

# The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life

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**Human activities continue to erode the tree of life, requiring us to prioritize research and conservation. Amphibians represent key victims and bellwethers of global change, and the need for action to conserve them is drastically outpacing knowledge. We provide a phylogeny incorporating nearly all extant amphibians (7,238 species). Current amphibian diversity is composed of both older, depauperate lineages and extensive, more recent tropical radiations found in select clades. Frog and salamander diversification increased strongly after the Cretaceous–Palaeogene boundary, preceded by a potential mass-extinction event in salamanders. Diversification rates of subterranean caecilians varied little over time. Biogeographically, the Afro- and Neotropics harbour a particularly high proportion of Gondwanan relicts, comprising species with high evolutionary distinctiveness (ED). These high-ED species represent a large portion of the branches in the present tree: around 28% of all phylogenetic diversity comes from species in the top 10% of ED. The association between ED and imperilment is weak, but many species with high ED are now imperilled or lack formal threat status, suggesting opportunities for integrating evolutionary position and phylogenetic heritage in addressing the current extinction crisis. By providing a phylogenetic estimate for extant amphibians and identifying their threats and ED, we offer a preliminary basis for a quantitatively informed global approach to conserving the amphibian tree of life.**

Biodiversity and its many functions are being lost rapidly worldwide, with significant but often poorly quantified implications for current and future ecosystems<sup>1,2</sup>. The breadth and magnitude of these declines has brought to the fore questions about how to prioritize research and conservation management in the face of these changes<sup>3,4</sup> and the recognition of ‘facets’ of biodiversity that go beyond species identities as targets for conservation<sup>5,6</sup>. Calls have been made to conserve the tree of life<sup>7–9</sup>, with potential for rapidly expanding phylogenetic coverage to help guide these actions<sup>10–14</sup>. Symbolizing these developments are amphibians, a fascinatingly ancient and near-global radiation of around 7,700 species, which are a prime casualty, bellwether and cause célèbre of global change<sup>15–19</sup>. The group is undergoing worldwide population declines and stands out among vertebrates as particularly impacted by anthropogenic activities<sup>19–21</sup>. Physiological, ecological and biogeographic characteristics such as a highly permeable skin, dependence on specific microclimates and habitats, and small geographic ranges all contribute to a high vulnerability to environmental change, infectious disease and exploitation for many amphibian species<sup>15,17,20,22–24</sup>.

Amphibians (<http://amphibiaweb.org>) today comprise ~6,700 frogs (Anura), ~700 salamanders (Caudata) and ~200 caecilians (Gymnophiona). Sister to all other terrestrial vertebrates, they constitute around one-fifth of all extant tetrapods<sup>25</sup>. With the origin and separation of the three major groups stretching back into the Palaeozoic<sup>26</sup>, amphibians were originally thought to have seen a gradual diversity increase through the Mesozoic and Cenozoic<sup>27</sup>. This view was modified by more recent molecular work that identified potential earlier extinction periods more in line with the environmental sensitivity of the group, followed by strong accumulation of lineages in the late Cretaceous and Palaeogene<sup>28,29</sup>. However, limited taxon sampling has left several fundamental questions about

the amphibian tree of life unanswered. Specifically, the relative contributions of early versus more recent species accumulation to extant diversity, and heterogeneity in diversification rates through time, have seen limited quantitative scrutiny to date. A fully sampled phylogeny for the group thus has the potential to provide a global characterization of the tempo and mode of diversification, and to pinpoint its effects on the global distribution of diversity within the group.

Quantifying diversification-rate heterogeneity in amphibians also has direct implications for conservation, specifically for understanding how phylogenetic diversity (that is, the sum of all branch lengths in the amphibian tree of life) is apportioned among extant species as evolutionary distinctiveness (ED; in Myr of branch length). This tip-level ED characterizes species’ evolutionary isolation on the tree and distinguishes recent radiations, resulting in high phylogenetic redundancy, from species subtended by long, isolated branches that are characteristic of more ancient divergence and subsequent stasis, extinctions or high turnover<sup>30</sup>. When complete sampling of extant taxa is available, the measure both supports a species-level interpretation of tree-wide rate heterogeneity and provides a transparent measure of a species’ unique contribution to the tree of life for prioritizing research and management<sup>10–12,31</sup>.

Are species of more ancient and evolutionarily isolated lineages particularly rare or susceptible to already ongoing change? Are such species concentrated in select parts of the world or geographically widespread? With extant members ranging from ‘living fossils’, such as the cave-dwelling European olm (*Proteus anguinus*, Caudata) to recently derived species such as the widely invasive cane toad (*Rhinella marina*, Anura), amphibians span a vast evolutionary and ecological spectrum enabling us to address these questions. Gauging the rapidity at which ongoing global change is poised to

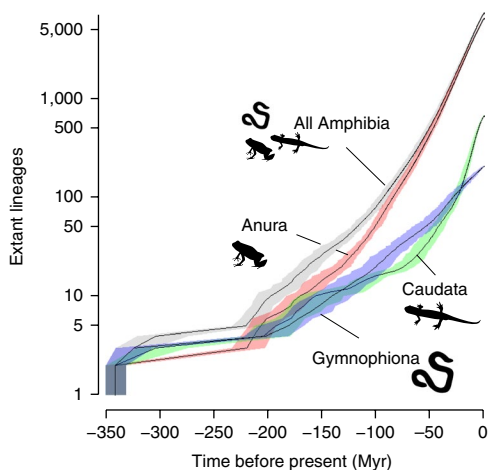
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erase millions of years of independent evolutionary history is of chief concern, with nearly one-third of amphibians considered threatened out of the ~4,850 species assessed by experts as of 2016, and an additional 35 or more species considered extinct in the wild<sup>18,19,32</sup>. Given the environmental sensitivity of most amphibians, threats are expected to increase under most future climate-change scenarios<sup>33–37</sup>. The need for conservation action stands in contrast with often substantial uncertainty about threat status<sup>15,16,38</sup> and the spatial distribution of diversity<sup>39–43</sup>. Information that can help prioritize research and management and guide the safeguarding of the amphibian tree of life thus has vital practical relevance.

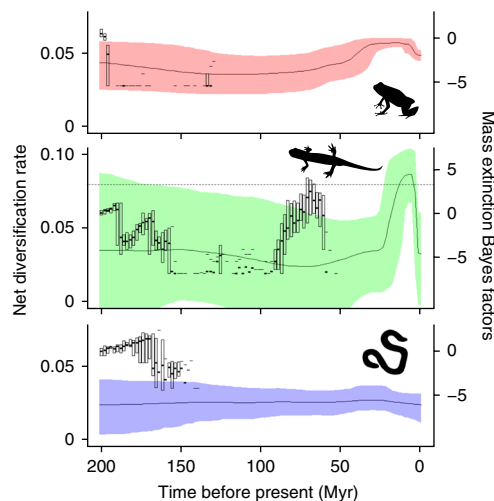
In this study, we develop a Bayesian time tree of 7,238 extant amphibian species (~94% of all described species) to assess the heterogeneous spatial and temporal diversification of the group and implications for a phylogenetically informed conservation. We investigate among- and within-clade variation in the rates and timing of lineage accumulation and examine how independent history (ED) is partitioned among species and the regions they occupy. We relate species' ED patterns to their current levels of imperilment and provide a basis for research and conservation prioritization informed by the macroevolutionary processes that have shaped this ancient radiation. We find that a small number of species, spread across all three groups, harbour a large portion of extant amphibian diversity and may be of particularly high priority for research and conservation. Yet, many thousands of species have still not been assessed for threat status, or are data deficient.

## Results and discussion

**Diversification across the amphibian tree of life.** Lineage-through-time analysis shows a biphasic diversification pattern in amphibians both as a whole and in frogs (Anura), with a lower rate of increase from ~200–100 million years ago (Ma) and a sharper uptick in diversity towards the present (Fig. 1). Bayesian variable birth–death rate estimates with possible mass extinctions<sup>44</sup> suggest that rates of net species diversification were consistently high in frogs throughout the Mesozoic (252–66 Ma; Fig. 2). Rates tend upwards after the Cretaceous–Palaeogene (K–Pg) boundary (~66 Ma), driven by shifts in both speciation and extinction rates (Supplementary Figs. 2–8). Salamanders (Caudata) show a slower and—among trees—more variable rate of lineage accumulation and net diversification during the Mesozoic. This is followed by an increase in



**Fig. 1 | Lineage-through-time plots of 100 time trees sampled from the Bayesian posterior distribution.** All extant amphibians (7,238 species), frogs (Anura, 6,380 species), salamanders (Caudata, 659 species) and caecilians (Gymnophiona, 199 species) are addressed. Black lines indicate the median trend and the coloured areas the 95% CIs of lineages at a given point in time.

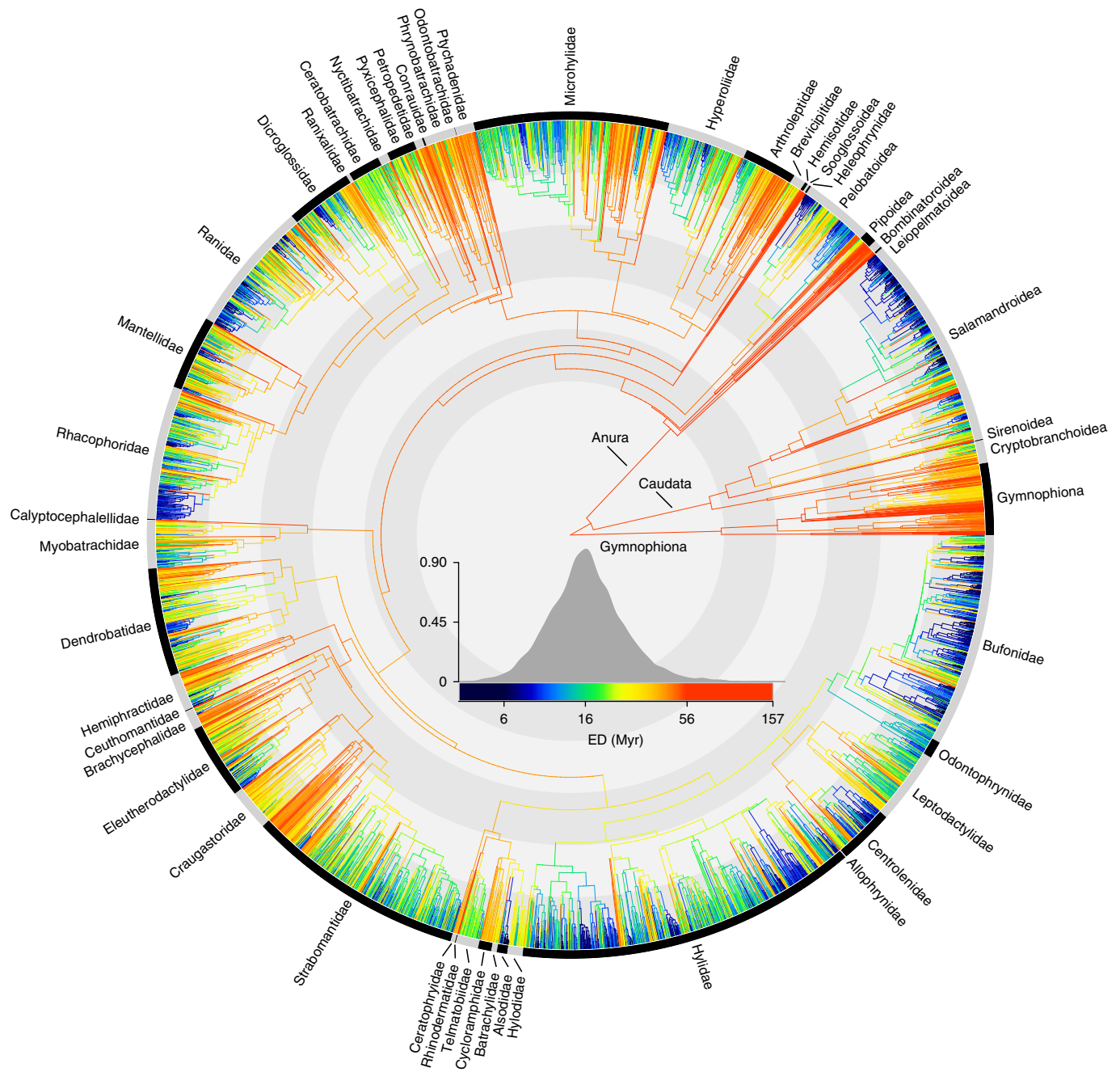


**Fig. 2 | Variation in net diversification rates of the three amphibian clades.**

Rate estimates (median and 95% CIs) are based on CoMet analysis of 10 trees sampled from the posterior. Boxplots (1.5 interquartile range boxes only) are included for Bayes factors for evidence of mass extinction in a given time interval. Bayes factors above 3.2 (marked with a dotted line for Caudata) are considered as providing 'substantial' evidence. For additional results, see Supplementary Figs. 2–8.

the diversification rate after the K–Pg boundary, stemming from an upshift in speciation rates and a downshift in extinction rates ~30–20 Ma. There is a subsequent steep drop in net diversification near the present, which may have multiple causes, including niche filling in the geographically limited regions of peak diversification in this group<sup>45</sup> or a high prevalence of cryptic and undescribed species<sup>46</sup>. Analyses using the CoMet<sup>47</sup> model yield substantial evidence of a mass extinction at the K–Pg boundary in Caudata, while there is no similar evidence in either Anura or Gymnophiona (Fig. 2). In contrast with both Caudata and Anura, the Gymnophiona lineage exhibits a nearly constant rate of lineage accumulation from ~200 Ma leading to its present diversity of ~200 species. The K–Pg mass-extinction event had strong effects on diversification in endothermic tetrapods<sup>48,49</sup> and signals of an even stronger impact may thus be expected for a group that is particularly environmentally sensitive and ectothermic. This is supported for frogs and especially salamanders, given the signs of a K–Pg mass extinction and increasing diversification rates over much of the Cenozoic. The diversification of Gymnophiona appears largely unaffected by the major climatic events in the geological past, potentially signalling relative robustness to long-term environmental change due to their subterranean lifestyle.

Examining the full amphibian tree in more detail (Fig. 3), we find strong heterogeneity in ancient lineages, either diversifying heavily or exhibiting long-term stasis. While many caecilian and salamander clades saw little net diversity increase over the past 50 Myr (for example, Rhinatrematidae and Proteidae), many salamanders (for example, Plethodontidae and Salamandridae), toads (Bufonidae) and tree frogs (Hylidae) are examples of more recent, explosive radiations. Across our distribution of time trees, half of extant amphibian species diverged from their closest extant relatives in the past 7.43 Myr (95% credible interval (CI): 7.0–8.03 Myr), with only 578 species (95% CI: 508–628) diverging less than 1 Ma. This suggests a relatively limited overall contribution of late Neogene or Quaternary radiations to extant amphibian diversity and high turnover. Accordingly, the median ED is 16.5 Myr and, thanks to the long interior branches in the amphibian tree of life, even the least evolutionarily distinctive species *Rhinella gnustae* still harbours 4.1 Myr of ED (this species is a close relative to the cane toad,



**Fig. 3 | Phylogeny of all extant amphibian species and their variation in ED.** One tree, randomly drawn from the posterior distribution, is shown.

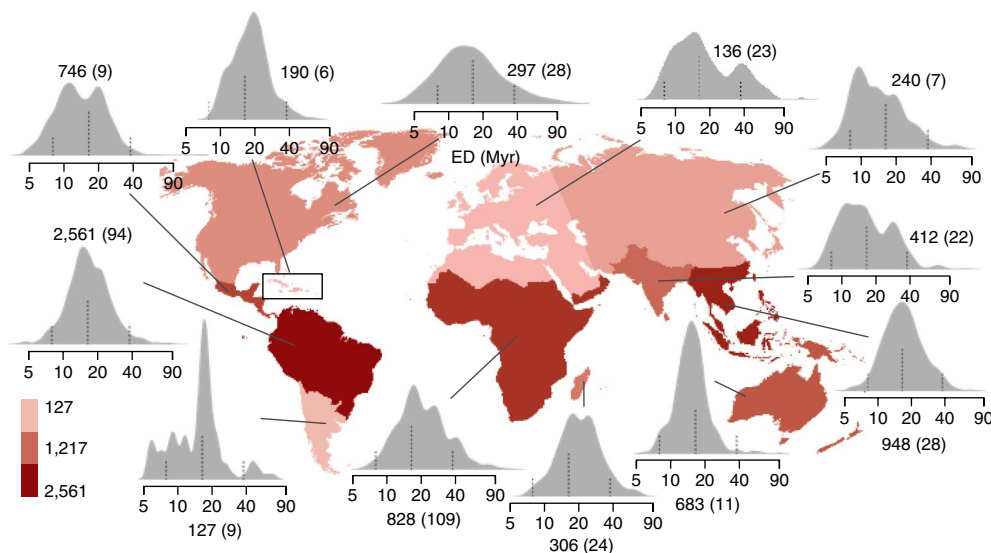
For visualization purposes, the branches are coloured according to the ancestral states estimated under Brownian motion using a least-squares algorithm. The graph in the centre is the probability density of ED values of all extant species. Circles in different grey scales represent 20 Myr intervals.

*R. marina* which has an ED of 4.9 Myr). This contrasts strongly with birds<sup>10,50</sup>, which have a median ED of 6.2 Myr and a minimum value of 0.8 Myr—that is, over five times lower in birds, with multiple conspicuous instances of recent rapid radiations. However, this may also partially represent a Linnaean shortfall in some poorly known, recently radiating groups of amphibians such as Microhylidae and Strabomantidae.

**Variation in ED.** Temporal and phylogenetic heterogeneity in diversification rates directly determines the evenness of ED, as distributed among extant species for any clade across the tree of life. Recent radiations in a given clade suggest less evolutionary information specific to individual species (lower ED) and, in the context

of conserving phylogenetic diversity, they imply greater redundancy among species. In contrast, more ancient diversification followed by slowdowns or extinctions results in individual species holding high ED and their conservation being of great relevance for safeguarding the tree of life, as each species is highly unique.

In Amphibia, we find that the variation in within- and among-clade diversification of extant amphibians results in a highly uneven apportionment of diversity among extant species in the amphibian tree of life (Fig. 3). Among extant species, ED appears almost symmetrically distributed in log space (Fig. 3 inset) with 95% of species in the 7.13–45.73 Myr range. As expected from their limited diversification since the late Cretaceous (Fig. 1), highly distinctive species (the top 10% of ED; that is, >30 Myr) are particularly prevalent



**Fig. 4 | Biogeographic variation in amphibian phylogenetic structure as characterized by the probability density of ED values.** Regions are coloured by their total richness, which is shown beside each density plot, followed (in brackets) by the count of global top 5% ED species in the region. Vertical dotted lines indicate 5, 50 and 95th percentile values of the global ED distribution. Region delineation follows ref. <sup>51</sup>. The label ‘ED (Myr)’ applies to all x axes.

in the ancient caecilian lineage (Gymnophiona), but are otherwise found throughout the tree in early-branching anuran and salamander lineages. Low-ED species (bottom 10%; <9.5 Myr) characteristic of recent radiations constitute the majority of salamanders (especially Plethodontidae and Salamandridae), toads (Bufonidae), true frogs (Ranoidea) and tree frogs (Hylidae). These groups consistently rank among the most recently diverged species, with very low ED. In most remaining parts of the tree, substantial heterogeneity in ED exists, with both low- and high-ED species occurring in most extant families. This result is robust to restricting the tree only to species supported by molecular evidence (Supplementary Fig. 10). However, a large number of amphibian species probably remain to be discovered and described, particularly from tropical areas.

**Geographic variation in phylogenetic structure.** Our coverage of ED variation among described, extant species also enables a biogeographic analysis of different macroevolutionary regimes. We expect major biogeographic patterns in ED to reflect the originally Pangaean distribution of major lineages<sup>51</sup>, combined with the overall relatively low dispersal ability of amphibians to result in all major regions harbouring at least some high-ED lineages. In line with this expectation, all major regions of the world span the spectrum of high- and low-ED species (Fig. 4), with each region harbouring members of the bottom and top 5% ED groups (each comprised of 362 species). However, regions are distinguished by strongly differing concentrations and among-species variation in ED scores. The Afrotropics in particular stands out for hosting nearly one-third of the top 5% ED species, despite only ranking third in overall species richness. The Neotropics—by far the most species-rich region—holds a similarly high number of most distinct species, but they only constitute 4% of all species in the region, compared with the high-ED species representing 13% of all species in the Afrotropics.

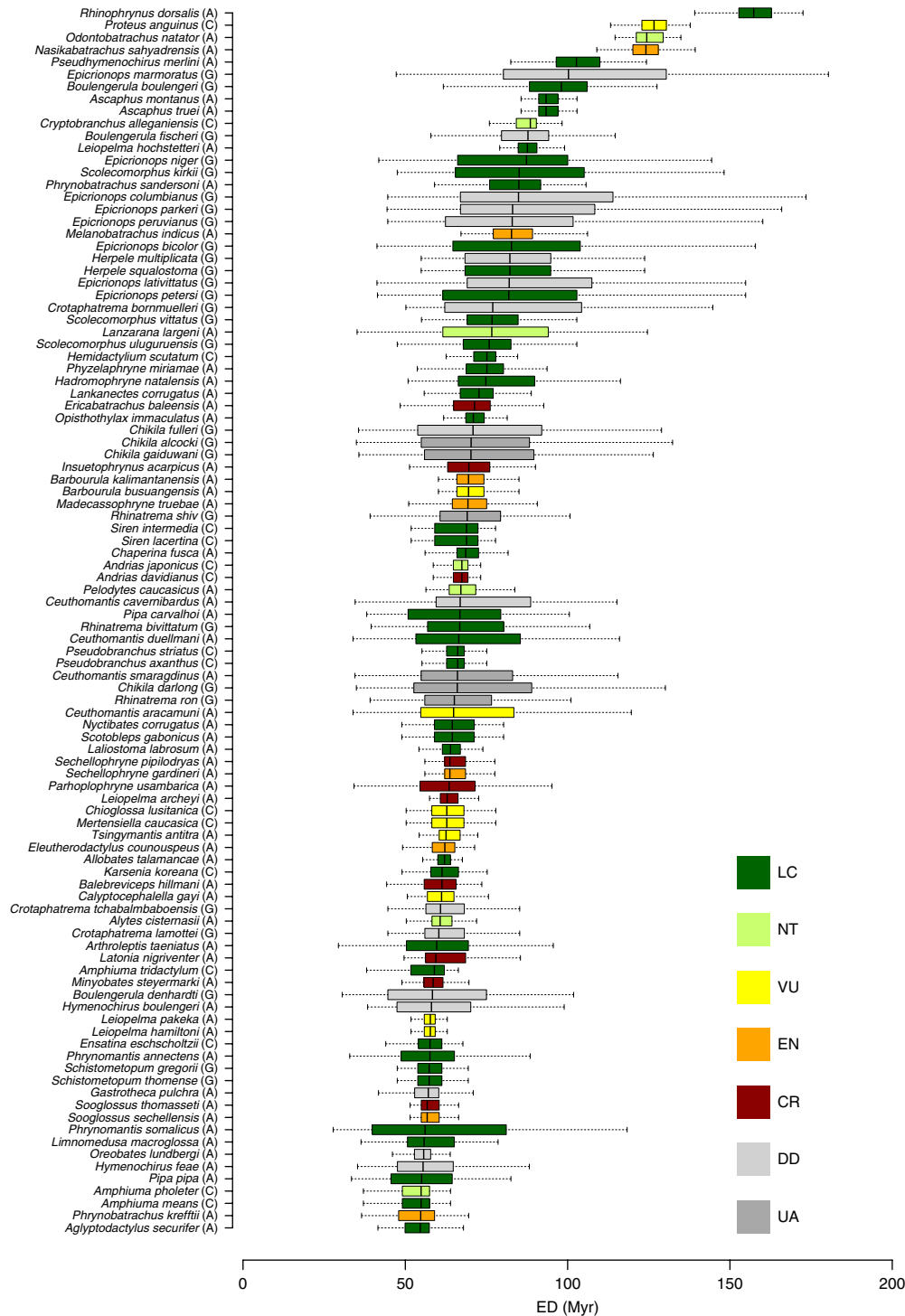
In contrast, the ED of species in other regions is more central or skewed towards having more recently diverged, low-ED species. This is particularly true for the Eastern Palearctic, West Indies and Middle America, as well as Australasia, which hold only a handful of high-ED species that constitute  $\leq 3\%$  of all species in those regions. Generally, temperate South America, tropical Middle America and the three northern latitude regions are all characterized by a particularly large proportion of more recent divergences, although all contain at least one ancient, high-ED lineage

(for example, Calyptocephalellidae, Rhinophrynidae, Proteidae and Cryptobranchidae, respectively). In contrast, tropical regions with high diversity show more centred distributions, peaking only slightly above the global median.

This regional perspective characterizing independent units provides some nuances compared with gridded patterns<sup>52,53</sup> where small-ranged taxa may see diminished representation. Spatial patterns of amphibian richness and turnover have been shown to strongly follow contemporary environmental gradients in factors such as net primary productivity<sup>54–56</sup> and diversification rates increase towards lower latitudes<sup>56,57</sup>. Overall, we find no obvious association between a region’s median or shape of ED distribution and the total number of species that it supports.

**Conservation priorities.** Total amphibian phylogenetic diversity; that is, the sum of all branch lengths in the amphibian tree of life, represents 136 Gyr of combined evolutionary history, but is captured by some species much more than others. Four species from four different continents stand out in particular, with their posterior distribution suggesting an ED significantly greater than 100 Myr (Fig. 5). The Mexican burrowing toad *Rhinophrynus dorsalis* (Mexico)—the only living member of Rhinophrynidae—stands first with 157 Myr of evolutionary information unique to this species alone. It is followed by the European olm *P. anguinus* (median 126 Myr; Central Europe), the sabre-toothed frog *Odontobatrachus nator* (124 Myr; West Africa) and the only recently (re-)discovered purple frog *Nasikabatrachus sahyadrensis* (124 Myr; Southern India). The top 100 amphibian ED species (Fig. 5) represent  $\sim 7.2\%$  of the total amphibian phylogenetic diversity, compared with only 1.5% represented by the 100 lowest-ED species. Similarly, the top 5 and 10% of highest-ED species capture  $\sim 17$  and 28% of the total phylogenetic diversity, compared with  $\sim 4$  and 8% of diversity represented by 5 and 10% of the lowest-ED species.

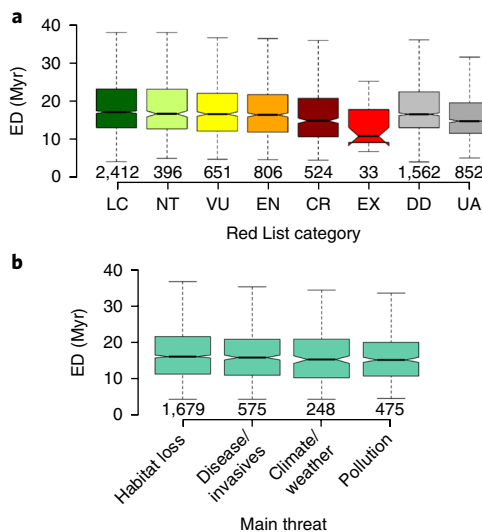
We were able to link  $\sim 88\%$  of extant species in the current taxonomy to a recent IUCN Red List threat assessment, and to place imperilled species and their ED on the amphibian tree of life (Supplementary Fig. 12). Crucially, much of the phylogenetic diversity represented by high-ED species is now at risk of extinction: among the top 100 ED species, 34 are considered threatened (27) or near threatened (7), while almost one-quarter (23) remain unassessed or data deficient (Fig. 4). Species assessed as not imperilled



**Fig. 5 | ED of the top 100 most evolutionarily distinct amphibian species (median ED: >54 Myr) and their 2016 IUCN Red List threat status and clade affinity.** Box colour indicates threat status. Clade affinities are marked 'A' for Anura, 'C' for Caudata and 'G' for Gymnophiona. Boxplots show the variation (minimum, 25th percentile, median, 75th percentile and maximum) in metrics among 1,000 randomly sampled trees from the posterior distribution of 10,000 trees. CR, critically endangered; DD, data deficient; EN, endangered; LC, least concern; NT, near threatened; UA, species that remain unassessed; VU, vulnerable.

share overall similar, but slightly higher, ED compared with imperilled species (Fig. 6a; geometric mean EDs of 17.42 Myr versus 16.11 Myr, respectively;  $F=38.11$ ,  $P<0.001$ ). Remarkably, the 559 species that are closest to the brink of extinction (that is, critically endangered) or recently extinct have slightly lower ED (geometric mean: 14.91 Myr), suggesting that recent extinction pressures

are highest in recent radiations located in habitats and regions now under human pressure. However, their imminent disappearance would still lead to significant losses in phylogenetic diversity and elevate the ED of their extant sisters<sup>58</sup>. A remarkable number of amphibian species (2,414 species; 33% of the total) remain unassessed or data deficient, although their ED (geometric mean:



**Fig. 6 | Variation in ED by threat status categorization and main threat.**

**a**, ED for various 2016 IUCN threat status categories: CR, critically endangered; DD, data deficient; EN, endangered; EX, extinct in the wild; LC, least concern; NT, near threatened; UA, species that remain unassessed; VU, vulnerable. **b**, ED for each main threat for imperilled species. Boxplots show medians (horizontal line), an approximation of 95% CIs suitable for comparing two medians (notches), 25th and 75th percentiles (boxes), and the most extreme data points that are no more than 1.5 times the length of the box away from the box (whiskers). Numbers below boxes are sample sizes (species) per category. Threat information was available for 1,748 species assessed as imperilled (VU, EN or CR), and a single species may have multiple threats listed. See Methods for further details.

16.46 Myr) is on average only marginally different from those of assessed species (geometric mean ED: 16.96 Myr;  $F = 6.64$ ,  $P < 0.01$ ). A large suite of factors is known to contribute to the high levels of amphibian imperilment, including habitat encroachment, infectious diseases and pollution<sup>15,17,20,22–24,59,60</sup>. We use an evaluation of major threats contributing to amphibian imperilment to test the hypothesis that threat types are associated with ED. We find that species suffering from habitat loss—by far the most frequently listed threat in amphibians—show no noticeable difference in ED compared with those currently affected by disease (such as chytridiomycosis), pollution or changing climate conditions, and no significant ED differences among the four groups emerge (Fig. 6b;  $F = 0.72$ ).

Our analysis also provides a global list of amphibian species that are both highly evolutionarily distinct and recognized as imperilled (that is, globally endangered)—so-called ‘EDGE’ species<sup>11</sup>. The results advance on previous work, benefitting from more complete molecular sampling and by placing all species in a single Bayesian framework that better addresses uncertainty in the available evidence. Our work highlights the Bale Mountains frog *Ericabatrachus baleensis* (Ethiopia), Barrio’s frog *Insuetophrynus acarpicus* (Chile) and Chinese giant salamander *Andrias davidianus*—all critically endangered and occurring in threatened habitats—as the top three most imperilled and distinctive according to the EDGE score (Fig. 5). Only *A. davidianus* has previously been identified as being among the top 10 EDGE species. Only 27% (19/71) of the species in the new top 100 EDGE list that in name and threat status matched a previous assessment<sup>31,52</sup> had previously been recognized as top 100 (Supplementary Table 1). Furthermore, 64% (45/71) of the previous top 100 list are not retained. This mirrors major differences in ED values in the two analyses, with the 4,307 species’ ED values in the earlier work linked to our tree only showing a very limited correlation (0.33; Supplementary Fig 9). For 59% of these species, the

previously estimated ED falls significantly outside the 95% posterior CI (48% were underestimated; 11% overestimated).

### Tree uncertainty and phylogenetically informed prioritization.

We characterized global ED for amphibians with an approach that uses taxonomic constraints for the placement of the 44% of species without genetic data, and captured the resulting topological and branch length uncertainty arising in a posterior distribution<sup>61</sup>. Without this approach, calculating and comparing ED values would not have been possible, as missing species skew the ED of their neighbours in the tree. This resulting uncertainty adds to that from imperfect molecular information. The missingness of molecular data is geographically highly uneven, ranging from under 10% of species in the Nearctic, western Palearctic and Madagascar to over 50% in the Afrotropics, South Asia, Neotropics and Oceania. Our approach to completing the tree was thus critical to enable an appropriate regional comparison. Of the top 100 ED species (Fig. 5), 23 lack any genetic data. Species lacking molecular data (see Supplementary Fig. 10) have mean ED values (geometric mean ED = 16.48 Myr) that are only slightly different from those represented by sequences (geometric mean ED = 17.04 Myr,  $F = 9.20$ ,  $P < 0.01$ ). As expected, species lacking molecular data have much greater uncertainty (geometric mean 95% CI of ED = 22.93 Myr versus 10.44 Myr;  $F = 3,170$ ;  $P < 0.001$ ; see Supplementary Fig. 11).

Future species discoveries, as well as taxonomic lumps and splits, will also affect the phylogeny and estimated ED values and rankings. For example, a proposal to split the monotypic of the West African genus *Odontobatrachus* into five species<sup>62</sup> will result in *O. natator* losing its current high-ED rank. Notably, even in the case of taxonomic splits, the current call for attention to these species and their spatial range remains valid, as the joint phylogenetic diversity they represent over their range remains high regardless of nomenclatural division. There is a new generation of phylogenies that include nearly all known extant species<sup>50</sup> and quantitatively address uncertainties arising from molecular and/or taxonomic placement constraints<sup>61</sup>, or increasingly just molecular uncertainty (that is, full molecular taxon sampling). While there is some cause for caution in the downstream application of these trees for estimating character evolution<sup>63</sup>, we suggest that these now provide a much-improved basis for the calculation and use of ED in conservation and decision-making over previous efforts that used incomplete trees or other information such as taxonomic classifications alone. Nevertheless, phylogenies and any phylogenetically informed conservation prioritization will require careful future updates as species are discovered or go extinct, taxonomies change, or new insights arise from fossil data.

### Conclusion

Global phylogenies that include all extant members of a clade provide a number of novel avenues for evolutionary inference and conservation application. Here, we elucidate the differing evolutionary paths of the three major amphibian clades, with all extant lineages accounted for. Including all branches in the amphibian tree of life enables us to quantify tree shape in species-level metrics that can be connected to species attributes, such as traits or their geographic distribution. Specifically, by capturing the portion of a clade’s phylogenetic diversity (or Myr of evolutionary information) distinct to a single species from across all species, the ED metric allowed us to develop several key findings. We found some support for a K–Pg mass extinction in Caudata, which is a novel result to our knowledge. We show that high- and low-ED species, in rapidly or slowly radiating parts of the tree, are distributed heterogeneously throughout the phylogeny and the world. While clusters of particularly recent or old divergences exist, heterogeneity in ED across the tree is substantial and comparable to that recently documented in birds<sup>10</sup>. Amphibians thus differ substantially from squamates<sup>64</sup> where large

portions of the tree are dominated by either very recent (snakes) or ancient (geckos) divergences, and only parts of the tree show large heterogeneity. This heterogeneity is also geographically repeated in all major regions of the world, but distinct among-region variation also exists. Both temperate and tropical regions contain imperilled, high-ED amphibian lineages, but more diversity (and thus greater total phylogenetic diversity and more ED species) is found in the tropics. Finally, imperilled species are not strongly different in phylogenetic location or ED values. These data, standardized across the full tree, offer a tangible tool to assist research or management actions. Over 2,400 amphibian species remain unassessed for their threat status or are data deficient. Uncertainty in the current tree remains, and additional systematics work and updates to the phylogeny are needed as many tropical amphibian species remain yet to be described. Nevertheless, we now know the closest relatives and approximate ED of otherwise often data-limited species, supporting both phylogenetic imputation of threat status and conservation prioritization<sup>14</sup>. As global change is poised to bring many members of this ancient radiation even closer to the brink of extinction, an understanding and use of their ED has the potential to assist in appreciating and safeguarding the amphibian tree of life.

## Methods

**Tree construction and ED calculation.** We used the Phylogenetic Assembly with Soft Taxonomic Inferences approach<sup>50,61,64</sup>—a combination of phylogenetic inference and taxonomic assignment—to generate a posterior distribution of phylogenies for Amphibia including nearly all extant species. Complete data and methods are presented in the Supplementary Information and briefly outlined here. We used the 19 February 2014 edition of AmphibiaWeb (<http://amphibiaweb.org>) for a reference taxonomy containing 7,238 species. We revised an existing molecular supermatrix<sup>31</sup> to include all available sequence data for 15 genes (5 mitochondrial and 10 nuclear) for 4,061 (56%) amphibians plus the outgroup *Homo sapiens*. Using the Exascale Maximum Likelihood and Randomized Accelerated Maximum Likelihood programmes<sup>65</sup>, we estimated the maximum-likelihood topology for these species. This topology was enforced as a backbone constraint for all subsequent analyses for those species with data.

We identified 174 subclades, which accounted for all 7,238 species. We then extracted a subset of the matrix containing those 174 species representing each subclade, which we dated separately using MrBayes 3.2 (ref. <sup>66</sup>) under a relaxed-clock model. Node-age calibrations were taken from fossils and recent consensus estimates for the amphibian time tree (see Supplementary Fig. 1). While this approach potentially limits the maximum age of subclades, the estimated backbone ages are highly congruent with essentially all recently published studies for higher-level amphibian divergences<sup>28,29</sup> and thus should not represent a skewed estimate of diversification processes. For the 174 individual subclades, we estimated trees scaled to relative time under the same relaxed-clock model. In these analyses, the topology of species with DNA-sequence data was fixed to the global maximum-likelihood analysis (as an extracted subclade), and the remaining unsampled species were assigned randomly within their genus or species group, with branch lengths drawn from the overall subclade distribution.

From these subclade analyses, one tree from each subclade distribution was grafted onto its parent lineage on the backbone tree, with the root age then re-scaled to absolute time. In sum, this yielded a distribution of 10,000 trees containing 7,238 species. In each of these trees, the maximum-likelihood topology for 4,061 species was constant and the placement of the unsampled species was drawn from the posterior distribution of their possible locations within each genus- or species-group clade. Thus, the distribution of 10,000 trees represents a limited region of treespace, accounting for both known phylogenetic relationships of species with molecular data and the taxonomic classification of unsampled species based on morphology.

Estimating fully sampled phylogenies in this manner is increasingly used for applications involving evolutionary rates and distinctiveness of threatened species, even for unsampled taxa<sup>50,61,64</sup>. Such estimates may not be suitable for estimating rates of continuous-character evolution or other trait-based metrics, but simulations have shown them to be appropriate for assessing branch-length-related measures such as the diversification rate and, by extension, ED<sup>67</sup>. They are therefore useful for creating null models of the distribution of threat status while remaining conservative with respect to phylogenetic uncertainty.

With these trees, we calculated ED based on the fair proportion metric<sup>11,68,69</sup> using the R package 'picante'<sup>70</sup>. The fair proportion metric is commonly used to characterize evolutionary isolation<sup>10,30,69,71</sup> and has, in the past, been employed by the EDGE programme to identify evolutionarily distinct species of particular conservation concern<sup>11</sup>. Fair proportion estimates ED as the weighted sum of the branch lengths along the path from the root of an ultrametric tree to the tip,

with weights determined as the inverse of the number of tips sharing that branch. This measure of ED is closely inversely related to the equal splits metric used to characterize tip-level diversification rates<sup>10,69</sup> (Spearman's  $r = -0.89$  for  $n = 7,238$  species means calculated across 1,000 trees from the posterior). All ED values are provided in Supplementary Table 1.

**Tree-wide diversification rates.** To illustrate the variation in lineage accumulation, we used lineage-through-time plots as implemented in the R package 'ape', summarized across 100 trees drawn from the posterior distribution of trees. For a more quantitative evaluation of amphibian diversification over time, we inferred rate estimates for speciation and extinction with a Bayesian variable 'birth-death', as implemented in the 'TESS' R package<sup>44</sup>. Jointly with this analysis, we also searched for potential evidence of past mass extinctions using the 'compound Poisson process on mass-extinction analysis' (CoMet<sup>68</sup>). Specifically, for ten trees from the posterior of each of the three amphibian clades, we modelled a variable 'birth-death processes' with explicit mass-extinction events following the guidelines in ref. <sup>72</sup>, with the sampling fraction set to 1. We generated empirical hyperpriors through an initial Bayesian Markov chain Monte Carlo analysis under a constant-rate birth-death-process model to determine reasonable hyperparameter values for the diversification priors<sup>72</sup>. We allowed up to two mass-extinction events and two rate change events (varying these parameters yielded qualitatively similar results). After burn in, we ran final analyses for 100,000 generations (200,000 in the case of Caudata) and diagnosed models using effective sample size (which as a rule of thumb should exceed 200) and the Geweke statistic<sup>72</sup>. Figure 2 shows the combined rate variation of ten trees drawn from the posterior. For detailed results for a single tree, see Supplementary Figs. 3–8.

**Distribution and threat.** We used the assignment of species to the 12 biogeographic regions developed from previous studies (ref. <sup>31</sup> and the references therein). In total, 230 species occur in 2 regions and only 6 species in 3, confirming the overall high independence of regions and supporting a comparison of ED densities among them with only negligible pseudoreplication. The IUCN Red List of Amphibians consisted of 6,460 species accounts on 3 April 2016 (ref. <sup>32</sup>). Of these, 528 were critically endangered, 1,604 were data deficient, 810 were endangered, 2 were extinct in the wild, 33 were recently extinct, 2,427 were of least concern, 400 were near threatened and 656 were vulnerable. Thus, 2,029 were threatened, 2,827 were non-threatened and 1,604 were data deficient. Matching these to our list of 7,238 extant amphibian species, 71 were considered synonyms of taxa in our list and 5 have only been described recently; thus, 6,384 matched taxa that we recognized. Thus, in our reference taxonomy of 7,238 species, 2,015 were threatened, 2,807 were non-threatened and 1,562 were data deficient, with an additional 854 species unassessed (see Fig. 6 for details). From these, we used the median estimated ED values to calculate EDGE scores for the 4,787 extant (excluding extinct in the wild and recently extinct) assessed taxa (see Supplementary Table 1) to aid the Edge of Existence Programme<sup>11</sup>. Following their methodology, extinction probability is expected to increase with every IUCN category by a factor of 2, with species of least concern having a probability of extinction of 0. EDGE is then given as  $\ln(\text{ED} + 1) + \text{GE}(\ln(2))$ , where the scaling factor GE is given as: least concern, 0; near threatened, 1; vulnerable, 2; endangered, 3; and critically endangered, 4. We also compared these with the 4,331 ED and EDGE scores provided by ref. <sup>31</sup>, of which 4,307 matched our reference taxonomy, excluding 24 synonyms. For 4,142 species—and specifically the 1,748 species assessed and classified as imperilled (categories vulnerable, endangered and critically endangered)—we were able to taxonomically link the threats classification<sup>73</sup> developed by Red List assessment experts<sup>74</sup> to more directly address key threats recognized for amphibians. We reclassified its 12 main threat categories<sup>75,76</sup> as follows: 'habitat loss' (classes: residential and commercial development; biological resource use (including logging); agriculture and aquaculture; energy production and mining; transportation and service corridors; and natural system modifications); 'disease/invasives' (classes: invasive and other problematic species; and genes and diseases); 'pollution' (class: pollution); and 'climate/weather' (class: climate change and severe weather). We lumped the remaining categories, which only marginally applied to amphibians, as 'other' (classes: human intrusions and disturbance; geological events; and other options) and did not analyse them further. We used this information to evaluate whether certain types of threat faced by imperilled species are associated with higher or lower ED.

**Life Sciences Reporting Summary.** Further information on experimental design is available in the Life Sciences Reporting Summary.

**Data availability.** See Supplementary Table 1 for all species data analysed. The phylogenetic datasets generated and/or analysed during the current study, including a posterior distribution of 10,000 trees, are available at <https://vertlife.org/data> and will also be made available on the Dryad Digital Repository (<https://doi.org/10.5061/dryad.cc3n6j5>).

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### Competing interests

The authors declare no competing interests.

### Additional information

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### ▶ Experimental design

#### 1. Sample size

Describe how sample size was determined.

We included all amphibian species described by early 2014 (when we began the phylogeny construction) according to the authoritative community resource, [amphibiaweb.org](#). Since then additional species have been discovered and described. In terms of genes, we included all genes with sufficient sample size among species to enable tree building.

#### 2. Data exclusions

Describe any data exclusions.

To our knowledge, no species recognized in 2014 were excluded. All species included in the tree building were included in subsequent analyses.

#### 3. Replication

Describe whether the experimental findings were reliably reproduced.

We produced a posterior distribution of 10,000 trees. We ran macroevolutionary analyses over a random set of 10, 100, 1,000 trees and list the number of trees used with each analysis, respectively.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

NA

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R packages ape, TESS, phytools, picante, pastis, ExaML, RAxML, MrBayes

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no restrictions

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Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

NA

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a. State the source of each eukaryotic cell line used.

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