Is body shape of varanid lizards linked with retreat choice?

Graham G. Thompson[A,F], *Christofer J. Clemente*[B], *Philip C. Withers*[C], *Bryan G. Fry*[D]
and Janette A. Norman[E]

[A]Centre for Ecosystem Management, Edith Cowan University, 100 Joondalup Drive, Joondalup,
 WA 6027, Australia.

[B]Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

[C]Zoology, School of Animal Biology (M092), University of Western Australia, Crawley, WA 6009, Australia.

[D]Department of Biochemistry, Bio21 Institute, University of Melbourne, Vic. 3010, Australia.

[E]Population and Evolutionary Genetics Unit, Sciences Department, Museum Victoria, GPO Box 666,
 Melbourne, Vic. 3001, Australia.

[F]Corresponding author. Email: g.thompson@ecu.edu.au

Abstract.   In our earlier analysis of *Varanus* body shape, size was a dominating factor with some qualitative phylogenetic patterns and grouping of species into ecological categories. With a phylogeny and an improved capacity to account for the effects of size, we have reanalysed our morphometric data for male Australian goannas (*Varanus* spp.) using an increased number of specimens and species to examine whether variations in body shape can be accounted for by retreat choice, as it can for Western Australian *Ctenophorus* dragon lizards. After accounting for body size in the current analysis, four ecotypes based on retreat choice (i.e. those that retreat to oblique crevices between large rocks or rock faces, those that retreat to burrows dug into the ground, those that retreat to spaces under rocks or in tree hollows, and those that retreat to trees but not tree hollows) accounted for much of the variation in body shape. There is a phylogenetic pattern to the ecotypes, but accounting for phylogenetic effects did not weaken the link between body shape and ecotype based on retreat choice. This suggests that there are large differences in body shape among ecotypes, and shape is relatively independent of phylogeny. The strong link between shape and choice of retreat site in *Varanus* spp. is consistent with that for *Ctenophorus* spp. We speculate on why there might be a strong link between retreat choice and body shape for both *Varanus* and *Ctenophorus*.

Introduction

Body shape is related to variation in body size for lizards (Mosimann 1970; LaBarbera 1989; Hews 1996; Malhotra and Thorpe 1997). In addition to the effects of body size, variations in body shape have also been related to ancestry, performance traits, behaviour and microhabitat use (Losos 1990*a*, 1990*b*; Miles 1994; Bauwens *et al*. 1995; Melville and Swain 2000; Kohlsdorf *et al*. 2001). Herrel *et al*. (2002) reported that, with the exception of the well documented case of the relationship between morphology and habitat for anoline lizards (Losos 1990*a*, 1990*b*; Harmon *et al*. 2005), and for *Niveoscincus* between limb length and habitat openness (Melville and Swain 2000), there had been few examples of the relationship between morphology and habitat use. Vitt *et al*. (1997) reported relatively rapid morphological changes in response to changes in habitat for *Tropidurus* lizards. Herrel *et al*. (2002) went on to report a correlation between habitat use and limb length for phrynosomatid lizards. More recently, Thompson and Withers (2005) reported that much of the variation in the body shape of Western Australian *Ctenophorus* dragon lizards could be explained by their choice of retreat. Size-free residuals of body shape variables placed *Ctenophorus* into two groups – those that dig burrows and those that do not. Within the group that do not dig burrows there is a subgroup that retreat under

rocks or into rock crevices. The other species in this group retreat to various locations, such as log hollows, depressions in the ground and under brushes. We could find no other studies that linked lizard body shape with retreat choice, although the link might seem intuitive. For example, it might be anticipated that lizards that retreat to narrow rock crevices would be dorso-ventrally compressed, and those lizards that dug narrow burrows would have shorter limb lengths.

Unlike *Ctenophorus*, Australian varanids (*Varanus* spp.) vary appreciably in body size (Thompson and Withers 1997), and we know that when there is an effect of size on shape, and there are large variations in body size, the size factor can dominate the analysis and mask other relationships (Thompson and Withers 1997). In our earlier study on varanid morphology, size was accounted for by using residuals from regression on body length. However, we can now more effectively remove size effects using Somers' (1986, 1989) method, which loads the variance due to size into the first principal component and provides residuals for subsequent analysis. In addition, a robust phylogeny for Australian varanids was not available for our earlier investigation so no attempt was made to statistically account for phylogenetic effects. In the absence of tools to adequately remove size effects and an inability to account for phylogenetic effects, Thompson

and Withers (1997) reported that body shape variations were dominated by size effects and species separated into phylogenetic groups, but there was evidence to suggest that shape was also related to habitat and dominant foraging mode. The link between morphology and retreat choice was not examined in that analysis.

A phylogeny is now available for Australian varanids, as is an improved capacity to analyse morphometric data from taxa with widely varying size. We therefore examined whether the strong relationship between size-free body shape of Western Australian *Ctenophorus* dragon lizards and retreat choice holds true for Australian varanids, and, second, to what extent variations in body shape could be accounted for by phylogeny. We have enlarged our previous morphological dataset (Thompson and Withers 1997) by including an additional 46 specimens and 15 species or subspecies.

Methods

Measurement of body shape

Various morphological dimensions were measured for 26 species of Australian varanid from the Western Australian and Queensland Museum collections. Tail length (TAIL), head length (HL), head width (HW), head depth (HD), upper fore-limb length (UFL), lower fore-limb length (LFL), upper hind-limb length (UHL), lower hind-limb length (LHL) and body length (BL) were measured (Fig. 1). All measurements were made to ±1 mm, and we used species means in the analysis. If we were analysing performance-related traits with body shape, then separating the lower limb and the foot in recording body measurement is probably appropriate, but for an analysis of body shape and retreat types, Clemente (2007) indicated that this separation made little difference to the results.

Thompson and Withers (1997) documented sexual dimorphism for many species of Australian varanids, so we included data for males only in this analysis. The sex of each varanid specimen was determined by an examination of the gonads. Some specimens were from disjunct populations with appreciable intervening distances, and some will be designated as subspecies or even new species after further taxonomic study (King and Green 1999). We have treated these geographically separated individuals as 'species' for the purpose of our analyses (some morphological differences among these geographically isolated populations are greater than differences among recognised species).

Phylogenetic analysis

We recovered a phylogenetic tree using data from 56 species and subspecies of varanid, then we removed those species for which no morphological data were available as this provided a more accurate estimate of phylogenetic relationships. For 29 species and subspecies (with a focus on the Australian species and subspecies), we extracted sequences from skeletal muscle. Skeletal muscle tissue was collected from dead, frozen or alcohol-preserved specimens. Sequences were then lodged with GenBank. The remaining 27 species were included in the phylogeny using sequences previously published on GenBank. Details of sample sources and sample collection localities are presented in Appendix 1.

DNA sequences were obtained for 1038 base pairs of the NADH2-gene. DNA was extracted using the salt : chloroform procedure described in Norman *et al.* (1998). The NADH-2 gene (ND2) was amplified using primers L4437 (5′-AAG CAG TTG GGC CCA TRC C: Macey *et al.* 1997) and ND2.2H (5′-AAA GTG TCT GAG TTG CAW TCA G: J. Norman, Museum Victoria). PCR amplifications were performed in 25-μL volumes consisting of 12.5-μL dilutes DNA, 1.5 mM MgCl$_2$, 0.2 mM dNTP, 0.28 μM of each primer, 2.5 μL commercial reaction buffer (Qiagen) and 0.5 unit Taq polymerase (Qiagen HotStar). The following cycle parameters were used: a hot start at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 20 s, annealing at 56°C for 20 s and extension at 72°C for 140 s. Products were purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) and eluted in 35 μL of 10 mM Tris. Sequencing reactions were performed in 10-μL reactions consisting of 5.75 μL of purified PCR product, 0.25 μL forward (L4437) or reverse (ND2.2H) primer and 4 μL ET Terminators (Amersham Biosciences). Cycling consisted of 25 cycles of 95°C for 20 s, 55°C for 20 s, 60°C for 1 min. Reaction products were purified using AutoSEqn 96 plates and injected
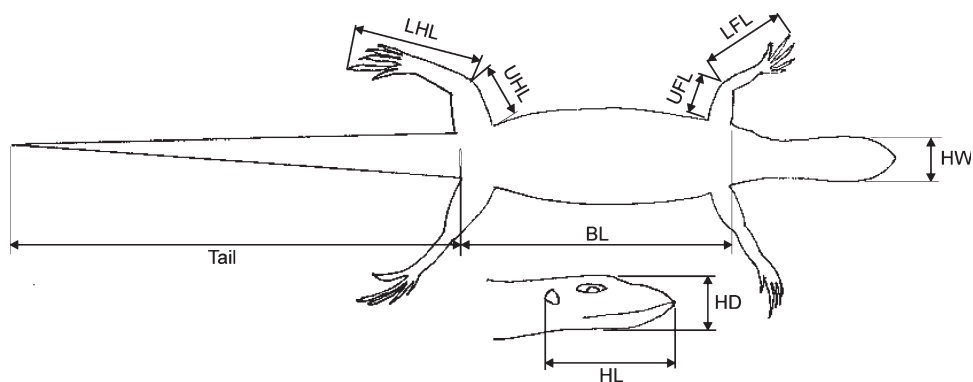

Fig. 1. Measures for varanids, with TAIL = tail length; HL = head length; HW = head width; HD = head depth; UFL = upper fore-limb length; LFL = lower fore-limb length; UHL = upper hind-limb length; LHL = lower hind-limb length; BL = body length.

onto a MegaBACE 1000 capillary sequencer at 3 kVA for 80 s and then electrophoresed at 9 kVA for 100 min.

DNA sequences were manually checked and edited using the program ProSeq (Filatov 2002). Edited DNA sequences were aligned using Clustal X (Thompson *et al.* 1997). The phylogenetic tree was constructed using the maximum-likelihood algorithm in the computer program PAUP* (v4.0b2a Swofford 2000). An appropriate model of molecular evolution based on maximum-likelihood analysis was evaluated by the likelihood ratio test using Modelltest 3.7 software (Posada and Crandall 1998). This test justified the use of the GTR + I + Γ model of molecular evolution based upon Akaike's information criterion (AIC). The estimated proportion of invariable sites was 0.28, the α shape parameter was 0.80, and the assumed nucleotide frequencies were A = 0.36, C = 0.39, G = 0.05, and T = 0.20. Starting branch lengths were obtained using the Rogers–Swofford approximation method. Trees with approximate likelihoods 5% or further from the target score were rejected without additional iteration. For branch-length optimisation, a one-dimensional Newton–Raphson model was used with a pass limit of 20, and a delta of 1e–06. Starting trees were obtained via stepwise addition, with a random addition sequence, three replicates and a random starting seed. One tree was held at each step during stepwise addition, and a tree–bisection–reconnection branch-swapping algorithm was used. Branches were collapsed, creating polytomies, if branch lengths were less than or equal to 1e–08. On the basis of Ast (2001), African *V. griseus* is basal to the Australasian varanids and was used as an outgroup. The robustness of the resulting topology was assessed by applying the bootstrap method with heuristic search and 100 bootstrap replicates.

Retreat cluster analysis

Each species was classified as belonging to one of four retreat types: (a) oblique crevices between large rocks or rock faces, (b) burrows dug into the ground, (c) spaces under rocks or in tree hollows, and (d) trees but not tree hollows. Classification of retreat types were based on literature and observational data (Table 1). Species that retreat to spaces under rocks and tree hollows were grouped as some of species utilised both locations in different parts of their range (e.g. *V. caudolineatus*, *V. tristis*) and we considered body-shape requirements for utilising tree hollows to be similar to those utilising existing spaces under rocks. The logic to support this grouping is that both spaces are irregular and roughly cylindrical and often restrict movement to forwards and backwards. Most often, lizards entering these retreats have to exit the retreat in the same orientation that they entered it. In addition, alternative classifications of retreat types explained a lower proportion of the variance on body shape.

To determine whether retreat types were evenly distributed across the phylogenetic tree, or whether they tended to cluster in parts of the tree, we used 'Clustering' analysis. This approach was similar to that proposed by Vanhooydonck and Van Damme (1999). The sum of distances between species with the same retreat types was used as a measure of clustering. To determine whether retreat type was significantly clustered, we generated 10 000 trees with an identical branching pattern, but with retreat type randomly assigned to the tip nodes. If 95% of the clustering values obtained from the randomly assigned trees were higher than the actual clustering value, then retreat type was deemed to be significantly clustered. The result of this analysis is expressed as a *P* value.

Data analysis

We could find no commentary in the literature on whether size or phylogenetic effects should be removed first, or if the order in which effects were removed influenced the placement of species in morphometric space in discriminant analysis. We did, as we did in our analysis of *Ctenophorus* spp. (Thompson and Withers 2005), remove size effects first, as our data for *Ctenophorus* provided an outcome that made biological sense.

We used a program written by Somers (1986, 1989) to remove 'size' effects (see our analysis of shape for Western Australian dragon lizards: Thompson and Withers 2005). We rewrote Somers' program in Visual Basic so it could handle larger data files. A primary reason for using the Somers' method was that having removed the effects of size, size-free measurements are available for each individual for subsequent analysis.

We used an autocorrelation procedure (Rohlf 2001) to account for phylogenetic effects, using a custom-written Visual Basic program (available from PCW). Sequence data were not available for two species of varanid: *V. pellewensis* and *V. primordius*. Because of this our analyses are based on two datasets: (a) all species measured are included in the size-free analysis, and (b) those for which we had phylogenetic data were included in the size-free and phylogenetically corrected analysis. We appreciated that deleting or including species in a discriminant analysis can influence the clustering of species as the internal weighting for each morphological variable will alter. However, highly similar results were obtained when analysis was performed including morphological data only for species for which phylogenetic information was available, thus we are confident that the impact is relatively small in the discriminant analysis undertaken here as the clustering of species remains similar for both datasets (see Results below). Discriminant analysis was undertaken for size-free, and size-free and phylogenetically corrected datasets using StatistiXL (http://www.statistixl.com/) after being log$_{10}$-transformed.

Results

Table 2 provides a summary of the number of individuals measured for each of the 26 species and the mean linear dimensions for each appendage, head and abdomen length.

Phylogeny

Maximum-likelihood analysis found a single optimum tree (lnL of –20987.692) (Fig. 2). Similar topologies were produced from this analysis as with previous hypotheses of varanid evolution (Ast 2001; Fitch *et al.* 2006). The likelihood analysis shows a well supported ingroup for the Indo-Australian group, consistent with the findings of Baverstock *et al.* (1993), Card and Kluge (1995), Fuller *et al.* (1998), Ast (2001) and Fitch *et al.* (2006). Our analysis recovered three major clades which were also apparent in Ast's (2001) phylogeny: the Indo-Asian A group (*V. dumerilii*, *V. bengalensis*, *V. salvator* and *V. rudicollis*), the Indo-Asian B group (*V. olivaceus*, *V. prasinus*, *V. keithhornei*, *V. beccari*,

Table 1. Species' preferred habitat and retreat
WA, Western Australia; NT, Northern Territory

Species	Habitat	Retreat	Sources
V. acanthurus	Stony ridges and rock outcrops, spinifex meadows, terrestrial	Burrows under rocks and spinifex	Dryden 2004; GGT pers. obs.
V. bushi	Mulga woodlands, arboreal	Tree hollows	Thompson 2004*a*; GGT pers. obs.
V. brevicauda	Hummock grass, sand plains, terrestrial	Burrows under spinifex and rocks	Pianka 2004*a*; GGT pers. obs.
V. caudolineatus	Treed and mulga plains, arboreal	Mostly in tree hollows but also under rocks	Pianka 1969; Thompson 2004*a*; GGT pers. obs.
V. eremius	Hummock grass, sand plains, terrestrial	Burrows	Pianka 2004*b*; GGT pers. obs.
V. giganteus	Rocky outcrops, eucalypt woodlands, sand plains, terrestrial	Burrows in the ground or rock holes	Horn and King 2004; GGT pers. obs.
V. gilleni	Sand plains, treed areas, arboreal	Tree hollows	Pianka 1969; Horn 2004; GGT pers. obs.
V. glauerti	Rocky outcrops in WA, trees in NT	Oblique rock crevices in WA, tree hollows in NT	Sweet 1999, 2004*a*; GGT pers. obs.
V. glebopalma	Rocky cliff faces	Oblique rock crevices	Sweet 1999, 2004*b*; GGT pers. obs.
V. gouldii	Sand plains, woodlands, terrestrial	Burrows in the ground, hollow logs	Thompson 1994, 1995, 2004*b*; GGT pers. obs.
V. indicus	Estuarine and coastal areas, mangroves, arboreal	Trees??	Dryden and Ziegler 2004
V. keithhornei	Mesophyll vine rainforest, arboreal	Tree hollows	Irwin 1996, 2004
V. kingorum	Rock outcrops	Rock crevices	King 2004*b*; GGT pers. obs.
V. mertensi	Waterways and adjacent riparian vegetation, terrestrial, semiaquatic	Burrows in the banks of waterways, rock holes	GGT pers. obs.
V. mitchelli	River and water way edges, trees, arboreal, occasional aquatic	Rock holes, crevices	Schultz and Doody 2004; GGT pers. obs.
V. panoptes	Wide variety of habitats including riparian, sand plains and woodlands	Burrows in the ground	Christian 2004; GGT pers. obs.
V. pellewensis[A]	Unknown, possibly arboreal		
V. pilbarensis	Rocky outcrops, terrestrial	Oblique rock crevices	King 2004*a*; GGT pers. obs.
V. primordius	Rocky outcrops, terrestrial	Burrows in the ground	Husband and Christian 2004
V. rosenbergi	Woodlands, terrestrial	Burrows	King and King 2004; GGT pers. obs.
V. scalaris	Savannah woodlands, arboreal	Tree hollows	Smith *et al.* 2004; GGT pers. obs.
V. semiremex	Mangrove swamps, creeks and waterways and adjacent woodlands, arboreal	Trees??	Pianka 2004*c*
V. spenceri	Plains and grasslands, terrestrial	Burrows in the ground	Lemm and Bedford 2004
V. storri	Rocky grasslands and open woodlands, terrestrial	Burrows under rocks	Eidenmuller 2004
V. tristis	Open woodlands, rocky areas	Tree hollows	Pianka 2004*d*; Thompson *et al.* 1999
V. varius	Open forested areas and woodlands terrestrial/arboreal	Burrows or log hollows	Weavers 1993, 2004; GGT pers. obs.

[A]*V.* 'pellewensis' was the name assigned to this species in the Queensland Museum; its taxonomic status was not known and it has been treated as a 'separate group'.

V. yuwonoi, *V. doreanus*, *V. jobensis*, *V. melinus* and *V. indicus*) and the Indo-Australian group. The Indo-Asian B group appears to be most basal with the Indo-Asian A group (including *V. salvator*) forming a sister clade to the Indo-Australian clades, supporting the findings of Sprackland (1991), Fuller *et al.* (1998) and Ast (2001). The well supported Indo-Australian clade can be further divided into three major clades: the *gouldii* clade (*V. mertensi*, *V. spenceri*, *V. giganteus*, *V. rosenbergi*, *V. gouldii* and *V. panoptes*), the *Odatria* clade (*V. eremius*, *V. brevicauda*, *V. caudolineatus*, *V. gilleni*, *V. bushi*, *V. storri*, *V. kingorum*, *V. baritji*, *V. acanthurus*, *V. glebopalma*, *V. pilbarensis*, *V. glauerti*, *V. tristis*, *V. timorensis*, *V. mitchelli*, *V. semiremex* and *V. scalaris*) and the *varius* clade (*V. salvadorii*, *V. komodoensis* and *V. varius*). Within the *Odatria* clade there is some support for two groups: the *acanthurus* group (*V. eremius*, *V. brevicauda*, *V. caudolineatus*, *V. gilleni*, *V. bushi*, *V. storri*, *V. kingorum*, *V. baritji* and *V. acanthurus*) and the *tristis* group (*V. glebopalma*,

V. pilbarensis, *V. glauerti*, *V. tristis*, *V. timorensis*, *V. mitchelli*, *V. semiremex* and *V. scalaris*), consistent with the findings of Ast (2001) and Fitch *et al.* (2006).

Non-phylogenetically corrected size-free shape

A discriminant analysis, using four categories of retreat choice showed separation of species using size-free scores (Table 3; Fig. 3). The first discriminant function loaded most positively on LHL and HD and most negatively on UHL and TAIL (Table 3). This function accounted for 80% of total variance and separated species that retreat to oblique rock crevices from the three other retreat types. This suggested that species that retreated to oblique crevices showed greater dorso-ventral flattening (as characterised by head depth) and longer tails.

The second discriminant function, which reduced variance by 16.2%, was most positively loaded on UFL, BL, HL and UHL and most negatively on LFL. This function separated burrowing

Table 2. Mean morphometric measurements for Australian *Varanus* species
The number examined (*N*) and the mean dimensions for each appendage, head and body (mm) are shown. Qld, Queensland; WA, Western Australia

Species	Species no.	*N*	TAIL	HL	HW	HD	UFL	LFL	UHL	LHL	BL	Neck
V. acanthurus	1	13	313.2	32.3	15.9	10.9	14.2	35.4	20.5	47.2	111.2	36.6
V. brevicauda	2	18	90.9	17.3	9.3	6.4	5.9	15.8	7.4	17.8	65.3	15.8
V. caudolineatus	3	31	134.7	20.1	10.6	6.8	7.8	20.1	11.3	25.1	67.9	21.1
V. eremius	4	30	248.5	27.9	13.4	9.6	11.3	26.1	16.8	39.4	90.4	26.7
V. giganteus	5	7	639.6	79.8	34.3	25.7	43.6	95.3	55.9	126.9	245.6	114.4
V. gilleni	6	12	161.3	22.9	12.1	7.3	8.7	22.3	11.7	27.8	79.8	25.7
V. glauerti	7	17	465.2	37.6	16.4	9.6	19.6	42.8	26.8	56.7	121.2	51.2
V. glebopalma	8	13	580.7	54.6	25.3	17.8	32.8	63.5	48.2	89.5	181.9	91.4
V. gouldii (Qld)	9	4	620.0	67.1	34.4	25.6	43.6	95.7	59.0	130.4	273.0	101.9
V. gouldii (WA)	10	43	433.5	50.8	23.6	17.6	25.5	59.0	36.6	82.7	182.0	60.9
V. indicus	11	4	368.8	48.3	21.4	16.6	21.4	59.7	33.7	79.2	150.8	57.1
V. keithhornei	12	2	525.5	51.4	24.3	19.5	32.1	77.6	42.0	91.8	173.5	58.7
V. kingorum	13	10	162.8	20.1	10.9	6.5	8.8	20.0	11.2	26.8	67.0	22.5
V. mertensi	14	10	419.2	45.0	22.7	15.5	22.0	53.6	32.6	74.5	165.8	68.6
V. mitchelli	15	11	308.6	32.4	14.4	9.4	12.1	33.6	18.8	44.9	109.5	38.3
V. p. panoptes (Qld)	16	3	626.0	76.2	36.6	28.5	43.4	112.8	63.2	136.2	305.3	110.8
V. p. panoptes (WA)	17	5	249.0	37.0	16.6	12.0	15.1	36.2	19.8	48.7	99.2	36.2
V. p. rubidus (WA)	18	11	625.5	62.1	29.0	22.8	37.5	83.6	55.1	122.3	238.5	86.2
V. pellewensis	19	1	292.0	34.0	16.2	12.9	21.8	41.5	22.4	53.3	109.0	39.3
V. pilbarensis	20	6	260.8	29.3	14.3	8.3	14.3	31.3	20.0	42.2	88.5	33.2
V. primordius	21	4	139.3	18.8	9.4	6.6	6.6	17.5	8.2	21.4	59.8	14.7
V. rosenbergi	23	17	485.4	58.1	28.0	21.3	29.7	68.7	41.1	91.9	193.1	67.4
V. scalaris	24	22	239.9	28.5	13.3	9.7	11.8	30.5	16.8	39.6	97.8	34.2
V. scalaris (Qld)	25	1	420.0	44.6	23.5	16.3	22.0	50.3	29.6	73.2	157.0	54.7
V. semiremex	26	2	294.0	35.3	17.1	12.9	13.2	36.6	18.9	51.2	129.0	34.4
V. bushi	31	6	162.0	20.9	11.1	7.1	8.6	21.4	11.3	26.9	72.7	23.0
V. spenceri	34	6	456.7	67.4	34.9	24.4	39.5	90.1	55.8	122.3	266.8	111.8
V. storri (WA)	38	10	192.0	24.5	12.7	8.9	10.0	23.4	13.1	31.2	70.7	20.7
V. storri (Qld)	41	5	171.4	23.6	12.7	8.8	9.4	23.4	14.8	31.9	72.4	21.1
V. tristis (Qld a)	44	5	326.2	33.4	15.9	10.9	13.4	37.4	21.9	52.3	113.0	40.5
V. tristis (Qld b)	48	2	359.0	36.9	18.0	12.6	15.1	40.8	25.0	58.0	112.0	41.9
V. tristis (WA)	50	20	399.2	39.8	18.1	12.6	18.4	44.3	26.0	60.6	132.3	49.7
V. varius	58	2	635.0	69.1	31.8	22.8	35.2	91.4	55.9	115.5	226.0	85.6

species, which showed longer bodies and heads, from species that retreat into spaces under rocks or tree hollows. Further, burrowing species appear to be associated with a lengthening of the upper hind-limb and fore-limb, but a reduction in the length of the lower fore-limb.

Eigenvalues for the first two functions were greater than 1.0 and considered to be significant. The third and subsequent discriminant functions contributed less than 4% of total variance, had eigenvalues of less that 1.0, and were not considered significant.

Size-free and phylogenetically corrected shape

A discriminant analysis provided good separation of the four retreat categories (Fig. 4). The first discriminant function reduced variance by 72.7% (Table 4) and loaded most positively on HW and LHL, and most negatively on UHL and UFL. The second discriminant function, which reduced variance by 19.9%, was loaded most positively on HW, HL, UHL and BL and most negatively on LFL (Table 4). Eigenvalues of Discriminant Functions 1 and 2 were greater than 1.0, and were considered significant. The grouping of species in the phylogenetically

corrected and non-phylogenetically corrected discriminant analysis is similar (Figs 3, 4). Only *V. varius* swaps groups and *V. mertensi* is out of its group. Three species that retreat to oblique crevices in rocks (*V. glebopalma*, *V. glauerti* and *V. pilbarensis*) are the most different in morphometric space. *V. semiremex* and *V. indicus* are also quite separate from the other species.

A comparison of the discriminating power among the species used in both size-free, and size-free and phylogenetically corrected analysis was undertaken by comparing Wilks' lambda scores. The Wilks' lambda score ($\Lambda_{10,3,80}$) for the first discriminant function for size-corrected data was 0.035; for the size- and phylogenetically corrected analysis, the Wilks' lambda score ($\Lambda_{10,3,80}$) for the first discriminant function was 0.041. The Wilks' lambda score for the size- and phylogenetically corrected value was not significantly different ($F_{3,17} = 0.97$, $P = 0.429$), indicating that phylogenetic correction did not weaken the discriminting power among species.

Retreat clustering

The tendency for retreat types to cluster in parts of the phylogenetic tree was tested. If the sum of the branch lengths
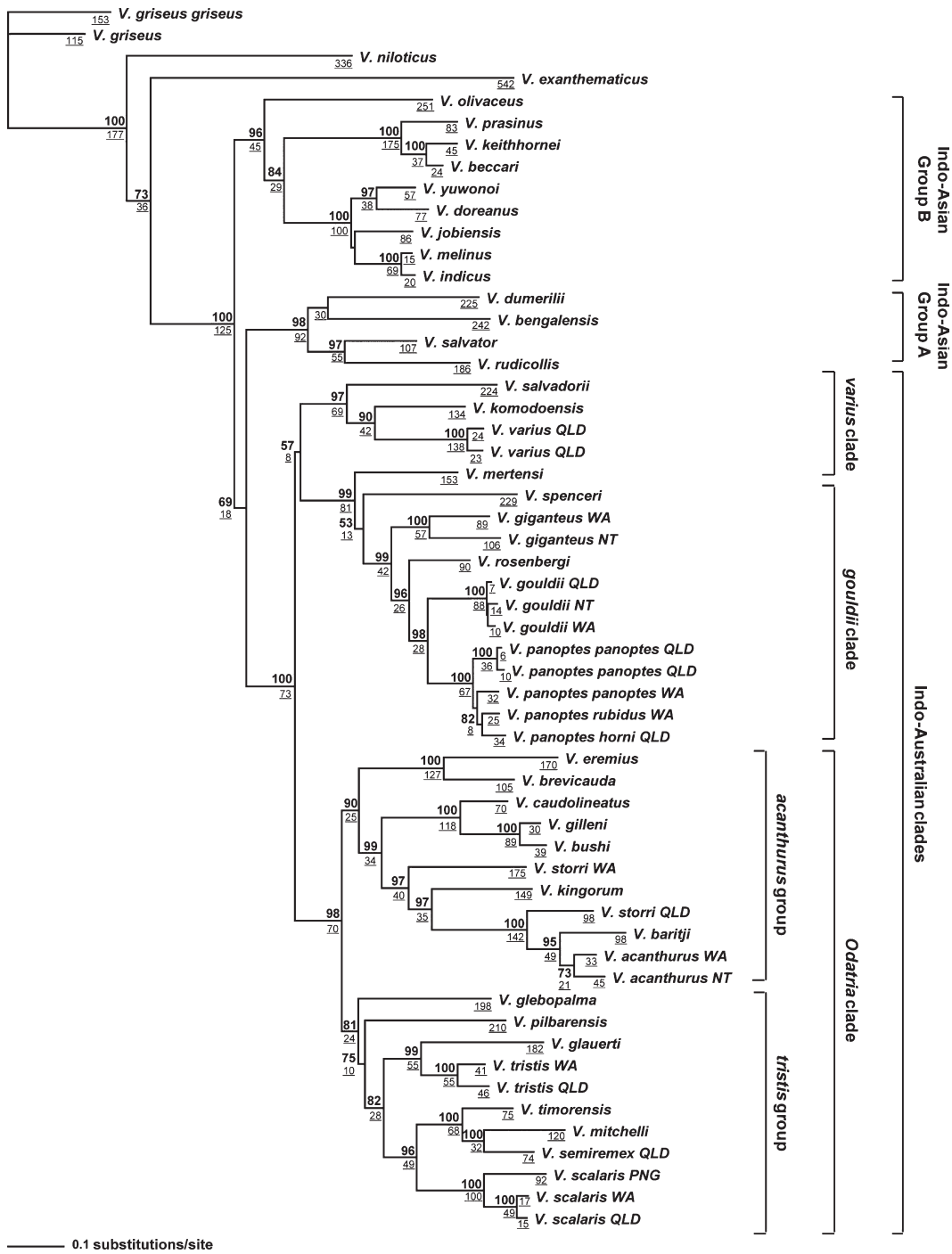
Fig. 2. Phylogenetic relationships amongst varanids based on the ND2 gene showing the maximum-likelihood hypothesis (lnL = −20987.69). Bootstrap values >50% (percentages of 100 pseudoreplicates) are shown above the branches. Branch lengths are shown underlined below branches (substitutions per site × 1000).

among species with similar retreat types was less than the sum of the branch lengths when retreat types were randomly assigned to the tree, then retreat type would be considered to be clustered. The sum of the branch lengths between species with similar retreat types was 149.89. Of 10 000 randomly assigned trees, only four

had stronger clustering than was recorded for varanids, giving $P = 0.0004$. This suggests that retreat type is significantly clustered within this varanid phylogeny. Similar results were obtained when the sum of the nodes between species with similar retreat types was used instead of the sum of branch lengths.

Table 3. Eigenvalues and standardised discriminant function coefficients for male Australian varanids using size-free data

Variables	Function 1	Function 2
Eigenvalues	7.45	1.51
% of variance	80.0	16.2
Wilks' lambda	0.035	0.294
χ^2	83.9	30.6
d.f.	30	18
P	0.000	0.032
Standardised discriminant function coefficients		
TAIL	−1.059	1.142
HL	−0.175	1.548
HW	−0.547	0.568
HD	0.658	1.047
UFL	−0.656	1.928
LFL	0.053	−0.239
UHL	−1.116	1.235
LHL	1.148	0.719
BL	−0.116	1.926
Neck	−0.088	0.788
Group centroids		
Spaces beneath rocks and in tree hollows	−0.390	−1.407
Ground burrows	1.096	0.961
Oblique crevices between rocks	−7.037	1.169
Trees	4.875	0.179

Fig. 4. Plot of Discriminant Functions 1 and 2 for size-free and phylogenetically corrected varanid morphometric dimensions.

Table 4. Eigenvalues and standardised discriminant function coefficients for male Australian varanids using size-free and phylogenetically corrected data

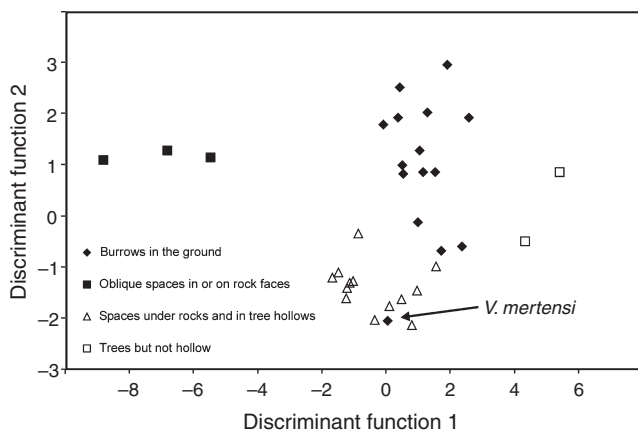| Variables	Function 1	Function 2
Eigenvalue	5.42	1.48
% of variance	72.7	19.9
Wilks' lambda	0.041	0.260
χ^2	70.5	29.6
d.f.	30	18
P	0.000	0.041
Standardised discriminant function coefficients		
TAIL	−0.292	0.980
HL	−0.047	1.123
HW	0.465	1.092
HD	0.324	0.200
UFL	−0.692	0.555
LFL	0.247	−0.291
UHL	−0.930	1.112
LHL	0.984	0.401
BL	−0.014	1.245
Neck	−0.301	0.074
Group centroids		
Ground burrows	0.893	1.148
Spaces beneath rocks and in tree hollows	−0.198	−1.263
Oblique crevices between rocks	−5.728	0.594
Trees	3.974	−0.770

Fig. 3. Discriminant Function Scores 1 and 2 for size-free varanid morphometric dimensions.

Discussion

Thompson and Withers (1997) separated Western Australian varanids into Mertens' two subgenera (*Odatria* and *Varanus*) using canonical variate analysis for logarithmically transformed morphometric data. Their analysis was heavily influenced by the argument of Bookstein *et al*. (1985) that size ought not be removed from morphometric data, as it often explains meaningful covariance, and they did not remove phylogenetic effects as a robust phylogenetic tree was not then available. Thompson and Withers (1997) reported no obvious overall placement of *Varanus* species in morphometric space on the basis of known performance traits, ecology or preferred habitats, although some
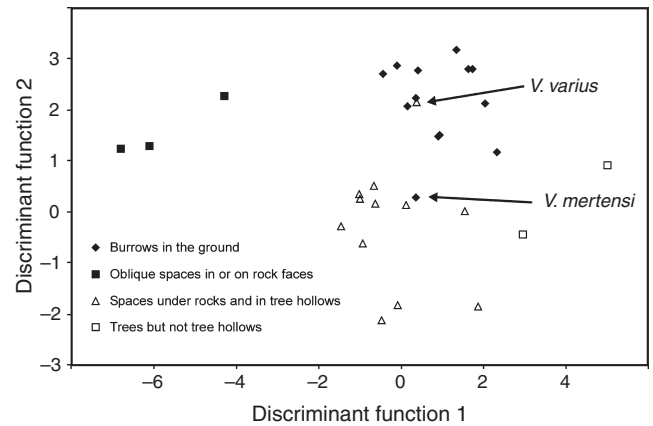
species of similar size and ecology (e.g. *V. caudolineatus* and *V. gilleni*) were consistently grouped together. Body size and phylogenetic placement dominated shape comparisons in their analysis.

A comparison of the results and conclusions reached by Thompson and Withers (1997) from non-size-corrected morphological data and results obtained here using size-free morphological data suggested that body size ought to be removed in shape analysis, at least for varanids and probably other genera with widely varying sizes. When size effects are removed using Somers' (1986, 1989) approach, species separate in morphometric space using discriminant analysis based on retreat

categories (Figs 3, 4). Two species appear to be out of place in the grouping based on retreat choice. *V. mertensi* is grouped with species that utilise spaces under rocks and in tree hollows in the non-phylogenetically corrected data (Fig. 3), and *V. varius* is grouped with species that utilise a burrow in the phylogenetically corrected dataset. So, although not a perfect fit, retreat choice can explain a substantial amount of the variance in body shape for Australian varanids.

Thompson and Withers (2005) reported that when body size was accounted for, retreat choice (burrows versus no burrows) for *Ctenophorus* dragon lizards was closely associated with body shape, particularly tail and hind limb length. Phylogeny of *Ctenophorus* spp. seemed much less important than retreat type when establishing correlates with body shape for this genus (Thompson and Withers 2005). A very similar clustering of varanids was obtained when the data were and were not corrected for phylogenetic effects, but arrangement of species in morphometric space was better delineated for phylogenetically corrected data (Fig. 4). Despite strong clustering of retreat type within the phylogeny (Fig. 5), removing the phylogenetic effect had little effect on the discriminating power of the four ecotypes, suggesting large differences between ecotypes. These data suggest that there is a strong link between body shape and retreat type for *Ctenophorus* and *Varanus* and phylogenetic effects are not strong.

Retreat choice

Why would variations in body shape for two large genera of Australian reptiles be associated with retreat choice? Changes in body shape in an evolutionary context should bestow a selective advantage. There are at least two possible scenarios to explain the relationship between body shape and retreat choice.

The first scenario is that choice of retreat directly affects body shape. If individuals are susceptible to predation when in their retreats, then those that minimise predator access when in their retreat will have improved chances of survival. For example, dorso-ventral compression, as measured by head depth, was associated with species that retreat to oblique rock crevices. It is likely that these species can squeeze deeper into narrow crevices and will avoid a range of predators while resting. Similarly, the elongation of the head and body of burrowing species may be coupled with a narrowing of these morphological features (though this is yet to be verified), and may allow species to retreat deeper into narrower burrows to avoid predators.

A second scenario is that body shape varies among *Varanus* and *Ctenophorus* lizards in response to improvement in some performance trait such as speed, endurance, or stability, which enables individuals to avoid predation, secure prey, and locate mates (Arnold 1983; Irschick and Garland 2001). This scenario has been widely supported in the literature; for example, longer hind limbs may improve speed and acceleration (see Garland and Losos 1994 for a review), which in turn might be favourable in certain habitats (e.g. open habitats: Vanhooydonck and Van Damme 2003).

Varanids species that retreat to narrow crevices were characterised by long tails. Tail length has been associated with speed, although often in the context of tail loss (Arnold 1988; Russell and Bauer 1992). As increased tail length is unlikely to be associated with increased speed for these species, increased tail length may be associated with other performance variables. Recently, tails have been shown to play an important role in stability while climbing vertical surfaces. For example, Jusufi *et al*. (2008) showed that the tail of geckos acts as an emergency fifth leg during climbing, by pushing into the surface in response to a slip, which prevents the pitch back of the head and upper body. Further, during a fall the tail can implement an in-air righting response, returning the lizard to a sprawling posture, and further movements of the tail control yaw and pitch as the lizard descends. These results suggested that a large active tail can function as an effective control appendage (Jusufi *et al*. 2008). Narrow crevices into which these varanids retreat are typically found in high vertical stone-wall cliff-faces. As varanids rely primarily on their sharp claws for adhesion on vertical and inclined surfaces, these steep stone vertical cliff-faces may be more difficult to adhere to than more deformable substrates (such as tree bark) into which the claws can penetrate. Thus, the lengthening of the tail may be a response to prevent slipping or to control falling in the more unstable habitat in which narrow crevices are typically found.

Within these varanids there was also an association between species that retreat to burrows with an elongation of the upper fore- and hind-limbs, and a shortening of the lower fore-limb. Elongated limbs are often associated with greater sprint speeds (Garland and Losos 1994), which in turn are related to open habitats (Vanhooydonck and Van Damme 2003). Burrowing species are often found in open habitats as they do not rely on naturally occurring retreat sites. The association between the elongated upper limb measurements may then be related to the open habitats where burrowing species typically occur, mediated via increased speeds. A second possibility is that the association of limb dimensions with burrow retreats is mediated by the performance variable of burrowing itself; however, the exact reasoning for this remains ambiguous as biomechanical models
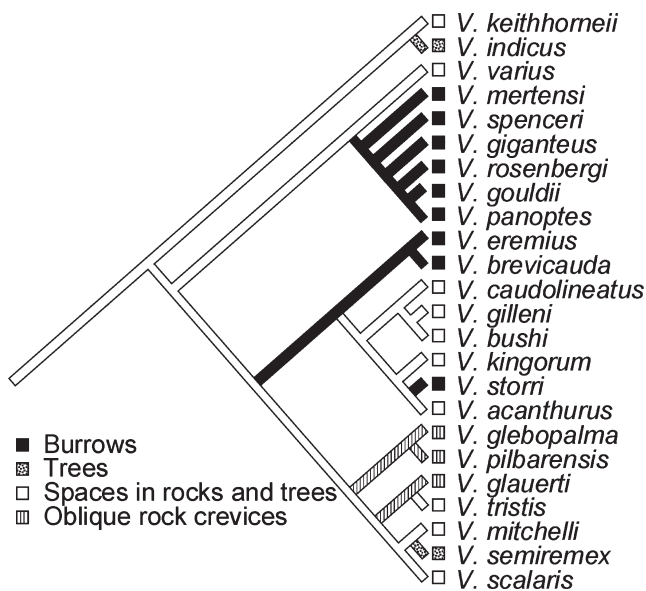

Fig. 5. Cladogram of Australian varanids based on the maximum-likelihood hypothesis presented, with retreat type mapped over the top. Note the tendency for retreat type to cluster in the phylogeny.

for burrowing species have been poorly studied (Hildebrand 1985). It is possible that the shortening of the lower forelimb is associated with increased strength and stability of the distal portion of the limb during burrowing as varanids typically burrow head-first.

However, these relationships should be interpreted with caution until they can be confirmed with rigorous biomechanical modelling. We do not completely understand the cause and outcome relationships that drive the evolution of body shape of *Ctenophorus* and *Varanus*, but it is likely to be a complex combination of factors, and whether retreat choice is a 'cause' or an 'outcome' can only be speculated on.

Species comparisons

The two species that are out of place in the grouping based on retreat choice are *V. mertensi* and *V. varius*. *V. mertensi* will retreat to holes between the rocks around water holes and along waterways, but will also retreat to burrows dug in the banks of rivers and irrigation channels (Mayes 2007). So, in this regard its placement in both analyses is correct. Weavers (2004) reported that *V. varius* used burrows and hollow logs as retreats, but that it also readily climbed trees to forage. It does not appear to dig a burrow. Many varanids are very plastic in their habitat requirements, e.g. *V. giganteus* is generally reported as retreating to holes in rock outcrops (Cogger 1992; Wilson and Knowles 1995); however, it will also retreat to burrows in the sand far removed from rock outcrops (Pianka 1994). *V. caudolineatus* is generally described as arboreal, living in tree hollows; however, it is found almost exclusively under rocks in one habitat (29°22′S, 121°17′E) when there are many suitable tree hollows to use as retreats. Therefore, any categorisation of varanids (and possibly other reptile species) based on habitat type (and possibly performance traits) has exceptions within and among species. This makes classification problematic.

References

Arnold, E. N. (1988). Caudal autotomy as a defense. In 'Biology of the Reptilia. Vol. 16'. (Eds C. Gans and R. B. Huey.) pp. 235–274. (Liss: New York.)

Arnold, S. J. (1983). Morphology, performance and fitness. *Integrative and Comparative Biology* **23**, 347–361. doi: 10.1093/icb/23.2.347

Ast, J. C. (2001). Mitochondrial DNA evidence and evolution in Varanoidea (Squamata). *Cladistics* **17**, 211–226. doi: 10.1006/clad.2001.0169

Bauwens, D., Garland, T., Castilla, A. M., and van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution* **49**, 848–863. doi: 10.2307/2410408

Baverstock, P., King, D., King, M., Birrell, J., and Krieg, M. (1993). Evolution of species of Varanidae: microcomplement fixation analysis of serum albumins. *Australian Journal of Zoology* **41**, 621–638. doi: 10.1071/ZO9930621

Bookstein, F., Chernoff, B., Elder, R., Humphries, J., Smith, G., and Strauss, R. (1985). 'Morphometrics in Evolutionary Biology: The Geometry of Size and Shape Change, with Examples from Fishes.' (Academy of Natural Sciences of Philadelphia: Philadelphia, PA.)

Card, W., and Kluge, A. G. (1995). Hemipeneal skeleton and varanid lizard systematics. *Journal of Herpetology* **29**, 275–280. doi: 10.2307/1564567

Christian, K. (2004). *Varanus panoptes*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 423–429. (Indiana University Press: Bloomington, IN.)

Clemente, C. (2007). Evolution of locomotion in Australian varanid lizards (Reptilia: Squamata: Varanidae): ecomorphological and ecophysiological considerations. Ph.D. Thesis, University of Western Australia, Perth.

Cogger, H. (1992). 'Reptiles and Amphibians of Australia.' (Reed: Sydney.)

Dryden, G. (2004). *Varanus acanthurus*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 298–307. (Indiana University Press: Bloomington, IN.)

Dryden, G., and Ziegler, T. (2004). *Varanus indicus*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 184–188. (Indiana University Press: Bloomington, IN.)

Eidenmuller, B. (2004). *Varanus storri*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 472–476. (Indiana University Press: Bloomington, IN.)

Filatov, D. A. (2002). Analysis of DNA sequence data sets. *Molecular Ecology Notes* **2**, 621–624. doi: 10.1046/j.1471-8286. 2002.00313.x

Fitch, A. J., Goodman, A. E., and Donnellan, S. C. (2006). A molecular phylogeny of the Australian monitor lizards (Squamata: Varanidae) inferred from mitochondrial DNA sequences. *Australian Journal of Zoology* **54**, 253–269. doi: 10.1071/ZO05038

Fuller, S., Baverstock, P., and King, D. (1998). Biogeographic origins of goannas (Varanidae): a molecular perspective. *Molecular Phylogenetics and Evolution* **9**, 294–307. doi: 10.1006/mpev.1997.0476

Garland, T., and Losos, J. B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In 'Ecological Morphology: Integrative Organismal Biology'. (Eds P. C. Wainwright and S. M. Reilly.) pp. 240–302. (University of Chicago Press: Chicago, IL.)

Harmon, L. J., Kolbe, J. J., Cheverud, J. M., and Losos, J. B. (2005). Convergence and the multidimensional niche. *Evolution* **59**, 409–421.

Herrel, A., Meyers, J. J., and Vanhooydonck, B. (2002). Relationship between microhabitat use and limb shape in phrynosomatid lizards. *Biological Journal of the Linnean Society* **77**, 149–163. doi: 10.1046/j.1095-8312.2002.00101.x

Hews, D. K. (1996). Size and scaling of sexually-selected traits in the lizard, *Uta palmeri. Journal of Zoology* **238**, 743–757.

Hildebrand, M. (1985). Digging of quadrupeds. In 'Functional Vertebrate Morphology'. (Eds M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake.) pp. 89–109. (Harvard University Press: Cambridge, MA.)

Horn, G.-H. (2004). *Varanus gilleni*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 355–365. (Indiana University Press: Bloomington, IN.)

Horn, G.-H., and King, D. R. (2004). *Varanus giganteus*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 335–354. (Indiana University Press: Bloomington, IN.)

Husband, G., and Christian, K. (2004). *Varanus primordius*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 434–437. (Indiana University Press: Bloomington, IN.)

Irschick, D. J., and Garland, T. (2001). Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annual Review of Ecology and Systematics* **32**, 367–396. doi: 10.1146/annurev.ecolsys.32.081501.114048

Irwin, S. (1996). Capture, field observations and husbandry of the rare canopy varanid. *Thylacinus* **21**, 12–19.

Irwin, S. (2004). *Varanus keithhornei*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 401–405. (Indiana University Press: Bloomington, IN.)

Jusufi, A., Goldman, D. I., and Revzen, S. (2008). Active tails enhance arboreal acrobatics in geckos. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 4215–4219. doi: 10.1073/pnas.0711944105

King, D. R. (2004*a*). *Varanus pilbarensis*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 430–433. (Indiana University Press: Bloomington, IN.)

King, D., and Green, B. (1999). 'Varanid: The Biology of Varanid Lizards.' (UNSW Press: Sydney.)

King, D. R., and King, R. A. (2004). *Varanus rosenbergi*. In 'Varanoid lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 438–450. (Indiana University Press: Bloomington, IN.)

King, M. (2004*b*). *Varanus kingorum*. In 'Varanoid lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 406–409. (Indiana University Press: Bloomington, IN.)

Kohlsdorf, T., Garland, T., and Navas, C. A. (2001). Limb and tail lengths in relation to substrate usage in *Tropidurus* lizards. *Journal of Morphology* **248**, 151–164. doi: 10.1002/jmor.1026

LaBarbera, M. (1989). Analyzing body size as a factor in ecology and evolution. *Annual Review of Ecology and Systematics* **20**, 97–117. doi: 10.1146/annurev.es.20.110189.000525

Lemm, J. M., and Bedford, G. (2004). *Varanus spenceri*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 466–471. (Indiana University Press: Bloomington, IN.)

Losos, J. B. (1990*a*). Ecomorphology, performance capability and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecological Monographs* **60**, 369–388. doi: 10.2307/1943062

Losos, J. B. (1990*b*). The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* **44**, 1189–1203. doi: 10.2307/2409282

Macey, J. R., Larson, A., Ananjeva, N. B., Fang, Z., and Papenfuss, T. J. (1997). Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* **14**, 91–104.

Malhotra, A., and Thorpe, R. S. (1997). Size and shape variation in a Lesser Antillean anole, *Anolis oculatus* (Sauria: Iguanidae) in relation to habitat. *Biological Journal of the Linnean Society* **60**, 53–72.

Mayes, P. J. (2007). The ecology and behaviour of *Varanus mertensi* (Reptilia: Varanidae). Ph.D. Thesis, Edith Cowan University, Perth.

Melville, J., and Swain, R. (2000). Evolutionary relationships between morphology, performance and habitat openness in the lizard genus *Niveoscincus* (Scincidae: Lygosominae). *Biological Journal of the Linnean Society* **70**, 667–683.

Miles, D. B. (1994). Covariation between morphology and locomotory performance in sceloporine lizards. In 'Lizard Ecology: Historical and Experimental Perspectives'. (Eds L. J. Vitt and E. R. Pianka.) pp. 207–235. (Princeton University Press: Princeton, NJ.)

Mosimann, J. E. (1970). Size allometry: size and shape variables with characterizations of the lognormal and generalised gamma distributions. *Journal of the American Statistical Association* **65**, 930–945. doi: 10.2307/2284599

Norman, J. A., Christidis, L., Westerman, M., and Hill, F. A. R. (1998). Molecular data confirms the species status of the Christmas Island hawk-owl *Ninox natalis*. *Emu* **98**, 197–208. doi: 10.1071/MU98028

Pianka, E. R. (1969). Notes on the biology of *Varanus caudolineatus* and *Varanus gilleni*. *Western Australian Naturalist* **11**, 76–82.

Pianka, E. R. (1994). Comparative ecology of *Varanus* in the Great Victoria Desert. *Australian Journal of Ecology* **19**, 395–408. doi: 10.1111/j.1442-9993.1994.tb00505.x

Pianka, E. R. (2004*a*). *Varanus brevicauda*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 312–317. (Indiana University Press: Bloomington, IN.)

Pianka, E. R. (2004*b*). *Varanus eremius*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 328–334. (Indiana University Press: Bloomington, IN.)

Pianka, E. R. (2004*c*). *Varanus semiremex*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 462–465. (Indiana University Press: Bloomington, IN.)

Pianka, E. R. (2004*d*). *Varanus tristis*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 477–487. (Indiana University Press: Bloomington, IN.)

Posada, D., and Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. doi: 10.1093/bioinformatics/14.9.817

Rohlf, F. J. (2001). Comparative method for the analysis of continuous variables: geometric interpretations. *Evolution* **55**, 2143–2160.

Russell, A. P., and Bauer, A. M. (1992). The *m. caudifemoralis longus* and its relationship to caudal autotomy and locomotion in lizards (Reptilia: Sauria). *Journal of Zoology* **227**, 127–143.

Schultz, T., and Doody, S. (2004). *Varanus mitchelli*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 416–422. (Indiana University Press: Bloomington, IN.)

Smith, L. A., Sweet, S. S., and King, D. R. (2004). *Varanus scalaris*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 451–461. (Indiana University Press: Bloomington, IN.)

Somers, K. M. (1986). Multivariate allometry and removal of size with principal components analysis. *Systematic Zoology* **35**, 359–368. doi: 10.2307/2413387

Somers, K. M. (1989). Allometry, isometry and shape in principal component analysis. *Systematic Zoology* **38**, 169–173. doi: 10.2307/2992386

Sprackland, R. G. (1991). The origin and zoogeography of the monitor lizards of the subgenus *Odatria* Gray (Sauria: Varanidae): a re-evaluation. *Mertensiella* **2**, 240–252.

Sweet, S. S. (1999). Spatial ecology of *Varanus glauerti* and *V. glebopalma* in Northern Australia. *Mertensiella* **11**, 317–366.

Sweet, S. S. (2004*a*). *Varanus glauerti*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 366–372. (Indiana University Press: Bloomington, IN.)

Sweet, S. S. (2004*b*). *Varanus glebopalma*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 373–379. (Indiana University Press: Bloomington, IN.)

Swofford, D. L. (2000). 'PAUP*: Phylogenetic Analysis using Parsimony (* And Other Methods).' Version 4.0. (Sinauer: Sunderland, MA.)

Thompson, G. G. (1994). Activity area during the breeding season of *Varanus gouldii* (Reptilia: Varanidae) in an urban environment. *Wildlife Research* **21**, 633–641. doi: 10.1071/WR9940633

Thompson, G. G. (1995). Foraging patterns and behaviours, body postures and movement speed for varanids, *Varanus gouldii* (Reptilia: Varanidae), in a semi-urban environment. *Journal of the Royal Society of Western Australia* **78**, 107–114.

Thompson, G. G. (2004*a*). *Varanus caudolineatus*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 318–327. (Indiana University Press: Bloomington, IN.)

Thompson, G. G. (2004*b*). *Varanus gouldii*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 380–400. (Indiana University Press: Bloomington, IN.)

Thompson, G. G., and Withers, P. C. (1997). Comparative morphology of Western Australian varanid lizards (Squamata: Varanidae). *Journal of Morphology* **233**, 127–152. doi: 10.1002/(SICI)1097-4687(199708)233:2<127::AID-JMOR4>3.0.CO;2-3

Thompson, G. G., and Withers, P. C. (2005). The relationship between size-free body shape and choice of retreat for Western Australian *Ctenophorus* (Agamidae) lizards. *Amphibia-Reptilia* **26**, 65–72. doi: 10.1163/1568538053693323

Thompson, G. G., de Boer, M., and Pianka, E. R. (1999). Activity areas and daily movements of an arboreal monitor lizard, *Varanus tristis* (Squamata: Varanidae) during the breeding season. *Australian Journal of Ecology* **24**, 117–122. doi: 10.1046/j.1442-9993.1999.241952.x

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality tools. *Nucleic Acids Research* **25**, 4876–4882. doi: 10.1093/nar/25.24.4876

Vanhooydonck, B., and Van Damme, R. (1999). Evolutionary relationships between body shape and habitat use in lacertid lizards. *Evolutionary Ecology Research* **1**, 785–805.

Vanhooydonck, B., and Van Damme, R. (2003). Relationships between locomotor performance, microhabitat use and antipredator behaviour in lacertid lizards. *Ecology* **17**, 160–169.

Vitt, L. J., Caldwell, J. P., Zani, P. A., and Titus, T. A. (1997). The role of habitat shift in the evolution of lizard morphology: evidence from tropical *Tropidurus*. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 3828–3832. doi: 10.1073/pnas.94.8.3828

Weavers, B. W. (1993). Home range of male lace monitors, *Varanus varius* (Reptilia: Varanidae), in south eastern Australia. *Wildlife Research* **20**, 303–313. doi: 10.1071/WR9930303

Weavers, B. (2004). *Varanus varius*. In 'Varanoid Lizards of the World'. (Eds E. R. Pianka and D. R. King.) pp. 488–502. (Indiana University Press: Bloomington, IN.)

Wilson, S. K., and Knowles, D. G. (1995). 'Australia's Reptiles: A Photographic Reference to the Terrestrial Reptiles of Australia.' (Cornstalk: Sydney.)

Appendix 1. List of taxa sequenced, source and voucher number for tissue or specimen, GenBank accession number and location
WC = wild-caught, AM = Australian Museum, QM = Queensland Museum

Species	Species no.	Voucher no.	GenBank ID	Collection locality
V. acanthurus	1	WC-V5	EU920057	Newman, WA
V. acanthurus	–		AF407488	N and Central Aust.
V. baritiji	–		AF407489	N Australia
V. beccari	–		AF407490	Aru Islands
V. bengalensis	–		AF407491	Indonesian Islands
V. brevicauda	2		AY264940	Australia
V. bushi	3	WC-V20	EU920129	Auski Roadhouse, WA
V. caudolineatus	4	WC-V12	EU920058	Leinster, WA
V. doreanus	–		AF407493	Papua New Guinea
V. dumerilli	–		AF407494	SE Asia
V. eremius	5	WC-V23	EU920060	Giralia Stn, WA
V. exanthermaticus	–		AF407496	Central Africa
V. giganteus (WA)	6	WC-V8	EU920061	Newman, WA
V. giganteus (NT)	–	WC-V26	EU920062	Alice Springs, NT
V. gilleni	7	WC-V18	EU920063	Kiwirrakurra, WA
V. glauerti	8	WC-V22	EU920064	Kununarra, WA
V. glebopalma	9		AF407501	NW Australia
V. gouldii (Qld)	10	R138112	EU920065	Wyandra, Qld
V. gouldii (WA)	11	WC-Vgouldi1	EU920074	Perth, WA
V. gouldii (NT)	–	AM-R156899	EU920072	Curtin Springs, NT
V. griseus griseus	–		AF407503	N Africa/Central Asia
V. griseus	–		U71334	N Africa/Central Asia
V. indicus	12		AF407506	N Australia
V. jobensis	–		AF407507	Papua New Guinea
V. keithhornei	13	QM-J63906	EU920084	Australia Zoo, Qld
V. kingorum	14	WC-V14	EU920085	Warnum, WA
V. komodoensis	–		AF407510	Indonesian Islands
V. melinus	–		AF407511	Indonesian Islands
V. mertensi	15	AM-R123877	EU920087	Mitchell Plateau, WA
V. mitchelli	16	WC-V3	EU920088	Kununarra, WA
V. niloticus	–		AF407514	S and Central Africa
V. olivaceus	–		AF407515	Luzon Island
V. p. panoptes (Qld)	17	QM-J79717	EU920095	Wandoan, Qld
V. p. panoptes (Qld)	–	J76100	EU920080	Queensland
V. p. horni (Qld)	–	R143871	EU920097	Northern Qld
V. p. panoptes (WA)	18	WC-Vpano1	EU920101	Kununarra, WA
V. p. rubidus (WA)	19	WC-Vprub2	EU920102	Sandstone, WA
V. pilbarensis	21		AF407518	W Australia
V. prasinus	–		AF407519	Papua New Guinea
V. rosenbergi	23		AY26941	S Australia
V. rudicollis	–		AF407521	SE Asia
V. salvadorii	–		AF407522	Papua New Guinea
V. salvator	–		AF407526	SE Asia
V. scalaris (Qld)	24		AF407527	N Australia
V. scalaris (WA)	25	WC-V1	EU920108	Kununarra, WA
V. scalaris (???)	–		AF407528	Papua New Guinea
V. semiremex	26	QM-J76101	EU920111	Townsville, Qld
V. spenceri	27	QM-J76102	EU920112	Cannington, Qld
V. storri (Qld)	28	AM-EBU5139	EU920115	Croydon, Qld
V. storri (WA)	29	WC-V17	EU920114	Kununarra, WA
V. timorensis	–		AF407532	Timor
V. tristis (Qld a)	30	QM-J81072	EU920118	Moranbah, Qld
V. tristis (WA)	32	WC-Vtri/V19	EU920117	Kununarra, WA
V. varius	33	QM-J80572	EU920126	Brookfield, Qld
V. varius	–	QM-J76106	EU920120	Ravenshoe, Qld
V. yuwonoi	–		AF407535	Halmahera Island