Dear Robert and Jérémy,

We completed the revisions; thank you (Robert) for the careful editorial comments, which we implemented as documented below. Below, our responses are in bold type.

Best wishes,

Michel (on behalf of all co-authors).

Round #2

Decision

by Robert Asher, 2019-09-30 15:44

Manuscript: https://doi.org/10.1101/352609 version 1

What do ossification sequences tell us about the origin of extant amphibians?

I agree with the reviewer that this manuscript is just about ready for publication. I've made a number of minor comments for the authors to consider, below, so am obliged to tick "revision". However these are all minor and the authors can incorporate as they see fit.

line 14: I'd slightly edit the first sentence. "Controversial" is a value judgement; I'd delete this term. The authors say as much in the end of this sentence regarding current lack of consensus. You might also add some text to make clear that the lack of consensus is about lissamphibian affinities among fossil groups, not (for example) that they are tetrapods or are the sister taxon to extant amniotes.

Good idea. Done.

line 37: There's only one phylogeny (at least of vertebrate high level taxa) and it's neither "molecular" nor "paleontological". Rather, we use these kinds of data (among others) to reconstruct what it is. So here & throughout reserve adjectives like "molecular" to describe data, not phylogenies (at least when you're talking about species trees and not gene trees). The authors already use this style nicely in (for example) lines 58, 82, 93, 97.

Done.

line 44: Note that Gill (1872 Smithsonian Misc Collec, p. xliii [p. 44 of the pdf]) shows a very modern-looking tree with well-corroborated clades like gnathosomtes, cyclostomes, bony fish, actinopts, sarcopts including lungfish & coelacanth, and tetrapods with "reptiles" originating out of "batrachians" (see also discussion in Asher & Müller 2012 chap 1, p.2 in From Clone to Bone CUP)

> **Indeed, but I am explicitly referring to a supposed link between extant amphibians and temnospondyls, which Cope (1888) made. Gill (1872) does not appear to discuss temnospondyls at all. He just placed Batrachia in an apparently ancestral position to Reptilia. Thus, this is not really relevant to the present draft and we did not add a reference to Gill (1872).**

lines 111-112: This sentence is a bit long. I'd recommend "...extant amphibians. Recently, Danto et al.

(2019) …"

Indeed, thanks!

line 134: We may need some guidance from our PCI colleagues regarding "supplementary material". At present, this is mentioned in the main text but without a URL or other precise description of exactly where this is (or will be) available. Reference to the URL should eventually be added either as an appendix or directly in the text whenever "supplementary data" or "supplement" is mentioned (e.g., line 270). Relatedly, ensure that the wording for these data is the same throughout, as opposed to writing "supplementary material" on line 134 and "supplement" on line 270.

Good point. We have indicated, in line 138: "The sequences themselves and the phylogenetic trees corresponding to the tested hypotheses are included in the supplements, which are posted on the bioRχiv page from which this paper is available."

We have uniformized to "supplements".

At the end of the paper, I have added the URL where the supplements should be available:

https://www.biorxiv.org/content/10.1101/352609v3.supplementary-material

lines 182-183: The comment about lungfish "seem mostly impossible to homologize" is ambiguous. Ideally the authors might add a bit more justification or background to this statement. I'd recommend adding at least some references to guide readers to previous efforts at recognizing cranial homologies in these groups.

We added a citation of Criswell (2015), who provides a historical review of such attempts and summarizes the current situation well.

Also please respond to the comment from R1 regarding line 133 in the first version: "133- this is incorrect. Firstly, Schoch 2006 used the actinopt Amia with fairly few homology problems.

He dealt with those problems as follows (p. 527): "all those bones only present in tetrapods were given the final rank in the *Amia* sequence, which resulted in the identification of apparent evolutionary shifts of these elements in all tetrapods; these shifts are all artifacts of coding, which was throughout considered in the event-pair cracking analyses." We preferred not to introduce such artefacts, which could for instance have seriously distorted the fit of the branch lengths that represent the models of evolution we tested. While it may sometimes make sense to consider the appearance of a bone to be so far delayed that it never happens, there is no reason to think the ancestors of *Amia* ever had frontals or nasals in the first place. (The frontal and the nasal were included in Schoch's analysis, as seen in his Table A1, and are included in our 8-character cranial dataset; the nasal is also included in our other two cranial datasets.)

Secondly, some part of the development of Eusthenopteran were published (Cote, 2002; Schultze 1984), though admittedly little about cranial development. It would provide some data about postcranial though."

We have reformulated ("As cranial ossification sequences remain poorly documented for extant finned sarcopterygians"). And we already use the appendicular *Eusthenopteron* sequence in some of our analyses; the dermal skull is complete even in the tiniest known individuals, so the sequence of those bones remains unknown.

To justify the absence of actinopterygian data, we added that this results also from missing data (which our method cannot handle). This was admittedly not clear in the initial draft, but should now be clear.

line 217: "see below" regarding missing data might also pertain to the text *above*

> **Indeed, so noted.**

line 237: the sentence here can end with "...events in ontogeny" without the "would". The following sentence is confusing; it has too many clauses (between commas) and two occurrences of "because". Please rewrite.

> **Indeed, thanks for catching this. Done.**

line 241: "simple" regarding your formula is a value judgment and should be deleted.

> **Done.**

lines 260-261: change to "...only with sequences standardized by position" (or otherwise simplify so that "sequence" isn't repeated). Also "data" as a plural should be modified by "few" rather than "little".

> **Done.**

line 292-93: change "maximal" to "maximum".

> **Done.**

The fact that the analysis took so long is presumably because with few characters the treespace is relatively flat and the algorithm gets bogged down on many local optima. I've found the recent builds of PAUP (including 4.0a165 used here) are faster than 4.0b10 from a few years ago, but (unless you explicitly limit PAUP, e.g., time or iterations per replicate) it'll get stuck among the huge number of possible topologies in a flat-treespace with few characters. Default in TNT is faster; you can limit the tree buffer in advance via "hold" and (in my experience) it will more quickly escape local optima, only filling up the tree buffer upon branch swapping & after finding many local optima. There's no need to redo analyses as far as I can see but for future reference, the authors might consider using TNT: http://phylo.wikidot.com/tntwiki http://phylobotanist.blogspot.com/2015/03/parsimony-analysis-in-tnt-using-command.html https://groups.google.com/forum/#!forum/tnt-tree-analysis-using-new-technology

> **Thank you for the information. We are aware of TNT's capabilities and have used it on occasion, on much larger matrices. In this case, we don't think that it would make much difference because the consensus of trees one or two steps longer looked very similar to the results that we obtained.**

line 329: It's not quite clear to me why "branch lenghts... set to the same length" is an expectation of punctuated equilibria. The latter is (despite occasional Gouldian hyperbole) is just an application of peripatric (= "allopatric" in Eldredge & Gould 1972) speciation to the fossil record & the consequent expectation that small populations will tend not to leave behind a fossil record. "Stasis is data", as the saying goes, & is essentially an indication of population size. Perhaps another sentence or two explaining how punk-eek leads to particular expectations regarding branch lengths would be helpful.

> **We are discussing the evolutionary patterns rather than the underlying causes at the population genetics level. We have added both a clarification and a supporting reference:**
>
> **The second is the speciational model, in which all branches are set to the same length because changes are thought to occur at speciation events, which are typically equated with cladogeneses in evolutionary models (Bokma et al. 2016).**

line 350: "mentioned" is more appropriate than "evoked"

> **Changed.**

line 359: change to "...drawbacks that led us not to use them".

Done.

line 361: I'd break this sentence into at least two, e.g., "...can be summarized briefly as follows:..." [new sentence]

Done.

line 368: "documented previously" in Germain & Laurin (2009)? Please add ref.

Done (Schulmeister and Wheeler 2004).

line 386-90: "established consensus" should come with a list of references behind the branching pattern and divergence estimates (ahh I see this is from line 396).

We added here "see the next paragraphs for the sources" because they are quite numerous; it would be awkward to list them here in bulk.

Please also add these to the fig. 2 caption.

Done, though this adds six lines to the legend.

Also "molecular divergence dates" are themselves contigent upon paleontological calibrations, and hopefully you've picked estimates that do not recycle other clock dates as calibrations themselves (see Graur & Martin 2004, Reisz & Muller 2004, both in Trends Genetics).

Indeed, we read these papers and are aware of the potential problem. We believe that most divergence dates that we used don't rely on secondary calibration constraints.

line 415: as you've assembled a (very impressive) ossification dataset across osteichthyans, data from birds and mammals are relevant. However stating here with "for the birds" is a bit sudden, and you might add a sentence here to remind readers the importance of amniote data for your study of lissamphibian origins.

Done. We added this sentence here: "We sampled many extant amniotes to achieve broad coverage of Tetrapoda."

Also I'm not sure about the topologies in Pons et al., Wang et al & Gonzales et al., but the Prum et al. 2015 (very large) dataset shows topological conflict with other, large genomic studies, in particular Jarvis et al. 2014 reflecting what remains a stubborn polytomy at the base of Neoaves (nicely reanalyzed & discussed in Reddy et al. 2017 Syst Bio). How might the competing phylogenies in (say) Prum et al. 2015 vs Jarvis et al. (2014) influence your interpretations of amniote ossification sequences?

We doubt that changes deep within amniotes would strongly affect the fit of our various hypotheses, which differ in the position of Paleozoic stegocephalians, but we have not performed such tests because we already performed so many that summary tables became necessary. This would be worth looking into in a future paper, if this polytomy persists. We think that this paper includes sufficient new data and results to stand on its own; we leave some work for the future! In any case, the methods section would not be the appropriate place to discuss this.

line 419: A good summary & rationale for mammalian divergences (and why some estimates may be too old) is Phillips & Fruciano 2018 BMC Ev Biol, also Dos Reis et al. 2012 (Proc Roy Soc B). A good compendium of vertebrate divergence dates in Benton et al. 2015 Paleont Electr.

We knew about the Benton et al. 2015 paper, which is of course already outdated in several respects; we think that for constraints within lissamphibians, our timetree (Marjanovic and Laurin 2013b "2014") is more comprehensive and reliable.

Thank you for alerting us to Phillips and Fruciano (2018). That paper, which supersedes dos Reis et al. (2012), says the same for placentals as Berv and Field (2017, already cited) said for birds, so we now cite the two together as cautioning against overestimates of mammalian divergence dates, and we cite Phillips and Fruciano (2018) together with others on the absence of Cretaceous placentals.

line 477 (and elsewhere): paragraphs like this that have frequent references to acronyms (DH, LH, PH2, etc.) are hard to follow. It's fine to shorten the text w/ such acronyms but perhaps you could add parentheticals to remind your readers that "PH2" etc. are shown in your fig. 1a, b, c, etc.

We have added a reminder at the end of the paragraph that this is explained in the legend of Figure 1. The same information is given a page and a half earlier, and in the introduction. Hopefully, when the paper is formatted using your template, this will be fairly obvious.

line 516: This sentence would be easier to follow if you broke it up, e.g., rephrase text from "but it is weaker..." as a new sentence. Ditto for the long sentence in lines 518-523.

Done.

The Fig. 1 caption is very long. Perhaps move a few qualitative phrases (e.g., "very cautiously Froebisch et al. 2017...") to the main text. The detailed attributions of which authors are associated with which trees are important, but again could probably be moved to the main text while still making reference to those details with a single phrase in the caption, e.g., "See Methods for details on support for these competing topologies".

Done. In fact, nearly all that information was already clearly stated in the introduction, so very little had to be transferred there.

Fig. 2 caption is too short & makes no mention of the data behind this topology or divergence estimates. Please provide citations to make the caption self-contained and enable your readers to know the data & publications behind this tree. Please state what the horizontal colored lines represent (I guess marine stages?). Also they're garish and make the branches harder to read compared to (for example) grayscale, dotted lines, or similar.

There are no data from this paper behind the tree; it is a supertree compiled from the literature. Thus, we added the supporting references, which makes the legend quite long (this adds 6 lines and a bit).

We added this explanation: "The colored bands represent geological stages from the international geological timescale (Ogg et al. 2016)."

line 1041: Just write "are" rather than "appear to be" (also line 1049). Again this caption has interpretation & detail (e.g., "...there is clearly a phylogenetic signal...") that is more appropriate for Results or Discussion than a figure caption.

Done. Much of the text was transferred to the results, and a smaller part to the discussion.

line 1044: As noted previously RE: suppdata, write out what "SM 1" means (also line 1050) and add a statement (somewhere) indicating where this can be downloaded.

Done. See above about how we referred to the supplements and how to access them.

Appendix 1: without lines to indicate columns & rows, and without a repeating header at the top of each page, this appendix is hard to read. I would recommend moving this to online supplementary data in the form of a spreadsheet. Alternatively, reduce the font size and make this a table or figure with repeating a header and lines to delineate cells.

We will reformat this using the template if possible, or convert it to an online appendix otherwise.

Reviews

Reviewed by anonymous reviewer, 2019-09-29 21:31

The authors have greatly improved this manuscript. The authors approach the results of this analysis with much more caution and acknowledgement of limitations. As such, the data and interpretations appear appropriate.

Thank you to the uthors for taking my comments and questions into consideration and clarifying issues found in the first draft of this manuscript.

Thank you for the many detailed comments on the previous review.