

Early evolution of the angiosperm clade Asteraceae in the Cretaceous of Antarctica

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The Asteraceae (sunflowers and daisies) are the most diverse family of flowering plants. Despite their prominent role in extant terrestrial ecosystems, the early evolutionary history of this family remains poorly understood. Here we report the discovery of a number of fossil pollen grains preserved in dinosaur-bearing deposits from the Late Cretaceous of Antarctica that drastically pushes back the timing of assumed origin of the family. Reliably dated to ~76–66 Mya, these specimens are about 20 million years older than previously known records for the Asteraceae. Using a phylogenetic approach, we interpreted these fossil specimens as members of an extinct early diverging clade of the family, associated with subfamily Barnadesioideae. Based on a molecular phylogenetic tree calibrated using fossils, including the ones reported here, we estimated that the most recent common ancestor of the family lived at least 80 Mya in Gondwana, well before the thermal and biogeographical isolation of Antarctica. Most of the early diverging lineages of the family originated in a narrow time interval after the K/P boundary, 60–50 Mya, coinciding with a pronounced climatic warming during the Late Paleocene and Early Eocene, and the scene of a dramatic rise in flowering plant diversity. Our age estimates reduce earlier discrepancies between the age of the fossil record and previous molecular estimates for the origin of the family, bearing important implications in the evolution of flowering plants in general.

Asteraceae | evolution | Antarctica | fossil | phylogenetics

Flowering plants underwent a rapid ecological radiation and taxonomic diversification in the Early Cretaceous, about 121–99 Mya (1). Asterids, in particular, represent an extraordinarily diverse clade of extant angiosperms that includes more than 80,000 species. This clade contains the most species-rich angiosperm family, the Asteraceae, with 23,000 species, many of which are economically important taxa, such as sunflowers, lettuce, and gerberas. The origin and early diversification of family Asteraceae were important events in the history of life largely because this lineage has been a dominant component for the past several millions of years in numerous biomes around the world, primarily in open habitat ecosystems. Particularly, the evolution of Asteraceae, typically characterized by bearing attractive inflorescences (or capitula), may have promoted the radiation of insect pollinators (e.g., solitary bees) that heavily rely on this family to feed and reproduce (2). To date, the oldest fossil confidently assigned to Asteraceae is from the Middle Eocene of Patagonia. It consists of an inflorescence and associated pollen grains assigned to an extinct clade of Asteraceae, phylogenetically placed at a moderately derived position within the phylogenetic tree of the family (3). The discovery of these Eocene specimens indicated that the crucial split between subfamily Barnadesioideae, the earliest diverging branch of the family, and the rest of Asteraceae occurred even earlier, either during the early Paleogene or Late Cretaceous (4, 5). Recent molecular dating analyses support a Late Cretaceous origin for the crown group Asterales (4, 6), whereas the emergence of Asteraceae was estimated to have occurred in the Early Eocene (4).

Here we report fossil pollen evidence from exposed Campanian/Maastrichtian sediments from the Antarctic Peninsula (Fig. 1, Fig. S1, and *SI Materials and Methods, Fossiliferous Localities*) (7) that radically changes our understanding of the early evolution of Asteraceae.

Results and Discussion

The pollen grains reported here and discovered in the Late Cretaceous of Antarctica are tricolporate, microechinate, with long colpi and rimmed margins. We placed these specimens within the wide-ranging variable fossil species *Tubulifloridites lilliei* (Couper) Farabee and Canright previously recorded in a restricted time interval within the Late Cretaceous of western Gondwana (8, 9) (see also *Supporting Data, Systematic Remarks*). It has been botanically related to a number of eudicot families (*Supporting Data, Systematic Remarks*; see also Figs. S2B and S3F for comparison) based on superficial similarities of the pollen grains or considered as an angiosperm of uncertain position (9). We assembled our specimens from Antarctica as a subgroup of the polymorphic *T. lilliei* that here we informally denominate as *T. lilliei* type A, which is distinguished from other *T. lilliei* specimens by several specific morphological characters (e.g., clearly tricolporate pollen grains with well-defined lalongate ora and intercolpal depressions) (see *Supporting Data, Systematic Remarks* for a full description). Morphologically identical specimens of *T. lilliei* type A were also recovered in the Late Cretaceous of New Zealand (Fig. S4). *Tubulifloridites lilliei*, including *T. lilliei* type A, disappeared almost simultaneously from Antarctica, Australia, Patagonia, and New Zealand about 66 Mya (K/P boundary) (see *Supporting Data, Systematic Remarks*).

Significance

The flowering plant family Asteraceae (e.g. sunflowers, daisies, chrysanthemums), with about 23,000 species, is found almost everywhere in the world except in Antarctica. Asteraceae (or Compositae) are regarded as one of the most influential families in the diversification and evolution of a large number of animals that heavily depends on their inflorescences to survive (e.g. bees, hummingbirds, wasps). Here we report the discovery of pollen grains unambiguously assigned to Asteraceae that remained buried in Antarctic deposits for more than 65 million years along with other extinct groups (e.g. Dinosaurs, Ammonites). Our discovery drastically pushes back the assumed origin of Asteraceae, because these pollen grains are the oldest fossils ever found for the family.

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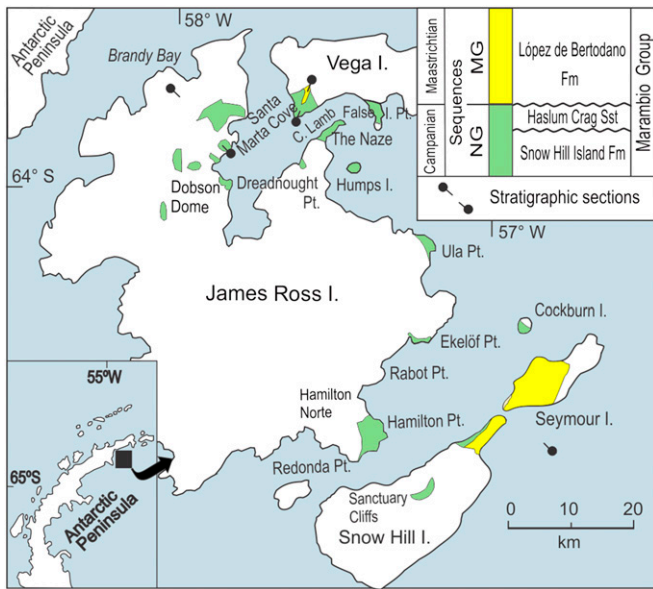


Fig. 1. Map showing distribution of Upper Cretaceous rocks of the Snow Hill Island and López de Bertodano Formations. The studied sections in Brandy Bay–Santa Marta Cove (James Ross Island) and Cape Lamb (Vega Island) are also indicated. Adapted from Olivero (7).

Using an apomorphy-based method [in the sense of Sauquet et al. (10)] as a first attempt at comparing the Antarctic fossils (*T. lilliei* type A) and the pollen produced by extant eudicots (all

supported by a single morphological synapomorphy: triaperturate pollen), we found strong morphological similarities between *T. lilliei* type A and some members of Asterales (Supporting Data and Figs. S24 and S3C). We explored further the phylogenetic placement of *T. lilliei* type A within Asterales in a parsimonious framework by using a matrix of pollen morphological characters (Supporting Data, List of Characters and Character State Definitions Used to Compile a Matrix Used as Input in Parsimony Analyses Aimed at Placing the Fossil Taxa and Table S1) and a phylogenetic tree of Asterales as backbone constraint (Fig. 2). After conducting a sensitivity analysis (see SI Materials and Methods, Estimation of Divergence Times) we found one position suitable for calibration based on the single most-parsimonious tree (188 steps). This single most-parsimonious tree places *T. lilliei* type A within *Dasyphyllum* of the Barnadesioideae (Fig. 2), the earliest diverging subfamily of the Asteraceae; the fossil possesses most of the derived morphological character states of the *Dasyphyllum* pollen (Figs. 3 and 4 and Fig. S3 A, B, D, and E). We also explored other scenarios, assuming *T. lilliei* type A was either an extinct stem relative of Asteraceae or more closely related to other members of the Asterales (Fig. S5 and Table S2). Here, we discuss the age of the origin of the daisy family considering *T. lilliei* type A as a crown group member (i.e., nested within *Dasyphyllum*).

The crown of Asteraceae [i.e., the most recent common ancestor (MRCA) of the family plus all extant and extinct lineages that descended from it] is inferred to have been present from the Late Cretaceous, estimated here at 85.9 Mya [95% highest posterior density (HPD) interval: 82.3–91.5 Mya] (Fig. 5), coinciding in part with the expansion of other eudicot lineages, herbivorous and social insects, birds, mammals, and some dinosaur groups (1, 11–14). The MRCA of Asteraceae other than

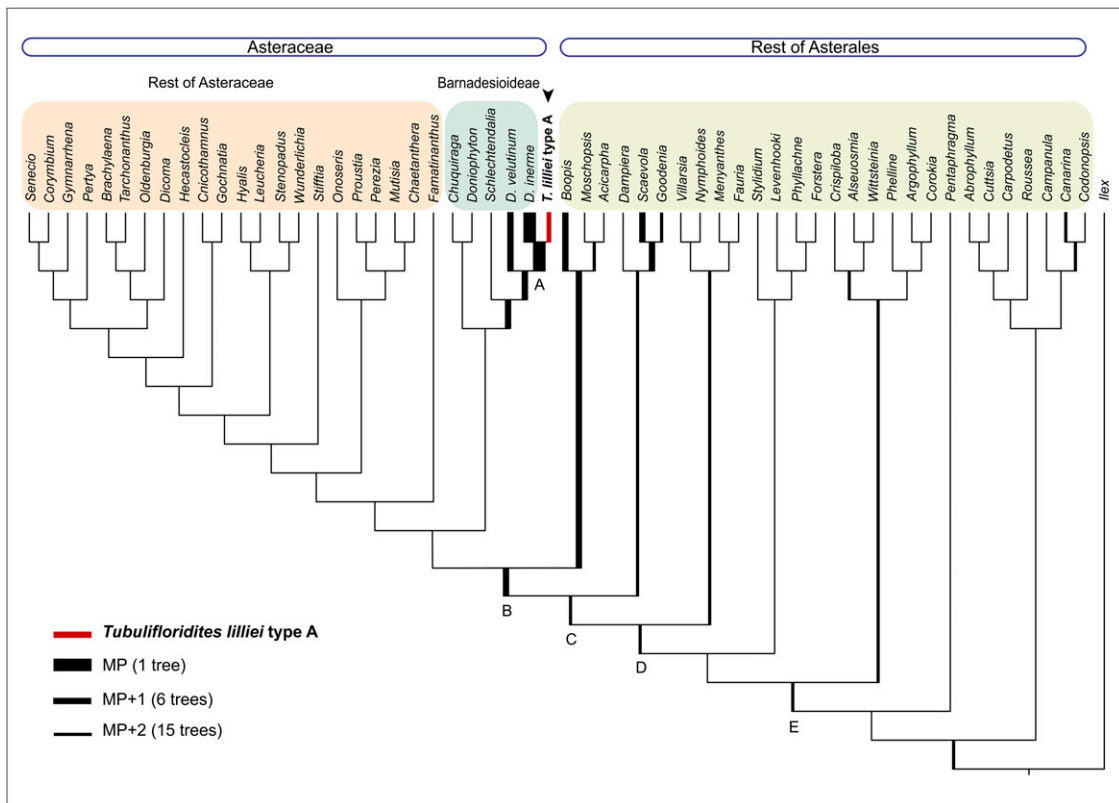


Fig. 2. Phylogenetic analyses of the fossil taxa. Branching positions of the fossil *T. lilliei* type A mapped onto a backbone tree derived from a molecular analysis of Beaulieu et al. (4), with some asteracean taxa added, following a recent comprehensive analyses of Panero et al. (24). Thicker black lines indicate the most parsimonious (MP), one step less parsimonious (MP + 1), and two steps less parsimonious (MP + 2) positions for *T. lilliei* type A. Letters indicate the nodes used to calibrate alternative scenarios, A: Fig. 5; B–E: Fig. S5 and Table S2.

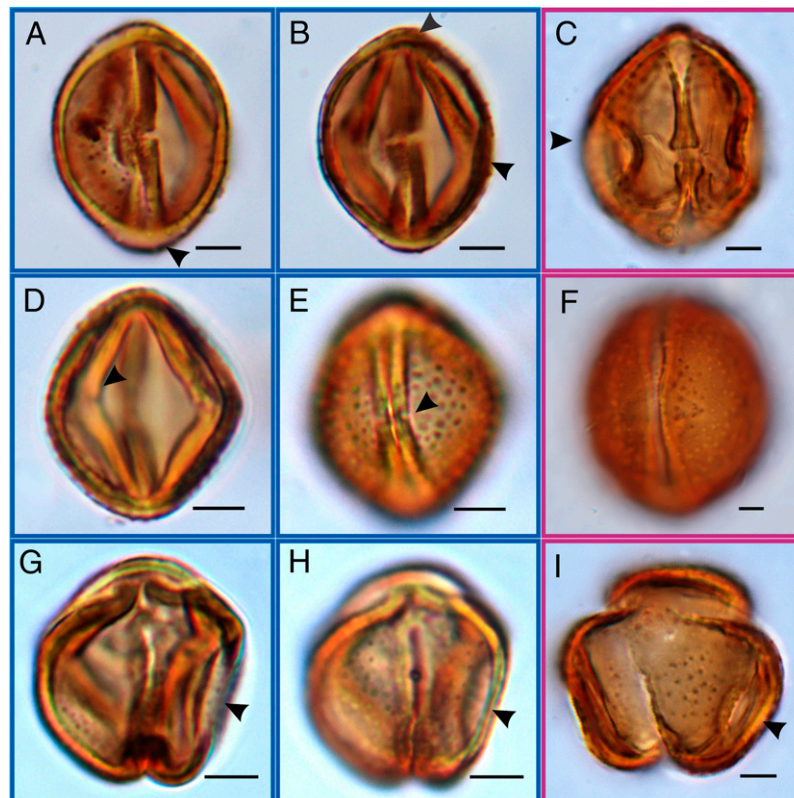


Fig. 3. Fossil and extant representatives of Asteraceae observed by light microscopy. (A, B, D, E, G, H) Specimens of *Tubulifloridites lilliei* type A from the Late Cretaceous of Antarctica (blue frames). Specimens on slide BAPal. ex CIRGEO Palin 963b; (A and B) N42(4); (D and E) L36(0); (G and H) P57(1). (A, B, D, E) Equatorial view. (G and H) Subpolar view. (A and B) Exine thickened at the poles (arrowhead). (A, E, and H) Microechinate-baculate sculpture. (D and E) Thickened exine at apertures level (arrowhead). (B, G, and H) Poorly defined intercolpal depressions (arrowhead). (G and H) Rounded colpi ends. (C, F, and I) Pollen of extant species for comparison (pink frames). (C and I) Extant *Dasyphyllum inerme* (Rusby) Cabrera, with well-defined intercolpal depressions and rounded colpi ends comparable to those of *T. lilliei* type A (arrowheads). (F) Extant *Dasyphyllum velutinum* (Baker) Cabrera, with microechinate-baculate exine surface similar to that of *T. lilliei* type A. (Scale bars, 5 μ m.)

Barnadesioideae is estimated to have evolved about 60 Mya during the Paleocene. Interestingly, the major clades of the family diverged from this common ancestor after the K–P mass extinction event and during a relatively short time interval during the late Paleocene–early Eocene, the Cenozoic’s most pronounced warm interval (59–52 Mya) (15), which was in turn associated with a dramatic rise in flowering plant diversity and a sharp increase in insect herbivory (6, 16, 17). The analysis, assuming that the fossil is a stem relative of Asteraceae, indicated an age for Asteraceae of 67.9 Mya, also within the Late Cretaceous (Fig. S54 and Table S2).

The tolerance of some of the early diverging taxa of Asteraceae, and most members of its sister family Calyceraceae, to extreme environmental and ecological conditions leads us to believe that this resistance might have played a major role in the early evolution of Asteraceae. The earliest lineage of Asteraceae and Calyceraceae occur today in a limited number of restricted regions in South America (18), and several of their members can tolerate the extreme climatic conditions that characterize the Patagonian desert of today (e.g., intense winds, droughts, salt-sprays). Assuming that *T. lilliei* type A pollen grains might represent a member of the crown Barnadesioideae, their parent plants may have been able to cope with environmental stress. We infer that *T. lilliei* type A parent plants occupied a wide geographic range, as suggested by their distribution across western Gondwana during the Late Cretaceous, but may have become drastically reduced close to the K/P boundary, with persistence only in some areas of western Gondwana. Their descendants survived and expanded in South America, probably during the Miocene, as indicated by several fossil pollen

records (19). It is assumed that plant lineages characterized by higher adaptability and increased tolerance to harsh environmental conditions (e.g., earliest branches of Asteraceae and sister Calyceraceae) were probably less affected during global extinction events. It has also been observed that the survival probability in these severe conditions would have been better for plants with polyploid genomes (20). Polyploidy is common in Asteraceae and occurs in virtually all species of subfamily Barnadesioideae (21) and family Calyceraceae (22); thus, polyploidization in the early-diverging lineages of Asteraceae may also have contributed to the survival of this group across the K–P extinction event. The pronounced climatic warming during the Late Paleocene and the Early Eocene Climatic Optimum might have also influenced the diversification of Asteraceae. We show here that most of the major lineages of Asteraceae, which mainly occur today in South America, diverged during this period of global warmth (Fig. 5) and later became isolated when cool-temperate conditions were established in the more austral regions during the Oligocene. For example, in the Guyana Highlands of northeastern South America some species of the earliest-diverging lineages (e.g., *Stenopadus* group) co-exist as relictual patches (23). The presence in Patagonia of an Eocene inflorescence and pollen grains displaying some of the characters of this *Stenopadus* group (5) supports the notion that the MRCA of Asteraceae, excluding Barnadesioideae, existed in the southernmost latitudes of South America, and began to diverge and disperse northward following the equable conditions of the early Cenozoic. The global drop in temperatures during the late Cenozoic may have caused the local extinction of these Guyana

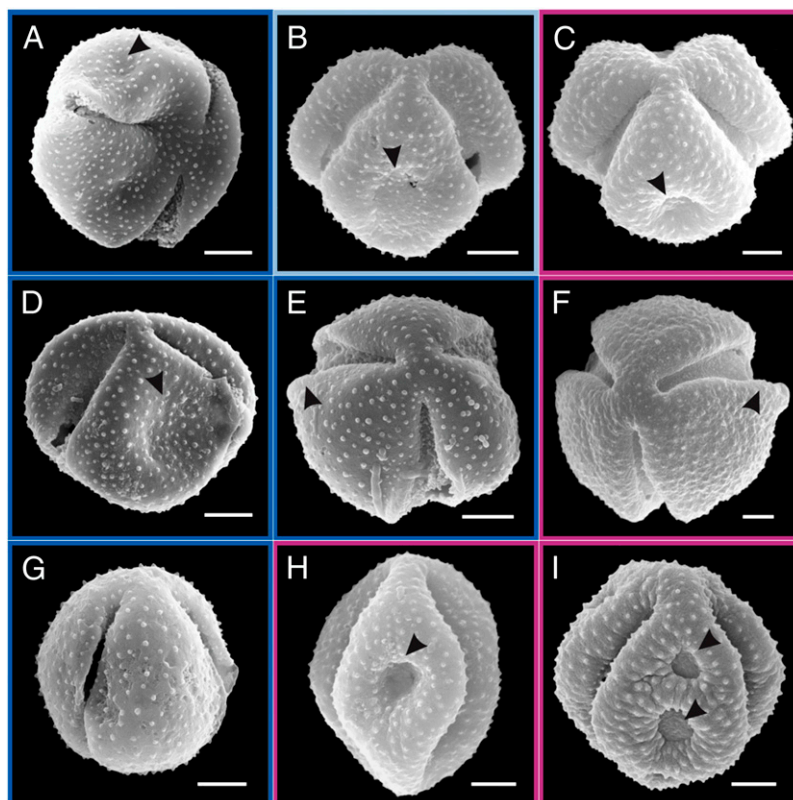


Fig. 4. Fossil and extant representatives of Asteraceae observed by scanning electron microscopy. (A, D, E, G) Specimens of *Tubulfloridites lilliei* type A from the Late Cretaceous of Antarctica (blue frames). (A) Subpolar view showing details of sculpture and poorly defined depressions (arrowhead); note the microgranulate apertural membrane. (D) Subequatorial view showing a poorly defined depression (arrowhead). (E) Polar view with small apocolpium and thickened colpi margins. (G) Equatorial view showing the microechinate-baculate-verrucate sculpture. (B) Specimen of *Quilembaypollis tayuoides* Barreda and Palazzesi from the Miocene of Patagonia (light blue frame) that shares morphological features with both the Cretaceous and extant asteraceous specimens; note the microechinate-baculate sculpture. (C, F, H, I) Extant species of *Dasyphyllum* (pink frames) showing variations in the development and number of intercolpal depressions. (C and H) *Dasyphyllum inerme* (Rusby) Cabrera. (F) *Dasyphyllum latifolium* (Gardner) Cabrera. (I) *Dasyphyllum leptacanthum* (Gardner) Cabrera. (Scale bars, 5 μ m.)

Highland-centered genera from the higher latitudes and their consequent restriction in low latitudes of South America.

Our new divergence time-estimate analysis contradicts some previous assumptions about a geologically recent origin of the Asteraceae (18), indicating instead that the MRCA of the family existed far back into the Late Cretaceous. However, we also infer that the vast majority of the present-day diversity of the Asteraceae is the result of a radiation event that took place during the early Cenozoic, several millions of years after the origin of the family. This finding has important implications for our understanding of the evolution of this highly diverse and ecologically important family. The Cretaceous record from Antarctica is still poorly explored and much evidence on the early evolution of the Asterales, and potentially other groups, probably remains buried beneath present-day ice sheets. From our present knowledge, however, we estimate that the world's highest Southern Hemisphere latitudes (i.e., Patagonia, New Zealand, Antarctica, and Australia) witnessed the emergence and early evolution of what is today the most diverse flowering plant family.

Materials and Methods

Fossil Samples. Rock samples were recovered from the Campanian/Maastrichtian Snow Hill and López de Bertodano Formations on the James Ross and Vega islands, in Antarctica by E.B.O. Samples were chemically treated following standard palynological techniques (*SI Materials and Methods, Fossiliferous Localities and Fossil Pollen Morphotypes*, and Fig. S1). The slides are housed in the palynological collection of the Museo Argentino de

Ciencias Naturales (Buenos Aires, Argentina): BAPal, ex CIRGEO Palin 605–613, 962–965.

Phylogenetic Placement of the Fossil. The apomorphy-based method was used first to compare the fossil *T. lilliei* type A with extant angiosperm families, particularly those having triaperturate microechinate pollen grains (e.g., Ranunculaceae, Rubiaceae, Euphorbiaceae, Campanulaceae, Calyceraceae, Asteraceae) by using information available in the literature. We observed strong morphological similarities between *T. lilliei* type A and some members of Asterales. To increase the taxonomic resolution of this assignment we conducted a parsimony analysis to evaluate the placement of the fossils from Antarctica within the order. Pollen characters for 55 extant species of Asterales were scored (*SI Materials and Methods, Extant Reference Samples, and Supporting Data, List of Characters and Character State Definitions Used to Compile a Matrix Used as Input in Parsimony Analyses Aimed at Placing the Fossil Taxa and Details of the Extant Material Examined for Morphological Characters Provided in Data Matrix and References for Scoring*). The morphological matrix comprises 26 binary and multistate pollen characters, and 55 taxa chosen to represent all families and tribes in Asterales, along with one outgroup taxon, *Ilex* from family Aquifoliaceae (*Supporting Data, Details of the Extant Material Examined for Morphological Characters Provided in Data Matrix and References for Scoring* and Table S1). We used a backbone tree derived from a molecular analysis of Beaulieu et al. (4), with some additional taxa, following the recent comprehensive analysis of Panero et al. (24). We conducted the analyses using the parsimony criterion as implemented in the software PAUP (25), enforcing the topological constraint, with the heuristic search option of 1,000 random addition replicates and tree bisection and reconnection branch swapping. Alternative phylogenetic positions of *T. lilliei* type A were evaluated by searching for the bootstrap consensus tree, the most parsimonious tree, and by searching for trees one and two steps longer than the most parsimonious tree

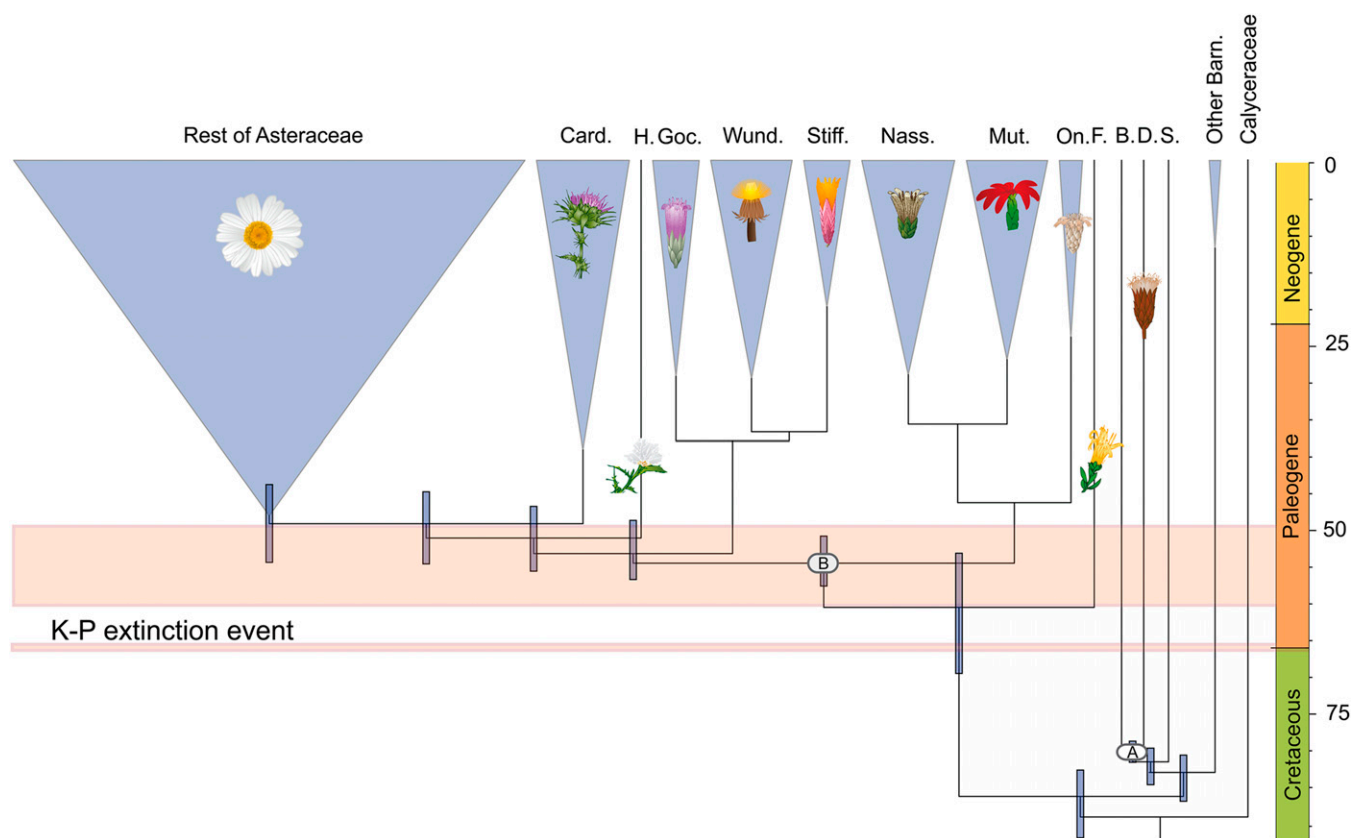


Fig. 5. Evolutionary timescale of the diversification of Asteraceae. Chronogram (scale on the right in Mya) estimated using a Bayesian relaxed clock calibrated with a previously described fossil inflorescence from the Eocene ("B") and our newly discovered specimens from the Cretaceous of Antarctica ("A"). We assume that this Cretaceous species (*T. lilliei* type A) represents an extinct branch nested within *Dasyphyllum* (crown representative). Other possible calibration scenarios are illustrated in Fig. S5. Light-blue bars at nodes represent 95% credibility intervals on estimates of divergence times. Orange horizontal lines indicate the timing of the K-P extinction event and the Cenozoic's warmest interval. Most subfamilies of Asteraceae diverged during the Paleogene, but the earliest divergence occurred in the Late Cretaceous. B., *Barnadesia*; Barn., Barnadesioideae (91 species); Card., Carduoideae (2,500+ species); D., *Dasyphyllum*; F., Famatinanthoideae (1 species); Goc., Gochnatioideae (90 species); H., Hecastocleidoideae (1 species); Mut., Mutisieae (254 species); Nass., Nassauvieae (313 species); On., Onoserideae (52 species); S., *Schlechtendalia*; Stiff., Stiffitioideae (44 species); Wund., Wunderlichioideae (41 species); rest of Asteraceae (19,600 + species).

(SI Materials and Methods, Estimation of Divergence Times) and by assigning the fossil manually to different branches with MacClade (26), following the approach of Doyle and Endress (27).

Divergence Time Estimates. We selected DNA sequences of 101 species of Asteraceae, with an additional 36 species used as outgroup taxa. Three protein-coding genes from the plastid genome (*ndhF*, *rbcL*, *matK*) were obtained for all taxa from GenBank (Table S3). Alignment of individual regions was completed using default settings in MAFFT v.7 (28).

Divergence time estimates and phylogenetic relationships were inferred using Markov Chain-Monte Carlo methods implemented in BEAST2 (29). A GTR + Γ substitution model applied to the entire dataset, and the birth-death model of speciation and an uncorrelated lognormal-relaxed molecular clock model were used. Prior distributions on the root and two other nodes were applied based on the interpretation from the fossil record of Asteraceae. A complete list of the fossil species used to calibrate the tree, geologic ages, and citations is given in Table S4 and Fig. S6, and a list of the explored calibration scenarios is given in Table S2 and illustrated in Fig. S5. We ran

four independent chains for each calibration scenario, each for 100 million iterations, sampling every 1,000th generation using the CIPRES Science Gateway. The program Tracer (29) was used to confirm that the four independent runs converged on the same stationary distribution. Post burn-in samples from the marginal posterior distribution were combined using LogCombiner v1.5.4 (29) and trees summarized with TreeAnnotator (29). The topology of the tree broadly corresponds with that obtained by Panero et al. (24).

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- Friis EM, Crane PR, Pedersen KR (2011) *Early Flowers and Angiosperm Evolution* (Cambridge Univ Press, Cambridge, UK).
- Funk VA, et al. (2009) Compositae metatrees: The next generation. *Systematics, Evolution, and Biogeography of Compositae*, eds Funk VA, Susanna A, Stuessy TF, Bayer RJ (IAPT, Vienna, Austria), pp 747–777.
- Barreda VD, et al. (2010) Eocene Patagonia fossils of the daisy family. *Science* 329(5999):1621.
- Beaulieu JM, Tank DC, Donoghue MJ (2013) A Southern Hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. *BMC Evol Biol* 13:80.
- Barreda VD, et al. (2012) An extinct Eocene taxon of the daisy family (Asteraceae): Evolutionary, ecological and biogeographical implications. *Ann Bot (Lond)* 109(1):127–134.
- Magallón S, Castillo A (2009) Angiosperm diversification through time. *Am J Bot* 96(1):349–365.
- Olivero EB (2012) Sedimentary cycles, ammonite diversity and palaeoenvironmental changes in the Upper Cretaceous Marambio Group, Antarctica. *Cretac Res* 34(4):348–366.
- Raine JL, Speden IG, Strong CP (1981) New Zealand. *Aspects of Mid-Cretaceous Regional Geology*, eds Reymont RA, Bengtson P (Academic, London), pp 221–267.
- Dettmann ME, Jarzen DM (1988) Angiosperm pollen from uppermost Cretaceous strata of southeastern Australia and the Antarctic Peninsula. *Memoir of the Association Australasian Palaeontologists* 5:217–237.
- Sauquet H, et al. (2012) Testing the impact of calibration on molecular divergence times using a fossil-rich group: The case of *Nothofagus* (Fagales). *Syst Biol* 61(2):289–313.

11. Cardinal S, Danforth BN (2013) Bees diversified in the age of eudicots. *Proc Biol Sci* 280(1755):20122686.
12. Ericson PG, et al. (2006) Diversification of Neoaves: Integration of molecular sequence data and fossils. *Biol Lett* 2(4):543–547.
13. Lloyd GT, et al. (2008) Dinosaurs and the Cretaceous terrestrial revolution. *Proc Biol Sci* 275(1650):2483–2490.
14. Ho SYW (2014) The changing face of the molecular evolutionary clock. *Trends Ecol Evol* 29(9):496–503.
15. Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292(5517):686–693.
16. Wilf P, et al. (2005) Eocene plant diversity at Laguna del Hunco and Río Pichileufú, Patagonia, Argentina. *Am Nat* 165(6):634–650.
17. Currano ED, et al. (2008) Sharply increased insect herbivory during the Paleocene-Eocene Thermal Maximum. *Proc Natl Acad Sci USA* 105(6):1960–1964.
18. Stuessy TF, Sang T, DeVore ML (1996) Phylogeny and biogeography of the subfamily Barnadesioideae with implications for early evolution of the Compositae. *Compositae: Systematic*, eds Hind DNN, Beentje H (Royal Botanical Gardens, Kew, UK), pp 463–490.
19. Palazzesi L, Barreda VD, Telleria MC (2009) Fossil pollen grains of Asteraceae from the Miocene of Patagonia: Barnadesioideae affinity. *Rev Palaeobot Palynol* 155:83–88.
20. Fawcett JA, Maere S, Van de Peer Y (2009) Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. *Proc Natl Acad Sci USA* 106(14):5737–5742.
21. Semple JC, Watanabe K (2009) A review of chromosome numbers in Asteraceae with hypotheses on chromosomal base number evolution. *Systematics, Evolution and Biogeography of Compositae*, eds Funk VA, Susanna A, Stuessy TF, Bayer RJ (IAPT, Vienna), pp 61–72.
22. Hellwig FH (2007) Calyceraceae. *The Families and Genera of Vascular Plants. Flowering Plants—Eudicots. Asterales*, eds Kubitzki K, Kadereit JW, Jeffrey C (Springer, Berlin), pp 19–25.
23. Bremer K (1993) Intercontinental relationships of African and South American Asteraceae: A cladistic biogeographic analysis. *Biological Relationships Between Africa and South America*, ed Goldblatt P (Yale Univ Press, New Haven, CT), pp 104–135.
24. Panero JL, et al. (2014) Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae. *Mol Phylogenet Evol* 80:43–53.
25. Swofford D (2002) *PAUP* Phylogenetic Analysis Using Parsimony (* and other methods). Version 4* (Sinauer Associates, Sunderland, MA).
26. Maddison DR, Maddison WP (2003) *MacClade 4: Analysis of Phylogeny and Character Evolution, Version 4.06* (Sinauer Associates, Sunderland, MA).
27. Doyle JA, Endress PK (2010) Integrating Early Cretaceous fossils into the phylogeny of living angiosperms: Magnoliidae and eudicots. *J Syst Evol* 48(1):1–35.
28. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30(4):772–780.
29. Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. Available at bio.ed.ac.uk/Tracer. Accessed July 15, 2014.
30. Salgado L, Gasparini Z (2006) Reappraisal of an ankylosaurian dinosaur from the Upper Cretaceous of James Ross Island (Antarctica). *Geodiversitas* 28(1):119–135.
31. Coria RA, Moly JJ, Reguero M, Santillana S, Marensi S (2013) A new ornithomimid (Dinosauria, Ornithischia) from Antarctica. *Cretac Res* 41:186–193.
32. Pirrie D, Crame JA, Riding JB (1991) Late Cretaceous stratigraphy and sedimentology of Cape Lamb, Vega Island, Antarctica. *Cretac Res* 12(3):227–258.
33. Roberts EM, et al. (2014) Stratigraphy and vertebrate paleoecology of Upper Cretaceous—? Lowest Paleogene strata on Vega Island, Antarctica. *Palaeogeogr Palaeoclimatol Palaeoecol* 402:55–72.
34. Traverso A (1988) *Paleopalynology* (Unwin Hyman, Boston).
35. Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. *Rev Palaeobot Palynol* 143(1-2):1–81.
36. Urtubey E, Telleria MC (1998) Pollen morphology of the subfamily Barnadesioideae (Asteraceae) and its phylogenetic and taxonomic significance. *Rev Palaeobot Palynol* 104(1):19–37.
37. Telleria MC (2008) Taxonomic significance of pollen types in the Guayana Highland-centred composite genera of Mutisioideae (Asteraceae). *Bot J Linn Soc* 156(2):327–340.
38. Telleria MC, Katinas L (2004) A palynologic and comparative study of *Chaetanthera* (Asteraceae, Mutisieae) and allied genera. *Syst Bot* 29(3):752–773.
39. Telleria MC, Katinas L (2005) The unusual occurrence of tricolpate pollen within Mutisieae (Asteraceae). *Grana* 44(2):91–97.
40. Telleria MC, Katinas L (2009) New insights into the pollen morphology of *Mutisia* (Asteraceae, Mutisieae). *Plant Syst Evol* 280(3-4):229–241.
41. Telleria MC, Sancho G, Funk VA, Ventosa I, Roque N (2013) Pollen morphology and its taxonomic significance in the tribe Gochnatieae (Compositae, Gochnatioideae). *Plant Syst Evol* 299(5):935–948.
42. Erdtman G (1960) The acetolysis method, a revised description. *Sven Bot Tidskr* 54:561–564.
43. Doyle J (2012) Molecular and fossil evidence on the origin of Angiosperms. *Annu Rev Earth Planet Sci* 40(1):301–326.
44. Gradstein FM, Ogg JG, Schmitz MD, Ogg GM (2012) *The Geologic Time Scale 2012* (Elsevier, Amsterdam, The Netherlands).
45. Couper RA (1953) Upper Mesozoic and Cainozoic spores and pollen grains from New Zealand. *New Zealand Geological Survey Palaeontological Bulletin* 22:1–77.
46. Wilson GJ (1975) Palynology of deep-sea cores from DSDP site 275, southeast Campbell Plateau. *Initial Reports Deep Sea Drilling Project 29*, eds Kennett JP, et al. (US Government Printing Office, Washington, DC), pp 1031–1035.
47. Barreda VD, et al. (2012) Cretaceous/Paleogene floral turnover in Patagonia: Drop in diversity, low extinction, and a *Classopollis* spike. *PLoS One* 7(12):e52455.
48. Specht RL, Dettmann ME, Jarzen DM (1992) Community associations and structure in the Late Cretaceous vegetation of southeast Australasia and Antarctica. *Palaeogeogr Palaeoclimatol Palaeoecol* 94(3-4):283–309.
49. Macphail M, Hill RS, Partridge AD, Jordan GJ (2014) Geo-botany of the Cretaceous to Neogene. *Geological Evolution of Tasmania*, eds Corbett KD, Quilty PG, Calver CR (Geological Society of Australia Inc, Special Publication 24, Canberra, Australia), pp 495–507.
50. Heusser CJ (1971) *Pollen and Spores of Chile* (Univ of Arizona Press, Tucson, AZ).
51. Skvarla JJ, Turner BL, Patel VC, Tomb AS (1977) Pollen morphology in the Compositae and in morphologically related families. *The Biology and Chemistry of the Compositae*, eds Heywood VH, Harborne JB, Turner BL (Academic, London), pp 141–265.
52. Clarke GCS, Punt W, Hoen PP (1991) The Northwest European pollen flora, Ranunculaceae. *Rev Palaeobot Palynol* 69(1-3):117–271.
53. Raj B (1983) A contribution to the pollen morphology of Verbenaceae. *Rev Palaeobot Palynol* 39(3-4):343–422.
54. Moar NT (1993) *Pollen Grains of New Zealand Dicotyledonous Plants* (Manaaki Whenua Press, Lincoln, New Zealand).
55. Xie L, Li LQ (2012) Variation of pollen morphology, and its implications in the phylogeny of *Clematis* (Ranunculaceae). *Plant Syst Evol* 298(8):1437–1453.
56. Huysmans S, Robbrecht E, Delprete P, Smets E (1999) Pollen morphological support for the Gataebaeae-Chiococceae-Exostema-complex (Rubiaceae). *Grana* 38(6):325–338.
57. Takahashi M, Nowicki JW, Webster GLA (1995) Note on remarkable exines in Acylphoideae (Euphorbiaceae). *Grana* 34(5):282–290.
58. De-Yuan H, Kai-Yu P (2012) Pollen morphology of the platycodonoid group (Campanulaceae s. str.) and its systematic implications. *J Integr Plant Biol* 54(10):773–789.
59. Gustafsson MHG, Grafström E, Nilsson S (1997) Pollen morphology of the Goodeniaceae and comparisons with related families. *Grana* 36(4):185–207.
60. DeVore ML, Zhao Z, Jansen RK, Skvarla JJ (2007) Pollen morphology and ultrastructure of Calyceraceae. *Lundellia (Austin, Tex)* 10:32–48.
61. Karehed J (1965) Alseuosmiaceae Airy Shaw. *Kew Bull* 18(249):7–12.
62. Karehed J, Lundberg J, Bremer B, Bremer K (1999) Evolution of the Australasian families Alseuosmiaceae, Argophyllaceae, and Phellinaceae. *Syst Bot* 24(4):660–682.
63. Rowley JR, Nilsson S (1972) Structural stabilization for electron microscopy of pollen from herbarium specimens. *Grana* 12(1):23–30.
64. Bronckers F, Stainier F (1972) Contribution à l'étude morphologique du pollen de la famille des Styliidiaceae. *Grana* 12(1):1–22.
65. Nilsson S (1973) Menyanthaceae Dum. Taxonomy by Robert Ornduff. *World Pollen and Spore Flora* 2:1–20.
66. Martin HA (1977) The history of *Ilex* (Aquifoliaceae) with special reference to Australia: Evidence from Pollen. *Aust J Bot* 25(6):655–673.
67. Lobreau-Callen D (1977) Les pollens des Celastrales. *Mémoires et Travaux de l'Institut de Montpellier (l'École Pratique des Hautes Études, Montpellier, France)* 3:1–116.
68. Ferguson H, Hideux MJ (1978) Some aspects of the pollen morphology and its taxonomic significance in Cornaceae sens. lat. *Proceedings of the Fourth International Palynological Conference, Lucknow, India (1976–1977)*. 1:240–249.
69. Dunbar A (1978) Pollen morphology and taxonomic position of the genus *Pentaphragma* Wall. (Pentaphragmataceae). *Grana* 17(3):141–147.
70. Pragłowski J, Grafström E (1985) The genus *Carpodetus* (Escalloniaceae): A pollen morphological enigma. *Grana* 24(1):11–22.
71. Cilliers SS (1991) Pollen morphology and its taxonomic value in *Brachylaena* (Asteraceae) in southern Africa. *S Afr J Bot* 57(6):325–330.
72. Hansen HV (1991) SEM-studies and general comments on pollen in tribe Mutisieae (Compositae) sensu Cabrera. *Nord J Bot* 10(6):607–623.
73. Lundberg J (2001) The asteralean affinity of the Mauritian *Roussea* (Rousseaceae). *Bot J Linn Soc* 137(4):267–276.
74. Telleria MC, Urtubey E, Katinas L (2003) *Proustia* and *Lophopappus* (Asteraceae, Mutisieae): Generic and subtribal relationships based on pollen morphology. *Rev Palaeobot Palynol* 123(3-4):237–246.
75. Polevova SV (2006) Review of the sporoderm ultrastructure of members of the Asterales. *Paleontol J* 40(5):656–663.
76. Wortley AH, et al. (2007) A search for pollen morphological synapomorphies to classify roge genera in Compositae (Asteraceae). *Rev Palaeobot Palynol* 146(1-4):169–181.
77. Blackmore S, Wortley AH, Skvarla JJ, Gabarayeva NI, Rowley JR (2010) Developmental origins of structural diversity in pollen walls of Compositae. *Plant Syst Evol* 284(1-2):17–32.
78. Pereira Coutinho A, Almeida da Silva R, Sá da Bandeira D, Ortiz S (2012) Pollen morphology in tribe Dicomeae Panero and Funk (Asteraceae). *Plant Syst Evol* 298(10):1851–1865.
79. Freire SE, Barboza GE, Cantero JJ, Ariza Espinar L (2014) *Famatinanthus*, a new Andean genus segregated from *Aphyllocladus* (Asteraceae). *Syst Bot* 39(1):349–360.
80. Crame JA, Francis JE, Cantrill DJ, Pirrie D (2004) Maastrichtian stratigraphy of Antarctica. *Cretac Res* 25(5):411–423.
81. Crampton JS, et al. (2000) Revision of the Piripauan and Haumurian local stages and correlation of the Santonian-Maastrichtian (Late Cretaceous) in New Zealand. *NZ J Geol Geophys* 43(3):309–333.
82. Partridge AD (2006) *Australian Mesozoic and Cenozoic Palynology Zonations—Updated to the 2004 Geologic Time*, coord. Monteil E (Geoscience Australia Record 2006/23 Chart 4, Canberra, Australia).