Reply to Panero: Robust phylogenetic placement of fossil pollen grains: The case of Asteraceae

We thank Panero (1) for his interest in our paper (2). However, we consider his interpretations (1) somewhat incomplete and misleading, principally because he reports results using methods that we did not apply in our study, and underestimates the importance of pollen morphological characters in phylogenetic studies. We respond to each of these points with the aim of clarifying his misinterpretations about our paper.

Firstly, Panero (1) questions our estimate of 85.9 Ma for the crown node of Asteraceae and claims that the assignment to Asteraceae of the Cretaceous fossils we reported is premature based on a "bootstrap majority consensus topology" (BMCT) that he could not reproduce from the data. We did indeed estimate the age of the Asteraceae crown node to be 85.9 Ma, but we used the single-most parsimonious tree to assign a position for this calibration, not a BMCT, as he erroneously understood. We have not reported any BMCT in our work. However, we conducted a sensitivity analysis to explore the impact of different calibration scenarios (SI of ref. 2); one of them (our second scenario) consisted of a bootstrap consensus tree (instead of a BMCT) that placed our pollen fossil sister to the remainder of Asteraceae.

Panero (1) debates this misunderstanding of the second calibration scenario in our sensitivity analysis, and favors the calibration at the Asteraceae + Calyceraceae + Goodeniaceae node (our third scenario), also on the basis of his BMCT mentioned above. His second point demonstrates that he misunderstood our analyses.

Panero (1) then questions the validity of inferring the phylogenetic position of taxa using only a few pollen characters. The phylogenetic significance of pollen grain morphology is supported by numerous studies; the 26 characters used in our analysis represent an improvement relative to other well-accepted studies (e.g., 22 and 12 characters, in refs. 3 and 4, respectively). Character 19 state 1 (columellae poorly distinguishable) was scored in several genera within Asterales (e.g., *Dasyphyllum*, *Stylidium*, *Argophyllum*), so no character weighting exists. Character 21, the bilayered condition of the *Dasyphyllum* ectexine, is only observed under TEM analysis. Our fossil specimens were observed under LM, so we have not included TEM observations to standardize our analysis. Character 22, the exine thickness of most of *Dasyphyllum* is around 3 μm (5). Character 17, the "concavities" in the fossils, are interpreted as intercolpal depressions due to structural modifications in the exine, rather than postmortem compaction as Panero (1) suggests. There are species of *Dasyphyllum* with and without intercolpal depressions; hence, our phylogenetic results will not be affected by the state of this character.

Panero (1) concludes his critique of our results by commenting on the diversification of South American lineages that was not primary due to the *Dasyphyllum* + *Barnadesia* calibration. We used both fossils (the Cretaceous and the Eocene) in our interpretations. The stem length observed (figure 5 of ref. 2) results from our *Tubulifloridites lilliei* constraint; its alternative placements will have a determinant influence on this pattern. Our careful assignment of both fossils, particularly of *T. lilliei*, as outlined in our paper and reiterated here, produces what we believe to be the most robust molecular age estimates for the family Asteraceae to date.

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1 Panero JL (2016) Phylogenetic uncertainty and fossil calibration of Asteraceae chronograms. *Proc Natl Acad Sci USA*, 10.1073/pnas.1517649113.

2 Barreda VD, et al. (2015) Early evolution of the angiosperm clade Asteraceae in the Cretaceous of Antarctica. *Proc Natl Acad Sci USA* 112(35):10989–10994.

3 Sauquet H, et al. (2009) Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proc Natl Acad Sci USA* 106(1):221–225.

4 Crepet WL, Nixon KC, Daghlian CP (2013) Fossil Ericales from the Upper Cretaceous of New Jersey. *Int J Plant Sci* 174(3):572–584.

5 Urtubey E, Tellería MC (1998) Pollen morphology of the subfamily Barnadesioideae (Asteraceae) and its phylogenetic and taxonomic significance. *Rev Palaeobot Palynol* 104(1):19–37.

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The authors declare no conflict of interest.

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