

Dear Dr. Sobral,

Many thanks for your time and prompt attention to our manuscript on armadillo physiology and morphology, submitted for peer-review at PCI-Ecology. Our responses to all reviewer comments are embedded below in bold type. All of the edits to our manuscript are similarly highlighted with track-changes on a revised version now available on the osf.io site, along with our figures and tables. We have also made our former "appendix 1" (now supplementary appendix S2) part of our online supplementary data and provided the URL where these may be found (line 645).

We hope these changes are sufficient to address the reviewer comments which have helped to improve our manuscript.

On behalf of my coauthors, I am very grateful to you and the reviewers for your time in reviewing and editing our manuscript.

Sincere regards,

Robert Asher, Department of Zoology, University of Cambridge

Our revised manuscript, tables, figures, supplementary data, and reviewer responses are available here:

<https://osf.io/dpw69/>

Round #1

Decision

/by Mar Sobral, 2020-01-08 11:54/
Manuscript: <https://osf.io/q8ndp>

Thanks for your interesting preprint

Two reviewers and myself have thought the manuscript is clear and interesting.

Some comments have been made by reviewers which are easy to implement and will improve the manuscript.

Please send a revision having into account the comments made by the two reviewers.

Best regards!

We're very grateful for your speedy and constructive review of our manuscript.

Reviews

/Reviewed by Alexandra Panyutina, 2019-12-28 17:43/

On the manuscript

«Body temperatures, life history, and skeletal morphology in the nine-banded armadillo»
by Frank Knight, Cristin Connor, Ramji Venkataramanan, Robert J. Asher

Very interesting animal: belongs to one of the earliest-diverged placental lineages, is one of the few mammals that invaded North America from South America, is one of the few placentals withstanding considerable decrease of body temperature without torpor stance, is the only mammal (and not only mammal) which normally gives birth to monozygotic quadruplets.

Very important problem: the body temperature fluctuations during gestation, their inherent periodism, association with ambient temperature fluctuations, gestation. The separate task was checking an ad hoc hypothesis that the gestation temperature influences the vertebral numbers.

Shortcoming of the subject which greatly complicates solution of the problem is delayed implantation. The duration of the delay is unknown. Therefore the implantation date is derived from the birth day based on an approximate estimate of 4.5 month gestation. This time is subtracted back from the date of birth which is, in turn, not known outdoors since the birth occurs under ground. However, the authors did all possible precautions in their analysis.

Again we're grateful for R1's thoughtful assessment.

Methods. A wide variety of advanced experimental and analytical techniques is employed. They include intra-abdominal implantation of long-term temperature recorders, analysis of body temperature periodism by approximation of the recordings with Fourier series, and CT-scanning of fetuses. Results. Pronounced 12 and/or 24 periods in body temperature fluctuations. Counter-intuitive finding that the body temperature fluctuates in the 9-banded armadillo as if inversely proportional to the ambient temperature — less outdoors than indoors. Gestation is accompanied with a not much pronounced reduction of the range of body temperature fluctuations. BUT... nothing important on the vertebral variation in pups: the variation is inside the range known from the earlier research and

We would argue here that the documentation of variation in genetically identical quadruplets (as shown in Figs. 9 & 10) is a novel & important result. We have therefore underscored this result in our text (e.g., lines 390-92 & 518-519). Previous studies have documented variability in both temperatures and vertebral counts, but to our knowledge none has yet presented CT scans & corresponding anatomical details for all pups from individual litters or conclusively demonstrated divergent morphologies among armadillo littermates.

does not depend from any factor taken into account — neither from genetic identity, nor from gestation temperature. In general, the manuscript supplies very thoroughly collected, very rare, interesting and important data on temperatures which should be definitely published as a preprint. However, the vertebrologic part is premature. I suggest to remove it from the main text and possibly include the CT results as supplementary material but without any theoretic considerations which look too huge relative to the facts.

We're sympathetic to R1's concern, but in our view the results derived from vertebral morphology among littermates (and from dams with known body temperatures over many weeks) is among the most important in our paper. Our discussion of morphology is indeed derived from the facts and figured & discussed in the text, and furthermore provides a basis upon which further research can proceed. The original hypothesis for this project was the notion that temperature variation drives high-levels of intraspecific variation in vertebral counts in xenarthrans, including armadillos. As discussed in the text, the link with body temperatures remains elusive (lines 499-516), but the divergent vertebral morphologies in some litters of genetically identical individuals is unambiguous. This is a novel result which comprises a key part of our research, and we would very much like to retain it in our main text (e.g., revised abstract and lines 518-519). We have edited our abstract to further highlight the important & novel results derived from our examination of vertebral morphologies.

If the authors will continue the vertebrology research, I would advice to take into account two sources which they missed:

Jenkins, Farish A., Jr. 1970. Anatomy and Function of Expanded Ribs in Certain Edentates and Primates. *Journal of Mammalogy*, Voi. 51, No. 2 (May,), pp. 288-301
<http://www.jstor.org/stable/1378479?origin=JSTOR-pdf>

Kuznetsov, Alexander N. 2007. The Definitive Variation of the Vertebral Column in *Rhea americana* L.: the Concept of Morphogenesis Precision. (Conference Poster)
<https://www.researchgate.net/publication/337732771/Precision/of/morphogenesis/concept>

These are helpful comments and we have incorporated the Jenkins 1970 citation into our Discussion, lines 460-463. We would like to also cite Kuznetsov 2007, but the researchgate.net link above ("DOI: 10.13140/RG.2.2.10864.8192") does not appear to link to any journal articles or books from a source indexed on scholar.google.com. We would prefer not to cite just a researchgate.net URL without a full citation for a journal article, edited book, thesis, or conference proceeding. Our search has likely just missed the citation as it is in Russian so if the reviewer or editor can advise what the correct journal/book citation is (preferably peer-reviewed), we'd be happy to incorporate it.

/Reviewed by Darin Croft, 2019-12-16 17:28/

This is a clearly-written, well-crafted, hypothesis-driven manuscript. With the caveat that my expertise lies in anatomy rather than physiology, I found no major issues with the

manuscript. The experimental design and execution strike me as reasonable, and the authors are forthright about the potential shortcomings in their data and interpretations. The result is new information that is of clear value to the scientific community.

We're grateful for these constructive comments.

The only minor issue I found is one that can easily be resolved: information should be provided in the methods section about how different vertebral categories are recognized. Perhaps the criteria of Asher et al. (2011) were used; if so, that should be stated explicitly. I suspect some modification of those methods was used, as the counts of Asher et al. (2011) did not deal with “half vertebrate” (i.e., those with single ribs).

This is now fixed (lines 220-227)

The only typographical modification to the manuscript I would suggest is clarifying that the values listed on line 352 represent hours.

Fixed (lines 360-362)

There are a few typos in the figure captions. In Figure 1, *D. novemcinctus* should be italicized (though this may be an artifact of how captions are uploaded) and scalebars should be two words (this also applies to Figures 9-10). Also in Figure 10, the initial word should be capitalized.

Fixed.