

# Current Biology

## Long-Proboscid Flies as Pollinators of Cretaceous Gymnosperms

### Highlights

- A highly rare, direct evidence of insect pollination is reported in Cretaceous amber
- Extinct zhangsolvids were a widespread and highly specialized group of flies
- One specimen has a clump of gymnosperm pollen grains attached to its body
- Detailed anatomy of zhangsolvid mouthparts reveals feeding on nectar-like secretions

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### In Brief

Direct evidence of insect-mediated pollination is extremely scarce in the fossil record. Peñalver et al. present Cretaceous amber flies that pollinated gymnosperms—as one fly carries a pollen clump—and fed on nectar-like secretions. The finding strongly supports insect pollination already being well established before angiosperms diversified.

# Long-Proboscid Flies as Pollinators of Cretaceous Gymnosperms

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<http://dx.doi.org/10.1016/j.cub.2015.05.062>

## SUMMARY

The great evolutionary success of angiosperms has traditionally been explained, in part, by the partnership of these plants with insect pollinators [1–6]. The main approach to understanding the origins of this pervasive relationship has been study of the pollinators of living cycads, gnetaleans, and basal angiosperms [7]. Among the most morphologically specialized living pollinators are diverse, long-proboscid flies. Early such flies include the brachyceran family Zhangsolvidae, previously known only as compression fossils from the Early Cretaceous of China and Brazil. It belongs to the infraorder Stratiomyomorpha, a group that includes the flower-visiting families Xylomyidae and Stratiomyidae. New zhangsolvid specimens in amber from Spain (ca. 105 mega-annum [Ma]) and Myanmar (100 Ma) reveal a detailed proboscis structure adapted to nectivory. Pollen clumped on a specimen from Spain is *Exesipollenites*, attributed to a Mesozoic gymnosperm, most likely the Bennettitales. Late Mesozoic scorpionflies with a long proboscis have been proposed as specialized pollinators of various extinct gymnosperms, but pollen has never been observed on or in their bodies [8]. The new discovery is a very rare co-occurrence of pollen with its insect vector and provides substantiating evidence that other long-proboscid Mesozoic insects were gymnosperm pollinators. Evidence is thus now gathering that visitors and probable pollinators of early angiosperms, or seed plants, involved some insects with highly specialized morphological adaptations, which has consequences for interpreting the reproductive modes of Mesozoic gymnosperms and the significance of insect pollination in angiosperm success.

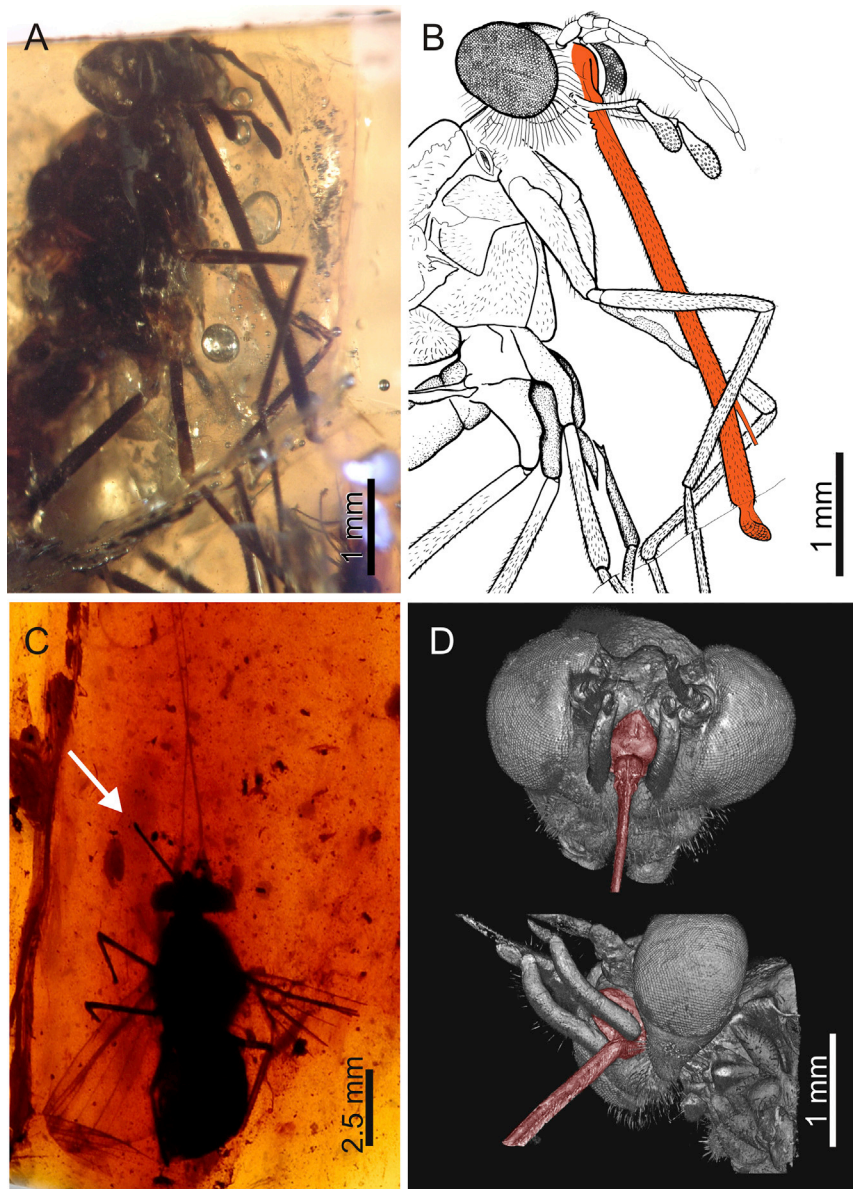
## RESULTS

Herein, we report rare direct evidence for a plant-insect interaction: well-preserved *Exesipollenites*-like pollen on the body of a

pollinator with a long proboscis, a true fly in the Cretaceous family Zhangsolvidae. *Exesipollenites* is pollen of a plant that is probably in the Bennettitales, cycad-like gymnosperms that lived approximately 250 to 70 Ma [6, 9]. Zhangsolvid flies lived at least between 125 and 100 Ma, and three of the five known species, which are the subjects of this report, were discovered recently in Cretaceous amber [10]: *Buccinatoromyia magnifica*, *B. soplensis*, and *Linguatoromyia teletacta*. All species including the specimens reported herein possessed a long proboscis, and other features indicate their flight was well maneuvered.

## Nectivorous Proboscis

Microscopic preservation in amber has revealed a fine structure of the long zhangsolvid proboscis that is adapted for nectar and possibly pollen feeding, the most common reward or “primary attractants” for attracting pollinators to flowers [11], but not for predation or blood feeding. The new specimens in amber have a forward-projecting, straight proboscis and an inflated face (clypeus), beneath which must reside a large cibarial sucking pump (Figure 1). These siphonate proboscides are very long: 3.85 mm and 4.37 mm in Spanish and Burmese specimens, respectively. The base of the rostrum has an annulated, accordion-like membrane. Otherwise, the proboscis is formed primarily of a sclerotized, tube-like haustellum that is laterally compressed and opened dorsally (= theca) (Figures 1, 2A, and 2B). The haustellum is covered with a dense vestiture of microtrichia and large, stiff setae having deep longitudinal ribbing. The dorsal groove of the proboscis is internally closed by a long, narrow labrum. The food canal is a fine tubule within the haustellum, comprised of a very thin cuticle in transmission electron microscopy (TEM) cross-section (Figures 2C–2E) and surrounded by numerous longitudinal, rod-like ligaments (Figures 2D and 2E). Cuticular layers of the theca and food canal are preserved (Figures 2D and 2E). No muscle bundles were observed under TEM. The labellum is comprised of two small, fleshy lobes at the apex of the proboscis; each lobe has 10–12 pseudotracheae with 30–40 transverse pseudotracheal rings (Figures 2F and 2G). General anatomy of the proboscis indicates it was rigid along its length, probably without flexure along the haustellum. Lateral flattening prevents dorso-ventral flexion, and the lack of longitudinal musculature indicates no lateral flexion. Thus, proboscis movement was controlled by head mobility and by the accordion-like basal membrane, which in modern long-proboscid flies



**Figure 1. External Anatomy of the Zhangsolvid Flies and Their Nectivorous Proboscides Preserved in Cretaceous Amber from Spain and Myanmar**

(A and B) Head of the Spanish species *Buccinatormyia magnifica* (holotype; accession number CES-349.1). In (B) and (D), proboscis emerging from the bulbous clypeus is colored in red.

(C) Habitus of the holotype of the Burmese species *Linguatormyia teletacta* (arrow indicates the proboscis tip); photograph from [10].

(D) Head nano-CT scans of *Linguatormyia teletacta*. See also Figure S1.

shredding from the intense forces generated at very high wing-beat frequencies [4, 14]. A robust thorax with large flight muscles (viewed for *Linguatormyia* in high-resolution computed tomography [CT] scans), the short, flattened abdomens, and the curvature of wing veins  $R_4$ ,  $R_5$ , and  $M_1$  near the apex of the wing (Figure S1) indicate zhangsolvids were capable of at least modest hovering. The zhangsolvid antennae are unique among Brachycera, being fine, flagellate structures much longer than the body in one species [10]. Like the palps and proboscis, they project forward and may have been used for guidance while hovering for feeding. Zhangsolvid eyes are large and bulging, occupying nearly the entire lateral surface of the head, and they have a broad frontal surface, which optimizes frontal resolution (Figure 2A). Unlike the apparently rigid zhangsolvid proboscides, the flexible long proboscides described for Mesozoic anthophilous scorpionflies [8] are associated with insects that alight on plant reproductive organs and maneuver their proboscides into deep corolla-like tubes, as seen in many extant Lepidoptera, like butterflies.

allows slight elongation and dorso-ventral tilting [12, 13]. The palps also project forward; basal palpomeres are covered with abundant, fine, stiff setae (Figure 2A) and most likely sensed proboscis position.

### Hovering Flight

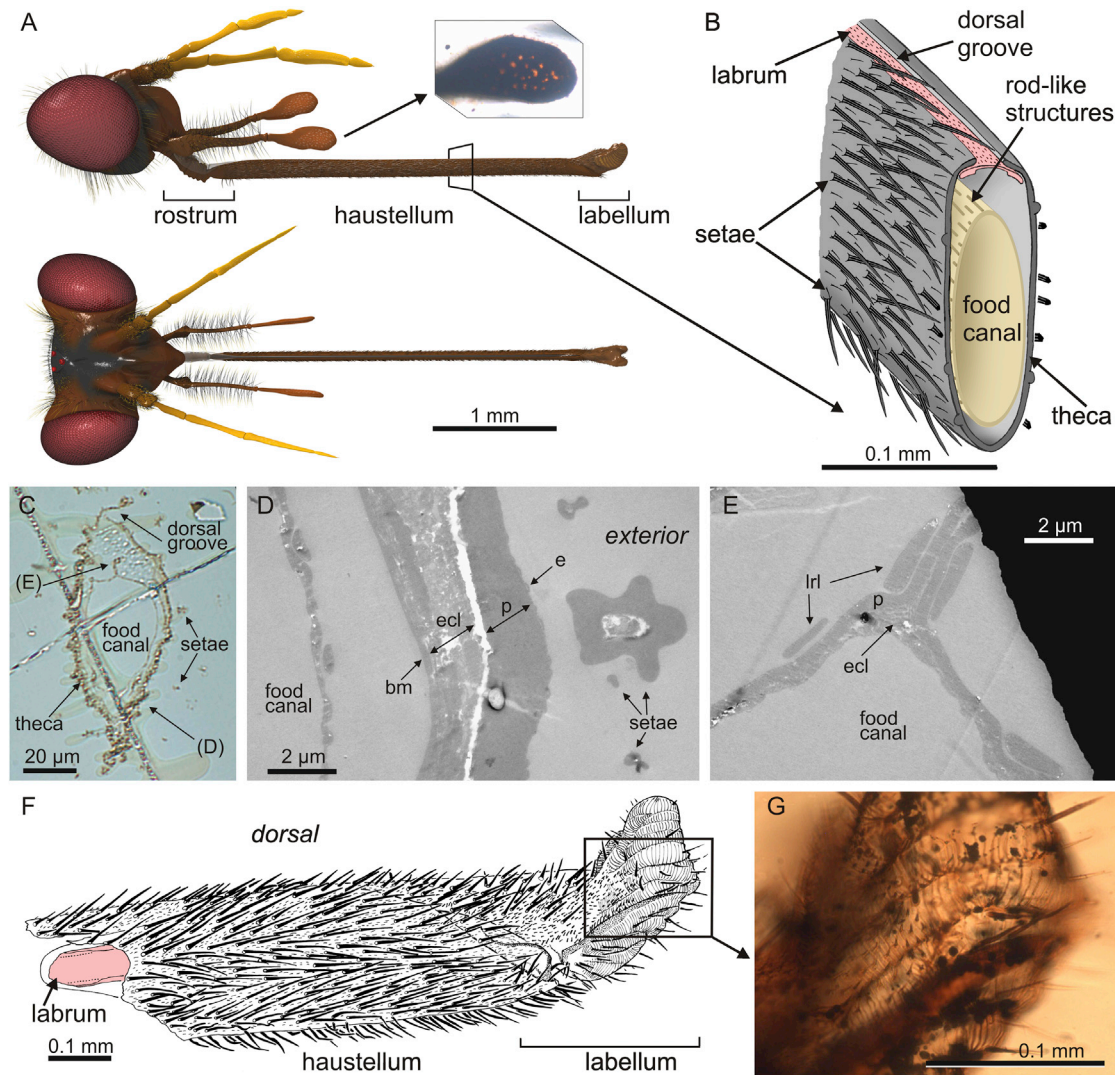
Adaptations for obligate nectivory vary with the animal group. In insects and birds, hovering is always associated with nectivorous species having a long proboscis, haustellum, or bill (e.g., masarine wasps, sphingid moths, hummingbirds, as well as various families of flies), serving to stabilize the body while guiding the proboscis tip into a narrow opening [14] (Movie S1). As hovering is energetically expensive, rapid siphoning of highly caloric nectar from many flowers is an optimal strategy. Slender wings with a narrow tip and, for insects, vein apices abruptly turned away from the tip maintain rigidity and prevent apical

The dense sensillar foveae on the palps (Figure 2A) and antennae most likely detected scents from plant reproductive organs.

### Pollen Load and Palynology

A total of 421 pollen grains (~500 estimated) were observed on one of the specimens of *Buccinatormyia magnifica*. The pollen is adhering to the dorso-lateral surfaces of abdominal segments IV and V (Figure 3A), in contact mainly with unusual radial setae [10]. Pollen grains are discoidal in polar view and lentoid in equatorial view, with subcircular to circular amb (Figures 3B–3D), and have an operculate pore (Movie S2). Average polar diameter is  $11.94 \mu\text{m}$  ( $n = 50$ ; range 7.72–18.64) with the proximal face psilate and distal face porate with an average diameter of  $3.28 \mu\text{m}$  ( $n = 50$ ; range 2.04–5.05) (Table S1). The aperture is perforate with a broken margin in some specimens; exine is psilate and less than  $1 \mu\text{m}$  thick. The pollen grains are clumped and were





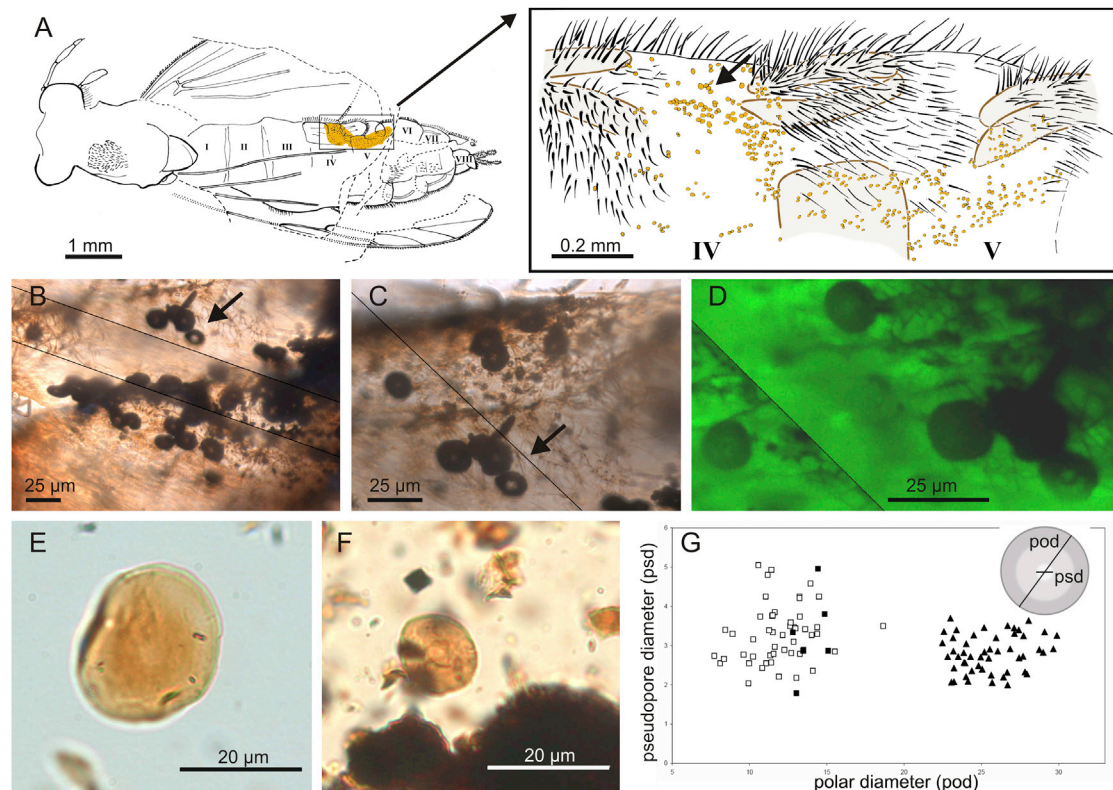
**Figure 2. External and Internal—Ultrastructural—Anatomy of the Nectivorous Proboscis in Zhangsolvid Flies in Cretaceous Spanish Amber**

- (A) Reconstructed head of *Buccinatoromyia magnifica* (artist: J.A. Peñas); the palpal sensillar foveae are shown in inset.  
 (B) Reconstruction of the mid-proboscis region in cross-section.  
 (C) Proboscis thin section at the middle of the haustellum in the paratype specimen of *Buccinatoromyia magnifica* (accession numbers CES-015.1 and CES-392.3; arrows indicate the location of the images D and E from another consecutive thin section).  
 (D) TEM image of the food canal membrane and the theca showing their cuticular layers. bm, basement membrane; ecl, epidermal cell layer; p, procuticle; e, epicuticle.  
 (E) TEM image of the dorsal part of the food canal membrane at the base of a preservational fold, showing the cuticular layers and external reinforcement by longitudinal rod-like ligaments (lrl).  
 (F) Preserved apical part of the proboscis of the holotype specimen of the Spanish species *Buccinatoromyia soplensis*, showing a portion of the labrum (red color) and one labellar lobe of the paired labellum.  
 (G) Photomicrograph showing pseudotracheae with transverse pseudotracheal rings and some dark, minute pyrite crystals; photomicrograph from [10].

probably sticky (Figures 3B and 3C), which together with small size suggest entomophilous pollen [5]. The pollen grains are of the gymnospermous form genus *Exesipollenites*, which had a widespread geographical distribution throughout the Jurassic–Cretaceous [15]. *Exesipollenites* has been related to both taxodioid conifers and Bennettitales [16]. The few studies of in situ pollen from cones of taxodioid conifers, purported to be *Exesipollenites*, report larger grains with granulate to papillate exines [17], suggesting anemophily. Taxodioids are exclusively

anemophilous and can be ruled out as a source of the pollen grains on the fly considering their substantial structural difference.

Bennettitales were widespread and conspicuous from the Late Triassic to the mid-Cretaceous [6]. Bennettitalean cones usually produce boat-shaped, monosulcate pollen grains [9, 18], except for *Williamsoniella lignieri* (Nathorst) Harris (family Williamsoniaceae), from the early Bajocian of the UK, which has pollen attributed to *Exesipollenites* [19]. This pollen has a circular amb,



**Figure 3. *Exesipollenites* Pollen Grains on Abdominal Segments of the Holotype of the Spanish Amber Species *Buccinatormyia magnifica* and Those from the Amber-bearing Sediments in Spain**

(A) Dorsal habitus schema with pollen grains colored in orange (radial setae not depicted for clarity).

(B and C) Photomicrographs of pollen grains from the assemblage (arrows in A–C indicate the same cluster; see also Figure S4).

(D) Confocal laser scanning microscopy image of the same cluster (see also Movie S2).

(E and F) Pollen grains from the amber-bearing rock (see Figure S2), larger than grains on the fly identified as *Exesipollenites tumulus* (E), and ones of the same size (F) identified as *Exesipollenites* sp. (see also Figure S4).

(G) Bivariate plot of pollen sizes, in microns (see Table S1), of pollen grains on the fly (white square) and from the amber-bearing sediments (black square: *Exesipollenites* sp.; black triangle: *E. tumulus*) (see also Figure S3).

although grains were originally nearly spherical, 22–29 μm diameter, with a rounded to oval pore 6 × 8–10 μm (sometimes larger, up to 15 × 12 μm), and with a thin, micro-reticulate exine up to 1 μm thick. The reproductive structures of Williamsoniaceae had fleshy pollen-producing structures and a whorl of bracts that gave them a flower-like appearance [6] (Figure 4). The pollen grains of *W. lignieri* are most similar to those on the zhangsolvid fly (the former differing by a more spherical shape, larger size, and micro-reticulation). In general, Bennettitales have been largely considered to be entomophilous plants [20], and some of their ovulate organs possessed elongate micropyles, most likely a feature for pollination by long-proboscid insects [21]; however, especially for some Williamsoniaceae, wind pollination has also been discussed [6, 20, 22].

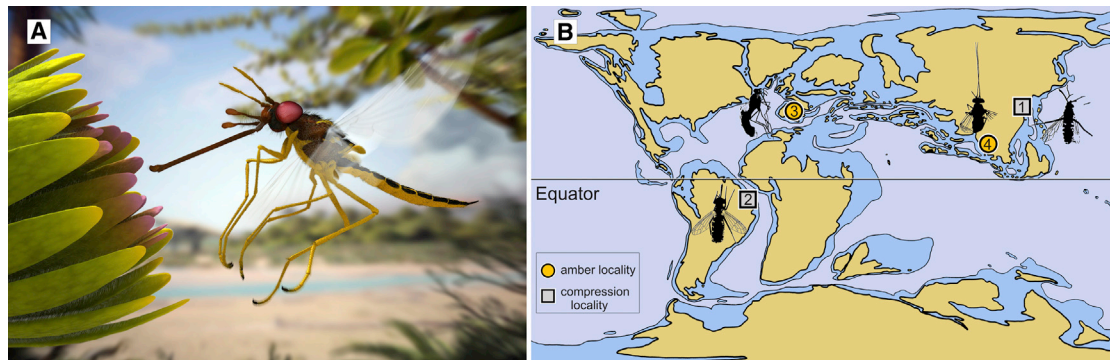
Abundant dispersed *Exesipollenites* pollen grains found in all matrix samples from the Spanish amber outcrop (Figures 3E, S2, and S3) are larger and show anemophilous features (i.e., dispersion in monads, diameter greater than 25 μm, and presence of Übisch bodies on the exine [Figure S4], as occurs in pollen of extant Cupressaceae s.l.). This pollen belongs to the species *E. tumulus*. However, a different morphotype of

*Exesipollenites* in the rock samples (Figures 3F and S4) has the same size as that found on the fly body (Figure 3G; Table S1), but it constitutes only 0.18% of the entire palynological assemblage and only 3.5% of the *Exesipollenites* records, a scarcity typical of entomophilous pollen. These smaller, rare pollen grains are indistinguishable from those on the fly.

## DISCUSSION

Angiosperms are arguably the defining form of terrestrial life, characterizing most terrestrial biomes and biological communities, from tundra to tropical rain forests. The 352,000 estimated extant species are the result of approximately 130 million years of evolution, a radiation at least partly attributed to partnership with animal pollinators, particularly insects [1–5, 23]. Highly vectored outcrossing efficiently promotes genetic diversity (with consequent adaptive advantages); it is a primary mode of pre-zygotic isolation, it insures fertilization in species with brief flowering periods, and it allows individual plants to be dispersed, thus better exploiting optimal habitats while also avoiding intra-specific competition and herbivore and disease outbreaks [6].





**Figure 4. Reconstruction and Paleodistribution of Zhangsolvid Flies**

(A) Reconstruction of a female of *Buccinatoromyia magnifica* hovering to consume nectar-like secretions at a bennettitalean ovulate reproductive organ. Reconstruction of the plant is based on williamsonian reproductive structures; colors of the fly are conjectural but based on the common yellow and black coloration seen in the related flower-visiting families Xylomyiidae and Stratiomyidae (artist: J.A. Peñas; see also [Movie S1](#)).

(B) Cosmopolitan paleodistribution of the five known species on a mid-Albian paleogeographic map: (1) China (Early Cretaceous), (2) Brazil (Aptian), (3) Spain (Albian), two species, and (4) Myanmar (Albian–Cenomanian).

The main approach to understanding the origins of this pervasive partnership has been the study of the pollinators of living basal angiosperms and their close relatives.

Studies have been made on the pollinators of *Zamia* cycads, the three highly disparate genera of living gnetaleans, and most of the basal angiosperms [7]. Where known, their visitors are essentially small, morphologically generalized flies, beetles, primitive moths, wasps, and thrips [4]. Although diverse Mesozoic insect taxa have been previously considered pollinators [4, 8, 14], direct and definitive evidence of specimens carrying pollen grains has been found only a few times, once a Cretaceous thrips that had specialized setae probably for transporting pollen [24]. A fossil fly identified as the extant family Therevidae is reported with pollen on its body of *Classopollis* (a genus of the Mesozoic conifer family Cheirolepidiaceae) [20]. As discussed in [25], definitive Therevidae are known only from the Tertiary, though definitive fossils of the closely related family Apsilcephalidae extend into the Cretaceous. Therevid flies are inhabitants of mainly dry areas, and adults feed on fluids, including plant sap and secretions; the flies are “rarely collected at flowers” [26]. Moreover, none of them (fossil or living) have long proboscides or other obvious structures specialized for pollination, so the association of this fossil fly with conifer pollen is likely incidental. The evidence described herein establishes in detail a rare, direct plant-insect association in the fossil record [27], although in this case, specialized insect anatomical adaptations are also involved.

Zhangsolvid flies belong to the infraorder Stratiomyomorpha [10], a group that includes the flower-visiting families Xylomyiidae and Stratiomyidae. Zhangsolvidae are the only members, living and extinct, of this infraorder that possess a long, rigid proboscis. Given the habits of stratiomyomorphans, the functional structure of the zhangsolvid proboscis, and the presence of pollen on one specimen, these extinct flies were clearly obligate nectivores and possibly pollenivores. Moreover, they were ecologically successful, now known to have been widespread from the Early to mid-Cretaceous of Laurasia and Gondwana (Figure 4).

Obligate nectivory using a long proboscis occurs in 14 families of extant Diptera, especially in lower (orthorrhaphous) Brachy-

cera [13, 28], a grade that includes stratiomyomorphans. Significant such taxa include the pangoniine horseflies (Tabanidae), certain mydas flies (e.g., *Rhaphiomidas*: Mydidae), various spider parasites (e.g., *Lasia*, *Eulonchus*, *Megalybus*, etc.: Acroceridae), tangle-veined flies (Nemestrinidae), and many beeflies (Bombyliidae, especially Bombyliinae), among others [28]. Studies, for example, on the common Holarctic beeflies *Bombilyus* show that individual species are not restricted to flowers with corolla tubes [29], but they are locally oligolectic and even monolectic on deep, narrow corolla flowers, such as the heterostylus flowers primrose (*Primula*: Primulaceae) and bluets (*Houstonia*: Rubiaceae) [12, 30]. In the phloxes (Polemoniaceae), species and populations with the longest corolla tubes are pollinated exclusively by long-proboscid flies, such as the acrocerid fly *Eulonchus smaragdinus*, which has a 2-cm-long proboscis and feeds from corollas up to 3 cm deep [31]. The most impressive fly pollination system is in the Cape flora of southern Africa, where *Moegistorrhynchus longirostris* (Nemestrinidae), appropriately named for its 8-cm proboscis, pollinates certain irises with very deep, narrow corollas [32].

We do not suggest that Zhangsolvidae were obligate pollinators of Bennettitales, but it is reasonable to assume that they were common visitors to some gymnosperms. Late Mesozoic scorpionflies with siphonate proboscides are another exception to the “small-and-generalized” syndrome of primitive pollination, although fossilized pollen has yet to be observed on or in the bodies of the scorpionflies [8]. There is great range in the length of zhangsolvid proboscides, approximately 1.3 to 7 mm, including specimens in compression rocks; in the ecologically analogous scorpionflies, proboscides ranged from approximately 1 to 10 mm. The diversity of proboscis lengths strongly suggests diverse plant hosts. Each of the three clades of Mesozoic long-proboscid scorpionflies has different proboscis features that have been tentatively related to feeding from Cheirolepidiaceae and/or Gnetales, Caytoniaceae and/or Czekanowskiceae, and Pentoxylaceae and/or Bennettitales [8]. The proboscides of the scorpionflies and the micropyle tubes in the plant strobili are similar in lengths and diameters, suggesting exclusive mutualism [8]. Other, unrelated Cretaceous flies from Liaoning, China, had

proboscides 2.5–5.2 mm in length and were inferred to have fed from early angiosperms [33]. In lieu of direct evidence (e.g., pollen presence), it is possible that these flies also fed from gymnosperms [14].

If in the Cretaceous there were efficient and morphologically specialized pollinators of certain gymnosperms, and pollination by insects is an adaptively superior mode of dispersing pollen, the question arises as to why Bennettitales and many Gnetales became extinct. There may be other biological features of these plants that account for their demise, or, alternatively, insect pollination may not confer the fitness advantages that are traditionally assumed. If pollination adaptations became highly specialized in insects as a result of their interactions with gymnosperms for tens of millions of years, this may have predisposed insects into developing with angiosperms one of the most pervasive symbiotic relationships on earth.

### EXPERIMENTAL PROCEDURES

The specimens are housed at the laboratory of the El Soplao cave in Cantabria, Spain, and at the American Museum of Natural History in New York, USA. They were prepared and polished; Spanish specimens were first embedded in synthetic epoxy resin. CT scanning was performed using a ZEISS Xradia 520 Versa instrument at the Cornell University Institute of Biotechnology. A cross-section of the proboscis was stained with 2% uranyl acetate in water and lead citrate and observed using a JEM-1010 electron microscope. The three-dimensional models of the fly and plant host were performed with Light-Wave 3D computer graphics program (NewTek). Pollen grains on one fly specimen were imaged using confocal laser scanning microscopy. Rock samples were prepared in accordance with palynological methods using various acids at high temperatures, and palynomorph percentage diagram was created using Tilia 2 and TGView 2.0.2 software (see [Supplemental Information](#) for further details of the experimental procedures).

### SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, one table, and two movies and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.05.062>.

### AUTHOR CONTRIBUTIONS

E.P., A.A., and D.A.G. designed research. E.P., A.A., R.P.-d.I.F., X.D., E.B., and D.A.G. performed research on insect paleobiology and plant-insect interaction. E.B. performed research on paleobotany. E.P. and X.D. performed transmission electronic microscope observations. M.L.R. performed CT scanning. E.P., A.A., R.P.-d.I.F., X.D., E.B., and D.A.G. analyzed and discussed data. E.P., E.B., and D.A.G. wrote the paper.

### ACKNOWLEDGMENTS

We thank the El Soplao cave, SIEC S.A., and the Government of Cantabria. We are grateful to Carmen López and Yolanda Muelas (Serveis Científicotècnics, Barcelona University), Alberto Jorge García (MNCN), Rafael López del Valle (MCNA), and José Antonio Peñas. We thank Dr. Jeff Ollerton (University of Northampton) for his comments on an earlier version of the manuscript. This study is supported by the IGME project 491-CANOA 35015, the Spanish Ministry of Economy and Competitiveness projects CGL2011-23948/BTE and CGL2014-52163, NIH grant S10OD012287, and NSF grant 1305066 and by funding from Robert G. Goelert, Trustee and Chairman Emeritus of the AMNH.

Received: March 3, 2015

Revised: April 25, 2015

Accepted: May 31, 2015

Published: July 9, 2015

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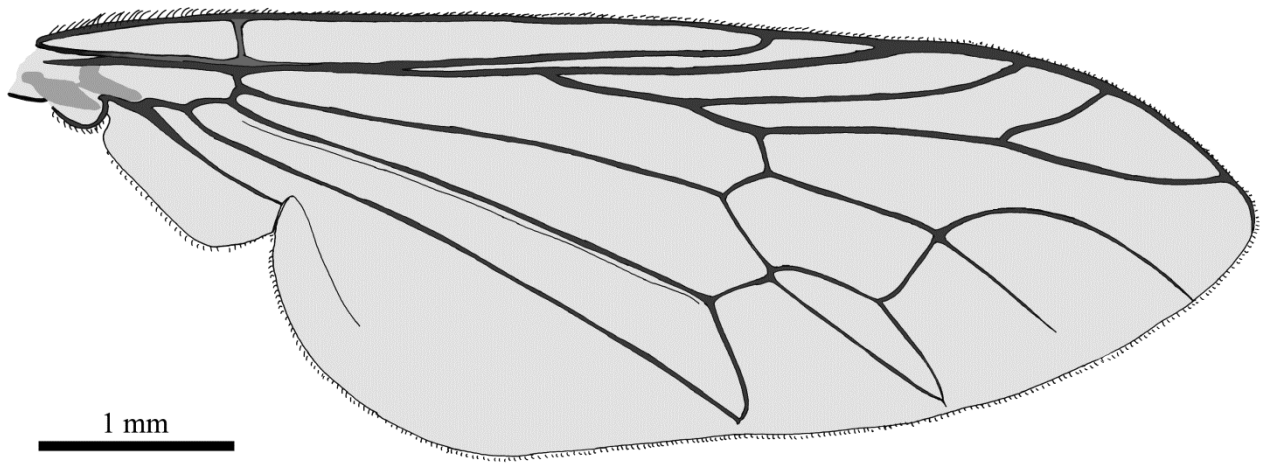


**Current Biology**

**Supplemental Information**

# **Long-Proboscid Flies as Pollinators of Cretaceous Gymnosperms**

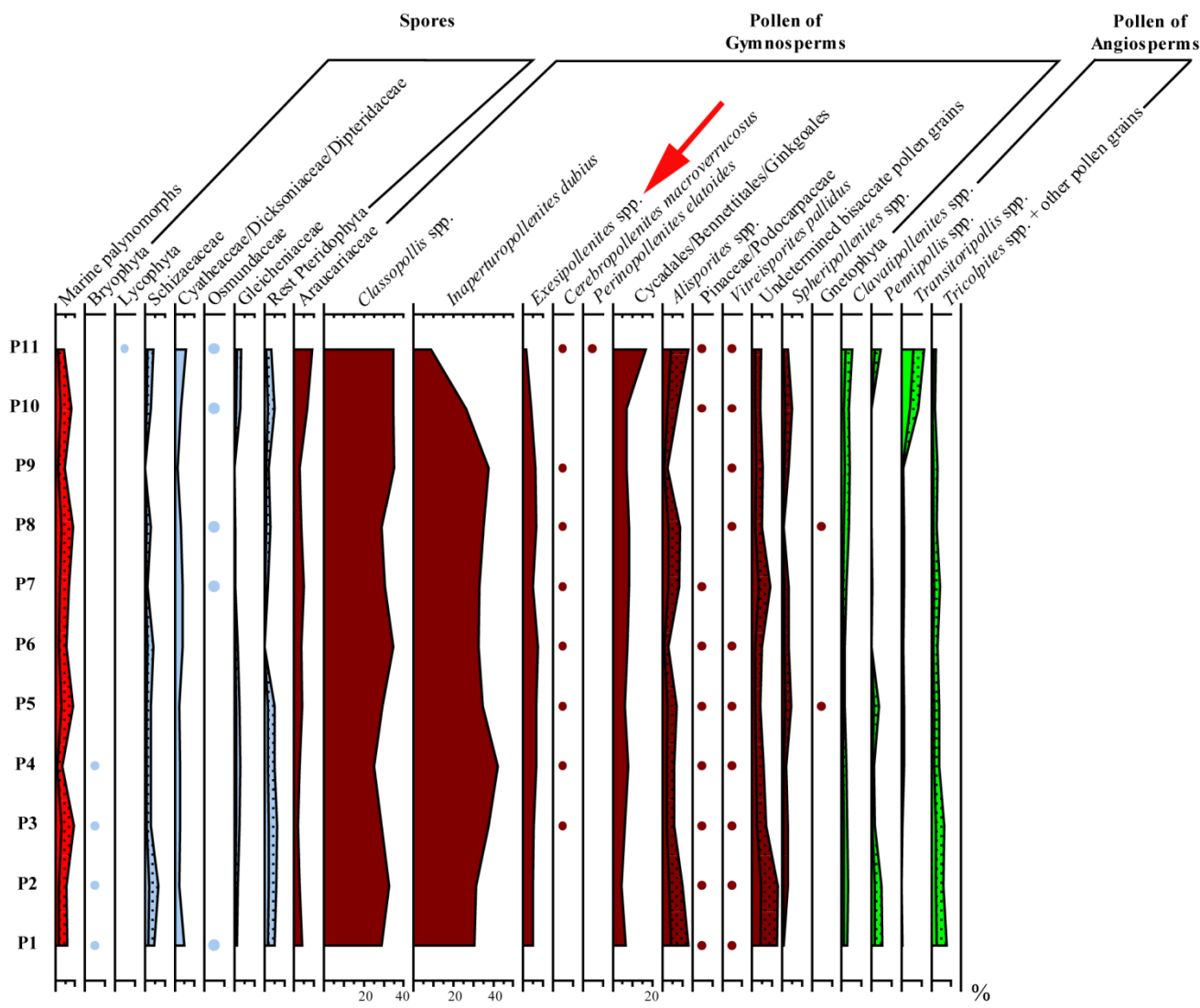
**Enrique Peñalver, Antonio Arillo, Ricardo Pérez-de la Fuente, Mark L. Riccio, Xavier Delclòs, Eduardo Barrón, and David A. Grimaldi**



**Figure S1. Wing of the Spanish amber zhangsolvid species *Buccinatormyia magnifica* (female) (related to Figure 1).** Wing morphology indicates hovering flight, particularly the apical veins that diverge near the wing tip.

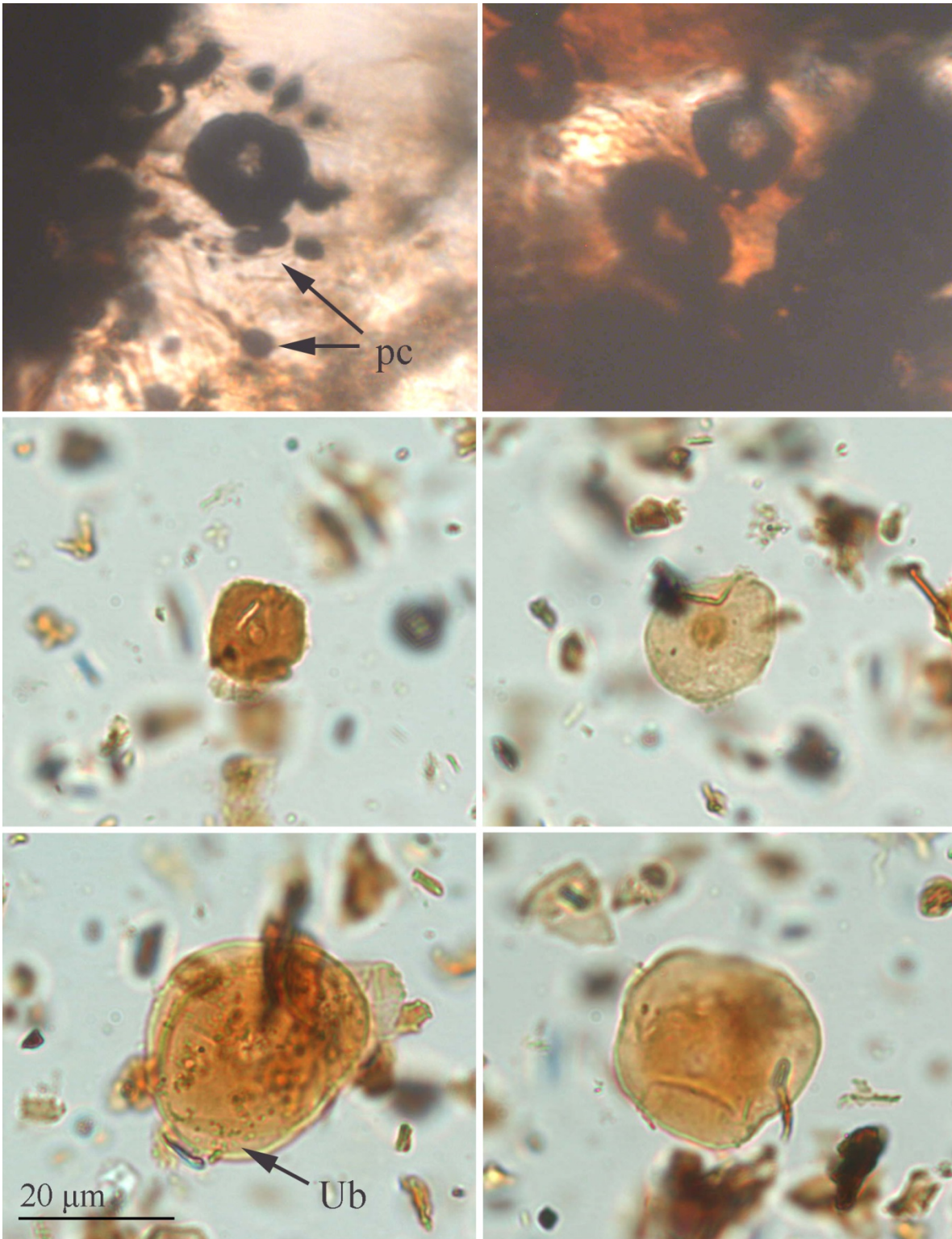


**Figure S2. El Soplao amber outcrop (related to Figure 3).** This excavation was in October 2008 and a section of the amber-bearing level was sampled for palynological study.



**Figure S3. Palynomorph percentage diagram of the El Soplao amber outcrop (related to Figure 3).** All samples in this diagram are from the level with amber at the section having 2.5 m thickness – see Figure S2–. The main groups of taxa are classified according to their botanical affinities and abundance. The red arrow indicates the percentage of abundance of *Exesipollenites tumulus* Balme plus scarce occurrences of the smaller *Exesipollenites* morphotype. Points indicate other taxa whose presence was sporadic. Dotted areas highlight palynomorph percentage variation for some scarce taxa.





**Figure S4.** *Exesipollenites* pollen grains from El Soplao amber outcrop in Cantabria, Spain (related to Figure 3). Pollen on the fly preserved in amber (upper) and pollen from the amber-bearing rock showing smaller morphotype (middle) and *E. tumulus* Balme (lower), to compare features and size. All to the same scale (pc= pyrite crystals, Ub= Übisch bodies).

**Table S1. Measurements in microns,  $\mu\text{m}$ , of the *Exesipollenites* pollen grains (related to Figure 3). Polar diameter and pseudopore diameter of *Exesipollenites* pollen grains on the fly and from the amber-bearing rock. In bold are the maximum and minimum measurements.**

Amber		Amber-bearing rock			
<i>Exesipollenites</i> on fly (n=50)		Small <i>Exesipollenites</i> (n=6)		<i>E. tumulus</i> Balme (n=50)	
polar diameter	pseudopore diameter	polar diameter	pseudopore diameter	polar diameter	pseudopore diameter
14.38	3.47	14.42	<b>4.96</b>	27.12	3.45
15.52	2.85	<b>12.79</b>	3.34	<b>22.48</b>	3.07
10.56	<b>5.05</b>	13.47	2.87	23.19	2.86
12.14	3.27	<b>15.08</b>	2.87	27.14	3.31
11.40	2.57	14.86	3.81	25.55	2.91
11.63	3.85	13.02	<b>1.79</b>	26.06	2.86
13.26	2.79			22.82	2.72
12.70	2.81			24.51	3.27
11.34	2.75			23.23	2.08
14.49	4.25			25.70	2.06
13.26	3.74			23.86	2.82
<b>18.64</b>	3.05			22.58	3.36
11.47	3.76			23.39	3.40
11.56	2.79			26.70	3.51
11.50	3.77			23.17	3.20
12.87	3.47			23.50	2.72
11.90	2.21			24.14	2.09
12.62	3.50			24.02	3.05
12.85	3.10			25.95	3.20
13.60	3.42			27.73	3.25
14.02	3.27			27.42	3.40
13.95	4.58			23.88	2.57
13.03	2.18			27.44	2.69
11.41	4.93			24.82	2.49

11.05	4.25			27.70	2.73
10.22	2.72			25.18	3.46
10.70	3.74			26.70	<b>2.00</b>
13.49	2.89			23.95	2.56
13.23	4.25			24.01	2.21
11.18	4.80			23.23	2.43
8.10	2.55			24.30	2.72
8.43	3.40			24.38	2.36
<b>7.72</b>	2.74			28.75	2.90
8.90	3.30			26.86	2.92
8.34	2.66			29.09	3.35
11.51	3.34			25.20	2.84
14.10	2.36			26.67	2.32
12.67	3.59			26.43	2.20
11.08	2.55			24.96	2.71
11.63	2.97			29.00	2.83
10.24	3.16			25.48	2.68
12.96	3.44			25.64	2.25
10.82	2.43			23.06	2.07
14.38	3.30			23.00	<b>3.70</b>
11.26	3.39			28.06	3.64
9.93	<b>2.04</b>			25.05	2.40
9.97	2.55			27.95	2.28
13.23	4.21			29.66	2.92
12.26	2.89			<b>29.89</b>	3.26
9.61	2.77			27.82	2.33
Mean 11.94	Mean 3.28	Mean 13.94	Mean 3.27	Mean 25.57	Mean 2.81



## Supplemental Experimental Procedures

### 1. Paleoentomological study

Three Spanish zhangsolvid specimens, along with other numerous specimens belonging to various arthropod taxa, were found in a large piece of amber from the El Soplao outcrop (Cantabria), Albian in age, and separated into several sub-pieces, subsequently embedded in synthetic epoxy resin (EPO-TEK 301) and polished [S1] to allow proper examination. All these specimens are housed at the laboratory of the El Soplao Cave, Cantabria (Spain), encompassing the Institutional Collection from the El Soplao outcrop. They have accession numbers CES-349.1 and CES-015.1/CES-392.3 (female holotype and paratype specimens of *Buccinatormyia magnifica*, respectively) and CES-015.2/CES-392.2 (holotype specimen of *Buccinatormyia soplaisensis*, sex unknown). All of the inclusions are well-preserved, without any evidence of fungal decomposition. The specimens were examined with an Olympus BX51 compound microscope; photomicrographs and drawings were made using a digital camera and a camera lucida, respectively, both attached to the compound microscope.

The Burmese specimen (accession number AMNH Bu-SE13) is preserved in amber from the Albian–Cenomanian boundary in Kachin State, northern Myanmar, and is the holotype of *Linguatormyia teletacta*. It is virtually completely preserved in a piece of dark, turbid amber containing fine particulate debris and several additional insects. It was photomicrographed with an Infinity© K-2 long distance microscope and is housed at the American Museum of Natural History, New York (USA).

Reconstruction of the *Buccinatormyia magnifica* female was undertaken to depict the entire body aspect and head habitus in life. The three-dimensional models of the fly and plant host (picture in Figure 4 and Movie S1) were performed with LightWave 3D computer graphics program (NewTek). Head and body colorations are conjectural but based on the common black and yellow patterns found in Recent, flower-visiting relatives in the Xylomyidae and Stratiomyidae. Wing angle

motion and body angle are based on the literature [S2]. A bennettitalean host and its general aspect were inferred based on williamsonian reproductive organs [6, 9, S3], a group that produced *Exesipollenites* pollen grains very similar that those found on the zhangsolvid fly.

The cross-sectioned proboscis of *Buccinatormyia magnifica* was gently isolated from the paratype specimen using a scalpel and then infiltrated with Spurr resin for 2 days, embedded in the same resin and polymerized at 60°C for 48 hours. Semithin and ultrathin sections were obtained using a Leica Ultracut UC6 ultramicrotome (Leica Microsystems, Vienna) and mounted on Formvar-coated copper grids. They were stained with 2% uranyl acetate in water and lead citrate and were observed in a JEM-1010 electron microscope (Jeol, Japan). These procedures were performed at the Serveis Científicotècnics of the University of Barcelona.

CT scanning of *Linguatormyia teletacta* was performed at the Cornell Biotechnology Resource Center Imaging facility using a Zeiss VERSA XRM-520 instrument. For each dataset, 2400 X-Ray projections were obtained at 0.15° intervals over 360° using 60kV, 32 μA, and 5000 ms exposure time with a 2048x2048 pixel detector using a 1.5 μm pixel size that was 2x2 binned into an effective pixel size of 3.0 μm. Proper placement of the specimen was accomplished with the assistance of X-Ray fluoroscopy mode, to insure that the area of interest was included within the scanning field of view. The projection views were used to reconstruct a CT image using a convolution back-projection approach implemented in 3D, giving a 3 x 3 x 3 mm<sup>3</sup> volume of image data with 3 μm isotropic voxels in arbitrary density units (ADU) and exported as DICOM or TIFF stacks. Reconstructed data files were imported into the software programs OsiriX (64 bit, version 5.5) and Avizo 8.0 (VSG), where they were visualized and analyzed using a variety of 2D and 3D techniques.

The middle Albian (ca. 105 Ma) paleogeographic map in Figure 4 was redrawn from the bibliography [S4].

## 2. Palynological study

The Spanish zhangsolvid specimens, including the specimen carrying pollen grains, and the samples of the palynological study came from the same level of the amber outcrop, named El Soplao (Upper Albian: Early Cretaceous), in the western Basque-Cantabrian Basin, northern Spain [S5–6]. The El Soplao deposit occurs in a non-marine to transitional marine siliclastic unit (Las Peñas Formation), which is a deltaic-estuarine environment developed in the regressive stage of a regressive-transgressive cycle [S5]. During a paleontological excavation carried out in October 2008, eleven rock samples were collected from the main amber-bearing level of the El Soplao outcrop for examination of their pollen grains content. The studied level (Figure S2) is comprised of organic-rich clays, 0.7–2.5 m of variable thickness, containing a rich fossil record of amber with abundant bioinclusions, gymnosperm plant cuticles, fusainized wood, marine or brackish-water invertebrates and diverse palynomorphs [S7] (Figure S3). All samples were prepared in accordance with palynological methods using various acids at high temperatures (HCl, HF, HNO<sub>3</sub>) [S8] and then were aliquoted. Slides were prepared by mounting palynomorph concentrates in glycerine jelly. These preparations were performed at ALICONTROL, Madrid. All the samples were fertile. Palynomorph percentage diagram was created using *Tilia 2* and *TGView 2.0.2* software [S9–10].

We examined the gut contents of two specimens of *Cratomyia* (Zhangsolvidae) from the Crato Formation of Brazil (housed in the AMNH) for the presence of pollen, but without success. From the new zhangsolvids in amber only the holotype of *Buccinatormyia magnifica* showed pollen grains. An area near the pollen grains was polished very closely, while avoiding damage to the wing, in order to permit suitable resolution and magnification; isolation of some pollen grains from the body was not performed in order to avoid damaging this holotype and unique specimen. We are confident that the presence of abundant pollen grains on the body of this specimen was not accidental due to the syndrome of entomophilous features of the pollen, its occurrence as a discrete spot on the



abdomen (Figure 3A), and the nectivorous habit of zhangsolvids. Due to the hovering flight of these flies (Figure 4A), we expected to find pollen grains on their heads, mainly on their proboscides; however, the plant reproductive organs involved and their pollen transferring strategies are largely unknown. Extant hovering insects commonly stabilize themselves with their forelegs on flowers when feeding on nectar, and sometimes fully alight, so different parts of their bodies can be covered by pollen grains.

The pollen grains are completely preserved, apparently in their original volume; they were probably quickly dehydrated and show an organic layer in contact with the amber. The pollen grain cavity is likely empty, as is typical of fossils preserved in amber [S11] and the organic remains are likely no more than the exine layer composed by sporopollenin. Only ten of the 421 pollen grains are slightly separated from the abdominal cuticle. It was not possible to observe any pollen grain at 1000x magnification, and both their opacity and contact with abdominal cuticle made their study difficult even with intense illumination (Figure S4).

However, seven of these pollen grains on the holotype specimen of *Buccinatormyia magnifica* have been 2D and 3D imaged using a confocal microscope to visualize them at a higher magnification. Were imaged using CLSM analyses at the Museo Nacional de Ciencias Naturales, Madrid. The confocal microscope used was a Leica TCS SPE-DM 2500 CSQ V-Vis instrument equipped with four solid-state lasers operating at 488 nm, 532 nm and 635 nm, a 10× eyepiece, an ACS APO 10×/0.3 objective, ACS APO 40×/1.15 CS and ACS APO 63×/1.3 CS oil immersion objectives, and the Leica Application Suite Advanced Fluorescence software. Fluorescence emission was collected from approximately 10 nm above the excitation wavelength up to 750 nm. Laser power for acquisition was set by viewing the fluorescence emission and increasing the power until the rate of increase in fluorescence appeared slowed. The photomultiplier gain for acquisition was then set by viewing the image and increasing the gain until signal overload was detected, at which point the gain was reduced slightly. Pixels matrices of 512 × 512 or 512 × 128 were acquired for each Z-step at a

zoom setting of 1. An Airy unit setting of 1 was routinely used for the observation pinhole; this resulted in a software assigned pinhole diameter of 67.5  $\mu\text{m}$  when using 405 nm illumination and the 63 $\times$ /1.3 objective. No attempts were made to optimize image quality by minimization of the confocal pinhole diameter, variation of the mounting medium, high levels of signal averaging, high pixel resolution, or by executing very small Z axis steps.

CLSM images revealed that this pollen has an operculate pore and the grains are lentoid in equatorial view (Movie S2). In some areas of the pollen spot there are minute pyrite crystals, which are homogeneous in size, completely opaque, glossy, having angulate surfaces (ie, crystalline shape), and so are easily distinguished from pollen (Figure S4).

*Exesipollenites* assemblages from the sediments of the El Soplao amber outcrop fossilized under very different taphonomic processes than did the pollen grains adhering to the fly in amber. The two morphotypes of *Exesipollenites* pollen grains in the amber-bearing sediments are preserved mainly complete, but partially collapsed, as semitransparent remains of their sporopollenin exine layer and showing external morphological features. They were treated and isolated using conventional palynological methods, thus their observation was optimal (Figure S4, middle and lower photomicrographs). Figure S3 shows the palynomorph percentage diagram of the 11 samples studied.

*Exesipollenites tumulus* Balme from the amber-bearing sediments are discoidal in polar view and lentoid in equatorial view with a subcircular to circular amb [S12]. The studied pollen grains from El Soplao have an average polar diameter 25.57  $\mu\text{m}$  (n=50; range 22.48–29.89  $\mu\text{m}$ ). The proximal face is psilate with exine around 1  $\mu\text{m}$  thick that is not columellate, sometimes having dispersed Übisch bodies (Figure S4). The distal face is pseudoporate having an averaged diameter of 2.81  $\mu\text{m}$  (n=50; range 2.00–3.70  $\mu\text{m}$ ). The smaller *Exesipollenites* morphotype is discoidal in polar view and lentoid in equatorial view, with a circular amb –having an averaged polar diameter 13.94  $\mu\text{m}$  (n=6; range 12.79–15.08  $\mu\text{m}$ )–, proximal face psilate, and exine psilate not columellate 0.5–1  $\mu\text{m}$

thick. This morphotype is monoporate (the pore occurs in the central part of the distal face) having an average diameter of 3.27  $\mu\text{m}$  (n=6; range 1.79–4.96  $\mu\text{m}$ ); sometimes specimens with an operculate pore are present.

From the 11 palynological samples, samples P1, P3, P4, P7 and P10 were screened especially carefully in order to find the scarce smaller morphotype (Figure S4, middle photomicrographs). From the 166 *Exesipollenites* grains that they contained, only six of them (3.5 %) are of the smaller morphotype (the grains measured in Table S1). We recently detected these small pollen grains in another Spanish amber outcrop, the San Just locality; most likely, they are present in other Cretaceous amber outcrops but have been under-detected or not observed. Commonly, entomophilous pollen grains, unlike anemophilous ones, are scarce in rocks due to low production by plants (as animal pollination is highly targeted and hence efficient in terms of pollen production) and infrequent wind dispersion.

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