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Ecological and Evolutionary Drivers of Chameleon Forelimb Variation

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Ecological and Evolutionary Drivers of Chameleon Forelimb Variation

by

Ellie Schley

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The members of the Honors Thesis Committee appointed

to examine the thesis of Ellie Schley

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ABSTRACT

Ecological and Evolutionary Drivers of Chameleon Forelimb Variation

Ellie Schley

Director: Christopher Anderson, Ph. D.

Skeletal anatomy can vary greatly among individuals of the same family that share a common ancestor. Differences in skeletal anatomy and morphology allow species to be better suited to their environments. The study of skeletal anatomy variation as it pertains to species relatedness and habitat variation can provide useful insight into what may be driving evolutionary patterns among species. Specifically, studying skeletal anatomy of the forelimb could allow for better understanding of how the forelimb anatomy differs based on the arboreality of the species, which allows for better understanding of how habitat can affect morphology. To study the skeletal structure of the forelimb, I used micro computed tomography scans (microCT scans) of various chameleon species to isolate and analyze the skeletal anatomy of the chameleon forelimb. A total of 12 species from 6 genera were used in this analysis and include species with varying levels of arboreality. Measurements of the proximal, medial, and distal widths of the radius, ulna, and metacarpals 1-5, along with the angle of curvature for the radius and the ulna, were taken and analyzed using a principal component analysis (PCA) and phylogenetic logistic regression.

Surprisingly, there was no difference between any of the forelimb measurements based on arboreality, either with or without phylogeny. However, qualitative observations of the metacarpals of the forelimb revealed a pattern based on arboreality. All arboreal

species had some separation between metacarpals 1-3, whereas terrestrial species had none. The pattern shown in the metacarpals based on arboreality reveal that there seems to be a difference between chameleon forelimbs based on arboreality.

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Introduction

Since Charles Darwin first presented his findings on the evolution of Galapagos finches, it has been theorized that habitat can lead to anatomical changes in species as well as species differentiation (Fay, 2008). Individuals of a species are selected for or against depending on whether they have traits that are advantageous for survival in a given habitat. Individuals that possess these advantageous survival traits are more likely to breed, and therefore pass on those traits (Gregory, 2009). Within certain habitats, these traits can lead to morphological differences between individuals (Fay, 2008). Using the example of Galapagos finches, when the larger beak of one bird within the species gave that individual better access to a particular variety of food sources, that bird had a better chance of survival and therefore a higher likelihood of breeding and passing on the gene that allowed for the larger beak size (Abzhanov, 2010). As this continued through generations, it led to the evolution and differentiation among species of finch that were able to occupy different habitats and feed on different food sources.

Darwin's work provided a basis to our current working theory of evolution. With all life as we currently know it having evolved from common ancestors, understanding what drives evolutionary diversification processes can help inform our understanding of life in general (Ashraf, 2016). One large contributor to evolution is variation in habitat and food availability. Species that possess traits that allow them to occupy new habitats or better utilize resources available in a new habitat will have for greater likelihood of survival due to lower predation and increased food opportunities (Menezes, 2020). For

instance, species that diversified from a terrestrial to a more arboreal life may also experience benefits such as increased food opportunities and decreased predation (Losos, 2009).

Chameleons (Family Chamaeleonidae) are a diverse lineage of lizards with numerous morphological and ecological specializations. There are 12 genera of chameleons and more than 200 species, which occupy a multitude of different habitats (Glaw, 2015). Many chameleon species are native to sub-Saharan Africa and India, areas that have experienced significant habitat turnover in the last 10 million years (Giles and Arbuckle, 2022). Rapid changes in the environment have led to morphological changes in species that occupy these habitats, resulting in species that are better suited for the new environments. Indeed, research suggests that the majority of the diversification of chameleon species is due to this habitat turnover, which increased diversification rates (Giles and Arbuckle, 2022). Additionally, the lengths of time chameleons have been in an area is positively related to chameleon diversity in that area. Madagascar has a large diversity of chameleon species, likely due to the fact that chameleons have inhabited Madagascar for a long enough time that species have naturally diversified to fit the various niches of the island and decrease competition for one habitat type (Giles and Arbuckle, 2022). Throughout time, chameleons have likely become morphologically diversified to better fit arboreal or terrestrial habitats, to allow for greater opportunities in an area with such a large population of chameleons.

For this study, I examined the morphological variation of the forelimb among chameleons from six genera with both terrestrial and arboreal species to determine how arboreality affects variation in chameleon forelimbs. I hypothesized there would be

differences between the forelimbs of terrestrial and arboreal species, specifically of the radius and ulna.

Understanding how habitat and evolutionary lineage impacts chameleon skeletal anatomy is important to better understanding the mechanisms of evolution within a species or family. Analyzing changes and variation within and across genera can help build a better understanding of the factors that drive variation and evolution across a family. Further, studies on the driving forces of such evolutionary patterns can broadly inform trends observed among other taxa to better understand how habitat can lead to variation at different scales.

Species Selection

Species selected for this study were chosen from a database of chameleon micro computed tomography (CT) scans collected previously in the Anderson Lab at USD. This database includes CT scans of 75% of chameleon species across all major chameleon lineages. I selected a more terrestrial and more arboreal species from six genera of chameleons from this dataset. As many species are not strictly arboreal or strictly terrestrial, for the purpose of this study, the most terrestrial and most arboreal species from each major lineage was chosen.

In the *Bradypodion* genera, I used scans of *B. damaranum*, an arboreal species from montane forest habitats along the southern coast of South Africa (Stuart-Fox et. al., 2007) and *B. occidentale*, a more terrestrial species from the southwestern cost of South Africa that utilizes small vegetation and leaf litter for its primary habitat (Segall et. al., 2013). The *Calumma* genus is more strictly arboreal, therefore I used *C. hilleniusi,* which inhabits the mountain forests and leaf litter habitats in Madagascar (Randrianantoandro

et. al., 2010) and *C. crypticum* from the mountain forests of Madagascar (Jenkins et. al.,

2011). I then examined the highly terrestrial *Chamaeleo namaquensis* from the desert

sand dunes Southern Africa (Herrel et. al., 2012) and the arboreal *Chamaeleo zeylanicus*

from the tropical dry forests of India and Sri Lanka (Sharma and Koli, 2018). Among

Furcifer species, I examined the highly arboreal *F. balteatus* from the humid rainforests

of Madagascar (Jenkins et. al., 2020) and the more terrestrial *F. campani* from the dry,

savannah habitats of eastern Madagascar (Vences et. al., 2002). Although most

Rhampholeon species are regarded as terrestrial, I examined the arboreal *R. spinosus*

from forest patches throughout the Usambara mountains of Tanzania (Shirk et. al., 2014)

along with the terrestrial *R. temporalis* which inhabits leaf litter on the forest floor of the

Usambara mountains of Tanzania (Shirk et. al., 2014). Finally, I examined the

semiterrestrial *Trioceros goetzei goetzei* which resides in the mountain grasslands of

Tanzania and Malawi (Tolley, 2014), and the arboreal *Trioceros werneri* from the

montane forests of Tanzania (Tolley and Menegon, 2014).

Methodology

To analyze the forelimbs of each of the chameleon species, microCT scans were

used to isolate the bones of the forelimb of one individual per species for observation and

measurement. Amira was used to segment (isolate) the bones of one forelimb (left or

right) per specimen. The bones used for segmentation and measurement were the radius,

ulna, carpals (the number of which varied by species), metacarpals, and phalanges. After

segmenting the forelimb, measurements were taken using the measurement tool in the

Amira software program. For the radius and ulna, measurements were taken of the bone

length, the proximal, medial, and distal diameter, as well as the angle of curvature of each bone. For metacarpals 1-5, measurements were taken of the proximal, medial, and distal widths, as well as the length of each metacarpal. The carpals and phalanges were not measured in this study.

Bone length measurements were normalized to account for the fact that larger species would naturally have larger forelimb bones and permit analysis of bone measurements of chameleons of different sizes. To do this, the snout vent length (SVL) was measured for each species using the Slicer software program. Each species used in this experiment was measured from the tip of the snout to the beginning of the first sacral vertebrae along the curvature of the vertebral column. All length measurements for the radius, ulna, and metacarpals were then divided by the SVL to normalize the data to allow for further analysis.

For qualitative analysis, photos were taken of the medial, lateral, dorsal, and plantar views of the forelimbs to observe the general structure of the forelimb for each species, and allow for comparison of forelimb structure across species and genera. The photos were also used for observation regarding curvature of the radius and ulna. Photos were also taken of the metacarpals alone for comparison across species. Using the photos of the metacarpals, observations regarding their fusion and degree of association were made and compared based on genera and arboreality.

A principal component analysis (PCA) was performed in R studio running R version 4.2.2, to reduce the dimensionality of all quantitative measurements. PC score 1, which accounted for 38% of the variation, was used in subsequent analyses to test for an

effect of the arboreality on the quantitative measurements that loaded significantly on that PC score.

To analyze the effect of phylogeny, a pruned phylogeny (Figure 1) including only the species involved in this study was created based on a published, comprehensive chameleon phylogeny (Tolley et. al., 2013). A phylogenetic logistic (binomial) regression was then used to model the relationship between PC-1 and arboreality. Another binomial regression was done without use of the phylogeny to determine how phylogenetic relationships affected the trends observed.
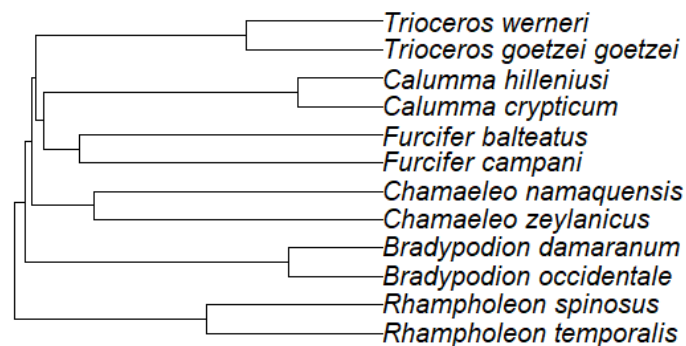

Figure 1: A pruned phylogeny based on Tolley et. al. (2013) depicting the relationships among species from this study.

<center>**Results**</center>

Quantitative forelimb measurements were collected from one specimen of each of 12 chameleon species (Table 1). The results of the PCA revealed that PC-1 accounted for 38% of the variance found between the variables measured (Figure 2). PC's 2 and 3 accounted for 16% and 14% of the variance, respectively. Due to the far greater contribution of PC-1, only PC-1 was used in the analysis based on arboreality and phylogeny. Each PC score was loaded to different levels by each variable, however PC-1 was most strongly loaded by the normalized values for metacarpal 4 medial width, metacarpal 4 length, metacarpal 1 length, metacarpal 5 length, metacarpal 3 length, metacarpal 1 distal width, metacarpal 4 distal width, radius distal width, ulna distal width, metacarpal 5 medial width, metacarpal 2 length, metacarpal 3 distal width, ulna medial width, metacarpal 4 proximal width, metacarpal 3 proximal width, metacarpal 5 proximal width, metacarpal 5 distal width, and radius proximal width (Figure 3; Table 2). The arboreal and terrestrial species exhibited largely overlapping distribution within the morphospace of PC-1 and PC-2 (Figure 3,4).

Table 1: Forelimb and body length measurements collected from each specimen. Snout-vent length, SVL; ulna, U; radius, R;

metacarpal, M; proximal width, pw; medial width, mw; distal width, dw; length, L.

	T. werneri	*T. g. goetzei*	*R. spinosus*	*R. temporalis*	*F. balteatus*	*F. campani*	*C. namaquensis*	*C. zeylanicus*	*C. hilleniusi*	*C. crypticum*	*B. occidentale*	*B. damaranum*
SVL	90.66	73.19	40.71	49.45	167.52	58.76	131	101.73	65.05	110.45	76.67	73.38
U L	13.56	10.84	5.02	7.27	26.65	8.92	22.79	16.89	8.39	15.67	9.98	10.86
U pw	3.3	1.65	0.92	0.917	4.42	1.45	3.6	2.09	1.46	2.1	1.62	1.44
U mw	1.81	0.942	0.67	0.634	2.5	0.733	1.27	1.54	0.705	1.38	1.25	1.22
U dw	2.49	1.86	0.842	1.05	3.57	1.22	3.31	2.42	1.44	2.53	1.95	2.03
R L	14.34	10.75	5.15	7.29	27.44	9.09	22.32	16.04	8.5	15.94	10.51	10.88
R pw	1.59	1.41	0.611	0.869	3.1	1.39	3.51	2.49	1.22	2.04	1.35	1.26
R mw	1.4	0.935	0.434	0.598	2.19	0.706	1.85	1.62	0.684	1.28	0.84	0.943
R dw	2.1	1.27	0.372	0.612	2.47	0.773	2.85	1.77	1.09	1.55	1.1	1.08
M 1 pw	1.01	0.827	0.353	0.503	2.05	0.619	1.48	1.27	0.647	1.56	0.629	0.741
M 1 mw	1.08	0.862	0.381	0.467	1.53	0.76	1.61	1.27	0.954	1	0.681	0.742
M 1 dw	1.49	0.979	0.555	0.483	1.92	0.8	1.87	1.43	0.853	1.54	0.907	1.13
M 1 L	2.84	1.77	0.853	0.794	3.75	1.61	2.9	2.06	1.18	2.77	1.72	2.26
M 2 pw	0.962	0.606	0.295	0.284	1.33	0.512	1.33	1.04	0.844	1.02	0.516	0.67
M 2 mw	1.05	0.683	0.292	0.413	1.33	0.64	1.25	1.14	1.24	0.94	0.722	0.82
M 2 dw	1.28	0.934	0.386	0.449	1.93	0.631	1.55	1.29	1.07	1.3	0.75	0.987
M 2 L	2.64	1.64	0.815	0.908	3.85	1.62	2.51	2.16	1.28	2.75	1.78	2.01
M 3 pw	1.28	1.07	0.32	0.49	2.15	0.514	1.62	1.3	0.643	1.39	0.937	0.839
M 3 mw	1.23	0.96	0.492	0.519	1.66	0.91	2	1.63	0.859	1.38	0.981	1.17
M 3 dw	1.79	1.45	0.414	0.684	2.83	0.777	1.99	1.73	1.16	1.98	1.2	1.49
M 3 L	3.04	1.91	0.549	1	4.24	1.78	2.93	2.57	1.62	3.05	1.96	2.25
M 4 pw	1.49	0.965	0.6	0.348	2.53	0.62	1.82	1.32	0.575	1.48	0.908	1.24
M 4 mw	2.24	1.14	0.539	0.606	2.67	0.957	2.2	1.66	0.555	1.92	1.12	1.81
M 4 dw	2.28	1.46	0.599	0.797	2.81	0.968	2.15	1.58	0.597	1.74	1.28	1.6
M 4 L	3.05	2.02	0.777	1.04	4.7	1.45	2.8	2.37	1.48	3.04	1.89	2.39
M 5 pw	1.49	0.973	0.396	0.451	1.71	0.792	2.04	1.01	0.624	1.41	0.388	0.776
M 5 mw	1.6	0.941	0.542	0.551	2.65	0.858	2.1	1.6	0.558	1.9	0.923	1.16
M 5 dw	1.65	1.04	0.437	0.612	2.39	0.737	2.08	1.42	0.757	1.46	1.32	0.958
M 5 L	2.62	1.65	0.771	0.855	4.05	1.18	2.51	1.92	1.37	2.45	1.5	2.04
R curvature	162.93	153.72	159.48	161.5	164.01	167.34	165.87	172.35	164.01	162.19	167.96	165.18
U curvature	160.46	157.2	160.02	172.78	168.95	164.57	165.41	167.43	157.73	168.29	164.58	160.06

Table 2: Loading values for each variables of the first principal component; metacarpal, M. Bolded values indicate a variable loads more heavily on PC-1 than if all variable contributed equally.

| Measurement	PC 1
Normalized M 4 Medial Width	**-0.25481580**
Normalized M 4 Length	**-0.24394632**
Normalized M 1 Length	**-0.24047987**
Normalized M 5 Length	**-0.23647882**
Normalized M 3 Length	**-0.23026968**
Normalized M 1 Distal Width	**-0.22695605**
Normalized M 4 Distal Width	**-0.22595398**
Normalized Distal Radius Width	**-0.22405162**
Normalized Distal Ulna Width	**-0.21685177**
Normalized M 5 Medial Width	**-0.21180883**
Normalized M 2 Length	**-0.21153983**
Normalized M 3 Distal Width	**-0.20914912**
Normalized Medial Ulna Width	**-0.20729804**
Normalized M 4 Proximal Width	**-0.20391176**
Normalized M 3 Proximal Width	**-0.19904250**
Normalized M 5 Proximal Width	**-0.18756817**
Normalized M 5 Distal Width	**-0.18741895**
Normalized Proximal Radius Width	**-0.18172885**
Normalized Ulna Length	-0.16868125
Normalized M 2 Distal Width	-0.14690385
Normalized Radius Length	-0.13969508
Normalized M 3 Medial Width	-0.13801051
Normalized M 2 Proximal Width	-0.12591433
Normalized Medial Radius Width	-0.12221821
Normalized M 1 Proximal Width	-0.11579299
Normalized M 1 Medial Width	-0.06036160
Normalized Proximal Ulna Width	-0.05802950
Radius Curvature Angle	-0.03292573
Normalized M 2 Medial Width	-0.02812623
Number of Carpals	0.01959551
Ulna Curvature Angle	0.07790800
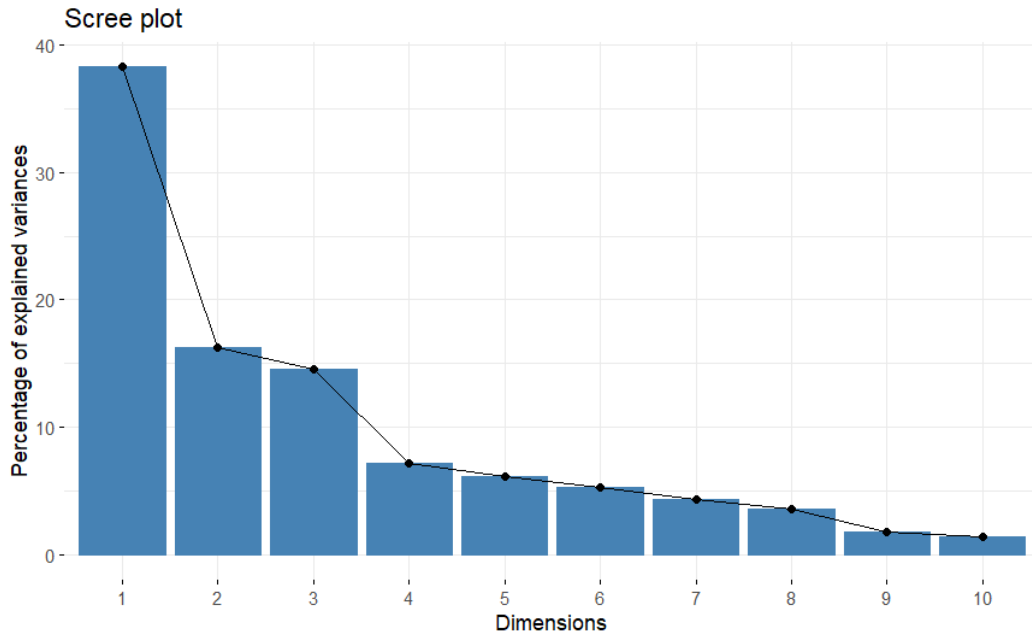
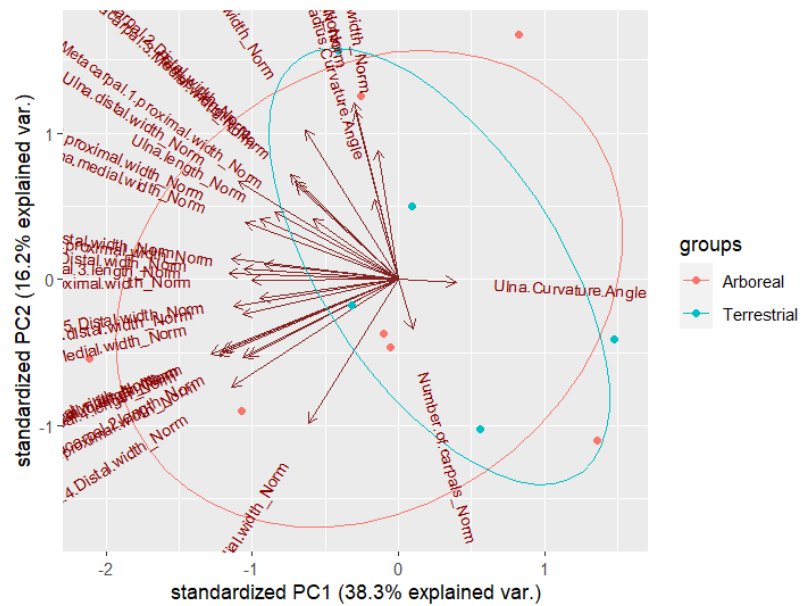Figure 2: Scree plot depicting the percentage of variance explained by each PC
dimension.

Figure 3: Biplot for PC1 and PC2 showing species PC scores grouped by arboreality
designations and loading vectors representing the contribution of each measurement to
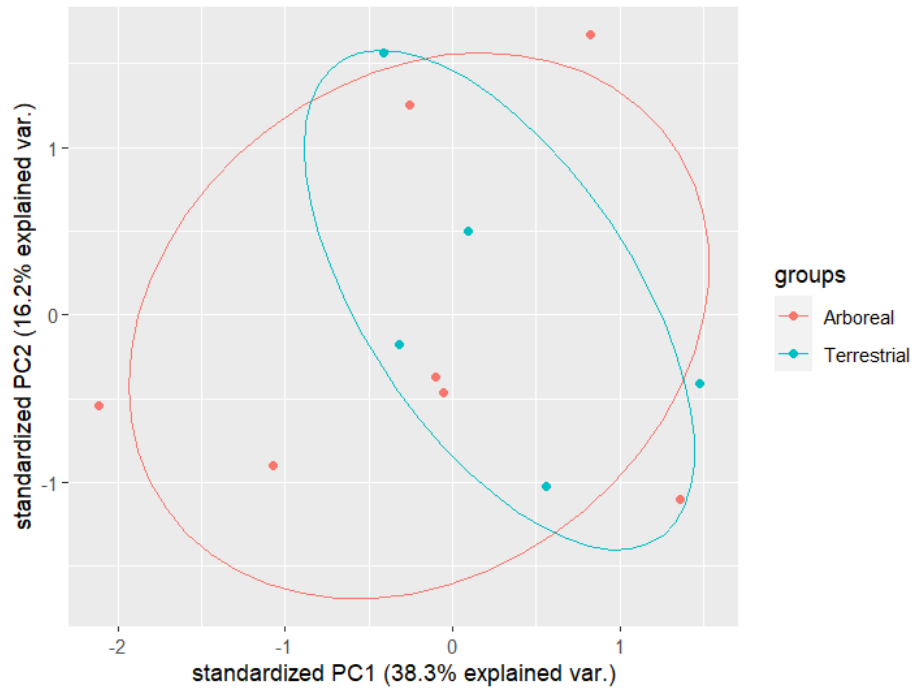the respective PC dimension.

Figure 4: Score plot for PC1 and PC2 without loading vectors. Groupings as in Fig. 2.

Arboreality was not a significant predictor of quantitative forelimb measurements as summarized by PC1 when analyzed with (p = 0.091) or without (p = -0.405) phylogenetic relatedness included in the models.

Qualitative analysis of the forelimbs showed greater separation between metacarpal elements, specifically in the medial region between metacarpals 1-3, in arboreal species (Figure 5). Terrestrial species, on the other hand, showed no separation between metacarpal elements, and metacarpals were fused throughout the length of bone.
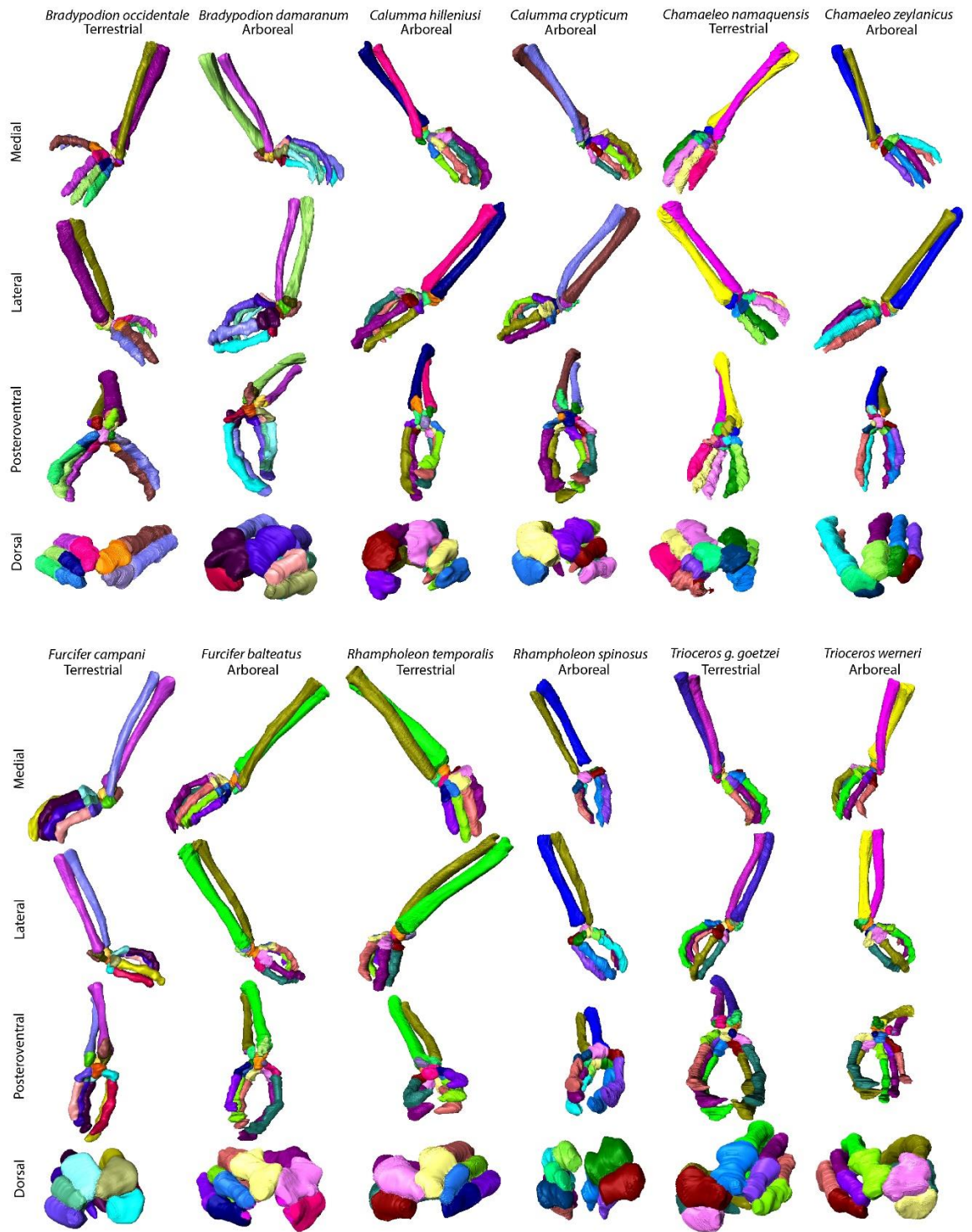
Figure 5: Compiled photos of medial, lateral, and posteroventral views of the forelimb of each species, as well as dorsal views of the metacarpals alone.

Discussion

Although one might expect arboreal and terrestrial chameleon species to exhibit differences in their forelimb anatomy to perform better in their respective environments, analyses of quantitative measurements of the forelimb as characterized by a PCA, indicated that the degree of arboreality does not predict observed forelimb variation among the species examined in this study. This was true whether phylogenetic relatedness was included in the statistical model or not. However, there is a strong trend (p = 0.09) toward statistical differences between the forelimbs based on arboreality. The results of this study contrast with studies that have found various differences in locomotor performance, and even morphology among chameleons based on habitat.

A previous study found that two arboreal species had greater grip strength in their forelimbs and tails than two terrestrial congeners, but slower sprint speeds (Herrel et. al, 2012). This study also found that arboreal species had larger lateral forefoot lengths than terrestrial species (Herrel et. al., 2012). Curiously, three of the four species they used were also examined for this study: *C. namaquensis, B. occidentale,* and *B. damaranum.* The findings of the study performed by Herrel et. al. (2012) may have been influenced by a small sample size, because the study only focused on four species from two different genera, rather than examining broader evolutionary patterns.

The aforementioned study also somewhat contradicted the qualitative findings of this experiment. Herrel et. al. (2012) showed that the two terrestrial species had no fusion between any metacarpals in any region of the bone, whereas arboreal species showed fusion at the proximal end of the bone, with one of the two species showing fusion at the proximal and distal ends as well. However, through qualitative analysis of the

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metacarpals in the study I performed, there seems to be a clear pattern that the terrestrial species have little to no separation between the metacarpals, whereas the arboreal species have much greater separation between metacarpals, primarily in the medial region of the bone. Interestingly, the Herrel et. al. (2012) study found that terrestrial species showed non-fused carpal elements, and the metacarpals of these species had separation between each metacarpal, with the most separation occurring in the medial region with less separation in the proximal and distal regions. That study focused primarily on the degree of fusion at the proximal end of the metacarpals, with figures showing a greater amount of separation throughout the rest of the bone in the two terrestrial species. It is also important to note that the majority of the species in the study that I performed that showed separation in the metacarpals had separation occurring somewhere in metacarpals 1-3, with little to no separation between metacarpals 4 and 5. Herrel et. al., (2012) also showed the most separation and lack of fusion between metacarpals 1-3, with metacarpals 4 and 5 also having less separation throughout. These findings could be because species may utilize their more medial fused set of digits (where metacarpals 1-3 are located) when griping things, and less association between the metacarpals would allow for greater range of movement. However, further study would need to be done to accurately predict why this trend would occur.

In addition to the differences in performance (where arboreal species had greater grip strengths and terrestrial species had faster sprint speeds) between arboreal and terrestrial species, there are also differences in locomotor patterns that allow arboreal species to be better climbers (Higham et. al., 2015). A study by Higham et. al. (2015) showed extensive differences in locomotion kinematics between one species

(*Bradypodion pumilum*) with two different morphs, where one morph was much more arboreal (woodland morph), and one morph was much more terrestrial (fynbos morph). This study tested the speed, stride frequency, humerus and femur retraction, elbow angle, wrist angle, and other factors relating to forelimb and hindlimb movement as the specimens moved across large and small perch diameters at angles of 0° and 90°. The data showed the fynbos morph had greater sprint speeds on small and large diameter perch sizes when the perch angle was at 90°, and slower speeds than the woodland morph on small and large diameters when the perch angle was 0°. The fynbos morph also had smaller wrist angles at footfall and middle stance when moving across smaller perch diameters than the woodland morph; this is likely because the fynbos morph has much smaller perch sizes available, and therefore the species has adapted better techniques to utilize the perches. In summary, this study highlights the vast differences between the two morphs of the same species, showing how greatly habitat can impact locomotion and kinematics of movement within one species (Higham et. al., 2015).

These studies suggest extensive differences in locomotor kinematics and performance of species or morphs inhabiting and utilizing different habitats. Primarily, arboreal species tend to have greater grip strength for gripping branches, are better clingers/climbers, are able to maintain performance as elevation increases, and are generally slower that terrestrial species when moving on completely flat surfaces (Herrel et. al., 2012) (Higham et. al., 2015). These differences indicate that there is a large potential for differences in skeletal anatomy of species of different habitats in order to best maximize performance in each habitat. However, previous studies were conducted with limited taxonomic diversity, effectively comparing either a single or two pairs of

species from different habitats rather than looking for broader evolutionary trends. Here, looking more broadly across the family, the results of this research suggest there is a trend of metacarpal size and arrangement differences in the forelimbs of species of different habitats. I suggest that with additional data points (species) and/or additional measurements (digit lengths, quantitative measures on metacarpal fusions, etc.), a significant difference between arboreal and terrestrial species forelimbs may appear. I also suggest that analyzing several individuals of two different species within one genera could yield significant results as well. Further, it could be that some of the species examined from different habitats have not yet adapted to the point of affecting forelimb skeletal structure, or that there is variation among species in how that adaptation presents itself. For instance rather than skeletal characteristics, these traits could include greater muscle mass in the forelimb and hindlimb might allow increased grip strength to better hold tree branches or more flexible wrists or strong wrists which could allow them to maintain hold on steeper inclines. Some of these changes and traits may have occurred somewhat recently, which is why there have not yet been changes in the skeletal anatomy of these species. It is possible that as these species continue to evolve to better fit their habitat, there will be an increase in variation between the forelimbs and this number could become significant. Alternatively, it may be that changes in skeletal anatomy are not needed to sufficiently increase performance of arboreal species in arboreal habitats. It is also possible that multiple factors have caused there to be no significant differences in skeletal anatomy, and there may not be one single answer.

In summary, this study shows a trend in the toward possible differences between species based on arboreality, specifically within the metacarpal elements. Qualitative analysis also showed a pattern in metacarpal elements based on arboreality, where arboreal species showed separation between metacarpals and terrestrial species had metacarpals that were fused throughout. This data supports the hypothesis that environments can impact morphology and select for traits that are more advantageous to each specific environment. Studies like these are important to better understand how environments can impact species, and how species can change to be better suited for their environment.

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