Title: Evidence for an ancient association between leaf mining flies and herbaceous eudicot angiosperms

Authors: Nathan A. Jud^a and Jae-Cheon Sohn^{b,c*}

Author Affiliations:

^a Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611, USA

^b Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20013-7012 USA

^c Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20013-7012 USA

***Corresponding Author:** Jae-Cheon Sohn email: jay.c.sohn@gmail.com

14

15 ABSTRACT

16 We describe an early angiosperm and a leaf mine ichnofossil from the Lower Cretaceous
17 Potomac Group of Virginia, USA. The descriptions are based on a fossil leaf that was first
18 reported in 1895 but identified as a fragment of a fossil fern. The new genus and species of
19 angiosperm, *Vernifolium tenuiloba* Jud and Sohn, gen. et sp. nov., was likely herbaceous and is
20 based on a twice odd-pinnatifid leaf with glandular teeth at the tips of the lobes, and minute resin
21 bodies covering the lamina. Leaf architectural features and sedimentological context indicate that
22 this leaf was produced by an herbaceous eudicot angiosperm, possibly associated with
23 Ranunculales. The leaf mine *Phytomyzites wardi* Sohn and Jud, ichnosp. n. is a full depth linear-
24 blotch mine with frass, a trace of puparium inside the blotch mine section, and
25 feeding/oviposition-related puncture marks. The features of the mine are most consistent with
26 those produced by agromyzid flies. This fossil extends the record of agromyzid flies by about 40
27 myr. Furthermore, data from molecular studies suggests that the ancestral hosts for agromyzid
28 flies are asterids, with several later host-shifts to feeding on Ranunculaceae, but this fossil
29 provides evidence that agromyzid flies or their ancestors were feeding on herbaceous basal
30 eudicots similar to modern herbaceous ranunculids during the Early Cretaceous, prior to the
31 appearance and diversification of asterids.

32

33 Keywords: Cretaceous, Albian, Potomac Group, eudicot, Schizophora, Agromyzidae

34

35

52

53 **1. Introduction**

54

55 Agromyzid flies are globally distributed and include thousands of species that feed on
56 leaf mesophyll as larvae; these are the leaf mining flies. (Scheffer et al., 2007; Spencer, 1972).
57 The diversity of agromyzid flies is shaped largely by evolutionary radiations following host
58 shifts (Spencer, 1990), but the importance of extinction on the phylogenetic structure of host
59 modern associations may be under appreciated (Winkler et al., 2010). Most extant agromyzid
60 flies mine the leaves of herbaceous plants (Spencer, 1990), but some (including members of the
61 early-diverging genus *Agromyza*) specialize on distantly related woody plants (Scheffer et al.,
62 2007; Winkler et al., 2010). This pattern may reflect an ancestral association between leaf
63 mining flies and ancient woody plant lineages followed by more recent radiations on species-rich
64 herbaceous host lineages, or alternatively early agromyzid flies may have diversified primarily
65 on herbaceous hosts, with rare host-shifts to woody plants (Winkler et al., 2010). Fossil evidence
66 of extinct associations with woody Platanaceae and Rutaceae during the Paleocene appears to
67 support to the former hypothesis (Winkler et al., 2010); however, the paucity of fossil mines on
68 herbs could be the result of failure to detect the fossil leaves of herbaceous plants (Winkler et al.,
69 2010).

70 The taphonomic megabias against herbaceous plants in litter assemblages is well known
71 (Behrensmeyer et al., 2000); however, fine-grained sediment deposited under low-energy
72 conditions on floodplains can preserve herbaceous plants in-situ or nearly in-situ, providing a
73 “snapshot” of floodplain communities (Upchurch and Doyle, 1981; Wing et al., 2011). In the
74 Potomac Group (Gp), these types of assemblages are generally found in local, organic-rich
75 deposits of clay or mud with planar fabric and little or no lamination (Upchurch and Doyle,
76 1981; Jud, 2014).

77 Here, we describe an herbaceous eudicot angiosperm with evidence of leaf mining based
78 on a fossil from the Lower Cretaceous (Albian) Potomac Group in Virginia. The preserved leaf
79 architecture indicates a close relationship to eudicot angiosperms, and the mine has a
80 combination of features produced by agromyzid flies. The occurrence of an agromyzid fly in the
81 Lower Cretaceous extends the fossil record of the family by ~40 million years ago (Ma) and
82 supports the hypothesis that agromyzid flies have an ancient and ancestral association with

herbaceous basal eudicots rather than with Cenozoic woody angiosperms or herbaceous rosids or asterids (Winkler et al., 2010).

2. Methods

Collecting locality. The fossil was collected from a carbonaceous silty clay bed along the bank of the Potomac River.

Stratigraphic position and age. The Mount Vernon locality

The leaf is preserved as an adpression in two pieces and was collected from a light brown clay bed exposed along the Potomac River in Northern Virginia, USA (USGS locality 499), on what was historically the Mount Vernon estate, near Fort Belvoir, Virginia, USA. (38.675N, 77.131W). (Fig. 1). The bed from which the leaf was collected belongs to pollen zone IIB of the Potomac Group (Patapsco Formation) (Brenner, 1963; Doyle and Hickey, 1976; Doyle and Robbins, 1977) (Fig. 2). Previous work showed that pollen and spore flora at the Mount Vernon site is more than 50% angiosperm pollen and includes diverse tricolpate grains indicative of eudicot angiosperms (Doyle and Hickey, 1976). Pollen zone IIB is now thought to correspond to the middle-upper Albian (Hochuli et al., 2006). Ward (1895) reported on three collections from this site that include fossils of ferns, gymnosperms, and angiosperms. We re-examined the specimens that were identified as ferns for herbaceous angiosperms and recognized extensive insect damage on one specimen. We examined the specimen using low-angle light under a dissecting scope and photographed the specimen using a Canon EOS digital camera with a 100mm EF macro lens and a Nikon D70 digital camera with a Macro-Nikkor 65mm lens. We processed the images with Adobe Photoshop (San Jose, California, USA) and applied whole-image manipulations to improve the contrast between the fossil and the matrix. The leaf architectural character definitions used are based on those outlined in the Manual of Leaf Architecture (Ellis et al., 2009) with some of modifications (Jud and Hickey, 2013). The leaf mine trace was observed and photographed using an Olympus SZX12 stereomicroscope accompanied with image capture system and an Image-Pro 6.1 (Media Cybernetics) platform.

The leaf mine was illustrated using Adobe Illustrator CC (Adobe systems). The morphological terms for the leaf mine follow Hering (1951) and Winkler et al. (2010).

3. Systematics

3.1 The host plant

Clade: Angiosperms

Group: Eudicots

Order: cf. Ranunculales Jussieu ex Berchtold & J. Presl, 1820.

Family: *incertae sedis*

Genus *Vernifolium* Jud and Sohn, gen. nov.

Vernifolium tenuiloba Jud and Sohn, sp. nov. Fig. 3

Diagnosis. Leaves simple, petiolate. Blade a microphyll, twice odd-pinnatifid, wider than long (l:w ratio ~ 1:2); primary lobes in sub-opposite arrangement, secondary lobes alternate; sinuses between primary lobes deepening proximally; sinuses between secondary lobes deeply rounded, deepening proximally; apex unknown, lobe apices acute; base straight, reflex. Primary venation palmate, with three basal veins; secondary vein framework craspedodromous; craspedodromous tertiary veins present, intercostal tertiary veins not observed; glandular teeth sparse, present at apices of lobes; teeth supplied medially by principle vein and laterally by perimarginal veins; tooth margins slightly convex, forming acute apex; lamina covered in minute oil bodies at a density of ~15 per square millimeter.

Holotype. USNM 5380 (Figs. 3A-C) is curated in the Paleobotanical Type and Illustrated Collections, Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, DC, U.S.A.

Etymology. *Vernifolium* comes from the Mount Vernon, the name of George Washington's estate where the fossil was found, and folium, the latin for leaf; *tenuiloba* comes from the latin for narrow, and means narrow-lobed.

Detailed description. A single incompletely preserved leaf. In some areas cracks, preparation marks, and abrasion marks make it difficult to distinguish the leaf margin using the naked eye; however, minute amber-like resin spherules that are scattered across the lamina aid in

recognizing where the fossil ends and the matrix begins (Figs. 3A–C). The leaf is marginal petiolate and the blade is a small microphyll, at least >25mm long, >54mm wide (Fig. 3A). The leaf is lamina simple and deeply lobed (Fig. 4). The margin is twice odd-pinnatifid and sparsely toothed. This means that the leaf blade is pinnately divided and has a single terminal lobe, but divisions of the blade do not incise all the way to the midvein; the lobes formed by these sinuses are again pinnately divided, but again not all the way to the secondary vein. There are three pairs of major (first-order) lobes preserved, and they are subopposite. The minor (second-order) lobes are narrow and alternate. The minor lobes are separated by deeply rounded sinuses and teeth are present at the lobe apices (Fig. 3B). Major lobe size and the depth of the sinuses between them all decrease gradually toward the leaf apex. Minor lobe size gradually decreases distally as well. The frequency of minor lobes per major lobe decreases rapidly toward the apex of the leaf- the first pair of primary lobes bear at least five minor lobes, and the second pair of major lobes bear only two minor lobes. The apex of the leaf is not preserved, but the apices of some of the minor lobes are preserved and they are uniformly acute. The leaf base is reflex and straight. The primary vein framework is pinnate, with three basal veins. The secondary vein framework is craspedodromous, and secondary vein spacing smoothly decreases distally. The attachment of the secondary veins to the primary vein is acute and decurrent, except at the base where the first pair of secondary veins is acute and excurrent. The angle of attachment gradually increases proximally. Secondary veins terminate in teeth at the apices of the major lobes. Craspedodromous tertiary veins terminate in teeth at the apices of the minor lobes. Intercostal tertiary veins poorly preserved, but possibly irregular reticulate. Higher-order veins are not visible. One to three glandular teeth are present at the apices of the lobes. The teeth are supplied medially by a principle vein, and possibly laterally by perimarginal veins that, if present, are difficult to distinguish.

Remarks. The type material of *Sphenopteris grevillioides* was described in Flora Fossilis Arctica Vol. III, part 2 (Heer, 1874: 34, pl. XI, figs. 10, 11). These small fragments of fern pinnae were collected from the Lower Cretaceous Kome Formation in western Greenland. Evidently, Heer gave this species the specific epithet “*grevillioides*” because of superficial similarity to the southern hemisphere eudicot angiosperm genus *Grevillea* (Proteaceae). Ward (1895) identified the specimens in the Mount Vernon collections from the Potomac Group as this species presumably based on the similar size, alternate lobes, and acute apices. In a subsequent

publication Ward reported seven specimens (Ward, 1905: 491) from the Mount Vernon collections, but only one remains in the Type and Illustrated Collections in the Department of Paleobiology at the Smithsonian Institution. This specimen cannot be a fern because it has glandular structures at the lobe apices that are supplied medially by a principle vein and laterally by perimarginal conjunctal veins. Based on the arrangement the primary, secondary and tertiary veins and the shape and arrangement of the lobes, we interpret the mode of leaf dissection is intermediate polyternate/acropeta/basipetal-pedate, which is typical of herbaceous eudicots angiosperms (Gleissberg and Kadereit, 1999; Jud and Hickey, 2013). Taken together, the herbaceous habit, glandular teeth, mode of dissection, and age suggest a close relationship to ranunculalean eudicot angiosperms in particular.

3.2 The leaf mine

Ichnogenus *Phytomyzites* Straus, 1977

Remarks.— *Phytomyzites* was originally proposed by Straus (1977) for the leaf mine fossils associated with extant *Phytomyza*. The generic name has been redefined by Winkler et al. (2010) as an ichnotaxon to accommodate all fossil leaf mines caused by agromyzid flies.

Phytomyzites wardi Sohn and Jud, ichnosp. n. Figs. 5, 6A–D

Classification. This mine can be classified as a phyllonome (leaf mine), partially full-depth mine, and ophistigmatome (linear-blotch mine).

Diagnosis. This ichnospecies differs from three previously known congeners in host plants: *P. biliapchaensis* Winkler, Lanandeira et Wilf, 2010 from Platanaceae; *P. corni* Straus, 1977 from Cornaceae; and *P. schaarschmidtii* Wappler, 2010 from Rutaceae. The leaf mine of *P. wardi* ends with a blotch, differing from those of the other three species which are serpentine or weakly waving linear mines, gradually broadened over the path.

Etymology. This ichnospecies is named after Lester F. Ward who mistakenly described the plant host as a fern.

Holotype. On the holotype (USNM 5830 with original & counter part) of *Vernifolium tenuiloba* gen. et sp. nov.

Damage type. DT297 added to Labandeira et al. (2007). This type of feeding damage can be characterized by linear-blotch mine whose linear section is inverted one time and in which alternating frass grains are present.

Description. Minute punctures with darkened tissue around the margins are scattered across the lamina (Figs. 5A and 5C). The mine trace commences near the distal end of lobe margin on the proximal side, continues toward the midvein of the leaflet and gradually broadens along leaf margin, turns to the opposite direction in short distance, and then expands into elliptical blotch (Figs. 5C–E). The blotch mine expands crossing the primary midvein. Frass is present as grains and dots in irregular size and shape except the distal 3/5 of blotch section. The frass trail runs nearly at center in the narrow linear mine section but it is situated close to one side in the expanded linear and blotch mine sections. The linear mine and the portion of the blotch mine with frass trail have paler discoloration than the resting portion of the blotch mine. The paler portion may be due to full-depth feeding of the leaf miner. Alternatively, the darker portion may be due to contamination by exudate of frass (Fig. 5D). A possible puparium embedded within mine is present at the distal 1/6 of the blotch on the main leaf vein, elliptical, broken into parts, some of which are preserved on counter fossil piece (Figs. 6A–D).

Measurements. Linear mine from initiation to a turning point – 12.7 mm; linear mine from a turning point to blotch – 6.3 mm; blotch mine section – 6.9 mm in max. length; possible puparium – 1.0 mm in max. length, 0.31 mm in max. width; feeding/oviposition scars – ca. 0.27 mm in diameter.

Modern analogs. No exact match with the leaf mine trace on our specimen was found among the extant examples of leaf mining damages on Ranunculales. The frass trail somewhat resembles those in the leaf mines of *Phytomyza hendeli* Hering [Diptera: Agromyzidae] on *Anemone nemorosa* L. (Edmunds, 2014, electronic source), but there are substantial differences in the course of the mine track. The leaf mine trace also resembles those by *Liriomyza pusilla* (Meigen) [Diptera: Agromyzidae] on *Solidago* spp. (Csóka, 2003) in starting at and progressing along the leaf margin, but differs from the latter in having the shorter linear mine section and the longer blotch mine section. The overall shape of the leaf mine on our fossil is similar to those of *Rhynchaenus fagi* Fabricius [Coleoptera: Curculionidae] on *Fagus* spp. (Csóka, 2003), but

differs in the frass trail and where the mine starts (leaf margin in our fossil vs. near mid-vein in *R. fagi*).

4. Discussion

4.1 Comparisons with extant and fossil plants

The combination of leaf shape with the presence glandular teeth indicate that *Vernifolium tenuiloba* is an angiosperm rather than a fern or gymnosperm (Jud and Hickey, 2013). The presence of minute reddish resin bodies dispersed across the leaf may also support the interpretation that *Vernifolium* is an angiosperm, as they have been observed on a variety of fossil angiosperm leaves, but not on fern foliage (N.A. Jud, *personal observation*).

In the leaves of extant Ranunculales, the arrangement and relative size of lobes can be used to infer the order in which the lobes were initiated (Gleissberg and Kadereit, 1999; Jud and Hickey, 2013). The arrangement and relative sizes of the leaflets and lobes in *Verniphyllum* match the pattern expected for leaves with an intermediate polyternate/acropetal/basipetal-pedate arrangement typical of ranunculalean angiosperms (Gleissberg and Kadereit, 1999).

The fossil record of tricolpate pollen grains demonstrates that eudicot angiosperms were globally distributed by the late Albian (Brenner, 1976). Many Lower Cretaceous eudicot genera known primarily from leaf fossils can be distinguished based on the mode and depth of leaf dissection, as well as leaf size (e.g. Fontaine, 1889; Sun et al. 2011; Barral et al. 2013; Jud and Hickey, 2013; Jud, 2015). Leaf architectural differences like these can often be used to distinguish modern ranunculalean genera as well (e.g. Gleissberg and Kadereit, 1999). Among the diversity of Lower Cretaceous eudicot angiosperms, the leaf of *Vernifolium* is superficially similar to those of *Iterophyllum* (Barral et al., 2013) because the two taxa share narrow lobes and glandular teeth at the lobe apices. However *Iterophyllum* has distinct intersecondary veins, a principally acropetal mode of leaf dissection, an acute base, and it has distinct glands in the sinuses. By contrast the leaves of *Vitiphyllum* (Doyle and Hickey, 1976; Fontaine, 1889), has a principally basipetal-pedate mode of leaf dissection with obtuse and rounded lobes and a reflex and lobate base.

Although the stem is not preserved, *Vernifolium* was likely an herbaceous basal eudicot rather than a woody shrub or tree. The deeply lobed margin, the reflex base, and the thin laminar texture (i.e. coriaceous) are features generally associated with herbaceous plants and with adaptation to shade (Givnish, 1987; Upchurch and Wolfe, 1993). The small size, and apparently slim but winged petiole are also consistent with the herbaceous habit. Poor organization of thin intercostal tertiary veins is characteristic of early herbaceous eudicot angiosperms and may have contributed to their poor preservation. Together these features are rare in trees but common in herbs because they do not confer tolerance to the sheer stress experienced by canopy leaves exposed to wind and rain (Upchurch and Wolfe, 1993). Furthermore, while there is mounting evidence for diverse herbaceous basal eudicots during the Early Cretaceous (Jud, 2015), and evidence of eudicot angiosperm trees is sparse until the Late Cretaceous (Peralta-Medina and Falcon-Lang, 2012).

4.2 Ecology of the site

The sedimentological context of the collections from USGS locality 499 provides additional support for our interpretation that *Vernifolium* was a small herb. The site occurs in a local carbonaceous silty clay bed (Ward, 1995). The sediment has a planar fabric and is poorly laminated except near its basal contact with an underlying bed of poorly lithified cross-bedded sand. The most common megafossils are foliage of the bennittitalean plant *Dioonites buchianus* (Ward, 1995). Fossil fern and conifer foliage is also common. Angiosperm remains from the site are generally small, and include small peltate leaves, cordate leaves, and a five-parted perianth ("*Antholithus*" *gaudium-roseae*). There are also several specimens of an enigmatic herbaceous axis with minute, whorled leaves and attached lateral organs that may be reproductive structures (Ward, 1995). Together, this sedimentological information suggests preservation of the fossils in a low-energy depositional environment favorable for the preservation of herbaceous angiosperms.

4.3 Identification of the miner

Leaf mines are produced by members of four orders of holometabolous insects: Lepidoptera, Diptera, Hymenoptera, and Coleoptera. Although leaf mines are complex structures

with several independent evolutionary origins, it is often difficult to determine the taxonomic identity of miners based on mine morphology alone because there are few features that reflect synapomorphies of miner clades above the species level. However, the mines produced by agromyzid flies are one exception to this general pattern. Henrig (1951) and Winkler et al. (2010, 2009a) identified features that when found in combination provide strong evidence that a particular mine was produced by an agromyzid fly. These features are (1) serpentine-blotch-intermediate, (2) fluidized frass, (3) a pattern of frass deposition that reflects the side-to-side feeding mode of the maggots (alternating bands along the margin, or a herringbone pattern in blotch mine), (4) puncture marks with reaction tissue on the leaf lamina associated with female feeding/oviposition behavior, and (5) a semicircular exit slit at the end of a linear mine or along the edge of a blotch mine (Dr. Sonja Scheffer, *personal communication*).

In the specimen described above, the frass is primarily deposited along the center of the course, differing from those of the coleopteran and hymenopteran leaf mines which are usually a simple pile-up at bottom. Our specimen also clearly shows a frass trail along nearly the entire path of the mine. This characteristic excludes many gracillariid leaf miners (Lepidoptera), notably *Phyllocnister* and *Phyllorycter*, which are sap-feeders in early or entire larval stages; several lepidopteran leaf miners, such as *Atemelia* (Praydidae), Bedellidae, Coleophoridae, *Cosmopterix* (Cosmopterigidae), and Tischeriidae, which expel or hang frass out of the leaf mine; and some agromyzid leaf miners (Diptera) which produce fluidized frass and thus leave no apparent frass trail.

Dipteran association of our leaf-mine fossil is substantiated by its frass pattern. The frass trail in this fossil is composed of grains which are somewhat disarrayed but alternated in some parts. The linear mine section is too narrow to allow a space for side-by-side deposition of frass grains, or for alternate deposition of frass grains along the margins of the mine. Once enlarged, the linear mine shows approximately alternating pattern of frass grains, although there seems to be some disturbed or ambiguous parts in the frass trail. Partly alternating frass pattern is often observed from extant dipteran leaf mines (Winkler et al., 2010).

The presence of small feeding punctures with leaf reaction tissue associated with the mine near the site of oviposition is characteristic of agromyzid leaf mines. These feeding punctures are made by the agromyzid females just prior to oviposition (Parrella, 1987; Zoenisch and Schuster, 1987). The number of those marks greatly varies depending on species and

preservation. In our leaf mine specimen, there were at least ten possible feeding/oviposition punctures scattered on four leaf lobes (white arrows in Fig. 5C). These holes are somewhat scattered across the lamina, but two of those are on the lobe that is supradjacent to the lobe where the mine is situated. The holes are situated near one another and are surrounded with reaction tissue (Fig. 5A). These punctures are similar in size to those associated with other agromyzid leaf mine fossils, including *Phytomyzites biliapchaensis* Winkler, Labandeira and Wilf (especially figure 7.3 in Winkler et al., 2010).

Hering (1930, 1951) indicated the presence of a semicircular exit slit near the pupation chamber as an additional character of dipteran leaf mines. Winkler et al. (2010) found that this character is present in most extant agromyzid leaf mines. In the blotch mine section of our specimen, there is a small elliptical hole which cuts through the mid-vein of the major lobe. This area does not seem to be associated with an exit hole, as it appears as a protuberance (Figs. 6A–C). More likely, it represents an agromyzid puparium, although no traces of segmentation are observed. This lack could be explained by poor preservation. Some species of agromyzids pupate inside the mine with anterior portion of the pupa embedded through the leaf surface (Dr. Sonja Scheffer, *personal communications*). The position and shape of the protuberant area on our specimen seems remarkably similar to an extant example associated with *Phytomyza ditmani* Kulp (Fig. 6E). Ding *et al.* (2014) characterized that dipteran leaf mines typically are not full-depth in terms of tissue consumption. There are, however, some species of Agromyzidae exhibiting full-depth mine (Spencer, 1972; Dr. Sonja Scheffer, *personal communication*). Therefore, the full-depth mine section of our specimen does not exclude its dipteran origin.

5. Conclusions

To date, the oldest reliably identified agromyzid fossils are two leaf mines from the lower Paleocene of Montana, USA described as *Phytomyzites biliapchaensis* (Wilf et al., 2006). Using the early Paleocene agromyzid fossils, Winkler et al. (2009b) estimated the origin of Agromyzidae back to the earliest Paleocene and the origin of Schizophoran (“higher”) flies, including Agromyzidae, back to possibly the Late Cretaceous. This result was consistent with earlier conclusions that Schizophora and Agromyzidae in particular diversified primarily during

the Cenozoic (Grimaldi and Engel, 2005; Rohdendorf et al., 1974; Wiegmann et al., 2003; Wilson, 1978). By contrast, the mid-late Albian leaf mine fossil described here significantly predates the oldest known agromyzid evidence and thus suggests that Agromyzidae and Schizophora evolved earlier than previously thought (Fig. 7). This occurrence of an agromyzid leaf mine from the Lower Cretaceous extends the fossil record of Agromyzidae by over 40 myr, and also represents the earliest evidence of the clade Schizophora. It also reduces a long gap between the earliest known Cyclorrhapha (~127Ma) and Schizophora (Wiegmann et al., 2011). There have been no confirmed body fossils of Schizophora from Mesozoic deposits, likely because of insufficient sampling of deposits with suitable preservation.

This fossil also has important implications for understanding the ecological history of agromyzid flies. Winkler et al. (2009a, 2009b) traced the evolution of host plant usage by Agromyzidae, especially *Phytomyza*, the largest genus within the family (a summary in Fig. 7). The majority species of *Phytomyza* feed on either asterid angiosperms or ranunculid angiosperms (Spencer, 1973). (Spencer, 1990) suggested the original host association of *Phytomyza* with Ranunculaceae, based on the morphological diversity exhibited by different host-feeding groups; however, using phylogenetic ancestral state reconstruction, Winkler et al. (2009a) concluded that the ancestral lineages of *Phytomyza* were more likely associated with asterids and that there were at least two host-switches from asterids to ranunculids (Fig. 7). If *P. wardi* belongs to one of the two modern clades of *Phytomyza* that feed on ranunculids identified by Winkler (the *Phytomyza* PML clade, or the *Phytomyza miniscula* clade) then many deeper splits in the Schizophora phylogeny are much older than previously thought and the associations with asterids in Agromyzidae must represent many independent adaptations to asterids. Alternatively, if the miner belongs outside of those clades, near the base of *Phytomyza* or any of the other leaf mining genera, or perhaps along the agromyzid stem lineage for the clade that includes both *Agromyza* and *Phytomyza* (Scheffer et al., 2007), then we have evidence that the ancestral host plants were herbaceous basal eudicots that lived long before the appearance and diversification of the asterids. In either case this occurrence indicates that at least some ancient associations are lost and therefore extinction has played an important role in shaping the modern diversity of agromyzid associations.

Acknowledgments

We appreciate of two anonymous reviewers who gave several useful comments for improving our manuscript. We would like to thank Dr. Conrad Labandeira (National Museum of Natural History) for giving several thoughtful suggestions for our early manuscript. We are also indebted to Drs. Donald R. Davis (National Museum of Natural History) and Sonja J. Scheffer (ARS-United States Department of Agriculture, Beltsville, Maryland) who helped our identification of the fossil specimen and provided photos of extant examples. This work was supported from the Peter Buck Pre-Doctoral (for NAJ) and Postdoctoral (for JCS) Fellowships, Smithsonian Institution, from the Korea Research Fellowship program funded by the Ministry of Science, ICT and Future Planning through National Research Foundation of Korea (2015035581), and by the University of Maryland Graduate School; the University of Maryland Program in Behavior, Ecology, Evolution, and Systematics; the Maryland Agricultural Experiment Station.

References

- Barral, A., Gomez, B., Feild, T.S., Coiffard, C., Daviero-Gomez, V., 2013. Leaf architecture and ecophysiology of an early basal eudicot from the Early Cretaceous of Spain. *Bot. J. Linn. Soc.* 173, 594–605. doi:10.1111/boj.12095
- Behrensmeyer, A.K., Kidwell, S.M., Gastaldo, R.A., 2000. Taphonomy and paleobiology. *Paleobiology* 26, 103–147. doi:10.1666/0094-8373(2000)26[103:TAP]2.0.CO;2
- Brenner, G.J., 1976. Middle Cretaceous floral provinces and early migrations of angiosperms, in: Beck, C.B. (Ed.), *Origin and Early Evolution of Angiosperms*. Columbia University Press, New York, pp. 23–47.
- Brenner, G.J., 1963. The spores and pollen of the Potomac Group of Maryland. *Md. Dep. Geol. Mines Water Resour. Bull.* 27, 1–215.
- Csóka, G., 2003. *Leaf mines and leaf miners*. Agroinform Kiadó, Budapest.
- Ding QiaoLing, Labandeira, C.C., Ren Dong, 2014. Biology of a leaf miner (Coleoptera) on *Liaoningocladus boii* (Coniferales) from the Early Cretaceous of northeastern China and the leaf-mining biology of possible insect culprit clades. *Arthropod Syst. Phylogeny* 72, 281–308.

421 Doyle, J.A., Hickey, L.J., 1976. Pollen and leaves from the mid-Cretaceous Potomac Group and their
 422 bearing on early angiosperm evolution, in: Beck, C.B. (Ed.), Origin and Early Evolution of
 423 Angiosperms. Columbia University Press, New York, New York, U.S.A., pp. 139–206.
 424 Doyle, J.A., Robbins, E.I., 1977. Angiosperm pollen zonation of the continental Cretaceous of the
 425 Atlantic Coastal Plain and its application to deep wells in the Salisbury Embayment. Palynology
 426 1, 43–78. doi:10.2307/3687316
 427 Ellis, B., Daly, D.C., Hickey, L.J., Mitchell, J.D., Johnson, K.R., Wilf, P., Wing, S.L., 2009. Manual of
 428 Leaf Architecture, First Edition. ed. Comstock Publishing Associates.
 429 Fontaine, W.M., 1889. The Potomac or younger Mesozoic flora. U.S. Geological Survey Monograph 15,
 430 Washington D.C.
 431 Givnish, T.J., 1987. Comparative studies of leaf form: Assessing the relative roles of selective pressures
 432 and phylogenetic constraints. New Phytol. 106, 131–160. doi:10.1111/j.1469-
 433 8137.1987.tb04687.x
 434 Gleissberg, S., Kadereit, J.W., 1999. Evolution of leaf morphogenesis: Evidence from developmental and
 435 phylogenetic data in Papaveraceae. Int. J. Plant Sci. 160, 787–794. doi:10.1086/314165
 436 Gradstein, F.M., Ogg, J.G., Schmitz, M., Ogg, G. (Eds.), 2012. The Geologic Time Scale 2012, 1st ed.
 437 Elsevier.
 438 Grimaldi, D., Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press.
 439 Heer, O., 1874. Flora Fossilis Arctica. Zurich J. Wurster.
 440 Hering, M., 1930. A mining Fly as a Pest of Pea Plants. Anz. Schadlingskunde 6, 61–64 pp.
 441 Hering, P.E.M., 1951. Mine Producers, in: Biology of the Leaf Miners. Springer Netherlands, pp. 39–53.
 442 Hochuli, P.A., Heimhofer, U., Weissert, H., 2006. Timing of early angiosperm radiation: recalibrating the
 443 classical succession. J. Geol. Soc. 163, 587–594. doi:10.1144/0016-764905-135
 444 Jud, N.A. 2015. Fossil evidence for an herbaceous diversification of early eudicot angiosperms. Proc.
 445 Roy. Soc. B 202: 20151045
 446 Jud, N.A., 2014. Morphotype Catalog of a Zone I (Aptian—Earliest Albian) Flora from Fairlington,
 447 Virginia, USA. Bull. Peabody Mus. Nat. Hist. 55, 135–152. doi:10.3374/014.055.0204
 448 Jud, N.A., Hickey, L.J., 2013. *Potomacapnos apeleutheron* gen. et sp. nov., a new Early Cretaceous
 449 angiosperm from the Potomac Group and its implications for the evolution of eudicot leaf
 450 architecture. Am. J. Bot. 100, 2437–2449. doi:10.3732/ajb.1300250
 451 Labandeira, C.C., Wilf, P., Johnson, K.R., Marsh, F., 2007. Guide to insect (and other) damage types on
 452 compressed plant fossils.
 453 Parrella, M.P., 1987. Biology of Liriomyza. Annu. Rev. Entomol. 32, 201–224.

454 Peralta-Medina, E., Falcon-Lang, H.J., 2012. Cretaceous forest composition and productivity inferred
 455 from a global fossil wood database. *Geology* 40, 219–222. doi:10.1130/G32733.1
 456 Rohdendorf, B.B., Hocking, B., Oldroyd, H., Ball, G.E., 1974. The Historical Development of Diptera.
 457 University of Alberta.
 458 Scheffer, S.J., Winkler, I.S., Wiegmann, B.M., 2007. Phylogenetic relationships within the leaf-mining
 459 flies (Diptera: Agromyzidae) inferred from sequence data from multiple genes. *Mol. Phylogenet.*
 460 *Evol.* 42, 756–775. doi:10.1016/j.ympev.2006.12.018
 461 Spencer, K.A., 1990. Host specialization in the world Agromyzidae (Diptera). xi + 444 pp.
 462 Spencer, K.A., 1973. Agromyzidae (Diptera) of Economic Importance. Ser. Entomol. 9.
 463 Spencer, K.A., 1972. Diptera: Family Agromyzidae.
 464 Straus, A., 1977. Gallen, Minen und andere Fraßspuren im Pliozän von Willershausen am Harz, in:
 465 Verhandlungen Des Botanischen Vereins Der Provinz Brandenburg. pp. 43–80.
 466 Sun, G. D.L. Dilcher, H. Wang, and Z. Chen, 2011. A eudicot from the Early Cretaceous of China. *Nature*
 467 471: 25-628
 468 Upchurch, G.R., Doyle, J.A., 1981. Paleoecology of the conifers *Frenelopsis* and *Pseudofrenelopsis*
 469 (Cheirolepidiaceae) from the Cretaceous Potomac Group of Maryland and Virginia, in: Romans,
 470 R.C. (Ed.), *Geobotany II*. Plenum Press, New York, USA, pp. 167–202.
 471 Upchurch, G.R., Wolfe, J.A., 1993. Cretaceous vegetation of the Western Interior and adjacent regions of
 472 North America. *Evol. West. Inter. Basin* 39, 243–281.
 473 Ward, L.F., 1895. The Potomac Formation. Govt. Print. Off., Washington D.C.
 474 Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.-W., Lambkin, C., Bertone, M.A.,
 475 Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair,
 476 B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier,
 477 G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich, M., Meier,
 478 R., Yeates, D.K., 2011. Episodic radiations in the fly tree of life. *Proc. Natl. Acad. Sci.* 108,
 479 5690–5695. doi:10.1073/pnas.1012675108
 480 Wiegmann, B.M., Yeates, D.K., Thorne, J.L., Kishino, H., 2003. Time Flies, a new molecular time-scale
 481 for Brachyceran fly evolution without a clock. *Syst. Biol.* 52, 745–756.
 482 doi:10.1093/sysbio/52.6.745
 483 Wilf, P., Labandeira, C.C., Johnson, K.R., Ellis, B., 2006. Decoupled plant and insect diversity after the
 484 end-Cretaceous extinction. *Science* 313, 1112–1115. doi:10.1126/science.1129569
 485 Wilson, M.V.H., 1978. Paleogene insect faunas of western North America. *Quaest. Entomol.* 14, 13–34.

- Wing, S.L., Strömberg, C.A.E., Hickey, L.J., Tiver, F., Willis, B., Burnham, R.J., Behrensmeyer, A.K.,
2011. Floral and environmental gradients on a Late Cretaceous landscape. *Ecol. Monogr.* 82, 23–
47. doi:10.1890/11-0870.1
- Winkler, I.S., Labandeira, C.C., Wappler, T., Wilf, P., 2010. Distinguishing Agromyzidae (Diptera) leaf
mines in the fossil record: New taxa from the Paleogene of North America and Germany and their
evolutionary implications. *J. Paleontol.* 84, 935–954. doi:10.1666/09-163.1
- Winkler, I.S., Mitter, C., Scheffer, S.J., 2009a. Repeated climate-linked host shifts have promoted
diversification in a temperate clade of leaf-mining flies. *Proc. Natl. Acad. Sci.* 106, 18103–18108.
doi:10.1073/pnas.0904852106
- Winkler, I.S., Scheffer, S.J., Mitter, C., 2009b. Molecular phylogeny and systematics of leaf-mining flies
(Diptera: Agromyzidae): delimitation of *Phytomyza* Fallén sensu lato and included species
groups, with new insights on morphological and host-use evolution. *Syst. Entomol.* 34, 260–292.
doi:10.1111/j.1365-3113.2008.00462.x
- Zoebisch, T.G., Schuster, D.J., 1987. Suitability of foliage of tomatoes and three weed hosts for
oviposition and development of *Liriomyza trifolii* (Diptera: Agromyzidae). *J. Econ. Entomol.* 80,
758–762. doi:10.1093/jee/80.4.758

FIGURE LEGENDS

Fig. 1. Map of Lower Cretaceous Potomac Group outcrop in Virginia and Maryland, USA. The Mount Vernon locality is indicated by an arrow on the western bank of the Potomac River.

Fig. 2. Age of the lower part of the Potomac Group (modified from Doyle, 1992; Hochuli et al., 2006; dates on the left are from Gradstein et al. (2012). The Mount Vernon locality is in the Patapsco Fm, or the upper part of pollen zone IIA/B, indicated by the leaf icon. Abbreviations: Barr. = Barremian age, Haut. = Hauterivian age.

Fig. 3. *Vernifolium tenuloba* gen. et sp. nov. USNM 5380. A, Holotype. Note the gently arching primary midvein with and the large major lobe with pinnate minor lobes on the left side of the midvein. Scale bar = 10 mm; B, Counterpart of the only preserved proximal major lobe in A. Scale bar = 10 mm; C, Close up view of the surface of the distal portion of the leaf fossil showing the punctate surface of the lamina where minute resin-like bodies are occasionally preserved (abbreviations: mx = matrix; mv = midvein; lv = lateral vein; id = insect damage). Scale bar = 2 mm.

Fig. 4. Idealized diagram of leaf architecture in *Vernifolium tenuloba* based on the holotype. Thick black line, primary midvein; dark green, secondary venation; brown, tertiary venation; red, glandular structures in the lobe apices.

Fig. 5. *Phytomyzites wardi* ichnosp. n. A, Close up of two feeding punctures with dark wound response tissue (red box and arrow in figure B); B, Enlarged section of the frass trail in the mine (marked with 'f' in figure D). Green arrows indicate alternating pattern of frass deposition; C, Insect damage of the leaf fossil. White arrows indicate possible feeding/oviposition punctures scattered across the leaf blade (also see figure A). Black arrow indicates hole feeding. Gray arrow indicates the leaf mine (see figure E). Scale bar = 10 mm; D, Close up of the leaf mine, submerged in alcohol (p – possible puparium, f – a part of frass trail); E. Line drawing of the leaf mine, corresponding to figure D.

Fig. 6. Possible puparium of *Phytomyzites wardi* ichnosp. n. (A–D) and pupation of extant agromyzid in leaf mine (E). A, Position of possible puparium within leaf mine (at arrow). Scale unit = 1 mm; B–C, Trace of possible puparium, close up of figure 4D with color added for protuberant areas from original (pink) and counter (green) parts; D, Combined fragments from figures B and C, reconstructed actual contour indicated by dotted line; E, Leaf mine of *Phytomyza ditmani* Kulp on *Illex decidua* (photo credit: Dr. Sonja J. Scheffer). Red arrow indicates a puparium.

Fig. 7. Summary of host use evolution in Agromyzidae. Stars indicate fossil taxa and most recent common ancestors (MRCA). Lines and star-marks in green or purple indicates Ranunculaceae- or asteroid-feeders respectively. Colored circles connected with arrows represent host-shifts between Ranunculaceae and Asterid clade. Open star indicates a fossil taxon feeding on a host plant belonging to neither Ranunculaceae nor Asterid clade. ‘Ma’ stands for “million years ago.” Divergence estimation data extrapolated from Winkler et al. (2009a).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7