

Phylogenetic history, allometry and disparate functional pressures influence the morphological diversification of the gekkotan quadrate, a keystone cranial element

DANIEL J. PALUH*¹ and AARON M. BAUER

Department of Biology, Villanova University, Villanova, Pennsylvania, USA

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The functional components of the vertebrate skull—including the chondrocranial braincase, protective dermatocranium and lower jaw—are interconnected and become operational through the quadrate bone. This latter element is critical for cranial biomechanics and support of the auditory system in squamate reptiles, but the interspecific variation of quadrate anatomy has not been studied in detail. Our objectives were to determine the relative influence of phylogenetic history, allometry and functional selection pressures on the morphological diversification of this keystone cranial element across gecko genera using high-resolution X-ray micro-computed tomography, three-dimensional geometric morphometrics and phylogenetic comparative methods. Our results demonstrate substantial variation in gecko quadrate morphology. Two families possess highly derived quadrate morphologies, while the remaining gekkotans retain extensive overlap in quadrate shape. Allometric scaling has influenced shape across species; distantly related miniaturized taxa possess elongate, slender quadrate morphologies while large taxa have robust, laterally expanded quadrates. The relative height of the coronoid eminence and the loss of the peripheral auditory system co-vary with quadrate anatomy, indicating that multiple disparate functional pressures may act on this element. Our study has identified the disparity of quadrate morphology within geckos and has highlighted the importance of considering multiple factors that may influence the diversification of phenotypes.

ADDITIONAL KEYWORDS: anatomy – computed tomography – cranial evolution – gecko – geometric morphometrics – skull.

INTRODUCTION

A long-standing challenge in evolutionary biology is the classification and interpretation of phenotypic diversity. Historically, researchers have experienced limitations in sampling taxa broadly, quantifying complex morphologies and disentangling the complex mechanisms responsible for generating macroevolutionary patterns. The diversification of phenotypes within a clade may be an adaptive response to ecological and functional pressures that can lead to divergence or convergence, depending on the selective regime (Losos, 1990; Grant & Grant, 2002). Phylogenetic and architectural constraints can have a restrictive influence, however, yielding phenotypic conservatism or allometric scaling of structures (*Konstruktions-Morphologie*; Seilacher, 1970; Gould & Lewontin,

1979). Furthermore, complex phenotypes, including the vertebrate skull, may be modular and partitioned into semi-independent blocks of traits, and these modules may be differentially influenced by historical, architectural and adaptive mechanisms (Drake & Klingenberg, 2010; Goswami & Polly, 2010; Felice & Goswami, 2017). By employing recent advances of micro-computed tomography, three-dimensional (3D) geometric morphometrics and phylogenetic comparative methods, we investigated the relative roles of phylogenetic history, allometry and functional pressures in the morphological diversification of a single unit of the lepidosaurian skull across the majority of gecko genera.

Gekkotans are one of the most successful vertebrate lineages, comprising more than 25% of all described lizard species. They are phenotypically diverse, varying greatly in body size, habitat and activity preferences, and diet (Daza *et al.*, 2009; Gamble *et al.*, 2012, 2015). Furthermore, the interrelationships of geckos are well understood and are highly supported in recent

*Corresponding author. Current address: Department of Biology and Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA. E-mail: dpaluh@ufl.edu

molecular phylogenies (Gamble *et al.*, 2012, 2015). The skull anatomy of geckos has been studied in multiple taxa (see Daza *et al.*, 2008: appendix), and a general reduction in the cranial bones occurs in nearly all species relative to the plesiomorphic squamate condition (e.g. loss of the postorbital and supratemporal bars), although the paired quadrate bones remain prominent and central to skull structure (Fig. 1).

The functional units of the lizard skull are divided into the chondrocranial braincase, protective dermatocranium and lower jaw (Rieppel, 1993; Evans, 2008). These regions are interconnected and become operational through a fourth functional unit, the quadrate bone. The quadrate is a paired bone that is located posterolaterally in the skull, flanking either side of the braincase. The overall structure of the quadrate is complex and has been variously described as auricle- (Oelrich, 1956), conch- (Earle, 1961), shell- (Daza *et al.*, 2008), lamina- (Evans, 2008) or even taco-shaped (J. D. Daza, pers. comm.). Generally, there is a medial column that supports dorsal (cephalic) and ventral (mandibular) condyles (Fig. 2). The lateral wing of the quadrate forms a posterior concavity (conch), and the midline of this structure supports the anterior edge of the tympanic membrane of the ear (Fig. 1), whereas the rest of the membrane circumference is supported by connective tissue (Wever, 1973). The stapes and extracolumella also possess either a direct contact or a soft-tissue connection with the quadrate. The cephalic condyle articulates with the paroccipital process of the otooccipital (chondrocranial braincase), as well as the supratemporal and squamosal (protective dermatocranium; although these two articulations are often lost in geckos), while the mandibular condyle articulates with the articular of the lower jaw. The medial surface has a reduced facet for articulation with

the pterygoid, dorsal to the mandibular condyle (Fig. 1). The anterior surface of the quadrate also acts as an attachment area for multiple cranial muscles, including the massive adductors, which function to close the jaw (Rieppel, 1984; Daza *et al.*, 2011). Because of these many connections, the quadrate is an important functional unit for support of the peripheral auditory system and for cranial biomechanics. The quadrate also plays a critical role in squamate cranial kinesis: anteroposterior translation of this element, or streptostyly, pulls the palatal bones of the skull posteriorly, which depresses the muzzle bones at the mesokinetic joint (ventroflexion between the parietal and frontal; Versluys, 1912; Frazzetta, 1962). Multiple hypotheses exist regarding the function of squamate cranial kinesis (Metzger, 2002), and of these, improved prey manipulation is the most well supported (Rieppel, 1978).

The functional demands placed on the quadrate are likely particularly high in gekkotans, as these animals are known to exhibit pronounced hearing sensitivity (Werner *et al.*, 2002, 2005) and cranial kinesis (Herrel *et al.*, 1999, 2000, 2007). While this element has been morphologically described for a few individual gekkotan taxa, a large-scale, comparative analysis has yet to be undertaken. The objectives of this study were to answer three main questions: (1) Are there differences in the shape of the quadrate among the different family-level clades of geckos, indicating an influence of phylogenetic history? (2) Do similarly sized species show comparable patterns in quadrate morphology across clades, demonstrating architectural constraints related to allometry? (3) Do hypothesized functional traits vary among species relative to quadrate morphology, suggesting that quadrate shape changes in response to functional selection pressures? Answering these questions will allow us to identify the disparity of

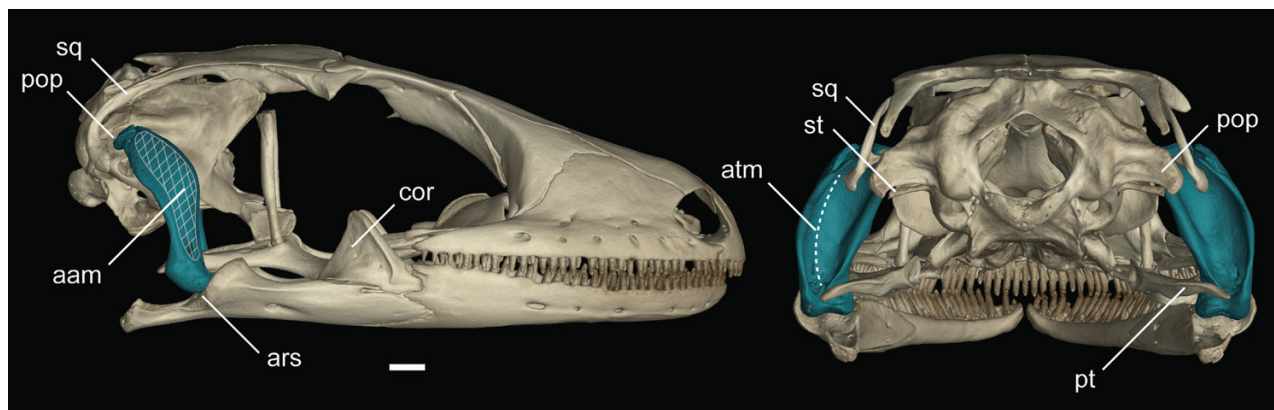


Figure 1. Skull of *Toropuku stephensi* (Diplodactylidae; CAS 47986) in lateral (left) and posterior (right) view. Quadrate is highlighted in blue. Abbreviations: aam, attachment area for adductor mandibulae muscles; ars, articular surface; atm, attachment area for tympanic membrane; cor, coronoid; pop, paroccipital process; pt, pterygoid; sq, squamosal; st, stapes. Scale bar = 1 mm.

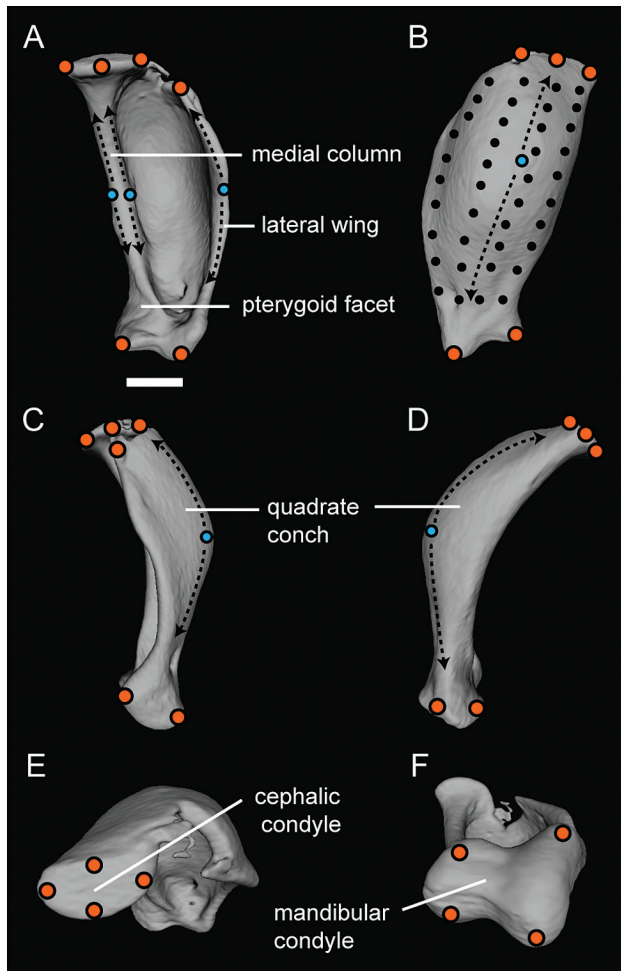


Figure 2. Quadrate of *Toropuku stephensi* (Diplodactylidae; CAS 47986) in posterior (A), anterior (B), lateral (C), medial (D), dorsal (E) and ventral (F) views. Three-dimensional landmarks digitized on quadrate shape files: nine fixed landmarks (orange points) that correspond to the cephalic condyle, mandibular condyle and dorsolateral edge of the lateral wing; four sliding semi-landmarks (blue points) that correspond to the maximal lateral edge of the quadrate conch, relative width of the medial column and anterior extent of the conch (dashed lines indicate the direction of the sliding semi-landmarks); and 40 surface semi-landmarks (black points) that correspond to the anterior surface of the quadrate conch. Scale bar = 1 mm.

quadrate morphology across geckos and better elucidate which processes have influenced the phenotypic diversification of this keystone cranial element.

MATERIAL AND METHODS

We investigated quadrate morphology within the Gekkota using high-resolution X-ray micro-computed tomography (CT). Specimens were scanned on an

Xradia MicroCT scanner (Pleasanton, CA, USA) at the University of Texas at Austin High Resolution X-ray CT-Facility (<http://www.ctlab.geo.utexas.edu/>). In total, 132 specimens representing all families and nearly all genera (116 of 125) were CT-scanned and included in our analyses (Supporting Information, Table S1). All individuals examined were believed to be adults – although a few subadult specimens may be included due to intraspecific variation in size at sexual maturity – as we have demonstrated elsewhere that ontogeny, but not sexual dimorphism, drives the intraspecific variation of quadrate morphology in a single species of gecko (Paluh *et al.*, 2018). Three-dimensional stereolithography (STL) files and CT settings are available to view and download from Duke University’s morphological data archive (<http://www.morphosource.org>).

A 3D model of the right quadrate was generated for each specimen using the segmentation tools in the software Avizo v.9.1 (VSG, Visualization Sciences Group, Burlington, MA, USA) and exported in polygon file format (PLY) for subsequent analyses. Three-dimensional geometric morphometric analyses were used to quantify variation in quadrate shape using the R package geomorph v.3.0.3 (Adams & Otarola-Castillo, 2013). Nine fixed landmarks, four sliding semi-landmarks and 40 surface semi-landmarks were digitized on each quadrate using geomorph. The fixed landmarks correspond to homologous and repeatable points of the quadrate: the cephalic condyle, mandibular condyle and dorsolateral edge of the lateral wing; the sliding semi-landmarks correspond to the medial column, maximal lateral edge of the lateral wing and maximal anterior extent of the conch; and the surface semi-landmarks correspond to the anterior surface of the quadrate conch (Fig. 2). A generalized Procrustes analysis was performed to align, rotate and scale specimens to a common coordinate system and unit-centroid size to remove variation in their position, orientation and size (Rohlf & Slice, 1990), resulting in a set of shape variables, or Procrustes tangent coordinates, for all specimens.

PHYLOGENETIC HISTORY

To visualize quadrate shape variation among all gecko species examined, we performed a principal component analysis (PCA) using the Procrustes tangent coordinates, and the Procrustes-aligned specimens were plotted in two dimensions of tangent space (PC1 and PC2) and grouped by clade membership. A multi-locus, genus-level molecular phylogeny for Gekkota (Gamble *et al.*, 2015) was pruned to correspond to the taxa in the shape variables data set and provided an estimate of the evolutionary relationships. Head length measurements and principal component scores were mapped onto the phylogeny using the R package phytools (Revell, 2012). We calculated the *K*-statistic’s

generalization for multivariate data (Kmult; Adams, 2014a) to determine the strength of phylogenetic signal in quadrature shape. The *P*-value evaluates the magnitude of phylogenetic signal in the set of shape variables and the Kmult statistic assesses the fit of a Brownian motion (BM) evolutionary model of trait evolution (Blomberg *et al.*, 2003). The magnitude of phylogenetic signal was evaluated for all taxa combined (Gekkota), as well as for each familial clade separately. To visualize the degree of phylogenetic signal, the gekkotan phylogeny was projected into quadrature morphospace by calculating ancestral states of the internal nodes through maximum likelihood, as implemented in geomorph. Analysis of covariance (ANCOVA; Collyer *et al.*, 2015) and phylogenetic least squares (PGLS; Adams, 2014b) tests were performed using the aligned Procrustes tangent coordinates to determine whether the seven families of geckos occupy different regions of quadrature morphospace (Table 1). Post-hoc pairwise comparisons of group means were calculated to determine which gekkotan clades have diverged from one another in quadrature morphology.

ALLOMETRY

We examined the influence of allometry by assessing the multivariate and phylogenetic regression between quadrature shape and quadrature centroid size (CS; Bookstein, 1991; method RegScore) based on Procrustes distances. A homogeneity of slopes (HOS) test and post-hoc pairwise slope comparisons were performed to determine if the seven families of geckos have diverged from one another in the allometric relationship of quadrature shape to size. A regression plot was generated to visualize the multivariate relationship between size and shape of gekkotan quadrates using 'RegScore' shape scores (Drake & Klingenberg, 2008) and ordinary least squares regression lines were generated for each family to visualize differences in slope. We further compared the relationship between quadrature size (centroid size) and quadrature shape (Regscore) to linear measurements of the head (log-transformed length, width and depth). Lastly, the interaction of quadrature centroid size and the main effects of the other ANCOVAs and PGLSs (clade membership, coronoid height, head depth, external ear presence) were also tested (Table 1).

FUNCTIONAL ASSOCIATIONS

Due to the lack of ecological (e.g. diet) and biomechanical (e.g. bite force) data available for geckos across all genera, we tested if quantifiable hypothesized functional traits co-vary among species relative to quadrature shape. Previous research has demonstrated that relative head depth and relative coronoid eminence

height may be a strong predictor of bite force and diet across many squamate groups (Herrel *et al.*, 2001; Herrel *et al.*, 2004; McBrayer, 2004; Metzger & Herrel, 2005; Lappin *et al.*, 2006), as skull height influences the orientation and length of the jaw adductors (which influences torque) and coronoid height corresponds to the available insertion area for the jaw adductors (Daza *et al.*, 2011). We measured head depth, coronoid height and head length of all taxa using the CT data and measurement tools in VGStudio Max 3.0 (Volume Graphics, Heidelberg, Germany). Both head depth and coronoid height were first divided by head length to remove the influence of overall skull size. An ANCOVA and PGLS were performed to determine whether a predicted relationship exists between quadrature shape and relative head depth and between quadrature shape and relative coronoid height (Table 1).

To determine whether a predicted relationship exists between quadrature shape and the peripheral auditory system of geckos, we conducted a survey of the presence/absence of the external ear system (external auditory meatus and tympanum) through examination of specimen photographs and transverse cross-sections of the X-ray tomograms. The pygopodids in the genus *Aprasia* have been previously described as possessing a rudimentary auditory system, lacking an external ear, tympanum and stapes (Shute & Bellairs, 1953; Manley & Kraus, 2010; Daza & Bauer, 2015), but this has yet to be reported in other gekkotans. An ANCOVA and PGLS were performed between gekkotans that possess the external auditory system and those that lack these structures (Table 1).

RESULTS

Substantial shape variation was observed across the 132 gekkotan quadrates we examined (Fig. 3). The carphodactylids possess a relatively large and robust quadrature that has a laterally expanded wing, wide medial column and expanded condyles (Fig. 3A–D), while the pygopodids have dorsoventrally inflected quadrates that contain either a large posterior concavity (Fig. 3F, H) or a bilaterally compressed column-like structure (Fig. 3E, G, I). The remaining five families possess quadrature shapes that are less distinct than the Carphodactylidae and Pygopodidae but vary in relative size of the cephalic and mandibular condyles, width of the medial column and lateral wing, and size of the posterior concavity (Fig. 3J–AR). The diversity of quadrature shapes observed in the Eublepharidae (Fig. 3S–W) and Phyllodactylidae (Fig. 3AD–AH) is lower than that in the Diplodactylidae (Fig. 3J–R), Sphaerodactylidae (Fig. 3X–AC) and Gekkonidae (Fig. 3AI–AR). The first two axes of the PCA explain 43% of the total shape variation observed in the

Table 1. Results from ANCOVA and PGLS tests comparing trends in quadrate shape divergence using familial clades, coronoid height, head depth and ear presence as the main effects, centroid size as the co-variate, and the interaction between each main effect and centroid size

Model	d.f.	SS	MS	R ²	F	Z	P value
Multivariation regression							
Centroid size	1	0.2114	0.21140	0.16103	24.952	7.3725	<0.001
Phylogenetic regression							
Centroid size	1	0.8210	0.82105	0.088493	12.621	5.3333	<0.001
ANCOVA (clade)							
Clade	6	0.28211	0.047019	0.214892	6.8294	4.5685	<0.001
Centroid size	1	0.12480	0.124802	0.095065	18.1272	14.0408	<0.001
Clade:centroid size	6	0.09349	0.015582	0.071213	2.2632	2.2093	<0.001
Residuals	118	0.8124	0.006885				
Total	131	1.3128					
PGLS (clade)							
Clade	6	0.3015	0.05025	0.032496	0.7991	7.1322	<0.001
Centroid size	1	0.7451	0.74507	0.080304	11.8492	12.3826	<0.001
Clade:centroid size	6	0.8118	0.1353	0.087497	2.1518	3.2744	<0.001
Residuals	118	7.4198	0.06288				
Total	131	9.2781					
ANCOVA (coronoid)							
Coronoid	1	0.09350	0.093497	0.071219	11.0621	8.2875	<0.001
Centroid size	1	0.114835	0.114835	0.087473	13.5866	10.9951	<0.001
Coronoid:centroid size	1	0.02261	0.02261	0.017223	2.6751	2.4515	0.008
Residuals	128	1.08186	0.008452				
Total	131	1.3128					
PGLS (coronoid)							
Coronoid	1	0.3586	0.35855	0.038645	5.6412	2.702	0.003
Centroid size	1	0.5768	0.57678	0.062116	9.0747	8.6154	<0.001
Coronoid:centroid size	1	0.2072	0.20716	0.022327	3.2593	1.3188	0.119
Residuals	128	8.1357	0.06356				
Total	131	9.2781					
ANCOVA (head depth)							
Head depth	1	0.01670	0.016698	0.012719	1.9545	1.4823	0.098
Centroid size	1	0.19222	0.192224	0.146422	22.5003	7.0006	<0.001
Head depth:centroid size	1	0.01036	0.010357	0.00789	1.2124	1.118	0.24
Residuals	128	1.09353	0.008543				
Total	131	1.3128					
PGLS (head depth)							
Head depth	1	0.596	0.59596	0.064233	10.579	0.3719	0.955
Centroid size	1	0.8235	0.82351	0.088758	14.618	5.8346	<0.001
Head depth:centroid size	1	0.648	0.64799	0.069841	11.503	0.495	0.926
Residuals	128	7.2107	0.05633				
Total	131	9.2781					
ANCOVA (ear)							
Ear	1	0.12362	0.123621	0.094165	15.9039	10.0102	<0.001
Centroid size	1	0.18551	0.185508	0.141307	23.8658	17.6793	<0.001
Ear:centroid size	1	0.00873	0.008734	0.006653	1.1237	0.9857	0.275
Residuals	128	0.99494	0.007773				
Total	131	1.3128					
PGLS (ear)							
Ear	1	0.7015	0.70152	0.075610	11.6539	8.2447	<0.001
Centroid size	1	0.7500	0.75000	0.080835	12.4592	12.1547	<0.001
Ear:centroid size	1	0.1215	0.12151	0.013096	2.0186	1.5736	0.089
Residuals	128	7.7051	0.0602				
Total	131	9.2781					

Boldfaced values indicate statistical significance.

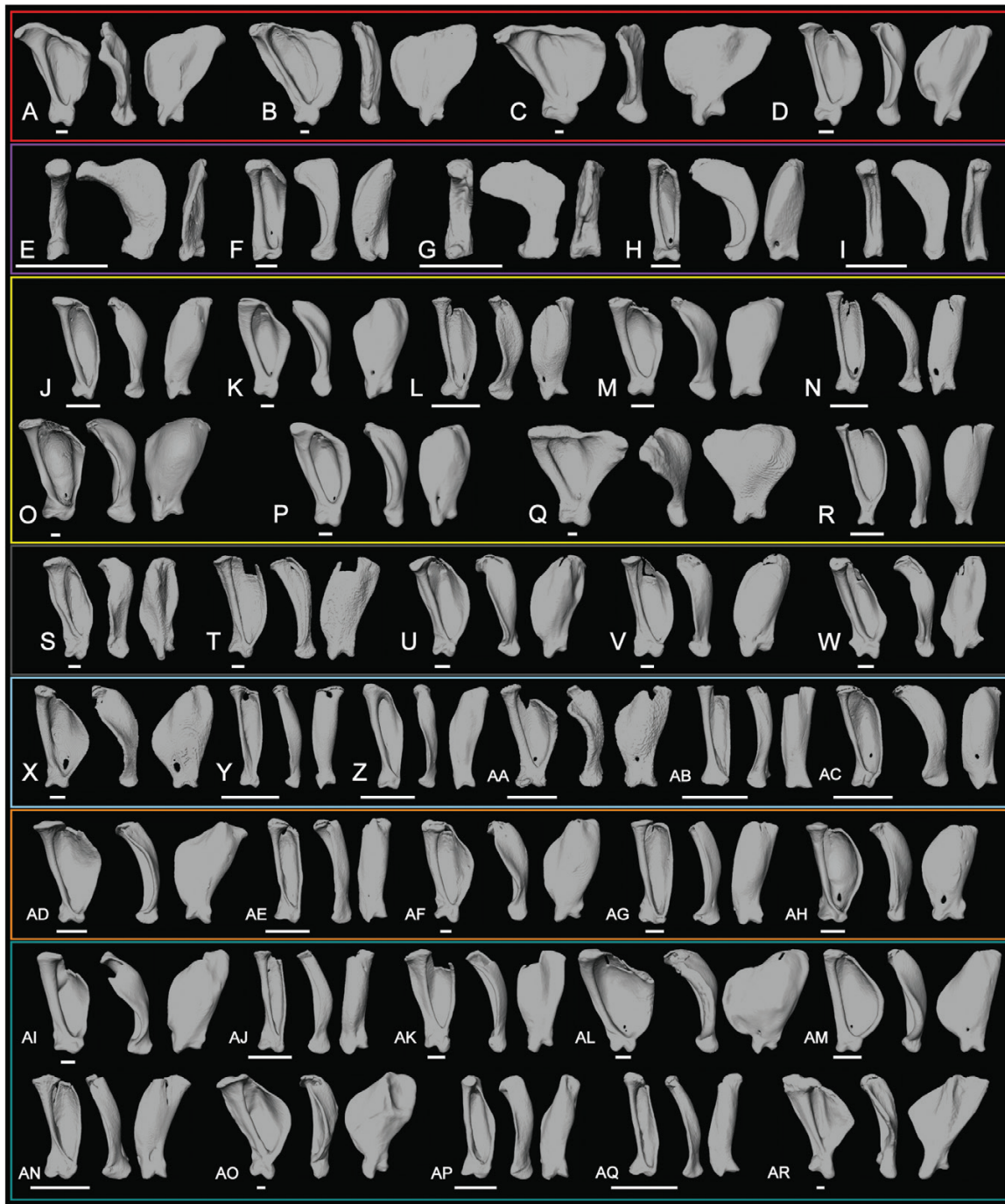


Figure 3. Representative quadrates from the seven families of geckos in posterior, lateral and anterior views from left to right. Carphodactylidae: A, *Carphodactylus laevis* (MCZ R-35114); B, *Nephrurus asper* (CAS 74733); C, *Saltuarius cornutus* (FMNH 57503); D, *Underwoodisaurus milii* (CAS 74744). Pygopodidae: E, *Aprasia repens* (CAS 104382); F, *Lialis burtonis* (FMNH 166958); G, *Ophidiocephalus taeniatus* (AMS R45179); H, *Paradelma orientalis* (CAS 77652); I, *Pletholax gracilis* (MCZ R-187676). Diplodactylidae: J, *Amalosa rhombifer* (CAS 100919); K, *Correlophus belepensis* (CAS 250865); L, *Crenadactylus ocellatus* (CAS 95287); M, *Dactylocnemis pacificus* (CAS 47979); N, *Dierogecko insularis* (AMS R161070); O, *Hoplodactylus duvaucelii* (CM 51270); P, *Mniarogecko jalu* (CAS 250858); Q, *Rhacodactylus auriculatus* (CAS 205486); R, *Rhynchoedura ornata* (UMMZ 124484). Eublepharidae: S, *Aeluroscalabotes felinus* (FMNH 146141); T, *Eublepharis macularius* (CM 67524); U, *Goniurosaurus kuroiwae* (CAS 198810); V, *Hemitheconyx caudicinctus* (CAS 165588); W, *Holodactylus africanus* (CAS 198932). Sphaerodactylidae: X, *Aristelliger georgeensis* (CAS 176485); Y, *Coleodactylus brachystoma* (UMMZ

quadrate across geckos (PC1 = 30% and PC2 = 13%). The PC1 axis describes shape differences driven by the relative expansion of the quadrate lateral wing: species with a negative PC1 score possess broad quadrates that have large lateral expansions while species with a positive PC1 score have narrow quadrates and no lateral expansion. The PC2 axis describes the shape differences driven by the degree of quadrate inflection: taxa with a negative PC2 score possess vertical quadrates and species with a positive PC2 score have dorsoventrally inflected quadrates.

PHYLOGENETIC HISTORY

Mapping these principal component scores onto the phylogeny of [Gamble *et al.* \(2015\)](#) indicates that there is generally little correspondence between the direction of PC values and clade membership, with the exception of the Carphodactylidae (red clade; [Fig. 4A](#)) and Pygopodidae (purple clade; [Fig. 4A](#)). The PCA plot further demonstrates that the gekkotan families are largely overlapping in morphospace, except for the Pygopodidae and Carphodactylidae, which are separated by the PC2 axis and PC1 axis, respectively ([Fig. 4B](#)). The pygopodids possess a dorsoventrally inflected quadrate, and the carphodactylids possess a robust, vertical quadrate that is characterized by a laterally expanded wing. The ANCOVA and PGLS indicated that quadrate shape divergence across the gekkotan families is significant ([Table 1](#)) and post-hoc pairwise comparisons of group means indicate that the Carphodactylidae and Pygopodidae possess derived quadrates, while the quadrate shapes of the remaining five families are not different from one another ([Table 2](#)). The *K*-statistic's generalization for multivariate data suggested that less phylogenetic signal is present than expected under a BM evolutionary model of trait evolution for Gekkota as a whole and the individual familial clades, although the Carphodactylidae and Eublepharidae approach significance ([Table S2](#), [Fig. S1](#)).

ALLOMETRY

The multivariate regression and phylogenetic regression between quadrate shape and centroid size exhibited significant allometry, indicating there is a quadrate shape to size relationship within the Gekkota ([Table 1](#); [Fig. 4C](#)). In general, small geckos

have narrow, slender quadrates and large geckos have robust, laterally expanded quadrates ([Fig. 5](#)). The HOS test suggested that the gekkotan families display differing patterns of allometry and post-hoc pairwise slope comparisons indicate that the Carphodactylidae have a distinct slope vector length from all other families, whereas the Pygopodidae have a different slope vector orientation compared to four of the families ([Table 3](#); [Fig. 4C](#)). The linear measurements of the head (log-transformed length, width and depth) were highly correlated with one another across all specimens in this study ($R^2 > 0.9$), and quadrate centroid size was highly correlated with these linear measurements ($R^2 > 0.9$; [Fig. 6](#)). This is in contrast to quadrate shape (Regscore), which was only weakly correlated with the head measurements ($R^2 < 0.7$; [Fig. 6](#)). A significant interaction between centroid size and clade membership was identified in both the ANCOVA and the PGLS ([Table 1](#)).

FUNCTIONAL ASSOCIATIONS

A predicted relationship was found between quadrate shape and size-corrected coronoid height ([Table 1](#)), suggesting that the morphology of the quadrate and coronoid may co-vary, independent of size, in response to functional selection pressures related to bite force and diet. As the relative height of the coronoid increases, the relative width of the quadrate increases. A significant interaction between centroid size and coronoid height was identified in the ANCOVA, but not in the PGLS. The size-corrected head depth ANCOVA and PGLS indicated that no relationship exists between quadrate shape variation and relative head depth variation ([Table 1](#)). External ears, tympana and stapes were present in all gekkotans, with the exception of three miniaturized pygopodid genera. *Aprasia repens* lacks all three of these structures, whereas *Ophidiocephalus taeniatus* and *Pletholax gracilis* possess a reduced stapes (footplate only) and lack a tympanum and external auditory meatus. The ANCOVA and PGLS indicated that quadrate shape divergence between non-tympanic pygopodids and the remaining gekkotans is significant ([Table 1](#)). The non-tympanic pygopodids possess dorsoventrally inflected, columnar quadrates that are mediolaterally compressed and lack a posterior concavity and lateral wing ([Fig. 3E, G, I](#)).

103051); Z, *Lepidoblepharis xanthostigma* (CAS 178104); AA, *Pristurus carteri* (CAS 225349); AB, *Saurodactylus fasciatus* (CAS 92404); AC, *Sphaerodactylus semasiops* (MCZ R-55766). Phyllodactylidae: AD, *Asaccus elisae* (CAS 218137A); AE, *Garthia gaudichaudii* (UMMZ 111574); AF, *Haemodracon riebeckii* (MCZ A-27255); AG, *Phyllodactylus baurii* (CAS 9501); AH, *Tarentola mauritanica* (CAS 87112). Gekkonidae: AI, *Ailuronyx seychellensis* (CAS 167459); AJ, *Alsophyllax pipiens* (CAS 143679); AK, *Calodactylodes aureus* (MCZ R-3918); AL, *Chondrodactylus bibronii* (CAS 173299); AM, *Crossobamon eversmanni* (CAS 180001); AN, *Ebenavia inunguis* (CAS 66195); AO, *Gekko gekko* (SHSVM-H-0001-2014); AP, *Hemiphyllodactylus typus* (CAS 174223); AQ, *Microgecko helenae* (CAS 120795); AR, *Uroplatus fimbriatus* (CAS-SU 13469). Scale bars = 1 mm.

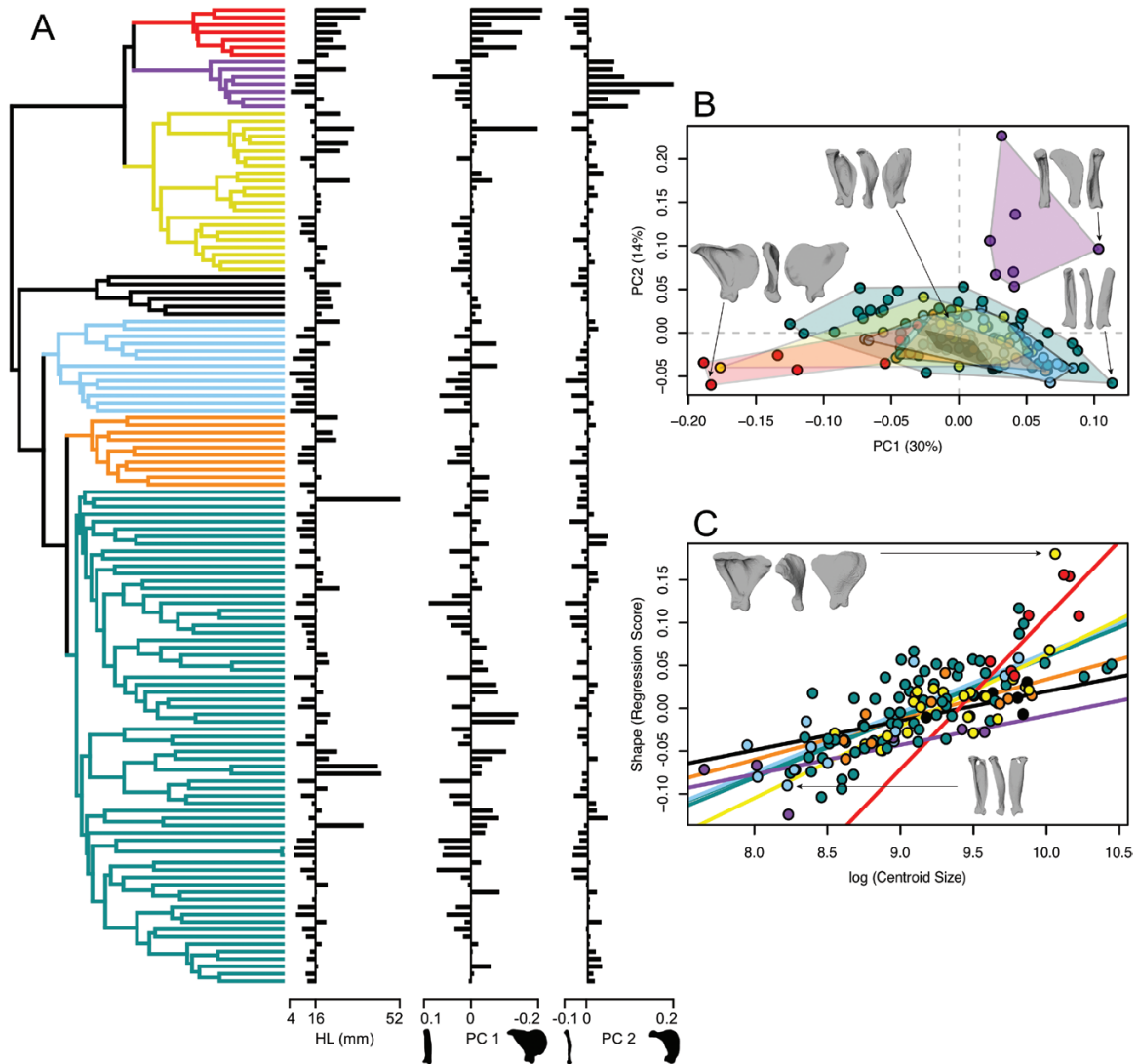


Figure 4. A, Head length (HL, mm) and quadrate principal component scores (PC1 and PC2) of all specimens mapped onto the genus-level phylogeny of Gamble *et al.* (2015) demonstrating little correspondence between clade membership and the direction of PC values, with the exception of the Carphodactylidae and Pygopodidae. Family-level clades are colour-coded: red = Carphodactylidae, purple = Pygopodidae, yellow = Diplodactylidae, black = Eublepharidae, blue = Sphaerodactylidae, orange = Phyllodactylidae, cyan = Gekkonidae. B, PCA plot of shape variation exhibiting the diversity of quadrate morphologies within geckos. Divergent quadrates are shown in posterior (left), lateral (centre) and anterior (right) views: *Saltuarius salebrosus* (CAS 74742; Carphodactylidae; bottom left), *Naultinus elegans* (CAS 47976; Diplodactylidae; centre), *Pletholax orientalis* (MCZ R-187676; Pygopodidae; top right), *Matoatoa breviceps* (MCZ R-190009; Gekkonidae; bottom right). C, multivariate regression between quadrate shape (RegScore) and quadrate centroid size. Ordinary least squares regression lines are displayed for each family to demonstrate the slope differences in Carphodactylidae and Pygopodidae. Quadrates are shown from a large species (*Rhacodactylus auriculatus*; CAS 205486; Diplodactylidae; top right) and a small species (*Coleodactylus brachystoma*; UMMZ 103051; Sphaerodactylidae; bottom left).

DISCUSSION

Through the use of micro-CT, 3D geometric morphometrics and phylogenetic comparative methods, we were able to begin disentangling the complex mechanisms responsible for generating the phenotypic diversity in a single

functional unit of the gecko skull. Our results indicate that phylogenetic history, allometry and functional demands have differentially influenced the diversification of gekkotan quadrate morphology. We discuss the results for our three main questions in further detail below.

Table 2. P-values for pairwise distances of group means associated with ANCOVA examining quadrate shape differences across gecko families

	Carph	Diplo	Euble	Gekk	Phyllo	Pygo	Sphaer
Carph	1	*	*	*	*	*	*
Diplo	<0.01	1	*	*	*	*	*
Euble	<0.01	0.1	1	*	*	*	*
Gekk	<0.01	0.09	0.3	1	*	*	*
Phyllo	<0.01	0.5	0.3	0.8	1	*	*
Pygo	<0.01	<0.01	<0.01	<0.01	<0.01	1	*
Sphaer	<0.01	0.06	0.2	0.2	0.6	<0.01	1

Carph, Carphodactylidae; Diplo, Diplodactylidae; Euble, Eublepharidae; Gekk, Gekkonidae; Phyllo, Phyllodactylidae; Pygo, Pygopodidae; Sphaer, Sphaerodactylidae.

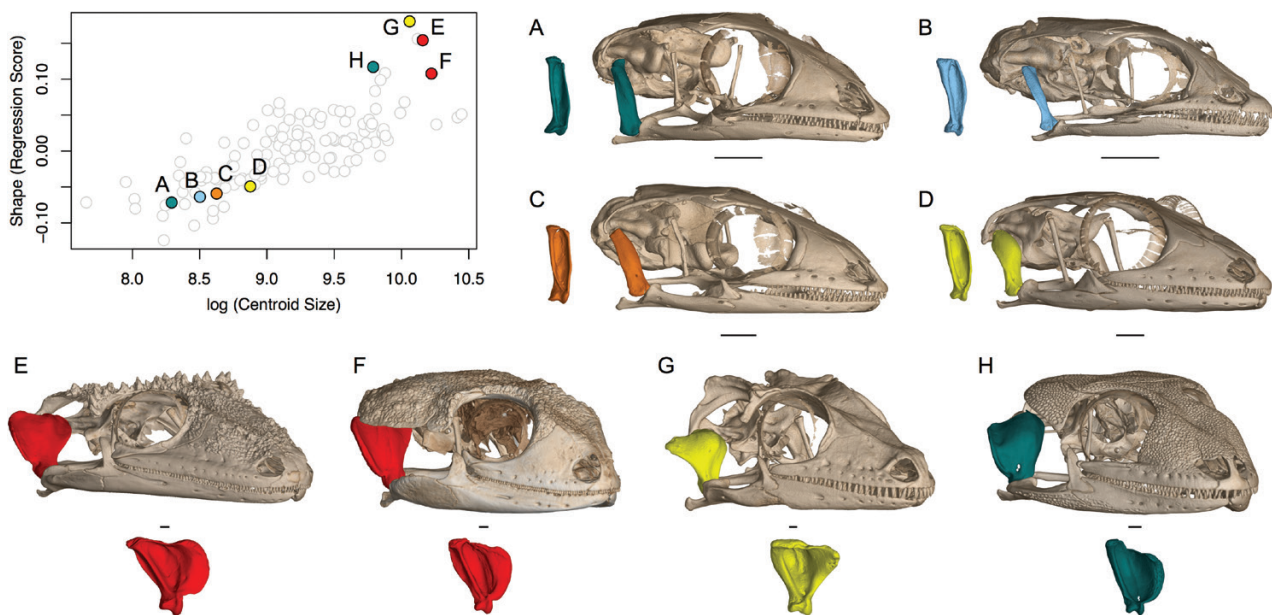


Figure 5. A–D, four miniaturized taxa from different families that possess elongate, slender quadrates: A, *Microgecko heleanae* [CAS 120795; Gekkonidae; 6.05 mm head length (HL)]; B, *Pseudogonatodes barbouri* (MCZR 14385; Sphaerodactylidae; 4.24 mm HL); C, *Garthia gaudichaudii* (UMMZ 111574; Phyllodactylidae; 7.88 mm HL); D, *Amalosa rhombifer* (CAS 100919; Diplodactylidae; 10.61 mm HL). E–H, four large taxa that possess robust, laterally expanded quadrates: E, *Saltuarius cornutus* (FMNH 57503; Carphodactylidae; 37.38 mm HL); F, *Nephrurus asper* (CAS 74733; Carphodactylidae; 28.77 mm HL); G, *Rhacodactylus auriculatus* (CAS 205486; Diplodactylidae; 32.27 mm HL); H, *Chondrodactylus bibronii* (CAS 173299; Gekkonidae; 21.63 mm HL). Inset: multivariate regression between quadrate shape and quadrate centroid size highlighting taxa A–H. See colour designations of Figure 4. Scale bars = 1 mm.

ARE THERE QUADRATE SHAPE DIFFERENCES AMONG DIFFERENT CLADES OF GECKOS?

The Pygopodidae and Carphodactylidae occupy unique areas of quadrate morphospace and are significantly divergent from other gekkotans in shape, suggesting that the quadrate structure of these two families are derived and have been influenced by their respective phylogenetic histories. All pygopodids possess a dorsoventrally inflected quadrate (Fig. 3E–I), while all carphodactylids possess a robust, vertical quadrate that is characterized by a laterally expanded crest

(Fig. 3A–D). Interestingly, these two families are sister lineages that are endemic to Australia (with one species of pygopodid in New Guinea), and both clades have also been shown to have an enlarged squamosal that participates in the formation of the posttemporal bar and may contact the quadrate more strongly (Stephenson, 1960; Bauer, 1986; Daza & Bauer, 2012). Phylogenetic signal was absent or less than expected under BM for Gekkota as a whole, as well as within each family. As might be expected, particularly given our dense sampling at the generic level, clades with

Table 3. *P*-values for pairwise slope comparisons associated with homogeneity of slopes tests examining quadrate allometry differences across gecko families; the first set of *P*-values is for differences in slope vector length (magnitude) and the second set is for slope vector orientation differences

	Carph	Diplo	Euble	Gekk	Phyllo	Pygo	Sphaer
Carph	1	*	*	*	*	*	*
Diplo	<0.01	1	*	*	*	*	*
Euble	<0.01	0.98	1	*	*	*	*
Gekk	<0.01	0.20	0.99	1	*	*	*
Phyllo	<0.01	0.22	0.87	0.75	1	*	*
Pygo	0.01	0.21	0.84	0.02	0.02	1	*
Sphaer	<0.01	0.70	0.99	0.36	0.36	0.11	1
Carph	1	*	*	*	*	*	*
Diplo	0.31	1	*	*	*	*	*
Euble	0.17	0.63	1	*	*	*	*
Gekk	0.57	0.22	0.32	1	*	*	*
Phyllo	0.23	0.50	0.52	0.62	1	*	*
Pygo	0.14	0.02	0.24	<0.01	<0.01	1	*
Sphaer	0.54	0.24	0.33	0.36	0.32	<0.01	1

Carph, Carphodactylidae; Diplo, Diplodactylidae; Euble, Eublepharidae; Gekk, Gekkonidae; Phyllo, Phyllodactylidae; Pygo, Pygopodidae; Sphaer, Sphaerodactylidae.

the most genera and sampled specimens (Gekkonidae, Diplodactylidae) occupy a larger amount of tangent space (convex hull area > 0.01) than the less speciose clades (convex hull area < 0.008) within the PCA plot (Fig. 4B).

Daza *et al.* (2009) conducted a 2D geometric morphometric analysis of the entire gekkotan skull, and similarly determined that pygopodids occupy a divergent region of morphospace due to lateral and dorsoventral compression of the skull. The disparity between pygopodids and other geckos in cranial anatomy may be due to many factors; however, the most likely influence is the possession of a highly derived, limb-reduced, serpentine form. Despite this extreme morphological modification, the Pygopodidae vary considerably in habitat specificity (Underwood, 1957; Gans, 1986), jaw mechanics (Patchell & Shine, 1986a), diet and foraging behaviour (Shine, 1986; Patchell & Shine, 1986b), and body size (Meiri, 2008). Therefore, we hypothesize that limb loss in this group was coupled with modifications to the skull as a whole, as well as to the quadrate specifically. Although subsequent morphological, functional and ecological diversification events took place, the Pygopodidae retain a quadrate morphology that is more similar among these species than to other gekkotans. The quadrates of the Carphodactylidae occupy a divergent region of morphospace that is characterized by a robust cephalic condyle, mandibular condyle and medial column, as well as a laterally expanded wing. We hypothesize that the disparity between carphodactylids and other gekkotans in quadrate morphology has been influenced by their phylogenetic history in relation to

both unique allometric scaling and cranial function related to foraging ecology. Members of this family are characterized by possessing relatively large bodies and disproportionately large, co-ossified heads (Bauer, 1986). It is likely that the robustness of the condyles and medial column are coupled with increases in ossification of the entire skull. Carphodactylids lack toepads, yet they have diversified into terrestrial, arboreal and rock outcrop habitat specialists (Cogger, 2014). Despite this diversity in habitat use, members of the Carphodactylidae retain similar dietary preferences in that they incorporate robust prey into their diet, including large scorpions, centipedes, cockroaches and even other lizards (Bauer, 1986, 1990a; How *et al.*, 1990; Doughty & Shine, 1995). The flaring lateral crest in this group is likely utilized to increase the insertion area available for cranial musculature, which would permit an increased bite force and ability to consume large prey. This is in contrast to most geckos, which typically forage on small-bodied insect groups and spiders and rarely take larger prey (Daza *et al.*, 2009). An alternative hypothesis is that the expanded lateral wing may be used to enhance sound detection, as for the pinna in mammals (Webster, 1966); however, auditory detection capabilities in carphodactylid geckos are completely unknown (Rohtla, 2016) but warrant investigation.

DO SIMILARLY SIZED SPECIES SHOW SIMILAR MORPHOLOGICAL PATTERNS ACROSS CLADES?

Our results indicate that gekkotan quadrate morphology is influenced by allometric scaling, as a positive relationship was identified between quadrate

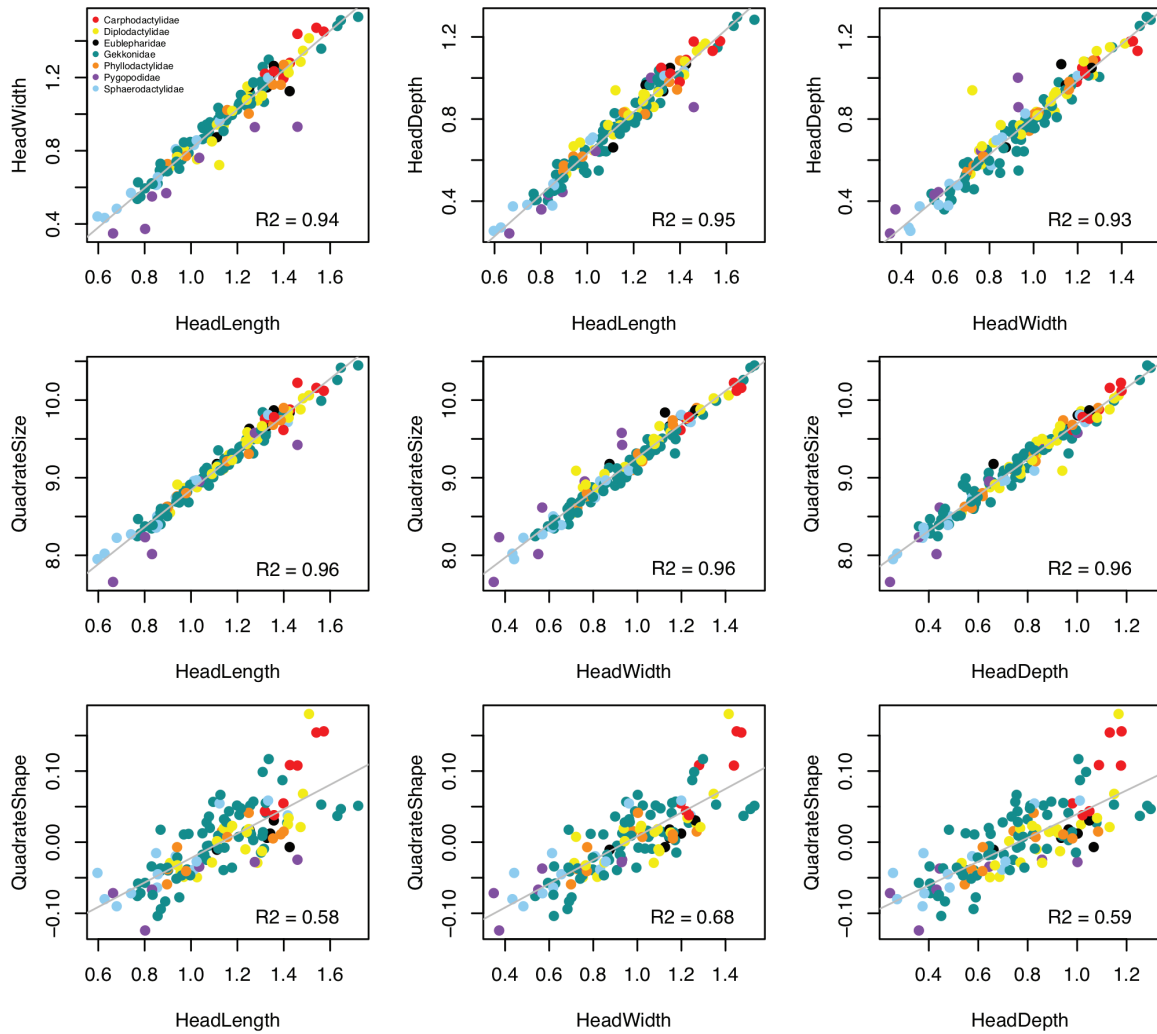


Figure 6. Regression relationships and ordinary least squares regression lines between: log-transformed head length, width and depth (top row), quadrate size (log-transformed centroid size) and log-transformed linear measurements of the head (middle row), and quadrate shape (RegScore) and log-transformed linear measurements of the head (bottom row).

shape and centroid size. The Carphodactylidae and Pygopodidae display differing patterns of allometry (Table 3, Fig. 4C), which may further explain the unique quadrate shape of these two families. We have previously shown that intraspecific quadrate shape variation in the gekkonid *Hemidactylus turcicus* is driven largely by allometric and ontogenetic change (Paluh *et al.*, 2018). The direction of shape change through ontogeny in *H. turcicus* is similar to the general pattern we have identified across differently sized species: small geckos have narrow, slender quadrates while large geckos possess laterally expanded quadrates (Fig. 5). The amount of quadrate shape diversity across all species examined in this study (in specimens ranging from 4.0 to 52.6 mm head length) is much greater than the shape change observed during the development of *H. turcicus* (in specimens ranging

from 8.9 to 16.4 mm head length; Paluh *et al.*, 2018). Most species in our current analyses are represented by one individual due to the broad taxonomic sampling across gekkotan genera; therefore, further work is needed to verify that ontogeny is the primary influence on intraspecific variation in quadrate morphology across other gecko species. The influence of allometric scaling on overall head shape has been previously identified in other lizards, including *Anolis* (Sanger *et al.*, 2011) and *Varanus* (Openshaw & Keogh, 2014). Furthermore, allometric and ontogenetic scaling have been identified in lizard functional traits supported by the quadrate, including auditory sensitivity (Werner & Igic, 2002; Werner *et al.*, 2002, 2005) and bite force (Meyers *et al.*, 2002; Verwajen *et al.*, 2002; Herrel & O'Reilly, 2006). Multiple studies have also identified ontogenetic variation in the diets of lizards, in which

juveniles eat smaller and softer prey than adults (Saenz, 1996; Angelici *et al.*, 1997; Herrel *et al.*, 2006; Whitfield & Donnelly, 2006). If allometric scaling in bite force exists across geckos due to diet variation, this may explain the significant association found between quadrate shape and the interaction of relative coronoid height and centroid size (Table 1). The relationship between quadrate centroid size and head dimensions is stronger than the association between quadrate shape and head dimensions (Fig. 6), indicating that head size alone can largely predict quadrate size, but that other factors are influencing quadrate shape. For example, there are miniaturized gecko genera across multiple families that possess a slender quadrate structure that lacks a laterally expanded wing, but variation remains between these taxa, including the relative size of the conch, cephalic condyle and mandibular condyle (Fig. 5A–D).

DO FUNCTIONAL TRAITS VARY AMONG SPECIES RELATIVE TO QUADRATE SHAPE?

A significant association was found between quadrate shape and relative coronoid height, suggesting that variation in quadrate morphology may be a response to selection pressures related to bite force and diet. The most extreme carphodactylid quadrates occupy a region of morphospace that is shared with one diplodactylid and two gekkonids that have converged on similar morphologies (Fig. 4B), including a large coronoid eminence (Fig. 5E–H). These taxa are also characterized by having disproportionately large heads and large body sizes. *Rhacodactylus auriculatus* (Diplodactylidae) has an extremely large quadrate lateral wing that flares anterolaterally into a triangular shape (Bauer, 1990b) and is very distant from any other diplodactylids in morphospace (Figs 3Q, 4B). Similar to the carphodactylids, multiple reports indicate that large prey items, including other lizards, are a substantial part of the *R. auriculatus* diet (Bauer & Sadlier, 1994; Snyder *et al.*, 2010). *Chondrodactylus angulifer* and *C. bibronii* (Gekkonidae) possess a lateral expansion that is rounded (Figs 3AL, 5H), and previous work has documented that these taxa also commonly forage on geckos and other large prey (Loveridge, 1947; Pianka & Huey, 1978). These data suggest that the unique quadrate morphologies of the Carphodactylidae, *Rhacodactylus* and *Chondrodactylus* may be the result of unique cranial functional demands. Although significant, the relationship between quadrate shape and relative coronoid height was weak (Table 1), and no relationship was found between quadrate shape and head depth. To better investigate if variation in quadrate morphology is driven by cranial biomechanical pressures, we suggest future work

quantifies cranial myology and bite force variation, as well as diet variation, across gekkotan genera.

A unique pattern was identified within the Pygopodidae in relation to quadrate morphology and the presence/absence of the external auditory system elements. Four surface-dwelling species (*Delma borea*, *Lialis burtonis*, *Paradelma orientalis* and *Pygopus lepidopodus*) possessed an external ear, tympanic membrane and fully formed stapes, while the remaining three fossorial species (*Aprasia repens*, *Ophidiocephalus taeniatus* and *Pletholax gracilis*) lacked the external ear and tympanum. The stapes and extracolumella are absent in *Aprasia* (Daza & Bauer, 2015) and are highly reduced in *Pletholax* and *Ophidiocephalus* (Figs 7A, S2). The rudimentary ear and derived quadrate morphology of *Aprasia* have previously been described (Shute &

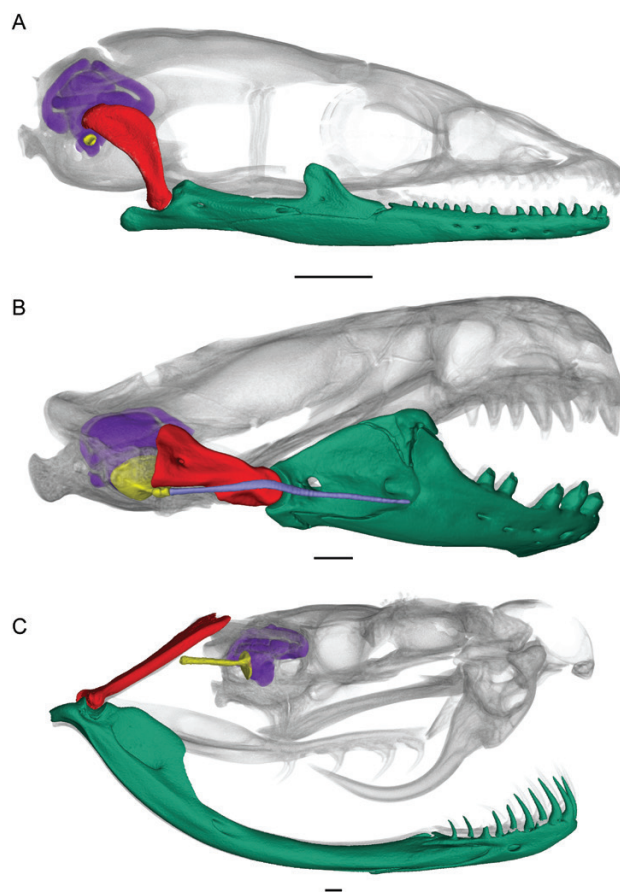


Figure 7. Three squamate groups that may use non-tympanic reception of substrate vibrations using divergent structural pathways between the lower jaw and inner ear: A, *Pletholax orientalis* (MCZ R-187676; Pygopodidae); B, *Amphisbaena fuliginosa* (UF 63167; Amphisbaenia); C, *Crotalus adamanteus* (UF 103268; Serpentes). Purple = inner ear endocast, yellow = stapes, blue = extracolumella, red = quadrate, green = lower jaw. Scale bars = 1 mm.

Bellairs, 1953; Manley & Kraus, 2010; Daza & Bauer, 2015), but these modifications have not been reported in *Pletholax gracilis* or *Ophidiocephalus taeniatus*. The tympanic pygopodids have dorsoventrally inflected quadrates that are characterized by a large posterior concavity and lateral crest, whereas the non-tympanic pygopodids possess columnar quadrates that are mediolaterally compressed and lack a posterior concavity and lateral expansion (Figs 3E–I, S2). Daza & Bauer (2015) suggested that *Aprasia repens* may possess poor sound-pressure detection but is sensitive to substrate vibrations if the modified quadrate is able to act as a structural link in transmitting low-frequency vibrations from the jaw to the inner ear. We hypothesize that *Aprasia*, *Pletholax* and *Ophidiocephalus* all utilize their quadrate for this function due to their anatomical similarities in quadrate structure, reductions in ear morphology, and tight articulation between the quadrate and paroccipital process. These miniaturized non-tympanic pygopodids are burrowing species (Rieppel, 1985; Shea & Peterson, 1993; Cogger, 2014); therefore, a shift from sound-pressure detection to substrate vibration sensitivity would be advantageous (Christensen *et al.*, 2012).

Non-tympanic reception of substrate vibrations has been confirmed in other squamates, including in snakes and amphisbaenians (Gans & Wever, 1972; Wever, 1979; Christensen *et al.*, 2012). However, the structural link between the lower jaw and inner ear appears to be highly variable in these non-tympanic taxa (Fig. 7). The stapes in snakes are generally anchored to the quadrate via one or more intervening cartilages (McDowell, 1967; Young, 2015; Fig. 7C), whereas in most amphisbaenians, an elongated stapes and extracolumella extend anteriorly, bypassing the quadrate, and contacting the lateral surface of the lower jaw directly (Wever, 1979; Fig. 7B). The non-tympanic pygopodids appear to be unique in reducing the size and function of the stapes and extracolumella as part of the structural link and may utilize an alternative, quadrate-only mechanism because of the close association between the quadrate, paroccipital process and inner ear (Figs 7A, S2). These different anatomical mechanisms may be explained by alternative foraging strategies. *Aprasia*, *Pletholax* and *Ophidiocephalus* primarily forage on very small invertebrates, such as ants and termites, and their larvae (Patchell & Shine, 1986b; Shea & Peterson, 1993; Webb & Shine 1994), which probably require little cranial kinesis (including little streptostyly) and a relatively small gape, permitting the quadrate to be shifted anteriorly and abut the otic capsule and inner ear. Macrostromatan snakes generally forage on very large prey, utilizing extreme cranial kinesis capabilities and a large gape, thereby constraining the quadrate in a posterior position, suspended from

an extended supratemporal (Gans, 1961; Fig. 7C). The quadrate in snakes is distant from the inner ear and requires the structural link of the stapes. However, a number of snake species that occupy underground macrohabitats (e.g. leptotyphlopids, anomalepidids, uropeltids, some colubroids) possess an anteriorly shifted quadrate, a reduced stapedia shaft (but large footplate) and an insectivorous diet (Rieppel, 1979; Rieppel *et al.*, 2009; Olori & Bell, 2012; Daza & Bauer, 2015; Scanferla, 2016), suggesting that many fossorial squamate lineages have independently evolved these traits.

CONCLUSIONS

Our study has highlighted the importance of considering multiple factors that may influence the evolution of morphological phenotypes. Although we emphasized the gekkotan taxa that demonstrate particularly strong influences of phylogenetic history, allometry or potential functional demands, most taxa exhibit intermediate forms that have likely been influenced by the interaction of these three factors, as well as others that were not explored in this study. It is also important to note that the quadrate is a single functional unit within an integrated skull and studying the mechanisms of diversification in the remaining cranial elements will provide a more comprehensive understanding of gekkotan skull evolution as a whole. The evolution of the quadrate bone remains understudied in all squamates, and its role in jaw mechanics, cranial kinesis and support of the auditory system requires further investigation in a comparative and evolutionary framework.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Phylomorphospace plots of gekkotan quadrate shape variation. Black points are specimens projected into tangent space and white points are estimated ancestral states.

Figure S2. Posterior view of quadrate (left) and lateral view of skull (right). A, *Paradelma orientalis* (CAS 77652); B, *Aprasia repens* (CAS 104382); C, *Pletholax gracilis* (MCZ R-187676); D, *Ophidiocephalus taeniatus* (AM R-45179). E, Phylomorphospace plot illustrating the divergence in quadrate shape between tympanic pygopodids (orange) and nontympanic pygopodids (purple). Quadrates in phylomorphospace plot are in lateral view.

Figure S3. Labelled PCA plot of gekkotan quadrates in tangent space (see Table S1 for plot numbers). See colour designations in [Figure 4](#).

Figure S4. Labelled multivariate regression plot between quadrate shape and quadrate centroid size (see Table S1 for plot numbers). See colour designations in [Figure 4](#).

Table S1. Gekkotan specimens examined in this study and data associated with them. Institutional abbreviations for the specimens studied are as follows: AMNH, American Museum of Natural History; AMS, Australian Museum; CAS, California Academy of Sciences; CM, Carnegie Museum of Natural History; FMNH, Field Museum of Natural History; MCZ, Museum of Comparative Zoology, Harvard University; QM, Queensland Museum; SAMA, South Australian Museum; UMMZ, University of Michigan Museum of Zoology; USNM, National Museum of Natural History, USA; YPM, Yale Peabody Museum of Natural History.

Table S2. Kappa statistic and *P* value associated with phylogenetic signal assessment. Groups with phylogenetic signal approaching significance are marked with an asterisk (*).