

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 hylids,
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 Asian immigrants and include arguably the continent's oldest living vertebrate
12 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.

17

Keywords: Anuran; adaptive radiation; Gondwana; phylogenetics

18 *Introduction*

19 Frogs are an ancient vertebrate radiation originating in the Permian more than
20 250 million years ago (Hime et al. 2021). They share a unique and unusual morphology
21 yet are a spectacularly successful group, with more than 7,500 extant species spread
22 across most of the world (AmphibiaWeb 2022). Despite their age, much of this diversity,
23 potentially more than 95%, has developed since the Cretaceous-Paleogene mass
24 extinction (65 mya) (Feng et al. 2017). Australia is one of the driest continents on Earth
25 yet, surprisingly, it is home to nearly 250 frog species. Australia's frogs belong to just
26 four anuran groups spread widely across the “modern frog” suborder Neobatrachia: (1)
27 Myobatrachoidea comprising the Limnodynastidae (66 species) and Myobatrachidae (70
28 spp.); (2) Hyloidea represented by the family Pelodryadidae (91 spp.); (3) the
29 Microhylidae subfamily Asterophryinae (24 spp.); and (4) a single Ranidae species in the
30 genus *Papurana*. These groups show very different levels of species richness and
31 geographic spread across the continent (Fig.1). However, together they have radiated to
32 inhabit almost every part of Australia including tropical rainforests, alpine streams,
33 featureless boulder piles, and hyper-arid deserts.

34 While we know a great deal about many aspects of Australian frog biology (Tyler
35 1998; Anstis 2017), the age of each of the major groups and the timing of their
36 subsequent diversification, is poorly understood. Since the origin of frogs over 250
37 million years ago, the landmass that is now Australia has traveled extensively. Long ago
38 it was part of the supercontinent Pangea before separating as a component of
39 Gondwana alongside South America, Africa, Antarctica, and India. Sometime around 50
40 million years ago Australia separated from Antarctica and began drifting alone towards
41 Asia (Hall 2002; Bijl et al. 2013). Given the long evolutionary history of frogs, and
42 Australia's varied geographic affinities with other landmasses, we ask three related
43 questions: (1) Where did Australia's frogs originate? (2) When did they get to

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

47

48 *Materials and Methods*

49 We assembled an exon-capture dataset comprising 99 frog species spanning all
50 major anuran clades and with particular focus on the families Pelodryadidae,
51 Microhylidae, Limnodynastidae and Myobatrachidae (Table S1). This dataset includes
52 near-complete (92%) genus-level sampling of Australia's frogs. We generated new
53 Anchored Hybrid Enrichment (AHE—Lemmon et al. 2012) data for 83 samples and
54 combined these with outgroup samples from Hime et al.'s (2021) amphibian
55 phylogenomic dataset. Outgroup sampling was designed around maximizing commonly
56 used anuran fossil calibrations to provide a consistent time-calibrated phylogenomic
57 estimate of Australian frogs. Data from different AHE projects were combined using
58 custom scripts which relied on *metablastr* to identify orthologous loci
59 (*blast_best_reciprocal_hit*) (Benoit & Drost 2021), *mafft* to align them (*--add*, --
60 *keeplength*) (Katoh et al. 2013), and *AMAS* to manipulate alignments (Borowiec 2016).
61 We reconstructed individual genealogies for our exon-capture data (n = 450) under
62 maximum-likelihood in IQTREE (Nguyen et al. 2015), allowing the program to assign
63 the best fitting model of nucleotide substitution using ModelFinder (Kalyaanamoorthy
64 et al. 2017) and then perform 1,000 ultrafast bootstraps (Minh et al. 2013). We then
65 estimated a species tree using the quartet-based summary method ASTRAL III (Zhang
66 et al. 2018) with IQTREE gene trees as input. To estimate divergence times among taxa
67 we applied a series of fossil calibrations first compiled by Feng et al. (2019) (Table S2)
68 and used the Bayesian divergence time software MCMCTree (Rannala & Young 2007).
69 We started by concatenating all loci and partitioning them into two partitions, first and

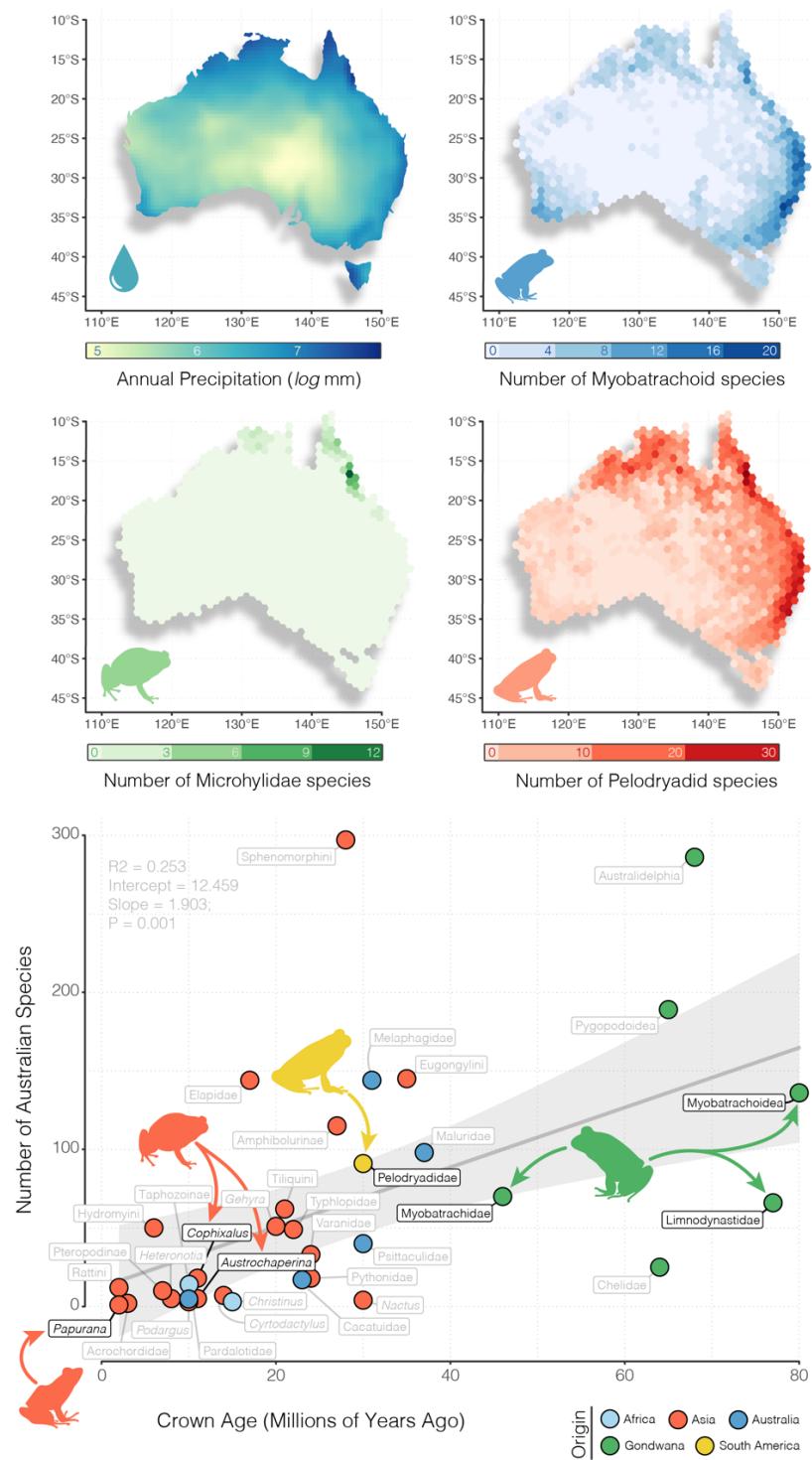
70 second codons together, and third codons separately. We then used *baseML* to estimate
71 approximate likelihoods (dos Reis & Yang 2011) and branch lengths before running
72 *mcmcTree* on the gradient and Hessian (in.BV file) for ten replicate analyses. We
73 inspected mcmc files for stationarity and compared for convergence, then combined
74 them using logCombiner, and used this combined mcmc file to summarize divergence
75 times on our tree (*print* = -1 in .ctl file). Sample, alignment, and gene tree summary
76 statistics are presented in Supplementary Material (Fig.S1-3) and are available alongside
77 all other materials on Dryad (NNNN) and GitHub
78 (https://github.com/IanGBrennan/Crown_Frogs).

79

80 *Results*

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

96 Australia and Chile is ancient, occurring more than 100 million years ago. The
97 remaining phylogenomic topology and divergence estimates of frogs are broadly
98 consistent with previous results (Feng et al. 2017; Hime et al. 2021) (Fig.2, S4, S5).
99



100

101

102 Figure 1. Australian frogs show a pattern of increasing species richness with
 103 precipitation, and with time. Above, maps of richness for the three focal radiations
 104 (with Limnodynastidae and Myobatrachidae presented together as Myobatrachoidea)

105 are plotted alongside a map showing total annual precipitation. Greatest richness is
106 concentrated along Australia's east coast, however this pattern falls away in cold areas
107 of the far southeast, particularly in southwest Tasmania. Species occurrence records
108 were collated from the Atlas of Living Australia (<https://ala.org.au>). Below, Australian
109 radiations can be divided broadly into (1) relictual Gondwanan clades >40 myo (green),
110 (2) ancient colonizing groups (>20 myo, <40 myo; varied colors), or (3) immigrant
111 clades of Asian origin (orange). Each point is colored according to the region of
112 hypothesized origin and labeled by the narrowest phylogenetic taxonomy. Black labels
113 indicate focal groups and grey labels indicate other Australian vertebrate clades.
114 Regression in background is fit to all points and shows a general pattern of increasing
115 species richness with age.

116

117 *Discussion*

118 Here we present the first reliable estimates of relationships among all of
119 Australia's frog genera and major clades of the mega-genus *Litoria*. Our investigation
120 into the timing and origins of the Australian frog fauna reveals that each radiation
121 originated or arrived during different periods in Australia's history, demonstrating a
122 staggered colonization and population of the continent. This stratified arrival means
123 each radiation established itself in a very different incarnation of the continent. Across
124 these eras Australia has flourished through a warm and wet Eocene, cooling and drying
125 following the onset of Antarctic glaciation in the Oligocene, warm and forested Miocene,
126 and a gradual aridification leading to its present status (Byrne et al. 2011, Pross et al.
127 2012, Macphail & Hill 2018, Mao & Retallack 2019).

128

129 *Origins and Biogeography*

130 The Myobatrachidae and Limnodynastidae (together—myobatrachoids) represent
131 the oldest, most diverse (136 spp.), and only near-endemic of Australia’s frog radiations
132 (one species—*Lechriodus aganoposis* is found in New Guinea). They share a long history
133 with South America and its Gondwanan past. Anchored by a deep split with the South
134 American *Calyptocephallela* (roughly 100 mya; Fig.2), early divergences among the
135 Australian myobatrachoids, principally between myobatrachids and limnodynastids,
136 occurred in the late Cretaceous (80–70 mya), preceding the isolation of Australia from
137 Antarctica. This dates to a time when South America, Antarctica, and Australia were a
138 continuous landmass that was likely temperate in climate (Palazzi & Barreda 2007;
139 Mörs et al. 2020). The phylogenetic depth and distribution of myobatrachoids (including
140 *Calyptocephallela*) across these now widely disjunct continents suggests a historically
141 continuous distribution across southern Gondwana, including Antarctica. This idea is
142 supported by the recent discovery of an extinct calyptocephallelid from mid-Miocene
143 Antarctica that lived more than 40 mya (Mörs et al. 2020). The persistence of
144 calyptocephallelids in Antarctica into the Late Eocene highlights the dichotomy between
145 young extant myobatrachid and limnodynastid diversity (most species < 30 mya) and
146 ancient splits between limnodynastids and myobatrachids and within limnodynastids (>
147 70 mya). These long branches are likely the survivors of a much greater southern
148 Gondwanan myobatrachoid diversity, potentially mirroring the diversity of extinct
149 calyptocephallelids through southern South America and Patagonia (Nicoli et al. 2022).

150 Australian myobatrachoids however are not the only group with close
151 connections to South America. The Pelodryadidae are a species rich (91 spp.) and
152 morphologically diverse clade of Australasian frogs. Embedded within the primarily
153 Neotropical treefrogs, they show a more recent late-Eocene divergence from their South
154 American relatives the Phyllomedusidae, some 40 mya. Crown divergence of the
155 pelodryadids occurred in the mid-to-late Oligocene (30 mya) before erupting into a

radiation across Australia and New Guinea in the early Miocene. This timing has spurred speculation about the origins of pelodryadids either as part of a young Gondwanan group or more recent over-water dispersers from South America (Pyron 2014). Divergence between phyllomedusids and pelodryadids 40 mya aligns with the opening of the Drake Passage and separation of South America from Antarctica (Toumoulin 2020). Unfortunately this does not provide any certainty about how pelodryadids arrived in Australia. While the Brazil Current would have provided a favorable trajectory for rafting frogs, the over-water distance between South America and Australia remained immense. A more likely scenario is that pelodryadids dispersed from South America through Antarctica and into Australia. Climate reconstructions suggest warm temperate/tropical habitats across Antarctica which would have been suitable through a long period of the Eocene (Pross et al. 2012). Dispersal via Antarctic land bridges would have had to occur prior to the Eocene-Oligocene cooling (34 mya) that blanketed Antarctica beneath an ice sheet (van den Ende et al. 2017).

Contrasting with the comparatively ancient limnodynastids, myobatrachids, and pelodryadids, Australia's youngest anuran radiation are the microhylids. Embedded deeply in the Asterophryinae subfamily, the two temporally adjacent clades (12–13 mya) of *Austrochaperina* and *Cophixalus* crossed the gap from New Guinea to Australia in the mid Miocene. This time frame coincides with a period of increased variation in sea surface levels driven by cooling global temperatures following the mid Miocene climatic optimum. Dropping sea levels likely repeatedly exposed a landbridge between southern New Guinea and northern Australia (both Cape York and the Top End) and facilitated biotic exchange between these landmasses (Mitchell et al. 2014). The young age of these clades, and existence of two other species-rich incumbent frog clades in the pelodryadids and myobatrachoids potentially explains why Australian microhylids are relatively species poor (*Austrochaperina*—5 spp., *Cophixalus*—18 spp.) and morphologically

182 conservative compared to their New Guinean neighbors (200+ spp.), reflecting a pattern
183 seen in monitor lizards (Pavón-Vázquez et al. 2021).

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

194 The staggered temporal origins of Australian frogs exemplifies the general
195 colonization history of Australian vertebrates. Radiations of mammals, birds, frogs, and
196 reptiles fall into discretized temporal groups broadly identified as (1) Gondwanan relicts
197 >40 myo, (2) old colonizers (>20 myo, <40 myo) with varied origins, or (3) recent
198 Asian immigrants (<20 myo). The Limnodynastidae and Myobatrachidae fall
199 undoubtedly into the Gondwanan group alongside ancient Australian radiations like
200 Australidelphian marsupial mammals which include koalas, kangaroos, and Tasmanian
201 devils; side-necked chelid turtles; and pygopodoid geckos which include the bizarre
202 limbless pygopodids. These groups—with the exception of pygopodoids—have close links
203 to South American relatives based on molecular and fossil evidence (Georges et al. 1999;
204 Mitchell et al. 2014). While a Pelodryadidae link with South America is clear, they are
205 perhaps the sole radiation to have emigrated from South America to Australia since the
206 continental breakup. Most other similarly aged Australian groups instead show signal of
207 Asian or Australian origins. In comparison, the Australian microhylids

208 (*Austrochaperina*, *Cophixalus*) and the ranid *Papurana daemeli* are relatively young
209 colonizers from New Guinea with deeper origins in Asian groups. Both the
210 Asterophryinae and Ranidae, to which these species belong, have a long history in the
211 Sunda and Wallacean regions, reflecting patterns of old diversity in this tectonically
212 active area. Alongside a number of other groups such as pythons (Esquerré et al. 2020),
213 monitor lizards (Brennan et al. 2021), honeyeater birds (Marki et al. 2017), dragon
214 lizards (Tallowin et al. 2020), elapid snakes (Keogh 1998), various gekkonid gecko
215 genera (Heinicke et al. 2011), megabats, frogmouth birds (Oliver et al. 2020), cockatoos
216 and parrots (Schweizer et al. 2011), several skink subfamilies (Skinner et al. 2011), and
217 two rodent groups (Roycroft et al. 2020), they share diversity across Australia and New
218 Guinea with repeated exchange between the two islands. Many of these groups show a
219 telltale stepping stone biogeographic pattern that links them back to mainland Asian
220 ancestors, with Australo-Papuan members deeply phylogenetically nested. In general,
221 these Australian clades show a pattern of increasing species richness with clade age,
222 however the drivers of such a pattern are likely idiosyncratic (Fig.1).

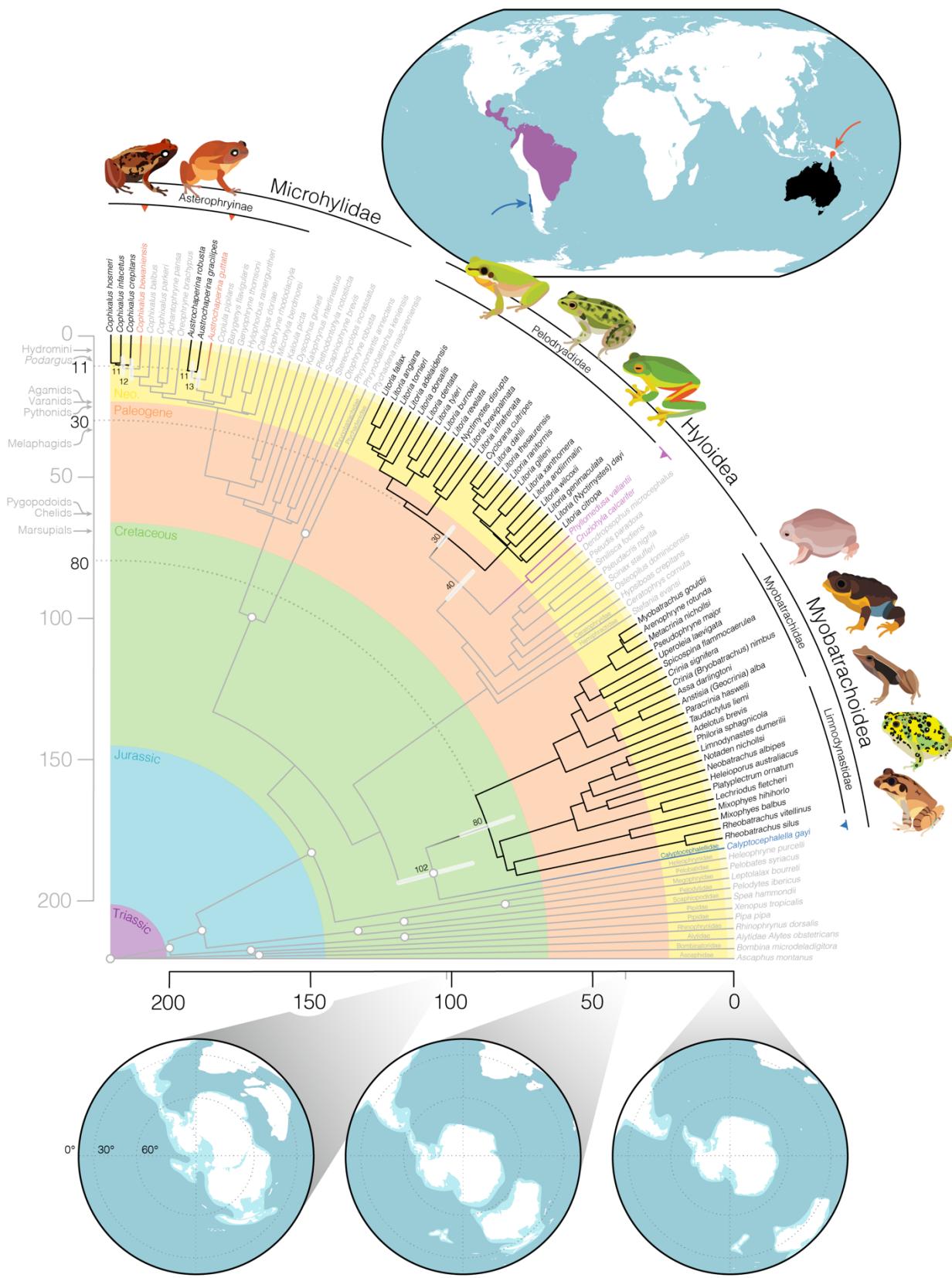
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224 *Macroevolutionary Patterns*

225 The radiation of frogs in Australia has occurred over a deep timescale and across
226 a changing climatic landscape. Old species-poor lineages have become confined to the
227 mesic-temperate fringes of the continent, while new niches and species have popped up
228 in the expanding arid zone. And while frogs are found across most of the Australian
229 continent, their basic moisture requirements and desiccation sensitivity mean that
230 Australian amphibian diversity shows a stark mesic-arid gradient (Fig.1), similar to that
231 seen for birds and mammals, and the inverse of lizards (Powney et al. 2010). Not all has
232 been lost in the red center though—several independent clades of dry-country
233 inhabitants have evolved among Australia’s harsh sandy and stony deserts.

234 *Neobatrachus*, *Notaden*, and *Cyclorana* have all evolved to aestivate through the hottest
235 and driest seasons. These genera (commonly known as the water-holding frogs) are
236 capable of growing epidermal cocoons to retain moisture that may see them through
237 periods of extreme drought lasting from months to years (van Beurden 1980).

238 Along with changes in habitat and ecology, Australia's frogs have also
239 accumulated vast diversity in reproductive strategy, ontogenetic trajectory, and
240 morphology (Crump 2015, Duellman 1992, Sherratt et al. 2018). While we do not
241 present data on these topics, our well-resolved phylogenetic hypothesis provides new
242 context for the macroevolution of some of these extreme traits. Bizarre rearing habits
243 such as raising young in stomachs (*Rheobatrachus*), hip-pockets (*Assa*), or subterranean
244 nests (*Myobatrachus*) exist on both long branches and deeply nested taxa suggesting a
245 remarkable frequency of transition among states. Similarly, morphological variation has
246 rapidly evolved to dramatic extremes. The long limbed highly aquatic *Litoria dahlii*
247 with webbed feet and dorsally situated eyes is sister to the short-limbed burrowing
248 water-holding frogs *Cyclorana* (Vidal-Garcia & Keogh 2015). Together these frogs are
249 embedded deeply within the otherwise toe-padded and arboreal tree frogs, highlighting
250 the adaptive capacity of pelodryadids. Myobatrachoids too have taken ecomorphology to
251 the extreme, offering us what is perhaps the world's strangest living anuran, the turtle
252 frog *Myobatrachus gouldii*. In pursuit of their backwards burrowing lifestyle and termite-
253 heavy diet, *Myobatrachus* lack many of the characteristics we typically associate with
254 frogs. Their beady black eyes are set in small heads and, alongside their sister taxon
255 *Arenophryne*, they crawl—not jump—across the ground on short limbs that are
256 incapable of hopping (Vidal-Garcia et al. 2014).



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

275

276 *Conclusion*

277 Australian frogs offer important insights into colonization, persistence, and
278 diversification of a major continental group through deep time. The varied species
279 richness, timing of diversification, and ecomorphological diversity among replicate
280 radiations provides evidence of the processes dictating the accumulation of biodiversity.
281 Beyond the temperate and tropical forests of the east and north coast, the Australian
282 continent is an open country of habitat scarcely welcoming to frogs. Despite this,
283 anurans have a long history in Australia and have diversified into an amazing array of

284 forms, colors, and lifestyles. This success is potentially the result of the stratified
285 temporal arrival of the three main frog clades and possibly exaggerated by their
286 ecological differences. Our phylogenetic framework provides a foundation for examining
287 how temporal changes to climate, habitat, and niche space have influenced the
288 diversification of one of Australia's richest and most unique vertebrate faunas.

289

290

291 *Data Accessibility*

292 Sequence alignments, analysis control files, and phylogenetic trees can be
293 downloaded from Dryad: *link to be updated upon submission*.

294

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464 *Figure Captions*

465 Figure 1. Australian frogs show a pattern of increasing species richness with
466 precipitation, and with time. Above, maps of richness for the three focal radiations
467 (with Limnodynastidae and Myobatrachidae presented together as Myobatrachoidea)
468 are plotted alongside a map showing total annual precipitation. Greatest richness is
469 concentrated along Australia's east coast, however this pattern falls away in cold areas
470 of the far southeast, particularly in southwest Tasmania. Species occurrence records
471 were collated from the Atlas of Living Australia (<https://ala.org.au>). Below, Australian
472 radiations can be divided broadly into (1) relictual Gondwanan clades >40 myo (green),
473 (2) ancient colonizing groups (>20 myo, <40 myo; varied colors), or (3) immigrant
474 clades of Asian origin (orange). Each point is colored according to the region of
475 hypothesized origin and labeled by the narrowest phylogenetic taxonomy. Black labels
476 indicate focal groups and grey labels indicate other Australian vertebrate clades.
477 Regression in background is fit to all points and shows a general pattern of increasing
478 species richness with age.

479

480 Figure 2. Time-calibrated frog phylogeny highlights the varied origins and staggered
481 arrival of the four major frog families that comprise the Australian anuran fauna.
482 Primarily Australian clades are identified by black branches and text, their closest living
483 relatives outside of Australia are noted by colored branches and text, and outgroup taxa
484 are grey. White circles at nodes identify the location of fossil calibrations (see Table S2).
485 Upper inset map shows the general geographic location of: (red) closely related
486 microhylids in New Guinea, (purple) phyllomedusid hylids in South America, and (dark
487 blue) *Calyptocephallela* in Chile. Lower inset maps show the connection and proximity
488 of Australia to other Gondwanan continents as Australia drifted away over the past 100
489 million years. White indicates contemporary coastlines, light blue the continental plates,

490 and dark blue the oceans. Maps were generated using GPlates and input files modified
491 from Landis (2017). Partial fan phylogeny was plotted using *phytools* in the R
492 programming environment. Annotations on vertical time axis show the age of crown
493 divergences of other notable Australian groups for temporal context (see Fig.1). Species
494 illustrated clockwise from top left: *Cophixalus infacetus*, *Austrochaperina robusta*,
495 *Litoria fallax*, *Litoria dahlii*, *Litoria xanthomera*, *Myobatrachus gouldii*, *Spicospina*
496 *flammoecaerulea*, *Taudactylus acutirostris*, *Notaden bennettii*, *Mixophyes balbus*.
497
498

499 Supplementary Materials

500

501 Data available from the Dryad Digital Repository:

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

503 and from the GitHub repository: https://github.com/IanGBrennan/Crown_Frogs

504

505 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>Rhinophrynus dorsalis</i>	MVZ 164756
Outgroup	Leiopelmatoidae	Ascaphidae	—	<i>Ascaphus montanus</i>	REF AscMon
Outgroup	Discoglossoidea	Bombinatoridae	—	<i>Bombina microdelinqitora</i>	CAS 242112
Outgroup	Discoglossoidea	Alytidae	—	<i>Alytes obstetricans</i>	MVZ 231914
Outgroup	Pelobatoidea	Scaphiopodidae	—	<i>Spea hammondii</i>	MVZ 145187
Outgroup	Pelobatoidea	Pelodrytidae	—	<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>Kalophrynus interlineatus</i>	ABTC 105933
Outgroup	Ranoidea	Microhylidae	Dyscophinae	<i>Dyscophus quinetti</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 rainierguntheri</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>Copula 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

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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	Rheobatrachidae	—	<i>Rheobatrachus silus</i>	ABTC 7324
Australian Clade	Myobatrachoidea	Rheobatrachidae	—	<i>Rheobatrachus vitellinus</i>	ABTC 105698
Australian Clade	Myobatrachoidea	Mixophyidae	—	<i>Mixophyes balbus</i>	ABTC 25323
Australian Clade	Myobatrachoidea	Mixophyidae	—	<i>Mixophyes hihihorlo</i>	ABTC 45861
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Lechriodus fletcheri</i>	ABTC 24892
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Opisthodon ornatus</i>	ABTC 15543
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Heleioporos 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	Mvobatrachoidea	Limnodynastidae	—	<i>Philaria sphaanicola</i>	ABTC 25832
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Adelotus brevis</i>	ABTC 24210
Australian Clade	Mvobatrachoidea	Mvobatrachidae	—	<i>Taudactylus liemi</i>	ABTC 50947
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Paracrinia haswelli</i>	ABTC 26441
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Geocrinia alba</i>	ABTC 106079
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Assa darlingtoni</i>	ABTC 136278
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Bryobatrachus nimbus</i>	ABTC 25297
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Crinia signifera</i>	ABTC 25676
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Spicospina flammoecaerulea</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	Mvobatrachoidea	Mvobatrachidae	—	<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	Hyoioidea	Hemiphractidae	—	<i>Stefania evansi</i>	BNP1286
Outgroup	Hyoioidea	Ceratophryidae	—	<i>Ceratophrys cornuta</i>	MVZ 247561
Outgroup	Hyoioidea	Hylidae	Cophomantinae	<i>Hypsiboas crepitans</i>	YPM 10666
Outgroup	Hyoioidea	Hylidae	Lophohylinae	<i>Osteopilus dominicensis</i>	MCZA148702
Outgroup	Hyoioidea	Hylidae	Scinaxinae	<i>Scinax staufferi</i>	MVZ 257781
Outgroup	Hyoioidea	Hylidae	Pseudinae	<i>Pseudis paradoxa</i>	LSUMNS 12511
Outgroup	Hyoioidea	Hylidae	Dendropsophinae	<i>Dendropsophus microcephalus</i>	MVZ 264263
Outgroup	Hyoioidea	Hylidae	Acrisinae	<i>Pseudacris nigrita</i>	REF PseNig
Outgroup	Hyoioidea	Hylidae	Hylinae	<i>Smilisca fodiens</i>	YPM 014191
Outgroup	Hyoioidea	Phyllomedusidae	—	<i>Cruziohyla calcarifer</i>	QCAZ 48552
Outgroup	Hyoioidea	Phyllomedusidae	—	<i>Phyllomedusa vallantii</i>	QCAZ 48818
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Litoria citropa</i>	ABTC 7146
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Nyctimystes dayi</i>	ABTC 15997
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Litoria genimaculata</i>	ABTC 42824
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Litoria wilcoxii</i>	ABTC 16804
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Litoria andirrrmalin</i>	ABTC 142651
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Litoria xanthomera</i>	ABTC 102385
Australian Clade	Hyoioidea	Pelodryadidae	—	<i>Litoria ailleni</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
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

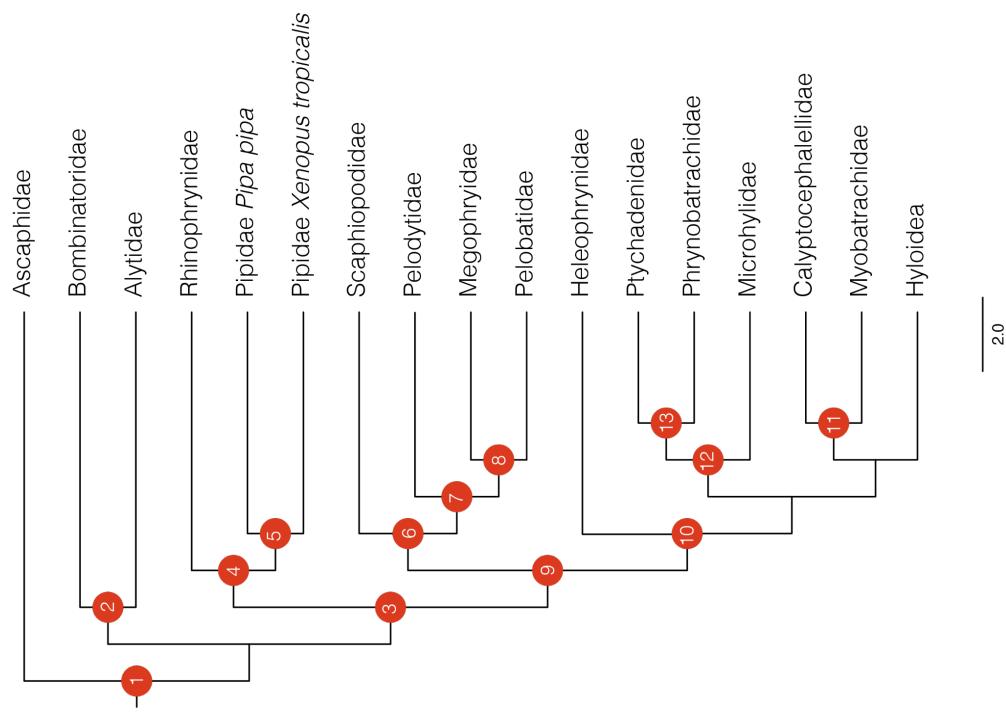
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508 Table S2. Fossil calibrations implemented in MCMCTree analysis of frog divergence
 509 dates. Node number (#) corresponds to nodes in supplementary figure below.
 510

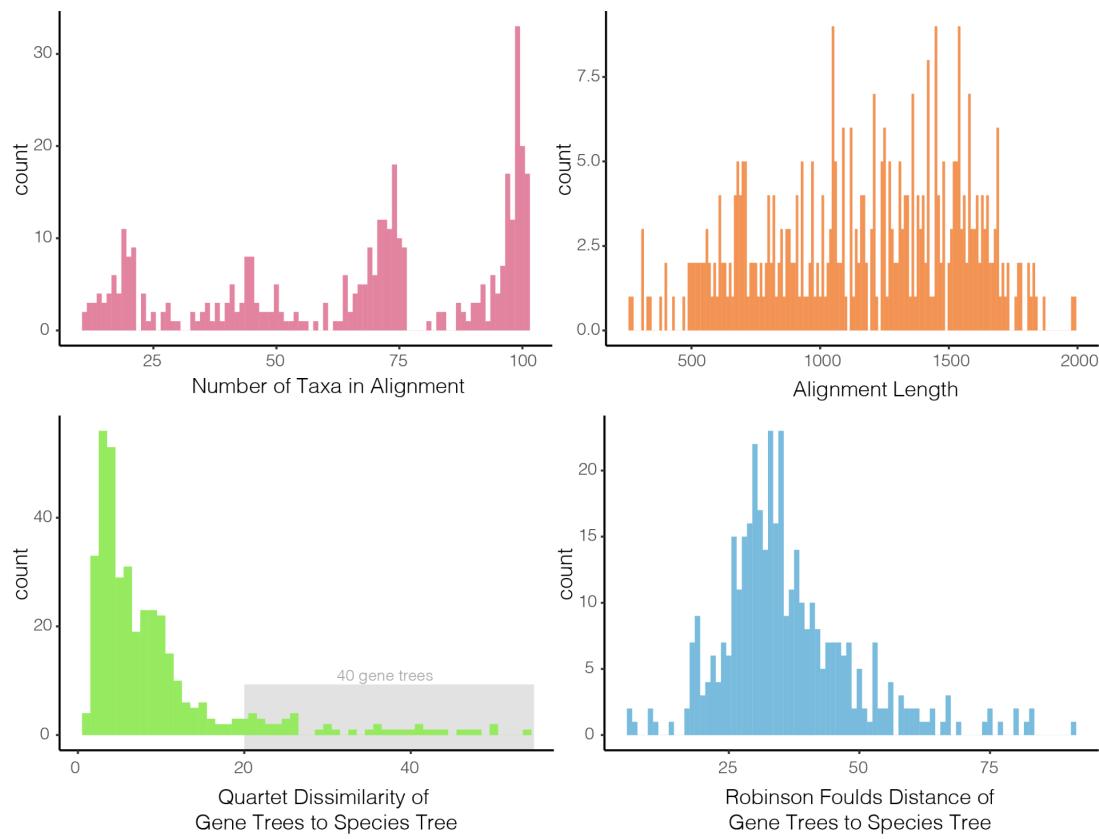
#	Node Calibrated	Fossil	Minimum	Soft Max.	Source (see Feng et al. 2017)
1	Anura	\dagger <i>Liaobatrachus zhaoi</i>	129.7	252	Chang et al. (2009)
2	Alytoidea	\dagger <i>Iberobatrachus angelae</i>	125	252	Gomez et al. (2016)
3	Pipanura	\dagger <i>Rhadinosteus parvus</i>	148.1	252	Cannatella (2015)
4	Pipoidea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

511

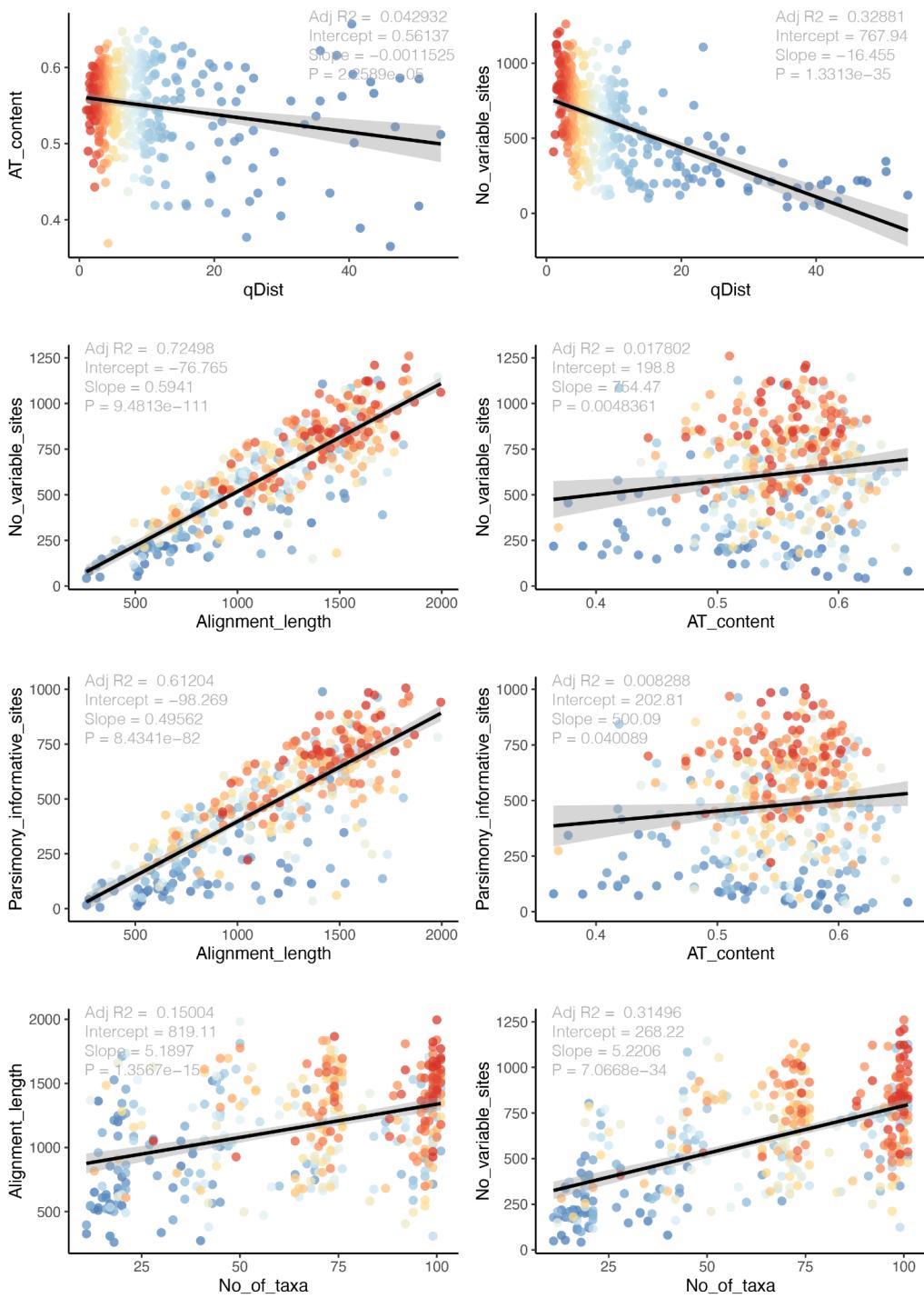
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516 Figure S1. Data completeness across all samples. Left histogram shows data
517 completeness as percent of bases in total alignment (concatenated alignment length
518 523,036 bp) exclusive of gaps (-) and missing bases (N). Right histogram shows data
519 completeness as the absolute number of loci included per sample, as a representation of
520 the number of gene trees per sample.



521
522 Figure S2. Basic summary statistics of the 450 locus alignments and gene trees. Top row
523 shows histograms of the number of taxa in (max=101, min=11) and length of each
524 alignment. Bottom row shows gene tree--species tree distances as quartet dissimilarity
525 scores and Robinson Foulds distances, two different measures of topological similarity.
526 Both quartet dissimilarity and RF scores are estimated by first subsetting the species
527 tree to match gene tree sampling.
528

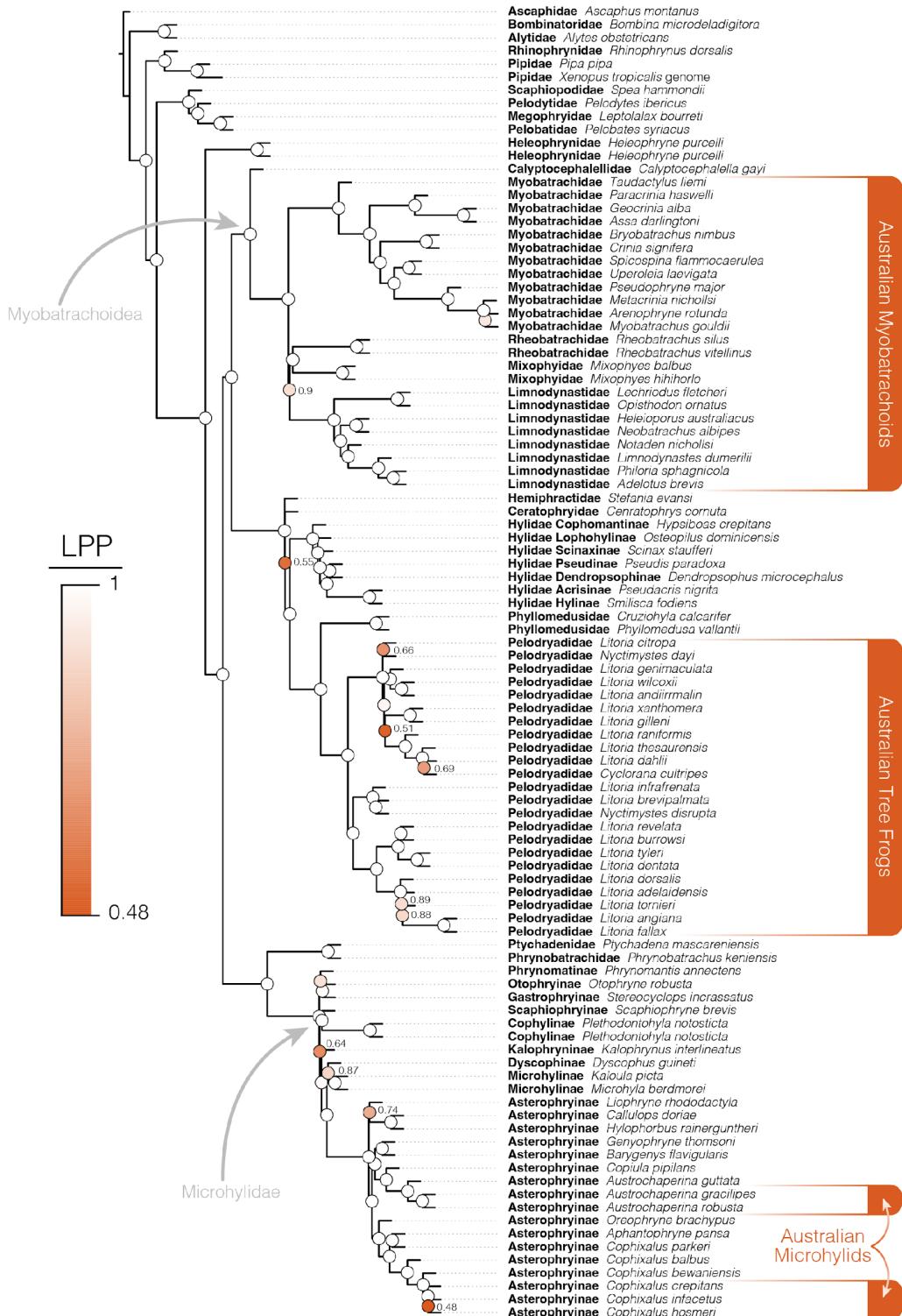


529

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

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

538



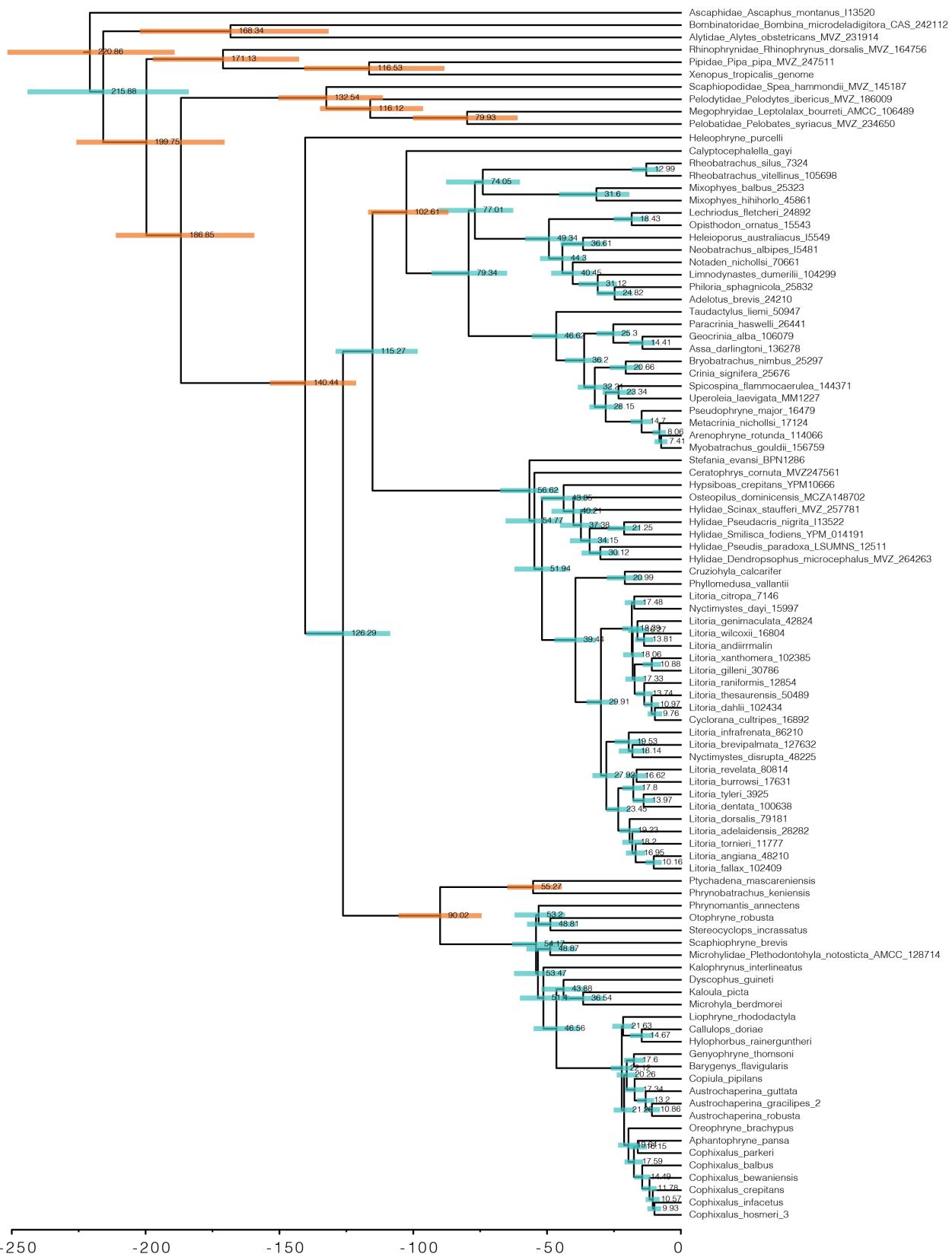
539

540 Figure S4. Species tree of Australian frogs and appropriate outgroup taxa estimated using
 541 ASTRAL with locus trees estimated by IQTREE as input. Phylogenetic resolution among
 542 major frog groups and within Australian frog clades is consistently high. Support values

543 are shown at nodes and colored according to local posterior probabilities (LPP), values

544 >0.9 are considered strongly supported and not indicated at nodes (white circles).

545



547 Figure S5. Species tree of Australian and outgroup frogs estimated with ASTRAL from
548 IQTREE genetrees and time-calibrated with MCMCTree. Shaded bars at nodes indicate
549 95% confidence estimates on ages and numbers indicate mean age estimates. Orange
550 shaded bars indicate nodes which were calibrated with fossil evidence (see Table
551 S2).