## Response to Reviewer Comments on: Populating a Continent: Phylogenomics Reveal the Timing of Australian Frog Diversification

We would like to begin by thanking the two anonymous reviewers, as well as Drs. Bell and Sanmartín for their thoughtful and explicit input. All reviewers provided carefully considered comments and we have done our best to accommodate those suggestions here. On behalf of myself and my coauthors, we appreciate your time and contributions.

Below please find reviewer comments in green, and our responses in black. We have formatted a page break between each reviewer. Where necessary, we have interjected our responses among reviewer comments.

Thank you for your time and consideration, Ian Brennan (on behalf of all coauthors)

24-Mar-2023

Dear Dr Brennan

Decision on USYB-2023-002: "Populating a Continent: Phylogenomics Reveal the Timing of Australian Frog Diversification".

Reject; resubmission encouraged

Thank you for your Systematic Biology submission. It has been reviewed by Associate Editor Dr Rayna Bell and two expert reviewers. Their comments are listed at the end of this letter.

The reviewers and the AE agree that this is an interesting empirical system, and the dataset is impressive. However, the analytical approach falls somewhat short of what is expected from Systematic Biology. Even if this is a Spotlight submission, a careful approach to data collection and analysis is required. I agree with the reviewers' comment that the biogeographic conclusions (and the discussion on ecomorphological diversity) are not supported by an explicit analysis (only visual assessment). For example, the authors mention Landis (2017) as a the source of the paleogeological maps in Figure 2. Landis (2017, Syst. Biol., 66, 128–144) proposed a paleogeography-informed Bayesian biogeographic model, in which a time-heterogeneous Markov model informed by empirical paleogeographic scenarios, is used, alongside molecular and distribution data, to jointly infer speciation times and ancestral ranges. If the authors do not wish to go that further, the simple or stratified DEC models implemented in RevBayes (Landis et al. 2018; Evolution 72-11: 2343–2359; Lavor et al. 2019, J. Biogeography 46, 238-250) allow inference of the number and timing of dispersal/migration events by stochastic mapping without rejection sampling: stochastic mapping simulations are estimated jointly with ancestral ranges. This would allow the authors to explicitly assess the tempo and mode of continent colonization and biogeographic assembly within a Bayesian framework (accounting for uncertainty in the form of marginal probabilities).

We thank Dr. Sanmartín for highlighting the paleo-biogeographic study of Landis (2017)—of which we're very big fans—and agree that a more rigorous testing of the

biogeographic history of our focal group is warranted. To address this, we have undertaken an ancestral range reconstruction via Maximum Likelihood using the R package *BioGeoBEARS*. An abbreviated description of our methodology is included in the *Materials and Methods* section of the main text and a much more complete description of the models and data construction is included in the Supplementary Materials and Methods section *Biogeography*.

In brief, we have estimated the biogeographic history of frogs with a focus on Australian clades using the DEC model of Ree & Smith (2008) in a time-stratified context. This while accounting for dispersal probability as a function of vicariant 'jump' events (*j*), distance between areas (*x*), and area adjacency type (*w*). This required building time-stratified distance and adjacency matrices informed by the paleogeographic reconstructions of Landis (2017). Undertaking this exercise also allowed us to explicitly test the competing hypotheses of overwater versus overland (through Antarctica) dispersal for the pelodryadid tree frogs. Ultimately our results are consistent with our previous hypotheses (Pelodryadidae and Myobatrachoidea originate from South America; Asterophryinae microhylids from Asia), however we now include explicit model estimates and a biogeographic reconstruction, which we include as Figures 3 and S7. All materials, model fit objects, and visualizations are included in the supplementary material in a directory labelled *Biogeography*.

I also agree with the AE's comment that precipitation data at present is not very informative for these ancient diversification events (especially given Australia's complex history of geological drift and biome changes).

Our intention in presenting the precipitation data alongside species richness maps was not to propose an explanation for patterns of Australian frog diversification. While we find the topic interesting, environmental explanations for patterns of Australian vertebrate species richness have been addressed expertly by Coops et al. (2018) and Powney et al. (2010). Instead, our aim was to simply provide context for readers about the imbalance in richness and range of the three focal clades, and show how contemporary richness largely tracks contemporary rainfall.

We have amended the figure caption to clarify this, and have shifted the focus to visualizing differences in continental distributions among the three clades. In response to a request from R2 we have also included an explanation in the Supplementary Materials and Methods section titled *Developing Figure 1* that outlines our process and goals.

In addition, I was surprised that all phylogenomic analyses employ site-homogeneous molecular models (except for the attempt to partition the datasets by codon). Given the ancient age of these families, with a most recent common ancestor dating back to the Mesozoic (150 Ma), the authors could evaluate site-heterogeneous in which the properties of the substitution model (e.g., the equilibrium frequencies) can change across sites in the sequence (CAT-GTR, Lartillot et al. 2007, BMC Evolutionary Biology 7: S4), or more complex, heterotachous (non-stationary) models, which allow the substitution process to change across edges in the tree (e.g., IQ-TREE GHOST model, Crotty et al. 2020, Syst Biol, 69(2):249-264). Heterotachy and compositional heterogeneity (e.g., GC bias) may play a bigger role than incomplete lineage sorting (ASTRAL analyses) for these phylogenetic depths.

We agree that heterotachy is potentially an important process to account for in phylogenetic estimation at this temporal scale. To address this we applied the GHOST model in IQTREE to our concatenated phylogenomic alignment. GHOST is an edge-unlinked partition model which does not require *a priori* data partitioning, but does need the number of

mixture classes specified by the user. We have added this method to the *Materials and Methods* in the main text. Specifics of how we implemented the method are discussed in the Supplementary Materials and Methods section *Phylogenetics*.

In brief, after discussion with two of the method's designers (Drs. Minh Bui and Rob Lanfear) we applied a GTR model with 2, 3, and 4 rate classes and identified the best fit via AICc comparison. The preferred model GTR\*H4 fit to our data resulted in a topology almost identical to our ASTRAL analysis of genetrees estimated under site-homogenous molecular models. Differences are limited to the position of Hemiphractidae and Ceratophryidae as sister to the 'Hylidae', subfamilial relationships of the Microhylidae (position of *Phrynomantis*), and the interspecific relationships among Australian *Cophixalus*. These limited topological differences are not entirely surprising as they exist in areas of low support in both trees, and on series of short nested internal branches. This scenario is where we would expect concatenation to potentially be misled, and so we continue to prefer to present the ASTRAL species tree. The concatenated species tree topology is presented as Figure S5.

While we do not find strong evidence of among-lineage rate heterogeneity in our GHOST results, among-site heterogeneity is high (up to 50x). This validates the use of codon-partitioning in our divergence dating analysis and provides a valuable comparison against our quartet-based summary method (ASTRAL).

All materials, model fit objects, and resulting tree estimates are included in the supplementary material in a directory labelled *Heterotachy*.

Finally, the authors are encouraged to provide further explanation for methodological decisions and interpretation of the results; in the current version the results section does not really connect results to methods because the latter is very short. Given that the original manuscript is only 3,200 words long, there is ample room for adding more details.

To improve the connection between our methods and results we have reformatted and expanded the results section to better reflect the chronology of our findings. This includes additional information on branching patterns within Australian groups to highlight the major phylogenetic findings. We also provide detailed reports on new analyses including the results of the GHOST heterotachy-partitioned analysis, and our biogeographic ancestral range reconstructions. Additional details that are relevant but not essential have been included in the supplementary materials.

If you choose to submit an entirely re-worked, new paper on this topic to Systematic Biology please address each point made by the Editor, AE and reviewers. Include the responses in the ScholarOne Manuscripts field under "Response to Decision Letter." The best way to address each point would be to copy this file, and insert your comments after each point made. Please do not change the order of or delete any of the comments because this makes it difficult to review again and would slow the review process. Be sure to clearly distinguish between your comments and the reviewers' comments. Feel free to argue your case with careful documentation if you disagree with any of the suggestions.

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Would you please acknowledge receipt of the reviews by email to <a href="mailto:sysbio.editorialoffice@oup.com">sysbio.editorialoffice@oup.com</a> and let us know if you plan to submit a new paper on this topic?

Thank you very much for your submission.

Sincerely,

Dr Isabel Sanmartín
Editor in Chief
Systematic Biology
isanmartin@rjb.csic.es
Associate Editor Dr Rayna Bell

Recommendation: Reject; resubmission encouraged after revisions

#### Comments to the Author:

This submission has been reviewed by two experts who are enthusiastic about the study system and dataset, but note several areas for improvement with respect to analyses and the presentation of results. In particular, Reviewer 1 notes that a quantitative approach for the biogeographic inference portion of the manuscript would better support the conclusions the authors wish to draw from their data.

We agree with all reviewers who requested a quantitative approach to biogeographic inference. We have undertaken ancestral state reconstruction using *BioGeoBEARS* and outline our methodology above in response to comments from Dr. Sanmartín. Further specifics can be found in the Supplementary Materials and Methods section *Biogeography*. Ultimately we believe this has strengthened our manuscript considerably and appreciate the encouragement.

Likewise, Reviewer 2 notes that much of the information displayed in Figure 1 is not described in the methods/results of the manuscript and these data are not really analyzed in a statistical framework, though from the discussion it appears the authors do wish to draw conclusions from the qualitative patterns they observe. I suggest the authors consider both of these key points in a revised manuscript. This will likely require streamlining the discussion. Although taxonomic revision does not appear to be an aim of the study, Reviewer 2 highlights a pertinent taxonomic discussion point that should be addressed head-on for reader clarity.

We regret that the data and information presented in Figure 1 were not introduced sufficiently in the methods section of the manuscript. As discussed above in response to Dr. Sanmartín, our intention with Figure 1 was not to present explanations of the processes dictating clade diversification or spatial distribution, but instead to orient readers who may not be familiar with specifics of the focal groups (continental distribution, patterns of richness), Australian climate, or the Australian vertebrate fauna. Including specific statistical analyses of these patterns are possible, but not a central focus of this manuscript, and so we believe a basic visual summary is appropriate. We have modified the figure legend and text to reflect this, and provide detailed information on data collection and figure generation in the

supplementary materials and methods.

### A couple minor points:

## Line 118: "among nearly all of" (I believe 92% of genera were included?)

Yes, thank you for the correction, we have amended the text to include this. Following the Australian Society of Herpetologists Recognized Species List (July 2022; included in the Supplementary Materials directory *Scripts*) we're missing the native genera *Papurana* and *Geocrinia*. The genus *Anistisia* was described while we were undertaking this work, rendering our existing *Geocrinia* sampling moot. This makes the sampling 92.59% complete (25 of 27 genera). We exclude the introduced Cane Toad *Rhinella* for obvious reasons. Taxonomic changes are extremely common, particularly in Australian herpetology, however we're happy with our effort.

Lines 122–123: given the long history of these radiations on the continent and this statement, it was less clear to me why an association of species diversity with contemporary estimates of annual precipitation would be insightful. Instead this sentence makes me wonder about the longer-term variation in climate across the continent and what the climate looked like when each of the radiations first became established and began to diversify.

Thank you, this is a fair comment and one which we certainly agree with. As mentioned above, comparing contemporary diversity against rainfall is an overly simple, but we think, potentially effective way of introducing where frogs live in Australia and one contributing factor to their richness patterns, particularly for those that may not be familiar with this group or the contemporary Australian climate. Australia and its climate have changed dramatically over the last 200 million years, with particularly marked changes since the middle Miocene and even within the last million years. Modelling the evolution of frog richness and diversity in response to this climatic change would make for a project, but it is unfortunately outside the scope of this current work. Regardless, we believe that identifying that each group established itself in a very different version of Australia (100 mya vs. 30 mya vs. 10 mya) remains relevant and appropriate. We have changed the legend for Figure 1 to reflect that the figure is a visual representation of contemporary diversity and not an indication of the processes generating spatial diversity over deep time.

#### Lines 195–198: it might be helpful to introduce these temporal groups as part of Figure 1.

The legend for Figure 1 includes the same text presented on lines 195—198, introducing the three general groups. We believe this is sufficient, but if not, we would appreciate further instructions for how best to clarify it. The text in the figure caption reads:

Australian radiations can be divided broadly into (1) relictual Gondwanan clades >40 myo (green), (2) ancient colonizing groups (>20 myo, <40 myo; varied colors), or (3) immigrant clades of Asian origin (orange).

# Lines 226–228: I don't think the authors have really demonstrated this with their data/analyses

Thank you for catching this. These are ideas that have been proposed and studied elsewhere, and should have been cited. We have corrected this in the text.

Reviewer(s)' comments to author (if any): Reviewer: 1

#### Comments to the Author

The authors examine the origins and phylogenetic positions of representatives of clades which include the approximately 250 species of Australian frogs. Brennan et al. use AHEs to sequence representatives from 4/5 of these families and use species derived from Hime et al. (2019) as outgroups to reveal the origins and age of frog invasions.

I think this is a great subject for a Spotlight article and both of the figures are beautiful, especially Figure 2 with the wonderful illustrations.

We appreciate the kind words about our manuscript and the figures. We hope readers appreciate the time and energy we've put into the figures as well.

My major critique of this article involves the fact that the authors have not used traditional methodology for biogeographic analysis to evaluate assumptions of the origins of Australian invasions. As this is a major focus, I think this needs to be done. In my opinion, I don't think a sister relationship with another taxon is enough to infer origin. In some situations, such as Asterophryinae, the authors may just need to color many of the other species in red, as they are mostly from New Guinea. However, I think using something like BioGEOBEARS or another framework would go a long way to make this a better paper. For this reason, I am suggesting a major revision.

As mentioned previously we agree with the reviewer and have incorporated biogeographic range reconstruction via BioGeoBEARS. We direct attention to the comments we have made in response to Dr. Sanmartín and to the materials and methods section, as well as the Supplementary Materials and Methods section *Biogeography*.

Other than that, I don't have too many issues with most of the methodology. But there are a few things on which I would like some clarification:

1. Lines 69-70. Please clarify the origins of the loci. In Lemmon et al. 2012, loci can have segments that come from exons, introns, and other sequences (Figure 2d). Were all sites used just protein-coding portions of loci? How was this confirmed?

We appreciate this question and the opportunity to explain our methodology. To assess locus identity (exon, intron, other) we blasted our AHE loci against gene sequences from *Xenopus* downloaded from Ensemble. 390 of the 450 total AHE loci mapped to *Xenopus* exons and so were used for the only downstream analysis which relied on codon partitioning, divergence time estimation. We have incorporated this information into the materials and methods as well as the Supplementary Materials and Methods section *Sequence Identity*.

2. In addition to splitting your data into 1st/2nd and 3rd codon positions, were there any differences in the evolutionary rate among your loci? It might also be useful to partition your data in MCMCTree by slow/fast or some other method that incapsulates rate (see Mario dos Reis et al., 2012, Proc B)

This is a great point to consider in any phylogenomic study. Sampling any number of loci from across the genome will ultimately result in rate variation among loci. While there are many different takes on how to optimize subsampling in a way that might reduce rate-variation induced artifacts, it's arguable that such subsampling may ultimately do more harm than good (Mongiardino, 2021). dos Reis et al. (2012) and dos Reis et al. (2014) show

evidence that partitioning by rate has little overall effect, but that divergence time uncertainty is sensitive to the number of partitions applied. Unfortunately increasing the number of partitions (but not their length) dramatically increases the computational costs associated with estimated divergence dates. We opted for two partitions as a conservative approach, and have now added text to the Methods section to explain our reasoning. While we do find this an interesting topic, our general consistency of divergence date estimates with other major amphibian phylogenomic studies (Feng et al. 2017; Hime et al., 2021) suggests to us that rate variation among loci in our dataset is not resulting in adverse effects.

3. If you need to cut anything, I would cut the paragraph about Papurana as it was not in your phylogenomic analysis and does not necessarily have to be discussed in more detail.

Thank you. Currently we're under the maximum word limit. We decided to include the discussion of *Papurana* because, although not represented in our dataset, it is a divergent member of the Australian frog community and as such provides interesting considerations for the accumulation of a continental amphibian fauna.

#### Reviewer: 2

Comments to the Author

Populating a Continent: Phylogenomics reveal the timing of Australian Frog Diversification by Brennan et al.

Summary: The phylogenomic dataset and taxonomic focus seem highly appropriate for the Spotlight section of Systematic Biology. Overall, I found the figures aesthetically pleasing and easy to interpret. The paper is well written and the authors are obviously experts in their fields. However, I identified several areas for improvement, both in terms of clarity in the writing and quality of the conclusions. Below I have provided some major and minor suggestions that I hope will be helpful in revising the manuscript. After revision, I think this manuscript will be of great interest to the readership of Systematic Biology.

Thank you to R2 for their positivity. We hope that this manuscript will be of broad interest too.

#### Major suggestions:

1. Missing analytical details: The manuscript presents analyses that are not described with methods. The methods section only reports how the phylogeny was inferred + divergence times estimated, yet there are many other (presumably novel) analyses that are reported in the manuscript. For example, Figure 1 shows datasets related to geographical distribution and precipitation (top) and a bivariate plot with Number of species vs. crown age for Australian vertebrates clades (bottom). While the figure caption reveals where the species occurrence records were obtained, there is no information (in the caption or Materials + Methods) about where the environmental data come from or the information about crown ages for nonfrog crown ages. That leaves many important things unclear - for example how were the different vertebrate clades selected? Was this arbitrary or was there some criterion for defining different vertebrate clades. Also Myobatrachoidea includes all species belonging to the clades Limnodynastidae and Myobatrachidae, however, these two clades are also present in the figure - does this cause issues with statistical independence? These (and other) details need to be added.

We appreciate R2's thorough comments regarding the visualizations in Figure 1. As mentioned above (in response to the EIC and AE), our intention was to provide the reader context on the diversity of Australian frogs across the continent and among the many other terrestrial vertebrate radiations in Australia. We appear to have failed in our first attempt to convey that these are visual representations of patterns of diversity and not comments on the processes they've accumulated by. To correct this we've adjusted the figure caption to reflect this.

2. Interpretation of Figure 1: The caption states 'Australian frogs show a pattern of increasing species richness with precipitation, and with time.' Regarding precipitation, while the maps are lovely, I think a regression comparing number of species and annual precipitation for each grid cell might make the author's point more clearly. Regarding time, I do not think this is really a fair statement because what is being displayed in the regression is not the relationship between frog diversity and time - but rather vertebrate diversity and time. I think the authors either need to re-run the regression with only frog clades or modify the figure caption.

To address these comments and those made above, we have rewritten the figure caption for Figure 1. We agree that a regression of species richness against precipitation

would have the desired effect. However, as mentioned previously in this document the relationship between these variables is a contemporary one and likely not reflective of the evolutionary history of the group or the Australian climate. Instead, we aimed to show how the three frog clades show different continental distributions, and how generally this conforms to Australian rainfall patterns (though contemporary distribution may be better modelled by actual evapotranspiration, see Powney et al. 2010; Coops et al. 2018). With regards to the regression, we have modified the figure caption to better reflect what we intended to show (the pattern of Australian vertebrate richness increasing with age, to which frogs contribute). This pattern holds when with a regression of species richness against clade age of just Australian frogs (n=5; Myobatrachoidea, Pelodryadidae, *Cophixalus*, *Austrochaperina*, *Papurana*) results in a similar pattern with (R<sup>2</sup>=0.849, intercept=1.827, slope=1.805, p=0.016).

3. Limnodynastidae and Myobatrachidae: As an amphibian biologist/taxonomist I was particularly interested to see how the authors dealt with these two families because their content and definition is controversial. This is because what many authors call Myobatrachidae (e.g. Frost , 2023, Amphibian Species of the World) is non-monophyletic, with "Limnodynastidae" being a clade nested within Myobatrachidae. I can see that the authors likely grappled with this issue and considered possible solutions (i.e. there are two "new" families "Mixophyidae" and "Rheobatrachidae" listed in Table S1 and Fig. S4 - but these are not mentioned elsewhere), however, this issue is not discussed anywhere in the text. Please clarify the higher level taxonomic decisions used in the manuscript.

Though slightly embarrassing to admit, this was an oversight during the submission stage. As many of us are taxonomists as well, we recognize that this is an important topic that must be addressed adequately, and we are actively working that. However, we feel that this manuscript is not the appropriate avenue to suggest taxonomic changes, as the short format means they are likely to be lost among the broader diversification themes. We have a substantial phylogenomic dataset of the Myobatrachoidea representing complete species-level sampling which we hope to use to revise the family-level taxonomy of the group. In preparing this current manuscript we inadvertently included a taxonomy that we have been using informally, but have now made the necessary corrections to follow family names as listed by Frost and the Australian Society of Herpetologists *Official List of Recognized Species*.

4. Opportunity to make the writing more evergreen: Throughout the manuscript there are many terms used to describe Australian frogs radiations that could be interpreted as insensitive to broader discussions happening in evolutionary biology at the moment. For example, 'Asian immigrants', 'Recent Asian immigrants', 'Colonizers', and subjective alienating terms like 'world's strangest', and 'bizarre'. I suggest the authors read a recent paper by Cheng et al. 2023, Trends in Ecology & Evolution (<a href="https://www.cell.com/trends/ecology-evolution/fulltext/S0169-5347(22)00359-7">https://www.cell.com/trends/ecology-evolution/fulltext/S0169-5347(22)00359-7</a>) for some possible guidance on rethinking some terminology.

As scientists working in the 21<sup>st</sup> century we understand the importance of inclusivity and learning from past insensitivities. We certainly meant no offense in our phrasing, but feel limited by the common language available to discuss many concepts, including immigration and colonization. To address some of the phrases flagged by R2 we consulted the EEB Language Project (<a href="https://www.eeblanguageproject.com/repository">https://www.eeblanguageproject.com/repository</a>) which aims to provide a list of troublesome words/phrases and potential alternatives. The suggested alternatives for "colonization / colonizer" are "established /early successional species". We endeavoured to switch towards this phrasing where possible (e.g. changing "invasion" to "dispersal"),

however the terms are not always entirely synonymous. For example, Merriam-Webster's suggested synonyms for "immigrant" contain far more troublesome terms (refugee, settler, foreigner, alien, colonist). Where possible we have tried to alleviate awkwardness by changing "Asian immigrants" to "immigrants from Asia", which may have the desired effect. While we acknowledge this is an area that we are all working to improve in, we are happy to entertain more specific suggestions for alternatives to our terminology.

With regards to descriptors considered "subjective alienating terms", we appreciate the sentiment but respectfully disagree that they detract from the manuscript. We expect that the word "bizarre" could be swapped for the synonym "unusual" and likely would not attract the same negative attention. But stripping adjectives from the discussion of a manuscript serves to make it drier and denser, not more objective or scientific. The animals these terms are used in reference to (pygopodid geckos and *Myobatrachus*—the turtle frog) are both objectively morphological outliers from their respective groups. We aim to highlight their uniqueness as a mark of appreciation—flagging the extent of their distinctiveness—not a remark of derision.

#### Minor suggestions:

Line 93: closest relatives should be closest living relatives

Thank you for catching this, we have changed it to follow your suggestion.

Line 119: What is a mega-genus?

It's a genus that's mega-sized. We have changed this to "diverse genus Litoria."

Line 123: I am not sure that "incarnation" works here. Perhaps instead use "...each radiation established itself during different eras and under environmental conditions."

We have replaced this sentence, which now reads "This stratified arrival took place under the varied environmental conditions of vastly different eras."

Line 147: I think the 'The tips of these long branches are likely the survivors' would be clearer than 'These long branches are likely the survivors...'

Changed to "the tips of these long branches..."

## Line 155: How can you differentiate "erupting" into a radiation from high levels of recent extinction?

Regardless of if there have been high levels of extinction prior-to or post the Miocene radiation of pelodryadids, the divergence of species in this period remains remarkably rapid. Successive branches in the pelodryadid tree (branches 154, 157, 158) are 0.9, 0.3, and 0.7 million years long, suggesting a very fast pace of diversification that we believe the phrase "erupting" is appropriate for.

Line 172: What is a temporally adjacent clade? Sorry, having trouble understanding this term.

We were referring to the similarity in ages of these clades, but perhaps the phrasing is awkward. We have changed this to "two similarly aged clades"

Line 197: Could you say  $\sim$ 30 myo instead of >20 myo, <40 myo? Could also use a range 20-40 myo.

Changed to "(20—40 myo)"

# Line 222: What is the evidence that the drivers of the pattern are idiosyncratic - are there citation that can be added here?

We suggest that the pattern is potentially due to idiosyncratic drivers because this pattern is highly variable across the tree of life. We now provide some citations for this topic in the manuscript.

#### Line 232: I think 'arid center' might be clearer than 'red center'

We have changed "red center" to "arid center"

### Line 242: I suggest using 'unique' or 'rarely observed' instead of bizarre.

Thank you for your suggestion. We have changed "bizarre" to "unique".

#### Line 251: Again, I would consider using an alternative term instead of 'world's strangest'.

Thank you for your valued input. We appreciate your suggestion, but as noted above the turtle frog is an outlier among other myobatrachoids and frogs. Here we choose to celebrate the uniqueness of what we consider "perhaps the world's strangest anuran".

# Figure 2: I love the frog images! I might use a different term for the clade of hyloids, as it only contains a subset of Hyloidea. Another name for the clade depicted here is Amazorana (e.g. Womack et al. 2022, Ichthyology & Herpetology).

We're glad you enjoy the frog drawings, we hope everyone enjoys them as much. We appreciate the suggestion of using Amazorana (Streicher et al. 2018) to refer to the hyloid subclade consisting of Hemiphractidae, Ceratophryidae, and "Hylidae". While this is clearly a valuable taxonomic term, we believe that the ever-finer labelling of clades above the genus level is more appropriate in a taxonomic or strictly phylogenetic context and perhaps not useful for the macroevolutionary focus of this manuscript. To accommodate the suggestion, we have amended the "Hyloidea" labelling on the tree to read "Hyloidea (in part)", which we believe preserves the valuable reference to a broader identifiable clade but draws attention to the fact that it is not entirely represented here.

Figure S4: Features "Mixophyidae" and "Rheobatrachidae" yet these are not mentioned in the main text. Also, why were two individuals of Heleophryne purcelli used when there are no other duplicated species in the phylogeny (that I noticed at least)? Other trees only have one H. purcelli (e.g Fig S5).

We apologize for including the duplicate sample of *Heleophryne* in this supplementary figure. During our exercise of combining alignments from different frog phylogenomics projects we duplicated the *Heleophryne* sequences as a quality control check to make sure there were not batch effects in the downstream alignments and trees. Ultimately we removed the duplicate sample prior to our divergence dating analysis, and we have since corrected this supplementary figure. We have also amended family names as discussed above.

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