

What's my age again? On the ambiguity of histology-based skeletochronology

Pia J. Schucht, Nicole Klein and Markus Lambertz

Article citation details

Proc. R. Soc. B **288**: 20211166.

<http://dx.doi.org/10.1098/rspb.2021.1166>

Review timeline

Original submission: 14 January 2021

1st revised submission: 21 May 2021

2nd revised submission: 24 June 2021

Final acceptance: 24 June 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

Decision letter (RSPB-2021-0102.R0)

17-Jan-2021

Dear Dr Lambertz:

Thank you for submitting your manuscript RSPB-2021-0102 entitled "What's my age again? – On the ambiguity of histology-based skeletochronology" to Proceedings B.

All manuscripts are assessed by a specialist member of the Editorial Board, who decides whether the manuscript is suitable for Proceedings B.

Unfortunately, your manuscript has been rejected at this stage of the assessment process. Competition for space is currently extremely severe, and we receive many more manuscripts than we are able to publish. On this occasion it was felt that your manuscript was unlikely to be able to compete successfully for a space in the journal.

Please find below the specialist Board member's comments. I hope you may find these useful should you wish to submit your manuscript elsewhere.

Sincerely,

The Proceedings B Team

<mailto:proceedingsb@royalsociety.org>

Board Member

Comments to Author(s):

This study addresses an important question: how accurate are estimates of specimen age based on histology-based skeletochronology? The results show varying degrees of correspondence with the actual age of the specimen across taxonomic groups and specimen ages, and between petrographic ground sections and microtomed sections. These results will be valuable to the research community in that they sound a cautionary note for researchers who rely on this type of data, but they are not particularly surprising. As the authors acknowledge, previous studies (e.g., Castanet et al. 1992) have shown comparable variation and inaccuracies in results from skeletochronology, especially in endothermic and older animals. A method for quantifying the uncertainty of these estimates in different types of samples would be useful.

RSPB-2021-1166.R0

Review form: Reviewer 1 (Alexandra Houssaye)

Recommendation

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

Dear Editor,

This paper proposes a critical analysis to estimate the accuracy of skeletochronology. The authors carefully analyze results obtained from petrographic ground sections versus stained microtomed sections made in the same bones of various tetrapods. The study is done with caution (I notably appreciate the effort to scan bones first in order to precisely locate the area with the thickest cortex); results are clearly presented (great suppl. Data!). Results highlight difficulties in using LAGs to estimate age. This is a major result since skeletochronology is often used with closed eyes without questioning enough the possibility of bias.

The paper is well written; I do not have an attached pdf with comments, which is rare ;)

I have minor comments:

L63 "but not in endotherms"; please adapt the sentence since you know that growth marks are also found in endotherms (e.g. Köhler et al 2012; Castanet et al 2004, already cited);

L84 please add ref. about the observation modes.

Figures 1 and 2 are mixed!! This was disturbing and maybe strengthened the fact that I do not find it straightforward for the reader to understand why for *Lacerta* there is only one lag in (1), whereas 2 are counted for (4)-(5) in *Pogona*. Maybe a few sentences should be added about splitting since, beyond the mouse, it might be more widespread (being also observed in *Varanus*; Buffrénil & Castanet 2001; also cited), even in your sample.

Generally the discussion might benefit from a few sentences about the cases where skeletochronology seemed to work "perfectly". There might be interest also in citing Castanet et al. 1992 (Castanet, J., Meunier, F. J., & Francillon-Vieillot, H. (1992). Squeletteochronologie à partir des os et des dents chez les vertébrés. ; though in French, easily translatable online now ;) where limits are already highlighted, to insist on the fact that even historically it was never "perfect" and that even the "fathers of skeletochronology" discussed about limits, their causes, and how to possibly bypass them.

Except from these few comments, I have nothing to add about this paper, which is a great contribution to our field! Skeletochronology is more and more used, especially in paleontology. Recently more and more doubts are raised about skeletochronology and this study offers a great and an unprecedented analysis to highlight problems, raise questions, and stimulate more careful works and inferences in the future.

I thus strongly recommend publication.

Best regards,

Alexandra Houssaye

Review form: Reviewer 2**Recommendation**

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

In this piece, Schucht et al. make an extensive assessment of the most prominent methods in histology-based skeletochronology. The study is well executed and the results relevant to a broad range of researchers, raising a critical note of caution on these methods.

My only comments focus on the discussion. As the authors mention, there is lots of research to be done before histological skeletochronology can be used broadly for age estimation. However, it seems like the authors fall short of suggesting specific research avenues with reference to our knowledge of osteology, development, chemistry, and alternative methods. In other words, the discussion does not give a clear outlook of where the field should go.

For example, presumably the rings observed are associated with chemistry or porosity, and some speculation or research on these should be added. If this can be linked to ecology it would give proper hypotheses that can be examined in the future. Similarly, the chemistry of dyes must be discussed at least briefly in past literature, such that the authors could speculate on the signals exposed. Lastly, given the negative outlook on these methods, it would be ideal to mention suitable alternatives, and raise critical strengths and weaknesses of those alternatives (e.g., predictive growth curves per species). It seems fundamental to propose an outlook to researchers, truly synthesising the results with existing work.

Decision letter (RSPB-2021-1166.R0)

21-Jun-2021

Dear Dr Lambertz

I am pleased to inform you that your manuscript RSPB-2021-1166 entitled "What's my age again? – On the ambiguity of histology-based skeletochronology" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Before uploading your revised files please make sure that you have:

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- 2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.
- 3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository (<https://royalsociety.org/journals/authors/author-guidelines/#data>).

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[http://datadryad.org/submit?journalID=RSPB&manu=\(Document not available\)](http://datadryad.org/submit?journalID=RSPB&manu=(Document not available)) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/> for more details.

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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,

Dr Maurine Neiman

mailto:proceedingsb@royalsociety.org

Associate Editor

Comments to Author:

The reviewers agree that the study is well conducted and important, and they have only minor comments. These comments mainly relate to how researchers should incorporate these results into their methods in the future, so even these minor changes could substantially increase the impact on the field. In particular, Reviewer 2 requests some sort of recommendation for future studies, and Reviewer 1 suggests discussing past successful skeletochronological studies and why they might have succeeded where others failed.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

Dear Editor,

This paper proposes a critical analysis to estimate the accuracy of skeletochronology. The authors carefully analyze results obtained from petrographic ground sections versus stained microtomed sections made in the same bones of various tetrapods. The study is done with caution (I notably appreciate the effort to scan bones first in order to precisely locate the area with the thickest cortex); results are clearly presented (great suppl. Data!). Results highlight difficulties in using LAGs to estimate age. This is a major result since skeletochronology is often used with closed eyes without questioning enough the possibility of bias.

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l.84 please add ref. about the observation modes.

Figures 1 and 2 are mixed!! This was disturbing and maybe strengthened the fact that I do not find it straightforward for the reader to understand why for *Lacerta* there is only one lag in (1), whereas 2 are counted for (4)-(5) in *Pogona*. Maybe a few sentences should be added about splitting since, beyond the mouse, it might be more widespread (being also observed in *Varanus*; Buffrénil & Castanet 2001; also cited), even in your sample.

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I thus strongly recommend publication.

Best regards,

Alexandra Houssaye

Referee: 2

Comments to the Author(s).

In this piece, Schucht et al. make an extensive assessment of the most prominent methods in histology-based skeletochronology. The study is well executed and the results relevant to a broad range of researchers, raising a critical note of caution on these methods.

My only comments focus on the discussion. As the authors mention, there is lots of research to be done before histological skeletochronology can be used broadly for age estimation. However, it seems like the authors fall short of suggesting specific research avenues with reference to our knowledge of osteology, development, chemistry, and alternative methods. In other words, the discussion does not give a clear outlook of where the field should go.

For example, presumably the rings observed are associated with chemistry or porosity, and some speculation or research on these should be added. If this can be linked to ecology it would give proper hypotheses that can be examined in the future. Similarly, the chemistry of dyes must be discussed at least briefly in past literature, such that the authors could speculate on the signals exposed. Lastly, given the negative outlook on these methods, it would be ideal to mention suitable alternatives, and raise critical strengths and weaknesses of those alternatives (e.g., predictive growth curves per species). It seems fundamental to propose an outlook to researchers, truly synthesising the results with existing work.

Author's Response to Decision Letter for (RSPB-2021-1166.R0)

See Appendix A.

Decision letter (RSPB-2021-1166.R1)

24-Jun-2021

Dear Dr Lambertz

I am pleased to inform you that your manuscript entitled "What's my age again? – On the ambiguity of histology-based skeletochronology" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

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Electronic supplementary material:

All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit <https://royalsociety.org/journals/ethics-policies/media-embargo> for more information.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,

Editor, Proceedings B

mailto: proceedingsb@royalsociety.org

Appendix A

Response to the reviewers

Reviewer 1

I.63 "but not in endotherms"; please adapt the sentence since you know that growth marks are also found in endotherms (e.g. Köhler et al 2012; Castanet et al 2004, already cited);

Of course. This was our original intention to be stated here and we clarified the sentence.

I.84 please add ref. about the observation modes.

There is no meaningful reference that could be cited here – except for each and every study that used one or the other approach. It's simply what people do: using polarized microscopy for the rather thick ground sections and normal microscopy for the thinner stained sections. We therefore feel that adding any randomly selected references would not contribute to the value of our manuscript.

Figures 1 and 2 are mixed!! This was disturbing and maybe strengthened the fact that I do not find it straightforward for the reader to understand why for *Lacerta* there is only one lag in (1), whereas 2 are counted for (4)-(5) in *Pogona*. Maybe a few sentences should be added about splitting since, beyond the mouse, it might be more widespread (being also observed in *Varanus*; Buffrénil & Castanet 2001; also cited), even in your sample.

Thanks for discovering this flaw! We changed the numbering of the figures. We also agree on the "splitting problem". We now mention the above-mentioned *Lacerta* case also in the main manuscript where we point towards these problems, but this is furthermore already more extensively discussed in the taxon-specific sections of the supplementary data.

Generally the discussion might benefit from a few sentences about the cases where skeletochronology seemed to work "perfectly". There might be interest also in citing Castanet et al. 1992 (Castanet, J., Meunier, F. J., & Francillon-Vieillot, H. (1992). Squelettechronologie à partir des os et des dents chez les vertébrés. ; though in French, easily translatable online now ;) where limits are

already highlighted, to insist on the fact that even historically it was never “perfect” and that even the “fathers of skeletochronology” discussed about limits, their causes, and how to possibly bypass them.

We agree that the “fathers and mothers” of skeletochronology were aware of certain limitations and our manuscript already refers particularly to this group of authors when discussing the subject, though not the 1992 paper (l. 77ff): “a frame of reference for each species (or even population) remains strongly advised [9, 19, 27]. However, this is rarely applied in practice and impossible for fossils.” The “perfect” cases differ among the taxa, and we discuss this in general in the extensive supplementary data. However, we also added a brief discussion on these to the main manuscript now.

Reviewer 2

My only comments focus on the discussion. As the authors mention, there is lots of research to be done before histological skeletochronology can be used broadly for age estimation. However, it seems like the authors fall short of suggesting specific research avenues with reference to our knowledge of osteology, development, chemistry, and alternative methods. In other words, the discussion does not give a clear outlook of where the field should go.

We agree with the statement that there will be “lots of research to be done before histological skeletochronology can be used broadly”. However, this will span over a huge number of – as a matter of fact – not only biological disciplines. We feel that pointing out the major problems/areas of shortage of knowledge (as per l. 186ff) was our duty here, but we feel unqualified to propose, for instance, a chemist a specific avenue how to tackle these problems. We ourselves intend to focus next on the ultrastructure of bone growth, and are convinced that other researchers will be stimulated to set up their own research programmes as well, based on our study and then lead by

their expertise.

For example, presumably the rings observed are associated with chemistry or porosity, and some speculation or research on these should be added. If this can be linked to ecology it would give proper hypotheses that can be examined in the future. Similarly, the chemistry of dyes must be discussed at least briefly in past literature, such that the authors could speculate on the signals exposed. Lastly, given the negative outlook on these methods, it would be ideal to mention suitable alternatives, and raise critical strengths and weaknesses of those alternatives (e.g., predictive growth curves per species). It seems fundamental to propose an outlook to researchers, truly synthesising the results with existing work.

In general, we simply do not like to speculate. We are morphologists and neither chemists nor ecologists. We therefore feel that it would be particularly inappropriate to speculate about fields we are not even remotely experts in. We agree that these are critical topics that urgently need to be tackled by the respective experts, and are absolutely convinced that our study will stimulate such future research.

Concerning a viable alternative: if we had one, please believe us that we would have proposed it. However, we are convinced that, although we cannot provide an alternative route out of this dilemma, this does not diminish the value of our study that clearly highlights the fundamental problems with the existing approach.