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Interactive comment on "Phylogeny of the Stipa and implications for grassland evolution in China: based on biogeographic evidence" *by* Qing Zhang et al.

Anonymous Referee #2

Received and published: 19 June 2018

The authors use a time calibrated phylogenetic reconstruction of 20 Stipa species to reconstruct the origins and spread of grasslands in China. The premise of this study is based on the observation that several Stipa species are restricted to different regions and the idea that the evolutionary history of these species can serve as a proxy for the history of grassland development. There are several issues with the premise of the study. 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

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[Discussion paper]

evolved after the respective grasslands and subsequently invaded and became domi-nant. 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. 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 geograph-ical 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. A third ma-jor 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 fam-ily 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. For the ancestral area analysis to be meaningful, the phylogeny esti-mate 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). Specific Comments Abstract – The abstract is clear – the dates given for grassland formation are very precise some indication of the variance is needed here. Introduction - The grammar needs to be corrected in several places. 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 Line 58: "sporopollen" should be "pollen" Lines 58-61: Studies focusing on the Holocene probably are too recent to be important for understanding the origins of grasslands. Line 99: "fragments of chlorophyll" should be "chloroplast fragments" Methods Section 2.2.1 - Good Section 2.2.2 – Combine this section with the previous section. Section 2.2.3 Line 120: Change "assembled" to "aligned". Line 133-134: The average evolution rate of chloroplast gene of herbaceous plants ($3.46 \times 10\text{-}9\text{s/s/y}$) 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. Lines 133-134 – What are the other parameters of the BEAST run? Lines145-146: "The S-DIVA analysis used all 100 trees and combined trees in the Bayesian collection." - Where did the 100 trees come from? 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," Lines 155-156: The GC content of the chloroplast is typically much lower that the AT content. Lines 158: No results given for the phylogeny estimation. Discussion The main conclusions can not be supported given the above listed deficiencies in the data. Table 1. Not clear why the species name is repeated. Also, the Table caption is a bit redundant. Only a brief title is needed. 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.

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.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140, 2018.

C4