

Evolution of pedal digit orientation and morphology in relation to acquisition and secondary loss of the adhesive system in geckos

Mingna V. Zhuang¹  | Anthony P. Russell²  | Timothy E. Higham¹ 

¹Department of Evolution, Ecology, and Organismal Biology, University of California Riverside, Riverside, California

²Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada

Correspondence

Mingna V. Zhuang, University of Texas at El Paso, 500 University Ave, El Paso, TX 79912.
Email: mzhuang@utep.edu

Present address

University of Texas at El Paso, 500 University Ave., El Paso, TX 79912

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Abstract

Among geckos, the acquisition of the adhesive system is associated with several morphological changes of the feet that are involved in the operation of the adhesive apparatus. However, analyses using a comparative framework are lacking. We applied traditional morphometrics and geometric morphometric analysis with phylogenetic comparative methods to morphological data, collected from X-ray scans, to examine patterns of morphological evolution of the pes in association with the gain and loss of adhesive capabilities, and with habitat occupancy among 102 species of gecko. Padbearing gecko lineages tend to have shorter digits and greater inter-digital angles than padless ones. Arboreal and saxicolous species have shorter digits than terrestrial species. Our results suggest repeated shifts that converge upon a similar padbearing morphology, with some modifications being associated with the habitat occupied. We demonstrate that functional innovation and habitat can operate on, and influence, different components of foot morphology.

KEYWORDS

comparative methods, Gekkonidae, geometric morphometrics, radiography, shape evolution

1 | INTRODUCTION

The gecko adhesive system, which facilitates locomotion on smooth surfaces (Autumn 2000), is purported to have originated at least 11 times (Gamble, Greenbaum, Jackman, Russell, & Bauer, 2012, 2017), with at least one origin in four of the six gecko families. Previous studies have suggested how gecko foot morphology is expected to change with the acquisition of an adhesive system (Russell, 1975, 1979; Russell, Bauer, & Laroia, 1997). For padbearing lineages, in addition to changes in phalangeal form and proportions, digits are likely to be shorter and the arc subtended by them should increase to attain a fan-like configuration (Russell et al., 1997), facilitating the ability of at least some of the digits to always be aligned with the vector of gravitational acceleration while moving on vertical surfaces (Russell & Oetelaar, 2015). Such enhanced digital spreading also provides increased interdigital space for the laterally expanded individual

toepads, preventing overlap (Russell & Oetelaar, 2015). Trends toward skeletal changes appear to have preceded the acquisition of morphologically differentiated gecko toe pads (Russell, Baskerville, Gamble, & Higham, 2015), indicating that relatively small morphological changes are sufficient for the initial expression of a seta-based dry adhesion attachment system reliant upon a combination of van der Waals interactions and frictional adhesion (Autumn et al., 1999). This is evidenced by the morphology exhibited by key gekkotan and non-gekkotan species (e.g., *Gonatodes*, anoles, certain skinks; Autumn, Dittmore, Santos, Spenko, & Cutkosky, 2006; Dalla Valle et al., 2010). The more fully elaborated adhesive apparatus, generally taken to be typical of geckos, is more structurally complex than that of the aforementioned taxa and incorporates wholesale morphological changes of the skeleton, musculotendinous systems, and vasculature (Russell, 1975, 1979; Russell et al., 1997; Russell & Bauer, 1988, 1990; Zaaf, Herrel, Aerts, & De Vree, 1999) that are associated with the operation of setae.

For gecko species that exhibit secondary reduction or loss of toepads, there has been an increase in the rate of morphological change, suggesting that constraints on foot morphology are relaxed in association with this evolutionary reversal (Higham, Birn-Jeffery, Collins, Hulsey, & Russell, 2015). Although this implies that the function of the adhesive system imposes constraints on foot morphology and, therefore, should be associated with decreased morphological diversity, it is unclear whether constraints are imposed on the entire foot complex. Furthermore, it is unclear how changes in the morphology inherent in adhesive system acquisition are associated with habitat occupancy.

We use a phylogenetic framework in which to explore not only the principles of how gecko feet evolve and how morphology might be associated with the evolution of the adhesive system, but also how habitat might be associated with the expression of this padbearing morphology. We combine data of Russell et al. (1997) and those collected by us from museum specimens to test the predictions of Russell et al. (1997) about the differences between padbearing and padless geckos, namely that interdigital angles are greater, digits are shorter, and the pes is more symmetrical in padbearing geckos. We also predict that the occupation of morphospace will be less extensive, and that the rates of morphological change will be slower in padbearing geckos due to functional constraints imposed by the adhesive apparatus (Higham et al., 2015). We use a geometric morphometric framework to capture shape evolution so as to better assess the aforementioned aspects of the evolution of pedal symmetry, including the incorporation of data from the tarsals that is seldom considered in the analysis of foot form.

2 | MATERIALS AND METHODS

2.1 | Phylogeny

We pruned a tree of Zheng and Wiens (2016) and added additional species (Figure 1). *Rhoptropus taeniostictus* and species of *Aristelliger* were added on the basis of taxonomic and morphological information (Bauer & Good, 1996); *Cyrtodactylus*, *Goggia*, *Pseudothecadactylus*, *Rhacodactylus*, *Cnemaspis psychedelica*, and *Hoplodactylus pacificus* were added to the tree by combining the information from the trees of Bauer and Good (1996); Bauer, Good, Bauer, Good, and Branch (1997); Jacobsen, Kuhn, Jackman, and Bauer (2014); Pyron, Burbrink, and Wiens (2013); Wood, Heinicke, Jackman, and Bauer (2012); and Grismer, Wood, Onn, Anuar, and Muin (2014).

2.2 | Sampling

We used data from Russell et al. (1997) for 137 preserved specimens representing 100 species and collected additional data (that we use in our geometric morphometric analysis) from a further 65 specimens (54 species, three of which are additional to the species included in the original dataset, Figure 2) housed in the collections of the California Academy of Sciences (CAS; RRID: SCR_011132), San Francisco, California; the Museum of Vertebrate Zoology (MVZ; RRID: SCR_010608), University of California at Berkeley, Berkeley,

California; La Sierra University (LSUHC), Riverside, California; University of Texas at El Paso (UTEP), El Paso, Texas; University of Calgary Museum of Zoology, Calgary, Alberta; James Ford Bell Museum of Natural History, Falcon Heights, Minnesota; and the private collections of Anthony P. Russell, Timothy E. Higham, and Raul Diaz. Some uncatalogued specimens will be deposited at the University of California Riverside's zoological collection and the Los Angeles Natural History Museum (see Supplementary Online Material, Table S1 for a listing of specimens examined). Data for all components of the study were compiled from radiographs obtained using a Kubtec XPert 80 Radiography Cabinet (Kubtec, Fairfield, CT). Specimens were sandwiched between two sheets of Plexiglass before radiography to control for orientation and parallax. Only specimens that could be appropriately oriented were used for obtaining individual interdigital angles, digit lengths, and metatarsal lengths, as per the procedures of Russell et al. (1997) and Powell, Osgood, and Russell (2018) (Figure 3).

For the traditional morphometric analysis, the following variables were measured in ImageJ (RRID: SCR_003070) and calculated for the 65 specimens used for the geometric morphometric analysis and combined with data from Russell et al. (1997). To obtain the sum of total phalangeal lengths within each digital ray (wherein a digital ray consists of two moieties: the metatarsal moiety and the phalangeal moiety comprising the sum of the phalangeal lengths—see Powell et al., 2018), we subtracted metatarsal length from length of the digital ray. Interdigital angle data were collected by extending the longitudinal axes of the metatarsals proximally until they intersected with the axis of their neighboring metapodials (Russell et al., 1997) and with those of more distant digital rays, including the intersection of the axes of digits I and V (Figure 3). Three measurements describing the spread of the digits were used: the sum of the first three interdigital angles (IDA I–IV); the sum of all interdigital angles (sum of IDA: I–II, II–III, III–IV, and IV–V); and the angle between the first and fifth digit (IDA I–V; Figure 3). We used these three measures to account for any errors that may be inherent by using only a single measure, as elaborated upon in the next paragraph.

The first four metatarsals are sheathed in a common plantar compartment (Robinson, 1975), so that their axes diverge from each other at the location projected proximally from the regions at which adjacent metatarsal heads contact one another. However, the position of the fifth metatarsal is more variable, and the angle between digits IV and V depends upon the position in which the digits of the specimen were fixed. Because of the potential unreliability of the angle between digits IV and V (Russell et al., 1997), the sum of the first three interdigital angles (IDA I–IV) was calculated in addition to the sum of all of the interdigital angles. Because the axis of the fifth metatarsal often does not intersect with the axes of the other metatarsals, an additional measure describing total digital spread was obtained by measuring the angle between digits I and V, at the intersection of the lines projected proximally from the long axes of metatarsals I and V. To obtain an assessment of the relative equitability of the total of the spread of all digits lying on either side of the long axis of the third metatarsal (representing the mesaxonic digit), we subtracted the sum of the angles between digits I and III from the sum of the angles between digits III–V, using the absolute value of this measure.

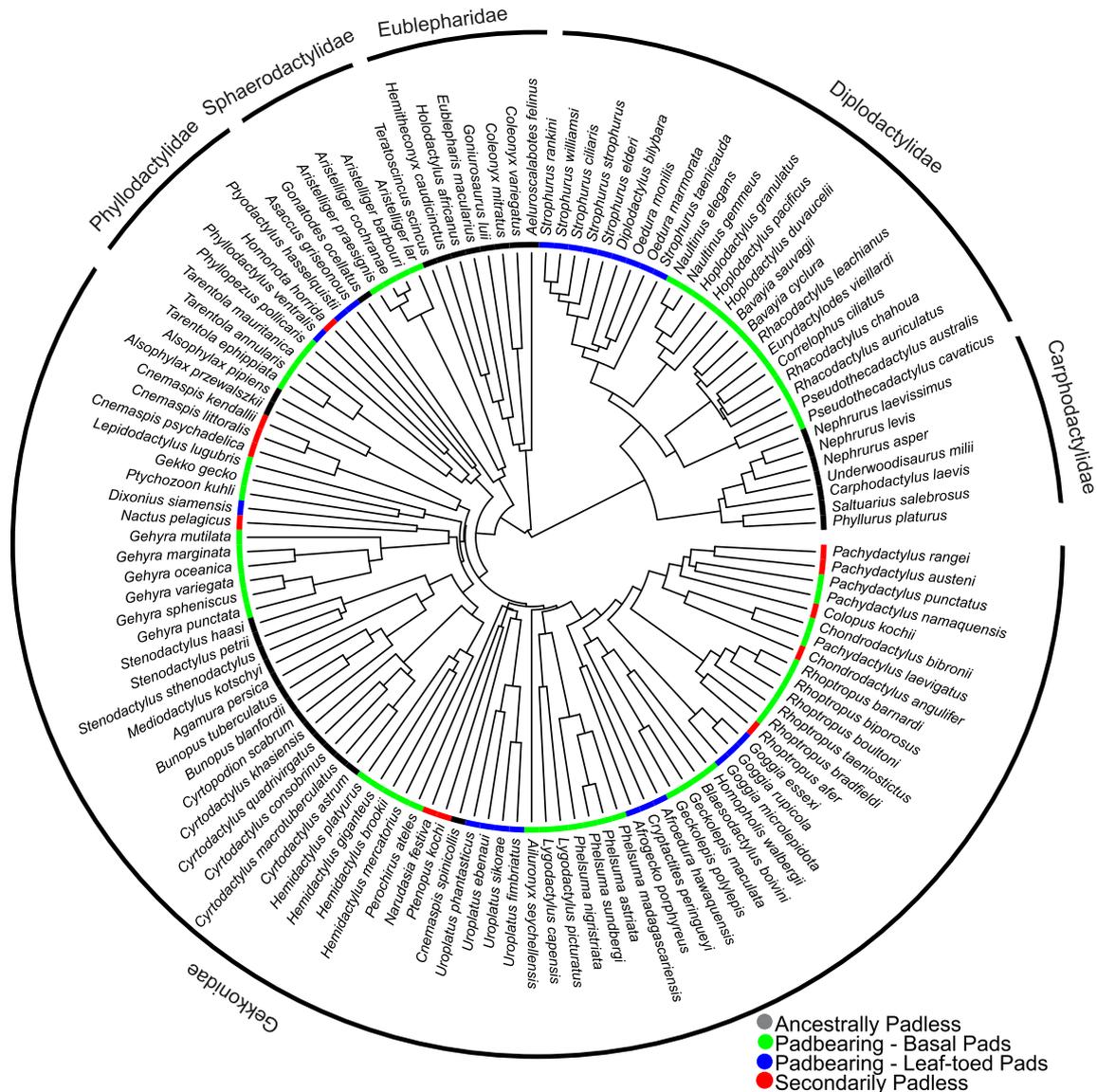


FIGURE 1 Phylogenetic tree of geckos used for our statistical analyses. The tree was pruned from the phylogeny of Zheng and Wiens (2016). Sampling covers at least five origins of the adhesive system (see Gamble et al., 2012) and includes ancestrally padless geckos (black), padbearing geckos (green), leaf-toed padbearing geckos (blue), and geckos exhibiting secondarily reduced or lost toepads (red)

Padstate was coded as padless or padbearing, as indicated by Gamble et al. (2012; see Figure 1). Secondarily padless or pad-reduced geckos were coded as padless. Information about the presence of the leaf-toed configuration of the toe pads was gleaned from the literature (Gamble et al., 2012; Higham et al., 2015), allowing us to make a distinction between the presence of basally derived or situated, and terminally derived or situated, pads (Gamble et al., 2017; Russell & Gamble, 2019). Habitat data were gleaned from the literature, and geckos were classified as being either arboreal, saxicolous, or terrestrial (Supplementary Online Material, Table S2).

2.3 | Data collection for geometric morphometric analysis

For the newly added 65 specimens, encompassing 54 species (Figure 2), eleven landmarks describing the arrangement of the tarsals

and metatarsals were digitized on each X-ray image (Figure 3; Table 1) using tpsDig2 (Rohlf, 2005). Because digit position can depend on the quality of preservational arrangement of the digits during fixation, landmarks were placed only on the metatarsals and tarsals, which are more constrained in position by the unified structure of the plantar region of the metapodium; these elements not being free to displace in this region (Robinson, 1975; Russell et al., 1997). Landmarks were superimposed using General Procrustes Analysis (Rohlf & Slice, 1990) in the “geomorph” package (Adams & Otárola-Castillo, 2013), and a mean shape per species was calculated for further analysis. For this analysis, centroid size was highly correlated with body size ($r^2 = 0.71$, $df = 102$, $p = 0.001$ with phylogenetic correction).

Relative bilateral asymmetry of the shape of the autopodium was calculated by measuring the Procrustes distance from the original shape of a species and its symmetric consensus (Klingenberg, Barluenga, & Meyer, 2002). Greater values of bilateral asymmetry

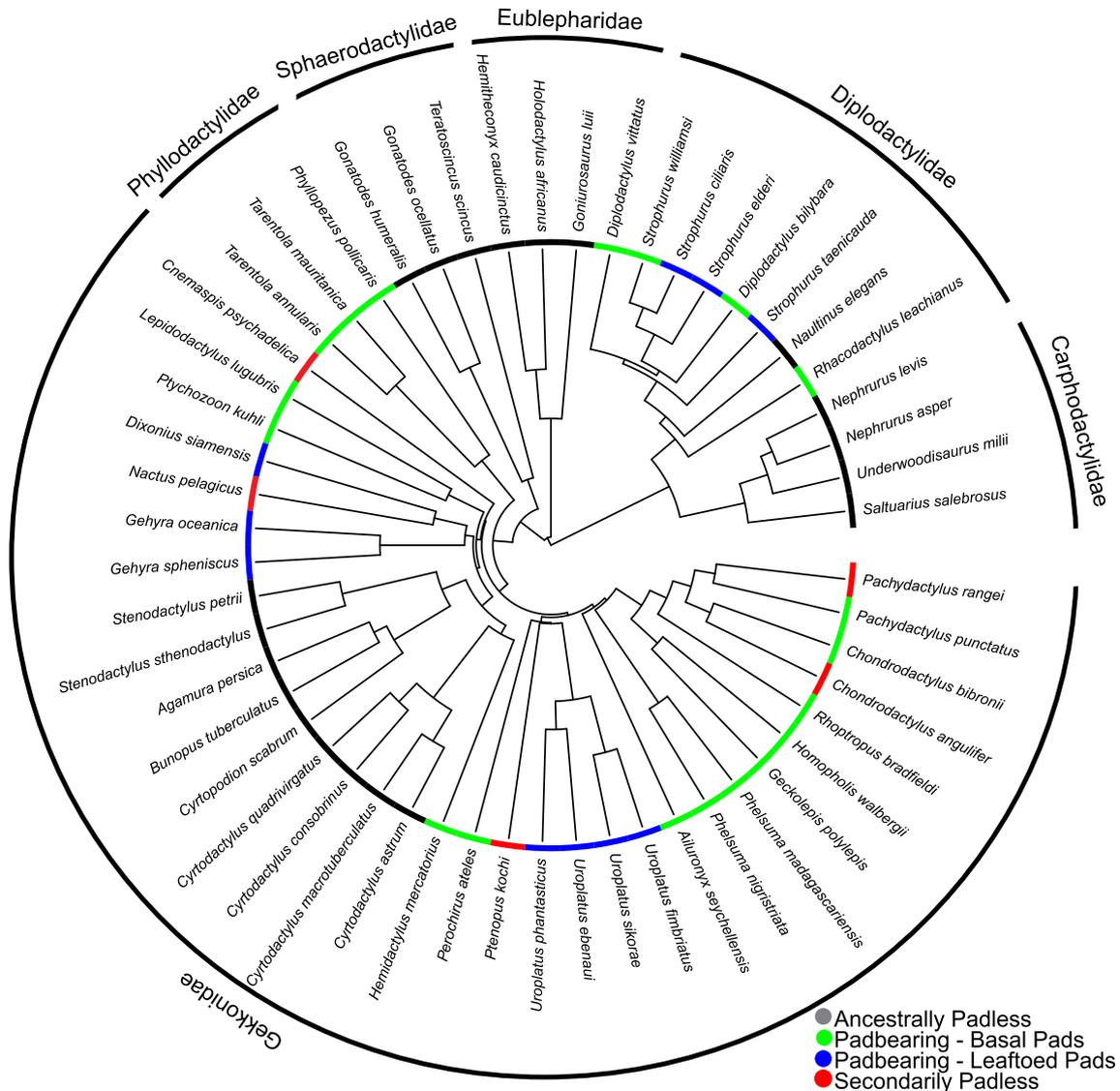


FIGURE 2 Phylogenetic tree of geckos used in the geometric morphometric analysis. Sampling covers up to three origins of the adhesive system (see Gamble et al., 2012) and includes ancestrally padless geckos (black), padbearing geckos (green), leaf-toed padbearing geckos (blue), and geckos exhibiting secondarily reduced or lost toepads (red)

indicate greater asymmetry, whereas smaller values indicate an object with greater within-object symmetry. This variable is a more comprehensive measure than that obtained by examining asymmetry of interdigital angles because it incorporates the asymmetry of digit lengths and interdigital angles simultaneously (Figure 4).

2.4 | Statistical analysis

To correct for size and examine its effect on the variables, we regressed each length variable against body size (snout-vent-length) using a linear-model regression and phylogenetic generalized least squares regression (PGLS) for the total dataset (194 specimens representing 123 species). We then applied Equation 1.1, where b is the slope of the regression line from the PGLS (Blomberg, Garland, & Ives, 2003). For most of the analyses, we found that padbearing geckos

with the leaf-toe morphology did not differ significantly from geckos with the basal pattern of toepad shape; therefore, we pooled their data together to represent a single padbearing state.

$$\log\left(\frac{\text{variable}}{\text{SVL}^b}\right) \quad (1.1)$$

To assess the interactive effect of padstate and habitat, we performed two-way ANCOVAs, using type III sum of squares, on variables that loaded highly in the principal components analysis (PCA). For these analyses, we used the “car” and “nlme” packages. If the interaction effect was not significant, we repeated the model with type II sum of squares and without the interaction effect. We first performed the analyses assuming Brownian motion, then took the residuals from the model and fitted them to both Brownian motion (BM) models and the Ornstein-Uhlenbeck (OU) models in the “geiger”

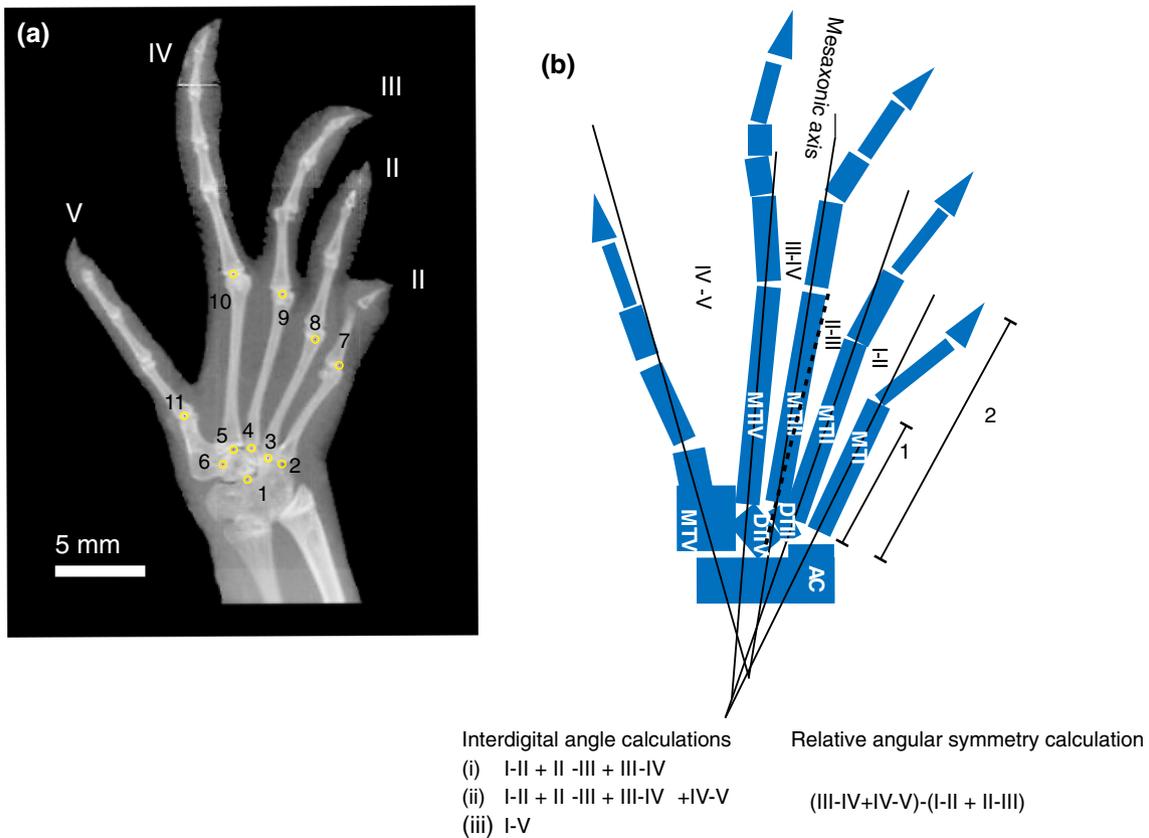


FIGURE 3 Landmarks used to describe the metapodial compartment of the pes. The shape of the pes, as represented by the elements contained within this compartment, was assessed by digitizing landmarks primarily on the left pes as seen in 2D X-rays (A). Schematic representation of the left pes of a gecko in dorsal view (based on a radiograph of the left pes of *Eublepharis macularius*) showing the skeletal elements and the angular measurements taken (B). The solid black lines represent the median axes of the metatarsals (labelled MTI–MTV). The mesaxonic axis is represented by the midline longitudinal axis of digit III. Black lines represent the metatarsal length measured (1) and total digit length (2). Angles between the metatarsals are represented in black between the digits (I and II, II and III, III and IV, IV and V). The measurements taken to calculate interdigital angles and the relative symmetry of the pes are indicated below the diagram (sum of the first three interdigital angles [i], sum of all the interdigital angles [ii], angle between digits I and V [iii]). Other abbreviations: AC, astragalocalcaneum; DTIII, third distal tarsal; DTIV, fourth distal tarsal

package (Revell, 2010). We then chose a model based on the lowest Akaike information criterion, unless the difference was less than 4, in which case the Brownian motion model was used. If the OU-model was a better fit, we repeated the analysis using it. Phylogenetic correction was used only if the residuals of the PGLS model had a significant phylogenetic signal. If we found a significant interaction effect or effect of habitat, we performed a Tukey post hoc test using the “lsmeans” package in R 3.4.1 (Length, 2016).

Phylogenetic signal for residuals and morphological traits was estimated with Pagel's lambda in the “phytools” package (Pagel, 1999; Revell, 2012). The value of Pagel's lambda spans from 0 to 1. A strong phylogenetic signal is represented by values closer to 1, indicating the trait among closely related species is more similar than in those species that are distantly related. A value of 0 indicates the lack of a phylogenetic signal.

Because some data from some of the variables measured were missing from the original data set, we used a subset of the total data, which resulted in 102 species being included in the PCA, which was performed using size-corrected measures of all of the sums of

TABLE 1 Definition of landmarks for the geometric morphometric analysis

Landmark #	Description
1	The most central point between the articulation of the fourth distal tarsal and the astragalocalcaneum
2	The most proximal point of the first metatarsal
3	The most proximal point of the second metatarsal
4	The most proximal point of the third metatarsal
5	The most proximal point of the fourth metatarsal
6	The most proximal point of the fifth metatarsal
7	The most distal point of the first metatarsal
8	The most distal point of the second metatarsal
9	The most distal point of the third metatarsal
10	The most distal point of the fourth metatarsal
11	The most distal proximal point of the fifth metatarsal

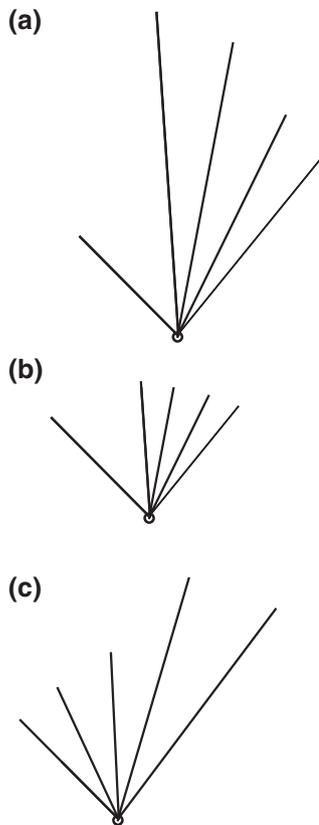


FIGURE 4 Representations of different ways of achieving bilateral asymmetry on either side of the third digit axis. Asymmetry can be achieved by having unequal interdigital angles and metapodial lengths (A), unequal interdigital angles and equal metapodial lengths (B), or equal interdigital angles and unequal metapodial lengths (C)

phalangeal lengths, metatarsal lengths, interdigital angles, the sum of the interdigital angles, and the bilateral asymmetry score. The phylogeny was then projected onto the morphospace to create a phylomorphospace using the “phytools” package to seek any patterns of morphological diversification and convergence (Sidlauskas, 2008) that may be present. With this method, PC-scores are computed at internal nodes, permitting us to assess the general direction of shifts in morphospace when transition in pad states has occurred.

To assess whether the patterns revealed when all six gecko families were analyzed together were the product of a combination of patterns idiosyncratic to one or more of these, we repeated our analyses using species from the Gekkonidae only. That family is represented in our dataset by the greatest number of species and incorporates all pad state types (Figure 2). The results for the analyses of the Gekkonidae alone are presented as Supplemental Online Material Figures S1 and S2.

2.5 | Geometric morphometric analysis

All statistical analyses pertaining to the geometric morphometric data were performed using a combination of the “geomorph” and “phytools” packages (Adams & Otárola-Castillo, 2013; Revell, 2012). We

performed a PCA on the landmarks and then conducted two-way ANOVAs on the first two PC axes, as well as the bilateral asymmetry measure, using padstate and habitat as the independent variables. To understand the evolution of foot shape, we fitted the shape data to single-rate Brownian motion and OU-models, using the fitContinuousMV function in geiger (Harmon, Weir, Brock, Glor, & Challenger, 2008). To estimate the phylogenetic signal of foot shape, we used the multivariate version of Blomberg’s K statistic, using the physignal function in “geomorph.” A lack of phylogenetic signal is indicated by values of K closer to 0, whereas a value of 1 or higher indicates a strong phylogenetic signal (Blomberg et al., 2003).

3 | RESULTS

3.1 | Effect of size

The metatarsal moiety and phalangeal moiety increase with body size with scaling exponents ranging from 0.87 to 1.0 (Table 2; Figure 5). The individual interdigital angles between digits I and V, I and II, II and III, as well as the sum of interdigital increase with body size. Bilateral asymmetry and the last two interdigital angles (III–IV and IV–V) are not affected by body size.

3.2 | Effect of padstate and habitat on angle measures

Angular measurements and digital spread were usually greater for padbearing lineages than padless lineages (Table 3; Figure 6). The digital spread variables (IDA I–V, IDA I–IV, and sum of total interdigital angles) are not associated with habitat. Bilateral asymmetry is not associated with padstate but is greater for arboreal species than saxicolous ones ($F_{(2,4)} = 5.82$, $p = 0.00394$, post hoc Tukey test $p < 0.05$). Each interdigital angle is greater for padbearing lineages than for padless lineages. The effect of padstate depends on habitat for interdigital angle (III–IV).

3.3 | Effect of padstate and habitat on digital morphology

Overall, the phalangeal and metatarsal moieties are shorter in padbearing lineages than in padless lineages (Table 3; Figure 7). Only the first two metatarsals are shorter in padbearing lineages than padless lineages. The effect of habitat depends on padstate for the first and third metatarsals. The sum of the phalangeal lengths within each digit is lesser in padbearing lineages than in padless lineages but does not depend on habitat. For all digits, the total length of each digital ray (the composite of the metapodials and serially contiguous phalanges) was lesser for padbearing lineages than for padless lineages. For means and ranges of measurements among padbearing and padless species, see Supplementary Online Material, Table S3.

TABLE 2 Results of linear regression of variables against the effect of size and phylogenetic signal

Variable	df	R ²	p-value	Exponent	R ² ^a	p-value ^a	Exponent ^a	λ
Sum of interdigital angles (I–IV)	115	0.10	<0.01		0.10	4.42E-05 ^{b****}	0.01	0.66
Bilateral asymmetry	113	<0.01	0.99		<0.01	0.97 ^b	<0.01	0.66
Sum of interdigital angles	114	0.07	3.44E-03 ^{***}		0.07	0.01 ^{b*}	<0.01	0.59
Interdigital angle (I–V)	109	0.09	1.21E-03		0.09	1.63E-03 ^{b****}	<0.01	0.69
Interdigital angle (I–II)	112	0.14	3.97E-05 ^{***}	11.26	0.14	2.26E-05 ^{b****}	0.01	0.63
Interdigital angle (II–III)	116	0.06	0.01 [*]	6.56	0.06	2.88E-03 ^{b****}	0.01	0.65
Interdigital angle (III–IV)	115	0.01	0.21	4.40	0.01	0.08	<0.01	0.63
Interdigital angle (IV–V)	116	0.02	0.14	14.23	0.02	0.35	<0.01	0.64
Metatarsal I	116	0.70	3.23E-32 ^{***}	0.94	0.7	9.02E-40 ^{b****}	0.74	0.92
Metatarsal II	118	0.69	9.08E-32 ^{***}	0.90	0.69	5.13E-44 ^{b****}	0.80	0.97
Metatarsal III	118	0.69	7.97E-32 ^{***}	0.87	0.69	4.00E-42 ^{b****}	0.84	0.93
Metatarsal IV	118	0.74	6.50E-36 ^{***}	0.91	0.74	1.12E-42 ^{b****}	0.84	0.95
Metatarsal V	117	0.68	8.35E-31 ^{***}	0.92	0.68	3.09E-31 ^{b****}	0.73	0.75
Phalanges of digit I	111	0.58	2.08E-22	1.07	0.58	2.35E-06 ^{b****}	0.51	0.88
Phalanges of digit II	115	0.68	4.79E-30 ^{***}	1.08	0.68	1.80E-35 ^{b****}	0.64	0.88
Phalanges of digit III	115	0.70	6.66E-32 ^{***}	1.02	0.7	5.23E-40 ^{b****}	0.65	0.93
Phalanges of digit IV	113	0.66	1.72E-28 ^{***}	0.96	0.66	1.58E-32 ^{b****}	0.69	0.73
Phalanges of digit V	114	0.58	3.56E-23 ^{***}	1.03	0.58	3.35E-23 ^{b****}	0.56	0.60

^aPhylogenetically corrected results.

^bWhere phylogenetic correction was necessary.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

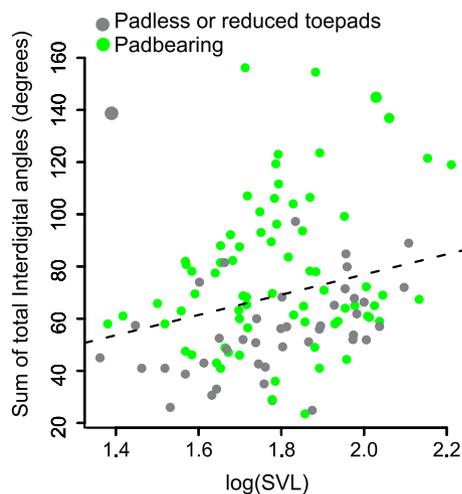


FIGURE 5 Scaling of the sum of total interdigital angles with size log(SVL) for padless (grey) and padbearing (green) lineages

3.4 | Predictors of angular measurements

Angular bilateral asymmetry and digital spread are not determined by a single interdigital angle. Bilateral asymmetry increases with an increase in the interdigital angles between digit III and V ($R^2 = 0.49$, $df = 117$, $p < 0.05$ and $t_{(1,117)} = 22.81$, $p < 0.05$, respectively). Bilateral asymmetry is not associated with changes in interdigital angles between the more medial digits (I–III) ($t_{(1,117)} = -0.36$, $p = 0.721$, $R^2 = 0.07$, $df = 101$, $p = 0.483$). Bilateral asymmetry increases with an

increase in digital spread (Digital Spread (I–IV): $t_{(1,117)} = 2.4$, $p = 0.018$). All interdigital angles increase with an increase in digital spread as measured by IDA I–V ($t_{(1,101)} = 2.4$ to 12.64 , $p < 0.001$). All interdigital angles are intercorrelated with the exception of the last (IV–V), which is only correlated with interdigital angle (III–IV) (Table 4).

3.5 | PCA of traditional morphometric measurements

Of the 123 species for which we had data, a total of 102 of them had complete data sets. Therefore, we used these for the PCA. Using the broken stick model (Hammer, Harper, & Ryan, 2001), the first three principal components (PC) were retained for analysis. These collectively explain 74.83% of the variance (Figure 8; see Supplementary Online Material, Table S4 for PC-scores of species means). The first PC-axis (40.80%) describes changes in lengths of the metatarsal and phalangeal moieties within the digit (Table 5). Higher scores along the first PC-axis correspond to longer metatarsals and sums of phalangeal lengths (Table 5). The second PC-axis (23.38%) describes changes in interdigital angles between the second and fifth digits and angular descriptions, such that higher scores along this axis correspond to lesser bilateral asymmetry, smaller interdigital angles (III–IV and IV–V), and a smaller sum of total interdigital angles. Finally, the third PC axis (10.65%) describes changes in the interdigital angles of the more medial (preaxial) digits (I–II and II–III), the last interdigital angle (IV–V), and bilateral asymmetry. Higher scores along the third PC axis

TABLE 3 Results of ANOVAs performed on variables that loaded highly on the first two PCs of the traditional morphometric dataset

Principal component	Effect	df	F	p-value	F	p-value
Metatarsal I	Padstate	1,111	18.75	3.21E-05***	4.62	0.03 [‡] *
	Habitat	2,111	1.67	0.19	9.38	0.01 [‡] *
	Interaction effect	2, 111			6.43	0.04 [‡] *
Metatarsal II	Padstate	1,113	16.18	1.03E-04***	6.72	0.01 [‡] *
	Habitat	2,113	5.40	0.01*	6.26	0.04 [‡] *
Metatarsal III	Padstate	1,112	9.04	3.24E-03**	0.76	0.38 [‡]
	Habitat	2,112	6.49	2.13E-03**	21.95	<0.01 [‡] **
		2,112			8.90	0.01 [‡] *
Metatarsal IV	Padstate	1,115	7.88	0.01*	2.62	0.11 [‡]
	Habitat	2,115	11.60	2.56E-05***	23.29	<0.01 [‡] **
Metatarsal V	Padstate	1,114	5.25	0.02*	2.73	0.10 [‡]
	Habitat	2,114	3.12	0.05	5.98	0.05 [‡]
Phalanges of digit I	Padstate	1,114	10.13	1.90E-03***	10.13	<0.01**
	Habitat	2,114	1.14	0.32	2.27	0.32
Phalanges of digit II	Padstate	1,115	21.72	8.71E-06***	8.22	<0.01 [‡] **
	Habitat	2,115	3.93	0.02*	2.90	0.23 [‡]
Phalanges of digit III	Padstate	1,116	15.48	1.44E-04***	15.48	<0.01**
	Habitat	2,116	1.03	0.36	2.06	0.36
Phalanges of digit IV	Padstate	1,114	16.02	1.14E-04***	8.41	<0.01 [‡] **
	Habitat	2,114	2.48	0.09	2.36	0.31 [‡]
Phalanges of digit V	Padstate	1,114	10.82	1.35E-03**	4.07	0.04 [‡] *
	Habitat	2,114	2.89	0.06	2.63	0.27 [‡]
Sum of interdigital angles	Padstate	1,114	9.07	3.22E-03**	10.00	1.57E-03 [‡] **
	Habitat	2,114	0.61	0.54	0.93	0.63 [‡]
Interdigital angle (II–III)	Padstate	1,114	11.52	9.47E-04***	11.52	6.88E-04 [‡] ***
	Habitat	2,114	0.96	0.39	1.92	0.38 [‡]
Interdigital angle (III–IV)	Padstate	1,112	0.83	0.36	1.56	0.21 [‡]
	Habitat	2,112	1.33	0.27	2.50	0.29 [‡]
	Interaction effect	2,112	5.40	0.01*	8.61	0.01 [‡] *
Interdigital angle (IV–V)	Padstate	1,113	9.63	2.41E-03**	9.63	1.91E-03**
	Habitat	2,113	2.32	0.10	4.65	0.10
Interdigital angle (I–V)	Padstate	1,113	6.97	0.01*	7.67	0.01*
	Habitat	2,113	2.79	0.07	5.56	0.06
Bilateral asymmetry	Padstate	1,113	1.01	0.32	1.13	0.29
	Habitat	2,113	5.86	3.80E-03**	9.04	0.01*
Sum of interdigital angles (I–IV)	Padstate	1,113	7.74	0.01*	7.75	0.01 [‡] *
	Habitat	2,113	0.50	0.61	0.80	0.67 [‡]

[‡] Indicates where phylogenetic correction was necessary.

* $p < 0.5$, ** $p < 0.05$, *** $p < 0.005$.

represent greater angles between digits I and II and between digits III and IV and a smaller interdigital angle between digits IV and V, as well as less divergence from bilateral symmetry. With the exception of the most lateral (postaxial) interdigital angle (IV–V), all traits have phylogenetic signal (Table 2). Angular approximations of bilateral asymmetry have weak phylogenetic signal. Body size (SVL), and the remainder of the interdigital angles, angular measurements, and the sum of phalangeal lengths, have moderate signals ($0.3 < \lambda < 0.6$). Metatarsal

lengths, digit lengths, and the sum of angles between digits I and IV have strong phylogenetic signals ($\lambda > 0.6$).

The relationship of habitat to the phalangeal and metatarsal moieties depends on padstate (PC1; Table 6; Figure 9). For the arboreal and saxicolous habitats, padbearing lineages have shorter metatarsal and phalangeal moieties than padless lineages ($p < 0.05$, Tukey post hoc test). When only padbearing lineages are examined, geckos with arboreal habits have shorter metatarsal and phalangeal moieties than

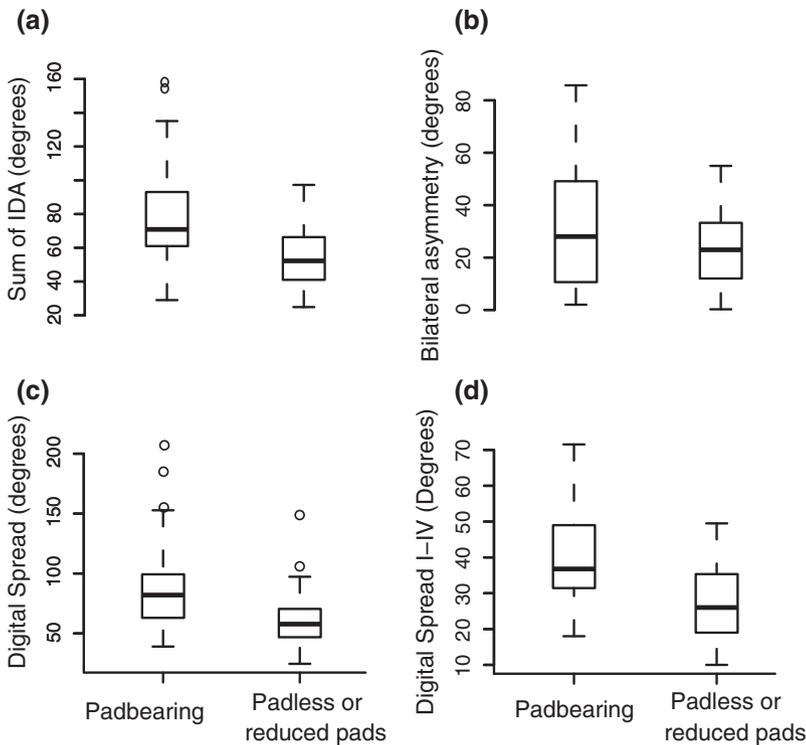


FIGURE 6 Box plots of the sum of total interdigital angles (A), bilateral asymmetry (B), digital spread (I-V) (C), and digital spread (I-IV) (D). Error bars indicate one standard error (SE)

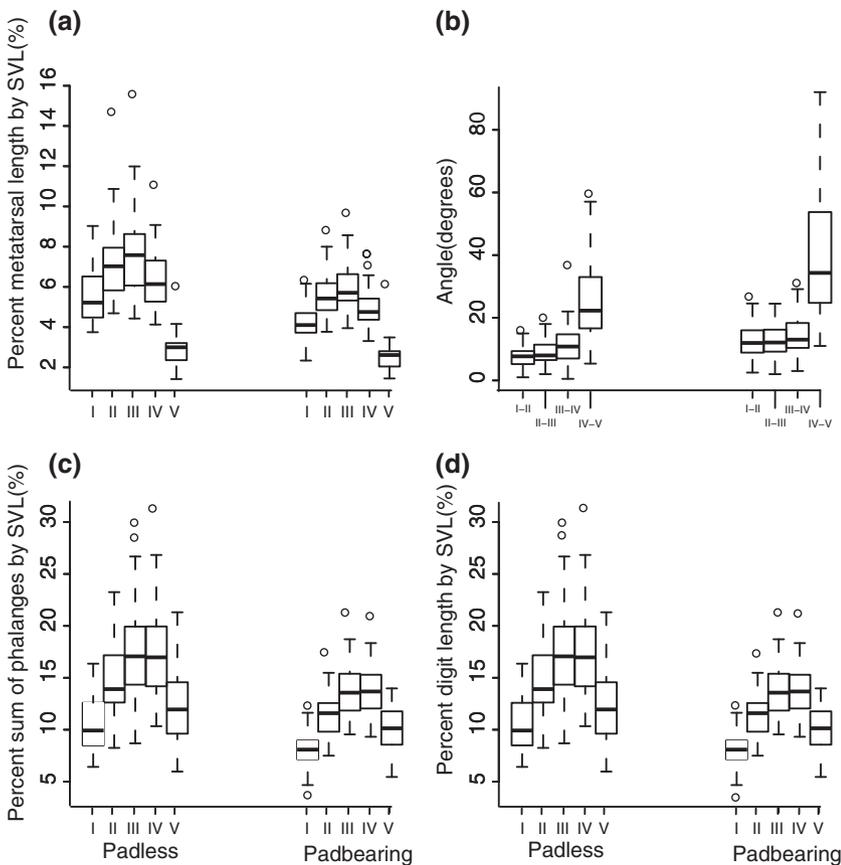


FIGURE 7 Box plots of relative metatarsal lengths (A), interdigital angles (B), sum of phalangeal lengths (C), and total digit length (D) for each digit and interdigital angle in padless and padbearing lineages

those occupying saxicolous habitats and padbearing arboreal lineages differ from all padless lineages (Figure 9). Neither habitat nor padstate is correlated with the magnitude of interdigital angles (PC2; Figure 9; Table 6).

Analysis of our Gekkonidae only data (Supplementary Online Material, Document S1) revealed nearly identical distributions of species along each PC to that of the complete dataset but permits clarification of several of the patterns of morphological evolution suggested

TABLE 4 Intercorrelations of interdigital angles

Interdigital angle	IDA (I–II)	IDA (II–III)	IDA (III–IV)
IDA (I–II)			
IDA (II–III)	0.32***		
IDA (III–IV)	0.09***	0.1***	
IDA (IV–V)	0.04	0.06	0.15***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 5 PCA loading scores of the traditional morphometric dataset

Variable	PC1	PC2	PC3
Interdigital angle (I–II)	–0.05	–0.27	0.51
Interdigital angle (II–III)	–0.09	–0.31	0.44
Interdigital angle (III–IV)	–0.01	–0.39	–0.07
Interdigital angle (IV–V)	–0.08	–0.41	–0.34
Bilateral asymmetry	–0.05	–0.36	–0.52
Sum of interdigital angles	–0.08	–0.47	0.06
Metatarsal I	0.34	–0.06	–0.08
Metatarsal II	0.33	–0.01	–0.18
Metatarsal III	0.32	0.06	–0.2
Metatarsal IV	0.28	0.2	0.01
Metatarsal V	0.28	0.1	0.15
Phalanges of digit I	0.29	–0.1	0.1
Phalanges of digit II	0.32	–0.13	0.08
Phalanges of digit III	0.36	–0.14	0.03
Phalanges of digit IV	0.31	–0.13	0.09
Phalanges of digit V	0.28	–0.19	0.12

Bolded values indicate a loading score > 0.3 .

by the latter and allows for an enhanced appreciation of trends associated with phylogeny.

3.6 | Geometric morphometric analysis

Using the broken stick model, the first two PCs were retained for analysis, and collectively, these account for 77.24% of the variance. The first PC (45.91%) describes changes in the interdigital angles, shortening of the metatarsals, and an increase in the distance between the heads of the metatarsals (Figure 10). Positive scores along the first PC axis describe shapes in which the landmarks demarcating the proximal metatarsal heads are closer together and the interdigital angles are smaller. The second PC axis (19.73%) similarly describes changes in the interdigital angles, the spacing of the metatarsal heads, and changes in the relative length of the fourth metatarsal. Higher scores on the second axis correspond to greater distances between the landmarks demarcating the proximal metatarsal heads, larger interdigital angles, and a relatively longer fourth metatarsal in

comparison to the other metatarsals. Foot shape has a relatively strong phylogenetic signal (Blomberg's $K = 0.853$, $p = 0.001$).

Padstate segregates along the first PC axis, but habitat does not (Table 6). For PC2, the effect of habitat depends upon padstate. Only arboreal padbearing species have a more asymmetrical foot shape and greater interdigital angles than do arboreal padless species. Among padless species, arboreal species have greater scores along PC2 than do saxicolous species ($p < 0.05$, post hoc Tukey test). Within padbearing species, arboreal ones have higher scores along PC2 than terrestrial species but are not different from saxicolous ones. Finally, saxicolous padbearing species have higher scores on PC2 than do saxicolous padless species ($p < 0.05$, post hoc Tukey test).

Centroid size is a good indicator of overall size (SVL; $R^2 = 0.71$, $p = 0.001$ with phylogenetic correction), thus allowing us to examine changes in shape with size. Shape is not associated with changes in size ($t = 1.152$, $p = 0.35$ with phylogenetic correction). Foot size, as assessed by the centroid size of foot shape, is also not associated with padstate or habitat ($X^2_{(2,5)} = -0.99$, $p = 0.325$ with phylogenetic correction and $X^2_{(2,5)} = 1.42$, $p = 0.163$ with phylogenetic correction, respectively).

When we controlled for centroid size as a covariate, padbearing species are, overall, different from padless lineages (Procrustes ANCOVA: $F_{(2,5)} = 4.56$, $p = 0.001$). Padbearing species have more equitably distributed interdigital angles. Foot shape also differs depending on habitat ($F_{(2,5)} = 3.69$, $p = 0.001$). A post hoc test at $p < 0.005$ shows that saxicolous and arboreal species are not significantly different from each other, but that both are different from terrestrial lineages. Rate ratios of morphological evolution are not significantly different between padstates ($R = 1.07$, $p = 0.64$).

When we controlled for centroid size as a covariate, padbearing lineages exhibit less asymmetry than padless lineages, and a posthoc test shows that saxicolous lineages have lesser degrees of asymmetry than do arboreal or terrestrial species, as assessed using geometric morphometric methods ($F_{(2,5)} = 7.3232$, $p = 0.000$ and $F_{(2,5)} = 7.43$, $p = 0.002$, respectively, post hoc Tukey test $p < 0.05$). However, arboreal padbearing species have approximately the same degree of symmetry as saxicolous padbearing species. There are few terrestrial padbearing species in our sample; however, therefore, padless lineages may be driving the observable differences in symmetry among arboreal, saxicolous, and terrestrial lineages. Furthermore, PC1 is not correlated with asymmetry ($R^2 = 0.01$, $p < 0.451$). PC2 scores are negatively correlated with asymmetry ($R^2 = 0.4553$, $p < 0.0001$).

Analysis of Gekkonidae only data (Supplementary Online Material, Document S1) remains generally the same as that for the entire dataset, but the separation of padstates is clearer and significant differences in symmetry among padstates are not evident.

4 | DISCUSSION

We expected the morphology of the gecko pes to be impacted by the functional demands imposed by the adhesive system, as well as the demands imposed by the habitat in which they live. We used a

TABLE 6 Results of ANOVAs performed on the first two or three principal components

	Principal component	Variance explained (%)	Effect	df	F	p-value	F ^a	p-value ^a
Traditional morphometric analysis	PC 1	40.8	Padstate	1,96	25.3	<0.00001 ***	5.11	0.02 ^{b*}
			Habitat	2,96	0.21	0.81	2.96	0.23 ^b
			Interaction effect	2,96			6.82	0.03
	PC 2	23.38	Padstate	1,98	2.6	0.11	3.96	0.047*
			Habitat	2,98	3.08	0.05	4.48	0.11
	PC 3	10.65	Padstate	1,98	1.2	0.28	1.15	0.28
Habitat			2,98	7.96	0.0006***	9.19	0.01*	
Geometric morphometric analysis	PC 1	45.91	Padstate	1,50	8.54	6.32E-04***	18.53	9.47E-05 ^{b***}
			Habitat	2,50	5.23	0.03*	2.61	0.11 ^b
	PC 2	19.73	Padstate	2,48	17.59	1.14E-04***	8.49	3.57E-03**
			Habitat	1,48	16.16	4.07E-06***	22.86	1.08E-05***
			Interaction effect	2,48	4.66	1.40E-02**	7.92	0.02*

^aPhylogenetically corrected results.

^bIndicates where phylogenetic correction was necessary.

* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

phylogenetic framework and geometric morphometrics to characterize the morphology of the gecko pes in species that (i) have never had an adhesive system, (ii) have a well-developed adhesive system, and (iii) have secondarily reduced or lost adhesive capabilities. We found that both habitat and the presence or absence of an adhesive system influence how the foot is shaped. Thus, the results support our hypothesis that the acquisition of a morphological novelty can be accompanied by different trajectories of morphological change. Shifts to a padbearing state are accompanied by different trends in changes of digital morphology and arrangement.

4.1 | Evolution of digit morphology as revealed by traditional morphometric analysis

We predicted that padbearing species would express digit morphologies that facilitate the function of the adhesive system. Specifically, we expected that, in comparison to padless lineages, padbearing lineages would have shorter digits and a greater digital spread, features that would enhance the functionality of a directional adhesive system (Autumn, Niewiarowski, & Puthoff, 2014) and furnish space to circumvent the potential for the overlapping of expanded toepads, thereby allowing all toes to adhere simultaneously (Russell et al., 1997; Russell & Oetelaar, 2015; Webster, Johnson, & Russell, 2009). Our results generally support this prediction, but we found considerable overlap in morphology between padbearing and padless lineages across the entirety of the Gekkota. Padless lineages residing in the region of overlap belong to the Carphodactylidae, some species of the Eublepharidae, and several species of the *Pachydactylus* radiation thought to be secondarily padless.

By tracing the traits along phylogenetic branches (Bergmann, 2015), we identified multiple trajectories that have led to a padbearing

morphology (Figure 8). Within the Pygopodoidea, diplodactylids do not have larger interdigital angles than those of their sister clade, the carphodactylids and primarily differ from carphodactylids by having shorter digits. Simultaneous decrease in digit lengths and increase in digital spreading, in contrast, occurs in the Gekkonidae and Phyllodactylidae. The leaf-toed geckos in the Diplodactylidae appear to have shorter digits and have less variation than the basally padded geckos within the family. The placement of the four species of the Sphaerodactylidae suggests that padbearing lineages within this family have slightly larger interdigital angles than padless lineages, but little change in digit lengths within the family. Finally, within the gekkonid clade containing *Stenodactylus*, *Agamura*, *Bunopus*, *Cyrtopodion*, *Cyrtodactylus*, and *Hemidactylus* (Figure 1), the latter overlaps with the padless lineages of this group but has relatively shorter digits. In comparison to padless lineages of other families, padless lineages of this group exhibit a large variation in interdigital angles. *Hemidactylus* is the only padbearing lineage within this clade, and the three sampled species occupy a much smaller region of the morphospace than the padless lineages. Examination of more species of *Hemidactylus* would help to clarify the direction of this transition from a padless state to a padbearing state. In each trajectory leading to a padbearing, morphology shifts in a similar direction are evident, indicating convergence in digit morphology. However, changes in the interdigital angles have not all occurred in the same direction. These results demonstrate different changes in morphology with transitions in padstate. Shortening of digit length is a key and common component associated with the origin of the gekkotan adhesive system, whereas changes in interdigital angles may or may not occur.

The morphological variation of pedal form among padbearing lineages is likely impacted by the function of the gecko adhesive system (Higham et al., 2015). When examined on a broad scale, however,

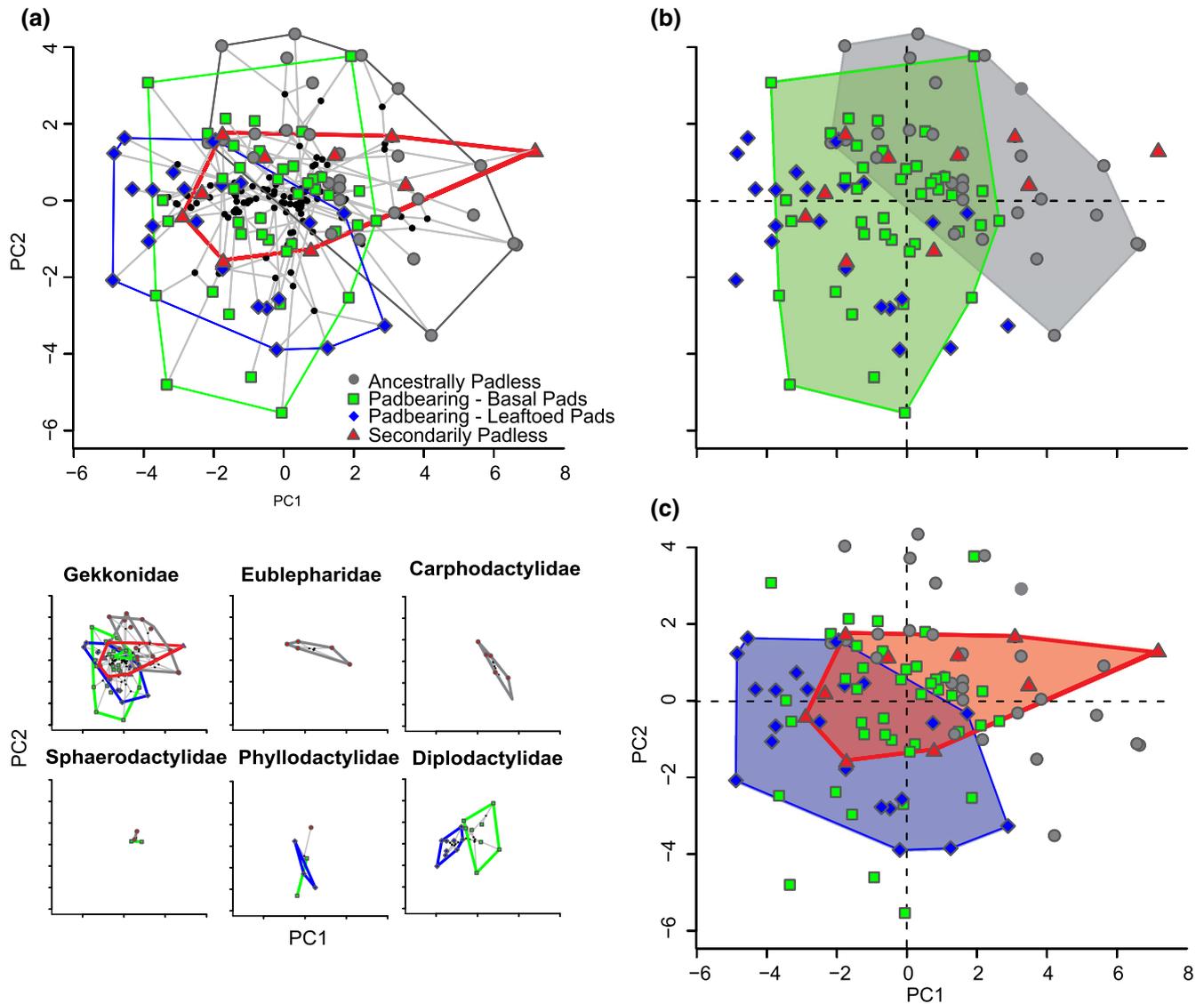


FIGURE 8 Principal components analysis (PCA) of 16 variables describing digit morphology. The phylogeny (light grey) is projected onto the morphospace. Minimum convex polygons outline ancestrally padless (dark grey), padbearing – basal pad (green), padbearing – leaf-toed (blue), and secondarily padless or reduced toepad lineages (red) (A). Morphospaces are shaded to illustrate overlap among ancestrally padless and padbearing – basal pad lineages (B) and padbearing – leaf-toed and secondarily padless or reduced toe pad lineages (C). Small graphs and polygons below the composite plot show the general distribution of the members of each family relative to the combined morphospace

there appear to be multiple outcomes to the acquisition of an adhesive system. This might explain the large region of morphospace occupied by padbearing lineages (Figure 8) and the lack of difference in the rates of morphological change between padbearing and padless lineages, in contrast to our prediction. Within the *Pachydactylus* radiation (as represented here by the clade bracketed by *Pachydactylus rangei* and *Rhoptropus afer*, Figure 1), the rate of morphological and kinematic evolution increases following the secondary loss of adhesion and reversion to a cursorial lifestyle (Higham et al., 2015). Perhaps, the ability to detect a rate shift relates to the relative age of the group and the fact that all species are relatively closely related. Although we did not find evidence for the relaxation of functional constraints, it is apparent that pad reduction or loss in gekkonids may lead to a regaining of a morphology similar to that of ancestrally padless

lineages. This is most obvious in *Rhoptropus afer*. The latter diverges from its congeners by having relatively longer digits and is unusual in that it is highly cursorial (Collins, Russell, & Higham, 2015; Higham & Russell, 2010; Johnson, Russell, & Bauer, 2005). However, longer digits may be achieved through differential lengths of the individual phalanges, which was not examined in our study (because there are differences in phalangeal formulae across the phylogeny of geckos; Bauer, Russell, & Powell, 1996).

In our study, the measurements of the pes of leaf-toed geckos do not differ significantly from those of basally padded lineages, despite apparent differences in toepad morphology. In comparison to basally padded geckos, the vasculature and muscular control systems of the digits in leaf-toed geckos are evidently different (Russell, 1979; Russell & Delaugerre, 2017). It is noteworthy that within the

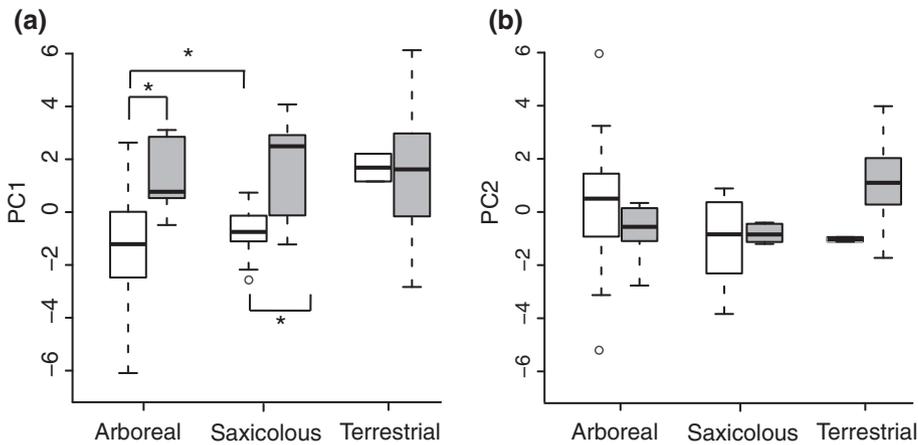


FIGURE 9 Box plots of the first two principal component axes (A and B, respectively) relative to habitat use (arboreal, saxicolous, and terrestrial) for padbearing (white) and padless (grey) lineages. Asterisks indicate that the bracketed groups are significantly different from each other based on a Tukey post hoc test ($p < 0.05$)

Diplodactylidae, *Strophurus*, *Oedura*, and *Diplodactylus* appear to segregate from the basally padded geckos within that family by having shorter digits, but such a pattern is not repeated in every family and, therefore, this shift might be attributable to the relatedness of these genera. Alternatively, differences between leaf-toed lineages and basally padded lineages may not have been captured by the measurements taken in this study. Instead, widths and shapes of the metatarsals, phalanges, and other elements of the pes may better distinguish the pes of leaf-toed geckos from those that are basally padded.

Unlike more proximal sectors of the limb, the foot interacts intimately with the substrate and is directly impacted by the functional demands of the locomotor surface imposed upon it (Higham, 2015). Foot width, which may serve as a proxy for the digital spread measured in this study, is narrower in arboreal lineages than terrestrial lineages of tropidurines (Grizante, Navas, Garland, & Kohlsdorf, 2010). Narrower soles may aid the pes in grasping in non-gekkotan lizards. However, because a large digital spread is beneficial to the deployment of the adhesive system, the hind feet of padbearing geckos do not exhibit a similar trend, but may instead exhibit similar changes to those found in the manus of several non-gekkotan lizards (*Anolis* and *Physignathus*) and geckos (Fontanarrosa & Abdala, 2016). Padbearing gekkotans have a greater interdigital angle I-V, which generally is associated with the ability to grasp in arboreal habitats. In our study, interdigital angle I-V of the pes was not found to be associated with habitat but was found to be greater in padbearing lineages than padless lineages. These findings together suggest similarities in morphological change of the manus and pes with transitions in padstate and indicate that the larger interdigital angles in the pes may relate to pedal grasping. They also indicate that the manus and pes in gekkotans serve similar functions in adhesive system deployment.

The trends in foot width we observed are also similar to those encountered in other arboreal taxa, such as squirrels and several lineages of frog (Mintsa, Berge, & Picard, 2009; Simons, 2008). In squirrels, foot length is also shorter in arboreal taxa. According to our analyses, traditional morphometric measurements reveal that within padbearing lineages, arboreal geckos have shorter metatarsals and summed lengths of phalanges than do other geckos. Abdala, Tulli, Russell, Powell, and Cruz (2014) did not find an association between

metatarsal dimensions and habitat in neotropical iguanian lizards. Therefore, our findings may be peculiar to geckos that are associated with the functional demands of the adhesive system. The results of other studies of gecko lineages are somewhat consistent with our findings. Differences between climbers and non-climbers were not able to be clearly delineated by Johnson et al. (2005) in the *Pachydactylus* radiation, but among neotropical geckos, climbing geckos have shorter digits and a shorter fifth metatarsal when compared to nonclimbing lineages (Rothier, Brandt, & Kohlsdorf, 2017). These latter results are compatible with our findings and suggest that this pattern is repeated across the gekkotan phylogeny. With the exception of *Rhoptropus afer*, the examined members of the genus *Rhoptropus* cluster together in morphospace, and this genus does not appear to differ greatly from the rest of the *Pachydactylus* radiation, which contrasts with the findings of Johnson et al. (2005) and Higham et al. (2015). This difference in outcomes may be attributable to the inclusion of interdigital angle data and slightly different length measurements, given that Johnson et al. (2005) measured segments of digits IV and V instead of all five digits. In other words, this broad-scale variation may wash out any variation within radiations of geckos. A generally consistent pattern, however, is that the sum of the lengths of the phalanges describes most of the variation in foot morphology among geckos. We therefore propose this to be a key aspect of functional divergence which requires further examination in future studies, especially with regard to proportions of phalangeal lengths within the digit, which were not examined in this study.

Instances exhibiting a markedly or completely reduced adhesive system (*Rhoptropus afer*, *Pachydactylus namaquenensis*, *Pachydactylus austeni*, and *P. rangei*) are situated in a region of morphospace that overlaps with both padless and padbearing lineages. These findings suggest that such lineages differ somewhat from ancestrally padless lineages in digit morphology and arrangement. They may retain shortened digits despite reducing or losing the adhesive system. Specifically, *P. rangei*, *P. namaquenensis*, *P. austeni*, *Colopus kochii*, and arguably *Cnemaspis psychadelica* (see below for further discussion) retain smaller interdigital angles and shorter digit lengths. Features associated with the adhesive system that are retained among lineages with a reduced or completely expunged adhesive apparatus also

include modifications of the phalanges associated with digital hyperextension. This pattern may be further reflected in skeletal, muscular, and external morphology (Russell & Rewcastle, 1979). However, *Rhoptropus afer* and arguably *Ptenopus* have markedly elongate digits even for ancestrally padless geckos.

In some respects, the expressions of padbearing or padless pedal morphologies depend on the context in which the adhesive system evolved. Within padbearing lineages, arboreal species tend to have shorter digits than saxicolous ones. How an evolutionary novelty is expressed is thought to be modulated in association with changes in habitat due to the intense selection pressure imposed by the latter (Hunter, 1998). Furthermore, within padless lineages, arboreal geckos have a different morphology than saxicolous ones. Therefore, it is likely that the change across this transition may be exaggerated in association with the evolution of the adhesive system.

4.2 | Evolution of the metapodium as revealed by geometric morphometric analysis

Results of our geometric morphometric analyses echo many of the findings revealed by our traditional morphometric approach, but we were also able to characterize the shape of the foot by encompassing the metatarsals and interdigital angles simultaneously, as well as by being able to incorporate data from the tarsals. Most importantly, this approach enabled us to identify changes in the distance between the articulation of the mesotarsal joint and the divergence of the metatarsal heads and to relate these to changes in padstate and habitat. An increase in this distance suggests that the third and fourth distal tarsal may be relatively larger in padbearing and arboreal lineages than in padless and terrestrial lineages. Change in the shape of the fourth distal tarsal is especially important because of its involvement in the mesotarsal ankle joint (Brinkman, 1980; Rewcastle, 1983). A relatively enlarged fourth distal tarsal could provide for a more expansive articular surface between it and the astragalocalcaneum and, therefore, increase the possible range of motion at this joint by providing a larger surface across which it can translate over the astragalocalcaneum (Rewcastle, 1980). Spacing of the landmarks on the proximal metatarsal heads could also change as a result of alterations to the fourth distal tarsal and thus underlie the shift to increased interdigital angles. Increased distance between the landmarks demarcating the proximal metatarsal heads is also a potential indicator of their lateromedial expansion, which may be an additional mechanism for increasing interdigital angles in padbearing lineages, and thereby a means of increasing the area of muscle and/or tendon insertion. For example, the plantar aponeuroses, responsible for supinating the limb and controlling sensors of digit five, has one branch that inserts onto the head of the fifth metatarsal (Russell, 1975; Russell, 1993). Further analyses of the shape of both the metatarsal heads and the elements involved in the mesotarsal joint are needed to clarify this.

Geometric morphometric analysis also enables a more comprehensive assessment of metapodium shape symmetry by considering not only the interdigital angles, but also the lengths of the metatarsals. This is important because asymmetry of the metapodium can be

brought about by changing the angles and/or the lengths of the digits (Figure 4). For example, if the fourth and fifth metatarsals are longer than the first and second, the shape of the metapodium is asymmetrical even if all interdigital angles are equal (Figure 4C). Therefore, asymmetry, when assessed by measuring angles only, may not be the most effective means of describing the distribution of the toepads overall and, instead, may only be a measure of the symmetry of the radiation of the digits. Our geometric morphometric analysis provides support for our prediction that the pes of padbearing species has a greater degree of symmetry than that of padless lineages when all gecko lineages are considered. These results also support the suggestions of Russell et al. (1997) that trends toward symmetry are present in ancestrally padless lineages.

We found that PC2 correlates negatively with our symmetry measure. However, this does not necessarily indicate that species located toward the positive end of PC2 have symmetrical metapodia. Instead, our analysis reveals several ways that asymmetry can be achieved. Species located at the negative end of PC2 achieve asymmetry by having a large postaxialmost (IV–V) interdigital angle, whereas on the positive side of PC2, asymmetry, which has a lower value, results from a longer fourth digit (Figure 10B). This latter region of the morphospace is occupied mostly by arboreal padbearing lineages, indicating shifts to secondary asymmetry.

4.2.1 | Patterns of metapodium asymmetry

The observation that secondary asymmetry of digit lengths occurs in some lineages, such as *Phelsuma*, may be a correlate of an adhesive system operating in the arboreal environment (Russell et al., 1997). This secondary asymmetry in digit length is different from that evident in ancestrally padless lizards in that it is associated with interdigital angles that are much greater than those exemplified by the latter and may also be associated with the vestigialization of the first digit (Russell et al., 1997; Russell & Bauer, 1990). Asymmetry resulting from the postaxialmost interdigital angle being the largest of the interdigital angles and the fourth digit being elongated, has several possible functional correlates, including the permitting of grasping between the first four digits and the fifth and/or facilitating propulsion and positioning the fifth metatarsal such that it can function like the calcaneal tuber of mammals (Robinson, 1975; Russell & Rewcastle, 1979; Zhuang & Higham, 2016). In the case of *Phelsuma*, grasping small perch diameters does not appear to function as described by Robinson (1975). Based on high-speed video of *Phelsuma* running across branches of small diameter, the longer fourth digit appears to facilitate the wrapping of the foot around the branch, such that grasping of the branch occurs between both hind feet (Zhuang & Higham, 2016).

In lizards with an asymmetrical pes, the distal tips of metatarsals I–III are aligned such that they form the metatarsophalangeal line, oriented perpendicular to the long axis of the body during stance (Rewcastle, 1983). This serves as an important contact with the substrate at the end of the propulsive phase of locomotion. Although symmetry within the foot reduces the number of digits involved in establishing the metatarsophalangeal line, secondary asymmetry may

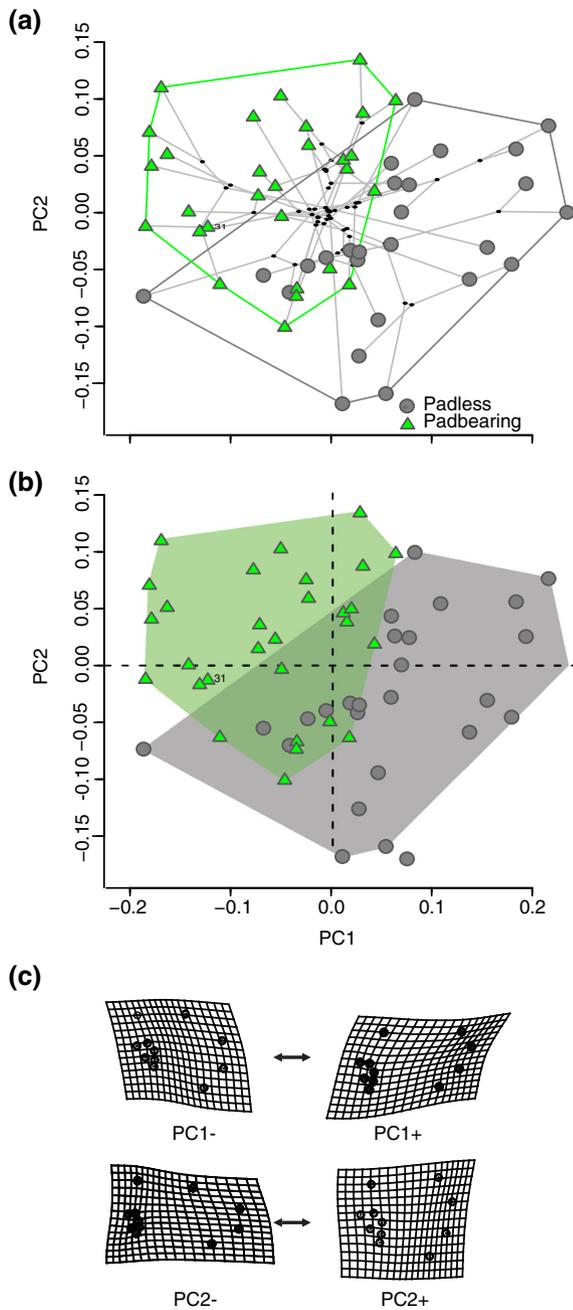


FIGURE 10 PCA performed on the 11 landmarks placed on the left hind foot (see Figure 2A), for 54 species of padless (dark grey) and padbearing (green) lineages. The phylogeny is projected onto the morphospace (light grey). Minimum convex polygons group the padless and padbearing lineages in the phylomorphospace (A) and are shaded to display the overlap in lineages in the original morphospace (B). Warp grids on each axis illustrate the shape of the minimum and the maximum PC score of that axis (C)

increase the number of distal metatarsal tips involved, thereby promoting an increase in propulsive forces. Thus, secondary asymmetry may facilitate propulsion on relatively broad substrates that are characteristic of many arboreal microhabitats, such as tree trunks.

Asymmetry resulting from a longer third digit and an unequal distribution of interdigital angles appears to be characteristic of more saxicolous lineages among both padbearing and padless species. However, it

is clear that arboreal padbearing species also have asymmetrical metapodia, albeit to a lesser extent than saxicolous species. When only padbearing lineages are considered, arboreal and saxicolous species exhibit similar values for asymmetry, but these are achieved via different pathways, as mentioned above. Our results indicate that the symmetry measure should be interpreted in the context of the region of the morphospace occupied, because similar values can be achieved in different ways (Johnson et al., 2005). It should also be noted that the geometric morphometric assessments only consider the arrangement of the tarsals and metatarsals and not the digit tips. Therefore, the shape analyzed is a descriptor of the shape of the proximal part of the autopodium (including the metapodium) rather than the entire foot.

4.3 | Secondarily padless species

Special consideration must be given to certain taxa that may be equivocal for their categorization as being secondarily padless. According to Gamble et al. (2012), the ancestral padstate for the following is considered equivocal: *Ptenopus*, *Narudasia*, and *Nactus*. Furthermore, a more recent ancestral state reconstruction analysis (Gamble et al., 2017) interprets the ancestral padstate for *Homonota* as equivocal, whereas Gamble et al., 2012 indicate *Homonota* to be secondarily padless. The more recent ancestral state reconstruction analysis (Gamble et al., 2017) also indicates that *Narudasia festiva*, *Ptenopus*, and SE Asian *Cnemaspis* are secondarily padless. There does not, however, appear to be any morphological evidence that these purported “secondarily padless species” once had an adhesive system (Russell & Gamble, 2019). Therefore, all of these taxa can be alternatively interpreted as being either ancestrally or secondarily padless. In the traditional morphometric morphospace (Figure 8), *Ptenopus kochi* is characterized as having the longest digits (PC1) of any of the padless species in our sample, including the ancestrally padless species. If *P. kochi* is secondarily padless, it would represent an extreme case of reversal because it resides squarely within the region of the majority of the ancestrally padless species. Our results do not refute the hypothesis that *Narudasia festiva* and *Cnemaspis psychadelica* are secondarily padless species because both reside in the morphospacial area of overlap between padbearing and secondarily padless lineages. However, *Cnemaspis kendalii*, *Nactus*, and *Homonota* are located in the region occupied by the majority of the ancestrally padless species. These results do not eliminate the possibility that these species are secondarily padless because increased digit lengths and smaller interdigital angles occur in *Rhoptropus afer*, a definitively secondarily padless species. If they are secondarily padless, this would support the hypothesis that the secondary loss of the adhesive system drives increased digit lengths and decrease in interdigital angles.

4.4 | Effect of size

We identified a positive relationship between the magnitude of digital spreading, mediated by the increase in the first two interdigital angles (I–II and II–III), and body size (Figure 5). As body size increases, body mass and the forces acting on a lizard are expected to increase

disproportionately due to geometric similarity (Biewener, 2005). Therefore, an increased spread of the digits, corresponding to an increased range of the substratum in the vicinity over which adhesive and clinging mechanisms can be applied, would facilitate counteracting gravity in climbing situations. Therefore, an increased surface area of the foot overall does not necessarily equate to an increased surface area that is engaged at one time (i.e., the functional area; Imburgia, Kuo, Briggs, Irschick, & Crosby, 2019). Instead, for padbearing lineages, a greater spread of the digits may increase the potential zone into which a particular toepad may intrude without encroaching upon those of its neighboring digits (Webster et al., 2009). Thus, digital spreading permits the ability to increase the size of individual toepads (Webster et al., 2009). The importance of the spread of the digits for padbearing lineages is apparent, because it permits the engagement of the adhesive apparatus in a variety of orientations simultaneously (Birn-Jeffery & Higham, 2014; Russell & Oetelaar, 2015; Zhuang & Higham, 2016). Larger species may benefit from greater support provided by toepads applied in opposing directions, which may also facilitate changing directions quickly (Imburgia et al., 2019; Russell & Oetelaar, 2015).

This relationship between the magnitude of spreading of the digits and body size was also found among padless lineages, suggesting that it may be characteristic of gekkotans overall. One possible explanation is that the interdigital angles, which are affected by the shape of the metatarsal heads, may change with size due to their expansion. If this is so, we would expect a similar relationship to be found in non-gekkotans. Interdigital angles in non-gekkotans, with few exceptions (Kubo, 2010), have not been measured or documented, and their relationship to size has not been examined. Padless lineages rely primarily on claws when climbing, which are also directional, albeit with an interlocking clinging mechanism (Arnold, 1998; Cartmill, 1985; Zani, 2000). A greater spread of the digits would reduce the amount of support that the lizard could obtain from its claws, but this could be compensated for by the lizard's behavioral modulation of the digits, so that all digits are abducted and oriented as much as possible in the same direction.

As demonstrated by our geometric morphometric analysis, arboreal, ancestrally padless species, such as *Saltuarius salebrosus*, often have a highly symmetrical foot despite the absence of toepads. A study similar to that of Powell et al. (2018), examining ontogenetic growth in such species, with the simultaneous measurement of metatarsal head shape and interdigital angle measurement, could help determine the mechanisms underlying changes in interdigital angles. In addition, behavioral analyses, similar to that of Russell and Oetelaar (2015), would reveal how such species use their claws in combination with the broad digital spread that they possess (Naylor & Higham, 2019).

Morphological evolution of the pes of geckos is shaped by a number of factors. We have demonstrated not only repeated shifts to a padbearing morphology that are correlated with environment but also several cases of shifts to a reduction or total loss of toepads. We also characterize important principles of pedal morphology and demonstrate that symmetry and digital spreading are important components of the padbearing morphology when all limbed gekkotan families are considered. Geometric morphometric analysis further reveals

potential changes in more proximal elements of the foot (the tarsals and proximal heads of the metatarsals) that are highly relevant to locomotion in padbearing lineages and that affect the more distal elements of the foot. These results provide evidence of not only changes at the proximal end of the foot that may affect the morphology of distal elements but also the link between internal skeletal structure and external morphology. For both of these analyses, our examination of the data for the Gekkonidae alone (the most speciose family and the family with the greatest representation in our total dataset; see Supplementary Online Material, Document S3) allowed us to explore whether the results for other families obscured patterns unique to the Gekkonidae. As a result of these analyses, we found that symmetry may not be as important to padbearing lineages of the Gekkonidae and that, overall, there is less overlap between the morphologies of padbearing and padless lineages. We focused on preserved specimens in this study, but it is possible that geckos can, to some extent, actively modulate pedal symmetry. However, future studies are needed to determine the extent and functional implications of this.

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AUTHORS' CONTRIBUTIONS

Zhuang, Higham, and Russell developed the ideas for this study. Zhuang and Russell collected the data. Zhuang wrote the first draft of the manuscript, and Higham and Russell provided extensive revisions. The authors declare that there is no conflict of interest.

ORCID

Mingna V. Zhuang  <https://orcid.org/0000-0001-7928-4269>
 Anthony P. Russell  <https://orcid.org/0000-0001-6659-6765>
 Timothy E. Higham  <https://orcid.org/0000-0003-3538-6671>

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