

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

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## Abstract

Australian frogs comprise a remarkable ecological and morphological diversity categorized into a small number of distantly-related radiations. We present a phylogenomic hypothesis based on an exon-capture dataset that spans the main clades of Australian myobatrachoid, pelodryadid, and microhylid frogs. Our time-calibrated phylogeny identifies great disparity in the relative ages of these groups which vary from Gondwanan relics to recent Asian immigrants. This stratification provides insight into the population of the Australian continent despite dramatic climatic changes. Contemporary Australian frog diversity highlights the adaptive capacity of anurans particularly in response to heat and aridity and explains why they are one of the world's most visible and unique faunas.

## Introduction

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

While we know a great deal about many aspects of Australian frog biology (Tyler 1998; Anistis 2017), the age of each of the major groups, and the timing of their subsequent diversification, is poorly understood. Since the origin of frogs over 250 million years ago, the landmass that is now Australia has traveled extensively. It was part of the supercontinent Pangea before separating with what is now South America, Africa, Antarctica, and India as a component of Gondwana. Australia separated from Antarctica approximately 40 million years ago and then began drifting alone towards Asia. Given the long evolutionary history of frogs, and Australia's varied geographic affinities with other landmasses, we ask three related questions: where did Australia's frogs originate, when did they get to Australia, and which lineages, and where, are their closest relatives? The relative timing of these immigrant groups, their varied species richness, and ecological diversity offer important insight into the evolution of a continental fauna.

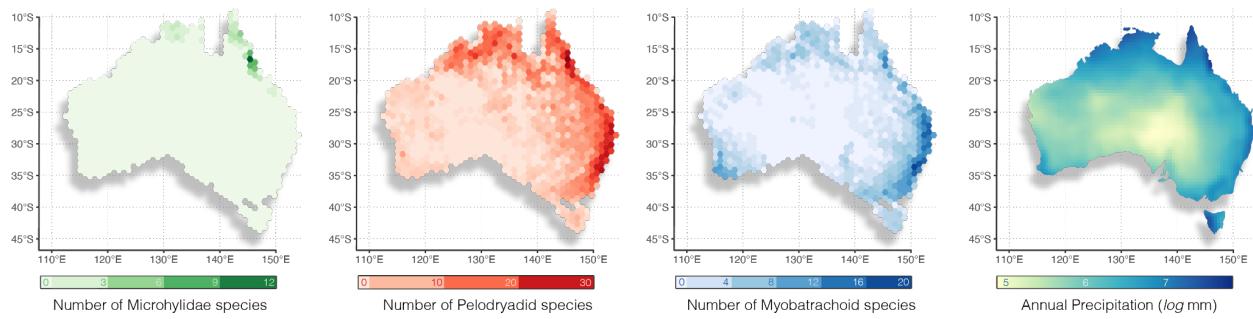


Figure 1. Species richness maps (left and middle plots) for the three largest Australian frog radiations show a pattern of increasing richness with precipitation with greatest richness along Australia's east coast. Annual total precipitation is plotted at far right. Species occurrence records were collated from the Atlas of Living Australia <https://ala.org.au>.

### Materials and Methods

We assembled an exon-capture dataset comprising 99 frog species spanning all major anuran clades and with particular focus on the families Hylidae, Microhylidae, Limnodynastidae and Myobatrachidae (Table S1). We generated new Anchored Hybrid Enrichment (Lemmon et al. 2012) data for 83 samples and combined these with outgroup samples from Hime et al.'s (2021) amphibian phylogenomic dataset. Outgroup sampling was designed around maximizing commonly used anuran fossil calibrations to provide a consistent time-calibrated phylogenomic estimate of Australian frogs. Data from different AHE projects were combined using custom scripts which relied on *metablastr* to identify orthologous loci (*blast\_best\_reciprocal\_hit*) (Benoit & Drost 2021), *mafft* to align them (*--add*, *--keeplength*) (Katoh et al. 2013), and *AMAS* to manipulate alignments (Borowiec 2016). We reconstructed individual genealogies for our exon-capture data (n=450) under maximum-likelihood in IQTREE (Nguyen et al. 2015), allowing the program to assign the best fitting model of nucleotide substitution using ModelFinder (Kalyaanamoorthy et al. 2017), then perform 1,000 ultrafast bootstraps (Minh et al. 2013). We then estimated a species tree using the shortcut coalescent method ASTRAL III (Zhang et al. 2018) with IQTREE gene trees as input. To estimate divergence times among taxa we applied a series of fossil calibrations first compiled by Feng et al. (2019) (Table S2) and used the Bayesian divergence time software

MCMCTree (Rannala & Young 2007). We started by concatenating all loci and partitioning them into two partitions, first and second codons together, and third codons separately. We then used *baseml* to estimate approximate likelihoods (dos Reis & Yang 2011) and branch lengths before running *mcmctree* on the gradient and Hessian (in.BV file) for ten replicate analyses. We inspected mcmc files for stationarity and compared for convergence, then combined them using logCombiner, and used this combined mcmc file to summarize divergence times on our tree (*print* = -1 in .ctl file). Sample, alignment, and gene tree summary statistics are presented in Supplementary Material (Fig. S1-3).

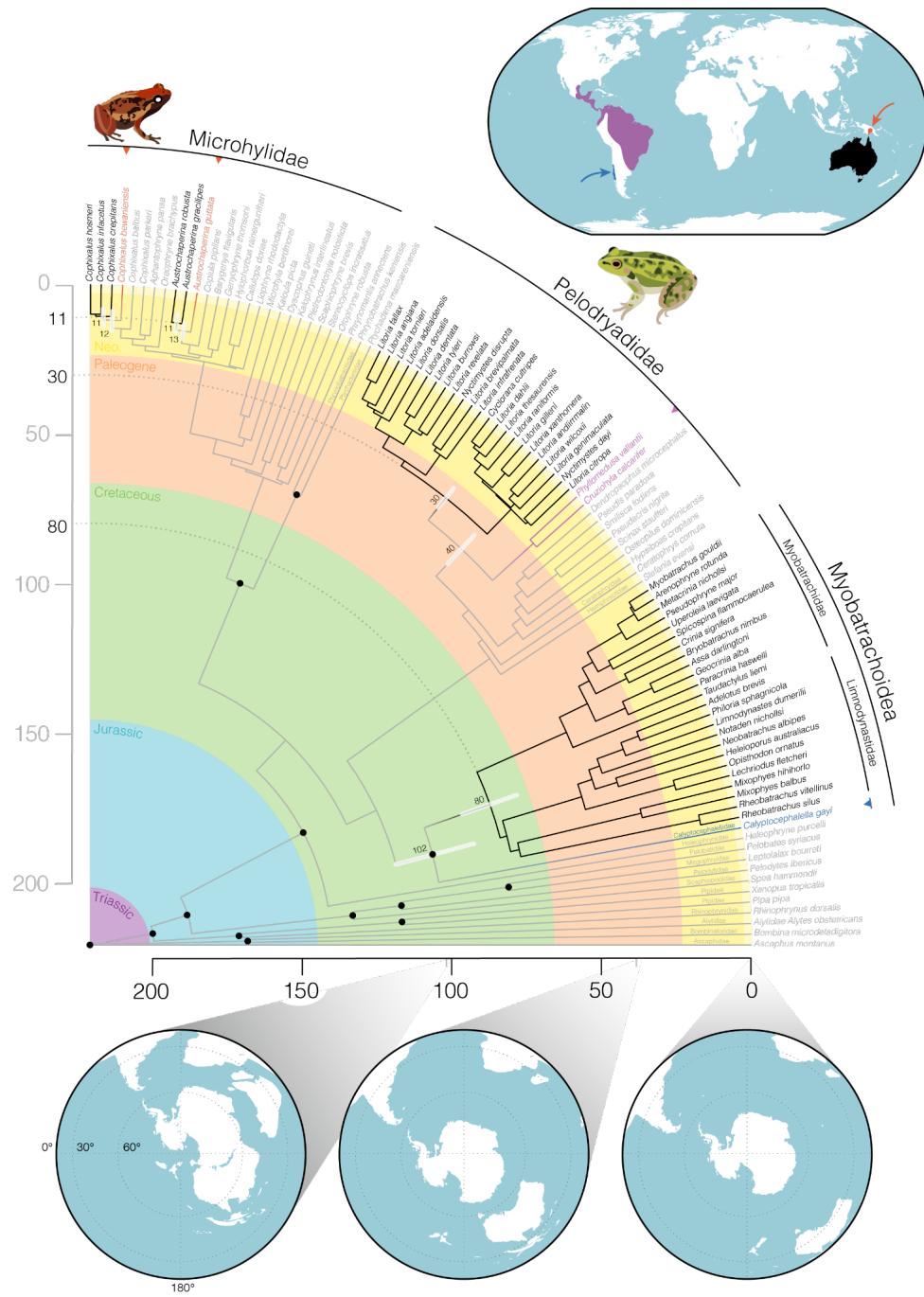


Figure 2. Time-calibrated frog phylogeny highlights the staggered arrival of the four major frog families that comprise the Australian anuran fauna. Primarily Australian clades are identified by black branches and text, their closest living relatives outside of Australia are noted by colored branches and text, and outgroup taxa are grey. Black circles at nodes identify the location of fossil calibrations (see Table X). 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) *Calyptocephallela* 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. Maps were generated using GPlates and input files modified from Landis (2017). Partial fan phylogeny was plotted using *phytools* in the R programming environment.

### Results

Our phylogenomic topology and divergence estimates of frogs are broadly consistent with previous results (Feng et al. 2017; Hime et al. 2021) (Fig.2, S4). Crown divergences of the three Australian frog radiations can be clearly separated into old (Myobatrachoidae–80mya), intermediate (Pelodryadidae–30 mya), and young (Asterophryinae–11 mya). The youngest Australian group, microhylids in the genera *Austrochaperina* and *Cophixalus*, are embedded deeply within the Asterophryinae and appear to represent two separate invasions of Australia from New Guinea. Pelodryadidae tree frogs also share a complex biogeographic history across Australasia, with several species groups split across the Torres Strait, suggesting frequent biotic exchange. The closest relatives of these Australasian tree frogs however are the iconic Phyllomedusidae found throughout Central and South America. The crown split between extant Pelodryadidae in Australia/New Guinea and South America occurred approximately 40 million years ago. Australian Myobatrachoida also have their closest relatives in South America—the Calyptocephalidae, represented here by *Calyptocephallela*, the Helmeted Water Toad of Chile. The crown split between extant myobatrachoids in Australia and Chile is ancient, occurring more than 100 million years ago.

### Discussion

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

### Origins and Biogeography

The oldest, most species diverse (136 spp.), and only endemic of Australia's frog radiations, the myobatrachoids, share a long history with South America and its Gondwanan past. Anchored by a deep split with the South American *Calyptocephallela*, early divergences among the

Australian myobatrachoids occur in the late Cretaceous (80–70 mya) and long precede the isolation of Australia from Antarctica. This dates to a time when South America, Antarctica, and Australia were a continuous landmass that was likely temperate in climate (Palazzi & Barreda 2007; Mörs et al. 2020). The phylogenetic depth and distribution of myobatrachoids across these now widely disjunct continents suggests a historically continuous distribution across southern Gondwana, including Antarctica. This idea is supported by the recent discovery of an extinct calyptocephallelid from mid-Miocene Antarctica that lived more than 40 mya (Mörs et al. 2020). The persistence of calyptocephallelids in Antarctica into the Late Eocene highlights the dichotomy between young extant myobatrachoid diversity (most <30 mya) and ancient splits among Australian myobatrachoids (>70 mya). These long bare branches are likely the survivors of a much greater southern Gondwanan myobatrachoid diversity, potentially mirroring the diversity of calyptocephallelids through southern South America and Patagonia (Nicoli et al. 2022).

Australian myobatrachoids however are not the only group with close connections to South America. The Pelodryadidae are a species rich (91 spp.) and morphologically diverse clade of Australasian frogs. Embedded within the primarily Neotropical treefrogs, they show a more recent late-Eocene divergence from their South American relatives the Phyllomedusidae some 40 mya. Crown divergence of the pelodryadids occurred in the mid-to-late Oligocene (30 mya) before erupting into a radiation across Australian 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 the comparatively ancient myobatrachoids 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 *Astrochaperina* 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, has meant that Australian microhylids are relatively species poor (*Astrochaperina*—5 spp., *Cophixalus*—18 spp.) and morphologically conservative compared to

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

### *Macroevolutionary Patterns*

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

With changes in habitat and ecology Australia's frogs have also accumulated vast diversity in reproductive strategy, ontogeny, and morphology (Crump 2015, Duellman 1992, Sherratt et al. 2018). Many pelodryadid and myobatrachoids have decoupled their larval and adult ecomorphologies highlighting the plasticity of genotype-phenotype relationships (Sherratt et al. 2017). Others have evolved bizarre rearing habits such as raising young in their stomachs (*Rheobatrachus*), hip-pockets (*Assa*), or subterranean nests (*Pseudophryne*). But most obvious is their morphological variety. In general aquatic and burrowing frogs have maximized and minimized limb proportions respectively, but these ecomorphs have evolved into dramatic extremes (Vidal-Garcia & Keogh 2015). The long limbed highly aquatic *Litoria dahlii* with webbed feet and dorsally situated eyes is sister to the burrowing water-holding frogs *Cyclorana*. Together these frogs are embedded deeply within the otherwise toe-padded and arboreal tree frogs, highlighting the adaptive capacity of pelodryadids. Myobatrachoids too have taken ecomorphology to the extreme, offering us what is perhaps the world's strangest living anuran, the turtle frog *Myobatrachus gouldii*. In pursuit of their backwards burrowing lifestyle and termite heavy diet, *Myobatrachus* have dispensed with many of the characteristics we typically associate with frogs. Their beady black eyes are set in small heads and, alongside their sister taxon *Arenophryne*, they crawl---not jump---across the ground on short limbs that are incapable of hopping (Vidal-Garcia et al. 2014). Extreme morphological change is also evident in the youngest radiation in Australia. While generally morphologically conservative, *Cophixalus* frogs have evolved a novel giant ecomorph capable of living amongst Queensland's boulder fields (Hoskin & Aland 2011; Hoskin 2013). For these *Cophixalus* the repeated transition from rainforest to boulder piles is suggested to have facilitated diversification and niche expansion.

### *Conclusion*

Australian frogs offer important insight into how a continent becomes populated. The varied species richness, timing of diversification, and ecomorphological diversity among replicate

radiations provides evidence of the processes dictating the accumulation of biodiversity. Beyond the temperate and tropical forests of the east coast, the Australian continent is an open country of habitat scarcely welcoming to frogs. Despite this, anurans have a long history in Australia and have diversified into an amazing array of forms, colors, and lifestyles. This success is potentially the result of the stratified temporal arrival of the three main frog clades and exaggerated by their ecological differences. Our phylogenetic framework provides a foundation for asking further questions about how temporal changes to climate, habitat, and niche space have influenced the diversification of one of Australia's richest and most unique vertebrate faunas.

#### *Data Accessibility*

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

#### *Acknowledgments*

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#### *Instructions:*

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## Supplementary Materials

Table S1. Taxon sampling for this project.

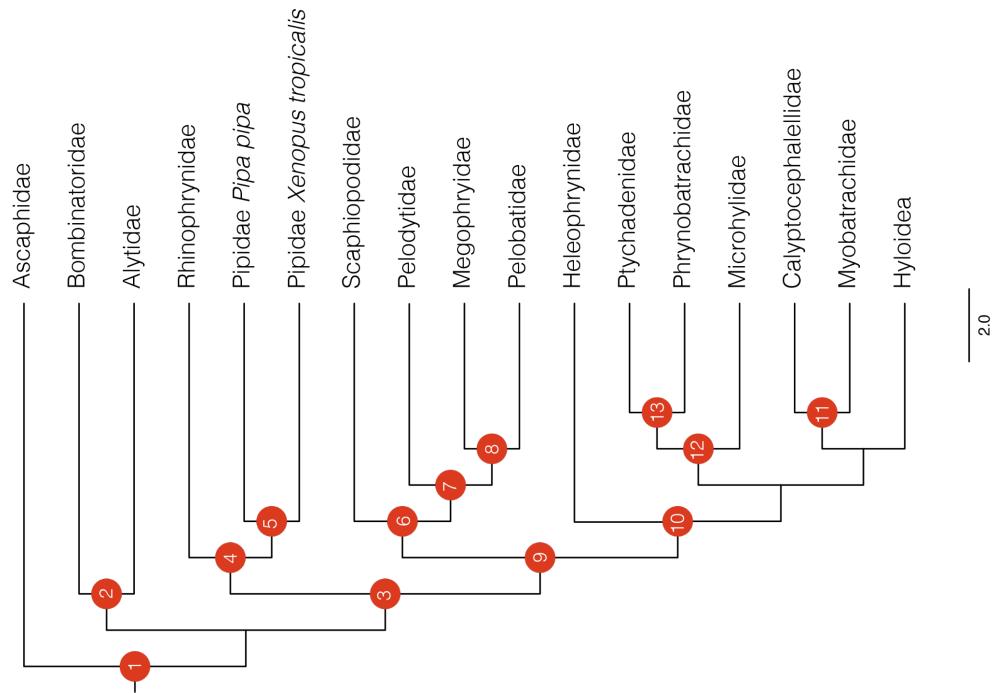
Geography	Superfamily/Clade	Family	Subfamily	Genus species	Registration
Outgroup	Pipoidea	Pipidae	—	<i>Xenopus tropicalis</i>	NCBI Genome
Outgroup	Pipoidea	Pipidae	—	<i>Pipidae Pipa pipa</i>	MVZ 247511
Outgroup	Pipoidea	Rhinophrynidiae	—	<i>Rhinophrynum dorsalis</i>	MVZ 164756
Outgroup	Leiopelmatoidae	Ascaphidae	—	<i>Ascaphus montanus</i>	REF AscMon
Outgroup	Discoglossoidea	Bombinatoridae	—	<i>Bombina microdeladigitora</i>	CAS 242112
Outgroup	Discoglossoidea	Alvtidae	—	<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	Otophryvinae	<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	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 rainerquntheri</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>Astrochaperina guttata</i>	ABTC 141506
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Astrochaperina gracilipes</i>	ABTC 79186
Australian Clade	Ranoidea	Microhylidae	Asterophryinae	<i>Astrochaperina robusta</i>	conx5153
Outgroup	Ranoidea	Microhylidae	Asterophryinae	<i>Oreophryne brachynus</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>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	Mvobatrachoidea	Limnodynastidae	—	<i>Limnodunastes dumerilii</i>	ABTC 104299
Australian Clade	Myobatrachoidea	Limnodynastidae	—	<i>Philoria 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 flammocaerulea</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>Cruziophyla calcarifer</i>	QCAZ 48552
Outgroup	Hvloidea	Phyllomedusidae	—	<i>Phyllomedusa vallantii</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 qenimaculata</i>	ABTC 42824
Australian Clade	Hyloidea	Pelodryadidae	—	<i>Litoria wilcoxi</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	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 angiana</i>	ABTC 48210
Australian Clade Hyloidea	Pelodryadidae	—	<i>Litoria fallax</i>	ABTC 102409
Australian Clade Hyloidea	Pelodryadidae	—	<i>Litoria tornieri</i>	ABTC 11777

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

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



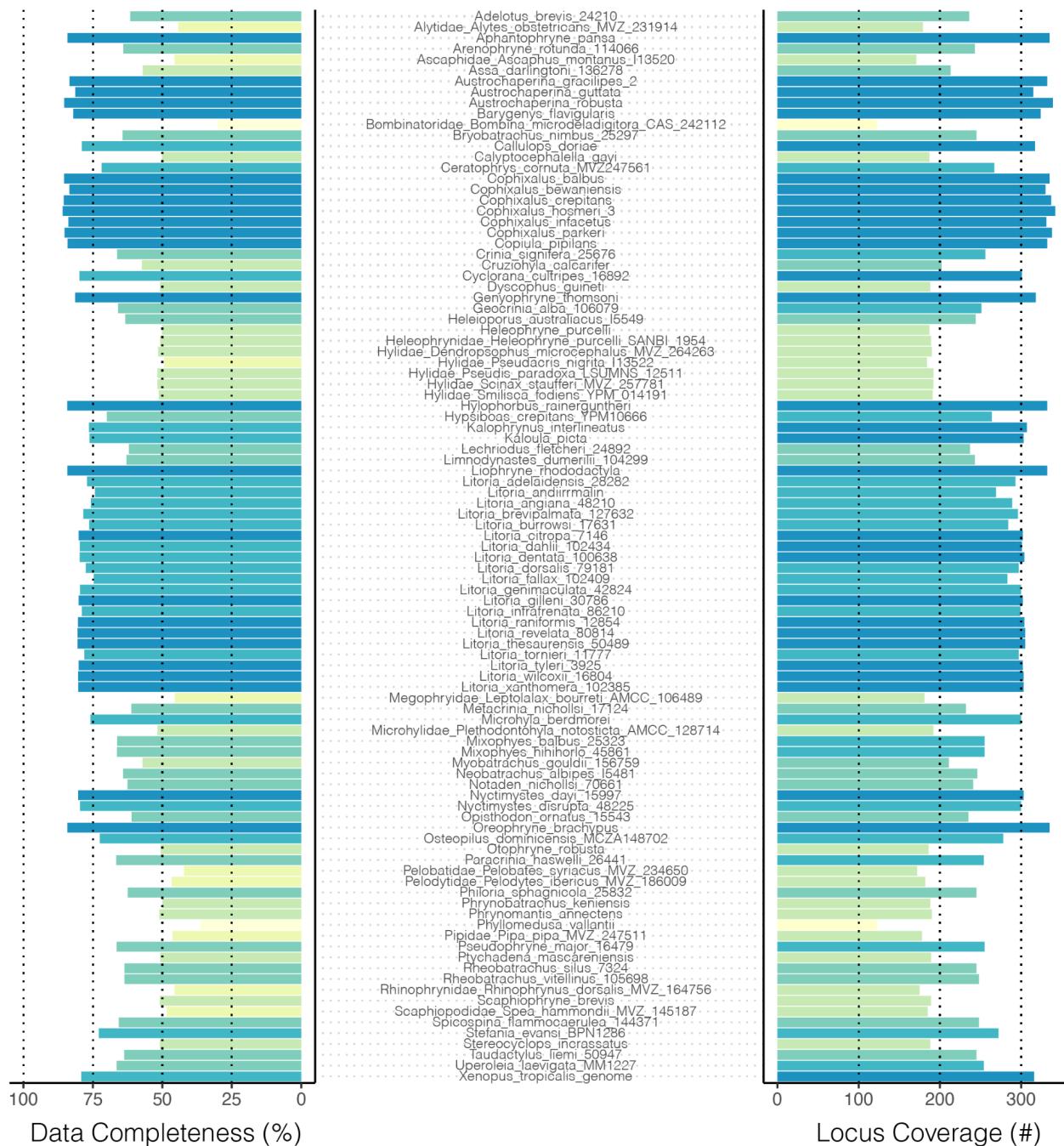


Figure S1. Data completeness across all samples. Left histogram shows data completeness as percent of bases in total alignment (concatenated alignment length 523,036 bp) exclusive of gaps (-) and missing bases (N). Right histogram shows data completeness as the absolute number of loci included per sample,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

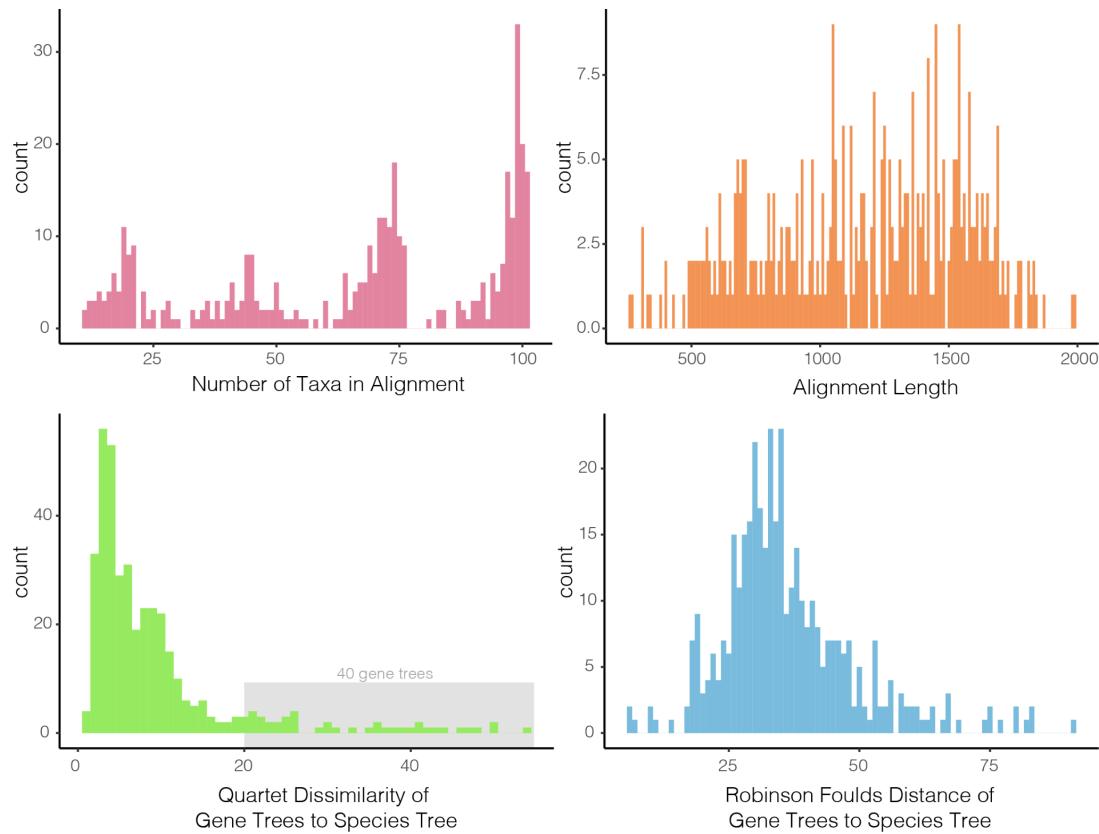


Figure S2. Basic summary statistics of the 450 locus alignments and gene trees. Top row shows histograms of the number of taxa in (max=101, min=11) and length of each alignment. Bottom row shows gene tree--species tree distances as quartet dissimilarity scores and Robinson Foulds distances, two different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

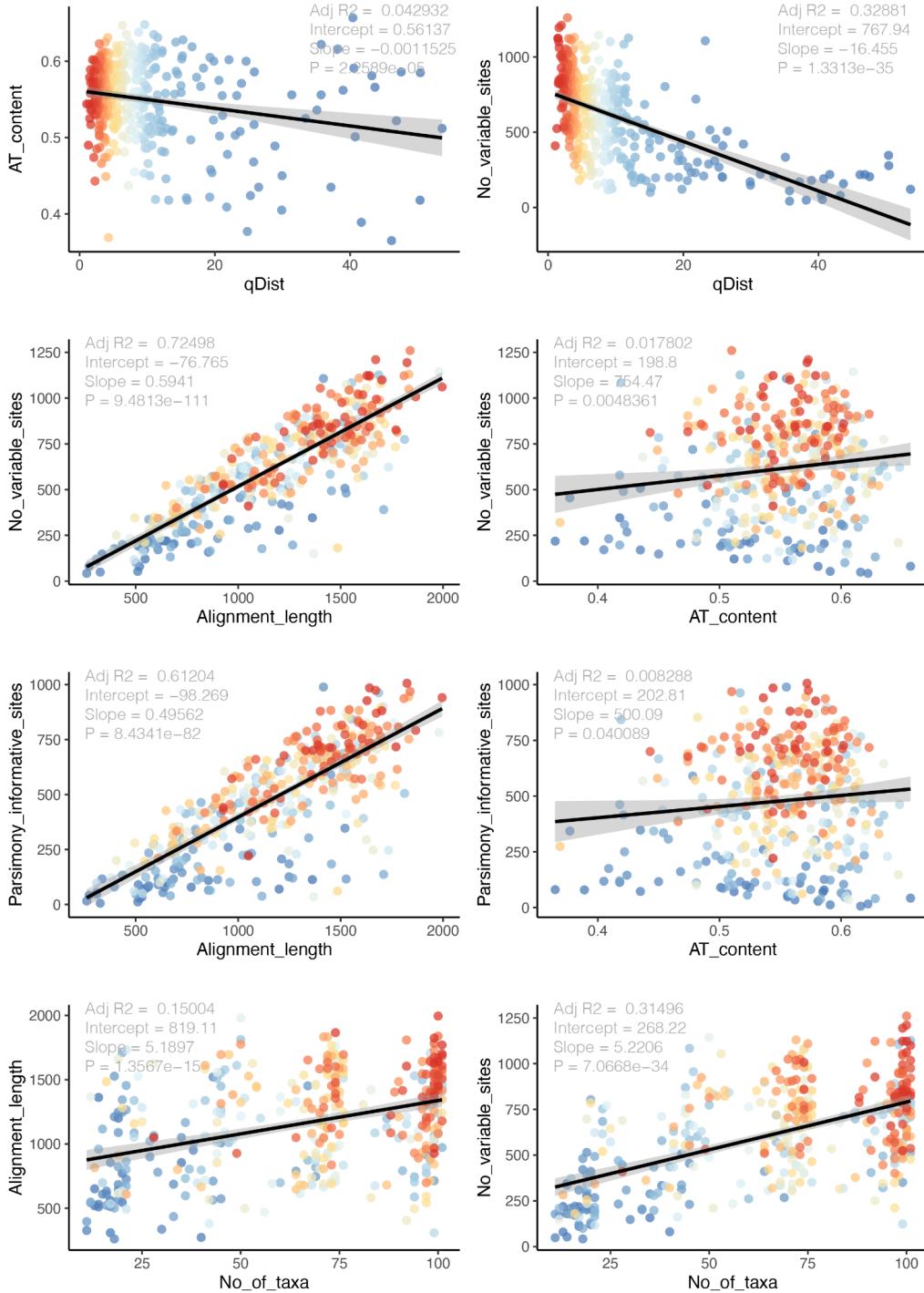


Figure S3. Detailed summary statistics of the 450 locus alignments and gene trees. Top row compares AT content and number of variable sites against quartet distance between each gene tree and the species tree (a measure of topological similarity). The second and third rows compare measures of locus informativeness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

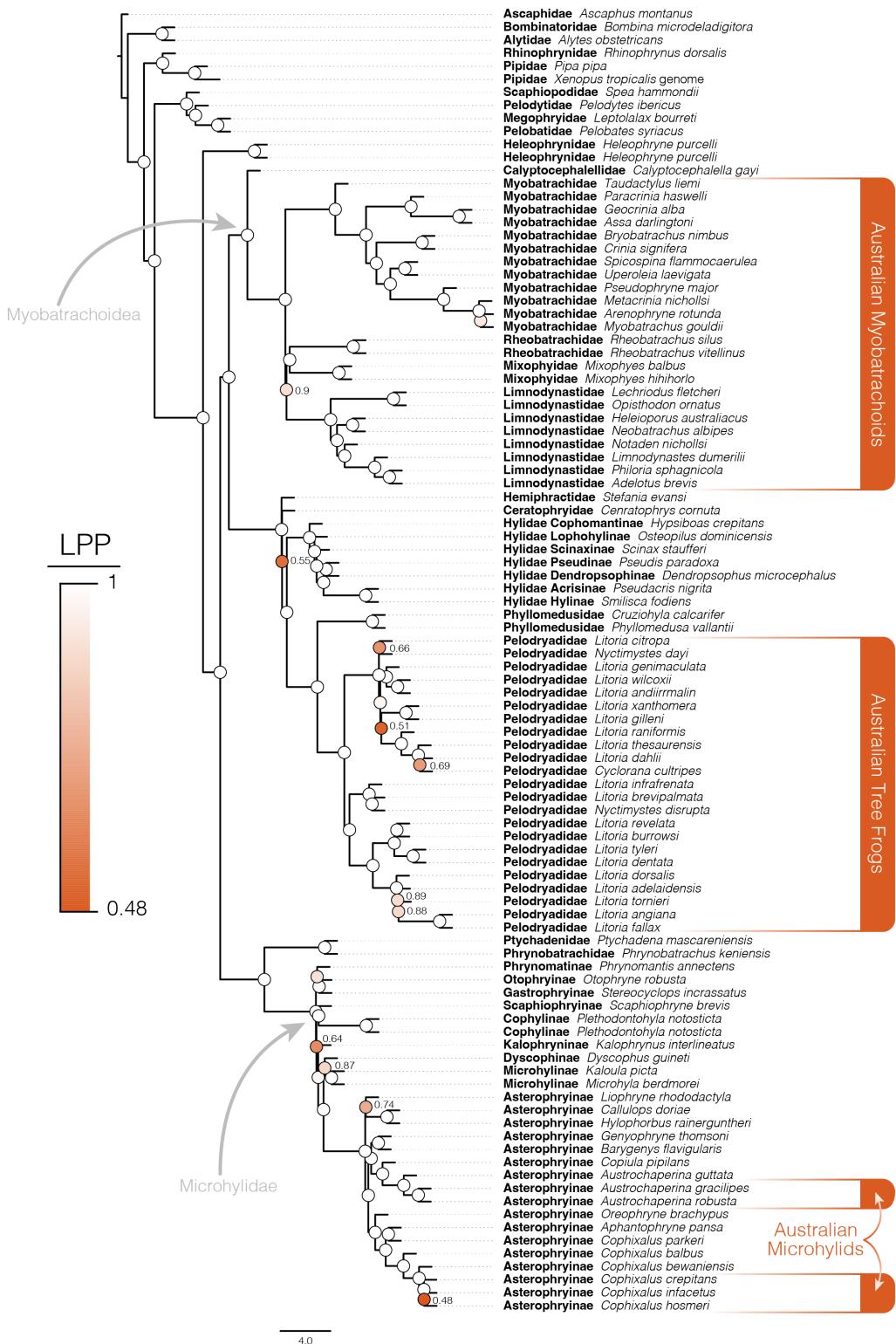


Figure S4. Species tree of Australian frogs and appropriate outgroup taxa estimated using ASTRAL with locus trees estimated by IQTREE as input. Phylogenetic resolution among major frog groups and within Australian frog clades is consistently high. Support values are shown at nodes and colored according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