

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 – RC2:

Issue 1. *First, the evolutionary history of one lineage is not enough evidence to draw conclusions about the history of a community. While Stipa is a dominant grass species in these habitats it is not the only grassland species and the authors do not discuss any paleontological evidence to suggest that Stipa has always been a major component of these grasslands. For example, the species of Stipa included in the study may have evolved after the respective grasslands and subsequently invaded and became dominant.*

However, if various grassland species in the region were found to have a common origination time frame then one could conclude that the community began to assemble at that time. This issue could be resolved by restating the goals of the study to focus on the evolutionary history of Stipa without the assumption that the history of Stipa is a good proxy for the evolution of the grasslands that they are found in.

Response: We appreciate the constructive comment and agree with it. We will delete all the content of grassland evolution indicated by the evolution of Stipa species.

Issue 2. *A second major issue is that the researchers only consider 20 species of Stipa in a genus with over 100 species and there is no indication that the 20 species represents a monophyletic group. Since there are potentially many missing taxa, each with unsampled geographical distributions, the ancestral area analysis and any inference about dispersal routes and timing are not reliable. Each of the taxa included in the study could have a sister species from a different geographical region perhaps from outside the study area. If so, that would affect both the inference about the pathway of dispersal and the timing of when speciation events occurred. The authors should investigate the availability of additional Stipa species on Genbank or other public sequence databases.*

Response: We agree with the reviewer and are grateful for the valuable suggestion. 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 3. A third major issue relates to the methods used to calibrate the phylogeny. The parameters used for the BEAST analysis are not clearly stated; however, it appears that the authors assumed a strict molecular clock with a nucleotide substitution rate based on the “rate of chloroplast gene of herbaceous plants”, although the source of the substitution rate was not given. There are other studies which report substitution rates in the grass family which substantially from the rate used by the authors; however, calibrations based only on substitution rates are not very reliable unless there is a well-established rate for the group of organisms. A better method uses fossils to calibrate the stem nodes of clades to which the fossil is assigned. There are fossils of *Stipa*, or at least close relatives of *Stipa*, as well as other grass fossils which could be used to calibrate the phylogeny. This would require expanding the phylogeny to include outgroup clades for which fossils are available. Sequences are available on Genbank that could allow the authors to do this.*

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). Meanwhile, we also will try to consult fossils of *Stipa* or close species and corresponding available sequences date to further calibrate the Phylogeny.

*Issue 4. For the ancestral area analysis to be meaningful, the phylogeny estimate needs to be well supported. The authors did not clearly report BPP support from the BEAST analysis nor did they compare the results of their phylogeny estimate with previous phylogeny estimates of *Stipa* (i.e. Hamasha et al. 2012).*

Response: We thanks for the suggestion. Hamasha et al (2012) studied phylogeny of 109 Stipa species from Eurasia, Americas and Australia. It is very useful for us to further discuss the phylogeny of Stipa in our manuscript.

Issue 5. *The abstract is clear – the dates given for grassland formation are very precise some indication of the variance is needed here.*

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

Issue 6. *Lines 27-37: This paragraph is unnecessary since it describes fundamental assumptions that the readers should already be familiar with. Line 38: This paragraph is a better way to start the paper*

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

Issue 7. *Line 58: “sporopollen” should be “pollen”*

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

Issue 8. *Lines 58-61: Studies focusing on the Holocene probably are too recent to be important for understanding the origins of grasslands.*

Response: We appreciate the comment and agree with the point. We will revise it as the suggestion.

Issue 9. *Line 99: “fragments of chlorophyll” should be “chloroplast fragments”*

Response: Agreed. We will change “fragments of chlorophyll” to “chloroplast fragments”.

Issue 10. *Section 2.2.1 - Good Section 2.2.2 – Combine this section with the previous section.*

Response: Agreed. We will revise it as the comment.

Issue 11. *Section 2.2.3 Line 120: Change “assembled” to “aligned”.*

Response: Agreed. We will revise “assembled” to “aligned”.

Issue 12. *Line 133-134: The average evolution rate of chloroplast gene of herbaceous plants (3.4610-9s/s/y) was used to calculate the divergence times. The resulting 95% HPD of node age estimates is very wide indicating that crown divergence of Stipeae may have occurred between 15-34 million years ago. This wide confidence interval is not adequately discussed by the authors. There is no discussion of how well the topology is supported. There are what appear to be bootstrap results on the RASP analysis but there was no boot strap analysis reported.*

Response: As reply to comment 3, We will adopt a rate of chloroplast evolution of Stipeae to calculate the divergence times of Stipa species from this literature (Romaschenko et al. 2014). Meanwhile, we also will try to consult fossils of Stipa or close species and corresponding available sequences date to further calibrate the phylogeny.

Issue 13. *Lines 133-134 –What are the other parameters of the BEAST run?*

Response: The evolution rate of chloroplast gene was the crucial parameter in RASP analysis, and others parameters were basic.

Issue 14. *Lines 145-146: “The S-DIVA analysis used all 100 trees and combined trees in the Bayesian collection.” - Where did the 100 trees come from?*

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 15. *Lines 147-148: “The maximum number of distributions in 148 each distribution area was set as two,” : : : Revise this to: : : “The maximum number of ancestral areas was set at two,”*

Response: Agreed. We will revise this sentence as the suggestion.

Issue 16. *Lines 155-156: The GC content of the chloroplast is typically much lower that the AT content.*

Response: Thanks. We will revise the sentence as the comment “The GC content of the chloroplast is typically much lower that the AT content.”

Issue 17. *Lines 158: No results given for the phylogeny estimation.*

Response: We apologize for the confusion. The credibility of phylogeny is determined by the ESS value. If the ESS value is greater than 200, the result is credible. We will add some information of ESS values in the revised manuscript.

Issue 18. *Table 1. Not clear why the species name is repeated. Also, the Table caption is a bit redundant. Only a brief title is needed.*

Response: We thank the review for the valuable suggestion. We will only retain species names, and named Table 1 as “Relevant information about all *Stipa* species.” in the revised manuscript.

Issue 19. *Figure 3b: This figure is quite confusing. The colors on the node symbols do not seem to match the tip data and there are more than two ancestral areas represented at internal nodes.*

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 20. *References (mentioned in this review) Hamasha, H. R., von Hagen, K. B., & Röser, M.(2012). Stipa (Poaceae) and allies in the Old World: molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages. Plant Systematics and Evolution, 298(2), 351-367. Zhong, B., Yonezawa, T., Zhong, Y., & Hasegawa, M. (2009). Episodic evolution and adaptation of chloroplast genomes in ancestral grasses. PLoS One, 4(4), e5297.*

Response: Thanks to the reviewer for recommending these articles. These articles have provided a great help for us to revise our manuscript.

References

Hamasha HR, von Hagen KB, Roeser M. 2012. Stipa (Poaceae) and allies in the Old World: molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages. *Plant Syst. Evol.*, 298(2):351-367.

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.