20-Mar-2023  
  
Dear Dr. Tejero-Cicuéndez:

The manuscript EVO-23-0020 entitled "Evolution along allometric lines of least resistance: Morphological differentiation in *Pristurus* geckos", which you submitted to  Evolution has now been reviewed.  Associate Editor Dr. Brian Sidlauskas handled it, obtaining two reviews and writing an evaluation (all attached). Based on these evaluations, as well as my own, I regret to inform you that the manuscript is not suitable for publication in Evolution in its present form (these evaluations are below). However, I consider a new manuscript on this topic potentially appropriate for Evolution. Therefore, although the issues raised preclude the manuscript's acceptance, I am declining it "without prejudice" regarding future resubmission.

If you are certain that you can fully address the issues raised, I encourage you to revise the manuscript and resubmit it.  A revised version will be treated as a new submission and will be subject to the full peer review process. Resubmitting your manuscript does not guarantee eventual acceptance. Issues are sometimes identified in the resubmitted manuscript that were not observed in the original submission. Hence, there is no guarantee that the resubmission will be accepted even if the issues highlighted in the first round of review are fully addressed.

If you choose to resubmit this manuscript to Evolution, please include an itemized response to each of the comments or suggestions. For continuity, the manuscript is likely to be assigned the same Associate Editor, but it may be sent out to different reviewers. In any event, I hope you find the reviews constructive.

All revised manuscripts must include two versions of the main text: (1) a clean document (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.  
  
Once you have revised your manuscript, go to <http://mc.manuscriptcentral.com/evo> and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript. Though your original files are carried over to the resubmission, 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.

Thank you for submitting your work to Evolution.

Sincerely,  
Dr. Tim Connallon

Editor, Evolution

[tim.connallon@monash.edu](mailto:tim.connallon@monash.edu)

Associate Editor

Comments to the Author:

Thank you for submitting your work for consideration in Evolution.  I have received two high quality reviews, both from scientists with substantial expertise in evolutionary morphology.  Both of them found substantial merit in the manuscript, including the excellent dataset, the interest of the central questions and the suitability of the study system.   However, both reviewers also identified important concerns with the paper as currently written, and the most important of these is an analytical concern that may cause the paper's central conclusions to shift fundamentally once corrected.  As such, I think that the paper cannot be published in its current version, but a future version might well be publishable in Evolution. As such, I am recommending rejection of the the current version of the manuscript, with encouragement to submit a substantially revised version that addresses the concerns of the two reviewers.

In your revisions, pay the closest attention to Reviewer one's comments about the conflation of evolutionary, static and ontogenetic allometry in the analysis.  That reviewer is correct that  the current approach to the individual-level data (which uses a single pooled mean for all species) introduces a substantial amount of evolutionary allometry into the individual dataset, and that some of the paper's conclusions (such as the strong correspondence between evolutionary and "static" allometry) may be artifacts of that analytical decision.   This issue must be addressed before the paper could potentially move forward.

That reviewer also stresses concerns with the conflation of static and ontogenetic allometry, and indeed, it would be optimal if these can be separated.  If they can't be disentangled (for example, because of the lack of some ontogenetic stages in some species) then at a minimum this needs to be acknowledged and discussed, and terms clarified.

Reviewer two also makes several important critiques, of which the most important probably involves the insufficient connection to recent advances in the study of allometry, and incomplete explanation of how the results of this paper fits into current discussion about allometric theory, and perhaps the discussions surrounding concepts of modularity and integration. I recommend spending the most effort responding to that concern, though all of reviewer two's points should certainly be considered.

I hope that this decision does not come as too much of a disappointment, and I stress that both reviewers see potential in the manuscript, as do I.  I look forward to seeing a revised and resubmitted version of the work.

Sincerely,

Brian Sidlauskas

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author

General. This is an interesting study in a fascinating study system. As the authors state, this is a contribution that adds to a growing body of literature documenting the effect of habitats not only on mean phenotypes, but also on the patterns of allometry. The methodology used here is the traditional approach on linear measurements, combined with modern statistical analyses. The main finding is that the patterns of evolutionary allometry, as characterized by phylogenetic regression, are similar to the patterns of “static” allometry across individuals.

My main concern is with the levels of allometry, especially what the authors call “static” allometry. Static allometry, as usually understood, is the size-associated variation in response to variation of size among individuals within a single population/species and at the same ontogenetic stage. Some of the analyses of “static” allometry in this manuscript include multiple species, and therefore some evolutionary allometry. Considering this, the finding that the patterns of evolutionary and “static” allometry coincide appears considerably less surprising than it may be at face value.

Also, as far as I know, geckos have indeterminate growth and therefore increase in size as they get older, although probably at a progressively diminishing rate. Neither this manuscript, nor the paper by Tejero-Cicuéndez et al. (2021) mention any steps that were taken to standardize the ontogenetic stage of specimens used for measuring the traits. Therefore, it seems at least plausible ore even likely that what is called “static” allometry has also some ontogenetic component.

As a consequence, I think the analyses will need to be rerun in a manner that cleanly separates evolutionary allometry from intraspecific variation (e.g. through a pooled within-species regression). This should be fairly straightforward. In addition, every possible effort should be made to standardize for ontogenetic stage, so that the ontogenetic component of variation can at least be minimized. This may be harder, I imagine. At the least, however, the problem must be clearly acknowledged in the manuscript.  
  
Whether the main findings will hold up in these new analyses is unclear. Even if they won’t, chances are the new results will still be interesting, but the take-home message may change. This will also be an opportunity to clarify the explanations of many of the methods used in the analyses. As it stands, it is rather hard for the reader to understand what exactly was done.

Major points:

1. Lines 75-77. I agree that this is important and interesting. Yet, there is also the problem whether a feature of variation can be attributed confidently to habitat, or whether there are alternative explanations—for instance membership of a particular clade. This problem may be particularly acute for the ground-living species. If I understand correctly the right side of Fig. 1b in Tejero-Cicuéndez et al. (2021), the ground as a habitat had a single origin, so that the ground-living species are a subclade within the genus. Special features of size variation and allometry of ground-living species could therefore be a consequence of the ground as habitat and selective environment, or they could be due to some other clade-level feature that evolved on the same branch of the phylogeny as ground-living, but might not be causally connected to that habitat. It is impossible to infer which of these is true from comparative analyses, but we would need data on ecological and evolutionary factors to distinguish the two scenarios. Arboreal habitat seems to have evolved a few times, so the situation is a little better there, but some ambiguity still remains. Some more caution in the interpretations based on habitat therefore would be appropriate throughout the manuscript.

2. Lines 113-121: The description of how the morphological data were collected says nothing about the ontogenetic stages of the specimens that were used. I think this is important to know because the variation among individuals within species may be a combination of static and ontogenetic variation. As far as I was able to see when checking the literature on growth in geckos, the species that have been investigated seem to show indeterminate growth, where older individuals continue to grow, although at gradually diminishing rates. Were any steps taken to standardize the ontogenetic stages and thus sizes within species? Obviously, this has an effect on the variation among species in the average body sizes. Based on the available information, it may not be possible to separate static and ontogenetic allometry; in this case, perhaps more neutral terminology might be preferable (e.g. “intraspecific allometry”?), along with an explanation that it is a composite of static and ontogenetic allometry in unknown or unknowable degree.

3. Lines 131-132: A pooled analysis of individuals across multiple species will produce a characterization of some mix of static and evolutionary variation (allometry etc.). To the extent that the species are distinct from each other and individuals cluster fairly tightly around the respective species mean, evolutionary variation will actually dominate in this type of analysis. To characterize variation among individuals within species, which I guess is what the authors intended here, a pooled within-group analysis would be more appropriate, in this case a pooled within-species analysis. These analyses (regression, PCA, etc.) focus on the deviations of individuals of the respective species mean, combining those deviations across all groups/species (hence pooled within-group). The assumption underlying this approach is that the groups/species in the analysis share the same characteristics (regression slope, PC vectors etc.).

A further problem of pooling individuals across species, as stated in the manuscript, is that the measurement values in such combined data are not independently sampled from some underlying distribution, as it is assumed by the statistical tests done as part of regression analyses. Depending whether two measurements are from individuals of the same or two different species and how closely those species are related, the measurements will be related through a different degree of shared ancestry. By contrast, deviations of individuals from their respective species averages are independent of one another, provided the distributions within species are the same. Note that even permutation tests do make the assumption that observations are independent – this is the rationale for simulating the null hypothesis by randomly permuting values – although they do not make the other assumptions of parametric tests.

4. Lines 136-139: The same problem as above applies here. As long as the measurements of specimens from the different species are used, not deviations of specimens from the respective species averages, evolutionary variation will be a component in this analysis. Alternatively, species might be included as an additional factor, in the context of a (phylogenetic) generalized linear model to take into account the non-independence due to phylogeny. This is a bit awkward because habitat and species are interrelated, but I guess this will not be an insurmountable obstacle.

5. Lines 153-163: The analyses of integration, if they used all specimens from the set of species of a particular habitat type, use a mix of variation from different levels: evolutionary (if more than one species is included), static and ontogenetic (the intraspecific component). I guess this is actually not what the authors intended, and so suggesting a solution depends a bit on what the intention actually is.  
One sensible question would be whether the different habitats have effects on the static/ontogenetic integration within species. In this case, the analyses should be based again on the deviations of specimens from the respective species means. Alternatively, integration statistics could be computed species by species.

6. Lines 164-171: as far as “static” allometry is concerned, all the comments from above apply. Because “static” allometry on the basis of habitat groups with multiple species includes a component of evolutionary variation from the differences among species means, this sort of comparison would be expected to yield a strong concordance between “static” and evolutionary allometry, especially if a large number of species are included and a the range of size variation is large.

7. Lines 176-179: A PLS analysis where one of the blocks contains multiple variables and the other just a single one yields a single PLS axis that is a scaled version of the vector of covariances between the two blocks (scaled so that the squared coefficients sum up to 1.0). This is exactly proportional to the regression vector from the multivariate regression of the multivariate block on the univariate block (in this case, multivariate regression of head or limb measurements on SVL). So there is actually no need to introduce that PLS analysis. This might simplify the explanations of what the analyses in this paragraph are doing…

8. Lines 218-224: Note that least-squares regression coefficients depend to some extent on the range of variation of the independent variable (log-size). For a model with homoscedastic variation of the log-trait values (i.e. the same variation of traits in all groups and for any value of log-size) and the same true regression coefficients, one would expect that the estimates of regression coefficients decline with declining range of sizes. Therefore, one would expect the ground-living lizards, which have the greatest range of sizes (Fig. 2), to have the highest estimated regression coefficients and thus the most positive or least negative allometry, the rock-dwelling group to be intermediate, and the tree-dwelling group to have the least positive or most negative allometry. This is exactly the result that is being described here. So, the result could be in part or entirely a statistical artifact.

Using within-species analyses (the proper thing anyway if static allometry is of interest) would at least in large part address this problem.

9. Lines 230-238: The same concern applies to integration: greater size ranges yield greater integration because more of the variation is due to the dominant allometric trend. So the results are again in the direction one would expect if there were no biological effect of habitat at all.

The size-corrected analyses are a bit more tricky. Because of the bias in estimating regressions for the size corrections, the simplest expectation would be that groups with smaller size ranges would produce residuals that show greater under-correction than groups with greater size ranges. But I am not sure I have thought this through to the end.  
Again, pooled within-species analyses seem more appropriate, above all because it seems that static and not evolutionary integration is being addressed in this analysis, and also because problems because of variable size ranges would matter less.

10. Lines 239-253: Because “static” allometry in the analysis contains a component from differences between species (if the species are clearly distinct and intraspecific variation is relatively minor, this is an substantial or even dominant component), the comparison is partly a comparison of evolutionary allometry with itself. Again, a pooled within-species analysis for the ”static” level would rectify this problem. Even then, though, that intraspecific variation may still be a mix of the static and ontogenetic levels (see above).  
  
11. Discussion: Overall, this is rather longer than it needs to be. Nevertheless, the current version does not mention the problems with the levels of allometry or the possible statistical artifacts that may affect the interpretation of results.

I think much will need to be rewritten in light of different analyses that need to replace the current ones. I would suggest to the authors to adopt a somewhat more concise style, focussing the discussion to the main message(s) of the study. Readers will likely find this much clearer, so that the paper will have more impact.

Minor points:

1. Line 39: Fitness is not only survival, but also reproduction. Therefore, adaptation to habitats is expected to produce traits that enhance performance in tasks relevant to reproduction and/or survival in that habitat.

2. Line 39: Whether the selection pressures are unique for all habitats, or whether there can be different habitats that exert the same selection pressures on a given set of traits is questionable. I’d say one would expect the selection pressures to be different for different habitats, but not necessarily unique for each habitat.

3. Line 63: Allometric levels are not necessarily hierarchical. They are for evolutionary allometry (among species, at a particular ontogenetic stage) versus static allometry (among individuals within species and at a given ontogeneic stage), but ontogenetic allometry cuts across this in a sort of “orthogonal” manner.  
And the ontogenetic level is a point that is missing in this manuscript, but needs some consideration as geckos are organisms with indeterminate growth, even if it is only to assure readers that it is not of major concern.

4. Line 100: The phrase “… may play a determinant role…” is awkward. Overall, I think the whole sentence on lines 97-101 tries to say too many things at once, and therefore there is a danger that readers won’t get any of them.

5. Lines 112-113: How reliable and how consistent are the habitat descriptions “found in the literature”? These tend to be done without a consistent methodology, depending on the different authors and their respective aims, which would raise questions for this study. A few comments on how the quality of the habitat designations was assessed would be helpful.

6. Line 119: Instead of the Greek letter mu, the word “mean” (or maybe median, as appropriate) would be more helpful, because it would unambiguously inform the reader what statistic of central tendency was used (I guessed it is the mean, rounded to the nearest integer, but this is no more than a guess…).

7. Line 134 (and elsewhere throughout the manuscript): the expression “angular direction” is very odd (it seems to be a direct translation from another language that does not quite work in English). The difference between two directions can be quantified by an angle. But a single direction is not angular.

8. Lines 207-211. As mentioned above, this concordance is not too surprising because a (possibly substantial) component of the variation among all the individuals from the various species is evolutionary variation/allometry.

9. Line 227: The phrase “… these multivariate allometric trends…” is confusing because the preceding passage (lines 218-226) describes the findings of multiple bivariate allometric analyses.

10. Lines 249-253: Smaller individuals tend to belong to smaller species, so the congruence between individual variation (in a multispecies mix) and evolutionary allometry is not all that surprising.

11. Lines 264-267: I’m sorry, but I do not get that impression from looking at Figure 4. Some more rigorous type of analysis is required here.

12. Lines 270-279: Two points here:

(a) Note that overlap of groups in a scatter plot of PC scores does not mean that the groups necessarily overlap in the multidimensional space, as they may be separated in directions orthogonal to the PCs used in the plot (and possibly not well aligned with any of the PCs, but oblique to all of them).

(b) Note that residuals from a regression of some traits on size are not necessarily shape. That word may be tempting as a shorthand, but it leads to conceptual confusion. There have been repeated calls to avoid this, with very good arguments, in the literature at least since the 1960s.

13. Figure 6: these are four hand-picked examples. Yet, Figure 5 suggests that there is quite a lot of variation within the groups, raising the question what the point is to single out those four.

14. Lines 296-306: This is essentially a repeat from the Introduction. Is this necessary here?

Reviewer: 2

Comments to the Author

The study aims to investigate how allometry and integration may have driven ecomorphological evolution using Pristurus geckos. The phenotypic data include SVL and 8 linear measurements. The sampling is impressive with a total of 687 individuals, each of 25 species represented by 9+ individuals. Notable results include ground-dwelling taxa exhibiting most divergence in allometric signal and the strongest level of morphological integration. The overall conclusion of the study is that allometric trajectories and strength of integration differ between habitat types. In general, the manuscript is well written and overall easy to comprehend (some places missing details, which are noted in my comments below). At the same time, the novelty of the results, clear explanation of expected differences in allometric trajectories, extent of study's broader implications, and synthetic discussion on ecology-allometry-integration-and evolution may be deficient or could be better articulated. Below, I list my major concerns and comments, followed by more specific comments.

GENERAL COMMENTS:

1. The lack of our understanding of how allometry (and trait covariation) is implicated in ecomorphological evolution is mentioned as a motivation for the study (Ln 17–18; 60–62). There is quite a large corpus of work on this topic (e.g., work coming out of research groups like Chris Klingenberg, Jen Bright, Anjali Goswami, Dean Adams, Chris Cooney, to name a few). How this study is a breakthrough in the study of allometry compared to previous studies should be better articulated to justify the potential novelty of the study.

2. The genus Pristurus seems like a good system, with varying body sizes within ecologies. However, why this genus is ideal for investigating the macroevolutionary consequences of allometry (and integration) is not adequately justified in my view. As such, I was left wondering how extensible the results of this study is beyond this genus.

3. I recommend adding a morphospace of raw (and/or size-scaled) phenotypic values to compare with Fig. 5. The Fig. 5 morphospace uses variables that have been transformed multiple times, which make it more dificult to interpret biologically.

4. The study's aim is mentioned to be elucidating the mechanism of ecomorphological diversity (Ln 6), but this study is still on trends and patterns--not so much process. This study does not provide any information about how ecological selection has driven differences in allometric trends and/or integration and vice versa, as well as the mechanistic drivers of ecomorphological divergence. For example, what are the adaptive reasoning for higher integration in ground-dwelling species? To demonstrate that differences in allometric trajectories have shaped ecomorphological evolution or ecology shaping scaling relationships, a clear link between these properties need to be described (i.e., how one would influence the other). This connection is not addressed in a satisfactory way. For instance, how could we conclude that natural selection likely acted along the principle axis of allometric trends?

5. How phylogenetically structured is the habitat type? If there is signficant phylogenetic signal in the habitat data, then it would undermine statistical comparisons between the two habitat types that are not phylogenetically corrected (e.g., vector differences and trait covariation between habitat types).

6. In my view, the main conclusions of this study are not particularly novel or not presented as such. The concluding sentence: "Therefore, the phenotypic diversity observed in Pristurus is best explained as the result of a complex interplay between ecological selection, body size differentiation, and differing allometric trajectories across ecological habitats" (Ln 418–420) is already known and have been shown in various taxa. The authors should articulate the novelty of this study with respect to existing literature and/or provide unique insights stemming from the results.

SPECIFIC COMMENTS:

Abstract  
  
Ln 17–18: "However, how ecophenotypic patterns are affected by allometric trends and trait integration levels is less well understood". As indicated above, there is a large corpus of literature that have examined allometric signal in ecomorhological data. Therefore, the novelty of this study should be better justified.

Introduction

> Ln 39: "Evolutionary theory"--more precisely, theory of natural selection.

> Ln 60-62: "However, while the patterns of morphological differences in distinct ecological contexts have been well documented, less-well understood is how this differentiation has been influenced by trait covariation associated with body size differences (i.e., allometry)." As commented above, there is a large corpus of work on how allometric trends relate to ecomorphology.

> Ln 63: "comparing allometry at the individual level, or static allometry." Static allometry is allometry within a population or species at the same growth stage, not necessarily at the "individual level."

Materials and Methods

> Ln 108: "Time-dated" should be just "dated" or "time-calibrated"

> Ln 160–161: "following the procedure of (2022)". The in-text citaton is incomplete.

Results  
> Ln 264-267: "Visual inspection of static allometry trends within species (Figure 4) largely confirmed these patterns, illustrating that ground-dwelling species generally displayed steeper allometric patterns in head proportions as compared with rock-dwelling species". I believe that the color-coded allometric slope values are scaled within habitat groups; therefore, comparison of slope is challenging to do, given that the differences in slope are very subtle between groups. Fig. 4 could be more useful if all habitat groups were plotted in the same plot for ease of comparison.

> In the main text and/or in Fig 5, please report the proportion of total variation accounted for by the PC axes.

> Ln 271-272: "though arboreal (tree-dwelling) species were somwewhat more separated in morphospace". I do not observe this in Fig. 5. The different habitat groups, including arboreal taxa, are quite spread out with large overlap.

> Figure 3. In my view, the layout of this figure is confusing because the taxonomic names do not line up with the branches of the traitgrams. As such, which taxa are exhibiting higher or lower residual values are unclear, despite this figure being referenced as such in the main text (Ln 254–256). For a more intuitive and useful figure, I recommend mapping the residual values onto the time-calibrated phylogenies for head and limb measurements, separately.

> Figure 5. The magenta color for data points of arboreal species is lacking. Presumably they are the gray points. In addition, the data points exhibit different sizes. Do the sizes correspond to their SVL?