

Supplemental Appendix

Rapid diversification and time explain amphibian richness at different scales in the Tropical Andes, Earth's most biodiverse hotspot

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American Naturalist

Author-supplied Appendix

Analyses using an alternative topology

Overview

To assess the robustness of our results to reasonable variation in topology, we performed the major analyses described above on an alternative topology. To generate this alternative tree, we conducted a second set of generic-level BEAST analyses in which we constrained a clade of five families to be monophyletic (Leptodactylidae, Allophrynidae, Centrolenidae, Bufonidae, and Dendrobatidae). Note that the placement of dendrobatids as sister to other hyloids in our tree was the major disagreement with other recent studies at this phylogenetic scale, since other studies instead placed dendrobatids with bufonids (see Online Appendix Part A). Next, we repeated the relevant analyses from the main text following the methods in the previous sections. All results using the alternative topology remain broadly consistent with those from the main text, and details are included here.

Tree comparison

The phylogenetic results of the alternative topology are generally similar to those of other recent multi-locus analyses across hyloids (Roelants et al. 2007; Pyron and Wiens 2011; Pyron 2014), but one difference remains (figure S1). By constraining the topology (bufonids, dendrobatids, allophrynids, centrolenids, and leptodactylids), we find bufonids sister to dendrobatids, and allophrynids+centrolenids sister to leptodactylids, which is in agreement with other recent studies (Roelants et al. 2007; Pyron and Wiens 2011; Pyron 2014). However, these alternative analyses placed hemiphractids as sister to all other hyloids (but with low support), whereas

Pyron and Wiens (2011) and Pyron (2014) placed terraranans in this position, and Roelants et al. (2007) placed rhinodermatids as sister to all other hyloids. Our original tree also found terraranans as sister to hemiphractids, which differed from these other studies (Roelants et al. 2007; Pyron and Wiens 2011; Pyron 2014). The alternative tree here also supports the monophyly of a clade of eight small South American families, including ceratophryids, rhinodermatids, cycloramphids, odontophrynids, telmatobiids, alsodids, hyloidids, and batrachylids. Finally, we also find that all families and 98% of genera remained monophyletic, as in the initial tree.

Previous multi-locus time-calibrated analyses across hyloids suggested that most divergences among hyloid families occurred around 50–75 Mya (Roelants et al. 2007; Pyron and Wiens 2013; Pyron 2014). Our results for this alternative topology suggest more similar divergence dates to these previous studies than our unconstrained tree (which suggested somewhat older dates). This alternative topology finds splits among hyloid families occurring ~55–85 Mya (figure S2).

Diversification-rate hypothesis

Bayesian analyses of diversification rates across the alternative phylogeny of 1583 South American hyloid species detected 6 significant increases in diversification rates (figure S3). Four shifts correspond to the Andean clades: Centrolenidae, *Atelopus* (Bufonidae), *Pristimantis* (Pristimantinae), and *Telmatobius* (Telmatobiidae). The remaining shifts correspond to the colonization of the Atlantic Forest by *Rhinella* (Bufonidae) and one at the base of Terrarana. No shifts correspond to lowland colonization. The major difference between these results and those of the unconstrained analyses (figure 1 main text) is that in the constrained tree, the shift for

Gastrotheca (Hemiphractidae) that was detected in the unconstrained tree is not included in the best shift configuration. However, this shift is included in other shift configurations with a lower marginal probability ($f = 0.0095$ vs. $f = 0.014$ for the best configuration) and shows an increase in rates generally. This result may be due to the unconventional placement of Hemiphractidae as sister to all other hyloids. Finally, we find that diversification rates on the original tree are significantly correlated with rates on the alternative tree (Spearman's rank correlation: $r_s = 0.952$; $P < 0.001$; $n = 1583$; raw species rates in Supplemental table S3).

At the largest biogeographic scale, we again did not initially find a significant relationship between the mean diversification rates of species within each region and that region's species richness (table S4), using ordinary least-squares (OLS) regression and the alternative tree ($n = 7$ biogeographic regions; $R^2 = 0.428$; $P = 0.111$). However, these results were again strongly influenced by an outlier (the Atlantic Forest region), which has low rates and high richness. After removing this outlier, the relationship is significant ($n = 6$: $R^2 = 0.659$; $P = 0.046$), as it is in the unconstrained tree. Conversely, after removing the Andes, the relationship remains insignificant ($n = 6$: $R^2 = 0.019$; $P = 0.791$), supporting the Atlantic Forest as an outlier and not the Andes. Overall, these results from the constrained tree confirm that diversification is exceptionally fast in Andean lineages and that this faster diversification helps explain high Andean species richness relative to other South American regions.

As in the analyses from the unconstrained tree, we again found that these accelerated diversification rates do not explain patterns of elevational species richness within the Andes. We extracted diversification rates of genera from the BAMM analyses (table S5) and tested for a relationship between their current Andean elevational midpoints (excluding non-Andean montane species) and their diversification rates. The relationship was not significant using

phylogenetic generalized least squares (PGLS) regression ($n = 104$; $B_0 = 1160.2$; $B_1 = -1578.8$; $R^2 = 0.012$; $P = 0.188$), as in the unconstrained tree. Conversely, we again found a strong negative relationship between the mean diversification rate of species within an elevational band (table S6) and the band's species richness using ordinary least-squares (OLS) regression ($n = 11$; $R^2 = 0.825$; $P < 0.001$).

Time hypothesis

Using the alternative phylogeny, we again found a positive but non-significant relationship between colonization time and current richness for regions (table S4), using both constrained (such that ancestral regions could only be colonized after they first appear) and unconstrained biogeographic reconstructions, respectively ($R^2 = 0.010$ and 0.009 ; $P = 0.831$ and 0.840). Again, the Andes were an influential outlier, due to their high richness and late colonization time. However, the relationship remains non-significant if the Andes are removed ($R^2 = 0.246$ and 0.164 ; $P = 0.317$ and 0.427 ; constrained and unconstrained analyses, respectively). These results support the idea that richness of these regions is explained by diversification rates and not time, as in the original tree.

To address the causes of the mid-elevation peak in Andean richness, we again estimated the timing of the first colonization of each 500 m elevational band among Andean species (table S4), using species elevational midpoints and ancestral reconstructions on the species-level tree. We then tested for a relationship between colonization times and current species richness of each band using OLS regression and found a strong positive relationship ($R^2 = 0.604$; $P < 0.001$). Thus, these results support the idea that time generally explains richness patterns at smaller phylogenetic scales, as in the results from the unconstrained tree.

Drivers of Andean speciation

Using the alternative tree, we detected 41 significant increases in rates of evolution for elevational distributions on the tree, with one or more shifts occurring temporally near a diversification rate shift in Andean clades (figure S4). These results are somewhat different from those using the unconstrained tree, which estimated only 17 rate increases. As in the unconstrained tree, we found a significant and positive (but relatively weak) relationship between rates of change in elevational distributions and diversification rates of Andean genera using PGLS regression ($n = 102$; $B_0 = -5.5$; $B_1 = 4.1$; $R^2 = 0.041$; $P < 0.001$; data in table S5). Finally, we find that rates of change in elevational distributions on the original tree are significantly correlated with rates calculated from the alternative tree (Spearman's rank correlation: $r_s = 0.853$; $P < 0.001$; $n = 1583$; raw species rates in Supplemental table S3).

Tables and Figures

Table S4. Regional species richness patterns, diversification rates, and colonization times (Myr) for South American biogeographic regions for the alternative, constrained tree for Hyloidea. The constrained colonization timing prevents the Andes from being colonized before their uprising. However, we note that these analyses are intended to represent the colonization of a habitat type similar to their present day regions, and so unconstrained analyses are also potentially valid.

Region	Total richness	Mean diversification rate of species	First colonization (unconstrained)	First colonization (constrained)
Andes	1260	0.079	55.59	39.25
Amazonia	482	0.071	61.02	61.02
Choco	155	0.065	40.54	24.23
Guiana Shield	357	0.069	50.99	50.99
Cerrado	303	0.071	43.73	43.73
Atlantic Forest	566	0.059	82.18	82.18
Temperate	169	0.057	71.99	71.99

Table S5. Data for genera used in testing the diversification-rate hypothesis and for testing if there is a relationship between diversification rates (species/Myr.) and rates of change in elevational distributions (i.e. trait rate) analyses. These rates are derived from the alternative (constrained) tree topology. Rates are estimated for each genus from BAMM using the mean rates of the species included in each genus.

Genus	Diversification Rate	Trait Rate (meters)
<i>Adelophryne</i>	0.073	15973.6
<i>Adelphobates</i>	0.069	29763.4
<i>Adenomera</i>	0.088	2773.4
<i>Agalychnis</i>	0.064	4011.9
<i>Allobates</i>	0.076	8931.7
<i>Allophryne</i>	0.068	24557.9
<i>Alsodes</i>	0.126	227621.2
<i>Amazophrynella</i>	0.088	1819.9
<i>Ameerega</i>	0.070	30286.5
<i>Andinobates</i>	0.070	30024.8
<i>Anomaloglossus</i>	0.070	33758.0
<i>Aplastodiscus</i>	0.069	805.6
<i>Aromobates</i>	0.126	32057.3
<i>Atelognathus</i>	0.127	39789.9
<i>Atelopus</i>	0.086	140240.4
<i>Bokermannohyla</i>	0.070	12365.7
<i>Brachycephalus</i>	0.068	0.0
<i>Bryophryne</i>	0.068	24557.9
<i>Celsiella</i>	0.070	24919.6
<i>Centrolene</i>	0.070	27025.4
<i>Ceratophrys</i>	0.070	4002.1
<i>Chacophrys</i>	0.068	24557.9
<i>Chimerella</i>	0.070	24870.4
<i>Cochranella</i>	0.070	25080.6
<i>Colostethus</i>	0.070	30059.6
<i>Craugastor</i>	0.409	23219.4
<i>Crossodactylodes</i>	0.072	4051.1
<i>Cruziohyla</i>	0.068	24557.9
<i>Cryptobatrachus</i>	0.042	18143.8
<i>Cycloramphus</i>	0.126	0.0

<i>Dendrobates</i>	0.070	29926.9
<i>Dendrophryniscus</i>	0.086	1823.1
<i>Dendropsophus</i>	0.038	28516.1
<i>Diasporus</i>	0.068	24557.9
<i>Edalorhina</i>	0.068	24557.9
<i>Engystomops</i>	0.072	21172.1
<i>Epipedobates</i>	0.070	30498.9
<i>Espadarana</i>	0.070	25074.3
<i>Euparkerella</i>	0.407	0.0
<i>Eupsophus</i>	0.125	106017.1
<i>Excidobates</i>	0.070	30102.4
<i>Flectonotus</i>	0.042	18201.7
<i>Fritziana</i>	0.042	1731.4
<i>Frostius</i>	0.068	24557.9
<i>Gastrotheca</i>	0.069	18171.3
<i>Haddadus</i>	0.068	24557.9
<i>Hemiphractus</i>	0.069	18190.6
<i>Hyalinobatrachium</i>	0.070	25122.4
<i>Hydrolaetare</i>	0.068	24557.9
<i>Hylodes</i>	0.126	0.0
<i>Hyloscirtus</i>	0.071	19675.6
<i>Hyloxalus</i>	0.070	30099.7
<i>Hypodactylus</i>	0.058	22776.4
<i>Hypsiboas</i>	0.069	25798.5
<i>Ikakogi</i>	0.068	24557.9
<i>Ischnocnema</i>	0.068	3337.3
<i>Leptodactylus</i>	0.072	5719.5
<i>Lithodytes</i>	0.068	24557.9
<i>Lynchius</i>	0.081	21108.7
<i>Mannophryne</i>	0.069	32109.8
<i>Melanophryniscus</i>	0.086	37594.9
<i>Nannophryne</i>	0.084	37954.1
<i>Noblella</i>	0.052	56986.7
<i>Nymphargus</i>	0.070	25096.9
<i>Odontophrynus</i>	0.070	20031.5
<i>Oophaga</i>	0.070	29824.9
<i>Oreobates</i>	0.063	20180.5
<i>Osornophryne</i>	0.080	38861.9
<i>Osteocephalus</i>	0.035	30864.3
<i>Paratelmatobius</i>	0.072	4041.8
<i>Phasmahyla</i>	0.068	4134.4
<i>Phrynopus</i>	0.067	20651.6

<i>Phyllobates</i>	0.070	29531.8
<i>Phyllodytes</i>	0.070	4024.4
<i>Phyllomedusa</i>	0.072	7449.0
<i>Physalaemus</i>	0.069	4040.2
<i>Phyzelaphryne</i>	0.070	8173.3
<i>Pleurodema</i>	0.071	100854.5
<i>Pristimantis</i>	0.409	41684.7
<i>Proceratophrys</i>	0.070	3061.5
<i>Pseudis</i>	0.036	4018.0
<i>Pseudopaludicola</i>	0.070	3961.7
<i>Psychrophrynella</i>	0.068	21951.0
<i>Ranitomeya</i>	0.069	28620.2
<i>Rhaebo</i>	0.085	38705.3
<i>Rheobates</i>	0.068	24557.9
<i>Rhinella</i>	0.080	38304.1
<i>Rhinoderma</i>	0.069	5016.1
<i>Rulyrana</i>	0.070	25152.6
<i>Sachatamia</i>	0.070	25035.5
<i>Scarthyla</i>	0.037	3966.8
<i>Scinax</i>	0.065	8049.9
<i>Silverstoneia</i>	0.069	30354.2
<i>Smilisca</i>	0.069	3868.6
<i>Sphaenorhynchus</i>	0.039	4047.0
<i>Stefania</i>	0.043	18062.1
<i>Strabomantis</i>	0.408	22404.9
<i>Telmatobius</i>	0.069	81990.9
<i>Tepuihyla</i>	0.071	181709.0
<i>Teratohyla</i>	0.071	24974.5
<i>Trachycephalus</i>	0.037	4046.7
<i>Vitreorana</i>	0.071	24959.9
<i>Xenohyla</i>	0.068	24557.9
<i>Yunganastes</i>	0.067	22562.8

Table S6. Elevational species richness patterns, diversification rates, and colonization times (Myr) for Tropical Andean elevational regions for the alternative topology of Hyloidea.

Elevational band (m)	Total richness	Mean diversification rate of species	First colonization time (myr)
0–500	1141	0.066	74.9
501–1000	998	0.064	91.6
1001–1500	584	0.065	71.9
1501–2000	585	0.066	71.8
2001–2500	517	0.069	65.4
2501–3000	406	0.091	55.6
3001–3500	286	0.102	40.7
3501–4000	148	0.132	11.4
4001–4500	65	0.196	21.9
4501–5000	12	0.185	4.8
5501–5500	4	0.191	1.6

Figure S1. Alternative constrained backbone tree topology and posterior probabilities from BEAST.

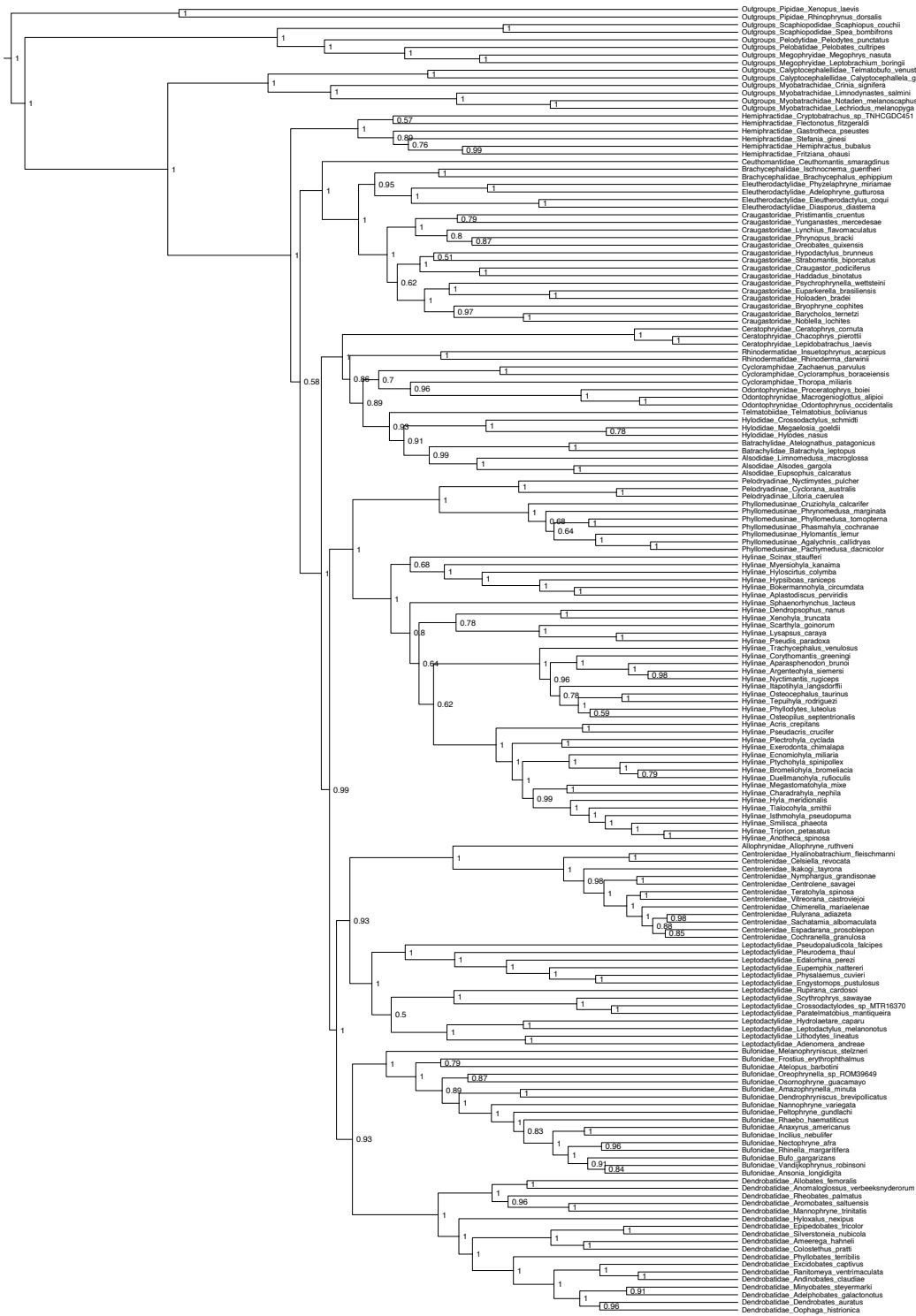


Figure S3. Significant diversification rate increases (green dots) within Hyloidea from BAMM analyses using the alternative, constrained phylogeny.

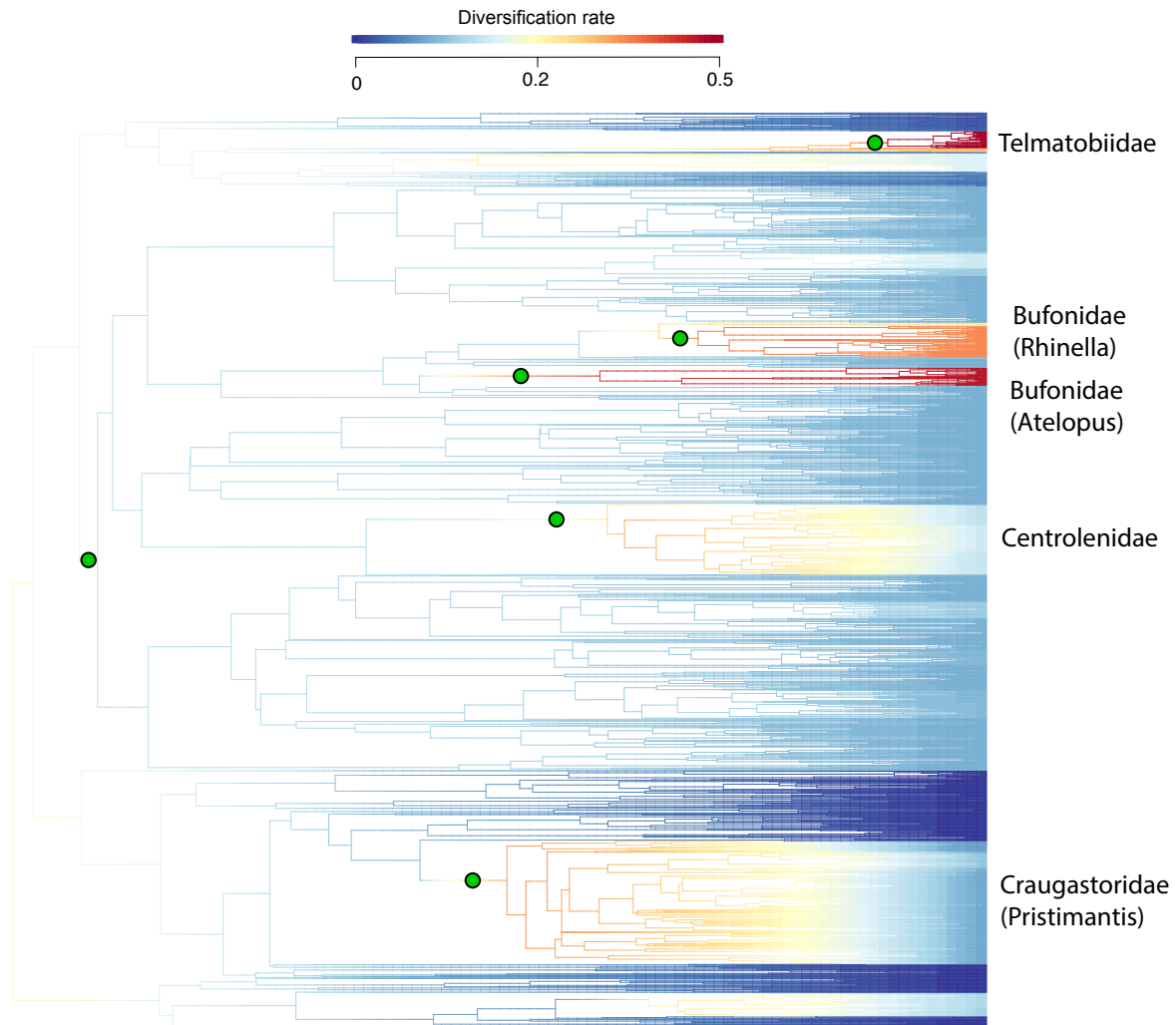


Figure S4. Rates of evolution for elevational distributions for Hyloidea from BAMM analyses using the alternative, constrained phylogeny. The green dots indicate significant increases in the rate.

