Response to Comment on "Eocene Fagaceae from Patagonia and Gondwanan legacy in Asian rainforests"

Peter Wilf[1*], Kevin C. Nixon[2], María A. Gandolfo[2], N. Rubén Cúneo[3]

[1]Department of Geosciences, Pennsylvania State University, University Park, PA 16802 USA. [2]Liberty Hyde Bailey Hortorium, Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853 USA. [3]CONICET, Museo Paleontológico Egidio Feruglio, 9100 Trelew, Chubut, Argentina.

***Corresponding author. Email: pwilf@psu.edu**

Denk *et al.* agree that we reported the first fossil Fagaceae from the Southern Hemisphere. We appreciate their general enthusiasm for our findings, but we reject their critiques, which we find misleading and biased. The new fossils unequivocally belong to *Castanopsis*, and significant evidence supports our Southern Route to Asia hypothesis.

We recently (*1*) reported two *Castanopsis rothwellii* fossil infructescences from the early Eocene (52 Ma) of Argentine Patagonia. These are (we maintain) the oldest fossils assigned to the genus by ca. eight million years (*2, 3*), and they co-occur with hundreds of fagaceous leaves indistinguishable from those of living *Castanopsis*. The same fossil beds contain numerous taxa whose close living relatives characteristically associate with *Castanopsis* in New Guinea and elsewhere, including *Papuacedrus*, *Agathis*, *Araucaria* Sect. *Eutacta*, *Dacrycarpus*, a *Phyllocladus* relative (*4*), *Podocarpus*, *Retrophyllum*, *Ripogonum*, *Eucalyptus*, *Ceratopetalum*, *Gymnostoma*, engelhardioid Juglandaceae, and *Todea*, as cited (*1*). Nearly all these lineages are well-known examples of the Southern Route to Asia confirmed by fossil evidence from one or more of Antarctica, Australasia, and Asia (*5-7*), and we concluded that *Castanopsis* most likely had similar biogeographic history. *Castanopsis* thrives on the Australian plate today in New Guinea, and its southern range is only a short distance over shallow water from Australia, with which New Guinea had frequent past land connections and biotic interchanges (*8*).

In short, we presented a suite of positive evidence for our Southern Route hypothesis that led us to favor the idea. Most notably, we reported the first remains of Fagaceae trees that grew on Gondwana, clearly identifiable as *Castanopsis* and found in a fossilized New Guinea-type association. However, Denk *et al.* (*9*) assert that "evidence for such a pathway is currently missing."

First, we reject Denk *et al.*'s appeal-to-authority argument: "…the southern route hypothesis would require that generations of palynologists had overlooked the characteristic pollen of Castaneoideae in Gondwanan records." This statement appears biased regarding both South America, an integral part of Gondwana at 52 Ma (*10*), and those who work there. The current "generation" has found fossil Castaneoideae in Gondwana (*1*), even though previous highly skilled colleagues had not.

Second, Denk *et al.* invoke a moving-the-goalposts argument. Even though we just reported Fagaceae fossils several thousand kilometers south of any previous occurrences (*1*), Denk *et al.* hold that the family did not range any farther south (their South American "dead end"). Theirs is a perilous position because there was no oceanic separation of South America and Antarctica at 52 Ma and thus no "end" to South America (*10*); instead, abundant austral biotic interchange took place at that time (*11*). The South American "dead end" would also require that *Castanopsis* went extinct in the Southern Hemisphere, then rejoined the same New Guinea-type lineages at a much later date via an entirely different Holarctic path after crossing several climate zones. This scenario seems far less likely than the Southern Route, which is supported by our finding the oldest *Castanopsis* already in a perhumid New Guinea-type rainforest association at 52 Ma in West Gondwana (*1*). It is irrelevant that *Castanopsis* occurs in other plant associations in its living range (*9*), as we discussed (*1*).

Third, Denk *et al.* wrongly assert that molecular data from living Fagaceae can be used to reject a hypothesis about the affinities of specific early Cenozoic fossils determined from paleobotany ("molecular data reject the notion that…"). In so doing, Denk *et al.* also mistakenly state that *Castanea* "lacks a fossil record outside Eurasia." The oldest fossil *Castanea*, as we cited (*1*), is from the middle Eocene of Tennessee, not Eurasia. Thus, shared sequences from the sister genera *Castanea* and *Castanopsis* in all likelihood reflect their common ancestry in the ancient New World, not the Old World as Denk *et al.* argue.

Fourth, Denk *et al.* erroneously contend that *Castanopsis rothwellii*, a fossil with so many diagnostic characters preserved that it could only be assigned to *Castanopsis* if 'found alive' today (*1*), has plesiomorphic features and cannot be placed confidently in the extant genus. Their idea rests on a misleading phylogenetic argument (see next paragraph), and it is unacceptable at face value because it ignores basic botany and our detailed taxonomic treatment (*1*). The diagnostic characters of *Castanopsis* in the fossils are in no way generalized for all Fagaceae, including the spike-like infructescence axes of numerous solitary, asymmetrical, valved and sutured lateral cupules that entirely enclose the single nut, which retains three short, linear, 'castaneoid' styles with unexpanded stigmas. These features match *Castanopsis* precisely and definitely exclude the fossils from placement with *Quercus*, *Fagus*, and the trigonobalanoids. Within the remaining, castaneoid genera, *C. rothwellii* only matches *Castanopsis,* and thus, there is no basis whatsoever for separating this fossil from *Castanopsis*. Denk *et al.* also pose an invalid syllogism by arguing that because *Castanopsoidea* and *C. rothwellii* have some similar features, and the former is an extinct genus, then our fossils do not belong in an extant genus.

Denk *et al.*'s phylogenetic conclusions from their emended tree and matrix are misleading, in that any morphological matrix includes characters that are relevant only for the taxa included in the analysis. Because the fossils are castaneoid in all features, we did not include all Fagaceae in our original analysis (*1*) and likewise did not include all characters relevant to non-castaneoid fagaceous taxa. Denk *et al.* (9) added several genera to their analysis without adding any morphological characters to resolve these additional taxa, then used their uninformative result to criticize our phylogenetic interpretation as uninformative. In their framework, even a living *Castanopsis* would not resolve in *Castanopsis*! By adding just three relevant characters to the Denk *et al.* (*9*) scaffold to accommodate the genera they added (Table 1), the fossil *Castanopsis rothwellii* is placed only with *Castanopsis* in the single most parsimonious tree (Fig. 1). We note that even when the same morphological data are used alone, without any scaffold, the fossil resolves with the *Castanopsis fissa* group. We acknowledge our miscoding of *Fagus* for flower number, a typographic error that does not affect the outcome of any of our analyses. The other character re-codings by Denk *et al.* only make the morphological data less precise.

We expected vigorous debate regarding the biogeographic implications of our Gondwanan *Castanopsis* fossils, which hold importance for understanding and conserving the imperiled southern-sourced associations that survive in Asian rainforests (*1, 6*). Unfortunately, Denk *et al.* (*9*) do not advance the discussion. Only time and many more fossils, not negative evidence and misleading assertions (*9*), will tell where else the Fagaceae occurred.

References

1. P. Wilf, K. C. Nixon, M. A. Gandolfo, N. R. Cúneo, Eocene Fagaceae from Patagonia and Gondwanan legacy in Asian rainforests. *Science* **364**, eaaw5139 (2019).
2. S. R. Manchester, Fruits and seeds of the middle Eocene Nut Beds flora, Clarno Formation, Oregon. *Palaeontogr. Am.* **58**, 1-205 (1994).
3. M. C. Mihlbachler, J. X. Samuels, A small-bodied species of Brontotheriidae from the middle Eocene Nut Beds of the Clarno Formation, John Day Basin, Oregon. *J. Paleontol.* **90**, 1233-1244 (2016).
4. A. Andruchow-Colombo, P. Wilf, I. H. Escapa, A South American fossil relative of *Phyllocladus*: *Huncocladus laubenfelsii* gen. et sp. nov. (Podocarpaceae), from the early Eocene of Laguna del Hunco, Patagonia, Argentina. *Aust. Syst. Bot.* **32**, 290-309 (2019).
5. R. S. Hill, "The history of selected Australian taxa," in *History of the Australian Vegetation: Cretaceous to Recent,* R. S. Hill, Ed. (Cambridge University Press, Cambridge, UK, 1994), pp. 390-419.
6. R. M. Kooyman *et al.*, Paleo-Antarctic rainforest into the modern Old World Tropics: the rich past and threatened future of the "southern wet forest survivors." *Am. J. Bot.* **101**, 2121-2135 (2014).
7. X.-K. Wu *et al.*, Northern Hemisphere megafossil of *Dacrycarpus* (Podocarpaceae) from Miocene of South China and its evolutionary and palaeoecological implication. *J Syst. Evol.*, doi: 10.1111/jse.12534 (2019).
8. D. M. J. S. Bowman *et al.*, Biogeography of the Australian monsoon tropics. *J. Biogeogr.* **37**, 201-216 (2010).
9. T. Denk *et al.*, Comment on “Eocene Fagaceae from Patagonia and Gondwanan legacy in Asian rainforests”. *Science* **365**, e______ (2019).
10. L. A. Lawver, L. M. Gahagan, I. W. D. Dalziel, "A different look at gateways: Drake Passage and Australia/Antarctica," in *Tectonic, Climatic, and Cryospheric Evolution of the Antarctic Peninsula,* J. B. Anderson, J. S. Wellner, Eds. (AGU, Washington, DC, 2011), pp. 5-33.
11. P. Wilf, N. R. Cúneo, I. H. Escapa, D. Pol, M. O. Woodburne, Splendid and seldom isolated: the paleobiogeography of Patagonia. *Annu. Rev. Earth Planet. Sci.* **41**, 561-603 (2013).

Acknowledgments

Funding: This work was funded by NSF grants DEB-1556666, DEB-1556136, EAR-1925755, and EAR-1925552 to P.W. and M.A.G. **Author contributions:** All authors contributed to researching and writing this response.

Table 1. Additional character scores for phylogenetic analysis. Scores shown left to right in the following order. Stigma: expanded = 0; unexpanded = 1. Nut in cross-section: triangular/flattened = 0; generally rounded = 1. Cupule symmetry: symmetrical = 0; asymmetrical = 1.

Taxon	**Score**
Castanopsis rothwellii fossils	111
Fagus	000
Castanea chestnut group	110
Castanea pumila group	110
Castanopsis fissa group	111
Castanopsis Castanopsis group	11[0,1]
Chrysolepis	100
Lithocarpus A	110
Lithocarpus B	110
Notholithocarpus	110
Colombobalanus	000
Formanodendron	00[0,1]
Trigonobalanus	000
Quercus	010

Fig. 1. Phylogenetic analysis. Consensus of the two most parsimonious trees, based on the Denk *et al.* (*9*) scaffold and emended morphological matrix with the addition of the three characters listed in Table 1, generated using the same analytical methods described previously (*1*). See text for discussion.