

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

16
17 *Introduction*

18 Frogs are an ancient vertebrate radiation originating in the Permian more than 250 million
19 years ago (Hime et al. 2021). They share a unique and unusual morphology yet are a spectacularly
20 successful group, with more than 7,500 extant species spread across most of the world
21 (AmphibiaWeb 2022). Despite their age, much of this diversity, potentially more than 95%, has
22 developed since the Cretaceous-Paleogene mass extinction (65 mya) (Feng et al. 2017). Australia
23 is one of the driest continents on Earth yet, surprisingly, it is home to nearly 250 frog species.
24 Australia's frogs belong to just four anuran groups spread widely across the "modern frog" suborder
25 Neobatrachia: (1) Myobatrachidae comprising the Limnodynastidae (66 species) and

26 Myobatrachidae (70 spp.); (2) Hyloidea represented by the family Pelodryadidae (91 spp.); (3)
27 the Microhylidae subfamily Asterophryinae (24 spp.); and (4) a single Ranidae species in the
28 genus *Papurana*. These groups show very different levels of species richness and geographic spread
29 across the continent (Fig.1). However, together they have radiated to inhabit almost every part
30 of Australia including tropical rainforests, alpine streams, featureless boulder piles, and hyper-arid
31 deserts.

32 While we know a great deal about many aspects of Australian frog biology (Tyler 1998;
33 Anstis 2017), the age of each of the major groups and the timing of their subsequent diversification,
34 is poorly understood. Since the origin of frogs over 250 million years ago, the landmass that is
35 now Australia has traveled extensively. It was part of the supercontinent Pangea before separating
36 with what is now South America, Africa, Antarctica, and India as a component of Gondwana.
37 Australia separated from Antarctica more than 50 million years ago and then began drifting alone
38 towards Asia (Hall 2002; Bijl et al. 2013). Given the long evolutionary history of frogs, and
39 Australia's varied geographic affinities with other landmasses, we ask three related questions:
40 Where did Australia's frogs originate? When did they get to Australia? Who and where are their
41 closest relatives? The relative timing of these frog groups, their varied species richness, and
42 ecological diversity offer important insight into the evolution of a continental fauna.

43

44 *Materials and Methods*

45 We assembled an exon-capture dataset comprising 99 frog species spanning all major
46 anuran clades and with particular focus on the families Pelodryadidae, Microhylidae,
47 Limnodynastidae and Myobatrachidae (Table S1). This includes complete genus-level sampling of
48 Australia's frogs. We generated new Anchored Hybrid Enrichment (AHE—Lemmon et al. 2012)
49 data for 83 samples and combined these with outgroup samples from Hime et al.'s (2021)
50 amphibian phylogenomic dataset. Outgroup sampling was designed around maximizing commonly
51 used anuran fossil calibrations to provide a consistent time-calibrated phylogenomic estimate of
52 Australian frogs. Data from different AHE projects were combined using custom scripts which
53 relied on *metablastr* to identify orthologous loci (*blast_best_reciprocal_hit*) (Benoit & Drost
54 2021), *mafft* to align them (*--add, --keeplength*) (Katoh et al. 2013), and *AMAS* to manipulate
55 alignments (Borowiec 2016). We reconstructed individual genealogies for our exon-capture data
56 ($n = 450$) under maximum-likelihood in IQTREE (Nguyen et al. 2015), allowing the program to
57 assign the best fitting model of nucleotide substitution using ModelFinder (Kalyaanamoorthy et
58 al. 2017) and then perform 1,000 ultrafast bootstraps (Minh et al. 2013). We then estimated a
59 species tree using the quartet-based summary method ASTRAL III (Zhang et al. 2018) with
60 IQTREE gene trees as input. To estimate divergence times among taxa we applied a series of
61 fossil calibrations first compiled by Feng et al. (2019) (Table S2) and used the Bayesian divergence
62 time software MCMCTree (Rannala & Young 2007). We started by concatenating all loci and
63 partitioning them into two partitions, first and second codons together, and third codons
64 separately. We then used *baseml* to estimate approximate likelihoods (dos Reis & Yang 2011) and
65 branch lengths before running *mcmcTree* on the gradient and Hessian (in.BV file) for ten replicate
66 analyses. We inspected mcmc files for stationarity and compared for convergence, then combined
67 them using logCombiner, and used this combined mcmc file to summarize divergence times on our

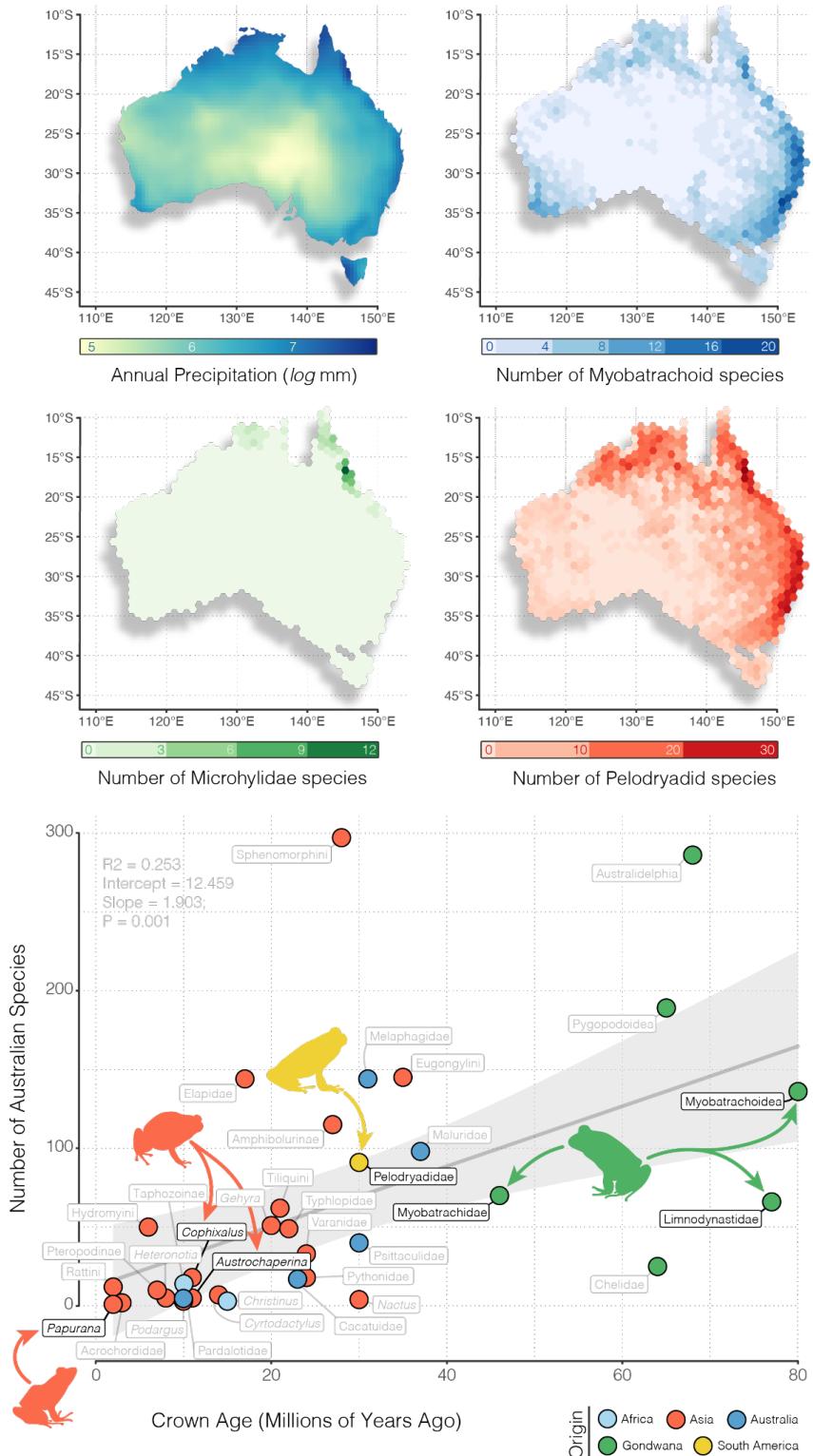
68 tree (*print* = -1 in .ctl file). Sample, alignment, and gene tree summary statistics are presented in
69 Supplementary Material (Fig.S1-3).

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71 *Results*

72 Crown divergences of the three Australian frog radiations can be clearly separated into old
73 (Myobatrachidae and Limnodynastidae—80 mya), intermediate (Pelodryadidae—30 mya), and
74 young (Asterophryinae—11 mya) (Fig.2). The youngest Australian group, microhylids in the genera
75 *Austrochaperina* and *Cophixalus*, are embedded deeply within the subfamily Asterophryinae and
76 appear to represent two separate, relatively recent (\simeq 11 mya) invasions of Australia from New
77 Guinea. Pelodryadidae tree frogs also share a complex biogeographic history across Australasia,
78 with several species groups split across the Torres Strait (separating Australia and New Guinea),
79 suggesting frequent biotic exchange. However, the origins of the Pelodryadidae are far older. Their
80 closest relatives are the iconic Phyllomedusidae found throughout Central and South America,
81 with the crown split between extant Pelodryadidae in Australia/New Guinea and South America
82 estimated at approximately 40 million years ago. Australian myobatrachids and limnodynastids
83 also have their closest relatives in South America—the Calyptocephalidae, represented here by
84 *Calyptocephallela*, the Helmeted Water Toad of Chile. The crown split between extant
85 myobatrachoids in Australia and Chile is ancient, occurring more than 100 million years ago. The
86 remaining phylogenomic topology and divergence estimates of frogs are broadly consistent with
87 previous results (Feng et al. 2017; Hime et al. 2021) (Fig.2, S4).

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91 Figure 1. Australian frogs show a pattern of increasing species richness with precipitation. Above, maps of
92 richness for the three focal radiations (with Limnodynastidae and Myobatrachidae presented together as
93 Myobatrachchoidea) are plotted alongside a map showing total annual precipitation. Greatest richness is
94 concentrated along Australia's east coast, however this pattern falls away in cold areas of the far southeast,

95 particularly in southwest Tasmania. Species occurrence records were collated from the Atlas of Living
96 Australia (<https://ala.org.au>). Below, Australian radiations can be divided broadly into (1) relictual
97 Gondwanan clades >40 myo, (2) ancient colonizing groups (>20 myo, <40 myo), or (3) immigrant clades
98 of Asian origin. Each point is labeled by the narrowest phylogenetic taxonomy and colored according to the
99 region of hypothesized origin. Overall, Australian vertebrate clades show a general pattern of increasing
100 species richness with age.

101

102

103 *Discussion*

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

113

114 *Origins and Biogeography*

115 The Myobatrachidae and Limnodynastidae (together—myobatrachoids) represent the
116 oldest, most diverse (136 spp.), and only endemic of Australia's frog radiations. They share a long
117 history with South America and its Gondwanan past. Anchored by a deep split with the South
118 American *Calyptocephallela* (roughly 100 mya; Fig.2), early divergences among the Australian
119 myobatrachoids, principally between myobatrachids and limnodynastids, occurred in the late
120 Cretaceous (80–70 mya), long preceding the isolation of Australia from Antarctica. This dates to
121 a time when South America, Antarctica, and Australia were a continuous landmass that was likely
122 temperate in climate (Palazzi & Barreda 2007; Mörs et al. 2020). The phylogenetic depth and
123 distribution of myobatrachoids across these now widely disjunct continents suggests a historically
124 continuous distribution across southern Gondwana, including Antarctica. This idea is supported
125 by the recent discovery of an extinct calyptocephallelid from mid-Miocene Antarctica that lived
126 more than 40 mya (Mörs et al. 2020). The persistence of calyptocephallelids in Antarctica into
127 the Late Eocene highlights the dichotomy between young extant myobatrachid and limnodynastid
128 diversity (most species < 30 mya) and ancient splits between limnodynastids and myobatrachids
129 and within limnodynastids (> 70 mya). These long branches are likely the survivors of a much
130 greater southern Gondwanan myobatrachoid diversity, potentially mirroring the diversity of
131 calyptocephallelids through southern South America and Patagonia (Nicoli et al. 2022).

132 Contrasting the comparatively ancient limnodynastids, myobatrachids, and pelodryadids,
133 Australia's youngest anuran radiation are the microhylids. Embedded deeply in the
134 Asterophryinae subfamily, the two temporally adjacent clades (12–13 mya) of *Astrochaperina*
135 and *Cophixalus* crossed the gap from New Guinea to Australia in the mid Miocene. This time
136 frame coincides with a period of increased variation in sea surface levels driven by cooling global
137 temperatures following the mid Miocene climatic optimum. Dropping sea levels likely repeatedly

138 exposed a landbridge between southern New Guinea and northern Australia (both Cape York and
139 the Top End) and facilitated biotic exchange between these landmasses (Mitchell et al. 2014). The
140 young age of these clades, and existence of two other species-rich incumbent frog clades in the
141 pelodryadids and myobatrachoids potentially explains why Australian microhylids are relatively
142 species poor (*Austrochaperina*—5 spp., *Cophixalus*—18 spp.) and morphologically conservative
143 compared to their New Guinean neighbors (200+ spp.), reflecting a pattern seen in monitor lizards
144 (Pavón-Vázquez et al. 2021).

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

155 The staggered arrival of Australian frogs exemplifies the general colonization history of
156 Australian vertebrates. Radiations of mammals, birds, frogs, and reptiles can be discretized into
157 temporal groups broadly identified as (1) Gondwanan relics >40 myo, (2) old colonizers (>20
158 myo, <40 myo) with varied origins, or (3) recent Asian immigrants (<20 myo). The
159 Limnodynastidae and Myobatrachidae fall undoubtedly into the Gondwanan group alongside
160 ancient Australian radiations like Australidelphian marsupial mammals which include koalas,
161 kangaroos, and Tasmanian devils; side-necked chelid turtles; and pygopodoid geckos which include
162 the bizarre limbless pygopodids. These groups—with the exception of pygopodoids—have close
163 links to South American relatives based on molecular and fossil evidence (Georges et al. 1999;
164 Mitchell et al. 2014). While a Pelodryadidae link with South America is clear, they are perhaps
165 the sole radiation to have emigrated from South America to Australia since the continental
166 breakup. Most other similarly aged Australian groups instead show signal of Asian or Australian
167 origins. In comparison, the Australian microhylids (*Austrochaperina*, *Cophixalus*) and *Papurana*
168 *daemeli* are relatively young colonizers from New Guinea with deeper origins in Asian groups.
169 Both the Asterophryinae and Ranidae, to which these species belong, have a long history in the
170 Sunda and Wallacean regions, reflecting patterns of old diversity in this tectonically active area.
171 Alongside a number of other groups such as pythons, monitor lizards, honeyeater birds, dragon
172 lizards, elapid snakes, various gekkonid gecko genera, megabats, frogmouth birds, cockatoos,
173 parrots, several skink subfamilies, and two rodent groups, they share diversity across Australia
174 and New Guinea with repeated exchange between the two islands. Many of these groups show a
175 telltale stepping stone biogeographic pattern that links them back to mainland Asian ancestors,
176 with Australo-Papuan members deeply phylogenetically nested. In general these Australian clades
177 show a pattern of increasing species richness with clade age, however the drivers of such a pattern
178 are likely idiosyncratic (Fig.1).

179

180 *Macroevolutionary Patterns*

181 The radiation of frogs in Australia has occurred on a deep timescale and across a changing
182 climatic landscape. Old species-poor lineages have found themselves confined to the mesic-
183 temperate fringes of the continent, while new niches and species have popped up in the expanding
184 arid zone. And while frogs are found across most of the Australian continent, their basic moisture
185 requirements and desiccation sensitivity mean that Australian amphibian diversity shows a stark
186 mesic-arid gradient (Fig.1), similar to that seen for birds and mammals, and the inverse of lizards
187 (Powney et al. 2010). Not all has been lost in the red center though—several independent clades
188 of dry-country inhabitants have evolved among Australia's harsh sandy and stony deserts.
189 *Neobatrachus*, *Notaden*, and *Cyclorana* have all evolved to aestivate through the hottest and
190 driest seasons. These genera (commonly known as the water-holding frogs) are capable of growing
191 epidermal cocoons to retain moisture that may see them through periods of extreme drought
192 lasting from months to years (van Beurden 1980).

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

210

211 *Conclusion*

212 Australian frogs offer important insights into colonization, persistence, and diversification
213 of a major continental group through deep time. The varied species richness, timing of
214 diversification, and ecomorphological diversity among replicate radiations provides evidence of the
215 processes dictating the accumulation of biodiversity. Beyond the temperate and tropical forests of
216 the east and north coast, the Australian continent is an open country of habitat scarcely welcoming
217 to frogs. Despite this, anurans have a long history in Australia and have diversified into an
218 amazing array of forms, colors, and lifestyles. This success is potentially the result of the stratified
219 temporal arrival of the three main frog clades and possibly exaggerated by their ecological
220 differences. Our phylogenetic framework provides a foundation for asking further questions about

221 how temporal changes to climate, habitat, and niche space have influenced the diversification of
222 one of Australia's richest and most unique vertebrate faunas.

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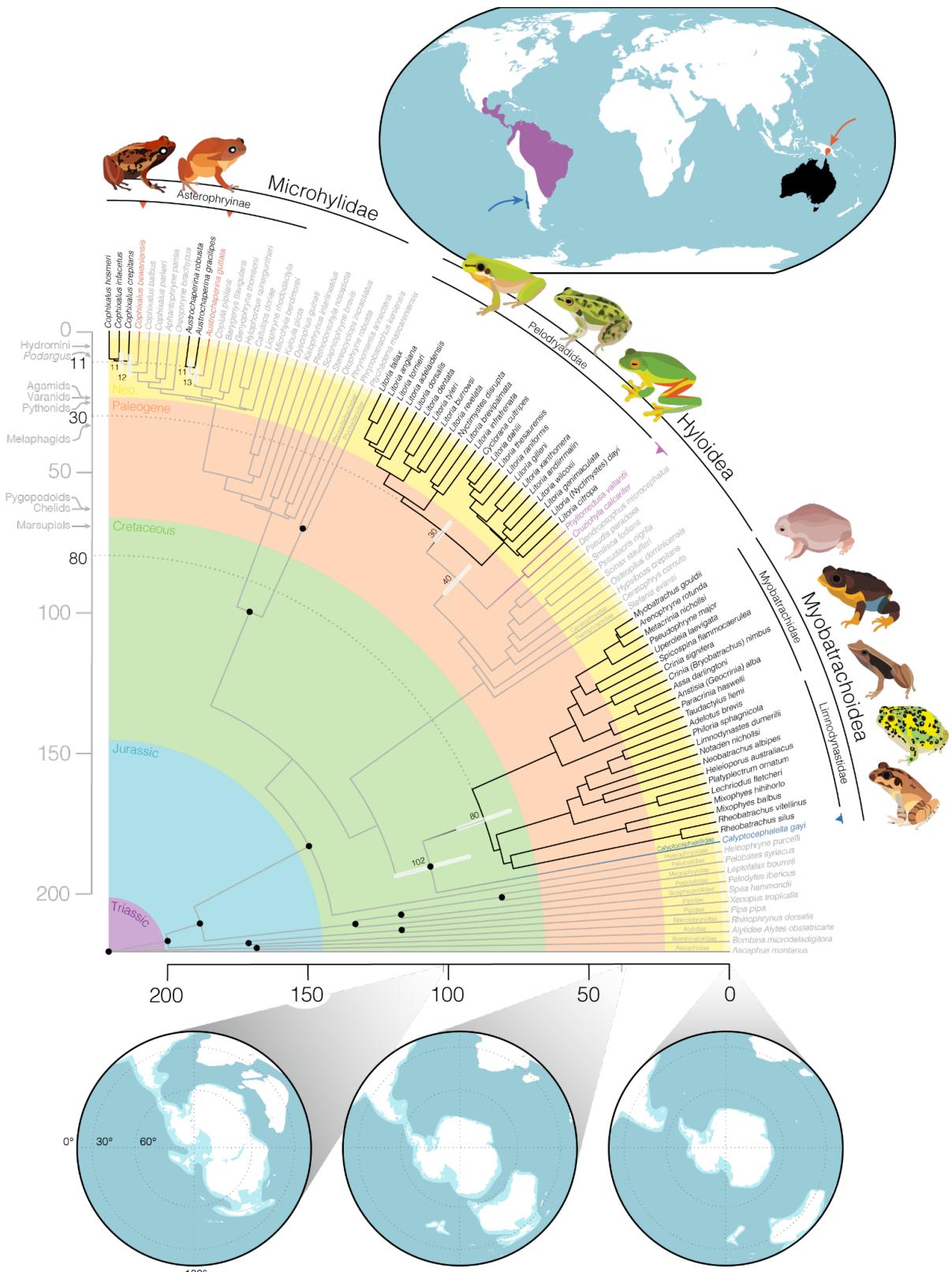
225 *Data Accessibility*

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

228

229 *Acknowledgments*

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231 locality information that made this work possible. JSK, CJH, and SCD thank the Australian
232 Research Council for ongoing support.



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235 Figure 2. Time-calibrated frog phylogeny highlights the staggered arrival of the four major frog families
236 that comprise the Australian anuran fauna. Primarily Australian clades are identified by black branches

237 and text, their closest living relatives outside of Australia are noted by colored branches and text, and
238 outgroup taxa are grey. Black circles at nodes identify the location of fossil calibrations (see Table S2).
239 Upper inset map shows the general geographic location of: (red) closely related microhylids in New Guinea,
240 (purple) phyllomedusid hylids in South America, and (dark blue) *Calyptocephalella* in Chile. Lower inset
241 maps show the connection and proximity of Australia to other Gondwanan continents as Australia drifted
242 away over the past 100 million years. White indicates contemporary coastlines, light blue the continental
243 plates, and dark blue the oceans. Maps were generated using GPlates and input files modified from Landis
244 (2017). Partial fan phylogeny was plotted using *phytools* in the R programming environment. Annotations
245 on vertical time axis show the age of crown divergences of other notable Australian groups for temporal
246 context (see Fig.1). Species illustrated clockwise from top left: *Cophixalus infacetus*, *Austrochaperina*
247 *robusta*, *Litoria fallax*, *Litoria dahlii*, *Litoria xanthomera*, *Myobatrachus gouldii*, *Spicospina*
248 *flammocaerulea*, *Taudactylus acutirostris*, *Notaden bennettii*, *Mixophyes balbus*.
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383 Supplementary Materials

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385 Table S1. Taxon sampling for this project.

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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>Rhinophryne dorsalis</i>	MVZ 164756
Outgroup	Leiopelmatoidae	Ascaphidae	—	<i>Ascaphus montanus</i>	REF AscMon
Outgroup	Discoglossoidea	Bombinatoridae	—	<i>Bombina microdeladigitora</i>	CAS 242112
Outgroup	Discoglossoidea	Alvidae	—	<i>Alutes 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	Phrynomatinae	<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>Kalophryns interlineatus</i>	ABTC 105933
Outgroup	Ranoidea	Microhylidae	Dyscophinae	<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 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 quittata</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>Oreophrune 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 qayi</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>Heleoporus 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>Limnodunastes 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	Myobatrachidae	—	<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	Mvobatrachoidea	Mvobatrachidae	—	<i>Spicospina flammoecaerulea</i>	ABTC 144371
Australian Clade	Myobatrachoidea	Myobatrachidae	—	<i>Uperoleia laevigata</i>	MM 1227
Australian Clade	Mvobatrachoidea	Mvobatrachidae	—	<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	Hvloidea	Hvlidae	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	Hvloidea	Hvlidae	Hylinae	<i>Smilisca fodiens</i>	YPM 014191
Outgroup	Hyloidea	Phyllomedusidae	—	<i>Cruziohyla calcarifer</i>	QCAZ 48552
Outgroup	Hyloidea	Phyllomedusidae	—	<i>Phyllomedusa vallantini</i>	QCAZ 48818
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria citropa</i>	ABTC 7146
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Nyctimystes dayi</i>	ABTC 15997
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria genimaculata</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	Hvloidea	Pelodrvadidae	—	<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 angiana</i>	ABTC 48210
Australian Clade Hyloidea	Pelodryadidae	—	<i>Litoria fallax</i>	ABTC 102409
Australian Clade Hyloidea	Pelodryadidae	—	<i>Litoria tornieri</i>	ABTC 11777

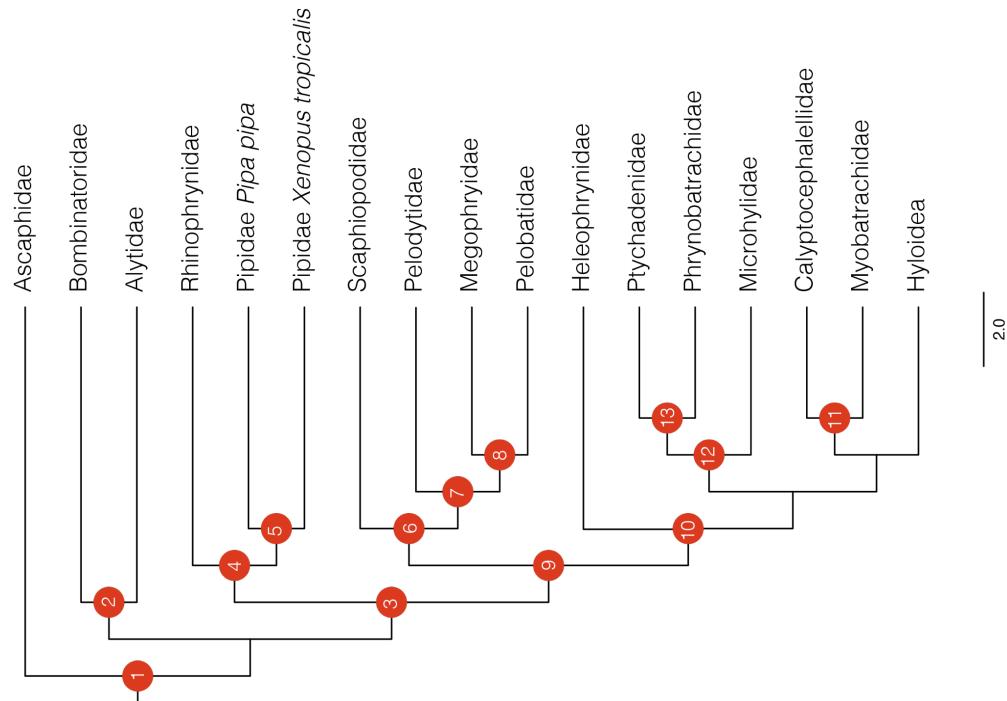
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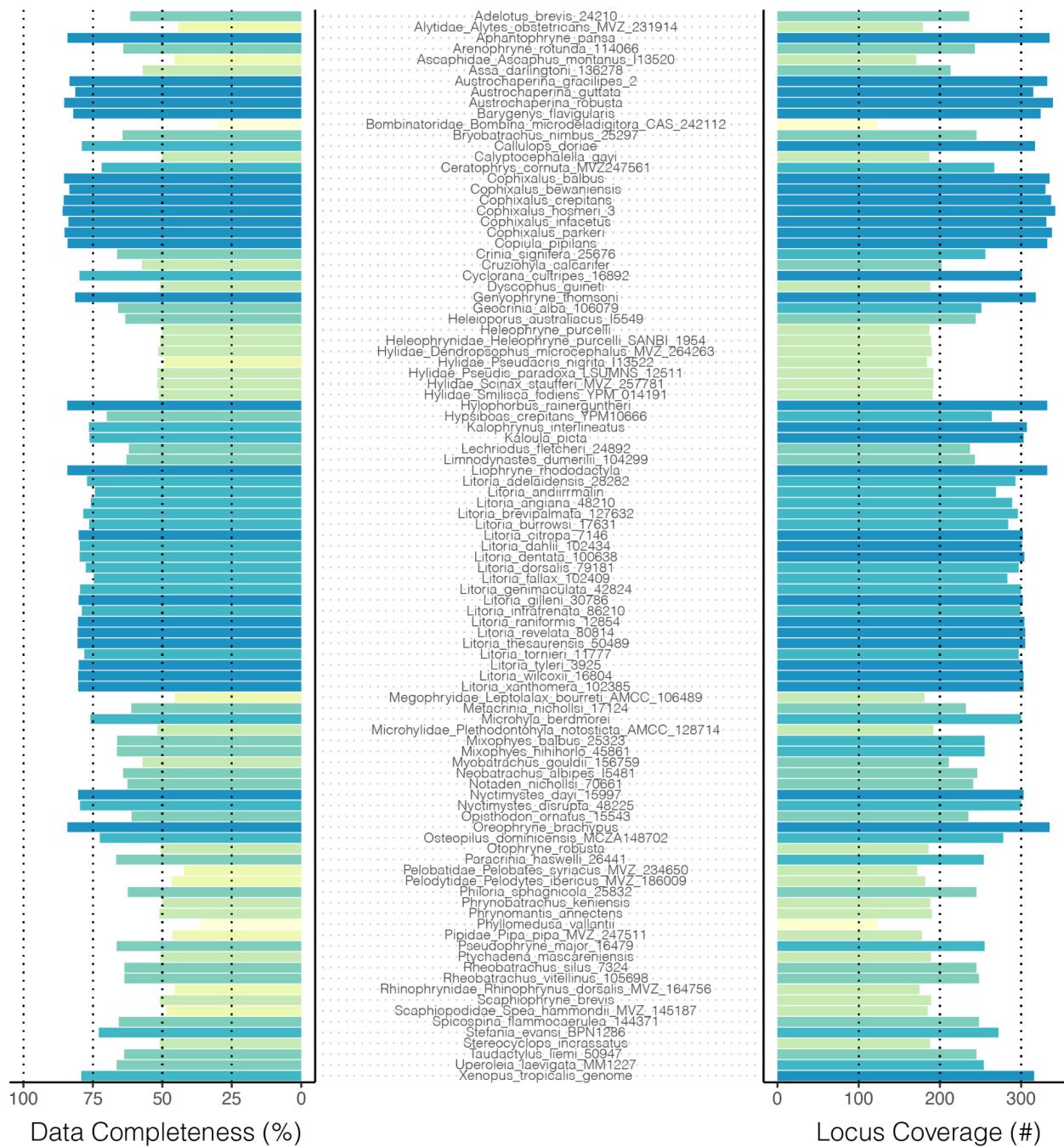
389 Table S2. Fossil calibrations implemented in MCMCTree analysis of frog divergence dates. Node
 390 number (#) corresponds to nodes in supplementary figure below.
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#	Node Calibrated	Fossil	Minimum	Soft Max.	Source (see Feng et al. 2017)
1	Anura	\dagger <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 taquetai</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)

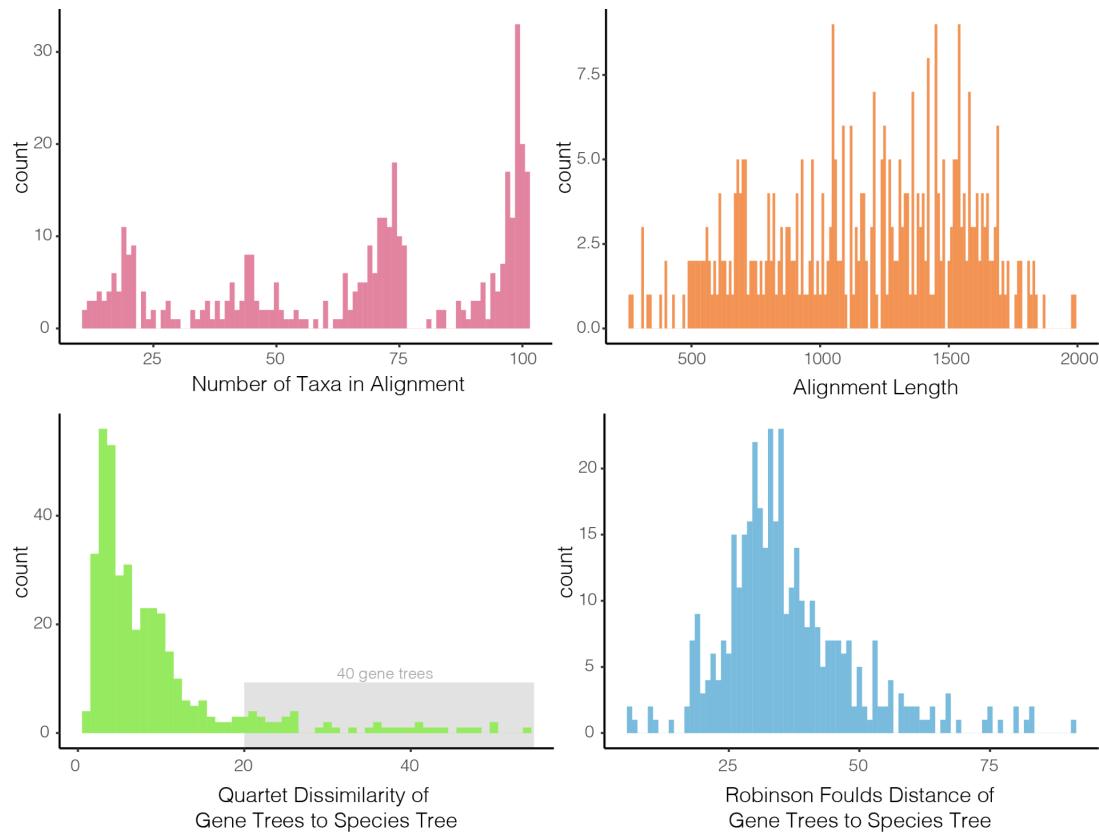
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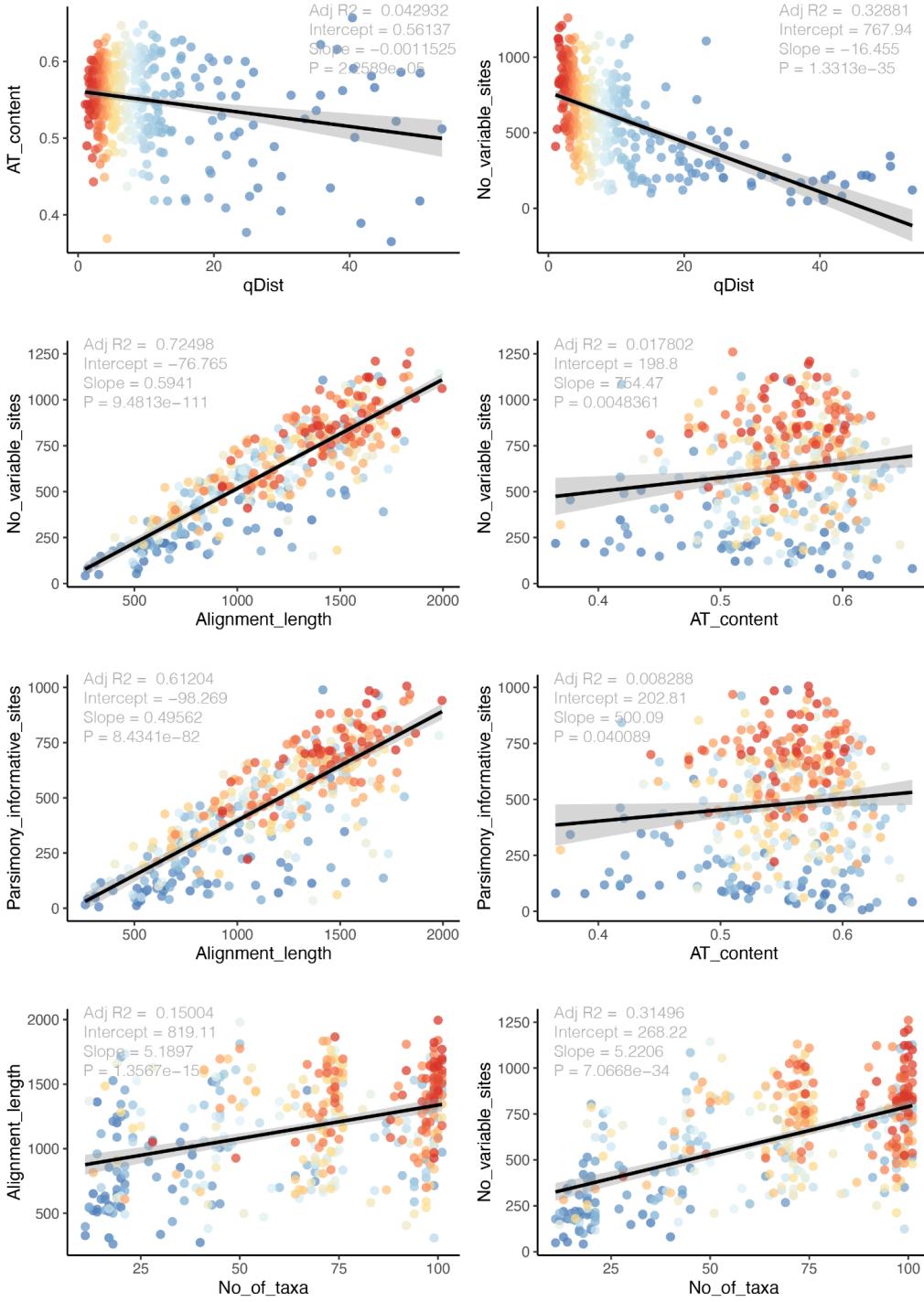


395 Data Completeness (%) Locus Coverage (#)
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397 Figure S1. Data completeness across all samples. Left histogram shows data completeness as percent of
398 bases in total alignment (concatenated alignment length 523,036 bp) exclusive of gaps (-) and missing bases
399 (N). Right histogram shows data completeness as the absolute number of loci included per sample, as a
400 representation of the number of gene trees per sample.



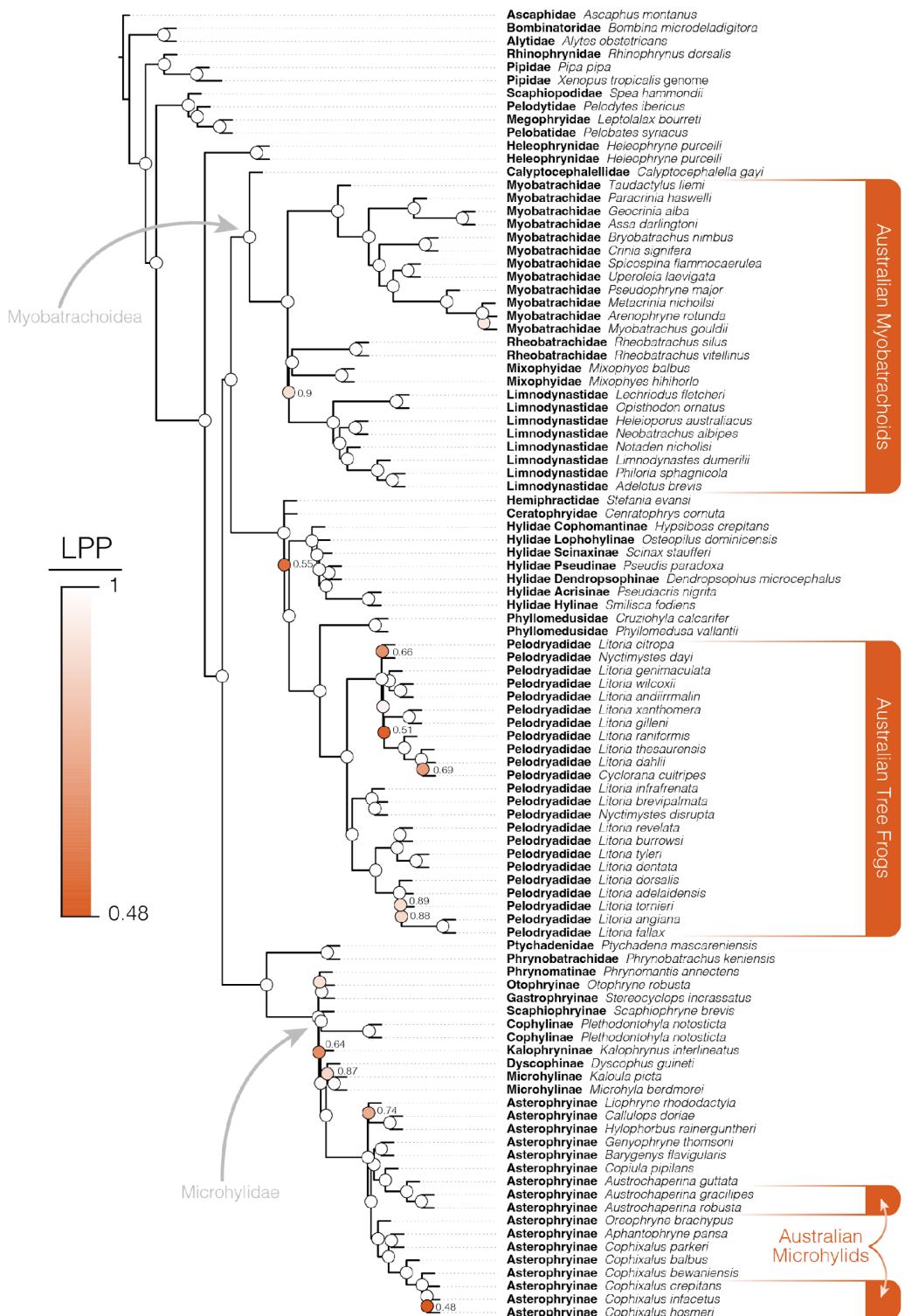
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Figure S2. Basic summary statistics of the 450 locus alignments and gene trees. Top row shows histograms of the number of taxa in (max=101, min=11) and length of each alignment. Bottom row shows gene tree--species tree distances as quartet dissimilarity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



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Figure S3. Detailed summary statistics of the 450 locus alignments and gene trees. Top row compares AT content and number of variable sites against quartet distance between each gene tree and the species tree (a measure of topological similarity). The second and third rows compare measures of locus informativeness (number of variable sites, number of parsimony informative sites) against alignment length and AT content. The bottom row shows alignment length and number of variable sites as a function of the number of taxa in the alignment. In all plots points (representing trees or alignments) are colored according to the quartet distance from the species tree.



417

418 Figure S4. Species tree of Australian frogs and appropriate outgroup taxa estimated using ASTRAL with
 419 locus trees estimated by IQTREE as input. Phylogenetic resolution among major frog groups and within
 420 Australian frog clades is consistently high. Support values are shown at nodes and colored according to
 421 local posterior probabilities (LPP), values >0.9 are considered strongly supported and not indicated at
 422 nodes (white circles).