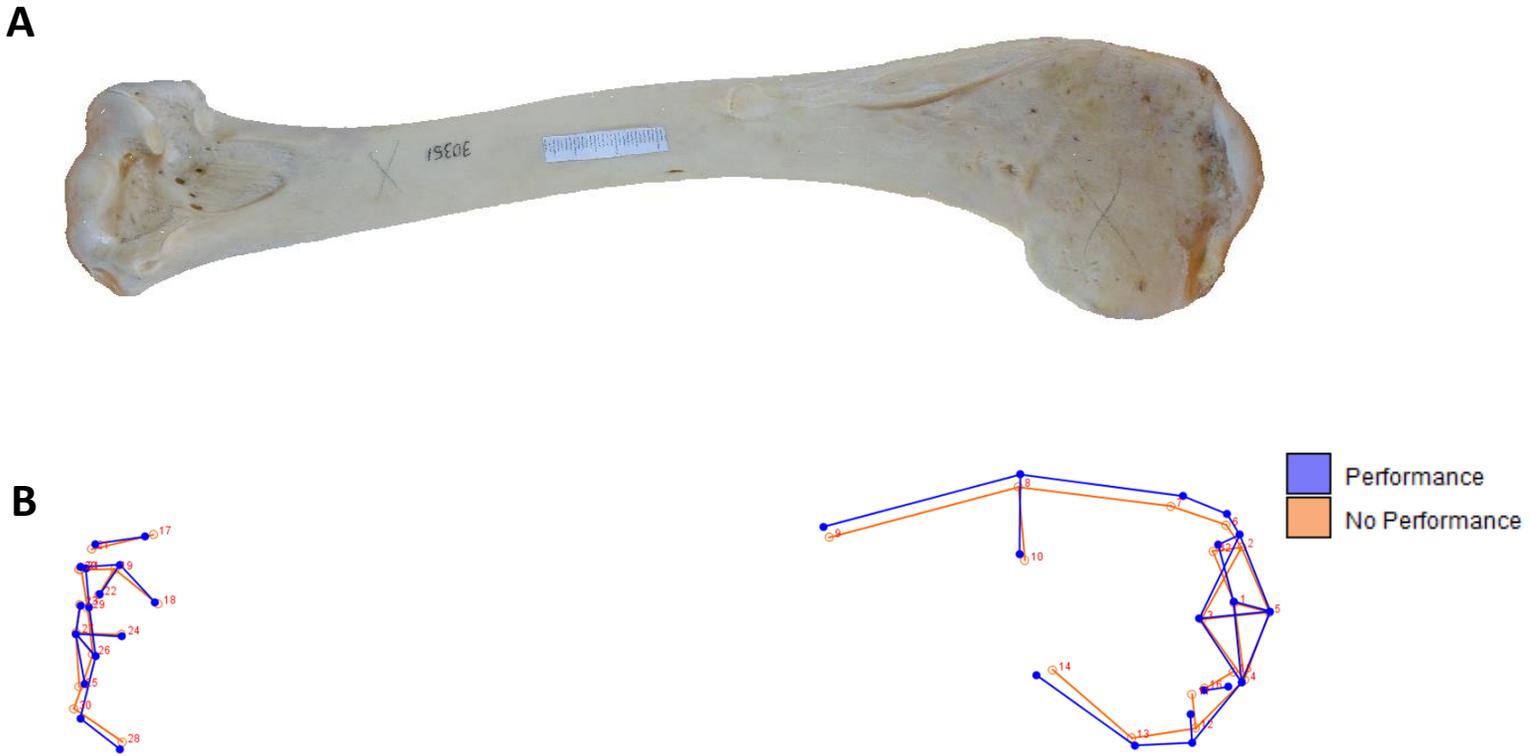


Figure 10. Shape changes between group centroids from the CVA on aerial courtship abilities. The superimposition views correspond to the orientation of the right humerus (A) in the x vs z dimension as shown. Comparisons shown correspond to the shape changes between (B) those that perform aerial courtship (blue) and species that do not (orange).

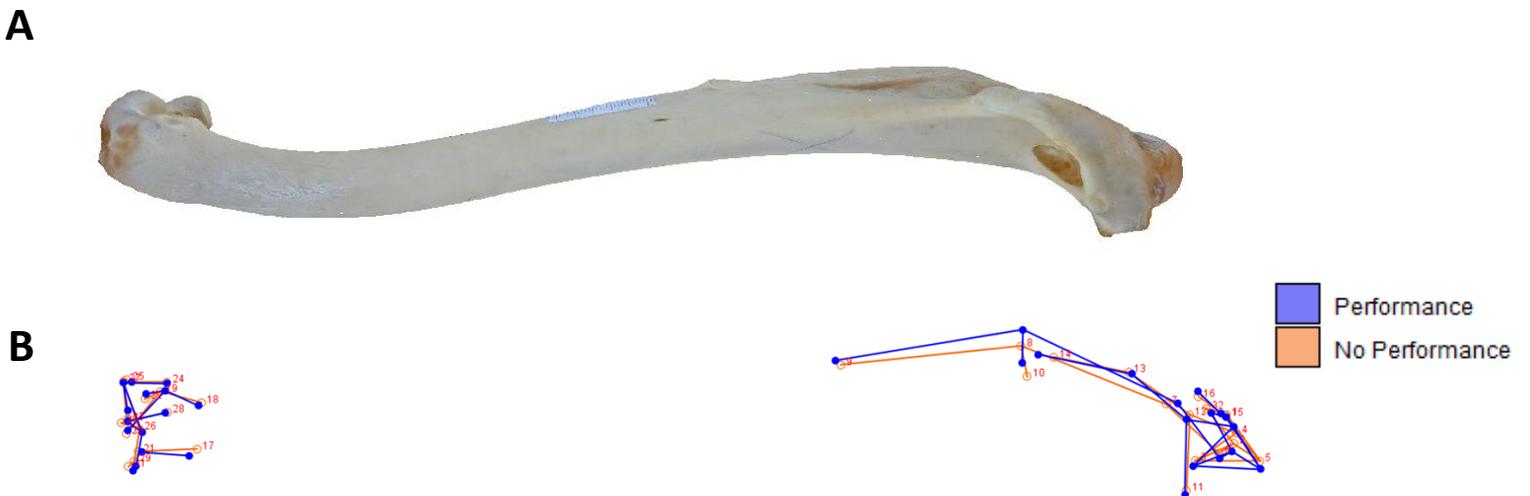


Figure 11. Shape changes between group centroids from the CVA on aerial courtship abilities. The superimposition views correspond to the orientation of the right humerus (A) in the y vs z dimension as shown. (1) The proximal view and (2) is the distal view. Comparisons shown correspond to the shape changes between (B) those that perform aerial courtship (blue) and species that do not (orange).

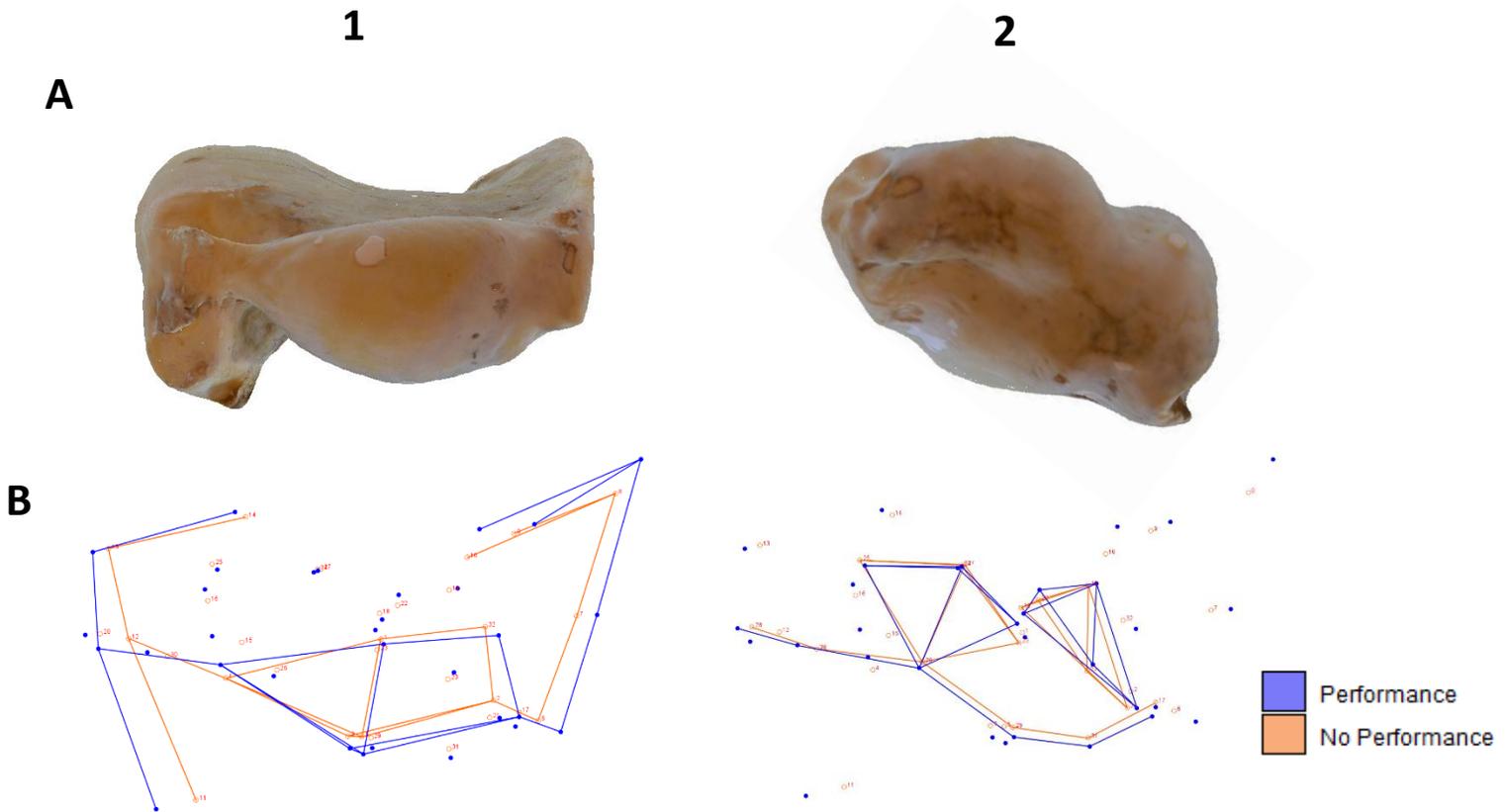


Figure 12. Shape changes between group centroids from the CVA on migration capabilities. The superimposition views correspond to the orientation of the right humerus (A) in the x vs y dimension as shown. Comparisons shown correspond to the shape changes between (B) those that migrate (blue) and species that are sedentary (orange).

A



B

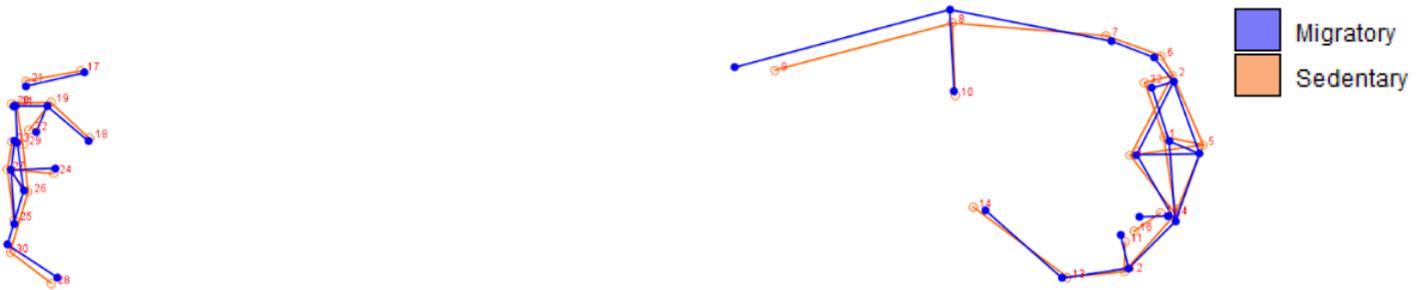


Figure 13. Shape changes between group centroids from the CVA on migration capabilities. The superimposition views correspond to the orientation of the right humerus (A) in the x vs z dimension as shown. Comparisons shown correspond to the shape changes between (B) those that migrate (blue) and species that are sedentary (orange).

A



B

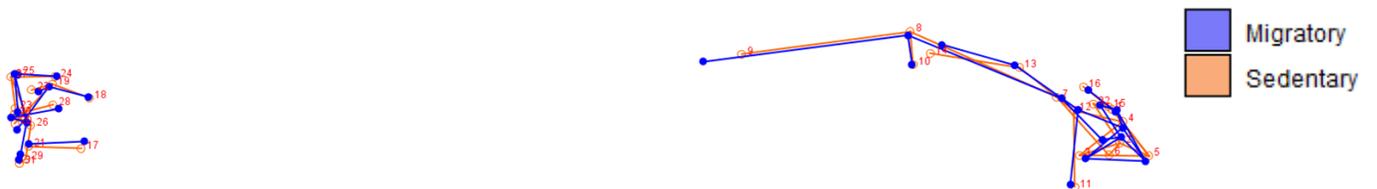
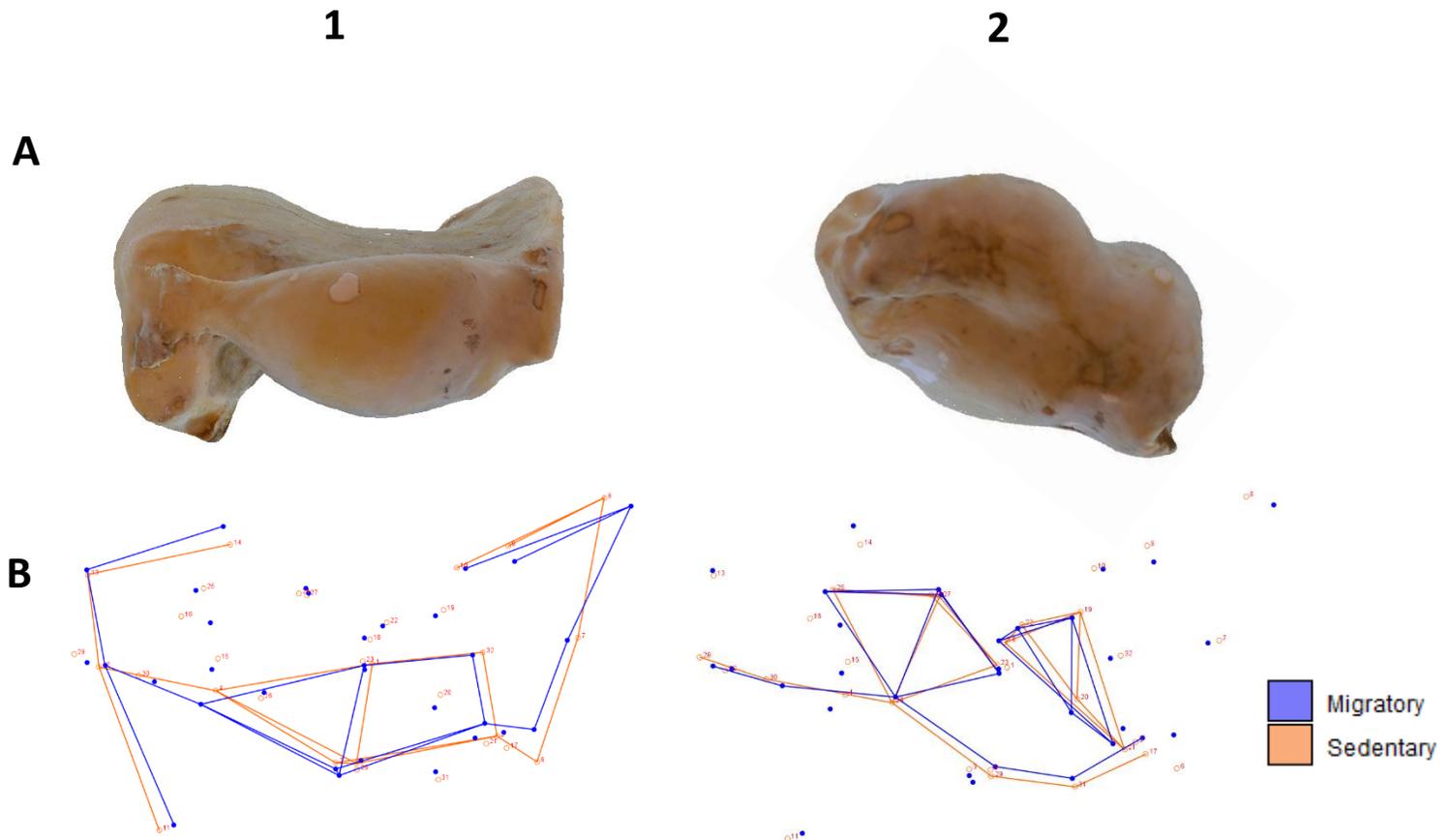


Figure 14. Shape changes between group centroids from the CVA on migration capabilities. The superimposition views correspond to the orientation of the right humerus (A) in the y vs z dimension as shown. (1) The proximal view and (2) is the distal view. Comparisons shown correspond to the shape changes between (B) those that migrate (blue) and species that are sedentary (orange).

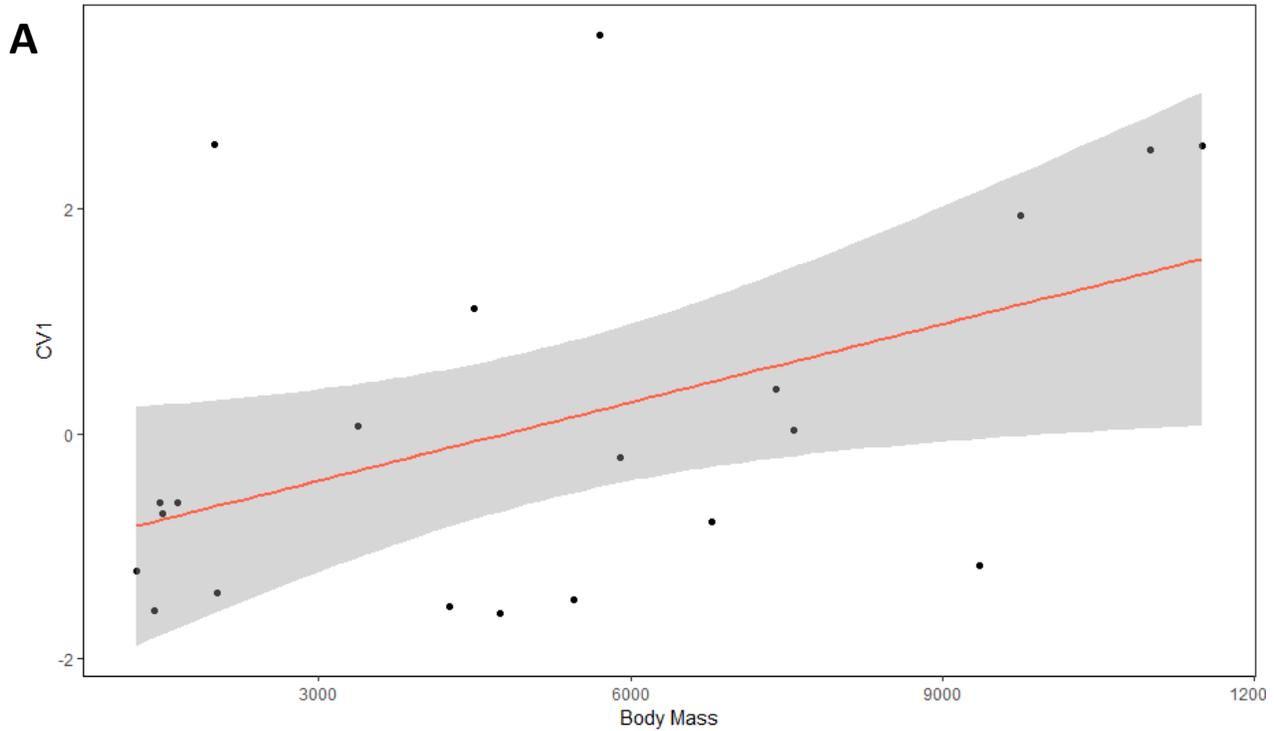


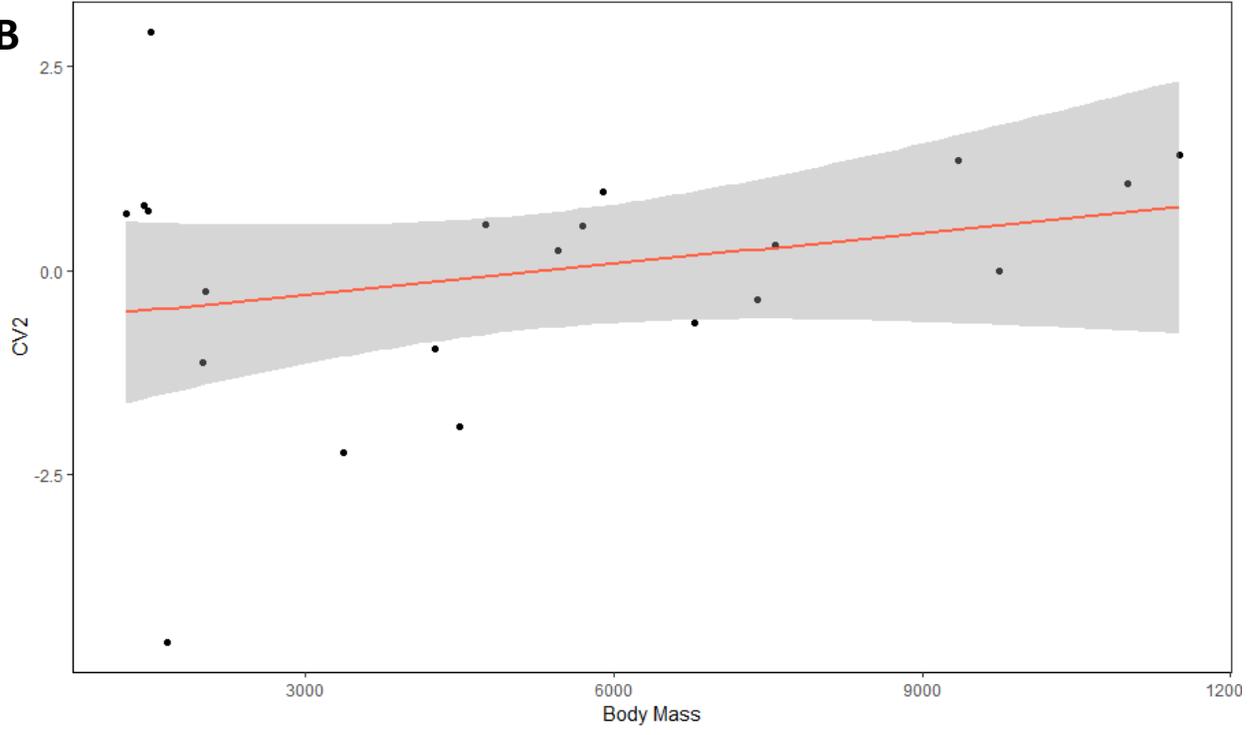
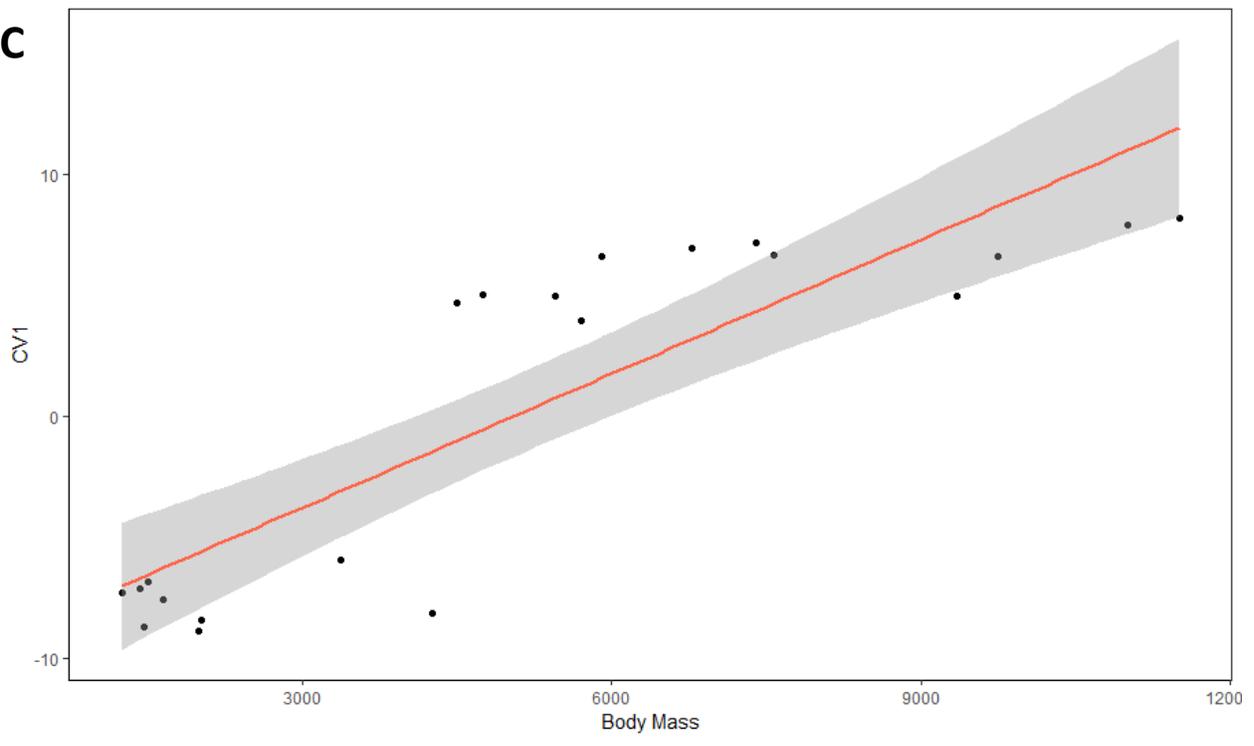
Appendix B

Table 9. Results obtained from regression analyses of shape (canonical variates of independent contrasts) on size (average body mass). Numbers indicate p -values and the percentages are the variance explained by allometry (based on R^2 values).

	CV1	CV2
Habitat	0.03121 (18%)	0.2415 (2.2%)
Weight	< 0.0001 (73%)	0.2066 (3.4%)
Courtship	0.4544 (0%)	N/A
Migration	0.8816 (0%)	N/A

Figure 15. Graphs for regressions performed from canonical variates of independent contrasts against average body mass for each category. (A) Habitat CV1, (B) Habitat CV2, (C) Weight CV1, (D) CV2, (E) Courtship, (F) Migration.



B**C**

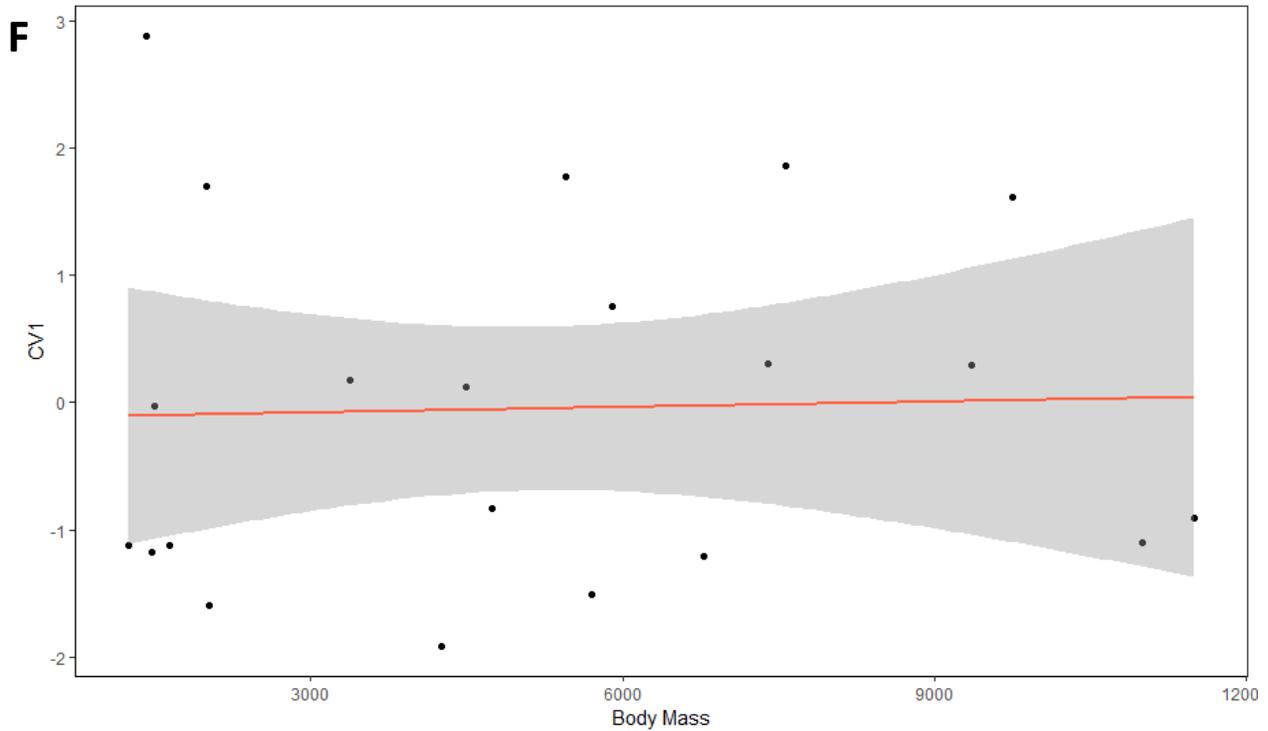


Table 10. Results obtained from regression analyses of shape (canonical variates of independent contrasts) on size (log centroid size). Numbers indicate p -values and the percentages are the variance explained by allometry (based on R^2 values).

	CV1	CV2
Habitat	0.206 (3.4%)	0.5018 (0%)
Weight	0.2625 (1.6%)	0.9435 (0%)
Courtship	0.5791 (0%)	N/A
Migration	0.8402 (0%)	N/A

Figure 16. Graphs for regressions performed from canonical variates of independent contrasts against log centroid size for each category. (A) Habitat CV1, (B) Habitat CV2, (C) Weight CV1, (D) CV2, (E) Courtship, (F) Migration.

