

In gray, editors' and reviewers' comments.

In bold black, our response.

06-Aug-2023

Dear Dr. Tejero-Cicuéndez:

Thank you for submitting your manuscript, "Evolution along allometric lines of least resistance: Morphological differentiation in *Pristurus geckos*" (ID EVO-23-0198), to Evolution. The evaluations of the Associate Editor Dr. Brian Sidlauskas and two Reviewers, as well as my own, indicate that your manuscript is potentially appropriate for Evolution. Nevertheless, some substantive issues need to be addressed before the manuscript can go forward.

The concerns of the Associate Editor and reviewer are detailed below, and include suggestions for how to improve the presentation in ways that will substantially improve the paper (e.g., writing and figures).

When submitting your revised manuscript please provide a cover letter documenting the changes that you have made to the original manuscript. To expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s). The manuscript length guidelines are expected to be adhered to as much as possible during the revision process.

Please note that issues are occasionally identified in the revision that were not observed in the original submission. Hence, there is no guarantee that the revised manuscript will be accepted even if the issues highlighted in the first round of review are addressed.

All revised manuscripts must include two versions of the main text: (1) a clean document and (2) a document with highlighted and/or tracked changes. If you are unable to do this (e.g., because your manuscript was created in LaTeX), please contact the editorial office.

DATA ARCHIVING: Please also remember that Evolution requires, as a condition for publication, that data supporting the results in the paper be archived in an appropriate public archive. Please ensure you have archived your data and included an updated link to the data in the Data Availability statement, if applicable.

If you indicated in your submission that you will be archiving data on Dryad, the journal will notify Dryad of your acceptance decision to initiate curation and release of your data. Once your data have been deposited and released, please include the Dryad identifier (DOI) in your updated Data Availability statement. Please email the editorial office at managingeditor@evolutionsociety.org if you have any questions about this process.

1. You may use the link below with no need to enter login details:

*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***

https://mc.manuscriptcentral.com/evo?URL_MASK=b70206104a83493890f8bab9631fefdd

OR

2. Log into <https://mc.manuscriptcentral.com/evo>, enter your Author Center, and click on the link "Manuscripts with Decisions"

Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. Though your original files are carried over to the revision, you will be unable to modify them. Please delete any redundant files before completing the submission. Any original files that do not require modifications may remain.

When we receive your revised manuscript, the Associate Editor will review it and your responses before deciding whether further revision or information is needed. The Associate Editor will then make a recommendation regarding final acceptance to me, and I will make a final decision on whether to accept the paper for *Evolution*.

In order to facilitate the timely publication of manuscripts submitted to *Evolution*, we ask that you resubmit your revised manuscript within 90 days. If this is impossible, please send an email to our Managing Editor (managingeditor@evolutionsociety.org) to request an extension. Otherwise, any paper returned after the 90-day limit may be treated as a new submission.

Once again, thank you for submitting your manuscript to *Evolution* and I look forward to receiving your revision.

We thank the editors and reviewers for their careful attention and thoughtful comments. As described below, we have incorporated the suggestions given to us, including a PCA of the size-standardized species means resulting from a non-phylogenetic regression (new Figure 5) as raised by Reviewer 2, and the multiple points of Reviewer 1: the correction of the terminology regarding static allometry, which, due to a potential conflation of static and ontogenetic components, we now refer to as ‘intraspecific allometry’, the addition of a new table showing the comparison between evolutionary and intraspecific allometry vectors as well as between habitats (new Table 1), the addition of a figure in the Supplementary Material showing a scatterplot of limb and head residuals, and the modification of multiple parts of the Discussion to underscore the need of a cautious interpretation of our results regarding adaptive processes, especially those involving the colonization of ground habitats.

Sincerely,
Dr. Tim Connallon
Editor, *Evolution*
Dr. Tim Connallon

Associate Editor
Comments to the Author:

I have received two reviews from experts in this paper's methodology, both of whom agree that the manuscript has improved substantially from the previous version. Both also indicate some need for additional revision.

Reviewer one asks for a phylomorphospace with fewer manipulations to the dataset prior to visualization, citing Polly (2013), who demonstrated that the use of a phylogenetic correction prior to comparative analysis can yield results that are difficult to interpret, or that differ substantially from results using

ordinary PCA. While the supplement does include a phylomorphospace using raw trait values, it primarily illustrates that some species are larger than the others (PC1) without telling us much about proportional shape. Presentation of a phylomorphospace based on ordinary principal components analysis of the size-standardized species means (e.g., without an underlying modification for the phylogenetic structure of the data) would better address this reviewer's concern and would reveal whether any of the core results of the paper depend on the choice of ordination method. The authors may want to place both visualizations in the main paper, or to place one in the supplement, but either way it would be valuable for readers to be able to see both versions of the phylomorphospace.

We appreciate the comment, and have now included in the main text a PCA of the size-adjusted data without use of the phylogeny (i.e. OLS regression, not phylogenetic regression, to obtain size-adjusted values). We do note however that our approach was not a phylogenetic PCA (sensu Revell 2009), which was the approach that Polly 2013 addressed. Nonetheless, we appreciate the reviewer's point that a simpler presentation via PCA would better facilitate interpretation. We now included the original phylomorphospace in the Supplementary Material.

Reviewer two penned a very thorough review and raised a concern about the possible conflation of static and ontogenetic allometry that needs to be addressed. Has growth really slowed enough at the ontogenetic stage examined herein for the variation to reflect pure static allometry? If the variation seen here instead reflects a conflation of static and ontogenetic allometry, the paper should call this "intraspecific" variation, and should be more cautious in some interpretations, as the reviewer outlines.

We thank the AE and Reviewer 1 for this point. Given that data were not available for juveniles, we are incapable of quantitatively disentangling static and ontogenetic allometry (though we agree with the reviewer that this doesn't fundamentally change any of our findings). However, because of this, we have referred to this level of allometry as 'intraspecific' throughout as suggested by the reviewer and AE.

Reviewer two also indicates that the discussion seems weighted too heavily toward an adaptive interpretation of the observed variation in allometric trajectories and raises several good points that support that position. While the authors of course have the leeway to argue for their preferred interpretation, they should consider these comments carefully. They may wish to rewrite the discussion to focus more strongly on comparisons to allometric studies in other groups, to reduce the speculation about the adaptive value of allometric differences in these geckos, and to consider alternative explanations.

We have carefully considered the reviewer's comments and have toned down some wording in the discussion as appropriate, adding multiple cautionary notes on the adaptive interpretation of our results, especially those regarding the colonization of ground habitats.

With respect to the second reviewer's comments about the figures, I agree that figure 3 is somewhat difficult to interpret and could be more effectively presented as a scatterplot. I don't share reviewer two's concerns about figure 6, which I thought helped to tether the paper's mathematical abstractions to the original observations nicely. That figure could, I suppose, be complemented with a more quantitative treatment, but I don't think that the authors need to remove it.

We have added a scatterplot of head and limb slope values as the reviewer suggests in the Supplementary Material. For the main text, however, we kept our original Figure 3 since we believe the evolutionary interpretation is much more intuitive with the values mapped onto the phylogeny, which also allows for this visualization to be framed in a temporal context. The aim of this figure is to visualize how the relative morphological proportions have changed during the evolutionary history of the genus and with respect to changes in body size. Therefore, we believe this information is better conveyed

through a phylogenetic tree mapped with the residuals, and separately for head and limbs. We have added additional description for clarification.

Overall, I think the manuscript is shaping up to be a fine contribution to evolutionary allometry and herpetology, but also agree with the reviewers that some items still need polish. Luckily, the remaining revisions should be relatively straightforward. I look forward to reading the next version of the work.

We thank the AE for their thoughtful comments and care with our paper. Their comments, and those of the reviewers, have greatly improved our work.

Literature Cited

Polly, P. D., Lawing, A. M., Fabre, A. C., & Goswami, A. (2013). Phylogenetic principal components analysis and geometric morphometrics. *Hystrix*, 24(1), 33.

Dr. Brian Sidlauskas

Reviewer(s)' Comments to Author:

Reviewer: 2

Comments to the Author

I thank the authors for revising the manuscript based on my previous comments, with most of the comments addressed in the updated version. There is one major aspect of the study that I'd like the authors to consider:

The conclusion that rock-dwelling and ground-dwelling species show contrasting allometric trends (e.g. Ln 28–30) is based on residuals from PGLS on body measurements and SVL. This statement implies that these two ecological groups undergo opposite allometric trends/slopes, but we don't observe this (e.g., Fig. 2). I suspect that this observation is being made due to the compounding corrections of the original morphometric data (for allometry and phylogenetic relationship) that modify the multivariate data in increasingly unpredictable ways. In other words, these corrections make it more difficult to biologically interpret the results (refer to Polly et al. 2013 *Hystrix*). What I strongly recommend, as I did in the previous round of reviews, is to show a phylomorphospace with PC scores on raw measurements (and perhaps another with allometry-corrected values). Otherwise, it is genuinely difficult for me (and many others) to reliably interpret the biological phenomena occurring in this system.

We appreciate the reviewer's point and have now performed the PCA on size-adjusted data from an OLS regression, not a phylogenetic regression. The general patterns we observed in our prior figure still hold, and thus so too do the biological interpretations. We do note that use of phylogenetic regression to obtain residuals is not mathematically the same as the issue Polly et al. 2013 discussed (that of incorporating the phylogeny into the PCA rotation via phylogenetic PCA; sensu Revell 2009). Nonetheless, we do acknowledge that fewer manipulations of the data to obtain a PCA facilitates a more straight-forward interpretation for the readers, which we implement here and present in the updated Figure 5.

Reviewer: 1

Comments to the Author

General. The revision has tackled the problems of the statistical analyses of allometry and of disentangling evolutionary from intraspecific allometry. As a result, the new version of the manuscript is clearly improved. I am less convinced by the way the revision has handled the problem of the relative contributions of static versus ontogenetic allometry to the observed intraspecific allometry, and I think this needs some further work. Finally, given that this essentially is a study about allometry and how it evolves in this group, possibly in response to habitat switching, I am somewhat surprised by how much of the Introduction and Discussion are about things other than allometry. To me, much of this seems rather speculative and relies on interpretations of the results that rely in part on assumptions that cannot be tested with the available data. In some instances, I actually doubt the authors' interpretations, even though I must admit that my knowledge of herpetology is very limited.

Overall, I recommend a further round of revision. I am confident that the authors can address the remaining problem concerning the intraspecific allometry. For the problems of interpretation, I am coming to (or already venturing beyond) the limits of my knowledge of lizards and herpetology in general. Unless reviewer 2 is a herpetologist, I think it might be sensible to use a different reviewer with expertise in this area for a next round of review instead of me.

We thank the reviewer for their comments, and address the points below.

Major points:

1. Response to my point 1 (single origin of ground living): In their response to my comment the authors say that they "have modified the text adding some cautionary notes in the interpretation, especially regarding the ground-living species". As far as I can see, this refers to the two sentences on lines 383–386 in the revised Discussion. As far as I can see in the remainder of the Discussion (overall 147 lines long), there are many places where associations of traits with ground living are interpreted as adaptation without any mention of this important caveat. I think this requires more changes throughout the Discussion.

We agree with the reviewer on the importance of being cautious when interpreting results in the context of adaptive processes. The parts of our discussion that involve the link of traits with habitats from an adaptive perspective, especially in ground habitats, are mainly paragraphs 3, 4, and 7. We included additional cautionary notes about this on such parts of the Discussion.

Paragraph 3: when discussing shifts along branches of the phylogeny leading to species exploiting different habitats: *"although it must be noted that all extant ground species have a single origin in the phylogeny and constitute a monophyletic group"*.

Paragraph 3, when talking about habitat-driven adaptive dynamics: *"even though the fact that all ground species belong to the same clade hinders our ability to draw stronger conclusions about adaptive dynamics involving the colonization of ground habitats"*.

Paragraph 3, instead of claiming that there is support for the hypothesis that colonization of the ground has triggered morphological change, the new version now states that our results are consistent with that idea, which stems from a previous paper on this genus (Tejero-Cicuéndez et al. 2021 PRS-B).

From this point, we focus our discussion on the "habitat-driven morphology" perspective, as stated at the beginning of paragraph 4, adding context from other examples in the literature, but always with a cautious position regarding the adaptive interpretation of our results. In order to be more cautious, following the reviewer's recommendations in this comment and in the major point 4 (see below), we have further modified the text in other parts of the discussion when we present our results in an adaptive context (paragraph 4 and 7).

At the end of paragraph 4, we now add: “The lack of repeated events of colonization of ground habitats in *Pristurus* makes it challenging to corroborate these adaptive explanations about phenotypic changes”.

Paragraph 7: When talking about ecological selection on body size derived from ground colonization. “This observation might be related to some level of ecological selection on body size” instead of “This observation implies some level of ecological selection on body size”.

Paragraph 7: “although this perspective would be further supported if there had been repeated instances of colonization of ground habitats in the genus”.

Paragraph 7: “From this adaptive perspective [...]”.

2. Response to my point 2 (ontogenetic stages): I think there was a bit of miscommunication between me and the authors here. I apologize if my original comments were unclear, and I encourage the authors to have another go at this problem. The authors have added a comment (lines 131-132) that all specimens included in the data were adults and that ontogenetic allometry therefore could not be considered in this study. This is true and I thank the authors for adding this comment.

The more serious concern, however, has not been addressed in the manuscript: the question whether what the authors call static allometry is indeed static allometry or some combination of static and ontogenetic allometry instead. In their response, the authors claim that “while geckos do display indeterminate growth, this diminishes precipitously post-maturation.” In the manuscript, there is no such explanation. If the observed intraspecific variation indeed is static variation, there should be no correlation between the sizes and ages of individuals within species. I am no herpetologist, and so I ran a simple literature search on Google Scholar with the keywords “gecko growth”; the very first paper that the search returned showed that there was just such a correlation in a different species of gecko and calculated growth rates seemed to decline rather gradually (https://brill.com/view/journals/amre/27/3/article-p393_10.xml). My point is not to start a debate about “precipitous” versus “gradual” decline of growth rate, but I am genuinely concerned about the interpretation of what is going on.

For instance, we might suppose that rock-dwelling species might be more limited in their growth than ground-living species, for which we might provide an adaptive speculation that this enables rock-dwellers to hide from predators in small crevices (I dimly remember reading something like this concerning other lizards) whereas larger individuals in open habitats can invest more resources in reproduction, or alternatively the more extensive growth might just be a non-adaptive feature of the ground-dwelling clade. In either way, intraspecific allometry would be clearer in the ground-living species because of the greater variation in body size from more extensive growth and therefore a greater contribution from ontogenetic allometry to the observed intraspecific allometry. For the same reason, under this scenario, the ground-living species would exhibit stronger intraspecific integration than the rock-dweller because the contribution of ontogenetic variation (expected to be concentrated mostly in the single direction of phenotypic space along the mean ontogenetic trajectory) produces higher integration.

The information I have seen is not about *Pristurus*, and so I cannot say whether such a scenario might be at all realistic. I see two possible ways to address this problem. If this information is available, the authors could provide convincing evidence in the manuscript (e.g. from literature) that in samples of adult *Pristurus*, such as those of this study, age and size are indeed uncorrelated (and therefore the bigger individuals do not tend to be the older ones). If this is impossible because there is no such information available (determining the age may require destructive sampling, as far as I understand), it would be sufficient to change the terminology by changing “static” to a more agnostic term such as “intraspecific” throughout the manuscript, and adding an explanation in the Methods section that, because of the

indeterminate growth of these lizards, it is not possible to separate static and ontogenetic variation completely. None of the main findings of the paper would change, as far as I can see.

See also comments to AE above. As age data was not available for our specimens, we were not able to quantitatively disentangle static from ontogenetic allometry in our dataset (though as the reviewer correctly points out, this does not alter any of our main findings). Therefore, and following the suggestion of both the reviewer and the AE, we refer to this level of allometry as ‘intraspecific’ allometry, so as not to inappropriately assign the pattern to static allometric trends, when it is possible that some ontogenetic scaling may also be included. We now make this issue clearer in the manuscript and explain the reasoning behind the wording.

3. Lines 228-235 and 237-252: For each of these comparisons, the manuscript does not present the actual patterns. I would be curious to see those, and there should be room in the manuscript (for evolutionary versus intraspecific allometry, it’s a table with just two columns of numbers, and manageably more for the habitat types...). Seeing the regression coefficients would enable readers to interpret what is going on in closer connection to anatomy, natural history, biomechanics etc....

We have now included the regression coefficients for each habitat type (which in the prior version of the manuscript appeared in the Supplementary Material Section 2) in the main manuscript (Table 1), along with the evolutionary and the intraspecific allometry vectors. We agree with the reviewer that this information is better found in the main manuscript.

4. To my taste, the Discussion contains too much adaptive speculation that is presented without sufficient (or even without any) explanation or evidence. This starts around line 372, where the manuscript interprets shifts in allometric trajectories as a result of habitat-induced selection, and goes on to the end of the Discussion. The manuscript presents no evidence that shifts in allometric trajectories are the result of selection. There are many similar instances where the existence of differences is assumed to be adaptive and the result of selection.

Related to this is also the selection of references, which is at least debatable in some places. The last instance of adaptive speculation in the Discussion is on the implications of arboreality, where the manuscript suggests that limited ranges in shapes and sizes of arboreal taxa are related to strong ecological selection (lines 459-461). The two references cited to support this are papers about the evolution of arboreality in salamanders. Even as an evolutionary biologist not particularly familiar with the study group, I can think of a closer example that provides a very different picture: *Anolis* lizards, where there is not just one but several types of arboreal niches and morphological ‘types’ of species inhabiting them. So the arboreal habitat seems to be very different for salamanders versus *Anolis* lizards; how it affects *Pristurus* is an interesting question that may go beyond what this study has to offer. I think a fundamental reworking of the Discussion to focus on questions of allometry, which is the subject of this paper, and removes adaptive speculation would greatly benefit this paper. Where adaptive interpretations are offered, this should come with substantially more detailed reasoning and specific evidence. I am not familiar enough with the biology of geckos to provide a competent review of such a revision; this is why I have suggested that a different reviewer should see a revised version.

We thank the reviewer for this comment that is aimed to improve the overall accuracy of our manuscript. We believe that what the reviewer points out as adaptive speculation in the Discussion is the interpretation of our results under an adaptive perspective. Although the reviewer states that this goes from line 372 (paragraph 3 of the Discussion) to the end, these explanations are mainly focalized in paragraphs 3, 4, and 7 of the Discussion. These parts of the Discussion are important to link the patterns observed in our data and the plausible processes generating them. Additionally, paragraph 4 about how the ecological context might have acted producing the observed patterns was included following suggestions of reviewer 2 in the previous round of revision.

Therefore, rather than removing these parts, we have softened the adaptive discussion, mainly related to the colonization of ground habitats (see our response to the related major point 1 above), and we believe that the new version of the manuscript presents a much more cautious interpretation of our results. Even though we acknowledge that we rely on speculation for discussing our results, we believe that our hypotheses and explanations are sufficiently justified by the broader context in which our discussion is framed, and also that, with the new comments added following the reviewer's suggestions, the interpretation of our results is appropriately cautious. Our results in this manuscript, together with other recent results on this system (Tejero-Cicuéndez et al. 2021 PRS-B), offer, in our view, a proper opportunity to focus the discussion on the adaptive processes that might underlie the patterns we found. However, we believe that our discussion also presents a detailed overview of the allometry-related processes that our analyses specifically revealed, in the broader context provided by the literature on allometry and integration in other systems.

For the case of the potential adaptation to the arboreal habitat, we thank the reviewer for pointing out the contrast between our results in *Pristurus* and those of other lizards such as *Anolis*, and we have added it in our Discussion: *"If that is the case, this contrasts with the evolutionary dynamics observed in other lizards such as the Anolis radiations, where there are multiple morphotypes for different strata of the arboreal habitat (Losos 2009)"*.

Minor points:

1. Line 55: The phrase "allometric patterns manifest widely" is awkward ("differ" instead of "manifest", or something like that?).

We changed the text, and it now reads "[...] allometric patterns are widely prominent across differing levels of biological organization [...]".

2. Line 268: The arrow is the wrong sign for indicating a range (I might simply spell out that angles ranged from 5.8° to 7.2°).

Done. It now reads " $5.8 < \theta < 7.2$ ".

3. Figure 3. What is the aim of this figure? If it is to show the connection between head residuals and limb residuals, why not produce a scatter plot of hward residuals versus limb residuals and possibly project the phylogeny into the scatter plot (phylomorphospace with scatter plot of residuals as the morphospace instead of a scatter plot of PC scores). You could use different colors of symbols for the dots to indicate habitat types. I think this would be clearer, as I find it a bit tedious to relate the colors of lines in the two otherwise identical graphs, and spare a thought for a reader who might print out the figure on a black-and-white printer....

The aim of Figure 3 is to visualize how the relative morphological proportions have changed during the evolutionary history of the genus and in the context of changes in body size. Since head and limb structures are potentially subjected to different evolutionary trajectories, as justified in the text, and the temporal component of this evolutionary pattern is relevant, we believe that the information about these changes is better transmitted separately for head and limbs with the residuals mapped onto the branches of the phylogeny. Following this reasoning, a scatterplot would be in our opinion somewhat harder to interpret in the sense that changes are not visualized in a temporal framework and the phylogenetic relationships and body size evolution are harder to grasp. However, we agree that the

visualization of the scatterplot with the phylogenetic relationships gives an additional perspective and might still be useful for some readers, and therefore we included it within the Supplementary Material.

4. Line 296: spell out that this is the phylomorphospace of the PCs of regression residuals.

Done.

5. Line 366: Allometric patterns aren't rock-like. Change the wording.

Done.

6. Figure 6 is not entirely helpful because it is not consistent with the quantitative approach used in the rest of this paper. Why not draw some diagram that is based on the measurements actually used in the analyses, but without uncontrollable differences in other aspects?

After considering the reviewer's comment, we still believe that Figure 6 is helpful for interpreting the interaction of body size and shape differentiation across the ground and rock habitats, showing the morphological similarities and differences between large and small species in different habitats.