Long-proboscid Flies as Pollinators of Cretaceous Gymnosperms

Enrique Peñalver[1,*], Antonio Arillo[2], Ricardo Pérez-de la Fuente[3], Mark L. Riccio[4], Xavier Delclòs[5], Eduardo Barrón[1] and David A. Grimaldi[6]

[1] Museo Geominero, Instituto Geológico y Minero de España, Madrid, E-28003, Spain
[2] Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense,
Madrid, E-28040, Spain
[3] Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA
[4] Institute of Biotechnology, Cornell University, Ithaca, NY 14853, USA
[5] Departament d'Estratigrafia, Paleontologia i Geociències Marines, Facultat de Geologia, Universitat de Barcelona, Barcelona, E-08028, Spain
[6] Division of Invertebrate Zoology, American Museum of Natural History, New York, NY 10021, USA
*Correspondence: e.penalver@igme.es

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 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 anthophytes, 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 were discovered recently in Cretaceous amber [10], which are the subjects of this report: *Buccinatormyia magnifica*, *B. soplaensis* and *Linguatormyia 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 rewards or "primary attractants" for attracting pollinators to flowers [11], not for predation or blood feeding (see Supplemental Information). 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 and 2A–B). 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 very thin cuticle in TEM x-section (Figure 2C–E) and surrounded by numerous longitudinal, rod-like ligaments (Figure 2D–E). Cuticular layers of the theca and food canal are preserved (Figure 2D–E). 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 (Figure 2F–G). 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 allows slight elongation and dorsoventral 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, veins apices abruptly turned away from the tip maintain rigidity and prevent apical shredding from the intense forces generated at very high wing-beat frequencies [4, 14]. A robust thorax with large flight muscles (viewed for *Linguatormyia i*n high resolution CT scans); the short, flattened abdomens; and 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] is 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. 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 (Figure 3B–D), and have an operculate pore (Movie S2). Average polar diameter is 11.94 µm (n=50; range 7.72–18.64) with the proximal face psilate and distal face porate with an average diameter of 3.28 µm (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 µm thick. The pollen grains are clumped and were probably sticky (Figure 3B–C), 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, although grains were originally nearly spherical, 22–29 µm diameter, with a rounded to oval pore 6 x 8–10µm (sometimes larger, up to 15x12 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 and 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 intraspecific competition and herbivore and disease outbreaks [6]. 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 Apsilocephalidae extend into the Cretaceous. Therevoid 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 Xylomyidae 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) Brachycera [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 *Bombylius* 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*: Primulacaeae) and bluets (*Houstonia*: Rubiaceae) [12, 30]. In the phloxes (Polemoniaceae), species and populations with the longest corolla tubes are pollinated exclusively by long-proboscis 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, ca. 1.3 to 7 mm, including specimens in compression rocks; in the ecologically analogous scorpionflies proboscides ranged from ca. 1 to 10 mm. The diversity of proboscis lengths strongly suggests diverse plant hosts. Each of the three clades of Mesozoic long-proboscid scorpionflies have different proboscis features that have been tentatively related to feeding from Cheirolepidiaceae/Gnetales, Caytoniaceae/Czekanowskiaceae and Pentoxylaceae/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, Cantabria (Spain) and at the American Museum of Natural History, New York (USA). They were prepared and polished; Spanish specimens were first embedded in synthetic epoxy resin. CT scanning was performed using a Zeiss VERSA XRM-520 instrument at the Cornell University Institute for 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 tri-dimensional models of the fly and plant host were performed with LightWave 3D computer graphics program (NewTek). Pollen grains on one fly specimen were imaged using confocal laster 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).

Author contributions

E.P., A.A., and D.A.G designed research; E.P., A.A., R.P.-d.l.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.l.F., X.D., E.B. and D.A.G. analyzed and discussed data; and E.P., E.B. and D.A.G. wrote the paper.

Supplemental Information

Supplemental Information includes, Supplemental Results and Discussion, Supplemental Experimental Procedures, four figures, one table, and two movies.

Acknowledgments

We thank the El Soplao cave, SIEC S.A. and the Government of Cantabria. Thanks to Carmen López and Yolanda Muelas (Serveis Cientificotècnics, 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 project CGL2011-23948/BTE, NSF grant 1305066, and by funding from Robert G. Goelet, Trustee and Chairman Emeritus of the AMNH. The authors declare no competing financial interests.

References

1. Darwin, C. (1862). The Various Contrivances by Which Orchids are Fertilized (London: John Murray).
2. Darwin, C. (1876). The Effects of Cross and Self Fertilization in the Vegetable Kingdom (London: John Murray).
3. Raven, P.H., Evert, R.F., and Eichhorn, S.E. (2005). Biology of Plants (New York: W.H. Freeman and Co.).
4. Grimaldi, D.A., and Engel, M.S. (2005). Evolution of the Insects (New York: Cambridge University Press).
5. Hu, S., Dilcher, D.L., and Taylor, D.W. (2012). Pollen evidence for the pollination biology of early flowering plants. In Evolution of Plant-Pollinator Relationships, S. Patiny, ed. (Cambridge: Cambridge University Press), pp 165–236.

6. Taylor, T.N., Taylor, E.L., and Krings, M. (2009). Paleobotany. The Biology and Evolution of Fossil Plants (Amsterdam: Academic Press-Elsevier).

7. Friedman, W.E. (1996). Biology and evolution of the Gnetales. Int. J. Plant Sci. *157*, S1–S125.

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

9. Harris, T.M. (1969). The Yorkshire Jurassic Flora. Volume III Bennettitales (London: British Museum (Natural History)).

10. Arillo, A., Peñalver, E., Pérez-de la Fuente, R., Delclòs, X., Criscione, J., Barden, P.M., Riccio, M.L., and Grimaldi, D.A. (2015). Long-proboscid brachyceran flies in Cretaceous amber (Diptera: Stratiomyomorpha: Zhangsolvidae). Syst. Entomol. *40*, 242–267.

11. Armbruster, W.S. (2012). Evolution and ecological implications of “specialized” pollinator rewards. In Evolution of Plant-Pollinator Relationships, S. Patiny, ed. (Cambridge: Cambridge University Press), pp 44–67.

12. Grimaldi, D.A. (1988). Beeflies and bluets: *Bombylius* (Diptera: Bombyliidae) flower-constant on the distylous species, *Hedyotis caerulea* (Rubiaceae), and the manner of foraging. J. Nat. Hist. *22*, 1–10.

13. Krenn, H.V., Plant, J.D., and Szucsich, N.U. (2005). Mouthparts of flower-visiting insects. Arthropod Struct. Dev. *34*, 1–40.

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

15. Guy-Ohlson, D. (1986). Jurassic palynology of the Vilhelmsfält Bore nº 1, Scania, Sweden, Toarcian-Aalenian (Stockholm: Swedish Museum of Natural History).

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

17. Van Konijnenburg-van Cittert, J.H.A. (1971). In situ gymnosperm pollen from the Middle Jurassic of Yorkshire. Acta Bot. Neerlandica *20*, 1–96.

18. Osborn, J.M., and Taylor, T.N. (1995). Pollen morphology and ultrastructure of the Bennettitales: In situ pollen of *Cycadeoidea*. Am. J. Bot. *82*, 1074–1081.

19. Harris, T.M. (1974). *Williamsoniella lignieri*: its pollen and the compression of spherical pollen grains. Palaeontology *17*, 125–148.

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

21. 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.

22. Osborn, J.M., and Taylor, M.L. (2010). Pollen and coprolite structure in Cycadeoidea (Bennettitales): implications for understanding pollination and mating systems in Mesozoic cycadeoids. In Plants in Mesozoic time, C.T. Gee ed. (Bloomington: Indiana University Press), pp. 35–49.

23. 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.

24. 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. U.S.A. *109*, 8623–8628.

25. Grimaldi, D., Arillo, A., Cumming, J.M., and Hauser, M. (2011). Brachyceran Diptera (Insecta) in Cretaceous ambers, Part IV. Significant new orthorrhaphous taxa. ZooKeys *148*, 293–332.

26. Gaimari, S., and Webb, D. (2009). Therevidae (stiletto flies). In Manual of Central American Diptera, vol. 1, B.V. Brown, A. Borkent, J.M. Cumming, D.M. Wood, N.E. Woodley, and M.A. Zumbado eds. (Ottawa: NRC Research Press), pp. 633–648.

27. Ollerton, J., and Coulthard, E. (2009). Evolution of animal pollination. Science *326*, 808–809.

28. Marshall S.A. (2012). Flies: The Natural History and Diversity of Diptera (New York: Firefly Books, Buffalo).

29. Proctor, M., Yeo, P., and Lack, A. (1996). The Natural History of Pollination (Portland: Timber Press).

30. Darwin, C. (1862). On the two forms, or dimorphic condition, in the species of *Primula* and on their remarkable sexual relations. J. Linn. Soc., Bot. *6*, 77–96.

31. Grant, V., and Grant, K.A. (1965). Flower Pollination in the Phlox family (New York: Columbia University Press).

32. Goldblatt, P., and Manning, J.C. (2000). The long-proboscid fly pollination system in southern Africa. Ann. Mo. Bot. Gard. *87*, 146–170.

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

Figure legends

Figure 1. External anatomy of the zhangsolvid flies and their nectivorous proboscides preserved in Cretaceous amber from Spain and Myanmar (see also Figure S1)
(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 CT-nanoscans of *Linguatormyia teletacta*.

Figure 2. External and internal (ultrastructural) anatomy of the nectivorous proboscis in zhangsolvid flies in Cretaceous Spanish amber. (A) Reconstructed head of *Buccinatormyia magnifica* (artist: J.A. Peñas); the palpal sensillar foveae are show 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 *Buccinatormyia magnifica* (accession number CES-015.1+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 its 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
Buccinatormyia soplaensis, 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].

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) CLSM 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).

Figure 4. Reconstruction and paleodistribution of zhangsolvid flies. (A) Reconstruction of a female of *Buccinatormyia 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 Xylomyidae 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); (4) Myanmar (Albian–Cenomanian).