

# Fossil-calibrated molecular phylogenies reveal that leaf-mining moths radiated millions of years after their host plants

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## Keywords:

coevolution;  
cospeciation;  
divergence times;  
evolutionary rates;  
fossil record;  
host shift;  
leaf-mining moth;  
penalized likelihood;  
plant–insect interactions;  
sequential evolution.

## Abstract

Coevolution has been hypothesized as the main driving force for the remarkable diversity of insect–plant associations. Dating of insect and plant phylogenies allows us to test coevolutionary hypotheses and distinguish between the contemporaneous radiation of interacting lineages vs. insect ‘host tracking’ of previously diversified plants. Here, we used nuclear DNA to reconstruct a molecular phylogeny for 100 species of *Phyllonorycter* leaf-mining moths and 36 outgroup taxa. Ages for nodes in the moth phylogeny were estimated using a combination of a penalized likelihood method and a Bayesian approach, which takes into account phylogenetic uncertainty. To convert the relative ages of the moths into dates, we used an absolute calibration point from the fossil record. The age estimates of (a selection of) moth clades were then compared with fossil-based age estimates of their host plants. Our results show that the principal radiation of *Phyllonorycter* leaf-mining moths occurred well after the main radiation of their host plants and may represent the dominant associational mode in the fossil record.

## Introduction

The associations between vascular plants and insects or their early arthropodan relatives have a fossil record dating back to the first known terrestrial ecosystems of the early Devonian, 415–400 Ma (Kevan *et al.*, 1975; Labandeira, 2002). More recently, during the Cretaceous, from 145 to 65.5 Ma, flowering plants and their insect herbivores have dominated terrestrial biomes and currently constitute a major fraction of the earth’s biodiversity, perhaps accounting for as much as 50% of all described species (excluding micro-organisms; Strong *et al.*, 1984). Since the pioneering work of Ehrlich & Raven (1964) on the coevolution of butterflies and their host plants, there has been great interest in trying to

detect and understand macroevolutionary patterns in insect–plant associations (Farrell, 1998b, 2001; Sequeira & Farrell, 2001; Percy *et al.*, 2004; Kergoat *et al.*, 2005). This research has received added impetus in recent years due to the increasing availability of molecular phylogenies of speciose groups of plants and their herbivores.

There are now many established examples of clades of herbivores that have radiated on phylogenetically related groups of plants (e.g. Farrell, 1998a; Ronquist & Liljeblad, 2001; Weiblen & Bush, 2002). Less clear is the temporal relationship between the plant and insect diversifications. At least three patterns are possible, but these should be seen as parts of a continuum rather than discrete categories. First, the insects might speciate contemporaneously with their host plants (cospeciation) so that their phylogenies are congruent. Secondly, insect diversification may follow rapidly (in geochronological terms) after plant diversification (‘fast colonization hypothesis’), but with the pattern of plant colonization influenced by some factors in addition to host-plant

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phylogeny, resulting in an insect radiation that is slightly younger and incongruent with that of the plants. Thirdly, colonization of plants may require an evolutionary innovation and occur only when this arises, some time after plant diversification ('delayed colonization hypothesis'). Again, an imperfect match between the phylogenies is predicted, but now we also predict a time lag before colonization.

Testing the above alternative scenarios ideally requires phylogenies of both taxa, along with fossil data for both groups, to provide absolute time calibrations. Recently, Farrell (1998a) studied the evolution of host-plant use among herbivorous Phytophaga beetles (weevils, long-horned and leaf beetles), using phylogenetic and fossil data. He showed two bursts of beetle speciation: one during the mid-Cretaceous (90 Ma) and the other in the later-Cainozoic (10 Ma). This bimodal distribution may be attributable to three events: (1) a major spurt of herbivore diversification coinciding with the mid-Cretaceous ecological expansion of angiosperms, (2) extinction of many specialized associations at the K/T boundary and (3) a gradual rebound to late-Cretaceous species diversity levels during the earlier Cainozoic (Wilf *et al.*, 2001; Labandeira *et al.*, 2002).

More recently, Percy *et al.* (2004) compared fossil-dated molecular phylogenies of both jumping plant-lice (Psylloidea) and their legume host plants. They found that there was significant topological congruence of the phylogenies, but that the psyllids radiated after their legume hosts, except in the geologically recent Canary Islands where psyllids and genistoid legumes radiated contemporaneously.

So far, attempts to time the diversifications of insect and plant groups have focused mainly on external feeders. Here, we explore this issue in a speciose genus of leaf-mining moths, *Phyllonorycter* (Lepidoptera: Gracillariidae). Radiations of internal feeders, such as leaf miners, may differ from those of external feeders. For example, the larvae of leaf-mining moths have very intimate associations with their host plants and this may mean that there are more host-imposed constraints on their ecology and evolution compared with externally feeding herbivores. Several authors have suggested that internal feeders show both greater host-plant specificity and greater phylogenetic conservatism in colonizing new species of plants (e.g. Powell, 1980; Mitter & Farrell, 1991; Lopez-Vaamonde *et al.*, 2003).

There are also some particular advantages to studying these issues with leaf-mining microlepidoptera. First, the earliest fossil evidence for leaf-mining moth clades extends to at least 99 Myr, almost to the start of the major explosion of angiosperm diversity during the Cretaceous (130–90 Myr; Powell, 1980; Powell *et al.*, 1999). Secondly, the fossil record preserves important host-plant associations of leaf-mining insects, often in exquisite detail, and replete with larval behavioural and developmental characters that often are assignable at

mid-level taxonomic ranks to extant taxa (Labandeira *et al.*, 1994). This facilitates the temporal calibration of the insect phylogeny, which is more difficult in other insect groups.

In a previous study, we found no evidence for cospeciation between *Phyllonorycter* and their host plants (Lopez-Vaamonde *et al.*, 2003). However, *Phyllonorycter* species from the same host-plant genus frequently form a monophyletic clade. Closely related moth species often feed on closely related plants, which we interpret as due to host switching (colonization followed by speciation) being more likely to happen amongst phylogenetically related plants.

The aim of this paper was to test whether the main diversification of *Phyllonorycter* moths occurred near-contemporaneously (on a geochronological time scale) with that of their host plants, or after a significant time lag. To do this we generated a molecular phylogeny of 100 species of *Phyllonorycter* leaf-mining moths using 28S rDNA sequence data. We then used a penalized likelihood method with a Bayesian approach to estimate divergence times, a method that takes into account phylogenetic uncertainty. We converted the relative ages of the moths into absolute dates using a calibration point from the fossil record. The absolute ages of the moths were then compared with fossil-based estimates of the dates of diversification of their host plants.

## Material and methods

### Insect sampling

Moths were reared and collected as described by Lopez-Vaamonde *et al.* (2003). We used several other genera of gracillariids (from the subfamilies Gracillariinae and Phyllocnistinae as well as the Lithocolletinae to which *Phyllonorycter* belongs), one species of Bucculatricidae (in the same suborder) and one species of Micropterigidae (a basal group of lepidopterans) as outgroups (see Appendix S1).

### Data generation and laboratory procedures

We estimated phylogenies using sequence data from an approximately 1000 bp fragment of the 28S nuclear ribosomal subunit gene. We added 21 new outgroup species and 22 new *Phyllonorycter* species to the published 28S rDNA phylogeny of 77 *Phyllonorycter* species in Lopez-Vaamonde *et al.* (2003). All new sequences have been deposited in GenBank (accession numbers: AY521484–AY521528; see Appendix S1). We used the polymerase chain reaction and sequencing procedures described by Lopez-Vaamonde *et al.* (2001). 28S sequences showed substantial length variation and were aligned following the procedure of Lopez-Vaamonde *et al.* (2003). The DNA alignments are available from TREEBASE (<http://www.treebase.org/treebase/>; study accession number = S1423).

### Estimating phylogenies

We used Markov Chain Monte Carlo (MCMC) methods (Larget & Simon, 1999) within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees. By adopting this Bayesian approach, we take account of phylogenetic uncertainty, both with respect to topology and to branch lengths, in our estimates of divergence age.

We used hierarchical likelihood ratio tests in the computer program *MODELTEST 3.0B6* (Posada & Crandall, 1998) to select the best model of nucleotide substitution, which was a general time reversible (GTR) model, allowing for rate heterogeneity across sites assuming a discrete  $\gamma$ -distribution and for a proportion of sites to be invariable (GTR + I +  $\Gamma$ ). The phylogenies were then estimated using *MRBAYES V3.0B4* (Huelsenbeck & Ronquist, 2001) and the MCMC was run for  $10^6$  cycles, sampling one tree every 1000th cycle. After filtering the trees for our defined nodes of interest, a total of 8368 trees remained for use in the age estimation analyses.

### Estimating divergence times

We estimated node ages for the moths using a penalized likelihood approach in the computer program *r8s v. 1.50* (Sanderson, 2003; <http://ginger.ucdavis.edu/r8s/>). For each node of interest (see below) the posterior distribution of the divergence time, conditioned on both the phylogenetic model and the calibration point obtained from the fossil record, was obtained by local density estimation using the program *LOCFIT* (Loader, 1999), implemented in the 'R' statistical package (Ihaka & Gentleman, 1996 see script in Fig. S1). To summarize the fitted distributions, we report their modes and 90% highest posterior density (HPD) limits (Table 1 and Fig. S1). The mode represents the most likely value for the divergence time of a particular node, and the HPD provides a confidence interval for this estimate. It is important to emphasize that we have inferred age from the entire posterior probability distribution of the 8368 trees sampled rather than using a single topology, so our age estimates account for both branch length and topological uncertainties.

The ages of the host-plant groups were obtained from the literature based on the fossil record (Table 2).

### Character mapping and phylogenetic uncertainty

We mapped the character 'host-plant taxa' onto the moth phylogeny using Bayesian methodology in order to incorporate phylogenetic and mapping uncertainty. Unfortunately, current software (*MRBAYES* and *SIMMAP*, <http://www.simmap.com>) does not allow sufficient character states for this analysis. Consequently, we reconstructed the evolution of host use manually on 100 trees drawn randomly from the Bayesian posterior distribution

**Table 1** Estimates of the age when particular plant groups were colonized by *Phyllonorycter* leaf-mining moths (column 3).

Taxon	Number of node	Mode (Myr)	90% LHPD	90% UHPD	Probability (%)
Fagales	2	37.8	27.3	50.8	32
Malpighiales	2	37.8	27.3	50.8	27
<i>Populus</i>	3	29.9	21.1	42.5	76
<i>Ulmus</i>	4	32.6	24.5	48.4	79
<i>Acer</i>	5	13	7	24.5	79
Fagaceae	6	24.4	14.6	34.1	51
<i>Lyonia</i>	7	18.8	10.9	27.2	68
<i>Malus</i>	8	11.4	7.6	17.7	43
<i>Alnus</i>	9	25.7	18.1	36.2	97
<i>Corylus</i>	10	11.3	7.4	17.5	98
Salicaceae	11	15.9	11.1	24.4	65
<i>Prunus</i>	12	9.6	6.2	16.2	98
<i>Acer</i>	13	18	12.1	27.8	73
<i>Carpinus</i>	14	14.8	9.8	22.2	81
<i>Quercus</i>	15	15.8	11	24.4	47

Number of node is indicated on the cladogram (see Fig. 3). LHPD, 90% lower highest posterior density; UHPD, 90% upper highest posterior density; probability (%), the figures represent the probability (as averaged over the 100 randomly selected trees from the posterior distribution) that a particular plant group is reconstructed for our node of interest and not reconstructed for the node below (i.e. the colonization event has occurred along the stem lineage to our node of interest).

(the 8368 trees sampled, filtered for our nodes of interest) using *MESQUITE* version 1.0 (build e58; <http://mesquiteproject.org>; see Table S1). We selected clades that grouped closely related moths feeding on closely related plants (same host-plant genus, family, order). On this basis, 15 nodes of interest, supported by posterior probabilities of at least 92%, were chosen for insect and plant clade age comparisons (Tables 1 and 2).

### Crown-group and stem-group ages

The distinction between crown and stem-group ages is crucial when dealing with colonization events. Consider a clade of insects that all feed on the same host-plant taxon. A colonization event is inferred to have occurred somewhere along the stem lineage to this clade. However, the date of this colonization event can be estimated either as the date of origin of the stem group or as the date of origin of the crown group (see Fig. 1). We have consistently used stem-group ages for both moth and host-plant groups in our analyses. In terms of testing for a significant delay between plant and insect radiations, insect stem-group ages are the conservative option as they provide the older date. For the host plants, crown-group ages (younger) would be more conservative for the hypothesis test. However, palaeobotanical reports of the first occurrence of particular plant groups rarely identify the taxon as a stem-group lineage or part of the crown

**Table 2** Earliest fossil occurrences of plant taxa that currently are hosts for *Phyllonorycter* leaf-mining moths.

Host-plant taxon	Earliest occurrence		Absolute age (Myr)*	Type	References
	Epoch	Stage			
<i>Acer</i>	Paleocene	Thanetian	56.5	Leaves, fruit	Crane <i>et al.</i> (1990)
<i>Alnus</i>	Eocene	[Middle] Lutetian	48.5	Leaves, fruits	Crane (1989)
<i>Carpinus</i> †	Eocene	[Middle] Lutetian	49.0	Fruit	Wilde & Frankenhäuser (1998)
<i>Corylus</i>	Eocene	[Middle] Lutetian	48.5	Fruit	Pigg <i>et al.</i> (2003)
Fagales‡	Gallic	Santonian	85.0	Pollen	Doyle & Robbins (1977), Romero (1986), Hill & Dettmann (1996), Sims <i>et al.</i> (1998), Herendeen <i>et al.</i> (1999)
Fagaceae	Gallic	Upper Santonian	84	Staminate inflorescences, flowers, cupules and fruits	Sims <i>et al.</i> (1998)
<i>Lyonia</i>	Miocene	[Middle] Langhian	15.5	Fruits, seeds	Friis (1985)
Malpighiales§	Late-Cretaceous	Turonian	91.5	Flowers	Crepet & Nixon (1998)
<i>Malus</i> ¶	Oligocene	[Early] Rupelian	31.0	Leaves	Meyer & Manchester (1997)
<i>Populus</i>	Paleocene	[Late] Thanetian	55.0	Leaves	S. R. Manchester, personal communication to CCL
<i>Prunus</i>	Eocene	[Middle] Lutetian	48.0	Fruits	Cevallos-Ferriz & Stockey (1991); Manchester (1994)
<i>Quercus</i>	Eocene	[Middle] Lutetian	48.5	Acorns	Manchester (1994)
Salicaceae	Middle-Eocene	[Middle] Lutetian	45	Reproductive foliage and leaves	Manchester <i>et al.</i> (1986)
<i>Ulmus</i>	Eocene	[Middle] Lutetian	48.0	Leaves, fruit	Manchester (1989, 1999)

\*To nearest 0.5 Myr; stage-interval data are given as midpoints; some are radioisotopic age dates. Time scale is from Gradstein & Ogg (2004).

†The assignment is 'aff. *Carpinus*'; of which the same deposit has the very closely related *Paleocarpinus*, also a member of the subfamily Coryleae.

‡Fagales comprises the Betulaceae, Casuariniaceae, Fagaceae, Juglandaceae, Myricaceae, Nothofagaceae and Rhoipteleaceae (Judd *et al.*, 1999).

§Malpighiales comprises the Malpigiaceae, Clusiaceae, Euphorbiaceae, Chrysobalanaceae, Rhizophoraceae, Salicaceae, Passifloraceae,

Violaceae and Flacourtiaceae (Judd *et al.*, 1999).

¶Leaves of *Malus* are difficult to distinguish from *Pyrus*.

group of living species. Generating crown-group age estimates would therefore require an extensive taxonomic evaluation of both fossil and living plant taxa, clearly beyond the scope of this study. Therefore, we use stem groups wherever possible; one exception being the estimate for the origin of *Phyllonorycter*. This taxon was used to establish a diversification rate estimate based only on numbers of extant species, so a crown-group estimate is more appropriate.

### Fossil record and calibration points

To convert the relative ages obtained through the penalized likelihood analysis into dates, we used an absolute calibration point from the fossil record. Mines of the gracillariid subfamily Phyllocnistinae have been recorded from magnoliid dicot leaves originally reported to be at least 97 Myr old (Labandeira *et al.*, 1994; Fig. 2), but this date has now been revised to at least 99 Myr, based on new evidence (Brenner *et al.*, 2000; Wang, 2002; Gradstein & Ogg, 2004).

These mines represent the oldest fossil evidence for the family Gracillariidae (Kristensen & Skalski, 1999; see

Table S2). However, it is unclear whether they should be assigned to the stem or crown group as the adult morphology is not preserved, and therefore cannot be compared with extant species. Consequently, we applied a 99 Myr calibration point to the Phyllocnistinae stem-group node (the time of divergence of the Phyllocnistinae from its sister taxon).

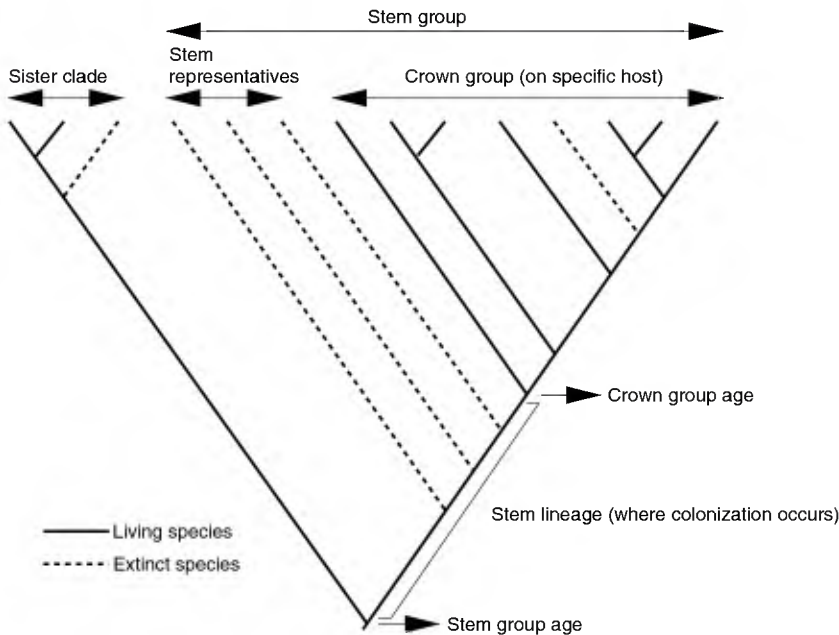
### Estimating diversification rates

We estimate rates of diversification ( $r$ ) for the genus *Phyllonorycter* as a whole by using Magallón & Sander's (2001) eqn (7) for a crown-group age

$$\hat{r}_e = \frac{1}{t} \left[ \log \left[ \frac{1}{2} n(1 - e^2) + 2e \right] + \frac{1}{2} (1 - e) \sqrt{n(ne^2 - 8e + 2ne + n)} \right] - \log 2,$$

where  $n$  is number of species,  $e$  is the extinction rate and  $t$  the crown-group age.

In addition, we estimate  $r$  using two extinction rate values at opposite ends of the spectrum of possible values: one corresponds to zero extinction ( $r_0$ ) and the



**Fig. 1** Distinction between crown-group and stem-group ages (based on Magallón, 2004).

other to a very high extinction rate ( $r_{0.9}$ ; for a similar approach see: Strathmann & Slatkin, 1983; Magallón & Sanderson, 2001). We previously estimated the number of extant *Phyllonorycter* species as 400 (Lopez-Vaamonde *et al.*, 2003) and used this estimate for the age of the crown group obtained in this study.

## Results

### Bayesian analysis

We obtained 28S rDNA sequences for 100 *Phyllonorycter* species and 36 outgroup taxa. The aligned D1 + D2 + D3 region sequences comprised 1130 bp. Results of the Bayesian analysis are presented as a 50% majority rule consensus tree in Fig. 3. As in a previous study (Lopez-Vaamonde *et al.*, 2003), *Cameraria* is the sister group of *Phyllonorycter*, and *Phyllonorycter* species are arranged in clades that reflect their host-plant relationships (Fig. 3). The *r8s* analysis suggests that the genus *Phyllonorycter* originated during the early Palaeocene (mode = 62.3 Myr, lower and higher 90% posterior density limits = 50.3 and 76.3 Myr).

### Character evolution and phylogenetic uncertainty

The mapping of host-plant use onto the 100 randomly chosen trees from the Bayesian posterior distribution (Fig. 3) enabled us to identify 15 host-plant colonization events. Nodes, indicating where these events occurred, are numbered from 1 to 15 on Fig. 3.

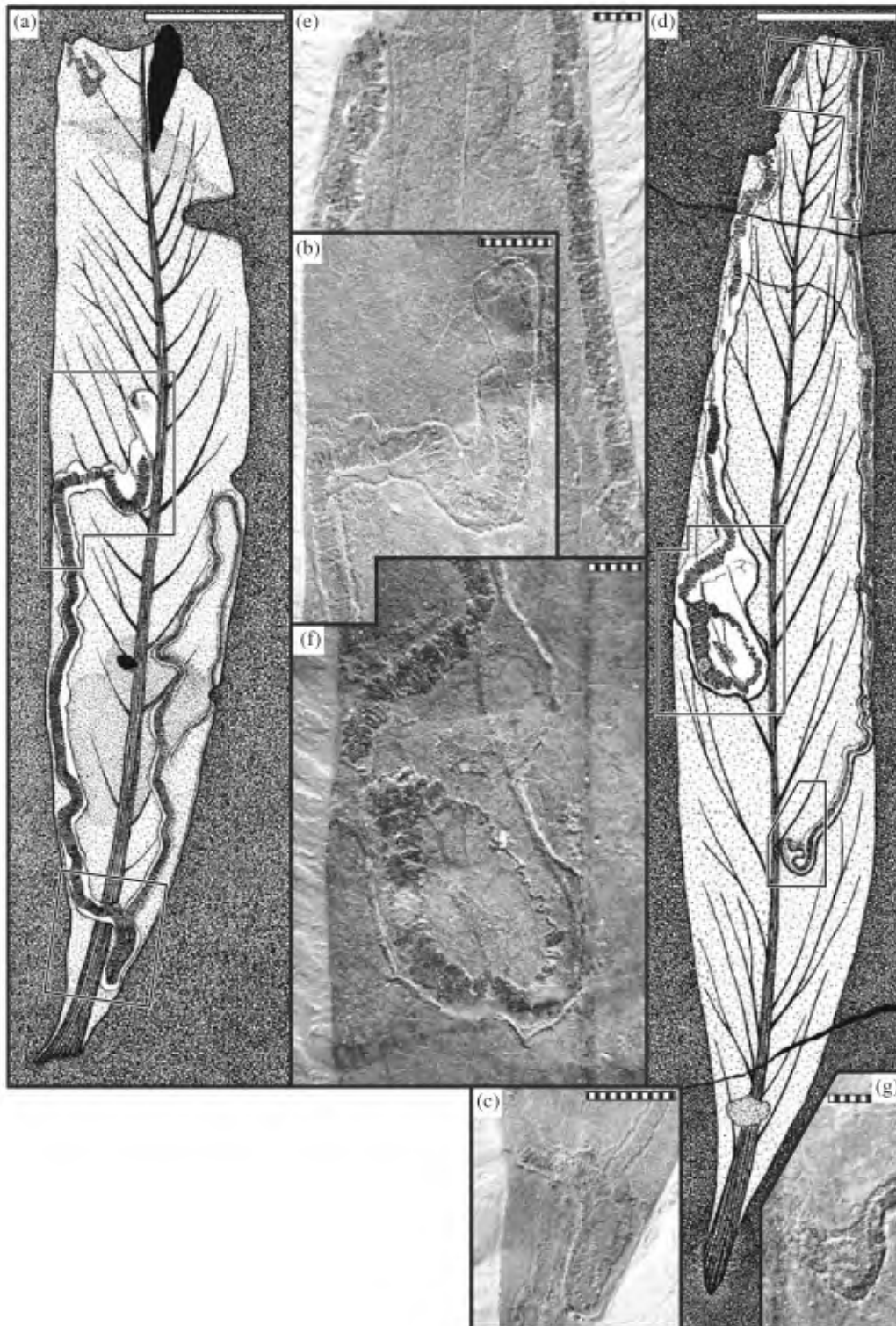
When host use was mapped onto Fig. 3, node 2 was matched to a host shift onto the plant order Fagales

whereas two other nodes were identified as colonizations of the plant family Fabaceae (results not shown). When phylogenetic uncertainty was taken into account, the robustness of these host-plant colonization events decreased. Thus, the mapping of host use on the 100 posterior trees showed node 2 as a colonization event of Fagales and Malpighiales (Table 1), but there was not a single tree out of the 100 sampled that supported the two nodes as colonization events of Fabaceae (see Table S1). This clearly indicates that using a single topology for reconstructing character evolution can yield misleading results.

### Comparing moth and plant age estimates

When we compare the age estimates of the 15 nodes (Table 1) with those of the associated host plants (Table 2), we observe, as expected, that the moths are younger than the plants. For example, we placed the origin of the genus *Phyllonorycter* in the Palaeocene (62.3 Myr), considerably postdating the origin of both extant angiosperm clades (Early Cretaceous, 130–110 Myr) and extant eudicot subclades (mid-Cretaceous, 110–90 Myr; Stuessy, 2004). Similarly, the colonization of the order Fagales (Fig. 3a, node 2), a major group of hosts for *Phyllonorycter*, occurred during the late-Eocene (37.8 Myr) whereas the order Fagales originated during the late-Cretaceous (84 Myr; Herendeen *et al.*, 1999; Table 2). From these data, on average, moths are  $32.3 \pm 16.6$  Myr younger than their host plants and this difference is significant (paired *t*-test:  $t_{14} = -7.52$ ,  $P < 0.0001$ ).

The ages of the host plants were obtained from the fossil record (Table 2) and compared with the



**Fig. 2** Gracillariidae (Phyllocnistinae) leaf mine on host plant *Rogersia parlatorii* Fontaine, whose affinities is closest to extant Lauraceae within the order Laurales cf. Lauraceae. (a–c) Specimen UF7351, showing a camera lucida drawing of the entire leaf (a), terminal mine chamber (b) and mine loop adjacent to midrib at the leaf base (c). (d–g) Specimen UF4818, displaying a camera lucida drawing of the entire leaf (d), mine course near leaf apex (e), terminal chamber (f) and oviposition site with adjacent frass trail from early instars (g). Both specimens are from the Cenomanian-Albian boundary interval of the Dakota Formation and were found at the Braun's Ranch Locality, Cloud Co., Kansas, USA.

sequence-derived ages of the moths. An alternative would be to estimate the ages of the plants from molecular data as well (with fossil data providing minimum age constraints). However, it has been shown that fossil-based ages are nearly always younger than the corresponding sequence-derived ages (Rodríguez-Trelles *et al.*, 2002; Wikström *et al.*, 2003) and hence this would simply increase the time span between host-plant origin and moth colonization.

**Rate of diversification**

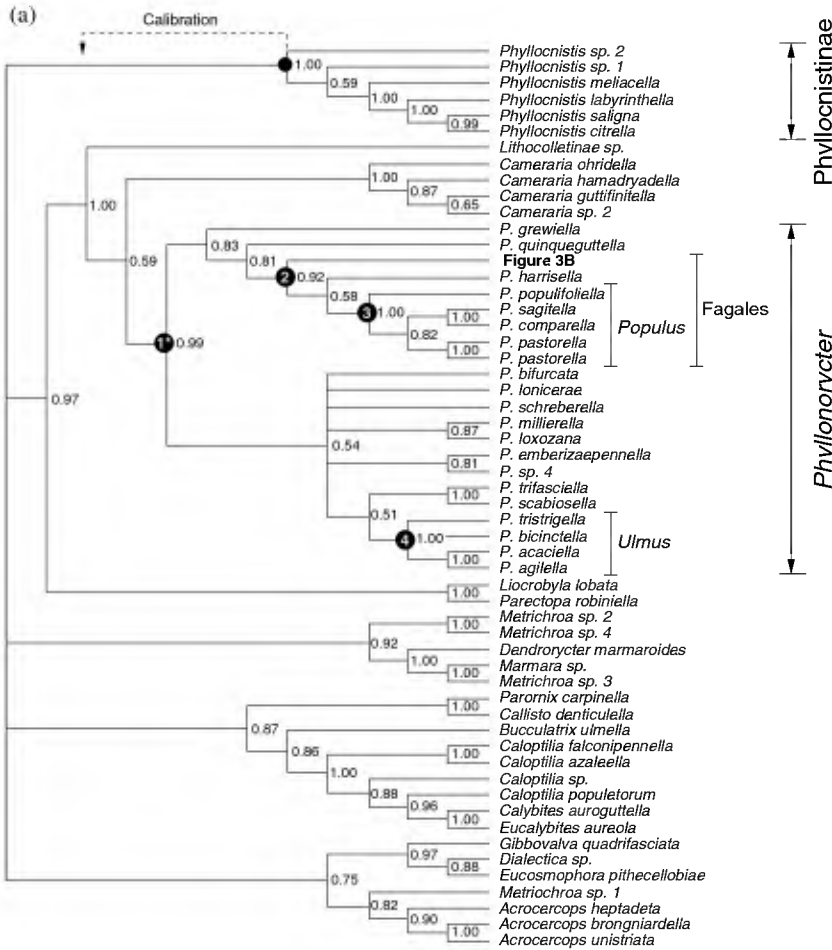
Given that there are about 400 *Phyllonorycter* species extant today (Lopez-Vaamonde *et al.*, 2003), we can use our estimate of the age of the crown group (62.3 Myr) to obtain a measure of the net diversification rate as a function of the relative extinction rate (*e*). The values we obtain are 0.04 net speciation events per million years in the absence of extinction (*e* = 0), and a minimum of 0.03 under the assumption of a high relative extinction rate (*e* = 0.9).

**Discussion**

**Contemporary or sequential radiations?**

This is the first study to make a rigorous quantitative comparison of the timing of radiations by internally feeding insects and their host plants. Our results provide strong evidence for sequential radiation of the insects onto an already diversified group of plants. The analyses suggest that the principal *Phyllonorycter* radiation was at least 27.3–50.8 Ma, when these moths colonized plants in the orders Fagales and/or Malpighiales (Fig. 3a, node 2). By contrast, the main radiation of Fagales occurred at least 84 Myr (Hill & Dettmann, 1996; Sims *et al.*, 1998) and that of Malpighiales at least 90 Ma (Crepet & Nixon, 1998).

On average moths took  $32.3 \pm 16.6$  Myr to colonize new plant clades. However, this figure is an underestimate of the true figure because we have no information about the number and age of suitable but as yet uncolonized plant clades. Moreover, fossil calibration



**Fig. 3** Bayesian tree with posterior probability values above the branches. Nodes of interest (where host-plant colonization events are inferred) are numbered from 1 to 15 and their stem-group age estimates are reported in Table 1.

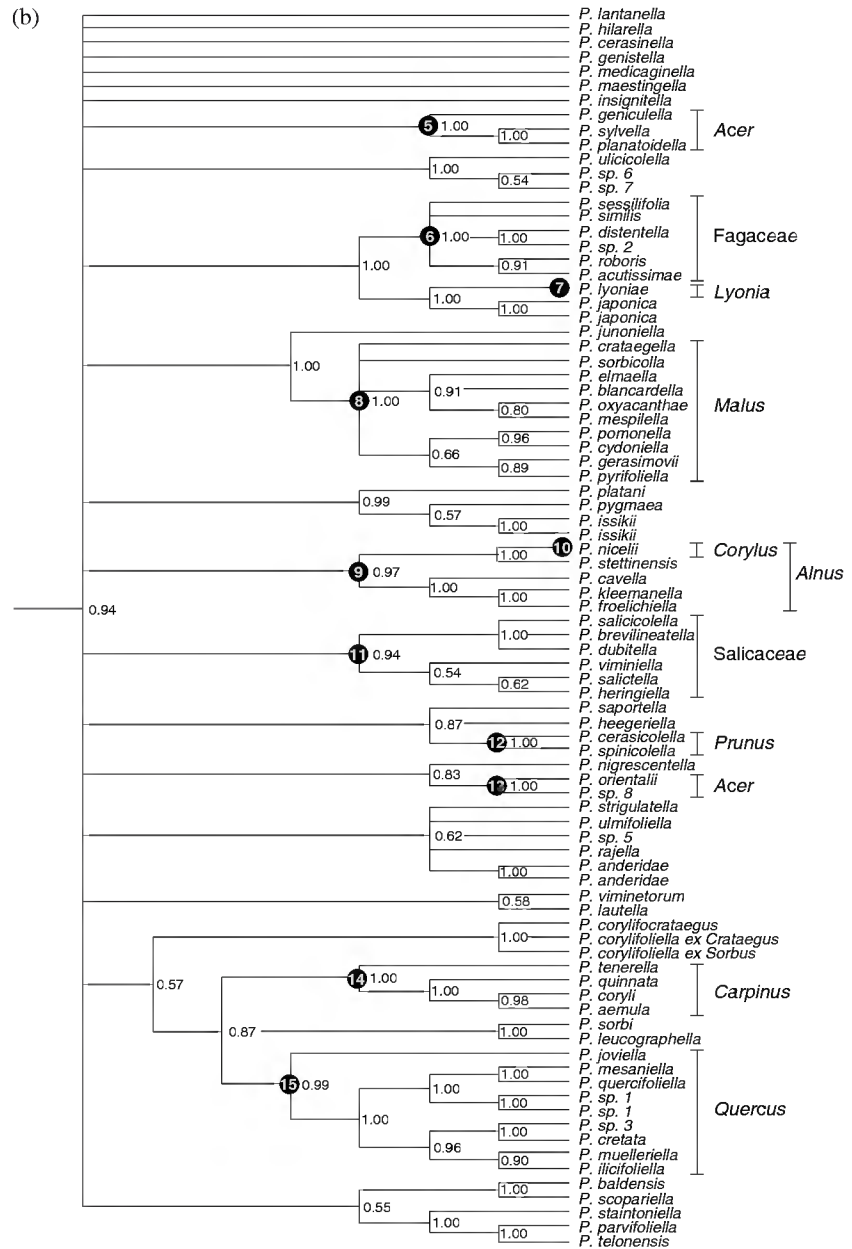


Fig. 3 Continued

dates are underestimates that are more likely to increase rather than decrease with additional fossil data. Regardless, our findings clearly suggest substantial delays (15–52 Myr) between the radiation of host-plant taxa and their colonization by *Phyllonorycter* moths, rejecting the hypothesis of ‘fast colonization’ and supporting the hypothesis of ‘delayed colonization’ or sequential evolution (Jermy, 1976; Percy *et al.*, 2004). Interestingly, the moth phylogeny is poorly resolved around the time of colonization of Fagales (internal branches are very short, Lopez-Vaamonde *et al.*, 2003) and this probably reflects rapid speciation following this key host shift.

Based on recent studies of the three major types of insect herbivore radiation on new host-plant clades – cospeciation, fast colonization and delayed or sequential radiation (see Labandeira, 2002) – it appears that the sequential mode is the most common. This pattern is supported by several other studies focusing on extant plant host and their insect herbivore clades (Schoonhoven *et al.*, 1998) as well as by our own study. Detection of sequential evolution (Jermy, 1976) in the fossil record requires geochronologically measurable time lags between the origin of a plant clade and its subsequent colonization by insects. In contrast, the rapid radiation of



angiosperms that occurred from the later-, early- and mid-Cretaceous is normally thought to be associated with cospeciation or rapid insect diversification (Grimaldi, 1999), based exclusively on insect fossil data. If further studies support this conclusion then our data and the other studies discussed above indicate a different mode of colonization in subsequent colonization events. Consequently, we suggest that delayed sequential colonization may represent the norm for insect herbivores during the overwhelmingly longer durations of geological time that are characterized by more typical levels of plant cladogenesis.

Our data suggest a substantial delay between the radiation of host plants and their colonization by host-specific *Phyllonorycter* moths. The moths, in turn, also harbour host-plant-specific parasites, namely parasitoid wasps of the genus *Achrysocharoides* (Hymenoptera: Eulophidae; Lopez-Vaamonde *et al.*, 2005). It is clear that these wasps have not cospeciated with either the plants or the moths (Lopez-Vaamonde *et al.*, 2005), but there has not been a formal comparison of the ages of moth and parasitoid radiations. However, comparisons of both nuclear and mitochondrial DNA reveal greater sequence divergences between pairs of moth species than matched pairs of their parasitoids, suggesting strongly that the parasitoids radiated across an already diversified group of moth hosts (Lopez-Vaamonde *et al.*, 2005). Consequently, this plant-leafminer-parasitoid system seems to be characterized by two waves of sequential colonization, which we might call 'bottom-up sequential radiation'.

### Dealing with uncertainty

Our analyses are subject to three potentially important sources of error. These occur in the procedures of phylogeny estimation, character mapping and node dating respectively.

Phylogeny estimation is difficult when based on a single genetic marker due to the stochastic nature of coalescence events (Hudson, 1990). Indeed, the use of a single locus to estimate divergence times can be problematic (Edwards & Beerli, 2000; Arbogast *et al.*, 2002). To address this issue, at least partly, we used a Bayesian framework for phylogenetic reconstruction. It is very probable that obtaining data from one or more additional genes would decrease the error bounds of our age estimates. However, our main result about the timing of speciation is sufficiently marked that it is very unlikely to change.

Any phylogeny generated from data is only an estimate of the true phylogeny and may be little better than some alternative estimates. Conclusions from mapping exercises using a single tree can depend crucially on a few influential (but not necessarily well supported) nodes. Our comparison between mapping on a single topology and on a sample of 100 posterior trees shows

that uncertainty in the estimate of a phylogeny estimate can lead to spurious mapping results. Mapping onto 100 trees sampled randomly from a posterior distribution is similar to using nonparametric bootstrapping to account for phylogenetic uncertainty (Ronquist & Liljeblad, 2001). However, neither approach addresses mapping uncertainty – i.e. the error associated with reconstructing the evolution of a character on a given tree – adequately (Ronquist, 2004). Only a full Bayesian approach can account simultaneously for both mapping and phylogenetic uncertainty, but such a procedure has yet to be applied in any empirical study. We hope that future software development will permit analysis of characters with more than 10 states, a constraint that prevented us from using the full Bayesian approach here.

### Molecular dating and uncertainty

It is well established that molecular rates vary across lineages, and that this makes it difficult to estimate divergence dates accurately using DNA sequence data (Arbogast *et al.*, 2002). There are two major approaches to dealing with this problem (Welch & Bromham, 2005). The first, nonparametric rate smoothing (NPRS) was developed by Sanderson (1997) and allows different rates on different branches of the tree. It assumes that rates are constant on individual branches and that branch length estimates are their true values. The penalized likelihood method is a superior modification of NPRS that relies on a parameter-rich model to estimate rates on branches and, like NPRS, employs a numerical penalty to avoid rapid changes between neighbouring branches. The relative importance of the model vs. the penalty function is determined by a smoothing parameter, the choice of which is based on the data using cross-validation (Sanderson, 2002).

A second approach was developed by Thorne *et al.* (1998), and uses Bayesian statistics to model explicitly the rate of evolution in a fully parametric setting. This is a more sophisticated technique, but it would be impractical to apply it to our data for two reasons. First, it is too computationally intensive to apply this approach to the full set of >8000 trees. Secondly, although in theory we could apply it to a suitable consensus tree, there is no satisfactory consensus tree for this data set, because of poor resolution (<50% bootstrap support) at many nodes. We thus chose to use the penalized likelihood method for practical reasons, but also note that this does not necessarily perform less well than the alternative approach (Welch & Bromham, 2005).

Age estimates may be poor if the fossil calibration is inaccurate, or if there is substantial variation in the rate of sequence evolution (Magallón, 2004). As we have shown a significant delay between host-plant radiation and moth colonization, we are most concerned here with factors that might lead us to accept the hypothesis of sequential radiation erroneously, i.e. to underestimate

the age of insect clades or overestimate the age of plant clades.

The low estimate of moth diversification rate (see below) might indicate that our calibration point is too old, resulting in *Phyllonorycter* crown-group age being overestimated. However, any younger age would further strengthen the result of sequential evolution, so this is not a great concern. To favour contemporaneous radiation, a considerably older *Phyllonorycter* origin would be necessary and there is no fossil evidence to support this. However, this might only reflect the general paucity of phyllocnistine leaf mines in the Cretaceous fossil record, a general concern in paleobiology (Marshall, 1997) and it has been shown recently that divergence dates based on single calibration points tend to be underestimates (Yoder & Yang, 2004). Unfortunately, no other calibration points are currently available, although further studies of plant–insect associations from the Dakota Formation and other Cretaceous biotas may provide new calibration points in the future. Particularly promising are a Cenomanian (approximately 97 Ma) deposit from Israel (Krassilov & Bacchia, 2000) and a younger Turonian (approximately 92 Ma) flora from Kyzyl Zhar, Kazakhstan (Kozlov, 1988), both yielding dicot leaf mines, and possibly older strata such as the Barremian (approximately 125 Ma) Yixian deposits from Hebei Province, China (Ren, 1995; Sun *et al.*, 2001) and the Hauterivian (approximately 135 Ma) Baissa deposits from Transbaikalia, Russia (Zherikhin *et al.*, 1999).

Additionally, variation in the rate of sequence evolution can bias both relative and absolute age estimates in a nonlinear way. The direction and magnitude of errors can only be evaluated on an individual node basis by direct comparison with an independent age estimate, typically a fossil. A recent comparison between fossil- and sequence divergence-based age estimates of land-plant lineages indicated that rate variation can impose large error bounds on age estimates (Soltis *et al.*, 2002).

When taken overall, the fossil record supports the hypothesis that the colonization of Fagales by *Phyllonorycter* moths occurred significantly after the diversification of major fagalean lineages (Table 2).

### Interpreting diversification rates

The estimated rate of diversification ( $r$ ) for *Phyllonorycter* leaf-mining moths (0.03–0.04) is very slow compared with estimates for other insect groups (i.e. Coleoptera,  $r \leq 0.20$ ; Diptera,  $r \leq 0.23$ ; Hymenoptera,  $r \leq 0.25$ ; Wilson, 1983) and also slower than typical rates in angiosperms [ $r \leq 0.077$  ( $e = 9$ ) to  $r \leq 0.089$  ( $e = 0$ ); Magallón & Sanderson, 2001]. However, this could be an artefact due to either an underestimate of the number of species (overlooked cryptic species, poor sampling in tropics) or a phylogeny calibration error. Nevertheless, such a slow rate is consistent with other plant host and dependent insect clades that have associations extending to the

Cretaceous, including leaf-rolling hispine beetles and their ginger hosts (Wilf *et al.*, 2000) as well as water lilies and their scarab beetle pollinators (Ervik & Knudsen, 2003) and seed-beetles and their legume hosts (Kergoat *et al.*, 2005).

In conclusion, determining the precise timing of phylogenies has become increasingly important to disentangle the evolution of interactions between insects and plants (Percy *et al.*, 2004; Kergoat *et al.*, 2005). Our dated phylogeny supports the hypothesis that the diversification of *Phyllonorycter* leaf-mining moths occurred well after the diversification of their host plants. This scenario of asynchronous radiations might apply to other predominantly monophagous insect–plant systems and has implications for the generation of biodiversity at geochronological time scales.

### Acknowledgments

The authors thank the colleagues listed in the Appendix S1 for providing specimens for DNA sequencing. CLV is most grateful to Tosio Kumata, Ohshima Issei and Kazuhiro Sugisima for help during field collections in Hokkaido. We also thank David Dilcher of the Florida Museum of Natural History for the loan and identification of the host-plant specimens (UF7351, UF4818) upon which one of our dates is based. The authors thank Elisabeth Herniou and Tim Barraclough for comments on earlier versions of this manuscript, and Finnegan Marsh for assistance with Fig. 2. This study has been supported by the National Environment Research Council Centre for Population Biology. This is contribution no. 121 of the Evolution of Terrestrial Ecosystems consortium at the National Museum of Natural History.

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## Supplementary Material

The following supplementary material is available for this article online:

**Fig. S1** Distributions of age estimates. Age: mode of the distribution. It represents the most likely value for the divergence time of a particular node; LHPD: 90% lower highest posterior density limit; UHPD: 90% upper highest posterior density limit. Nodes of interest are numbered from 1 to 15 and indicated on the cladogram (see Fig. 3a,b; script for 'R' statistical package).

**Table S1** Probability (as averaged over the 100 randomly selected trees from the posterior distribution) that a particular plant group is reconstructed for our node of interest and not reconstructed for the node below.

**Table S2** Body and trace fossil record of the Gracillarioidea (Lepidoptera: Ditrysia, Gracillariidae and Bucculatricidae).

**Appendix S1.** Specimens used in this study. They have been ordered according to the plant classification (APG, 1998).

This material is available as part of the online article from <http://www.blackwell-synergy.com>

*Received 15 June 2005; revised 18 October 2005; accepted 17 November 2005*

## Supplementary material to:

### **Fossil-calibrated molecular phylogenies reveal that leaf-mining moths radiated several million years after their host plants**

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## Figure Caption

Figure 4. Distributions of age estimates. Age = mode of the distribution. It represents the most likely value for the divergence time of a particular node. LHPD= 90% Lower Highest Posterior Density limit. UHPD= 90% Upper Highest Posterior Density limit. Nodes of interest are numbered from 1 to 15 and indicated on the cladogram (see figure 3a,b).

## Script for R' statistical package

```
# The authors accept no liability for the accuracy, use/misuse of this
script, or whether it is fit for purpose
# This script 'automatically' calculates mode and 90%HPD intervals for
Bayesian distributions given a matrix of values drawn from a posterior
distribution.
# The contributed package "Locfit" is required (see http://www.r-
project.org)
# A data file is required, consisting of a comma delimited ".csv" file,
exported from a spreadsheet such as Excel.
# The first row should be column headings with the name of each
variable
# The first column can be an index field, listing the iteration (or
draw number) for the posterior distribution
# Each column is the values for each variable and the rows are the
values from a given iteration.
# You will need to adjust the filename for the .csv file on the second
line to match your own data file.
# It is recommended that you perform some exploratory analysis with
locfit first to ensure the default settings allow unbiased estimation
of the distributions
# You should plot the locfit objects to check the shape of the
distributions, these should be smooth, not spiky!
# This is not a 'blackbox' analysis and users must be aware of the need
to check that it is appropriate for their datasets and to confirm their
own results.

library(locfit)
age<-read.csv("agesheet.csv") #reads in the file "agesheet.csv",
you'll need to change the file name to match your data file
attach(age) #makes age the main data frame

sum.stat<-summary(age) #summary stats for all columns
print(sum.stat)
lnam<-length(names(age))
```

```

res<-c(0,0,0) #sets up dummy variable for results
table, use this if the first the column of values is an index field
(i.e. contains the iteration number)
#res<-c() #sets up dummy variable for results
table, use this if there is instead of the above if there is no index
field
# in this case you also need to change
the 'for loop' below to (s in 1:lnam)

#Calculations begin

for (s in 2:lnam) {

for interval max.node<-max(age[s], na.rm=TRUE) #gets max value
for interval min.node<-min(age[s], na.rm=TRUE) #gets min value
for interval interval<-c(min.node,max.node)
x<-as.matrix(age[s])
lf <- locfit(~x) #fits density
distribution

mfd<-function(x,lf){return(predict(lf,x))}
#defines function used to get predicted values # next
line finds the mode of the distribution

mmaxd<-optimise(mfd, interval=c(min.node,max.node),
lower=min(interval), upper=max(interval), maximum=TRUE, tol=0.001, lf)

f<-fitted(lf) #next few lines find 90% HPD values
fi<-order(f)
j<-length(f)
j1<-0.1*j
k<-fi[j1:j]
r<-range(x[k])
# r #this is the 90% HPD range
m1<-as.numeric(mmaxd[1]) #converts mode value to
numeric object
res<-append(res,m1) #appends to results vector
res<-append(res,r) #appends to results vector

}

###Process results vector for output

cn<-c("Name","Mode","90% LHPD","90% UHPD") #column names for
results table

res.mat<-t(matrix(res,3,(length(res)/3))) #turns the results
vector in to a matrix
# res.tab<-data.frame(res.mat) #makes the results
matrix in to a table for ouput
res.tab<-data.frame(cbind(names(age),res.mat)) #does the same as
above but adds names of original data columns
colnames(res.tab)<-cn #assigns column names

```



```
res.tab
```

```
# write.table(res.tab,file="results.txt") #writes  
a table to file, you should set your own file name
```

```
write.table(res.tab,file="results.txt",quote=FALSE,sep="\t") #does the  
same but without quotes and tab delimited, more convenient for import  
into other programmes.
```

```
### End of script.
```

Table 3. Probability (as averaged over the 100 randomly selected trees from the posterior distribution) that a particular plant group is reconstructed for our node of interest and not reconstructed for the node below.

Tree Nr.	Node 2 Fagales	Node 2 Malpighiales	Node 3 <i>Populus</i>	Node 4 <i>Ulmus</i>	Node 5 <i>Acer</i>	Node 6 Fagaceae	Node 7 <i>Lyonia</i>	Node 8 <i>Malus</i>	Node 9 <i>Alnus</i>	Node 10 <i>Corylus</i>	Node 11 Salicaceae	Node 12 <i>Prunus</i>	Node 13 <i>Acer</i>	Node 14 <i>Carpinus</i>	Node 15 <i>Quercus</i>
1	0.4	0.4	1.0	0.5	0.7	0.0	0.7	0.8	0.4	0.4	0.4	1.0	0.5	0.75	0.5
2	0.4	0.4	0.5	0.9	0.7	1.0	0.7	0.2	1.0	0.4	0.4	0.5	0.9	0.75	0
3	0.4	0.4	1.0	0.9	0.0	1.0	0.7	0.7	1.0	0.4	0.4	1.0	0.9	0.667	0.67
4	0.4	0.4	0.5	0.9	0.7	0.8	0.9	0.1	0.8	0.4	0.4	0.5	0.9	0.75	0.5
5	0.4	0.4	1.0	0.9	0.0	0.5	0.7	0.4	1.0	0.4	0.4	1.0	0.9	0.667	0.67
6	0.4	0.4	1.0	0.9	1.0	0.8	0.8	0.7	1.0	0.4	0.4	1.0	0.9	0.75	0
7	0.4	0.4	0.3	0.8	1.0	1.0	0.7	0.7	0.0	0.4	0.4	0.3	0.8	0.667	0.67
8	0.4	0.4	1.0	0.9	0.5	0.5	0.7	0.2	0.7	0.4	0.4	1.0	0.9	0.75	0.5
9	0.4	0.4	1.0	0.5	1.0	0.5	0.7	0.1	0.0	0.4	0.4	1.0	0.5	0.75	0.5
10	0.3	0.3	1.0	0.9	0.8	0.7	0.7	0.7	1.0	0.3	0.3	1.0	0.9	1	0
11	0.4	0.4	0.3	0.9	0.7	0.0	0.7	0.8	1.0	0.4	0.4	0.3	0.9	0.75	0.5
12	0.3	0.3	1.0	0.8	1.0	1.0	0.7	0.2	0.5	0.3	0.3	1.0	0.8	0.75	0.5
13	0.0	0.0	0.4	1.0	1.0	0.8	0.8	0.4	0.5	0.0	0.0	0.4	1.0	1	0
14	0.0	0.0	0.4	0.9	0.0	0.5	0.7	0.7	1.0	0.0	0.0	0.4	0.9	0.75	0.5
15	0.8	0.0	0.4	0.9	0.5	0.5	0.7	0.8	1.0	0.8	0.0	0.4	0.9	0.75	0.5
16	0.4	0.4	1.0	0.9	0.7	0.5	0.7	0.4	0.8	0.4	0.4	1.0	0.9	0.75	0.5
17	0.0	0.0	0.4	0.9	0.0	0.5	0.7	0.7	1.0	0.0	0.0	0.4	0.9	1	0.5
18	0.0	0.0	0.4	1.0	0.7	0.0	0.7	0.2	1.0	0.0	0.0	0.4	1.0	0.75	0.5
19	0.4	0.4	1.0	0.9	0.7	1.0	0.7	0.4	1.0	0.4	0.4	1.0	0.9	0.857	0
20	0.0	0.0	0.4	0.7	1.0	0.0	0.7	0.7	1.0	0.0	0.0	0.4	0.7	0.667	0.667
21	0.4	0.4	1.0	1.0	1.0	0.8	0.7	0.8	0.0	0.4	0.4	1.0	1.0	0.75	0.5
22	0.3	0.3	0.3	0.9	0.7	0.0	0.7	0.0	1.0	0.3	0.3	0.3	0.9	1	1
23	0.4	0.4	1.0	0.9	0.7	0.5	0.7	0.7	1.0	0.4	0.4	1.0	0.9	0.75	0.5

24	0.3	0.3	1.0	0.8	1.0	0.8	0.7	0.8	1.0	0.3	0.3	1.0	0.8	0.75	0.5
25	0.0	0.0	0.4	0.7	1.0	0.5	0.7	0.1	1.0	0.0	0.0	0.4	0.7	1	0.5
26	0.4	0.4	0.3	0.9	1.0	0.8	0.8	0.2	1.0	0.4	0.4	0.3	0.9	0.75	0.5
27	0.0	0.0	0.4	1.0	0.8	0.8	0.7	0.7	0.4	0.0	0.0	0.4	1.0	0.75	0
28	0.4	0.4	1.0	0.9	1.0	0.5	0.7	0.1	1.0	0.4	0.4	1.0	0.9	1	0.5
29	0.0	0.0	0.4	0.8	1.0	0.0	0.7	0.8	0.0	0.0	0.0	0.4	0.8	0.75	0.5
30	0.4	0.4	1.0	0.9	0.8	0.5	0.7	0.4	1.0	0.4	0.4	1.0	0.9	1	0
31	0.0	0.0	0.4	0.8	0.5	0.5	0.7	0.1	1.0	0.0	0.0	0.4	0.8	0.667	0.667
32	0.3	0.2	0.0	0.7	1.0	0.0	0.7	0.1	0.0	0.3	0.2	0.0	0.7	1	0.5
33	0.4	0.4	1.0	0.8	0.0	1.0	0.7	0.8	1.0	0.4	0.4	1.0	0.8	0.75	0
34	0.0	0.0	0.4	0.8	0.5	0.5	0.7	0.1	0.5	0.0	0.0	0.4	0.8	0.75	0.5
35	0.4	0.4	1.0	0.5	1.0	0.5	0.7	0.8	0.0	0.4	0.4	1.0	0.5	0.75	0.5
36	0.4	0.4	0.3	0.9	1.0	0.0	0.7	0.2	1.0	0.4	0.4	0.3	0.9	0.667	0.5
37	0.4	0.4	1.0	0.7	0.5	1.0	0.7	0.8	1.0	0.4	0.4	1.0	0.7	0.667	0.667
38	0.3	0.3	0.3	0.8	1.0	0.8	0.8	0.2	1.0	0.3	0.3	0.3	0.8	0.667	0.667
39	0.0	0.0	0.4	0.9	1.0	0.5	0.7	0.2	1.0	0.0	0.0	0.4	0.9	1	0
40	0.3	0.3	1.0	0.8	0.8	0.8	0.8	0.8	0.4	0.3	0.3	1.0	0.8	1	0
41	0.4	0.4	1.0	0.9	1.0	0.8	0.7	0.0	1.0	0.4	0.4	1.0	0.9	1	0.5
42	0.4	0.4	1.0	0.5	1.0	0.8	0.7	0.4	1.0	0.4	0.4	1.0	0.5	0.75	0.5
43	0.4	0.4	0.3	0.9	1.0	0.0	0.7	0.7	0.7	0.4	0.4	0.3	0.9	0.667	0.667
44	0.4	0.4	1.0	0.5	0.8	0.5	0.7	0.1	0.5	0.4	0.4	1.0	0.5	0.667	0.667
45	0.3	0.2	0.4	0.8	0.7	0.5	0.7	0.7	1.0	0.3	0.2	0.4	0.8	0.75	0.5
46	0.4	0.4	1.0	0.9	1.0	1.0	0.7	0.4	1.0	0.4	0.4	1.0	0.9	0.75	0.5
47	0.4	0.4	1.0	0.9	1.0	0.5	0.7	0.2	1.0	0.4	0.4	1.0	0.9	1	0.5
48	0.3	0.3	1.0	0.5	0.0	0.5	0.7	0.8	1.0	0.3	0.3	1.0	0.5	0.75	0.5
49	0.4	0.4	1.0	0.5	0.0	0.8	0.7	0.7	1.0	0.4	0.4	1.0	0.5	0.667	0.667
50	0.4	0.4	1.0	0.9	1.0	0.8	0.7	0.4	1.0	0.4	0.4	1.0	0.9	1	0.5
51	0.4	0.4	1.0	0.7	1.0	0.5	0.7	0.7	1.0	0.4	0.4	1.0	0.7	1	0
52	0.4	0.4	1.0	0.9	1.0	0.5	0.7	0.2	1.0	0.4	0.4	1.0	0.9	0.75	0.5

53	0.0	0.0	0.4	0.9	0.7	0.5	0.7	0.2	1.0	0.0	0.0	0.4	0.9	1	0.5
54	0.4	0.4	1.0	0.9	1.0	0.0	0.7	0.4	1.0	0.4	0.4	1.0	0.9	0.75	0.5
55	0.0	0.0	0.4	0.8	0.5	0.0	0.7	0.1	1.0	0.0	0.0	0.4	0.8	1	0.5
56	0.0	0.0	0.3	0.9	1.0	0.0	0.7	0.2	1.0	0.0	0.0	0.3	0.9	1	0.5
57	0.0	0.0	0.4	0.9	1.0	0.0	0.7	0.8	1.0	0.0	0.0	0.4	0.9	0.75	0.5
58	0.3	0.3	1.0	0.9	1.0	0.5	0.7	0.7	0.0	0.3	0.3	1.0	0.9	0.667	0
59	0.4	0.4	1.0	0.9	1.0	0.8	0.7	0.2	1.0	0.4	0.4	1.0	0.9	1	0.5
60	0.4	0.4	1.0	1.0	1.0	1.0	0.7	0.2	1.0	0.4	0.4	1.0	1.0	1	0
61	0.4	0.4	1.0	0.8	1.0	0.5	0.7	0.7	0.4	0.4	0.4	1.0	0.8	0.75	0.5
62	0.4	0.4	1.0	0.9	1.0	0.8	0.7	0.2	1.0	0.4	0.4	1.0	0.9	0.75	0.5
63	0.3	0.3	1.0	0.8	1.0	1.0	0.7	0.8	0.5	0.3	0.3	1.0	0.8	0.667	0.667
64	0.3	0.3	1.0	0.9	0.8	1.0	0.7	0.4	0.5	0.3	0.3	1.0	0.9	0.75	0.5
65	0.8	0.0	0.8	0.8	1.0	0.0	0.7	0.7	1.0	0.8	0.0	0.8	0.8	0.667	0.667
66	0.4	0.4	1.0	1.0	1.0	0.0	0.7	0.8	0.0	0.4	0.4	1.0	1.0	1	0.5
67	0.4	0.4	1.0	0.0	0.7	1.0	0.7	0.2	0.5	0.4	0.4	1.0	0.0	0.75	0
68	0.4	0.4	1.0	0.5	0.7	1.0	0.7	0.8	0.5	0.4	0.4	1.0	0.5	0.667	0.667
69	0.4	0.4	1.0	0.9	1.0	0.8	0.7	0.2	1.0	0.4	0.4	1.0	0.9	0.75	0.5
70	0.4	0.4	1.0	1.0	0.8	0.8	0.7	0.8	0.7	0.4	0.4	1.0	1.0	1	0.5
71	0.4	0.4	0.3	0.9	1.0	1.0	0.7	0.7	1.0	0.4	0.4	0.3	0.9	0.75	0.5
72	0.4	0.4	1.0	0.8	1.0	0.5	0.7	0.7	0.0	0.4	0.4	1.0	0.8	0.75	0.5
73	0.3	0.3	1.0	0.7	1.0	0.5	0.7	0.2	1.0	0.3	0.3	1.0	0.7	0.667	0.667
74	0.4	0.4	1.0	0.5	1.0	1.0	0.7	0.2	0.5	0.4	0.4	1.0	0.5	0.75	0.5
75	0.4	0.4	1.0	0.8	1.0	0.0	0.7	0.2	0.0	0.4	0.4	1.0	0.8	0.667	0.667
76	0.4	0.4	0.3	1.0	0.8	1.0	0.8	0.7	0.4	0.4	0.4	0.3	1.0	0.75	0.5
77	0.0	0.0	0.4	0.7	0.5	0.0	0.7	0.2	0.7	0.0	0.0	0.4	0.7	0.667	0.667
78	0.4	0.4	0.3	0.9	0.7	0.0	0.7	0.2	0.7	0.4	0.4	0.3	0.9	0.75	0.5
79	0.4	0.4	1.0	0.8	0.0	1.0	0.7	0.0	1.0	0.4	0.4	1.0	0.8	0.667	0.667
80	0.0	0.0	0.4	0.8	0.8	0.5	0.7	0.8	1.0	0.0	0.0	0.4	0.8	1	0
81	0.4	0.4	1.0	0.9	0.5	0.0	0.7	0.7	1.0	0.4	0.4	1.0	0.9	0.667	0.667

<b>82</b>	0.0	0.0	0.4	0.9	1.0	0.0	0.7	0.4	0.0	0.0	0.0	0.4	0.9	0.667	0.667
<b>83</b>	0.4	0.4	1.0	0.8	0.5	0.8	0.7	0.2	1.0	0.4	0.4	1.0	0.8	0.667	0.667
<b>84</b>	0.4	0.4	1.0	1.0	1.0	0.5	0.7	0.2	0.0	0.4	0.4	1.0	1.0	1	0.5
<b>85</b>	0.3	0.3	1.0	0.9	1.0	0.0	0.7	0.8	1.0	0.3	0.3	1.0	0.9	0.75	0.5
<b>86</b>	0.4	0.4	1.0	1.0	0.7	1.0	0.7	0.4	1.0	0.4	0.4	1.0	1.0	0.75	0.5
<b>87</b>	0.8	0.0	0.4	0.8	0.7	0.0	0.7	0.4	1.0	0.8	0.0	0.4	0.8	1	0.5
<b>88</b>	0.3	0.3	1.0	0.8	1.0	0.5	0.7	0.7	1.0	0.3	0.3	1.0	0.8	1	0.5
<b>89</b>	0.4	0.4	1.0	0.8	1.0	0.5	0.7	0.7	0.0	0.4	0.4	1.0	0.8	0.75	0.5
<b>90</b>	1.0	0.0	1.0	0.8	1.0	0.8	0.8	0.0	1.0	1.0	0.0	1.0	0.8	1	1
<b>91</b>	0.0	0.0	0.5	0.9	1.0	0.5	0.7	0.4	1.0	0.0	0.0	0.5	0.9	0.75	0.5
<b>92</b>	0.0	0.0	0.4	0.5	0.5	0.5	0.7	0.2	1.0	0.0	0.0	0.4	0.5	0.75	0.5
<b>93</b>	1.0	0.0	1.0	0.7	1.0	0.0	0.7	0.4	1.0	1.0	0.0	1.0	0.7	1	0.5
<b>94</b>	0.0	0.0	0.4	0.9	1.0	0.0	0.7	0.0	1.0	0.0	0.0	0.4	0.9	0.667	0.667
<b>95</b>	0.4	0.4	0.3	0.9	0.5	0.0	0.7	0.8	1.0	0.4	0.4	0.3	0.9	0.75	0.5
<b>96</b>	0.8	0.0	0.4	0.5	1.0	0.0	0.7	0.8	1.0	0.8	0.0	0.4	0.5	0.8	0.8
<b>97</b>	0.3	0.3	1.0	0.7	1.0	0.5	0.7	0.7	1.0	0.3	0.3	1.0	0.7	1	0
<b>98</b>	0.4	0.4	1.0	0.8	1.0	0.0	0.7	0.2	1.0	0.4	0.4	1.0	0.8	1	0.5
<b>99</b>	0.4	0.4	1.0	0.8	0.7	1.0	0.7	0.2	1.0	0.4	0.4	1.0	0.8	1	0.5
<b>100</b>	0.4	0.4	1.0	0.9	1.0	0.0	0.7	0.4	0.7	0.4	0.4	1.0	0.9	0.75	0.5
<b>Total</b>	32.2	26.8	76.1	78.7	78.8	50.7	67.9	43.1	77.1	32.2	26.8	76.1	78.7	80.748	46.816

Table 4. Body and trace-fossil record of the Gracillarioidea (Lepidoptera: Ditrysiinae, Gracillariidae and Bucculatricidae).

<b>Leaf Miner</b>	<b>Host plant</b>	<b>Age (epoch, stage)</b>	<b>Formation and locality</b>	<b>References</b>
cf. <i>Bucculatrix thoracella</i> Thunburg	<i>Tilia</i> sp. (Malvales: Tiliaceae)	late Pliocene, (Piacenzian; ~2.7 m.y.)	Willershausen, Germany	(Strauss, 1977)
cf. <i>Caloptilia alchimiella</i> S.C.	<i>Fagus</i> cf. <i>occidentalis</i> (Fagales: Fagaceae)	late Pliocene, (Piacenzian; ~2.7 m.y.)	Willershausen, Germany	(Strauss, 1977)
cf. <i>Caloptilia roscipennis</i>	<i>Juglans</i> (Fagales: Juglandaceae)	late Pliocene, (Piacenzian; ~2.7 m.y.)	Willershausen, Germany	(Strauss, 1977)
cf. <i>Corsicium</i> sp.	<i>Magnolia</i> sp. (Magnoliales: Magnoliaceae) , or <i>Syringia</i> sp. (Lamiales: Oleaceae)	late Pliocene, (Piacenzian; ~2.7 m.y.)	Willershausen, Germany	(Strauss, 1977)
<i>Lithocolletis maestingella</i>	unknown host	late Pliocene, (Piacenzian; ~2.7 m.y.)	Willershausen, Germany	(Strauss, 1977)

Zetterstedt		m.y.)	Germany	
cf. <i>Parornix</i> sp.	<i>Amelanchier</i> sp. (Rosales: Rosaceae)	late Pliocene, (Piacenzian; ~2.7 m.y.)	Willershaus en, Germany	(Strauss, 1977)
cf. <i>Caloptilia</i> <i>roscipenella</i>	Fagales: Betulaceae	early Pliocene (Zanclian; ~4.4 m.y.)	Chuzbaia, Romania	(Givulescu, 1984)
<i>Caloptilia</i> sp.	? <i>Quercus</i> (Fagales: Fagaceae)	Miocene [undifferentiated]	Latah Formation; eastern	(Lewis, 1969)
<i>Acrocercops</i> sp.	<i>Quercus</i> <i>consimilis</i> Newberry (Fagales: Fagaceae)	late Miocene (Messinian; ~6.2 m.y.)	Trout Creek, Oregon, USA	(Opler, 1973)
" <i>Lithocolletis</i> " sp.	<i>Populus</i> <i>trichocarpa</i> var. <i>ingrata</i> (Malpighiales: Salicaceae)	late Miocene (Messinian; ~6.2 m.y.)	Stewart Valley, Nevada, USA	(Opler, 1973)
<i>Bucculatrix</i> sp.	<i>Quercus</i> <i>hanibalii</i> Dorf (Fagales: Fagaceae)	late Miocene (Messinian; ~6.2 m.y.)	Buffalo Canyon, Nevada,	(Opler, 1973)

	Fagaceae)		USA	
<i>Cameraria</i> sp.	<i>Quercus simulata</i> Knowlton (Fagales: Fagaceae)	early to late Miocene (Tortonian/(Serravv allian; ~13 m.y.)	Thorn Creek, Idaho, USA	(Opler, 1973)
" <i>Lithocolletis</i> "	<i>Quercus hanibalii</i> Dorf (Fagales: Fagaceae)	late middle Miocene, (Serravallian; ~13 m.y.)	Upper Goldyke, Cedar	(Opler, 1973)
Gracillariidae (body fossil)	— —	early Miocene, (Aquitanian; ~22 m.y.)	La Toca Formation amber;	(Poinar <i>et al.</i> , 1991)
<i>Phyllonorycter?</i> <i>Oliveirae</i> Martins-Neto	<i>Symplocos</i> sp. (Ericales: Symplocaceae)	early Oligocene (Rupelian; ~31 m.y.)	Tremembé Formation; Taubaté, São Paulo, Brazil	(Martins-Neto, 1989)
<i>Bucculatrix</i> sp.	" <i>Zelkova</i> " <i>drymeja</i> (Rosales: Ulmaceae)	late Eocene (Priabonian; ~34.5 m.y.)	Florissant Formation; Park, County, Colorado, USA	(Opler, 1982; Kozlov, 1988)



<i>Gracillariites</i> sp. (body fossil)	— —	late Eocene, (Priabonian; ~35 m.y.)	Bembridge Marls, Gunnard Bay, Isle of Wight, U.K.	(Jarzembowski, 1980; Kozlov, 1987)
" <i>Lithocolletis</i> " sp.	unknown dicot	late Eocene, (Priabonian; ~35 m.y.)	White Lake Basin, British Columbia, Canada	(Freeman, 1965; Kozlov, 1988)
<i>Gracillariites</i> <i>mixtus</i> * Kozlov (body fossil)	— —	middle Eocene, (Lutetian; ~44.1 m.y.)	Baltic amber	(Kozlov, 1987)
<i>Gracillariites</i> <i>lithuani-</i> Kozlov (body fossil)	— —	middle Eocene, (Lutetian; ~44.1 m.y.)	Baltic amber	(Kozlov, 1987)
Gracillariid [recent analog: <i>Lyonettia</i> <i>prunifoliella</i> Hübn	unknown dicot	middle Eocene, (?Barto	Bransome Sand Formation; Bournemouth,	(Lang <i>et al.</i> , 1995)

Gracillariid (recent analog: <i>Lithocolletis</i> <i>rajella</i> on <i>Alnus</i> )	unknown dicot	middle Eocene, (Bartonian)	Bransome Sand Formation; Bournemouth,	(Stevenson, 1992; Lang <i>et al.</i> , 1995)
Gracillariid (recent analog: <i>Phyllocnistis</i> <i>suffusella</i> Zeller on <i>Populus</i> (Salicaceae)	unknown dicot	middle Eocene, (Bartonian)	Bransome Sand Formation; Bournemouth,	(Stevenson, 1992; Stevenson & Scott, 1992; Lang <i>et al.</i> , 1995)
Gracillariid	unknown dicot	middle Eocene, (Lutetian; ~48 m.y.)	Republic, Ferry County, Washington, U	(Labandeira, 2002)
<i>Phyllocnistis</i> sp.	<i>Cedrela</i> sp. (Sapindales: Meliaceae)	early Eocene, (Ypresian; ~51 m.y.)	Wind River Formation; Dubois, Wyoming, USA	(Hickey & Hodges, 1975)
Gracillariid (recent analog: <i>Bucculatrix</i> <i>albella</i> Sft. on	<i>Marmarthia</i> <i>pearsoni</i> (Laurales)	Late Cretaceous, (late Maastrichtian; ~ 91 m.y.)	Hell Creek Formation; Willis ton Basin,	(Lang, 1996; Labandeira, 2002; Labandeira <i>et al.</i> , 2002)

<i>Paliurus</i> (Rhamnaceae)			North Dakota, USA	
? <i>Phyllocnistis</i>	“ <i>Staphylea?</i> <i>fremontii</i> ” [an unknown dicot]	Late Cretaceous (Turonian; ~91 m.y.)	Frontier Formation; Lincoln County, Wyoming, USA	(Knowlton, 1917)
<i>Bucculatrix</i> <i>platani</i> Kozlov	<i>Platanus</i> sp. (Proteales: Platanaceae)	Late Cretaceous (Turonian; ~91 m.y.)	Kizyl-Zhar, Kazakstan	(Kozlov, 1988)
<i>Phyllocnistis</i> sp.	<i>Rogersia</i> <i>parlatorii</i> Laurales: cf. Lauraceae); <i>Densinevum</i> sp. (cf. Chlor anthaceae); <i>Pabiana</i> <i>variloba</i> (Laurales)	Early Cretaceous (late Albian; ~99 m.y.)	Dakota Formation; Kansas and Nebraska, USA	(Labandeira <i>et al.</i> , 1994; Labandeira, 1998; Kristensen & Skalski, 1999)

1. Systematic placement of plant hosts follows Judd (1999)

2. Geochronologic dates are given as midpoints of stages (Gradstein & Ogg, 1996), unless specific isotopic dates are provided.
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Appendix 1. Specimens used in this study. They have been ordered according to the plant classification (APG, 1998)

Host Plant	Species name	Collection site	Voucher number	Collector	GenBank accession number 28S rDNA
<b>ORDER Proteales</b>					
<b>Family Platanaceae</b>					
<i>Platanus sp.</i>	<i>Phyllonorycter platani</i> (Staudinger, 1870)	Osterley Park, Middlesex, UK	49(91)	CLV	AF477565
<b>ASTERIDS</b>					
<b>ORDER Ericales</b>					
<b>Family Ericaceae</b>					
<i>Vaccinium vitis-idaea</i>	<i>Phyllonorycter junoniella</i> (Zeller, 1846)	Loch Garten, Inverness-shire, Scotland	67(150)	MS	AF477555
<i>Lyonia ovalifolia</i>	<i>Phyllonorycter lyoniae</i> Kumata, 1963	Asahi-Kogen, Honsyu, Japan	266	KS	<b>AY521508</b>
<b>Euasterid II</b>					
<b>ORDER Dipsacales</b>					
<b>Family Caprifoliaceae</b>					
<i>Lonicera sp.</i>	<i>Phyllonorycter loniceræ</i> Kumata, 1963	Oguni-Zinaya, Honsyu, Japan	268	KS	<b>AY521517</b>
<i>Lonicera sp.</i>	<i>Phyllonorycter trifasciella</i> (Haworth, 1828)	Hanwell, London, UK	13,14(142)	CLV	AF477580
<i>Symphoricarpos sp.</i>	<i>Phyllonorycter sp.4</i>	Santa Rosa, Guanajuato, Mexico	242	CLV	AY230759
<i>Symphoricarpos rivularis</i>	<i>Phyllonorycter emberizaepennella</i> (Bouche, 1834)	Pett's Wood, Kent, UK	104(182)	DO	AF477526
<b>Family Valerianaceae</b>					
<i>Viburnumx carlcephalum</i>	<i>Phyllonorycter lantanella</i> (Schrank, 1802)	Kew Gardens, UK	158	CLV	AF477502
<b>Family Dipsacaceae</b>					
<i>Scabiosa sp.</i>	<i>Phyllonorycter scabiosella</i> (Douglas, 1853)	Addington, Kent, UK	92(172)	DO	AF477518
<b>ROSIDS</b>					
<b>Eurosid II</b>					
<b>ORDER Malvales</b>					
<b>Family Malvaceae</b>					
<i>Grewia sp.</i>	<i>Phyllonorycter grewiella</i> (Vari, 1961)	Bontebok, South Africa	248	CLV	AY230753
<i>Tilia platyphylla</i>	<i>Phyllonorycter issikii</i> (Kumata, 1963)	Bialowiezca, Poland	246	EO	AY230754

<i>Tilia japonica</i>	“	Ishikari, Hokkaido, Japan	321	HK	<b>AY521521</b>
<i>Dombeya torrida</i>	<i>Phyllonorycter loxozana</i> (Meyrick, 1936)	Kenya: Zuni	126	DA	AF477550
<b>ORDER Sapindales</b>					
<b>Family Sapindaceae</b>					
<i>Acer pseudoplatanus</i>	<i>Phyllonorycter geniculella</i> (Ragonot, 1874)	Silwood Park, Ascot, Berkshire, UK	26(110)	CLV	AF477575
<i>Acer platanoides</i>	<i>Phyllonorycter platanoidella</i> (Joannis, 1920)	Silwood Park, Ascot, Berkshire, UK	41(128)	CLV	AF477563
<i>Acer campestre</i>	<i>Phyllonorycter sylvella</i> (Haworth, 1828)	Lepe, New Forest, UK	28(137)	CLV	AF477582
<i>Acer carpinifolium</i>	<i>Phyllonorycter orientalis</i> Kumata, 1963	Honsyu, Simasima-dani, Azumi Vill. Nagano Pref., Japan	JP16	KS	<b>AY521519</b>
<i>Acer mono</i>	<i>Phyllonorycter sp.</i> 8	Kannon-Zawa, Sapporo City, Hokkaido, Japan	JP15	KS	<b>AY521518</b>
<b>Eurosid I</b>					
<b>ORDER Malpighiales</b>					
<b>Family Salicaceae</b>					
<i>Salix repens</i>	<i>Phyllonorycter quinqueguttella</i> (Stainton, 1851)	La Chaume, Vendee, France	91(167)	CLV	AF477532
<i>Salix caprea</i>	<i>Phyllonorycter viminiella</i> (Sircom, 1848)	Lepe, Hampshire, UK	29(138)	CLV	AF477571
“	“	Silwood Park, Ascot, Berkshire, UK	42(114)	CLV	AF477571
<i>Salix cinerea.</i>	<i>Phyllonorycter salicicolella</i> (Sircom, 1848)	Kristianstad, Sweden	177	SV	AF477522
<i>Salix sp.</i>	<i>Phyllonorycter dubitella</i> (Herrich-Schaffer, 1855)	Silwood Park, Ascot, Berkshire, UK	40(129)	CLV	AF477572
<i>Salix fragilis.</i>	<i>Phyllonorycter salictella</i> (Zeller, 1846)	Kristianstad, Sweden	179	SV	AF477505
<i>Salix aurita</i>	<i>Phyllonorycter brevilineatella</i> (Benander, 1946)	Jamshog, Sweden	178	SV	AF477538
<i>Salix viminalis</i>	<i>Phyllonorycter viminetorum</i> (Stainton, 1854)	Woolhampton, Reading, UK	159	IS	AF477530
<i>Not reared</i>	<i>Phyllonorycter hilarella</i> (Zetterstedt, 1839)	Twolluvaara, Sweden	227	SV	AF477534
<i>Salix sp.</i>	<i>Phyllonorycter heringiella</i> Gronlien, 1932	Bjalbo, Marstad, Sweden	181	SV	AF477542
<i>Salix sp.</i>	<i>Phyllonorycter pastorella</i>	Trudovec, Bulgary	272	RT	AF477535



	(Zeller, 1846)				
<i>Salix miyabeana</i>	“	Sapporo, Hokkaido, Japan	323	HK	AF477509
<i>Populus canescens</i>	<i>Phyllonorycter comparella</i> (Duponchel, 1843)	Andreze, Maine Loire, France	86(164)	CLV	AF477539
<i>Populus tremula</i>	<i>Phyllonorycter sagitella</i> (Bjerkander, 1790)	Worcestershire, UK	209	MH	AF477532
<i>Populus sp.</i>	<i>Phyllonorycter populifolilella</i> (Treitschke, 1833)	Uglich, Russia	80	EO	AF477571
<b>ORDER Fabales</b>					
<b>Family Fabaceae</b>					
<i>Medicago sp.</i>	<i>Phyllonorycter medicaginella</i> (Gerasimov, 1930)	Butan, Bulgaria	275	RT	<b>AY521515</b>
<i>Vicia sp.</i>	<i>Phyllonorycter nigrescentella</i> (Logan, 1851)	Darenth Wood, Kent, UK	98(176)	DO	AF477515
<i>Trifolium sp.</i>	<i>Phyllonorycter insignitella</i> (Zeller, 1846)	La Gachere, Brem sur Mer, France	85(165)	CLV	AF477540
<i>Sarothamnus scoparius</i>	<i>Phyllonorycter scopariella</i> (Zeller, 1846)	Mandagout, Cevennes, France	71(153)	CLV	AF477552
<i>Ulex europaeus</i>	<i>Phyllonorycter ulicicolella</i> (Stainton, 1851)	Silwood Park, Ascot, Berkshire, UK	69(151)	CLV	AF477516
<i>Genista pilosa</i>	<i>Phyllonorycter staintoniella</i> (Nicelli, 1853)	Perranporth, Cornwall, UK	154	FHNS	AF477540
<i>Genista radiata</i>	<i>Phyllonorycter baldensis</i> Deschka, 1986	Corna Piana, , Monte Valdo, Verona, Italy	192	GD	AF477504
<i>Genista florida</i>	<i>Phyllonorycter genistella</i> (Rebel, 1900)	Miraflores de la Sierra, Madrid, Spain	258	EN	<b>AY521512</b>
<i>Adenocarpus complicatus</i>	<i>Phyllonorycter parvifoliella</i> (Ragonot, 1875)	Branza, Arzua, Galicia, Spain	175(218)	CLV	AF477510
<i>Chamaespartium tridentatum</i>	<i>Phyllonorycter cerasinella</i> (Reutti, 1852)	Alajar, Sierra de Aracena, Huelva, Spain	255	EN	<b>AY521509</b>
<i>Stauracanthus genistoides</i>	<i>Phyllonorycter sp.6</i>	Magazon, Huelva, Spain	256	EN	<b>AY521510</b>
<i>Genista polyantha</i>	<i>Phyllonorycter sp.7</i>	El Garrobo, Sevilla, Spain	257	EN	<b>AY521511</b>
<i>Adenocarpus telonensis</i>	<i>Phyllonorycter parvifoliella</i> (Ragonot, 1875)	Miraflores de la Sierra, Madrid, Spain	259	EN	<b>AY521513</b>
<b>ORDER Fagales</b>					
<b>Family Betulaceae</b>					
<i>Alnus sp.</i>	<i>Phyllonorycter sp.5</i>	Santa Rosa, Guanajuato, Mexico	244	CLV	AY230760
<i>Alnus glutinosa</i>	<i>Phyllonorycter stettinensis</i> (Nicelli, 1852)	Silwood Park, Ascot, Berkshire, UK	43(112)	CLV	AF477545
“	“	Virginia Water, Surrey,	5(6.2)	CLV	AF477545

		UK			
“	“	Silwood Park, Ascot, Berkshire, UK	111(189)	CLV	AF477545
<i>Alnus glutinosa</i>	<i>Phyllonorycter froelichiella</i> (Zeller, 1839)	Silwood Park, Ascot, Berkshire, UK	6(6.3)	CLV	AF477586
<i>Alnus glutinosa</i>	<i>Phyllonorycter rajella</i> (Linnaeus, 1758)	Silwood Park, Ascot, Berkshire, UK	55(113)	CLV	AF477568
<i>Alnus incana</i>	<i>Phyllonorycter strigulatella</i> (Lienig & Zeller, 1846)	Bookham, Surrey, UK	99(158)	CLV	AF477511
“	“	Medmenham, Berks, UK	162	IS	AF477511
<i>Alnus glutinosa</i>	<i>Phyllonorycter strigulatella</i> (Lienig & Zeller, 1846)	Blacknest, Brimpton Common, Berks, UK	161	IS	AF477511
“	<i>Phyllonorycter kleemannella</i> (Fabricius, 1781)	Rostock, Sweden	184	SV	AF477549
<i>Betula pendula</i>	<i>Phyllonorycter corylifoliella</i> (Hubner, 1796)	Val d'Aran, Pirineos, Spain	94	EO	AF477503
<i>Betula sp.</i>	<i>Phyllonorycter ulmifoliella</i> (Hubner, 1817)	Osterley Park, Middlesex, UK	38(101)	CLV	AF477508
“	“	Osterley Park, Middlesex, UK	56(101)	CLV	AF477508
“	“	Chateaufort sur Loire, Loiret, France	90(88)	CLV	AF477508
<i>Betula pubescens</i>	<i>Phyllonorycter anderidae</i> (W. Fletcher, 1875)	Dagsmosse, Sweden	163	SV	AF477519
<i>Betula sp.</i>	“	Medmenham, Berks, UK	232	IS	<b>AY521504</b>
<i>Betula pubescens</i>	<i>Phyllonorycter cavella</i> (Zeller, 1846)	Dagsmosse, Sweden	228	SV	AF477512
<i>Corylus avellana</i>	<i>Phyllonorycter nicellii</i> (Stainton, 1851)	Silwood Park, Ascot, Berkshire, UK	9(11.1)	CLV	AF477562
<i>Not reared</i>	“	Virginia water, Surrey, UK flying	66(149)	CLV	AF477562
<i>Corylus avellana</i>	<i>Phyllonorycter coryli</i> (Nicelli, 1851)	Silwood Park, Ascot, Berkshire, UK	110(188)	CLV	AF477507
<i>Ostrya carpinifolia</i>	<i>Phyllonorycter aemula</i> Triberti, Deschka & Huemer, 1997	Nago, Trento, Italy	193	GD	AF477559
<i>Carpinus betulus</i>	<i>Phyllonorycter tenerella</i> (Joannis, 1915)	Chateaufort sur Loire, Loiret, France	89(86)	CLV	AF477546

<i>Carpinus betulus</i>	<i>Phyllonorycter esperella</i> (Goeze, 1783)= <i>quinnata</i> (Geoffroy, 1785)	Osterley Park, Middlesex, UK	32(89)	CLV	AF477569
<i>Carpinus laxiflora</i>	<i>Phyllonorycter japonica</i> Kumata, 1963	Miyakonojo, Mizakazi, Japan	262	YS	<b>AY521524</b>
<i>Carpinus cordata</i>	“	Japan, ?	327	HK	<b>AY521525</b>
<b>Family Fagaceae</b>					
<i>Quercus robur</i>	<i>Phyllonorycter messaniella</i> (Zeller, 1846)	Silwood Park, Ascot, Berkshire, UK	1(2.1)	CLV	AF477584
<i>Quercus robur</i>	<i>Phyllonorycter distentella</i> (Zeller, 1846)	Thorden Wood, East Kent, UK	101(179)	DO	AF477541
<i>Quercus robur</i>	<i>Phyllonorycter harrisella</i> (Linnaeus, 1761)	Richmond Park, Middlesex, UK	51(125.3)	CLV	AF477567
“	“	Richmond Park, Middlesex, UK	52(125.1)	CLV	AF477567
<i>Quercus robur</i>	<i>Phyllonorycter lautella</i> (Zeller, 1846)	Pett's Wood, Kent, UK	214	DO	AF477579
<i>Quercus robur</i>	<i>Phyllonorycter quercifoliella</i> (Zeller, 1839)	Richmond Park, Middlesex, UK	57(125.1)	CLV	AF477561
<i>Quercus robur</i>	<i>Phyllonorycter saportella</i> (Duponchel, 1840)	South Lopham, UK	210(191)	CLV	AF477543
<i>Quercus robur</i>	<i>Phyllonorycter muelleriella</i> (Zeller, 1839)	Cyrencester park, Hateled	157	IS	AF477506
<i>Quercus robur</i>	<i>Phyllonorycter roboris</i> (Zeller, 1839)	Queen's wood, Dymock, Worcestershire, UK	211(196)	CLV	AF477560
<i>Not reared</i>	<i>Phyllonorycter heegeriella</i> (Zeller, 1846)	Torrings, Sweden	229	SV	AF477531
<i>Quercus</i>	<i>Phyllonorycter ilicifoliella</i> (Duponchel, 1843)	Val Lourian, Rougiens, Var, France	118	JN	AF477529
<i>Quercus ilex</i>	<i>Phyllonorycter joviella</i> (Constant, 1890)	Montseny, Barcelona, Spain	84	EO	AF477523
<i>Quercus crispula</i>	<i>Phyllonorycter cretata</i> Kumata, 1963	Ishikari, Japan	260/264	IT	<b>AY521505</b>
<i>Quercus acutissima</i>	<i>Phyllonorycter acutissimae</i> Kumata, 1963	Nara, Japan	263	SH	<b>AY521522</b>
<i>Quercus mongolica</i> var. <i>grosseserrata</i>	“	Ishikari, Hokkaido, Japan	320	HK	<b>AY521522</b>
<i>Quercus mongolica</i> var. <i>grosseserrata</i>	<i>Phyllonorycter similis</i> (Kumata)	Ishikari, Hokkaido, Japan	324	HK	<b>AY521528</b>
<i>Quercus sessilifolia</i>	<i>Phyllonorycter similis</i> (Kumata)	Guangdong, Shao-guan City, China	331	FH	<b>AY521527</b>
<i>Quercus dentata</i>	<i>Phyllonorycter pygmaea</i>	Kagoshima, Japan	265	YS	<b>AY521507</b>

<i>Fagus sylvatica</i>	Kumata, 1963 <i>Phyllonorycter maestingella</i> (Muller, 1764)	Kew Gardens, Middlesex, UK	73(156)	CLV	AF477520
<i>Quercus robur</i>	<i>Phyllonorycter</i> sp.1	O Carballal, Bueu, Spain	188	CLV	AF477537
“	“	O Carballal, Bueu, Spain	189	CLV	AF477551
<i>Quercus pyrenaica</i>	<i>Phyllonorycter</i> sp 2	Serra do Xures, Lovios, Spain	216	CLV	AF477553
<i>Quercus suber</i>	<i>Phyllonorycter</i> sp.3	Serra do Xures, Lovios, Spain	190	CLV	AY230761
<b>ORDER Rosales</b>					
<b>Family Rosaceae</b>					
<i>Pyrus</i> sp.	<i>Phyllonorycter cydoniella</i> (Denis & Schiffermuller, 1775)	Virginia water, Surrey, UK	54(115)	CLV	AF477573
<i>Sorbus torminalis</i>	<i>Phyllonorycter mespillella</i> (Hubner, 1805)	Bookham, Surrey, UK	75(162)	CLV	AF477548
<i>Sorbus torminalis</i>	<i>Phyllonorycter corylifoliella</i> (Hubner, 1796)	Bookham, Surrey, UK	76(161)	CLV	AF477536
<i>Sorbus aucuparia</i>	<i>Phyllonorycter sorbi</i> (Frey, 1855)	Silwood Park, Ascot, Berkshire, UK	7(8.1)	CLV	AF477585
<i>Sorbus commixta</i>	<i>Phyllonorycter sorbicola</i> (Kumata, 1963)	Ishikari, Hokkaido, Japan	322	HK	<b>AY521523</b>
<i>Crataegus monogyna</i>	<i>Phyllonorycter oxyacanthae</i> (Frey, 1856)	Silwood Park, Ascot, Berkshire, UK	112(190)	CLV	AF477570
<i>Crataegus monogyna</i>	<i>Phyllonorycter corylifoliella</i> (Hubner, 1796)	Silwood Park, Ascot, Berkshire, UK	106	CLV	AF477514
<i>Prunus capuli</i>	<i>Phyllonorycter crataegella</i> (Clemens, 1859)	Santa Rosa, Guanajuato, Mexico	241	CLV	AY230762
<i>Prunus spinosa</i>	<i>Phyllonorycter spinicolella</i> (Zeller, 1846)	Brent river park, Hanwell, London, UK	33(134)	CLV	AF477554
<i>Prunus</i> sp.	<i>Phyllonorycter cerasicolella</i> (Peyerimhoff, 1872)	Molins rei, Barcelona, Spain	81	EO	AF477528
“	“	Brent river park, Hanwell, London, UK	105(183)	CLV	AF477528
<i>Pyracantha</i> sp.	<i>Phyllonorycter leucographella</i> (Zeller, 1850)	Hanwell, London, UK	60(143)	CLV	AF477556
<i>Malus</i> sp.	<i>Phyllonorycter blancardella</i> (Fabricius, 1781)	Lepe, Hampshire, UK	34(135)	CLV	AF477564
<i>Malus</i> sp	<i>Phyllonorycter elmaella</i> Doganlar & Mutuurua, 1980	USA	31	TU	AF477558
<i>Malus</i> sp.	<i>Phyllonorycter pomonella</i> Zeller, 1846 nec. auct.	Emilia-Romagna, Spain	47	EO	AF477578

<i>Malus sp</i>	<i>Phyllonorycter gerasimovi</i> (M. Hering, 1930)	Bucharest, Romania	119	EO	AF477524
<i>Malus sp</i>	<i>Phyllonorycter pyrifoliella</i> (Gerasimov, 1933)	Silistra, Bulgaria	271	RT	<b>AY521514</b>
<b>Family Ulmaceae</b>					
<i>Ulmus glabra</i>	<i>Phyllonorycter schreberella</i> (Fabricius, 1781)	Silwood Park, Ascot, Berkshire, UK	35(100)	CLV	AF477566
<i>Ulmus glabra</i>	<i>Phyllonorycter tristrigella</i> (Haworth, 1828)	Virginia water, Surrey, UK	27(111)	CLV	AF477581
<i>Ulmus davidiana</i> var. <i>japonica</i>	<i>Phyllonorycter bicinctella</i> (Matsumura)	Ishikari, Hokkaido, Japan	328	HK	<b>AY521526</b>
<i>Ulmus sp.</i>	<i>Phyllonorycter acaciella</i> (Duponchel, 1843)	Sofia, Bulgaria	277	RT	<b>AY521516</b>
<i>Ulmus sp.</i>	<i>Phyllonorycter agilella</i> (Zeller, 1846)	Sofia, Bulgaria	279	RT	<b>AY521520</b>
<b>Family Celtidaceae</b>					
<i>Celtis australis</i>	<i>Phyllonorycter millierella</i> (Staudinger, 1871)	Barcelona, Spain	95	EO	AF477517
<i>Celtis sinensis</i>	<i>Phyllonorycter bifurcata</i> Kumata, 1967	Korimoto, Kyusyu, Japan	261/267	YS	<b>AY521506</b>
<b>OUTGROUPS</b>					
<b>Superfamily</b>					
<b>Family Micropterigidae</b>					
	<i>Micropteryx thunbergella</i> (Fabricius, 1794)	Burnham Beeches, Slough, England	54	IS	<b>AY521484</b>
<b>Superfamily Gracillarioidea</b>					
<b>Family Bucculatricidae</b>					
<i>Quercus robur</i>	<i>Bucculatrix ulmella</i> Zeller, 1848	Richmond Park, Middlesex, UK	53(125)	CLV	AF477574
<b>Family Gracillariidae</b>					
<b>Subfamily Gracillariinae</b>					
<i>Malus spp.</i>	<i>Callisto denticulella</i> (Thunberg, 1794)	Medmenham, Berks, UK	167	IS	AF477525
<i>Hypericum perforatum</i>	<i>Calybites auroguttella</i> (Stephens, 1835)	Bix Bottom, Oxon, UK	166	IS	AF477521
<i>Hypericum sp.</i>	<i>Eucalybites aureola</i> Kumata, 1982	Okusawa-suigenti, Otaru City, Hokkaido, Japan	JP10	KS	<b>AY521500</b>
<i>Margaritaria nobilis</i>	<i>Caloptilia sp.</i>	Chiquibul Forest, Belize	150	OL	<b>AY521501</b>
<i>Betula sp.</i>	<i>Caloptilia populetorum</i> (Zeller, 1839)	Chigwell Row, Essex, UK	175	IS	AF477513
<i>Rhododendron</i>	<i>Caloptilia azaleella</i> (Brants, 1913)	Virginia water, Surrey, UK	65(148)	CLV	AF477577

<i>Alnus glutinosa</i>	<i>Caloptilia falconipennella</i> (Hubner, 1813)	Medmenham, Marlow, Bucks, UK	170	IS	AF477533
<i>Carpinus betulus</i>	<i>Parornix carpinella</i> (Frey, 1863)	Silwood Park, Ascot, Berkshire, UK	37(90)	CLV	AF477583
<i>Tournefortia hirsutissima</i>	<i>Dialectica</i> sp.	Chiquibul Forest, Belize	141	OL	AF477527
<i>Mallotus philippensis</i>	<i>Acrocercops heptadeta</i> Meyrick, 1936	Ie-rindo, Okinawa-honto, Ryukyu, Japan	JP17	KS	<b>AY521489</b>
<i>Quercus ilex</i>	<i>Acrocercops brongniardella</i> (Fabricius, 1798)	La Gachere, Brem sur Mer, France	127(171)	CLV	AF477501
“	“	“	128(171)	CLV	AF477501
<i>Quercus robur</i>	“	Syderstone, Norfolk	168	IS	AF477501
<i>Quercus serrata</i>	<i>Acrocercops unistriata</i> Decheng, 1986	Oguni-zinya, Mori Town, Honsyu, Japan	JP14	KS	<b>AY521488</b>
<i>Malvaviscus arboreus</i>	<i>Eucosmophora pithecellobiae</i> nov.sp.	Chiquibul Forest, Belize	140-142	OL	<b>AY521495</b>
<i>Malachra fasciata</i>					
<i>Pisonia aculeata</i>	<i>Marmara</i> sp.	Chiquibul Forest, Belize	153	OL	<b>AY521485</b>
<i>Coccoloba belizensis</i>	<i>Metrichroa</i> sp.1	Chiquibul Forest, Belize	151	OL	<b>AY521498</b>
<i>Piper pseudofulgineum</i>	<i>Metrichroa</i> sp.2	Chiquibul Forest, Belize	145	OL	<b>AY521496</b>
<i>Psichotria nervosa</i>	<i>Metrichroa</i> sp.3	Chiquibul Forest, Belize	139	OL	<b>AY521499</b>
<i>Turpinia occidentalis</i>	<i>Metrichroa</i> sp.4	Chiquibul Forest, Belize	147	OL	<b>AY521497</b>
<i>Alnus hirsuta</i>	<i>Dendrorhycter marmaroides</i> Kumata, 1978	Eniwa-keikoku, Eniwa City, Hokkaido, Japan	JP11	KS	<b>AY521486</b>
<i>Pueraria lobata</i>	<i>Liocrobyla lobata</i> Kuroko, 1960	Ogasa-yama, Daito Town, Honsyu, Japan	JP12	KS	<b>AY521501</b>
<i>Machilus thunbergi</i>	<i>Gibbovalva quadrifasciata</i> (Stainton, 1863)	Hunaura, Uehara, Iriomote-zima, Ryukyu, Japan	JP13	KS	<b>AY521487</b>
<i>Robinia</i>	<i>Parectopa robiniella</i> Clemens, 1863	Primorsko, Bulgary	278	RT	<b>AY521502</b>
<b>Family Gracillariidae</b>					
<b>Subfamily Phyllocnistinae</b>					
<i>Citrus limon</i>	<i>Phyllocnistis citrella</i> Stainton, 1856	California, USA	68	JMA	AF477557
	<i>Phyllocnistis saligna</i> (Zeller, 1839)	Schinnen, Holland	281/284	EN	<b>AY521491</b>
	<i>Phyllocnistis labyrinthella</i> (Bjerkander, 1790)	Zwannenwater, Holland	283	EN	<b>AY521494</b>
<i>Stigmaphyllon lindenianum</i>	<i>Phyllocnistis</i> sp.1	Chiquibul Forest, Belize	133	OL	<b>AY521490</b>
<i>Swietenia macrophylla</i>	<i>Phyllocnistis meliacella</i>	Chiquibul Forest, Belize	134	OL	<b>AY521492</b>
<i>Nectandra</i> cf. <i>longicaudata</i>	<i>Phyllocnistis</i> sp.2	Chiquibul Forest, Belize	132-138	OL	<b>AY521493</b>

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*Licaria peckii*

Family Gracillariidae

Subfamily Lithocolletinae

<i>Aesculus flavax</i> * <i>pavia</i>	<i>Cameraria</i> sp 1	Virginia, USA	254	MW	AY230758
<i>Quercus</i> sp.	<i>Cameraria</i> sp 2	Santa Rosa, Guanajuato, Mexico	243	CLV	AY230756
	<i>Cameraria guttifinitella</i> (Clemens, 1859)	USA	247	DD	AY230755
<i>Quercus</i> sp.	<i>Cameraria hamadryadella</i> (Clemens, 1859)	Virginia, USA	250	MW	AY230757
<i>Aesculus hippocastanum</i>	<i>Cameraria ohridella</i> Deschka & Dimic, 1986	Austria	83	EO	AF477544
unidentified leguminosous tree	<i>Lithocolletinae</i> sp.	Bourbon Hotel park, Foz do Iguaçu, Parana, Brasil	282	EN	<b>AY521503</b>

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New sequences reported in this study are highlighted in bold.

Note. CLV: Carlos Lopez Vaamonde; EO: Elisenda Olivella; DO: Dennis O’Keeffe; MS: Mark Shaw; TU: Tom, Uhru; S: Sruogal; JN: Jacques Nel; FHNS: Frank Smith; FH: Frank Hsu; OL: Owen Lewis; DA: David Agassiz.; IS: Ian Simms; SV: Ingvar Svensson; GD: Gerfried Deschka; MH: Michael Harper; DD: Don Davis; MW: Mike Wise; EN: Erik J. Van Nieukerken; RT: Rumen Tomov; IT: Ishida T.; YS: Y. Sakamaki; SH: Sato H.; KS: K. Sugisima; JMA: Juan Manuel Alvarez; HK: Hodeki Kagata; MK: Marc Kenis; TK: Tosio Kumata.