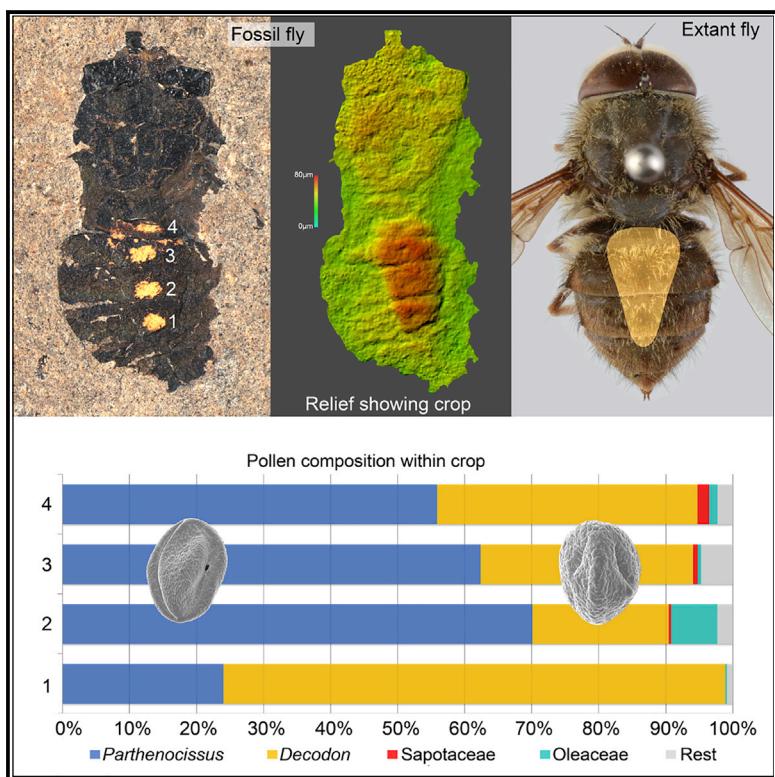


The last meal of an Eocene pollen-feeding fly

Graphical Abstract



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In brief

Direct evidence for pollen feeding is very rare in the fossil record. Wedmann et al. analyze the last meal of pollen of a fossil tangle-veined fly and infer on its ecology and behavior.

Highlights

- A newly described fossil nemestrinid fly preserves its last pollen meal in its crop
- Analysis of crop content reveals consumption of pollen of mainly two plant genera
- Inferences on foraging and feeding behavior can be drawn from the fossil
- Implications of similar feeding behavior can be deduced for extant relatives

Report

The last meal of an Eocene pollen-feeding fly

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SUMMARY

One of the most important trophic interactions today is that between insects and their floral hosts. This biotic association is believed to have been critical to the radiation of flowering plants and many pollinating insect lineages over the last 120 million years (Ma). Trophic interactions among fossil organisms are challenging to study, and most inferences are based on indirect evidence. Fossil records providing direct evidence for pollen feeding, i.e., fossil stomach and gut contents, are exceptionally rare.^{1,2} Such records have the potential to provide information on aspects of animal behavior and ecology as well as plant-animal interactions that are sometimes not yet recognized for their extant relatives. The dietary preferences of short-proboscid nemestrinids are unknown, and pollinivory has not been recorded for extant Nemestrinidae.³ We analyzed the contents of the conspicuously swollen abdomen of an ca. 47.5 Ma old nemestrinid fly of the genus *Hirmoneura* from Messel, Germany, with photogrammetry and state-of-the-art palynological methods. The fly fed on pollen from at least four plant families—Lythraceae, Vitaceae, Sapotaceae, and Oleaceae—and presumably pollinated flowers of two extant genera, *Decodon* and *Parthenocissus*. We interpret the feeding and foraging behavior of the fly, reconstruct its preferred habitat, and conclude about its pollination role and importance in paratropical environments. This represents the first evidence that short-proboscid nemestrinid flies fed, and possibly feed to this day, on pollen, demonstrating how fossils can provide vital information on the behavior of insects and their ecological relationships with plants.

RESULTS AND DISCUSSION

Systematic paleontology

Tangle-veined flies comprise 277 extant species in 17 genera.⁴ Their fossil record ranges from the Jurassic to the Eocene and includes about 25 species assigned to 13 genera.⁵ Most fossil nemestrinids have been described from Mesozoic sediments, with only seven species (including the record presented herein) reported from the Cenozoic of Florissant, USA,⁶ and Messel, Germany.⁷ Morphological terminology and vein nomenclature are as follows:⁸ Diptera Linnaeus, 1758 Nemestrinidae Macquart, 1834 *Hirmoneura* Meigen, 1820 *Hirmoneura messelense* sp. nov. (Figures 1A–1C and 2; <http://zoobank.org/NomenclaturalActs/07876388-5E17-4737-A4C9-FA4074A07427>).

Etymology

The specific epithet is derived from Messel, the fossil site where the specimen was found.

Holotype

Specimen SF-Mel7548. Slab A and counterslab B, deposited at Senckenberg Forschungsinstitut und Naturmuseum Frankfurt/M, Germany (SF).

Locality and horizon

Messel Pit (latitude 49°55'N, longitude 8°45'E) near Darmstadt, Hesse, Germany, grid square G8/9. Messel Formation, lower Middle Eocene (Lutetian, MP 11), 3.25 m–3.55 m above local stratigraphic marker level alpha. The sediments of the Messel Pit were deposited in a maar lake created by explosive volcanic activity.^{9,10} Radiometric dating of a basalt fragment underlying the fossiliferous sediments from the Messel Pit suggests an age for the eruption of about 48.2 Ma and an according age of the fossil of at least 47.5 Ma.^{11,12}

Diagnosis

Body length around 11 mm. Compound eyes close together dorsally, with no setae between ommatidia visible. Thorax has rather long setae. Wings slender, around 9 mm long, around 2.5 mm wide, and width/length ratio about 0.29. Wing membrane without microtrichia. Wing venation has very straight diagonal vein and diagonal vein distad middle of wing; Sc joining C relatively close to wing tip; R₁ and R₂ slightly convergent; R₃ present; cross-vein r-m absent; cell br reaching into distal half of wing; CuA₁ and M₂ confluent (meeting at single point); no supernumerary cross-veins present; apices of CuA₂ and A₁ highly convergent, thus cell cup closed, i.e., present; and CuP moderately developed.

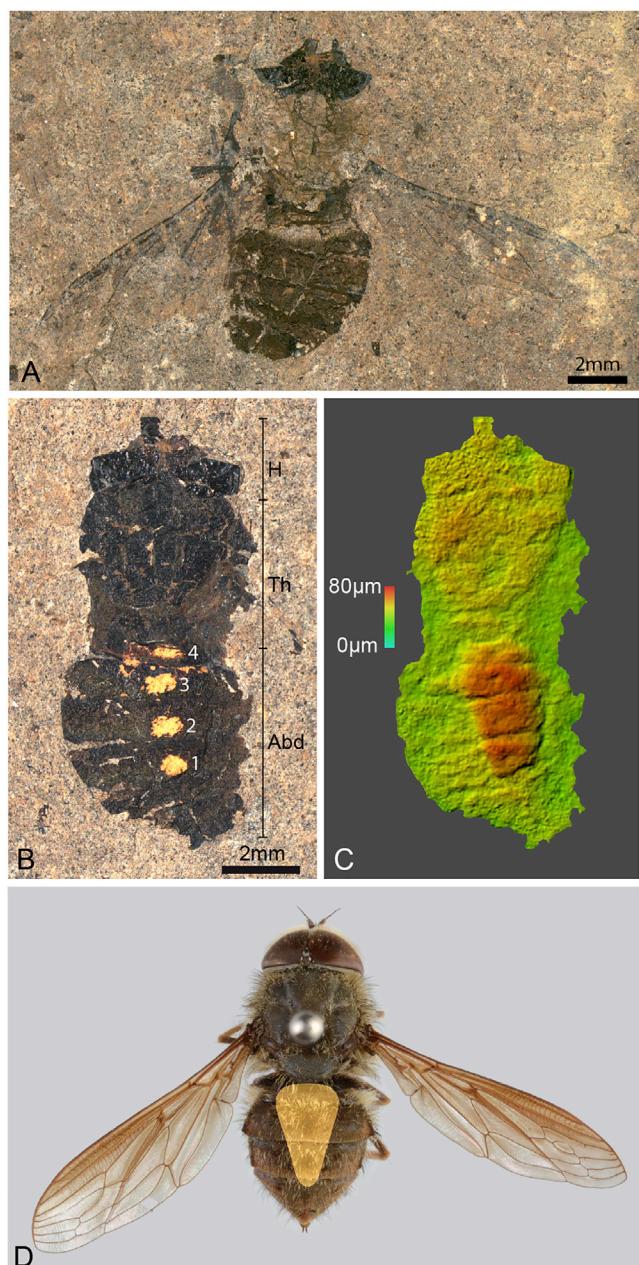


Figure 1. Fossil and extant tangle-veined flies in dorsal view

(A–C) *Hirmoneura messelense* sp. nov. (SF-Mel7548) from Grube Messel fossil site.

(A) Slab A showing body and wings.

(B) Counterslab B showing body and wing remains, numbers 1–4 on abdomen indicate position of samples (yellow areas) where cuticle has been removed to expose pollen content of crop.

(C) Color-coded 3D relief of SF-Mel7548 slab B, reconstructed from photogrammetry. Elevated areas, orange-red; lower areas, green-blue. Orange area shows engorged crop.

(D) Extant *Hirmoneura maculipennis* Macquart, 1850, with position of crop illustrated.

Abd, abdomen; H, head; Th, thorax.

See Table S1 for details on other animal fossils with pollen inside their guts.

Description

Gender unknown. Compression fossil preserved in dorsoventral position, split into two parts: slab A and counterslab B. Slab A (Figure 1A) contains parts of body, with an indentation in middle of abdomen, and large parts of both wings. Measurements for slab A are as follows: maximum preserved body length along mid-axis 10.9 mm; maximum preserved width of head 4.0 mm; maximum preserved width of abdomen 4.8 mm, measured at middle of abdomen; maximum length of right wing: ca. 8.8 mm; and maximum width of right wing 2.6 mm. Slab B preserves body (Figure 1B), including pollen load located inside abdomen (Figure 1C), and remains of one wing. Measurements for slab B are as follows: maximum preserved body length along mid-axis 11.2 mm; maximum preserved width of head 3.9 mm; and maximum preserved width of abdomen 4.8 mm, measured at middle of abdomen. In the head, compound eyes are well preserved, close together dorsally; most of ocular surface is shiny and smooth, without setae between ommatidia, not even at eye margins. No other parts of head are identifiable as preserved: no ocelli detected; face indiscernible; and proboscis undetected (suggesting that proboscis is short or vestigial and non-sclerotized, consistent with species of *Hirmoneura*). The thorax is preserved as an imprint on slab A, with setae on all sclerites; setae are up to 0.5 mm long. On slab B, scutum and scutellum are barely distinguishable. Lower part of scutum is diagenetically broken into four similar parts, corresponding to positions of thoracic muscles; no transverse suture of scutum is visible. The wings on slab A are spread out from both sides of the body; wing bases and proximal parts of wings are obscured; wings are slender, max. width of right wing 2.6 mm, length of right wing ca. 8.8 mm; width/length ratio 0.295; wing membrane hyaline, microtrichia not visible. Venation with complete, very straight diagonal vein (Figure 2); Sc joining C relatively close to wing tip; R₁ and R₂ slightly convergent; R₃ present; crossvein r-m absent (i.e., R₄₊₅ and M₁₊₂ confluent); cell br long, extending into distal half of wing; CuA₁ and M₂ confluent; no supernumerary crossveins present; apices of CuA₂ and A₁ closely convergent, cell cup closed, i.e., present; CuP moderately developed, visible until CuA splits into CuA₁ and CuA₂; alula not preserved. Only slab A preserves parts of the legs, and no specific podites are identifiable. Six abdominal tergites are observable on slab A; masses of setae are visible on all six tergites, especially on posterior portions. Indentation on abdominal segments I–IV corresponds to bulge observed in slab B (Figures 1A–1C), representing crop filled with pollen (Figure 1D). Samples for pollen analysis were taken from each of the four anterior segments of slab B (Figure 1B). Concerning the coloration, the body is uniformly dark brown, and no color pattern is preserved.

Systematic placement

The wing venation of the fossil shows generalized nemestrinid features, which according to Bernardi¹³ allow placement in the genera *Hirmoneura* Meigen, 1820 or *Nemestrinus* Latreille, 1802: R₃ present; crossvein r-m absent; and R₄₊₅ and M₁₊₂ confluent. Because no supernumerary crossveins are present in the wings, the compound eyes are relatively close together and bare and no proboscis is visible (the proboscis was probably shorter than the head, and is probably obscured by it), systematic placement within *Nemestrinus* is excluded. The fossil

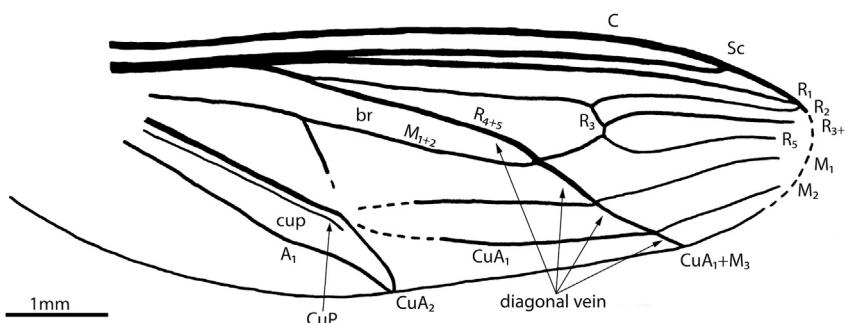


Figure 2. Reconstruction of the wing venation of *Hirmoneura messelense* sp. nov.

belongs to the morphologically diverse genus *Hirmoneura*, a group that is considered to be polyphyletic (p. 241 in Bernardi¹³), although this remains to be confirmed by comprehensive phylogenetic analyses.

Comparison with other species

The following differences between the fossil SF-Mel 7548 described herein and another nemestrinid fly from Messel, SF-Mel 6954, show that they belong to two different species: R_3 present in Mel 7548, absent in Mel 6954; Sc rather long in Mel 7548, distinctly shorter in Mel 6954; and cell *br* long in Mel 7548, shorter in Mel 6954. Comparison of the wing venation of Late Eocene nemestrinid flies from Florissant, USA⁶ also separates SF-Mel 7548 from other fossil species, especially *Hirmoneura willistoni*.¹⁴ In contrast to *H. messelense* sp. nov., a short cross-vein *r-m* is present in *H. willistoni*, and the diagonal vein is not straight, Sc is shorter, and a supernumerary crossvein is present between M_1 and M_2 .^{6,14} Two species of *Hirmoneura* from the mid-Cretaceous of Myanmar differ from the present Messel fossil by the absence of R_3 and in having an open cell *cup* (i.e., CuA_2 and A_1 are not strongly convergent to one another).⁵ The Lower Cretaceous species *H. richterae*,¹⁵ from Spain, and *H. elenae*,¹⁵ from Russia, also differ, owing to the absence of R_3 in these species. Because of differences in the wing venation placement in the subgenus *Hirmoneura* (*Eohirmoneura*) Rohdendorf, 1968 can be excluded.¹⁵ The cell *cup* is open, i.e., absent, in modern species of *Hirmoneura* (see p. 50 in Grimaldi⁵), which helps to distinguish *H. messelense* sp. nov. from extant species and suggests that the present fossil is perhaps part of a stem group, along with other fossil species, to the remainder of the genus.

Analysis of the fossil gut content

The present specimen of *H. messelense* (Figure 1) is unique in having preserved a distinct three-dimensional structure at the basal center of the abdomen (Figures 1B and 1C). No significant amounts of pollen could be found by fluorescence microscopy on the body surface or on the surrounding sediment. The hump in the abdomen is congruent with an engorged crop, in this case containing pollen grains, the fly's last meal. A crop is a dilated structure of the foregut where food can be stored before it passes to the gut for digestion;¹⁶ it has been documented that it may contain intact pollen from the crop of extant hover flies (Syrphidae).¹⁷

Comparison with the anatomy of extant flies shows that the crop is located in the first three abdominal segments,^{16,18} corresponding to the position of the hump in the fossil. The pollen

mass inside the fossil crop is visualized by a color-coded representation of the relief in Figure 1C. The position of the crop in extant flies is illustrated in Figure 1D. After removing the outer cuticle, we took four samples from inside the fossil crop for further analysis and determination. The sample sites are indicated in Figure 1B.

The state of pollen preservation allowed for reliable determination at either familial and/or generic levels. The pollen composition varies somewhat between the four sampled spots (Figure 3A), but the two main components are always pollen from *Decodon* J.F.Gmel., 1791 (water willow; Figure 3B) and *Parthenocissus* Planch., 1887 (creepers; Figure 3C). Pollen grains representing other plant taxa, including Sapotaceae (Figure 3D) and Oleaceae (Figure 3E), are rare. For detailed descriptions of the fossil pollen, refer to STAR Methods section Systematic palynology.

Photogrammetric analysis of the fossil provided an estimated volume of ca. $4.8 \times 10^8 \mu\text{m}^3$ for the pollen mass preserved in the fly's crop. Based on the three-dimensional extension of the crop and the sizes of the different pollen grains, it was possible to estimate the content of the crop in terms of number of pollen grains (Table 1). Given the parameters that influenced the crop (and its contents) prior to its present condition, such an estimate has a potentially wide error margin. However, the preservation of the pollen justifies such an approach, because the size and shape of the pollen has not been significantly modified during fossilization.

Today, a single flower of *Decodon* produces 70,000–110,000 pollen grains or 7,000–11,000 pollen per anther.¹⁹ Assuming that the fly consumed all pollen from a visited flower, a single flower could have provided all of the *Decodon* pollen found within its crop. This is highly unlikely, and the mixture of different pollen types (up to seven different taxa) occurring in each sample from one to four in the crop (Figures 1B and 2A) suggests interchanging and repeated visits to flowers of selected taxa.

Inferences of foraging and feeding behavior

Flies in general, and especially nemestrinids, are well-known flower visitors and important pollinators. For example, long-proboscid, tangle-veined flies in southern Africa form spectacular pollination systems with plants bearing tubular flowers,^{20,21} feeding exclusively on nectar and not on pollen.²² The ecological role of nemestrinids as pollinators is probably as old as the Jurassic.^{23,24}

For the short-proboscid, tangle-veined fly, to which *H. messelense* sp. nov. belongs, investigations on feeding mode are still lacking, and pollinivorous feeding behavior has so far not been recorded for any extant Nemestrinidae.³ The fossil tangle-veined fly presented herein clearly fed on angiosperm pollen and, consequently, represents the first direct evidence of a pollinivorous nemestrinid.

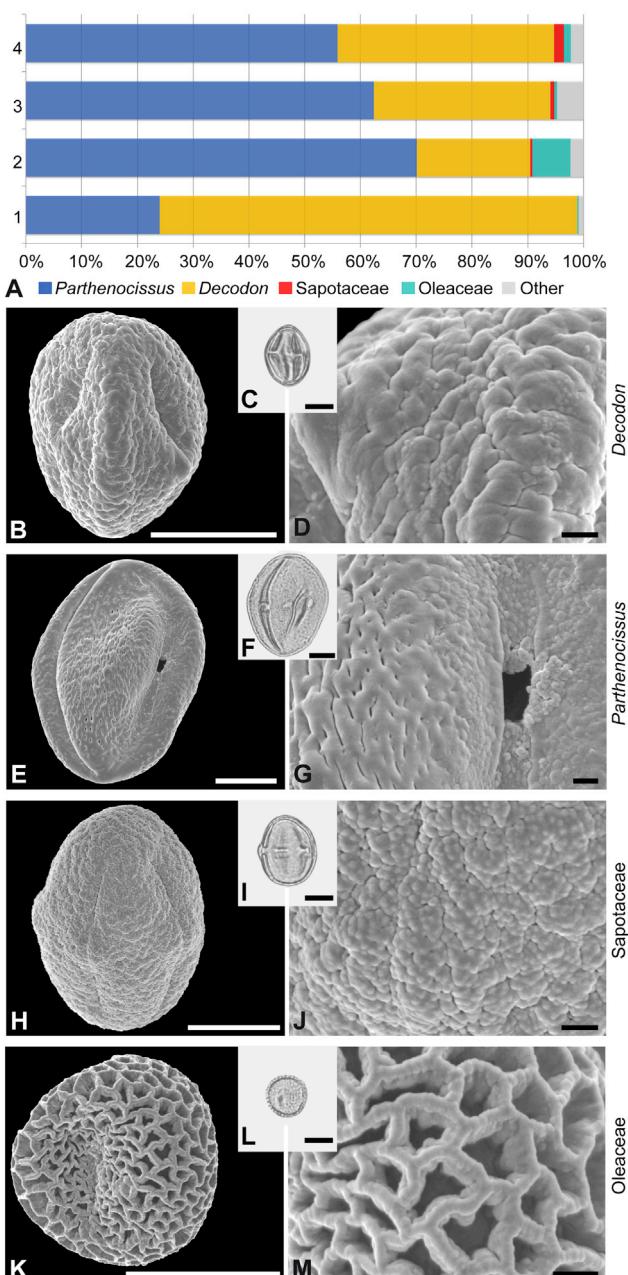


Figure 3. Fossil pollen from the crop of *Hirmoneura messelense* sp. nov. (SF-MeI7548; see Figure 1B for sample sites)

(A) Composition of crop content at the four sample sites, number of pollen grains analyzed: N(1) = 350; N(2) = 261; N(3) = 420; and N(4) = 399. (B-D) SEM and LM (C) images of fossil *Decodon* pollen from the sample. (E-G) SEM and LM images of *Parthenocissus* pollen from the sample. (H-J) SEM and LM images of *Sapotaceae* pollen from the sample. (K-M) SEM and LM images of *Oleaceae* pollen from the sample. Scale bars in (D), (G), (J), and (M), 1 μm; in other images, 10 μm. See Figures S1–S4 for details on pollen, Table S1 for details on animal fossils with pollen inside their guts, and Tables S2 and S3 for information on Messel plants.

Assuming that the flower structures and awards in extant plants, e.g., *Decodon*, *Parthenocissus*, *Sapotaceae*, and *Oleaceae*, represent the Eocene scenario at Messel, the presence

of nectaries suggests a direct attractant for pollinators encouraging regular and repeated visits to the flowers. However, in the present case, it is uncertain whether odor from nectaries, pollen, or both was acting as the primary attractant. Still, pollen attraction is more selective than nectar attraction,²⁵ and the number of different pollen types found in the gut of the fly implies a generalist (polyphagous) behavior when visiting pollen resources. Because nectaries occur in all nearest living relatives (genus or family) of the fossil plant taxa, it is likely they acted as primary attractants for the fly that then fed on pollen when visiting the flowers.

Even though the Messel flora (Tables S2 and S3) is dominated by woody elements typical of dense (sub)tropical forests, the stomach content of the fly indicates that its feeding grounds were not deep within a thick and enclosed forest or high up within a complex canopy. The large number of *Decodon* pollen found in all segments of the crop shows that the fly repeatedly visited flowers of this taxon. *Decodon* is an herbaceous plant, usually 0.5–2 m tall, and growing in shallow waters and along the borders of various waterbodies.^{26,27} The high number of *Parthenocissus* pollen also supports the reconstruction that the fly fed on plants growing at the forest margin and along lakes, because the greenery and flowers of this taxon typically occur on the sunny (open) canopy side of bushes and trees and on rocky surfaces at ground level.^{28–30} The *Oleaceae* and *Sapotaceae* pollen probably originate from small trees or bushes growing at the forest margin and close to water. Because *Sapotaceae* and *Oleaceae* pollen, as well as three additional unknown pollen types, occur mixed among the *Decodon* and *Parthenocissus* pollen, their parent plants must have grown in close vicinity. The fly would not have wasted energy on “long-distance” flights between flowers during feeding, and the mean interplant distance for flower-visiting flies is usually less than 1 m.³¹ A comparable open-habitat scenario has been documented for extant pollinivorous polyphagous hover flies (e.g., *Platycheirus* Lepeletier and Serville, 1828; *Epsyrhus* Matsumura and Adachi, 1917; and *Sphaerophoria* Lepeletier and Serville, 1828), with the polyphagous taxa common in open habitats, similar to that reconstructed for *H. messelense*, although oligophagous hover flies are more forest dwelling.³²

Shared properties among extant flowers of *Decodon* and *Parthenocissus* include symmetry, small size, presence of nectaries, and that flowers become open with recurved sepals/petals during anthesis, exposing nectaries and anthers to insect visitors. Flowers of both *Decodon* and *Parthenocissus* occur in clusters and fall within the “generalist fly flower” syndrome as defined by p. 308 in Willmer.³¹ Such flowers are favored by various flies but are also visited by other groups of insects (beetles, wasps, etc.). Flies are often flexible fliers capable of hovering mid-air and landing and taking off in any direction. The morphology, especially of the head, and the gut content of the fossil *Hirmoneura* suggests that it did not hover above flowers while feeding, as long-proboscis nemestrinids presently do,²² but landed on the flower before engulfing pollen from anthers. In both *Decodon* and *Parthenocissus*, flowers are closely spaced so the fly could easily have walked directly between flowers or across the inflorescence surface to a neighboring flower for further feeding.

Although no pollen grains were found here, the setae on all parts of the thorax and abdomen of the fossil likely sufficed for

Table 1. Estimated number of pollen grains in the crop of *Hirmoneura messelense* sp. nov. (SF-MeI7548)

	Volume per grain, ca. (μm^3)	Number of grains, ca.	Percentage of volume
Decodon	1,900	>103,000	41.42%
Parthenocissus	15,000	>17,000	53.1%
Oleaceae	1,300	ca. 8,200	2.23%
Sapotaceae	4,500	ca. 750	0.71%
Other	3,000	ca. 4,000	2.54%
Total		>133,000	

See Figures S1–S4 for details on pollen, Table S1 for details on animal fossils with pollen inside their guts, and Tables S2 and S3 for information on Messel plants.

pollen transport, suggesting the fly could have been a potential pollinator. At present, flies are frequent flower visitors and compensate for their “poor” pollen transport by large numbers.³³ Investigation of the role of flower-visiting flies has long been neglected, and their feeding behavior is currently understudied. The pollinivorous fossil *Hirmoneura* suggests that this lineage might have played an important role in pollen transport and the reproductive cycle of several plant taxa occupying open habitats along the paleo-Messel lake. This lends credibility to the hypothesis of Willmer³¹ that anthophilous flies may be more important flower visitors than bees in some of today’s tropical environments and that non-syrphid flies may be more important pollinators than previously assumed.³⁴ It also provides for a prediction that modern *Hirmoneura*, and potentially other short-proboscid Nemestrinidae, are pollinivorous, like their fossil progenitor, and have underappreciated roles as pollinators.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2021.02.025>.

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AUTHOR CONTRIBUTIONS

S.W. and F.G. designed research; S.W., T.H., R.Z., and F.G. performed research; S.W., T.H., and F.G. analyzed data; and S.W., T.H., M.S.E., and F.G. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Acetic anhydride (99% CH ₃ CO ₂ O)	https://www.sigmaaldrich.com	CAS-nr. 108-24-7
Sulfuric acid (95-97%, H ₂ SO ₄)	https://www.sigmaaldrich.com	CAS-nr. 7664-93-9
Glycerine	https://www.sigmaaldrich.com	CAS-nr. 56-81-5
SEM. Specimen Stubs	https://www.agarscientific.com	G301
LM Microscope Super Frost Slides, White	https://www.agarscientific.com	L4339
Software and algorithms		
PhotoScan Professional	Agisoft LLC, 11 Degtyarniy per., St. Petersburg, Russia, 191144	v. 1.4.3
Adobe Photoshop	Adobe Systems	CS3
Adobe Illustrator	Adobe Systems	CS3
Microsoft Excel	Microsoft Corporation	v. 16
ProgRes CapturePro Microscope Camera Software	Jenoptic	v. 2.9

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be answered by the Lead Contact, Sonja Wedmann (sonja.wedmann@senckenberg.de)

Materials availability

The holotype SF-MeI7548 (slab A and counterslab B) and the extracted pollen are deposited under the same number in the collection of the Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt/M., Germany (SF). The fossil originates from the Eocene maar lake sediments of the Messel Pit near Darmstadt, Hesse, Germany.

Data and code availability

A list of all fossil insects/animals with pollen preserved inside their gut or bowels, detailed descriptions of the pollen found on the fossil *Hirmoneura* fly, and information concerning the paleoflora of Messel can be found in the [Supplemental information](#).

This published work has been registered with ZooBank (<http://zoobank.org/References/0FE1EB15-4E20-433E-A069-475E0F10C9CA>) and the nomenclatural act is registered under <http://zoobank.org/NomenclaturalActs/07876388-5E17-4737-A4C9-FA4074A07427>

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The specimen described in this study is deposited and accessible at the Senckenberg Research Station Messel, Senckenberg Research Institute Frankfurt (SF), Germany under collection number SF-MeI7548. The fossil is stored permanently in glycerol to prevent damage by desiccation. All necessary permits were obtained for the described study, which complied with all relevant regulations. In all years in which SF dug for fossils in the Messel Pit Fossil Site, it had permission to do so issued by the following authority: Landesamt für Denkmalpflege Hessen, hessenARCHÄOLOGIE, Schloss Biebrich, 65203 Wiesbaden, Germany.

METHOD DETAILS

Taxonomy

The systematic classification of Nemestrinidae follows Bernardi¹³; wing venation terminology follows Teskey.⁸

Photogrammetry

Investigation of the fossil showed a distinctive relief, especially in the abdomen. To document and analyze this relief a set of 56 images of slab B were taken to be used in a photogrammetric reconstruction. 33 images were taken while rotating the specimen in front of the camera so each image encompassed the complete fossil (without wing remains). The remaining 23 images were taken while moving the specimen longitudinally and transversally in front of the camera with each image capturing $\geq 50\%$ of the fossil. An Olympus Tough TG-5 set to “Microscope” mode was used for taking the images with a slightly oblique view on the fossil. Reconstruction of a 3D-model and the color-coded image shown in Figure 1C was done with Agisoft PhotoScan Professional v. 1.4.3. The results of the 3D-reconstruction were used to estimate the volume of the engorged crop.

Initial search for pollen

The fossil and its surrounding matrix were checked for pollen with a Zeiss AXIO Imager.M2 fluorescence microscope with filter set 38 HE.

Pollen preparation

The fossil fly was examined with a dissecting microscope (stereomicroscope) and parts from the four main protruding segments of its digestive system (crop) were sampled. The cuticle was removed in four places, exposing the pollen mass preserved *inside* the crop of the abdomen of the fossil. Clumps of pollen were extracted using a teasing needle and transferred into a drop of glycerine on a glass microscope slide (L4339 Super frost slide, white, Agar Scientific). The pollen grains were studied with an erect image compound microscope (Nikon Eclipse 80i, Light Microscope), the pollen clumps were dismantled using a micromanipulator, and each pollen was assigned to a morphotype and counted. Representatives from each morphotype were transferred using a micromanipulator into a drop of readymade acetolysis fluid (nine to one mix of 99% acetic anhydride and 95%–97% sulfuric acid) on a glass microscope slide (see p. 103, Acetolysis the fast way, in Halbritter et al.³⁵). The slides were then heated over a candle flame for a short time to dissolve extra organic material on the pollen grain surfaces and to stain the grains for photography with LM. The pollen grains were then transferred into fresh drops of glycerine on a new glass slide and photographed with LM (using Jenoptik ProgRes SpeedXT^{core}5 CCD Camera with ProgRes CapturePro microscope camera software). The same individual pollen grains were then transported using a micromanipulator (human nasal hair attached to a teasing needle (see p. 122 in Halbritter et al.³⁵) onto scanning electron microscopy (SEM) stubs (G301 SEM specimen stubs, Agar Scientific), washed with ethanol, sputter coated with gold (Bio-Rad SC500, Sputter Coater), and photographed with SEM (Jeol JSM-6400, Scanning Electron Microscope) (see p. 122, Applying the single grain method, in Halbritter et al.³⁵).

Estimating pollen grain numbers

For calculating the volume of the pollen-filled crop its margin was assumed to be where the hump becomes level with the surrounding cuticle. This margin was marked in Agisoft PhotoScan and the enclosed area and volume calculated. For calculating the volume, it was assumed that the hump is a structure lying on a flat surface extending between the marked margins. This assumption certainly contains an error since it seems quite probable that the crop and its contents extend somewhat below this level and into the matrix. Therefore, the estimated number of pollen grains most likely represents a lower margin. Volumes of pollen grains were calculated assuming them to be approximate rotational ellipsoids with measurements taken from the microscopic images of actual fossil specimens. The size of “other” pollen was calculated from the means of the dimensions of the four identified groups.

Systematic palynology

Pollen descriptions include the most diagnostic features observed with LM and SEM. The pollen terminology for LM follows Punt et al.³⁷ and for SEM Halbritter et al.³⁵ The classification to family follows Chase et al.³⁸

Rosids

Order Vitales Reveal

Family Vitaceae Juss.

Genus *Parthenocissus* Planch.

Parthenocissus sp. (Figure S1)

Description

Pollen prolate (P/E ratio 1.1–1.5), outline triangular to loped in polar view, elliptic in equatorial view, shape triangular dipyramid to ellipsoid; polar axis 40–46.3 μm in LM, 34.2–42.8 μm in SEM, equatorial diameter 25–35 μm in LM, 24.8–28 μm in SEM; exine 1.5–2.8 μm thick (LM), nexine thinner than sexine, nexine thickened in area around endopori; pollen tricolporate, colpi long, endopori

lalongate elliptic; pollen wall semitectate (SEM); sculpture scabrate to reticulate in LM, heterobrochate reticulate to microreticulate, fossulate, and perforate in SEM, muri rounded, lumina irregular in outline, mostly narrow and slit-like, longest diameter of lumina oriented parallel to polar axis, lumina increasing in size toward polar areas and decreasing in size close to colpi, lumina fused along colpi and forming a margo, margo psilate, colpus membrane nanorugulate to nanoverrucate and granulate (SEM).

Remarks

Pollen from most of the extant species of *Parthenocissus* has been studied with LM and SEM by Lu et al.,²⁹ and also by Wang et al., Perveen and Qaiser, Li et al., and Miyoshi et al.^{39–42} *Parthenocissus* is a small temperate to tropical genus, comprising ca. 15 species of woody climbers and lianas, occurring in North America (extending to Central America and the Caribbean region) and East Asia (extending into the western Ghats of India and Sri Lanka, Java, and northern Thailand).^{28–30} *Parthenocissus* flowers are small, usually ≤5 mm, greenish, hermaphrodite, insect pollinated, and occur in clusters, on a much-branched inflorescence (panicle). The flower bears five sepals that form a circular collar around the base of the flower, five cucullate petals arise alternate to the sepals and opposite to the stamens. The anthers are tetrasporangiate and introrse. The gynoecium is composed of a basal disc, superior ovary, a short style, and short stigma. The stamens and gynoecium have a bright yellow color, and nectar can be found at the reduced basal disc. At anthesis, the petals reflex to reveal anthers, exposing the reproductive structures and nectar rewards for floral visitors.^{29,30,43} Fossil pollen grains of *Parthenocissus*, studied with combined LM and SEM, have been reported from the Eocene of Austria,^{44,45} the Oligocene of Germany,⁴⁶ the Miocene of Iceland,⁴⁷ Austria,^{48–51} and Greece,⁵² and the Pleistocene of Albania.⁵³ Similar fossil pollen, *Tricolporopollenites macrodurensis* Thomson et Pflug (Vitaceae), studied with LM were described from the dispersed pollen record of Messel by Thiele-Pfeiffer⁵⁴ (pl. 12, Figures 1–7). Fossil seeds of *Parthenocissus*, *P. britannica* (Heer) Chandler, have also been recorded from Messel by Collinson et al.⁵⁵ (pl. 42, figs i–k & m–r).

Malvids

Order Myrtales Juss. ex Bercht. et J.Presl.

Family Lythraceae J.St.-Hil.

Genus *Decodon* J.F. Gmel.

Decodon sp. ([Figure S2](#))

Description

Pollen prolate (P/E ratio 1.1–1.8), outline triangular to circular in polar view, elliptic in equatorial view, shape triangular dipyramid to ellipsoid, prominent meridional ridges in mid-mesocolpial areas; polar axis 17.5–25 µm in LM, 14.3–21.7 µm in SEM, equatorial diameter 15–20 µm in LM, 12.3–17.9 µm in SEM; exine 1.1–2.0 µm thick, nexine thinner than sexine, sexine thickened at meridional ridges and in polar regions; pollen tricolporate, colpi often broader and rounded at apices, usually constricted in the equatorial region, pori elliptic, sexine forming a bridge in area of endopori; pollen wall tectate (SEM); sculpture psilate to scabrate in LM, rugulate to microrugulate, microverrucate, fossulate, perforate in SEM, colpus membrane nanoverrucate (SEM).

Remarks

Pollen of *Decodon verticillatus* (L.) Ell. has been studied in detail with both LM and SEM by Lieux, Graham et al., and Grímsson et al.^{56–58} *Decodon* is a monotypic, herbaceous, perennial, which is restricted to eastern North America where it inhabits freshwater marshes, swamps, bogs, borders of lakes and rivers, and forested wetlands.^{26,27} Flowers of *Decodon* are usually 25–30 mm in size, pinkish to purple, hermaphrodite, insect pollinated, and occur in tufts in the axils of leaves. The flowers are actinomorphic, five-sided, with two whorls of stamens (usually five per whorl) and a superior ovary. Sepals, petals, and stamens are fused basally forming a calyx tube. Stamens of the lower whorl alternate with stamens of the upper whorl. Nectaries are gynoecial, rising up the ovary to a level above the locule bases. The plant produces 35,000–55,000 pollen per anther whorl, and 70,000–110,000 pollen per flower. The flowers are visited/pollinated by butterflies, bees, wasps, and flies.^{19,59–61} Fossil pollen of *Decodon*, identified using combined LM and SEM, has been reported from the Eocene of Canada⁵⁸ and Germany;^{62,63} the Oligocene of Austria;⁵⁸ the Miocene of Iceland, Spain, Germany, Austria, Turkey, and China;^{58,64–66} and the Pliocene of Germany.^{58,67} Fossil infructescences and fruits of cf. *Decodon* have been described from Messel by Collinson et al.⁵⁵ (pls. 25 & 26).

Asterids

Order Ericales Bercht. et J.Presl

Family Sapotaceae Juss.

Sapotaceae gen. et spec. indet. ([Figure S3](#))

Description

Pollen prolate (P/E ratio 1.1–1.2), outline convex-pentagonal in polar view, elliptic in equatorial view, shape ellipsoid; polar axis 25–27.5 µm in LM, 24.1–26.5 µm in SEM, equatorial diameter 22–23 µm in LM, 18.6–20.5 µm in SEM; exine 1.7–2.5 µm thick (LM), nexine as thick as sexine; pollen stephano(5)colporate, colpi narrow and short, colpi 13.6–17.5 µm long in SEM, endopori lalongate, margins of endopori perpendicular to polar axis thickened; pollen wall tectate; sculpture psilate in LM, nanoverrucate, fossulate, and perforate in SEM, nanoverrucae closely spaced and forming rugulae, fossulae and perforations more frequent in polar areas, colpus membrane nanoverrucate (SEM).

Remarks

Pollen morphology and ultrastructure of Sapotaceae has been investigated in detail by Harley.^{68–73} She identified 12 pollen types,⁷¹ subdivided into a total of 49 subtypes.⁷² The fossil Sapotaceae gen. et spec. indet. pollen falls within the range of 'Pollen Type 1A'⁷¹ and 'Subtype 1A'⁷² produced by plants in subfamilies Sapotoideae (*Madhuca*, *Manilkara*, *Mimusops*, *Palaquium*) and Chrysophylloideae (*Xantolis*). All genera producing pollen similar to the fossil type are insect pollinated, woody evergreen shrubs or trees, occurring in tropical or/to subtropical regions.^{74,75} Fossil Sapotaceae pollen grains have been reported from the late Cretaceous, but most records are confined to the Cenozoic, indicating a cosmopolitan distribution by the late Eocene (summarized in Harley, Mulley, Song et al., and Stuchlik et al.^{71,72,76–78}). Fossil Sapotaceae pollen similar to the type described herein have been reported from the middle Eocene of Germany,^{79,80} the late Oligocene/early Miocene of Germany,⁸¹ the early to middle Miocene of Turkey,^{66,82,83} and the middle Miocene of Austria.⁸⁴ Even though Thiele-Pfeiffer⁵⁴ (pl. 16, Figures 27–33) described different Sapotaceae pollen from the dispersed palynoflora of Messel, she did not come across the pentacolporate pollen type described herein.

Lamiids

Order Lamiales Bromhead
Family Oleaceae Hoffmanns. et Link
Oleaceae gen. et spec. indet. (Figure S4)

Description

Pollen isodiametric (P/E ratio 0.9–1.1), outline circular to lobate in polar view, circular in equatorial view, shape spheroidal; polar axis 15–17.5 µm in LM, 13.3–16 µm in SEM, equatorial diameter 15–17.5 µm in LM, 13–16.3 µm in SEM; exine 1.3–1.7 µm thick (LM), nexine thinner than sexine; pollen tricolpate, colpi short; pollen wall semitectate (SEM); sculpture scabrate to reticulate in LM, reticulate to microreticulate in SEM, muri narrow and segmented, lumina pentagonal to hexagonal in outline, diameter of lumina 0.7–2 µm in SEM, lumina decreasing in size close to colpi, lumina with 0–2 free-standing columellae; colpus membrane nanoverrucate to granulate (SEM).

Remarks

Oleaceae pollen has been studied by Li et al., Miyoshi et al., Renault-Miskovsky et al., Zhang, Cerceau-Larrivall et al., Nilsson, Punt et al., Sachse, and Xu et al.^{41,42,85–91} Oleaceae is a cosmopolitan family comprising 25 genera and about 600 species of trees, shrubs, and woody climbers. Pollination in most Oleaceae is entomophilous, except for *Fraxinus* and *Phillyrea*, and flowers of several taxa are known for their sweet scent produced by osmophores situated on the corolla.⁹² The different Oleaceae pollen types described by Thiele-Pfeiffer⁵⁴ (pl. 12, Figures 8–14 & 24–31) from the dispersed palynoflora of Messel do not correspond with the Oleaceae gen. et spec. indet. pollen type depicted herein. A fossil Sapotaceae seed, cf. *Sapotispernum*, has also been reported from Messel by Collinson et al.⁵⁵; pl. 38, figs j & k.

Incertae sedis

Three additional pollen types were observed during the combined LM and SEM study of the fly's gut content.

Paleobotanical background

There are currently three main publications available on the paleoflora of Messel. One on the palynoflora by Thiele-Pfeiffer,⁵⁴ one on the fossil leaves by Wilde,⁹³ and one on the seeds and fruits by Collinson et al.⁵⁵ For a detailed list of the angiosperm components reported in these studies consult Tables S2 and S3. The palynoflora is rich, with about 140 different angiosperm pollen types, but 66 of them have not been affiliated to any extant taxon with certainty. A similar scenario was observed from the mesofossil record that is composed of about 155 different angiosperm seeds/fruits, with 65 types of uncertain affinity. The macrofossil record comprises 70 different types of leaves that were all affiliated to extant or extinct taxa. Interestingly, of the 69 families reported in these studies only six families, that is, Nymphaeaceae, Arecaceae, Juglandaceae, Ulmaceae, Vitaceae, and Nyssaceae, are represented by all three organ types (pollen, fruit/seed, leaf). Just over 20%, or 16 families, are represented by two organ types (pollen and seed/fruit [6 families], pollen and leaf [5 families], or seed/fruit and leaf [5 families]). The majority of the taxa, 47 families, are represented by a single type of organ. Most of the taxa, especially the seed/fruit record, suggest a warm and humid environment, comparable to that of present day tropical to subtropical forest regions. This paratropical paleovegetation was dominated by plants whose modern living relatives are currently thriving under equatorial climates (mostly Af-climate sensu Köppen and Geiger⁹⁴). In short and simplified, the plant taxa discovered so far suggest a dense and complex tropical type of forest (zonal vegetation), rich in climbing plants and lianas, a more open lake margin vegetation (azonal), as well as aquatic plants (azonal). For a more complete assessment of the Messel biota see for example^{36,95,96} and references therein.