Insect pollination in deep time

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Abstract

The inference of insect pollination from the fossil record is a focus of current research, especially because of findings in Cretaceous ambers. However, the lack of consensus on the definition of an "insect pollinator" challenges the recognition of this plant-insect relationship in the fossil record. We propose a conceptual definition for fossil-insect pollinator and an operational classification for fossil insects into “pollinator” or “presumed pollinator”. Through this, we have identified 15 insect families that include pollinators in deep time and show pollination relationships have existed since at least the Upper Jurassic (~163 Ma). There is a clear need for more detailed studies about the development of plant-pollinator interactions that can be inferred from the fossil record. As a first step this work provides insights for understanding the origin and evolution of this relationships in the history of life on Earth, and its influence on the establishment and composition of modern ecosystems.

Pollination biology

Pollination by animals, particularly insects, is essential to the reproductive success, survival and evolution of most flowering plants (**angiosperms**) (see Glosary) and a significant proportion of **gymnosperms** [1–5]. The heterotrophic nature of animals makes them completely dependent on plants for food, directly or indirectly. This includes humans and the annual global market value of crop production directly linked to animal pollination is estimated to be $235–$577 billion [6]. Accordingly, the past two decades have seen unprecedented interest in pollinators and pollination ecology, especially related to anthropogenic intervention in ecosystems and the effects of global decline of pollinators [see 7–10].

Angiosperm pollination occurs by the transfer of a pollen grain (containing the male gametophyte) to a flower's stigma (the receptive surface of the female reproductive organs). There the pollen germinates, sends the gametophyte down through the style within a pollen tube, which fertilizes an ovule to produce a seed (Fig. 1.A). The pollination of gymnosperms is broadly similar except there is no stigma or style and pollen germinates in liquid "pollination drops" [11] (Fig. 1.B). Except in the case of autogamous self-pollination, the transfer of pollen between plants requires a vector, wich can be abiotic in the case of **anemophily** or **hydrophily**, or biotic (**zoophily)** or a mixture, **ambophily** [9,12–15].

Animals, especially insects, are the most common pollen vectors, with an estimated 350,000 described species interacting with almost 90% of flowering plants [1,9,10,16]. There has been no equivalent review of pollination in extant gymnosperms, but we estimate that perhaps 40% of the c. 900 species employ insect pollination, though the diversity of those pollinators is unclear.

The study of pollination ecology involves the analysis of the effectiveness and efficiency of the pollinator assemblage among flowering plants in relation to their searching behaviors, flight distances between flowers, and the number of pollen grains deposited on the stigma [17–19]. The Cox-Knox Postulates provide a systematic approach to the recognition of pollen vectors [10,20] but this kind of behavioral and ecological information is rarely, if ever, represented in the fossil record, though consumption of flowers without pollination (florivory) has recently been described by Xiao *et al*. [21].

Ancient pollination

Angiosperms currently dominate most terrestrial ecosystems, but this dominance has only existed during the last ~100–50 million years (My) [22]. Their relatively rapid rise starting in the Early **Cretaceous** (or perhaps earlier – see [23]), ultimately replaced the previously gymnosperm-dominated forests in most parts of the world. This floristic transition has been named the Angiosperm Terrestrial Revolution [24]. It is thought that flowering plants have interacted with insect pollinators since their beginning, and that the earliest pollen vectors were perhaps generalist insects like beetles, primitive short-tongued and mandibulate moths, apoid wasps, and flies [25–27]. Presumed pollinators have been reported from the Cretaceous fossil record [28] and examples such as the beetle families Oedemeridae and Kateretidae reveal evidence of shifts from gymnosperm to angiosperm pollination [28,29]. One reason for insects making this shift was that they fed on gymnosperm pollination drops [30,31]. By evolving nectar, angiosperms provided a more nutritious and efficient system for consuming surface fluids, allowing hosts switches and, inadvertently, pollen transfer among conspecifics [32].

The complexity of pollination is an obstacle when it comes to recognising plant-insect pollinator relationships in **deep time** particularly for gymnosperms which are currently less well studied but were much more diverse in the past. Pollination involves several stages, from a visit of the insect to a plant, attachment of pollen grains to the insect, transport of pollen, visit to a conspecific, and deposition of the pollen on a stigma (Fig. 1 (A, B)). Only some stages can be directly observed in the fossil record, for example pollen transport through grains attached to insect bodies preserved in **amber** or as compressions. Another source of information comes from fossil flowers with pollen in the anthers. If an insect is found at the same locality with identical pollen affixed to body parts, then it is possible to infer a pollination relationship [33]. However, such specimens are uncommon as it requires exceptional preservation and unique taphonomic conditions.

Because of the recent scientific interest in the evolution and ecology of pollination, we consider it imperative to conceptually define fossil pollinators, which has not been done previously. Our aims is to compare multidisciplinary ecological and paleontological research and propose a set of parameters that confidently recognize pollinating insects in the fossil record. We elaborate a conceptual definition and operational classification of fossil pollinators, using it to infer the origin and evolution of animal pollination in the past.

Defining fossil insect pollinators

We conducted a literature search in the ScienceDirect, PubMed, and Google Scholar databases using the keywords “insect pollinator/s”, “fossil pollinator/s”, “fossil insect pollinator/s”. The aim was to compile all extant and extinct families with known pollinating insect species by finding studies that describe insect pollinators or any relationship regarding pollination in deep time. Both **bioinclusions** in amber and specimens as compressions in rocks were considered. As well as the identity of the fossil, we recorded its age, type of preservation (bioinclusion vs. compression), country of location, and the type of evidence used to infer its role as a pollinator. This information is summarized in Supplementary Material 1 and 2.

Using this information, we developed a schematic key (Fig. 2.A) to allow the classification of a case study of fossil insect into one of two categories: “pollinator” and “presumed pollinator”.

Criteria to classify a fossil insect as a pollinator

To determine that a fossil insect was a pollinator, the following requirements must be met: (1) pollen grains attached to a part of the insect’s body surface that may subsequently contact the female reproductive organ of a plant; and (2) assignment to an extant lineage that is considered to include pollinators either exclusively or partially. Here we use ‘lineage’ to mean a family, although a monophyletic group at any taxonomic rank would be appropriate. These criteria must be considered together because each one by itself is not sufficiently robust to determine a pollinator role in the past. In the case of fossil insects that belong to an extinct lineage or modern counterparts that today are not considered pollinators but satisfy the first criteria, they must additionally meet one of the following attributes: i) long proboscis devoid of piercing elements that could be used to feed on nectar or its gymnosperm equivalent; or ii) any morphological specialization that indirectly implies pollination (e.g., specialized structures for capturing and/or carrying pollen, etc.).

There is an exception to the criteria described above for species belonging to a **crown-group** of monophyletic lineages with pollinating insects, for example the Hymenoptera: Corbiculata [34–38] and other hymenopteran families. These groups have specialized structures only for the transport of pollen (*i.e*., scopa, fiscina, and corbicula) and which strongly implies that they visit flowers and therefore have the potential to be pollinators. For example, fossil honey bees (genus *Apis* Linnaeus), in which neither pollen is preserved nor the pollen-gathering or transport structures are preserved (e.g., fragmentary specimens preserving synapomorphies for the genus but not pollen or the corbicula), would still be classified as a pollinator as *Apis* belongs to the lineage Corbiculata. To conclude otherwise would require evidence against its classification as a pollinator. In the case of fossil isolated wings of corbiculate bees or lepidopteran scales, the presence of pollinators in that geological time and place must be considered a possibility. These data were not included in our results, however, because we cannot always classify them with sufficient certainty (e.g., [39–42]). Excluding these few cases, however, does not alter our results.

Classifying fossil insects as pollinators through knowledge of extant pollinator relatives in a clade needs to be done cautiously as reversals from nectar or pollen feeding occur. One can build added confidence if the fossil is part of a crown group of insects that are predominantly pollinators or if the fossil also preserves evidence satisfying one of the other criteria, such as the presence of pollen grains attached to the body. This provides direct evidence of the diet [28,43], and pollen consumption has often been considered an evolutionary precursor to biotic pollination [13,44].

Pollen consumption alone, however, only allows us to infer a trophic relationship between the insect and some host plants, which can be achieved independent of pollination.

There are cases where some fossil insects with attached pollen grains are found, but belonging to extant lineages where no pollinators are known, or to extinct groups. In these cases, we can assess another series of alternative conditions that allow us to assess their role as pollinators. For such fossils, it is important that there are preserved pollen- or nectar-feeding specializations, such as long-proboscides with pollen-gathering setae [32,43,45], or mandibular setose specializations [46], among others. Also, the presence of structures exclusively related to pollen feeding, such as pollen baskets [37,47], specialized pollen-gathering setae (e.g., [48]), and specialized abdominal setae [37,49] can also be crucial. However, it must be unambiguously demonstrated that the structure has the function described and no alternative functions.

A fossil insect with a morphological adaptation that *indirectly* implies pollination may be only considered as a pollinator if it is also accompanied by pollen grains attached to the body surface. If not, the morphological trait by itself would not necessarily imply that it was a pollinator, because other alternatives would remain open. Thus, it will be considered a presumed pollinator. This is to be contrasted with morphological specializations that are exclusively known to be indirectly implied in pollination, and in fossils belonging to a crown-group of a clade of pollinators. In these rare cases, the structure indicates more than the mere potential to be implied in pollination and instead cannot serve any other purpose, and so the trait alone can serve as evidence of a pollinating role.

Criteria to classify a fossil insect as presumed pollinator

To determine that a fossil insect is a presumed pollinator requires to: (1) pollen grains attached to the insect's body, as this is indicative of visiting a plant reproductive structure (angiosperm or gymnosperm), however, the specimen cannot be assigned to an extant lineage of pollinators and no additional evidence is present; or (2) without pollen grains attached to the insect's body, however it belongs to an extant lineage that is considered to contain pollinators today. In the latter case the specimen must also meet one of the following conditions: i) coprolites with pollen grains associated with the anal region of the insect; or ii) pollen grains in the gut of the insect; or iii) a long proboscis devoid of any piercing elements; or iv) any morphological specialization that indirectly implies pollination (e.g., specialized structures for capturing and/or carrying pollen, etc.). Thus, we believe that co-occurrence of multiple conditions satisfying some combination of the above cited criteria is necessary.

With respect to coprolites with pollen grains, a taphonomic analysis is essential and the coprolite has to be attached to the anal region to infer a pollen diet, as there are limitations in attributing coprolite shape, size, and texture to insects beyond major taxonomic divisions [13]. Unfortunately, there is no comprehensive catalog of fecal pellets for living groups of pollinators [50]. The same can be true for pollen grains preserved in intestines, because this only suggests a pollinivorous habit [51], and the presence of a long proboscis by itself is only indicative of a fluid-feeding habit [52], for example haematophagy or phloem feeding.

If a fossil insect cannot be assigned to an extant lineage of pollinators, either because it belongs to an extinct group with no descendants or because its modern counterparts are today not considered pollinators, it must be determined using the criteria outlined in iii) otherwise, it cannot be classified.

The classification of fossil insects as pollinators (Fig. 2.B) should be complemented with additional information from the fossil record, such as the identity of the attached pollen grains. Modern methods for the analysis of fossil pollen grains, such as SEM and TEM imaging, permit the acquisition of far finer character data [53]. There are often differences between pollen produced by wind-pollinated and animal-pollinated plants. Pollen of wind-pollinated species tend to be smooth, dry, and of moderate size, while that of animal-pollinated species is usually sticky, with an ornamented surface, and is of variable size (see [27] and references therein). This kind of information can support different conclusions about early pollination relationships, but can lack precision. If the sample with pollen grains is too old, it is more difficult to determine which lower-level taxon (species or genus) it came from. This is the case for many Cretaceous amber pollen grains (e.g., [28,54]).

Fossil pollinators

At least 193 modern insect families belonging to the following 10 orders are considered pollinators: beetles (Coleoptera), flies (Diptera), butterflies and moths (Lepidoptera), true bugs (Hemiptera), bees, ants, wasps and relatives

(Hymenoptera), grasshoppers and crickets (Orthoptera), net-winged insects (Neuroptera), thrips (Thysanoptera), cockroaches (Blattodea), and earwigs (Dermaptera) (Fig. 3; see Supplementary Material 1).

Our literature search of the fossil record of pollinating lineages, resulted in 54 different insect families (five undetermined) belonging to 13 orders (one undetermined) for which there was evidence of their pollinating ability (Supplementary Material 2).

Using the criteria above, we classified 104 fossil insect species belonging to 15 families in six orders as pollinators (Supplementary Material 2); one species in the **Jurassic**, 12 in the Cretaceous, and 91 in the **Paleogene** plus **Neogene**. Moreover, 120 fossil species belonging to 32 families, and 17 species of undetermined families, from nine different orders (1 undetermined), are classified as presumed pollinators (Supplementary Material 2). In addition, six fossil species belonging to five families, and one of undetermined family, from five different orders, could not be classified (Supplementary Material 2).

Fossil specimens that meet our criteria are not necessarily the earliest representatives of their respective families in the fossil record. Therefore, pollinating fossil insects are temporally distributed between the Upper Jurassic (~163Ma) and **Miocene** (~20Ma) (Fig. 2.A). Also, based on the current data of pollinator families, there is evidence of a massive shift of the different insect clades from gymnosperms towards pollination of angiosperms (Fig. 4.B).

Evidence of pollination in the past

The fossil record provides exceptional opportunities to study and analyze the origin and co-evolution of different organisms and their interactions. Evidence of interactions between plants and insects that could promote pollination includes the study of: i) plant reproductive paleobiology, ii) plant damage, iii) dispersed coprolites, iv) insect gut contents, v) insect mouthparts, and vi) taxonomic assignment to a modern descendant for which reliable ecological data exist [13]. All of them can be differentiated between direct and indirect evidence of pollination, both for gymnosperms and angiosperms, considering the limits of the fossil record and the **taphonomy** of amber and compression deposits.

Indirect fossil evidence of pollination involves structures like tubes, funnels, channels, and ducts of gymnosperm reproductive organs related to the mouthparts of long-proboscid pollinating insects [30,55], or structures like nectary discs in angiosperms that attract mandibulate pollinating insects [56]. Additional evidence is provided by entomophilous features of fossil pollen such as size, morphology, sculpturing, stickiness, amount, and potential of clumping [57,58]. Among insects, the modification of elongate siphonate mouthparts, potentially flexible in some cases, can be utilized to access pollen drops and nectar offered by both gymnosperms and flowers with deep gynoecia in angiosperms [30,59,60]. But a long proboscis alone is not evidence of a pollinating habit or even feeding on plant fluids as evidenced by the elongate proboscides of protosiphonapterans (e.g., [61]).

Thus, fossil long-proboscid insects can only be considered pollinators if they have specific characters in their mouthparts, like flexible maxillary palps [60], combined with further evidence of specialized pollen-gathering structures (e.g., pollen-capturing setae, setal combs, etc.) or if belonging to a clade of known pollinators. Insect gut contents and coprolites are particularly exceptional indirect evidence [32,44,52,62], given that in such cases the plant visited and the feeding habits have been determined. Nonetheless, such evidence is insufficient to indicate that the insect was a pollinator as some may feed on pollen but not actually vector the pollen to a conspecific plant. Note that if such coprolites are not in contact with the anal region of the producer, then a conclusion of pollination can be quite controversial (e.g., [63,64]), and these species should not be interpreted as pollinators or presumed pollinators if further evidence is not gathered.

Direct evidence of insect pollination is becoming more widely known in the fossil record, particularly during the last 25 years (e.g., [28,34,45,47,65,66]). This type of evidence involves insects associated with pollen grains affixed to different parts of the body, particularly the mouthparts, head, and legs, but occasionally other parts, including specific pollen-capture or transport structures (e.g., corbiculae, scopae, or fiscinae among bees [34,37,38]). The latter let infer that these insects at least visited the male reproductive organs of the plants.

The newly proposed criteria for recognizing a pollinating insect in the fossil record (Fig. 4.A) reveals indisputable evidence of this relationship between insects and plants since at least the Upper Jurassic (~163 Ma). This is well prior to the radiation of angiosperms [24] as unequivocal fossil flowers date to only about 125 Ma [67], although recent molecular data estimate an earlier origin of crown angiosperms before the Cretaceous [23,68,69]. This relationship may be even older since the record of insects with pollen grains attached to the body and other insects with long probosces indicates their origin in the Lower **Permian** (Kungurian, ~276 Ma) [60,70]. Therefore, it should not be surprising that some groups of current gymnosperms, such as Cycadales and Gnetales, have entomophilous or ambophilous pollination rather than exclusively anemophilous pollination [3,4, K. Bolinder, PhD thesis, Stockholm University, 2014)]. Such relationships with insects may have persisted since the Mesozoic, which is evidenced through the remarkable interaction between different insect families with gymnosperm and angiosperm plants during the Cretaceous period (Fig. 5).

Concluding remarks

The search for fossil insect specimens carrying pollen grains attached to the body should be encouraged, both in amber bioinclusions and compressions, as they can give new information about pollination interactions in the past. Therefore, they can help to understand the impacts of this old relationship between insects and plants in the shaping of today’s ecosystems (see **Outstanding Questions**).

Description of new specimens with pollen attached to their bodies has increased in recent years (e.g., [49, 70]), and this trend is expected to continue. There are vast and virtually unexplored paleoentomological amber records, such as those from Lebanon and Myanmar, which may hold key information about the transition from gymnosperm to angiosperm pollination. The fossil record shows some evidence for this transition [29] but there are knowledge gaps for periods prior to the Albian.

The suggested criteria concerning the degree of certainty of the various sources of evidence shown by fossil insects related to pollination, are based on features that can be directly and easily observed in the fossils. This, together with the simple categorization proposed, will result in a better description and interpretation of fossils, and more rigorous knowledge of pollination in deep time.

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Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Ollerton, J. *et al*. (2011) How many flowering plants are pollinated by animals? *Oikos* 120, 321–326

2. Wang, X.Q. and Ran, J.H. (2014) Evolution and biogeography of gymnosperms. *Mol. Phylogenet. Evol.* 75, 24–40

3. Ickert-Bond, S.M. and Renner, S.S. (2016) The Gnetales: Recent insights on their morphology, reproductive biology, chromosome numbers, biogeography, and divergence times. *J. Syst. Evol.* 54, 1–16

4. Toon, A. *et al*. (2020) Insect pollination of cycads. *Austral Ecol.* 45, 1033–1058

5. Rodger, J.G. *et al*. (2021) Widespread vulnerability of flowering plant seed production to pollinator declines. *Sci. Adv*. 7, eabd3524

6. IPBES (2016) Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. (Potts, S.G. *et al*., eds). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany

7. Potts, S.G. *et al*. (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353

8. Millard, J. *et al*. (2021) Global effects of land-use intensity on local pollinator biodiversity. *Nat. Commun.* 12, 1–11

9. Ollerton, J. (2017) Pollinator Diversity: Distribution, Ecological Function, and Conservation. *Annu. Rev. Ecol. Evol. Syst.* 48, 353–376

10. Ollerton, J. (2021) *Pollinators & Pollination: Nature and Society*, Pelagic Publishing, Exeter, UK

11. Nepi, M. *et al*. (2009) Nectar and pollination drops: how different are they? *Ann. Bot.* 104, 205–219

12. Ackerman, J.D. (2000) Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Syst. Evol.* 222, 167–185

13. Labandeira, C.C. (2000) The Paleobiology of Pollination and its Precursors. *Pal. Soc. Papers* 6, 233–270

14. Nepi, M. *et al*. (2017) Phylogenetic and functional signals in gymnosperm ovular secretions. *Ann. Bot.* 120, 923–936

15. Schatz, B. *et al*. (2017) Plant–Insect Interactions: A Palaeontological and an Evolutionary Perspective. In *Advances in Botanical Research* (Sauvion, N., Thiéry, D. and Calatayud, P.A., eds), pp. 1–24, Elsevier

16. Wardhaugh, C.W. (2015) How many species of arthropods visit flowers? *Arthropod Plant Interact.* 9, 547–565

17. Herrera, C.M. (1987) Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos* 50, 79–90

18. Ollerton, J. (1999) La evolución de las relaciones polinizador-planta en los artrópodos. *Bol. Soc. Entomol. Arag.* 26, 741–758

19. Ne'eman, G. *et al*. (2010) A framework for comparing pollinator performance: effectiveness and efficiency. *Biol. Rev.* 85, 435–451

20. Cox, P.A. and Knox, R.B. (1988) Pollination postulates and two-dimensional pollination in hydrophilous monocotyledons. *Ann. Mo. Bot. Gard.* 75, 811–818

21. Xiao, L. *et al*. (2021) Florivory of Early Cretaceous flowers by functionally diverse insects: implications for early angiosperm pollination. *Proc. R. Soc. B.* 288, 20210320

22. Condamine, F.L. *et al*. (2020) The rise of angiosperms pushed conifers to decline during global cooling. *Proc. Natl. Acad. Sci. U. S. A.* 117, 28867–28875

23. van der Kooi, C.J. and Ollerton, J. (2020) The origins of flowering plants and pollinators. *Science* 368, 1306–1308

24. Benton, M.J. *et al*. (2022) The Angiosperm Terrestrial Revolution and the origins of modern biodiversity. *New Phytol.* 233, 2017–2035

25. Friis, E.M. *et al*. (1999) Early angiosperm diversification: the diversity of pollen associated with angiosperm reproductive structures in Early Cretaceous floras from Portugal. *Ann. Mo. Bot. Gard.* 86, 259–296

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

27. Hu, S. *et al*. (2008) Early steps of angiosperm–pollinator coevolution. *Proc. Natl. Acad. Sci. U. S. A.* 105, 240–245

28. Peris, D. *et al*. (2020) Generalist Pollen-Feeding Beetles during the Mid-Cretaceous. *iScience* 23, 100913

29. Peris, D. *et al*. (2017) The case of *Darwinylus marcosi* (Insecta: Coleoptera: Oedemeridae): A Cretaceous shift from a gymnosperm to an angiosperm pollinator mutualism. *Commun. Integr. Biol.* 10, e1325048

30. Ren, D. *et al*. (2009) A Probable Pollination Mode Before Angiosperms: Eurasian, Long-Proboscid Scorpionflies. *Science* 326, 840–847

31. Zhao, X. *et al*. (2020) Mouthpart homologies and life habits of Mesozoic long-proboscid scorpionflies. *Sci. Adv.* 6, eaay1259

32. Labandeira, C.C. *et al*. (2007) Pollination Drops, Pollen, and Insect Pollination of Mesozoic Gymnosperms. *Taxon* 56, 663–695

33. Geier, C. *et al*. (2023). Collecting in situ/adhered pollen from fossil compressed angiosperm flowers. *Rev. Palaeobot. Palynol*. 310, 104831

34. Engel, M.S. (2001) A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). *Bull. Am. Mus. Nat. Hist.* 259, 1–192

35. Engel, M.S. (2001) Monophyly and extensive extinction of advanced eusocial bees: Insights from an unexpected Eocene diversity. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1661–1664

36. Michener, C.D. (2007) *The Bees of the World* (2nd edn). Johns Hopkins University Press

37. Engel, M.S. *et al*. (2021) Stingless bees in Miocene amber of southeastern China (Hymenoptera: Apidae). *J. Melittology* 105, 1–83

38. Engel, M.S. and Rasmussen, C. (2021) Corbiculate bees. In *Encyclopedia of Social Insects* (Starr, C.K., ed.), pp. 302–310, Springer Nature

39. Engel, M.S. (1998) Fossil honey bees and evolution in the genus *Apis* (Hymenoptera: Apidae). *Apidologie* 29, 265–281

40. Prokop, J. *et al*. (2017) An Early Miocene bumble bee from northern Bohemia (Hymenoptera, Apidae). *Zookeys* 710, 43–63

41. Dehon, M. *et al*. (2019) Morphometric analysis of fossil bumble bees (Hymenoptera, Apidae, Bombini) reveals their taxonomic affinities. *Zookeys* 891, 71–118

42. van Eldijk, T.J.B. *et al*. (2018). A Triassic-Jurassic window into the evolution of Lepidoptera. *Sci. Adv*. 4, e1701568

43. Grimaldi, D.A. *et al*. (2019) Direct evidence for eudicot pollen-feeding in a Cretaceous stinging wasp (Angiospermae; Hymenoptera, Aculeata) preserved in Burmese amber. *Commun. Biol.* 2, 1–10

44. Labandeira, C.C. (1998) How Old Is the Flower and the Fly? *Science* 280, 57–59

45. Khramov, A.V. *et al*. (2020) The Fossil Record of Long-Proboscid Nectarivorous Insects. *Entomol. Rev.* 100, 881–968

46. Cai, C. *et al*. (2018) Beetle pollination of cycads in the Mesozoic. *Curr. Biol.* 28, 2806–2812

47. Wappler, T. *et al*. (2015) Specialized and Generalized Pollen-Collection Strategies in an Ancient Bee Lineage. *Curr. Biol.* 25, 3092–3098

48. Rasmussen, C. *et al*. (2020) A primer of host-plant specialization in bees. *Emerg. Top. Life Sci.* 4, 7–17

49. Peñalver, E. *et al*. (2012) Thrips pollination of Mesozoic gymnosperms. *Proc. Natl. Acad. Sci. U. S. A.* 109, 8623–8628

50. Lupia, R. *et al*. (2002) A New Fossil Flower and Associated Coprolites: Evidence for Angiosperm-Insect Interactions in the Santonian (Late Cretaceous) of Georgia, USA. *Int. J. Plant Sci.* 163, 675–686

51. Wedmann, S. *et al*. (2021) The last meal of an Eocene pollen feeding fly. *Curr. Biol.* 31, 1–7

52. Labandeira, C.C. (1997) Insect Mouthparts: Ascertaining the Paleobiology of Insect Feeding Strategies. *Annu. Rev. Ecol. Syst.* 28, 153–193

53. Grimsson, F. *et al*. (2021). How to extract and analyze pollen from internal organs and exoskeletons of fossil insects? *STAR Protoc*. 2, 100923

54. Peñalver, E. *et al*. (2015) Long-proboscid flies as pollinators of Cretaceous gymnosperms. *Curr. Biol.* 25, 1917–1923

55. 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

56. Friis, E.M. (1985) Structure and function in late Cretaceous angiosperm flowers. *Biol. Skr.* 25, 1–37

57. Taylor, D.W. and Hu, S. (2010) Coevolution of early angiosperms and their pollinators: Evidence from pollen. *Palaeontogr. Abt. B: Palaeophytologie.* 283, 103–135

58. Hu, S. *et al*. (2011) Pollen evidence for the pollination biology of early flowering plants. In *Evolution of Plant-Pollinator Relationships* (Patiny, S., ed.), pp. 165–236, Cambridge University Press

59. Labandeira, C.C. *et al*. (2016) The evolutionary convergence of mid-Mesozoic lacewings and Cenozoic butterflies. *Proc. R. Soc, B.* 283, 20152893

60. Khramov, A.V. *et al*. (2022) Possible long-proboscid insect pollinators from the Early Permian of Russia. *Curr. Biol*. 32, 1–6

61. Huang, D. *et al*. (2013) Mesozoic giant fleas from northeastern China (Siphonaptera): Taxonomy and implications for palaeodiversity. *Chin. Sci. Bull.* 58, 1682–1690

62. Huang, D.Y. *et al*. (2016) New fossil insect order Permopsocida elucidates major radiation and evolution of suction feeding in hemimetabolous insects (Hexapoda: Acercaria). *Sci. Rep.* 6, 23004.

63. Tihelka, E. *et al*. (2021) Angiosperm pollinivory in a Cretaceous beetle. *Nat. Plants* 7, 445–451

64. Bao, T. *et al*. (2022) Was the kateretid beetle *Pelretes* really a Cretaceous angiosperm pollinator? *Nat. Plants* 8, 38–40

65. Engel, M.S. (1995) *Neocorynura electra*, a New Fossil Bee Species from Dominican Amber (Hymenoptera: Halictidae). *J. N. Y. Entomol. Soc.* 103, 317–323

66. Peñalver, E. *et al*. (2006) Fig wasps in Dominican amber (Hymenoptera: Agaonidae). *Am. Mus. Novit.* 2006, 1–16

67. Friis, E.M. *et al*. (2011). *Early Flowers and Angiosperm Evolution.* Cambridge University Press

68. Barba-Montoya, J. *et al*. (2018) Constraining uncertainty in the timescale of angiosperm evolution and the veracity of a Cretaceous Terrestrial Revolution. *New Phytol.* 218, 819–834

69. Li, H.T. *et al*. (2019) Origin of angiosperms and the puzzle of the Jurassic gap. *Nat. Plants* 5, 461–470

70. Khramov, A.V. *et al*. (2023) The earliest pollen-loaded insects from the Lower Permian of Russia. *Biol. Lett.* 19, 20220523

71. Truman, J.W. (2019) The Evolution of Insect Metamorphosis. *Curr. Biol.* 29, R1252–R1268

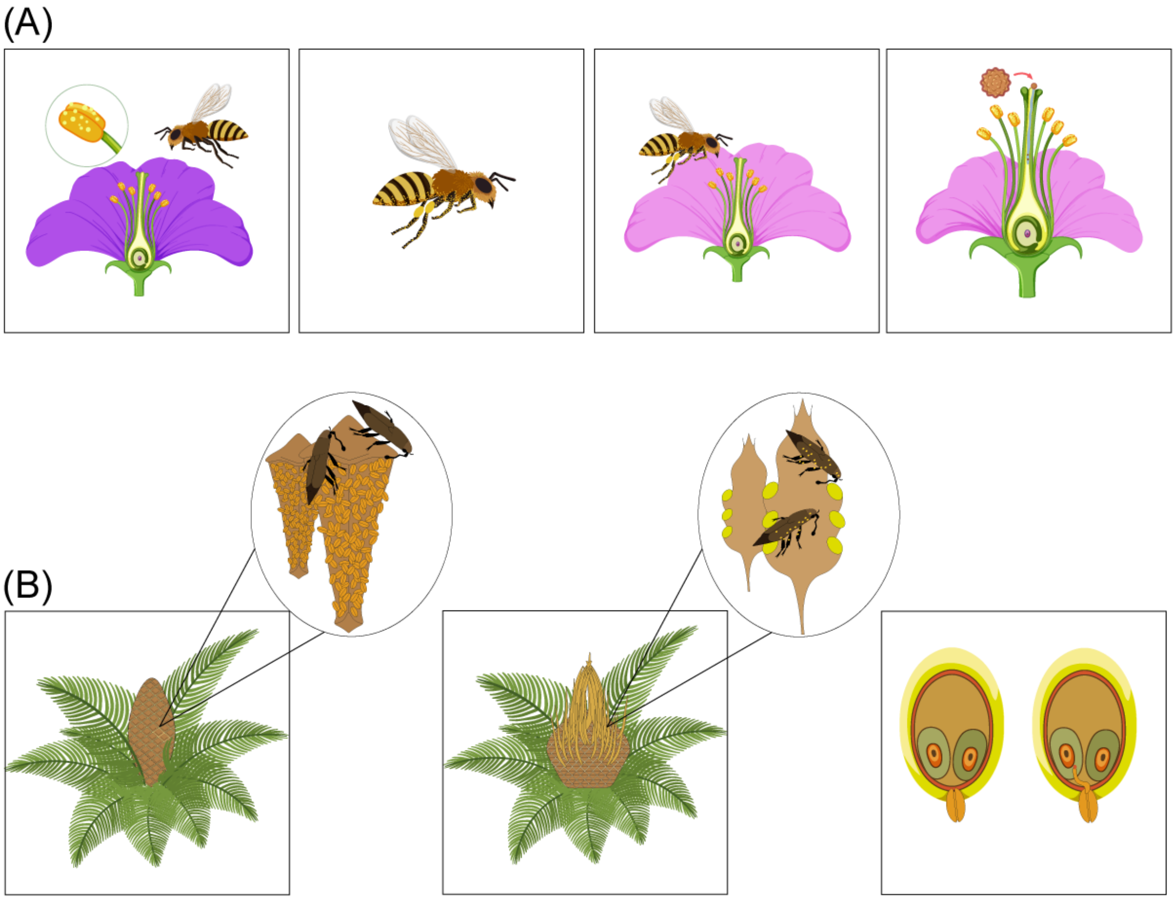

Figure 1. Pollination process. (A) Zoophilous pollination in angiosperms with a bee acting as pollen vector carrying the pollen from one flower to another; (B) gymnosperm with beetles acting as pollen vectors carrying the pollen from a male cone of a cycad (left) to a female cone (centre) with ovaries (right).

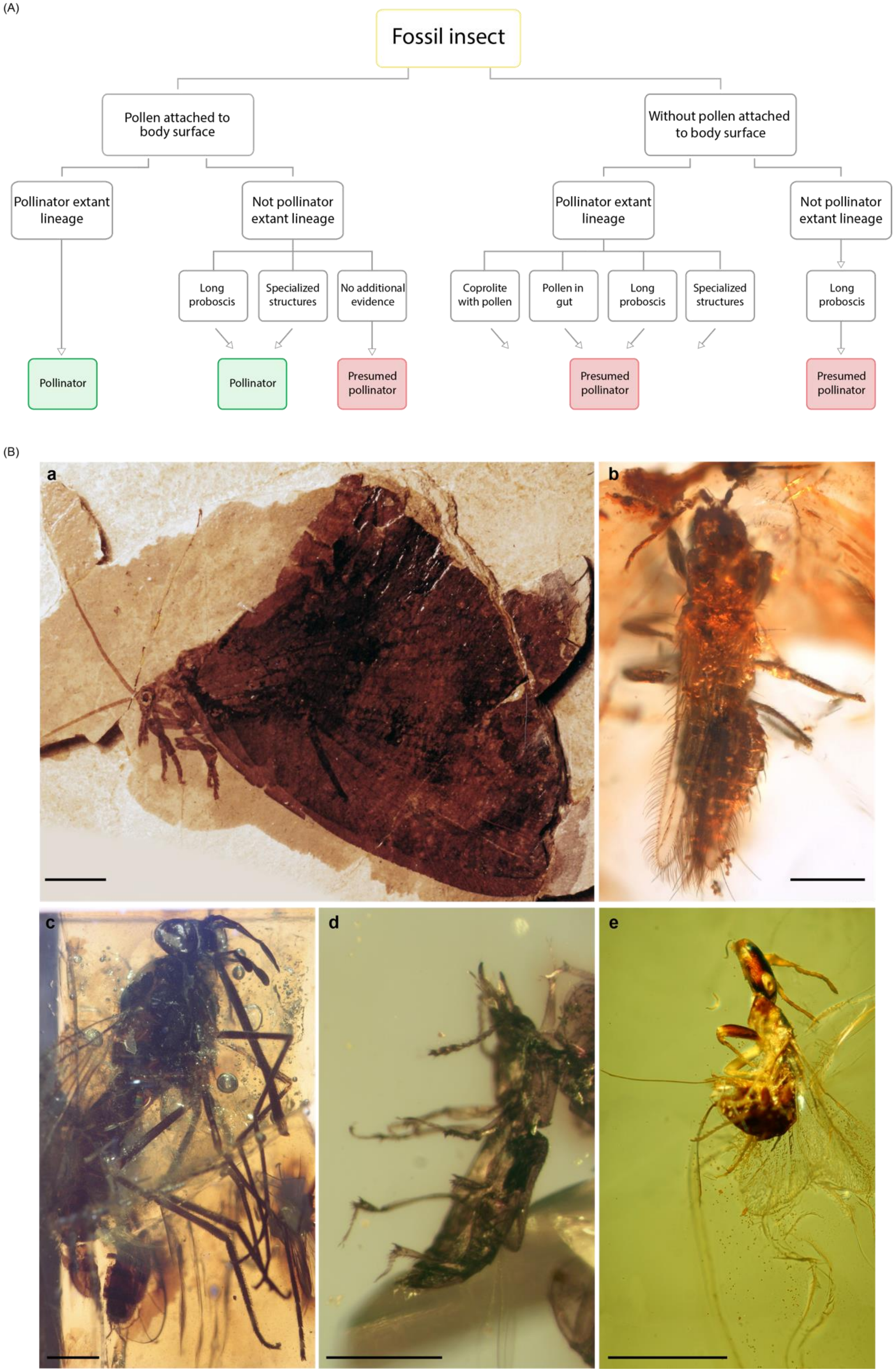
(A)
Fossil insect
Pollen attached to body surface
Without pollen attached to body surface
Pollinator extant lineage
Not pollinator extant lineage
Pollinator extant lineage
Not pollinator extant lineage
Long proboscis
Specialized structures
No additional evidence
Coprolite with pollen
Pollen in gut
Long proboscis
Specialized structures
Long proboscis
Pollinator
Pollinator
Presumed pollinator
Presumed pollinator
Presumed pollinator
(B)
a
b
c
d
e

Figure 2. Classification criteria. (A) Schematic key of the criteria that must be accomplished for a fossil insect to be considered as a pollinator or presumed pollinator. The “Long proboscis” criterion must follow the characteristics described in [48], specific for imbibing liquids. (B) Some pollinator fossil insects with extinct families marked † below: (a: Neuroptera: †Kalligrammatidae (Middle-Late Jurassic, Karatau, Kazakhstan), scale bar 1 cm; b: Thysanoptera: Melanthripidae (Early Cretaceous, Peñacerrada I, Spain), scale bar 0.2 mm; c: Diptera: †Zhangsolvidae (Early Cretaceous, El Soplao, Spain), scale bar 1 mm; d: Coleoptera: Kateretidae (Late Cretaceous, Myanmar), scale bar 0.5 mm; e: Hymenoptera: Agaonidae (Early Miocene, Dominican Republic) scale bar 1 mm).

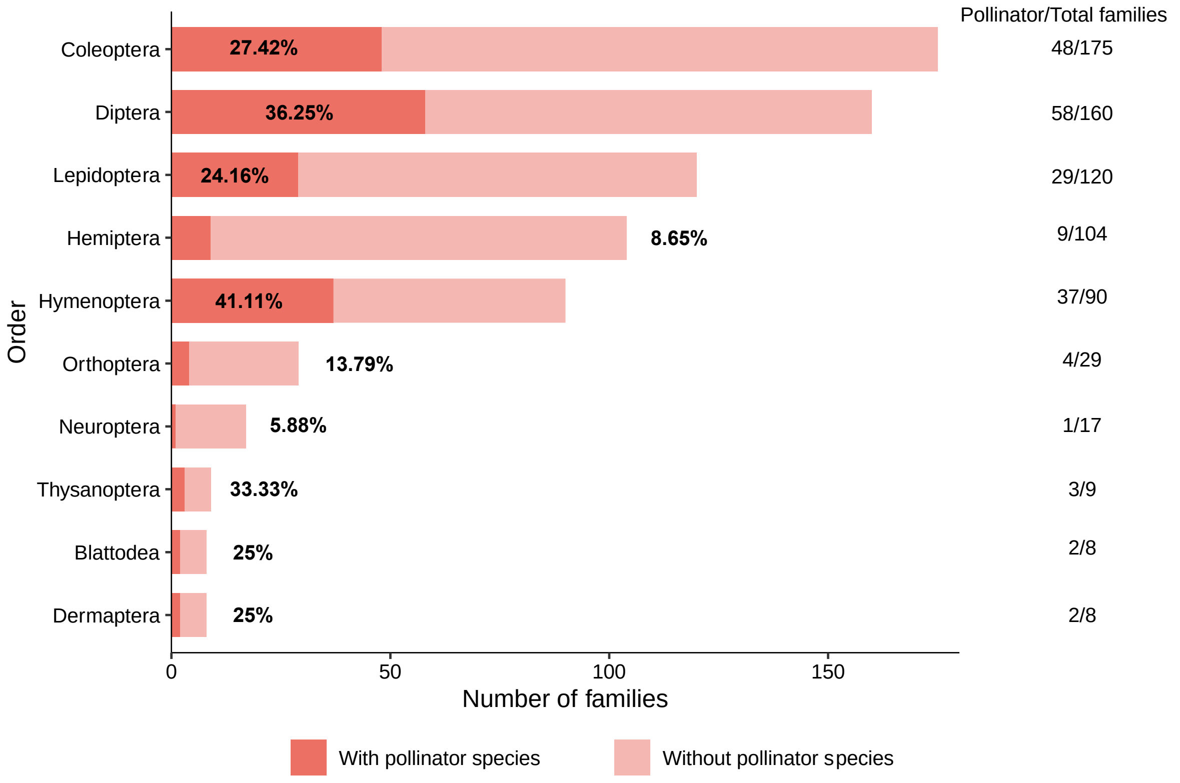

Figure 3. Distribution of extant pollinating insects. Extant insect families considered pollinators are arranged by order. Bars show the number of insect families in each order separated into families where pollinators are currently known to be present and those where they are not. The distribution from top to bottom is arranged by family diversity. Values used for percentage calculation of pollinator extant families were obtained from https://www.royensoc.co.uk/understanding-insects/classification-of-insects/.

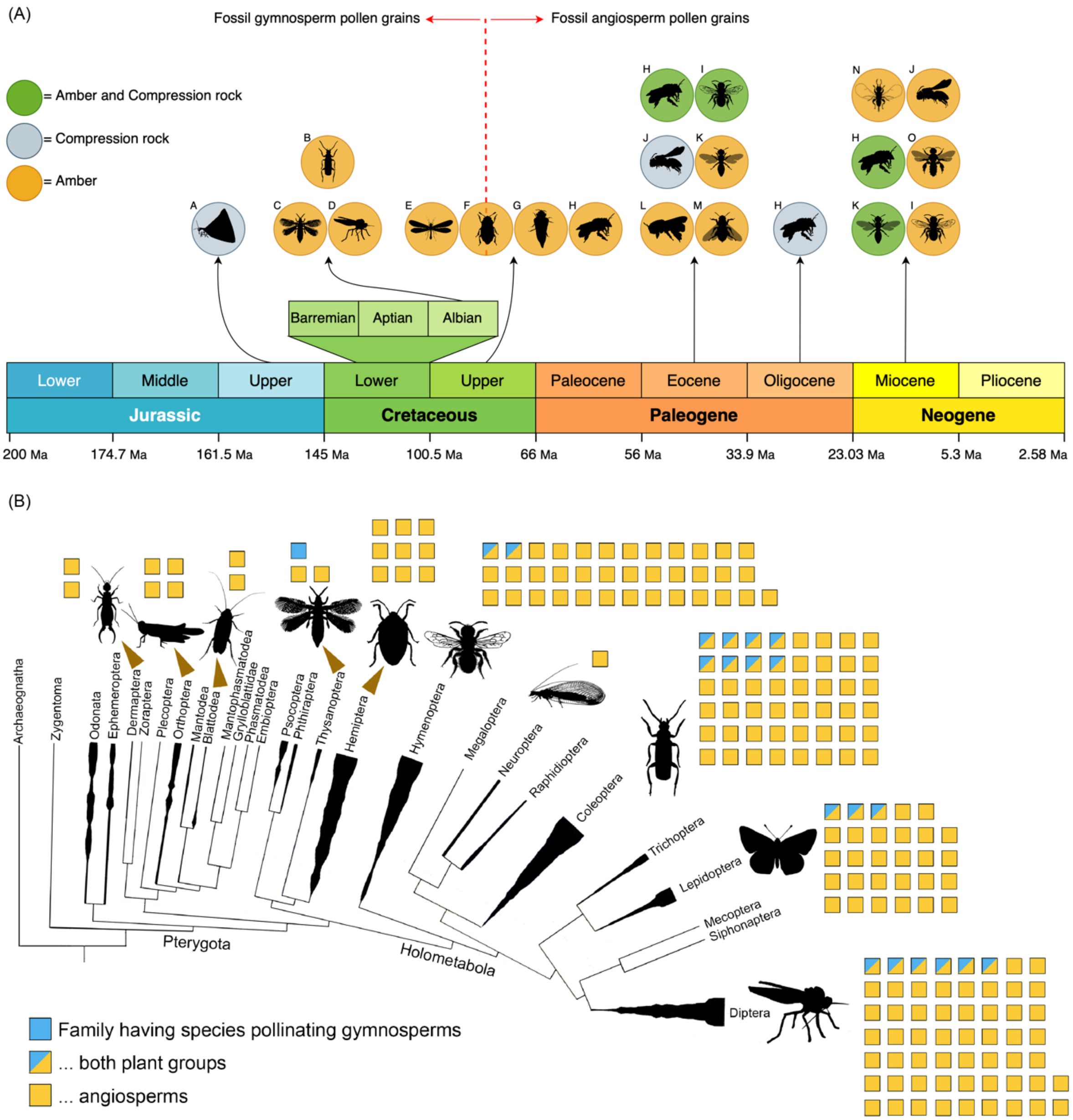

Figure 4. Insect pollinators in deep time. (A) Chronological distribution of the families containing any fossil insect species considered as pollinators according to the newly proposed criteria. Each circle shows a different family, with extinct families marked † below. On the left of the red dotted line are indicated families with a suggested gymnosperm host and on the right families with a suggested angiosperm host; the figure F corresponds to Coleoptera: Kateretidae, suggested from both host

groups. Only silhouettes A and F are newly created (Families are A: Neuroptera: †Kalligrammatidae; B: Coleoptera: Oedemeridae; C: Thysanoptera: Melanthripidae; D: Diptera: †Zhangsolvidae; E: Mecoptera: †Pseudopolycentropodidae; F: Coleoptera: Kateretidae; G: Coleoptera: Mordellidae; H: Hymenoptera: Apidae; I: Hymenoptera: Megachilidae; J: Hymenoptera: Andrenidae; K: Hymenoptera: Halictidae; L: Hymenoptera: Melittidae; M: Hymenoptera: †Paleomelittidae; N: Hymenoptera: Agaonidae; O: Hymenoptera: Colletidae). (B) Extant pollination of gymnosperms and angiosperms by insect families having pollinator species mapped onto the insect phylogeny. In the major orders the width of the lines show the number of families through time. Reprinted from The Evolution of Insect Metamorphosis, Truman, J.W, *Curr. Biol.* 29, R1252–R1268, 2019, with permission from Elsevier [71]. Silhouettes B–E, G–I used to illustrate Fig. 4.A, and some silhouettes in Fig 4.B were obtained from the public domain at http://www.phylopic.org.

Figure 5. Paleoecological reconstruction of insect pollination of gymnosperms and angiosperms during the Cretaceous. To the left of the resiniferous trunk is represented the Albian (Lower Cretaceous), with a ginkgolean with Melanthripidae thrips, a cycad host with Oedemeridae beetles, and a bennettitalean with Zhangsolvidae fly, based on Spanish amber fossil records. At the right of the trunk is represented the Cenomanian (Upper Cretaceous), with water lilies with Kateretidae beetles at the bottom, a cycad host with Kateretidae beetles, and a eudicot angiosperm with Mordellidae beetles at the front, based on Myanmar amber fossil records. Art by J.A. Peñas.