Morphological and functional changes in the vertebral column with increasing aquatic adaptation in crocodylomorphs

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Note: This manuscript was transferred from another Royal Society journal after peer review.

Review History

RSOS-150439.R0 (Original submission)

Review form: Reviewer 1 (Emma Schachner)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes, although I would prefer to see the raw CT data made available through a curated digital repository; however, I understand that some museums may refuse if they paid for the original scanning.

Do you have any ethical concerns with this paper?
No
I think this manuscript is much improved by incorporating the SI materials into the main text and I am very happy to see the dorsal views of the vertebral columns new version of Figure 1. It appears as though all of my suggestions were incorporated into this draft, and I have no further changes to request.

Review form: Reviewer 2 (Stephan Lautenschlager)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
The submitted manuscript by Molnar et al. describes the changes in form and function of crocodylomorph vertebrae in different extant and extinct taxa to elucidate locomotory capabilities and adaptations. As such, this study will be of interest to a broad audience of palaeontologist and biologists working on related topics ranging from biomechanical modelling, palaeoecology and comparative anatomy. The study has been well conceived, thoroughly executed and the manuscript is well thought-through and shows a high level of detail, which seems to be missing more and more in current publications. I welcome the inclusion of all methodological descriptions in the main text rather than having to search for those in a supplement and I am convinced the authors will benefit from this more the having the paper published in a seemingly higher-impact, but short-format journal.

As the manuscript has undergone review from two referees previously, I find only some minor points to improve this contribution. Concerns raised by the previous reviewers and suggestions have been addressed in the current version. I have a few more specific suggestions and comments listed below. Accordingly I would recommend this manuscript for publication pending minor revisions, which shouldn't take the authors very long to implement.
Page 2, line 43: Mention here, that Thalattosuchia only includes fossil representatives. Specialist readers will know that, but as the paper is intended for a broader audience as well, this information should be included.

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Figures 3 & 5: Similar to the suggestion above, the diagrams could do with units on the y-axes.

Figure 4: The plots for the lateral flexion for Terrestrisuchus are very conspicuous in being asymmetrical. Is there a reason for that (preservation, retrodeformation artefacts,...)?

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An additional figure complementary to Fig. 1 would be nice showing the full extent of dorsoventral and mediolateral flexion for the complete vertebral column for all taxa using the virtual models. This would allow to compare and evaluate the maximum flexion across all taxa.

I wonder if the authors have thought about using the experimental results of Crocodylus to estimate some kind of correction factor, calculated from the differences in range of motion and stiffness between the whole trunk setup and a skeletal setup. Such a correction factor could be used to estimate the effect of soft tissues for the fossil specimens. Although there is some degree of speculation involved regarding modifications of the musculature, etc. depending on
locomotory style, it would allow to approximate the degree of overestimation of RoM due to the lack of soft tissues. An experimentally derived correction factor would also be of interest for other studies of this kind.

Decision letter (RSOS-150439)

21-Sep-2015

Dear Professor Molnar

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-150439 entitled "Morphological and functional changes in the vertebral column with increasing aquatic adaptation in crocodylomorphs" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link:
http://datadryad.org/submit?journalID=RSOS&manu=RSOS-150439

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.
We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

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 (i.e. by the 30-Sep-2015). If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create 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 referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees.

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

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4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript
5) Included your supplementary files in a format you are happy with (no line numbers, vancouver referencing, track changes removed etc) as these files will NOT be edited in production

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

Best wishes
Emilie Aime
Senior Publishing Editor, Royal Society Open Science
on behalf of Kevin Padian
Subject Editor, Royal Society Open Science.
openscience@royalsociety.org
Author's Response to Decision Letter for (RSOS-150439)

See Appendix A.
Dear Editors,

We are very pleased that you have accepted our manuscript, and we have addressed all reviewer comments below. We also have arranged to submit our 3D data to Morphomuseum (doi: 10.18563/m3.1.3.e5) to make it accessible to readers.

Many thanks,
Julia Molnar, Stephanie Pierce, Bhart-Anjan Bhullar, Alan Turner, John Hutchinson

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
The submitted manuscript by Molnar et al. describes the changes in form and function of crocodylomorph vertebrae in different extant and extinct taxa to elucidate locomotory capabilities and adaptations. As such, this study will be of interest to a broad audience of palaeontologist and biologists working on related topics ranging from biomechanical modelling, palaeoecology and comparative anatomy. The study has been well conceived, thoroughly executed and the manuscript is well thought-through and shows a high level of detail, which seems to be missing more and more in current publications. I welcome the inclusion of all methodological descriptions in the main text rather than having to search for those in a supplement and I am convinced the authors will benefit from this more the having the paper published in a seemingly higher-impact, but short-format journal.

As the manuscript has undergone review from two referees previously, I find only some minor points to improve this contribution. Concerns raised by the previous reviewers and suggestions have been addressed in the current version. I have a few more specific suggestions and comments listed below. Accordingly I would recommend this manuscript for publication pending minor revisions, which shouldn’t take the authors very long to implement.

Page 2, line 43: Mention here, that Thalattosuchia only includes fossil representatives. Specialist readers will know that, but as the paper is intended for a broader audience as well, this information should be included.

We have changed the line to read, “one extinct clade, Thalattosuchia…”

Page 4, line 124: it would be useful if table 1 also contained the length (or length range) of the individual vertebra not just the column length. Also vertebral numbers for the subdivisions (thoracic, lumbar, etc.) in addition to the overall vertebral number would be good.

We have included this information and revised the caption accordingly. We also made a correction to this table: the number of vertebrae for Steneosaurus and Metriorhynchus in the table was switched.

Page 4, line 127: For completeness sake, the scan parameters or at least the scan resolution should be listed here. As there is no page limit this information can go in the main text rather than a supplement.

We have added this information to the first paragraph of the Methods section and Table 1.

Page 4, line 141: List average mesh size/number of faces or elements for the stl files so it can be evaluated whether artefacts from low resolution might have affected the results. Looking at fig 1, the resolution seems fine, but you want to be as thorough as possible.
We have added a column to Table 1 listing these statistics.

Page 5, line 167: "Using lumbar length rather than lumbar length was a necessary simplification due to the incompleteness of the Terrestrisuchus specimen", there is one "lumbar" too much.

We have changed this line to read, “Using lumbar length rather than thoracolumbar length…”

Page 6, line 176: Again, it would be very useful to list the length of the intervertebral spaces, for example in table 1, so that it is possible to compare dimensions across the different taxa.

We have added a column to Table 1 with this information and a paragraph to the Results section and a sentence to the discussion summarizing the differences and their implications:

“The amount of space between adjacent centra relative to centrum length was substantially greater in Crocodylus than the other taxa, and substantially smaller in Terrestrisuchus (Table 1). This difference might be related to preservation (in the case of Terrestrisuchus), or it might reflect different amounts of soft tissue within the joints (more likely in the case of Crocodylus because it is the only studied taxon with procoelous IVJs). If it is an artefact of preservation, the small amount of intervertebral space in Terrestrisuchus might cause our RoM estimates to be too low, particularly in lateral flexion because this movement was limited by contact between the centra (Table 4).” (lines 356-362)

“Our results showed that Crocodylus had substantially greater space between adjacent centra than the other taxa we studied (Table 1), suggesting that the shift to procoelous IVJs may have involved an increase in the amount of soft tissue within the joint, increasing RoM. Future studies should investigate this speculation with a larger dataset.” (lines 578-581)

Page 7, line 222: How were contacts (osteological stops) between successive vertebra determined? Manually. Or does 3D Studio include automatic collision detection?

We added the sentence, “Contacts between bones were detected visually, checking that the vertebrae did not touch or penetrate each other in 3D.”

Page 7, line 240: is there a reason why only dorsoventral flexion in one direction ("head and tail up, belly down") was measured and not in reverse direction as well? I might have missed it in the text, in which case list all experimental scenarios here clearly

We changed the first sentence of the paragraph to read, “Range of motion in ventral flexion, dorsal extension, and lateral flexion was estimated…”

Page 8, line 258: Reference #20, not #2

Thank you, we have corrected this.

Page 14, line 466: “agility” or “flexibility” rather than “athleticism”?

We have changed it to “agility”.

Figure 1: Having the taxon names below the respective 3D models would improve legibility, as the reader hasn't to refer to the caption constantly.

Good point; we have done so.
Figures 3 & 5: Similar to the suggestion above, the diagrams could do with units on the y-axes.

Figure 3 does not show standard length units. We have added the following explanatory sentence to the caption: “Length units not shown because measurements were normalized; see SI for non-normalized measurements.” Units have been added to Figure 5.

Figure 4: The plots for the lateral flexion for Terrestrisuchus are very conspicuous in being asymmetrical. Is there a reason for that (preservation, retrodeformation artefacts,...)?

Almost certainly this is caused by preservation. We have added an explanatory sentence to the discussion: “RoM in lateral flexion was generally fairly symmetrical, except in Terrestrisuchus where asymmetrical preservation caused the joints to reach osteological stops at different degrees of flexion to the left and right.”

Figure 5: I would consider combining the two plots to show the differences/similarities between the experimental and virtual manipulation results more clearly, using different colours or line styles. Also, I am a bit confused about the information provided in the caption. It states that 5a shows the average data from seven specimens, whereas the respective paragraph in the methods (page 7) only lists two specimens?

We tried combining the plots, but the smaller range of values in 5b made it difficult to see any difference in RoM by region or bending direction. Because we use this figure to discuss patterns of RoM within each specimen, we think that it would be better to keep the plots separate.

Regarding the second comment, the data in 5b were individual joint measurements taken from Molnar et al. 2014 (7 specimens), whereas the C. niloticus data in this manuscript represent the entire trunk (2 specimens). We have added an explanation to the caption: “...estimated from manipulation of virtual skeletons of Crocodylus allowing 0.45mm translation (A) and experimental measurements from individual cadaveric joints taken from Molnar et al. (20) (B).” We also corrected this figure; A and B were switched in the caption.

An additional figure complementary to Fig. 1 would be nice showing the full extent of dorsoventral and mediolateral flexion for the complete vertebral column for all taxa using the virtual models. This would allow to compare and evaluate the maximum flexion across all taxa.

We have done so; this figure is now Figure 8. We added a paragraph to the Discussion explaining the figure:

“Finally, the total number of thoracolumbar vertebrae affects the behaviour of the trunk during locomotion. If RoM of individual joints remains constant, a greater number of dorsal vertebrae translates into greater RoM along the trunk (16). Relative to Protosuchus, Pelagosaurus, and Crocodylus, Metriorhynchus has two more thoracolumbar vertebrae and Steneosaurus has two fewer (Table 1), presumably granting slightly greater total trunk RoM to the former and slightly less to the latter. Total trunk RoM for each taxon, extrapolated from the virtual joints we tested, is shown in Figure 8.”

I wonder if the authors have thought about using the experimental results of Crocodylus to estimate some kind of correction factor, calculated from the differences in range of motion and stiffness between the whole trunk setup and a skeletal setup. Such a correction factor could be used to estimate the effect of soft tissues for the fossil specimens. Although there is some degree of speculation involved regarding modifications of the musculature, etc. depending on locomotory style, it would allow to approximate the degree of overestimation of RoM due to the lack of soft tissues. An experimentally derived correction factor would also be of interest for other studies of this kind.
We have thought about this point and agree that a “correction factor” would be a helpful thing to have, but we are concerned that it involves too many assumptions (e.g. soft tissue properties may not be the same outside the crown group, and we are almost certain that properties of the osteoderms would be different). Moreover, we would need a larger number of specimens to give these numbers with any confidence, even for the modern taxa. We would certainly consider doing this in a future study.

Reviewer: 2

Comments to the Author(s)
I think this manuscript is much improved by incorporating the SI materials into the main text and I am very happy to see the dorsal views of the vertebral columns new version of Figure 1. It appears as though all of my suggestions were incorporated into this draft, and I have no further changes to request.

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Keywords: axial skeleton, locomotion, stiffness, range of motion, archosaur
Abstract: The lineage leading to modern Crocodylia has undergone dramatic evolutionary changes in morphology, ecology, and locomotion over the past 200+ million years. These functional innovations may be explained in part by morphological changes in the axial skeleton, which is an integral part of the vertebrate locomotor system. Our objective was to estimate changes in osteological range of motion and intervertebral joint stiffness of thoracic and lumbar vertebrae with increasing aquatic adaptation in crocodylomorphs. Using 3D virtual models and morphometrics, we compared the modern crocodile <i>Crocodylus</i> to five extinct crocodylomorphs: <i>Terrestrisuchus, Protosuchus, Pelagosaurus, Steneosaurus</i> and <i>Metriorhynchus</i>, which span the spectrum from mainly terrestrial to fully aquatic. In Crocodylus, we also experimentally measured changes in trunk flexibility with sequential removal of osteoderms and soft tissues. Our results for the more aquatic species matched our predictions fairly well, but those for the more terrestrial early crocodylomorphs did not. A likely explanation for this lack of correspondance is the influence of other axial structures, particularly the rigid series of dorsal osteoderms in early crocodylomorphs. The most important structures for determining range of motion and stiffness of the trunk in <i>Crocodylus</i> were different in dorsoventral versus mediolateral bending, suggesting that changes in osteoderm and rib morphology over crocodylomorph evolution would have affected movements in some directions more than others.

EndDryadContent