False Blister Beetles and the Expansion of Gymnosperm–Insect Pollination Modes before Angiosperm Dominance

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SUMMARY

During the mid-Cretaceous angiosperms diversified from several nondiverse lineages to their current global domination [1], replacing earlier gymnosperm lineages [2]. Several hypotheses explain this extensive radiation [3], one of which involves proliferation of insect pollinator associations in the transition from gymnosperm to angiosperm dominance. This hypothesis is supported by direct evidence of pollen occurring on insect bodies, currently established for four insect groups: Thysanoptera (thrips), Neuroptera (lacewings), Diptera (flies) and now Coleoptera (beetles). Each group represents a distinctive pollination mode linked to a unique mouthpart type and feeding guild (Table 1) [4–9]. Extensive indirect evidence, based on specialized head and mouthpart morphology, is present for one of these pollinator types, the long-proboscid pollination mode [10], representing minimally ten family-level lineages of Neuroptera, Mecoptera (scorpionflies) and Diptera [8, 11, 12] (Table S1). A recurring feature uniting these pollinator modes is host associations with ginkgoalean, cycad, conifer and bennettitalean gymnosperms (Table 1). Pollinator lineages bearing these pollination modes were categorized into four evolutionary cohorts during the 35 million-year-long angiosperm radiation. Each cohort is defined by its host-plant associations (gymnosperm, angiosperm) and evolutionary pattern (extinction, continuation, origination) during this interval [13]. Here, we provide the first, direct evidence for one cohort, exemplified by the beetle *Darwinylus marcosi*, family Oedemeridae (false blister beetles), that had an earlier gymnosperm (likely cycad) host association, later transitioning onto angiosperms [14]. This association constitutes one of four patterns explaining the plateau of family-level plant-lineages generally and pollinating insects in particular during the mid-Cretaceous angiosperm radiation [15, 16].

RESULTS

The holotype of *Darwinylus marcosi* from Spanish amber in the Basque-Cantabrian Basin of northern Spain was recently re-examined (Supplemental Information). This specimen represents the oldest definitive fossil so far described for the beetle family Oedemeridae (false blister beetles) and is phylogenetically basal within the subfamily Oedemerinae [9]. The amber piece containing the beetle revealed 126 associated pollen grains, some of which formed distinct clumps (Figures 1A, 1C–D, 1E–G). Five of these pollen grains are each in contact with different body regions of the specimen, including placement on a metatibia, left and right pronotal margins, and external aspect of the left and right elytra (Figures 1B; reconstructed in Figures 2 and S1). All pollen grains are distributed in the amber as a linearly expanding trail surrounded by a cone-like envelope wherein the beetle lies in the center of the cone base (Figure 1H–I). The stalactite-shaped amber piece exhibits multiple, superposed layers of resin deposition, which suggests that resin was extruded under aerial conditions. Overall, the pollen dispersion pattern and presence of multiple resin flows indicate that all pollen grains were attached to *Darwinylus marcosi* prior to resin entrapment, and that both the beetle and its pollen grains were jointly swept along by one of those resin flows (Supplemental Information).

Morphological and ultrastructural features of these pollen grains are attributable to the form-genus *Monosulcites* [17], described from the dispersed fossil-pollen record. The pollen grains are monosulcate with sulci generally broadest at their midpoints, boat shaped, psilate in ornamentation, atectate in wall ultrastructure, and 25.14 µm long by 16.56 µm wide in average size (Figures 1A, 1C–D). The *Monosulcites* exine is on average 2.50 µm thick at the distal pole of the grains, becoming very thin in the proximal pole beside the sulci. In addition, the structure of the grain exine exhibits an outer imperforate sexine, which is lacking in the proximal pole, and an inner sexine with alveolate elements. Based on these features of exine structure, the pollen grains can be confidently referred to a gymnosperm source plant [18]. This certainty is based on the erection of *Monosulcites* as a form-genus that integrated different species of Mesozoic gymnosperm pollen, and only in a few cases was an angiosperm affinity indicated, but these assignments were Cenozoic in age [17] (Supplemental Information).

Although the specific taxonomic affinities of the gymnospermous *Monosulcites* grain associated with *D*. *marcosi* are not known for sure, the botanical affinity of the Late Jurassic to earliest Cretaceous *Monosulcites* relates it broadly to the Ginkgoales, Cycadales and Bennettitales [17]. Some Mesozoic representatives of Cycadales and Bennettitales have been associated with beetle damage in vegetative tissues and reproductive strobili, such as coprolites associated with Middle Triassic cycads [5, 19]. The adhesive properties of the *Monosulcites* pollen grains, exemplified by the presence of clumps consisting of 16 to 24 grains in the specimen (Figure 1H), provide additional evidence for entomophily. Presently, many species of cycads and gnetaleans have entomophilous pollen that aggregate into clusters [5, 20], whereas anemophilous pollen overwhelmingly are dispersed as solitary monads [21] (Figure S2; Supplemental Information).

DISCUSSION

Animal-pollinated plants constitute 85 % of all extant angiosperm species globally [22] and are overwhelmingly pollinated by insects [2]. Insect pollination has had a tremendous impact on diversification of flowering plants, reflected in a significant fossil record [2, 23]. Entomophily has been regarded as the plesiotypic condition for angiosperms based on palynological data and phylogenetic inferences [23, 24], although ambophily, a combination of insect and either wind or water pollination, has been suggested for some early angiosperm taxa [2, 23]. Whereas the earliest fossil evidence for the appearance of angiosperms (as pollen) originates at about 130 Ma [3], and the earliest macrofloral record (as flowers) occurs at about 125 Ma [2], direct associational evidence for Cretaceous angiosperm pollination by insects is absent, currently known only from the Cenozoic [25, 26]. Occurrences with indirect evidence, such as floral morphology from the Turonian (ca. 92 Ma) of New Jersey, indicate specialized bee pollination [27].

Among approximately 30 extant insect orders, Thysanoptera (thrips), Coleoptera (beetles), Diptera (true flies), Lepidoptera (moths and butterflies) and Hymenoptera (wasps, bees and ants) presently are the most prominent pollinators [15, 24, 28]. Of these five major groups of pollinating insects, beetles probably played the earliest and longest lasting, key pollinator roles, and might have acted as pollinators since their first appearance during the Permian [5, 15, 19]. Beetles are considered among the earliest pollinators of extant basal angiosperms, particularly basal lineages with generalized flowers and unelaborated pollinator rewards emphasizing pollen [2, 24, 29]. Although Mesozoic diversification of Coleoptera is thought to result from the persistence of older lineages such as darkling beetles [29, 30] and colonization of multiple niches, other lineages that underwent radiation events, including weevils [31] and dung beetles [32], exploited new ecological and evolutionary opportunities related to the initial angiosperm radiation. The diversification of flowering plants represented a 35 million-year-long interval from 125 to 90 Ma [2, 13], during which pollinators colonized new hosts, habitats and ecosystems, as evidenced by early phylogenetic trajectories of diverse clades such as certain beetles [31, 33], ants [34], bees [27, 35] and moths [36] that further diversified into the Cenozoic [4, 25, 35,37]. Nevertheless, what were the specific patterns of insect pollination before angiosperm dominance?

The idea that insect pollination may have preceded the angiosperms was substantively hypothesized during the 1970’s [15], although it was disputed [38]. More recently, pollinator relationships between diverse insect and gymnosperm groups since the Late Paleozoic have been suggested by substantial, indirect, paleontological evidence, principally functional morphology applied to plant and insect fossils [10, 12, 40], evidence from insect gut contents and coprolites [19, 40], and entomophilous features of fossil pollen such as size, shape, ornamentation, stickiness, quantity and clumping ability [18, 23]. This evidence supports the hypothesis that insects were pollinating a variety of gymnosperm groups throughout the pre-angiosperm Mesozoic. Such early interactions likely acted as an evolutionary and ecological prelude to later interactions between early angiosperms and their insect pollinators during the Cretaceous [10, 12, 29, 37].

Darwinylus marcosi bore mandibulate-ectognathate mouthparts, a common type of mostly prognathous mouthparts that employ a chewing feeding style [41]. A related feature was a body surface laden with cycad-attributed *Monosulcites* pollen [9] (Figures 2, S2). These features constitute the first, direct evidence of pollination in the fossil record of beetles and the fourth, major gymnosperm–insect pollination mode documented in the fossil record during the mid-Mesozoic (Table 1). Three other major pollination modes reported from 165 to 105 million-year-old Eurasian deposits, each with a distinctive mouthpart type and attributed feeding guild [41], also involved gymnosperm pollen (Table 1). The second and oldest occurrence is the long-proboscid pollination mode, involving siphoning of surface fluids. One lineage of long-proboscid pollinators includes two kalligrammatid lacewings, *Kallihemerobius feroculus* from the late Middle Jurassic (165 Ma) of Inner Mongolia in Northeastern China and *Meioneurites spectabilis* from the Late Jurassic of Kazakhstan, associated with bisaccate pollen of an unknown gymnosperm and *Classopollis* pollen, respectively [8, 42] (Supplemental Information). A second lineage of the long proboscid pollination mode is represented by the zhangsolvid fly *Buccinatormyia magnifica* from late Early Cretaceous Spanish amber, carrying abundant *Exesipollenites* pollen attributed to bennettitaleans [7, 17, 43] The third major pollination mode involves labellate mouthparts [41], representing the absorptive sponging of surface fluids by a basal asilomorph fly, *Paroikus* sp., from Transbaikalian Russia [4], occurring in Early Cretaceous deposits ca. 130–120 Ma in age [2]. This fly bore distinctive tetrads of *Classopollis* pollen plastered onto the frontal aspect of the head adjacent the mouthpart base, typical of some modern pollinator flies [44] (Supplemental Information). The fourth major pollination mode are mouthcone mouthparts [41], engaged in a tissue-penetrating punch-and-suck feeding style involving the melanthripid thrips *Gymnopollisthrips minor* and *G. maior*, from Spanish Amber [6]. These thrips were covered with copious *Cycadopites* pollen grains attributed to a ginkgoalean, or more remotely a cycad, and possessed unique ring setae specialized for pollination [6, 43]. These four, basic pollination modes among major insect pollinator lineages (Figure 3A) and their gymnosperm hosts parallel the evolutionary dynamics of other plant-associated insect groups during the mid Mesozoic (Figure 3B), such as xylophages and particularly herbivores [13].

The proliferation of gymnosperm–insect associations during the mid Mesozoic involves evaluation of the effect that the transition from a gymnosperm to angiosperm dominated global flora had on lineage diversity of plant-associated insects in general and pollinators in particular. During a 178 million-year-long interval from the Triassic–Jurassic boundary (201 Ma) to the Paleogene–Neogene boundary (23 Ma), a plateau of total family-level diversity has consistently been documented for the mid Mesozoic by several studies [45–47]. This plateau also has been supported by a more finely-tuned study of 280 plant-associated families through this time interval, which also exhibits a flattening of diversity and, indeed, a temporary decrease of plant-associated diversity from the Barremian to Cenomanian interval, termed the Aptian–Albian gap (Figure 3B) [13]. By establishing the origin of angiosperms at their first appearance as fossils during the Hauterivian Stage at ca. 130 Ma [3], by allocating plant-associated insect families into four basic cohorts based on their gymnosperm or angiosperm host associations, and by assessing their diversification dynamics, an explanation is provided for the overall diversity plateau of these family-level insect lineages [13].

Insect pollinator response to the mid-Cretaceous angiosperm radiation provides an additional refinement that relates cohort persistence through time to host association (gymnosperm or angiosperm) and evolutionary pattern (extinction, continuation or origination) (Figure 3A). During the 35 million-year-long interval, these four cohorts of insect families were those with: (i) gymnosperm host associations that became extinct earlier in the interval, such as zhangsolvid flies [12]; (ii) gymnosperm host associations that survived the interval and continued largely to the present, exemplified by merothripid thrips [5]; (iii) gymnosperm host associations that transitioned laterally onto new angiosperm hosts, which included false blister beetles [9]; and (iv) new angiosperm host associations later in the interval, of which bees are an example [25,26] (Figure 3A). For pollinators, the Aptian–Albian gap is explained as a consequence of older insect lineages with gymnosperm hosts undergoing extinction (cohort i) that generally occurred before younger insect lineages originated on angiosperm hosts (cohort iv). Also contributing to this diversity minimum were diversity decreases attributable to those surviving lineages that retained their gymnosperm hosts (cohort ii) and other lineages undergoing gymnosperm to angiosperm host shifts including *Darwinlyus marcosi* (cohort iii). For pollinators, much of the Aptian–Albian gap likely consisted of cohorts (ii) and (iii) to fill the interval (Figure 3A).

The mid-Mesozoic record of insect pollinators provides evidence for a distinctive family-level diversity pattern based on their plant-host associations and evolutionary histories (Figure 3; Tables 1, S1). During the angiosperm radiation and the Aptian–Albian gap in particular, there were extinctions, reductions and host shifts of insect lineages on gymnosperm hosts [6, 13, 16] and the origination of new associations on angiosperms [24, 38]. Compression-impression deposits, such as the Yixian Formation (ca. 124 Ma) of Northeastern China have provided considerable documentation for these several, major, insect pollinator lineages that evidently became extinct during the Aptian–Albian gap [8, 10, 13] (Figure S2). However, the three most important amber deposits of this interval – Lebanese Amber (ca. 130–125 Ma, possibly somewhat older), Spanish Amber (105 Ma) and Myanmar Amber (99 Ma) – recently have provided a wealth of insect pollinator and plant host data [16]. The four major evolutionary patterns now include a gymnosperm to angiosperm host transition for mandibulate-feeding false blister beetles (Figures 2 and S1), the demise or significant reduction of earlier insect lineages with gymnosperm hosts, and the emergence of newer lineages on angiosperm hosts [16]. This time interval represents one of the most profound insect pollinator–plant host shifts in the fossil record.

EXPERIMENTAL PROCEDURES

The examined specimen was found in a large piece of amber that was cut, polished and subsequently embedded in synthetic epoxy resin (EPO-TEK 301) for preservation and optimal observation [48]. The holotype of *Darwinylus marcosi* is housed at the Museo de Ciencias Naturales de Álava (Vitoria-Gasteiz, Spain) under accession number MCNA–11229 [9]. The beetle and pollen grains were examined with a Leica MS5 stereomicroscope, Motic BA310 and Olympus BX51 compound microscopes. Macrophotographs were taken by a Leica DFC 420 camera attached to a Leica MS5 microscope with Leica IM1000 software. Microphotographs were taken using a Moticam 2500 camera on a Motic BA310 and a ColorView IIIu camera attached to an Olympus BX51 compound microscope. Drawings were made using a camera lucida attached to a Leica MS5 microscope.

SUPPLEMENTAL INFORMATION

The Supplemental Information consists of text sections on: (1) “Geological Context”, (2) “Preservational Bias and Taphonomy of the Amber Specimens”, (3) “Plant Affinities of the Pollen”, and (4) “The Relationship between *Classopollis* Pollen and Insects”; Figure S1 on a habitus reconstruction of *Darwinylus marcosi* and Figure S2 on the distribution of pollination types recorded in Spanish Amber; “Occurrence Data for Mid-Mesozoic Long-Proboscid Insects”, expressed as Table S1; and “Supplemental References”. These data can be found linked to this article online at http://dx.doi.org/10.1016j.cub-xxxxx).

AUTHOR CONTRIBUTIONS

D.P., E.P., X.D., R.P.F. and C.L. designed the research. D.P., R.P.F. and C.L. performed the study on plant–insect interactions. E.B and D.P. contributed to paleobotanical research. D.P., C.L. E.P. and E.B. conducted scanning electronic microscope and optical microscope observations and camera lucida drawings. All authors analyzed and discussed the data. D.P., R.P.F. and C.L. wrote the paper, with contributions from the remaining authors.

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REFERENCES

1. Benton, M.J. (2010). The origins of modern biodiversity on land. Phil. Trans. R. Soc. *365*, 3667–3679.
2. Friis, E.M., Pedersen, K.R., and Crane, P.R. (2006). Cretaceous angiosperm flowers: innovation and evolution in plant reproduction. Palaeogeogr. Palaeoclim. Palaeoecol. *232*, 251–293.
3. Augusto, L., Davies, T.J., Delzon, S., and Schrijver, A. (2014). The enigma of the rise of angiosperms: can we untie the knot? Ecol. Lett. *17*, 1326–1338.

4. Labandeira, C.C. (2005). Fossil history and evolutionary ecology of Diptera and their associations with plants. In The Evolutionary Biology of Flies, D.K. Yeates and B.M. Wiegmann, eds. (New York: Columbia University Press), pp. 217–273.

5 Labandeira, C.C., Kvaček, J., and Mostovski, M. (2007). Pollination drops, pollen, and insect pollination of Mesozoic gymnosperms. Taxon *56*, 663–695.

6. Peñalver, E., Labandeira, C.C., Barrón, E., Delclòs, X., Nel, P., Nel, A., Tafforeau, P., and Soriano, C. (2012). Thrips pollination of Mesozoic gymnosperms. Proc. Natl. Acad. Sci. USA *109*, 8623–8628.

7. Peñalver, E., Arillo, A., Pérez-de la Fuente, R., Riccio, M.L., Delclòs, X., Barrón, E., and Grimaldi, D.A. (2015). Long-proboscid flies as pollinators of Cretaceous gymnosperms. Curr. Biol. *14*, 1917–1923.

8. Labandeira, C.C., Yang, Q., Santiago-Blay, J.A., Hotton, C.L., Monteiro, A., Wang, Y., Goreva, Y., Shih, C., Siljeström, S., Rose, T.R., Dilcher, D.L., and Ren, D. (2016). The evolutionary convergence of mid-Mesozoic lacewings and Cenozoic butterflies. Proc. R. Soc. B *283*, 20152893.

9. Peris, D. (2016). Early Cretaceous origin of pollen-feeding beetles (Insecta: Coleoptera: Oedemeridae). Cladistics, doi: 10.1111/cla.12168.

10. Labandeira, C.C. (2010). The pollination of Mid Mesozoic seed plants and the early history of long-proboscid insects. Ann. Mo. Bot. Gard. *97*, 469–513.

11. Ren, D. (1998). Flower-associated Brachycera flies as fossil evidence for Jurassic angiosperm origins. Science *280*, 85–88.

12. Ren, D., Labandeira, C.C., Santiago-Blay, J.A., Rasnitsyn, A., Shih, C., Bashkuev, A., Logan, M.A., Hotton, C.L., and Dilcher, D. (2009). A probable pollination mode before angiosperms: Eurasian, long-proboscid scorpionflies. Science *326*, 840–847.

13. Labandeira, C.C. (2014). Why did terrestrial insect diversity not increase during the angiosperm radiation? Mid-Mesozoic, plant-associated insect lineages harbor clues. In Evolutionary Biology: Genome Evolution, Speciation, Coevolution and Origin of Life, P. Pontarotti, ed. (Cham, Switzerland: Springer), pp. 261–299.

14. Lawrence, J.F., and Ślipiński, S.A. (2010). Oedemeridae Latreille, 1810. In Handbook of Zoology. Volume 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim), R.A.B. Leschen and R.G. Beutel, Eds. In Handbook of Zoology, Arthropoda: Insecta, N.P. Kristensen and G. Beutel, eds. (Berlin and New York: Walter de Gruyter), pp. 674–681.

15. Crepet, W.L. (1979). Insect pollination: A paleontological perspective. Bioscience *29*, 102–108.

16. Labandeira, C.C. (2014). Amber. In Reading and Writing of the Fossil Record: Preservational pathways to Exceptional Fossilization, M. Laflamme, J. Schiffbauer, and S. Darroch, eds., Paleontol. Soc. Pap. *20*, 163–215.

17. Balme, B.E. (1995). Fossil in situ spores and pollen grains: an annotated catalogue. Rev. Palaeobot. Palynol. *87*, 81–323.

18. Walker, J.W. (1976). Evolutionary significance of the exine in the pollen of primitive angiosperms. In The Evolutionary Significance of the Exine, D.K. Ferguson and J. Miller, eds. J. Linn. Soc. Symp. Ser. *1*, 251–308.

19. Klavins, S.D., Kellogg, D.W., Krings, M., Taylor, E.L., and Taylor, T.N. (2005). Coprolites in a Middle Triassic cycad pollen cone: Evidence for insect pollination in early cycads? Evol. Ecol. Res. *7*, 479–488.

20. Hall, J.A., and Walter, G.H. (2011). Does pollen aerodynamics correlate with pollination vector? Pollen settling velocity as a test for wind versus insect pollination among cycads (Gymnospermae: Cycadaceae: Zamiaceae). Biol. J. Linn. Soc. *104*, 75–92.

21. Timerman, D., Greene, D.F., Ackerman, J.D., Kevan, P.G., and Nardone, E. (2014). Pollen aggregation in relation to pollination vector. Internat. J. Pl. Sci. *175*, 681–687.

22. Ollerton, J., Winfree, R., and Tarrant, S. (2011). How many flowering plants are pollinated by animals? Oikos *120*, 321–326.

23. Hu, S., Dilcher, D.L., and Taylor, D.W. (2012). Pollen evidence for the pollination biology of the early flowering plants. In Evolution of plant-pollinator relationships, S. Patiny ed. (Cambridge: Cambridge University Press), pp. 165–236.

24. Thien, L.B., Bernhardt, P., Devall, M.S., Chen, Z.-D., Luo, Y.-B., Fan, J.-H., Yuan, L.-C., and Williams, J.H. (2009). Pollination biology of basal angiosperms (ANITA grade). Am. J. Bot. *96*, 166–182.

25. Ramírez, S.R., Gravendeel, B., Singer, R.B., Marshall, C.R., and Pierce, N.E. (2007). Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. Nature 448, 1042–1045.

26. Wappler, T., Labandeira, C.C., Engel, M.S., Zetter, R., and Grimsson, F. (2015). Specialized and generalized pollen-collection strategies in an ancient bee lineage. Curr. Biol. *25*, 3092–3098.

27. Crepet, W.L., and Nixon, K.C. (1998). Fossil Clusiaceae from the Late Cretaceous (Turonian) of New Jersey and implications regarding the history of bee pollination. Am. J. Bot. *85*, 1122–1133,

28. Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P.D., Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K.S., et al. (2016). Non-bee insects are important contributors to global crop pollination. Proc. Natl. Acad. Sci. USA *113*, 146–151.

29. Wang, B., Zhang, H., and Jarzembowski, E.A. (2013). Early Cretaceous angiosperms and beetle evolution. Front. Plant Sci. *4*, 360 (doi: 10.3389/fpls.2013.00360).

30. Kergoat, G.J., Bouchard, P., Clamens, A.L., Abbate, J.L., Jourdan, H., Jabbour-Zahab, R., Genson, G., Soldati, L., and Condamine, F.L. (2014). Cretaceous environmental changes led to high extinction rates in a hyperdiverse beetle family. BMC Evol. Biol. *14*, 220 (doi: 10.1186/s12862).

31. McKenna, D.D., Sequeira, A.S., Marvaldi, A.E., and Farrell, B.D. (2009). Temporal lags and overlap in the diversification of weevils and flowering plants. Proc. Natl. Acad. Sci. USA *106*, 7083–7088.

32. Gunter, N.L., Weir, T.A., Ślipińksi, A., Bocak, L., and Cameron, S.L. (2016). If dung beetles (Scarabaeidae: Scarabaeinae) arose in association with dinosaurs, did they also suffer a mass co-extinction at the K-Pg boundary? PLoS ONE *11*(5), e0153570.

33. McKenna, D.D., Wild, A., Kanda, K., Bellamy, C., Beutel, R.G., Caterino, M., Farnum, C.W., Hawks, D.C., Ivie, M.A., Jameson, M.Z., et al. (2015). The beetle tree of life reveals that Coleoptera survived end-Permian mass extinction to diversify during the Cretaceous terrestrial revolution. Syst. Entomol. *40*, 835–880.

34. Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., and Pierce, N.E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. Science *312*, 101–104.

35. Cardinal, S., and Danforth, B.N. (2013). Bees diversified in the age of eudicots. Proc. R. Soc. B *280*, 20122686.

36. Wahlberg, N., Wheat, C.W., and Peña, C. (2013). Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). PLoS ONE *8*, e80875.

37. Grimaldi, D.A. (1999). The co-radiations of pollinating insects and angiosperms in the Cretaceous. Ann. Mo. Bot. Gard. *86*, 373–406.

38. Schneider, D., Wink, M., Sporer, F., and Lounibos, P. (2002). Cycads: their evolution, toxins, herbivores and insect pollinators. Naturwissenschaften *89*, 281–294.

39. Myskowiak, J., Azar, D., and Nel, A. (2015). The first fossil hilarimorphid fly (Diptera: Brachycera). Gond. Res. *35*, 192–197.

40. Krassilov, V.A., Rasnitsyn, A.P., and Afonin, S.A. (2007). Pollen eaters and pollen morphology: Co-evolution through the Permian and Mesozoic. Afr. Invert. *48*, 3–11.

41. Labandeira, C.C. (1997). Insect mouthparts: ascertaining the paleobiology of insect feeding strategies. Annu. Rev. Ecol. Syst. *28*, 153–193.

42. Traverse, A. (2008). Paleopalynology. Second edition (Springer).

43. Barrón, E., Peyrot, D., Rodríguez-López, J.P., Meléndez, N., López del Valle, R. Najarro, M., Rosales, I., and Comas-Rengifo, M.J. (2015). Palynology of Aptian and upper Albian (Lower Cretaceous) amber-bearing outcrops of the southern margin of the Basque–Cantabrian basin (northern Spain). Cret. Res. *52*, 292–312.

44. Nicholson, S.W. (1994). Pollen feeding in the eucalypt nectar fly, *Drosophila flavohirta*. Physiol. Entomol. *19*, 58–60.

45. Dmitriev, V.J., and Zherikhin, V.V. (1988). Changes in the diversity of insect families from data of first and last occurrences. In The Mesozoic–Cenozoic Crisis in the Evolution of Insects, A.G. Ponomarenko, ed. (Moscow: Nauka Press), pp. 208–215 [in Russian].

46. Labandeira, C.C., and Sepkoski, J.J., Jr. (1993). Insect diversity in the fossil record. Science *261*, 300–305.

47. Jarzembowski, E.A., and Ross, A.J. (1996). Insect origination and extinction in the Phanerozoic. In Biotic Recovery from Mass Extinction Events, M.B. Hart, ed. Geol. Soc. Spec. Publ. *102*, 65–78.

48. Nascimbene, P., and Silverstein, H. (2000). The preparation of fragile Cretaceous ambers for conservation and study of organismal inclusions. In Studies on Fossils in Amber, with

Particular Reference to the Cretaceous of New Jersey, D. Grimaldi, ed. (Leiden: Backhuys Publishers), 93–102.

49. Dejax, J. (1987). Une étude palynologique dans le Crétacé Inférieur du Congo. (Paris: Université Pierre et Marie Curie, thesis).

50. Ogg, J.G., Ogg, G.M., and Gradstein, F.M. (2016). A Concise Geologic Time Scale 2016. (Amsterdam: Elsevier), p. 234.

FIGURE CAPTIONS

Figure 1. Pollen associated with *Darwinylus marcosi*, an oedemerid beetle, showing *Monosulcites* pollen grains associated with the body surface and locomotory trajectory of the beetle within the resin. (A) Detached pollen grains, enlarged in (D) and (C), from the beetle's specimen, shown in (B). At (E)–(G) are pollen grains attached to various elements of the body surface. (H) shows the whole amber piece containing the beetle at lower left, which matches the camera lucida drawing in (I). According to our taphonomic interpretation, the dark blue arrow indicates the direction of resin flow that swept along the beetle and the pollen grains that originally adhered to its body, with the resulting widening cone-like pattern of pollen dispersion shown in dark green. Pollen grains are depicted in dark red.

Figure 2. A three-dimensional model of *Darwinylus marcosi* Peris 2016, with an associated *Monosulcites* pollen load. The distribution and density of pollen on the body is inferred from the density of pollen in the surrounding amber and is conjectural. Artwork by J.A. Peñas.

Figure 3. The evolutionary fates of the four insect pollinator cohorts and insect families hosting major vascular-plant hosts during the mid-Cretaceous angiosperm radiation.
(**A**) The four basic cohorts of insect pollinators, with family-level examples, during the mid-Cretaceous angiosperm radiation based on their gymnosperm or angiosperm host-plant associations and their evolutionary fates across the Aptian–Albian gap. No vertical or horizontal scale is implied, except for the duration of the Aptian–Albian gap.
(**B**) Diversity of 280 insect families that hosted ferns (purple), gymnosperms (dark green) or angiosperms (light green) during the 178 million-year-long interval from the Triassic–Jurassic to the Paleogene–Neogene period boundaries, emphasizing their evolutionary dynamics across the mid-Cretaceous angiosperm radiation. The data and modified figure are from [13] and assumes that angiosperms originated during the Hauterivian Stage as indicated by the fossil record [2].

Table 1. Mid-Mesozoic gymnosperm–insect pollination associations based on direct evidence of body, head and mouthpart associated pollen. The first four entries are from amber.

	Pollinated Plant				Insect Pollinator				
	Pollinator taxon	Affiliated clade	Mouthpart class[1]	Feeding guild[1]	Whole plant taxon[2]	Affiliated clade[2]	Insect associated pollen[3]	Locality, age and date[4]	References
1.	*Gymnopollisthrips minor*	**Thysanoptera**: Merothripidae	Mouthcone	Punch and sucking	*Nehvizdyella/ Eretmophyllum*	**Ginkgoales**: Ginkgoaceae	*Cycadopites* sp.[5]	Álava, Spain 105; Late Albian	[6, 49]
2.	*Gymnopollisthrips maior*	**Thysanoptera:** Merothripidae	Mouthcone	Punch and sucking	*Nehvizdyella/ Eretmophyllum*	**Ginkgoales**: Ginkgoaceae	*Cycadopites* sp.[5]	Álava, Spain 105; Late Albian	[6, 49]
3.	*Darwinylus marcosi*	**Coleoptera**: Oedemeridae	Mandibulate ectognathate	Chewing	Indeterminate cycad	?**Cycadales**	*Monosulcites* sp.[6]	Álava, Spain; 105; late Albian	[9, this report, 43]
4.	*Buccinatormyia magnifica*	**Diptera**: †Zhangsolvidae	Long-proboscid	Siphonate fluid feeding	Indeterminate bennettitalean	**Bennettitales**: †Williamsoniaceae	*Exesipollenites* sp.	El Soplao, Spain; 105; late Albian	[7; 17]
5.	*Meioneurites spectabilis*	**Neuroptera**: †Kalligrammatidae	Long-proboscid	Siphonate fluid feeding	*Frenelopsis* sp.	**Coniferales**: †Cheirolepidiaceae	*Classopollis* cf. *annulatus*	Karatau, Russia; 155; Kimmeridgian	[8, 46]
6.	*Kallihemerobius feroculus*	**Neuroptera**: †Kalligrammatidae	Long-proboscid	Siphonate fluid feeding	[unknown]	**Pinales**: ?Pinaceae	Undetermined bisaccate	Daohugou, China; 165; late Callovian	[8]
7.	*Paroikus* sp.	**Diptera**: Asilomorpha indet.	Labellate	Sponging fluid feeding	*Frenelopsis* sp.	**Coniferales** †Cheirolepidiaceae	*Classopollis* sp.	Baissa, Russia; 134 –139; Valanginian	[4, 5, 46]

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[1] See [42] for a phenetic classification of insect mouthpart classes and feeding guilds.

[2] Several sources were used for establishing the source plant clades based on pollen taxa identifications, notably Balme [17] and Traverse [41].

[3] Pollen form-genera identifications are from the primary source literature, cited at right.

[4] Geochronologic stage dates pegged to absolute age determinations are from Gradstein et al. [50].

[5] There is a remote possibility that this palynomorph is referable to a cycadophyte.

[6] There is a less probable possibility that this palynomorph is referable to Bennettitales.