

Peer Review File

Manuscript Title: The oldest tadpole reveals evolutionary stability of the anuran life cycle

Editorial Notes:

Redactions – Third Party Material

Parts of this Peer Review File have been redacted as indicated to remove third-party material.

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The manuscript entitled “THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE ANURAN LIFE CYCLE” by Chuliver et al. describes the thus far oldest record of an anuran tadpole in the fossil record, specifically the mid-late Jurassic of Patagonia.

This find is remarkable and interesting as the highly specialized and derived life history of anurans is in this extent unique amongst tetrapods and alongside the evolution of the derived body plan of anurans is thought to have played a pivotal role in the radiation and evolutionary success of frogs that began in the Mesozoic. Previously, the earliest known tadpoles were known from the Cretaceous, while adult anurans are comparatively well-represented as early as the early Jurassic.

Based on distinct morphological characters, the authors assign the tadpole to the stem anuran species *Notobatrachus degiustoi*, which is well known from adult specimens from the same locality. Their assessment is also supported by their careful phylogenetic analysis and I have no reservations against this taxonomic assignment.

While I think that this is an important and exciting find, I feel that the authors in the current form of the manuscript leave some important questions unanswered and undiscussed, which should be considered in the context of this find in a revised version of the study before considering its publication:

1. The authors report that *Notobatrachus* is well represented at the locality by a large number of specimens that have been recovered at the site since the 1950s. Yet, despite considerable collection effort at the site, the described tadpole is the only pre- metamorphic specimen from this locality? Why is that? If the lacustrine deposits represent an ecosystem that was used for breeding/egg deposition and this is where tadpoles lived, why were there no other tadpoles found at the locality? It also remains unclear in the text and supplementary information, if the specimen derived from the same horizon as the adult specimens or from a different horizon, hence possibly representing a slightly different time slice and/or sampling a different habitat than the layers that yielded the adult specimens. This needs to be clarified and explained.
2. If the tadpole derived from the same horizon as the adult specimens, the biological reasons for this specimen being the only tadpole amongst hundreds of adults need to be explored in the manuscript. In this context the supposedly giant size of the tadpole might be an important clue. Incidences in which individual tadpoles fail to metamorphose, but instead grow to giant size are well known from several

frog taxa (several species of *Rana*, but also from *Ascaphus*, the latter also often becoming cannibalistic). While these individuals are exceptions, they are not entirely uncommon and these giant tadpoles also often explore other regions of water bodies than their normally-sized larval conspecifics, which could explain why this specimen is the only tadpole found amongst adults.

3. The point above is also critical in the line of argumentation regarding the tadpoles of *Notobatrachus* being gigantic. I think this find cannot simply be considered a normal larval specimen, unless more tadpoles that size are found that could document that this was indeed the normal tadpole size for this taxon.

The authors also state that the giant size of this tadpole indicates that tadpole gigantism evolved in stem anurans. For the reasons outlined above I think one has to be very careful with this assumption.

Moreover, even if further finds should show that this taxon indeed had giant tadpoles as a rule, this could not demonstrate that gigantism in tadpoles evolved in stem anurans as it could alternatively indicate that stem anurans simply had large tadpoles initially and the much smaller larval size evolved secondarily in the crown group, possibly in concert with an even more condensed, specialized metamorphosis and the constraints the latter imposes on body size, cell cycles etc.

4. Since this paper focuses on (amphibian) metamorphosis as a core subject, the authors need to phrase more carefully what they mean with metamorphosis and a biphasic life cycle and clarify what they mean with “gradual changes” in this context. Arguably, anuran metamorphosis is the most extreme amongst extant tetrapods and tadpoles represent a highly derived larval stage. Nevertheless, salamanders and in specialized ways caecilians undergo a distinct metamorphosis that involves significant physiological and morphological changes. Moreover, there is a large number of salamander taxa that most definitively undergo a change of habitat, feeding mode, respiration and locomotion between larval and adult stage and therefore the opening sentence following the abstract is incorrect.

While metamorphosis is often considered the classic life cycle of amphibians, amphibian life histories are enormously diverse and highly derived in all three groups of lissamphibians and there are gradations of metamorphosis in all three modern clades, e.g. some neotenic salamanders still undergo quite significant physiological and morphological metamorphic changes, even though they never switch their habitat, while other taxa have a clear-cut, gill breathing larval stage that metamorphoses into a lung-breathing fully terrestrial adult within 3-4 weeks. Similarly, by far not all frogs have free swimming, aquatic tadpoles that metamorphose into terrestrial adult frogs, but show an impressive range of specializations as well.

I do not mean to say that the entire breadth of life history patterns in lissamphibians has to be discussed in detail in the context of the paper, but I think the use of terminology -metamorphosis vs. “gradual change” - has to be defined and clarified more rigorously here and a bit more credit should be given to the evolutionary complexity of this phenomenon (see also point below).

5. The evolution of early tetrapod life history patterns generally and a lissamphibian-like metamorphosis more specifically has been researched quite extensively, most notably by Rainer Schoch and colleagues and the statement that temnospondyl development progressed without major changes of habitat, morphology or ecology is incorrect.

For many temnospondyl taxa, metamorphic changes were more gradual, more drawn out and less condensed in terms of time than in lissamphibians, but this is not the same as no morphological changes or changes in habitat happening. Moreover, amphibamiforms have convincingly been demonstrated to

have had a very lissamphibian-like metamorphosis and the evolutionary groundwork for the extreme life history patterns as seen in lissamphibians, was likely laid down in this stem lineage of the modern forms. The authors cite a paper by Schoch 2002, but research on this topic has advanced significantly since then.

Depending on how the current manuscript is framed, this data might not be crucial in great detail. However, if the evolutionary history of the extreme anuran life history is included here or commented on, these more recent data should certainly be considered.

Generally, I think that the authors present a fascinating and very interesting find that has important implications for understanding the timing and evolution of the extreme anuran larval stage. Nevertheless I feel that in the current stage, some important questions are not sufficiently explored, which should be addressed in more detail.

Referee #2 (Remarks to the Author):

Review of “**THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE ANURAN LIFE CYCLE**”, by Mariana Chuliver et al.

Reviewer: Jim Gardner

Date: 19 March 2024 (first day of Spring in Northern Hemisphere)

Among the many unique features of salientians (frogs) is their primitive possession of a free-living tadpole (larval stage) that is dramatically different morphologically and ecologically from the adult (frog) stage. Studies of extant (i.e., crown clade) frogs agree that tadpoles are primitive for frogs and have led to constrained speculation about what the primitive or ancestral tadpole morph might have looked like and how it lived. Although tadpoles have a modest fossil record, to date that record only extends back to the Early Cretaceous (vs. modern aspect frogs appeared considerably earlier by at least the Early Jurassic) and all described fossil tadpoles are referable to crown-clade frogs. As such, the fossil record provides no direct evidence about the morphology and ecology of tadpoles belonging to the earliest frogs. This new report by Chuliver et al. of a tadpole dating to the Middle-Late Jurassic boundary and belonging to the stem anuran *Notobatrachus degiustoi* provides new and exciting insights into the morphology and ecology of stem anuran tadpoles. This report is interesting and significant enough to warrant publishing in a high-profile journal such as *Nature*. For reasons outlined below, **I recommend this MS be accepted pending minor revisions.**

I have no concerns with the authors' identification of the fossil or their interpretations of its structures. The amount of detail presented in the main text is adequate for *Nature* and, for those of us who crave more, additional information is presented in the Extended Data Figures and Supplemental Information. Although I am not an expert in phylogenetic analyses of tadpoles or morphometric analyses, the methodologies for those portions of the MS seem reasonable to me. The descriptions and analyses are complemented by informative figures (but see my comments on how those could be improved). The Abstract adequately summarizes the significance and key findings of the report and the References are comprehensive.

As is typical for me, I read the MS several times. Being unrepentantly ‘old school’, I marked up my review copy by hand. I spent about 12 hours on those tasks, plus another full day composing my written report. I am not willing to invest more of my time and energy in typing all my comments, questions, and suggested changes onto the digital version of the submitted MS or providing a list of those. Instead, I am providing a scanned PDF version of my hand-annotated copy of the complete main text, figures, and “Methods”, plus the Extended Data Figures and all of parts 1 to 3 and the first and last pages of part 4 for the Supplemental Information text. The authors can look those annotated pages of their MS and deal with my points as they see fit. If the

authors have trouble deciphering any of my printing or have questions, they are welcome to contact me at james.gardner@gov.ab.ca for clarification.

Below I summarize my most notable points about the MS. Some of these elaborate on comments, questions, and suggested changes that I made in my annotated copy of the MS. All my points are minor, and the authors should have no trouble dealing with them.

- 1) I like how the authors propose the first (and easy-to-understand) definition for “giant tadpoles” as those tadpoles having a total length greater than ca. 105 mm. Their definition seems reasonable to me. If nothing else, that definition provides an absolute value, which is preferable to the vague and unstated use of “giant” in a relative sense, for tadpoles that are somewhat larger (but how much is never stated) than is typical for a given species.
- 2) I suggest the authors also clearly indicate the sense in which they are using the term “giant”. From my perspective, there are two kinds of giant tadpoles. The first are naturally occurring giants that successfully metamorphose (e.g., the huge tadpoles of the extant Paradoxical Frog and tadpoles at the upper end of the normal size range for their species, such as the extant American Bullfrog). The other kind of giant tadpoles are abnormal ones (what Rot-Nikcevic & Wassersug, 2004 called “giant, non-metamorphosing tadpoles”) that cannot metamorphose and occur sporadically in some species (e.g., laboratory-raised African Clawed Frogs: see Rot-Nikcevic & Wassersug, 2004; Kerney et al. 2009). At least one species, the extant American Bullfrog, is known in the wild to have both naturally occurring giant tadpoles (e.g., Altig & McDiarmid, 2015) and rare, abnormal giants (e.g., Schooley et al. 2008; Burke 2018). A critical distinction between those two kinds of giant tadpoles is whether they can successfully metamorphose—that has implications for the significance of the large size of the sole *Notobatrachus degiustoi* tadpole. If your fossil tadpole is a non-metamorphosing giant, then your argument that *N. degiustoi* is a rare example of an anuran with both giant tadpoles and adults collapses. The challenge with fossil examples of giant tadpoles, such as the single reported tadpole of *N. degiustoi*, as well as rare examples of giant fossil tadpoles reported for palaeobatrachids (Miocene of Germany: Roček et al. 2006) and pelobatids (Miocene of Spain: BUSTILLO et al. 2017, 2019, TALAVERA et al. 2019; Miocene of Bulgaria: VERGILOV & TZANKOV 2021; Eocene of Germany: WUTTKE 1996, ROČEK & WUTTKE 2010, WUTTKE & ROČEK 2010, WUTTKE et al. 2022) is determining whether the individual was capable of metamorphosing and, thus, was either a normal giant or an abnormal giant. This can also be a problem for extant tadpoles: e.g., the giant, pre-metamorphic tadpole of the American Bullfrog reported by Bolek & Janovy (2004), which died soon after it was captured and before it potentially reached the stage where the onset of metamorphosis could be detected. At least one of the palaeobatrachid

tadpoles reported by Roček et al. (2006) and the Spanish pelobatid (BUSTILLO et al. 2017, 2019, TALAVERA et al. 2019) preserve rudimentary forearms, indicating they were well along in metamorphosis at the time of their deaths. That also appears to be the case with the *N. degiustoi* tadpole, which means you can legitimately argue it is a normal, giant tadpole. That point deserves to be emphasized in the MS. [References cited herein are at the end of my report.]

- 3) I am unclear on the geologic age of the *Notobatrachus degiustoi* tadpole. The statement (lines 29–30) “Here we report a late larva of the stem-anuran *Notobatrachus degiustoi* from the Middle-Late Jurassic of Patagonia (ca. 166–157 Mya) implies the tadpole is Middle–Late Jurassic in age. Yet on the next page is the statement (lines 55–56) “Here we describe the oldest-known tadpole from the Middle Jurassic La Matilde Formation (Callovian-Oxfordian; ca. 166–157 Mya)” —note here the age is given only as Middle Jurassic, yet both the reported stages (Callovian and Oxfordian) and absolute age range (ca. 166–157 Mya) fall within the late Early–early Middle Jurassic (i.e., more similar to what you reported in the Abstract). The geologic age is even less clear in the Supplemental Information, where (lines 29–37) an even broader range of Bathonian (late Middle Jurassic) to Valanginian (early Early Cretaceous) is reported. I realize age estimations can be imprecise, but considering you are describing the geologically oldest known tadpole, it is important to report its age as precisely and consistently as possible. If there is doubt about the precise age of the tadpole-bearing rocks, explicitly acknowledge that situation and then tell us which age or age range you prefer and why. Perhaps it is most conservative to simply regard its age as being around the Middle-Late Jurassic boundary?
- 4) I also am unclear what locality yielded the *Notobatrachus degiustoi* tadpole. In two places on page 3 (lines 57–59 and lines 66–67) you vaguely state that the tadpole was collected from “the same locality/quarry” that yielded fossils of metamorphosed individuals of the *N. degiustoi*. Yet when I look at your cited references, it is clear that metamorphosed individuals of *N.s degiustoi* have been collected from at least five localities (see especially the helpful list provided by Báez & Nicoli, 2004: appendix 1). That leaves me wondering which of those five localities yielded the tadpole? The caption for Extended Data Figure 1 states “... in the same levels w[h]ere *Notobatrachus*[insert space]*degiustoi* specimens are found in [the] La Matilde locality”, which implies that the tadpole was found at the “Cañadón de Estancia La Matilde” locality (sensu Báez & Nicoli, 2004: appendix 1). However, in the Supplemental Information (lines 21–22) we are told the tadpole “was found in a quarry close to [my emphasis] the “Estancia La Matilde”, a Jurassic locality”. If the tadpole was found in a quarry close to “Estancia La Matilde” (presumably equivalent to “Cañadón de Estancia La Matilde”), what is the name of the actual tadpole quarry? Is it the locality listed by Báez & Nicoli (2004: appendix 1) as “slopes west of Cañadón de Estancia La Matilde” or another locality?

Please be precise and consistent in how you report the tadpole locality. Assuming it is one of the localities listed by Báez & Nicoli (2004: appendix 1), for consistency and to make it easier for those of us not familiar with variants of locality names within the La Matilde Formation, I recommend you use their version of the locality name. If the tadpole locality is not one of the localities listed by Báez & Nicoli (2004: appendix 1), that should be clearly explained.

- 5) Throughout the MS, the fossil tadpole is variously identified as belonging to *Notobatrachus degiustoi* (e.g., Abstract line 29) or *Notobatrachus* (e.g., Abstract line 34). It is inaccurate to use only the genus name when referring to the tadpole, because two species currently are recognized for *Notobatrachus* (the type species *N. degiustoi* and a second species *N. reigi*), yet a tadpole is known only for *N. degiustoi*. In all cases, the tadpole should be identified as *Notobatrachus degiustoi* (first appearance in each paragraph) or *N. degiustoi* subsequent appearances in the same paragraph).
- 6) The fossil tadpole is referred to *Notobatrachus degiustoi* based on two lines of evidence (lines 66–72): a suite of osteological features it shares with metamorphosed individuals of that species and its co-occurrence in the quarry with “hundreds of adult specimens of *Notobatrachus degiustoi*”. I agree with that justification. Another line of evidence worth noting is that no other anuran species have been identified (to my knowledge) from the La Matilde Formation.
- 7) The ontogenetic age of the tadpole is estimated at Gosner stages 40–41. That seems reasonable considering the relative development of its skeleton. I am puzzled why the evidence for that ontogenetic age estimation is partitioned. In the main text (lines 87–88) the only feature listed to support your ontogenetic age estimate is “the forelimb is still contained within the body contour, likely covered by the operculum” whereas in the Supplemental Information (lines 135–147) a suite of other osteological features is listed, yet the position of the forelimb is omitted. I suggest you include the position of the forelimb in the Supplemental Information and, in the main text, qualify that the position of the forelimb is one of many features (which are listed in full in the Supplemental Information) that support your ontogenetic age estimate.
- 8) Specimen MPM-PV 23333 usually is identified as a tadpole (as in the title), yet in numerous places throughout the MS (main text, Extended Data Fig. 9 caption, and Supplemental Information) the terms “larva”, “larvae”, and “larval” are used. For consistency, I recommend you use only “tadpole”/“tadpoles” when referring to anuran larvae.
- 9) In the text (line 144) you imply that large adults of *Notobatrachus degiustoi*, with a stated SVL of 150 mm, are “by far the largest Mesozoic anurans”. I do not believe that is correct. In their re-description of the hyperossified, crown anuran *Beelzebufo ampinga* from the Maastrichtian of Madagascar, Evans et al. (2014: S1 text, “Size Estimation”) estimated the maximum SVL of *B. ampinga* as exceeding 300 mm; in other words, twice

the size of *N. degiustoi*. To my knowledge, *N. degiustoi* and *B. ampinga* are the largest Mesozoic frogs for which size can be directly measured or reasonably estimated, respectively. I am not aware of any other Mesozoic frogs of comparable size. You can legitimately state that *N. degiustoi* is the largest stem anuran, but not the largest Mesozoic anuran.

10) Comments on figures in main text:

- a. I like how the photographs in Figs. 1 & 2 are complemented by coloured, interpretive drawings. That combination of images nicely complements the descriptive text and makes it easy to see the skeletal and soft features of the tadpole.
- b. Those same two figures (Figs. 1 & 2) are adequate for the abbreviated description in the main text, yet as I noted in my annotated copy several features (mostly of the branchial basket) mentioned in the expanded description within the Supplemental Information are not labelled in the figures. I urge the authors to ensure that all mentioned features in the expanded description are clearly labelled to ensure readers can see the described features—that could be done by adding additional figures (e.g., enlarged version of the cranial closeup interpretive drawing) into the Supplemental Information.
- c. For your photographs, the descriptive passages “low exposure under flush light” (Fig 1 caption) and “high exposure under low vertical light” (Figs 1 & 2 captions) are unclear. For “low exposure” and “high exposure” do you mean you used a short or long exposure time with your camera when you took the photograph or that in Photoshop (or other image editing software) you manipulated the image by changing the exposure to the low or high end of the range? For “flush light” do you mean low angle light? For “low vertical light” do you mean soft or muted, high angle light? Please clarify what you mean, so readers are not left guessing.
- d. Ensure you use unique abbreviations for each feature. In Fig. 1, the abbreviation “n” is used for both nasal and neurocranium.
- e. Ensure all listed abbreviations appear in the corresponding figure and vice versa. In Fig. 2, the listed abbreviations “oc”, “pl”, and pp” are not labelled in the corresponding figure, whereas the structure labelled as “pph” is not defined in the caption.
- f. Ensure all abbreviations are listed alphabetically, as follows: all letter abbreviations, then all Roman numeral abbreviations, and finally all Arabic number abbreviations. In Fig. 1 caption the abbreviations “rb” and “up” are out of sequence, whereas in Fig. 2 caption the abbreviations “pl”, “pp”, and “ru” are out of sequence.
- g. For both Fig. 1 and 2 captions, your title sentence begins as “Overview and interpretive drawing ...”. What you call an “overview” is a photograph, so call it

that. Write “Photographs and interpretive drawing ...” (Fig. 1) and “Photograph and interpretive drawing ...” (Fig. 2).

- h. For Fig. 3b, the dark pink point plotted for the tadpole of *Notobatrachus degiustoi* is challenging to see against the dark pink background. I suggest making that point stand out more, perhaps by having a black arrow pointing at it.
- i. For Fig. 3c, in the upper right quadrant, two points are plotted. The point for *Notobatrachus degiustoi* is labelled, whereas the plot for *Aquarana catesbeiana* is not. For consistency and to reinforce the point that those are the only two anuran species having both giant tadpoles and adults, both points deserve to be labelled.

11) Extended Data Figures:

- a. Fig. 1—See my notes about the caption and figure in the annotated copy.
- b. Figs. 2 & 3—These appear to be larger sized images of the same photographs presented earlier as main text Fig. 1b and 1a, respectively. Why are essentially the same photographs repeated here? If you wish to retain these larger photographs, I urge you to (1) better explain the parameters for the photographs (currently vaguely described as “taken with short exposure” in Fig. 2 caption and “taken with superficial light” in Fig. 3) and ideally use the same wording as I suggested above (see point 10c) for the main text photographs and (2) include scale bars for both photographs.
- c. Fig. 4—Here in the caption you use XI, XII, and XIII for the three presacral neural arches, but in main text Fig. 1 you use 1, 2, and 3 for those structures. For consistency, do the same here. In the caption: (1) state the images are photographs; (2) provide parameters for those photographs (presumably taken with low angle light to enhance details); (3) replace “specimens” (plural) with “specimen” (singular); (4) delete “I–XIII, vertebrae”; and (5) as I recommended for main text Fig. 1 caption, at the end of the abbreviation list add “I–IX, presacral vertebrae; X, sacrum; 1–3, postsacral neural arches”.
- d. Figs. 5 & 6—In captions define the dashed grey, vertical lines. Presumably those denote the Permo-Triassic (left) and Cretaceous-Palaeogene (right) boundaries?
- e. Figs. 7 & 8—In captions define the pair of values separated by a forward slash at each node.
- f. Fig. 9—In the figure, (1) names of species with giant tadpoles should be italicized in Fig. 9b (as in your other figures) and (2) add “(mm)” behind “total length” (Fig. 9a & b) and “body length” (Fig. 9a) labels on the Y and X axes. In the caption, the phrase “double the media” is nonsensical. For clarity and consistency, it better to use the same wording as in the main text (line 440) and instead state “twice the mean”.

12) Supplemental Information text: As is typical for supplemental text, the writing in this section is far less polished than the main text. Please see my annotated copy of the MS

for numerous questions, corrections, and suggestions for improving the clarity and consistency of the supplemental text. See also comments raised in my previous points. I will reiterate that you are welcome to contact me at james.gardner@gov.ab.ca for clarification or if you need help deciphering my printing in the annotated MS.

13) Supplemental Tables:

- a. For Table 2, it is worth adding in its caption that full references for citations in “Source” column are provided in Table 1. It took me a few moments to figure that out.
- b. In the “Source” column for both tables, simply listing “MorphoSource” and “Xenopus webpage” without further details (e.g., exact urls) is unnecessarily vague. I recommend providing sufficient details so those sources can be reliably located.
- c. In Table 2, line 89, Source column, author name “Shrimpton” is misspelled as “Schrimpton”.

I hope my comments, questions, and suggested changes are helpful, even if they are not all welcome. Good luck with your revision. I was excited to get a sneak peek at this new fossil tadpole and I look forward to seeing your MS published in *Nature*.

As with all my reviews, I waive anonymity.

Regards and best wishes,

Jim Gardner, PhD
Curator of Palaeoherpetology
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TALavera,, R. R., BUSTILLO, M.-A. & B. SANCHIZ. (2019): Paleobiological forensic study of an upper Miocene *Pelobates* tadpole (Anura, Pelobatidae) from the Tresjuncos lagerstätte of central Spain. – Journal of Vertebrate Paleontology 39: e1669615.

VERGILOV, V. & N. TZANKOV (2021): Giant pelobatid fossil larva from the middle Miocene of Bulgaria. – *Historia naturalis bulgarica* 43(3): 29–42.

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For other reports of giant tadpoles, see one-page list of references by Ronn Altig (attached separately) and the following paper that reports on giant (although not by your definition) tadpoles of the extant Giant Cuba Tree frog: Lindsay, K., K. Orchard, & M. Yokoyama. 2019. Giant Cuban Treefrog (*Osteopilus septentrionalis*) tadpoles in an introduced population on Nevis (Lesser Antilles). *IRCF Reptiles & Amphibians*, 26(2): 125–126.

[REDACTED]

**THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE
ANURAN LIFE CYCLE**

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22 Anurans are characterised by a unique biphasic life cycle, with an aquatic larval
 23 (tadpole) stage followed by an adult (frog) stage, both connected through the
 24 metamorphic period in which dramatic morphological and physiological changes
 25 occur¹. Modern tadpoles exhibit a great morphological diversity and ecological
 26 relevance², but their absence in the pre-Cretaceous fossil record (older than 145
 27 Mya) makes their origins and early evolution enigmatic. This contrasts with the
 28 post-metamorphic anuran fossil record that dates back to the Early Jurassic (ca.
 29 191–183 Mya)³. Here we report a late ^{stage tadpole} larva of the stem-anuran *Notobatrachus*
 30 *degiustoi* from the Middle-Late Jurassic of Patagonia (ca. 166–157 Mya). This
 31 finding has a dual importance since it represents the oldest-known tadpole and the
 32 first stem-anuran larva described up to date. Its exquisite preservation, including
 33 soft tissues, reveals features associated with the filter-feeding mechanism present
 34 in extant tadpoles^{4,5}. Notably, both *Notobatrachus* larva and adult acquired large
 35 sizes, demonstrating that tadpole gigantism also evolved among stem-anurans. The
 36 new discovery reveals that a biphasic life cycle, with filter-feeding tadpoles
 37 inhabiting aquatic ephemeral environments, was already present in the early
 38 evolutionary history of stem-anurans, and has remained stable for 166 million
 39 years.

40

41 In most salamanders and extinct temnospondyl amphibians development
 42 progresses without major changes of habitat, morphology or feeding habits⁶. On the
 43 contrary, anurans are characterised by a biphasic life history with strikingly different
 44 larval and adult phases joined by a major metamorphic event¹. The general consensus is
 45 that a biphasic life cycle with metamorphosis is the plesiomorphic condition in
 46 anurans^{7–11}, and studies about evolution of the tadpole morphospace indicated that

Here +
 elsewhere
 use 1/2 dash
 to denote
 range.

larval characters may have evolved during the basal anuran radiation around the Triassic–Jurassic boundary¹². Nevertheless, some authors proposed that the larvae of Salientia (the stem-group of anurans) and early anurans were comparatively similar to the salamander larvae, with gradual changes through ontogeny^{13–16}. These hypotheses are based on evidence from extant anurans and remain untested because fossil larvae are completely absent in the Triassic and Jurassic fossil record^{17,18}. Thus, the timing and evolutionary transformations involved in the origins of the complex biphasic anuran life cycle remain enigmatic and matter of debate.

Here we describe the oldest-known tadpole from the Middle Jurassic La Matilde Formation (Callovian–Oxfordian; ca. 166–157 Mya) in southern Patagonia, Argentina, and it is referred to the stem-anuran *Notobatrachus degiustoi*. This species was previously represented by several, superbly preserved adult specimens ~~from~~^{from} the same locality^{19–21}. The exquisite preservation of this stem-anuran tadpole (MPM-PV 23333), including most of the hyobranchial apparatus and soft tissues, substantially enhances our knowledge of the origins and early evolution of the complex anuran life cycle.

"several" implies a small number
vs. "hundreds" is a greater number
why the discrepancy?

Description

The fossil MPM-PV (Museo Provincial Padre M.J. Molina) 23333 is a single individual that consists of a cranium and most of the postcranium of a tadpole in dorsal aspect, including the proximal part of the tail (Fig. 1). It was collected from the same quarry that has yielded hundreds of adult specimens of *Notobatrachus degiustoi*^{22,23}. Its occurrence and the presence of several skeletal characteristics shared with adult specimens of *Notobatrachus degiustoi* (i.e., 9 presacral vertebrae, free ribs associated with presacrals II–IV, transverse processes of posterior presacral vertebrae as long as sacral diapophysis, and uncinat process on the free rib III) support the referral of the

vague

72 fossil tadpole to this species. Geological data ^{for} of the locality (La Matilde Formation)
 73 indicate that sediments containing the specimen were deposited in lacustrine
 74 environments scattered in the floodplain with pyroclastic material input, under
 75 seasonally dry-winter wet biome^{24,25}. These sediments also contain diverse plants,
 76 branchiopod crustaceans, bivalves and insects (Extended Data Fig. 1)^{26–28}.

77 The larva was well chondrified as indicated by cartilage imprints, and the overall
 78 degree of ossification supports an advanced larval stage, prior to the beginning of
 79 metamorphosis (Extended Data Figs. 2–4). The cranial ossification that corresponds to
 80 the parasphenoid is flanked by the frontoparietals, which extend on both sides as
 81 longitudinal thin strips of bone, markedly pointed anteriorly and widely separated along
 82 the midline (Fig. 1). The right otic region preserves the crista parotica, represented by a
 83 subtriangular knob-like prominence (Fig. 1). The imprints of neural arches of nine
 84 presacral vertebrae, the sacrum and three postsacral vertebrae are present. The right
 85 forelimb is represented by the radius and ulna fused to each other, and the hand (Fig. 1). ^{"manus" in Fig. 1c}
 86 The right iliac shaft is preserved in lateral view, with the dorsal acetabular expansion
 87 already ossified (Fig. 1). As the forelimb is still contained within the body contour,
 88 likely covered by the operculum, we assign the tadpole to Gosner stages 40–41²⁹. The
 89 total length of the *Notobatrachus* tadpole is estimated at about 159 mm (see
 90 Supplementary Information).

91 MPM-PV 23333 is unique in the preservation of the hyobranchial skeleton,
 92 which was not preserved in any other fossil tadpole described up to date¹⁸. The
 93 expanded ceratohyal possesses well developed anterior, posterior, and lateral processes
 94 and a condylus articularis (Fig. 2), indicating the presence of an articulation with the
 95 palatoquadrate cartilage. Ceratobranchialia are preserved as four black ellipsoidal bands
 96 arranged in an anteroposteriorly oriented row (Fig. 2). Ceratobranchial I is medially

overlapped by the ceratohyal. Ceratobranchials II–IV are proximally connected by commissurae proximales and distally by commissurae terminales (Fig. 2). Medial and lateral projections for support of the gill filters are distinguished along the shafts of ceratobranchialia II and III. An oblique rod-like element is placed lateroventral to the ceratohyal and lateral to ceratobranchial I (Fig. 2); it could be assigned to the lateral projection of the hypobranchial plate, although its identity is not certain.

Soft parts including eyes and nerves are preserved as dark imprints, in their anatomical position. The eyes are dorsally positioned; the lens within the right eye is clearly distinguishable on the anterolateral region of the eyeball, which is indicative of an anterolaterally facing eye (Fig. 2). Several nerve impressions are distinguishable on the right side of the cranial region (Fig. 2; see Supplementary Information).

Analyses and discussion

We explored the phylogenetic relationships of the *Notobatrachus degiustoi* tadpole using a modified phylogenetic dataset based on tadpole traits¹². The new dataset is composed of 86 species scored for 152 characters (see Supplementary information). The tadpole of *Notobatrachus degiustoi* was recovered as a stem-Anura using both implied weighted maximum parsimony and Bayesian inference with a relaxed Markov k-state variable morphological clock model that incorporates taxon ages using a fossilized birth-death process (Fig. 3a). These results are consistent with those previously found exclusively based on the adult morphology of *Notobatrachus*^{21,30,31}. Despite the adaptive decoupling between larva and adult in anurans^{32,33}, the morphology of both semaphoronts (i.e. tadpole and adult) have congruent phylogenetic signals and retrieve consistent phylogenetic positions for both crown and stem anurans.

Several anatomical traits present in the *Notobatrachus degiustoi* tadpole demonstrate that key features of crown-anuran tadpoles have already evolved in stem taxa; particularly, the structures of the hyobranquial skeleton involved in the feeding mechanism. The filter feeding system of the vast majority of extant tadpoles consists of a complex buccopharyngeal filter apparatus that entraps food particles from water currents maintained by continuous action of a buccal pump^{4,34}. Commissurae terminales, commissurae proximales, and a ceratohyale with median horizontal plate lacking a distinct processus anterolateralis, and spiny projections on ceratobranchials are traits of the hyobranquial skeleton of extant larvae tightly related to the filter-feeding mechanism^{8,35,36}, which were already present in the *Notobatrachus* tadpole. Moreover, the phylomorphospace built based on the dataset of tadpole features also shows that the larva of *Notobatrachus* is positioned close in the morphospace to early-diverging crown anuran tadpoles, and has the lowest dissimilarity distance with respect to them (Fig. 3b). In other words, the general morphology described here for the tadpole of the stem-anuran *Notobatrachus degiustoi* is highly congruent with that present in extant anurans such as discoglossoids.

The estimated total length of the *Notobatrachus degiustoi* tadpole (about 159 mm; Extended Data Fig. 9) is comparable to the largest adults of the species, that reached a body size (snout-vent length) of 150 mm²³. This length falls within the range of giant adult anurans (>127 mm SVL)³⁷. Gigantism in tadpoles and postmetamorphic anurans have evolved several times in the history of the clade^{37,38}, but there are very few species for which tadpoles and postmetamorphic individuals are both giants (Fig. 3c). In fact, the occurrence of gigantism in both stages of the life cycle is only recorded in *Notobatrachus degiustoi*, by far the largest Mesozoic anuran (Supplementary Table S1), and the extant bullfrog *Aquarana catesbeiana* (Fig. 3c). Under regular conditions, there

are two ways in which a tadpole might become gigantic, namely a long larval development (deceleration)^{39,40} or accelerated developmental rates⁴¹. This depends on ecological factors such as the environmental stability (food availability, low risk of desiccation) and the absence of predators^{35,42}. According to fossil data of the locality interspecific competence and predator pressures were negligible, and therefore, the large size of the *Notobatrachus* tadpole could be the result of both factors, an increase in developmental time and growth in seasonally ephemeral ponds.

The paleoecology of the locality together with the anatomical information provided here indicate that an exotrophic larva, inhabiting lentic ephemeral microhabitats is the ancestral condition for anurans, in agreement to previous studies based on extant species^{7–10}. Therefore, the overall morphology of *Notobatrachus* indicates that the anuran biphasic life cycle with a dramatic metamorphosis evolved at least 160–180 million years ago, during the Early-Middle Jurassic. Furthermore, studies of the larval body plan of living anurans indicate that tadpole morphological novelties, such as those implicated in the filter feeding mechanism, have evolved in a radiation around the Triassic-Jurassic boundary¹². The striking anatomical similarity between the *Notobatrachus* tadpole and extant early-diverging crown anuran tadpoles supports this evolutionary scenario, which was probably promoted by the stable selective pressures of ephemeral freshwater habitats exploited by anurans since the early steps of their evolutionary history.

166

167 **References**

168

FIGURES

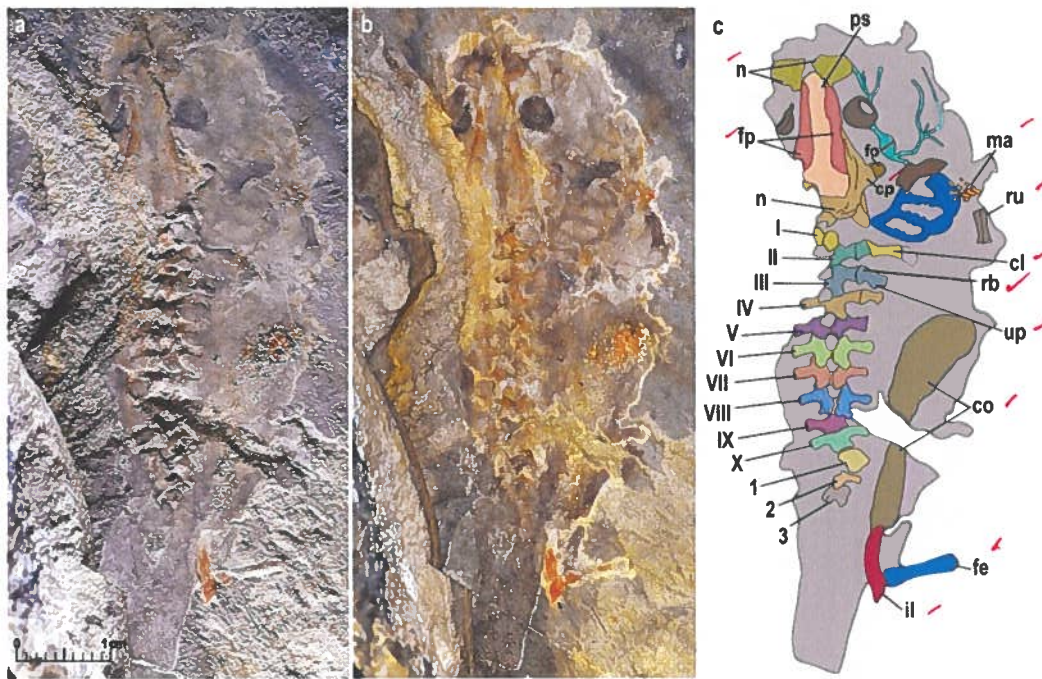
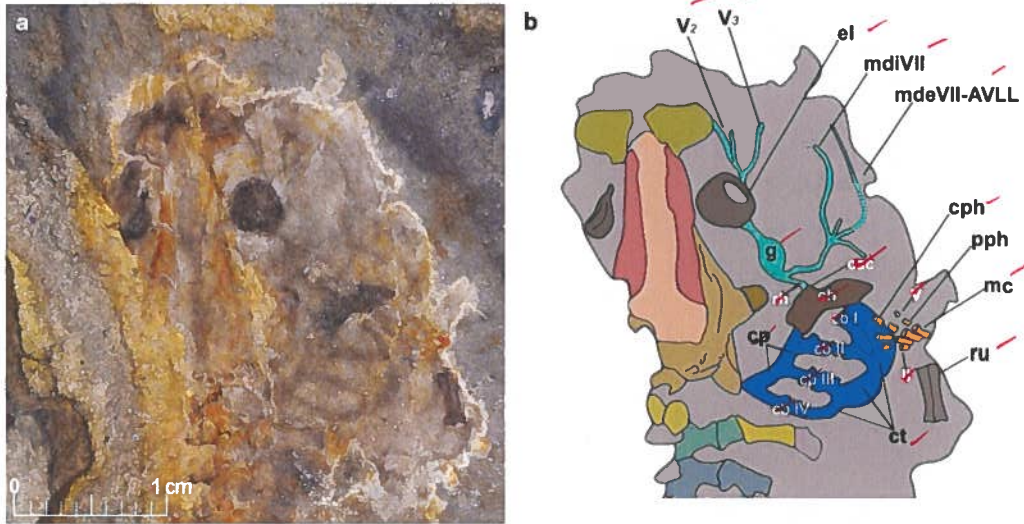


Figure 1. Overview and interpretative drawing of the specimen MPM-PV 23333. **a**, low exposure under flush light enhancing skeletal morphology. **b**, high exposure under low vertical light enhancing the soft tissues morphology. **c**, interpretative drawing of skeleton and soft tissues. Abbreviations: cl, cleithrum; co, cololite; cp, crista parotica; fe, femur; fo, fenestra ovalis; fp, frontoparietal; il, ilium; ma, manus; n, nasal; n, neurocranium; ps, parasphenoid; ru, radio-ulna; I-IX, presacral vertebrae; X, sacrum; 1-3, postsacral neural arches; rb, rib; up, uncinat process.

"n" also indicates nasal → need a unique abbreviation for nasal vs neurocranium

318



319 Figure 2. Overview and interpretative drawing of the head region of specimen MPM-PV
 320 23333. **a**, Picture of the head region taken with high exposure under low vertical light,
 321 enhancing ossifications, hyobranchial skeleton and soft organs. **b**, interpretative drawing
 322 depicting structures of the manus, hyobranchial skeleton and nerves. Abbreviations: cac,
 323 condylus articularis of ceratohyal; cb, ceratobranchialia I–IV; ch, ceratohyal; cp,
 324 commissurae proximales; cph, central phalanx; ct, commissurae terminales; el, eye lens;
 325 g, ganglion; mc, metacarpal; mdeVII-AVLL, ramus mandibularis externus of facial
 326 nerve and anteroventral lateral-line nerves; mdiVII, ramus mandibularis internus of
 327 facial nerve; oc, otic capsule; rh, ramus hyoideus of the facial nerve (VII); V₂, maxillary
 328 ramus of the trigeminal nerve; V₃, mandibular ramus of the trigeminal nerve; pl,
 329 processus lateralis of ceratohyal; pp, proximal phalanx; ru, radio-ulna; II, digit II; V,
 330 digit V.

Figure 3. Phylogenetic affinities of the tadpole of *Notobatrachus degiustoi*. **a**, Bayesian tip-dating tree based on tadpole anatomy characters and constrained following a molecular backbone of anuran phylogeny. **b**, phylomorphospace of the first two principal coordinates and dissimilarity distances (MORD) as heatmap, showing the larva of *Notobatrachus* (pink dot) positioned close to crown non-neobatrachian tadpoles (green dots). **c**, bivariate plot of the log-transformed tadpole total length against the log-transformed adult snout-vent length (residuals shown as a violin plot on the left-upper corner). Dotted red line represents the linear generalized least squares regression, dotted grey line represents the slope = 1, and the upper-right quadrante delimited by the yellow dotted lines indicate the area occupied by species with both giant tadpoles and adults, i.e., *Notobatrachus degiustoi* and *Aquarana catesbeiana*.

343 **Methods**

344 **Morphological description.** Osteological terminology followed Trueb^{43,44}, and Pugener
 345 & Maglia⁴⁵. Vertebrae are designated via Roman numerals in anteroposterior order.
 346 Vertebra X is referred to as the sacrum. Postsacral vertebrae are designated in an
 347 anterior to posterior sequence using Arabic numerals. Structures of the suspension filter-
 348 feeding system are referred according to Wassersug⁴⁶. Terms for hyobranchial skeleton
 349 follow Haas³⁶, ^{where} while nervous system structures follow terminology of Schlosser and
 350 Roth⁴⁷.

351

352 **Phylogenetic analyses.** The phylogenetic position of the *Notobatrachus* tadpole was
 353 tested using a phylogenetic data matrix including 86 taxa and 152 characters, modified
 354 from the analysis of Haas⁴⁸. A Bayesian tip-dating analysis was conducted in MrBayes
 355 3.2.7a⁴⁹. We used an Mkv substitution model, the same list of ordered characters as in
 356 Roelants et al.¹², and *Salamandrella keyserlingii* as the outgroup. We used an
 357 independent gamma rate relaxed clock model and uniform age priors modelled around
 358 the first appearance and last appearance dates for all tips of the tree. We implemented a
 359 node age calibration for Discoglossidae (uniform prior of 120.0–120.1 Ma⁵⁰), Pipioidea
 360 (uniform prior of 113.0–113.1 Ma⁵¹), Anomocoela (uniform prior of 52.0–52.1 Ma⁵²),
 361 and Pyxicephalidae (uniform prior of 39.5–39.6 Ma⁵³). We specified a fossilized-birth
 362 death process as the tree model using standard parameterizations and values. Fossils
 363 were specified to be tips and that the fraction of extant species represented 98.8% of the
 364 terminals. The deepest split within the tree was parameterized with a uniform tree age
 365 prior of 250.0–272.8 Ma, following the minimum and soft maximum ages of Benton et
 366 al.⁵⁴ for crown-Batrachia. We used Metropolis Coupling Markov chain Monte Carlo
 367 (MCMCMC) algorithms with two independent runs of 24 chains, using a heating

coefficient of 0.05 and 3 swap attempts per generation. Topological convergence, indicated by average standard deviation of split frequencies decreasing below 0.01, was achieved after 15,435,000 generations (Extended Data Fig. 5). Potential Scale Reduction Factors (PSRF) of 1.0, visual inspection of trace plot with Tracer 1.7.1⁵⁵, and estimated sample sizes (ESS) for all parameters >200 further indicated convergence. A second analysis was performed with the same settings, except that the topology was constrained following a molecular backbone of anuran phylogeny (Extended Data Fig. 6) that follows the results of Jetz and Pyron⁵⁶. This analysis reached average standard deviation of split frequencies below 0.001 after 4,010,000 generations.

The data matrix was also analysed under implied weighted maximum-parsimony with a range of concavity constant values (k) between 7 and 10 and using the program TNT 1.6⁵⁷. It was used the same list of ordered characters as in the Bayesian inference analysis. The search strategies started using a combination of the tree-search algorithms Wagner trees, tree bisection and reconnection (TBR) branch swapping, sectorial searches, Ratchet and Tree Fusing, until 100 hits of the same minimum tree length were achieved. The best trees obtained were subjected to a final round of TBR branch swapping. Zero-length branches in any of the recovered most parsimonious trees were collapsed and all the trees were rooted with *Salamandrella keyserlingii*. A global strict consensus tree (SCT) was generated from all the most parsimonious trees (MPTs) found in the analyses using the different k values (Extended Data Fig. 7). Homoplasy indices for each analysis under the different k values were calculated with a script that does not consider a priori deactivated terminals ('STATSb.run'). Group supports were quantified using no-zero weight symmetric resampling analyses, using 10,000 technical pseudo-replications and reporting both absolute and GC (group present/contradicted)

392 frequencies. The resampling frequencies were calculated from all the resampling trees
 393 recovered using the different k values and plotted on the branches of the global SCT.

394 Another analysis was conducted following the same matrix settings, tree search
 395 strategy, homoplasy indices calculation, and resampling analyses as before, but using a
 396 topological constrain based on the molecular backbone of anuran phylogeny (Extended
 397 Data Fig. 8) that follows the results of Jetz and Pyron⁵⁶. These parsimony analyses were
 398 implemented in two custom scripts written here for TNT
 399 ('treeSearches_protocol_Notobatrachus_unconstrained.run' and
 400 'treeSearches_protocol_Notobatrachus_unconstrained.run').

401

402 **Morphological disparity analysis.** Tadpole morphological diversity (disparity) was
 403 quantified on the taxon-character matrix modified from Roelants et al.¹² using the R
 404 package Claddis v0.6.3⁵⁸. This matrix is the same as that used for the phylogenetic
 405 analyses (86 species versus ¹⁵²discrete morphological ~~152~~ characters). The distance matrix
 406 was generated from the taxon-character matrix using the Maximum Observable
 407 Rescaled Distance (MORD)⁵⁸ because the commonly used Generalized Euclidean
 408 Distance (GED) may produce a strong methodological bias in matrices with a moderate
 409 to high amount of missing data^{59–61}. An ordination of the distance matrix was
 410 performed using a Principal Coordinates Analysis (PCoA) with Lingoes correction
 411 because of the presence of negative eigenvalues. No species had to be trimmed to
 412 conduct the ordination because of the absence of missing data in the distance matrix.
 413 The phylomorphospace was plotted using the first two Principal Coordinates, which
 414 account for a 14.47% of the total variance of the dataset, and the majority rule
 415 consensus tree with mean ages of the time-calibrated Bayesian inference analysis.
 416 Ancestral node estimations and their position in the morphospace were conducted with

417 the phylomorphospace function of the R package Phytools⁶². Extant anurans were
 418 graphically differentiated between non-neobatrachians and neobatrachians. Finally, a
 419 heatmap depicting the MORD of each species to *Notobatrachus degiustoi* was
 420 superimposed to the phylomorphospace biplot to show the row distance between
 421 tadpole morphologies and they evolutionary divergence through time. The heatmap was
 422 built with the function ‘filled.contour’ of the R package akima⁶³.

423

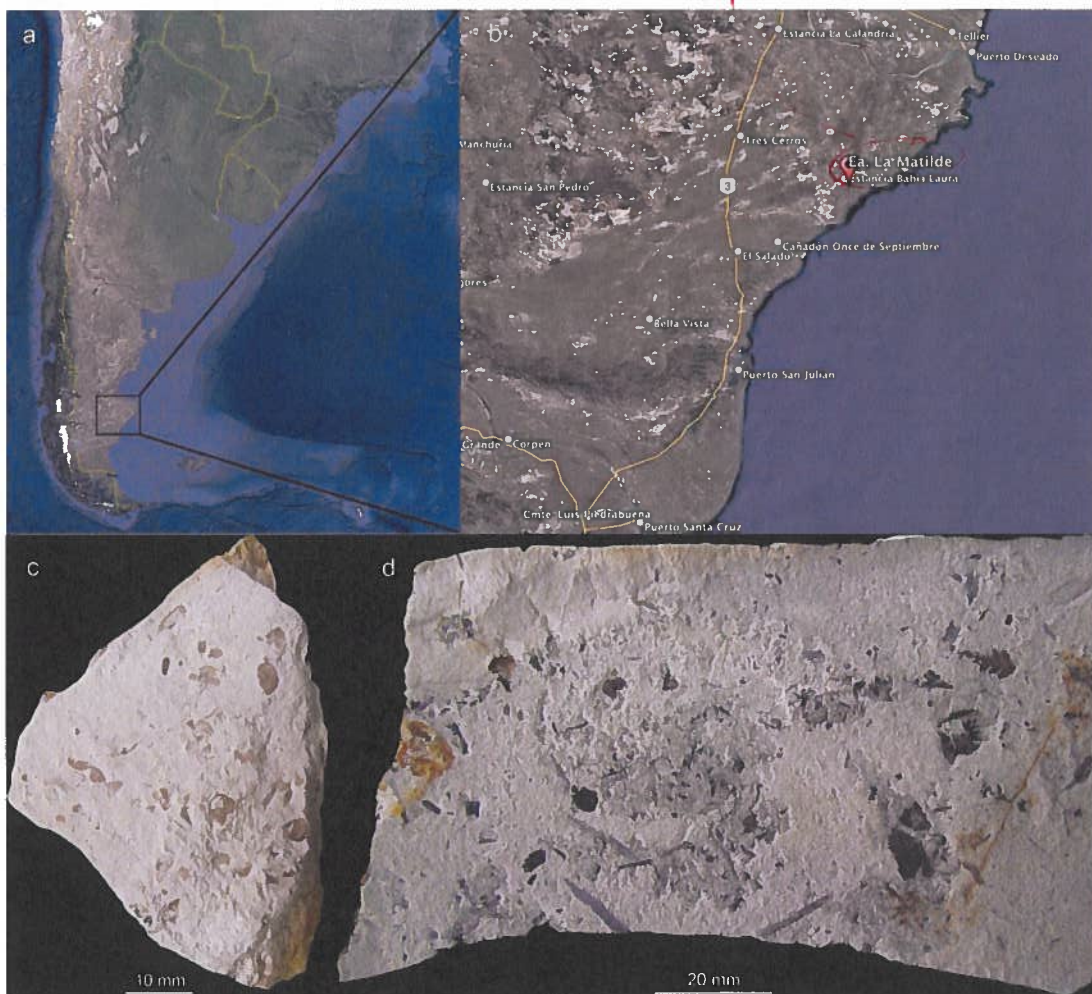
424 **Morphometric analyses.** We performed an exhaustive literature search for descriptions
 425 with measurements of tadpoles and adults of extant anuran species (Supplementary
 426 Table S1). For each species, we recorded tadpole body length (BL) and maximum total
 427 length (TL), and adult maximum snout-vent length (SVL). To reflect the size just prior
 428 to metamorphosis, we used measurements only from individuals at Gosner stages 39-41,
 429 the same range to which the *Notobatrachus degiustoi* tadpole is assigned. For adults we
 430 took the largest measurement found in the literature. Our sampling includes 35 of 50
 431 anuran families and a total of 191 species. This information is detailed along with
 432 literature resources in Supplementary Table S1.

433 In order to obtain an estimation of the total length of the *Notobatrachus*
 434 *degiustoi* tadpole, we built a linear regression model of body length (BL) against total
 435 length (TL). Data on tadpole BL were available for fewer species than TL, and therefore
 436 we had to reduce our dataset (Supplementary Table S2). A Shapiro–Wilk test was first
 437 performed to address whether each trait was normally distributed, under the null
 438 hypothesis that the distribution was normal.

439 As we found no agreement for the definition of a gigantic tadpole, we defined it
 440 as those specimens having a TL equal or higher than twice the mean of the TL of the
 441 species sampled in our dataset. According to our database, the average TL among

442 sampled taxa was 52.55 mm ([#]~~n~~ = 192); therefore, those tadpoles with a TL equal to or
 443 over 105.1 mm were classified as giants (Extended Data Fig. 9). Afterwards, to
 444 determine whether larval and adult body sizes were significantly related, and in
 445 consequence, if giant tadpoles were related to giant adults, we tested the relationship
 446 between tadpole and adult body size among species. We followed Womack & Bell³⁷ for
 447 description of giant adults, defined as those having SVL higher than 124 mm. We
 448 plotted the log-transformed tadpole TL versus the log-transformed adult SVL and we
 449 calculated a linear generalized least squares (GLS) regression between these variables.
 450 We have not conducted a phylogenetic GLS because available phylogenetic trees and
 451 our dataset of measurements do not share most of the species level taxa and to include
 452 phylogeny in the variance of the regression, we would have to prune most of the data.
 453 We also plotted a violin plot of the residuals of this regression and we highlighted the
 454 position of the residual of *Notobatrachus degiustoi*. All the graphics and the regression
 455 were done in R 4.2.3⁶⁴.
 456

EXTENDED DATA FIGURES



Extended Data Figure 1. a-b, geographic location of La Matilde locality, Santa Cruz province, Argentina. c-d, several specimens of the branchiopod *Eosolimnadiopsis? santacruensis* and plant debris typically found in the same levels were where *Notobatrachus degiustoi* specimens are found in La Matilde locality.

Is this the locality where the tadpole was found?

If so, ensure this name matches the name used throughout MS, see my point #4.



Extended Data Figure 2. High resolution photograph of the specimen MPM-PV 23333 taken with short exposure.

?
.

Angle of light?

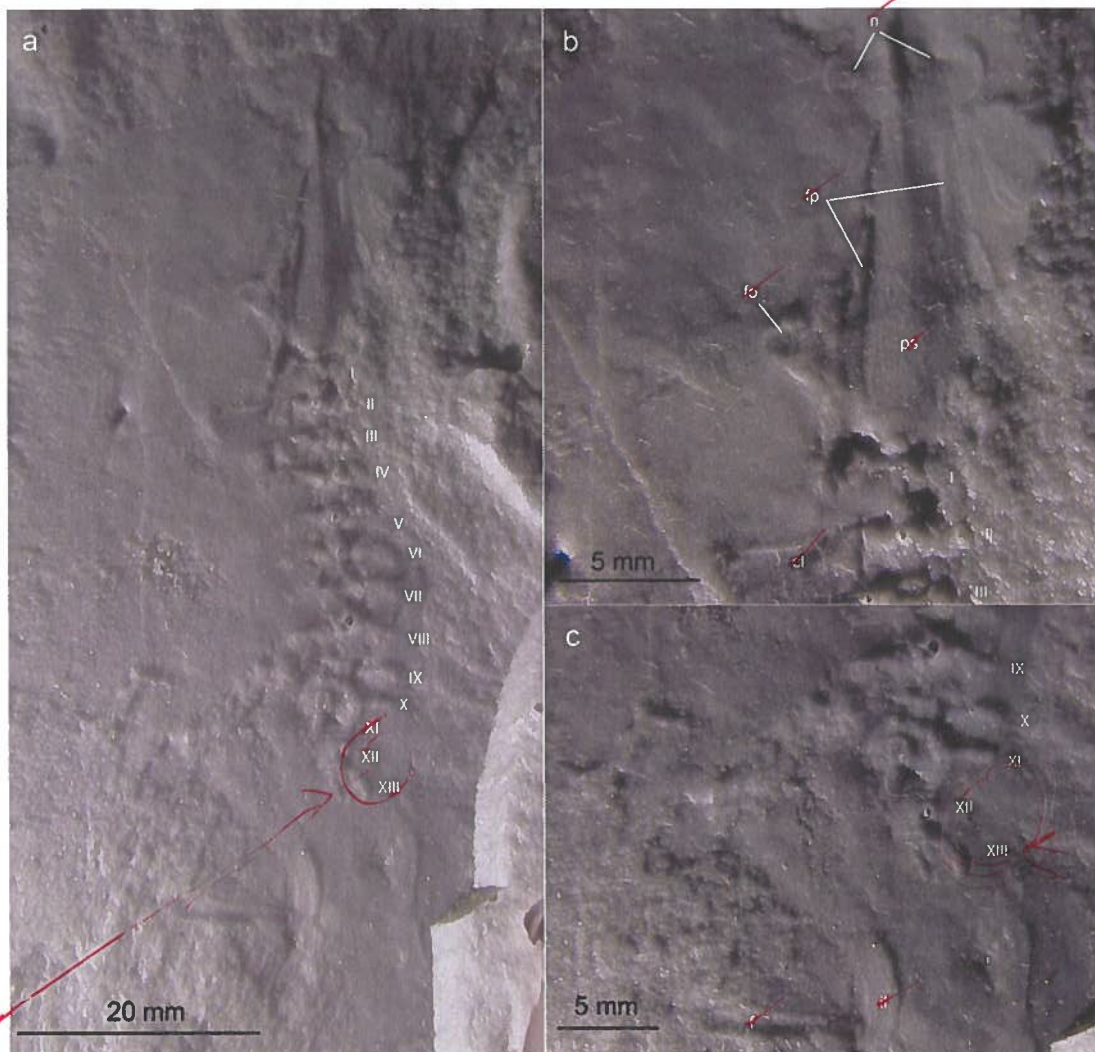
NO SCALE BAR



Extended Data Figure 3. High resolution photograph of the specimen MPM-PV 23333 taken with superficial light.

?

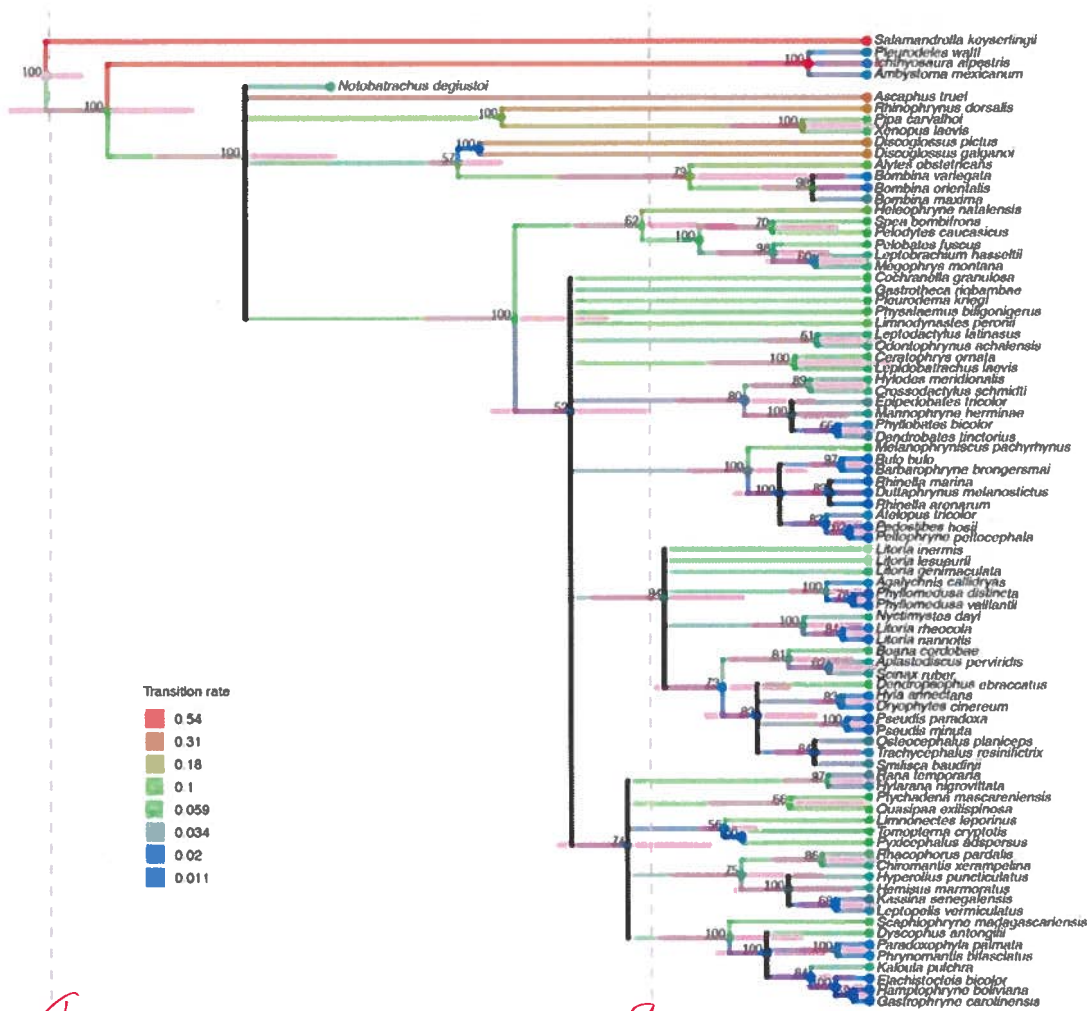
NO SCALE BAR



Extended Data Figure 4. Latex cast of specimen MPM-PV 23333. **a**, complete specimen showing the preserved vertebral elements; **b**, detail of the skull and first vertebrae; **c**, details of the last vertebrae and appendicular elements. Abbreviations: I-XIII, vertebrae; cl, cleithrum; f, femur; fo, fenestra ovalis; fp, frontoparietal; il, ilium; n, nasals; ps, parasphenoid.

↑
I-X, vertebrae
X, sacrum; I-XIII, post-sacral
neural arches

But in Fig. 1 you only
use Roman numerals for the
first 10 vertebrae + Arabic
numbers for the post-sacral
vertebrae!



Extended Data Figure 5. Unconstrained analysis.

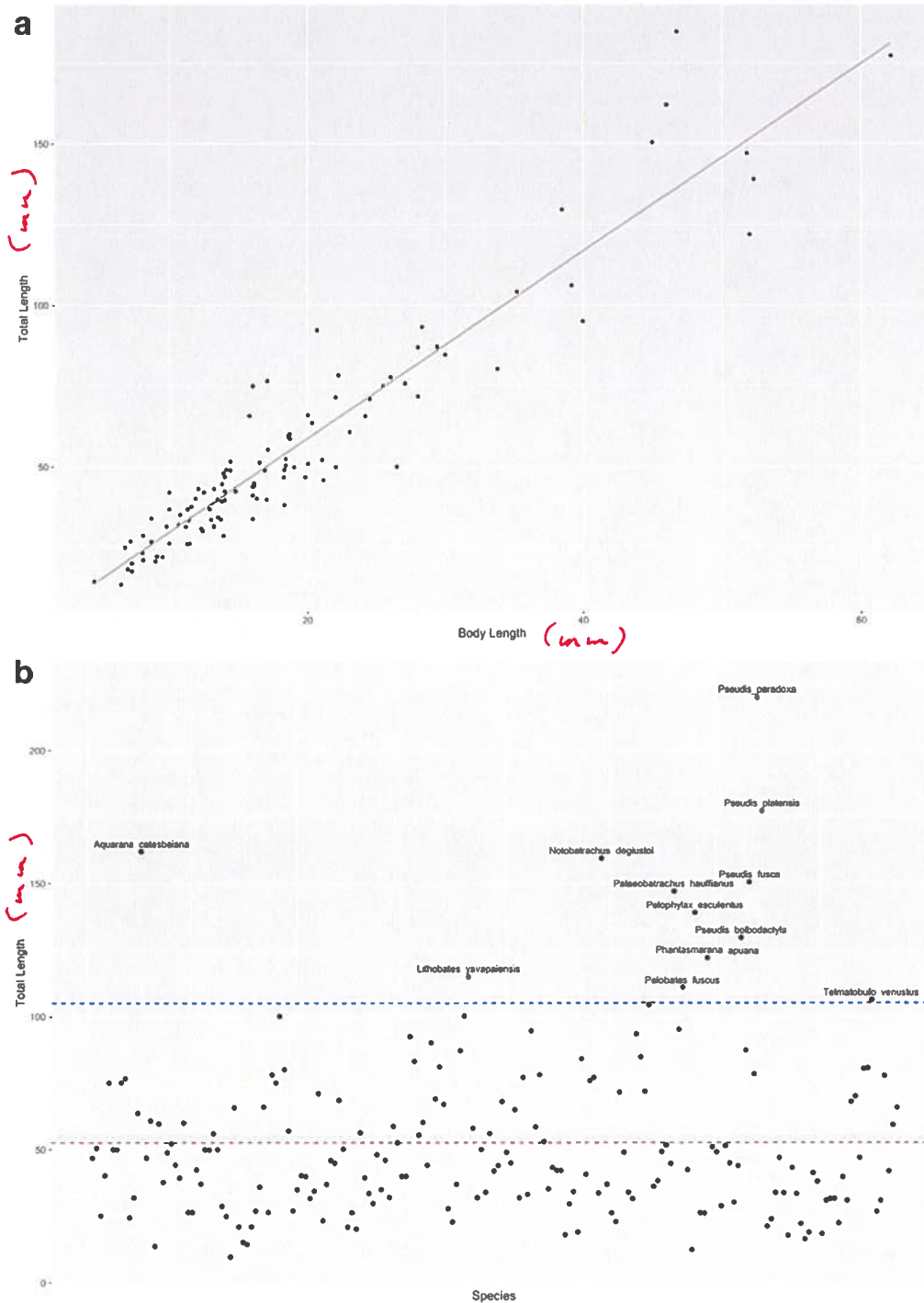
Define these



Define these values



Define these values



Extended Data Figure 9. a, Regression model of body length (BL) onto total length (TL) across 108 species of extant anuran larvae. **b**, Plot depicting the tadpole total length. Red line denotes the mean of the surveyed values (52.55 mm), whereas blue line denotes the double of the media (105.1 mm). Labeled species have gigantic tadpoles.

?

double the mean

Better choice
for consistency

or use same wording as
p. 20, line 440 "twice
the mean"

SUPPLEMENTARY INFORMATION

The oldest tadpole reveals evolutionary stability of the anuran life cycle

Mariana Chuliver, Federico L. Agnolín, Agustín Scanferla, Mauro Aranciaga Rolando, Martín D.

Ezcurra, Fernando E. Novas, Xing Xu

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2.2 Soft parts

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4.1 Character list

4.2 Calibration points

5. Supplementary references

1. Geology and paleoenvironment

Specimen MPM-PV 23333 was found in a quarry close to the “Estancia La Matilde”, a Jurassic locality in Santa Cruz province (Patagonia, Argentina) where hundreds of adult specimens of *Notobatrachus degiustoi* were found since 1950^{22,65}, thus supporting

Identifying SY
name

→ The type
locality for
the species
correct?

the taxonomic allocation of the specimen to this species. La Matilde Formation is a volcaniclastic unit that crops out in several localities in Santa Cruz province (Extended Data Fig. 1). Specifically, ~~the specimen~~ MPM-PV 23333 comes from fine grained, light-grey, tuffaceous sandstones deposited in alternating thin light and dark layers. The La Matilde Formation is laterally and vertically interbedded with the Chon Aike Formation, a pyroclastic unit covering an area of some 100000 km²(24, 66). The La Matilde Formation was considered as Bathonian-Callovian in age based on fossil floral remains by Stipanovic & Bonetti⁶⁷. However, the age of the La Matilde Formation is also established based on the radiometric dating of volcanic rocks of the interbedded Chon Aike Formation. Whole-rock K-Ar dates ranging from 161 ± 10 (Oxfordian, Jurassic) to 138 ± 10 Ma (Valanginian, Cretaceous) were obtained from samples at the Gran Bajo de San Julián area⁶⁸. Rb-Sr whole-rock analyses were also carried out on several lavas and associated ignimbrites from the eastern outcrops of the Chon Aike Formation, resulting in an isochron of 168.2 Ma (Bathonian, Jurassic^{69,70}). ← ~~Firmly dated Jurassic~~
~~to why reported~~

Geological data ~~of~~ ^{for the} La Matilde Formation indicate that these sediments were deposited in lacustrine environments, scattered ~~in~~ ^{across} the floodplain ~~with~~ ^{and} pyroclastic material input under ^a seasonally dry-winter wet biome^{24,25}. ^{The} La Matilde locality in particular has yielded many adult specimens of *Notobatrachus degiustoi* preserved as impressions, but also contains diverse plants, branchiopods, bivalves and insects (Extended Data Fig. 1). Carapaces of the clam shrimp *Eosolimnadiopsis*? ~~Santacrucensis~~²⁶ are frequently found. Currently, clam shrimps (Spinicaudata) live in temporary aquatic bodies^{71,72}. Interbedded between the frog-bearing levels ~~there~~ [?] are abundant vegetal detritus that evidence a high content of organic matter, provided by surrounding forests made up of Equisetales, Osmundales, Coniferales, Araucariaceae and Podocarpaceae^{27,28,73-75}. Taken together,

48 sedimentological and fossil ^{data} ~~content~~ suggest *Notobatrachus degiustoi* spent most of its life
49 cycle in shallow, ephemeral lentic environments with a high input of plant debris.

50

51

2. Additional anatomical information

52 Specimen MPM-PV 23333 represents an incomplete tadpole preserved in one slab
53 in dorsal aspect, with most of the right half of the body, including the proximal part of the
54 tail (Extended Data Figs. 2–3). There is a whitish area delimiting the contour of the
55 specimen which clearly exhibits a polished surface that contrasts with the rugose surface of
56 the slab, allowing ^{us} to distinguish soft parts of the body and tail (Extended Data Figs. 2–3).
57 In general aspect, preserved organs exhibit a high degree of anatomical relations, with
58 skeletal elements in articulation and soft parts in position. However, the disposition of the
59 hyobranchial skeleton in relation to the cranium, and the pelvic girdle and hindlimb indicate
60 a low degree of dorsoventral flattening oriented rightwards.

61 Skeletal elements are represented only by impressions, mirroring the taphonomic
62 mode observed in most adult specimens of *Notobatrachus degiustoi*. Some elements (e.g.,
63 cleithrum and femoral shaft) left a striated imprint, probably due to the incipient
64 ossification stage of these bones. Several soft parts including eyes and nerves were
65 preserved as dark imprints, easily identified ^{by respective} in their ^s anatomical position (Extended Data
66 Fig. 2).

67 Both nasals are present, each represented by an anteriorly concave ossification ^{lying close}
68 ~~closely related~~ to the anterior end of the frontoparietals ((Extended Data Fig. 3). ~~There are~~
69 ^{poorly defined,} dark areas ^{the} ~~poorly defined~~ anterior to ^{that} nasal ossifications which might correspond either to
70 nasal cartilages or to rostral cartilage. The ossification corresponding to the parasphenoid
71 bone is visible in the ^{median} ~~medial~~ region of the cranium ^{and} flanked by ^{the paired} frontoparietals, ^{the latter of which} which extend
^{worth describing as inverted T-shape with short laterally short lateral processes}

72 as longitudinal thin strips of bone widely separated ^{across} in the midline and markedly pointed
73 anteriorly. The right otic region is represented by a subtriangular ^{and} knob-like prominence, the
74 crista parotica. A large circular notch is present laterally in the crista parotica, which
75 corresponds with the fenestra ovalis (Extended Data Fig. 4). The crista parotica exhibits a
76 simple structure, lacking anterolateral (*processus anterolateralis*) and posterolateral
77 projections like in basal ^{crown-}anurans such as *Ascaphus* and discoglossids⁴⁸. Despite occipital
78 region does not display a conspicuous structure, and ossifications in the midposterior region
79 of the skull are not clearly distinguishable, the presence of exoccipitals ossifications might
80 be inferred in this position (Extended Data Figs. 3–4).

This does not make sense

81 The right hyobranchial skeleton is well preserved in ~~specimen~~ MPM-PV 23333. An
82 expanded ceratohyal is represented by a dark robust plate placed lateral to the otic region,
83 with ^{its} medial and lateral parts delimited by a slight constriction on its medial length. [?] Medial
84 part ^{of the ceratohyal} exhibits well developed processus anterior and processus posterior, ^{whereas} while the lateral
85 part shows a processus lateralis (Extended Data Figs. 2–3). A conspicuous condylus
86 articularis on the anterior rim of the ceratohyal is also distinguishable, ^{implying} inferring the
87 presence of an articulation with the ^{unpreserved} palatoquadrate cartilage. The overall morphology of the
88 ceratohyal strongly resembles that of extant tadpoles, ^{in which} whose processes ^{of the ceratohyal} provide attachment
89 surfaces for muscles that power the buccal pump^{5,8,36}.

Helpful to label these in Fig. 2 & 3.

This ceratohyal structure is labelled!

90 In ~~Notobatrachus~~ ^{MPM-PV 23333} tadpole slightly ornamented ceratobranchialia are preserved as
91 four black ellipsoidal bands arranged in an anteroposterior row (Extended Data Fig. 2). ~~The~~
92 ceratobranchial I is partially overlapped by the ceratohyal. ~~The~~ ceratobranchials II–IV are
93 proximally connected by commissurae proximales and distally by commissurae terminales.
94 Similar proximal fusions (commissurae proximales) are found in larvae of Discoglossoidea
95 and Pipoidae, in contrast to numerous species of Neobatrachia where some or all

But these are not labelled.

These are labelled.

These are not labelled

96 commissurae proximales are missing^{36,48,76,77}. Notably, medial and lateral projections are
97 distinguished along the shafts of ceratobranchialia II and III. These projections represent
98 novel structures of the anuran hyobranchial skeleton, which ~~give support to~~ the gill filters.
99 An oblique rod-like element is placed medioventral to ~~the~~ ceratobranchial I; it might be
100 assigned to the lateral projection of the hypobranchial plate although its identity could not
101 be ascertained.

102 The neural arches of nine presacral vertebrae, the sacrum and three postsacral
103 vertebrae are present (Extended Data Figs. 3–4). The ossified contralateral halves of the
104 neural arch laminae of vertebrae V–VIII contact at the midline, and their postzygapophyses
105 are well developed. Free ribs are present on the right side of presacrals II–IV, ~~its length~~. *The internal lengths of these ribs*
106 diminishes~~es~~ anteroposteriorly. Rib of vertebra III exhibits a posterior uncinat process at its
107 midlength like in adults of *Notobatrachus* ~~and~~ *degiusfoi* and basal crown anurans such as *Ascaphus*,
108 *Leiopelma* and discoglossids^{78,79}. Notably, adult specimens of *Notobatrachus* ~~bear~~ *degiusfoi* a fourth
109 pair of free ribs in vertebra V, although it was not still ossified in young postmetamorphic
110 individuals²³. Vertebrae V–VIII show short and distally rounded transverse processes on
111 both sides, ~~while~~ *whereas* the ninth vertebra preserve~~s~~ *the* only left transverse process, which is the
112 longest of all presacrals. The sacral diapophyses are narrow and not expanded distally.
113 There are three postsacral vertebrae represented by neural arches, those of postsacral 1
114 exhibit minute transverse processes (Extended Data Figs. 3–4). Extant anurans also bear
115 three postsacral vertebrae, which fuse during metamorphosis forming the coccyx, one of the
116 elements that constitute the urostyle⁸⁰. Thus, ~~the~~ *degiusfoi* *Notobatrachus* tadpole demonstrate~~s~~ *s* a
117 conservative morphogenetic developmental pathway for the urostyle, a key evolutionary
118 feature of the anuran *Bauplan*¹.

Is this correct? When I look at Fig. 1c, the laterally longest transverse processes appear to be on vertebrae VI + VII.

"many" in Fig. 1c

119 The pectoral girdle is only represented by the cleithrum, which is an elongated bar
120 overlapping the rib of vertebra II. The right forelimb is preserved within the body limits,
121 lateral to the hyobranchial skeleton (Extended Data Figs. 2–3). It is represented by the
122 radio-ulna and the hand. The radius and ulna are fused, although the suture is still clearly
123 distinguishable. Metacarpals II–V are represented as the imprints of cylindrical shafts, with
124 slight lateromedial overlapping between them. Metacarpal IV is the longest and stoutest.
125 The proximal phalanges of the four digits are short cylindrical elements with a slight
126 midlength constriction, whereas the central phalanges are only present in digits IV and V.

127 The posterior appendicular skeleton is represented by the ossifications of the right
128 ilium and femur, placed posterolaterally to the third postsacral (Extended Data Fig. 3). The
129 right iliac shaft is preserved in lateral view, with the dorsal acetabular expansion already
130 ossified. The femur extends laterally, with its long diaphysis oriented almost perpendicular
131 to the axial skeleton, and its proximal epiphysis slightly overlapped by the ilium (Extended
132 Data Fig. 3).

133

134 2.1 Ossification state and ontogenetic stage of *Notobatrachus* tadpole

135 MPM-PV 23333 is ~~on~~^{at} an advance^d stage of larval development, just prior to the
136 beginning of metamorphosis, as indicated by the state of development of cranial and
137 postcranial ossifications (Extended Data Fig. 4). During larval development, the
138 parasphenoid, frontoparietals and exoccipitals are the first skull ossifications to be
139 differentiated^{43,81–85}. These ossifications are present in the *Notobatrachus* tadpole,
140 demonstrating a conservative onset of ossification in anurans. Regarding the vertebral
141 column, ~~the~~ ossification of sacral diapophyses starts during metamorphic climax⁸⁶, while
142 the ossification of the coccyx is only established late during metamorphosis^{1,80}. This is

what about
normals?

degenerate

whereas

143 congruent with the developmental stage of the vertebral column of *Notobatrachus* tadpole,
144 allowing us to infer a larval stage previous to metamorphosis. Furthermore, the presence of
145 ossified central phalanx, the fused condition of the radio-ulna, and the ilium not contacting
146 the sacral vertebra allow us to refer specimen MPM-PV 23333 to a late larval stage. In sum,
147 we referred the tadpole of *Notobatrachus* to a larva likely on a Gosner Stage 40-41²⁹.

149 2.2 Soft parts

150 The exquisite preservation of specimen MPM-PV 23333 sheds light on some
151 remarkable aspects of the soft anatomy of *Notobatrachus* tadpoles. Both eyes are preserved,
152 but only the right is complete (Extended Data Fig. 2). The eyes are dorsally positioned; the
153 lens within the right eye is clearly distinguishable in the anterolateral region of the eyeball,
154 which is indicative of the eye's anterolateral facing direction.

155 Several nerve impressions are distinguishable on the right side of the cranial region
156 (Extended Data Fig. 2). We can differentiate three different rami of the trigeminal nerve
157 anterior to the eyeball. The truncus maxillo-mandibularis of the trigeminal nerve diverges
158 around the middle of the orbit into medial maxillary ramus (V₂) and lateral mandibular
159 ramus (V₃). Posterior to the eyeball, a thickened blackish area probably corresponds to a
160 ganglionic complex, whose neural components cannot be ascertained but likely corresponds
161 to ganglia of nerves V and VII. Posteriorly to this ganglionic complex, there is a prominent
162 hyomandibular trunk (truncus hyomandibularis) which extends toward the anterior element
163 of the hyobranchial skeleton in the ramus hyoideus of facial nerve (VII), and diverges
164 distally in the internal mandibular ramus of facial nerve, and the external mandibular ramus
165 (a composite of fibres belonging to the facial nerve and others belonging to the
166 anteroventral lateral line).

Here it is worth providing references for how you identify/interpret the preserved nerves. E.g. nerves described in which extant anurans & by who?

the *degiusfoi*
location of the rudimentary pharynx also relevant to inferring the ontogenetic age of the tadpole?
Don't the

Hard to follow when looking at Fig. 2c. Suggest breaking this into several smaller sentences.

167

at the level of vertebrae
V $\frac{1}{2}$ 3, isn't it?

168 **2.3 Bromalite**

169 On the right side of the axial skeleton, at the level of vertebrae VI, there is a
170 compact, yellowish accumulation of particles, enclosed within the tadpole body limits
171 (Extended Data Figs. 2–3). This accumulation has rounded boundaries and a roughly
172 ovoidal shape. It extends posteriorly as a narrow, diffuse accumulation that ends near the
173 base of the tail, at the level of the anterior end of the ilium. Several crystalline grains can be
174 observed, thus contrasting with the rest of the very fine-grained slab containing the
175 specimen. We consider ~~it represents~~ ^{the accumulation as representing} in situ intestinal matter, ~~which constitutes~~ ^{or a} a cololite⁸⁷.
176 The preserved cololite is not disposed on a spiral, ~~which might~~ ^{but instead} suggest a sack-like structure
177 ~~of the digestive system~~ ^{for}. This could be explained by the developmental stage of ~~the~~ ^{degiustoi}
178 *Notobatrachus* tadpole, which is ~~staged~~ at the end of prometamorphosis, when the coiled
179 intestine of filter-feeding tadpoles undergoes deep structural changes ~~becoming~~ ^{to become} a sac like
180 gut in adults⁴¹.

181

182 **3. Morphometric analyses**

183 We estimated the total length of *Notobatrachus* ^{the} tadpole ~~in~~ ^{degiustoi} 159 mm, ranging among ^{at placing it}
184 the size of extant giant tadpoles (Extended Data Fig. 9). Notably, ^{the} largest postmetamorphic
185 specimens of *Notobatrachus degiustoi* reached a body size (snout-vent length) of 150 mm²³, ^{which}
186 ~~also ranging among~~ ^{is within the range of} giant adult anurans (>127 mm SVL)³⁷. We found a weak correlation (r^2
187 = 0.44; $p < 2.825e-10$) between tadpole and adult body size in contrast to Emerson³⁹ but in
188 agreement with a recent comprehensive study about the evolution of body size in ^{tadpoles} larvae and
189 adult anurans⁸⁸.

190

4. Phylogenetic analyses

We employed the morphological matrix of Haas⁴⁸, excluding characters 12, 83, 116, and 117 following Roelants et al.¹², resulting in a matrix with 152 characters and 86 terminals for the inclusion of *Notobatrachus degiustoi* tadpole.

1.1 Character list (modified from Haas [2003])

- 1 Epidermal melanocytes elongated and forming orthogonal mesh (0); melanocytes of irregular shape not forming reticulation (1)
- 2 Larval subdermal serous glands absent (0) present (1).
- 3 Keratodonts absent (0) present (1).
- 4 Labial ridges with single row of keratodonts (0) ridges each with double (or more) rows of keratodonts (1).
- 5 Distal anterior labial ridge and keratodont-bearing row broad (0) very short and median (1).
- 6 Keratinized jaw sheaths absent (0) present (1).
- 7 Oral lips absent (0) present (1).
- 8 Upper lip papillation continuous (0) broad diastema present (1).
- 9 Lower lip papillation complete without gap (0) diastema in papillation (1).
- 10 Oral orifice wide (0) narrow (1).
- 11 Eye position dorsolateral (0) lateral (1).
- 12 Single-layered dermis (0) double-layered dermis (1).
- 13 Vena caudalis dorsalis absent (0) present (1).
- 14 Paired venae caudales laterales short (0) long (1).
- 15 Operculum not fused to abdominal wall (0) fused to abdominal wall (1).

430 Crato Formation, Early Cretaceous (113 ma)

431

432 Anomocoela-*Aerugoamnis paulus*⁵²

433 Fossil Butte Member of Green River Formation, ^{early}~~Lower~~ Eocene (52 ma)

434

435 Pyxicephalidae-*Thaumastosaurus servatus*⁵³

436 Phosphorites du Quercy, ^{late}~~upper~~ Eocene (39.5 ma)

437

438 5. Supplementary references

439

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445 volcanism in the Antarctic Peninsula and Patagonia by crustal melting. *J. Petrol.* **42**,
446 1043–1065 (2001).

447

448 67. Stipanovic, P.N. & Bonetti, M.I.R. Posiciones estratigráficas y edades de las principales
449 floras jurásicas argentinas. II Floras doggerianas y málmicas. *Ameghiniana* **7**, 101–118
450 (1970).

451

List of giant tadpole papers from Ronn Altig (July 2018)

Kerney R, RJ Wassersug, BK Hall@2006@Skeletal advance and arrest in giant non-metamorphosing African clawed frog tadpoles (*Xenopus laevis* Daudin)@Journal of Anatomy@216@1-12

Milto KD@2009@A giant tadpole record of *Rana esculenta* in northwestern Russia@Russian Journal of Herpetology@16@143-145

Tarkhnishvili DN, IA Serbinova@1999@Giant larvae of the Caucasian salamander (*Mertensiella caucasica*)@Advances in Amphibian Research in the Former Soviet Union@3@187-191

Rot-Nikcevic I, RJ Wassersug@2004@Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis@Journal of Experimental Biology@207@2133-2145

Rocek Z, R Bottcher, R Wassersug@2006@Gigantism in tadpoles of the Neogene frog *Palaeobatrachus*@Paleobiology@32@666-675

Doyle MJ, N Maclean@1978@Biochemical changes in developmentally retarded *Xenopus laevis* larvae: 1. The lens crystallin transition@Development@46@215-225

Boschwitz D@1957@Thyroidless tadpoles of *Pelobates syriacus* Boettger H@Copeia@1957@310-311

Boschwitz D@1960@A giant tadpole of *Hyla arborea* L.@Bulletin of the Research Council of Israel: Zoology@9

Undocumented specimen mentioned by Ronn: I know of a preserved giant tad of *Lithobates chiricahuensis*

Referee #3 (Remarks to the Author):

The manuscript reported a well-preserved tadpole specimen at stage of Gosner 40-41 of *Notobatrachus*, which is the oldest frog tadpole so far reported and the first tadpole of a stem frog, and therefore provides an excellent fossil evidence for how the larva form of stem frogs is, especially the exceptionally preserved hyobranchial apparatus, and some other soft tissue features.

The metamorphoses of frogs, intriguing to many people in general, are famous of those drastic changes in nearly all aspects of the animal, resulting in two difficult life stages, but we do not know when and how such a developmental feature originated due to the lack of the fossil tadpoles in early evolutionary history of frog. Therefore the discovery of this *Notobatrachus* tadpole from the Middle Jurassic of Argentina, together with the detailed morphological study of the *Notobatrachus* adults, proves the drastic metamorphosis which features the anurans among vertebrates, had occurred early during Middle Jurassic. I think this ms is of great interest to the people not only in my own discipline but also in general.

The fossil was prepared in a good condition and the photographs show clearly the preserved morphological information. The data for phylogenetic analyses and morphological disparity analyses are derived from a good previous work (Haas 2003), and new data based on the discovered fossil was included (but more may be done; see below). The data for body size analyses is good to give a rough estimate for the total length of the *Notobatrachus* tadpole. The results in this manuscript are mostly robust and reliable and there may be more to dig into this rare but important tadpole fossil (see below). The text is well written in a clear structure with a good collection of citations.

I listed my detailed comments and suggestions as below.

1. As for the morphological interpretation of the fossil tadpole, I agree mostly with the authors although there are some concerns as stated below.

SI Line 84-86: There is a confusion on two minor structures of ceratohyal - processus condylus articularis and processus anterior. If the processus condylus articularis was labelled correctly in Figure 2 (cac), there seems not to be a processus anterior medial to it.

Another concern is the identification of the cranial nerves V and VII. The two cranial nerves originate from the brain at a similar position, and branch in a similar way, and therefore it could be very different to say which one the preserved nerve in this *Notobatrachus* fossil tadpole is, especially considering the displacement the preservation causes. I think the interpretation, brought out herein by the author, that the anterior one is CN V and the posterior one is CV VII, is highly possible due to the possible ganglion. But if so, the ramus of hyoideus of CN VII branches off from the ramus of hyomandibularis, therefore being slenderer than the latter in many tadpoles (Schlosser & Roth 1976; Quinzio & Fabrizi 2019), which is different from the situation in the fossils.

2. Based on the forelimb remaining within the pocket, the integration of the hyobranchial apparatus (not yet broken up), and several well-defined bone elements (such as frontoparietal, parasphenoid, otic capsule, neural arches and ribs), it is reasonable to determine that the developmental stage of this

tadpole is at stage Gosner 40-41. However there are also some interesting points considering the stage of this fossil tadpole:

1) the early ossification of the full set of the manus phalanges (the hindlimb development is supposed to be at the same stage or slightly earlier than the forelimb, and therefore the foot phalanges were probably in its full configuration).

2) The early ossification and development of nasals (for example, nasals in *Xenopus laevis* typically do not appear until stage 58, but appear early in non-metamorphosing *Xenopus* tadpoles in earlier stages; Kerney et al. 2010)

Note Wassersug & Roth (1982) suggests some features in the larva of the primitive frogs appear closer to the state in adults. As *Notobatrachus* is the most primitive frog species, could it be possible that *Notobatrachus* tadpole may have some features even closer to the adult state?

This is possibility that this *Notobatrachus* tadpole was one which delayed its metamorphosis due to some environmental factor or intrinsic factor. Such delay may result in early development of some bones in spite of the premetamorphic stage. Could this possibility be ruled out? This may be related to the gigantism of the *Notobatrachus* tadpole.

3. The phylogenetic and morphological disparity analyses were conducted, in my opinion, in a routine and proper way, but I am not familiar with the detail of the morphological disparity analyses and therefore feel unable to fully assess.

As the *Notobatrachus* tadpole is the only fossil tadpole in the phylogenetic matrix, it seems a bit redundant to compare the constrained and unconstrained analyses (especially if the molecular backbone involves most of the extant species in the matrix). It would be interesting to include some of the well-studied fossil tadpoles of extinct species, such as *Paleobatrachus* tadpole from the Oligocene of Czech (Roček 2003), *Thoraciliacus rostriceps* and *Shomronella jordanica* (Roček & Van Dijk, 2006) from the Early Cretaceous, and to see where these fossil tadpoles are in the phylomorphospace.

4. The two datasets assembled for estimating the total length of the *Notobatrachus degiustioi* tadpoles and evaluating the larva and adult body sizes of the sampled frog species respectively, are reasonably large and covering various groups of Anura, although technically the sample is still small compared with the large number of anuran species. The gigantism of both *Notobatrachus* tadpole and adult is verified.

There are also some subtle issues I noticed when reading the ms and listed below.

Page 4, Line 91: 'hyobranchial' is misspelled as 'hyobranquial'.

Page 14, Figure 1: 'n' is used twice for different structures (nasal VS neurocranium). Different abbreviations should be used.

Page 18, Line 399: ('treeSearches_protocol_Notobatrachus_unconstrained.run' and 'treeSearches_protocol_Notobatrachus_unconstrained.run'), of which one should be 'treeSearches_protocol_Notobatrachus_constrained.run'

Page 21, Line 447: the giant adults are those having SVL higher than 127 mm.

SI, Line 43 'Eosolimnadiopsis? santacrucensis': the initial letter of the species name should be lowercase.

References

Haas, A. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19, 23–89 (2003).

Kerney, R., Wassersug, R., & Hall, B. K. (2010). Skeletal advance and arrest in giant non-metamorphosing African clawed frog tadpoles (*Xenopus laevis*: Daudin). *Journal of anatomy*, 216(1), 132-143. <https://doi.org/10.1111/j.1469-7580.2009.01176.x>

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Roček, Z. & Van Dijk, E. Patterns of larval development in Cretaceous pipid frogs. *Acta Palaeontol. Pol.* 51, 111–126 (2006).

Schlosser, G. & Roth, G. Distribution of cranial and rostral spinal nerves in tadpoles of the frog *Discoglossus pictus* (Discoglossidae). *J. Morphol.* 226, 189–212 (1995).

Wassersug, R.J. & Hoff, K. Developmental changes in the orientation of the anuran jaw suspension: a preliminary exploration into the evolution of metamorphosis. *Evol. Biol.* 15, 223–246 (1982).

Referee #4 (Remarks to the Author):

This is an important report on the earliest known anuran tadpole, previously unnoticed in the palaeontological collections of a regional museum in a remote region of southern Argentina. As such, this information will be of interest to a wide range of biologists. The illustrations are well done, although minor errors should be corrected.

Please check the size and therefore legibility of the text in Figure 3, especially the names of the taxa. Depending on the final size of this figure, some of its informational value may be lost.

Further remarks

Line 22: "Anurans are characterised by a unique biphasic life cycle".

An aquatic larval stage followed by a terrestrial adult stage also occurs in caudate amphibians and non-neotenic temnospondyls, so the term "unique" seems overstated (see also line 41).

Line 25: "Modern tadpoles"

I would recommend using "recent" instead of "modern".

Lines 28-29: "... anuran fossil record dating back to the Early Jurassic".

The earliest known fossil frogs are from the Late Triassic Chinle Formation (213-223 Mya) of Arizona, USA (Stocker et al. 2019).

Line 29: "late larva".

It might be better to use "advanced larva" (compare line 78).

Lines 47-48: "Larval characters may have evolved during the basal anuran radiation around the Triassic-Jurassic boundary"

The Late Triassic frogs from Texas (Stocker et al. 2019) with typical anuran ilia and probably also maxilla are derived from adults that had to be ontogenetically preceded by an anuran tadpole, not by a temnospondyl larva. Thus, anuran larval characters had to evolve earlier than around the Triassic-Jurassic boundary.

Line 50: "salamander larvae"

Do you mean the larvae of the genus *Salamandra* or salamandrid larvae?

Line 64: "Museo Provincial Padre M.J. Molina"

Add where the museum is located: in Rio Gallegos.

Line 67: "from the same quarry"

Be more precise: "from the Cañadón de Estancia La Matilde type locality near Puerto Deseado (La Matilde Formation)".

Line 72: Here you can remove: "(La Matilde Formation)".

Lines 91, 123, 129: hyobranchial" instead of "hyobranquial".

Line 96: "Ceratobranchialia are preserved as four black ellipsoidal bands arranged in an anteroposteriorly oriented row".

This can be misleading, because the dark stripes represent cartilaginous ceratobranchials, and the ellipsoid fields between them (especially the one between ceratobranchials III and IV) are branchial slits (cf. Fig. 2a). I would suggest: The ceratobranchials are preserved as four dark mediolaterally oriented bands with light ellipsoid fields between them representing branchial slits.

Line 144: "by far the largest Mesozoic anuran".

Please include: "so far known", because there are some fragmentary ilia (e.g. from the Late Cretaceous of Utah; Roček et al. 2010, fig. 11v) indicating the presence of other giant anurans in the Mesozoic.

Note to lines 158-161:

The anatomical transformation of the temnospondyl larva into the anuran tadpole is one of the key events in the evolutionary history of amphibians, because this event (long and gradual) represents the origin of frogs. Assuming that the appearance of tadpoles and adult frogs was a simultaneous process (as suggested by developmental processes in the skull and pelvic girdle of extant anurans), the presence of adult anurans in the Late Triassic suggests that tadpoles also existed at that time. Thus, the origin of both should be expected earlier than the Late Triassic.

Figure 1. fo should be marked with an arrow because the fenestra ovalis is not visible in the dorsal view. Also, the dark lateral extension of the otic capsule cannot be the fenestra ovalis, but rather the cartilaginous part of the crista parotica (as suggested by the same colour as the ceratohyal and the branchial basket).

Also remove the n representing the neurocranium and retain only the n representing the nasals. The posterior part of the neurocranium is not well preserved and is usually represented by the gap between the two otic capsules bridged by the cartilaginous tectum synoticum. The fontanella between the two frontoparietals and the gap between the two otic capsules undoubtedly reached the bottom of the braincase (i.e. the parasphenoid).

Figure 2.

Lines 328-329: pl processus lateralis of ceratohyal is not shown in Fig. 2b.

Line 329: pph should be complete as in Fig. 2b.

Figure 3. Please check the legibility of the text, especially the names of taxa.

Comment not directly related to the submitted text:

As this manuscript is an important report on the earliest known anuran tadpole, I would like to draw your attention to a disarticulated specimen CPBA-V-14003, which can also be attributed to *Notobatrachus* (see Báez and Nicoli 2004). It also comes from the type locality of *Notobatrachus* mentioned in the text above and is deposited in the collections of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, thus easily accessible to you.

Judging by the characters listed below, it may be of the similar ontogenetic age to the advanced larva described in the manuscript. These characters are:

- The epiphyses of the long bones are cartilaginous (not preserved = not ossified);
- Only the proximal part of the caput humeri is ossified;
- The ossified part of the iliac shaft is short (as in Fig. 1 above);
- Vertebral centra probably still separated from neural arches, but fused with transverse processes;
- Neural arches not yet fused dorsally;
- Only proximal part of ribs ossified;
- Frontoparietals in brief contact posteriorly, but separated anteriorly by a long fontanelle;
- Radioulna and tibiofibula both completely fused (as in Fig. 2);

I think it is worth mentioning this specimen in the present manuscript, at the cost of only moderately expanding the text.

Author Rebuttals to Initial Comments:

Please, find below detailed comments on each point raised by the referees (referee's comments in bold and italics).

REFEREE #1

The manuscript entitled "THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE ANURAN LIFE CYCLE" by Chuliver et al. describes the thus far oldest record of an anuran tadpole in the fossil record, specifically the mid-late Jurassic of Patagonia. This find is remarkable and interesting as the highly specialized and derived life history of anurans is in this extent unique amongst tetrapods and alongside the evolution of the derived body plan of anurans is thought to have played a pivotal role in the radiation and evolutionary success of frogs that began in the Mesozoic. Previously, the earliest known tadpoles were known from the Cretaceous, while adult anurans are comparatively well-represented as early as the early Jurassic.

*Based on distinct morphological characters, the authors assign the tadpole to the stem anuran species *Notobatrachus degiustoi*, which is well known from adult specimens from the same locality. Their assessment is also supported by their careful phylogenetic analysis and I have no reservations against this taxonomic assignment. While I think that this is an important and exciting find, I feel that the authors in the current form of the manuscript leave some important questions unanswered and undiscussed, which should be considered in the context of this find in a revised version of the study before considering its publication:*

*1. The authors report that *Notobatrachus* is well represented at the locality by a large number of specimens that have been recovered at the site since the 1950s. Yet, despite considerable collection effort at the site, the described tadpole is the only pre- metamorphic specimen from this locality? Why is that? If the lacustrine deposits represent an ecosystem that was used for breeding/egg deposition and this is where tadpoles lived, why were there no other tadpoles found at the locality? It also remains unclear in the text and supplementary information, if the specimen derived from the same horizon as the adult specimens or from a different horizon, hence possibly representing a slightly different time slice and/or sampling a different habitat than the layers that yielded the adult specimens. This needs to be clarified and explained.*

Tadpoles are delicate organisms with few or no ossified parts depending on their ontogenetic stage, and therefore, their preservation potential is very low. This is true even in renowned fossil sites with exceptional preservation and huge sampling efforts. A good example of that is the Messel lagerstätte, where many

postmetamorphic specimens of three different anuran species were recovered but only two tadpoles were discovered in more than forty years of continuous fieldwork (Wuttke, 2018). Despite autoecological or taphonomical filters that we have not identified yet, we speculate there is a sampling bias that explains the high number of adult (postmetamorphic) individuals of *Notobatrachus degiustoi* in contrast to the number of tadpoles discovered. This is due to the fact that rocks in the active front of the quarry of the Estancia La Matilde locality are more brittle and then easier to slice than rocks that are some centimeters into the bedrock. Thus, postmetamorphic *N. degiustoi* bones (their impressions actually) are identifiable as holes in the active front of the quarry, and people working on the quarry only slice those slabs containing the impressions, usually setting aside the rest of the rocks. Not surprisingly, most postmetamorphic individuals are large adults, probably because impressions of young postmetamorphics are less conspicuous. Despite we consider this conjecture a plausible explanation for the imbalance between pre- and postmetamorphic individuals in this fossil locality, it must be confirmed through other lines of evidence, and therefore we prefer to avoid discussing all these conjectures in the present study.

Most specimens of *Notobatrachus degiustoi*, including the type series and the tadpole described in this manuscript, were found in a quarry located in “Estancia La Matilde”, a farm located 100 km SW to Puerto Deseado city (Santa Cruz Province, Argentina), in rocks exposed near the main building of the farm. This fossil site was named as “Estancia La Matilde locality” and specimens were collected from the “fossiliferous level I” as originally described by Stipanovic and Reig (1957). We modified the text in the Supplementary Information accordingly in order to clarify this point, as suggested by referee 1.

Stipanovic, P.N. and Reig, O.A. 1957. El “complejo porfírico de la Patagonia Extraandina” y su fauna de anuros. *Acta Geológica Lilloana* 1: 185-298.
Wuttke M. 2018. Amphibians in Messel – in the Water and on Land. In: Messel-An Ancient Greenhouse Ecosystem, K.T. Smith, S. Schaal, y J. Habersetzer, J. (eds.). Frankfurt am Main: Senckenberg, pp. 113-120.

2. If the tadpole derived from the same horizon as the adult specimens, the biological reasons for this specimen being the only tadpole amongst hundreds of adults need to be explored in the manuscript. In this context the supposedly giant size of the tadpole might be an important clue. Incidences in which individual tadpoles fail to metamorphose, but instead grow to giant size are well known from several frog taxa (several species of Rana, but also from Ascaphus, the latter also often becoming cannibalistic). While these individuals are exceptions, they are not entirely uncommon and these giant tadpoles also

often explore other regions of water bodies than their normally-sized larval conspecifics, which could explain why this specimen is the only tadpole found amongst adults.

As was pointed out above, the scarcity of tadpoles in relation to postmetamorphic individuals seems to be common in other fossil sites, probably due to an interplay of different taphonomic filters, including autoecological traits and biostratinomic processes. However, we consider that the fact of being the only tadpole so far known is mainly due to a bias of the sampling methods. Hypothesizing that this specimen is an abnormal tadpole that failed to metamorphose and due to its large size is able to explore different areas of the pond implies too many *ad hoc* hypotheses and, therefore, is unlikely. Detailed arguments that justify why the tadpole of *Notobatrachus degiustoi* is a normal giant tadpole are given below.

3. The point above is also critical in the line of argumentation regarding the tadpoles of Notobatrachus being gigantic. I think this find cannot simply be considered a normal larval specimen, unless more tadpoles that size are found that could document that this was indeed the normal tadpole size for this taxon. The authors also state that the giant size of this tadpole indicates that tadpole gigantism evolved in stem anurans. For the reasons outlined above I think one has to be very careful with this assumption. Moreover, even if further finds should show that this taxon indeed had giant tadpoles as a rule, this could not demonstrate that gigantism in tadpoles evolved in stem anurans as it could alternatively indicate that stem anurans simply had large tadpoles initially and the much smaller larval size evolved secondarily in the crown group, possibly in concert with an even more condensed, specialized metamorphosis and the constraints the latter imposes on body size, cell cycles etc.

Regarding the size of the tadpole described in the manuscript, we consider that *Notobatrachus degiustoi* had normal giant tadpoles that naturally occurred and successfully metamorphosed. For those extant anuran species showing isolated cases of gigantism these are usually pathological, and more importantly they develop only up to Gosner Stages 34–37, ceasing development well before metamorphosis (e.g., Bolek and Janovy, 2004; Rot-Nikcevic and Wassersug, 2004; Bovero and Delmastro, 2009; Milto, 2009). On the other hand, frog species with normal giant tadpoles (e.g. *Pseudis* spp.) possess large sizes along their entire ontogenetic trajectory. In this line, the late developmental prometamorphic stage (Gosner Stage 40–41) of the *N. degiustoi* tadpole indicates it was close to metamorphose, thus allowing us to discard a pathological (e.g. athyroid) condition to explain gigantism. Further specimens will be necessary to explore causes

explaining this gigantism, such as “overwintering strategy” or other environmental factors.

The record of gigantism in *N. degiustoi* is noteworthy because this trait was only documented in few extant (e.g. *Pelobates spp.*, *Pseudis spp.*) and extinct (e.g. *Palaeobatrachus hauffianus*) crown anuran species. We agree with referee 1 that tadpole gigantism could represent a plesiomorphic character state for stem anurans. In order to test this hypothesis we optimized in the phylogeny the residuals of a linear regression between the tadpole total length and adult snout-vent length. The result shows that the larval gigantism recorded in *N. degiustoi* has been independently acquired with respect to those found in crown anurans. In order to avoid misinterpretations about the evolutionary origin of this trait we rewrote the corresponding paragraphs about gigantism and explicitly reported the results of the residuals optimization.

Bolek MG and J Janovy Jr. 2004. *Rana catesbeiana* (Bullfrog). Gigantic tadpole.

Herpetological Review 35:376-377.

Bovero S and GB Delmastro. 2009. Casi di gigantismo nel girino di rane piemontesi (Amphibia, Anura, Ranidae) RIV. PIEM. ST. NAT., 30: 193-208.

Milto KD. 2009. A giant tadpole record of *Rana esculenta* in northwestern Russia. Russian Journal of Herpetology Vol. 16: 143 – 145.

Rot-Nikcevic I., Wassersug R. J. 2004. Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis. The Journal of Experimental Biology 207, Nr. 12. P. 2133—2145.

4. Since this paper focuses on (amphibian) metamorphosis as a core subject, the authors need to phrase more carefully what they mean with metamorphosis and a biphasic life cycle and clarify what they mean with “gradual changes” in this context. Arguably, anuran metamorphosis is the most extreme amongst extant tetrapods and tadpoles represent a highly derived larval stage. Nevertheless, salamanders and in specialized ways caecilians undergo a distinct metamorphosis that involves significant physiological and morphological changes. Moreover, there is a large number of salamander taxa that most definitively undergo a change of habitat, feeding mode, respiration and locomotion between larval and adult stage and therefore the opening sentence following the abstract is incorrect. While metamorphosis is often considered the classic life cycle of amphibians, amphibian life histories are enormously diverse and highly derived in all three groups of lissamphibians and there are gradations of metamorphosis in all three modern clades, e.g. some neotenic salamanders still undergo quite significant physiological and morphological metamorphic changes, even though they never switch their habitat, while other

taxa have a clear-cut, gill breathing larval stage that metamorphoses into a lung-breathing fully terrestrial adult within 3-4 weeks. Similarly, by far not all frogs have free swimming, aquatic tadpoles that metamorphose into terrestrial adult frogs, but show an impressive range of specializations as well. I do not mean to say that the entire breadth of life history patterns in lissamphibians has to be discussed in detail in the context of the paper, but I think the use of terminology -metamorphosis vs. "gradual change" - has to be defined and clarified more rigorously here and a bit more credit should be given to the evolutionary complexity of this phenomenon (see also point below).

We employed the term "gradual change" in order to emphasize the differences between the metamorphosis experimented by anurans and that of other lissamphibian groups (salamanders, caecilians), being the former very recently referred to a "drastic metamorphosis" (Schoch and Witzmann, 2024). We agree with referee 1 about the flaws of using "gradual changes" when referring to metamorphosis of salamanders and temnospondyls. Accordingly, we defined metamorphosis in order to avoid vague terminology, and removed the term "gradual changes".

Schoch RR and F Witzmann. 2024. The evolution of larvae in temnospondyls and the stepwise origin of amphibian metamorphosis. Biol. Rev. doi: 10.1111/brv.13084

5. The evolution of early tetrapod life history patterns generally and a lissamphibian-like metamorphosis more specifically has been researched quite extensively, most notably by Rainer Schoch and colleagues and the statement that temnospondyl development progressed without major changes of habitat, morphology or ecology is incorrect. For many temnospondyl taxa, metamorphic changes were more gradual, more drawn out and less condensed in terms of time than in lissamphibians, but this is not the same as no morphological changes or changes in habitat happening. Moreover, amphibamiforms have convincingly been demonstrated to have had a very lissamphibian-like metamorphosis and the evolutionary groundwork for the extreme life history patterns as seen in lissamphibians, was likely laid down in this stem lineage of the modern forms. The authors cite a paper by Schoch 2002, but research on this topic has advanced significantly since then. Depending on how the current manuscript is framed, this data might not be crucial in great detail. However, if the evolutionary history of the extreme anuran life history is included here or commented on, these more recent data should certainly be considered. Generally, I think that the authors present a fascinating and very interesting find that has important implications for understanding the timing and evolution

of the extreme anuran larval stage. Nevertheless I feel that in the current stage, some important questions are not sufficiently explored, which should be addressed in more detail.

We agree with the referee and, as a result, we modified the statement about temnospondyl development and also added novel literature about amphibian metamorphosis. A recently published work about the larval development and metamorphosis in temnospondyls (Schoch and Witzmann, 2024) has been particularly useful to clarify the points exposed by referee 1. Thus, we modified the first paragraph of the main text in order to address the comment of the reviewer.

Schoch RR and F Witzmann. 2024. The evolution of larvae in temnospondyls and the stepwise origin of amphibian metamorphosis. Biol. Rev. doi: 10.1111/brv.13084

REFEREE #2

*Among the many unique features of salientians (frogs) is their primitive possession of a free-living tadpole (larval stage) that is dramatically different morphologically and ecologically from the adult (frog) stage. Studies of extant (i.e., crown clade) frogs agree that tadpoles are primitive for frogs and have led to constrained speculation about what the primitive or ancestral tadpole morph might have looked like and how it lived. Although tadpoles have a modest fossil record, to date that record only extends back to the Early Cretaceous (vs. modern aspect frogs appeared considerably earlier by at least the Early Jurassic) and all described fossil tadpoles are referable to crown-clade frogs. As such, the fossil record provides no direct evidence about the morphology and ecology of tadpoles belonging to the earliest frogs. This new report by Chuliver et al. of a tadpole dating to the Middle-Late Jurassic boundary and belonging to the stem anuran *Notobatrachus degiustoi* provides new and exciting insights into the morphology and ecology of stem anuran tadpoles. This report is interesting and significant enough to warrant publishing in a high-profile journal such as *Nature*. For reasons outlined below, I recommend this MS be accepted pending minor revisions.*

*I have no concerns with the authors' identification of the fossil or their interpretations of its structures. The amount of detail presented in the main text is adequate for *Nature* and, for those of us who crave more, additional information is presented in the Extended Data Figures and Supplemental Information. Although I am not an expert in phylogenetic analyses of tadpoles or morphometric analyses, the methodologies for those portions of the MS seem reasonable to me. The descriptions and analyses are complemented by informative figures (but see my comments on how those could be improved). The Abstract adequately summarizes the significance and key findings of the report and the References are comprehensive.*

All of the suggestions made by referee 2 in his hand-annotated copy of the main text, figures, and methods, plus the Extended Data Figures were addressed and modified accordingly.

Below I summarize my most notable points about the MS. Some of these elaborate on comments, questions, and suggested changes that I made in my annotated copy of the MS. All my points are minor, and the authors should have no trouble dealing with them.

1) I like how the authors propose the first (and easy-to-understand) definition for "giant tadpoles" as those tadpoles having a total length greater than ca. 105

mm. Their definition seems reasonable to me. If nothing else, that definition provides an absolute value, which is preferable to the vague and unstated use of “giant” in a relative sense, for tadpoles that are somewhat larger (but how much is never stated) than is typical for a given species.

We appreciate this comment. This was a contentious aspect in studies of extant anurans based on the ambiguous use of the term, therefore we followed the definition of Womack & Bell (2020) for adult anurans in their broad-scale analysis of anuran body-size evolution.

Womack, M.C. & Bell, R.C. Two-hundred million years of anuran body-size evolution in relation to geography, ecology and life history. *J. Evolution. Biol.* 33, 1417–1432 (2020).

2) I suggest the authors also clearly indicate the sense in which they are using the term “giant”. From my perspective, there are two kinds of giant tadpoles. The first are naturally occurring giants that successfully metamorphose (e.g., the huge tadpoles of the extant Paradoxical Frog and tadpoles at the upper end of the normal size range for their species, such as the extant American Bullfrog). The other kind of giant tadpoles are abnormal ones (what Rot-Nikcevic & Wassersug, 2004 called “giant, non-metamorphosing tadpoles”) that cannot metamorphose and occur sporadically in some species (e.g., laboratory-raised African Clawed Frogs: see Rot-Nikcevic & Wassersug, 2004; Kerney et al. 2009). At least one species, the extant American Bullfrog, is known in the wild to have both naturally occurring giant tadpoles (e.g., Altig & McDiarmid, 2015) and rare, abnormal giants (e.g., Schooley et al. 2008; Burke 2018). A critical distinction between those two kinds of giant tadpoles is whether they can successfully metamorphose—that has implications for the significance of the large size of the sole *Notobatrachus degiustoi* tadpole. If your fossil tadpole is a non-metamorphosing giant, then your argument that *N. degiustoi* is a rare example of an anuran with both giant tadpoles and adults collapses. The challenge with fossil examples of giant tadpoles, such as the single reported tadpole of *N. degiustoi*, as well as rare examples of giant fossil tadpoles reported for palaeobatrachids (Miocene of Germany: Roček et al. 2006) and pelobatids (Miocene of Spain: BUSTILLO et al. 2017, 2019, TALAVERA et al. 2019; Miocene of Bulgaria: VERGILOV & TZANKOV 2021; Eocene of Germany: WUTTKE 1996, ROČEK & WUTTKE 2010, WUTTKE & ROČEK 2010, WUTTKE et al. 2022) is determining whether the individual was capable of metamorphosing and, thus, was either a normal giant or an abnormal giant. This can also be a problem for extant tadpoles: e.g., the giant, pre-metamorphic tadpole of the American

Bullfrog reported by Bolek & Janovy (2004), which died soon after it was captured and before it potentially reached the stage where the onset of metamorphosis could be detected. At least one of the palaeobatrachid tadpoles reported by Roček et al. (2006) and the Spanish pelobatid (BUSTILLO et al. 2017, 2019, TALAVERA et al. 2019) preserve rudimentary forearms, indicating they were well along in metamorphosis at the time of their deaths. That also appears to be the case with the N. degiustoi tadpole, which means you can legitimately argue it is a normal, giant tadpole. That point deserves to be emphasized in the MS. [References cited herein are at the end of my report.]

We added the reference to the normal condition of the *Notobatrachus degiustoi* tadpole into the main text and thoroughly addressed these observations based on comments and suggestions made by referee 1 (see above).

3) I am unclear on the geologic age of the Notobatrachus degiustoi tadpole. The statement (lines 29–30) “Here we report a late larva of the stem-anuran Notobatrachus degiustoi from the Middle-Late Jurassic of Patagonia (ca. 166–157 Mya) implies the tadpole is Middle–Late Jurassic in age. Yet on the next page is the statement (lines 55–56) “Here we describe the oldest-known tadpole from the Middle Jurassic La Matilde Formation (Callovian-Oxfordian; ca. 166–157 Mya)”—note here the age is give only as Middle Jurassic, yet both the reported stages (Callovian and Oxfordian) and absolute age range (ca. 166–157 Mya) fall within the late Early–early Middle Jurassic (i.e., more similar to what you reported in the Abstract). The geologic age is even less clear in the Supplemental Information, where (lines 29–37) an even broader range of Bathonian (late Middle Jurassic) to Valanginian (early Early Cretaceous) is reported. I realize age estimations can be imprecise, but considering you are describing the geologically oldest known tadpole, it is important to report its age as precisely and consistently as possible. If there is doubt about the precise age of the tadpole-bearing rocks, explicitly acknowledge that situation and then tell us which age or age range you prefer and why. Perhaps it is most conservative to simply regard its age as being around the Middle-Late Jurassic boundary?

We agree with referee 2 and modified the main text and the Supplementary Information to properly clarify that the age of the tadpole is Middle Jurassic (Bathonian-Callovian; ca. 168–161 Mya), which is the age of deposition of the La Matilde Formation.

4) I also am unclear what locality yielded the Notobatrachus degiustoi tadpole. In two places on page 3 (lines 57–59 and lines 66–67) you vaguely state that the

tadpole was collected from “the same locality/quarry” that yielded fossils of metamorphosed individuals of the N. degiustoi. Yet when I look at your cited references, it is clear that metamorphosed individuals of N. degiustoi have been collected from at least five localities (see especially the helpful list provided by Báez & Nicoli, 2004: appendix 1). That leaves me wondering which of those five localities yielded the tadpole? The caption for Extended Data Figure 1 states “... in the same levels w[h]ere Notobatrachus[insert space]degiustoi specimens are found in [the] La Matilde locality”, which implies that the tadpole was found at the “Cañadón de Estancia La Matilde” locality (sensu Báez & Nicoli, 2004: appendix 1). However, in the Supplemental Information (lines 21–22) we are told the tadpole “was found in a quarry close to [my emphasis] the “Estancia La Matilde”, a Jurassic locality”. If the tadpole was found in a quarry close to “Estancia La Matilde” (presumably equivalent to “Cañadón de Estancia La Matilde”), what is the name of the actual tadpole quarry? Is it the locality listed by Báez & Nicoli (2004: appendix 1) as “slopes west of Cañadón de Estancia La Matilde” or another locality? Please be precise and consistent in how you report the tadpole locality. Assuming it is one of the localities listed by Báez & Nicoli (2004: appendix 1), for consistency and to make it easier for those of us not familiar with variants of locality names within the La Matilde Formation, I recommend you use their version of the locality name. If the tadpole locality is not one of the localities listed by Báez & Nicoli (2004: appendix 1), that should be clearly explained.

The fossil specimen was found in a quarry of the Estancia La Matilde (type locality), about 110 km SW of Puerto Deseado, Santa Cruz Province, Argentina. The La Matilde Formation (fossiliferous level I, section by Stipanivic and Reig, 1957) crops out in this place. We modified both the main text and the Supplementary Information in order to address this comment.

Stipanivic, P.N. and Reig, O.A. 1957. El “complejo porfírico de la Patagonia Extraandina” y su fauna de anuros. *Acta Geológica Lilloana* 1: 185-298.

5) Throughout the MS, the fossil tadpole is variously identified as belonging to Notobatrachus degiustoi (e.g., Abstract line 29) or Notobatrachus (e.g., Abstract line 34). It is inaccurate to use only the genus name when referring to the tadpole, because two species currently are recognized for Notobatrachus (the type species N. degiustoi and a second species N. reigi), yet a tadpole is known only for N. degiustoi. In all cases, the tadpole should be identified as Notobatrachus degiustoi (first appearance in each paragraph) or N. degiustoi subsequent appearances in the same paragraph).

We modified the text following the referee's suggestion and identified the fossil tadpole as *Notobatrachus degiustoi* when it appears for the first time in a paragraph or as *N. degiustoi* in its second and subsequent appearances.

6) The fossil tadpole is referred to *Notobatrachus degiustoi* based on two lines of evidence (lines 66–72): a suite of osteological features it shares with metamorphosed individuals of that species and its co-occurrence in the quarry with “hundreds of adult specimens of *Notobatrachus degiustoi*”. I agree with that justification. Another line of evidence worth noting is that no other anuran species have been identified (to my knowledge) from the La Matilde Formation.

We agree on this observation and we explicitly specified the absence of other anuran species in the formation for clarification.

7) The ontogenetic age of the tadpole is estimated at Gosner stages 40–41. That seems reasonable considering the relative development of its skeleton. I am puzzled why the evidence for that ontogenetic age estimation is partitioned. In the main text (lines 87–88) the only feature listed to support your ontogenetic age estimate is “the forelimb is still contained within the body contour, likely covered by the operculum” whereas in the Supplemental Information (lines 135–147) a suite of other osteological features is listed, yet the position of the forelimb is omitted. I suggest you include the position of the forelimb in the Supplemental Information and, in the main text, qualify that the position of the forelimb is one of many features (which are listed in full in the Supplemental Information) that support your ontogenetic age estimate.

We addressed this issue by including a sentence in the main text clarifying that the position of the forelimb and osteological features were both used for staging the specimen. Likewise, we added the reference to the position of the forelimb in the section “(2) Additional anatomical information of the Supplementary Information”.

8) Specimen MPM-PV 23333 usually is identified as a tadpole (as in the title), yet in numerous places throughout the MS (main text, Extended Data Fig. 9 caption, and Supplemental Information) the terms “larva”, “larvae”, and “larval” are used. For consistency, I recommend you use only “tadpole”/“tadpoles” where referring to anuran larvae.

We agree on this observation and replaced the pointed terms using 'tadpole' or 'tadpoles' where appropriate and whenever possible. However, in many sentences

of the main text the word larva has been used in order to avoid odd constructions and excessive repetition of the word tadpole.

9) In the text (line 144) you imply that large adults of *Notobatrachus degiustoi*, with a stated SVL of 150 mm, are “by far the largest Mesozoic anurans”. I do not believe that is correct. In their re-description of the hyperossified, crown anuran *Beelzebufo ampinga* from the Maastrichtian of Madagascar, Evans et al. (2014: S1 text, “Size Estimation”) estimated the maximum SVL of *B. ampinga* as exceeding 300 mm; in other words, twice the size of *N. degiustoi*. To my knowledge, *N. degiustoi* and *B. ampinga* are the largest Mesozoic frogs for which size can be directly measured or reasonably estimated, respectively. I am not aware of any other Mesozoic frogs of comparable size. You can legitimately state that *N. degiustoi* is the largest stem anuran, but not the largest Mesozoic anuran.

We modified the text following the referee’s suggestion and added the size information (body length) for adults of twenty-four Mesozoic anuran species to Supplementary Table S1 in order to clearly show the size differences among fossil taxa.

10) Comments on figures in main text:

- a. I like how the photographs in Figs. 1 & 2 are complemented by colored, interpretive drawings. That combination of images nicely complements the descriptive text and makes it easy to see the skeletal and soft features of the tadpole.**
- b. Those same two figures (Figs. 1 & 2) are adequate for the abbreviated description in the main text, yet as I noted in my annotated copy several features (mostly of the branchial basket) mentioned in the expanded description within the Supplemental Information are not labelled in the figures. I urge the authors to ensure that all mentioned features in the expanded description are clearly labelled to ensure readers can see the described features—that could be done by adding additional figures (e.g., enlarged version of the cranial closeup interpretive drawing) into the Supplemental Information.**
- c. For your photographs, the descriptive passages “low exposure under flush light” (Fig 1 caption) and “high exposure under low vertical light” (Figs 1 & 2 captions) are unclear. For “low exposure” and “high exposure” do you mean you used a short or long exposure time with your camera when you took the photograph or that in Photoshop (or other image editing software) you manipulated the image by changing the exposure to the low or high end of the range? For “flush light” do you mean low angle light? For “low vertical light” do**

you mean soft or muted, high angle light? Please clarify what you mean, so readers are not left guessing.

d. Ensure you use unique abbreviations for each feature. In Fig. 1, the abbreviation “n” is used for both nasal and neurocranium.

e. Ensure all listed abbreviations appear in the corresponding figure and vice versa. In Fig. 2, the listed abbreviations “oc”, “pl”, and pp” are not labelled in the corresponding figure, whereas the structure labelled as “pph” is not defined in the caption.

f. Ensure all abbreviations are listed alphabetically, as follows: all letter abbreviations, then all Roman numeral abbreviations, and finally all Arabic number abbreviations. In Fig. 1 caption the abbreviations “rb” and “up” are out of sequence, whereas in Fig. 2 caption the abbreviations “pl”, “pp”, and “ru” are out of sequence.

g. For both Fig. 1 and 2 captions, your title sentence begins as “Overview and interpretive drawing ...”. What you call an “overview” is a photograph, so call it that. Write “Photographs and interpretive drawing ...” (Fig. 1) and “Photograph and interpretive drawing ...” (Fig. 2).

*h. For Fig. 3b, the dark pink point plotted for the tadpole of *Notobatrachus degiustoi* is challenging to see against the dark pink background. I suggest making that point stand out more, perhaps by having a black arrow pointing at it.*

*i. For Fig. 3c, in the upper right quadrant, two points are plotted. The point for *Notobatrachus degiustoi* is labelled, whereas the plot for *Aquarana catesbeiana* is not. For consistency and to reinforce the point that those are the only two anuran species having both giant tadpoles and adults, both points deserve to be labelled.*

b. We labelled all the features described or mentioned in the Supplementary Information in Figures 1 and 2 of the Main Text. We did not add extra figures, such as a closeup of the cranial region and its interpretative drawing, in order to avoid redundancy or repetitiveness of figures and drawings.

c. Regarding Figures 1 and 2 captions we modified the text according to referee’s suggestion. For “low exposure” and “high exposure” we meant short and long exposure time with the photographic camera. Likewise, for “flush light” we meant low angle light, while for “vertical light” we meant high angle light. In this sense, we modified the text accordingly.

d. Abbreviation for nasals was kept as “n”, while abbreviation for neurocranium was changed for “cn” referring to chondrified neurocranium.

e. All listed abbreviations were included in the figure and vice versa.

f. All listed abbreviations were alphabetically ordered.

- g. Titles for both figures were modified following the referee's suggestion.
- h. We changed the color of the dot for a more contrasting light blue color, and also added an arrowhead to indicate the position of the *Notobatrachus degiustoi* tadpole in the phylomorphospace.
- i. We labelled the dot corresponding to *Aquarana catesbeiana* in the Figure 3c.

11) Extended Data Figures:

- a. Fig. 1—See my notes about the caption and figure in the annotated copy.**
- b. Figs. 2 & 3—These appear to be larger sized images of the same photographs presented earlier as main text Fig. 1b and 1a, respectively. Why are essentially the same photographs repeated here? If you wish to retain these larger photographs, I urge you to (1) better explain the parameters for the photographs (currently vaguely described as “taken with short exposure” in Fig. 2 caption and “taken with superficial light” in Fig. 3) and ideally use the same wording as I suggested above (see point 10c) for the main text photographs and (2) include scale bars for both photographs.**
- c. Fig. 4—Here in the caption you use XI, XII, and XIII for the three presacral neural arches, but in main text Fig. 1 you use 1, 2, and 3 for those structures. For consistency, do the same here. In the caption: (1) state the images are photographs; (2) provide parameters for those photographs (presumably taken with low angle light to enhance details); (3) replace “specimens” (plural) with “specimen” (singular); (4) delete “I–XIII, vertebrae”; and (5) as I recommended for main text Fig. 1 caption, at the end of the abbreviation list add “I–IX, presacral vertebrae; X, sacrum; 1–3, postsacral neural arches”.**
- d. Figs. 5 & 6—In captions define the dashed grey, vertical lines. Presumably those denote the Permo-Triassic (left) and Cretaceous-Palaeogene (right) boundaries?**
- e. Figs. 7 & 8—In captions define the pair of values separated by a forward slash at each node.**
- f. Fig. 9—In the figure, (1) names of species with giant tadpoles should be italicized in Fig. 9b (as in your other figures) and (2) add “(mm)” behind “total length” (Fig. 9a & b) and “body length” (Fig. 9a) labels on the Y and X axes. In the caption, the phrase “double the media” is nonsensical. For clarity and consistency, it better to use the same wording as in the main text (line 440) and instead state “twice the mean”.**

- a. The figure caption was modified according to the text in the Supplementary Information following the referee's observation.
- b. The aim of the Extended Data Figures 2 and 3 is to provide high definition images of the specimen without labeling any structure, so that the reader can appreciate in

detail different aspects of the anatomy of the tadpole under two different light settings. Therefore, we prefer not to add labels of the mentioned features in these supplementary images. We modified the figure caption according to the referee's observation in order to improve the explanation of the parameters used to take these photographs and using the same terminology as for the figures in the main text.

c. We modified the labelling of the postsacral neural arches in the Extended Data Figure 4 for consistency with figures in the main text, and we modified the figure caption according to the referee's suggestions.

d. Dotted grey vertical lines indicate the boundaries between the Permian and Triassic and Cretaceous and Palaeogene geological periods, respectively. We added this information to the figure captions.

e. Numbers at nodes indicate absolute (left) and GC (group present/contradicted) (right) no-zero weight symmetric resampling frequencies (values between squared brackets are negative values). We added this information to the figure captions.

f. Names of the species were changed and (mm) was added in Extended Data Figure 9. As the figure was modified, section (a) was removed from the new Extended Data Figure 9. The figure caption was also modified according to the reviewer's comment and to changes made to figure.

12) Supplemental Information text: As is typical for supplemental text, the writing in this section is far less polished than the main text. Please see my annotated copy of the MS for numerous questions, corrections, and suggestions for improving the clarity and consistency of the supplemental text. See also comments raised in my previous points. I will reiterate that you are welcome to contact me at james.gardner@gov.ab.ca for clarification or if you need help deciphering my printing in the annotated MS.

All corrections and suggestions made by referee 2 on his hand-annotated copy of the Supplementary Information were incorporated.

13) Supplemental Tables:

a. For Table 2, it is worth adding in its caption that full references for citations in "Source" column are provided in Table 1. It took me a few moments to figure that out.

b. In the "Source" column for both tables, simply listing "MorphoSource" and "Xenopus webpage" without further details (e.g., exact urls) is unnecessarily vague. I recommend providing sufficient details so those sources can be reliably located.

c. In Table 2, line 89, Source column, author name “Shrimpton” is misspelled as “Schrimpton”.

- a. We modified the caption of Table [now] S3 and added the information about cited references in Table [now] S2.
- b. We added the exact url of the mentioned webpages.
- c. Author name was changed according to the reviewer observation.

REFEREE #3

The manuscript reported a well-preserved tadpole specimen at stage of Gosner 40-41 of Notobatrachus, which is the oldest frog tadpole so far reported and the first tadpole of a stem frog, and therefore provides an excellent fossil evidence for how the larva form of stem frogs is, especially the exceptionally preserved hyobranchial apparatus, and some other soft tissue features. The metamorphoses of frogs, intriguing to many people in general, are famous of those drastic changes in nearly all aspects of the animal, resulting in two different life stages, but we do not know when and how such a developmental feature originated due to the lack of the fossil tadpoles in early evolutionary history frog. Therefore the discovery of this Notobatrachus tadpole from the Middle Jurassic of Argentina, together with the detailed morphological study of the Notobatrachus adults, proves the drastic metamorphosis which features the anurans among vertebrates, had occurred early during Middle Jurassic. I think this ms is of great interest to the people not only in my own discipline but also in general.

The fossil was prepared in a good condition and the photographs show clearly the preserved morphological information. The data for phylogenetic analyses and morphological disparity analyses are derived from a good previous work (Haas 2003), and new data based on the discovered fossil was included (but more may be done; see below). The data for body size analyses is good to give a rough estimate for the total length of the Notobatrachus tadpole. The results in this manuscript are mostly robust and reliable and there may be more to dig into this rare but important tadpole fossil (see below). The text is well written in a clear structure with a good collection of citations. I listed my detailed comments and suggestions as below.

1. As for the morphological interpretation of the fossil tadpole, I agree mostly with the authors although there are some concerns as stated below.

SI Line 84-86: There is a confusion on two minor structures of ceratohyale - processus condylus articularis and processus anterior. If the processus condylus articularis was labelled correctly in Figure 2 (cac), there seems not to be a processus anterior medial to it.

We modified the figure because the arrow pointing to the processus condylus articularis was not placed correctly. Thus, we labelled both structures (processus anterior and processus condylus articularis) in order to avoid confusions.

Another concern is the identification of the cranial nerves V and VII. The two cranial nerves originate from the brain at a similar position, and branch in a similar way, and therefore it could be very different to say which one the preserved nerve in this *Notobatrachus* fossil tadpole is, especially considering the displacement the preservation causes. I think the interpretation, brought out herein by the author, that the anterior one is CN V and the posterior one is CV VII, is highly possible due to the possible ganglion. But if so, the ramus of hyoideus of CN VII branches off from the ramus of hyomandibularis, therefore being slenderer than the latter in many tadpoles (Schlosser & Roth 1976; Quinzio & Fabrizi 2019), which is different from the situation in the fossils.

We agree with the referee and modified the drawing to accurately show the differences between both rami, expressing its hierarchy and following the interpretation of Schlosser & Roth (1995) for *Discoglossus pictus*.

Schlosser, G. & Roth, G. Distribution of cranial and rostral nerves in tadpoles of the frog *Discoglossus pictus* (Discoglossidae). J. Morphol. 226, 189–212 (1995).

2. Based on the forelimb remaining within the pocket, the integration of the hyobranchial apparatus (not yet broken up), and several well-defined bone elements (such as frontoparietal, parasphenoid, otic capsule, neural arches and ribs), it is reasonable to determine that the developmental stage of this tadpole is at stage Gosner 40-41. However there are also some interesting points considering the stage of this fossil tadpole:

1) the early ossification of the full set of the manus phalanges (the hindlimb development is supposed to be at the same stage or slightly earlier than the forelimb, and therefore the foot phalanges were probably in its full configuration).

2) The early ossification and development of nasals (for example, nasals in *Xenopus laevis* typically do not appear until stage 58, but appear early in non-metamorphosing *Xenopus* tadpoles in earlier stages; Kerney et al. 2010) Note Wassersug & Roth (1982) suggests some features in the larva of the primitive frogs appear closer to the state in adults. As *Notobatrachus* is the most primitive frog species, could it be possible that *Notobatrachus* tadpole may have some features even closer to the adult state?

This is possibility that this *Notobatrachus* tadpole was one which delayed its metamorphosis due to some environmental factor or intrinsic factor. Such delay may result in early development of some bones in spite of the premetamorphic stage. Could this possibility be ruled out? This may be related to the gigantism of the *Notobatrachus* tadpole.

- 1) We added a reference to the ossification of the phalanges of the manus and its relevance for staging the tadpole to section 2. Additional anatomical information of the Supplementary Information.
- 2) In their study about the skeletal ontogeny of the pipid *Pipa pipa*, Trueb et al. (2000) clearly synthesized the cranial ossification sequences for several early-diverging anuran species and the nasals ossify invariably just before metamorphosis, even in *Xenopus laevis* (Trueb and Hanken, 1992). Stage 58 of Nieuwkoop & Faber (1967) is equivalent to Gosner Stage 41. Thus, this is consistent with the assigned stage to the *Notobatrachus degiustoi* tadpole described in this study. As we pointed out before, we consider that the *N. degiustoi* tadpole was a normal giant tadpole, naturally occurring and successfully metamorphosing. Therefore, we consider that comparisons with abnormal *Xenopus laevis* (pathologic) tadpoles from Kerney et al. (2010) are not necessary.

Trueb, L. & Hanken, J. Skeletal development in *Xenopus laevis* (Anura: Pipidae). J. Morphol. 214, 1–41 (1992).

Trueb, L., Pugener, L.A & Maglia, A.M. Ontogeny of the bizarre: an osteological description of *Pipa pipa* (Anura: Pipidae), with an account of the skeletal development in the species. J. Morphol. 243, 75–104 (2000).

Nieuwkoop, P.D. & Faber, J. Normal table of *Xenopus laevis* (Daudin). A systematical and chronological survey of the development from fertilized egg till the end of metamorphosis. Amsterdam: North Holland. 252 pp. (1956).

Gosner KL. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190 (1960).

3. The phylogenetic and morphological disparity analyses were conducted, in my opinion, in a routine and proper way, but I am not familiar with the detail of the morphological disparity analyses and therefore feel unable to fully assess. As the Notobatrachus tadpole is the only fossil tadpole in the phylogenetic matrix, it seems a bit redundant to compare the constrained and unconstrained analyses (especially if the molecular backbone involves most of the extant species in the matrix). It would be interesting to include some of the well-studied fossil tadpoles of extinct species, such as Paleobatrachus tadpole from the Oligocene of Czech (Rocek 2003), Thoraciliacus rostriceps and Shomronella jordanica (Rocek & Van Dijk, 2006) from the Early Cretaceous, and to see where these fossil tadpoles are in the phylomorphospace.

The molecular backbone based on the Jetz and Pyron (2018) results includes most of the species sampled in the dataset of Hass (2003), but not all. Indeed, the

constrained and unconstrained Bayesian inference majority rule consensus trees are not identical, in which the tree of the unconstrained analysis is less resolved. The same occurs in the results of the parsimony analyses. Thus, although the results of the constrained and unconstrained analyses are congruent with each other, they are not redundant.

Regarding the inclusion of other fossil tadpoles in the morphological disparity analysis (which produces the phylomorphospace), this kind of analysis are prone to biases generated by high amount of missing data during the calculation of the distance matrix. For example, high proportions of missing data produce an artefactual attraction of the less informative terminals to the centroid of the morphospace using Generalized Euclidean Distances (Lehmann et al., 2019) and may produce a repulsion from the morphospace when using Maximum Observed Rescaled Distances (Lehmann and Ezcurra, 2019). In particular, the tadpole of *Notobatrachus* has 85% of missing entries in the Hass (2003) dataset and the tadpoles of *Paleobatrachus*, *Thoraciliacus rostriceps* and *Shomronella jordanica*, which are also incomplete (and notably lacking the hyobranchial apparatus), will produce higher amounts of missing data. The inclusion of terminals with very high proportions of missing entries (>90%) is very problematic in morphological disparity analyses, leading to artefactual results (Lloyd, 2016; Lehmann et al., 2019; Flannery Sutherland et al., 2019) and the problem seems to be exacerbated when terminals with high proportion of missing data are compared between each other (e.g. *Notobatrachus degiustoi* tadpole and other fossil tadpoles). Thus, we decided to keep the original taxon sampling of the disparity analysis that focuses on the comparison between the tadpole of *Notobatrachus degiustoi* and those of extant species.

- Flannery Sutherland, J. T., Moon, B. C., Stubbs, T. L., & Benton, M. J. 2019. Does exceptional preservation distort our view of disparity in the fossil record? *Proceedings of the Royal Society B*, 286(1897), 20190091.
- Lehmann O.E.R., Ezcurra M.D. 2019. Desafíos en los análisis de disparidad con taxones incompletos: el caso de la máxima distancia observada rescalada (MORD). Libro de Resúmenes Reunión de Comunicaciones de la Asociación Paleontológica Argentina 2019: 125–126.
- Lehmann, O. E., Ezcurra, M. D., Butler, R. J., & Lloyd, G. T. 2019. Biases with the Generalized Euclidean Distance measure in disparity analyses with high levels of missing data. *Palaeontology*, 62(5), 837-849.
- Lloyd, G. T. 2016. Estimating morphological diversity and tempo with discrete character-taxon matrices: implementation, challenges, progress, and future directions. *Biological Journal of the Linnean Society*, 118(1), 131-151.

4. The two datasets assembled for estimating the total length of the *Notobatrachus degiustioi* tadpoles and evaluating the larva and adult body sizes of the sampled frog species respectively, are reasonably large and covering various groups of Anura, although technically the sample is still small compared with the large number of anuran species. The giantism of both *Notobatrachus* tadpole and adult is verified.

We agree with the reviewer in that the assembled datasets comprise a small sample when compared with the large number of anuran species. Nevertheless, although there is a larger number of measurements available from the publication of Womack & Bell (2020), we had to strongly reduce the number of measurements to those close to the developmental stage of the *Notobatrachus degiustoi* tadpole, i.e. Gosner Stages 39–41.

There are also some subtle issues I noticed when reading the ms and listed below.

Page 4, Line 91: 'hyobranchial' is misspelled as 'hyobranquial'.

We modified the spelling according to the referee's correction.

Page 14, Figure 1: 'n' is used twice for different structures (nasal VS neurocranium). Different abbreviations should be used.

We modified the figure and the figure caption in order to avoid repetition of abbreviations.

Page 18, Line 399: ('treeSearches_protocol_Notobatrachus_unconstrained.run' and 'treeSearches_protocol_Notobatrachus_unconstrained.run'), of which one should be 'treeSearches_protocol_Notobatrachus_constrained.run'

We correct the name of the script according to the referee's suggestion.

Page21, Line 447: the giant adults are those having SVL higher than 127 mm.

We modified the value according to the referee's suggestion.

SI, Line 43 'Eosolimnadiopsis? santacrucensis': the initial letter of the species name should be lowercase.

We changed the initial letter of the species name.

REFEREE #4

This is an important report on the earliest known anuran tadpole, previously unnoticed in the palaeontological collections of a regional museum in a remote region of southern Argentina. As such, this information will be of interest to a wide range of biologists. The illustrations are well done, although minor errors should be corrected.

Please check the size and therefore legibility of the text in Figure 3, especially the names of the taxa. Depending on the final size of this figure, some of its informational value may be lost.

Further remarks

***Line 22: "Anurans are characterised by a unique biphasic life cycle".
An aquatic larval stage followed by a terrestrial adult stage also occurs in caudate amphibians and non-neotenic temnospondyls, so the term "unique" seems overstated (see also line 41).***

We modified the sentence according to the referee's suggestion.

Line 25: "Modern tadpoles"

I would recommend using "recent" instead of "modern".

We modified the text according to the referee's suggestion, avoiding the term "modern".

***Lines 28-29: "... anuran fossil record dating back to the Early Jurassic".
The earliest known fossil frogs are from the Late Triassic Chinle Formation (213-223 Mya) of Arizona, USA (Stocker et al. 2019).***

The work we referred to cite the oldest stem-anuran corresponds to *Prosalirus bitis* (Shubin and Jenkins, 1995), a species described on the basis of a complete specimen, that exhibits several crown-anuran features, and thus, allows a precise phylogenetic inference. Therefore, it is in the Early Jurassic that we have complete records of stem-anurans with the typical body plan of the group. Although Stocker et al. (2019) interpreted that the ilium from the Upper Triassic Chinle Formation bears certain features that suggest a closer affinity to crown-anurans than *Triadobatrachus* and *Czaktobatrachus*, this report lacks an explicit phylogenetic analysis. We therefore consider this isolated, fragmentary specimen as a poorly constrained phylogenetic record. In this way, we prefer to be cautious and date the

postmetamorphic fossil record of anurans to the Early Jurassic, with *Prosalirus bitis* described by Shubin and Jenkins (1995) as the earliest record.

Shubin, N.H. & Jenkins, F.A. An Early Jurassic jumping frog. *Nature* 377, 49–52 (1995).

Stocker MR, Nesbitt SJ, Kligman BT, Paluh DJ, Marsh AD, Blackburn DC, Parker WG. The earliest equatorial record of frogs from the Late Triassic of Arizona. *Biol. Lett.* 15: 20180922 (2019).

Line 29: "late larva".

It might be better to use "advanced larva" (compare line 78).

We rewrote the sentence according to the reviewer's suggestion and modified the text in order to avoid the term "late larva".

Lines 47-48: "Larval characters may have evolved during the basal anuran radiation around the Triassic-Jurassic boundary"

The Late Triassic frogs from Texas (Stocker et al. 2019) with typical anuran ilia and probably also maxilla are derived from adults that had to be ontogenetically preceded by an anuran tadpole, not by a temnospondyl larva. Thus, anuran larval characters had to evolve earlier than around the Triassic-Jurassic boundary.

The full sentence establishes: "Studies about evolution of the tadpole morphospace indicated that larval characters may have evolved during the basal anuran radiation around the Triassic–Jurassic boundary (Roelants et al., 2011)". We slightly modified the sentence in the revised text; however, it is referring to results of the work of Roelants et al. (2011) and is not a direct outcome of our observations on the fossil tadpole. The described specimen of *Notobatrachus degiustoi* is proving that at least in the Middle Jurassic a tadpole was already present among stem-anurans. We do not state that tadpoles as such appeared at the T-J boundary, but they might have had an evolutionary explosion in this period. We consider that proposing or discussing an earlier appearance of a tadpole-like larva for early-diverging salientians is beyond the scope of this article.

Roelants, K., Haas, A. & Bossuyt, F. Anuran radiations and the evolution of tadpole morphospace. *PNAS* 108, 8731–8736 (2011).

Line 50: "salamander larvae"

Do you mean the larvae of the genus Salamandra or salamandrid larvae?

We modified the text according to suggestions of referee 1 and therefore deleted this sentence.

Line 64: "Museo Provincial Padre M.J. Molina" Add where the museum is located: in Rio Gallegos.

We added the exact location of the museum in the text.

Line 67: "from the same quarry"

Be more precise: "from the Cañadón de Estancia La Matilde type locality near Puerto Deseado (La Matilde Formation)".

We agree with the reviewer and thoroughly address this point based also on observations of referee 2 (see above).

Line 72: Here you can remove: "(La Matilde Formation)".

We removed the reference to the formation.

Lines 91, 123, 129: hyobranchial" instead of "hyobranquial".

We rewrote the word correctly in the text.

Line 96: "Ceratobranchialia are preserved as four black ellipsoidal bands arranged in an anteroposteriorly oriented row".

This can be misleading, because the dark stripes represent cartilaginous ceratobranchials, and the ellipsoid fields between them (especially the one between ceratobranchials III and IV) are branchial slits (cf. Fig. 2a). I would suggest: The ceratobranchials are preserved as four dark mediolaterally oriented bands with light ellipsoid fields between them representing branchial slits.

We agree with the reviewer observation and rewrote the sentence in order to improve the description, both in Main Text and in Supplementary Information.

Line 144: "by far the largest Mesozoic anuran".

Please include: "so far known", because there are some fragmentary ilia (e.g. from the Late Cretaceous of Utah; Roček et al. 2010, fig. 11v) indicating the presence of other giant anurans in the Mesozoic.

We modified the statement based on the referee's suggestion and also when addressing the suggestions made by referee 2 (see above).

Note to lines 158-161:

The anatomical transformation of the temnospondyl larva into the anuran tadpole is one of the key events in the evolutionary history of amphibians, because this event (long and gradual) represents the origin of frogs. Assuming that the appearance of tadpoles and adult frogs was a simultaneous process (as suggested by developmental processes in the skull and pelvic girdle of extant anurans), the presence of adult anurans in the Late Triassic suggests that tadpoles also existed at that time. Thus, the origin of both should be expected earlier than the Late Triassic.

As we explained above, to date, we have no evidence of any salientian larva demonstrating the presence of a drastic metamorphosis, with a filter-feeding tadpole. The only positive evidence of a tadpole among stem-anurans is represented by the tadpole of *Notobatrachus degiustoi* reported here. In this sense, the main characteristics of the metamorphic period and larvae of salientians like *Triadobatrachus* or *Czaktobatrachus* are completely unknown, and hypothesizing about them is beyond the scope of our manuscript. Moreover, Roelants et al. (2011) proposed that there was an expansion of the tadpole morphospace during the early anuran radiation in the Triassic–Jurassic boundary, when key characters, such as those associated with the filter feeding mechanism, were probably established. Their results do not contradict the hypothesis of referee 4. The time period proposed by Roelants et al. (2011) for the establishment of key characters for the tadpole body plan also agrees with our findings and is expressed in the mentioned lines of the main text.

Figure 1. fo should be marked with an arrow because the fenestra ovalis is not visible in the dorsal view. Also, the dark lateral extension of the otic capsule cannot be the fenestra ovalis, but rather the cartilaginous part of the crista parotica (as suggested by the same colour as the ceratohyal and the branchial basket).

Also remove the n representing the neurocranium and retain only the n representing the nasals.

The posterior part of the neurocranium is not well preserved and is usually represented by the gap between the two otic capsules bridged by the cartilaginous tectum synoticum. The fontanella between the two frontoparietals

and the gap between the two otic capsules undoubtedly reached the bottom of the braincase (i.e. the parasphenoid).

Notobatrachus degiustoi specimens in the Estancia La Matilde locality are preserved as impressions, that is, skeletal elements (including the cartilaginous neurocranium) are represented as natural casts. The tadpole specimen depicted in Figure 1 shows the “negative” view of the skull region, while the latex casts depicted in Extended Data Figure 4 shows the “positive” relief. Due to the 2D condition of the specimen, we can observe simultaneously dorsal cranial elements (nasals, frontoparietals) along with the ventral view of the cartilaginous neurocranium. In this way, the deep rounded notch preserved in the slab corresponds to the rounded structure observed in the latex cast. This structure has the same shape and spatial relationship of the fenestra ovalis observed in the cartilaginous neurocranium of extant tadpoles (e.g. fig. 10 of Pugener and Maglia, 1997; figs. 9–10 of Maglia and Pugener, 1998). Because the fenestra ovalis can be observed in lateroventral view in extant forms, we infer that the ventral position of this structure in the fossil tadpole is due to the dorsoventral compression experimented during fossilization.

Abbreviation for nasals was kept as “n”, while the abbreviation for neurocranium was changed to “cn”, referring to chondrified neurocranium.

The posterior part of the neurocranium was probably represented by cartilage at this stage of development. Moreover, the texture of the posterior region of the skull is different from the texture of the parasphenoid, and, therefore, it is represented in this way in the interpretative drawing of Figure 1c.

Pugener LA, Maglia AM. Osteology and skeletal development of *Discoglossus sardus* (Anura: Discoglossidae). J. Morph. 233: 267–86 (1997).

Maglia, A.M. & Púgener L.A. Skeletal development and adult osteology of *Bombina orientalis* (Anura: Bombinatoridae). Herpetologica, 54: 344–363 (1998).

Figure 2.

Lines 328-329: *pl* processus lateralis of ceratohyal is not shown in Fig. 2b.

Line 329: *pph* should be complete as in Fig. 2b.

We added the label for the processus lateralis of ceratohyal (*pl*) to Figure 2, and added the corrected reference to proximal phalanx (*pph*) in the figure caption.

Figure 3. Please check the legibility of the text, especially the names of taxa.

We modified the text size within the figure in order to improve legibility.

Comment not directly related to the submitted text:

As this manuscript is an important report on the earliest known anuran tadpole, I would like to draw your attention to a disarticulated specimen CPBA-V-14003, which can also be attributed to Notobatrachus (see Báez and Nicoli 2004). It also comes from the type locality of Notobatrachus mentioned in the text above and is deposited in the collections of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, thus easily accessible to you.

Judging by the characters listed below, it may be of the similar ontogenetic age to the advanced larva described in the manuscript. These characters are:

- The epiphyses of the long bones are cartilaginous (not preserved = not ossified);***
- Only the proximal part of the caput humeri is ossified;***
- The ossified part of the iliac shaft is short (as in Fig. 1 above);***
- Vertebral centra probably still separated from neural arches, but fused with transverse processes;***
- Neural arches not yet fused dorsally;***
- Only proximal part of ribs ossified;***
- Frontoparietals in brief contact posteriorly, but separated anteriorly by a long fontanelle;***
- Radioulna and tibiofibula both completely fused (as in Fig. 2);***

I think it is worth mentioning this specimen in the present manuscript, at the cost of only moderately expanding the text.

We really appreciate this comment as this fossil specimen might be of great relevance for our understanding of the ontogenetic transformations that occurred in the development of *Notobatrachus degiustoi*, and could also provide deep insights into the species biology. Báez and Nicoli (2004) stated that “this specimen [CPBA-V-14003] may correspond either to a late metamorphosing tadpole or an early postmetamorphic individual.” Regrettably, we do not have access to this specimen because it is currently under embargo while being studied by other Argentinian researchers. Thus, we could not determine if it actually represents a tadpole or not.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The revised version of the manuscript “THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE ANURAN LIFE CYCLE” by Chuliver et al. is much improved and the authors responded to the suggestions adequately and to my satisfaction. They are reporting an important and exciting fossil find, which in my opinion is well worth of publication in Nature.

There are a few points that I think would improve the manuscript further prior to publication, all of which are minor and can be included quickly.

1. As I pointed out in the first review, it is intriguing that amongst so many very well-preserved adults, this is the only tadpole. In their response the authors state that this is a common pattern in fossil sites mentioning the Messel site. However, Messel is a rather peculiar fossil Lagerstätte with very unique and specific taphonomic conditions (being a caldera and fossils preserved in oil shale, which are very hard to recover and preserve) and there are a lot of taphonomic oddities at that site. There are other localities, however, that have yielded numerous adult frog fossils and abundant tadpoles (e.g. the Enspel lake or the Tayasir Formation that yielded *Shomronella*), therefore the question, why no further tadpole specimens have been found at the La Matilde locality does spring to mind immediately. To avoid misunderstanding: the argumentation by the authors that this is a taphonomic relic and probably also a collection bias is entirely conceivable, I just think it should be briefly, but clearly stated in the main text to clarify the point why this is the first tadpole find.

2. The authors did address the point that this tadpole is gigantic, but naturally so (rather than pathologically). Here also, I agree with and can follow their assessment and their interpretation is conceivable. The section, where this is briefly discussed in the main manuscript is a bit confusing though, which is primarily a matter of wording not of the content. I made a suggestion using track changes in word (see attached file) how these 1-2 sentences could be rephrased to make this point clear for readers.

Regarding the terminology of gigantism, I think this needs to be clarified some more than it currently is. This could be in the supplementary information if the authors do not want to include it in the main text, but it requires another short paragraph or so. This is currently only very briefly mentioned and the exact meaning in your paper remains a bit obscure, as it is not further explained in the main document and supplementary information beyond a reference to Womack and Bell (2020).

The reason why I think this is important here is that gigantism plays a crucial role in the presented manuscript, but the terms “gigantism” and the opposite “miniaturization” are used in the literature in a rather wide spectrum of meanings, often just loosely applied to organisms that are very large (or small) rather than referring to an explicit and testable evolutionary trend within a lineage. I think in this study,

you are referring to the latter and argue along these lines as well (e.g. lines 265-268). Gigantism in in this meaning and context has been the subject of detailed studies (including influential papers such as Hanken & Wake 1993, Gould & MacFadden 2004, Vermeij 2016 amongst others) and explicit definitions. I think it is necessary to clearly state and define your meaning of gigantism here.

3. Other than the points above, there are a few very minor points I noted related to wording/grammar in the paper, which I also indicated using track changes in the attached document.

Best wishes, Nadia Fröbisch

Referee #2 (Remarks to the Author):

Review of revised version of “THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE ANURAN LIFE CYCLE”, by Mariana Chuliver et al.

Reviewer: Jim Gardner

Date: 11 June March 2024

Having reviewed the original version of this MS, I was excited to see this revised version. I am pleased to see the authors satisfactorily dealt with my comments, queries, and suggestions. Their revised MS is much improved. They have done a better job of detailing where the fossil was collected and its geologic age, better supported their taxonomic identification and interpretation of the ontogenetic age of the fossil, improved the labelling of anatomical structures in their interpretive line drawings, and added a table of absolute size ranges for Mesozoic frogs that helps bolster their argument about tadpoles and adults of *Notobatrachus degiustoi* being unusually large compared to other Mesozoic and extant frogs. I continue to regard this report as interesting and significant enough to warrant publishing in a high-profile journal such as Nature. For reasons outlined below, I recommend this MS be accepted pending minor revisions.

Once again, I am providing a hand annotated copy of select portions of the MS (main text, captions, and figures; extended figure captions; and first nine pages of the Suppl. Info). As you will see from that document, most of my points deal with wording. Other minor points are as follows:

- 1) The colour used for the frontoparietals in the interpretive drawings in the first two figures differs: pink in Fig. 1c vs. red in Fig. 2b. For consistency, the same colour should be used in both and for clarity should be unique to that element. Regarding the latter point, note the same shade of red (or so it appears in my review copy) used for the frontoparietals in Fig. 2b also is used for the ilium in Fig. 1c and the same shade of blue (or so it appears in my review copy) is used for both the femur and hyobranchial skeleton in Fig. 1c.
- 2) For Fig. 2, the abbreviation “oc” appears and is defined in the caption, yet that abbreviation does not appear in the corresponding interpretive line drawing.
- 3) In my review copy, the three dotted lines in Fig. 3c are so small and faint that they are nearly invisible, especially to those of us with aging eyes. Maybe that is just an artifact of how the review copy prints? Regardless, it is worth trying to enhance those lines (e.g., make them thicker), so they are clearly visible in the final publication.
- 4) Three points for the “Morphometric analyses” section on p. 20:
 - a. It is unclear whether your “measurements of tadpoles and adults of anuran species” (line 434) is limited to extant species. I think that is the case, but it is worth clarifying in the text.
 - b. The statement that you “used measurements only from individuals at Gosner stages 39–41, the same range to which the tadpole of *Notobatrachus degiustoi* is assigned” (lines 437 and 438) needs to be qualified, because elsewhere you explicitly regard the fossil as being within a narrower range of Gosner stages 40–41. That qualification can be accomplished by adding “approximately” in front of “at the same

range”.

c. Discrepancy in number of species measured for size: 191 (line 440) vs. 193 in caption for Extended Data Fig. 9.

5) Discrepancy between how the locality for the fossil tadpole is presented in Extended Data Fig. 1: spelled out as “Estancia La Matilde” in caption vs. abbreviated (and not defined in caption) as “Ea. La Matilde” in corresponding figure.

Good luck with your final revision. This is an exciting fossil. I look forward to seeing it make a giant tadpole-sized splash in Nature.

As with all my reviews, I waive anonymity.

Regards and best wishes,

Jim Gardner, PhD
Curator of Palaeoherpetology
Royal Tyrrell Museum of Palaeontology
Drumheller, Alberta, Canada

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Jim Gardner, PhD
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Royal Tyrrell Museum of Palaeontology
Drumheller, Alberta, Canada

1 **THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE**
2 **ANURAN LIFE CYCLE**

3
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due 28 June 2024
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11 June 2024

23 Anurans are characterised by a biphasic life cycle, with an aquatic larval (tadpole)
 24 stage followed by an adult (frog) stage, both connected through the metamorphic
 25 period in which drastic morphological and physiological changes occur¹. Extant
 26 tadpoles exhibit a great morphological diversity and ecological relevance², but
 27 their absence in the pre-Cretaceous fossil record (older than 145 Mya) makes their
 28 origins and early evolution enigmatic. This contrasts with the post-metamorphic
 29 anuran fossil record that dates back to the Early Jurassic (ca. 191–183 Mya)³. Here
 30 we report a late stage tadpole of the stem-anuran *Notobatrachus degiustoi* from the
 31 Middle Jurassic of Patagonia (ca. 168–161 Mya). This finding has a dual
 32 importance since it represents the oldest-known tadpole and the first stem-anuran
 33 larva described up to date. Its exquisite preservation, including soft tissues, reveals
 34 features associated with the filter-feeding mechanism characteristic of extant
 35 tadpoles^{4,5}. Notably, both *Notobatrachus degiustoi* tadpole and adult reached large
 36 sizes, demonstrating that tadpole gigantism was also present among stem-anurans.
 37 The new discovery reveals that a biphasic life cycle, with filter-feeding tadpoles
 38 inhabiting aquatic ephemeral environments, was already present in the early
 39 evolutionary history of stem-anurans, and has remained stable for at least 161
 40 million years.

41

42 Tadpoles are the free-living, non-reproductive aquatic larvae of anurans, which
 43 have to go through a metamorphic phase of deep morphological and ecological changes
 44 within a short period of time in order to reach the adult reproductive stage^{1,2}. This
 45 drastic type of metamorphosis⁶ of anurans is the most extreme amongst extant tetrapods,
 46 and tadpoles represent a highly derived larval stage^{2,7}. Studies about the evolution of the
 47 tadpole morphospace indicated that key characters have evolved during the stem-anuran

48 radiation around the Triassic–Jurassic boundary⁸. Nevertheless, these hypotheses are
 49 solely based on evidence from extant anurans and remain untested because fossil
 50 tadpoles are completely absent in the Triassic and Jurassic fossil record⁹. Moreover,
 51 stem-anurans are only known from postmetamorphic individuals^{3,10–13}. Thus, the timing
 52 and evolutionary transformations involved in the origins of the tadpole body plan
 X 53 remain enigmatic and ^amatter of debate.

54 Here we describe the oldest-known tadpole from the Middle Jurassic La Matilde
 55 Formation (Bathonian–Callovian; ca. 168–161 Mya) based on a well-preserved
 56 specimen found in the Estancia La Matilde locality (Santa Cruz Province, Argentina),
 57 that is referred to the stem-anuran *Notobatrachus degiustoi*. This species is also
 58 represented by a large number of superbly preserved adult specimens from the same
 59 locality^{12,14}. The exquisite preservation of the new specimen, including most of the
 60 hyobranchial apparatus and soft tissues, allows us to infer the mode of life and feeding
 X 61 habits of ^{the}*N. degiustoi* tadpole. Therefore, this finding substantially enhances our
 62 knowledge of the origins and early evolution of the biphasic anuran life cycle.

63

64 Description

65 The fossil MPM-PV (Museo Provincial Padre M.J. Molina, Río Gallegos, Santa
 66 Cruz Province, Argentina) 23333 is a single individual that consists of a cranium and
 67 most of the postcranium of a tadpole in dorsal aspect, including the proximal part of the
 68 tail (Fig. 1). It was collected from a quarry at the Estancia La Matilde (the type locality ✓
 69 of *Notobatrachus degiustoi*)¹⁴, where the La Matilde Formation crops out and which
 70 also has yielded hundreds of adult specimens of the species^{12,14}. Its occurrence in the ✓
 71 same fossil levels as adults of *N. degiustoi*, the presence of several skeletal
 X 72 characteristics shared with adults of *N. degiustoi* (i.e., ^{nine}9 presacral vertebrae, free ribs

73 associated with presacrals II–IV, transverse processes of posterior presacral vertebrae as
 74 long as sacral diapophysis, and uncinat process on the free rib III), and the fact that no
 75 other anuran species have been identified from the La Matilde Formation support the
 76 referral of the fossil tadpole to this species. Geological data for the locality indicate that
 77 sediments containing the specimen were deposited in a low-energy lentic environment
 78 scattered in the floodplain with pyroclastic material input, under ~~seasonally~~ ^{a summer} dry-winter
 79 wet ~~biome~~ ^{climate} ^{15,16}. These sediments also contain diverse plants, branchiopod crustaceans,
 80 bivalves and insects (Extended Data Fig. 1)^{17–19}.

81 The tadpole was well chondrified as indicated by cartilage imprints, and the
 82 overall degree of ossification supports an advanced larval stage, prior to the beginning
 83 of metamorphosis (Extended Data Figs. 2–4). The cranial ossification that corresponds
 84 to the parasphenoid is flanked by the frontoparietals, which extend on both sides as
 85 longitudinal thin strips of bone, strongly tapering anteriorly, and widely separated from
 86 each other along the midline (Fig. 1). The right otic region preserves the crista parotica,
 87 represented by a subtriangular knob-like prominence (Fig. 1). The imprints of neural
 88 arches of nine presacral vertebrae, the sacrum, and three postsacral vertebrae are
 89 present. The right forelimb is represented by the radius and ulna fused to each other, and
 90 the manus (Fig. 1). The right iliac shaft is preserved in lateral view, with the dorsal
 91 acetabular expansion already ossified (Fig. 1). Based on the presence of the forelimb
 92 still contained within the body contour, likely covered by the operculum, and the state
 93 of development of cranial and postcranial ossifications^{20–22} (detailed in Supplementary
 94 Information) we assign the tadpole to Gosner stages 40–41²³.

95 MPM-PV 23333 is unique in the preservation of the hyobranchial skeleton,
 96 which was not preserved in any other fossil tadpole described up to date⁹. The expanded
 97 ceratohyal possesses well developed anterior, posterior, and lateral processes and a

Your meaning is clearer in the Suppl. Info.

Maybe re-write as "in a low-energy lentic environment (i.e., ponds) scattered across a floodplain..."

Not clear
what you
mean here.

condylus articularis (Fig. 2), indicating the presence of an articulation with the palatoquadrate cartilage. Ceratobranchialia are preserved as four dark mediolaterally oriented bands with ellipsoidal fields between them, representing branchial slits (Fig. 2). Ceratobranchial I is medially overlapped by the ceratohyal. Ceratobranchials II–IV are proximally connected by commissurae proximales and distally by commissurae terminales (Fig. 2). Medial and lateral projections for support of the gill filters are distinguished along the shafts of ceratobranchialia II and III. An oblique, rod-like element is positioned lateroventral to the ceratohyal and lateral to ceratobranchial I (Fig. 2); it ~~could be assigned to~~ ^{may represent} the lateral projection of the hypobranchial plate, although its identity is doubtful.

Soft parts, including eyes and nerves are preserved as dark imprints in their anatomical position. The eyes are dorsally positioned; the lens within the right eye is clearly distinguishable on the anterolateral region of the eyeball, which is indicative of an anterolaterally facing eye (Fig. 2). Several nerve impressions are distinguishable on the right side of the cranial region (Fig. 2; see Supplementary Information).

Analyses and discussion

We explored the phylogenetic relationships of the *Notobatrachus degiustoi* tadpole using a modified phylogenetic dataset based on tadpole traits⁸. The new dataset is composed of 86 species scored for 152 characters (see Supplementary information). The tadpole of *N. degiustoi* was recovered as a stem-Anura using both implied weighted maximum parsimony and Bayesian inference with a relaxed Markov k-state variable morphological clock model that incorporates taxon ages using a fossilized birth-death process (Fig. 3a). These results are consistent with those previously found exclusively based on the adult morphology of *N. degiustoi*^{11,24,25}. Despite the adaptive decoupling

123 between larva and adult in anurans^{26,27}, the morphology of both semaphoronts (i.e.
 124 tadpole and adult) have congruent phylogenetic signals and retrieve consistent
 125 phylogenetic positions for both crown and stem anurans.

126 Several anatomical traits present in the *Notobatrachus degiustoi* tadpole
 127 demonstrate that key features of crown-anuran tadpoles had already evolved in stem
 128 taxa; particularly, the structures of the hyobranquial skeleton involved in the feeding
 129 mechanism. The filter feeding system of the vast majority of extant tadpoles consists of
 130 a complex buccopharyngeal filter apparatus that entraps food particles from water
 131 currents maintained by continuous action of a buccal pump^{4,28}. Commissurae
 132 terminales, commissurae proximales and a ceratohyale with median horizontal plate
 133 lacking a distinct processus anterolateralis, and spiny projections on ceratobranchials
 134 are traits of the hyobranquial skeleton of extant tadpoles tightly related to the filter-
 135 feeding mechanism^{7,29,30}, which were already present in the *N. degiustoi* tadpole.
 136 Moreover, the phylomorphospace built based on the dataset of tadpole features also
 137 shows that *N. degiustoi* is positioned close in the morphospace to early-diverging crown
 138 anuran tadpoles, and has the lowest dissimilarity distance with respect to them (Fig. 3b).
 139 In other words, the general morphology described here for the tadpole of the stem-
 140 anuran *N. degiustoi* is highly congruent with that present in extant anurans such as
 141 discoglossoids.

142 The estimated total length of the *Notobatrachus degiustoi* tadpole is 159 mm
 143 (Extended Data Fig. 9; see Supplementary Information), which is above the threshold
 144 for giant tadpoles (>105.1 mm) and close to the total length of giant normal tadpoles of
 145 extant frog species, such as the paradoxical frog *Pseudis paradoxa*, whose tadpoles
 146 naturally occur and successfully metamorphose^{31,32}. The developmental stage of the *N.*
 147 *degiustoi* tadpole (Gosner Stage 40–41) indicates it was close to ^{reaching} ~~reach~~ the metamorphic

148 phase, thus allowing us to discard a pathological (e.g. athyroid) condition to explain
 149 gigantism in this specimen.

150 Notably, adults of ~~*N. degiustoi*~~ ^{*Notobatrachus*}—by far the largest stem-anuran and one of the ✓
 151 largest Mesozoic anurans (Supplementary Table S1)—reached a body size of 150
 152 mm¹², falling within the range of giant adult anurans (>127 mm SVL)³³. Gigantism in
 153 tadpoles and postmetamorphic anurans have evolved several times in the history of the
 154 clade^{33,34}, but there are few species for which tadpoles and postmetamorphic individuals
 155 are both giants (Fig. 3c; Supplementary Table S2). The optimization in the phylogeny of
 156 the residuals of a regression between the tadpole total length and adult snout-vent length
 157 shows that current data favours that the larval gigantism recorded in *N. degiustoi* has
 158 been independently acquired with respect to those found in crown anurans (Extended
 159 Data Fig. 9). Under regular conditions, there are two ways in which a normal tadpole
 160 might become gigantic, namely a long development (pre-displacement or
 161 hypermorphosis) or accelerated developmental rates³⁵. This depends on ecological
 162 factors such as the environmental stability (food availability, low risk of desiccation)
 163 and the absence of predators^{7,36}. According to fossil data of the locality, interspecific
 164 competence and predator pressures were negligible, and therefore, the large size of the
 165 *N. degiustoi* tadpole could be the result of both factors, an increase in developmental
 166 time and growth in seasonally ephemeral ponds.

167 The palaeoecology of the locality and the anatomical information provided here
 168 indicate that an exotrophic filter-feeding tadpole, inhabiting lentic ephemeral
 169 microhabitats is the ancestral condition for anurans, in agreement ^{with} ~~to~~ previous studies
 170 based on extant species^{30,37–39}. Therefore, the overall morphology of *Notobatrachus*
 171 *degiustoi* indicates that the anuran biphasic life cycle with a drastic metamorphosis was
 172 already present 161 million years ago, during the Middle Jurassic. These findings are

173 consistent with studies of the larval body plan of living anurans indicating that tadpole
 ✕ 174 morphological novelties, such as those implicated in the filter feeding mechanism, ~~have~~
 175 evolved in a radiation around the Triassic–Jurassic boundary⁸. The striking anatomical
 176 similarity between the *N. degiustoi* tadpole and extant early-diverging crown anuran
 177 tadpoles supports this evolutionary scenario, which was probably promoted by the
 178 stable selective pressures of ephemeral freshwater habitats exploited by anurans since
 179 the early steps of their evolutionary history.

180

181 **References**

182

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FIGURES

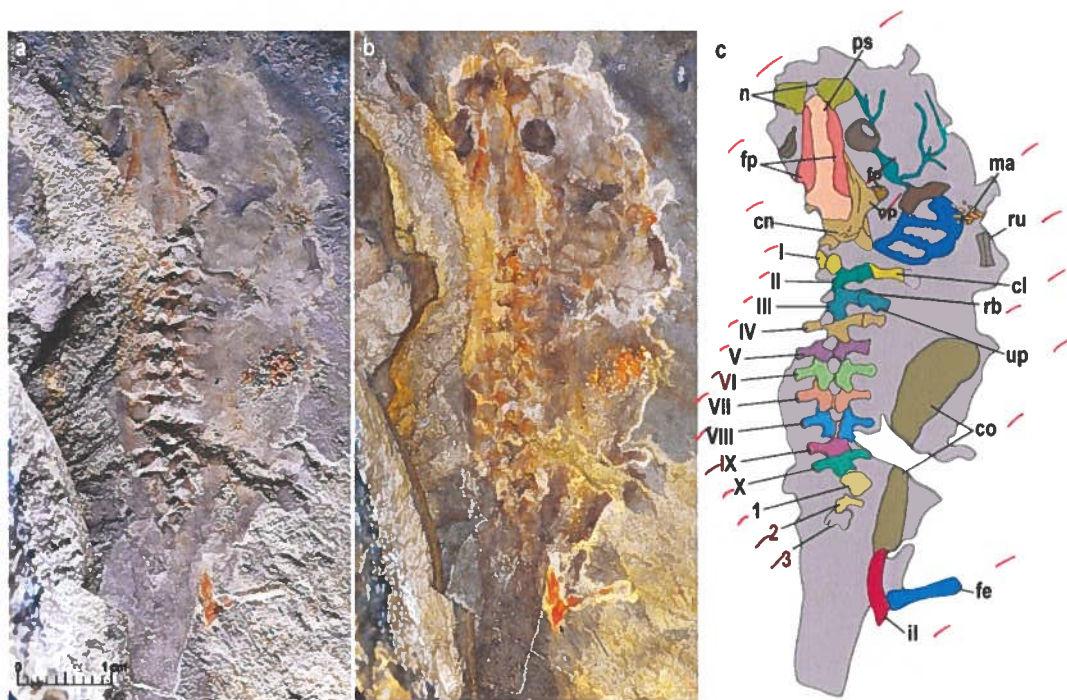
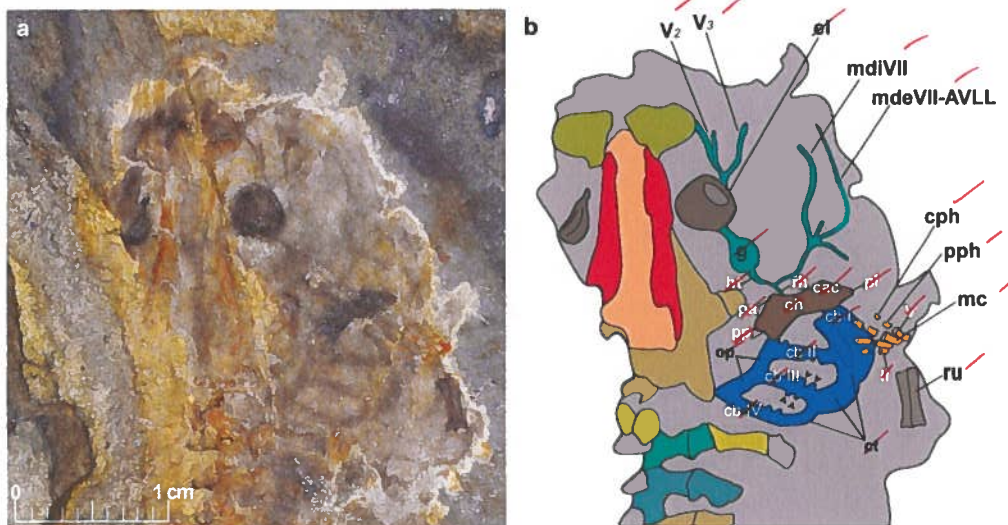


Figure 1. Photographs and interpretative drawing of the specimen MPM-PV 23333. **a**, photograph taken with short exposure time and low angle white light enhancing the skeletal morphology. **b**, photograph taken with long exposure time and high angle white light enhancing soft tissues morphology. **c**, interpretative drawing of the skeleton and soft tissues. Abbreviations: cl, cleithrum; cn, chondrified neurocranium; co, cololite; cp, crista parotica; fe, femur; fo, fenestra ovalis; fp, frontoparietal; il, ilium; ma, manus; n, nasal; ps, parasphenoid; rb, rib; ru, radio-ulna; up, uncinat process; I-IX, presacral vertebrae; X, sacrum; 1-3, postsacral neural arches.

Considering you mention eyes in the description, with labeling these structures in this otherwise well-labeled & presented figure. OK, may be less important considering that you label the eye lens in Fig-2!

324



325 Figure 2. Photograph and interpretative drawing of the head region of specimen MPM-

326 PV 23333. **a**, Picture of the head region taken with long exposure time under low

327 vertical light, enhancing ossifications, hyobranchial skeleton and soft organs. **b**,

328 interpretative drawing depicting structures of the manus, hyobranchial skeleton and

329 nerves. Medial and lateral projections of the ceratobranchial III are ~~pointed with~~ *indicated by*

330 arrowheads. Abbreviations: cac, condylus articularis of ceratohyal; cb, ceratobranchialia

331 I-IV; ch, ceratohyal; cp, commissurae proximales; cph, central phalanx; ct,

332 commissurae terminales; el, eye lens; g, ganglion; ht, hyomandibular trunk; mc,

333 metacarpal; mdeVII-AVLL, ramus mandibularis externus of facial nerve and

334 anteroventral lateral-line nerves; mdiVII, ramus mandibularis internus of facial nerve;

335 oc, otic capsule; pa, processus anterior; pl, processus lateralis; pp, processus posterior;

336 pph, proximal phalanx; rh, ramus hyoideus; ru, radio-ulna; II, digit II; V, digit V; V₂,

337 maxillary ramus of the trigeminal nerve; V₃, mandibular ramus of the trigeminal nerve.

338

Two points:

1) "oc" not labeled.

2) color for fpar is red in Fig. 2 b vs more pink in Fig. 1 c. For consistency, should be same color in both figures. Note red already used in Fig. 1 c for ilium.

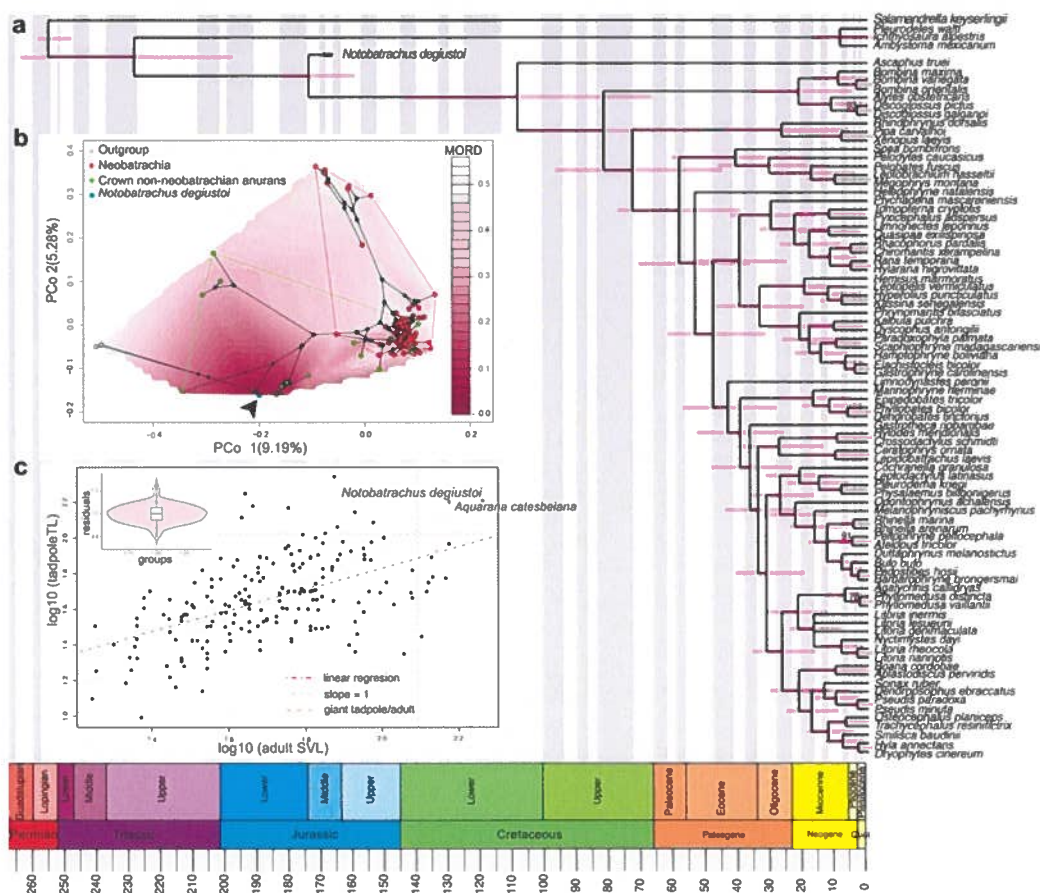


Figure 3. Phylogenetic affinities of the tadpole of *Notobatrachus degiustoi*. **a**, Bayesian tip-dating tree based on tadpole anatomy characters and constrained following a molecular backbone of anuran phylogeny. **b**, phylomorphospace of the first two principal coordinates and dissimilarity distances (MORD) as heatmap, showing the *Notobatrachus degiustoi* tadpole (light blue dot pointed with an arrowhead) close to crown non-neobatrachian tadpoles (green dots). **c**, bivariate plot of the log-transformed tadpole total length against the log-transformed adult snout-vent length (residuals shown as a violin plot on the left-upper corner). Dotted red line represents the linear generalized least squares regression, dotted grey line represents the slope = 1, and the upper-right quadrante delimited by the yellow dotted lines indicate the area occupied by species with both giant tadpoles and adults, i.e., *Notobatrachus degiustoi* and *Aquarana catesbeiana* (extant American bullfrog).

helpful to add this

NOTE in my review copy these three dotted lines (esp. grey + yellow) are so faint they are nearly invisible.

351 **Methods**

352 **Morphological description.** Osteological terminology followed Trueb^{20,40}, and Pugener
 353 & Maglia⁴¹. Vertebrae are designated via Roman numerals in anteroposterior order.
 354 Vertebra X is referred to as the sacrum. Postsacral vertebrae are designated in an
 355 anterior to posterior sequence using Arabic numerals. Structures of the suspension filter-
 356 feeding system are referred according to Wassersug⁴². Terms for hyobranchial skeleton
 357 follow Haas²⁹, whereas nervous system structures follow terminology of Schlosser and
 358 Roth⁴³.

359
 360 **Phylogenetic analyses.** The phylogenetic position of the *Notobatrachus degiustoi*
 361 tadpole was tested using a phylogenetic data matrix including 86 taxa and 152
 362 characters, modified from the analysis of Haas⁴⁴. A Bayesian tip-dating analysis was
 363 conducted in MrBayes 3.2.7a⁴⁵. We used an Mkv substitution model, the same list of
 364 ordered characters as in Roelants et al.⁸, and *Salamandrella keyserlingii* as the outgroup.
 365 We used an independent gamma rate relaxed clock model and uniform age priors
 366 modelled around the first appearance and last appearance dates for all tips of the tree.
 367 We implemented a node age calibration for Discoglossidae (uniform prior of
 368 120.0–120.1 Ma⁴⁶), Pipoidea (uniform prior of 113.0–113.1 Ma⁴⁷), Anomocoela
 369 (uniform prior of 52.0–52.1 Ma⁴⁸), and Pyxicephalidae (uniform prior of 39.5–39.6
 370 Ma⁴⁹). We specified a fossilized-birth death process as the tree model using standard
 371 parameterizations and values. Fossils were specified to be tips and that the fraction of
 372 extant species represented 98.8% of the terminals. The deepest split within the tree was
 373 parameterized with a uniform tree age prior of 250.0–272.8 Ma, following the minimum
 374 and soft maximum ages of Benton et al.⁵⁰ for crown-Batrachia. We used Metropolis
 375 Coupling Markov chain Monte Carlo (MCMCMC) algorithms with two independent

376 runs of 24 chains, using a heating coefficient of 0.05 and 3 swap attempts per
 377 generation. Topological convergence, indicated by average standard deviation of split
 378 frequencies decreasing below 0.01, was achieved after 15,435,000 generations
 379 (Extended Data Fig. 5). Potential Scale Reduction Factors (PSRF) of 1.0, visual
 380 inspection of trace plot with Tracer 1.7.1⁵¹, and estimated sample sizes (ESS) for all
 381 parameters >200 further indicated convergence. A second analysis was performed with
 382 the same settings, except that the topology was constrained following a molecular
 383 backbone of anuran phylogeny (Extended Data Fig. 6) that follows the results of Jetz
 384 and Pyron⁵². This analysis reached average standard deviation of split frequencies below
 385 0.001 after 4,010,000 generations.

386 The data matrix was also analysed under implied weighted maximum-parsimony
 387 with a range of concavity constant values (k) between 7 and 10⁵³ and using the program
 X 388 TNT 1.6⁵⁴. It ~~was~~ used the same list of ordered characters as in the Bayesian inference
 389 analysis. The search strategies started using a combination of the tree-search algorithms
 390 Wagner trees, tree bisection and reconnection (TBR) branch swapping, sectorial
 391 searches, Ratchet and Tree Fusing, until 100 hits of the same minimum tree length were
 392 achieved. The best trees obtained were subjected to a final round of TBR branch
 393 swapping. Zero-length branches in any of the recovered most parsimonious trees were
 394 collapsed and all the trees were rooted with *Salamandrella keyserlingii*. A global strict
 395 consensus tree (SCT) was generated from all the most parsimonious trees (MPTs) found
 396 in the analyses using the different k values (Extended Data Fig. 7). Homoplasy indices
 397 for each analysis under the different k values were calculated with a script that does not
 398 consider a priori deactivated terminals ('STATSb.run'). Group supports were quantified
 399 using no-zero weight symmetric resampling analyses, using 10,000 technical pseudo-
 400 replications and reporting both absolute and GC (group present/contradicted)

401 frequencies. The resampling frequencies were calculated from all the resampling trees
 402 recovered using the different k values and plotted on the branches of the global SCT.

403 Another analysis was conducted following the same matrix settings, tree search
 404 strategy, homoplasy indices calculation, and resampling analyses as before, but using a
 405 topological constrain based on the molecular backbone of anuran phylogeny (Extended
 406 Data Fig. 8) that follows the results of Jetz and Pyron⁵³. These parsimony analyses were
 407 implemented in two custom scripts written here for TNT
 408 ('treeSearches_protocol_Notobatrachus_constrained.run' and
 409 'treeSearches_protocol_Notobatrachus_unconstrained.run').

410

411 **Morphological disparity analysis.** Tadpole morphological diversity (disparity) was
 412 quantified on the taxon-character matrix modified from Roelants et al.⁸ using the R
 413 package Claddis v0.6.3⁵⁵. This matrix is the same as that used for the phylogenetic
 414 analyses (86 species versus 152 discrete morphological characters). The distance matrix
 415 was generated from the taxon-character matrix using the Maximum Observable
 416 Rescaled Distance (MORD)⁵⁵ because the commonly used Generalized Euclidean
 417 Distance (GED) may produce a strong methodological bias in matrices with a moderate
 418 to high amount of missing data^{56–58}. An ordination of the distance matrix was performed
 419 using a Principal Coordinates Analysis (PCoA) with Lingoes correction because of the
 420 presence of negative eigenvalues. No species had to be trimmed to conduct the
 421 ordination ~~because of the absence of missing data~~ ^{because no was missing} in the distance matrix. The
 422 phylomorphospace was plotted using the first two Principal Coordinates, which account
 423 for a 14.47% of the total variance of the dataset, and the majority rule consensus tree
 424 with mean ages of the time-calibrated Bayesian inference analysis. Ancestral node
 425 estimations and their position in the morphospace were conducted with the

426 phylomorphospace function of the R package Phytools⁵⁹. Extant anurans were
 427 graphically differentiated between non-neobatrachians and neobatrachians. Finally, a
 428 heatmap depicting the MORD of each species to *Notobatrachus degiustoi* was
 429 superimposed to the phylomorphospace biplot to show the row distance between
 X 430 tadpole morphologies and ^{their} ~~they~~ evolutionary divergence through time. The heatmap was
 431 built with the function 'filled.contour' of the R package akima⁶⁰.

432
 433 **Morphometric analyses.** We performed an exhaustive literature search for descriptions
 434 with measurements of tadpoles and adults of anuran species (Supplementary Table S2).
 435 For each species, we recorded tadpole body length (BL) and maximum total length
 436 (TL), and adult maximum snout-vent length (SVL). To reflect the size just prior to
 437 metamorphosis, we used measurements only from individuals at Gosner stages 39–41,
 438 ^{approximately} the same range to which the *Notobatrachus degiustoi* tadpole is assigned. For adults we
 439 took the largest measurement found in the literature. Our sampling includes 35 of 50
 440 anuran families and a total of 191 species. This information is detailed along with
 441 literature resources in Supplementary Table S2. > vs. 193 in caption for extended data figure 9

442 In order to obtain an estimation of the total length of the *Notobatrachus*
 443 *degiustoi* tadpole, we built a linear regression model of body length (BL) against total
 444 length (TL). Data on tadpole BL were available for fewer species than TL, and therefore
 445 we had to reduce our dataset (Supplementary Table S3). A Shapiro–Wilk test was first
 446 performed to address whether each trait was normally distributed, under the null
 447 hypothesis that the distribution was normal.

448 As we found no agreement for the definition of a gigantic tadpole, we defined it
 449 as those specimens having a TL equal or higher than twice the mean of the TL of the
 450 species sampled in our dataset. According to our database, the average TL among

Qualification needed because in main text (p. 4, line 94) you identify the fossil tadpole to a narrower range of Gosner stages 40–41. Also GS 40–41 in Suppl. Info, line 149

451 sampled taxa was 52.55 mm ($n = 193$); therefore, those tadpoles with a TL equal to or
452 over 105.1 mm were classified as giants (Extended Data Fig. 9). Afterwards, to
453 determine whether tadpole and adult body sizes were significantly related, and in
454 consequence, if giant tadpoles were related to giant adults, we tested the relationship
455 between tadpole and adult body size among species. We followed Womack & Bell³³ for
456 description of giant adults, defined as those having SVL higher than 127 mm. We
457 plotted the log-transformed tadpole TL versus the log-transformed adult SVL and we
458 calculated a linear generalized least squares (GLS) regression between these variables.
459 We have not conducted a phylogenetic GLS because available phylogenetic trees and
460 our dataset of measurements do not share most of the species level taxa and to include
461 phylogeny in the variance of the regression, we would have to prune most of the data. A
462 violin plot of the residuals of this regression was plotted and we highlighted the position
463 of the residual of *Notobatrachus degiustoi*. The residuals have been also optimized in
464 the Bayesian inference tree after the pruning of species that were not included in the
465 regression analysis and using maximum likelihood as optimality criterion (contMap
466 function of the R package Phytools⁵⁹). All the graphics and the regression were done in R
467 4.2.3⁶¹.

468

For clarity, this should be spelled out in Fig. 16, rather than using the undefined abbreviation "Ea"

596 **Extended Data legends**

597 **Extended Data Figure 1. a-b**, geographic location of the Estancia La Matilde locality X

598 (type locality of *Notobatrachus degiustoi*), in Santa Cruz Province, Argentina. c-d, ✓

599 several specimens of the branchiopod *Eosolimnadiopsis? santacruzensis* and plant

600 debris frequently found in the same levels as ~~*Notobatrachus*~~ *degiustoi* specimens in the X

601 Estancia La Matilde locality.

602

603 **Extended Data Figure 2.** High resolution photograph of MPM-PV 23333 taken under

604 long exposure time and sub-vertical artificial white light.

605

606 **Extended Data Figure 3.** High resolution photograph of MPM-PV 23333 taken under

607 short exposure time and low-angle artificial white light coming from the upper left

608 corner.

609

610 **Extended Data Figure 4.** Photography of the latex cast of MPM-PV 23333 taken under

611 low-angle light. **a**, cast of the complete specimen showing the preserved vertebral

612 elements; **b**, close-up of the skull and first vertebrae; **c**, close-up of the last vertebrae

613 and appendicular elements. Abbreviations: cl, cleithrum; cn, chondrified neurocranium,

614 f, femur; fo, fenestra ovalis; fp, frontoparietal; il, ilium; n, nasals; ps, parasphenoid; I- X

615 IX, presacral vertebrae; X, sacrum; 1–3 postsacral neural arches.

616

617 **Extended Data Figure 5.** Majority rule consensus tree recovered from the

618 unconstrained Bayesian phylogenetic analysis depicting the position of the

619 *Notobatrachus degiustoi* tadpole. Numbers at nodes indicate posterior probabilities and

620 dotted grey vertical lines indicate the boundaries between the Permian and Triassic and
 621 Cretaceous and Palaeogene geological periods, respectively.

622

623 **Extended Data Figure 6.** Majority rule consensus tree recovered from the constrained
 624 Bayesian phylogenetic analysis using a molecular backbone, depicting the position of
 625 the *Notobatrachus degiustoi* tadpole. Numbers at nodes indicate posterior probabilities
 626 and dotted grey vertical lines indicate the boundaries between the Permian and Triassic
 627 and Cretaceous and Palaeogene geological periods, respectively.

628

629 **Extended Data Figure 7.** Global strict consensus tree recovered from the unconstrained
 630 parsimony phylogenetic analysis depicting the position of the *Notobatrachus degiustoi*
 631 tadpole. Numbers above branches indicate absolute (left) and GC (group
 632 present/contradicted) (right) no-zero weight symmetric resampling frequencies (values
 633 between squared brackets are negative values).

634

635 **Extended Data Figure 8.** Global strict consensus tree recovered from the constrained
 636 parsimony phylogenetic analysis using a molecular backbone, depicting the position of
 637 the *Notobatrachus degiustoi* tadpole. Numbers above branches indicate absolute (left)
 638 and GC (group present/contradicted) (right) no-zero weight symmetric resampling
 639 frequencies.

640

641 **Extended Data Figure 9. a,** Plot depicting the total length of tadpoles across 193
 642 species. Red line denotes the mean of the surveyed values (52.55 mm), whereas blue
 643 line denotes twice the mean (105.1 mm). Species are alphabetically ordered. Labelled
 644 species are those with gigantic tadpoles. **b,** Optimization of the residuals of the linear

vs. 181 on p. 20,
line 440

645 regression between the tadpole total length and adult snout-vent length in the Bayesian
646 tip-dating tree (using mean ages), showing the independent acquisition of gigantism in
647 *Notobatrachus degiustoi* and some crown-anuran species.

SUPPLEMENTARY INFORMATION

The oldest tadpole reveals evolutionary stability of the anuran life cycle

Mariana Chuliver, Federico L. Agnolín, Agustín Scanferla, Mauro Aranciaga Rolando, Martín D.

Ezcurra, Fernando E. Novas, Xing Xu

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1. Geology and paleoenvironment

MPM-PV 23333 was found in a quarry of the Estancia La Matilde, located 100 km

SW of Puerto Deseado city, Santa Cruz Province, Argentina (Extended Data Fig. 1). This

quarry was named “Estancia La Matilde locality” in the literature¹⁴ and it is the type locality of *Notobatrachus degiustoi*. The rocks exposed at this locality belong to the La Matilde Formation¹⁴.

The La Matilde Formation, a volcanoclastic unit that crops out in several localities in Santa Cruz Province, ^{and} is laterally and vertically interbedded with the Chon Aike Formation, a pyroclastic unit covering an area of some 100000 km²(^{15, 62}). The La Matilde Formation was considered ~~as~~ ^{by} Bathonian–Callovian in age (Middle Jurassic) based on fossil floral remains by Stipanovic & Bonetti⁶³. However, the age of the La Matilde Formation is also established based on the radiometric dating of volcanic rocks of the interbedded Chon Aike Formation. Whole-rock K-Ar dates ranging from 161 ± 10 (Oxfordian, Late Jurassic) to 138 ± 10 Ma (Valanginian, Early Cretaceous) were obtained from samples ⁱⁿ at the Gran Bajo de San Julián area⁶⁴. Rb-Sr whole-rock analyses were also carried out on several lavas and associated ignimbrites from the eastern outcrops of the Chon Aike Formation, resulting in an isochron of 168.2 Ma (Bathonian, Middle Jurassic^{65,66}).

MPM-PV 23333 comes from fine grained, light-grey, tuffaceous sandstones deposited in alternating thin light and dark layers, which correspond to the “Fossiliferous level I” recognized by Stipanovic and Reig¹⁴ in their original description of the “Estancia La Matilde locality”. Geological data for the La Matilde Formation indicate that these sediments were deposited in lacustrine environments, scattered across the floodplain and with pyroclastic material input under ^{summer} ~~a seasonally~~ dry-winter wet ^{climatic conditions} biome^{15,16}. ^{In particular,} ~~the~~ Estancia La Matilde locality has yielded hundreds of adult specimens of *Notobatrachus* ^E *degiustoi* preserved as impressions, but also contains diverse plants, branchiopods, bivalves, and insects (Extended Data Fig. 1). Carapaces of the clam shrimp *Eosolimnadiopsis? santacruzensis*¹⁷ are frequently found in the sandstones along with adult

specimens of *N. degiustoi*. It is noteworthy that clam shrimps (Spinicaudata) currently live in temporary water bodies^{67,68}. Interbedded between the frog-bearing levels are abundant vegetal detritus, provided by surrounding forests composed of Equisetales, Osmundales, Coniferales, Araucariaceae, and Podocarpaceae^{18,19,69–71}. Taken together, sedimentological and fossil data suggest that the fossil-bearing levels at Estancia La Matilde locality were deposited in shallow, ephemeral lentic environments with a high input of plant debris.

2. Additional anatomical information

MPM-PV 23333 represents an incomplete tadpole preserved in one slab in dorsal aspect, with most of the right half of the body and the proximal part of the tail (Extended Data Figs. 2–3). There is a whitish area delimiting the contour of the specimen, which clearly has a polished surface that contrasts with the rugose surface of the slab, allowing us to distinguish soft parts of the body and tail (Extended Data Figs. 2–3). In general, preserved structures exhibit a high degree of anatomical relations, with skeletal elements in articulation and soft parts in natural in position.

Skeletal elements are represented only by impressions, mirroring the taphonomic mode observed in most adult specimens of *Notobatrachus degiustoi*. Some elements (e.g., cleithrum and femoral shaft) left a striated imprint, probably due to the incipient ossification stage of these bones. Several soft parts, including eyes and nerves, were preserved as dark imprints, easily identified by their respective anatomical positions (Extended Data Fig. 2).

Both nasals are present, each represented by an anteriorly concave ossification lying close to the anterior end of the frontoparietals (Extended Data Fig. 4). Poorly defined dark

71 areas anterior to the nasal ossifications ~~that~~ might correspond either to nasal cartilages or to
72 rostral cartilage. The ossification corresponding to the parasphenoid bone is visible in the
73 median region of the cranium, flanked by the paired frontoparietals, the latter of which
74 extend as longitudinal thin strips of bone widely separated from each other along the
75 midline and markedly pointed anteriorly. The posterior region of the chondrified
76 neurocranium is preserved as imprints, whereas the right otic region is represented by a
77 subtriangular and knob-like prominence, the crista parotica. A large circular notch is present
78 laterally in the crista parotica, which corresponds with the fenestra ovalis (Extended Data
79 Fig. 4). The crista parotica exhibits a simple structure, lacking anterolateral (*processus*
80 *anterolateralis*) and posterolateral projections, like in early-diverging crown-anurans such
81 as *Ascaphus* and discoglossids⁴⁴. ~~The exoccipitals ossifications might be inferred in the~~ ^{may be present}
82 midposterior region of the skull (Extended Data Fig. 4). ^{although their identity is not certain.}

83 The right hyobranchial skeleton is well preserved in MPM-PV 23333. A ceratohyal
84 is represented by a dark robust plate placed lateral to the otic region, with its medial and
85 lateral regions delimited by a slight constriction. The medial part of the ceratohyal exhibits
86 both a *processus anterior* and a *processus posterior*, whereas the lateral part shows a
87 *processus lateralis* (Fig. 2). A *condylus articularis* is also distinguishable, implying the
88 presence of an articulation with the unpreserved palatoquadrate cartilage. The overall
89 morphology of the ceratohyal strongly resembles that of extant tadpoles, in which processes
90 of this element provide attachment surfaces for muscles that power the buccal pump^{5,29,30}.

91 Slightly ornamented ceratobranchialia are preserved in MPM-PV 23333 as four dark
92 mediolaterally oriented bands, with bright ellipsoid fields between them representing
93 branchial slits (Extended Data Fig. 2). Ceratobranchial I is partially overlapped by the
94 ceratohyal. Ceratobranchials II–IV are proximally connected by commissurae proximales

↑
or similar
qualification

demonstrates a conservative morphogenetic developmental pathway for the urostyle, a key evolutionary feature of the anuran *bauplan*¹.

The pectoral girdle is only represented by the cleithrum, which is an elongate bar overlapping the rib of vertebra II. The right forelimb is preserved within the body limits, lateral to the hyobranchial skeleton (Extended Data Figs. 2–3). It is represented by the radio-ulna and the manus. The radius and ulna are fused, although the suture is still clearly distinguishable. Metacarpals II–V are represented as the imprints of cylindrical shafts, with slight lateromedial overlapping between them. Metacarpal IV is the longest and stoutest. The proximal phalanges of the four digits are short cylindrical elements with a slight constriction around mid-length, whereas the central phalanges are only present in digits IV and V.

The posterior appendicular skeleton is represented by the ossifications of the right ilium and femur, placed posterolaterally to the third postsacral vertebra (Extended Data Fig. 3). The right iliac shaft is preserved in lateral view, with the dorsal acetabular expansion already ossified. The femur extends laterally, with its diaphysis oriented almost perpendicular to the axial skeleton, and its proximal epiphysis is slightly overlapped by the ilium (Extended Data Fig. 3).

2.1 Ossification state and ontogenetic stage of *Notobatrachus* tadpole

MPM-PV 23333 is at an advanced stage of development, just prior to the beginning of metamorphosis, as indicated by the presence of the forelimb within the body contour and the state of development of cranial and postcranial ossifications (Extended Data Fig. 4). During development, the parasphenoid, frontoparietals and exoccipitals are the first skull ossifications to be differentiated^{77–81}. These ossifications are present in the *Notobatrachus*

142 *degiustoi* tadpole, demonstrating a conservative onset of ossification in anurans. Regarding
143 the vertebral column, ossification of sacral diapophyses starts during metamorphic
144 climax²¹, whereas the ossification of the coccyx is only established late during
145 metamorphosis^{1,76}. This is congruent with the developmental stage of the vertebral column
146 of the *N. degiustoi* tadpole, allowing us to infer a stage previous to metamorphosis.
147 Furthermore, the presence of an ossified central phalanx, fused radio-ulna, and ilium not
148 contacting the sacral vertebra allow us to refer MPM-PV 23333 to a late developmental
149 stage. In sum, we interpret the tadpole of *N. degiustoi* to be at Gosner Stage 40–41²³.

↪ see my comment
letter p. 20

151 2.2 Soft parts

152 The exquisite preservation of MPM-PV 23333 sheds light on some remarkable
153 aspects of the soft anatomy of the tadpole of *Notobatrachus degiustoi*. Both eyes are
154 preserved, but only the right is complete (Extended Data Fig. 2). The eyes are dorsally
155 positioned; the lens within the right eye is clearly distinguishable in the anterolateral region
156 of the eyeball, which is indicative of the eye's anterolateral facing direction.

157 Several nerve impressions are distinguishable on the right side of the cranial region
158 (Fig. 2). Based on comparisons with extant crown-anurans such as *Discoglossus pictus*⁴³
159 and several neobatrachians⁸², we differentiate three different rami of the trigeminal nerve
160 anterior to the eyeball. The truncus maxilo-mandibularis of the trigeminal nerve diverges
161 around the middle of the orbit into the medial maxillary ramus (V₂) and the lateral
162 mandibular ramus (V₃). Posterior to the eyeball, a thickened area is observed (Fig. 2). This
163 structure is identified as a ganglionic complex, whose neural components cannot be clearly
164 ascertained, but likely corresponds to ganglia of nerves V and VII^{72,83}. Posteriorly to this
165 ganglionic complex, there is a prominent hyomandibular trunk (truncus hyomandibularis)

166 that extends toward the ceratohyal in the ramus hyoideus of the facial nerve (VII). The
167 hyomandibular trunk also diverges distally into two main rami: the internal mandibular
168 ramus of the facial nerve and the external mandibular ramus. The latter corresponds to a
169 composite of fibres belonging to the facial nerve and fibres belonging to the anteroventral
170 lateral line in extant anuran tadpoles^{43,83,84}.

172 2.3 Bromalite

173 On the right side of the axial skeleton, from the level of vertebra V to postsacral 3,
174 there is a compact, yellowish accumulation of particles, enclosed within the tadpole body
175 limits (Extended Data Figs. 2–3). It has rounded boundaries and a roughly ovoidal shape. It
176 extends posteriorly as a narrow, diffuse accumulation that ends near the base of the tail, at
177 the level of the anterior end of the ilium. Several crystalline grains are observed, thus
178 contrasting with the rest of the very fine-grained slab containing the specimen. We consider
179 this accumulation as representing in situ intestinal matter or a cololite⁸⁵. The preserved
180 cololite is not ~~disposed on~~ ^{in the form of} a spiral, but instead suggests a sack-like structure for the
181 digestive system. This could be explained by the developmental stage of the *Notobatrachus*
182 *degiustoi* tadpole, which is at the end of prometamorphosis, when the coiled intestine of
183 filter-feeding tadpoles undergoes deep structural changes to become a sac like gut in
184 adults³⁵.

186 3. Morphometric analyses

187 We estimated the total length of the *Notobatrachus degiustoi* tadpole at 159 mm,
188 placing it within the size range of extant giant tadpoles (Extended Data Fig. 9). Notably, the
189 largest postmetamorphic specimens of *N. degiustoi* reached a body size (snout-vent length)

of 150 mm¹², which also is within the range of giant adult anurans (>127 mm SVL)³³. We found a weak correlation ($r^2 = 0.44$; $p < 2.825e-10$) between tadpole and adult body size in contrast to Emerson³¹, but in agreement with a recent comprehensive study about the evolution of body size in tadpoles and adult anurans⁸⁶.

4. Phylogenetic analyses

We employed the morphological matrix of Haas⁴⁴, excluding characters 12, 83, 116, and 117 following Roelants et al.⁸, resulting in a matrix of 152 characters and 86 terminals ~~for~~^{with} the inclusion of the *Notobatrachus degiustoi* tadpole.

1.1 Character list (modified from Haas [2003]⁴⁴)

- 1 Epidermal melanocytes elongated and forming orthogonal mesh (0); melanocytes of irregular shape not forming reticulation (1)
- 2 Larval subdermal serous glands absent (0) present (1).
- 3 Keratodonts absent (0) present (1).
- 4 Labial ridges with single row of keratodonts (0) ridges each with double (or more) rows of keratodonts (1).
- 5 Distal anterior labial ridge and keratodont-bearing row broad (0) very short and median (1).
- 6 Keratinized jaw sheaths absent (0) present (1).
- 7 Oral lips absent (0) present (1).
- 8 Upper lip papillation continuous (0) broad diastema present (1).
- 9 Lower lip papillation complete without gap (0) diastema in papillation (1).
- 10 Oral orifice wide (0) narrow (1).

Referee #3 (Remarks to the Author):

I am good with the revised version of this manuscript. The revised manuscript is now in good shape in terms of writing, figures and data, and the authors made corrections and changes according to my concerns on the morphological interpretation and gave good arguments on why more fossil tadpoles were not included in the morphological disparity analysis although I am still curious of the results when including *Paleobatrachus* and other fossil tadpoles, even though the results might be artefactual. Otherwise I have only two small concerns as stated below.

1. Figure 1c, fo – fenestra ovalis: as Dr. Roček commented that fenestra ovalis labelled as it is now, misleads the readers to take the lateral extension of the otic capsule as fenestra ovalis. Fenestra ovalis is encircled by bone of otic capsule whether is 'positive' or 'negative'. Maybe it is better not to make it in color.
2. 'hyobranquial' should be corrected all through the manuscript.

Referee #4 (Remarks to the Author):

I have only three comments, the most important is the first one:

Lines 28-29:

The statement 'the post-metamorphic anuran fossil record that dates back to the Early Jurassic', combined with the reference to the paper by Shubin and Jenkins (1995), is clear and straightforward, and may raise suspicion that the authors are unaware of the more recent paper by Stocker et al. (2019), especially as it is not included in the reference list. They should at least mention that such a paper has been published, but for the reasons given in their rebuttal, they do not consider this necessary.

Lines 128, 134: hyobranchial, instead of 'hyobranquial'.

Line 145: It would be worth repeating 'whose giant tadpoles naturally occur', not just tadpoles.

Zbyněk Roček

Author Rebuttals to First Revision:

Please, find below detailed comments on each point raised by the referees (referee's comments in bold and italics).

REFEREE #1

The revised version of the manuscript "THE OLDEST TADPOLE REVEALS EVOLUTIONARY STABILITY OF THE ANURAN LIFE CYCLE" by Chuliver et al. is much improved and the authors responded to the suggestions adequately and to my satisfaction. They are reporting an important and exciting fossil find, which in my opinion is well worth of publication in Nature.

There are a few points that I think would improve the manuscript further prior to publication, all of which are minor and can be included quickly.

1. As I pointed out in the first review, it is intriguing that amongst so many very well-preserved adults, this is the only tadpole. In their response the authors state that this is a common pattern in fossil sites mentioning the Messel site. However, Messel is a rather peculiar fossil Lagerstätte with very unique and specific taphonomic conditions (being a caldera and fossils preserved in oil shale, which are very hard to recover and preserve) and there are a lot of taphonomic oddities at that site. There are other localities, however, that have yielded numerous adult frog fossils and abundant tadpoles (e.g. the Enspel lake or the Tayasir Formation that yielded Shomronella), therefore the question, why no further tadpole specimens have been found at the La Matilde locality does spring to mind immediately. To avoid misunderstanding: the argumentation by the authors that this is a taphonomic relic and probably also a collection bias is entirely conceivable, I just think it should be briefly, but clearly stated in the main text to clarify the point why this is the first tadpole find.

We added a sentence in the main text stating that this specimen is the only pre-metamorphic specimen found at the locality, probably because a collection bias and/or a taphonomic issue.

2. The authors did address the point that this tadpole is gigantic, but naturally so (rather than pathologically). Here also, I agree with and can follow their assessment and their interpretation is conceivable. The section, where this is briefly discussed in the main manuscript is a bit confusing though, which is primarily a matter of wording not of the content. I made a suggestion using track changes in word (see attached file) how these 1-2 sentences could be rephrased to make this point clear for readers.

We modified the text following the referee's suggestions to rephrase the sentences stating that the giant tadpole of *N. degiustoi* naturally occurred and successfully metamorphosed. We also incorporated the suggestions made by the referee in the paragraph where is stated that larval gigantism in *N. degiustoi* evolved independently from gigantism in crown anurans.

Regarding the terminology of gigantism, I think this needs to be clarified some more than it currently is. This could be in the supplementary information if the authors do not want to include it in the main text, but it requires another short paragraph or so. This is currently only very briefly mentioned and the exact meaning in your paper remains a bit obscure, as it is not further explained in the main document and supplementary information beyond a reference to Womack and Bell (2020).

The reason why I think this is important here is that gigantism plays a crucial role in the presented manuscript, but the terms "gigantism" and the opposite "miniaturization" are used in the literature in a rather wide spectrum of meanings, often just loosely applied to organisms that are very large (or small) rather than referring to an explicit and testable evolutionary trend within a lineage. I think in this study, you are referring to the latter and argue along these lines as well (e.g. lines 265-268). Gigantism in this meaning and context has been the subject of detailed studies (including influential papers such as Hanken & Wake 1993, Gould & MacFadden 2004, Vermeij 2016 amongst others) and explicit definitions. I think it is necessary to clearly state and define your meaning of gigantism here.

The ambiguous use of the term 'gigantic' is a contentious aspect in studies of extant anurans, and therefore we followed the methodology employed by Womack & Bell (2020) for adult anurans in their broad-scale analysis of anuran body-size evolution. Accordingly, in the **Methods** section we stated: 'As we found no agreement for the definition of a gigantic tadpole, we defined it as those specimens having a TL equal or higher than twice the mean of the TL of the species sampled in our dataset. According to our database, the average TL among sampled taxa was 52.55 mm (n = 187); therefore, those tadpoles with a TL equal to or over 105.1 mm were classified as giants (Extended Data Fig. 9)'.

To make it clearer in the main text, we added a sentence about the definition of gigantism considered in our study (lines 155-157). In addition, we further explained definitions of adult and larval gigantism in the Supplementary Information (Section 3. Morphometric analyses).

3. Other than the points above, there are a few very minor points I noted related to wording/grammar in the paper, which I also indicated using track changes in the attached document.

We modified the wording and grammar throughout the entire manuscript based on the suggestions made by referee 1.

REFEREE #2

*Having reviewed the original version of this MS, I was excited to see this revised version. I am pleased to see the authors satisfactorily dealt with my comments, queries, and suggestions. Their revised MS is much improved. They have done a better job of detailing where the fossil was collected and its geologic age, better supported their taxonomic identification and interpretation of the ontogenetic age of the fossil, improved the labelling of anatomical structures in their interpretive line drawings, and added a table of absolute size ranges for Mesozoic frogs that helps bolster their argument about tadpoles and adults of *Notobatrachus degiustoi* being unusually large compared to other Mesozoic and extant frogs. I continue to regard this report as interesting and significant enough to warrant publishing in a high-profile journal such as *Nature*. For reasons outlined below, I recommend this MS be accepted pending minor revisions.*

Once again, I am providing a hand annotated copy of select portions of the MS (main text, captions, and figures; extended figure captions; and first nine pages of the Suppl. Info). As you will see from that document, most of my points deal with wording.

All of the suggestions made by referee 2 in his hand-annotated copy of the main text, figure captions, Extended Data Figures, and Supplementary Information were addressed, modifying the files accordingly.

Other minor points are as follows:

1) The colour used for the frontoparietals in the interpretive drawings in the first two figures differs: pink in Fig. 1c vs. red in Fig. 2b. For consistency, the same colour should be used in both and for clarity should be unique to that element. Regarding the latter point, note the same shade of red (or so it appears in my review copy) used for the frontoparietals in Fig. 2b also is used for the ilium in Fig. 1c and the same shade of blue (or so it appears in my review copy) is used for both the femur and hyobranchial skeleton in Fig. 1c.

We modified the colour used for the frontoparietals to use the same shade of red in both figures. Also, we modified the colours used for the ilium and femur in Fig. 1c.

2) For Fig. 2, the abbreviation “oc” appears and is defined in the caption, yet that abbreviation does not appear in the corresponding interpretive line drawing.

We removed the abbreviation from the figure caption to avoid adding more references to the interpretative drawing and address comments made by referees #3 and #4.

3) In my review copy, the three dotted lines in Fig. 3c are so small and faint that they are nearly invisible, especially to those of us with aging eyes. Maybe that is just an artifact of how the review copy prints? Regardless, it is worth trying to enhance those lines (e.g., make them thicker), so they are clearly visible in the final publication.

We made the three lines thicker and changed their colours so they are more clearly visible now.

4) Three points for the “Morphometric analyses” section on p. 20:

a. It is unclear whether your “measurements of tadpoles and adults of anuran species” (line 434) is limited to extant species. I think that is the case, but it is worth clarifying in the text.

b. The statement that you “used measurements only from individuals at Gosner stages 39–41, the same range to which the tadpole of *Notobatrachus degiustoi* is assigned” (lines 437 and 438) needs to be qualified, because elsewhere you explicitly regard the fossil as being within a narrower range of Gosner stages 40–41. That qualification can be accomplished by adding “approximately” in front of “at the same range”.

c. Discrepancy in number of species measured for size: 191 (line 440) vs. 193 in caption for Extended Data Fig. 9.

a. The measurements include species of extant tadpoles but one fossil species (*Palaeobatrachus hauffianus*). We added the information for clarification.

b. We modified the sentence according to the referee’s suggestion.

c. We amended the discrepancy in the number of species following the referee’s observation.

5) Discrepancy between how the locality for the fossil tadpole is presented in Extended Data Fig. 1: spelled out as “Estancia La Matilde” in caption vs. abbreviated (and not defined in caption) as “Ea. La Matilde” in corresponding figure.

We explained the abbreviation for “Estancia” in the figure caption according to the referee’s suggestion.

REFeree #3

*I am good with the revised version of this manuscript. The revised manuscript is now in good shape in terms of writing, figures and data, and the authors made corrections and changes according to my concerns on the morphological interpretation and gave good arguments on why more fossil tadpoles were not included in the morphological disparity analysis although I am still curious of the results when including *Paleobatrachus* and other fossil tadpoles, even though the results might be artefactual.*

Otherwise I have only two small concerns as stated below.

1. Figure 1c, fo – fenestra ovalis: as Dr. Rocek commented that fenestra ovalis labelled as it is now, misleads the readers to take the lateral extension of the otic capsule as fenestra ovalis. Fenestra ovalis is encircled by bone of otic capsule whether is 'positive' or 'negative. Maybe it is better not to make it in color.

The figure was modified according to the referee's observation.

2. 'hyobranquial' should be corrected all through the manuscript.

We modified the spelling throughout the manuscript according to the referee's correction.

REFeree #4

I have only three comments, the most important is the first one:

Lines 28-29:

The statement 'the post-metamorphic anuran fossil record that dates back to the Early Jurassic', combined with the reference to the paper by Shubin and Jenkins (1995), is clear and straightforward, and may raise suspicion that the authors are unaware of the more recent paper by Stocker et al. (2019), especially as it is not included in the reference list. They should at least mention that such a paper has been published, but for the reasons given in their rebuttal, they do not consider this necessary.

As we explained before, the work we cited corresponds to *Prosalirus bitis* (Shubin and Jenkins, 1995), a species described based on a complete specimen, that exhibits several crown-anuran features, thus allowing a precise phylogenetic inference. We consider that the isolated, fragmentary specimen from the Upper Triassic Chinle Formation described by Stocker et al. (2019) represents a poorly phylogenetically constrained record. However, to state that we are aware of that finding and we do not neglect the published information, we added the citation of the paper by Stocker et al. (2019).

Lines 128, 134: hyobranchial, instead of 'hyobranquial'.

We modified the spelling of the word throughout the manuscript according to the referee's correction.

Line 145: It would be worth repeating 'whose giant tadpoles naturally occur', not just tadpoles.

We added the word 'giant' to the sentence according to the referee's correction.