

1 Early origins of divergent patterns of morphological evolution on the mammal and reptile
2 stem-lineages

3 Running Head: EVOLUTION OF STEM MAMMALS AND REPTILES

4

5 Neil Brocklehurst^{1*}, David P. Ford², Roger B. J. Benson²

6 ¹Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge, UK

7 ²Department of Earth Sciences, University of Oxford, South Parks Road, Oxford, UK

8 * Corresponding Author. Email: nb661@cam.ac.uk

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25 ABSTRACT

26 The origin of amniotes 320 million years ago signalled independence from water in
27 vertebrates and was closely followed by divergences within the mammal and reptile stem
28 lineages (Synapsida and Reptilia). Early members of both groups had highly similar
29 morphologies, being superficially ‘lizard-like’ forms with many plesiomorphies. However,
30 the extent to which they might have exhibited divergent patterns of evolutionary change, with
31 potential to explain the large biological differences between their living members, is
32 unresolved. We use a new, comprehensive phylogenetic dataset to quantify variation in rates
33 and constraints of morphological evolution among Carboniferous–early Permian amniotes.
34 We find evidence for an early burst of evolutionary rates, resulting in the early origins of
35 morphologically distinctive subgroups that mostly persisted through the Cisuralian. Rates
36 declined substantially through time, especially in reptiles. Early reptile evolution was also
37 more constrained compared to early synapsids, exploring a more limited character state
38 space. Postcranial innovation in particular was important in early synapsids, potentially
39 related to their early origins of large body size. In contrast, early reptiles predominantly
40 varied the temporal region, suggesting disparity in skull and jaw kinematics, and
41 foreshadowing the variability of cranial biomechanics seen in reptiles today. Our results
42 demonstrate that synapsids and reptiles underwent an early divergence of macroevolutionary
43 patterns. This laid the foundation for subsequent evolutionary events and may be critical in
44 understanding the substantial differences between mammals and reptiles today. Potential
45 explanations include an early divergence of developmental processes or of ecological factors,
46 warranting cross-disciplinary investigation.

47 **Key Words:** Amniote; Phylogeny; Rate; Constraint; Body Size

48

49

50 INTRODUCTION

51 Amniotes, the terrestrialised vertebrates, are a diverse group comprising more than
52 25,0000 living species. Their earliest fossils occur around 318 million years ago, and already
53 include representatives of the two major subgroups that persist to the present day (Carroll
54 1964; Reisz 1972; Mann et al. 2020): Synapsida (mammal-line amniotes) and Reptilia, or
55 Sauropsida (the stem-lineage of reptiles, including birds; hereafter referred to as Reptilia or
56 ‘reptiles’). The earliest members of both groups were extremely similar in their general
57 morphology, being small and superficially lizard-like insectivores with sprawling limb
58 orientations. However, they rapidly radiated into a substantial ecomorphological diversity,
59 including diversification of diets (Sues & Reisz 1998, Brocklehurst & Benson 2021), body
60 sizes (Laurin 2004; Reisz & Fröbisch 2014; Brocklehurst 2016; Brocklehurst & Brink 2017;
61 Brocklehurst & Fröbisch 2018, Brocklehurst et al. 2020), habitat use (e.g. arboreality;
62 Spindler et al. 2018; Mann et al 2021), and diel activity patterns (Angielczyk & Schmitz
63 2014, Ford & Benson 2019). Their success has been attributed to a number of evolutionary
64 innovations, including musculoskeletal adaptations that freed the skull from its role in lung
65 ventilation, allowing greater skull versatility (Frazetta 1968; Janis & Keller 2001), the
66 evolution of temporal fenestration facilitating muscle attachment (Frazetta 1968, Wernberg
67 2019; Abel & Wernberg 2021), and the evolution of the amniotic egg (Romer 1957; Carroll
68 1970).

69 Early amniotes provide a classic example of diversification following adaptive zone
70 invasion, and various studies have sought to characterise macroevolutionary patterns during
71 this transition, with suggestions of little substantial change in rate or mode of morphological
72 evolution of body size or general anatomy at the origin of amniotes (Laurin 2004; Ruta et al.
73 2006, 2018), but substantial increases in functional disparity of the feeding apparatus
74 (Anderson & Friedman 2013) and in rates of tooth and jaw evolution (Brocklehurst & Benson
75 2021). However, understanding of the early radiation within amniotes is less well-

76 characterised. Analyses so far have been conducted at various phylogenetic scales, including
77 in larger analyses of tetrapod evolution that contain a more limited sampling of early
78 amniotes (e.g. Laurin 2004; Ruta et al. 2006, 2018; Anderson & Friedman 2013), as well as
79 restricted examinations of early amniote subgroups (Brocklehurst 2016, 2017; Brocklehurst
80 & Brink 2017; Romano et al. 2017, 2018; MacDougal et al. 2019). Studies have also focused
81 on different portions of the anatomy, including limbs (Ruta et al. 2018), jaws and teeth
82 (Anderson & Friedman 2013; Brocklehurst & Benson 2021), vertebrae (Jones et al. 2018,
83 2020), and body size (Laurin 2004). However, thus far there has been no study of
84 macroevolutionary patterns during the origin and early radiation of amniotes including a
85 broad selection of all clades, allowing direct comparison of the evolutionary patterns within
86 the major lineages, and across many anatomical regions.

87

88 Discrete character state matrices provide observations of morphological variation
89 from across the skeleton that may be used for large-scale macroevolutionary analyses. We
90 present a new phylogenetic dataset, substantially expanded from the most recent phylogenetic
91 assessment of early amniote evolution (Ford and Benson 2020), including species and
92 relevant anatomical variation from across all clades spanning the Carboniferous until the end
93 of the early Permian. We use this to assess rates of evolution and evolutionary constraints
94 during the earliest radiation of amniotes across their anatomy and within different partitions,
95 examining differences between early synapsids and early reptiles.

96

97 MATERIALS AND METHODS

98 *Dataset*

99 We analyse a new phylogenetic dataset of early amniotes, focused on coverage of late
100 Carboniferous (Pennsylvanian) and Early Permian (Cisuralian) taxa, and lineages that

101 survived into the middle Permian (Guadalupian), including Therapsida and Neodiapsida. We
102 attempted comprehensive coverage of phylogenetically-informative characters from previous
103 studies and our own observations (e.g. Benson 2012, Modesto et al 2014), expanded from the
104 analysis of Ford & Benson (2020) to achieve a broader sample of Paleozoic amniote lineages.
105 To this end, we added 31 new taxa, mostly pelycosaurian-grade synapsids, moradisaurine
106 captorhinids and acleistorhinid parareptiles. 72 additional characters were added, mostly
107 sourced from Benson (2012) and Modesto et al (2014). The final dataset contains 98 taxa and
108 366 characters (Supplementary Data 1 and 2).

109 Our study also includes analyses of a dataset of 144 dental traits in 534 taxa, taken
110 from Brocklehurst & Benson (2021). This was included to evaluate variation in dental traits
111 because it contains a greater sampling of dental characters from both jaws and the palate,
112 being designed to investigate macroevolutionary patterns within feeding apparatus. It also
113 contains a greater sampling of taxa, both within the interval of study and subsequent times
114 until the Early Triassic, allowing analysis over a longer time duration than available for our
115 primary matrix (Supplementary Data 3).

116

117 *Fossilised Birth Death Analysis*

118 We used Bayesian phylogenetic inference under a relaxed MkV model of character
119 state evolution with a Fossilised Birth Death (FBD) tree prior (Heath et al 2014) to infer a
120 time-scaled phylogeny and rates of character state evolution. To account for the uncertainty
121 in the time of the first appearances, the ages of taxa were represented by a uniform
122 probability distribution covering the full uncertainty of the age of the formation or
123 assemblage zone in which they first appear (See Supplementary Data 20 for origin of
124 formation ages). Net speciation rate (diversification) was drawn from a uniform prior, with
125 net extinction rate (turnover) and relative fossilisation rate drawn from beta priors. Temporal

126 variation in these parameters was not modelled independent gamma rates model was
127 employed to account for rate heterogeneity between branches (an uncorrelated clock model
128 where rates are drawn from a gamma distribution). Rate heterogeneity between characters
129 was also modelled as a gamma distribution. The analysis was carried out with two runs
130 containing four chains for 50 million generations, sampling every 1000, with 25% of trees
131 discarded as burn-in. The maximum clade credibility tree was used as the phylogenetic
132 framework for subsequent analyses. The analysis was implemented in MrBayes 3.2.6
133 (Ronquist & Huelsenbeck. 2003).

134

135 *Analysis of Rates*

136 Rates of character change along each branch were drawn from the results of the FBD
137 analysis (Supplementary Data 4). Variation in the rates of evolution through time was
138 assessed by time slicing the tree at intervals of one million years between 320 and 272 mya
139 (from the origin of amniotes until the end of the Cisuralian). The rates of all branches
140 crossing each time slice (not including non-amniote outgroups) were collated, and the median
141 rate of each time slice was calculated. In order to assess long-term trends in rate variation, a
142 Loess regression curve was fitted to the median rate values through time.

143

144 *Analysis of Constraint*

145 Variation in the strength of evolutionary constraint among lineages was assessed by
146 comparing patristic distances and morphological dissimilarities between pairs of taxa,
147 expanding on a procedure designed by Brocklehurst et al. (2021) to assess character state
148 saturation, or exhaustion: the point where further evolutionary change in morphology (i.e.
149 increasing patristic morphological distance) no longer results in an increase in the differences
150 between taxa (i.e. morphological dissimilarity, or disparity), but instead explores a pre-

151 established character state space, with a high prevalence of homoplasy. We indexed the
152 morphological dissimilarity between pairs of taxa as the proportion of character scores that
153 differ between them, calculated in the R v3.6.1 (R core team 2019) in the package Claddis
154 (Lloyd 2016) using the MORD distance metric. Evolutionary change (patristic morphological
155 distance) is represented by the total phylogenetic branch length between a pair of taxa,
156 representing the number of character state changes that evolved since divergence from their
157 common ancestor. To calculate this, the character/taxon matrix was reanalysed in MrBayes
158 using an a Mkv model of character state evolution, with no information on taxon ages,
159 constraining the topology to that found by the FBD analysis (for our primary matrix) or a
160 composite tree representing consensus from the literature (for our additional dental matrix;
161 see Brocklehurst & Benson [2021]). This Mkv analysis produced a phylogeny in which
162 branch lengths correspond to the inferred amount of morphological character state change
163 (Supplementary Data 5). The summed branch lengths between pairs of taxa were then used as
164 patristic morphological distances, extracted using the R package adephylo (Jombart et al
165 2010).

166 In general, morphological dissimilarity should increase with evolutionary state
167 changes (i.e. with increasing patristic morphological distance). However, this increase begins
168 to asymptote at higher patristic distances (Wagner 2000), indicating the a lack of further
169 exploration of novel character state space. This occurs because homoplastic state changes
170 and reversals can cause increases in similarity, and homoplasy becomes more frequent with
171 increasing patristic distance under constrained evolution (Brochu 1997, Wagner 2000). This
172 results in character state saturation (Foote 1994), or exhaustion (Wagner 2000), whereby
173 further increases in patristic morphological distance between taxon pairs does not, on
174 average, lead to greater morphological dissimilarity between them. Character state saturation,
175 indicated by the asymptote of the relationship between morphological dissimilarity and

176 patristic morphological distance, occurs at lower morphological dissimilarity when
177 constraints are strong, and higher dissimilarity when constraints are weak (Wagner 2000).

178 Individual groups were assessed for significant increases or decreases in constraint
179 using the procedure of Brocklehurst et al. (2021), in which a Michaelis-Menten curve was fit
180 to the comparisons of the patristic morphological distances and pairwise morphological
181 dissimilarities between all pairs of taxa within that clade, the V_{\max} (asymptote) parameter of
182 that curve being used to represent the point of character state saturation. The significance of
183 differences in V_{\max} between portions of the phylogeny was evaluated by comparison to
184 expectations given a uniform model of evolution, as described in Brocklehurst et al. (2021).
185 This was implemented by simulating null character/taxon matrices under an equal rates
186 model, with missing data scores added in the same location as in the empirical dataset. Null
187 morphological dissimilarities between the taxa were calculated from these matrices as
188 described above, which were compared to the patristic distances again by fitting a Michaelis-
189 Menten curve, showing whether the clade under study reached character state saturation at a
190 higher or lower level than in the null simulations. Character state saturation was assessed in
191 both reptiles and synapsids, restricting the comparisons using the primary matrix to pairs of
192 taxa that diverged within the interval of time under study: the Carboniferous–early Permian.
193 We omitted younger branches because they were incompletely sampled and had only been
194 included to ensure coverage of branches that originated in the early Permian, including some
195 that continued into later intervals. However, our analysis of the dental dataset of Brocklehurst
196 & Benson (2021) (Supplementary Data 6) extends up to the early Triassic allowing insights
197 into important later events such as the diversification of Therapsida, ankyromorph
198 parareptiles and Neodiapsida, at least for dental characters.

199 We expand on this method here to allow detection of variation in the strength of
200 constraint in the absence of prior hypotheses regarding the phylogenetic location of shifts.

201 This was done by applying the method to every node in the phylogeny, comparing the
202 patristic morphological distances and pairwise dissimilarities as described above, by fitting a
203 Michaelis Menten curve to estimate the V_{\max} parameter and 84% confidence intervals around
204 it. We then compared this to the null distribution resulting from analysis of null
205 character/taxon matrices resulting from 1000 iterations of our simulation approach.
206 Significance was determined when the 84% confidence interval of a node's observed V_{\max}
207 value lay entirely above the 84% quantile of the null simulations (significant release in
208 constraint), or entirely below the 84% quantile of the null simulations (significant
209 strengthening of constraints). We used 84% intervals because they are expected to overlap
210 95% of the time when two distributions are statistically identical, therefore representing a
211 significance threshold of 0.05 (Payton et al 2003). In contrast, two statistically identical 95%
212 intervals will overlap 99% of the time, resulting in increased frequency of false negatives.
213 The entire process is carried out in R using custom code (Supplementary Data 7 and 8)
214 written using functions from the packages Adephylo (Jombart et al 2010), Claddis (Lloyd
215 2016), and Phytools (Revell 2012).

216

217 *Analyses of Character Partitions*

218 Constraints within anatomical partitions were investigated by analysing subsets of the
219 full character list of each analysis, representing (i) the skull (including lower jaw and
220 mandible), (ii) postcranium (iii) snout (antorbital region of the skull, not including palate) (iv)
221 temporal (postorbital region of the skull) and (v) dentition (including palatal dentition; and
222 supplemented by analysis of the more extensive dataset of dental traits from Brocklehurst &
223 Benson 2021) (Supplementary Data 9-13). For analysing rates within the partitions,
224 characters from each to were subjected to an FBD analysis, with node ages and topology
225 constrained to those identified by the FBD analysis of the whole dataset. This produces a tree

226 identical to the MCC tree from analysis of the whole dataset, but where the rate values
227 represent only those of the character partition (Supplementary data 14-18). For analysis of
228 constraints, characters from each partition to were subjected to an undated Bayesian analysis
229 constraining the tree to that identified by the FBD analysis of the whole dataset. This
230 produces a tree whose branch lengths represent only character changes within the relevant
231 partition. Rates of evolution and character state saturation were then assessed as described
232 above.

233

234 *Analysis of Body Size*

235 Differences in patterns of trait evolution between reptiles and synapsids may result
236 from the macroevolutionary effects of large body sizes, which evolved frequently among
237 early synapsids but rarely among early reptiles (Modesto et al. 2015; Brocklehurst & Brink
238 2017; Brocklehurst & Fröbisch 2018). To evaluate this, taxa were assigned to one of four size
239 categories, each representing an order of magnitude: (small:<1kg, medium:1-10kg, large: 10-
240 100kg, very large: 100-1000kg) (Supplementary Data 19). Using discrete categories of body
241 size is less precise than estimates of size using continuous measurements (e.g. Alroy 1998;
242 Campione & Evans 2012; Brocklehurst & Brink 2017; Benson et al. 2018). Nevertheless, we
243 use discrete body size categories here because they allow the inclusion of the maximum
244 number of taxa, including those too fragmentary to make precise mass estimates. Constraint
245 within each size class was assessed as described above, comparing pairs of taxa within each
246 size category, limiting comparisons to pairs of taxa that diverged during the Carboniferous–
247 early Permian.

248 In order to examine how size evolution varied between synapsids and reptiles, four
249 models of discrete character evolution were fit to the phylogeny and body size categories:
250 Single regime, equal rates (all possible transitions between character state have a single rate,

251 and the rate is consistent between reptiles and synapsids); Single regime, all rates different
252 (all possible transitions between character state may a have a different rate, but the rates are
253 consistent between reptiles and synapsids); Multi regime, equal rates (all possible transitions
254 between character state have a single rate, but the rate varies between reptiles and synapsids);
255 Multi regime, all rates different (all possible transitions between character state may a have a
256 different rate, and the rates are vary between reptiles and synapsids). The model fitting was
257 carried out using the *fitMultiMk* function in phytools (Revell 2015). The fit of the models to
258 the data was assessed using the Akaike weights.

259

260 RESULTS

261

262 *Phylogeny*

263 Our phylogenetic analysis returns a tree topology that is consistent with that found by
264 Ford & Benson (2021), in spite of the addition of characters and taxa (Fig. 1; Fig S2).
265 Parareptiles are found as the sister to neodiapsids and varanopids are found as reptiles,
266 suggesting that support for this contentious phylogenetic hypothesis (e.g. Benoit et al. 2021;
267 Bazzana et al. 2021) remains high even given a larger sample of early synapsids. The
268 addition of further pelycosaurian-grade synapsids produced results broadly consistent with
269 consistent with recent analyses of this grouping (Brocklehurst & Fröbisch 2018, Maddin et al.
270 2020; Berman et al. 2020). The only noticeable discrepancy is that *Eocasea*, *Callibrachion*
271 and *Datheosaurus* are found as outgroups to other caseosaurs (Eothyrididae and Caseidae)
272 rather than being within caseids (as found by Resiz & Fröbisch 2014, Brocklehurst et al.
273 2016, Berman et al. 2020).

274

275 *Analysis of Rates*

276 Rates were highest during the earliest history of amniotes, decreasing through the late
277 Carboniferous until the middle of the early Permian (Cisuralian) (Fig. 1, 2a). Both synsapsids
278 and reptiles exhibit high evolutionary rates that decreased through time during the late
279 Pennsylvanian/earliest Cisuralian. However, rates of evolution in synsapsids remain about
280 twice as high as those of reptiles through the latter half of the Cisuralian (Fig. 2b). Among
281 subclades, rates are highest in Eupelycosauria (among synsapsids), and during the early
282 divergences of varanopids and parareptiles (among reptiles) (Fig. 1).

283

284 *Analysis of Constraints*

285 A relaxation of constraint relative to null expectations is observed at the base of
286 Synapsida, followed by their strengthening within individual lineages: caseids,
287 eupelycosaurs, and sphenacodontians (within Eupelycosauria) (Fig. 3a). Constraints
288 strengthen at the base of Reptilia but becomes relaxed around the earliest divergences of
289 Diapsida. Subsequent strengthening of constraints is observed within the mycterosaurine
290 varanopids and parareptiles. The overall pattern of relaxed constraints and elevated rates
291 during early amniote evolution, followed by slowdowns and increased constraints, are
292 consistent with models proposed by Simpson (1953) for phenotypic evolution during
293 adaptive radiations: rapid evolution between peaks in the adaptive landscape and the lineages
294 diverge into different regions of ecospace, followed by subsequent reductions in rate as
295 niches are filled and constraint increases within the adaptive optima.

296 Constraints played a more important role during early reptile evolution than in early
297 synsapsids. While both groups experienced initially relaxed constraints early in their
298 evolution, reptiles reached character state saturation before the end of the Cisuralian;
299 evidenced by asymptoting of the relationship between patristic distance and morphological
300 dissimilarity (Fig 3b, Table 1). Synsapsids, on the other hand, did not reach character state

301 saturation, and were therefore continuing to explore new areas of morphospace (Fig. 3b).
302 While the interquartile range and media of the morphological dissimilarities between taxa are
303 similar for reptiles and synapsids, the maximum dissimilarities observed are considerably
304 higher in synapsids, indicating their access to a larger area of morphospace (Fig 3c).

305

306 *Variation between anatomical partitions.*

307

308 Reptiles and synapsids differ in how patterns of evolution are expressed among different
309 anatomical partitions. Both lineages exhibit relaxed constraints on skull evolution, in particular
310 for the snout (Fig 4a,b). In contrast, constraints in the temporal region of the skull, which
311 houses the jaw closing muscles, and signifies important functional variation, are significantly
312 relaxed in reptiles but not synapsids (Fig 4c). This gave rise to higher median and maximum
313 values of morphological dissimilarity in this region among reptiles (Fig S3c). Synapsids
314 experienced elevated rates of skull evolution during their earliest evolution, which declined
315 through the late Carboniferous before recovering slightly during the late Cisuralian (Fig 5a).
316 In contrast, rates of skull evolution in reptiles remained low throughout the study interval.

317 Reptiles show high early rates of postcranial evolution compared to those of synapsids
318 (Fig 5b). Nevertheless, postcranial rates declined through time and exhibit significantly high
319 constraints in reptiles compared to synapsids, suggesting that high early rates did not result in
320 proliferation of a wide range of postcranial morphologies in reptiles (Fig 4d). Synapsids,
321 experienced a significant relaxation of constraints on postcranial evolution (Fig 4d),
322 suggesting that, although they evolved more slowly, they potentially acquired a wider
323 disparity of postcranial morphologies (Fig S3d).

324 Dental traits show an early release of constraints in both reptiles and synapsids.

325 However, while reptiles retained these relaxed constraints within Diapsida and Neoreptilia,

326 synapsids experienced a strengthening of constraints within eupelycosaurs (Fig S3a). This
327 was confirmed in our analyses of the larger dental dataset of Brocklehurst & Benson (2021),
328 which could be analysed over a longer study interval and provides evidence for a subsequent
329 relaxation of constraints on dental evolution in the synapsid subgroup Therapsida (Fig S3b).

330

331

332 *Constraint at different body sizes*

333 Small and medium sized amniotes were found to evolve under greater constraint than large
334 and very large taxa, reaching character state saturation at a lower morphological dissimilarity
335 (Fig. 6b). Interestingly, taxon-pairs within all three size classes are found to evolve under
336 greater constraint than expected from null simulations (Table 1). This suggests that
337 morphological innovation among early amniotes was predominantly associated with
338 evolutionary changes between size categories. Overall, therefore, taxa within different size
339 classes occupy different regions of morphospace, with larger taxa occupying larger regions of
340 morphospace (Fig S6).

341 Although reptiles and synapsids show different patterns of body size evolution (see
342 below), they nevertheless show similar patterns of overall morphological constraint within
343 the size categories. Large and very large taxa exhibit relaxed constraints in both synapsids
344 and reptiles, although early synapsids include more taxa of these sizes than do reptiles (Fig
345 7c, Table 1).

346 We also find evidence for different patterns of body size evolution among synapsids
347 compared to those in reptiles (see also Modesto et al. [2015]; Brocklehurst 2021). Evolution
348 of the discrete character representing body size best fits a model where the two lineages are
349 subject to different evolutionary regimes (Table 2). Within synapsids, only transitions from
350 smaller size categories to larger have positive rates; rates of transition from larger size

351 categories to smaller were all zero (Fig. 7). This indicates an evolutionary trend towards
352 larger body sizes in synapsids. Reptiles, on the other hands, have positive rate values for
353 transitions in both directions, and in some cases the rate of transition from larger to smaller
354 size categories is higher than from smaller to larger.

355

356 DISCUSSION

357 The evolution of full terrestriality, at the origin of amniotes, was a major event in
358 vertebrate evolution, providing insights into macroevolutionary patterns during ecospace
359 invasion. Moreover, the evolutionary divergences among early amniotes gave rise to the
360 mammal-line (Synapsida) and reptile-line (Reptilia), which persist to the present day and
361 show stark differences in their morphology, ecology, and biology. Previous studies indicated
362 variation in rates and constraint coinciding either with the origin of amniotes or their
363 subsequent diversification into different areas of terrestrial ecospace. This has been shown,
364 for example, during body size evolution (Laurin 2004; Reisz & Fröbisch 2014; Brocklehurst
365 2016; Brocklehurst & Brink 2017; Brocklehurst & Fröbisch 2018, Brocklehurst et al. 2020),
366 and the evolution of jaw (Anderson et al. 2013), tooth (Brocklehurst & Benson 2021) and
367 limb morphology (Ruta et al 2018). However, macroevolutionary patterns during this
368 transition have been unclear due to the variation in taxonomic scope of these studies and
369 regions of anatomy analysed. Our study represents the first analysis that samples broadly
370 across early members of the amniote crown-group and including morphological variation
371 from across the whole skeleton.

372 We find evidence for an early episode of high evolutionary rates across the skeleton,
373 coupled with relaxed constraints on cranial (especially snout and dental) evolution. This is
374 consistent with the early origins of a wide set of morphologically and ecologically distinctive
375 amniote subclades by the late Carboniferous, including herbivorous edaphosaurids (Sues &

376 Reisz 2000), macropredatory sphenacodontians (Fröbisch et al. 2010; Brocklehurst & Brink
377 2017), arboreal protorothyridids (Mann et al. 2021), and many other groups. These findings
378 contradict those of Ruta et al. (2006), who suggested that amniote origins did not give rise to
379 an increase in the rate of character state evolution. This may be an artefact resulting from
380 under-sampling of amniote taxa and characters in the dataset of Ruta et al. (2006), who
381 included only a very small sample of crown amniotes, from the reptile line only.
382 Nevertheless, future studies should examine whether the elevated rates and subsequent
383 decline observed here in early amniotes merely represents a subset of a longer-term decline in
384 rates across tetrapods.

385 Our findings of relaxed constraints on cranial, snout and dental evolution in the
386 earliest amniotes are consistent with the hypothesis that diet-related cranial variation was an
387 important axis of phenotypic diversification during their initial radiation (e.g. Janis & Keller
388 2001; Anderson & Friedman 2013; Brocklehurst & Benson 2021). We also show that rates of
389 evolution were also elevated for other anatomical regions, not strictly limited to dietary or
390 craniodental diversification. Nevertheless, those regions (postcrania, and the temporal region
391 of the skull) exhibit different patterns of variation in constraint between reptiles and
392 synapsids (Fig. 4), highlighting the divergent paths to morphological diversification that were
393 taken by these groups.

394 The initial diversification of reptiles appears to have been focused on the temporal
395 region. This is consistent with the qualitative observation that early reptiles exhibit
396 considerable evolutionary versatility of temporal fenestration, contrasting with more
397 conserved temporal anatomy in synapsids (Piñeiro et al. 20212; MacDougal & Reisz 2014;
398 Haridy et al. 2016; Ford & Benson 2020). Diversification of temporal fenestration among
399 early reptile groups likely corresponds to variation in muscle attachment and jaw function
400 (Frazzetta 1968; Wernberg 2019, Abel & Wernberg 2021), and may therefore reflect an early

401 diversification of cranial function. Reptiles also maintain relaxed constraints on their
402 dentition, whereas eupelycosaurian synapsids experience a strengthening of the constraints.
403 This may result from differences in the evolvability of palatal dentition between synapsids
404 and early reptiles. Reptiles exhibit a great diversity in the arrangement, size, density, and
405 patterns of loss of the palatal dentition, both today and in the past (Matsumoto and Evans
406 2017). Such variation is also present, to some extent, in caseosaurian synapsids (Brocklehurst
407 et al. 2016). However, eupelycosaurian synapsids show much less variability, with a trend to
408 simplification and ultimately loss of palatal teeth long before the origin of mammals
409 (Matsumoto and Evans 2017). This may have been compensated by a relaxation of
410 constraints on the marginal dentition along the line leading to mammals, which is evident
411 among the middle Permian divergences of therapsids. (Fig S3b). Increases in the evolutionary
412 versatility of marginal dentition among synapsids culminated in the development of strongly
413 heterodont and functionally differentiated marginal dentitions as a central innovation of later
414 cynodonts, including mammals (Compton & Jenkins 1968; Luo et al. 2015).

415 Postcranial data indicate a decoupling of rates and constraints. Reptiles show high
416 early rates coupled with significantly increased constraint suggesting that they rapidly
417 explored a relatively small postcranial character state space. In contrast, early synapsid
418 postcrania evolved at lower rates for much of the Carboniferous and early Permian, but under
419 significantly relaxed constraints that allowed them to gradually explore a much larger
420 character state space. Our findings are therefore potentially consistent with the limited
421 previous studies of synapsid postcranial variation, which found evidence for high disparity of
422 humerus shape and heterogeneity between vertebral regions in therapsids (Jones et al. 2018;
423 Lungmus & Angielczyk 2019).

424 Synapsid evolution is further distinct from that of reptiles in that synapsids rapidly
425 attained large body sizes during their early history, whereas reptiles did not. Ancestral

426 character state mapping suggests a small-bodied ancestor of the amniote crown-group (< 1
427 kg) (Fig 7a), from which multiple synapsid lineages independently evolved large body sizes
428 exceeding 40 kg before the end of the Carboniferous (Reisz & Fröbisch, 2014; Brocklehurst
429 & Brink, 2017; Brocklehurst & Fröbisch, 2018). In contrast, reptiles did not reach such sizes
430 until the latest Cisuralian origin of moradisaurine captorhinids (Brocklehurst, 2016). The
431 analysis of body size evolution presented here should be treated with caution due to the low
432 resolution of the size data, but it does demonstrate that synapsid body size was evolving
433 under a distinct regime to that of reptiles, and potentially exhibited a trend of increasing body
434 size that was absent in reptiles.

435 It has been suggested that the apparent increase in body size may have driven the
436 apparent greater diversity of synapsids during the Paleozoic, due to biases in either
437 preservation or collection: larger synapsids may be easier to find or have greater fossilisation
438 potential than smaller, more fragmentary reptiles (Modesto et al. 2015; Brocklehurst 2021).
439 The fossil reptiles named from the Carboniferous are smaller, but more complete than the
440 synapsids (Modesto et al. 2015). This potentially indicates that only the most complete
441 reptiles are collected or described, whereas synapsid material may be considered informative
442 even when it is more fragmentary, due to the larger body size of synapsids (Modesto et al.
443 2015). However, it is also possible that this signal is genuine, and reflects the earlier adoption
444 of herbivory and carnivory in synapsids (Modesto et al. 2015), reflecting a wider pattern of
445 divergence along ecological lines in early amniotes.

446 Irrespective of patterns of species diversification, larger body size appears to be
447 related to reduced constraint in morphological evolution, both in reptiles and synapsids (Fig.
448 6b,c). Therefore, the fact that synapsids show an early trend towards larger body size,
449 reaching larger sizes earlier and more frequently than the reptiles, may have permitted the
450 greater relaxation of constraints observed in early synapsids. A greater range of body sizes, as

451 observed in synapsids, has been linked to greater functional diversity (Woodward et al. 2005;
452 Rooney & McCann 2012). Larger body sizes allow access to a different range of ecotypes,
453 including macro-predation and high fibre herbivory (Clauss & Hummel 2005; Müller et al.
454 2013; Brocklehurst & Brink 2017), permitting further diversification within these distinct
455 regions of ecomorphospace. Moreover, the fact that synapsids rarely underwent reversals to
456 small body sizes could result in fewer homoplasies among size-dependent characters, and
457 therefore weaker apparent constraints on morphological evolution.

458 Early attainment of large body size is particularly relevant to the release in constraints
459 on postcranial evolution observed in synapsids, but not in reptiles. Scaling relationships
460 imply that the stresses experienced by the skeleton are relatively higher in larger-bodied
461 species (Stanley 1973; Biewener 1982). Thereby, ecological specialisation may require more
462 substantial postcranial specialisation among large-bodied species to exploit new niches.
463 Studies in mammals support these observations, demonstrating greater diversity within
464 different locomotor modes at larger sizes (Weaver & Grossnickle 2020), and greater
465 distinction between locomotor types in larger taxa (Jenkins 1974; Jenkins & Parrington 1976;
466 Runestad & Ruff 1995). The relaxed evolutionary constraints in the synapsid postcranium
467 may provide an explanation of why large body size evolved multiple times independently
468 among early synapsids, but not among early reptiles; synapsids' access to a wider region of
469 postcranial morphospace allowed the greater postcranial specialisation necessary for
470 ecological specialisation at large sizes. Alternatively, large body size may have evolved in
471 synapsids for other reasons (e.g. ecological), and necessitated postcranial specialisations that
472 are detected here as a release in constraint on postcranial evolution.

473 Early events in amniote evolution, documented here, set the stage for the origins of
474 major groups that comprise most of the extant diversity of land vertebrates. In particular,
475 Neodiapsida (including the reptile crown-group; 'Neo' in Fig. 1) and Therapsida (including

476 the mammalian crown-group; ‘The’ in Fig. 1) comprise the bulk of amniote diversity after the
477 early Permian and have highly distinct anatomy compared to their predecessors (Rubidge &
478 Sidor 2001). These groups evolved substantial morphological disparity (Ruta et al. 2013a,b;
479 Ezcurra & Butler 2018; Grunert et al. 2019; Lungmus & Angielczyk 2019). However, the
480 branches leading to them, which span most of the our early Permian study interval, show no
481 evidence of high rates of evolution. In fact both lineages represent evolutionary slowdowns
482 relative to the ‘backbones’ of the reptile and synapsid phylogenies, demonstrating that high
483 evolutionary rates are not required to explain the origins of these groups. This raises the
484 possibility that distinctive traits of both neodiapsids and therapsids assembled gradually
485 throughout the early Permian (Cisuralian) during a cryptic and poorly-sampled interval of
486 evolutionary history, before their rise to high abundances during latter intervals of Permian
487 and the Triassic. However, both groups exhibit a long interval in which direct evidence for
488 rates of accumulation of their derived characters is entirely missing the fossil record (late
489 Pennsylvanian–latest Cisuralian). Therefore, future fossil discoveries are required to test
490 hypotheses of their evolutionary patterns and could demonstrate, in reality, their traits
491 appeared abruptly, either late or early in this unsampled time window. This is particularly
492 relevant to discussions on the origin of therapsids, where it has been suggested that the
493 interval known as Olson’s Gap (late Kungurian-Roadian, latest Cisuralian-earliest
494 Guadalupian) (Lucas and Heckert 2001; Liu et al. 2009; Brocklehurst 2020) was an important
495 evolutionary interval and that better sampling at this time would shed light on would shed
496 light on the origins of therapsid anatomy (Abdala et al. 2008; Liu et al. 2009). Our results
497 show that current data are also consistent with a protracted origin, and that a great expansion
498 of fossil evidence throughout the early Permian could be required to fully test this.

499 Variation between macroevolutionary patterns among different anatomical partitions
500 for early synapsids and early reptiles may have underpinned the different evolutionary

501 trajectories of reptile- and mammal-line amniotes, and may ultimately have resulted in the
502 clear disparities between mammals and reptiles today. Our results potentially imply that a
503 deep divergence in patterns of evolutionary modularity (Vermeij 1973; 1973; Wagner and
504 Altenberg 1996), due to either developmental or ecofunctional drivers, might potentially
505 explain the different paths that reptiles and synapsids have taken during their ecological
506 diversification. For example, we provide evidence for relaxation of constraints on temporal
507 evolution i among early reptiles (Fig. 4). This is congruent with variation seen among extant
508 reptiles, which exhibit substantial disparity of temporal morphology and of regions associated
509 with jaws muscular and articulation (Watanabe et al. 2019; Rhoda et al. 2020), as well as
510 variation in the degree of cranial kinesis and the location of articulation points. This ranges
511 from relatively akinetic skulls in crocodiles, turtles and rhynchocephalians (Preuschoft &
512 Witzel 2002; Ferreira et al. 2020) to mobility of the frontoparietal suture, quadrate and palate
513 in many squamates, extreme forms of kinesis in snakes (Arnold 1989; Metzger 2002; Rhoda
514 et al. 2020), and kinesis of the beak relative to the braincase in birds (Bout & Zwiers 2001).
515 Variability in kinesis and articulation is noticeably more limited, or absent, in mammals.

516 Relaxation of postcranial evolution is shown here among early synapsids (Fig. 4).
517 This is congruent with considerable variation in the postcranial morphology of modern
518 mammals and their therapsid ancestors, including greater variability and distinction between
519 modules in the vertebral column in mammals compared to reptiles (Arnold et al. 2017; Jones
520 et al. 2018; Arnold 2021), and considerable increases in synapsid humeral disparity from the
521 middle Permian onwards (Lungmus & Angielczyk 2019). Our findings therefore suggest that
522 an initial release in postcranial evolution occurred at the origin of synapsids, with subsequent
523 increases occurring in therapsids and among mammals (Jones et al 2018; Lungmus &
524 Angielczyk 2019).

525

526 CONCLUSIONS

527 Synapsids and reptiles in the present day represent two highly divergent lineages, with
528 substantial differences in their morphology and physiology. During their earliest divergences
529 in the Carboniferous, however, they were morphologically and ecologically very similar, both
530 represented by small insectivores with a superficially ‘lizard-like’ body form. These
531 superficial similarities mask a deep evolutionary divergence in rates and modes of between
532 synapsids and reptiles, documented by our analyses. Analysis of constraint without *a priori*
533 assumptions on where regime shifts occur identify fundamental differences in patterns of
534 evolution between the two lineages; reduced constraint in the temporal region of the skull on
535 the reptile line, and reduced constraints in postcranial evolution on the synapsid line, which
536 may potentially be linked to a trend on increasing body size. This separation of evolutionary
537 patterns during the earliest divergence between synapsids and reptiles may be fundamental to
538 understanding the differences between these groups throughout their history, including stark
539 differences between members of these groups that are present today. Our observations of a
540 deep divergence of macroevolutionary modalities raises the possibility of a deep divergence
541 of either developmental processes or ecological factors very early on the mammal and reptile
542 lines. Further studies of morphological evolution, spanning the subsequent intervals of
543 amniote evolution are required to confirm this possibility. Moreover, developmental and
544 ecological or functional studies are also required to test the mechanisms that may have given
545 rise to this macroevolutionary divergence. Amniote origins and the long-term differentiation
546 of mammal and reptile phenotypes therefore provide a promising avenue for cross-
547 disciplinary investigation in evolutionary research.

548

549 ACKNOWLEDGEMENTS

550 We would like to thank Graeme Lloyd for assistance with Claddis, and Yara Haridy for
551 helpful discussion. Reviews by Kenneth Angielczyk, Peter Wagner and an anonymous
552 reviewer greatly improved this paper. This research was funded by the European Union's
553 Horizon 2020 research and innovation program 2014–2018 under grant agreement 677774
554 (European Research Council [ERC] Starting Grant: TEMPO) awarded to RBJB.

555

556 REFERENCES

- 557 Abdala, F., Rubidge, B. S. & Van Den Heever, J. (2008) The oldest therocephalians
558 (Therapsida, Eutheriodontia) and the early diversification of Therapsida.
559 *Palaeontology*, 51, 1011-1024
- 560 Abel, P., & Werneburg, I. (2021). Morphology of the temporal skull region in tetrapods:
561 research history, functional explanations, and a new comprehensive classification
562 scheme. *Biological Reviews*, In Press.
- 563 Alroy, J. (1998). Cope's rule and the dynamics of body mass evolution in North American
564 fossil mammals. *Science*, 280, 731-734
- 565 Anderson, P. S., Friedman, M., & Ruta, M. (2013). Late to the table: diversification of
566 tetrapod mandibular biomechanics lagged behind the evolution of terrestriality.
567 *Integrative & Comparative Biology*, 53, 197-208
- 568 Angielczyk, K. D., & Schmitz, L. (2014). Nocturnality in synapsids predates the origin of
569 mammals by over 100 million years. *Proceedings of the Royal Society B*, 281,
570 20141642.
- 571 Arnold, P. 2021. Evolution of the mammalian neck from developmental, morpho-functional,
572 and paleontological perspectives. *Journal of Mammalian Evolution*, 28, 173-183.
- 573 Arnold, P., Amson, E. and Fischer, M.S. 2017. Differential scaling patterns of vertebrae and
574 the evolution of neck length in mammals. *Evolution*, 71, 1587-1599.

- 575 Badyaev, A.V., Foresman, K.R. and Young, R.L. 2005. Evolution of morphological
576 integration: developmental accommodation of stress-induced variation. *The American*
577 *Naturalist*, 166, 382-395.
- 578 Bazzana, K. D., Evans, D. C., Bevitt, J. J. & Reisz, R. R. (2021). Neurosensory anatomy of
579 Varanopidae and its implications for early synapsid evolution. *Journal of Anatomy*, In
580 Press
- 581 Benoit, J., Ford, D. P., Miyamae, J. A., & Ruf, I. (2021). Can maxillary canal morphology
582 inform phylogenetic affinities? *Acta Palaeontologica Polonica*, 66, 389-393
- 583 Benson, R. B. (2012). Interrelationships of basal synapsids: cranial and postcranial
584 morphological partitions suggest different topologies. *Journal of Systematic*
585 *Palaeontology*, 10, 601-624.
- 586 Benson, R. B., Hunt, G., Carrano, M. T., & Campione, N. (2018). Cope's rule and the
587 adaptive landscape of dinosaur body size evolution. *Palaeontology*, 61, 13-48
- 588 Berman, D. S., Maddin, H. C., Henrici, A. C., Sumida, S. S., Scott, D., & Reisz, R. R. (2020).
589 New primitive caseid (Synapsida, Caseasauria) from the early Permian of Germany.
590 *Annals of the Carnegie Museum*, 86, 43-75
- 591 Biewener, A. A. (1982). Bone strength in small mammals and bipedal birds: do safety factors
592 change with body size? *Journal of Experimental Biology*, 98, 289-301.
- 593 Brochu, C. (1997). Morphology, fossils, divergence timing and the phylogenetic relationships
594 of *Gavialis*. *Systematic Biology*, 46, 479-522
- 595 Brocklehurst, N. (2016). Rates and modes of body size evolution in early carnivores and
596 herbivores: a case study from Captorhinidae. *PeerJ*, 4, e1555.
- 597 Brocklehurst, N. (2017). Rates of morphological evolution in Captorhinidae: an adaptive
598 radiation of Permian herbivores. *PeerJ*, 5, e3200.

- 599 Brocklehurst, N. (2020). Olson's Gap or Olson's Extinction? A Bayesian tip-dating approach
600 to resolving stratigraphic uncertainty. *Proceedings of the Royal Society B*, 287,
601 20200154
- 602 Brocklehurst, N. (2021). The First Age of Reptiles? Comparing reptile and synapsid
603 diversity, and the influence of Lagerstätten, during the Carboniferous and early
604 Permian. *Frontiers in Ecology and Evolution*, 9, 669765.
- 605 Brocklehurst, N., & Brink, K. S. (2017). Selection towards larger body size in both
606 herbivorous and carnivorous synapsids during the Carboniferous. *Facets*, 2, 68-84.
- 607 Brocklehurst, N., & Fröbisch, J. (2018). A reexamination of *Milosaurus mccordi*, and the
608 evolution of large body size in Carboniferous synapsids. *Journal of Vertebrate*
609 *Paleontology*, 38, e1508026.
- 610 Brocklehurst, N., & Benson, R. J. B. (2021). Multiple paths to morphological diversification
611 during the origin of amniotes. *Nature Ecology and evolution*, 5, 1243-1249
- 612 Brocklehurst, N., Reisz, R. R., Fernandez, V., Fröbisch, J. (2016). A Re-Description of
613 '*Mycterosaurus' smithae*, an Early Permian Eothyridid, and Its Impact on the
614 Phylogeny of Pelycosaurian-Grade Synapsids. *PLoS ONE*, 11, e0156810
- 615 Brocklehurst, N., Kammerer, C. F., & Benson, R. J. (2020). The origin of tetrapod herbivory:
616 effects on local plant diversity. *Proceedings of the Royal Society B*, 287, 20200124.
- 617 Brocklehurst, N., Panciroli, E., Benevento, G. L., & Benson, R. B. (2021). Mammaliaform
618 extinctions as a driver of the morphological radiation of Cenozoic mammals. *Current*
619 *Biology*, 31, 2955-2963
- 620 Campione, N. E., & Evans, D. C. (2012). A universal scaling relationship between body mass
621 and proximal limb bone dimensions in quadrupedal terrestrial tetrapods. *BMC*
622 *Biology*, 10, 1-22

- 623 Carroll R. L. (1970) Quantitative aspects of the amphibian-reptilian transition. *Forma et*
624 *Functio*, 3, 165-178
- 625 Clauss, M., & Hummel, J. (2005). The digestive performance of mammalian herbivores: why
626 big may not be that much better. *Mammal Review*, 35, 174-187
- 627 Compton, A. W., & Jenkins, F. A. (1968). Molar occlusion in Late Triassic mammals.
628 *Biological Reviews*, 43, 427-458
- 629 Ezcurra, M. D., & Butler, R. J. (2018). The rise of the ruling reptiles and ecosystem recovery
630 from the Permo-Triassic mass extinction. *Proceedings of the Royal Society B*, 285,
631 20180361.
- 632 Fabre, A.C., Perry, J.M., Hartstone-Rose, A., Lowie, A., Boens, A. and Dumont, M., 2018.
633 Do muscles constrain skull shape evolution in Strepsirrhines? *The Anatomical*
634 *Record*, 301, pp.291-310.
- 635 Felice, R.N. and Goswami, A., 2018. Developmental origins of mosaic evolution in the avian
636 cranium. *Proceedings of the National Academy of Sciences*, 115, 555-560.
- 637 Felice, R.N., Watanabe, A., Cuff, A.R., Noirault, E., Pol, D., Witmer, L.M., Norell, M.A.,
638 O'Connor, P.M. and Goswami, A. 2019. Evolutionary integration and modularity in
639 the archosaur cranium. *Integrative and comparative biology*, 59, 371-382.
- 640 Foote, M. (1994). Morphological disparity in Ordovician-Devonian crinoids and the early
641 saturation of morphological space. *Paleobiology*, 20, 320-344
- 642 Ford, D. P., & Benson, R. B. (2019). A redescription of *Orovenator mayorum* (Sauropsida,
643 Diapsida) using high-resolution μ CT, and the consequences for early amniote
644 phylogeny. *Papers in Palaeontology*, 5, 197-239.
- 645 Ford, D. P., & Benson, R. B. (2020). The phylogeny of early amniotes and the affinities of
646 Parareptilia and Varanopidae. *Nature Ecology & Evolution*, 4, 57-65.

- 647 Frazzetta, T. H. (1968). Adaptive problems and possibilities in the temporal fenestration of
648 tetrapod skulls. *Journal of Morphology*, 125, 145-157.
- 649 Fröbisch, J., Schoch, R.R., Müller, J., Schindler, T. and Schweiss, D., 2011. A new basal
650 sphenacodontid synapsid from the Late Carboniferous of the Saar-Nahe Basin,
651 Germany. *Acta Palaeontologica Polonica*, 56, 113-120.
- 652 Grunert, H. R., Brocklehurst, N., & Fröbisch, J. (2019). Diversity and disparity of
653 Therocephalia: Macroevolutionary patterns through two mass extinctions. *Scientific*
654 *reports*, 9(1), 1-11.
- 655 Haridy, Y., Macdougall, M. J., Scott, D., & Reisz, R. R. (2016). Ontogenetic change in the
656 temporal region of the Early Permian parareptile *Delorhynchus cifellii* and the
657 implications for closure of the temporal fenestra in amniotes. *PloS one*, 11, e0166819.
- 658 Heath, T. A., Huelsenbeck, J. P., & Stadler, T. (2014). The fossilized birth–death process for
659 coherent calibration of divergence-time estimates. *Proceedings of the National*
660 *Academy of Sciences*, 111, E2957-E2966.
- 661 Hotton, N.I.I.I., Olson, E.C., Beerbower, R., Sumida, S.S. and Martin, K.L.M. 1997. Amniote
662 origins and the discovery of herbivory. In *Amniote origins* (pp. 207-264). Academic
663 Press.
- 664 Janis, C. M., & Keller, J. C. (2001). Modes of ventilation in early tetrapods: Costal aspiration
665 as a key feature of amniotes. *Acta Palaeontologica Polonica*, 46, 137-170.
- 666 Jenkins Jr, F. A. (1974). The movement of the shoulder in clavicate and a clavicate
667 mammals. *Journal of Morphology*, 144, 71-83.
- 668 Jenkins, Jr, F. A., & Parrington, F. R. (1976). The postcranial skeletons of the Triassic
669 mammals *Eozostrodon*, *Megazostrodon* and *Erythrotherium*. *Philosophical*
670 *Transactions of the Royal Society of London. B*, 273, 387-431.

- 671 Jombart, T., Balloux, F., & Dray, S. (2010). Adephylo: new tools for investigating the
672 phylogenetic signal in biological traits. *Bioinformatics*, 26, 1907-1909.
- 673 Jones, K. E., Angielczyk, K. D., Polly, P. D., Head, J. J., Fernandez, V., Lungmus, J. K.,
674 Tulga, S. & Pierce, S. E. (2018). Fossils reveal the complex evolutionary history of
675 the mammalian regionalized spine. *Science*, 361, 1249-1252.
- 676 Jones, K. E., Gonzalez, S., Angielczyk, K. D., & Pierce, S. E. (2020). Regionalization of the
677 axial skeleton predates functional adaptation in the forerunners of mammals. *Nature*
678 *ecology & evolution*, 4, 470-478.
- 679 Jones, K. E., Dickson, B. V., Angielczyk, K. D., & Pierce, S. E. (2021). Adaptive landscapes
680 challenge the “lateral-to-sagittal” paradigm for mammalian vertebral evolution.
681 *Current Biology*, 31, 1883-1892.
- 682 Laurin, M. (2004). The evolution of body size, Cope's rule and the origin of amniotes.
683 *Systematic Biology*, 53, 594-622.
- 684 Lee, M.S. and Spencer, P.S., 1997. Crown-clades, key characters and taxonomic stability:
685 when is an amniote not an amniote? In *Amniote origins* (pp. 61-84). Academic Press.
- 686 Liu, J., Rubidge, B. S. & Li, J. (2009). New basal synapsid supports Laurasian origin for
687 therapsids. *Acta Palaeontologica Polonica*, 54, 393-400
- 688 Lloyd, G. T. (2016). Estimating morphological diversity and tempo with discrete character-
689 taxon matrices: implementation, challenges, progress, and future directions.
690 *Biological Journal of the Linnean Society*, 118, 131-151.
- 691 Lucas, S. G., & Heckert, A. B. (2001) Olson's gap: a global hiatus in the record of middle
692 Permian tetrapods. *Journal of Vertebrate Paleontology* 21, 75A
- 693 Lungmus, J. K., & Angielczyk, K. D. (2021). Phylogeny, function and ecology in the deep
694 evolutionary history of the mammalian forelimb. *Proceedings of the Royal Society B*,
695 288, 20210494.

- 696 Luo, Z. X., Gatesy, S. M., Jenkins, F. A., Amaral, W. W. & Shubin, N. H. (2015).
697 Mandibular and dental characteristics of Late Triassic mammaliaform Haramiyavia
698 and their ramifications for basal mammal evolution. *Proceedings of the National*
699 *Academy of Sciences*, 112, E7101-E7109
- 700 MacDougall, M. J., & Reisz, R. R. (2014). The first record of a nyctiphuretid parareptile
701 from the Early Permian of North America, with a discussion of parareptilian temporal
702 fenestration. *Zoological Journal of the Linnean Society*, 172(3), 616-630.
- 703 MacDougall, M. J., Brocklehurst, N., & Fröbisch, J. (2019). Species richness and disparity of
704 parareptiles across the end-Permian mass extinction. *Proceedings of the Royal Society*
705 *B*, 286, 20182572.
- 706 Maddin, H. C., Mann, A., Herbert, B. (2020). Varanopid from the Carboniferous of Nova
707 Scotia reveals the evidence of parental care in amniotes. *Nature Ecology and*
708 *Evolution*, 4, 50-56
- 709 Mann, A., Dudgeon, T.W., Henrici, A.C., Berman, D.S. and Pierce, S.E., 2021. Digit and
710 ungual morphology suggest adaptations for scansoriality in the late Carboniferous
711 eureptile *Anthracosaurus longipes*. *Frontiers in Earth Science*, 9, 440.
- 712 Modesto S. P. (1992). Did herbivory foster early amniote diversification? *Journal of*
713 *Vertebrate Paleontology*, 12 (Suppl.), 44A
- 714 Modesto, S. P., Lamb, A. J., & Reisz, R. R. (2014). The captorhinid reptile *Captorhinikos*
715 *valensis* from the lower Permian Vale Formation of Texas, and the evolution of
716 herbivory in eureptiles. *Journal of Vertebrate Paleontology*, 34, 291-302.
- 717 Modesto, S. P., Scott, D. M., MacDougall, M. J., Sues, H. D., Evans, D. C., & Reisz, R. R.
718 (2015). The oldest parareptile and the early diversification of reptiles. *Proceedings of*
719 *the Royal Society B: Biological Sciences*, 282, 20141912.

- 720 Müller, D. W., Codron, D., Meloro, C., Munn, A., Schwarm, A., Hummel, J., & Clauss, M.
721 (2013). Assessing the Jarman-Bell principle: scaling of intake, digestibility, retention
722 time and gut fill with body mass in mammalian herbivores. *Comparative*
723 *Biochemistry and Physiology A*, 164, 129-140
- 724 Payton, M. E., Greenstone, M. H., & Schenker, N. (2003). Overlapping confidence intervals
725 or standard error intervals: what do they mean in terms of statistical significance?
726 *Journal of Insect Science*, 3, 34.
- 727 Piñeiro, G., Ferigolo, J., Ramos, A., & Laurin, M. (2012). Cranial morphology of the Early
728 Permian mesosaurid *Mesosaurus tenuidens* and the evolution of the lower temporal
729 fenestration reassessed. *Comptes Rendus Palevol*, 11, 379-391.
- 730 R Core Team (2019). *R: A language and environment for statistical computing*. R
731 Foundation for Statistical Computing, Vienna, Austria.
- 732 Reisz, R. R., & Fröbisch, J. (2014). The oldest caseid synapsid from the Late Pennsylvanian
733 of Kansas, and the evolution of herbivory in terrestrial vertebrates. *PloS one*, 9,
734 e94518.
- 735 Revell, L. J. (2012). *phytools: an R package for phylogenetic comparative biology (and other*
736 *things)*. *Methods in Ecology and Evolution*, 3, 217-223.
- 737 Rhoda, D., Polly, P.D., Raxworthy, C. and Segall, M. 2021. Morphological integration and
738 modularity in the hyperkinetic feeding system of aquatic-foraging snakes. *Evolution*,
739 75, 56-72.
- 740 Romano, M., Brocklehurst, N., & Fröbisch, J. (2017). Discrete and continuous character-
741 based disparity analyses converge to the same macroevolutionary signal: a case study
742 from captorhinids. *Scientific reports*, 7, 1-9.
- 743 Romer A. S. (1957) Origin of the amniote egg. *The Scientific Monthly*, 85, 57-63

- 744 Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference
745 under mixed models. *Bioinformatics*, 19, 1572-1574.
- 746 Rooney, N., & McCann, K. S. (2012). Integrating food web diversity, structure and stability.
747 *Trends in Ecology and Evolution*, 27, 40-46
- 748 Rubidge, B. S. & Sidor, C. A. (2001). Evolutionary patterns among Permo-Triassic
749 therapsids. *Annual Review of Ecology and Systematics*, 32, 449-480
- 750 Runestad, J. A., & Ruff, C. B. (1995). Structural adaptations for gliding in mammals with
751 implications for locomotor behavior in paromomyids. *American Journal of Physical*
752 *Anthropology*, 98, 101-119.
- 753 Ruta, M., Wagner, P. J., & Coates, M. I. (2006). Evolutionary patterns in early tetrapods. I.
754 Rapid initial diversification followed by decrease in rates of character change.
755 *Proceedings of the Royal Society B*, 273, 2107-2111.
- 756 Ruta, M., Angielczyk, K. D., Fröbisch, J., & Benton, M. J. (2013a). Decoupling of
757 morphological disparity and taxic diversity during the adaptive radiation of
758 anomodont therapsids. *Proceedings of the Royal Society B: Biological Sciences*, 280,
759 20131071.
- 760 Ruta, M., Botha-Brink, J., Mitchell, S. A., & Benton, M. J. (2013b). The radiation of
761 cynodonts and the ground plan of mammalian morphological diversity. *Proceedings*
762 *of the Royal Society B: Biological Sciences*, 280, 20131865.
- 763 Ruta, M., Krieger, J., Angielczyk, K. D., & Wills, M. A. (2018). The evolution of the
764 tetrapod humerus: Morphometrics, disparity, and evolutionary rates. *Earth and*
765 *Environmental Science Transactions of the Royal Society of Edinburgh*, 109, 351-
766 369.
- 767 Simpson, G. G. (1953). *The major features of evolution*. Columbia University Press.

- 768 Spindler, F., Werneburg, R., Schneider, J. W., Luthardt, L., Annacker, V., Rößler, R. (2018).
769 First arboreal ‘pelycosaurs’ (Synapsida: Varanopidae) from the early Permian
770 Chemnitz fossil lagerstätte, SE Germany, with a review of varanopid phylogeny.
771 *Paläontologische Zeitschrift*, 92, 315-364
- 772 Stanley, S.M., 1973. An explanation for Cope's rule. *Evolution*, 27, 1-26.
- 773 Sues, H.D. and Reisz, R.R., 1998. Origins and early evolution of herbivory in tetrapods.
774 *Trends in Ecology & Evolution*, 13, 141-145.
- 775 Watanabe, A., Fabre, A.C., Felice, R.N., Maisano, J.A., Müller, J., Herrel, A. and Goswami,
776 A. 2019. Ecomorphological diversification in squamates from conserved pattern of
777 cranial integration. *Proceedings of the National Academy of Sciences*, 116, 14688-
778 14697.
- 779 Weaver, L. N., & Grossnickle, D. M. (2020). Functional diversity of small-mammal
780 postcrania is linked to both substrate preference and body size. *Current zoology*, 66,
781 539-553.
- 782 Werneburg, I. (2019). Morphofunctional categories and ontogenetic origin of temporal skull
783 openings in amniotes. *Frontiers in Earth Science*, 7, 13.
- 784 Woodward, G., Ebenman, B., Emmerson, M., Montoya, J. M., Olesen, J. M., Valido, A.,
785 Warren, P. H. (2005). Body size in ecological networks. *Trends in Ecology and*
786 *Evolution*, 20, 402-409
- 787 Young, R.L. and Badyaev, A.V. 2006. Evolutionary persistence of phenotypic integration:
788 influence of developmental and functional relationships on complex trait evolution.
789 *Evolution*, 60, 1291-1299.

790

791

792 **Figure Captions**

793 Figure 1: The maximum clade credibility tree produced from the Fossilised Birth Death
794 analysis, with branches colour coded according to log transformed evolutionary rates. Nodes
795 discussed in the text labelled: Eup. – Eupelycosauria; Var. – Varanopidae; Par. – Parareptilia;
796 Sph. – Sphenacodontia; Cas. – Caseidae; Ther. – Therapsida; Neo. – Neodiapsida

797

798 Figure 2: Rates of evolution through time. Narrow lines represent rates of individual
799 branches. Mid Weight line represents the median rate of each 1 million year time slice. Thick
800 line represents loess fitted regression between median rate and time. A) All amniotes; B)
801 reptiles and synapsids compared

802

803 Figure 3: Patterns of constraint in amniotes. A) Significant variation in constraint plotted over
804 a phylogeny where branch lengths represent the amount of character change along the branch.
805 Nodes in red experience a significant relaxation of constraint. Nodes in blue experience a
806 significant strengthening of constraints. B) Comparison of Patristic distances and
807 morphological dissimilarity in Synapsids (red) and Reptiles (blue). Each point represents a
808 pairwise comparison of two taxa. The curves represent Loess fitted regression curves.

809

810 Figure 4: Patterns of constraint in amniotes within different anatomical partitions plotted over
811 phlogenies where branch lengths represent the amount of character change within the
812 character prtition along that branch. Nodes in red experience a significant relaxation of
813 constraint. Nodes in blue experience a significant strengthening of constraints. A) Skull; B)
814 Snout; C) Temporal region; D) Postcranium.

815

816 Figure 5: Rates of evolution in reptiles and synapsids within anatomical partotions through
817 time. Narrow lines represent rates of individual branches. Mid Weight line represents the

818 median rate of each 1 million year time slice. Thick line represents loess fitted regression
819 between median rate and time. A) Skull; B) Postcranium

820

821

822 Figure 6: A) Body size category assigned to each taxon, and likelihood ancestral state
823 reconstruction of body size categories over the time calibrated tree. Colour of tip represents
824 size assigned to that tip. Pie charts at each node represent relative probability of each size
825 category being the ancestral state of that node. B) Patterns of constraint within each size
826 category. Each point represents a pairwise comparison of two taxa within a size category. The
827 curves represent Loess fitted regression curve. C) Patterns of constraint within reptiles and
828 synapsids assigned to the large or very large categories.

829

830 Figure 7: Rates of body size evolution in Synapsids and Reptiles. Numbers alongside arrows
831 represent the rate of transition between the size categories (the instantaneous probability of
832 transition) in the direction indicated by the arrow. Transitions with a rate of 0 are not shown.
833 The colour of the arrow represents the log transformed rates. Silhouettes open source from
834 phylopic.org (not to scale)

835