

Populating a Continent:  
Phylogenomics Reveal the Timing of Australian Frog Diversification

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1    *Abstract*

2       The Australian continent's size and isolation make it an ideal place for studying  
3       the accumulation and evolution of biodiversity. Long separated from the ancient  
4       supercontinent Gondwana, most of Australia's plants and animals are unique and  
5       endemic, including the continent's frogs. Australian frogs comprise a remarkable  
6       ecological and morphological diversity categorized into a small number of distantly  
7       related radiations. We present a phylogenomic hypothesis based on an exon-capture  
8       dataset that spans the main clades of Australian myobatrachoid, pelodryadid hyloid,  
9       and microhylid frogs. Our time-calibrated phylogenomic-scale phylogeny identifies great  
10      disparity in the relative ages of these groups which vary from Gondwanan relics to  
11      recent immigrants from Asia and include arguably the continent's oldest living  
12      vertebrate radiation. This age stratification provides insight into the colonization of, and  
13      diversification on, the Australian continent through deep time, during periods of  
14      dramatic climatic and community changes. Contemporary Australian frog diversity  
15      highlights the adaptive capacity of anurans, particularly in response to heat and aridity,  
16      and explains why they are one of the continent's most visible faunas.  
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*Keywords:* Anuran; adaptive radiation; Gondwana; phylogenetics

## AUSTRALIAN FROG PHYLOGENOMICS

### 21     Introduction

22       Frogs are an ancient vertebrate radiation originating in the Permian more than  
23      250 million years ago (Hime et al. 2021). They share a unique and unusual morphology  
24      yet are a spectacularly successful group, with more than 7,500 extant species spread  
25      across most of the world (AmphibiaWeb 2022). Despite their age, much of this diversity,  
26      potentially more than 95%, has developed since the Cretaceous-Paleogene mass  
27      extinction (65 mya) (Feng et al. 2017). Australia is one of the driest continents on Earth  
28      yet, surprisingly, it is home to nearly 250 frog species. Australia's frogs belong to just  
29      four anuran groups spread widely across the "modern frog" suborder Neobatrachia: (1)  
30      Myobatrachoidea comprising the Limnodynastidae (66 species) and Myobatrachidae (70  
31      spp.); (2) Hyloidea represented by the family Pelodryadidae (91 spp.); (3) the  
32      Microhylidae subfamily Asterophryinae (24 spp.); and (4) a single Ranidae species in the  
33      genus *Papurana*. These groups show very different levels of species richness and  
34      geographic spread across the continent (Fig.1). However, together they have radiated to  
35      inhabit almost every part of Australia including tropical rainforests, alpine streams,  
36      featureless boulder piles, and hyper-arid deserts.

37       While we know a great deal about many aspects of Australian frog biology (Tyler  
38      1998; Anstis 2017), the age of each of the major groups and the timing of their  
39      subsequent diversification, is poorly understood. Since the origin of frogs over 250  
40      million years ago, the landmass that is now Australia has traveled extensively. Long ago  
41      it was part of the supercontinent Pangea before separating as a component of  
42      Gondwana alongside South America, Africa, Antarctica, and India. Sometime around 50  
43      million years ago Australia separated from Antarctica and began drifting alone towards  
44      Asia (Hall 2002; Bijl et al. 2013). Given the long evolutionary history of frogs, and  
45      Australia's varied geographic affinities with other landmasses, we ask three related  
46      questions: (1) Where did Australia's frogs originate? (2) When did they get to

47 Australia? and (3) Who and where are their closest relatives? Answering these questions  
48 provides context for the varied species richness and ecological diversity of these groups  
49 and offers important insight into the evolution of a continental fauna.

50

51 *Materials and Methods*

52 We assembled an exon-capture dataset comprising 99 frog species spanning all  
53 major anuran clades and with particular focus on the families Pelodryadidae,  
54 Microhylidae, Limnodynastidae and Myobatrachidae (Table S1). This dataset includes  
55 near-complete (92%) genus-level sampling of Australia's frogs. We generated new  
56 Anchored Hybrid Enrichment (AHE—Lemmon et al. 2012) data for 83 samples and  
57 combined these with outgroup samples from Hime et al.'s (2021) amphibian  
58 phylogenomic dataset. Outgroup sampling was designed around maximizing commonly  
59 used anuran fossil calibrations to provide a consistent time-calibrated phylogenomic  
60 estimate of Australian frogs. Data from different AHE projects were combined using  
61 custom scripts which relied on *metablastr* to identify orthologous loci  
62 (*blast\_best\_reciprocal\_hit*) (Benoit & Drost 2021), *mafft* to align them (–add, –  
63 *keeplength*) (Katoh et al. 2013), and *AMAS* to manipulate alignments (Borowiec 2016).  
64 We reconstructed individual genealogies for our exon-capture data (n = 450) under  
65 maximum-likelihood in IQTREE (Nguyen et al. 2015), allowing the program to assign  
66 the best fitting model of nucleotide substitution using ModelFinder (Kalyaanamoorthy  
67 et al. 2017) and then perform 1,000 ultrafast bootstraps (Minh et al. 2013). We then  
68 estimated a species tree using the quartet-based summary method ASTRAL III (Zhang  
69 et al. 2018) with IQTREE gene trees as input. To complement our coalescent-consistent  
70 summary method we also estimated a species tree from the concatenated alignment  
71 using the edge-unlinked partition model GHOST implemented in IQTREE. This allowed  
72 us to more accurately model rate variation among sites and samples. To estimate

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74 divergence times among taxa on the ASTRAL species tree we applied a series of fossil  
75 calibrations first compiled by Feng et al. (2019) (Table S2) and used the Bayesian  
76 divergence time software MCMCTree (Rannala & Young 2007). We started by  
77 concatenating all exonic loci (n=390; Supp. Sequence Identity) and partitioning them  
78 into two partitions, first and second codons together, and third codons separately.  
79 following the strategy of dos Reis et al. (2018). Complex partitioning strategies such as  
80 filtering by evolutionary rate are possible but less influential than the absolute number  
81 of partitions (dos Reis et al. 2012). Additional data partitions ultimately incur  
82 substantial computational costs for modest increases in dating precision, and so we  
83 opted instead for a more conservative approach. We then used *baseml* to estimate  
84 approximate likelihoods (dos Reis & Yang 2011) and branch lengths before running  
85 *mcmctree* on the gradient and Hessian (in.BV file) for ten replicate analyses. We  
86 inspected mcmc files for stationarity and compared for convergence, then combined  
87 them using logCombiner, and used this combined mcmc file to summarize divergence  
88 times on our tree (*print = -1* in .ctl file). Sample, alignment, and gene tree summary  
89 statistics are presented in Supplementary Material (Fig.S1-3) and are available alongside  
90 all other materials on Dryad (doi:10.5061/dryad.zpc866tcj) and GitHub  
91 ([https://github.com/IanGBrennan/Crown\\_Frogs](https://github.com/IanGBrennan/Crown_Frogs)).

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92 To investigate the biogeographic origins of Australian frogs we reconstructed  
93 ancestral ranges using BioGeoBEARS (Matzke 2014). The deep timescale of frog  
94 evolutionary history necessitates accounting for continental rearrangement and dispersal  
95 barriers by incorporating time-stratified information from plate tectonics. To accomplish  
96 this we designed a series of models that augment dispersal probability as a function of  
97 distance among areas and adjacency. Briefly, these models penalize dispersal probability  
98 as distance between areas increases, and as the type of distance changes (e.g. over-land  
99 vs. over-water dispersal). To identify the dispersal path of the pelodryadid tree frogs

102 and how they arrived in Australia from a South American ancestor (Pyron 2014), we  
103 designed two data sets. The first requires the Pelodryadidae to have travelled from  
104 South America through Antarctica and into Australia (*H*1) and the second allows an  
105 overwater dispersal directly from South America to Australia (*H*2). Comparative model  
106 fit was assessed via AIC. Model specifics can be found in the *Supplementary Materials*  
107 and *Methods*.

108

109 *Results*

110 Species tree topologies are nearly identical across the quartet-based coalescent  
111 method (ASTRAL) and concatenation under the GHOST heterotachy model  
112 (IQTREE), and are broadly consistent with previously published phylogenomic frog  
113 hypotheses (Feng et al. 2017; Streicher et al 2018; Streicher et al. 2020; Hime et al.  
114 2021) (Fig.2, S4—S6). We estimate well-supported phylogenies with few unresolved  
115 nodes among Australian taxa. Australian microhylids fall into two non-sister clades,  
116 each nested within the primarily New Guinean Asterophryinae. Pelodryadids have  
117 diverged into two to three deep groups, with *Cyclorana* and *Nyctimystes* embedded  
118 within divergent clades of *Litoria*. Ancient splits among myobatrachoids show some  
119 uncertainty with a paraphyletic estimation of the Myobatrachidae. There is strong  
120 support uniting the genera *Mixophyes* and *Rheobatrachus*, and moderate support (LPP  
121 90) places this myobatrachid clade as sister to the Limnodynastidae, to the exclusion of  
122 remaining myobatrachid genera.

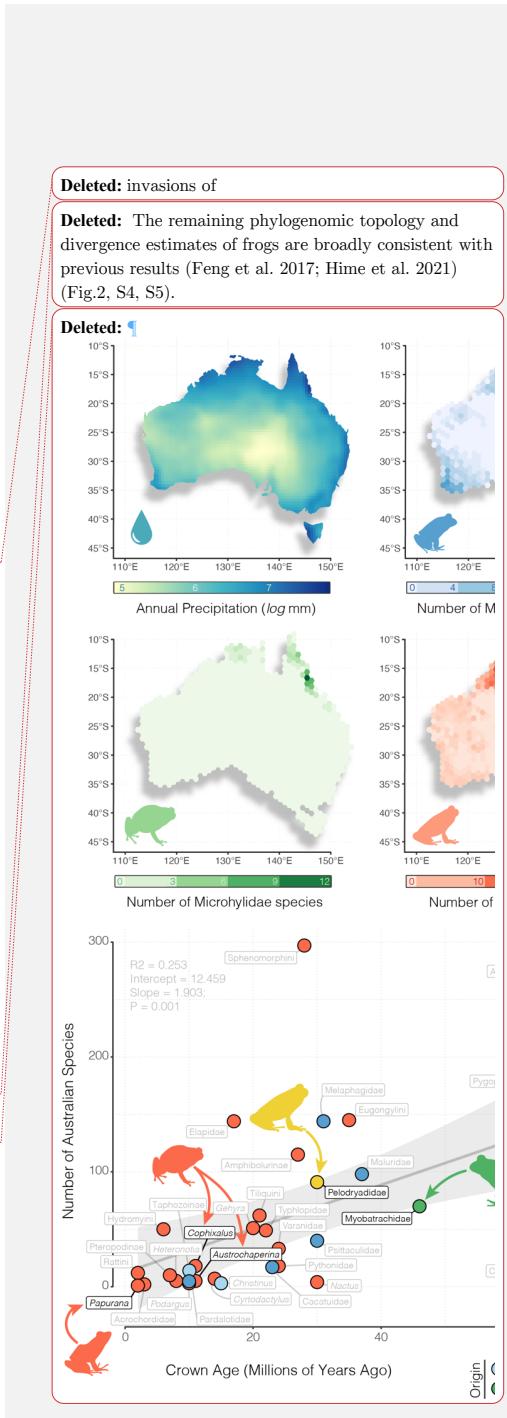
123 Concatenated and coalescent topologies differ at three very short branches which  
124 bear no significant implications for our understanding of the relationships of Australian  
125 frogs (Fig.S5). Successive short branching events such as these are known to mislead  
126 tree inference from concatenated data, and so are not surprising (Linkem et al. 2016).  
127 We find support in the GHOST model for four distinct rate classes, which vary in total

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128 tree length (TTL) by more than 50x, providing evidence of strong heterotachy among  
129 sites. The distribution of TTL among branches across the four trees however, is largely  
130 consistent suggesting little effect of heterotachy among lineages.

Crown divergences of the three Australian frog radiations can be clearly separated into old (Myobatrachidae and Limnodynastidae—80 mya), intermediate (Pelodryadidae—30 mya), and young (Asterophryinae—11 mya) (Fig.2). The youngest Australian group, microhylids in the genera *Austrochaperina* and *Cophixalus*, are embedded deeply within the subfamily Asterophryinae and appear to represent two separate, relatively recent ( $\approx$ 11 mya) dispersals into Australia from New Guinea. Pelodryadidae tree frogs also share a complex biogeographic history across Australasia, with several species groups split across the Torres Strait (separating Australia and New Guinea), suggesting frequent biotic exchange. However, the origins of the Pelodryadidae are far older. Their closest extant relatives are the iconic Phyllomedusidae found throughout Central and South America, with the crown split between extant Pelodryadidae in Australia/New Guinea and South America estimated at approximately 40 million years ago. Australian myobatrachids and limnodynastids also have their closest living relatives in South America—the Calyptocephalidae, represented here by *Calyptocephalella*, the Helmeted Water Toad of Chile. The crown split between extant myobatrachoids in Australia and calyptocephalellids in Chile is ancient, occurring more than 100 million years ago.

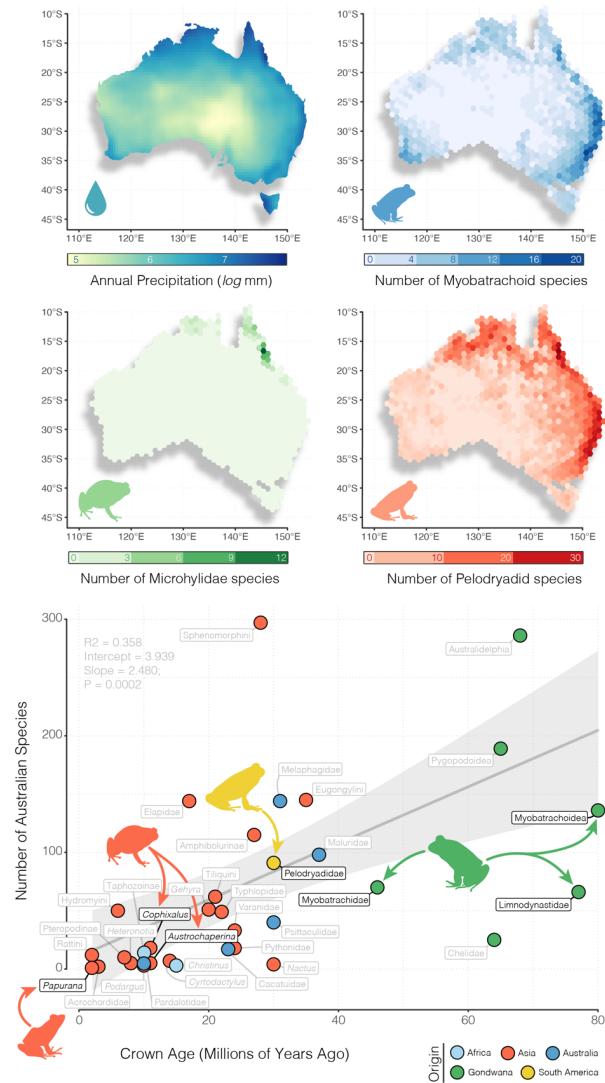
Biogeographic modelling provides support for a diversification scenario in which the dispersal of frogs was influenced by vicariant events (parameter  $j$ ), distance among biogeographic regions ( $x$ ), and dispersal type ( $w$ ; over-land vs. over-water) (Table S3). The top two models account for more than 80% of AIC weight, and both correspond to pelodryadid dispersal Hypothesis 1 in which treefrogs dispersed through Antarctica to reach Australia (DEC+ $j+x+w$  H1, AICw 59.7; DEC+ $j+x$  H1, AICw 21.5). The



161 preferred model represents a meaningful improvement over similar models under a  
162 pelodryadid dispersal Hypothesis 2 (Fig.3, S7; Table S3). Parameter estimates of  $x$   
163 under the top two models suggest that doubling the distance between areas reduces  
164 dispersal probability by one-third to one-half. Parameter estimation of  $w$  under the  
165 preferred model suggests that overland dispersal probability among non-adjacent areas  
166 is one-third that of between adjacent areas, and overwater dispersal probability is just  
167 one-tenth.

168 Ancestral range reconstructions provide evidence that both myobatrachoid and  
169 pelodryadid frogs are descended from South American ancestors. Asterophryinae  
170 microhylids, in which the Australian microhylids are embedded, likely diverged from an  
171 ancestor found in Asia.

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179 and Myobatrachidae presented together as Myobatrachoidea) represent visually how  
 180 contemporary patterns of frog richness reflect water availability, and are highest in the  
 181 wet temperate, subtropical, and tropical rainforests of the east coast. We show annual  
 182 precipitation here for ease of interpretation but Australian frog richness is potentially  
 183 better explained by actual evapotranspiration (Coops et al. 2018). Species occurrence  
 184 records were collated from the Atlas of Living Australia (<https://ala.org.au>). Below,  
 185 Australian radiations can be divided broadly into (1) relictual Gondwanan clades >40  
 186 myo (green), (2) ancient colonizing groups (>20 myo, <40 myo; varied colors), or (3)  
 187 immigrant clades of Asian origin (orange). Each point is colored according to the region  
 188 of hypothesized origin and labeled by the narrowest phylogenetic taxonomy. Black labels  
 189 indicate focal groups and grey labels indicate other Australian vertebrate clades.  
 190 Regression in background is fit to all points with the exception of Limnodynastidae and  
 191 Myobatrachidae (included jointly as Myobatrachoidea) and shows a general pattern of  
 192 increasing species richness with age. This pattern holds equally for a regression of just  
 193 frog clades ( $R^2=0.849$ , intercept=1.827, slope=1.805, p=0.016).

194

195 *Discussion*

196 Here we present the first reliable estimates of relationships among nearly all of  
 197 Australia's native frog genera (25 of 27) and major clades of the diverse genus *Litoria*.  
 198 Our investigation into the timing and origins of the Australian frog fauna reveals a  
 199 staggered colonization and population of the continent. This stratified arrival and  
 200 radiation of Australian frogs took place under the varied environmental conditions of  
 201 vastly different eras. Across these eras Australia has flourished through a warm and wet  
 202 Eocene, cooling and drying following the onset of Antarctic glaciation in the Oligocene,  
 203 warm and forested Miocene, and a gradual aridification leading to its present status  
 204 (Byrne et al. 2011, Pross et al. 2012, Macphail & Hill 2018, Mao & Retallack 2019).

**Deleted:** are plotted alongside a map showing total annual precipitation. Greatest richness is concentrated along Australia's east coast, however this pattern falls away in cold areas of the far southeast, particularly in southwest Tasmania.

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## AUSTRALIAN FROG PHYLOGENOMICS

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### 217 *Origins and Biogeography*

218 The Myobatrachidae and Limnodynastidae (together—myobatrachoids) represent  
219 the oldest, most diverse (136 spp.), and only near-endemic of Australia's frog radiations  
220 (~~4 spp. are~~ found in New Guinea). They share a long history with South America and  
221 its Gondwanan past. Anchored by a deep split with the South American  
222 *Calyptocephalella* (roughly 100 mya; Fig.2), early divergences among the  
223 myobatrachoids, principally between *Mixophyes*, *Rheobatrachus*, and ~~the~~  
224 limnodynastids, occurred in the late Cretaceous (80–70 mya), preceding the isolation of  
225 Australia from Antarctica. This dates to a time when South America, Antarctica, and  
226 Australia were a continuous landmass that was likely temperate in climate (Palazzi &  
227 Barreda 2007; Mörs et al. 2020). The phylogenetic depth and distribution of  
228 myobatrachoids ~~and calyptocephalellids~~ across these now widely disjunct continents  
229 suggests a historically continuous distribution across southern Gondwana, including  
230 Antarctica. This idea is supported by the recent discovery of an extinct  
231 calyptocephallelid from mid-Miocene Antarctica that lived more than 40 mya (Mörs et  
232 al. 2020). The persistence of *calyptocephalellids* in Antarctica into the Late Eocene  
233 highlights the dichotomy between young extant myobatrachid and limnodynastid  
234 diversity (most species < 30 mya) and ancient splits between limnodynastids and  
235 myobatrachids and within *myobatrachids* (> 70 mya). *The tips of these* long branches  
236 are likely the survivors of a much greater southern Gondwanan myobatrachoid  
237 diversity, potentially mirroring the diversity of extinct *calyptocephalellids* through  
238 southern South America and Patagonia (Nicoli et al. 2022).

239 Australian myobatrachoids however are not the only group with close  
240 connections to South America. The Pelodryadidae are a species rich (~~>220~~ spp.) and  
241 morphologically diverse clade of Australasian frogs. Embedded within the primarily

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**Deleted:** *Calyptocephalella*

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252 Neotropical treefrogs, they show a more recent late-Eocene divergence from their South  
 253 American relatives the Phyllomedusidae, some 40 mya. Crown divergence of the  
 254 pelodryadids occurred in the mid-to-late Oligocene (30 mya) before erupting into a  
 255 radiation across Australia and New Guinea in the early Miocene. This timing has  
 256 spurred speculation about the origins of pelodryadids either as part of a young  
 257 Gondwanan group or more recent over-water dispersers from South America (Pyron  
 258 2014). Divergence between phyllomedusids and pelodryadids 40 mya aligns with the  
 259 opening of the Drake Passage and separation of South America from Antarctica  
 260 (Toumoulin 2020). Unfortunately, this does not provide any certainty about how  
 261 pelodryadids arrived in Australia. While the Brazil Current would have provided a  
 262 favorable trajectory for rafting frogs, the over-water distance between South America  
 263 and Australia remained immense. Our biogeographic modelling indicates that the  
probability of overwater dispersal is just a fraction of that overland, making rafting  
seem improbable. Instead, we suggest a more likely scenario is that pelodryadids  
 264 dispersed from South America through Antarctica and into Australia. (Fig.3). Climate  
 265 reconstructions suggest warm temperate/tropical habitats across Antarctica which  
 266 would have been suitable through a long period of the Eocene (Pross et al. 2012).  
 267 Dispersal via Antarctic land bridges would have had to occur prior to the Eocene-  
 268 Oligocene cooling (34 mya) that blanketed Antarctica beneath an ice sheet (van den  
 269 Ende et al. 2017).

272 Contrasting with the comparatively ancient limnodynastids, myobatrachids, and  
 273 pelodryadids, Australia's youngest anuran radiation are the microhylids. Embedded  
 274 deeply in the Asterophryinae subfamily, two similarly aged clades (12–13 mya) of  
 275 *Astrochaperina* and *Cophixalus* crossed the gap from New Guinea to Australia in the  
 276 mid Miocene. This time frame coincides with a period of increased variation in sea  
 277 surface levels driven by cooling global temperatures following the mid Miocene climatic

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282 optimum. Dropping sea levels likely repeatedly exposed a landbridge between southern  
283 New Guinea and northern Australia (both Cape York and the Top End) and facilitated  
284 biotic exchange between these landmasses (Mitchell et al. 2014). The young age of these  
285 clades, and existence of two other species-rich incumbent frog clades in the pelodryadids  
286 and myobatrachoids potentially explains why Australian microhylids are relatively  
287 species poor (*Astrochaperina*—5 spp., *Cophixalus*—18 spp.) and morphologically  
288 conservative compared to their New Guinean neighbors (200+ spp.), reflecting a pattern  
289 seen in monitor lizards (Pavón-Vázquez et al. 2021).

290 The sole Australian ranid *Papurana daemeli* is native but not endemic to the  
291 continent, and can be found broadly across Australo-Papua, extending to just beyond  
292 the edge of the Sahul shelf (Reilly et al. 2022). It belongs to a clade of frogs distributed  
293 throughout southeast Asia, Wallacea, and Sahul, with other *Papurana* species found in  
294 New Guinea and the Solomon Islands (Oliver et al. 2015; Chan et al. 2020). Though not  
295 included in our phylogenomic sampling, *Papurana daemeli* is likely a relatively young  
296 species (<7 mya) with limited divergence between populations found in Wallacea and  
297 Sahul (Reilly et al. 2022). The broad distribution of *P. daemeli* across Australo-Papua  
298 suggests either a very recent colonization of Australia or vicariant speciation followed by  
299 subsequent dispersal out of Australia and back into New Guinea and Wallacea.

300 The staggered temporal origins of Australian frogs exemplifies the general  
301 colonization history of Australian vertebrates. Radiations of mammals, birds, frogs, and  
302 reptiles fall into discretized temporal groups broadly identified as (1) Gondwanan relics  
303 >40 myo, (2) old established clades (20–40 myo) with varied origins, or (3) recent  
304 immigrants from Asia (<20 myo). The Limnodynastidae and Myobatrachidae fall  
305 undoubtedly into the Gondwanan group alongside ancient Australian radiations like  
306 Australidelphian marsupial mammals which include koalas, kangaroos, and Tasmanian  
307 devils; side-necked chelid turtles; and pygopodoid geckos which include the bizarre

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311 limbless pygopodids. These groups—with the exception of pygopodoids—have close links  
 312 to South American relatives based on molecular and fossil evidence (Georges et al. 1999;  
 313 Mitchell et al. 2014). While a Pelodryadidae link with South America is clear, they are  
 314 perhaps the sole radiation to have emigrated from South America to Australia since the  
 315 continental breakup. Most other similarly aged Australian groups instead show signal of  
 316 Asian or Australian origins. In comparison, the Australian microhylids  
 317 (*Austrochaperina*, *Cophixalus*) and the ranid *Papurana daemeli* are relatively young  
 318 arrivals from New Guinea with deeper origins in Asian groups. Both the *Asterophryinae*  
 319 and Ranidae, to which these species belong, have a long history in the Sunda and  
 320 Wallacean regions, reflecting patterns of old diversity in this tectonically active area.  
 321 Alongside a number of other groups such as pythons (Esquerré et al. 2020), monitor  
 322 lizards (Brennan et al. 2021), honeyeater birds (Marki et al. 2017), dragon lizards  
 323 (Tallowin et al. 2020), elapid snakes (Keogh 1998), various gekkonid gecko genera  
 324 (Heinicke et al. 2011), megabats, (Tsang et al. 2020), frogmouth birds (Oliver et al.  
 325 2020), cockatoos and parrots (Schweizer et al. 2011), several skink subfamilies (Skinner  
 326 et al. 2011), and two rodent groups (Roycroft et al. 2020), they share diversity across  
 327 Australia and New Guinea with repeated exchange between the two islands. Many of  
 328 these groups show a telltale stepping stone biogeographic pattern that links them back  
 329 to mainland Asian ancestors, with Australo-Papuan members deeply phylogenetically  
 330 nested. In general, these Australian clades show a pattern of increasing species richness  
 331 with clade age, however the drivers of such a pattern are potentially idiosyncratic  
 332 (Fig.1) (Wiens 2011; Rabosky et al. 2012).

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### 334 *Macroevolutionary Patterns*

335 The radiation of frogs in Australia has occurred over a deep timescale and across  
 336 a changing climatic landscape. Old species-poor lineages have become confined to the

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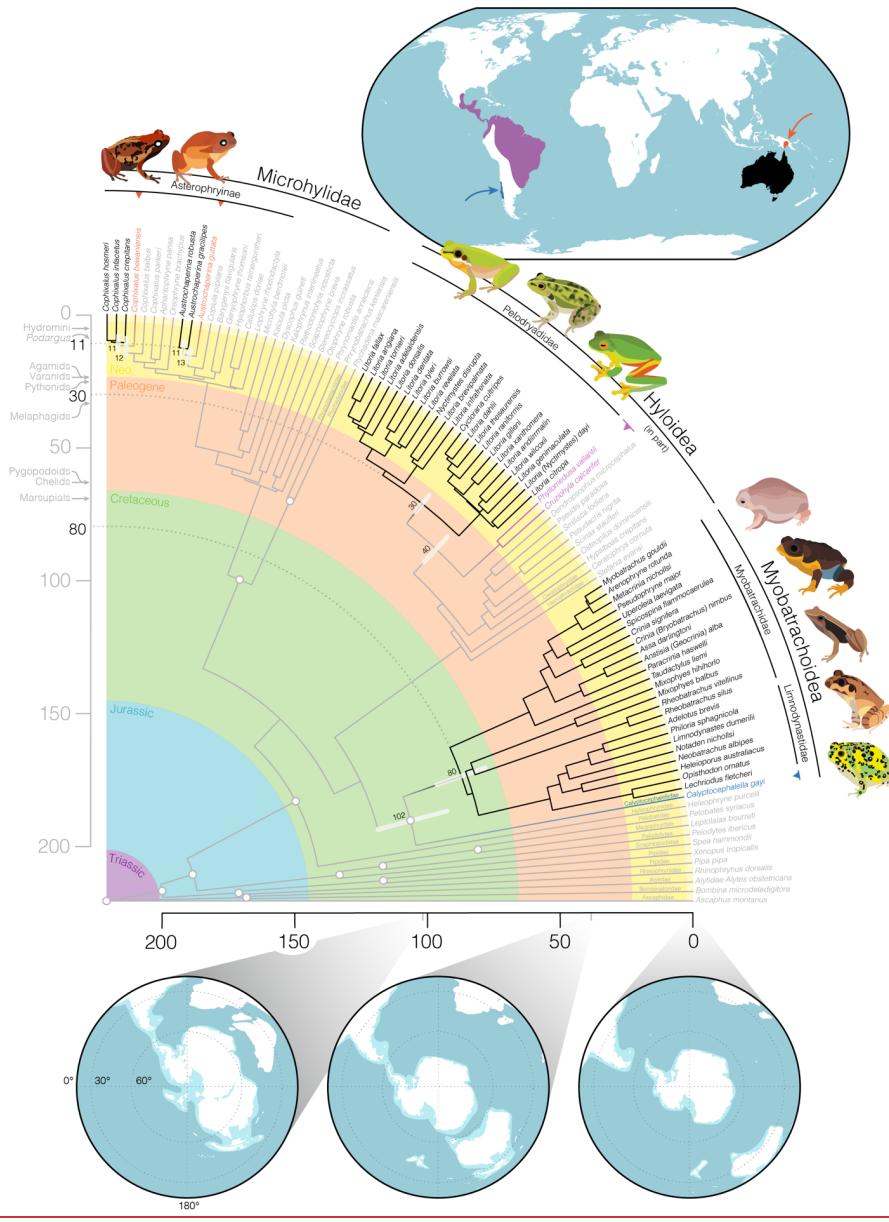
340 mesic-temperate fringes of the continent, while new niches and species have popped up  
341 in the expanding arid zone ([Morgan et al. 2007; Novikova et al. 2020](#)). And while frogs | Deleted: .  
342 are found across most of the Australian continent, their basic moisture requirements and  
343 desiccation sensitivity mean that Australian amphibian diversity shows a stark mesic-  
344 arid gradient (Fig.1), similar to that seen for birds and mammals, and the inverse of  
345 lizards (Powney et al. 2010; [Coops et al. 2018](#)). Not all has been lost in the ~~arid~~ center | Deleted: ).  
346 though—several independent clades of dry-country inhabitants have evolved among | Deleted: red  
347 Australia's harsh sandy and stony deserts. *Neobatrachus*, *Notaden*, and *Cyclorana* have  
348 all evolved to aestivate through the hottest and driest seasons. These genera (commonly  
349 known as the water-holding frogs) are capable of growing epidermal cocoons to retain  
350 moisture that may see them through periods of extreme drought lasting from months to  
351 years (van Beurden 1980).

352 Along with changes in habitat and ecology, Australia's frogs have also  
353 accumulated vast diversity in reproductive strategy, ontogenetic trajectory, and  
354 morphology (Crump 2015, Duellman 1992, Sherratt et al. 2018). While we do not  
355 present data on these topics, our well-resolved phylogenetic hypothesis provides new  
356 context for the macroevolution of some of these extreme traits. Unique rearing habits | Deleted: Bizarre  
357 such as raising young in stomachs (*Rheobatrachus*), hip-pockets (*Assa*), or subterranean  
358 nests (*Myobatrachus*) exist on both long branches and deeply nested taxa suggesting a  
359 remarkable frequency of transition among states. Similarly, morphological variation has  
360 rapidly evolved to dramatic extremes. The long limbed highly aquatic *Litoria dahlii*  
361 with webbed feet and dorsally situated eyes is sister to the short-limbed burrowing  
362 water-holding frogs *Cyclorana* (Vidal-Garcia & Keogh 2015). Together these frogs are  
363 embedded deeply within the otherwise toe-padded and arboreal tree frogs, highlighting  
364 the adaptive capacity of pelodryadids. Myobatrachoids too have taken ecomorphology to  
365 the extreme, offering us what is perhaps the world's strangest living anuran, the turtle

Brennan et al.

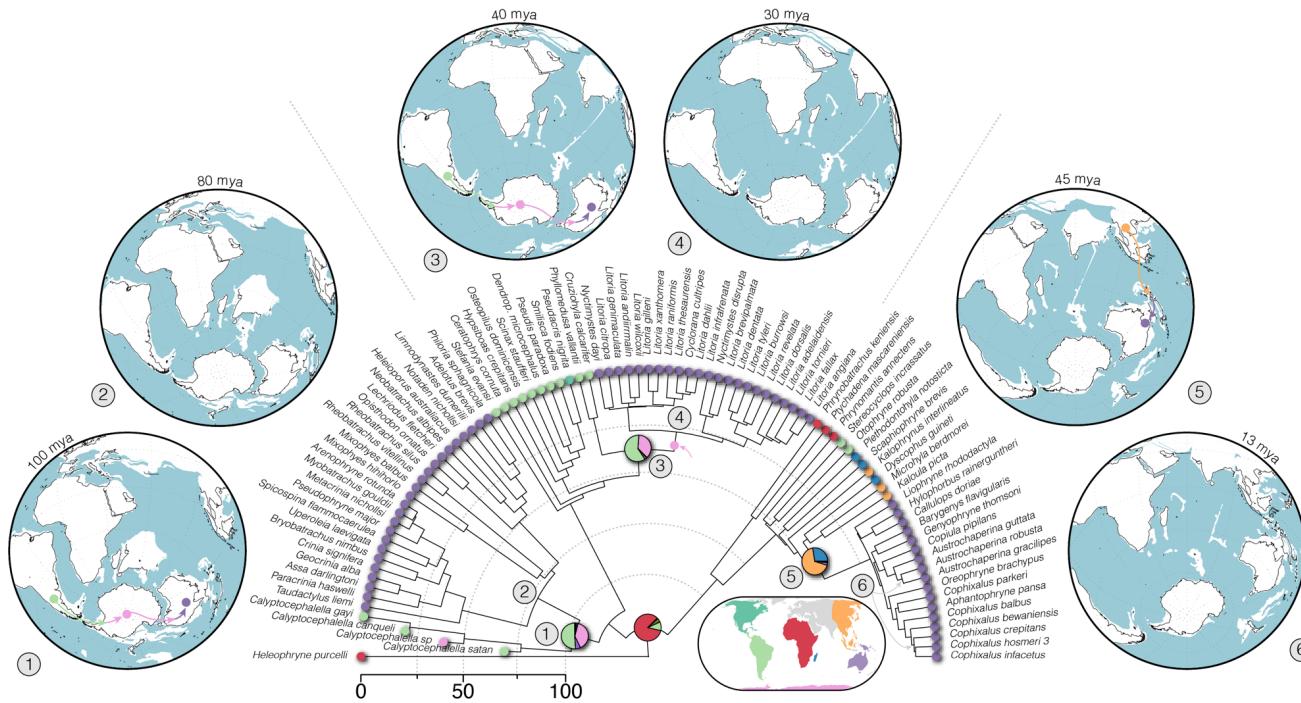
370 frog *Myobatrachus gouldii*. In pursuit of their backwards burrowing lifestyle and termite-  
371 heavy diet, *Myobatrachus* lack many of the characteristics we typically associate with  
372 frogs. Their beady black eyes are set in small heads and, alongside their sister taxon  
373 *Arenophryne*, they crawl—not jump—across the ground on short limbs that are  
374 incapable of hopping (Vidal-Garcia et al. 2014).

AUSTRALIAN FROG PHYLOGENOMICS



376 Figure 2. Time-calibrated frog phylogeny highlights the varied origins and staggered  
377 arrival of the four major frog families that comprise the Australian anuran fauna.  
378 Primarily Australian clades are identified by black branches and text, their closest living  
379 relatives outside of Australia are noted by colored branches and text, and outgroup taxa  
380 are grey. White circles at nodes identify the location of fossil calibrations (see Table S2).  
381 Upper inset map shows the general geographic location of: (red) closely related  
382 microhylids in New Guinea, (purple) phyllomedusid hylids in South America, and (dark  
383 blue) *Calyptocephalella* in Chile. Lower inset maps show the connection and proximity  
384 of Australia to other Gondwanan continents as Australia drifted away over the past 100  
385 million years. White indicates contemporary coastlines, light blue the continental plates,  
386 and dark blue the oceans. Maps were generated using GPlates and input files modified  
387 from Landis (2017). Partial fan phylogeny was plotted using *phytools* in the R  
388 programming environment. Annotations on vertical time axis show the age of crown  
389 divergences of other notable Australian groups for temporal context (see Fig.1). Species  
390 illustrated clockwise from top left: *Cophixalus infacetus*, *Astrochaperina robusta*,  
391 *Litoria fallax*, *Litoria dahlii*, *Litoria xanthomera*, *Myobatrachus gouldii*, *Spicospina*  
392 *flammodaeerulea*, *Taudactylus acutirostris*, *Mixophyes balbus*, *Notaden bennettii*.  
393

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399 Circular world maps show geological reconstructions at relevant time points, with numbers mapped to nodes of interest.  
400 Colored arrows indicate hypothesized dispersal paths for each clade. Under this biogeographic model the ancestors of both  
401 the Myobatrachioidea and Pelodryadidae lived in South America, and Australo-Papuan microhylids (Asterophryinae)  
402 originate from an Asian ancestor. The most likely dispersal path for the Pelodryadidae included expansion across  
403 Antarctica after divergence from the Phyllomedusidae. Phylogeny plotted with *phytools*, maps generated by the Ocean  
404 Drilling Stratigraphic Network (<https://www.odsn.de/odsn/services/paleomap/paleomap.html>).  
405

406 *Conclusion*

407     Australian frogs offer important insights into colonization, persistence, and  
408     diversification of a major continental group through deep time. The varied species  
409     richness, timing of diversification, and ecomorphological diversity among replicate  
410     radiations provides evidence of the processes dictating the accumulation of biodiversity.  
411     Beyond the temperate and tropical forests of the east and north coast, the Australian  
412     continent is an open country of habitat scarcely welcoming to frogs. Despite this,  
413     anurans have a long history in Australia and have diversified into an amazing array of  
414     forms, colors, and lifestyles. This success is potentially the result of the stratified  
415     temporal arrival of the three main frog clades and possibly exaggerated by their  
416     ecological differences. Our phylogenetic framework provides a foundation for further  
417     examining how temporal changes to climate, habitat, and niche space have influenced  
418     the diversification of one of Australia's richest and most unique vertebrate faunas.

419

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420 *Data Accessibility*

421     Sequence alignments, analysis control files, and phylogenetic trees can be  
422     downloaded from Dryad (doi:10.5061/dryad.zpc866tcj) and GitHub  
423     ([https://github.com/IanGBrennan/Crown\\_Frogs](https://github.com/IanGBrennan/Crown_Frogs)). We also provide a temporary link to  
424     these files suitable for peer review:

425     <https://datadryad.org/stash/share/gZnvJUP7Ow7xlQjrJr3Z9IYlMWqshquiWGGahkNRji4>

426

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427 *Conflicts of Interest*

428     The authors recognize no conflicts of interest, either direct or indirect, that might  
429     bias the conclusions, implications, or opinions stated in this work.

430

431

Field Code Changed

432 Acknowledgments

433 Thank you to colleagues and staff at Australian museums and more generally  
 434 across Australia for generously donating tissues and locality information for many frogs.  
 435 We also thank the technical staff at our institutions for their support and hard work  
 436 generating the genetic data presented here. The contributions of our many communities  
 437 have made this work possible. JSK, CJH, and SCD thank the Australian Research  
 438 Council for ongoing support. We appreciate comments from Isabel Sanmartín, Rayna  
 439 Bell, and two anonymous reviewers that helped to improve a previous version of this  
 440 manuscript.

**Moved (insertion) [4]****Moved (insertion) [5]****Moved down [6]:**

Figure 2. Time-calibrated frog phylogeny highlights the varied origins and staggered arrival of the four major frog families that comprise the Australian anuran fauna. Primarily Australian clades are identified by black branches and text, their closest living relatives outside of Australia are noted by colored branches and text, and outgroup taxa are grey. White circles at nodes identify the location of fossil calibrations (see Table S2). Upper inset map shows the general geographic location of: (red) closely related microhylids in New Guinea, (purple) phyllomedusid hylids in South America, and (dark blue) *Calyptocephalella* in Chile. Lower inset maps show the connection and proximity of Australia to other Gondwanan continents as Australia drifted away over the past 100 million years. White indicates contemporary coastlines, light blue the continental plates, and dark blue the oceans. Maps were generated using GPlates and input files modified from Landis (2017). Partial fan phylogeny was plotted using *phytools* in the R programming environment. Annotations on vertical time axis show the age of

**Moved up [2]: Conclusion**

→ Australian frogs offer important insights into colonization, persistence, and diversification of a major continental group through deep time. The

**Moved up [3]:** examining how temporal changes to climate, habitat, and niche space have influenced the diversification of one of Australia's richest and most unique vertebrate faunas.**Moved up [4]: Acknowledgments**

Thank you to colleagues and staff at Australian museums and more generally across Australia for generously donating tissues and locality information

**Moved up [5]:** We also thank the technical staff at our institutions for their support and hard work generating the genetic data presented here. The contributions of our many communities have made**Deleted:** *Notaden bennetti*, *Mixophyes balbus*.**Deleted:** Our phylogenetic framework provides a foundation for**Deleted:** .**Formatted:** Font colour: Custom Colour (RGB(34,34,34)), Highlight

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762 *Figure Captions*

763 Figure 1. Australian frogs show an imbalance in species richness, age, and geographic  
 764 spread. Above, maps of richness for the three focal radiations (with Limnodynastidae  
 765 and Myobatrachidae presented together as Myobatrachoidea) represent visually how  
 766 contemporary patterns of frog richness reflect water availability, and are highest in the  
 767 wet temperate, subtropical, and tropical rainforests of the east coast. We show annual  
 768 precipitation here for ease of interpretation but Australian frog richness is potentially  
 769 better explained by actual evapotranspiration (Coops et al. 2018). Species occurrence  
 770 records were collated from the Atlas of Living Australia (<https://ala.org.au>). Below,  
 771 Australian radiations can be divided broadly into (1) relictual Gondwanan clades >40  
 772 myo (green), (2) ancient colonizing groups (>20 myo, <40 myo; varied colors), or (3)  
 773 immigrant clades of Asian origin (orange). Each point is colored according to the region  
 774 of hypothesized origin and labeled by the narrowest phylogenetic taxonomy. Black labels  
 775 indicate focal groups and grey labels indicate other Australian vertebrate clades.  
 776 Regression in background is fit to all points with the exception of Limnodynastidae and  
 777 Myobatrachidae (included jointly as Myobatrachoidea) and shows a general pattern of  
 778 increasing species richness with age. This pattern holds equally for a regression of just  
 779 frog clades ( $R^2=0.849$ , intercept=1.827, slope=1.805,  $p=0.016$ ).

780  
 781 Figure 2. Time-calibrated frog phylogeny highlights the varied origins and staggered  
 782 arrival of the four major frog families that comprise the Australian anuran fauna.  
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 785 are grey. White circles at nodes identify the location of fossil calibrations (see Table S2).  
 786 Upper inset map shows the general geographic location of: (red) closely related  
 787 microhylids in New Guinea, (purple) phyllomedusid hylids in South America, and (dark

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**Deleted:** are plotted alongside a map showing total annual precipitation. Greatest richness is concentrated along Australia's east coast, however this pattern falls away in cold areas of the far southeast, particularly in southwest Tasmania.

**Moved (insertion) [6]**

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## AUSTRALIAN FROG PHYLOGENOMICS

796 blue) *Calyptocephalella* in Chile. Lower inset maps show the connection and proximity  
797 of Australia to other Gondwanan continents as Australia drifted away over the past 100  
798 million years. White indicates contemporary coastlines, light blue the continental plates  
799 and dark blue the oceans. Maps were generated using GPlates and input files modified  
800 from Landis (2017). Partial fan phylogeny was plotted using *phytools* in the R  
801 programming environment. Annotations on vertical time axis show the age of crown  
802 divergences of other notable Australian groups for temporal context (see Fig.1). Species  
803 illustrated clockwise from top left: *Cophixalus infacetus*, *Austrochaperina robusta*,  
804 *Litoria fallax*, *Litoria dahlii*, *Litoria xanthomera*, *Myobatrachus gouldii*, *Spicospina*  
805 *flammoecaerulea*, *Taudactylus acutirostris*, *Mixophyes balbus*, *Notaden bennettii*.  
806 ▲  
807 Figure 3. Simplified biogeographic history of Australian frogs with a focus on the range  
808 reconstruction of their immediate ancestors (complete figure in Fig.S7). Ranges have  
809 been estimated under the preferred model DEC+j+x+w supporting Hypothesis 1  
810 (Antarctic dispersal of Pelodryadidae frogs; pink arrow on tree indicates ancestral  
811 pelodryadid constrained to Antarctica) in BioGeoBEARS. Pie charts represent range  
812 probability at nodes with colors corresponding to inset map. Circular world maps show  
813 geological reconstructions at relevant time points, with numbers mapped to nodes of  
814 interest. Colored arrows indicate hypothesized dispersal paths for each clade. Under this  
815 biogeographic model the ancestors of both the Myobatrachoidea and Pelodryadidae lived  
816 in South America, and Australo-Papuan microhylids (Asterophryinae) originate from an  
817 Asian ancestor. The most likely dispersal path for the Pelodryadidae included expansion  
818 across Antarctica after divergence from the Phyllomedusidae. Phylogeny plotted with  
819 *phytools*, maps generated by the Ocean Drilling Stratigraphic Network  
820 (<https://www.odsn.de/odsn/services/paleomap/paleomap.html>).  
821

**Moved up [1]:** Figure 2. Time-calibrated frog phylogeny highlights the varied origins and staggered arrival of the four major frog families that comprise the Australian anuran fauna. Primarily Australian clades are identified by black branches and text, their closest living relatives outside of Australia are noted by colored branches and text, and outgroup taxa are grey. White circles at nodes identify the location of fossil calibrations (see Table S2). Upper inset map shows the general geographic location of: (red) closely related microhylids in New Guinea, (purple) phyllomedusid hylids in South America, and (dark blue) *Calyptocephalella* in Chile. Lower inset maps show the connection and proximity of Australia to other Gondwanan continents as Australia drifted away over the past 100 million years. White indicates contemporary coastlines, light blue the continental plates, and dark blue the oceans. Maps were generated using GPlates and input files modified from Landis (2017). Partial fan phylogeny was plotted using *phytools* in the R programming environment. Annotations on vertical time axis show the age of crown divergences of other notable Australian groups for temporal context (see Fig.1). Species illustrated clockwise from top left: *Cophixalus infacetus*, *Austrochaperina robusta*, *Litoria fallax*, *Litoria dahlii*, *Litoria xanthomera*, *Myobatrachus gouldii*, *Spicospina flammoecaerulea*, *Taudactylus acutirostris*.

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354 *Supplementary Materials and Methods*

855

856 Data available from the Dryad Digital Repository:

857 [http://dx.doi.org/10.5061/dryad.\[NNNN\]](http://dx.doi.org/10.5061/dryad.[NNNN])

858 and from the GitHub repository: [https://github.com/IanGBrennan/Crown\\_Frogs](https://github.com/IanGBrennan/Crown_Frogs)

859

360 *Developing Figure 1*

361 Figure 1 aims to provide background on the richness and spatial distribution of  
362 the focal frog clades, alongside evolutionary context for the accumulation of vertebrate  
363 biodiversity on the Australian continent. Neither the top or bottom visualizations are  
364 intended to provide an explanation of the *processes* dictating Australian vertebrate  
365 diversity. Instead they are visualizations of the *patterns* of contemporary Australian  
366 vertebrate diversity.

367 We downloaded Australian annual rainfall data from NASA using the R package  
368 *nasapower*, and combined this with species occurrence records downloaded from the  
369 Atlas of Living Australia. Annual rainfall is an easily interpretable measure of water  
370 availability in an environment, and as such provides a reflection of habitat suitability  
371 for frogs. However, we acknowledge that composite environmental variables such as  
372 actual evapotranspiration (AET) may be a better predictor of contemporary frog  
373 richness patterns (Powney et al., 2010; Coops et al., 2018).

374 To plot the relationship between clade age and richness of Australian terrestrial  
375 vertebrates we collected data from all available non-nested (each clade is only  
376 represented once) clades from the literature. Data are compiled in the supplement  
377 *Comparative\_Radiations.csv* and can be plotted using the script  
378 *Comparative\_Radiations.R*. We also incorporated information where available about  
379 the biogeographic origin of each group to visualize the contrast between young clades

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## AUSTRALIAN FROG PHYLOGENOMICS

880 from Asia and old Gondwanan groups. The included regression helps to visualize an  
881 interesting *pattern* in the data: species richness increases with clade age. However, we do  
882 not present this as an evolutionary explanation for varied richness among Australian  
883 terrestrial vertebrate groups.

884

### 885 *Sequence Identity*

886 To confirm sequence identity we downloaded a fasta file of *Xenopus* genes from  
887 Ensembl (UCB\_Xtro\_10.0) and used *metablastr* to do a reciprocal blast against the  
888 Anchored Hybrid Enrichment loci. Of the 450 loci, 390 matched to *Xenopus* exons, and  
889 the remainder to intronic and flanking sequences (see *RBH\_AHE\_Xenopus.csv* in  
890 Supplementary Material for list). Downstream divergence time analysis relied on  
891 partitioning loci by codon position and so only exonic targets were retained for this  
892 exercise. AHE exons are listed under the column *query\_id* and *Xenopus* matches under  
893 *subject\_id* with gene name indicated by *subject\_id\_name*.

894

### 895 *Phylogenetics*

896 Phylogeny reconstruction in the era of phylogenomics has simultaneously resolved  
897 many longstanding systematic questions and instigated new ones. The search for the  
898 most accurate species tree has reignited debates about concatenation versus coalescent  
899 methods and their pros and cons. Here we address two common issues resulting in  
900 phylogenetic error: incomplete lineage sorting (ILS) and rate variation among lineages  
901 and sites (heterotachy). Identifying and modelling heterotachy generally requires long  
902 alignments to accurately model rate variation, so most methods rely on concatenated  
903 sequence alignments. Because of the ancient age of our focal group and sparse sampling  
904 among major groups we risk biases due to heterotachy. To estimate a species tree from  
905 our concatenated alignment we used the General Heterogeneous evolution On a Single

906 Topology (GHOST) method. GHOST is implemented in IQTREE and requires a user  
907 specified number of mixture (rate) classes and model. We separately fit unlinked GTR  
908 models with 2–5 mixture classes (e.g.: -m GTR\*H<sub>4</sub>). AIC comparison identified the 4-  
909 class model as preferred ( $H^*2$  AICc = 13754122;  $H^*3$  AICc = 13604562;  $H^*4$  AICc =  
910 13500200;  $H^*5$  AICc = 13523685).

911 Concatenation methods are however expected to perform poorly when the true  
912 branching pattern includes nested rapid divergence events. In this case high rates of ILS  
913 may bias phylogenetic signal, trapping concatenation in the anomaly zone. To counter  
914 this we estimated a species tree using ASTRAL with IQTREE genetrees as input.

915

#### 916 *Biogeography*

917 To assess the biogeographic history of Australian frogs we combined our phylogenetic  
918 hypothesis with known fossil information and reconstructed ancestral ranges in  
919 *BioGeoBEARS* (Matzke 2014). We started by dividing the geographic distribution of  
920 our sampled taxa into eight discrete areas that (1) summarize the general biogeographic  
921 history of frogs, (2) are relevant to our sampling and questions, and (3) make sense on a  
922 geological timescale with reference to plate tectonics over the last 220 million years.  
923 These areas correspond to Africa, Asia (excluding the Indian subcontinent), Australo-  
924 Papua, Europe, Madagascar, North America, South America, and Antarctica. For single  
925 tips that represent a genus or subfamily we coded their geographic range accordingly,  
926 however this never resulted in an overrepresentation of areas that might inflate dispersal  
927 estimates. Our primary objective was to identify the ancestral distributions of each  
928 Australian frog clade to provide an estimate of their origins.

929 While Antarctica seems a strange inclusion in our discrete bioregions owing to its  
930 current climate and lack of frogs, a recent discovery has identified the continent's first  
931 anuran (Mörs et al. 2020). This information is vital to our understanding of the

## AUSTRALIAN FROG PHYLOGENOMICS

932 connectivity of the Gondwanan supercontinent as well as the biogeographic history of  
933 Australian frogs. To incorporate this sample we added a tip to our tree with an  
934 appropriate estimated age following Mörs et al. (2020). Due to our limited sampling of  
935 extant Calyptocephalellidae however, the addition of this taxon dramatically imbalances  
936 range reconstruction. To correct for this and account for the ancient known history of  
937 calyptocephalellids in South America (Moura et al. 2021; Nicoli et al. 2022) we included  
938 two additional South American fossil taxa, one younger—*Calyptocephalella canqueli*  
939 (following Muzzopappa & Báez 2009) and one older—*Calyptocephalella satan* (following  
940 Nicoli et al. 2022). Note, here we consider *C.satan* as interchangeable with the similarly  
941 aged *Baurubatrachus pricei* (following Báez & Gómez 2018), being representative of a  
942 broader extinct South American calyptocephalellid diversity (Nicoli et al. 2022). While  
943 the taxonomy and phylogenetic relationships of extant (*Calyptocephalella gayi*,  
944 *Telmatobufo spp.*) and extinct (*C. canqueli*, *C. satan*, et al.) calyptocephalellids is  
945 unresolved, we believe this sampling strategy is an appropriate solution for the question  
946 at hand.

947 In addition to the origins of Australian frogs we were interested in identifying  
948 how pelodryadids arrived in Australia. Specifically we aimed to test if they arrived via  
949 dispersal through Antarctica or overwater dispersal from South America. To test these  
950 hypotheses we added an ancestor (*Pelodryadidae Ancestor*) to our tree along the stem  
951 leading to the Pelodryadidae. BioGeoBEARS accommodates sampled ancestors as  
952 “hooks”, which are represented by a non-zero terminal edge length shorter than an  
953 arbitrary threshold (here: 0.000001 million years). This allowed us to force the ancestral  
954 pelodryadid to either have had a range in Antarctica (Hypothesis 1; H1; South  
955 America→Antarctica→Australia), or have remained in South America prior to an  
956 overwater dispersal event (Hypothesis 2; H2; South America→Australia).

957     The biogeographic history of frogs has played out on a very long timescale (>200  
958     million years) and across continents that have moved dramatically relative to one  
959     another. To capture the complex interplay of plate tectonics and biogeography we  
960     incorporated several elements that might make this scenario more realistic. We first  
961     divided the anuran tree into six equal slices of 30 million years (0–30, 30–60, ... 150–  
962     180) and one slice of 40 million years (180–220). At the upper bound of each time slice  
963     (30, 60 ... 180, 220) we then reconstructed continental positions in GPlates following  
964     Landis (2017) and extracted pairwise distances (in km) among areas from the closest  
965     points of two areas, using the measuring tool in GPlates. Additionally, we characterized  
966     regions as (a) in contact with one another, (b) separated by ocean, or (c) separated by  
967     another landmass. We used the area distances through time to construct distance  
968     matrices following Van Dam & Matzke (2016), and the area adjacency information to  
969     construct dispersal matrices.

970     Constructing these time-specific matrices allowed us to compare a set of scenarios  
971     that include the traditional DEC model (Dispersal Extinction Cladogenesis), DEC+j  
972     which allows jumps in range expansion (range discontinuity), DEC+x which estimates a  
973     parameter  $x$  corresponding to a correction for dispersal probability as a function of  
974     distance between areas (dispersal \* relative\_distance $^x$ ), DEC+j+x which allows jumps  
975     and corrects for distance among areas, DEC+x+w which estimates  $x$  (correcting for  
976     distance) in addition to a parameter  $w$  which can be interpreted as correcting for  
977     different levels of area adjacency (dispersal \* dispersal\_multiplier $^w$ ), and finally  
978     DEC+j+x+w which can be interpreted as allowing for jumps in range expansion ( $j$ )  
979     while correcting for geographic distance between areas ( $x$ ) and types of  
980     adjacency/separation ( $w$ ). Ultimately the most complex model (DEC+j+x+w) is an  
981     attempt to account for differences in the geographic distance between areas ( $x$ ) as well  
982     as what separates them ( $w$ ), through time, while allowing taxa to make rapid dispersal

AUSTRALIAN FROG PHYLOGENOMICS

983 events ( $j$ ). Estimating  $w$  unfortunately necessitates the manual input of dispersal  
984 multipliers which scale dispersal probability, however these are ultimately corrected by  
985 estimating their relationship via  $w$ . We established conservative manual dispersal  
986 multipliers for adjacent areas (1), areas split by another contiguous landmass (0.5), and  
987 areas split by ocean (0.25). Finally, we fit all six models to both the H1 and H2  
988 datasets. We compared models by calculating AIC values, delta AIC against the best fit,  
989 and AIC weights as the relative contribution to the pool of models.

990

991 Table S1. Taxon sampling for this project.

Geography	Superfamily/Clade	Family	Subfamily	Genus species	Registration
Outgroup	Pipoidea	Pipidae	—	<i>Xenopus tropicalis</i>	NCBI Genome
Outgroup	Pipoidea	Pipidae	—	<i>Pipidae Pipa pipa</i>	MVZ 247511
Outgroup	Pipoidea	Rhinophrynidiae	—	<i>Rhinophryns dorsalis</i>	MVZ 164756
Outgroup	Leiopelmoidea	Ascaphidae	—	<i>Ascaphus montanus</i>	REF AscMon
Outgroup	Discoglossoidea	Bombinatoridae	—	<i>Bombina microdeladigitora</i>	CAS 242112
Outgroup	Discoglossoidea	Alvitudae	—	<i>Alytes obstetricans</i>	MVZ 231914
Outgroup	Pelobatoidea	Scaphiopodidae	—	<i>Spea hammondii</i>	MVZ 145187
Outgroup	Pelobatoidea	Pelodytidae	—	<i>Pelodytes ibericus</i>	MVZ 186009
Outgroup	Pelobatoidea	Megophryidae	—	<i>Leptolalax bourreti</i>	AMCC 106489
Outgroup	Pelobatoidea	Pelobatidae	—	<i>Pelobates syriacus</i>	MVZ 234650
Outgroup	—	Heleophrynidiae	—	<i>Heleophryne purcelli</i>	SANBI 1954
Outgroup	Ranoidea	Ptychadenidae	—	<i>Ptychadena mascareniensis</i>	ESP/CJR R1068
Outgroup	Ranoidea	Phrynobatrachidae	—	<i>Phrynobatrachus keniensis</i>	MVZ 226261
Outgroup	Ranoidea	Microhylidae	Phrynomatiniae	<i>Phrynomantis annectens</i>	ESP/CJR R1330
Outgroup	Ranoidea	Microhylidae	Otophryinae	<i>Otophryne robusta</i>	PLVP PT459
Outgroup	Ranoidea	Microhylidae	Gastrophryinae	<i>Stereocyclops incrassatus</i>	PLVP PT273
Outgroup	Ranoidea	Microhylidae	Scaphiophryinae	<i>Scaphiophryne brevis</i>	PLVP PT312
Outgroup	Ranoidea	Microhylidae	Cophylinae	<i>Plethodontohyla notosticta</i>	AMCC 128714
Outgroup	Ranoidea	Microhylidae	Kalophryinae	<i>Kalophrymus interlineatus</i>	ABTC 105933
Outgroup	Ranoidea	Microhylidae	Dyscophiniae	<i>Dyscophus quineti</i>	MVZ 238744
Outgroup	Ranoidea	Microhylidae	Microhylinae	<i>Kaloula picta</i>	ABTC 76311
Outgroup	Ranoidea	Microhylidae	Microhylinae	<i>Microhyla berdmorei</i>	ABTC 106005
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Liophryne rhododactyla</i>	ABTC 49542
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Callulops doriae</i>	ABTC 98415
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Hylophorus rainerguntheri</i>	ABTC 98304
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Genyophryne thomsoni</i>	PLVP PT452
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Barygenys flavigularis</i>	PLVP PT439
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Copiuula pipilans</i>	ABTC 114698
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Austrochaperina guttata</i>	ABTC 141506
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Austrochaperina gracilipes</i>	ABTC 79186
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Austrochaperina robusta</i>	conx5153
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Oreophryne brachypus</i>	ABTC 104804
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Aphantophryne pansa</i>	ABTC 49605
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Cophixalus parkeri</i>	ABTC 49557
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Cophixalus balbus</i>	ABTC 114884
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Cophixalus bewaniensis</i>	ABTC 112107
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Cophixalus crepitans</i>	conx1112
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Cophixalus infacetus</i>	conx5295
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Cophixalus hosmeri</i>	conx5267
Outgroup	Myobatrachoidea	Calyptocephalellidae	—	<i>Calyptocephalella gayi</i>	PMH 1
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Rheobatrachus silus</i>	ABTC 7324
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Rheobatrachus vitellinus</i>	ABTC 105698
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Mixophyes balbus</i>	ABTC 25323
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Mixophyes hibhorlo</i>	ABTC 45861
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Lechriodus fletcheri</i>	ABTC 24892

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## AUSTRALIAN FROG PHYLOGENOMICS

Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Opisthodon ornatus</i>	ABTC 15543
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Heleioporus australiacus</i>	ABTC 67742
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Neobatrachus albipes</i>	ABTC 15833
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Notaden nichollsi</i>	ABTC 15833
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Limnodynastes dumerilii</i>	ABTC 104299
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Philaria sphagnicola</i>	ABTC 25832
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Adelotus brevis</i>	ABTC 24210
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Taudactylus liemi</i>	ABTC 50947
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Paracrinia haswelli</i>	ABTC 26441
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Anistisia (Geocrinia) alba</i>	ABTC 106079
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Assa darlingtoni</i>	ABTC 136278
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Crinia (Bruobatrachus) nimbus</i>	ABTC 25297
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Crinia signifera</i>	ABTC 25676
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Spicosina flammocaerulea</i>	ABTC 144371
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Uperoleia laevigata</i>	MM 1227
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Pseudophryne major</i>	ABTC 16479
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Metacrinia nichollsi</i>	ABTC 17124
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Arenophryne rotunda</i>	ABTC 114066
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Myobatrachus gouldii</i>	WAM R156759
Outgroup	Hyloidea	Hemiphractidae	—	<i>Stefania evansi</i>	BNP1286
Outgroup	Hyloidea	Ceratophryidae	—	<i>Ceratophrys cornuta</i>	MVZ 247561
Outgroup	Hyloidea	Hylidae	Cophomantinae	<i>Hypsiboas crepitans</i>	YPM 10666
Outgroup	Hyloidea	Hylidae	Lophohylinae	<i>Osteopilus dominicensis</i>	MCZA148702
Outgroup	Hyloidea	Hylidae	Scinaxinae	<i>Scinax staufferi</i>	MVZ 257781
Outgroup	Hyloidea	Hylidae	Pseudinae	<i>Pseudis paradoxa</i>	LSUMNS 12511
Outgroup	Hyloidea	Hylidae	Dendropsophinae	<i>Dendropsophus microcephalus</i>	MVZ 264263
Outgroup	Hyloidea	Hylidae	Acrisinae	<i>Pseudacris nigrita</i>	REF PseNig
Outgroup	Hyloidea	Hylidae	Hylinae	<i>Smilisca sordida</i>	YPM 014191
Outgroup	Hyloidea	Phyllomedusidae	—	<i>Cruziohyla calcarifer</i>	QCAZ 48552
Outgroup	Hyloidea	Phyllomedusidae	—	<i>Phyllomedusa vallantii</i>	QCAZ 48818
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria citropa</i>	ABTC 7146
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria (Nyctimystes) dayi</i>	ABTC 15997
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria geminamaculata</i>	ABTC 42824
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria wilcoxii</i>	ABTC 16804
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria andiirrmalin</i>	ABTC 142651
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria xanthomera</i>	ABTC 102385
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria gilleni</i>	ABTC 30786
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria raniformis</i>	ABTC 12854
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria thesaurensis</i>	ABTC 50489
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria dahlii</i>	ABTC 102434
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Cyclorana cultripes</i>	ABTC 16892
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria infrafrenata</i>	ABTC 86210
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria brevipalmata</i>	ABTC 127632
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Nyctimystes disrupta</i>	ABTC 48225
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria revelata</i>	ABTC 80814
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria burrowsi</i>	ABTC 17631
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria tyleri</i>	ABTC 3925
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria balatus</i>	ABTC 100638

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Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria dorsalis</i>	ABTC 79181
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria adelaidensis</i>	ABTC 28282
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria anqiana</i>	ABTC 48210
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria fallax</i>	ABTC 102409
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria tornieri</i>	ABTC 11777

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## AUSTRALIAN FROG PHYLOGENOMICS

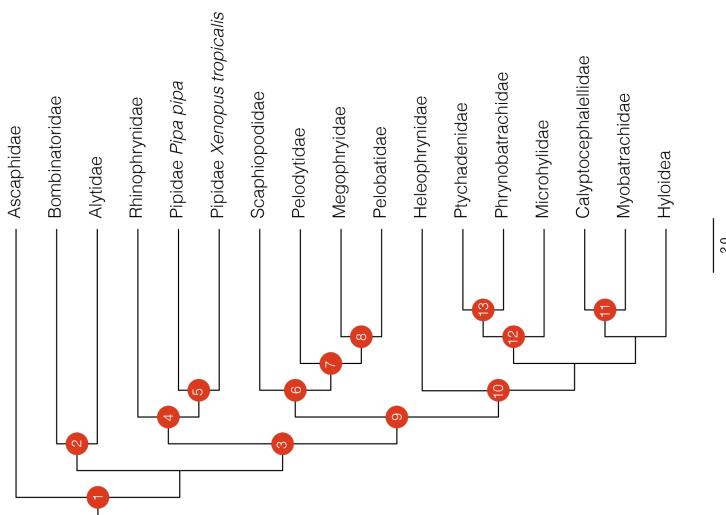
998 Table S2. Fossil calibrations implemented in MCMCTree analysis of frog divergence  
 999 dates. Node number (#) corresponds to nodes in supplementary figure below.

1000

#	Node Calibrated	Fossil	Minimum	Soft Max.	Source (see Feng et al. 2017)
1	Anura	<i>f Liaobatrachus zhaoi</i>	129.7	252	Chang et al. (2009)
2	Alytoidae	<i>f Iberobatrachus angelae</i>	125	252	Gomez et al. (2016)
3	Pipanura	<i>f Rhadinosteus parvus</i>	148.1	252	Cannatella (2015)
4	Pipoidea	<i>f Neusibatrachus wilferti</i>	127.2	52	Gomez et al. (2016)
5	Pipidae	<i>f Pachycentra taqueti</i>	83.6	48.1	Cannatella (2015)
6	Pelabatoidea	<i>f Elkobatrachus brocki</i>	46.1	148.1	Henrici and Haynes (2006)
7	Pelodytes + (Pelobatidae + Megophryidae)	<i>f Miopelodytes gilmorei</i>	38.9	148.1	Henrici and Haynes (2006)
8	Pelobatidae + Megophryidae	<i>f Macropelobates osborni</i>	28.1	148.1	Cohen et al. (2013)
9	Acosmanura	<i>f Eurycephalella alcinae</i>	113	252	Baez (2009)
10	Neobatrachia	<i>f Beelzebufo ampinga</i>	66	148.1	Rogers et al. (2013)
11	Myobatrachoidea	<i>f Calyptocephalella pichileufensis</i>	47.5	48.1	Gomez et al. (2011)
12	Ranoidea	<i>f Thamnostaurus gezei</i>	33.9	148.1	Rage and Roček (2007)
13	Ptychadenidae + Phrynobatrachus	<i>Ptychadenidae fossil</i>	25	148.1	Blackburn et al. (2015)

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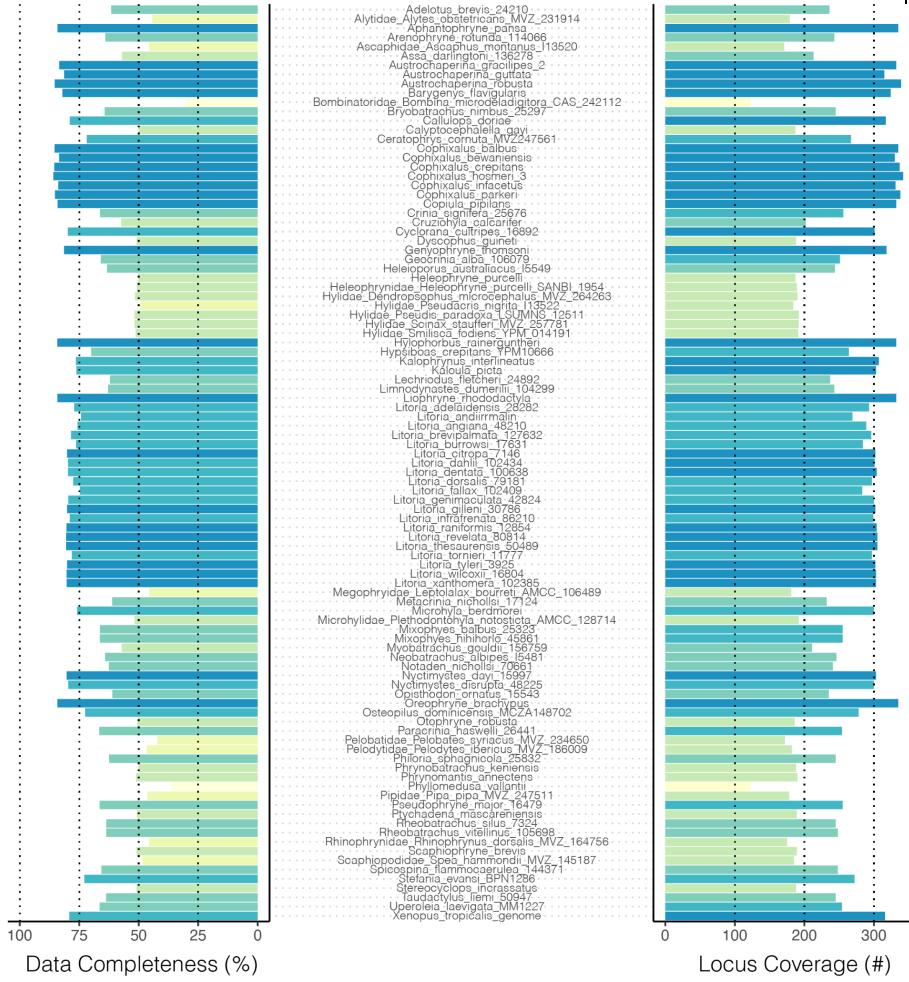
1010  
 1011 Table S3. Results of biogeographic ancestral range reconstruction in *BioGeoBEARS*. Hypothesis  
 1012 *H1* refers to the dispersal of pelodryadid frogs from South America through Antarctica to  
 1013 Australia, whereas *H2* refers to the over water dispersal of pelodryadid frogs from South  
 1014 America directly to Australia. Models are sorted according to deltaAIC scores, indicating the  
 1015 preferred model at the top.

Model	Hypothesis	No. Param.	LnL	AIC	deltaAIC	AICw
<u>DEC+j+x+w</u>	<u>H1</u>	<u>5</u>	<u>-91.47</u>	<u>192.94</u>	<u>0</u>	<u>59.7</u>
<u>DEC+j+x</u>	<u>H1</u>	<u>4</u>	<u>-93.49</u>	<u>194.98</u>	<u>2.04</u>	<u>21.5</u>
<u>DEC+j+x+w</u>	<u>H2</u>	<u>5</u>	<u>-93.08</u>	<u>196.16</u>	<u>3.22</u>	<u>11.9</u>
<u>DEC+j+x</u>	<u>H2</u>	<u>4</u>	<u>-94.66</u>	<u>197.32</u>	<u>4.38</u>	<u>6.69</u>
<u>DEC+x+w</u>	<u>H2</u>	<u>4</u>	<u>-102.71</u>	<u>213.42</u>	<u>20.48</u>	<u>0</u>
<u>DEC+j</u>	<u>H2</u>	<u>3</u>	<u>-104.91</u>	<u>215.82</u>	<u>22.88</u>	<u>0</u>
<u>DEC+x+w</u>	<u>H1</u>	<u>4</u>	<u>-105</u>	<u>218</u>	<u>25.06</u>	<u>0</u>
<u>DEC+x</u>	<u>H2</u>	<u>3</u>	<u>-106.47</u>	<u>218.94</u>	<u>26</u>	<u>0</u>
<u>DEC+x</u>	<u>H1</u>	<u>3</u>	<u>-107.85</u>	<u>221.7</u>	<u>28.76</u>	<u>0</u>
<u>DEC+j</u>	<u>H1</u>	<u>3</u>	<u>-109.33</u>	<u>224.66</u>	<u>31.72</u>	<u>0</u>
<u>DEC</u>	<u>H2</u>	<u>2</u>	<u>-114.67</u>	<u>233.34</u>	<u>40.4</u>	<u>0</u>
<u>DEC</u>	<u>H1</u>	<u>2</u>	<u>-121.56</u>	<u>247.12</u>	<u>54.18</u>	<u>0</u>

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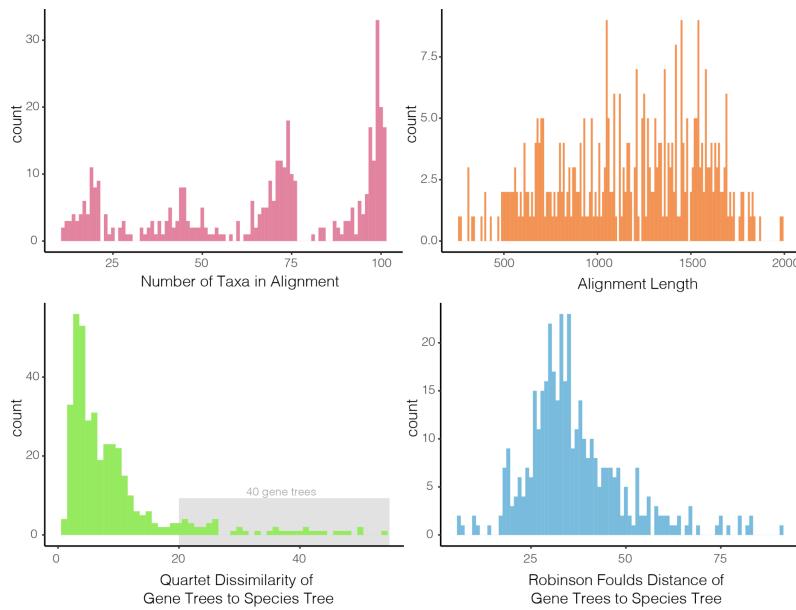
## AUSTRALIAN FROG PHYLOGENOMICS

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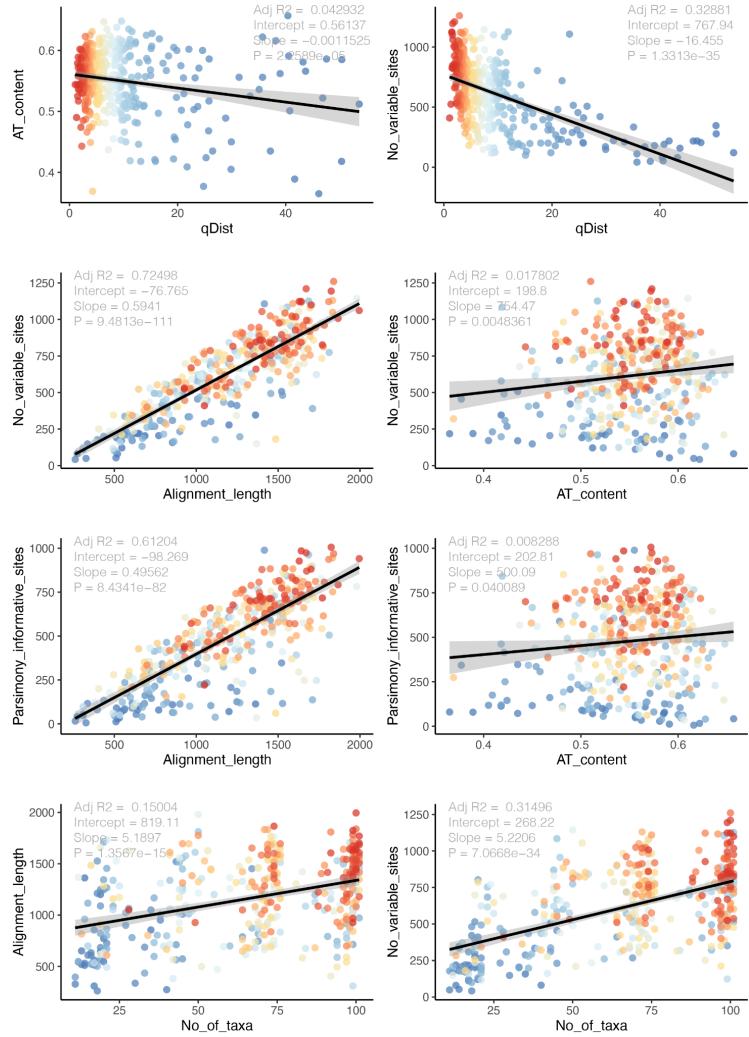
1020 Data Completeness (%)  
1021  
1022 Figure S1. Data completeness across all samples. Left histogram shows data  
1023 completeness as percent of bases in total alignment (concatenated alignment length  
1024 523,036 bp) exclusive of gaps (-) and missing bases (N). Right histogram shows data

1025 completeness as the absolute number of loci included per sample, as a representation of  
 1026 the number of gene trees per sample.



1027  
 1028 Figure S2. Basic summary statistics of the 450 locus alignments and gene trees. Top row  
 1029 shows histograms of the number of taxa in (max=101, min=11) and length of each  
 1030 alignment. Bottom row shows gene tree--species tree distances as quartet dissimilarity  
 1031 scores and Robinson Foulds distances, two different measures of topological similarity.  
 1032 Both quartet dissimilarity and RF scores are estimated by first subsetting the species  
 1033 tree to match gene tree sampling.  
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## AUSTRALIAN FROG PHYLOGENOMICS



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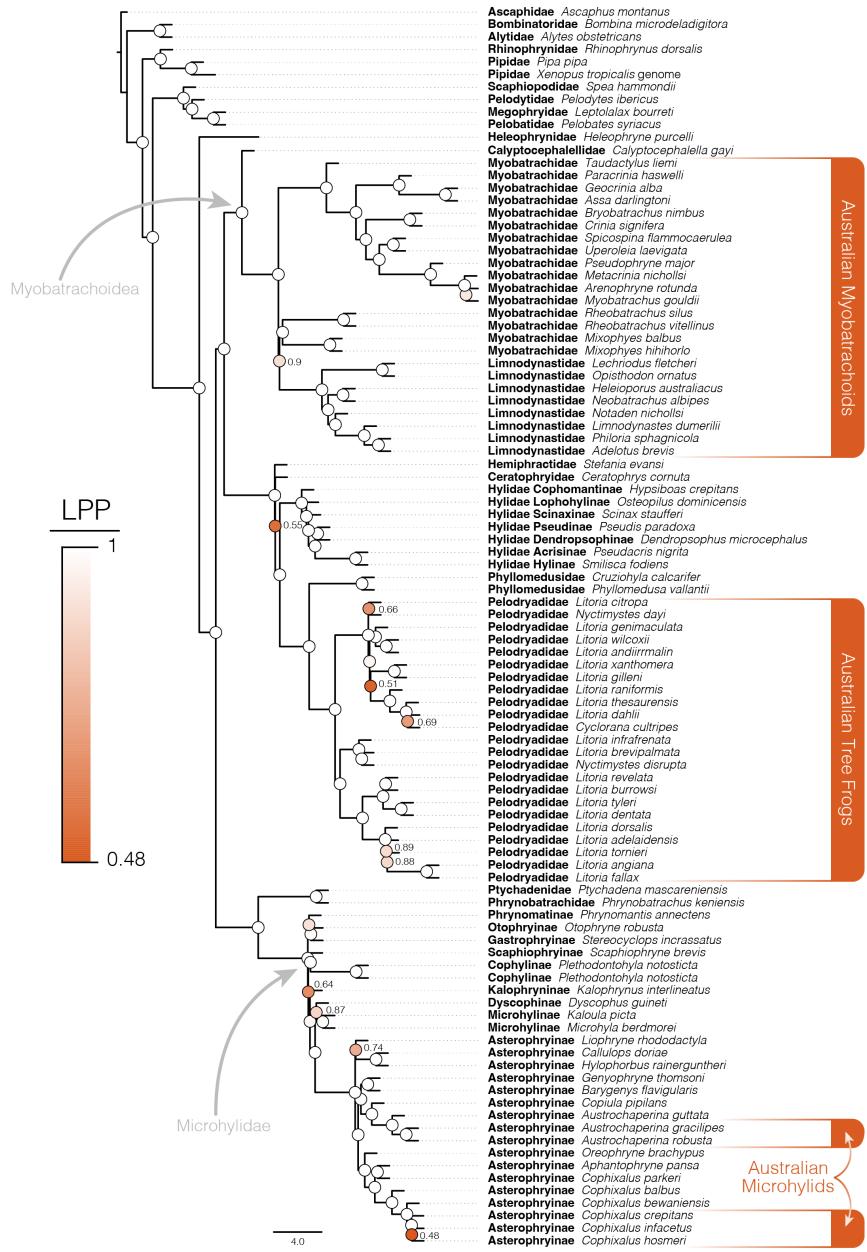
1036 Figure S3. Detailed summary statistics of the 450 locus alignments and gene trees. Top  
 1037 row compares AT content and number of variable sites against quartet distance between  
 1038 each gene tree and the species tree (a measure of topological similarity). The second and  
 1039 third rows compare measures of locus informativeness (number of variable sites, number

Brennan et al.

1040 of parsimony informative sites) against alignment length and AT content. The bottom  
1041 row shows alignment length and number of variable sites as a function of the number of  
1042 taxa in the alignment. In all plots points (representing trees or alignments) are colored  
1043 according to the quartet distance from the species tree.

1044

## AUSTRALIAN FROG PHYLOGENOMICS



1046 Figure S4. Species tree of Australian frogs and appropriate outgroup taxa estimated using  
1047 ASTRAL with locus trees estimated by IQTREE as input. Phylogenetic resolution among  
1048 major frog groups and within Australian frog clades is consistently high. Ultrafast  
1049 bootstrap support values (Hoang et al. 2018) are shown at nodes and colored according  
1050 to local posterior probabilities (LPP), values >0.9 are considered strongly supported and  
1051 not indicated at nodes (white circles).

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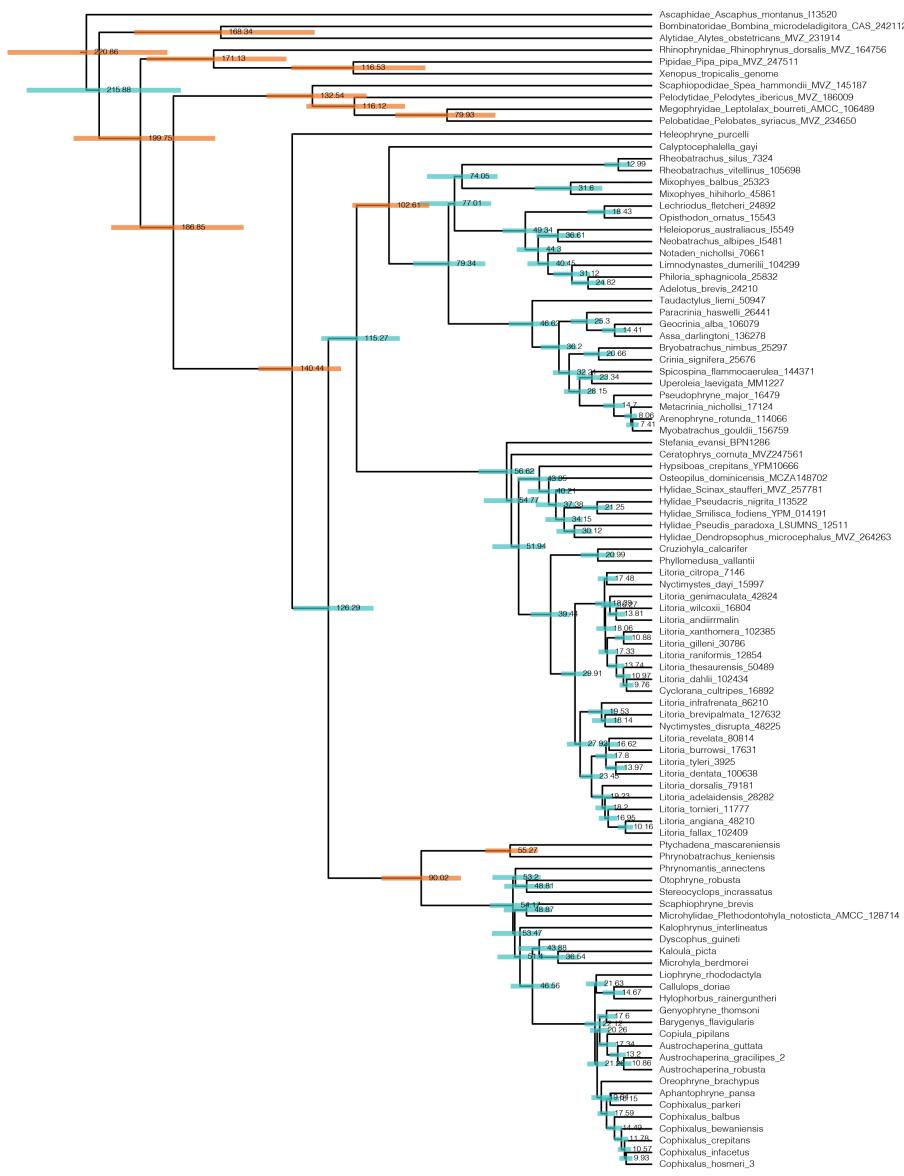
## AUSTRALIAN FROG PHYLOGENOMICS



1058 Figure S5. Species tree of Australian frogs and appropriate outgroup taxa estimated  
1059 from the concatenated sequence alignment under the GHOST model implemented in  
1060 IQTREE. Phylogenetic resolution among major frog groups and within Australian frog  
1061 clades is consistently high. Only ultrafast bootstrap support values less than 100 are  
1062 noted, here by grey branches and text (Hoang et al. 2018). This topology is highly  
1063 consistent with the phylogeny estimated using ASTRAL (Fig.2, S4), however three  
1064 differences are highlighted by orange branches and arrows indicating their location.  
1065 Branch lengths are weighted averages over four heterotachy classes.

## AUSTRALIAN FROG PHYLOGENOMICS

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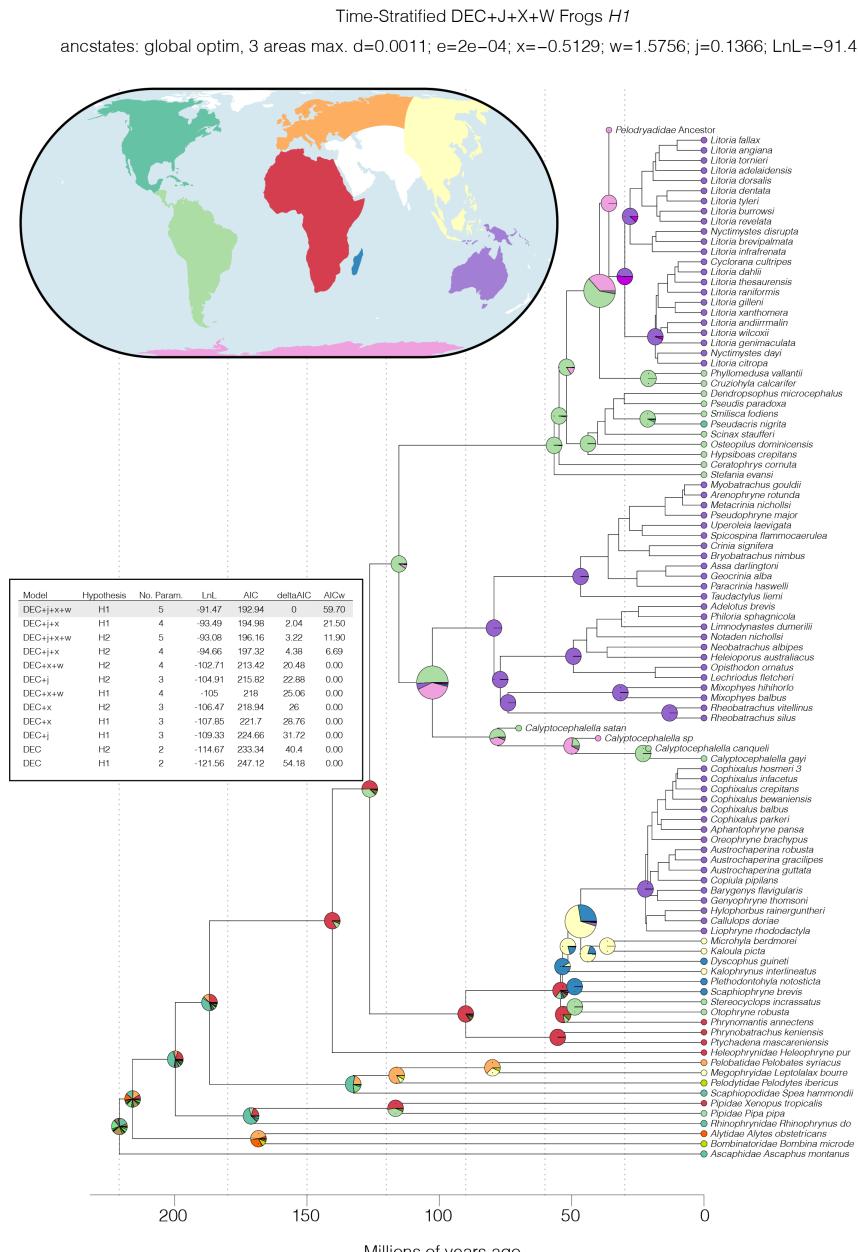


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1068 Figure S6. Species tree of Australian and outgroup frogs estimated with ASTRAL from  
1069 IQTREE genetrees and time-calibrated with MCMCTree. Shaded bars at nodes indicate  
1070 95% confidence estimates on ages and numbers indicate mean age estimates. Orange  
1071 shaded bars indicate nodes which were calibrated with fossil evidence (see Table  
1072 S2).  
1073

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## AUSTRALIAN FROG PHYLOGENOMICS



1076 Figure S7. Biogeographic history of frogs with a focus on the range reconstruction of  
1077 Australian clades. Inset table shows the 12 models fit to the data (6 models across two  
1078 'datasets'), ordered by deltaAIC. Ancestral range estimates under the preferred model  
1079 DEC+j+x+w H1 are shown at right as pie charts on the phylogenomic tree with several  
1080 fossil taxa added. Pie chart for the most recent common ancestor of each Australian  
1081 clade is enlarged to enhance visualization. The eight bioregions are shown in the inset  
1082 map and colors correspond to the tip state of taxa on the tree. Additional colors in the  
1083 pie charts correspond to combinations of areas, but are not discussed further.

AUSTRALIAN FROG PHYLOGENOMICS

- 1084  
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