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**Adult frogs and tadpoles have different macroevolutionary patterns across the Australian continent**

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2 **the Australian continent**

3

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20

## Abstract

21        Developmental changes through an animal's life are generally understood to contribute  
22 to the resulting adult morphology. A possible exception are species with complex life cycles,  
23 where individuals pass through distinct ecological and morphological life stages during their  
24 ontogeny, ending with metamorphosis to the adult form. Antagonistic selection is expected to  
25 drive low genetic correlations between life stages, theoretically permitting stages to evolve  
26 independently. Using the Australian frog radiation, we examine the evolutionary  
27 consequences on morphological evolution when life stages are under different selective  
28 pressures. We use morphometrics to characterise body shape of tadpoles and adults across  
29 166 species of frog and investigate similarities in the two resulting morphological spaces  
30 (morphospaces) to test for concerted evolution across metamorphosis in trait variation during  
31 speciation. A clear pattern emerges: Australian frogs and their tadpoles are evolving  
32 independently; their drastically different morphospaces and contrasting estimated  
33 evolutionary histories of body shape diversification indicate that different processes are  
34 driving morphological diversification at each stage. Tadpole morphospace is characterised by  
35 rampant homoplasy, convergent evolution and high lineage density; the adult morphospace  
36 by contrast shows greater phylogenetic signal, low lineage density and divergent evolution  
37 between the main clades. Our results provide insight into the macroevolutionary  
38 consequences of a biphasic life cycle.

39        Key words: complex life cycles; larval frogs; Anura; macroevolution; geometric  
40 morphometrics

41

43           The importance of development in shaping the morphological diversity observed  
44 among adult forms has long been recognised<sup>1-3</sup>. Perhaps the most pervasive idea in  
45 evolutionary developmental biology is that phenotypic variation among species arises  
46 through relative timing of developmental events along a continuous ontogenetic sequence,  
47 i.e., from embryo to juvenile to adult<sup>4-6</sup>. Indeed, subtle alterations to species-specific  
48 ontogenetic trajectories among closely related species can result in divergent or convergent  
49 phenotypes at the adult stage e.g.,<sup>7-9</sup>. However, the majority of animal phyla do not develop  
50 along a continuous ontogenetic sequence, but instead have distinct body plans at different  
51 stages of their life history<sup>10</sup>. This is a phenomenon known as a *complex life cycle* (CLC)  
52 where individuals pass through two or more distinct ecological and morphological phases  
53 during their ontogeny, ending with metamorphosis into the adult form. Distinct life-history  
54 stages are a solution to the problem of different, conflicting selection pressures arising during  
55 an animal's lifetime. These distinct stages, which are derived from the same genome, can be  
56 so different that larval stages of some taxa have been misclassified as entirely different  
57 organisms than the adult forms (e.g., the leptocephalus larvae of eels, or nauplius larvae of  
58 barnacles). Since the adult phenotype is not simply a change in size or proportion from  
59 previous life stages (as in simple life cycle taxa), but instead manifests through a complex  
60 metamorphosis, it is less apparent whether earlier life stages contribute to or constrain  
61 patterns of adult phenotypic diversity in taxa with CLCs.

62           Antagonistic selection at each life-history stage is hypothesised to maintain low genetic  
63 correlations across the metamorphic boundary, i.e., between larval and adult traits, known as  
64 the 'adaptive decoupling hypothesis'<sup>11,12</sup>. Within species, the adaptive decoupling hypothesis  
65 has been examined using a variety of CLC taxa, e.g.,<sup>13-21</sup>, with mixed support. Two  
66 approaches are generally taken to address this hypothesis: either examine genetic and/or  
67 phenotypic correlations between equivalent traits in pre- and post-metamorphic stages<sup>13,15-  
68 18,20</sup>, e.g., locomotive behaviour in larval and adult stages; or investigate larval traits that  
69 correlate with different, functionally-relevant post-metamorphic traits<sup>14,21</sup>, e.g., the  
70 relationship between larval life history and adult morphology. These studies suggest that it is  
71 not an all-or-nothing process but is trait dependent. The evolutionary consequence of  
72 adaptive decoupling is that the different life-history stages are theoretically free to evolve  
73 independently of each other, and would result in markedly different macroevolutionary  
74 patterns at each stage. Such discordance of interspecific phenotypic evolution in CLCs has

75 been referred to as a form of ‘mosaic evolution’, the decoupling of traits during evolution  
76 <sup>22,23</sup>. Yet, at the macroevolutionary level, relatively little is known about whether patterns of  
77 phenotypic evolution are congruent between life history stages. So far studies in biphasic taxa  
78 have not supported concerted morphological evolution across metamorphosis <sup>23-25</sup>. The  
79 degree of independent evolution between life-history stages of species with CLCs is  
80 important for understanding macroevolutionary diversity because it allows us to  
81 quantitatively assess the role that development, specifically preceding developmental stages,  
82 has in shaping adult diversity.

83 Anurans, frogs and toads, are a model system for studying the phenomena of CLCs.  
84 They are a species-rich group with remarkable levels of morphological, ecological and  
85 reproductive diversity yet most species have a biphasic lifecycle with ecologically and  
86 morphologically distinct life stages: the larval tadpole form is always tied to an aquatic or  
87 very moist environment, e.g., streams, permanent ponds, ephemeral water bodies, inundated  
88 burrows, even the vocal sacs and stomachs of adults in some species; adult frogs, conversely,  
89 occupy a wide range of terrestrial and freshwater environments, e.g., deserts and semi-arid  
90 areas, alpine regions, rainforest, and even permafrost in Alaska . Metamorphosis between  
91 these stages is an extraordinary transformation from an organism with a composite head and  
92 body, and a muscular tail (usually without vertebrae), to an adult with a distinct head and  
93 body, no tail but long limbs and fused vertebrae (urostyle).

94 In anurans, the degree of decoupling between larval and adult traits across  
95 metamorphosis is trait dependent, and thus support for the adaptive decoupling hypothesis  
96 varies. For example, behavioural traits (e.g., activity and exploration) are consistent and  
97 moderately correlated across metamorphosis <sup>19</sup>. Conversely, there are low genetic and  
98 phenotypic correlations in morphometric traits between recently metamorphosed juveniles  
99 and adult wood frogs <sup>17</sup>, and in locomotor performance and morphometric traits between  
100 larval and post-metamorphic frogs <sup>15,18</sup>. Finally, larval life-history traits show varying degrees  
101 of correlation with adult morphology <sup>14</sup>. The decoupling of larval and adult stages in anurans  
102 in relation to evolutionary diversification above the species level has only recently been  
103 investigated in Madagascan mantellid frogs <sup>26</sup>. Studies examining interspecific phenotypic  
104 evolution at a single life stage show that morphological diversification in tadpoles is  
105 predominantly related to locomotive specialisations <sup>27-29</sup>. There is also rampant homoplasy<sup>30</sup>,  
106 which is why tadpoles are notoriously difficult to identify to species. Morphological  
107 diversification of adult frogs often shows convergent evolution in many environments, such

108 as burrowing, arboreal or terrestrial niches e.g.,<sup>31,32-34</sup>, although adult morphology appears to  
109 have more phylogenetic signal than larval<sup>35</sup>. Thus, the trait of whole body form at *each* stage  
110 is known to reflect convergent ecomorphological adaptations to different locomotive  
111 strategies and the niches they inhabit, but are the patterns similar between the stages?

112 Here we investigate whether there has been a correlated response, across  
113 metamorphosis, to stage-specific processes whereby larval and adult evolution has proceeded  
114 in tandem along branches of the phylogenetic tree, or if the tadpole and adult frog life stages  
115 are evolving independently. On the one hand, stage-specific processes (such as ecological  
116 specialisation acting on the tadpole stage, or speciation acting on the adult reproductive  
117 stage) may result in correlated changes in some traits during the biphasic lifecycle, because  
118 the survival of one stage directly influences the next. However, the adaptive decoupling  
119 hypothesis suggests that low genetic correlations maintained between life stages by  
120 antagonistic selection will result in the two life stages evolving somewhat independently. We  
121 use frogs to examine the relationship between the morphospaces and inferred histories of  
122 morphological diversification of the larval and adult body plans. We use a comprehensive  
123 morphological dataset of Australian anurans, built from traditional and geometric  
124 morphometric data representing adult and tadpole body shape (Figure 1), to test the null  
125 hypothesis that there is no concordance of interspecific body shape evolution. Furthermore,  
126 we place the observed phenotypic variation in an evolutionary context using phylogenetic  
127 comparative methods to infer the history of morphological diversification at each stage  
128 (evolutionary morphospaces) to characterise the mode of evolution and whether it differs  
129 between the life stages.

130

## Results

131

### *Evolution of larval anuran morphospace*

132 Among species shape variation is often the result of an evolutionarily association with  
133 size variation, known as evolutionary allometry. In tadpole body shape, evolutionary  
134 allometry is weak and not significant (phylogenetic generalised least squares regression  
135 [PGLS],  $R^2 = 0.09$ ,  $P = 0.076$ ). The tadpole morphospace of all 166 species is summarised by  
136 principal components (PC) analysis, and four PCs describe a 80.1% of the total shape  
137 variation among species (depicted in Supplementary Figure 1); the remaining PCs each  
138 describe less than 5% of the shape variation. The main axes explain changes in shape  
139 regarding the whole tadpole (head/body and tail). The first axis describes variation in the

140 length of the tail relative to the head/body region, ranging from a tail that is equal length to  
141 the head/body, to a tail that is elongate and twice long as the head/body. The second axis  
142 describes shape variation arising from dorsoventral compression of the head/body and tail;  
143 tadpoles with high-arching tail fins and a deep head/body are contrasted with shallow tail fins  
144 and a flattened head/body. The phylomorphospace (PC morphospace into which a  
145 phylogenetic tree is projected) defined by the first two axes (61.1%) is shown in Figure 2.  
146 There is substantial homoplasy in tadpole shape, as demonstrated by the ‘bird’s nest’  
147 configuration; there are many crisscrossing branches, and closely related species are often  
148 widely divergent in morphospace. Consequently, phylogenetic signal is very low in tadpole  
149 body shape ( $K = 0.2215$ ,  $P = 0.001$ ). Lineage density of the tadpole morphospace is high  
150 (0.92), indicating there are many long branches packed within the morphospace enclosed by  
151 the observed species, which suggests low morphological innovation.

### 152 *Evolution of adult anuran morphospace*

153 There is significant evolutionary allometry in the adult shape variables with body size  
154 (snout-to-vent length) (PGLS  $R^2 = 0.27$ ,  $P = 0.001$ ). The adult frog morphospace of all 166  
155 species can be summarised by four PCs describing a total of 82.5% of the total variation  
156 (depicted in Supplementary Figure 2); the remaining PCs each describe less than 5% of the  
157 shape variation. Overall, the main axes explain changes to measurements of the head and  
158 limbs of the adult frogs. The first axis corresponds to the length of the digits (5<sup>th</sup> toe, 1<sup>st</sup> toe,  
159 4<sup>th</sup> finger and thumb) shortening with an increase in eye size (eye length), such that longer  
160 digits coincide with relatively smaller eyes. The second axis corresponds to the face length  
161 (naris-snout length) changing negatively with thumb length, such that longer snouts coincide  
162 with short thumbs. The phylomorphospace defined by the first two axes (67.2 %) is shown in  
163 Figure 2. The adult morphospace is distinctly divided by clades: hylids and myobatrachids  
164 are divided strongly along PC1, with partial overlap of the myobatrachid subfamilies  
165 Limnodynastinae and Myobatrachinae. Phylogenetic signal is moderate ( $K = 0.455$ ,  $P =$   
166  $0.001$ ), but much higher than in tadpole body shape. Lineage density of the adult  
167 morphospace is very low (0.18) indicating branches in the morphospace are relatively short  
168 and not densely packed, suggesting higher morphological innovation than in tadpoles.

### 169 *Dissimilarity in larval and adult anuran evolution*

170 The morphospaces of tadpoles and adults are strikingly different, particularly in that the  
171 clades are well separated in adults and highly overlapping in tadpoles (Figure 2). There is  
172 little correspondence and low statistical correlation in the patterns of morphological disparity

173 described by the two datasets; permuted partial disparity of adults and tadpole datasets have a  
174 low correlation (All data: Spearman's rank correlation,  $\rho = 0.026$ ,  $P = 0.4053$ ; only PCs1-4,  $\rho$   
175  $= 0.053$ ,  $P = 0.0961$ ; Supplementary Figure 3). In terms of dispersion of species in  
176 morphospace, the correlation between the tadpole and adult datasets is also low ( $\rho = 0.169$ ,  $P$   
177  $= 0.001$ ; only PCs1-4,  $\rho = 0.176$ ,  $P = 0.001$ ). These results indicate negligible  
178 correspondence between larval and adult evolutionary histories and resultant morphospaces.

179

## Discussion

180 Ontogeny undoubtedly plays an important role in creating phenotypic diversity, yet  
181 research in this area commonly focusses upon organisms with continuous, simple life-cycles,  
182 where the adult stages manifests as a change in size and allometric proportions from previous  
183 life stages. Comparatively less is understood about how much, if at all, early life history  
184 stages in animals with complex life cycles influence the diversity we observe in the adult  
185 phenotype. Using a model vertebrate system with a biphasic lifecycle, and taking a  
186 macroevolutionary perspective, we investigated whether the outcomes of stage-specific  
187 processes determining body shape diversity in the larval stage or the reproductive stage (i.e.,  
188 adult) are carried over to the other stage, or if these stages are evolving independently. Our  
189 results indicate that there is extreme discordance between the body plan morphospaces of  
190 larval and adult Australian anurans, where morphological variation (disparity) among species  
191 in one stage is not indicative of disparity at the other stage. The inferred evolutionary history  
192 of morphological diversification of each life-history stage is also very different: the tadpole  
193 morphospace shows substantial homoplasy, and a clear pattern of convergent evolution,  
194 whereas the adult morphospace has more phylogenetic signal and shows divergent evolution  
195 among the families and subfamilies. These findings suggest the larval and adult life-stages  
196 are evolving, for the most part, independently.

197 Differences in the evolutionary history of larval and adult traits often have been  
198 investigated with respect to their use for cladistic analyses, and consequently larval forms of  
199 many animals are found to be homoplastic compared to adults e.g.,<sup>23,25</sup>. Larval anurans are  
200 no exception to this pattern; anyone who has attempted to use taxonomic keys to identify  
201 tadpoles to species can attest. Our results from Australian anurans clearly show low  
202 phylogenetic signal and substantial homoplasy in tadpole body shape, which is consistent  
203 with other studies with broader taxonomic sampling<sup>30,35</sup>. A wide-scale study into the  
204 phylogenetic signal of microhabitat use in tadpoles and adult frogs found similar patterns to



205 ours: there is low phylogenetic signal and extreme plasticity in tadpoles, and more structure  
206 in adults<sup>36</sup>. Therefore, the substantial homoplasy in tadpoles may be due to this apparent ease  
207 in which microhabitat-shifts of tadpoles occur during speciation, since tadpole body shape is  
208 strongly linked to ecology<sup>37</sup>.

209 The efficiency of morphological innovation (inferred from lineage density in  
210 morphospace) was low for tadpoles, and higher for adult frogs. Although we cannot test it  
211 directly, this result may indicate greater disparity in adult morphology compared to tadpoles.  
212 If adult morphology has greater disparity, this may simply be because a body plan with four  
213 limbs with digits has more scope for shape variation than a ‘finless, fish-like’ body plan of a  
214 tadpole. Yet when the fish body plan is preserved through metamorphosis, Katz and Hale<sup>24</sup>  
215 found greater interspecific variation in body elongation in adults compared to larvae of bony  
216 fishes, which they attributed to different locomotor demands and physiological constraints  
217 (e.g., oxygen uptake through skin). Conversely, some studies of species with simple life  
218 cycles have found a greater disparity among similarly-aged juveniles relative to adult  
219 phenotypes, because of convergent evolution (e.g.,<sup>9,38</sup>). In newts, which also have a biphasic  
220 lifecycle, low disparity in body shape was found in early larval stages as well as in adults, but  
221 greater disparity in stages nearer to metamorphosis<sup>39</sup>. Future research into the phenomenon  
222 of differing temporal disparity across ontogeny should investigate the processes behind  
223 greater diversity at one stage or another, that is whether it is due to biological constraints or  
224 natural selection.

225 Our study focussed on whole body shape in tadpoles and adults because it is a complex  
226 trait that strongly relates to functional performance and niche specialisation<sup>27-29,31-34</sup>. In this  
227 way, comparing body shape in larval and adult stages is analogous to comparing tadpole  
228 swimming and adult frog jumping performance e.g.,<sup>15,18</sup>, which are traits that also have been  
229 shown to have little correlation across the metamorphosis boundary. Animal morphology is  
230 only one aspect of the animal, however, and previous studies testing adaptive decoupling in  
231 animals with CLCs have examined a great variety of traits, i.e., behavioural, morphological,  
232 performance, life-history and even colouration, with varying support for the adaptive  
233 decoupling hypothesis<sup>13,15-21</sup>. Traits that are genetically determined and directly passed over  
234 through metamorphosis represent the ideal test for decoupling and consequently independent  
235 evolution. However, the nature of metamorphosis and its complete overhaul of the body plan  
236 makes this a significant challenge. The suit of developmentally homologous traits at each  
237 stage is limited, and this is perhaps why the adaptive decoupling is often inferred from

238 correlated responses in different but functionally-related traits e.g.,<sup>14,21</sup>, or investigated in  
239 non-morphological traits e.g.,<sup>15,16,18</sup>. If morphology is the focus, a character-based approach  
240 to describing trait variation (as in<sup>23</sup>, see below) permits a greater range of traits to be  
241 captured, particularly presence and absence of a trait, in a way that morphometric methods  
242 usually cannot (although character and morphometric datasets do often provide congruent  
243 results<sup>40-42</sup>). Regardless of the trait, this study adds to a growing body of evidence that larval  
244 and adult life-stages of CLC species are distinct and have the propensity to evolve along their  
245 own macroevolutionary trajectories.

246 Early literature discussing the discordance of interspecific phenotypic evolution in  
247 CLCs co-opted the term ‘mosaic evolution’<sup>22,23</sup>, which generally refers to the decoupling of  
248 traits during development and evolution<sup>2</sup> (also known as modularity<sup>43</sup>), but in this case  
249 refers to larval and adult morphology evolving independently within a lifecycle. Few other  
250 studies have considered the evolutionary consequences of maintaining a CLC, and the  
251 potential adaptive-decoupling of life stages, by taking a macroevolutionary approach to  
252 explicitly test whether patterns of interspecific phenotypic evolution are congruent between  
253 life history stages. Smith and Littlewood<sup>23</sup> embarked upon a broad cladistic analysis of  
254 patterns of morphological evolution in echinoids (reanalysing data from<sup>22</sup>) and found there to  
255 be little evidence for concerted morphological evolution between larval and adult life-history  
256 stages. Recently, Wollenberg Valero *et al.*<sup>26</sup> used a transcriptomic approach in the  
257 Madagascan mantellid frog radiation and demonstrated that genes associated with  
258 morphological structure formation are expressed in a life stage-specific pattern, thus arriving  
259 at the same conclusion of the decoupling of phenotypic evolution across metamorphosis.  
260 Although not organisms with a CLC, Gerber *et al.*<sup>38</sup> examined shell shape morphospace  
261 occupation of juveniles and adults, and found substantially higher morphological disparity in  
262 adults, but they did not explicitly examine similarity of dispersion patterns or inferred  
263 evolutionary diversification as done here. CLC is the most common life history strategy in  
264 the animal kingdom, yet only few studies have considered evolution in these taxa at multiple  
265 life stages. The association of larval and adult morphospaces in CLC taxa, which Eble<sup>44</sup>  
266 referred to as developmental and non-developmental morphospaces, is important for  
267 understanding the genotype-phenotype map and address the fundamental question of how  
268 ontogeny relates to phylogeny. Further investigations using the plethora of CLC taxa,  
269 invertebrate and vertebrate, are encouraged.

270 Research on the evolution of taxa with CLCs has predominantly focused on why  
271 distinct life stages are maintained<sup>11,12</sup>, and the evolutionary consequences of losing the free-  
272 living larvae and instead directly developing into the adult form<sup>45-47</sup>. In amphibians,  
273 evolutionary shifts from a biphasic lifestyle to direct development are numerous<sup>48</sup>. The loss  
274 of a free-living larval stage in plethodontid salamanders has been implicated in the high  
275 morphological and ecological diversity, and species richness, of this clade, which forms the  
276 basis for a hypothesis that a biphasic lifestyle could represent an ontogenetic constraint on the  
277 adult phenotype<sup>49,50</sup>. In marine invertebrates, where developmental strategy is extremely  
278 evolutionarily labile, the degree to which a CLC affects adult phenotype, compared to direct  
279 developing species, appears to vary from clade to clade<sup>22,47</sup>. Whether the presence of a free-  
280 living larval stage provides an ontogenetic constraint on adult phenotypic diversity has yet to  
281 be explicitly examined in most CLC taxa. We propose that Australia's frogs are a potentially  
282 good system to examine this hypothesis, given that 12% of the continent's native anuran taxa  
283 are direct developers. The results of this study suggest that the ontogenetic constraint  
284 hypothesis may not apply to Australian frogs, or to Anura generally. Future research is  
285 encouraged along this avenue.

286

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292

### **Author Contributions**

293 E.S. and J.S.K. conceived the study. E.S., M.A. and M.V.G. collected the data. E.S.  
294 performed the analyses. E.S., J.S.K. and M.V.G. wrote the paper. All authors read and  
295 approved the final manuscript.

296

### **Competing financial interests**

297 The authors declare no competing financial interests.

298

## Materials & Correspondence

299

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301

## Materials and Methods

302

### *Samples and morphometric body shape data*

303

Australia's indigenous anuran biodiversity comprises three Neobatrachian families. We  
304 sampled only species that have a free-living larval stage from Hylidae (68 spp.) and  
305 Myobatrachidae (99 spp.), totalling 166 species (70% of the total amphibian diversity in  
306 Australia; Supplementary Table 1). We excluded all microhylid species and *Arenophryne*,  
307 *Metacrinia* and *Myobatrachus* (Myobatrachidae) because these species do not have a free-  
308 living larval stage (they are direct developers). We also excluded the single Ranidae species  
309 *Papurana daemeli* (formerly *Hylarana*) because it is a comparatively more recent migrant  
310 into Australia from New Guinea<sup>51</sup>. All the following procedures and analyses were  
311 performed in the R statistical environment v.3.3.1<sup>52</sup> unless otherwise stated.

312

Tadpoles were sampled from the comprehensive work of Anstis<sup>53</sup> and comprised  
313 animals that at the mean Gosner stage 35.4 ( $\pm 3.09$ ; hindlimb bud started to grow, toes  
314 gradually develop on hindlimbs). For each species she produced a detailed and accurate  
315 drawing of a tadpole in lateral view from multiple specimens where the position was  
316 standardised. The difference in morphology between photographs and the high-quality  
317 drawings is negligible relative to among species variation (Supplementary Figure 4). To  
318 circumvent phenotypic plasticity to environmental conditions, Anstis' tadpole drawings were,  
319 where possible, based on specimens reared in captivity under consistent conditions and  
320 therefore represent the phenotype without ecological influences. We used 2-dimensional (2D)  
321 landmarks and semilandmarks to characterise tadpole body shape (Figure 1A, Supplementary  
322 Table 2). Landmarks were digitised using tpsDig2 v.2.26<sup>54</sup> on published lateral view  
323 photographs and drawings of tadpoles<sup>53</sup>, and semilandmarks were automatically digitised by  
324 routines written in R and ImageJ<sup>55</sup>. Details of the digitizing process are given in the  
325 Supplementary Materials. The landmark and semilandmark coordinates were aligned using a  
326 generalized Procrustes superimposition<sup>56</sup> implemented in the R package *geomorph* v.3.0.2<sup>57</sup>,  
327 where the semilandmarks were permitted to slide along their tangent directions to minimise  
328 bending energy<sup>58</sup>. The resulting Procrustes shape coordinates were used as tadpole body  
329 shape variables in the subsequent analyses.

330 To characterise adult frog body shape we used published species-average morphometric  
331 data (5 female specimens per species) comprising 23 external linear measurements collected  
332 using digital calipers on alcohol-preserved museum specimens<sup>33</sup> (Figure 1B, Supplementary  
333 Table 3). The 22 variables (excluding snout-to-vent length, SVL) were transformed using the  
334 log-shape ratios method<sup>59</sup>, where the species data are divided by species size (as geometric  
335 mean) and ln-transformed, producing shape variables to be used in the subsequent analyses.  
336 This approach is analogous to the size correction to the Procrustes superimposition method  
337 for landmark data, and both approaches correct for size while retaining the allometric shape  
338 variation<sup>60</sup>. It is important to note that the juvenile and adult body shape variables are on a  
339 different scale.

#### 340 *Statistical analyses*

341 In order to do phylogenetically informed statistical analyses we generated a Bayesian  
342 molecular phylogeny, with branch lengths, for all 166 species of Australian hylids and  
343 myobatrachids used in this study. The phylogeny was consistent with previously published  
344 molecular phylogenies that have been used for macroevolutionary analyses in these groups  
345<sup>51,61</sup> (phylogeny available on Dryad, doi:10.5061/dryad.23j6t).

346 We investigated allometry in both the tadpole and adult datasets using multivariate  
347 regressions based on a phylogenetic generalised least squares (PGLS) model for multivariate  
348 data<sup>62</sup>, implemented in *geomorph*. Size in the tadpole dataset was centroid size (calculated  
349 from the landmark configurations), and SVL in the adult dataset. Statistical significance was  
350 assessed by permutation (1,000 iterations).

351 To visualise the tadpole and adult body shape morphospaces, we performed principal  
352 components analysis (PCA) of the Procrustes shape coordinates (herein tadpole shape  
353 variables) and the regression residuals of log-shape ratios (herein adult shape variables). The  
354 shape variation described by each PC axis of the tadpole morphospace was visualised using  
355 thin-plate spline warp grids of the PC loadings, implemented in *geomorph*, which plots the  
356 eigenvectors of each landmark coordinate simultaneously to represent the overall shape  
357 change from the mean shape of the sample to the minima, and maxima, of the axes. For the  
358 adult morphospace, the importance of the variable loadings on the first four axes were  
359 interpreted from biplots. We then used a complementary suite of analyses to compare these  
360 two morphospaces.

361 To estimate the overall strength of correlation between the two morphospaces and  
362 estimate the similarity of species dispersion, we performed a Mantel test between adult and  
363 tadpole matrices of the pairwise Euclidean dissimilarities (distances) between species  
364 implemented in *vegan* v.2.3-5<sup>63</sup>. Scale differences between the datasets are irrelevant for this  
365 approach. The null hypothesis of a Mantel test is the absence of relationship between the  
366 dissimilarities in the two matrices, and statistical significance was assessed using a  
367 permutation approach (1,000 iterations) on the Spearman's rank correlation coefficient.

368 To examine whether the two datasets describe similar morphological disparity, we  
369 applied a disparity permutation procedure designed to compare different datasets of the same  
370 taxa sensu<sup>40</sup>. Disparity was measured as variance, calculated as the trace of the covariance  
371 matrix divided by the number of observations<sup>8</sup> implemented in *geomorph*. We randomly  
372 subsampled sets of 84 species (half the total sample) and calculated disparity for each dataset,  
373 repeating 1000 times. Correlation between the subsampled disparities of the two datasets was  
374 calculated using Spearman's rank correlation coefficient. Here and above, the Spearman  
375 correlation is most appropriate here because it evaluates the monotonic relationship between  
376 the two matrices by using the ranked values for each, and as such does not assume a linear  
377 relationship. To account for the difference in the number of variables (110 in tadpole shape  
378 dataset, 22 in adult) we repeated this disparity permutation procedure using only the first four  
379 PC axes of each dataset.

380 To estimate the evolutionary history of body shape diversification in these  
381 morphospaces, we used a phylomorphospace approach<sup>64</sup>. The phylogeny was projected into  
382 the morphospaces by calculating ancestral states of the internal nodes through maximum  
383 likelihood, implemented in *geomorph*. This approach allows inference of the magnitude and  
384 direction of shape change along any branch. From the phylomorphospaces, we investigated  
385 the 'efficiency in morphological innovation' during evolution, which is the degree that taxa  
386 explored more novel regions of morphospace and expanded out from their inferred ancestral  
387 positions, or have remained relatively clumped within morphospace. This is done by  
388 calculating the lineage density (LD)<sup>64</sup>, which describes the degree of packing by the  
389 branches of the phylogeny in the morphospace, and is calculated as the sum of morphometric  
390 branch lengths divided by the volume occupied in the morphospace. LD is a ratio and  
391 therefore can be compared between datasets irrespective of scale. Higher LD values indicate  
392 that the morphospace is filled with long branches that are more densely folded to pack into  
393 the total occupied space, suggesting low morphological innovation, while lower LD values

394 indicate shorter branches, less packing, and suggests higher morphological innovation. The  
395 volume was calculated as minimum bounding hyper-ellipsoid of the space defined by the first  
396 four PC axes of each morphospace, implemented in *cluster* v.2.0.4<sup>65</sup>. The absolute  
397 magnitude of change per branch (to estimate whether there is more morphological variation  
398 at one life stage versus another) was not possible to estimate because of the difference in  
399 scale of the two datasets.

400 Finally, we measured phylogenetic signal, the degree to which related species resemble  
401 each other phenotypically, using the multivariate extension of the generalized  $K$  statistic<sup>66</sup>  
402 implemented in *geomorph*. A  $K$  value greater than 1 implies that closely related species are  
403 more similar than expected under a null model of Brownian Motion (more phylogenetic  
404 signal), while less than 1 suggests relatives resemble each other less than expected (less  
405 phylogenetic signal). Rather than absolute values of  $K$  on this scale, we focus on how  $K$   
406 differs between tadpoles and adults. Statistical significance was assessed by permutation  
407 (1,000 iterations).

#### 408 ***Data availability***

409 The digitizing protocol (including R computer code) is provided in Supplementary  
410 Methods, and R computer code for analyses, all data and phylogenetic tree are available on  
411 Dryad (doi:10.5061/dryad.23j6t).

### 412 **Figure Captions**

413 **Figure 1** Morphometric variables characterising body shape in tadpoles (A) and adult  
414 frogs (B). A: Landmarks and semilandmarks for characterizing the body shape of tadpoles  
415 (see Supplementary Table 2 for descriptions). B: Linear measurements for characterizing the  
416 body shape of adult frogs (see Supplementary Table 3 for descriptions). Note: numbers in A  
417 and B are not analogous.

418 **Figure 2** The evolutionary morphospaces of tadpoles (top) and adult (bottom) frogs,  
419 into which a phylogenetic tree has been projected to visualise the relationships among species  
420 (points). Drawings of 16 representative species as both stages demonstrate the difference in  
421 body shape diversity; scale bars are 10mm. Tadpole drawings modified from Anstis<sup>53</sup>, and  
422 adult drawings by E. Walsh. Species of the family Hylidae are denoted by squares (shades  
423 correspond to the two genera), and Myobatrachidae species are denoted by circles (shades  
424 correspond to the two subfamilies, and *Rheobatrachus* with *Mixophyes*).

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