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Macroevolutionary pattern of *Saussurea* (Asteraceae) provides insights into the drivers of radiating diversification

Xu Zhang, Jacob B. Landis, Yanxia Sun, Huajie Zhang, Nan Lin, Tianhui Kuang, Xianhan Huang, Tao Deng, Hengchang Wang and Hang Sun

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1st revised submission:	3 August 2021
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3rd revised submission:	2 October 2021
Final acceptance:	13 October 2021

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

Review History

RSPB-2021-1147.R0 (Original submission)

Review form: Reviewer 1 (Weston Testo)

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?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is an interesting paper examining the evolution of Saussurea, with a focus on the diversification of the group in the QTP. I appreciate the authors' effort to incorporate several complementary approaches to characterize drivers of the groups diversification, rather than relying on a single story. With that said, I do think this work would benefit from a stronger conclusion that attempts to synthesize the various topics examined into a cohesive framework for understanding the diversification of this clade (how do the niche- climatic- and trait-related drivers of diversification interact?).

More specific comments are provided below and are indicated with the line number at which they occur.

Line 20: Change "driver" to "drivers"

Line 131: Change "the divergence time" to "divergence times"

144: I don't think "high confident" is the right choice; perhaps "reliable"?

144-151: There are a few issues here. First, it is unclear what hyperpriors are specified for the calibration distributions – these can have very strong effects on divergence time estimates and should be specified here. In addition, this text is out of place – it should be described along with the other parameters of the BEAST analysis (~ line 136).

182: change "environmental variables" to "environmental variable"

200 (also in various figures): replace "haired" with "hairy"

205 (also in various figures): replace "appendage" with "appendaged"

220: there appears to be a superfluous "the" before "DIVERSITREE"

229: replace "GPS" with "latitude and "longitude"

231: Please cite the DOI of the GBIF dataset. This is made available when you download the data. In addition, was any attempt made to resolve the taxonomy of the records? This was done for the chloroplast genome accessions but I do not see any evidence here. In addition, further cleaning of the records using a semi-automated approach (e.g., CoordinateCleaner, Zizka et al., 2019) might help improve the reliability of these data, given the well-known problems with GBIF records. Finally, there are many duplicate records on GBIF – was any attempt made to remove these? Retaining these records can bias estimates of niche breadth. They could be removed by deleting records with identical latitude and longitude or alternatively by thinning (for example, with the 'spThin' R package [Aiello-Lammens 2019]).

243: How was the predictive power of the different bioclim variables assessed?

279: It is problematic to compare speciation rates of these subgenera, given that you have just demonstrated that they are non-monophyletic. These subgenera were defined on morphological characteristics that largely match those used in your trait-based analyses (as you indicate in lines 304-305); it makes sense to just retain those analyses and remove these from here and in Figure 1D.

302 (and line 416): "cauliferous plant" is a bit awkward, as it doesn't really refer to the trait, but to the plant as a whole. There are multiple options for how to reword this, but perhaps just "with stems" or "stemmed" would be sufficient.

308: This is the first time that I see FiSSE mentioned – this belongs in the methods.

447-451: This sentence doesn't seem to fit to me. How does this work provide a basis for mitigating biodiversity loss? Certainly not every paper has to be framed as relevant for conservation.

Figure 4: The analysis of niche breadth is interesting, but I wonder if there is more than can be done with this to provide a more meaningful insight into the evolution of this group. For example, evolution of niche breadth/range size or overlap of these amongst sister species? Given the differences observed amongst the three major clades, some interesting patterns should be expected.

Weston Testo

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions 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?
Excellent

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

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

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Yes

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Please see .docx for comments. (See Appendix A)

Decision letter (RSPB-2021-1147.R0)

17-Jun-2021

Dear Professor Wang:

I am writing to inform you that your manuscript RSPB-2021-1147 entitled "Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.

3) Line numbers in your main document.
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Sincerely,
Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
The manuscript under consideration was reviewed by two experts and myself. We all felt this is a valuable study based on an interesting dataset of a species-rich radiation in the Qinghai-Tibet Plateau region. The authors test various hypotheses about potential abiotic and biotic factors that may have influenced rates of diversification across the radiation of Saussurea. The authors have compiled an impressive dataset with plastome sequence data, paleoenvironmental inference, eight morphological characters, and ecological distribution data from over 200 species. The reviewers raise some important questions and concerns that I believe should be addressed before this manuscript can be published. In general, we all felt there were many analyzes performed and there needs to be a stronger integration of these various methods and the results with each other. Specifically: 1) Each of the many analyses should be explicitly justified, explained, and statistical results reported in the main text; 2) The results from the various analyzes should be compared and cross correlated and explained in context of the whole study; 3) Given the paraphyly of the group, a clear discussion of clade distinction and classification should be included; 4) The discussion should synthesize the results and describe the overall story of why this radiation occurred and how the results come together to form a complete story. The discussion should not simply repeat the results but instead develop the story.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
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Weston Testo

Referee: 2
Comments to the Author(s)
please see .docx for comments

Author's Response to Decision Letter for (RSPB-2021-1147.R0)

See Appendix B.

RSPB-2021-1575.R0

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions 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?
Good

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

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

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
I have read the revised manuscript "Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification" and I think the manuscript reads much clearer now with appropriate references to the methods used and key numerical results in text. As I stated earlier, I really like the general approach and the system is very interesting, so I congratrulate the authors. However, I have several remaining concerns that I believe the authors

did not address sufficiently and I am hesistant to accept the authors conclusions based on the results.

Firstly, I believe the estimated parameter value of Infinity for the carrying capacity of a diversity dependant model does not make sense and I do not understand the author's explanation for it. Under a model of diversity dependence, a clade-based carrying capacity (K) is approached in one of three different ways: speciation can decrease with diversity, extinction can increase with diversity, or both speciation can decreases and extinction increases with diversity. The authors found that a model where extinction increases is the best fit. However, they state that K=Inf is because speciation is constant – but diversity dependant extinction rates should still lead to identifiable value of K. Perhaps I am missing something crucial, and I apologise if that is the case, but I believe that this model is best supported because it was not compared to a constant rates model. I think a solution is to bring all the diversification models into the same framework which can be done in RPANDA. Here, the authors could compare a constant rates birth death model, environmental dependant models, time dependant models AND diversity dependant models and perform model selection.

The second point again relates to model selection, you found no evidence that temperature dependant diversification is a better fit to the data than a constant rates model, because even though this was the best model a delta AIC < 2 means the models are equivocal. You added part of the results to address this, but the discussion ignores this point completely which is misleading. The only evidence is that some particular clades (not the genus overall) show shifts in diversification rate in the Miocene and Pleistocene (from BAMM).

Third, the authors did not justify their decision to use three phylogenetic clades and three morphological clades to compare speciation rates. In their response they write "Comparisons of diversification rate among these morphological-based subgenera can provide helpful insights into the evolution and adaptation of key morphological innovations" this is true and the trait-based SSE analyses address this, but why did the authors compare speciation rates between the three arbitrary phylogenetic clades? In particular, there is no information on why looking at these three phylogenetic clades was done and the only mention in the methods is on line 276 "Three phylogenetic clades were resolved (clade-1, -2 and -3, Figure S1)". Without a justification, I don't think looking at the phylogenetic clades adds anything additional to the manuscript except unnecessary complexity. I also believe comparing the morphological sub-genera directly is not appropriate and instead this can be discussed in the context of trait dependant SSE models – a point also suggested by Reviewer 1 which i do not beleive was adequately addressed.

Decision letter (RSPB-2021-1575.R0)

31-Aug-2021

Dear Professor Wang:

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.

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.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article (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 http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

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

Associate Editor
Comments to Author:
I appreciate the authors' work to significantly revise and improve their study in response to reviewer comments. Please note the three remaining concerns of the reviewer about the analyzes that have been performed. I encourage the authors to address and explain these points.

Reviewer(s)' Comments to Author:
Referee: 2
Comments to the Author(s).
I have read the revised manuscript “Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification” and I think the manuscript reads much clearer now with appropriate references to the methods used and key numerical results in text. As I stated earlier, I really like the general approach and the system is very interesting, so I congratrulate the authors. However, I have several remaining concerns that I believe the authors did not address sufficiently and I am hesistant to accept the authors conclusions based on the results.

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Author's Response to Decision Letter for (RSPB-2021-1575.R0)

See Appendix C.

RSPB-2021-1575.R1

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions 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?
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?
No

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

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
I have re-reviewed the manuscript and I am mostly very happy with authors responses. Again, I really like the paper and the approach. I do have one main remaining point that was not sufficiently addressed in the revisions:

Main point:
I'm truly sorry if I am being pedantic but I must insist here that when K = Inf, the model is the same as a constant rates model of diversification, because diversity never approaches the asymptote and therefore diversification never slows down. I clarified this with the authors of the DDD package to make sure I was not misled. You can easily compare the diversity dependent models with a constant rates model within the DDD package using the bd_ml, bd_loglik functions, and I believe if you do this you find the constant rates model supported. If you only compare diversity dependant models you will always find support for diversity dependence, even when it does not exist! I don't think it is too much of a problem for your interpretation or discussion as you rely more heavily on the results from the RPANDA analysis than the DDD analysis anyway.

"The best-fit DDD model was one in which the extinction rate increased with diversity (DD+EL, AICc = 649.577, Table S7) while estimating an unlimited carrying capacity (K = Inf); indicating that accumulating diversity did not influence speciation rates of Saussurea." – here it is incorrect to state that the extinction rate increased with diversity if K = Inf.

"In addition, although extinction may increase with diversity, speciation of Saussurea is not limited by a species diversity carrying capacity, implying that the genus is possibly still in the evolutionary process of rapid speciation." – here it is unclear why speciation would be rapid based on this result?

A few minor points:
Will the phylogenetic data and trait data used in this study be published with the article?
Can the colour figures be made colour blind friendly. Each figure contains red and green as the main contrasting colours which is problematic for many.

Decision letter (RSPB-2021-1575.R1)

27-Sep-2021

Dear Professor Wang:

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.

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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.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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Data accessibility and data citation:
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policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

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Electronic supplementary material:
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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,
Professor Gary Carvalho
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
I am pleased to inform you that your revision of your manuscript RSPB-2021-1575 titled "Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification" is close to being acceptable for publication in Proceedings B. However, there are still some minor revisions that should be addressed expeditiously so I would appreciate if you could please deal with these in a revised version. First, please temper your language regarding your conclusions from your DDD analyses as there are still some interpretation concerns from the reviewer. And second, I would like you to make every effort possible to generate color figures using colorblind-friendly palettes. This minor revision will not be sent out for further review but will be checked by our Editorial staff to make sure that the paper is suitable to be sent to Production.

Reviewer(s)' Comments to Author:
Referee: 2
Comments to the Author(s)
I have re-reviewed the manuscript and I am mostly very happy with authors responses. Again, I really like the paper and the approach. I do have one main remaining point that was not sufficiently addressed in the revisions:

Main point:
I'm truly sorry if I am being pedantic but I must insist here that when K = Inf, the model is the same as a constant rates model of diversification, because diversity never approaches the asymptote and therefore diversification never slows down. I clarified this with the authors of the DDD package to make sure I was not misled. You can easily compare the diversity dependent models with a constant rates model within the DDD package using the bd_ml, bd_loglik functions, and I believe if you do this you find the constant rates model supported. If you only compare diversity dependant models you will always find support for diversity dependence, even when it does not exist! I don't think it is too much of a problem for your interpretation or discussion as you rely more heavily on the results from the RPANDA analysis than the DDD analysis anyway.

"The best-fit DDD model was one in which the extinction rate increased with diversity (DD+EL, AICc = 649.577, Table S7) while estimating an unlimited carrying capacity (K = Inf); indicating that accumulating diversity did not influence speciation rates of Saussurea." – here it is incorrect to state that the extinction rate increased with diversity if K = Inf.

"In addition, although extinction may increase with diversity, speciation of Saussurea is not limited by a species diversity carrying capacity, implying that the genus is possibly still in the evolutionary process of rapid speciation." – here it is unclear why speciation would be rapid based on this result?

A few minor points:
Will the phylogenetic data and trait data used in this study be published with the article?
Can the colour figures be made colour blind friendly. Each figure contains red and green as the main contrasting colours which is problematic for many.

Author's Response to Decision Letter for (RSPB-2021-1575.R1)

See Appendix D

Decision letter (RSPB-2021-1575.R2)

13-Oct-2021

Dear Professor Wang

I am pleased to inform you that your manuscript entitled "Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification" has been accepted for publication in Proceedings B.

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

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

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Please remember to make any data sets live prior to publication, and update any links as needed when you receive a proof to check. It is good practice to also add data sets to your reference list.

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

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,
Professor Gary Carvalho
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
(There are no comments.)

Appendix A

Zhang et al. present an exploration of the diversification of the cool temperate genus *Saussurea* in the QTP using macroevolutionary models based on molecular phylogenies, paleoenvironmental reconstructions and ecological trait data.

I liked the study, and it was interesting to see so many macroevolutionary models applied to an interesting and diverse group, using different kinds of data. These kinds of studies help us understand temporally dynamic diversification processes and make important contributions, particularly for regions like the QTP which often receive less attention than other biodiverse ranges like the Andes.

The methods are highly reproducible and the code on GitHub makes this accessible to readers, so I commend the authors on this.

I have a few main points as I felt unconvinced by the main conclusions, partly because I felt the results were under-reported, and also because the results seemed disparate so it was difficult to see how they all connected. Although it looks like a lot, I think my comments could all be easily addressed by the authors and I have made some specific suggestions as to how, but these could be addressed in a number of ways.

Main points:

1. My first main point is about validation and scrutiny of the main results. Lots of analyses were used but there was no assessment on whether they agree with one another. Where possible the authors should cross validate results, for example are the DR and BAMM tip rates positively correlated? Or if they give different answers – why? Also the authors found the best fitting RPANDA model to be temperature dependant, however the AICc score is < 2 AIC from time dependant or constant-rates – this suggests you actually can't reject a constant rates model. This is also supported by the DDD analysis – you found support for diversity dependence, but the K estimate was infinity – which suggests no diversity dependence at all! Support for this model was likely only preferred because it wasn't compared to a constant rates BD model (also for DDD analysis we need the model names in Table S8 explained). The TESS models are also not well explained, it seems like the posterior probability of a rate shift is very low, how do we interpret this?

   Also, and this might be a bit nit-picky (apologies), but the clades selected do not represent any singular taxonomic, ecological or morphological radiations (because the main morpho clades were paraphyletic right?), they are just three large clades that began to radiate at the same time. While it might be interesting to look at the differences between these groups, the selection of these clades is somewhat arbitrary. The three radiations therefore of course occurred in parallel, because you selected three clades from the same time.

2. Lots of different analyses were used in this study (BAMM, DR, TESS, RPANDA, DDD, HiSSE, MuSSE, FiSSE, QUaSSE, ES-sim, etc.). I understand that the macroevolutionary biologists toolkit requires lots of different models because we are yet to develop more holistic models that look at different aspects of trait evolution and diversification simultaneously. I use similar approaches in my work and can see the necessity of this. However, I think it would be important to take stock of why each of these models are used so the reader can understand the relevance of each one. Perhaps a table that specifies each model, the question it

addresses, and what kind of data it uses would be helpful. On this note, more clearly setting up the hypotheses and their predictions in the introduction might be helpful to see why the different models were used, e.g., if climate was more important than biotic interactions in generating QTP diversity then we would expect a stronger correlation between diversification and paleotemperature than species diversity….

On this note perhaps a justification of the assumptions should be made. How well does your data fit these assumptions? Similarly, how does taxon samplingaffect the results, given only 2/3 sampling.

3. My next point is that I believe that the results (and to a lesser degree the methods) were underexplained and most results were shifted to the supplementary material which makes it very hard for the reader to assess the work. For example, the authors should be reporting the tests used to get results – e.g., if you are reporting the results of an ANOVA (Line 278 or 280) then state this. Also all test statistics, parameter estimates, likelihood, AIC weights or deltas or whichever method was used to perform model selection, should be reported. This is done for the QuaSSE analysis but should do it for all analyses.

4. My final main point is that the results are interesting, but I was left wanting to know more about them and how they all fit together therefore I think the discussion needs more attention to the *why*. This would be easier if the hypotheses were set up more clearly, then the authors could state what is the relative support from each one based on the results. For example, I don't quite see how the range size and niche breadth analysis fit the overall story – this would have been considerable amount of work for the authors so it would be nice to place it more firmly in the overall "story".

   For example, the trait dependant diversification results were not deeply explained - why would these traits show different rates? What is the ecological significance of these traits? Do species in the clades which showed higher rates of diversification (from BAMM) have these trait values? The authors mention the snow lotus exhibit these traits, but did these lineages undergo more rapid diversification? Perhaps the trait values should be plotted on the tree next to the rates in figure 1 so the readers can actually see the distribution of these traits.

   For the paleotemperature driven rates, you are describing QTP uplift but using results from a global average temperature, but can you really make any inference about the uplift using this other data? If it was uplift, wouldn't a time-dependant model be best supported? And how well does the global average temperature reflect the local temperatures of the QTP?

Minor comments:

Line 86: I don't understand the use of attractive here – adaptive?

Line 113: These should be cited

Line 117: how was trait data combined – the mean, median?

Line 165: CLaDS is the latest tool for assessing branch specific rates and looks promising.

Line 166: Should use Phylogenetic anova as clades may be more similar due to common ancestry

Line 179: still a bit unclear what alpha and beta are – is one for the relationship with speciation and one for extinction?

Line 182: Please explain in more detail what these paleotemperature data are so the reader does not have to refer to another paper – also these are data for global paleotemperature not the QTP region – however local orogenesis probably means there is a big disconnect between local and global temperatures. Maybe justify the assumptions here.

Line 183: Nice to see the analysis replicated across the posterior!

Line 242: What does most predictive mean in this context?

Line 247: Can you please explain what is meant by climate lability?

Line 250: Which ENM models were fit? We need much more detail on this hyere as it seems integral to how you estimate species geographic ranges.

Line 274 & Fig S4: Figure S4 caption not well explained. What are the units on the right-hand side of the Y-axis? Also the posterior probability is very small for mass extinctions – its not clear why this was tested and the result is not explained.

Line 276-278: What test was done here? How was significance assessed? There is no context to this result. Same for DR statistic below.

Line 287: Why not report the ML value for alpha here?

Line 289: Which RPANDA model was this – linear or exponential, speciation or extinction constant?

Line 308: Why not describe this in the methods section?

Table S3 explain parameters

Line 342: ES-sim?

Appendix B

Associate Editor

Comments to Author:

The manuscript under consideration was reviewed by two experts and myself. We all felt this is a valuable study based on an interesting dataset of a species-rich radiation in the Qinghai-Tibet Plateau region. The authors test various hypotheses about potential abiotic and biotic factors that may have influenced rates of diversification across the radiation of Saussurea. The authors have compiled an impressive dataset with plastome sequence data, paleoenvironmental inference, eight morphological characters, and ecological distribution data from over 200 species.

RE: Thank you very much for the positive feedback on our study.

The reviewers raise some important questions and concerns that I believe should be addressed before this manuscript can be published.

RE: We greatly appreciate the constructive comments from the associate editor and the reviewers. Thank you very much for allowing us to address your concerns.

In general, we all felt there were many analyzes performed and there needs to be a stronger integration of these various methods and the results with each other. Specifically: 1) Each of the many analyses should be explicitly justified, explained, and statistical results reported in the main text; 2) The results from the various analyzes should be compared and cross correlated and explained in context of the whole study; 3) Given the paraphyly of the group, a clear discussion of clade distinction and classification should be included; 4) The discussion should synthesize the results and describe the overall story of why this radiation occurred and how the results come together to form a complete story. The discussion should not simply repeat the results but instead develop the story.

RE: We appreciate the comments from the editor and the reviewers. We have carefully addressed all points. We have listed our responses to the points raised by all reviewers below, along with line numbers within each response. We included a manuscript with our modifications colored in red. We are completely aware there are a lot of analyses involved, some complement previous analyses while others investigate different associations. We have included a summary of all analyses and major findings in Supplementary Material Table S1. We have intended to put this summary table in the main text to illustrate the relevance of each diversification analysis as suggested by both reviewers. However, we have to move the table to the supplementary file due to the strict page limit. Throughout the results and the discussion, we try to highlight when specific analyses complement others and show the same pattern, or when analyses are addressing new points. Paraphyly can be a more issue indeed; therefore, we attempted to frame the results both in terms of three major phylogenetic clades that were recovered as well as on the morphology-based subclades. The majority of analyses rely either on groupings of certain traits or within the three phylogenetic clades. We do make mention of the paraphyly observed, but that is predominately framed to show that many traits appear to be adaptive and form in the process of convergent evolution. For the discussion, we modified the previously submitted version to highlight the answers to our four main questions and when analyses supported similar patterns. We then integrated our findings with those found in the literature.

Referee: 1

This is an interesting paper examining the evolution of Saussurea, with a focus on the diversification of the group in the QTP. I appreciate the authors' effort to incorporate several complementary approaches to characterize drivers of the groups diversification, rather than relying on a single story. With that said, I do think this work would benefit from a stronger conclusion that attempts to synthesize the various topics examined into a cohesive framework for understanding the diversification of this clade (how do the niche- climatic- and trait-related drivers of diversification interact?).

RE: We thank the reviewer for the positive feedback on our study and these helpful comments. We agree that we need to synthesize the various topics examined into a cohesive framework for understanding the diversification of this clade. We added a table (Table S1, Line 104) for a summary of all diversification analyses conducted in the study and set up more clear hypotheses in the introduction (Line 92-102). These do make it clear and helpful for understanding how do the niche- climatic- and trait-related drivers of diversification interact.

Line 20: Change “driver” to “drivers”

RE: Done. Changed as suggested (Line 19).

Line 131: Change “the divergence time” to “divergence times”

RE: Done. Changed as suggested (Line 131).

144: I don’t think “high confident” is the right choice; perhaps “reliable”?

RE: Thanks, we changed “high confident” to “reliable” (Line 137).

144-151: There are a few issues here. First, it is unclear what hyperpriors are specified for the calibration distributions – these can have very strong effects on divergence time estimates and should be specified here. In addition, this text is out of place – it should be described along with the other parameters of the BEAST analysis (~ line 136).

RE: We thank the reviewer for the comments. We added detailed information on the calibration distributions. Both fossil calibrations were defined with lognormal distributions, which placed the 95% height posterior density (HPD) for the node age at 14.19-19.18 Mya and 5.19 -10.18 Mya, respectively (Line 141-143). For the crown age of Cardueae, we used a secondary calibration based on the estimation by Barres et al. (2013; Am J Bot, doi:10.3732/ajb.1200058). This calibration was defined with a normal distribution centered on 39.2 Mya and given a standard deviation of 2.0, which placed the 95% HPD for the node age at 35.91 - 43.49 Mya (Line 144-146). In addition, we replaced the position of these descriptions. Please see Line 137-146 in the revised manuscript.

182: change “environmental variables” to “environmental variable”

RE: Done. Changed as suggested (line 184).

200 (also in various figures): replace “haired” with “hairy”

RE: Done. All these wordings were replaced (Line 206, 209-211, 644, and figure 2 and S7).

205 (also in various figures): replace “appendage” with “appendaged”

RE: Done. All these wordings were replaced (Line 211 and figure S7).

220: there appears to be a superfluous “the” before “DIVERSITREE”

RE: Done. We removed “the” before “DIVERSITREE” here (Line 229).

229: replace “GPS” with “latitude and “longitude”

RE: Done. Replaced as suggested (Line 237-238).

231: Please cite the DOI of the GBIF dataset. This is made available when you download the data. In addition, was any attempt made to resolve the taxonomy of the records? This was done for the chloroplast genome accessions but I do not see any evidence here. In addition, further cleaning of the records using a semi-automated approach (e.g., CoordinateCleaner, Zizka et al., 2019) might help improve the reliability of these data, given the well-known problems with GBIF records. Finally, there are many duplicate records on GBIF – was any attempt made to remove these? Retaining these records can bias estimates of niche breadth. They could be removed by deleting records with identical latitude and longitude or alternatively by thinning (for example, with the ‘spThin’ R package [Aiello-Lammens 2019]).

RE: We thank the reviewer for very helpful comments. Indeed, we have included a full citation version of the GBIF datasets in our Supplementary Material-Supplementary Data S1. It includes the names of organizations or individuals, the database name and version, DOI of the dataset, and the access date. We appreciate the recommendations of useful tools by the reviewer. We resolved the taxonomy of the records as same as we did for the chloroplast genome accessions. We deleted duplicate collections with identical latitude and longitude and records with the coordinate system problems by manually checking all distribution records to improve the reliability of these data. We included a statement for the validation of the reliability of distribution data in the revised manuscript. Please see Line 241-243.

243: How was the predictive power of the different bioclim variables assessed?

RE: We removed highly correlated variables by calculating Pearson's correlation coefficient (> 0.75), and eight bioclimatic variables remained. We removed "most predictive" here (Line 252-253).

279: It is problematic to compare speciation rates of these subgenera, given that you have just demonstrated that they are non-monophyletic. These subgenera were defined on morphological characteristics that largely match those used in your trait-based analyses (as you indicate in lines 304-305); it makes sense to just retain those analyses and remove these from here and in Figure 1D.

RE: We appreciate these comments. While these subgenera are non-monophyletic, the morphological characteristics defining the subgenera have originated multiple times and are hypothesized to be adaptive. Comparisons of diversification rates among these morphological-based subgenera can provide helpful insight into the evolution and adaptation of key morphological innovations. In addition, our trait-dependent analyses showed that some trait syndromes observed in the subgenus *Amphilaena* (snow lotus) have higher diversification rates. We discussed the significance of snow lotus morphology in the divergence history of *Saussurea*.

302 (and line 416): "cauliferous plant" is a bit awkward, as it doesn't really refer to the trait, but to the plant as a whole. There are multiple options for how to reword this, but perhaps just "with stems" or "stemmed" would be sufficient.

RE: Done. We changed "with cauliferous plant" to "with stems" (Line 317).

308: This is the first time that I see FiSSE mentioned – this belongs in the methods.

RE: Thank you for this suggestion, we moved this sentence to the methods section. Please see Line 224-228.

451: This sentence doesn't seem to fit to me. How does this work provide a basis for mitigating biodiversity loss? Certainly not every paper has to be framed as relevant for conservation.

RE: We appreciate these comments. We removed this sentence.

Figure 4: The analysis of niche breadth is interesting, but I wonder if there is more than can be done with this to provide a more meaningful insight into the evolution of this group. For example, evolution of niche breadth/range size or overlap of these amongst sister species? Given the differences observed amongst the three major clades, some interesting patterns should be expected.

RE: We agree with the reviewer that it would be interesting to investigate the evolution of niche breadth/range size or overlap of these amongst sister species. However, our aim in this part of our study is to investigate how ecological opportunities contribute to the rapid diversification of *Saussurea* species. Moreover, because our taxonomic sampling of the whole genus is incomplete, the sister species on the phylogenetic clades might not be "real" sisters.

Referee: 2

Zhang et al. present an exploration of the diversification of the cool temperate genus Saussurea in the QTP using macroevolutionary models based on molecular phylogenies, paleoenvironmental reconstructions and ecological trait data. I liked the study, and it was interesting to see so many macroevolutionary models applied to an interesting and diverse group, using different kinds of data. These kinds of studies help us understand temporally dynamic diversification processes and make important contributions, particularly for regions like the QTP which often receive less attention than other biodiverse ranges like the Andes. The methods are highly reproducible and the code on GitHub makes this accessible to readers, so I commend the authors on this. I have a few main points as I felt unconvinced by the main conclusions, partly because I felt the results were under-reported, and also because the results seemed disparate so it was difficult to see how they all connected. Although it looks like a lot, I think my comments could all be easily addressed by the authors and I have made some specific suggestions as to how, but these could be addressed in a number of ways.

RE: We thank the reviewer for his constructive comments and relevant questions that improve the manuscript. We believe we've addressed all the concerns the reviewer raised. We added extensive information about the validation of the main results, detailed parameters of model comparison, and some additional explanations of results. We added a table for summary diversification analyses and evolutionary hypotheses to connect each analysis and the results. Moreover, we carefully revised the discussion to what we think is better story for all the findings from the different analyses.

Main points:

1. My first main point is about validation and scrutiny of the main results. Lots of analyses were used but there was no assessment on whether they agree with one another. Where possible the authors should cross validate results, for example are the DR and BAMM tip rates positively correlated? Or if they give different answers – why? Also the authors found the best fitting RPANDA model to be temperature dependant, however the AICc score is < 2 AIC from time dependant or constant-rates – this suggests you actually can't reject a constant rates model. This is also supported by the DDD analysis – you found support for diversity dependence, but the K estimate was infinity – which suggests no diversity dependence at all! Support for this model was likely only preferred because it wasn't compared to a constant rates BD model (also for DDD analysis we need the model names in Table S8 explained). The TESS models are also not well explained, it seems like the posterior probability of a rate shift is very low, how do we interpret this?

RE: We appreciate these comments. We completely agree with the reviewer on the questions of validation and scrutiny of the main results. We fitted our BAMM tip rates and DR results into a linear model using phylogenetic generalized least squares (PGLS) under a Brownian motion model in R-packages APE v5.5 to validate the correlation between these two methods (Line 169-172). The result shows a significant positive correlation between BAMM tip rates and DR results with a correlation coefficient: 0.313 and p-value < 0.001 (Line 285-286). This positive correlation is also supported by Pearson correlation analysis (Pearson's correlation coefficient: 0.571, p-value < 0.001; data not shown). We added the PGLS analysis in the revised manuscript for validation of the results. For the RPANDA analysis, we added additional information for model comparison. "However, the constant birth-death model cannot be rejected because the difference in AICc between the best-fit model and the constant birth-death model is small (ΔAICc = 0.672; Table S6)" (Line 304-307). For the DDD analysis, the best fitting model of the diversity-dependent process is that speciation does not depend on diversity and extinction depends linearly on diversity (DD+EL). The K estimate was infinity indicating accumulating diversity did not influence speciation rates, consistent with the no-dependent model of speciation. We added the abbreviations for models corresponding to Table S7 in the Method (Line 195-199, and 309), and also explained the full names of models in Table S8. For TESS analysis, we added a detailed explanation for TESS analysis (Line 162-165) in Figure S4 caption and rephrased the results part (Line 283-285).

Also, and this might be a bit nit-picky (apologies), but the clades selected do not represent any singular taxonomic, ecological or morphological radiations (because the main morpho clades were paraphyletic right?), they are just three large clades that began to radiate at the same time. While it might be interesting to look at the differences between these groups, the selection of these clades is somewhat arbitrary. The three radiations therefore of course occurred in parallel, because you selected three clades from the same time.

RE: We thank the viewer for these comments. While these subgenera are non-monophyletic, the morphological characteristics defining the subgenera have originated multiple times and are hypothesized to be adaptive. Comparisons of diversification rate among these morphological-based subgenera can provide helpful insights into the evolution and adaptation of key morphological innovations. For example, our trait-dependent analyses showed that some trait syndromes observed in the subgenus *Amphilaena* (snow lotus) have higher diversification rates. We discussed the significance of snow lotus morphology in the divergence history of *Saussurea*.

2. Lots of different analyses were used in this study (BAMM, DR, TESS, RPANDA, DDD, HiSSE, MuSSE, FiSSE, QUaSSE, ES-sim, etc.). I understand that the macroevolutionary biologists toolkit requires lots of different models because we are yet to develop more holistic models that look at different aspects of trait evolution and diversification simultaneously. I use similar approaches in my work and can see the necessity of this. However, I think it would be important to take stock of why each of these models are used so the reader can understand the relevance of each one. Perhaps a table that specifies each model, the question it addresses, and what kind of data it uses would be helpful. On this note, more clearly setting up the hypotheses and their predictions in the introduction might be helpful to see why the different models were used, e.g., if climate was more important than biotic interactions in generating QTP diversity then we would expect a stronger correlation between diversification and paleotemperature than species diversity. On this note perhaps a justification of the assumptions should be made. How well does your data fit these assumptions? Similarly, how does taxon sampling affect the results, given only 2/3 sampling.
RE: We appreciate these very helpful suggestions. We agree with the reviewer that it would be important to take stock of why each of these evolutionary models are used. We added a table (Supplementary Material Table S1, Line 104) for a summary of these diversification analyses conducted in our study. This table includes the hypothesis each model tested, the data is used, the setting of each method, and the main results from the method. We have intended to put this summary table in the main text; however, due to the strict page limit, we have to move the table to the

supplementary file. In addition, as the reviewer suggested, we posed the hypotheses and their predictions in the introduction of the revised manuscript (Line 92-102). In Table S1, we connected each method to a hypothesis it tested. By doing this, it is more clear to understand why the different models were used. For all analyses we used, we included a global sampling fraction to account for the incomplete sampling effect (Line 156-157). We recognize that our sampling of the Saussurea species remains limited, introducing possible biases. Nevertheless, these biases are inherent in any large-scale biodiversity analysis. We included this caveat in the discussion (Line 382-485).

3. My next point is that I believe that the results (and to a lesser degree the methods) were underexplained and most results were shifted to the supplementary material which makes it very hard for the reader to assess the work. For example, the authors should be reporting the tests used to get results – e.g., if you are reporting the results of an ANOVA (Line 278 or 280) then state this. Also all test statistics, parameter estimates, likelihood, AIC weights or deltas or whichever method was used to perform model selection, should be reported. This is done for the QuaSSE analysis but should do it for all analyses.

RE: We appreciate these very helpful suggestions. The consensus of not enough explanation for some aspects stems from a restriction on the word length for the submission. For the tests of model selection (i.e. RPANDA, DDD, HiSSE, MuSSE, and QUaSSE), we reported the value of ΔAICc, the difference in AICc between the model with the lowest AICc and the others, to access the significance of the model comparison. Moreover, we added the detailed test statistics and parameters for all the methods we used in the results.

4. My final main point is that the results are interesting, but I was left wanting to know more about them and how they all fit together therefore I think the discussion needs more attention to the why. This would be easier if the hypotheses were set up more clearly, then the authors could state what is the relative support from each one

based on the results. For example, I don’t quite see how the range size and niche breadth analysis fit the overall story – this would have been considerable amount of work for the authors so it would be nice to place it more firmly in the overall “story”. For example, the trait dependant diversification results were not deeply explained - why would these traits show different rates? What is the ecological significance of these traits? Do species in the clades which showed higher rates of diversification (from BAMM) have these trait values? The authors mention the snow lotus exhibit these traits, but did these lineages undergo more rapid diversification? Perhaps the trait values should be plotted on the tree next to the rates in figure 1 so the readers can actually see the distribution of these traits.

RE: This is a very constructive point. We added a table (Line 104) for a summary of these diversification analyses and the hypotheses in the introduction (92-102), as stated above (Table 1). These do make it clear and helpful for understanding how different models fit the overall story. We carefully made many modifications to the discussion of the submitted manuscript. We start the discussion with the major findings (Line 365-378) and main conclusions (Line 378-387) to complete the overall “story”, and then move into the discussion by stating the evolutionary scenario supported by each analysis based on the results. The discussion follows the order of three hypotheses posed in the introduction to answer our four main questions. For the trait-dependent diversification results, we added additional discussion about the ecological significance of the traits that contribute to the high diversification rate of Saussurea (Line 424-427 and 429-431). Additionally, we plotted traits values on the tree next to the rates in Figure 1 in order to show the distribution of these traits more clearly (Figure 1 and Line 629-637).

For the paleotemperature driven rates, you are describing QTP uplift but using results from a global average temperature, but can you really make any inference about the uplift using this other data? If it was uplift, wouldn’t a time-dependant model be best supported? And how well does the global average temperature reflect the local temperatures of the QTP?

RE: Thank you for the comments. For the QTP uplift, unfortunately, we failed to confidently use a single data set or a convincing model to represent the uplift process. Paleontologists recently concluded that the QTP is not a monolithic entity but assembled piecemeal during the Mesozoic by successive terrane accretions (reviewed by Spicer et al., 2020, National Science Review, doi: 10.1093/nsr/nwaa091; 2021, Palaeobiodiversity and Palaeoenvironments, doi: 10.1007/s12549-020-00452-1). Hence, a single model representing this complex geographic event seems to be inappropriate and may introduce bias. However, it has long been recognized that the southeast edge of QTP experienced a significant acceleration of growth during the Late Miocene and the Pliocene, i.e. the eastern Tibet and the Hengduan Mountains, which harbors a high level of biodiversity and is inferred as the diversity center of many plant lineages including *Saussurea* (reviewed by Favre et al., 2015, Biological Reviews, doi: 10.1111/brv.12107). In our study, we primarily infer the QTP uplift-driven diversification by the divergence time of BEAST result and rate shifts through the time of BAMM and TESS results. The latter two analyses are under a time-dependent model and can be good supports for the uplift process. For the RPANDA analysis, we used a global average temperature as an environmental variable to investigate how past climate change drive the diversification of Saussurea. While most species of Saussurea are distributed in the QTP area, many species have a wide distribution range. And the samples in our study include species from most lands of Eurasia and North America. Therefore, we used the global average temperature, which also was used in the local diversification studies, such as the study of Condamine et al. (2018, Systematic Biology, doi:10.1093/sysbio/syy009) on the radiation of Apollo butterflies in the QTP, and the study of Lagomarsino et al. (2016, New Phytologist, doi: 10.1111/nph.13920) on the rapid diversification of Andean bellflowers.

Minor comments:

Line 86: I don’t understand the use of attractive here – adaptive?

RE: Done. We changed “attractive” to “adaptive” (Line 82).

Line 113: These should be cited

RE: Done. We added citations here (Line 116).

Line 117: how was trait data combined – the mean, median?

RE: For the trait data, as we coded the character state but not continuous traits, we used the states of original variants as representatives for species with subspecific classification.

Line 165: CLaDS is the latest tool for assessing branch specific rates and looks promising.

RE: We appreciate the comment, especially in regards to new software to address questions of interest. We did not explore ClaDS, even though it is only a few years old. Other studies have shown that it can be computationally infeasible for certain analyses (Cooney and Thomas 2021; Nat Ecol Evol, doi: 10.1038/s41559-020-01321-y) and the accuracy may be only marginally better than BAMM. Even though BAMM does have its previous controversies, the authors of CLaDS state that any variance in rate heterogeneity may actually be underestimated in BAMM (Maliet et al 2019; Nat Ecol Evol, doi: 10.1038/s41559-019-0908-0). In fact, in the supplement to Maliet et al (2019), they acknowledge that CLaDS will find rate heterogeneity on any phylogeny even simulated under a homogenous constant rate model, which could lead to overestimation of intraclade variation in rates. Since we are not dealing with a large-scale evolutionary data set, and the multiple methods already employed support one another in most instances, we have strong confidence in our interpretations. At some point, it will be nice to see a thorough comparison of all standard methods to detect changes in diversification across multiple scales. Our study cannot do that at this time sadly. One of the big advantages that we see to using ClaDS would be specific hypothesis testing, which would overcome some of the limitations raised by Louca and Pennell (2020; Nature, doi: 10.1038/s41586-020-2176-1); however, since we are relating changes in diversification (including speciation and extinction) to abiotic and biotic factors with other analyses, we feel that we have successfully addressed limitations raised for diversification studies.

Line 166: Should use Phylogenetic anova as clades may be more similar due to common ancestry

RE: We thank the reviewer for the helpful suggestion. We performed phylogenetic ANOVA and reported the results of it (Line 172-175, and 287-295).

Line 179: still a bit unclear what alpha and beta are – is one for the relationship with speciation and one for extinction?

RE: Yes, alpha and beta are two parameters that control the variation of speciation (alpha) and extinction (beta) with the paleoenvironment.

Line 182: Please explain in more detail what these paleotemperature data are so the reader does not have to refer to another paper – also these are data for global paleotemperature not the QTP region – however local orogenesis probably means there is a big disconnect between local and global temperatures. Maybe justify the assumptions here.

RE: Thank you for the comments. We rephrased this sentence and added details for the paleotemperature data (Line 187-189). As we stated above, many Saussurea species have a wide distribution range, and the samples in our study include species from most areas of Eurasia and North America. In addition, the global average temperature was widely used in the previous studies of local diversification, such as the radiation of Apollo butterflies in the QTP (Condamine et al., 2018, Systematic Biology, doi:10.1093/sysbio/syy009) and the rapid diversification of Andean bellflowers (Lagomarsino et al., 2016, New Phytologist, doi: 10.1111/nph.13920).

Line 183: Nice to see the analysis replicated across the posterior!

RE: Thank you for your positive feedback.

Line 242: What does most predictive mean in this context?

RE: We removed “most predictive” here (Line 252-253).

Line 247: Can you please explain what is meant by climate lability?

RE: In our study, we used the phrase “climate lability” to represent the variation of bioclimatic variables retrieved across the distribution range for each species. This measure was calculated by extracting the first two principal components (PC1 and PC2) from the principal component analysis.

Line 250: Which ENM models were fit? We need much more detail on this here as it seems integral to how you estimate species geographic ranges.

RE: We used GLM models for estimating environmental niches. We added this statement in the revised manuscript (Line 260).

Line 274 & Fig S4: Figure S4 caption not well explained. What are the units on the right-hand side of the Y-axis? Also the posterior probability is very small for mass extinctions – its not clear why this was tested and the result is not explained.

RE: The TESS analysis was used as a compliment for the BAMM “PlotRateThroughTime” result to detect the changes in speciation and extinction rates through evolutionary time. We rephrased the statement of TESS analysis in Line 162-165 of the revised manuscript. The units on the right-hand side of the Y-axis is 2ln BF (Bayes factors). Each bar indicates the posterior probability of at least one rate shift within that interval. Bars that exceed the specified significance threshold (2ln BF > 6) indicate significant rate shifts. Bars that exceed the specified significance threshold (2ln BF > 6) indicate significant rate shifts. We added a detailed explanation for TESS analysis in the Figure S4 caption and removed the result of mass extinctions (Figure S4).

Line 276-278: What test was done here? How was significance assessed? There is no context to this result. Same for DR statistic below.

RE: Phylogenetic ANOVA was used to test whether differences of species diversification rates inferred from BAMM tip rate and DR statistic among three phylogenetic clades and four traditional subgenera were significant. We rewrote this part (Line 287-295)

Line 287: Why not report the ML value for alpha here?

RE: Done. We changed it to the alpha value (Line 299).

Line 289: Which RPANDA model was this – linear or exponential, speciation or extinction constant?

RE: The exponentially dependent model. We added this information (Line 299).

Line 308: Why not describe this in the methods section? Table S3 explain parameters

RE: We moved this sentence to the methods section (Line 224-228). We have added explanations of parameters and abbreviations in Table S4.

Line 342: ES-sim?

RE: Yes. We corrected it (Line 356).

Appendix C

Associate Editor

Comments to Author:

I appreciate the authors' work to significantly revise and improve their study in response to reviewer comments. Please note the three remaining concerns of the reviewer about the analyzes that have been performed. I encourage the authors to address and explain these points.

RE: Thank you very much for the positive feedback on our revised manuscript. We sincerely appreciate the editor and reviewers for their thoughtful comments and recommendations on our manuscript. We have studied the comments carefully and revised the manuscript accordingly. We believed that the three remaining concerns raised by the reviewer have been addressed and hope this revised manuscript will meet the journal's high standards. The amendments are highlighted in red font in the revised manuscript and the reply to the reviewers' comments is present as follows.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).

I have read the revised manuscript "Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification" and I think the manuscript reads much clearer now with appropriate references to the methods used and key numerical results in text. As I stated earlier, I really like the general approach and the system is very interesting, so I congratrulate the authors. However, I have several remaining concerns that I believe the authors did not address sufficiently and I am hesistant to accept the authors conclusions based on the results.

RE: We appreciate the reviewer for his positive feedback and interest in our study. We are thankful to the reviewer for his constructive comments that will substantially improve our research. We believed that the three remaining concerns raised by the reviewer have been well addressed. Below are the point-by-point replies to the comments.

Firstly, I believe the estimated parameter value of Infinity for the carrying capacity of a diversity dependent model does not make sense and I do not understand the author's explanation for it. Under a model of diversity dependence, a clade-based carrying capacity (K) is approached in one of three different ways: speciation can decrease with diversity, extinction can increase with diversity, or both speciation can decrease and extinction increases with diversity. The authors found that a model where extinction increases is the best fit. However, they state that K=Inf is because speciation is constant – but diversity dependent extinction rates should still lead to identifiable value of K. Perhaps I am missing something crucial, and I apologies if that is the case, but I believe that this model is best supported because it was not compared to a constant rates model. I think a solution is to bring all the diversification models into the same framework which can be done in RPANDA. Here, the authors could compare a constant rates birth death model, environmental dependent models, time dependent models AND diversity dependent models and perform model selection.

RE: Thanks for this helpful comment. We apologize for the confusing explanations of the diversity dependent result. The best-fit DDD model was one in which the extinction rate increased with diversity (DD+EL), and it estimated an unlimited carrying capacity (K = Inf). We have rewritten the descriptions of the diversity dependent result in the revised manuscript, including the Results and Discussion part as well as text in Table S1. We appreciate the recommendations of the reviewer using RPANDA to perform model selection of diversity dependent models. We used the DDD package in R to evaluate the likelihood of a phylogeny under a model that accommodates diversity-dependence and extinction. As proposed by Etienne et al., (2012, *Proc. R. Soc. B;* doi:10.1098/rspb.2011.1439), the diversity-dependence model with extinction should be used as the standard model for macro-evolutionary dynamics because of its biological realism and flexibility. We believe, after amendments, our results of the diversity-dependence analysis are robust. Additionally, our results are similar to those of Condamine et al., (2018, *Syst. Biol.*; doi:10.1093/sysbio/syy009) investigating the diversity dependence of Apollo Butterflies, in which they also found an unlimited carrying capacity. Since these results are not unknown or completely novel suggest that they can be trusted, especially in consideration of the other results found in our study. The revised explanations of the diversity-dependence result can be found in Line 302-305, 367 and 409-412.

The second point again relates to model selection, you found no evidence that temperature dependent diversification is a better fit to the data than a constant rates model, because even though this was the best model a delta AIC < 2 means the models are equivocal. You added part of the results to address this, but the discussion ignores this point completely which is misleading. The only evidence is that some particular clades (not the genus overall) show shifts in diversification rate in the Miocene and Pleistocene (from BAMM).

RE: Thanks for the suggestion. We have added some sentences to address this part in the discussion. *'While a constant rates model cannot be rejected from RPANDA analysis, it is evident that some clades show shifts in diversification rate in the Miocene and Pleistocene from BAMM and TESS results. The lack of ability to reject a constant rate model may be due to the heterogenous geography of the QTP which could be obscuring larger evolutionary patterns within the Asteraceae [55]'*. Please see Line 395-400 in the revised manuscript.

Third, the authors did not justify their decision to use three phylogenetic clades and three morphological clades to compare speciation rates. In their response they write "Comparisons of diversification rate among these morphological-based subgenera can provide helpful insights into the evolution and adaptation of key morphological innovations" this is true and the trait-based SSE analyses address this, but why did the authors compare speciation rates between the three arbitrary phylogenetic clades? In particular, there is no information on why looking at these three phylogenetic clades was done and the only mention in the methods is on line 276 “Three phylogenetic clades were resolved (clade-1, -2 and -3, Figure S1)”. Without a justification, I don’t think looking at the phylogenetic clades adds anything additional to the manuscript except unnecessary complexity. I also believe comparing the morphological sub-genera directly is not appropriate and instead this can be discussed in the context of trait dependent SSE models – a point also suggested by Reviewer 1 which i do not believe was adequately addressed.

RE: We agree with the reviewer that comparing differences of speciation rates among phylogenetic clades adds unnecessary complexity to our study. We have removed the comparison of speciation rates from our manuscript and revised the Figure 1 accordingly. However, we do believe that showing rate-through-time of the three major phylogenetic clades makes sense because those are the encompassing groups that the BAMM analyses indicated had shifts in diversification. For comparing the speciation rate between morphological sub-genera, we do believe this is necessary. Some traits/syndromes that have a higher diversification rate identified by the trait dependent analysis are commonly found in the subgenus *Amphilaena*. Although the subgenus *Amphilaena* is a non-monophyletic group, the results indicate that the morphological innovations have originated multiple times across the whole genus. As suggested by the reviewer, we discussed the comparison of the diversification rate of the morphological subgenera in the context of trait dependent SSE models (Line 424-427).

Appendix D

Associate Editor

Comments to Author:

I am pleased to inform you that your revision of your manuscript RSPB-2021-1575 titled "Macroevolutionary pattern of Saussurea (Asteraceae) provides insights into the drivers of radiating diversification" is close to being acceptable for publication in Proceedings B. However, there are still some minor revisions that should be addressed expeditiously so I would appreciate if you could please deal with these in a revised version. First, please temper your language regarding your conclusions from your DDD analyses as there are still some interpretation concerns from the reviewer. And second, I would like you to make every effort possible to generate color figures using colorblind-friendly palettes. This minor revision will not be sent out for further review but will be checked by our Editorial staff to make sure that the paper is suitable to be sent to Production.

RE: Thank you very much for the consideration for accepting our manuscript. We sincerely appreciate the editor and reviewer for their additional comments. We have revised the manuscript carefully. We believe that we have addressed all the concerns of the reviewer and look forward to the publication of our paper. The amendments are highlighted in red font in the revised manuscript and the reply to the reviewers' comments is present as follows.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s)

I have re-reviewed the manuscript and I am mostly very happy with authors responses. Again, I really like the paper and the approach. I do have one main remaining point that was not sufficiently addressed in the revisions:

RE: We thank the reviewer for their positive feedback and the additional comments on our study. We believe that the remaining point raised by the reviewer have been addressed. Below are the point-by-point replies to the comments.

Main point:

I'm truly sorry if I am being pedantic but I must insist here that when K = Inf, the model is the same as a constant rates model of diversification, because diversity never approaches the asymptote and therefore diversification never slows down. I clarified this with the authors of the DDD package to make sure I was not misled. You can easily compare the diversity dependent models with a constant rates model within the DDD package using the bd_ml, bd_loglik functions, and I believe if you do this you find the constant rates model supported. If you only compare diversity dependant models you will always find support for diversity dependence, even when it does not exist! I don't think it is too much of a problem for your interpretation or discussion as you rely more heavily on the results from the RPANDA analysis than the DDD analysis anyway.

RE: We really appreciate the responsible attitude of the reviewer to our study, especially with corresponding to the authors of one of the methods to make sure everything is correct. We have added a constant rates model in the DDD analysis as suggested by the reviewer (line 193-195). The DDD results showed that the constant speciation and extinction rates model (CSE) obtained the best support compared to the five diversity-dependence models (AICc =639.556, Table S7). We have revised this part of results and the relevant discussions. Please see lines 304-307, 369 and 411-413 in the revised manuscript.

"The best-fit DDD model was one in which the extinction rate increased with diversity (DD+EL, AICc = 649.577, Table S7) while estimating an unlimited carrying capacity (K = Inf); indicating that accumulating diversity did not influence speciation rates of Saussurea." – here it is incorrect to state that the extinction rate increased with diversity if K = Inf.

RE: Thank you for the comments. We revised this sentence to read: *The diversity-*

dependence analysis showed that the constant speciation and extinction rates model (CSE) obtained the best support compared to five diversity-dependence models (AICc =639.556, Table S7), indicating that accumulating diversity did not influence diversification rates of Saussurea.

"In addition, although extinction may increase with diversity, speciation of Saussurea is not limited by a species diversity carrying capacity, implying that the genus is possibly still in the evolutionary process of rapid speciation." – here it is unclear why speciation would be rapid based on this result?

RE: Thank you for the comments. We removed "rapid" here and modified this sentence to "*In addition, the diversification of Saussurea is not limited by the accumulation of species diversity, implying that the genus is possibly still in the evolutionary process of speciation*".

A few minor points:

Will the phylogenetic data and trait data used in this study be published with the article?

RE: Yes, the plastome sequences used for phylogenetic analysis have been deposited in the National Center for Biotechnology Information (NCBI) database with accession numbers provided in Supplementary Information – Table S2, and the trait data are included in the Supplementary Information -- Table S3.

Can the colour figures be made colour blind friendly. Each figure contains red and green as the main contrasting colours which is problematic for many.

RE: Thank you for the suggestion. We have changed the colour schemes of all figures present in the study.