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1 **The skull roof tracks the brain during the evolution and development of reptiles**  
2 **including birds**

3

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24

25 **Major transformations in brain size and proportions, such as the enlargement of the brain**  
26 **during the evolution of birds, are accompanied by profound modifications to the skull roof.**  
27 **However, the hypothesis of concerted evolution of shape between brain and skull roof over**  
28 **major phylogenetic transitions, and in particular of an ontogenetic relationship between specific**  
29 **regions of the brain and the skull roof, has never been formally tested. We performed 3D**

30 morphometric analyses to examine the deep history of brain and skull-roof morphology in  
31 Reptilia, focusing on changes during the well-documented transition from early reptiles through  
32 archosauromorphs including nonavian dinosaurs to birds. Non-avian taxa cluster tightly  
33 together in morphospace, whereas *Archaeopteryx* and crown birds occupy a separate region.  
34 There is a one-to-one correspondence between the forebrain and frontal and the midbrain and  
35 parietal. Furthermore, the position of the forebrain–midbrain boundary correlates significantly  
36 with the position of the frontoparietal suture in across the phylogenetic breadth of Reptilia and  
37 during the ontogeny of individual taxa. Conservation of position and identity in the skull roof is  
38 apparent, and there is no support for prior hypotheses that the avian parietal is a transformed  
39 postparietal. The correlation and apparent developmental link between regions of the brain and  
40 bony skull elements are likely ancestral to Tetrapoda and may be fundamental to all of  
41 Osteichthyes, coeval with the origin of the dermatocranium.

42

### 43 Introduction

44 The brain is often considered to have a peculiar primacy in the development of the head<sup>e.g.1-4</sup>. A  
45 general developmental relationship between brain and skull, with an emphasis on the facial region,  
46 has been well documented across tetrapods<sup>1-12</sup> – however, associations between particular regions of  
47 the brain and the primordia of individual elements of the skull roof have not been shown, despite  
48 the fact that these associations speak to both the patterning and the identity of the bones of the head.  
49 The skull roof or cranial vault directly overlies the brain; its largest constituents are the frontal  
50 bone anteriorly, between the orbits, and the parietal bone posteriorly, between the adductor muscle  
51 chambers. The homologies of these bones are a subject of recent contention based on developmental  
52 evidence, especially along the transition from small-brained nonavian reptile ancestors to large-  
53 brained birds (Aves)<sup>13-15</sup>. Early studies concluded that the entire frontal in chicken was  
54 developmentally derived from cells of the neural crest<sup>e.g. 16</sup>, as is the case in mouse and axolotl<sup>5-6,17</sup>,  
55 whereas later works suggested a composite germ-layer origin, with cranial neural crest contributing  
56 to the anterior part of the frontal and mesoderm to the posterior<sup>17-18</sup>. The latter interpretation  
57 inspired a hypothesis that the avian frontal actually represents a fusion of the frontal and parietal

58 bones of other reptiles, and that the avian parietal is in fact the postparietal or interparietal, an  
59 ossification or pair of ossifications seen in primitive Amniota<sup>18</sup>. This brings into question the  
60 identity, homology (defined as homology=synapomorphy for this study, or secondary homology *sensu*  
61 De Pinna<sup>19</sup>), and nomenclature of the skull elements in Aves relative to its successive sister taxa.  
62 We qualitatively and quantitatively examined the relationship of the brain to the skull roof from a  
63 combined phylogenetic and ontogenetic perspective. We wished in particular to trace the evolution  
64 of brain and skull roof through the entire history of Archosauromorpha and to resolve the  
65 conundrum concerning the identity and homology of skull-roof elements in crown birds. Contrary to  
66 previous studies<sup>17</sup>, we found no support for a fused origin of the avian frontal and a resulting shift  
67 in skull-roof element identity in birds. Our data instead suggest that the dominant developmental  
68 influence on the identity of individual skull-roof elements is the organization of the brain at its  
69 three-vesicle stage, and that a strict correlation between regions of the brain and particular skull-roof  
70 elements, specifically between the forebrain (derived from the embryonic prosencephalon) and the  
71 frontal and the midbrain (derived from the embryonic mesencephalon) and the parietal, is present at  
72 least across all amniotes. Morphological correlation of course is not sufficient to demonstrate  
73 developmental mechanism, and we hope that our findings inspire evolutionarily informed searches  
74 for the molecular patterning responsible for correspondences between early embryonic structures and  
75 the later-appearing ossified skeleton.

76 To test the hypothesis that there is a close relationship between brain and skull roof, we used 3D  
77 geometric morphometrics and comparative embryology to explore the association between these  
78 structures along the entire stem and crown reptile lineage, including Lepidosauria (lizards, snakes,  
79 and tuataras), Crocodylia, and Avialae, as well as stem-group Crocodylia, stem-group Archosauria,  
80 and stem-group reptiles (Fig. 1a). This sample includes pivotal taxa whose endocranial spaces and  
81 surrounding bones have never been examined (see Table S1 for a list of taxa included in this  
82 study)<sup>20-21</sup>. Extant taxa included embryonic series for alligator (*Alligator mississippiensis*) and chicken  
83 (*Gallus gallus*), which were stained to reveal soft tissues. When embryonic skull roofs were not  
84 sufficiently ossified, we used contrast-stained brains to extract brain shapes directly (see  
85 supplemental material for general methods used and protocols in staining).

86 **Results and Discussion**

87 Our data revealed an overall conservation of skull-roof architecture across Reptilia, as well as  
88 conservation in the relationship between the skull roof and the brain. The adult frontal always lies  
89 over the forebrain, whereas the adult parietal lies either over the midbrain or over a combination of  
90 midbrain and posterior forebrain (Fig. 1b-d, Fig. 2). The postparietal, when present in extinct  
91 reptiles, is a diminutive dermal element that does not directly overlie the brain. It is generally  
92 excluded from contacting the *dura mater* by the parietals and supraoccipital (Fig. 1b, Fig. 2). Its  
93 most consistent relationship is with the nuchal musculature, which can be reconstructed to have  
94 attached broadly to its posterior surface (Fig. 1b).

95 The postparietal is lost in Lepidosauria<sup>22</sup> (Fig. 1a, Fig. 2). It is commonly present, with some  
96 exceptions, along the archosaurian stem, and then almost completely disappears prior to the  
97 divergence of Archosauria<sup>23</sup>. We confirmed, however, one case of reappearance of the postparietal  
98 within the crocodile lineage: our CT scans of *Gracilisuchus stipanicorum* show clear sutures  
99 between the parietals and the element posited to be a secondarily acquired postparietal<sup>24</sup> (Fig. 1a-b,  
100 Fig. 2). Previous work has questioned the identity of the element and its distinction from the  
101 parietal<sup>23</sup>. The reacquired postparietal of *G. stipanicorum* is small and, as expected, does not border  
102 the endocranial space. In all the postparietals present in the extinct reptiles we examined, we could  
103 find neither anatomical nor topological points of similarity that could be used to suggest primary  
104 homology with dinosaurian, including avian, parietals, though it is possible that in some cases the  
105 small postparietal was absorbed by the parietals to form a minute posterior eminence.

106 Despite an overall conservation of organization in the reptile cranium, we also detected major  
107 evolutionary alterations to its architecture. Three-dimensional morphometric comparative analyses  
108 (Fig. 3, Fig. S1-S2, Table S1 and list of landmarks used in SI) yield a tight cluster that comprises  
109 non-avian dinosaurs, crocodile-line (pseudosuchian) archosaurs, stem archosaurs, lepidosaurs, and  
110 stem reptiles. This clustering arises despite an evolutionary divergence of 250 million years, an  
111 extremely wide range of apparent ecological niches, and a size range of several orders of  
112 magnitude, from small *Anolis* lizards (Squamata) to giant tyrannosaurs. PC 1 captures the transverse  
113 expansion of the brain and skull roof as well as the posterior shift of the forebrain–midbrain

114 boundary and the frontoparietal suture, while PC 2 represents the reduction of the parietal relative  
115 to the frontal, the expansion of the frontal, and the relative inflation of the forebrain and the  
116 cerebellum (Fig. S3). The presence or absence of a postparietal does not seem to influence the  
117 clustering of taxa in morphospace. One of the most divergent clusters is composed of the giant  
118 allosauroids *Allosaurus* and *Acrocanthosaurus*. Their aberrant position appears to be driven solely by  
119 the depth of the skull roof, possibly for the attachment of jaw muscles, as the deepening drastically  
120 increases the surface area of lateral adductor attachment sites on the parietal<sup>20,23</sup>. Brain endocasts of  
121 the giant allosauroids fall out with those of the other conservative taxa (Fig. 3c, Fig. S2).  
122 *Archaeopteryx* and crown birds diverge from the more conservative cluster along PC 1 (Fig. 3-S2);  
123 *Archaeopteryx* is closer to crown birds than to non-avian maniraptorans in brain and skull-roof  
124 shape despite having a plesiomorphic endocranial volume<sup>2</sup>.  
125 The inclusion of ontogenetic series for chicken and alligator revealed that, relative to alligator  
126 ontogeny, chicken ontogeny is morphologically short. Brains and skull roofs of chicken embryos are  
127 similar to those of adults, despite a sample that extends from early embryos to large adults. The  
128 ontogenetic trajectory of alligator is longer, traversing a distance in morphospace equivalent to 134%  
129 that of chicken. Alligator embryos clustered with crown birds and *Archaeopteryx* in the combined  
130 and skull-roof analyses, whereas they fell within the cluster of more conservative taxa when we  
131 included only the brain (Fig. 3b-c). We noted a negative allometry between the brain and skull  
132 during the development of alligators: the brain is relatively large in the early stages of development  
133 and becomes smaller with respect to the skull during growth. On the other hand, birds have a very  
134 large brain at hatching relative to the skull, and the brain continues to expand during ontogeny,  
135 growing with positive allometry. We suggest that the brain in Aves should be considered  
136 peramorphic in recognition of earlier onset of growth, faster sustained growth, and absolutely larger  
137 adult size in comparison with all but the most crownward non-avian avialans.  
138 Given the generally consistent clustering of taxa on the brain and skull-roof morphometric plots, we  
139 expected to find a correspondence between regions of the brain and bony elements of the skull  
140 roof. First, we tested and failed to reject the hypothesis of integration (similar levels of covariation  
141 for morphological traits between and within modules) between the skull roof and brain across the

142 evolutionary history of reptiles (CR = 0.982,  $P = 0.092$ ), a finding contrary to previous studies<sup>e-g</sup>  
143 <sup>3</sup>. This result reveals that, despite major morphological changes throughout the evolutionary history  
144 of the major reptile lineages, the general morphologies of the skull roof and brain regions are  
145 integrated across the entire clade. Second, as we wanted to test for a direct relationship between  
146 frontal and forebrain and parietal and midbrain, we compared the anteroposterior positions of the  
147 forebrain–midbrain boundary and the frontoparietal suture and demonstrated a significant correlation  
148 between the two ( $P = 0.014$ ), but with an unexpected pattern (Fig. 4, Table S2-S3). The best-fitting  
149 linear regression model also included a categorical variable subdividing sampled taxa into two  
150 groups ( $P = 8.0^{-5}$ ). The first group includes non-archosaurian reptiles, stem reptiles, and non-  
151 coelurosaurian archosaurs. In this sample, some of the taxa have a frontoparietal contact positioned  
152 anterior to the forebrain–midbrain boundary. The second group consists of coelurosaurian dinosaurs,  
153 including tyrannosaurs and crown birds. In these taxa, the forebrain–midbrain boundary and the  
154 overlying suture are nearly aligned (Fig. S4). Conspicuously, alligators shift from a bird-like  
155 configuration, with the brain and skull boundaries aligned, toward a non-coelurosaurian configuration,  
156 with an offset between the two, during their ontogeny.

157 Thus, in coelurosaurs, compared with their successive sister taxa, the adult frontoparietal suture  
158 shifts posteriorly relative to brain compartmental boundaries in a paedomorphic retention of the  
159 original embryonic relationship (Fig. 1a, Fig. 2, Fig. 4, Fig. S4). We propose that the maintenance  
160 of the posterior sutural position in adults is accompanied by the incorporation of additional and  
161 more posteriorly located sources of skeletal precursor cells into the frontal, as suggested by cell-  
162 lineage labeling experiments in chicken embryos<sup>25</sup>. The exact germ-layer origin of these cells is  
163 unknown, but is most likely mesodermal, and may correspond to mesodermal precursors that  
164 contribute to the parietal bone in non-avian reptiles. We note, however, that if this is the case,  
165 there was no intrinsic morphological information contained in these precursors: the back of the  
166 crown-avian frontal does *not* begin to look like the front of the ancestral dinosaurian parietal during  
167 the evolutionary transition. Instead, despite altered proportions, it flares outward and has the same  
168 proportional shape and articulations as other reptilian frontals (Fig. 1). The alternative explanation is  
169 a novel contribution of more posteriorly located mesenchymal precursor cells, also resulting in a

170 largely mesodermal but potentially mixed composition of the “new” posterior part of the avian  
171 frontal. This configuration could explain the ongoing confusion and debate regarding the exact  
172 developmental origin of this structure. Some studies still suggest that the entire avian frontal is  
173 derived from neural crest cells, a migratory population of neuroectodermal derivation; other  
174 experiments suggest a mesodermal contribution<sup>e.g. 17-18, 26</sup>. Germ-layer origins have been used to argue  
175 that the avian frontal must be a fused frontoparietal because it contains both neural crest– and  
176 mesoderm-derived cells, and that the avian parietal, generally accepted as being mesodermally  
177 derived, is a postparietal. However, the central part of the interparietal of mammals, which is  
178 homologous to the ancestral amniote postparietal, has been described as being derived from neural  
179 crest<sup>5</sup>. This means that the avian frontoparietal hypothesis must invoke a shift in germ-layer origins,  
180 the avoidance of which was its core inspiration. Moreover, the germ-layer origins of cranial roof  
181 bones are more varied than previously reported. The parietal in mammals, for instance, is also  
182 reported to be of dual origin, with its central part derived from neural crest and its more lateral  
183 portions from mesoderm<sup>3,6,27</sup>. In amphibians, the parietal is reportedly either mesodermally derived  
184 (in the axolotl) or of mixed neural crest–mesodermal origin (in the fused frontoparietal element of  
185 *Xenopus* frogs)<sup>17,26</sup>. More notably still, the frontal of zebrafish, like that of chicken, is reported to  
186 be of composite neural crest and mesodermal origin<sup>28</sup>. This raises the possibility that a composite  
187 frontal is in fact the ancestral osteichthyan condition. Data from additional groups, especially from  
188 reptiles such as representatives of Crocodylia and Squamata, and from other non-tetrapod vertebrates,  
189 are needed to establish both the ancestral osteichthyan condition and the polarity of change.

190

191 The topology of all other structures in the avian head remains consistent with our conclusion that  
192 the entire enlarged avian frontal bone is homologous to the smaller frontal in more conservative  
193 groups, and that the constricted and rotated avian parietal is nonetheless homologous to the ancestral  
194 reptilian parietal. The positional relations of these two elements to other cranial structures, skeletal  
195 and non-skeletal, are conserved, both in adulthood and during ontogeny (Fig. 5). In particular, the  
196 parietal is always broadly flanked by the squamosals and contacts the supraoccipital posteriorly (Fig.  
197 5). The eyes remain encircled by the frontals despite the greater size of eyes in crown birds, and



198 the pseudotemporalis or adductor internus group of the jaw muscles remains attached to the side of  
199 the parietal, even as this bone becomes smaller and rotates to assume a more vertical orientation  
200 (Fig. S5). The midbrain, including the optic lobes, remains subjacent to the parietal, though it is  
201 shifted ventrally in crown birds. This conservation of topology stands in contrast to recent claims  
202 that spatial relationships of the avian frontal and parietal are modified from the ancestral reptilian  
203 condition<sup>19,26</sup>. Thus, in terms of adult anatomical homology, there is no evidence that the avian  
204 frontal bone is a composite frontoparietal. Also, there is no evidence that the parietal is either a  
205 “semiparietal” or a reacquired and radically transformed postparietal. A proposed novel contribution  
206 to the posterior part of the avian frontal and a potential shift in germ-layer contribution appear to  
207 have occurred without violating the structural homology of the bone.

208 The ontogenetic shift in developing alligator from close alignment of the forebrain–midbrain  
209 boundary and frontoparietal suture to a displacement of those structures suggests that a decoupling  
210 between the brain and skull roof occurs later during development, following what we hypothesized  
211 to be an early close association of the initial ossifications of the skull roof with the divisions of  
212 the brain. This bone-to-brain relationship had not previously been examined in embryos, so we used  
213 contrast-stained CT scanning to simultaneously visualize the developing brain and skull roof in  
214 *Alligator mississippiensis* and *Gallus gallus* (Fig. 6). We supplemented these data with a  
215 developmental series of the lizard *Chalcides chalcides*. In all three taxa examined there is a direct,  
216 one-to-one correspondence between the developing forebrain and the frontal bone primordium and  
217 the developing midbrain and the parietal bone primordium. Moreover, this relationship can be seen  
218 to exist in mouse and opossum, though it has attracted little comment<sup>29</sup>. Contrary to a recent  
219 report based on 1937 data from de Beer<sup>30</sup>, the initial parietal ossifications in chicken and mammals,  
220 as well as in non-avian reptiles, appear in the same topological position relative to the brain, the  
221 other bones of the skull (notably the squamosal), and the chondrocranium (Fig. 5). Our results  
222 therefore support the notion that the brain plays an important role in patterning the skull roof.

## 223 **Conclusions**

224 We have shown that across the great change in brain size and shape in the evolution of birds from  
225 the reptile ancestor, the skull roof tracks the brain early in ontogeny and then becomes decoupled,

226 with a truncation of this decoupling occurring in Coelurosauria. The skull roof is remarkably  
227 conservative across reptiles, and we found no evidence that the avian parietal is anything other than  
228 a structure homologous to the ancestral reptilian parietal. The novel and dramatic posterior  
229 expansion of the avian frontal may be stimulated by the contribution of additional skeletal  
230 progenitor cells, most likely of mesodermal origin. Overall evidence indicates that this expansion of  
231 the frontal is not a product of fusion with the parietal. Finally, we have shown for the first time a  
232 one-to-one correspondence in embryos between major parts of the brain and the early ossifications  
233 of the skull-roof elements, a condition likely ancestral to all amniotes and possibly to all  
234 osteichthyans. This relationship, however, is dynamic during ontogeny, and the nature of the relation  
235 in adults shifts during the evolution of the avian lineage. This result serves as an example of  
236 character non-independence: the enlargement of the brain had widespread consequences on  
237 surrounding cranial elements, affecting the entire architecture of the skull. It also highlights the fact  
238 that developmental data by themselves are not sufficient to determine homology and must be  
239 interpreted within a phylogenetic framework provided by the fossil record and comparative  
240 morphology. Finally, it raises the question of whether the intimate association of the frontal and  
241 parietal with the brain, which is known to act as a major signaling center at least in terms of  
242 facial development, is the reason for their universal conservation in bony vertebrates; and whether  
243 the repeated losses of posterior cranial elements such as the postparietal, tabulars, and  
244 supratemporals have to do with their more peripheral positions with respect to an influential source  
245 of molecular developmental patterning information.

246

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262 M.F., N.M.K., A.C.P., M.H., and B.-A.S.B. placed landmarks and performed morphometric analyses.  
263 M.F. and N.M.K. performed correlation tests. M.F., N.M.K., A.C.P., M.H., E.H., G.S.B., T.B.R.,  
264 A.A., and B.-A.S.B., wrote the paper. M.A.N. and R.M.S. provided CT data and assisted in  
265 anatomical interpretation.

266

## 267 **Materials and Methods**

268 Original CT scan data of the taxa included in this analysis were acquired at the University of  
269 Texas High-Resolution X-Ray Scanning Facility (UTCT), at the University of the Witwatersrand  
270 Bernard Price Institute for Palaeontological Research (BPI), and at the Harvard Center for Nanoscale  
271 Systems (CNS). Endocasts of the brain and the skull elements were segmented using the software  
272 VGStudio. The taxa stem-ward of *Proterosuchus* do not ossify the anteroventral portion of their  
273 braincases, such that endocasts were necessarily less complete. However, we only analyzed those  
274 parts of the endocasts that were directly in contact with the skull roof (Fig. S1). The braincases of  
275 the coelurosaur *Garudimimus* and of the stem archosaur *Euparkeria* were partially disarticulated and  
276 required digital re-articulation. The restoration was performed in VGStudio. Normal developmental  
277 series for *Gallus gallus* (four stages, including E12, E15, E17 and E19), *Alligator mississippiensis*  
278 (four stages, including E32, E40, E46 and hatchling) and *Chalcides chalcides* (YPM R 15063) were  
279 included in the analyses for investigating the pattern of skull ossification and to test the one-to-one  
280 correlation between frontal and forebrain on the one hand and parietal and midbrain on the other.  
281 Embryos were stained in a 5% (by mass) phosphomolybdic acid, before CT scanning them.

282 The 3D configurations of landmarks were digitized with VGStudio on the CT scan data for all the  
283 taxa included in this study. Only one side of the braincase was landmarked. The right or left side  
284 of the braincase was chosen on the base of the presence or absence of deformation, quality of  
285 preservation and completeness of the specimen. In the case of *Garudimimus*, we placed the  
286 landmarks on the right side of the braincase, because it was less deformed as suggested by the  
287 more rounded morphology of the orbit<sup>31</sup>.

288 Generalized Procrustes analysis (GPA)<sup>32</sup> was applied in order to remove information relating to the  
289 location, size and orientation of the landmark configurations. The three-dimensional coordinates of  
290 landmarks were subjected to a full GPA, given the reduced sensitivity to outliers of this approach<sup>33</sup>.

291 Analysis were performed using MorphoJ v. 1.03b<sup>34</sup>, which automatically reflects specimens that were  
292 digitized on alternative sides. Major patterns of morphological variability were then extracted using  
293 Principal component analysis (PCA) on the Procrustes-aligned coordinates.

294 Delineation of clusters (that is, groups of organisms with similar morphology) was done using *k*-  
295 means clustering<sup>35</sup>. The method aims at partitioning all observations into *k* groups such that the sum  
296 of squares from all observations to their assigned cluster centers is minimized. Given the heuristic  
297 nature of the method, results shown derive from initiating the process from 1000 different randomly  
298 located cluster centers. Number of clusters in each case was determined using R package *NbClust*<sup>36</sup>  
299 that provides 30 metrics to evaluate the optimal number of clusters in a dataset. In all cases (Fig.  
300 3, Fig. S2), clustering was performed using all principal components that explained a variance >  
301 1%, and the number of clusters chosen was that supported by the majority of metrics.

302 We used the R package *geomorph*<sup>37-38</sup> to test the hypothesis of modularity between skull roof and  
303 brain. The Covariance Ratio (CR) was chosen as a measure to characterize the degree of covariance  
304 between these two *a priori* defined modules<sup>39</sup>. CR was preferred over the widely used RV  
305 coefficient<sup>40</sup>, because it is not influenced by attributes of the data such as the sample size and the  
306 number of variables<sup>39</sup>. Briefly, this metric represents the overall covariation between defined modules  
307 relative to the overall covariation found within them. For random sets of variables, the CR  
308 coefficient has an expected value of one; significant departures towards lower values are indicative  
309 of modularity. Significance was tested using a permutation test which randomly reassigns landmarks

310 into groups of equal size as the original partitions, and calculates the CR value for the generated  
311 subdivisions of landmarks. The degree of modularity between the skull roof and the brain was  
312 tested using: A) the full set of digitized landmark configurations ( $n = 21$ ), and B) only those  
313 corresponding to adult specimens ( $n = 17$ ). The sampling employed in the first set leads to  
314 difficulties interpreting the results, since they are determined by a mix of ontogenetic and  
315 evolutionary signals of integration across structures<sup>41</sup>. Therefore, results in the main text correspond  
316 to those of set B, which only assesses the degree of evolutionary integration. In both cases, we  
317 performed  $10^4$  permutations, and  $P$  values were empirically calculated as the fraction of  
318 permutations with CR values lower than the original. The analysis of set A (i.e., including the  
319 landmark configurations of the chicken and crocodile embryos), results in a significant degree of  
320 modularity between skull roof and brain (CR = 0.967,  $P = 0.031$ ). This might provide further  
321 evidence for the morphological decoupling between the two structures during ontogeny, as discussed  
322 in the main text, but once again caution should be taken when interpreting this result.

323 In order to further explore the morphological covariance between skull roof elements and brain  
324 regions, we measured the relative positions of the fronto-parietal and forebrain-midbrain sutures.  
325 Sagittal sections of the braincase and brain of all adults were extracted from the CT scans using  
326 VGStudio. Images were then imported into tpsDIG2 v. 2.22<sup>42</sup>, where the distance between the  
327 anterior tip of the olfactory bulbs and the foramen magnum was measured as the curved line  
328 joining those points along the internal side of the skull. This measure was used as a proxy for the  
329 overall skull roof length. The relative positions of both fronto-parietal and fore-mid brain sutures  
330 along the same line was quantified as their distance to the tip of the olfactory bulbs divided by the  
331 overall length, thus eliminating differences due to specimen size.

332 A relationship between the positions of the fronto-parietal suture and forebrain-midbrain suture was  
333 explored using a combination of ordinary and phylogenetic linear models. For the latter, a  
334 phylogenetic supertree was created in the software Mesquite v 3.04<sup>43</sup>. The topology of the tree was  
335 based on Pinheiro et al.<sup>44</sup> for early diapsids and Archosauromorpha, Gauthier et al.<sup>45</sup> for Squamata,  
336 Nesbitt<sup>46</sup> for Archosauriformes and Archosauria, Carrano et al.<sup>47</sup> for Tetanurae, Brusatte et al.<sup>48</sup> for  
337 Coelurosauria and Prum et al.<sup>49</sup> for Aves. A time-scaled version of the phylogenetic tree was built

338 using the calibration method described in Brusatte<sup>50</sup>. First and last appearances of all fossil taxa  
339 were recorded from the primary literature (See Table S2 for ages of the taxa and corresponding  
340 citations) and used to estimate the time of divergence of the clades represented in the analysis. This  
341 was done using the timePaleoPhy function in the R package *paleotree*<sup>51</sup>. Molecular estimates for the  
342 divergence of crown clades were drawn from Shedlock & Edwards<sup>52</sup> and Prum *et al.*<sup>49</sup>, and used to  
343 constrain minimum ages for the respective nodes. To account for uncertainty in both branch length  
344 estimates and phylogenetic relationships among paravian lineages, 100 different trees were generated  
345 by assigning an age for each taxon through random sampling between its first and last appearance  
346 in the fossil record, as well as randomly resolving the polytomy at the base of Paraves. The  
347 resulting 100 trees were subsequently used in all following analysis.

348 We considered three increasingly complex least-squares regression models: a model of simple  
349 allometry, a model incorporating different intercepts for coelurosaur and non-coelurosaur diapsids but  
350 with same slope, and a model with both different intercepts and slopes (shown in Table S3 as  
351 models A, B and C, respectively). Division of the included taxa into a coelurosaur and a non-  
352 coelurosaur group was applied on the base of the shift in the organization of braincase-brain found  
353 through the analyses performed in this study. As already stated, ordinary least squares (OLS) and  
354 phylogenetic generalized least squares (PGLS) approaches of these three models were performed (for  
355 the later, we used the R package *caper*<sup>53</sup>). A dummy variable representing clade membership was  
356 coded and included as categorical factor<sup>as in.54-55</sup>. Given the sensitivity of methods to deviations from  
357 a strict Brownian motion model<sup>56</sup>, branch length modifying parameters ( $\lambda$ ,  $\delta$  and  $\kappa$ <sup>57-58</sup>) were  
358 simultaneously estimated along the regression parameters, following the recommendations of Revell<sup>59</sup>.

359 For each of the three models, the fit of the OLS and 8 different PGLS (resulting from all  
360 combinations including the estimation of branch length modifying parameters, see Table S3) were  
361 compared using AIC weights, given that different scenarios are not nested within each other. On the  
362 other hand, the fit of progressively more complex models (i.e. simple allometry, allometry with  
363 clade-specific intercepts and allometry with clade-specific intercepts and slopes) was analyzed using  
364 log-likelihood ratio tests (LRT). The model with the best fit was considered to be the one with the  
365 lowest AIC value overall.

366 Several lines of evidence favored the OLS model including different intercepts but equal slopes for  
367 the two clades as the preferred one, the results of which are shown in Fig. 4 of the main text.  
368 This model had the overall smallest AIC value, and represented a significant improvement with  
369 respect to the OLS simple allometry ( $P = 0.000$ ); while the addition of different slopes for each  
370 clade was not considered to further improve the model ( $P = 0.394$ ). This was also confirmed  
371 through the use of a partial  $F$ -test ( $F = 0.57$ ,  $P = 0.464$ ). In fact, the regression residuals were  
372 found to lack phylogenetic signal, with a value of  $K = 0.20$ <sup>60</sup> and  $\lambda = 0.73$  ( $P = 0.64$  and  $0.37$ ,  
373 respectively, using 100 simulations with function `phylosig` of R package *phytools*<sup>61</sup>). Under such  
374 situation, OLS approaches have an estimation accuracy substantially higher than PGLS<sup>59</sup>. Allowing  
375 for the simultaneous estimation of branch length modifying parameters also confirmed this result.  
376 The second best model including a clade effect with equal slopes is a PGLS approach that includes  
377 a parameter  $\lambda = 0$  and  $\delta = 0.019$ , transforming the tree into a star phylogeny and (almost  
378 completely) homogenizing terminal branch lengths. This transformation effectively eliminates residual  
379 correlation due to shared evolutionary history from the variance-covariance matrix of the linear  
380 model (analogous to the PGLS $_{\lambda}$  approach discussed by Revell<sup>59</sup>, with the addition of a parameter  $\delta$   
381  $\approx 0$  to eliminate differences in terminal branch lengths), resulting in an approach equivalent to an  
382 OLS.

383

384 Data availability. CT data are in part publicly available in [www.digimorph.org](http://www.digimorph.org). The remnant CT  
385 data are available through the corresponding author, upon reasonable request. Landmarks are  
386 available as supplementary files in [www.nature.com](http://www.nature.com).

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521 **Figure 1.** Summary of skull roof evolution and relationship to soft tissue structures in Reptilia. a,  
522 To the left, phylogenetic tree showing presence (orange) or absence (green) of separate postparietal.  
523 The postparietal is ancestral for Reptilia and was lost several times within it, notably at the base of  
524 crown Archosauria with one reversal in *Gracilisuchus*. To the right, dorsal views of the segmented  
525 skull roof and brain endocast of selected taxa, anterior to the left, demonstrating the uniformly  
526 small size of the postparietal and the gradual transformation of the skull roof toward the avian  
527 lineage. b, Sagittal cutaway through the skull of the stem crocodylian *Gracilisuchus stipanicorum*  
528 including brain endocast, showing the relationship of the skull roof bones to the endocranium and  
529 the separation of the postparietal from the brain by the skull roof, braincase, and nuchal  
530 musculature. Anterior to the left. c, Sagittal cutaway as in b, but of near-hatching (E46) *Alligator*

531 *mississippiensis*, showing relation of contrast-stained brain and nuchal musculature to skull roof and  
532 braincase. d, Sagittal cutaway of contrast-stained chicken, *Gallus gallus*, showing relation of  
533 contrast-stained brain and nuchal musculature to skull roof and braincase. Frontal in fuchsia; parietal  
534 in green; postparietal in orange; brain endocast in blue.

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538 **Figure 2.** Oblique dorsolateral views of skull roofs, braincases, and brain endocasts of selected newly  
539 sampled fossil taxa, anterior to the lower left. Skull elements are cut along the sagittal plane  
540 leaving only the right sides, but endocasts are entire. These are the first reported endocasts of the  
541 early stem reptile *Youngina*, the stem archosaur *Proterosuchus*, the near-crown stem archosaur  
542 *Euparkeria*, the stem crocodylian *Gracilisuchus*, and the early-diverging dinosaur *Herrerasaurus*. The  
543 postparietal, where present, is uniformly small and restricted to a superficial, posterior position on  
544 the skull. The skull roof in *Zanabazar* is characteristic of coelurosaurs in that the frontoparietal  
545 suture is shifted backward, closer to the forebrain–midbrain boundary.

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549 **Figure 3.** 3D geometric morphometric principal component analyses (PCAs) of brains and skull  
550 roofs in reptiles. a, Phylogenetic tree showing included taxa and indicating color coding in plots. b,  
551 PCA plot including brain and skull roof landmarks. c, PCA plot including only skull roof  
552 landmarks. d, PCA plot including only brain landmarks. Taxa are grouped following k-means  
553 clustering with automatic selection of the appropriate number of clusters. A cluster of all avialan  
554 specimens is always found, sometimes also including alligator embryos. The remaining non-avialan  
555 reptiles are recovered as one cluster when analyzing brain morphology, or two clusters when  
556 including skull roof morphology, with allosauroids exhibiting a divergent morphology. Gray cluster is  
557 adult non-dinosaurian reptiles and non-avialan reptiles. Red cluster is Avialae. Blue arrows indicate  
558 alligator ontogeny. Red arrows indicate chicken ontogeny. See Supplementary Information for silhouette  
559 sources. Silhouettes from <http://phylopic.org>.

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563 **Figure 4.** Relationship between the position of frontoparietal suture and position of forebrain–  
564 midbrain boundary. Results derive from a linear model including clade as a categorical dummy  
565 variable and equal slopes ( $P=3.0^{-4}$ ,  $R^2=0.69$ ). Upper black line is regression for non-coelurosaurian  
566 reptiles, lower line is the regression for coelurosaurs. Diameters of the dots represent relative  
567 distance between the two sutures. For a comparison with phylogenetic generalized-least squares  
568 approaches, as well as method justification, refer to the Materials and Methods session. Embryos are  
569 plotted, although only adults were used in the analysis. Blue indicates all non-coelurosaur taxa. Red  
570 indicates the coelurosaur clade. Gray indicates alligator and chicken embryos and their ontogenetic  
571 trajectories. Silhouettes from <http://phylopic.org>.  
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575 **Figure 5.** Ossification of cranial elements in (a), (b) embryonic alligator (sample size=10) and (c),  
576 (d) embryonic chicken (sample size=10). Frontals are fuchsia, parietals green, and in the rightmost  
577 column, squamosals are turquoise, supraoccipitals yellow, and exoccipitals red. Note that the parietal  
578 primordia in E32 alligators and E15 chickens are similar in form and relative location, taking into  
579 account the compressed and rotated temporal region of the cranium in birds. Note also the  
580 homologous topological position of the parietals in both taxa with respect to the squamosals,  
581 flanking them and the supraoccipitals and exoccipitals behind.

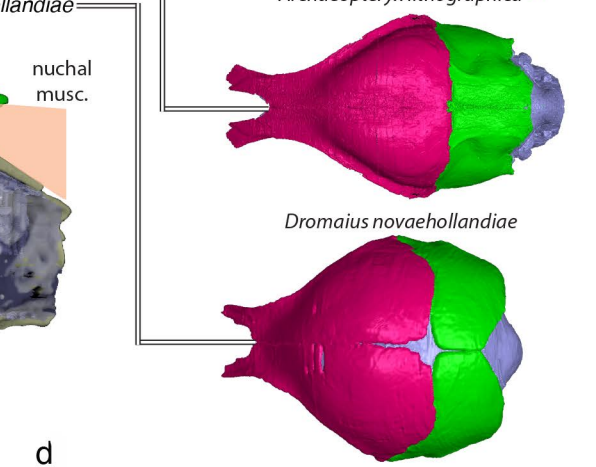
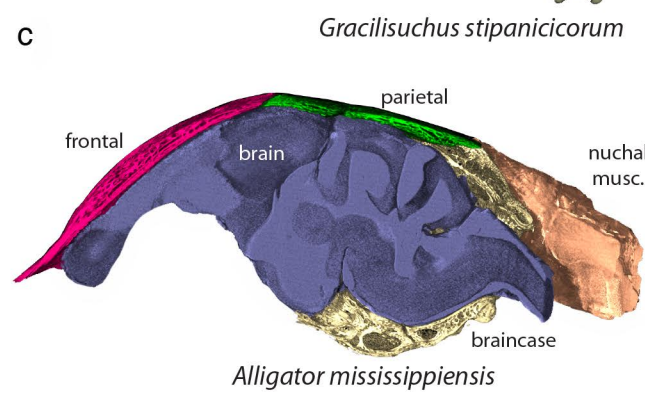
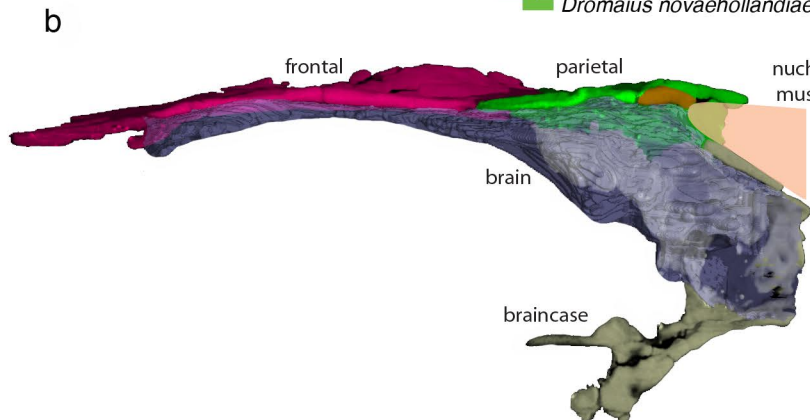
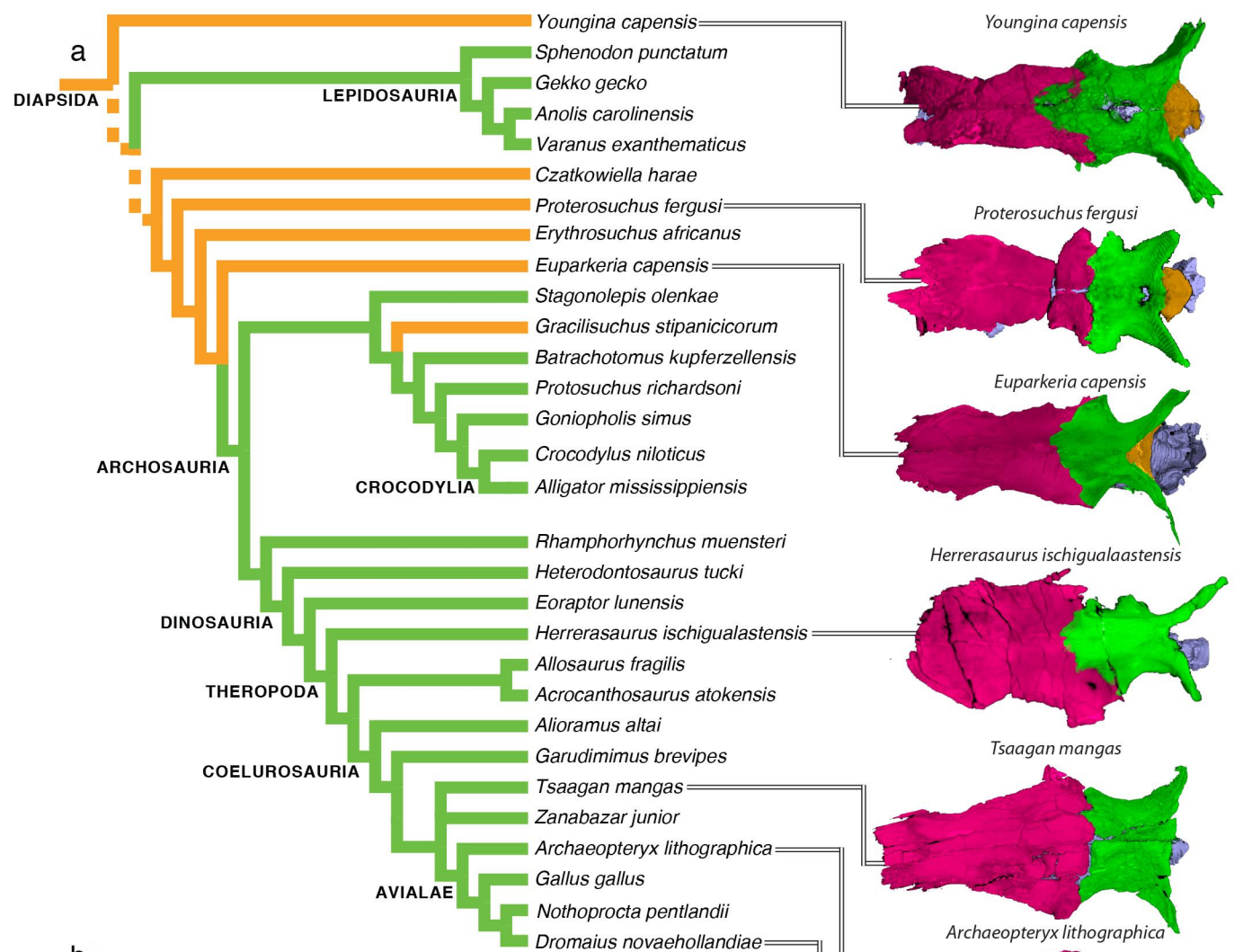
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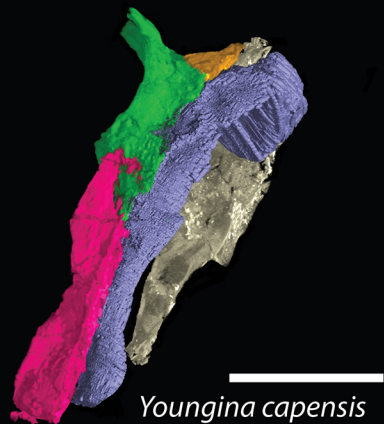
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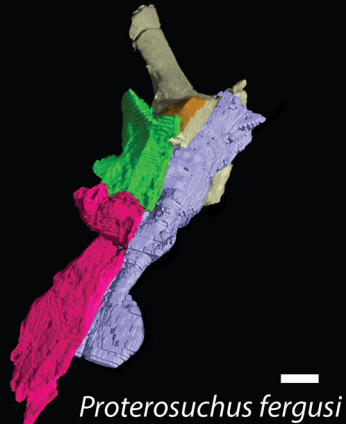
585 **Figure 6.** Above: Ossification of the frontal (pink) and parietal (green) over the brain (blue) in  
586 embryonic alligator (sample size=6) and chicken (sample size=6), with the eyes (white) and nuchal  
587 musculature (orange) shown in place. Below: Ossification of the frontal and parietal in an  
588 embryonic *Chalcides* lizard (Squamata) (sample size=2). In all of these taxa, bracketing Reptilia, the  
589 frontal first forms over the forebrain (fb) and the parietal first over the midbrain (mb).

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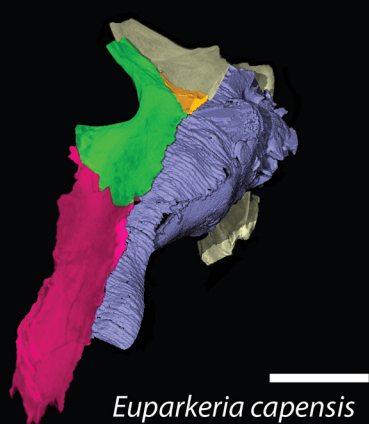




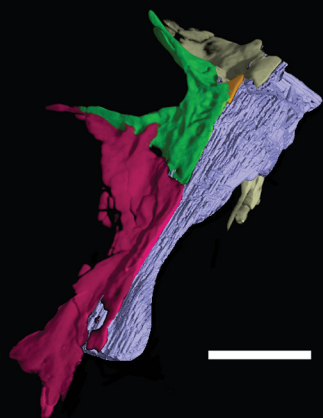
*Youngina capensis*



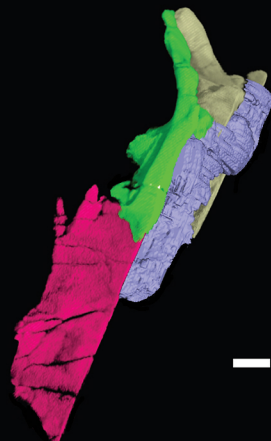
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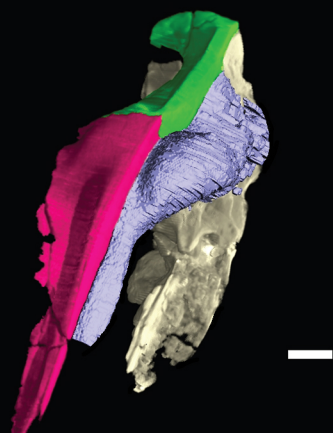
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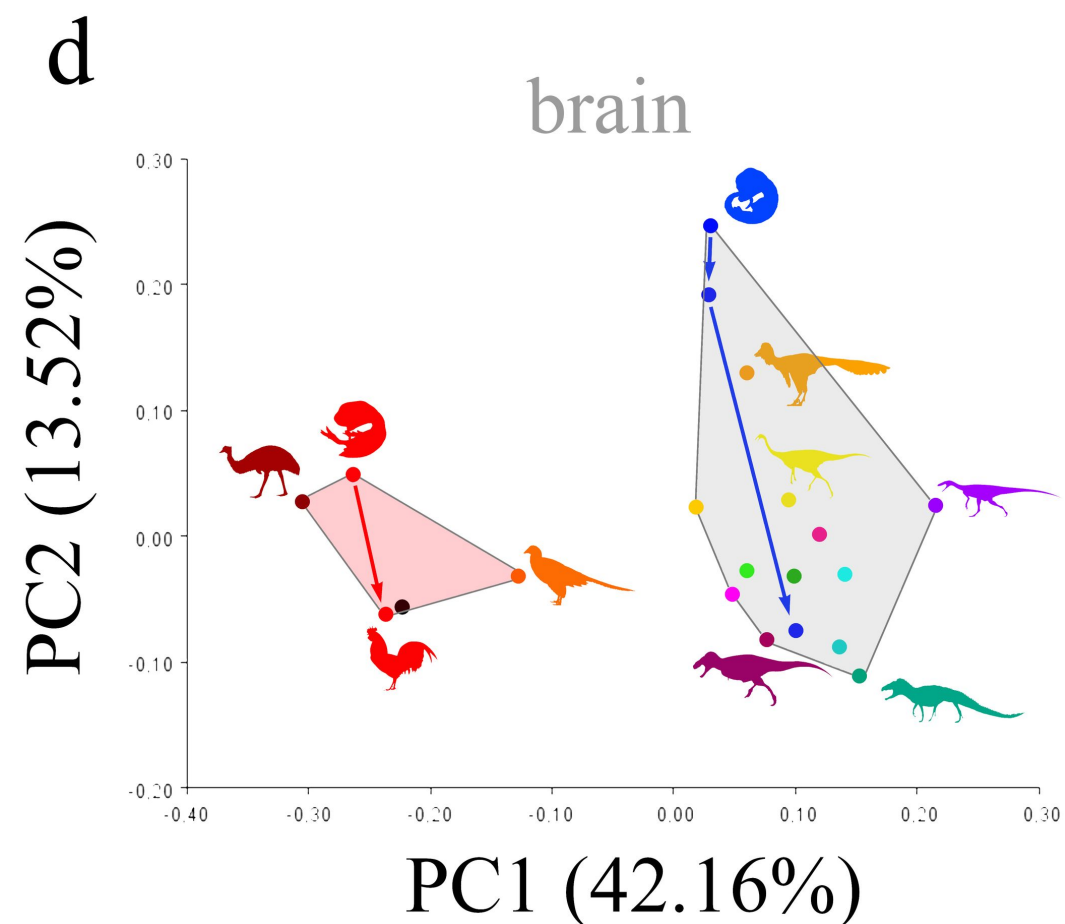
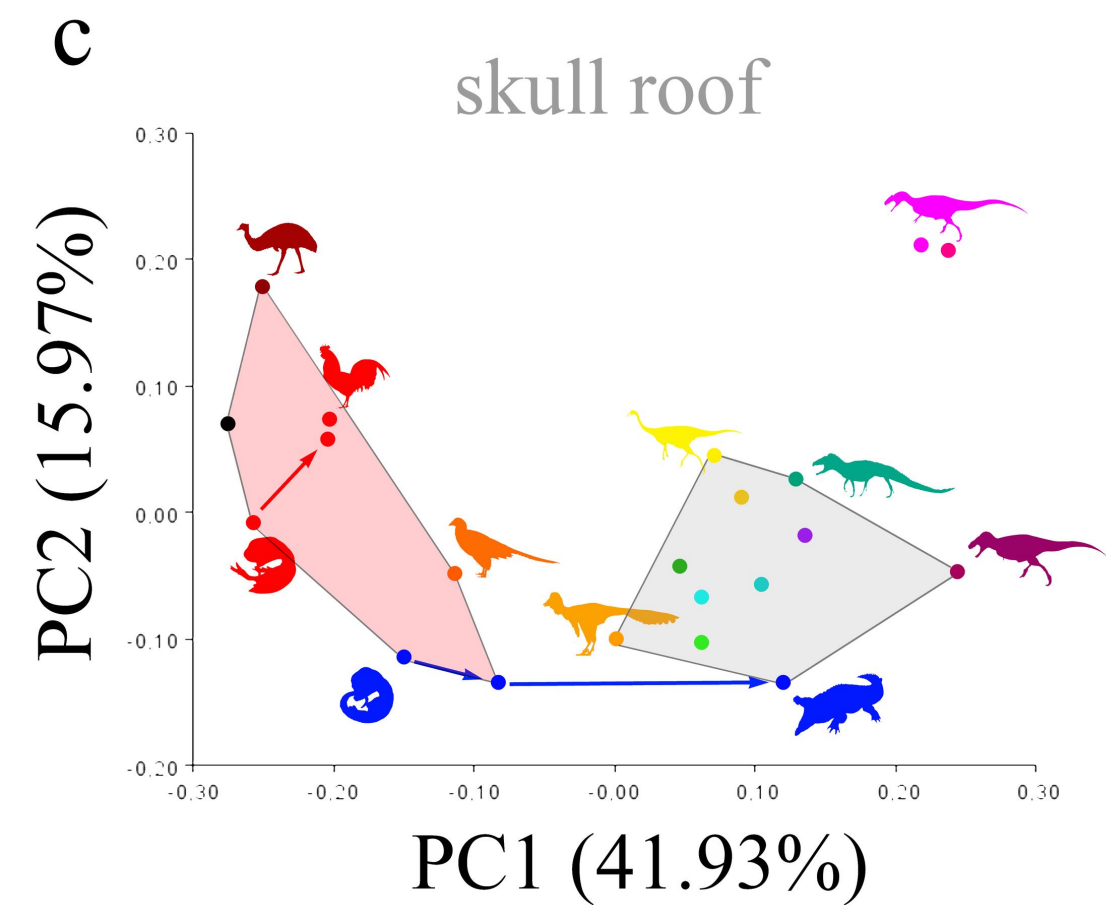
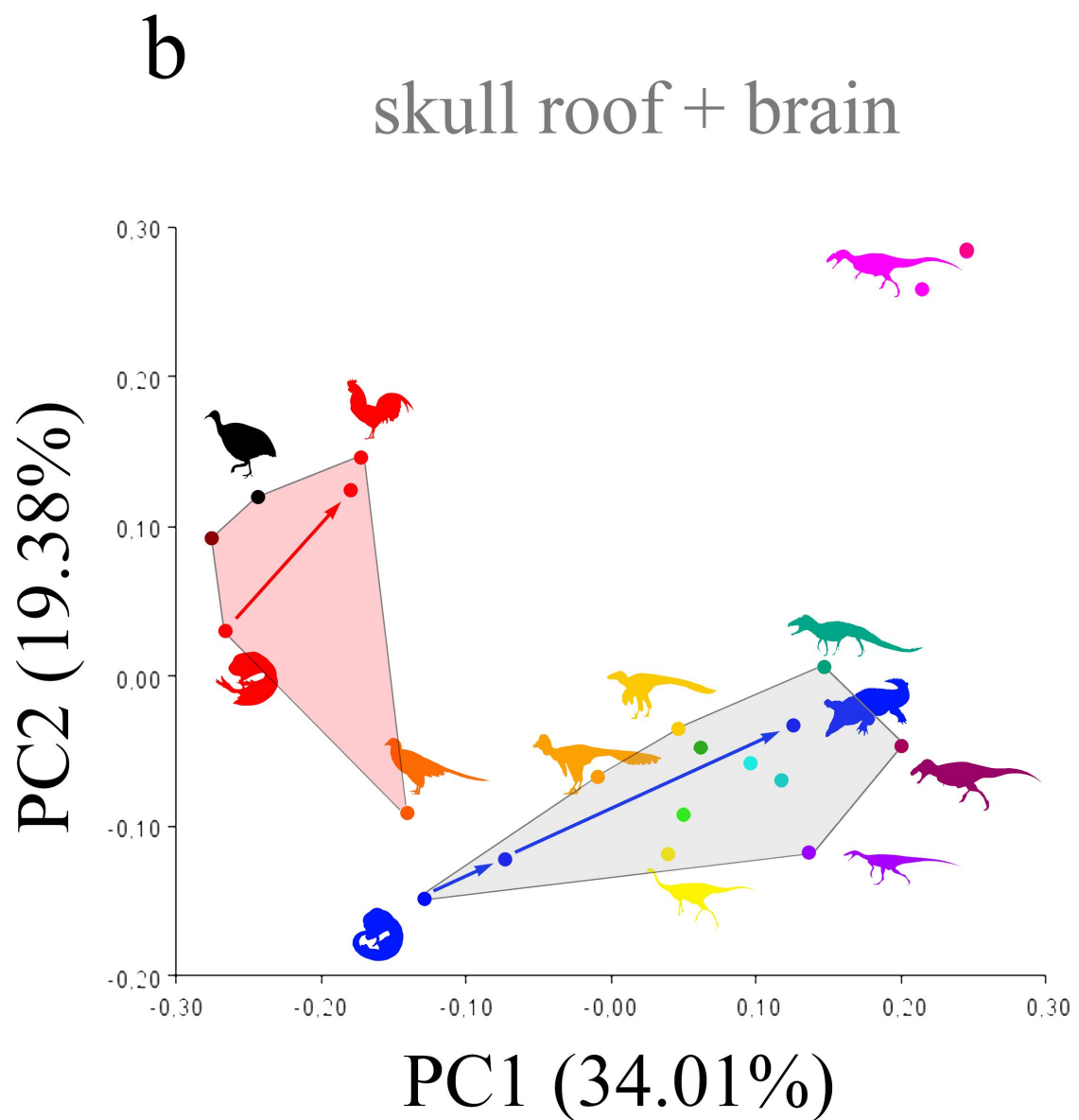
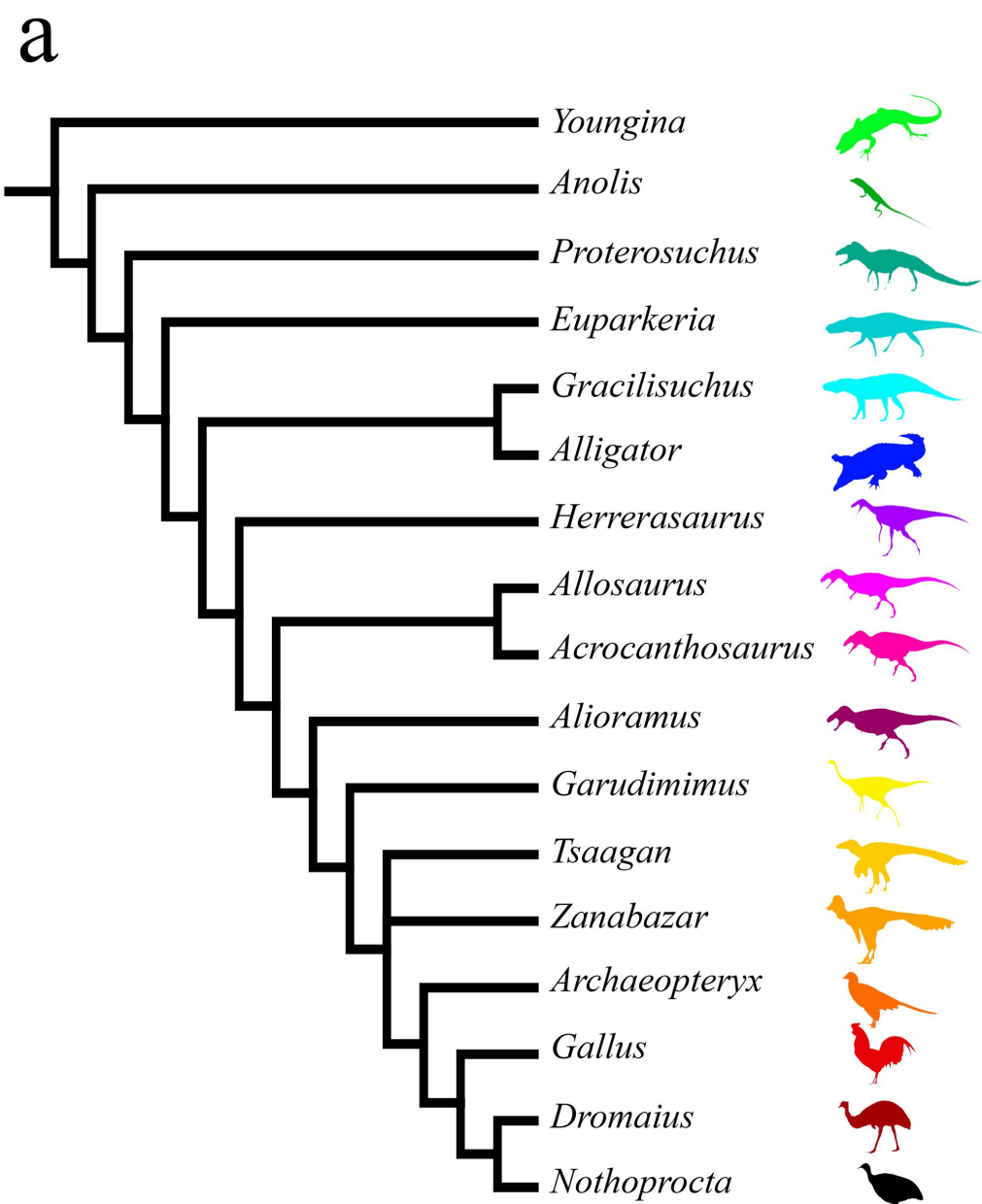
*Gracilisuchus stipanichorum*

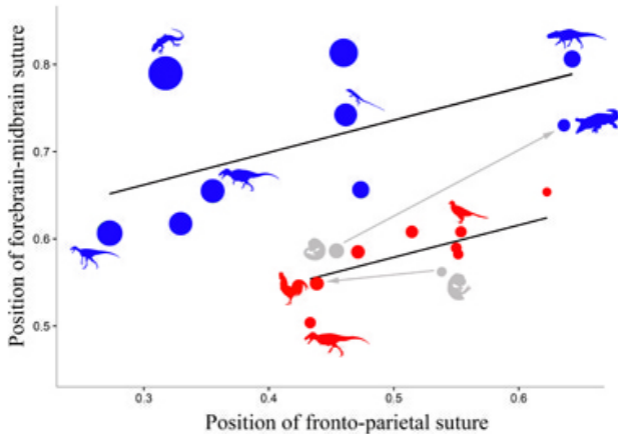


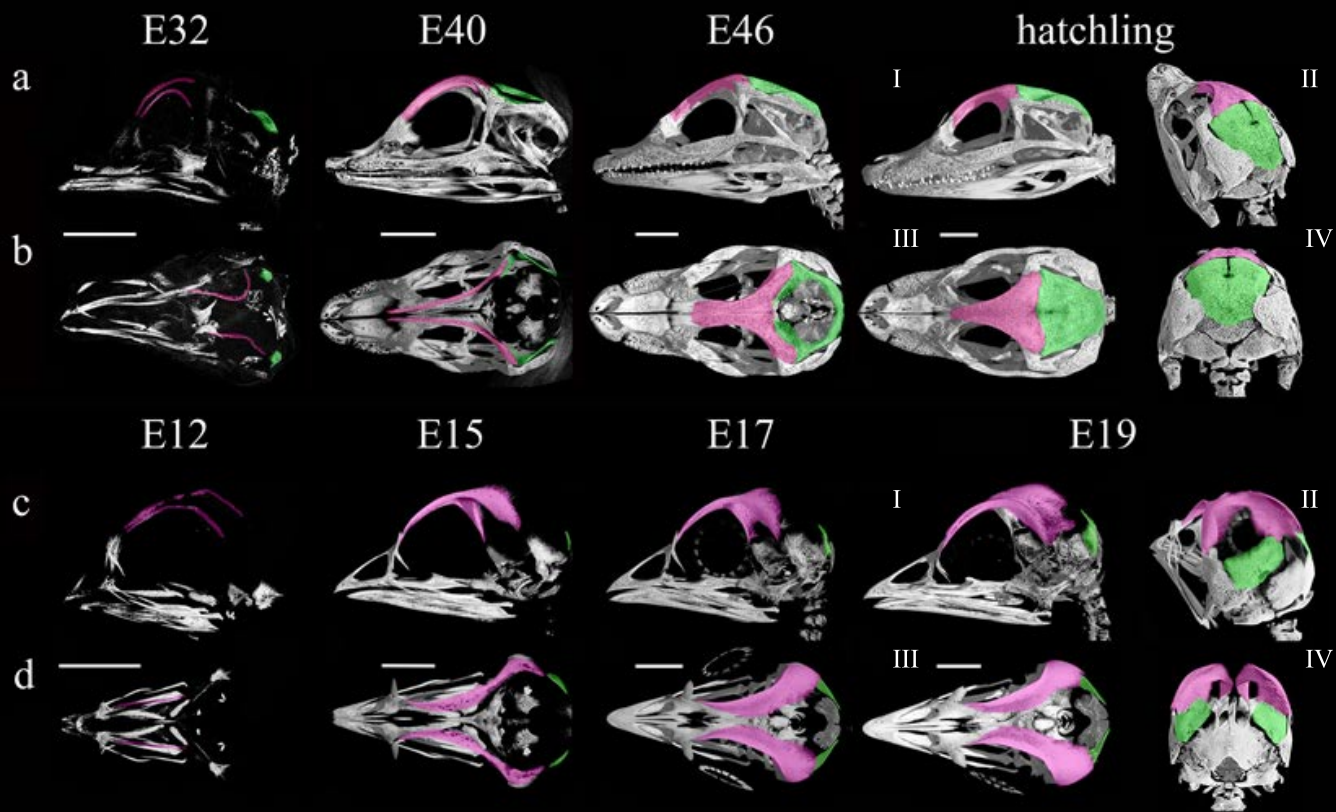
*Herrerasaurus ischigualastensis*

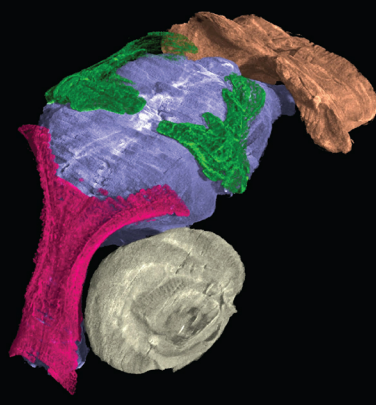


*Zanabazar junior*

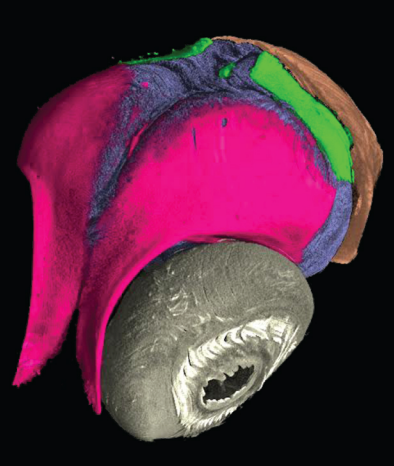




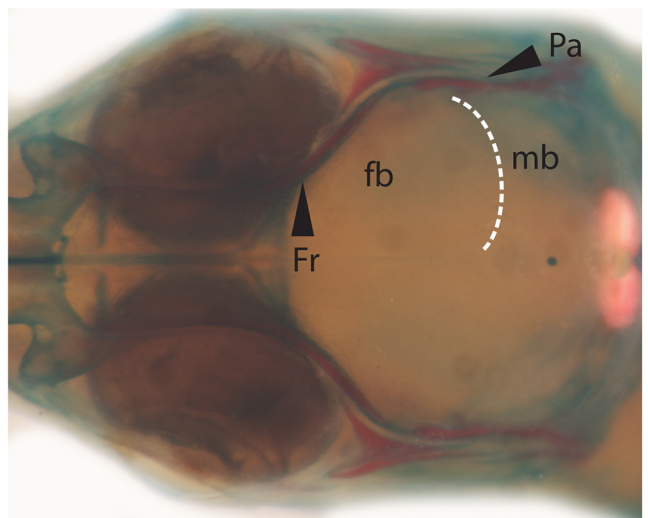
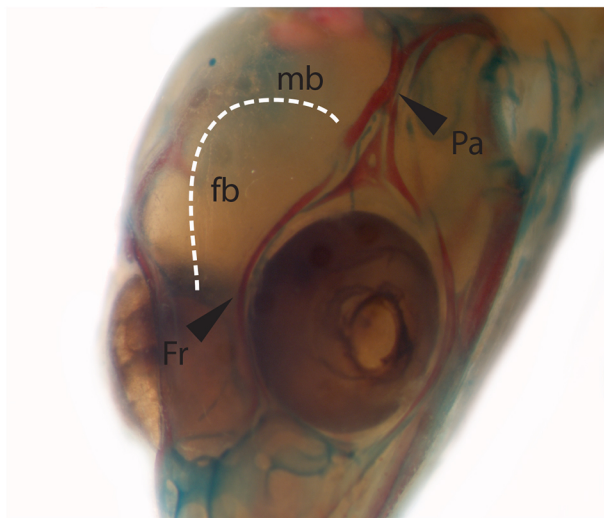




*Alligator mississippiensis*



*Gallus gallus*



*Chalcides chalcides*