July 4, 2018

Prof. Christopher Still

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Dear Prof. Christopher Still,

We would like to thank you for the opportunity to discuss our manuscript ID bg-2018-140 entitled 'Phylogeny of the Stipa and implications for grassland evolution in China: based on biogeographic evidence'. We are grateful to you and the two reviewers for their constructive comments and thoughtful suggestions that are very helpful in improving significantly the quality of our manuscript. We have analyzed all the comments carefully. All major replies are described in detail point-to-point. Please let us know should you have any questions regarding the manuscript. We are looking forward to hearing from you.

Sincerely yours,

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Response to bg-2018-140 – RC1:

Issue 1. *The biogeographic analysis that is presented relies on the assumption that the Chinese Stipa species form a clade and assumes that these lineages have not arrived in China from other regions e.g. Southeast Asia or Europe. For instance it seems possible that some of the Chinese Stipa might actually have sister species or other close relatives outside of China. There are sequences for more than 75 different species of Stipa available on GenBank and as many of these as possible should be included. With a more complete (or global) phylogeny of Stipa, inferences about dispersal and vicariance events would be much more reliable. I also do not think the data in this manuscript are sufficient to test anything about the diversification rates of Stipa. Again a broader phylogeny that includes Stipa from outside of China would be necessary for such an analysis.*

Response: We appreciate for the constructive suggestion and agree with the point. We will investigate the availability of additional *Stipa* species (global) on GenBank and other public sequence databases and conduct analysis about phylogeny, dispersal and vicariance events.

Issue 2. *Aside from sampling issues, I think that the results of this paper are stated too strongly. There is uncertainty in the phylogentic reconstruction, the inferred divergence dates, and in the biogeographic reconstruction, and very little of this uncertainty is presented or discussed in the current manuscript.*

Response: We agree with the point. We will add the corresponding uncertainty discussion of phylogenetic reconstruction, the inferred divergence dates, and the biogeographic reconstruction.

Issue 3. *Finally, there are grammatical mistakes throughout the manuscript and overall this paper needs to be thoroughly edited for clarity. One of the most common errors is "grasslands" should be plural rather than singular.*

Response: We apologize for the grammatical mistakes. We will ask for a native English speaker to check the revised manuscript.

Issue 4. *There should be a section in the introduction with background information about Stipa. How many species are there? What is its distribution outside of China? Maybe also something about the ecology of the clade could be added.*

Response: We appreciate the suggestion and agree with it. We will review more literature and add an overview of *Stipa* species numbers, distribution and clade.

Issue 5. *Line 49. See also Liu et al. 2018 "Phylogeny of Orinus (Poaceae), a dominant grass genus on the Qinghai-Tibet Plateau"*

Response: Thanks to the reviewer for recommending this article. This article has provided a great help for us to revise our manuscript.

Issue 6. *Lines 51-57. "Late Tertiary" seems like a misleading description of when Chinese grasslands emerged. "Mid tertiary" would be more appropriate for 33MaBP. Also see Edwards et al. 2010.*

Response: We also apologize for the confusion and agree with the point. It seems more appropriate that Chinese grasslands emerged about 33 MaBP during the Mid tertiary.

Issue 7. *Line 73. What are "constructive species?"*

Response: Constructive species is also called edificato, or edificator species. It is the most dominant species in a community, and also plays a significant control role in community structure and function.

Issue 8. *Line 99. Chloroplast not chlorophyll*

Response: We apologize for the mistake. We will modify it as the suggestion.

Issue 9. *105. Why is Ramirez et al. 2017 cited related to diluting DNA samples?*

Response: We also apologize for the confusion. We will change it to the appropriate literature.

Issue 10. *Line 134 where is this rate from? Is it possible to get a rate of chloroplast evolution that is specific to grasses?*

Response: We thank the reviewer for the valuable suggestion. We found it is feasible to get a rate of chloroplast evolution of Stipeae to calculate the divergence times of Stipa species from this literature (Romaschenko et al. 2014).

Issue 11. *Line 138-139. The sentence structure makes it sound as though FigTree was used to infer divergence times. Instead "Finally the tree and divergence times were visualized in FigTree."*

Response: We agree with the reviewer and will revise it as suggestion.

Issue 12. *Lines 145-147. I'm confused whether 100 or 2500 posterior trees were used for this analysis.*

Response: We apologize for the confusion. The number of 100 should be 10000. The S-DIVA analysis used 2500 random trees which were selected in the Bayesian analysis with a total of 10000 trees.

Issue 13. *Line 148. Setting the maximum number of areas a species can occupy to 2 seems justifiable but needs more context. How many areas do the most widespread Stipa species today occupy?*

Response: *Stipa krylovii* is the most widespread species, mainly distributes in Inner Mongolia, Xinjiang, Loess Plateau.

Issue 14. *More details are needed on the biogeographic analysis. How were results summarized across the posterior trees?*

Response: We apologize for the confusion. We will add more details of biogeographic analysis on revised manuscript.

Issue 15. *What analysis was used to generate Figure 4?*

Response: We also apologize for the confusion. It is generated by BEAST software. We will add the details in the revised manuscript.

Issue 16. *Lines 153-155. This isn't hugely important, but these numbers don't really add up in a way that makes sense. With 2988 conserved and 1102 variable sites there would only be 4090 sites total - what happened to the other three? I'm guessing it has to do with idels or gaps in the alignment? I'm not sure this information is necessary to report, but if you're going to include it, it would make sense to include something about indels as well.*

Response: We also apologize for the confusion. We only showed the length of the combined four gene fragments. We will add corresponding information of each gene fragment.

Issue 17. *Did the BEAST analysis converge? Were all ESS values >200?*

Response: The BEAST analysis was converge, and all ESS values were greater than 200.

Issue 18. *Was the tree well supported? The posterior probability for each node should be added to Figure 2.*

Response: Yes. The tree was well supported. We will add posterior probability for each node to Figure 2 in the revised manuscript.

Issue 19. *Line 160. What are the confidence intervals for these ages?*

Response: Agreed. We will add all age with 95% highest posterior density in the revised manuscript.

Issue 20. *Line 162. The tree alone does not provide adequate evidence for an "explosive radiation" of Stipa. This could be tested using BAMM (Rabosky et al. 2013) or other diversification methods.*

Response: We thank the review for the constructive suggestion. We will add the BAMM analysis to verify explosive radiation according to the literature(Rabosky et al. 2013).

Issue 21. *Lines 165-179. This scenario could be plausible if these species form a clade, but there are other possibilities. Moderate support for other scenarios seem to be indicated by multiple colors in the backbone nodes in Figure 3.*

Response: Thanks to reviewer for the value suggestion. Because we will add additional Stipa species sequence database from GenBank, we will explore other scenarios based on multiple colors in the backbone nodes in Figure 3.

Issue 22. *Line 182. What are "larger expansion and isolation events"? How are these different from the expansion and isolation events described in the previous section?*

Response: We also apologize for the unclear description. There is no difference between "larger expansion and isolation events" and "expansion and isolation events". We will unified these two the same as "expansion and isolation events".

Issue 23. *How do the age estimates from this study compare to estimates from Poaceae-wide analyses?*

Response: We appreciate the valuable comment. We will consult the appropriate literature to explore the age estimates compare to Poaceae.

Issue 24. *Line 200. See Xing and Ree 2017 for a nuanced perspective on this. "Uplift-driven diversification in the Hengduan Mountains, a temperate biodiversity hotspot"*

Response: We thanks the reviewer for recommending this article. This article has provided a great help for us to revise our manuscript.

Issue 25. *Line 213-4. These lines are confusing because the entire tree is dichotomous. For the second part maybe it should read "the inner branches are short compared to the tip branches?"*

Response: We apologize for the confusion. We will revise it as suggestion "the inner branches are short compared to the tip branches".

Issue 26. *Line 216-225. The discussion of rapid radiation in Stipa should either be qualified or removed since it is not explicitly tested in this paper.*

Response: As the reply to comment 20, we will add the BAMM analysis to verify explosive radiation according to the literature(Rabosky et al. 2013). Based on the result of BAMM, we will conduct the discussion.

Issue 27. *Lines 296-301. Because the Chinese grasslands pre-dated Stipa (last section of Discussion), it doesn't seem possible to infer the order of Chinese grassland formation from biogeographic patterns of Stipa.*

Response: We are grateful for the constructive suggestion and agree with this point. We will delete all the content about the order of Chinese grasslands formation.

Issue 28. *Figure 3. What are the colors in 3B? I'm confused by what the "largest possible distribution area of Stipa species" means? Largest compared to what? I think it would be clearer to color the map in part A to match the phylogeny in part B. Then the letters and numbers might not be necessary at all? I'm also confused by the fact that part A and the letters on part B seem to tell a very clear story, but the colors of the nodes suggest that there is a lot of uncertainty in the deeper nodes of the tree. That amount of uncertainty would be expected for this kind of an analysis and it seems like it should be reflected more clearly here and in the text of the paper.*

Response: We apologize for the confusion. In Figure 3B, each color refers to different distribution area of Stipa species. Based on RASP, we explored the ancestral distribution areas of Stipa species. Then, on the circle node, the ratio of each color represented the proportion of the ancestor distribution area.

Issue 29 *Figure 4. How was this graph generated? The colors on this graph aren't very Clear. A red/pink/purple all look similar to me. Maybe the lines could be thicker or a green/light blue color could be used instead of one of the other colors.*

Response: Thanks for the useful suggestion. We will modify the Figure 4 as the suggestion.

References

Rabosky DL, Santini F, Eastman J et al. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nature Communications, 4

Romaschenko K, Garciajacas N, Peterson PM et al. 2014. Miocene-Pliocene speciation, introgression, and migration of Patis and Ptilagrostis (Poaceae: Stipeae). Molecular Phylogenetics & Evolution, 70(1):244-259.