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

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Abstract:	<p>SUMMARY</p> <p>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. However, most evidence supports gymnosperm-insect pollinator associations, supported by direct evidence of pollen on insect bodies, currently established for four 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 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 <i>Darwinylus marcosi</i>, 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 specifically during the mid-Cretaceous angiosperm radiation [15, 16].</p>

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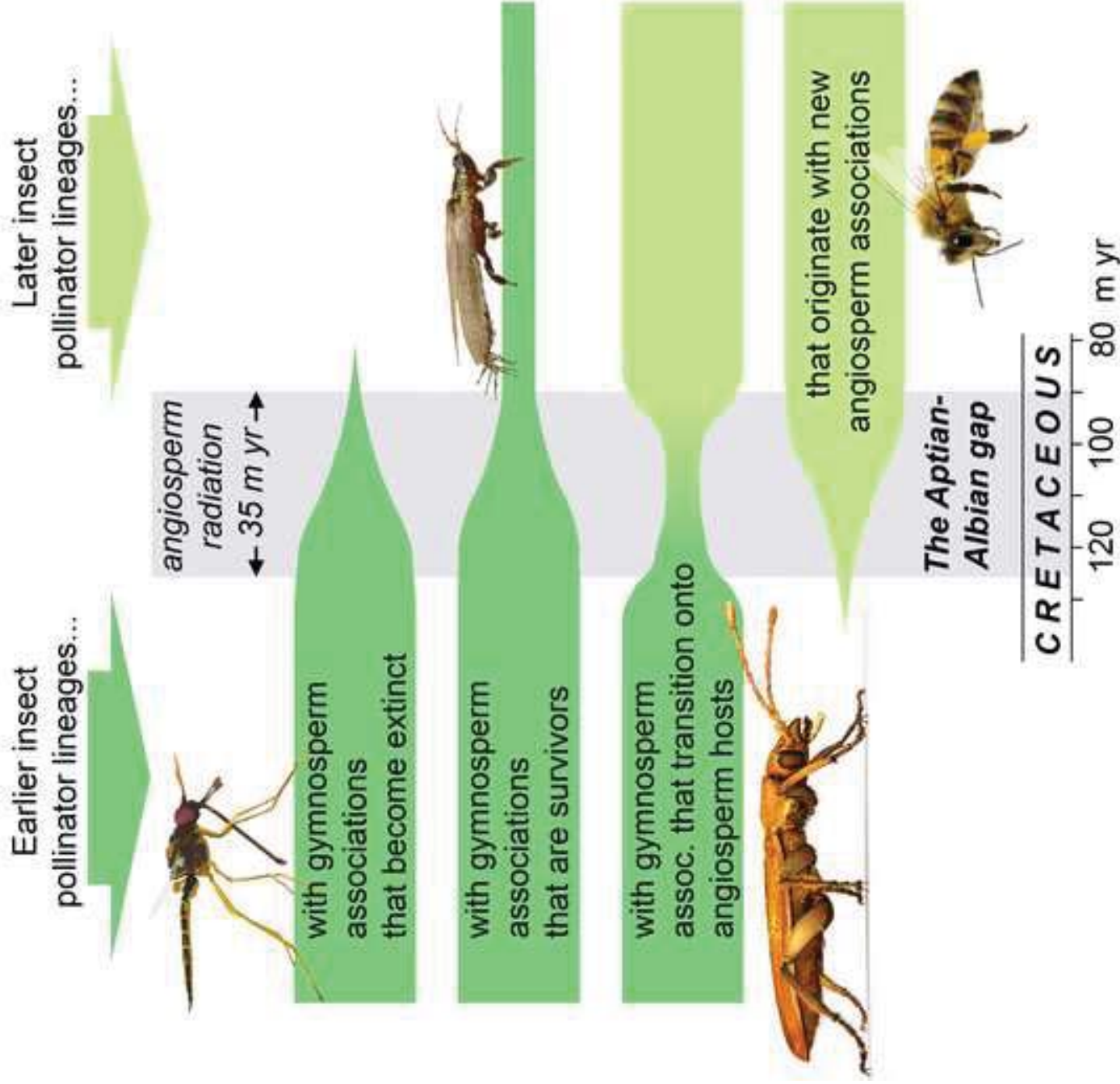
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False Blister Beetles and the Expansion of Gymnosperm–Insect Pollination Modes before Angiosperm Dominance

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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. However, most evidence supports gymnosperm–insect pollinator associations, supported by direct evidence of pollen on insect bodies, currently established for four 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, 10, 11] (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 defined by its host-plant associations (gymnosperm, angiosperm) and evolutionary pattern (extinction, continuation, origination) during this interval [12]. 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 [13]. This association constitutes one of four patterns explaining the plateau of family-level plant-lineages generally and pollinating insects specifically during the mid-Cretaceous angiosperm radiation [12].

RESULTS

The holotype of *Darwinylus marcosi* from Spanish amber in the Basque-Cantabrian Basin of northern Spain recently was 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, 1E–G; 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* [14], 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 [15]. 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 [14] (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* is related broadly to the Ginkgoales, Cycadales and Bennettitales [14]. 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]. 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, 16], whereas anemophilous pollen overwhelmingly are dispersed as solitary monads [17] (Figure S2; Supplemental Information).

DISCUSSION

Animal-pollinated plants constitute 85 % of all extant angiosperm species globally [18] 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, 19]. Entomophily has been regarded as the plesiotypic condition for angiosperms based on palynological data and phylogenetic inferences [19, 20], although ambophily, a combination of insect and either wind or water pollination, has been suggested for some early angiosperm taxa [2, 19]. 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 [2, 21]. Occurrences with indirect evidence, such as floral morphology from the Turonian (ca. 92 Ma) of New Jersey, indicate specialized bee pollination [22].

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 [20, 23]. 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]. Beetles are considered among the earliest pollinators of extant basal angiosperms, particularly basal lineages with generalized flowers and unelaborated pollinator rewards emphasizing pollen [2, 20, 24]. Although Mesozoic diversification of Coleoptera is thought to result from the persistence of older lineages such as darkling beetles [25, 26] and colonization of multiple niches, other lineages that underwent radiation events, including weevils [26] and dung beetles [27], 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, 12], during which pollinators colonized new hosts, habitats and ecosystems, as evidenced by early phylogenetic trajectories of diverse clades such as certain beetles [26, 28], ants [29], bees [30] and moths [32] that further diversified into the Cenozoic [4, 21, 30, 32]. 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 [10], although it was disputed [33]. 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, 11, 36], evidence from insect gut contents and coprolites [5], and entomophilous features of fossil pollen such as size, shape, ornamentation, stickiness, quantity and clumping

ability [14, 15, 19]. This evidence supports the hypothesis that insects were pollinating a variety of gymnosperm groups throughout the mid Mesozoic, a life-habit that evidently originated during the Permian [10] (Table S1). 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, 11, 24, 32].

Darwinylus marcosi bore mandibulate-ectognathate mouthparts, a common type of mostly prognathous mouthparts that employ a chewing feeding style [35]. 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 [35], 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] (Supplemental Information). A second lineage of the long proboscid pollination mode is represented by the zhangsolvid fly *Buccinatormyia magnifica* from Early Cretaceous Spanish amber, ca. 105 Ma in age, carrying abundant *Exesipollenites* pollen attributed to bennettitaleans [7, 14]. The third major pollination mode is characterized by labellate mouthparts [36], involving the absorptive sponging of surface fluids by a basal asilomorph fly, *Paroikus* sp., from Transbaikalian Russia [4], occurring in poorly dated Early Cretaceous deposits ca. 130 to 105 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 [37] (Supplemental

Information). The fourth major pollination mode are mouthcone mouthparts [35], engaged in a tissue-penetrating punch-and-suck feeding style involving the melanthripid thrips *Gymnopolisthrips 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]. 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 [12].

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 [38, 39]. 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) [12]. 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 [12].

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 [11]; (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 [21] (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, 12, 14] and the origination of new associations on angiosperms [2, 34]. 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, 12] (Figure S2). However, the three most important amber deposits of this interval – Lebanese amber (ca. 129 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 [12]. 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 [12]. This time interval represents one of the most profound insect pollinator–plant host shifts in the fossil record.

SUPPLEMENTAL INFORMATION

The Supplemental Information consists of the following sections.

Figure S1: Reconstruction of the habitus of *Darwinylus marcosi* on a cycad host.

Figure S2: The distribution of pollination types recorded in Spanish amber, indicating the presence of three distinctive pollination modes.

Occurrence data for mid-Mesozoic long-proboscid insects.

Table S1. Probable mid-Mesozoic long-proboscid insect pollinators based on the indirect evidence of head and mouthpart structure.

Geological context.

Plant affinities of the pollen.

The relationship between *Classopollis* pollen and insects.

Preservational bias and taphonomy of the amber specimens.

Experimental procedures.

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., R.P.F., E.P., X.D. and C.L. designed the research. D.P., R.P.F., E.P. and C.L. performed the study on plant–insect interactions. D.P. and E.B. contributed to paleobotanical research. D.P., E.P., E.B. and C.L. 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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FIGURES

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 (C) and (D), from the beetle specimen at (B). At (E)–(G) are pollen grains attached to various elements on body surfaces. At (H) the entire amber piece contains the beetle at lower left, which matches the camera lucida drawing in (I). Based on our taphonomic interpretation, the dark green arrow indicates the direction of resin flow that swept the beetle along and the pollen grains that originally adhered to its body, with the resulting widening cone-like pattern of pollen dispersion shown in orange. Pollen grains are depicted in dark red. The related Figure S1 shows the distribution of pollen on this beetle in its inferred habitat; Figure S2 shows the relationship of *D. marcosi* to *Monosulcites* pollen, which likely is attributable to a cycad.

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. Related to Figure S1, which shows the probable cycad habitus of *D. marcosi*. 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. Related Figure S2 shows the gymnosperm–insect interactions of three of the four pollinator cohorts whereas Table S1 provides biological data for 38 species of long-proboscid insect taxa, now documented from the mid Mesozoic.

(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 [12] 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 pollen¹.

Pollinator taxon	Insect Pollinator				Pollinated Plant				Sources
	Affiliated clade	Mouthpart class ²	Feeding guild ²	Whole plant taxon ³	Affiliated clade ³	Insect associated pollen ⁴	Locality, age and date ⁵		
1. <i>Gymnopollisthrips minor</i>	Thysanoptera: Merothripidae	Mouthcone	Punch and sucking	<i>Nehvizdyella/ Eretmophyllum</i>	Ginkgoales: Ginkgoaceae	<i>Cycadopites</i> sp. ⁶	Álava, Spain; 105, late Albian	[6, 14]	
2. <i>Gymnopollisthrips maior</i>	Thysanoptera: Merothripidae	Mouthcone	Punch and sucking	<i>Nehvizdyella/ Eretmophyllum</i>	Ginkgoales: Ginkgoaceae	<i>Cycadopites</i> sp. ⁶	Álava, Spain; 105, late Albian	[6, 14]	
3. <i>Darwinylus marcosi</i>	Coleoptera: Oedemeridae	Mandibulate ectognathate	Chewing	Indeterminate cycad	? Cycadales	<i>Monosulcites</i> sp. ⁷	Álava, Spain; 105, late Albian	[9, this report]	
4. <i>Buccinatormyia magnifica</i> ⁸	Diptera: †Zhangsolvidae	Long-proboscid	Siphonate feeding	Indeterminate bennettitalean	Bennettitales: †Williamsoniaceae	<i>Exesipollenites</i> sp.	El Soplao, Spain; 105, late Albian	[7]	
5. <i>Meioneurites spectabilis</i> ⁸	Neuroptera: †Kalligrammatidae	Long-proboscid	Siphonate feeding	<i>Frenelopsis</i> sp.	Coniferales: †Cheirolepidiaceae	<i>Classopollis</i> cf. <i>annulatus</i>	Karatau, Russia; 155, Kimmeridgian	[8]	
6. <i>Kallihemerobius feroculus</i> ⁸	Neuroptera: †Kalligrammatidae	Long-proboscid	Siphonate feeding	[unknown]	Pinales: ?Pinaceae	Undetermined bisaccate	Daohugou, China; 165, late Callovian	[8]	
7. <i>Paroikus</i> sp.	Diptera: Asilomorpha indet.	Labellate	Sponging feeding	<i>Frenelopsis</i> sp.	Coniferales †Cheirolepidiaceae	<i>Classopollis</i> sp.	Baissa, Russia; 130–105 Barremian to Albian	[4, 5]	

¹The first four entries are from amber.

²See [42] for a phenetic classification of insect mouthpart classes and feeding guilds.

³Several sources were used for establishing the source plant clades based on pollen taxa identifications, notably Traverse [14].

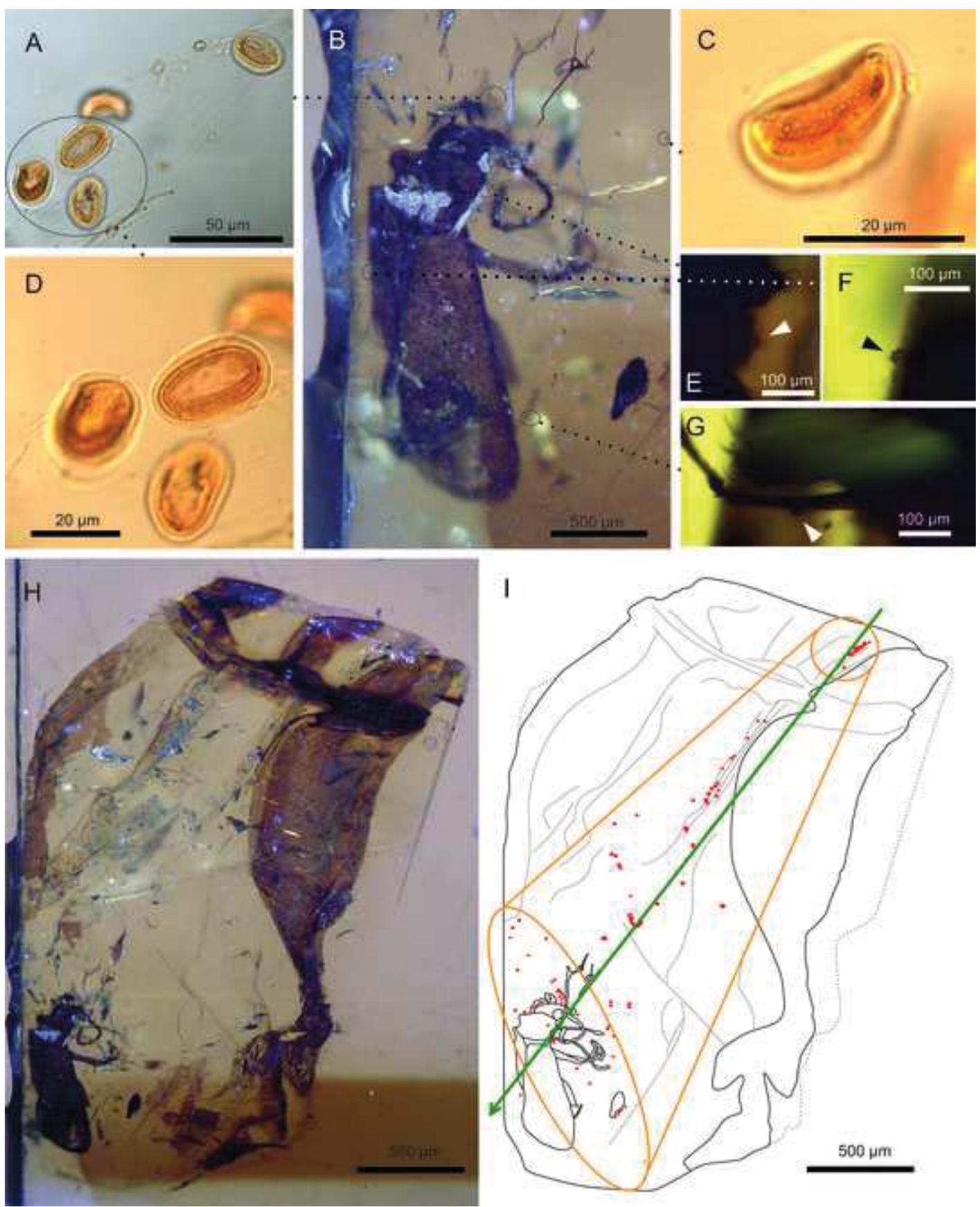
⁴Pollen form-genera identifications are from the primary source literature, cited at right.

⁵Geochronologic stage dates pegged to absolute age determinations are from Ogg et al. [41].

⁶There is a remote possibility that this palynomorph is referable to a cycadophyte.

⁷There is a less probable possibility that this palynomorph is referable to Bennettitales.

⁸See Table S1 (Supplemental Information) for an extensive list of additional mid-Mesozoic long-proboscid taxa based on indirect evidence.





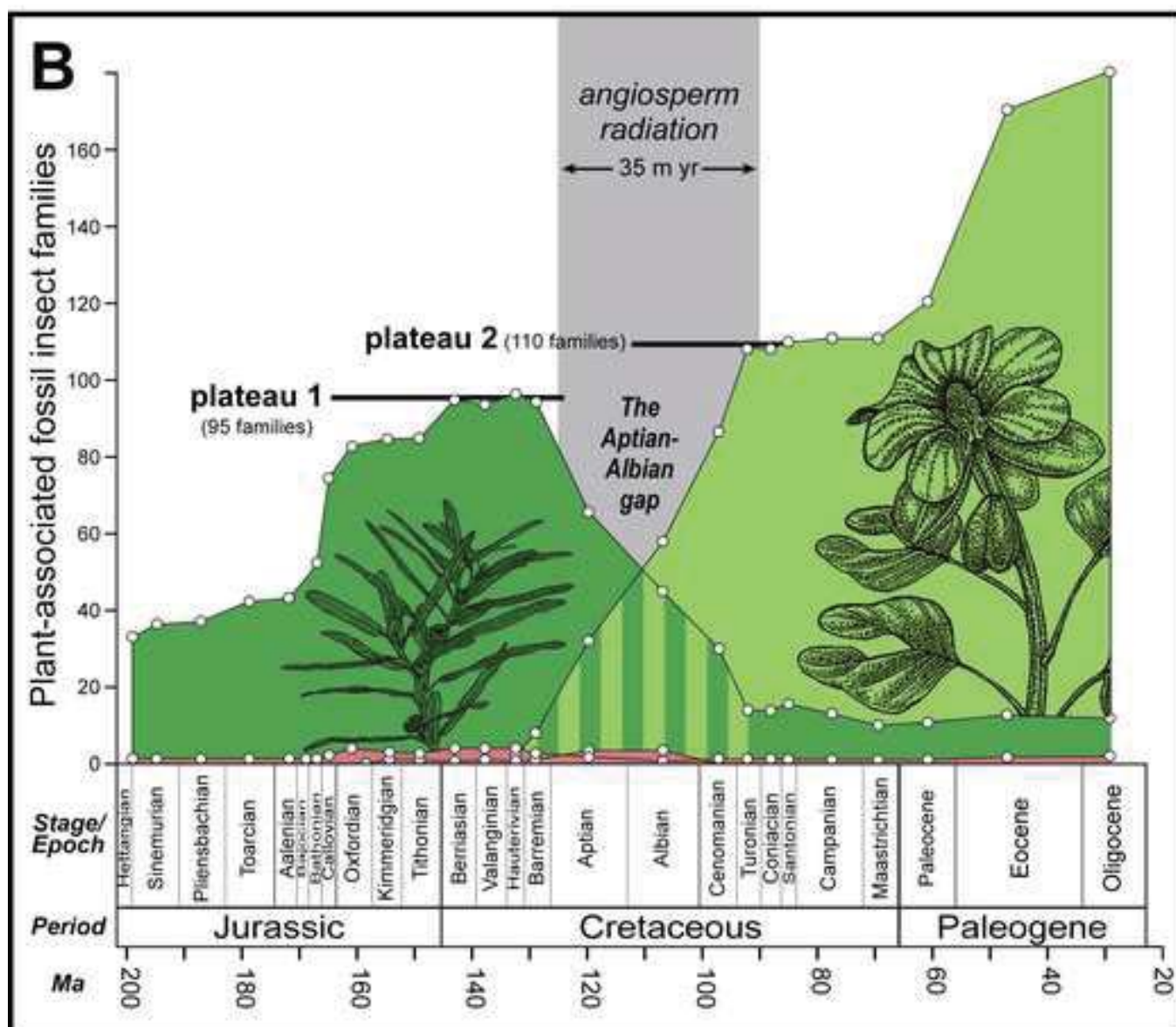
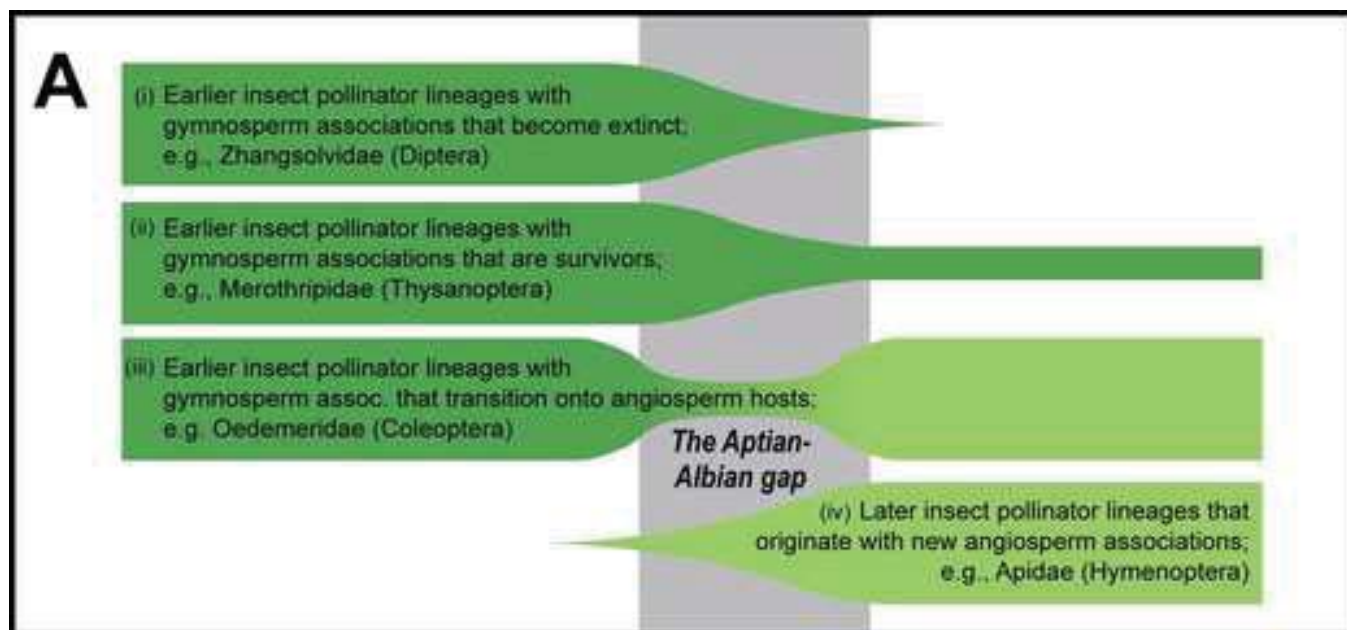




Figure S1 (above). Reconstruction of the habitus of *Darwinylus marcosi* on a cycad host. Related to Figure 2 which shows a more expanded, reconstructed view of *D. marcosi*, with the inferred distribution of pollen on body contact surfaces, see Figure 2 Artwork by J.A. Peñas..

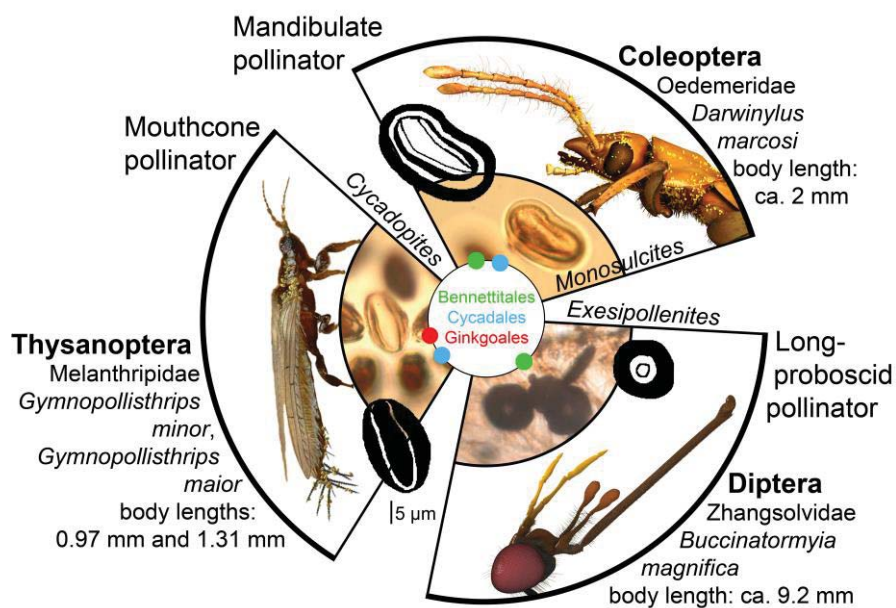


Figure S2 (above). Distribution of pollination types currently recorded in Spanish Amber, indicating the presence of three distinctive pollination modes. Related to Figure 3 and Table 1. Each pollinator insect lineage bears a distinctive mouthpart class and membership in a feeding guild [S55]. Excluded are labellate mouthparts found in older compression deposits from Transbaikalian Russia [S20, S56], discussed in the main text. Beetle and thysanopteran records are from Álava amber and the zhangsolvid record is from El Soplao amber. Pollen grain silhouettes are at the same scale.

Occurrence data for mid-Mesozoic long-proboscid insects (related to Table S1 below).

Table S1 provides data for 39 mid-Mesozoic long-proboscid taxa, considered probable pollinators of gymnosperms based on indirect evidence. This evidence includes mouthpart macro- and micromorphology, specialized feeding structures, head shape and other features such as forwardly deflected major wing veins indicating hovering flight. Because of the mid-Mesozoic timing of these occurrences and the delay of deep-throated angiosperm flowers until considerably later in the Cretaceous [S8], it is virtually assured that the hosts of these long-proboscid insects were gymnosperms. These data support the discussion in the main text, to which reference is made.

Table S1. Probable mid-Mesozoic long-proboscid insect pollinators based on the indirect evidence of head and mouthpart structure¹

Pollinator taxon	Affiliated clade	Mouthpart class ²	Feeding guild ²	Locality, date (Ma) and stage ³	Sources
1. <i>Tschekardithonopsis ?oblivius</i>	Neuroptera: †Permithonidae	Long proboscid	Siphonate fluid feeding	Chekarda, 276, Kungurian	[S38]
2. <i>Affinigramma myrioneura</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
3. <i>Kallihemerobius acidentatus</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
4. <i>Kallihemerobius almacellus</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
5. Kallihemerobiinae gen. indet.	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
6. <i>Kalligramma brachyrhyncha</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
7. <i>Kalligramma circularia</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
8. <i>Kalligramma</i> sp.	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
9. <i>Abrigramma calophleba</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
10. <i>Ithigramma</i> sp.	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S23, S39]
11. <i>Oregramma aureolusa</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Liutiaogou, 125, Late Barremian	[S23, S39]
12. <i>Oregramma Illicebrosa</i>	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid-feeding	Beipiao, 125, Late Barremian	[S23, S39]
13. <i>Oregramma</i> sp.	Neuroptera: †Kalligrammatidae	Long proboscid	Siphonate fluid feeding	Liutiaogou, 125, Late Barremian	[S23, S39]
14. <i>Fiaponeura penghiani</i>	Neuroptera: †Incertae sedis ⁴	Long proboscid	Siphonate fluid feeding	Myanmar amber, 99, Early Cenomanian	[S40]

15. <i>Cretanallachus magnificus</i>	Neuroptera: †Incertae sedis ⁴	Long proboscid	Siphonate fluid feeding	Myanmar amber, 99, Early Cenomanian	[S40]
16. <i>Burmopsychope limoae</i>	Neuroptera: †Incertae sedis ⁴	Long-proboscid	Siphonate fluid feeding	Myanmar amber, 99, Early Cenomanian	[S40]
17. <i>Paradoxosisyra groehni</i>	Neuroptera: Sisyridae	Long proboscid; ? piercing and sucking	Siphonate and penetrative (?) fluid feeding ⁵	Myanmar amber, 99; Early Cenomanian ⁴	[S41]
18. <i>Lichnomesopsyche gloriae</i>	Mecoptera: †Mesopsychidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S42]
19. <i>Lichnomesopsyche daohugouensis</i>	Mecoptera: †Mesopsychidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S42]
20. <i>Vitimopsyche kozlovi</i>	Mecoptera: †Mesopsychidae	Long proboscid	Siphonate fluid feeding	Pingquan, 125, Late Barremian	[S42]
21. <i>Lichnomesopsyche prochorista</i>	Mecoptera: †Mesopsychidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S43]
22. <i>Aneuretopsyche rostrata</i>	Mecoptera: †Aneuretopsychidae	Long proboscid	Siphonate fluid feeding	Karatau, 155, Kimmeridgian	[S44]
23. <i>Aneuretopsyche minima</i>	Mecoptera: †Aneuretopsychidae	Long proboscid	Siphonate fluid feeding	Karatau, 155, Kimmeridgian	[S44]
24. <i>Jeholopsyche liaoningensis</i>	Mecoptera: †Aneuretopsychidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S45]
25. <i>Pseudopolycentropus latipennis</i>	Mecoptera: †Pseudopolycentropodidae	Long proboscid	Siphonate fluid feeding	Karatau, 155, Kimmeridgian	[S46]
26. <i>Pseudopolycentropus janeannae</i>	Mecoptera: †Pseudopolycentropodidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S47]
27. <i>Pseudopolycentropus novokshonovi</i>	Mecoptera: †Pseudopolycentropodidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S47]
28. <i>Sinopolycentropus rasnitsyni</i>	Mecoptera: †Pseudopolycentropodidae	Long proboscid	Siphonate fluid feeding	Daohugou, 165, Late Callovian	[S48]
29. <i>Cretahilarimorpha lebanensis</i>	Diptera: Hilarimorphidae	Long proboscid; ?piercing and sucking ³	Siphonate and penetrative (?) fluid feeding ⁵	Lebanese amber, 123, Late Barremian	[S49]
30. <i>Linguatormyia teletacta</i>	Diptera: †Zhangsolvidae	Long proboscid	Siphonate fluid feeding	Myanmar amber, 99, Early Cenomanian	[S33]
31. <i>Buccinatormyia magnifica</i>	Diptera: †Zhangsolvidae	Long proboscid	Siphonate fluid feeding	El Soplao amber 105, Late Albian	[S50]
32. <i>Buccinatormyia soplensis</i>	Diptera: †Zhangsolvidae	Long proboscid	Siphonate fluid feeding	El Soplao amber 105, Late Albian	[S50]
33. <i>Cratomyia</i>	Diptera:	Long proboscid	Siphonate	Crato Fm., ca.	[S51]

<i>macrorrhyncha</i>	†Zhangsolvidae		fluid feeding	113, Early Albian	
34. <i>Palaepangonius eupterus</i>	Diptera: Tabanidae	Long proboscis	Siphonate fluid feeding	Yixian Fm., 125, Late Barremian	[S52]
35. <i>Florinemestrius pulcherrimus</i>	Diptera: Nemestrinidae	Long proboscis	Siphonate fluid feeding	Yixian Fm., 125, Late Barremian	[S52]
36. <i>Protonemestrius jurassicus</i>	Diptera: Nemestrinidae	Long proboscis	Siphonate fluid feeding	Yixian Fm., 125, Late Barremian	[S52]
37. <i>Protonemestrius rohdendorfi</i>	Diptera: Nemestrinidae	Long proboscis	Siphonate fluid feeding	Baissa, Transbaikalian Russia, 129, Early Barremian	[S53]
38. <i>Protapiocera megista</i>	Diptera: Apioceridae	Long proboscis	Siphonate fluid feeding	Yixian Fm., 125, Late Barremian	[S52]
39. <i>Prosoeca (Palembolus) Saxea</i>	Diptera: Nemestrinidae	Long proboscis	Siphonate fluid feeding	La Huerguina Fm., 126, Late Barremian	[S54]

¹This is not necessarily an exhaustive list; note the first occurrence is Permian in age. See related Table 1 for additional examples of gymnosperm-insect pollination associations other than the long-proboscis pollination mode.

²See Labandeira [S55] for a classification of insect mouthpart classes and feeding guilds.

³Geochronologic stage dates are pegged to absolute age determinations from [S57].

⁴The family-level clade assignment probably is Aetheogrammatidae [S31].

Geologic context

Peñacerrada I, located in the Basque-Cantabrian Basin (BCB) in northern Spain, is one of the ten Spanish amber-bearing deposits, out of 130 discovered in total, that has provided bioinclusions thus far. The Peñacerrada I locality occurs within the upper strata of the historically defined Escucha Formation [S1, S2], which in the Maestrazgo Basin that is divided into three lithostratigraphically defined members initially formed as a result of a mid-Albian progradation–retrogradation–progradation episode that formed a deltaic-swamp environment. An alternative interpretation of the Escucha Formation and the superjacent Utrillas Formation recently has been suggested by Rodríguez-López [S3], who proposed that the upper member of the Escucha Formation is genetically related to the lower strata of the Utrillas Formation, rather than to its two lower members. Both the upper member of the Escucha Formation and the Utrillas Formation comprise a broad, desert dune system that formed as a result of regional aridification coinciding with an overall retrogradation of the Tethys Sea. This new, emergent, lithostratigraphic unit associated with the progradation–retrogradation events was informally defined as the Utrillas Group by Rodríguez-López [S3]. The contact between the remaining Escucha Formation and the Utrillas Group was described as regional discontinuity surface (RDS), which expressed a regional angular unconformity associated with syn-sedimentary extensional tectonics, subsequent erosion and sedimentary input into extra-regional settings. The RDS is included within the historically-defined Escucha Formation and demarcates earlier forested environments of mostly gymnosperms under tropical to subtropical climate that produced lignite deposits during the early Albian, referred to as the coal bearing system [S4], from a well-developed, desert environment during the later early Albian to an early Cenomanian desert [S4, S5].

At the southern margin of the BCB in the Peñacerrada area, the Escucha Formation and the Utrillas Group crop out where the RDS discontinuity was identified [S6]. These lithostratigraphic units generally are comprised of siliciclastic strata that were accumulated in continental to shallow marine environments [S7, S8]. The Peñacerrada I and II amber outcrops also are included in the organic-rich mudstones and siltstones that are rich in organic matter at the base of the Utrillas Group – that is, in the upper levels of

the classical Escucha Formation. In the BinCB, the Utrillas Group is dated as late Albian to early Cenomanian and the amber-bearing strata has a late Albian age based on palynomorph associations. Recent work [S7] indicates that the Peñacerrada I outcrop, occurring at Moraza, in Burgos Province and presently not exposed, consists of three stratigraphic intervals from the base to the top of the unit. Basalmost are interbedded mudstones, fine-to coarse-grained sandstones, and coal seams with bivalves and coal fragments. Superposing the basalmost unit is an intervening tabular unit of coarse-grained sandstones containing carbonaceous fragments and macrofloral remains with encrusted parting surfaces towards the top. The uppermost unit bears fine-grained, well-sorted, non-cemented sandstones covered by an interval of heterolithic mudstones containing macrofloral remains along with interbedded sandstones. The absence of significant differences between the palynofloral assemblages of the Peñacerrada I and Peñacerrada II localities (the latter at Montoria, in Álava Province), suggests a similar age of late Albian for both and the lack of a significant stratigraphic gap between those floras [S6].

Plant affinities of the pollen

Pollen grains associated with the beetle *Darwinylus marcosi* [S9] are boat-shaped, prolate, atectate and monosulcate. For this palynospecies, average polar axis length and equatorial diameter are 25.14 μm ($n=62$; range 38.57–18.85 μm) and 16.56 μm ($n=69$; range 28.11–11.22 μm), respectively. The sulcus is elongate, more or less elliptic, and extends along the polar axis, approximately one-third shorter than the entire length of the grain. The sulcus shows rounded ends and it is broader at its mid-point (ca. 5.71–6.31 μm) than at their ends. The sulcus margin is simple, but in a few specimens appears folded. The ornamentation is psilate. The exine is ca. 5.68–3.74 μm thick. The sexine constitutes an outer, imperforate tectal layer 2.04–3.98 μm thick that is very patinate in the distal pole, and an inner sexine ca. 1.7 μm thick with alveolate elements that can be recognized at their proximal side where the outer sexine is thinner. The nexine is ca. 0.1 μm in thickness. Specific features of these pollen grains based on their shape, sulcus structure, and ornamentation attribute the specimens to the form-genus *Monosulcites* Cookson 1947 ex Couper 1953 [S10]. The stratigraphic range of this pollen type is extensive, ranging from the Late Jurassic [S11] to the Recent. It is difficult to affiliate the genus *Monosulcites* to a particular group of plants, as several of its species are comparable to pollen grains of various gymnosperm groups, and a few of them are referable to angiosperms [S12]. Nevertheless, the lack of columellae clearly is attributable to pollen grains of gymnosperms. There are neither SEM nor ultrastructural studies on *Monosulcites* pollen grains that allow attribution to a more precise taxonomic category.

According to Jarzen and Nichols [S13], features of the studied grains as well as the structure of their exines may be compared with those of several particular gymnosperm groups. These grains may be considered “atectate with some alveolate elements”, considering the evolutionary trends in sporopollenin exine structure of vascular plants, as proposed by Walker [S14]. The structure of the exine based on the examined pollen grains presents similarities with those of the Early Jurassic monosulcate species *Chasmatosporites apertus* Nilsson, 1958, and with *C. hians* Nilsson, 1958 and *C. major* Nilsson, 1958 [S15]. However, the inner sexine of these species presents irregular rod-like elements instead of alveoli. According to the latter authors and Balme [S12], these species may be affiliated to the Cycadales, or perhaps to the Bennettitales. When compared to the fossil taxa, the pollen grains of the extant Cycadales clearly differ by pollen-wall structures that typically are tectate with an alveolate-spongy exine [S16]. By contrast, the studied pollen grains clearly are different from those of extant *Ginkgo biloba* L., 1771, by the presence of a reticulum-like sculpturing in the inner part of the exine. Specifically, this structure closely resembles the net-like or reticulum-like sculpturing of the inner layer from the bladder or saccus in winged pollen grains, such as those from *Pinus* L., 1753 and *Podocarpus* Labill., 1806 [S17]. However, the exine structure of the examined grains resembles that of the pollen grains of the gymnosperm genus *Sahnia* Vishnu-Mitre, 1953 (Pentoxylales), although the grains exhibit a thinner exine and a conspicuous nexine [S18]. The pollen grain ultrastructure of the gymnosperm *Eucommiidites* (Erdtman, 1948) Potonié 1958 emend. Hughes, 1961 (Erdtmanithecales) also displays similar features, but exhibits a granular exine structure instead of an alveolar one [S19]. In summary, the bulk of the evidence suggests, albeit without certainty, an affiliation with the Cycadales (Figure S1).

The relationship between *Classopollis* pollen and insects

Another type of gymnosperm pollen, *Classopollis*, had a very important role in varied associations with insects during the mid Mesozoic. The biology of this distinctive pollen type is instructive for understanding pollination by forms such as *Meioneurites* and *Paroikus*. In addition to several pollinivorous insect lineages possessing gut contents laden with *Classopollis* pollen [S20, S21], *Classopollis* also has been found associated with the heads and mouthparts of insects [S22, S23], and indicates the presence of a distinctive pollination mode based on several lines of evidence. *Classopollis* evidence for a pollination mutualism includes: (i) large size of individual grains and their frequent presence as adherent tetrads [S24]; (ii) occasional occurrence as larger clumps that aerodynamically would be inconsistent with wind dispersal [25]; (iii) association of larger pollen size and clumped aggregations with insect pollination [S20, S25, S26]; (iv) presence of an exine similar to the tectate condition in insect-pollinated angiosperm pollen [S27, S28]; (v) surface sculptural patterns found in the exine, including spinules, hooks and ridges that may be involved in inter-grain attachment [S24]; and (vi) characteristic ubisch bodies, the spinose spheroidal structures adhering to the pollen grain surface perhaps serving a role similar to the sticky layer of pollenkit in angiosperms [S24, S28]. These features of *Classopollis* pollen parallel entomophilous angiosperm pollen that typically appear later in the fossil record, suggesting pollinator functional convergence [S29].

Preservational bias and taphonomy of the amber specimens

The amber record is biased ecologically towards the preservation of certain organisms that are spatially proximal to resin-producing plants, which are identified as gymnosperms for Cretaceous ambers [S30]. Those elements of the biota that are not exposed to or intercepted by such resin sources generally are underrepresented in amber [S31]. However, we posit that the current lack of insects associated with angiosperm pollen in Spanish Amber – a pattern typical of Cretaceous ambers worldwide – is not only due to an inherent bias, but also to the low relative abundance and diversity of angiosperms compared to gymnosperms during the late Albian (ca. 105 Ma). This observation is based on the Iberian palynological record that typifies the most prominent Spanish amber-bearing localities, such as Peñacerrada I, San Just and El Soplao [S6, S31–S33]. The presence of angiosperm pollen grains is documented in Iberia since the Valanginian [S34], and this record increases both qualitatively and quantitatively to later Albian deposits.

A cone-like dispersion pattern of the pollen grains within the amber piece contains the beetle located approximately at the center of the cone base. The most plausible explanation indicates that initially the beetle carried a considerable load of pollen grains as it became entrapped in a resin flow under aerial conditions. Subsequently, the pollen grains became gradually detached from the beetle's body due to its movement and gravitational forces within the semiliquid resin (Figure 1). A contributing factor accounting for the discharge of pollen from the beetle's body may have been the interaction of the resin with the electrostatic and adhesive properties of the pollen grains. Extant gymnosperm, wind-dispersed pollen grains lack adhesive surface compounds that would enable adhesion to insect bodies or result in the formation of pollen-grain clumps [S35]. However, there now is evidence of at least three lineages of Cretaceous insect pollinators from Spanish amber that vectored gymnosperm pollen grains with clearly adhesive properties (Figure S1). These lineages exhibited different pollination mechanisms: (i) punch-and-suck feeding thrips bearing ring setae in Álava Amber [S36]; (ii) long-proboscid siphonate mouthparts in zhangsolvid flies in El Soplao Amber [S33]; and (iii) mandibulate mouthparts of oedemerid beetles in Álava Amber [S9] (Figure S2). Pollen grains involved with these fossil associations are, without doubt, of entomophilous nature.

Similar trails of detached pollen grains have been reported in fig wasps carrying fig pollen grains from Miocene Dominican amber [S37], and in the thysanopteran species *Gymnopollisthrips major* bearing gymnospermous pollen grains from Cretaceous Spanish amber (see figure 2A in [S36]). Moreover, two undetermined plant fibers present in the amber piece of our study also are oriented following the inferred direction of the resin flux, based on the pollen dispersion pattern (Figure 1). Additionally, there is consideration of the improbable event of an insect collision with a pollen clump after its entrapment in resin. Although such a possibility cannot be completely ruled out, its potential

incidence is extremely low, particularly taking into account that isolated pollen aggregations are virtually absent from the Cretaceous amber 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 [39]. 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.

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