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Head shape evolution in monitor lizards (*Varanus*): interactions between extreme size disparity, phylogeny and ecology

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Abstract

Characterizing patterns of observed current variation, and testing hypotheses concerning the potential drivers of this variation, is fundamental to understanding how morphology evolves. Phylogenetic history, size and ecology are all central components driving the evolution of morphological variation, but only recently have methods become available to tease these aspects apart for particular body structures. Extant monitor lizards (*Varanus*) have radiated into an incredible range of habitats and display the largest body size range of any terrestrial vertebrate genus. Although their body morphology remains remarkably conservative, they have obvious head shape variation. We use two-dimensional geometric morphometric techniques to characterize the patterns of dorsal head shape variation in 36 species (375 specimens) of varanid, and test how this variation relates to size, phylogenetic history and ecology as represented by habitat. Interspecific head shape disparity is strongly allometric. Once size effects are removed, principal component analysis shows that most shape variation relates to changes in the snout and head width. Size-corrected head shape variation has strong phylogenetic signal at a broad level, but habitat use is predictive of shape disparity within phylogenetic lineages. Size often explains shape disparity among organisms; however, the ability to separate size and shape variation using geometric morphometrics has enabled the identification of phylogenetic history and habitat as additional key factors contributing to the evolution of head shape disparity among varanid lizards.

Introduction

The extent to which structures maintain a particular shape over a range of sizes, and the consequences of doing so, has long been of interest (Huxley, 1950), but only recently have the analytical tools become available to directly test alternative hypotheses (Klingenberg, 2010). Allometric scaling is generally the null hypothesis and suggests that when the size of a structure is changed, shape must be altered in a compensatory fashion to preserve function (Sweet, 1980; Smith, 1984). Allometric scaling can be established in an ontogenetic growth series, over evolutionary time, among related species, populations and sexes (Blackstone, 1987; Gerber *et al.*, 2008; Klingenberg, 2010; Wilson & Sánchez-Villagra, 2010; Suzuki *et al.*, 2011). The absence of size-required shape change is often termed 'geometric similarity' or 'isometry'. Geometric similarity predicts stresses on limbs to increase with body size; however, changes in posture, biomaterial properties of bones and muscles and physiological alterations may counter size-related increases in stress, enabling geometric scaling (Sweet, 1980; Bertram & Biewener, 1990; Clemente *et al.*, 2011).

The various ecological roles of a structure, in addition to its size, play a central role in the evolution of morphology. In squamates (lizards including snakes), the cranium is fundamental to foraging, prey capture, refuge use, defence and aggressive and sexual behaviours (Herrel *et al.*, 2007; Shirai & Marroig, 2010; Barros *et al.*, 2011). For most carnivorous squamates, head size limits the maximum size of prey that can be consumed ('gape-limited' predators). This has been studied with

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particular reference to sexual dimorphism in head size (Kaliontzopoulou *et al.*, 2012; Kikukawa & Hikida, 2012; Meik *et al.*, 2012) and ontogenetic shifts in diet accompanied by allometric head shape changes (Monteiro & Abe, 1997; Meyers *et al.*, 2002; Schaerlaeken *et al.*, 2012). There is also a rich literature examining the form–function relationship between squamate head morphology and bite performance (Meyers *et al.*, 2002; Brecko *et al.*, 2008; Huyghe *et al.*, 2009; Schaerlaeken *et al.*, 2012). Although fossorial and saxicolous habits are both known to directly impact squamate head morphology (Lappin *et al.*, 2006; Revell *et al.*, 2007; Barros *et al.*, 2011; Olori & Bell, 2012), how head shape and size variation reflect ecological diversification in squamates more broadly is not well understood.

Monitor lizards (*Varanus*: Varanidae) are widely regarded as a model system for morphological and ecological studies (Pianka, 1995; Collar *et al.*, 2011). Extant monitors have a very wide body size range, spanning adult total body lengths from 23 cm (*Varanus brevicauda*) to 3 m (*V. komodoensis*). The recently extinct *Varanus* 'Megalania' *priscus* is estimated to have reached between 6 and 9 m in total length, extending dramatically the body size range in the genus *Varanus*. In terms of body shape, monitors have been viewed as 'morphologically conservative' (Pianka, 1995), which implies geometric similarity or isometry, where interspecific variations in appendage length are proportional to changes in body length. However, various studies have shown nonisometric variation in limb dimensions for *Varanus*, with positive allometric scaling between body size and limbs (Christian & Garland, 1996; Thompson & Withers, 1997; Collar *et al.*, 2011). This suggests that size-required shape changes occur in the monitor lizard body plan. Throughout their range, *Varanus* have successfully invaded terrestrial, rocky, aquatic and arboreal habitats. This habitat diversification in monitors has been tightly linked with body size evolution with terrestrial monitors evolving large body size, arboreal monitors evolving intermediate sizes and rock-dwelling monitors evolving small body size (Collar *et al.*, 2011). However, it is not clear whether the pattern extends to the head shape variation clearly present in the group (see examples of varanid head shapes in Fig. 1).

Although some phylogenetic signal is expected, variation in head morphology among monitors may reflect interspecific size differences, or the ecological variation present in the genus. Here, we use geometric morphometric and phylogenetic comparative techniques to (1) determine the extent to which size variation explains interspecific head shape variation; (2) assess the phylogenetic structuring of head shape using an independent molecular phylogeny; and (3) examine ecology, as represented by habitat use, as an influence on head shape evolution. Although each of these aspects is analysed separately, we highlight the importance of interacting influences on the evolution of morphological variation.

Materials and methods

Geometric morphometrics

We examined external head morphology of 375 preserved adult monitor lizards from 36 species (Table 1). Subtle sexual size dimorphism in head size has been shown in some of the larger varanid species. Therefore, we tried to only use well-preserved males, but for some of the smaller species with no obvious dimorphism, we included some females. Including some females is unlikely to influence our results because we corrected for head size in our analyses. We based species-level sampling on a five-locus, dated molecular phylogeny that included representatives of all *Varanus* lineages and every described Australian species (Vidal *et al.*, 2012). Two species (*Varanus niloticus* and *Varanus rudicollis*) in the molecular phylogeny were not included here as they were not available in any Australian museums. Photographs of each specimen's head in a dorsal view were taken using a Nikon D5100 DSLR and a Tamron SP AF 90-mm macro lens. The camera was attached to a Kaiser ReproKid stand facing directly down to ensure that heads were consistently photographed from above, minimizing orientation error. A scale bar (ruler) was included in each photograph to enable scaling of each head. A configuration of 24 discrete landmarks (Fig. 2) was digitized on each head, and a scale set equating to 10 mm on the ruler included in the photograph, using tpsDig v2.16 (Rohlf, 2010). The landmarks are defined by anatomical features and scale types. Additionally, a snout-to-vent length (SVL) measurement was taken for each specimen.

We used geometric morphometric analytical techniques as implemented in MorphoJ v1.05e (Klingenberg, 2011) to characterize the primary variations in dorsal head shape among monitor lizards. Eight specimens were excluded based on a Mahalanobis distance squared > 0.003 from the average of the species, using raw coordinates, as recommended by Klingenberg & Monteiro (2005). The final data set therefore comprised information obtained from 367 specimens. Shape information was extracted from the scaled coordinate data by full Procrustes superimposition and projection into the shape-tangent space, taking into account object symmetry (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2002). Only the symmetric component of shape variation was analysed in this study. An initial Procrustes ANOVA confirmed a significant interspecific head shape variation, corrected for any effects of asymmetry ($P < 0.0001$) (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2002).

Quantifying the combined effect of size and phylogeny on head shape

We tested for allometry by performing a multivariate regression of shape on natural log-transformed centroid

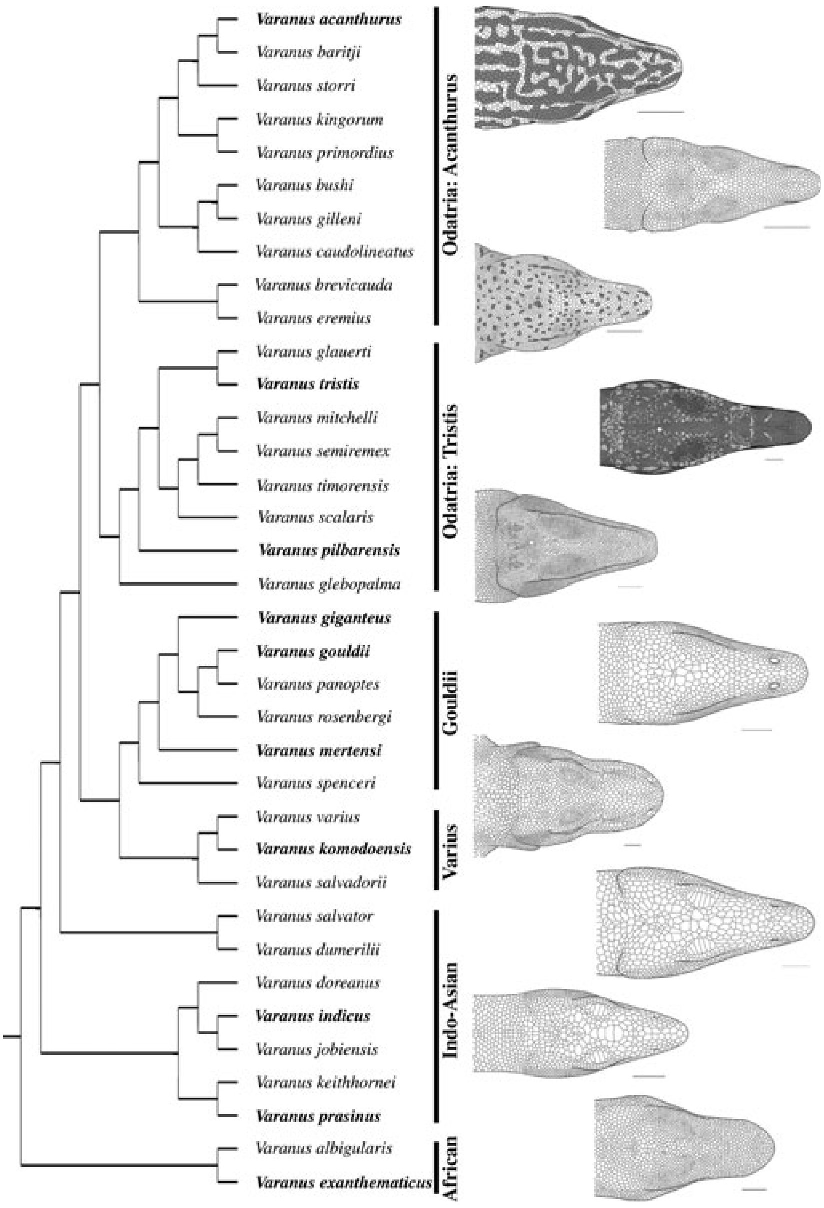

Fig. 1 Phylogenetic tree of the 36 *Varanus* species included in this study (based on the full phylogeny in Vidal *et al.*, 2012). Representative species heads for each phylogenetic group are shown with a scale bar equal to 1 cm and correspond with bold names on the phylogeny.

size (Monteiro, 1999). We performed this test first without considering phylogenetic group structuring and secondly including a pooled within-phylogenetic group component. We included a permutation test with 10 000 iterations to assess the statistical significance of both regressions. Using the residuals of a regression of shape on centroid size is a common method of correcting for size in geometric morphometrics and is used here in all 'size-corrected head shape' analyses (Monteiro, 1999; Klingenberg & Monteiro, 2005; Drake & Klingenberg, 2010; Sanger *et al.*, 2011; Klingenberg *et al.*, 2012).

To test hypotheses in a phylogenetic framework, we used the detailed molecular phylogeny with branch lengths based on three nuclear loci (BDNF, BMP2 and NT3) and two mitochondrial loci (ND1 and ND2) in Vidal *et al.* (2012). The topology we used was identical to the phylogeny presented in Vidal *et al.* (2012) except that it has slightly fewer taxa to match the 36 taxa represented in our morphometric data set. Phylogenetic structuring in the head shape data was then tested in two main ways. We performed an initial permutation test with 10 000 iterations, implemented in MorphoJ as part of the phylogenetic mapping analysis (Klingenberg & Gidaszewski, 2010). We then tested for strength of phylogenetic signal using Pagel's λ for both head shape (represented as mean size-corrected PC1 score for each species) and body size (mean SVL), using BayesTraits

Table 1 A list of the 36 species included in the study, ordered according to phylogenetic lineage with ample sizes, primary habitat and average snout-vent length (SVL) shown.

Phylogenetic lineage	*Varanus* sp.	*n*	Habitat	Average SVL (cm)
African group	*albigularis*	1	Terrestrial	41.1
	exanthematicus	1	Arboreal	27.9
Indo-Asian group	*doreanus*	2	Terrestrial	34.7 (32.4–37)
	dumerilii	1	Arboreal	34.5
	indicus	18	Terrestrial	39.9 (33.3–51.6)
	jobiensis	3	Terrestrial	36.3 (30–45.1)
	keithhornei	5	Arboreal	25.6 (23.4–27.8)
	prasinus	10	Arboreal	26.07 (23.1–30.2)
	salvator	4	Amphibious	43.2 (35.5–54)
Varius group	*komodoensis*	1	Terrestrial	128
	salvadorii	1	Arboreal	54.5
	varius	13	Arboreal	53.8 (37–64.2)
Gouldii group	*giganteus*	7	Terrestrial	67.8 (56.3–79.4)
	gouldii	24	Terrestrial	35.7 (26.7–50.5)
	mertensi	13	Amphibious	42.3 (33.8–54.2)
	panoptes	14	Terrestrial	50.7 (34.6–64.5)
	rosenbergi	16	Terrestrial	38.9 (31.1–51.8)
	spenceri	4	Terrestrial	46 (42.6–49.1)
Odatria: Tristis group	*glauerti*	17	Saxicolous	21.1 (16.8–24.9)
	glebopalma	12	Saxicolous	33.3 (29–39.1)
	mitchelli	14	Amphibious	23.2 (19.1–28.5)
	pilbarensis	11	Saxicolous	15.7 (13–18.5)
	scalaris	29	Arboreal	20.3 (17–24.9)
	semiremex	9	Amphibious	24.6 (22–27.9)
	timorensis	13	Arboreal	21.0 (17.8–26.1)
	tristis	15	Arboreal	23.9 (18.2–29.4)
Odatria: Acanthurus group	*acanthurus*	9	Saxicolous	20.7 (17.6–24.2)
	baritji	2	Saxicolous	17.3 (15.7–18.8)
	brevicauda	20	Terrestrial	10.9 (9.4–12.6)
	bushi	8	Arboreal	13.7 (11.6–15.8)
	caudolineatus	16	Arboreal	11.2 (10.4–12.6)
	eremius	17	Terrestrial	15.8 (12.1–20.2)
	gilleni	15	Arboreal	14.5 (13–19.4)
	kingorum	3	Saxicolous	10.3 (9.2–11.9)
	primordius	7	Saxicolous	10.0 (8.8–11.8)
	storri	11	Saxicolous	12.3 (10.6–13.9)

(Pagel & Meade, 2007). Pagel's λ can take a value from 0 to 1, measuring how well a phylogeny predicts the interspecific covariance in a trait, or traits, of interest (Villemereuil *et al.*, 2012). If $\lambda = 0$, then phylogeny has no impact on the distribution of the trait, whereas if $\lambda = 1$, then phylogeny predicts the distribution of the trait. For each set of variables, we calculated the log-likelihood of a model when $\lambda = 1$ (strong phylogenetic signal), $\lambda = 0$ (no phylogenetic signal) and when λ was estimated from the data. We then performed likelihood ratio tests to test for statistically significant differences between the likelihood based on the estimated λ and the likelihood based on $\lambda = 1$ or 0.

Describing and visualizing head shape disparity among monitors

Each species in our data set was assigned to their phylogenetic lineage as identified in Vidal *et al.* (2012): African (two species), Indo-Asian (seven species), Varius (three species), Gouldii (six species), Odatria: Tristis (eight species) and Odatria: Acanthurus (10 species). Habitat categories were assigned based on expert species accounts in 'Varanoid Lizards of the World' (eds: Pianka & King, 2004) and Wilson & Swan (2010) as: terrestrial (12 species), arboreal (12 species), saxicolous (rock-dwelling) (eight species) and amphibious (four species). See Table 1 for details.

Two metrics were computed to assess head shape disparity among phylogenetic and habitat groups. First, Procrustes distances were calculated using a canonical variate analysis (CVA) and including a permutation test with 10 000 iterations, to test the disparity *among* average shapes for each group. CVA was chosen because it maximizes the differences between taxa relative to the variation within taxa (Klingenberg *et al.*, 2012). Second, we used Procrustes variance, calculated during principal component analysis (PCA), to examine the

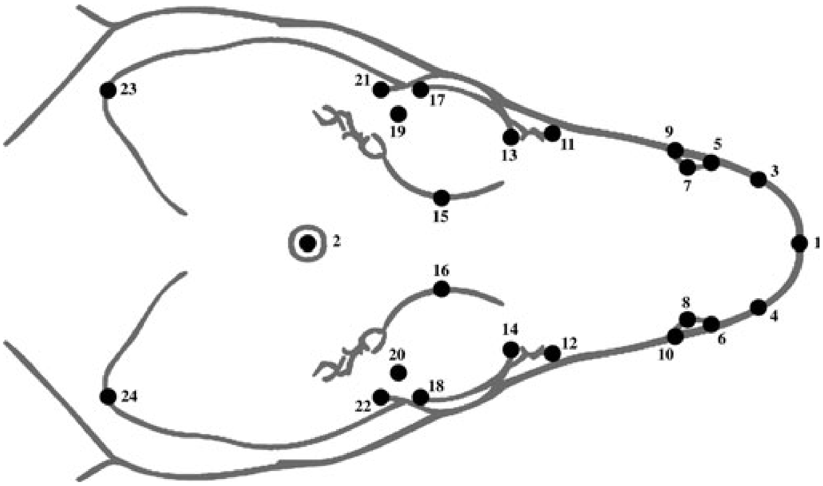

Fig. 2 Two-dimensional landmark configuration used in geometric morphometric analysis.

amount of head shape disparity *within* groups. Procrustes variance quantifies the average dispersion of data points around the mean shape and can be interpreted as the amount of shape space occupied by each group (Drake & Klingenberg, 2010). Both metrics were calculated before and after size correction, because allometry has been considered an important factor in the evolution of morphological variation for the group (Collar *et al.*, 2011; our multivariate regression results).

To visualize the majority of dorsal head shape variation among monitors, we performed a PCA on the covariance matrix of size-corrected head shape variation for all individuals in the data set, and generated deformation grids of the shape changes associated with the positive and negative directions for PC1–PC3. To visualize head morphology as it relates to phylogenetic relationships, we used the phylogenetic mapping technique implemented in MorphoJ, adapted from a test described by Laurin (2004) for scalar traits. The morphometric data were mapped onto the independent molecular phylogeny using squared-change parsimony (unweighted, because branch lengths were not included) and then projected into a shape space containing a single average value for each species, with the phylogeny shown over the top.

Results

Quantifying the combined effect of size and phylogeny on head shape

There is a strong, significant relationship between head size and shape, whereby head size accounts for 30.7% ($P < 0.0001$) of head shape variation after Procrustes superimposition. The second regression, which included a pooled within-phylogenetic group component, accounts for a further 5.2% ($P < 0.0001$) of shape variation. Phylogenetic relationships are an extremely good predictor of size-corrected head shape. The initial permutation test confirmed a significant phylogenetic structure ($P < 0.0001$). Head shape displayed a strong phylogenetic signal with the log-likelihood under an estimated λ not significantly different to the log-likelihood when $\lambda = 1$ ($\chi^2 = -1.223$, $P = 0.2687$), but significantly different when $\lambda = 0$ ($\chi^2 = -13.163$, $P = 0.0002$). Similarly, body size displayed a strong phylogenetic signal with the log-likelihood under an estimated λ not significantly different from the log-likelihood when $\lambda = 1$ ($\chi^2 = -1.228$, $P = 0.2677$), but significantly different from $\lambda = 0$ ($\chi^2 = -5.810$, $P = 0.0159$). Both head shape and body size evolution are consistent with a Brownian motion or random-walk model where, after divergence, each daughter lineage evolves in their own direction and more recently diverged lineages are more similar phenotypically than deeply diverged lineages.

Describing and visualizing head shape disparity among monitors

The greatest Procrustes distance among phylogenetic groups is between the Odatria: Acanthurus and Varius groups before size correction (0.1686; $P < 0.0001$) and between the African and Odatria: Tristis groups after size correction (0.2295; $P < 0.0001$). For habitat groups, the greatest Procrustes distance is between the saxicolous and terrestrial groups before size correction (0.0465; $P < 0.0001$) and between the amphibious and terrestrial groups after size correction (0.0408; $P < 0.0001$).

Procrustes variance values before and after size correction are given in Fig. 3. The Odatria: Acanthurus group has the largest Procrustes variance both before and after size correction. Size correction results in a slight increase in total variance for the Odatria: Tristis and Varius groups, most likely due to better separation

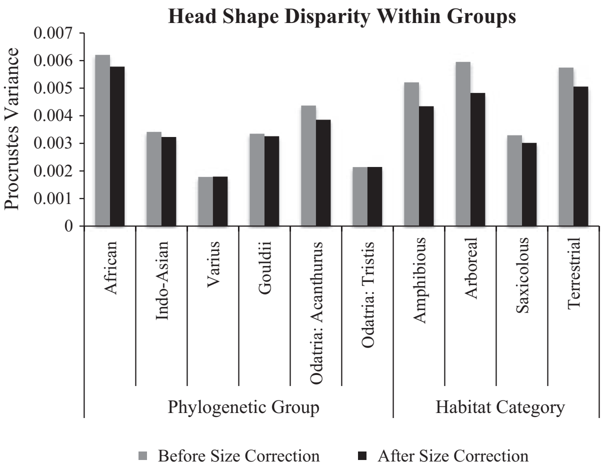

Fig. 3 Dorsal head shape disparity within each of the phylogenetic groups and habitat categories, quantified by Procrustes variance both before and after size correction.

of shapes in shape space. For habitat categories, the largest Procrustes variance is in the arboreal group. The saxicolous group has the smallest Procrustes variance and shows the smallest decrease after size correction.

The first three PCs of size-corrected shape variation account for almost 70% of the total variance in the data set. Deformation grids showing the shape changes captured by PC1-3 (both positive and negative directions) are shown in Fig. 4. All subsequent PCs account for less than 7% each and are likely due to a small number of species with unique head morphology, so they are not discussed further. PC1 (34% of total head shape variation) describes shape changes that account for the differences between snout length and proximity of the nares to the snout tip or eyes. PC1 changes in the positive and negative directions contrast an elongate snout and extreme separation of the eyes and nares, with a shortened snout and close proximity of the nares and eyes. PC2 (19% of total head shape variation) primarily contrasts a broadened head and consequent separation of eye scales laterally (positive shape change) with a narrow head and close proximity of eye scales to the middle of the head (negative shape change). PC3 (15% of total head shape variation) shape changes are associated with a contrast between shortening and lengthening/widening of the cranium. Other shape changes described by PC3 are more subtle, including nares and eye shape changes.

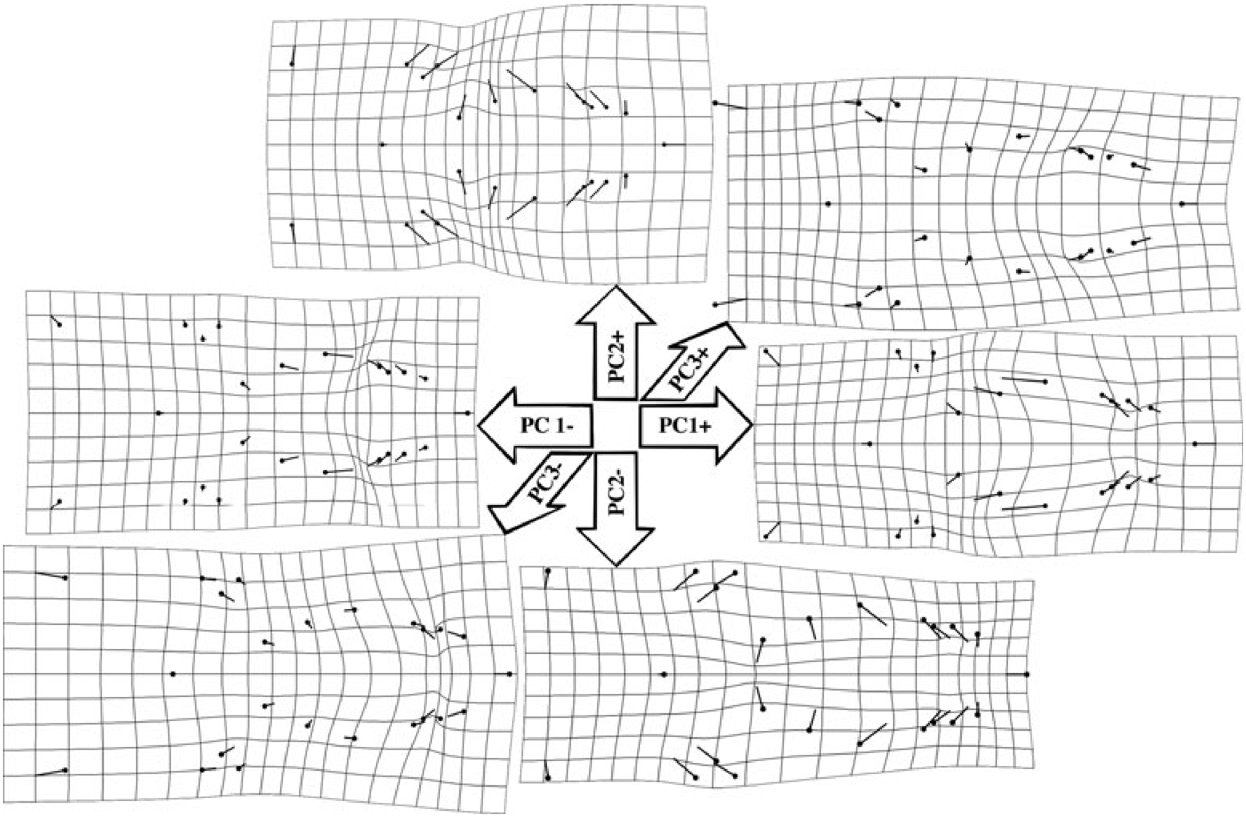

Fig. 4 Major shape changes in dorsal head shape among *Varanus*, calculated using a principal component (PC) analysis based on size-corrected values. The deformation grids correspond to the observed extremes in the positive and negative directions for each PC.

To visualize the phylogenetic and morphological relationships simultaneously, we projected the phylogeny onto the shape space containing species mean shapes (Fig. 5). Closely related species generally cluster together in shape space, corroborating our finding that phylogenetic relationships are a good predictor of size-corrected head shape. PC1 broadly separates species head shapes in accordance with their phylogenetic groups. The dominant shape change captured by PC1 is a shift in nares position (Fig. 4), indicating that phylogenetic groups with more positive PC1 scores have nares closer to the tip of the snout and phylogenetic groups with more negative PC1 scores have nares closer to the eyes. PC2 describes shape changes associated with head width (Fig. 4), indicating that species with more positive PC2 scores have broad heads, whereas species with more negative PC2 scores have comparatively narrow heads. If the highly distinct African species and *Varanus dumerilii* are excluded, some general habitat patterns can be seen on PC2. Arboreal species occupy a narrow range on PC2, more positive than most terrestrial species. Saxicolous species occupy a small cluster on both PC1 and PC2 (reflecting the low Procrustes variance value), with the same head width as the strongly terrestrial Gouldii species.

Discussion

Monitor lizards have long been recognized for their ecological diversity and body size disparity (Pianka, 1995; Collar *et al.*, 2011). Using geometric morphometrics, in combination with the most recent molecular phylogeny for the group, our study demonstrates that head shape also shows substantial variation among monitors. Head size accounts for a significant proportion of head shape disparity, and there is a strong phylogenetic signal in both size-corrected head shape and body size. Substantial interspecific head shape disparity remains after size correction, over half of which (PC1–2) describes shape changes concentrated in the snout and associated with head width. Species belonging to the Odatria: Acanthurus and Varius groups have the most disparate head shapes in the data set. Shape variance within phylogenetic lineages differs, likely

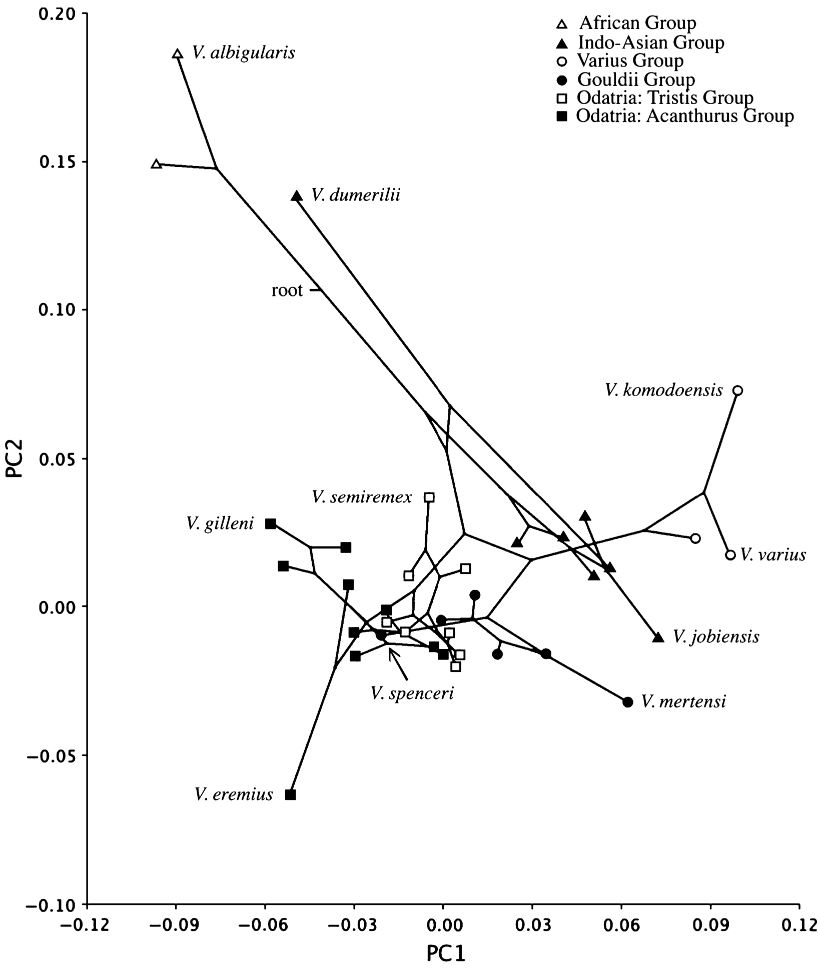

Fig. 5 Two-dimensional *Varanus* head shape morphospace, based on the first two principal component (PC) axes for size-corrected values, including every species in the data set. The molecular phylogeny is mapped onto the morphospace, with internal nodes reconstructed according to squared-change parsimony. Symbols correspond with species' phylogenetic lineage.

reflecting the additional influence of ecology on head shape, represented here by habitat.

The impact of head size on head shape

There is a strong, positive relationship between head size and shape among monitors, suggesting that a large proportion of initial head shape disparity is explained by allometric scaling. This is in accordance with previous studies showing an allometric relationship between body size and body shape among monitor lizards (Christian & Garland, 1996; Thompson & Withers, 1997; Collar *et al.*, 2011). The dependence of shape on size is a dominant factor contributing to morphological variation (Klingenberg, 2010), and such a strong correlation between size and shape has been documented in a range of other organisms, including domestic dogs (Drake & Klingenberg, 2010; Drake, 2011), *Anolis* lizards (Sanger *et al.*, 2011), crocodiles (Pierce *et al.*, 2009) and even plants (Klingenberg *et al.*, 2012).

A significant proportion of the allometry examined herein is phylogenetically structured (5.2%, $P < 0.0001$), suggesting the dwarf species scale differently, but still allometrically, to the larger species. This supports the idea that within- and between-group allometries cannot be expected to coincide where clades have different sizes, as has been demonstrated in cats (Sakamoto & Ruta, 2012). There are complex within-group variations contributing to interspecific scaling trends commonly identified among adults, making the integration of evolutionary history into the study of size and shape important (Gerber *et al.*, 2008). Furthermore, studies integrating phylogeny now suggest that allometries can evolve and that this evolution likely has an adaptive and functional basis that it is not simply a case of physics requiring shape change (Klingenberg, 2010).

But what does allometric scaling of shape reflect in monitor lizards? The size range of monitors suggests that their general body plan is successful in a wide variety of ecological niches. Evidence of positive allometry for the body suggests that necessary shape changes may help avoid the stresses on limbs associated with a body size increase. Larger monitors also mitigate stress on limb bones and muscles by increasing the percentage of time the hindfeet are grounded during movement, and reducing femur rotation (Clemente *et al.*, 2011). This likely has a negative impact on sprint speed, and it appears larger lizards sacrifice locomotor performance (Clemente *et al.*, 2009, 2011). Our results of positive allometric scaling in head shape and size might similarly reflect selection on the biomechanical function of the head across body size. The largest extant monitor, *V. komodoensis*, is known to have a low bite force relative to its body size (Moreno *et al.*, 2008). However, it also has increased neck movement and pulling power (changed muscle loading), presumably to aid in taking down large prey items (D'Amore *et al.*, 2011). Size-required shape changes are therefore accompanied by physiological, biomechanical and behavioural changes to optimize functioning at larger sizes, in both the body and head. This, taken into consideration with the different allometries between dwarf and giant species, suggests that head function between these groups may differ fundamentally in some aspect of ecology.

Phylogenetic structuring in body size and size-corrected head shape

There is a strong phylogenetic signal in size-corrected head shape among *Varanus*, and closely related species generally cluster together in the shape space, suggesting that head morphology and phylogenetic relationships broadly agree. There is, however, an overlap among Australian lineages in the shape space, which may reflect a relatively shorter time since divergence (~32 Mya, Vidal *et al.*, 2012). Because the lineages are separated in shape space after size correction, phylogenetic lineages must have distinct head shapes independent of size differences. That is, substantial head shape disparity has evolved within *Varanus* and is not simply due to size variation.

An overarching phylogenetic imprint on morphology has been widely documented and is generally expected (Blomberg *et al.*, 2003). For example, *Anolis* lizards are an extensively studied radiation in the Caribbean, Central and South America (Losos *et al.*, 1997; Mahler *et al.*, 2010; Yoder *et al.*, 2010). Sanger *et al.* (2011) confirmed a 'significant' phylogenetic signal in *Anolis* head shape (although its strength was not quantified); however, a strong ecological influence of habitat resulted in convergent head shapes among species sharing the same habitat independent of phylogenetic lineage. The clear separation of *Varanus* lineages in the head shape space and large Procrustes distances between average head shapes found here indicate that selective pressures among phylogenetic lineages must vary to result in the evolution of distinct head shapes.

Impact of ecology on head shape

Morphological diversity is often suggested to be a function of habitat use, because habitats differ in their complexity, imposition of functional constraints, predators, prey, competitors and other selective pressures (Losos, 1990; Collar *et al.*, 2011). In monitor lizards, the evolution of both body size and shape disparity is driven by habitat choice, retreat choice, foraging type and locomotor performance (Bedford & Christian, 1996; Christian & Garland, 1996; Thompson & Withers, 1997; Thompson *et al.*, 2008; Clemente *et al.*, 2009). Our results show that ecology as represented by habitat use is also contributing to within-phylogenetic lineage head shape variation among monitors; however, the aspects of habitat responsible, and how they specifically influence head shape, remain unclear.

Before size correction, rock-dwelling and terrestrial species have the most disparate head shapes. Terrestrial lineages of monitors have evolved larger body sizes, and as a consequence, become larger-limbed and shorter-tailed, whereas rock-dwellers have evolved pygmy sizes, wider bodies and longer tails (Bedford & Christian, 1996; Christian & Garland, 1996; Thompson & Withers, 1997; Collar *et al.*, 2011). Head shape varies least among rock-dwelling monitor lizards, and there is convergence in head shape among rock-dwelling species from the Odatria: Tristis and Odatria: Acanthurus lineages. This suggests selection for a particular head shape suited to rocky habitats. Monitors that retreat to oblique rock crevices are additionally known to have dorsoventrally compressed heads (Thompson *et al.*, 2008), an adaptation common to many rock-dwelling lizards (Revell *et al.*, 2007). Once size is accounted for, however, terrestrial and amphibious species have the most disparate head shapes. This suggests that previous inclusion of amphibious species into the terrestrial habitat type on the basis of moving across land where they do not encounter water may be incorrect (e.g. Collar *et al.*, 2011). Additionally, the tails of semiaquatic monitors across all sizes and phylogenetic lineages are strongly laterally compressed, with a ‘fin’ comprising the dorsal one-third of the tail (Bedford & Christian, 1996).

Arboreality is suggested to either represent a separate selective regime to terrestriality and rock-dwelling, with an intermediate body size selected, or a similar selective regime to rock-dwelling, where small body size is favoured (Collar *et al.*, 2011). Arboreality has evolved in multiple phylogenetic lineages; however, they have converged on similar head shapes (indicated by narrow shape space occupation on PC2 in Fig. 5). Although species currently sharing the same habitat type may have similar head shapes, they have likely experienced different histories of selection (Collar *et al.*, 2011). This leads to broad convergence in head shape among species utilizing the same habitat; however, subtle differences reflect evolutionary history or other factors.

Conclusions

In summary, our geometric morphometric analysis suggests that substantial head shape disparity has evolved through interaction between three key sources: head and body size evolution, phylogenetic relationships and habitat use. Size impacts shape not only through required changes that mitigate stress on the structure where a size increase has occurred (allometric scaling), but it also likely alters the function of the structure. There is an overarching imprint of phylogenetic history on extant *Varanus* head shapes; however, variation within lineages exists, reflecting the adaptation of closely related species to different habitats. How specific areas of habitat directly impact interspecific head shape variation patterns requires further examination.

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References

Barros, F.C., Herrel, A. & Kohlsdorf, T. 2011. Head shape evolution in Gymnophthalmidae: does habitat use constrain the evolution of cranial design in fossorial lizards? *J. Evol. Biol.* **24**: 2423–2433.

Bedford, G.S. & Christian, K.A. 1996. Tail morphology related to habitat of varanid lizards and some other reptiles. *Amphibia-Reptilia* **17**: 131–140.

Bertram, J.E.A. & Biewener, A.A. 1990. Differential scaling of the long bones in the terrestrial Carnivora and other mammals. *J. Morphol.* **204**: 157–169.

Blackstone, N.W. 1987. Allometry and relative growth: pattern and process in evolutionary studies. *Syst. Zool.* **36**: 76–78.

Blomberg, S.P., Garland, T. Jr & Ives, A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Brecko, J., Huyghe, K., Vanhooydonck, B., Herel, A., Grbac, I. & Van Damme, R. 2008. Functional and ecological relevance of intraspecific variation in body size and shape in the lizard *Podarcis melisellensis* (Lacertidae). *Biol. J. Linn. Soc. Lond.* **94**: 251–264.

Christian, A. & Garland, T. Jr 1996. Scaling of limb proportions in monitor lizards (Squamata: Varanidae). *J. Herpetol.* **30**: 219–230.

Clemente, C.J., Thompson, G.G. & Withers, P.C. 2009. Evolutionary relationships of sprint speed in Australian varanid lizards. *J. Zool.* **278**: 270–280.

Clemente, C.J., Withers, P.C., Thompson, G. & Lloyd, D. 2011. Evolution of limb bone loading and body size in varanid lizards. *J. Exp. Biol.* **214**: 3013–3020.

Collar, D.C., Schulte, J.A. & Losos, J.B. 2011. Evolution of extreme body size disparity in monitor lizards (Varanus). *Evolution* **65**: 2664–2680.

D'Amore, D.C., Moreno, K., McHenry, C.R. & Wroe, S. 2011. The effects of biting and pulling on the forces generated during feeding in the Komodo dragon (*Varanus komodoensis*). *PLoS ONE* **6**: e26226.

Drake, A.G. 2011. Dispelling dog dogma: an investigation of heterochrony in dogs using 3D geometric morphometric analysis of skull shape. *Evol. Dev.* **13**: 204–213.

Drake, A.G. & Klingenberg, C.P. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. *Am. Nat.* **175**: 289–301.

Gerber, S., Eble, G.J. & Neige, P. 2008. Allometric space and allometric disparity: a developmental perspective in the macroevolutionary analysis of morphological disparity. *Evolution* **62**: 1450–1457.

Herrel, A., Schaerlaeken, V., Meyers, J.J., Metzger, K.A. & Ross, C.F. 2007. The evolution of cranial design and performance in squamates: consequences of skull-bone reduction on feeding behavior. *Integr. Comp. Biol.* **47**: 107–117.

Huxley, J.S. 1950. A discussion on the measurement of growth and form; relative growth and form transformation. *Proc. R. Soc. Lond., B, Biol. Sci.* **137**: 465–469.

Huyghe, K., Herrel, A., Adriaens, D., Tadić, Z. & Van Damme, R. 2009. It is all in the head: morphological basis for differences in bite force among colour morphs of the Dalmatian wall lizard. *Biol. J. Linn. Soc. Lond.* **96**: 13–22.

Kaliontzopoulou, A., Adams, D.C., Van Der Meijden, A., Perera, A. & Carretero, M.A. 2012. Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. *Evol. Ecol.* **26**: 825–845.

Kikukawa, N. & Hikida, T. 2012. Geometric morphometric analysis of sexual dimorphism and allometric growth in the skull of the Japanese grass lizard, *Takydromus tachydromoides* (Reptilia: Lacertidae). *Curr. Herpetol.* **31**: 97–106.

Klingenberg, C.P. 2010. Evolution and development of shape: integrating quantitative approaches. *Nat. Rev. Genet.* **11**: 623–635.

Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**: 353–357.

Klingenberg, C.P. & Gidaszewski, N.A. 2010. Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst. Biol.* **59**: 245–261.

Klingenberg, C.P. & McIntyre, G.S. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with procrustes methods. *Evolution* **52**: 1363–1375.

Klingenberg, C.P. & Monteiro, L.R. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Syst. Biol.* **54**: 678–688.

Klingenberg, C.P., Barluenga, M. & Meyer, A. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* **56**: 1909–1920.

Klingenberg, C.P., Duttke, S., Whelan, S. & Kim, M. 2012. Developmental plasticity, morphological variation and evolvability: a multilevel analysis of morphometric integration in the shape of compound leaves. *J. Evol. Biol.* **25**: 115–129.

Lappin, A.K., Hamilton, P.S. & Sullivan, B.K. 2006. Bite-force performance and head shape in a sexually dimorphic crevice-dwelling lizard, the common chuckwalla [*Sauromalus ater* (= obesus)]. *Biol. J. Linn. Soc. Lond.* **88**: 215–222.

Laurin, M. 2004. The evolution of body size, Cope's rule and the origin of amniotes. *Syst. Biol.* **53**: 594–622.

Losos, J.B. 1990. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monogr.* **60**: 369–388.

Losos, J.B., Warheilt, K.I. & Schoener, T.W. 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* **387**: 70–73.

Mahler, D.L., Revell, L.J., Glor, R.E. & Losos, J.B. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of greater Antillean anoles. *Evolution* **64**: 2731–2745.

Meik, J.M., Setser, K., Mociño-Deloya, E. & Lawing, A.M. 2012. Sexual differences in head form and diet in a population of Mexican lance-headed rattlesnakes, *Crotalus polystictus*. *Biol. J. Linn. Soc. Lond.* **106**: 633–640.

Meyers, J., Herrel, A. & Birch, J. 2002. Scaling of morphology, bite force and feeding kinematics in an iguanian and a scleroglossan lizard In: *Topics in Functional and Ecological Vertebrate Morphology* (P. Aerts, K.D. D'Août, A. Herrel & R. Van Damme, eds), pp. 47–62. Shaker Publishing, Maastricht.

Monteiro, L.R. 1999. Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Syst. Biol.* **48**: 192–199.

Monteiro, L.R. & Abe, A.S. 1997. Allometry and morphological integration in the skull of *Tupinambis merianae* (Lacertilia: Teiidae). *Amphibia-Reptilia* **18**: 397–405.

Moreno, K., Wroe, S., Clausen, P., McHenry, C., D'Amore, D.C., Rayfield, E.J. *et al.* 2008. Cranial performance in the Komodo dragon (*Varanus komodoensis*) as revealed by high-resolution 3-D finite element analysis. *J. Anat.* **212**: 736–746.

Olori, J.C. & Bell, C.J. 2012. Comparative skull morphology of uropeltid snakes (Alethinophidia: Uropeltidae) with special reference to disarticulated elements and variation. *PLoS ONE* **7**: e32450.

Pagel, M. & Meade, A. 2007. BayesTraits. University of Reading. http://www.evolution.rdg.ac.uk/BayesTraits.html.

Pianka, E.R. 1995. Evolution of body size: varanid lizards as a model system. *Am. Nat.* **146**: 398–414.

Pianka, E.R. & King, D.R. 2004. *Varanoid Lizards of the World*. Indiana University Press, Bloomington, Indianapolis.

Pierce, S.E., Angielczyk, K.D. & Rayfield, E.J. 2009. Morphospace occupation in thalattosuchian crocodylomorphs: skull shape variation, species delineation and temporal patterns. *Palaeontology* **52**: 1057–1097.

Revell, L.J., Johnson, M.A., Schulte, J.A., Kolbe, J.J. & Losos, J.B. 2007. A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution* **61**: 2898–2912.

Rohlf, F.J. 2010. tpsDig. Department of Ecology and Evolution, State University of New York. http://life.bio.sunysb.edu/morph/

Sakamoto, M. & Ruta, M. 2012. Convergence and divergence in the evolution of cat skulls: temporal and spatial patterns of morphological diversity. *PLoS ONE* **7**: e39752.

Sanger, T.J., Mahler, D.L., Abzhanov, A. & Losos, J.B. 2011. Roles for modularity and constraint in the evolution of cranial diversity among anolis lizards. *Evolution* **66**: 1525–1542.

Schaerlaeken, V., Holanova, V., Boistel, R., Aerts, P., Velensky, P., Rehak, I. *et al.* 2012. Built to bite: feeding kinematics, bite forces, and head shape of a specialized durophagous lizard, *Dracaena guianensis* (Teiidae). *J. Exp. Zool. Ecol. Genet. Physiol. Part A* **317**: 371–381.

Shirai, L.T. & Marroig, G. 2010. Skull modularity in neotropical marsupials and monkeys: size variation and evolutionary constraint and flexibility. *J. Exp. Zool. Part B* **314**: 663–683.

Smith, R.J. 1984. Allometric scaling in comparative biology: problems of concept and method. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **15**: R152–R160.

Suzuki, S., Abe, M. & Motokawa, M. 2011. Allometric comparison of skulls from two closely related weasels, *Mustela itatsi* and *M. sibirica*. *Zool. Sci.* **28**: 676–688.

Sweet, S.S. 1980. Allometric inference in morphology. *Integr. Comp. Biol.* **20**: 643–652.

Thompson, G.G. & Withers, P.C. 1997. Comparative morphology of Western Australian varanid lizards (Squamata: Varanidae). *J. Morphol.* **233**: 127–152.

Thompson, G.G., Clemente, C.J., Withers, P.C., Fry, B.G. & Norman, J.A. 2008. Is body shape of varanid lizards linked with retreat choice? *Aust. J. Zool.* **56**: 351–362.

Vidal, N., Marin, J., Sassi, J., Battistuzzi, F.U., Donnellan, S., Fitch, A. *et al.* 2012. Molecular evidence for an Asian origin of monitor lizards followed by Tertiary dispersals to Africa and Australasia. *Biol. Lett.* **8**: 853–855.

Villemereuil, P., Wells, J., Edwards, R. & Blomberg, S. 2012. Bayesian models for comparative analysis integrating phylogenetic uncertainty. *BMC Evol. Biol.* **12**: 102.

Wilson, L.A.B. & Sánchez-Villagra, M.R. 2010. Diversity trends and their ontogenetic basis: an exploration of allometric disparity in rodents. *Proc. R. Soc. B.* **277**: 1227–1234.

Wilson, S. & Swan, G. 2010. *A Complete Guide to Reptiles of Australia*, 3rd edn. New Holland Publishers (Australia) Pty Ltd, Sydney.

Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, E. *et al.* 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evol. Biol.* **23**: 1581–1596.

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