1 **Combining geometric morphometric analyses of multiple 2D**

2 **observation views improves interpretation of evolutionary allometry**

3 **and shape diversification in monitor lizard (*Varanus*) crania**

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6

9 **ABSTRACT**

10 Geometric morphometrics is a powerful tool for the quantification, visualization and analysis of

11 morphological variation and change. This approach is being applied more frequently in a

12 phylogenetic comparative context to assess the relative influence of size, ecology, function, and

13 developmental constraints on morphological evolution. Geometric morphometric methods rely on

14 homologous landmarks as the source of shape data, and the level of detail and accuracy increases

15 with the amount of information contained in a landmark configuration. However, it may be possible

16 to capture particular elements of shape variation by concentrating on different observation angles of

17 a complex structure like the vertebrate cranium. Our study examines how observation view (dorsal,

18 ventral and lateral) influences 2D geometric morphometric analysis of interspecific cranial shape

19 variation in monitor lizards. We recover strong phylogenetic signal in all three views and general

20 concordance in patterns of size-corrected shape diversification within the genus. However, we also

21 find subtle but important differences among views in analyses of evolutionary allometry and shape

22 variation, which may reflect both landmark configuration design and adaptive functional trends of

23 the study system. Our study shows that studies restricted to a 2D geometric morphometric analysis

24 of a complex 3D biological structure can combine carefully designed 2D landmark configurations

25 describing alternative planes to maximize shape coverage.

INTRODUCTION

The field of morphometrics is a central component of biology that quantifies biological shape, shape variation, and its correlation with other variables (Bookstein, 1991; Adams, Rohlf & Slice, 2004; Webster & Sheets, 2010; Adams, Rohlf & Slice, 2013). Geometric morphometrics are a popular set of methods that restrict data to locations of discrete anatomical points (landmarks), and rely on a constellation of points (the landmark configuration) to sample morphology (Bookstein, 1986; Bookstein, 1993; Corti, 1993; O'Higgins, 2000). Designing an appropriate landmark configuration is a critical early step in geometric morphometric analysis and ideally, three broad goals are achieved through appropriate selection of landmarks - a configuration should cover the variation clearly observable among objects, provide a sufficiently comprehensive and biologically significant sampling of morphology, and deliberately test the hypotheses proposed (Roth, 1993; O'Higgins, 2000; Oxnard & O'Higgins, 2009; Zelditch, Swiderski & Sheets, 2013).

Geometric morphometric studies frequently use a two-dimensional landmark configuration to quantify form, even though the structure of interest is typically three-dimensional (Roth, 1993; Cardini, 2014; Klingenberg, 2015). While the 3D approach is becoming more accessible, for many it is still a more expensive and time consuming processes that requires large data storage facilities. The 2D approach has several advantages over 3D, in particular the ease of capturing 2D coordinates by placing points on a photograph using freely available, user-friendly software such as tpsDig (Rohlf, 2010a), and the intuitively understood visualizations of shape variation produced using the thin-plate spline (Klingenberg, 2013a). It is also much faster and so it is easier to obtain much larger sample sizes to quantify variation. The obvious disadvantage of a 2D approach is the loss of information regarding depth, and both correlational and comparative studies of 2D versus 3D datasets suggest that including the third coordinate may lead to different results for *some* biological structures (Cardini & Thorington, 2006; Álvarez & Perez, 2013; Cardini, 2014). Despite the fact that 2D morphometric analyses of 3D structures mean the loss of tridimensional information, the

52 relative increased costs, time and difficulty associated with 3D data collection, storage and analysis

53 still make 2D geometric morphometrics a realistic, inexpensive, and time-effective choice for many

54 researchers (Cardini, 2014; and references therein).

55

56 The vertebrate head is a complex structure that has received much attention in morphometric

57 studies, perhaps due to the enormous disparity in form that has evolved among groups. At the

58 broadest scale, the skull is comprised of three parts: the cranial vault, the cranial base, and face (de

59 Beer, 1937). Although these different parts originate in embryologically distinct regions, they

60 apparently grow in a morphologically integrated manner through numerous developmental and

61 functional interactions (Lieberman, Ross & Ravosa, 2000; Bookstein *et al.* 2003; Sanger *et al.*

62 2012). This has implications for 2D geometric morphometric analyses of head shape diversification

63 regarding which observation view (or combination of views) is most informative for any vertebrate

64 group. An observation view is the orientation of the specimen in relationship to observer, and the

65 view(s) chosen by a researcher may influence the nature of the morphometric data yielded from a

66 given specimen. This is problematic as the numerous structural subunits of the head observable

67 from different views may be semi-independent in ontogeny or function, and are thus expected to

68 vary in conservative properties and respond differently to ecological and evolutionary factors

69 (Klingenberg, 1996; Lu *et al.* 2014). It is not understood how profoundly 2D shape analyses of the

70 vertebrate head are influenced by observation view(s) and the landmark configuration used to

71 characterize them.

72

73 Lizards are ideal for such examination, as they have a remarkable diversity in cranial form that is

74 tightly linked to the functional and constructional demands of the skull, and show a unique

75 evolutionary trend toward increased fenestration and reduced cranial ossification (Evans, 2003;

76 Stayton, 2005; Herrel *et al.* 2007; Daza *et al.* 2008). The ventral morphology of lizard crania,

77 formed by parts of the dermatocranium and neurocranium, is directly involved in the mechanics of

feeding, affecting bite force through connections to the jaw musculature (Herrel *et al.* 2007). Jaw musculature is also associated with structures most clearly observed from a lateral view (for example the postorbital-postfrontal, quadrate, coronoid and jugal). Consequently, much shape variation observed in lateral view is strongly linked with diet, for example herbivorous lizards tend to have short snouts and high skulls, which is consistent with a slow but powerful bite relative to carnivorous lizards (Evans, 2003; Stayton, 2005). Palate morphology and the septomaxilla also reflect the evolution of the chemosensory system in general, and the vomeronasal system in particular (Rieppel, Gauthier & Maisano, 2008), and the skull roofing bones are greatly influenced by the development of the brain and other sensory organs (Haas, 1973). The most highly fenestrated cranial morphology belongs to the monitor lizards (family: Varanidae), and is known as a 'space frame' type of construction in engineering terms (McCurry *et al.* 2015). The monitor cranium has a noteworthy mixture of extremely reduced bones (such as those forming the skull roof, particularly the snout region), derived bones (such as the palpebral and dermal palate elements), and composite bones (such as the fused postorbitofrontal) (Bellairs, 1949; Frazzetta, 1962; Auffenberg, 1988; Rieppel *et al.* 2008; Werneburg *et al.* 2015). Further, 2D geometric morphometric analysis of lateral cranial shape among lizard families reveals monitors have conspicuous patterns of interspecific cranial shape disparity (Stayton, 2005).

Our study examines how observation view and landmark configuration design influence 2D geometric morphometrics analysis of interspecific cranial shape variation in monitor lizards. Specifically, we quantify the covariation of species cranial shapes with phylogeny and allometry, and characterize major patterns of interspecific cranial shape variation and size-corrected diversification in morphospace. We then identify and evaluate differences among 2D views to determine the utility of combining multiple views, and to assess whether choosing different views for landmark-based morphometric analyses could influence our quantification of shape differences among specimens or species, providing complementary morphological information to each other.

104 We expect congruence among views for quantification of shape covariation with phylogeny to

105 reflect developmental constraints within the cranium. A pervasive influence of allometry on shape

106 is predicted, and should be exemplified in monitor lizard morphology, as species differ in body

107 mass over four orders of magnitude (Pianka, 1995). However, we predict the anatomical points

108 sampled to reflect functionally different characters among views, and therefore to recover

109 discrepant patterns of interspecific cranial shape variation and size-corrected diversification.

110

MATERIALS AND METHODS

Morphometric data

113 Our morphometric dataset comprises images of the cranium for 29 *Varanus* species (n = 152

114 specimens) in lateral, dorsal and ventral views (Supp. Table S1). We only used cranial material of

115 adult specimens (skeletal maturity judged by specimen size) and we did not know the sex of the

116 specimens. Within a species, male and female varanids have the same shaped heads but subtle

117 sexual dimorphism in head size has been detected in some of the larger monitor species (Frýdlová,

118 Velenský, Šimková *et al.* 2011). Not knowing the sex in our study is unlikely to influence our

119 results because we correct for head size and our primary interest is in interspecific shape variation

120 rather than intraspecific size variation. A majority of the dataset is photographs of preserved crania,

121 obtained from collections in the American Museum of Natural History (AMNH), the Florida

122 Museum of Natural History (FLMNH), the Field Museum of Natural History (FMNH), the National

123 Museum of Victoria (NMV), the University of Michigan Museum of Zoology (UMMZ) and the

124 Smithsonian Institution National Museum of Natural History (USNM). Photographs were taken

125 with a mounted and leveled Olympus ® C-765 Ultra Zoom. Skulls were situated against a dark

126 background with a scale bar in the same plane. The lateral view involved positioning the crania so

127 the camera was perpendicular to the mid-sagittal plane. The dorsal view involved placing the

128 cranium ventral side down with the teeth and palate against the surface. The ventral view involved

129 positioning the skull so the palate was parallel to the camera lens. The dataset was supplemented

with 2D images obtained from micro X-ray computed tomography (CT) scans of crania from whole, alcohol-preserved specimens from the Western Australian Museum (WAM) (11 species). All scans were made with the Xradia microCT x400 system at the Australian Microscopy and Microanalysis Research Facility (University of Sydney). CT scan data were rendered using Drishti v2.0 (http://anusf.anu.edu.au/Vizlab/drishti/). Non-cranial volumes were digitally removed, and images of the lateral, dorsal and ventral views of the cranium were then saved (including a scale bar).

Phylogeny

The phylogenetic hypothesis used in this study is based on the published molecular phylogeny presented in Vidal *et al.* (2012). We assembled the published data for 26 species included in Vidal *et al.* (2012) and added three additional species from the published work of Welton *et al.* (2014) (total: 29 species) (Fig. 1; Supp. Table S1). In order to include the three additional species we generated a maximum-likelihood phylogenetic tree based on mitochondrial protein-coding genes (ND3 and ND4) and nuclear (brain-derived neurotrophic factor, bone morphogenetic protein 2, and neurotrophin 3) sequence data and constrained the major clades so that the resultant phylogeny would be consistent with the phylogeny presented in Vidal *et al.* (2012). The resulting phylogeny was used for visualizing shape diversification across *Varanus*, identifying shape differences among different groups in a phylogenetic context, and to test for phylogenetic signal.

2D Geometric morphometrics

We used a 2D geometric morphometric approach for all views, also using a 2D sliding semilandmark procedure for the ventral view (Bookstein, 1997). Landmarks and semilandmarks were collected using tpsDig v. 2.16 (Rohlf, 2010a (Fig. 2; Supp. Table S2). The semilandmarks were collected as a curve outlining the fused pre-maxillae and paired maxillae in ventral view (Fig. 2). These data were subsequently reduced to equidistant landmarks (four on the pre-maxillae and

156 ten on each maxilla), and subsequently defined as semilandmarks using the software tpsUtil v. 1.46

157 (Rohlf, 2010c). We then slid the landmarks using the bending energy method (Bookstein, 1997;

158 Gunz & Mitteroecker, 2013) implemented in the R statistics package *geomorph* (Adams & Otárola-

159 Castillo, 2013). The original landmark coordinate data were aligned using a generalized Procrustes

160 superimposition analysis (GPA), providing the centroid size and shape coordinates (Adams *et al.*

161 2013). The monitor cranium has matching symmetry in lateral view and object symmetry in dorsal

162 and ventral views. Although shape analysis procedures for both types of symmetry can separate the

163 symmetric (left-right averages) component of variation from left-right asymmetries within

164 individuals (Klingenberg, Barluenga & Meyer, 2002), damage to specimens in lateral view meant

165 we could only digitize landmarks on one side of the cranium. Shape data therefore refers to

166 Procrustes coordinates in lateral view, and the symmetric component of shape variation in dorsal

167 and ventral views. We then calculated the mean of the Procrustes coordinates and centroid sizes of

168 each species, in order to generate phylomorphospace plots and allow phylogenetic comparative

169 analyses on the shape data.

170

171 **Cranium size and shape variation**

172 Ignoring the phylogeny in preliminary transformations of species data results in substantially

173 elevated variance and type I error (false positive) in statistical estimators (Revell, 2009). To

174 quantify the cranial shape variation evolutionarily associated with allometry in the monitor lizard

175 cranium, we performed a Procrustes regression of shape on size, based on the Procrustes

176 coordinates averaged by species, using the software *geomorph* (Adams & Otárola-Castillo, 2013).

177 We also used the residuals from the averaged Procrustes coordinates and centroid sizes in order to

178 perform a phylogenetic regression of shape on size in a phylogenetic context, in order to get

179 'evolutionary allometry'-corrected shape data, also using *geomorph* (Adams & Otárola-Castillo,

180 2013). Analyzing the residuals from a regression of the size-dependent trait against size is a

181   common method of correcting for allometry in evolutionary studies (Gould, 1966; Monteiro, 1999;

182   Revell, 2009).

183

184   **Phylogenetic signal**

185   To quantify phylogenetic signal in *Varanus* crania for each of the views (dorsal, ventral, and

186   lateral), we used the K_{mult} method, which is Blomberg's K statistic generalization appropriate for

187   high-dimensional and/or multivariate data (Adams, 2014), using the R package *geomorph* (Adams

188   & Otárola-Castillo 2013). We determined the statistical significance of K_{mult} using phylogenetic

189   permutation with 10,000 iterations, which is calculated by permuting the shape data of the

190   Procrustes-aligned specimens among all tips of the phylogenetic tree. We also simulated the

191   confidence intervals of K under a Brownian Motion (BM) model of evolution using *phytools*

192   (Revell, 2012), with 10,000 iterations. Under BM, K_{mult} has an approximate expected value of 1.0,

193   and the higher the K_{mult} value is, the stronger the phylogenetic signal, indicating higher

194   morphological variance among clades instead of within clades (Adams, 2014).

195

196   **Patterns of cranial shape variation and diversification**

197   To examine patterns of cranial shape variation among *Varanus*, we subjected the averaged shape

198   coordinates to a Principal Component Analysis (PCA), both before and after size correction, for all

199   three views. To characterize the evolutionary patterns of cranial shape diversity, we projected the

200   phylogeny into the shape tangent space and projected it on plots species mean size-corrected cranial

201   shapes. This approach maps PC scores of the species cranial shapes to the phylogeny, computes the

202   PC scores at internal nodes, and subsequently projects the branches of the phylogenetic tree onto

203   the morphospace. Evaluating this 'phylomorphospace' provides insights into the history of

204   morphological diversification for complex traits and allows a multivariate visualization of shape

205   differences between species (Sidlauskas, 2008; Sherratt *et al.* 2014). To quantify the amount of

206   shape variation and dispersion between original and size-corrected shape data, we measured the

207 dispersion of all the individuals at three levels: (a) for the whole data set – by calculating the

208 dispersion around the mean shape for all the specimens, (b) by species, and (c) by the phylogenetic

209 groups identified in Vidal *et al.* (2012). Finally, we tested the integration between the three views,

210 in order to assess the strength of dependency among each landmark configuration, with *geomorph*

211 (Adams & Otárola-Castillo 2013).

212

RESULTS

Shape variation among *Varanus* crania

215 In dorsal view, original shape variation captured by the two first Principal Components (PC 1-2)

216 relates to cranium width, relative length of the snout and parietal units, and angularity of the fronto-

217 parietal suture and nasal-frontal boundary (Fig. 3; Fig. 4). PC 1 (accounting for 54.9% of the overall

218 morphological variance) (Table 1) contrasts crania with a shortened snout complex and widened

219 parietal unit in the negative direction, and crania with a lengthened snout complex and narrowed

220 parietal unit in the positive direction. PC 2 (19.0% variance) contrasts narrow crania with a

221 relatively short snout in the negative direction, and broad crania with a relatively long snout in the

222 positive direction.

223

224 In ventral view, over 70% of the cranial shape variation among monitors relates to cranium width,

225 curvature of the snout, robusticity of the palatine and ectopterygoid, and length of the pterygoid

226 (Fig. 3; Fig. 4). For PC 1 (47.7% variance), a negative change from the mean describes crania with

227 a short and broad snout, distally shifted and broadened suborbital bones, and a broad and long

228 pterygoid. A positive change from the mean describes crania with an elongate and narrow snout,

229 mesially shifted and narrowed suborbital region, and a shortened pterygoid. PC 2 (24.7%) captures

230 relative shifts in landmarks altering pointedness of the snout, width of the posterior maxilla region,

231 mesial-distal shifting in the palatine and length of the pterygoid.

232

In lateral view, shape variation captured by PC 1-2 relates to overall cranium height, length and angularity of the snout complex, convex-concave bending and length of the basal units, and robustness of the parietal unit (Fig. 3; Fig. 4). For PC 1 (31.1%), a negative change from the mean shape describes relative shifts in landmarks that flatten the cranium, lengthen the snout complex, and both shorten and project the parietal and basal units mesially. A positive change from the mean shape corresponds to a heightened cranium with a short but steeply rising snout complex, and enlarged parietal and basal units. PC 2 (22.5%) describes more subtle shape changes, contrasting crania with a lengthened snout complex, ventrally projected pterygoid, and steeply rising cranial roof in the negative direction, and crania with a shortened snout complex, mesially projected elongate pterygoid, and flattened cranial roof in the positive direction.

Phylogenetic signal

The results of the multivariate K-statistic calculated on the shape data are significant for each view of the *Varanus* cranium (dorsal: K_{mult} = 0.99, P < 0.001; ventral: K_{mult} = 0.85; P <0.001; lateral: K_{mult} = 0.83, P < 0.001). K 95% confidence interval for values expected under a Brownian Motion model of trait evolution = [0.658, 1.612].

Cranial size and shape variation

The multivariate Procrustes regressions of shape data on centroid size were statistically significant (P <0.0001) in dorsal, ventral and lateral view, indicating the presence of allometry in the *Varanus* cranium. In the quantification of evolutionary allometry (when phylogenetic relatedness is incorporated in the regression), the r^2 values decrease substantially but remain significant for all three views (Table 2). The dorsal view recovers the largest r^2 values, followed by lateral view then ventral view, suggesting that shape variation in the monitor lizard cranium is most heavily constrained by size in the skull roof. The amount of shape variation accounted for by PC 1-5 is substantially less after evolutionary allometry is removed from average species cranial shapes

259 (Table 1). The amount of dispersion and shape variation was very similar between original and size-

260 corrected shape data for each of the views, for all three levels (all individuals, by species, and by

261 phylogenetic groups; Table 3). The two-block PLS between different views pointed out very high

262 integration between dorsal and lateral (r-PLS = 0.943, P < 0.001) and between ventral and lateral

263 landmark configurations (r-PLS = 0.921, P < 0.001), and moderately high between dorsal and

264 ventral views (r-PLS = 0.878, P < 0.001).

265

266 **Size-corrected shape diversification in *Varanus* crania**

267 Superimposing the phylogeny onto a plot defined by PC 1-2 of evolutionary allometry-corrected

268 shape variation shows cranial shape diversification across species, depicting their great

269 morphological differences (Fig. S1). There is a moderate clustering of size-corrected species cranial

270 shapes according to phylogenetic group in 'phylomorphospace' for all three-observation views.

271 These clusters are most clearly separated from each other in dorsal view. The major difference in

272 diversification patterns among views is attributed to the African species, which occupy a positive

273 position on PC 2 in dorsal view, but a negative PC 2 position in ventral and lateral views. Cranial

274 shapes of species in the Indo-Australian C phylogenetic group show the greatest divergence from

275 all other species cranial shapes, occupying a distinct region of each phylomorphospace. Cranial

276 shapes of individual species from all other phylogenetic groups have diversified varying amounts in

277 each view, as shown by branches that traverse a large proportion of the region occupied by the

278 whole sample. These include the African species *Varanus exanthematicus* and *Varanus albigularis*,

279 the Indo-Asian species *Varanus olivaceus* and *Varanus rudicollis*, and the Indo-Australian species

280 *Varanus komodoensis* and *Varanus giganteus*.

281

282 **DISCUSSION**

283 The highly fenestrated space frame construction of monitor lizard crania creates clear differences in

284 abundance and spread of candidate landmarks among alternative views of the structure. It follows

that the design of each 2D landmark configuration inevitably describes shape in a slightly different way. Phylogeny underpins patterns of gross morphological variation among monitor lizard crania, even when diluted by size-related constraints and presumably adaptive trends, which might be also phylogenetically constrained. The strength of association between cranial size and shape differs among observation angles; however, it is unclear whether these results reflect genuine evolutionary trends or slight differences on the morphological information captured by each landmark configuration design. The patterns of cranial shape variation are broadly concordant among views, suggesting dietary variation has contributed to shape diversification across the entire cranium at a higher phylogenetic level. Below, we evaluate 2D characterization of the monitor cranium, present an overview of cranial shape evolution in monitors, and discuss more broadly how 2D geometric morphometric analyses of vertebrate head shape can be improved through analysis of multiple observation views and careful landmark configuration design.

The evolution of cranial morphology in monitor lizards

A careful consideration of landmark configuration design is required when interpreting geometric morphometric results. Our landmark configurations capture a dorsal-ventral 'silhouette' in lateral view, the medial paired and fused bones in dorsal view, and palatopterygoid morphology in ventral view (Fig. 2). A 2D characterization of monitor lizard cranial morphology is most comprehensive in ventral view, as abundant type I and type II landmarks (Bookstein, 1991) are reliably digitized, and four curves are traced to outline the fused pre-maxillae and paired maxillae. We find the lateral view to be the least reliable, because of the difficulty in accurately orienting specimens when taking photographs (Klingenberg, 2015). The requirement of 2D landmarks to be coplanar (Zelditch *et al.* 2013) is most limiting for landmark configuration design in lateral and dorsal view, and is the key reason why semilandmarks outlining the pre-maxillae and maxillae were only added in ventral view. There are also morphological differences clearly observable among species for numerous

bones (such as the palpebral); however, such variation should not be characterized using geometric morphometric methods (Zelditch *et al.* 2004).

The major monitor lizard clades match biogeographic distribution well, dividing the African, Indo-Asian, and Indo-Australian species (Fig. 1). An Asian origin for crown *Varanus* is likely, with major dispersal events occurring to Africa approximately 41 million years ago (mya), and to Australia approximately 32 mya (Vidal *et al.* 2012). Significant phylogenetic signal is found across observation angles, and interspecific cranial shape variation still retains substantial phylogenetic structure after correcting for evolutionary allometry; Fig. 4 shows that cranial shapes of closely related species form moderate clusters in phylomorphospace. The importance of phylogenetic affinity for explaining interspecific variation is recovered in geometric morphometric analyses of lateral, dorsal and/or ventral cranial shape in other lizard studies (Stayton, 2005; Daza *et al.* 2009; Openshaw & Keogh, 2014). Such clear phylogenetic structure in the morphometric data across observation angles may indicate a role for developmental constraints in diversification of monitor lizard cranial shape.

Monitors occupy a wide range of environments across their distribution, and the selective demands imposed by habitat have driven the evolution of extreme body size disparity in the genus (with extant species ranging in total body length as adults from 23 cm to 3 m) (Collar, Schulte & Losos, 2011). Strong size-shape relationships are known for monitor lizard body and head morphology (Christian & Garland, 1996; Thompson & Withers, 1997; Collar *et al.* 2011; Openshaw & Keogh, 2014). Initially, we found strong and significant allometry in each view of the cranium; however, controlling for non-independence of shape data due to shared ancestry reduced the estimation of evolutionary allometry in all views. This also reflects the strong phylogenetic signal in body size evolution for the group (Collar *et al.* 2011; Openshaw & Keogh, 2014). The inconsistency in strength of evolutionary allometry among views could reflect discrepancy in the way each

observation view quantifies size: dorsal view recovers the strongest influence of evolutionary allometry ($r^2 = 0.2072$), and is described by a landmark configuration that effectively captures cranium length, but only covers cranium width at the posterior-most edges of the cranial table (Fig. 2).

The three observation views share notable similarities in patterns of interspecific cranial shape variation (Fig. 3) readily linked with diversity in diet through particular influences on cranial performance and strength. First, the shape changes captured by the primary axis of shape variation (PC 1) reveals two cranial phenotypes: (1) relatively tall and broad crania with a short and steeply rising snout; and (2) relatively flat and gracile crania with an elongate, slender snout. Monitor lizard crania with greater widths and heights perform better at biting, pulling and shaking (as exemplified by *V. komodoensis*) (Moreno *et al.* 2008; D'Amore *et al.* 2011). The most extreme examples of this morphotype belong to durophagous species like *V. exanthematicus*, which are characteristically short and wide with a tall snout (McCurry *et al.* 2015). These traits are often coupled with deep mandibles and robust dentition (Rieppel and Lambhardt 1979; D'Amore 2015; McCurry *et al.* 2015). The gracile morphotype is most clearly observed in 2D in *V. giganteus*. The elongate cranial shape likely reflects a diet consisting of large prey and carrion (McCurry *et al.* 2015). Second, there are finer scale shape differences that are expected to be important in determining the strength of a space frame construction. For example, a greater ventral projection of the palatopterygoid articulation to form an obtuse angle, and a more vertical positioning of the epipterygoid increases structural support, and is observed in semi-frugivorous species feeding like *V. olivaceous* (Auffenberg, 1988). We also recover variation in angularity of the frontoparietal suture or mesokinetic joint. Cranial sutures are forms of articulation in which the bones are rigidly joined by fibrous tissue (synarthroses) (Di Ieva *et al.* 2013) and are expected to have important roles in cranial kinesis and reducing stresses during biting feeding (Moazen *et al.* 2009). The concordance among observation angles and links with diet suggests feeding performance imposes selective pressure

across each view, resulting in high integration between the different views, and to certain degree among the cranial subunits described by each landmark configuration (Harmon *et al.* 2005; Perez, Bernal & Gonzalez, 2006; Klingenberg, 2008; Meloro *et al.* 2011).

Our analysis of dorsal, ventral and lateral cranium shape in monitors leads to subtle differences in interpretation of morphological diversification of the cranium as a whole. Given the dorsal landmark configuration describes fused and paired bones, we expect to recover a more conserved pattern of morphological diversification. Increased conservatism is evident through the strong phylogenetic signal, the greatest size-shape constraint among views, and a more clear separation of phylogenetic groups in phylomorphospace. The interplay of lateral and ventral views is apparent, further supporting a role for some developmental constraints in morphological diversification of the monitor cranium, but also highlighting the role of diet.

Implications for 2D landmark configuration design

Geometric morphometric methods have been widely applied in studies of head shape diversification for numerous vertebrate groups (Slice, 2007). External head morphology is primarily landmarked using the facial features in primates, and the scales of lizards, snakes and fish (Kaliontzopoulou 2011; Kerschbaumer & Sturmbauer, 2011; Baab, McNulty & Rohlf, 2012). The skull is frequently broken down into its constituent parts (including dentition), depending on the question of interest, and described by landmarks at the contacts between bones, tips of processes, origins and attachments of muscles, locations of joints and tips of lever systems (Slice, 2007). Although landmarks are frequently collected as surface marks on one tissue (i.e. scales or bone), they may relate to different tissues whose relationships vary with evolution, development, and growth (Slice, 2007; Oxnard & O'Higgins, 2009). For example, 2D landmark configurations on the surface of bone have been utilized in marmot skulls for all three cranial observation views, and the mandible. The ventral cranium seems to be the best morphological feature for recovering phylogenetic

relationships in varanids, and it seems that the largest shape modifications occur in regions directly involved in the mechanics of mastication in several other groups (Cardini & O'Higgins, 2004; Caumul & Polly, 2004; Cardini & Thorington, 2006). The lateral cranium and mandible are least strongly correlated with phylogeny, and dorsal and lateral cranium morphology together reflects brain size and dermal bones (Caumul & Polly, 2004; Cardini, Hoffman & Thorington, 2005).

The efficiency of geometric morphometrics methods to provide a detailed and accurate quantitative description of form ultimately increases with the amount of information contained within a landmark configuration (Cardini & Thorington, 2006). Outline-based geometric morphometric techniques are therefore a powerful addition to a 2D landmark configuration, as they can capture structural information on large areas of morphology only represented by surfaces, curves or outlines (Oxnard, 1978; Roth, 1993; Adams *et al.* 2004; Perez *et al.* 2006; Webster & Sheets, 2010). The outline-based approach is less widely applied than the landmark-based approach, having mainly been utilized in anthropological studies (Slice, 2007; Baab *et al.* 2012). However, three limitations of outline-based methods should be considered: (1) protrusions on one individual that do not appear on another can be poorly captured by the resulting semilandmark alignment; (2) it is often not clear how many semilandmarks should be used for any given outline; and (3) outline methods only define relative features (MacLeod, 1999; McCane, 2013; Finlay & Cooper, 2015). Regardless, their utility is highlighted in studies like ours, where the clarity of results is considerably improved in the landmark configuration(s) that utilize both landmarks and outlines (Baab *et al.* 2012; Finlay & Cooper, 2015). By increasing the number of coordinates along a homologous contour or surface, it is ultimately possible to obtain more information on the shape of a structure. The optimal 2D observation view for analyzing cranial shape variation may thus be the view in which both landmarks and semilandmarks may be utilized.

413 In conclusion, our study adds to a growing body of evidence suggesting multiple carefully designed

414 2D landmark configurations can provide fairly accurate descriptions of interspecific variation in

415 complex structures like the vertebrate head (Cardini & Thorington, 2006; Perez *et al.* 2009; Álvarez

416 & Perez, 2013; Cardini, 2014; Finlay & Cooper, 2015; Klingenberg, 2015). The key benefits of 3D

417 techniques may instead be in the extensions to assessing morphological patterns in internal

418 structures (e.g. brain case), functional morphology (finite element modeling and mechanical strain)

419 and reconstruction of ancestral shapes (evolutionary warping or morphing) that they offer (Wiley *et*

420 *al.* 2007; Parr *et al.* 2012).

421

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FIGURE CAPTIONS

619 **Fig. 1.** Phylogenetic relationships of the 26 *Varanus* species included in this study. The tree is

620 primarily based on the phylogeny of Vidal *et al.* (2012), with additional information from Welton *et*

621 *al.* (2014) (see text for details).

622

623 **Fig. 2.** The three 2D landmark configurations used in this study to characterize cranial shape for (a)

624 dorsal, (b) ventral, and (c) lateral views. A scale bar equal to 10 mm is shown, and numbers refer to

625 the anatomical definitions of landmarks in Supp. Table S2. The example skull is *Varanus glauerti*

626 specimen [WAM_R77266].

627

628 **Fig. 3.** The two major principal axes of cranial shape variation in *Varanus*, visualized as

629 deformation grids, in dorsal, ventral and lateral views. PC axes are from a PCA of species means,

630 and shape changes associated with the PCs are shown as extreme cranial shapes representative of

631 the positive and negative ends of each axis. In each case the magnitude of the shape change from

632 the mean is indicated by the extent of deformation.

633

634 **Fig. 4.** Cranial shape diversification among *Varanus*, in (a) dorsal, (b) ventral, and (c) lateral views,

635 for original shape data. The phylogenetic tree is superimposed onto a plot of the first two PCs of the

636 covariance matrix based on residuals computed using the regression vector of independent

637 contrasts. The tips of terminal branches are at the locations of species means, and are coloured

638 according to phylogenetic group.

TABLES

Table 1. Cranial shape variation in the three observation views captured in PC 1-5, both for the original and size-corrected shape data. The tabled values are the percentages of total variance (%) for which each principal component accounts.

Axis	Original Shape Data			Size-corrected Shape Data		
	Dorsal	**Ventral**	**Lateral**	**Dorsal**	**Ventral**	**Lateral**
PC 1	54.939	47.742	31.077	15.242	21.246	14.443
PC 2	18.995	24.666	22.514	12.078	11.425	10.838
PC 3	6.361	7.426	13.142	9.283	8.190	8.719
PC 4	5.094	5.803	6.781	7.330	6.924	6.810
PC 5	3.745	3.174	5.734	6.319	6.367	6.510
Cumulative (PC1-5)	89.133	88.811	79.248	50.252	54.152	47.320

Table 2. Summary table of multivariate Procrustes regressions and phylogenetic regressions of shape data on centroid size, for each of the three views, using *geomorph* (Adams & Otárola-Castillo 2013).

	Dorsal		Ventral		Lateral	
	R^2	*p*	R^2	*p*	R^2	*p*
Regression	0.3052	<0.0001	0.1666	<0.0001	0.2294	<0.0001
Phylogenetic regression	0.2072	<0.0001	0.1121	<0.0001	0.1367	<0.0001

Table 3. Summary of Procrustes Variances, accounting for the morphological disparity of all the samples for the whole data set, grouped by species (dispersion calculated within species), and grouped by regions (dispersion within each geographical region). Procustes Variances were calculated for both the original morphological GPA (Generalised Procrustes Analysis)-aligned morphological dataset, and the size-corrected one (accounting for allometry on the GPA-aligned dataset). For each analysis, we performed 1000 permutations, using the R-package *geomorph* (Adams & Otárola‑ Castillo, 2013).

Groups	Original shape data			Size-corrected shape data		
	Dorsal	**Ventral**	**Lateral**	**Dorsal**	**Ventral**	**Lateral**
All specimens	0.0054	0.0057	0.0068	0.0038	0.0048	0.0052
Species:						
Varanus acanthurus	0.0088	0.0062	0.0085	0.0036	0.0038	0.0041
Varanus albigularis	0.0080	0.0066	0.0120	0.0078	0.0066	0.0120
Varanus bengalensis	0.0053	0.0041	0.0069	0.0057	0.0039	0.0068
Varanus brevicauda	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus bushi	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus caudolineatus	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus dumerilii	0.0066	0.0032	0.0056	0.0076	0.0031	0.0061
Varanus eremius	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus exanthematicus	0.0085	0.0125	0.0097	0.0072	0.0122	0.0092
Varanus giganteus	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus gilleni	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus glauerti	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus gouldii	0.0028	0.0039	0.0051	0.0023	0.0033	0.0052
Varanus griseus	0.0028	0.0034	0.0032	0.0027	0.0028	0.0026
Varanus indicus	0.0021	0.0020	0.0040	0.0017	0.0022	0.0050
Varanus komodoensis	0.0094	0.0098	0.0087	0.0016	0.0043	0.0036
Varanus mertensi	0.0014	<0.0001	<0.0001	0.0013	<0.0001	<0.0001
Varanus mitchelli	0.0024	0.0021	0.0058	0.0013	0.0011	0.0037
Varanus niloticus	0.0022	0.0035	0.0038	0.0025	0.0032	0.0033
Varanus olivaceus	0.0042	0.0030	0.0037	0.0053	0.0028	0.0038
Varanus prasinus	0.0041	0.0035	0.0047	0.0023	0.0036	0.0043
Varanus rosenbergi	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Varanus rudicollis	0.0183	0.0146	0.0150	0.0158	0.0145	0.0148
Varanus salvadorii	0.0055	0.0068	0.0096	0.0048	0.0075	0.0090
Varanus salvator	0.0048	0.0042	0.0054	0.0030	0.0049	0.0049
Varanus scalaris	0.0056	0.0036	0.0087	0.0018	0.0024	0.0040
Varanus storri	0.0127	<0.0001	<0.0001	0.0059	<0.0001	<0.0001
Varanus tristis	0.0041	0.0050	<0.0001	0.0031	0.0025	<0.0001
Varanus varius	0.0034	0.0027	0.0044	0.0045	0.0029	0.0045
Region:						
African	0.0043	0.0056	0.0065	0.0042	0.0053	0.0060
Indo-Asian A	0.0062	0.0055	0.0069	0.0055	0.0056	0.0066
Indo-Asian B	0.0038	0.0030	0.0041	0.0037	0.0029	0.0041
Indo-Australian A	0.0070	0.0076	0.0077	0.0029	0.0044	0.0046
Indo-Australian B	0.0029	0.0045	0.0053	0.0024	0.0040	0.0051
Indo-Australian C	0.0071	0.0078	0.0094	0.0032	0.0060	0.0049