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Locomotion and paleoclimate explain the re-evolution of quadrupedal body form in *Brachymeles* lizards

Philip J. Bergmann, Gen Morinaga, Elyse S. Freitas, Duncan J. Irschick, Günter P. Wagner and Cameron D. Siler

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Original submission: 14 August 2020
Revised submission: 15 October 2020
Final acceptance: 19 October 2020

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

Review History

RSPB-2020-1994.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

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

General interest: Is the paper of sufficient general interest?
Good

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?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
I thought this was an interesting and well written manuscript. Please see my comments in the attached file. (See Appendix A)

Review form: Reviewer 2 (Juan Daza)

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?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

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

Is it clear?
Yes

Is it adequate?
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Do you have any ethical concerns with this paper?
No

Comments to the Author
This is an interesting paper about the re-gain of limbs and the correlation with burrowing capabilities in a group of lizards. This genus is an excellent example to study transition from cursorial, surface active to ground dwelling, fossorial animals. There are not many examples of re-gain in lizards, and this could be a good case of study that might be relevant for understanding better the origin of snakes.
The authors also did a correlation of body shapes with temperature and use that data to explain the transition from "snakelike to limbed forms. I made a few comments on the pdf, which the authors are free to ignore. I have once comment about the phylomoprhospace analysis, and I must confess that I am not the most versed person on statistics (so I could be wrong). Did you remove the effect of size from the analysis, I have the impression that if you did not, then size alone would be explaining a high percentage of the PC1. If you remove it, then you will be testing body proportions (form) alone. I think this ca be done easily by scaling the data, but once again, I could be wrong.
I also have an appreciation, and this is from my personal experience working on areas that are dry, but where I have collected a variety of fossorial forms (blind snakes and amphisbaenians). In these environments, fossorial forms are not usually find in hard, dry compacted soils, but in mesic environments within these dry areas (e.g. shades areas which are relatively wet, almost like little oasis). Are these small patches of microhabitats important to consider in the big picture analysis of thermal data used here. Maybe no need to be incorporated in the analysis, but maybe just discuss it as many fossorial animals in dry areas, prefer these mesic patches, maybe you saw this also during your field season.

Juan D. Daza

Decision letter (RSPB-2020-1994.R0)

05-Oct-2020

Dear Dr Bergmann:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

As a scientist interested in this area of study, I thought the title might be altered to better reflect the focus. I expected the paper to be about all lizards but only Brachymeles were studied, so it would be maximally honest to end the title as "Brachymeles lizards". I am not sure if the macro vs. micro environment question can truly be answered but text could better address it. Please ensure that the full code and data are made available as per our rules for open science; ideally a private Dryad link could be made available to reviewers + AE.

To submit your revision please log into http://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.

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It is a condition of publication that you make available the data and research materials supporting the results in the article. Please see our Data Sharing Policies (https://royalsociety.org/journals/authors/author-guidelines/#data). Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
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If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

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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. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr John Hutchinson, Editor
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Thank you for the opportunity to read this study. Herein the authors examine the re-evolution of more typically quadrupedal body forms from snake-like body forms in Brachymeles lizards from multiple perspectives, including morphological, functional and environmental. The links they find between morphology, locomotor performance and environment lead them to suggest that a strong link between the re-evolution of longer limbs and climate, wherein the evolution of quadrupedal taxa occurred under more humid climates where efficient fossorial locomotion in packed soils was selectively beneficial. These conclusions are certainly important and of broad biological interest, and the expert reviewers agree that they are largely substantiated by the data presented. However, the reviewers do raise a number of issues that require attention. In particular, I would highlight the comments of reviewer 1 about the moisture content of soils, inclusion of all analyses/data in supplementary information, and consideration of predator escape in the discussion. My own personal uncertainty (and it is an uncertainty rather than an informed criticism) when reading the paper was, how good is the palaeoclimate and environmental data? Should any uncertainty in this be explicitly acknowledged in the paper? Reviewer 2 extends this point, in an insightful way I think, by asking how relevant more large-scale reconstructions of climate are to the more micro-scale environments that the lizards evolved in. Some discussion of this is warranted, I think. Overall though I am very positive about the paper and would welcome a resubmission that addresses the reviewer comments.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
I thought this was an interesting and well written manuscript. {lease see my comments in the attached file.

Referee: 2

Comments to the Author(s)
This is an interesting paper about the re-gain of limbs and the correlation with burrowing capabilities in a group of lizards. This genus is an excellent example to study transition from cursorial, surface active to ground dwelling, fossorial animals. There are not many examples of re-gain in lizards, and this could be a good case of study that might be relevant for understanding better the origin of snakes.
The authors also did a correlation of body shapes with temperature and use that data to explain the transition from "snakelike to limbed forms. I made a few comments on the pdf, which the authors are free to ignore. I have once comment about the phylomoprhospace analysis, and I must confess that I am not the most versed person on statistics (so I could be wrong). Did you remove the effect of size from the analysis, I have the impression that if you did not, then size alone would be explaining a high percentage of the PC1. If you remove it, then you will be testing body proportions (form) alone. I think this ca be done easily by scaling the data, but once again, I could be wrong.
I also have an appreciation, and this is from my personal experience working on areas that are dry, but where I have collected a variety of fossorial forms (blind snakes and amphisbaenians). In these environments, fossorial forms are not usually find in hard, dry compacted soils, but in mesic environments within these dry areas (e.g. shades areas which are relatively wet, almost like little oasis). Are these small patches of microhabitats important to consider in the big picture analysis of thermal data used here. Maybe no need to be incorporated in the analysis, but maybe just discuss it as many fossorial animals in dry areas, prefer these mesic patches, maybe you saw this also during your field season.

Juan D. Daza

Author's Response to Decision Letter for (RSPB-2020-1994.R0)

See Appendix B.

Decision letter (RSPB-2020-1994.R1)

19-Oct-2020

Dear Dr Bergmann

I am pleased to inform you that your manuscript entitled "Locomotion and paleoclimate explain the re-evolution of quadrupedal body form in *Brachymeles* lizards" has been accepted for publication in Proceedings B. Congratulations!!

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.

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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,
Dr John Hutchinson
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
I'm very happy that the authors have satisfactorily addressed the minor comments from the previous reviews.

Appendix A

Review of "Locomotor facilitation and paleoclimate explain the re-evolution of quadrupedal body form in lizards"

This manuscript examines the potential functional mechanisms underlying the revolution of limbs in a clade of skinks (Brachymeles) in an attempt to better understand evolutionary reversals. It explores the relationship between locomotor performance (burrowing and running velocity), body shape, and paleoclimate. The manuscript is well written, and I think the study was well conducted and employs appropriate methods to address the proposed questions. The authors employ appropriate evolutionary analyses and explain the rationale behind their choice of analyses. Overall, I think the manuscript is an interesting and quality submission that will be of interests to the readers of Proceedings of the Royal Society B. Having said that, I think there are some minor points that should be clarified prior to publication. I highlight these minor points below.

Specific suggestions/comments:

Page 3, line 30: Just a suggestion, change "with soils that were easier for digging in" to "with soil in which it was easier to dig".

Page 5, line 97

Page 6, lines 127-128: Did you measure the moisture content of the soil? How did you know it was the same between the two substrates? This may be picky, but seems important given your question.

Page 6, line 133-134: For the running trials, how wide is the field of view? Is it the full meter or are you measuring the speed over 10 cm?

Page 8, line 169-170: Readers may not want to look up the phylogeny while reading this paper. It would be nice to include a brief statement about this phylogeny. Is it well supported? Are there other evolutionary hypotheses that differ in a significant way?

Page 9, line 183-185: Are these analyses included in the supplement (I did not see them). They may be of interest to the readers.

Line 215: "Species with high pPC-1 scores" should read "Species with <u>low</u> pPC-1 scores". As it reads both snake-like and limbed terrestrial shapes have high pPC-1 scores.

Line 228: How do you define preferred? Just the lowest score regardless of difference (which seems to be the case)? I only ask because in many cases a threshold of 4 (and sometimes 2) is used. It may be helpful here to clarify why you made you decision.

Page 16, Line 323-328: The persistence of snake-like forms that are syntopic with pentadactyl species suggests that perhaps behavior plays an important role in the re-evoution of limbs. One factor you left out is predator escape. I believe at least one of the coauthors has published data demonstrating that lizards really use their maximum locomotor capabilities during predator escape, not foraging. Perhaps this is worth mentioning here as it could play an important role in the functional mechanism of reversal here, and strengthen the argument for the syntopic persistence of snake-like forms.

Figure 2. It would be helpful to the reader to include a brief description of what body shapes correspond to high and low pPC-1 scores in this legend. In the supplement you have pictures on the plot to show representative body shapes. I don't think you need to do that here, but including a sentence indicating that high scores are more snake-like would be helpful.

Figures 3 & 4: Most of these figure legends are the same as the figure legend for figure 1. You may want to consider just referring to the figure 1 legend (just a suggestion).

Appendix B

Dr. John Hutchinson
Editor
Proceedings of the Royal Society B

October 14, 2020

Dear Dr. Hutchinson:

My coauthors and I have now revised our manuscript (RSPB-2020-1994) entitled, "**Locomotion and paleoclimate explain the re-evolution of quadrupedal body form in *Brachymeles* lizards**" following editor and reviewer comments, and would like to re-submit it to *Proceedings of the Royal Society Series B*. In the manuscript, we integrate morphological, functional, ecological, and paleoclimatological data to gain an understanding of how a group of lizards re-evolved four pentadactyl limbs and short bodies from a limbless, elongate ancestor.

We are excited about the positive reviews that we received, and much appreciate the time and effort that went into them, as well as the opportunity to resubmit. We have addressed all of the comments, and explain how we addressed each below, followed by a tracked changes version of the manuscript. For ease of reading, we included each editorial/reviewer comment in black font, immediately followed by our response in blue. In our responses, we refer to line numbers where possible, and these correspond to the tracked document that follows (line numbers end up slightly different in the clean version). We feel that our resubmission is much strengthened and look forward to hearing back from you regarding the publication of our work in the journal. If you have any questions about our re-submission, please don't hesitate to contact me.

Sincerely,

Philip Bergmann
pbergmann@clarku.edu

Response to Editor and Reviewer Comments

Comment from Dr. Hutchinson:
As a scientist interested in this area of study, I thought the title might be altered to better reflect the focus. I expected the paper to be about all lizards but only Brachymeles were studied, so it would be maximally honest to end the title as "Brachymeles lizards". I am not sure if the macro vs. micro environment question can truly be answered but text could better address it. Please ensure that the full code and data are made available as per our rules for open science; ideally a private Dryad link could be made available to reviewers + AE.
→ We have modified the title to include the genus, but given the limit of 100 characters, were forced to change "Locomotor facilitation" to the somewhat less-informative "Locomotion". We have added discussion of the macro scale of the paleoclimatological data versus the possibility of microhabitat selection by the animals on the ground in lines (347-351), and addressed the reviewer's comment about this below.
→ We have now submitted our dataset, including phylogenies, and a text file containing code that we used to conduct our analyses and construct figures to Dryad Digital Repository. We provide this private link to the data for you and the reviewers: https://datadryad.org/stash/share/cmSfHSZu9lBrKWW7_VHah4hVAjsdB5_fE3jBkdAHS8c.

Comments from Associate Editor, Board Member 1:
Thank you for the opportunity to read this study. Herein the authors examine the re-evolution of more typically quadrupedal body forms from snake-like body forms in Brachymeles lizards from multiple perspectives, including morphological, functional and environmental. The links they find between morphology, locomotor performance and environment lead them to suggest that a strong link between the re-evolution of longer limbs and climate, wherein the evolution of quadrupedal taxa occurred under more humid climates where efficient fossorial locomotion in packed soils was selectively beneficial. These conclusions are certainly important and of broad biological interest, and the expert reviewers agree that they are largely substantiated by the data presented. However, the reviewers do raise a number of issues that require attention. In particular, I would highlight the comments of reviewer 1 about the moisture content of soils, inclusion of all analyses/data in supplementary information, and consideration of predator escape in the discussion. My own personal uncertainty (and it is an uncertainty rather than an informed criticism) when reading the paper was, how good is the palaeoclimate and environmental data? Should any uncertainty in this be explicitly acknowledged in the paper? Reviewer 2 extends this point, in an insightful way I think, by asking how relevant more large-scale reconstructions of climate are to the more micro-scale environments that the lizards evolved in. Some discussion of this is warranted, I think. Overall though I am very positive about the paper and would welcome a resubmission that addresses the reviewer comments.
→ Thank you for your positive view of our work, we are very excited about it, and for your constructive feedback. We address all of the reviewer comments below. The issue of the reliability of the paleoclimatological data is a valid and important one. In our original submission we had a short phrase saying that these data are sparse prior to 50Ma, but became more plentiful and reliable after that time. We have replaced the "plentiful and reliable" with a sentence describing the data in more detail. In particular, from 50Ma to present, there is a wealth of data supporting the paleoclimatological reconstructions, including oxygen isotopes, temperature, pollen, and algal fossils. Hence, we don't put a lot of emphasis on reconstructions before that

time, but can note here that those reconstructions are consistent with the later ones. Essentially, the climate was relatively dry and seasonal before 30Ma, and became very wet throughout the year (monsoonal) by about 25Ma to present. We now note the statement about what types of data the reconstructions are based on to make them reliable in Lines 329-331.

Comments from Referee 1:
I thought this was an interesting and well written manuscript. This manuscript examines the potential functional mechanisms underlying the revolution of limbs in a clade of skinks (Brachymeles) in an attempt to better understand evolutionary reversals. It explores the relationship between locomotor performance (burrowing and running velocity), body shape, and paleoclimate. The manuscript is well written, and I think the study was well conducted and employs appropriate methods to address the proposed questions. The authors employ appropriate evolutionary analyses and explain the rationale behind their choice of analyses. Overall, I think the manuscript is an interesting and quality submission that will be of interests to the readers of Proceedings of the Royal Society B. Having said that, I think there are some minor points that should be clarified prior to publication. I highlight these minor points below.
→ We thank you for your comments and feedback.

Specific suggestions/comments:
Page 3, line 30: Just a suggestion, change "with soils that were easier for digging in" to "with soil in which it was easier to dig".
→ Done.

Page 5, line 97
→ No comment was provided. We have read this area of the manuscript to ensure everything was clear. We split the sentence describing load-bearing capacity and soil moisture in two so that they would be clearer.

Page 6, lines 127-128: Did you measure the moisture content of the soil? How did you know it was the same between the two substrates? This may be picky, but seems important given your question.
→ We did not measure soil moisture of the substrates we used in trials, but have clarified the sentence to indicate that both experimental substrates were first dried and then sieved to ensure that they had the same moisture content. Therefore, soil moisture of the experimental substrates do not confound the locomotion data (Line 134).

Page 6, line 133-134: For the running trials, how wide is the field of view? Is it the full meter or are you measuring the speed over 10 cm?
→ We have now clarified that the field of view was approximately 30cm long (line 139). Addressing the second question, given that we calculated speed using frame-by-frame data and spline fitting, maximal speed was for the instant of fastest movement, while average speed is over the course of the animal's continuous movement (clarified on lines 166-167)

Page 8, line 169-170: Readers may not want to look up the phylogeny while reading this paper. It would be nice to include a brief statement about this phylogeny. Is it well supported? Are there other evolutionary hypotheses that differ in a significant way?

→ We have added a sentence indicating that the phylogeny is based on DNA sequences from multiple loci, and with most clades supported with posterior probabilities of one (lines 180-181). With *Brachymeles* being an obscure and poorly studied genus, the only evolutionary hypotheses available have been published by one of us (Siler), and have been refined with multiple iterations that have added sequence data and taxa to reflect complete taxonomic sampling.

Page 9, line 183-185: Are these analyses included in the supplement (I did not see them). They may be of interest to the readers.
→ We apologize for the omission. We have now revised Supplementary Table S3 to include these analyses and reference the table in the main text (Line 197).

Line 215: "Species with high pPC-1 scores" should read "Species with low pPC-1 scores". As it reads both snake-like and limbed terrestrial shapes have high pPC-1 scores.
→ Thank you for catching that! We have corrected it.

Line 228: How do you define preferred? Just the lowest score regardless of difference (which seems to be the case)? I only ask because in many cases a threshold of 4 (and sometimes 2) is used. It may be helpful here to clarify why you made you decision.
→ We have now clarified that we used a threshold of 2 in the methods (Line 202), and also clarify the wording in the results (Line 241-242). Although we are unaware of thresholds of 4 being used, this is an important issue because all cut-offs are arbitrary, and the AIC is ideally interpreted as a continuous variable. In this case, our conclusions are not substantially affected by threshold choice because the second-best model is more complex and also represents the hypothesis that the pentadactyl clade has larger body sizes. That model assigned different optima for limbless, limb-reduced, and pentadactyl species, instead of lumping limbless and limb-reduced together. The closest model that is inconsistent with our stated results is 6.6 AIC units worse than the best, and this is universally regarded as quite strong support (also supported by the model weights that we present in the supplementary table). On line 242 of the results, we now have also added wording that states that both of the top two models give the same conclusion.

Page 16, Line 323-328: The persistence of snake-like forms that are syntopic with pentadactyl species suggests that perhaps behavior plays an important role in the re-evoution of limbs. One factor you left out is predator escape. I believe at least one of the coauthors has published data demonstrating that lizards really use their maximum locomotor capabilities during predator escape, not foraging. Perhaps this is worth mentioning here as it could play an important role in the functional mechanism of reversal here, and strengthen the argument for the syntopic persistence of snake-like forms.
→ We have added phrasing toward the end of the paragraph to add predator escape to the narrative, including citing the suggested reference (Lines 362-364).

Figure 2. It would be helpful to the reader to include a brief description of what body shapes correspond to high and low pPC-1 scores in this legend. In the supplement you have pictures on the plot to show representative body shapes. I don't think you need to do that here, but including a sentence indicating that high scores are more snake-like would be helpful.

Figures 3 & 4: Most of these figure legends are the same as the figure legend for figure 1. You may want to consider just referring to the figure 1 legend (just a suggestion).
→ These are great suggestions. Since we already had silhouettes of snake-like and lizard-like species, we decided to include these in figures with pPC-1 to show this visually. We think this makes for more striking figures. In figure 2, we also indicated this in the text. Then we reduced the captions for figures 3 and 4, instead referencing figure 2, where the information first appears.

Comments from Referee 2:
This is an interesting paper about the re-gain of limbs and the correlation with burrowing capabilities in a group of lizards. This genus is an excellent example to study transition from cursorial, surface active to ground dwelling, fossorial animals. There are not many examples of re-gain in lizards, and this could be a good case of study that might be relevant for understanding better the origin of snakes.
→ Thank you for the kind comments.

The authors also did a correlation of body shapes with temperature and use that data to explain the transition from "snakelike to limbed forms. I made a few comments on the pdf, which the authors are free to ignore. I have once comment about the phylomoprhospace analysis, and I must confess that I am not the most versed person on statistics (so I could be wrong). Did you remove the effect of size from the analysis, I have the impression that if you did not, then size alone would be explaining a high percentage of the PC1. If you remove it, then you will be testing body proportions (form) alone. I think this ca be done easily by scaling the data, but once again, I could be wrong.
→ This was a complex issue because we were interested in body proportions or body form along the pentadactyl/lizard-like to snake-like axis, yet size is correlated with this transition in *Brachymeles*. Therefore, what we did with the phylogenetic PCA was include variables with the effects of size removed, plus head length, which we left unadjusted for size and was our measure of overall size. We also included the number of digits. Head length is typically used as a proxy for size in elongate lizards because their body (snout-vent length) has elongated during the evolution of a snake-like form, so is not a good measure of size. The list of variables can be found in Supplementary Table S3. We describe and justify these choices in the first paragraph of the "Statistical Analysis" of our methods. We have reread these to ensure that these elements of the analysis are clear.

I also have an appreciation, and this is from my personal experience working on areas that are dry, but where I have collected a variety of fossorial forms (blind snakes and amphisbaenians). In these environments, fossorial forms are not usually find in hard, dry compacted soils, but in mesic environments within these dry areas (e.g. shades areas which are relatively wet, almost like little oasis). Are these small patches of microhabitats important to consider in the big picture analysis of thermal data used here. Maybe no need to be incorporated in the analysis, but maybe just discuss it as many fossorial animals in dry areas, prefer these mesic patches, maybe you saw this also during your field season.
→ This is a fascinating insight, and demonstrates how variation in what "fossorial" means to different animals is often overlooked. We did not notice any microhabitat selection in *Brachymeles*, but we did measure how resistant to penetration (load-bearing capacity) the soil was and how moist it was. We found no relationship between these variables and body form

(how snake-like species were). An interesting thing about *Brachymeles* is, that unlike many fossorial lizards, they live in rainforests that have very wet soil. There certainly is variation in terms of how wet the soil is, but we saw no evidence of species with different body forms selecting their microhabitat based on this. We now include discussion of this issue at the end of the section on paleoclimate (Lines 347-351).

Juan D. Daza

Dr. Daza also provided some comments directly on the PDF of the manuscript, which he indicated were not critical. Nevertheless, we wish to address those here. We organize them by section:

Abstract:

- We have used the term "re-evolution" in favor of "regain", reaquisition", or "reversal" because we feel the term most clearly reflects the evolutionary component of what we are describing. A similar comment is made in the background section, where the term "reversal" is suggested. Reversal could include loss after gain of a structure, while we are interested in the gain after loss of a structure in particular – a subset of reversal.

- We used the term "burying" in favor of "burrowing" because it more accurately describes what we were able to observe and quantify, given that the animals disappear in the substrate.

- The point about drier soils being more compact is an interesting one. Certainly Barros et al. found this when studying gymnophthalmids in Brazil. However, in other habitats, dry substrate is actually less packed because it lacks cohesive forces, and this has been our observation in Australia with *Lerista* skinks, and reflects physics studies of granular media. We cite some of that work in our results/discussion.

Background:

- We have more completely described what we mean by reduction of limbs, by including "loss and reduction in size of digits and limbs" (Lines 47-48).

- On line 51, we changed "dominant hypothesis" to "prevalent hypothesis" as suggested.

- We revised wording about the "full range of body forms" to "ranging from pentadactyl to limbless" so that we do not imply that we sampled every possible combination of features (Lines 85-86).

- We changed "expect" to "predict" on line 87.

Results & Discussion:

- We changed "relationship" to "correlation" on line 250. We feel that relationship is appropriate in many of our usages, given that we essentially conducted a natural experiment by running animals with different body forms in a controlled setting. However, correlation seems more appropriate for some of the analyses that we conducted.

- On lines 275-276, we modified the language to reflect that the snake-like ancestor of pentadactyl *Brachymeles* is an estimate, rather than a certainty.

- On lines 300-301, we have revised the sentences to include reference to a shovel-like snout and also cite the Anatomical Record reference about *Calyptommatus*.

- We have proofed and corrected inconsistencies in the literature cited.