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Evolutionary diversification of the gall midge genus *Asteromyia* (Cecidomyiidae) in a multitrophic ecological context

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ABSTRACT

Gall-forming insects provide ideal systems to analyze the evolution of host–parasite interactions and understand the ecological interactions that contribute to evolutionary diversification. Flies in the family Cecidomyiidae represent the largest radiation of gall-forming insects and are characterized by complex trophic interactions with plants, fungal symbionts, and predators. We analyzed the phylogenetic history and evolutionary associations of the North American cecidomyiid genus *Asteromyia*, which is engaged in a complex and perhaps co-evolving community of interactions with host-plants, fungi, and parasitoids. Mitochondrial gene trees generally support current classifications, but reveal extensive cryptic diversity within the eight named species. *Asteromyia* likely radiated after their associated host-plants in the Astereae, but species groups exhibit strong associations with specific lineages of Astereae. Evolutionary associations with fungal mutualists are dynamic, however, and suggest rapid and perhaps coordinated changes across trophic levels.

1. Introduction

Recent phylogenetic and ecological analyses of endophytic insects are providing novel insights into the intricate ecological interactions between parasites and their hosts and the causes of evolutionary diversification (e.g., *Eurosta* gall flies, Craig et al., 2001; Waring et al., 1990; *Andricus* gall wasps, Cook et al., 2002; Nematine sawflies; Nyman et al., 2006). These insects—gall-formers, leaf-miners and stem borers—experience intimate interactions with host-plant tissues, and possess traits that, when polymorphic, can incur intense trade-offs in fitness among alternative hosts or host-plant modules (Yukawa, 2000; Karban and Agrawal, 2002; Harris et al., 2003; Wool, 2004), leading to ecologically-mediated genetic divergence and speciation (Craig et al., 1993, 1997; Stireman et al., 2005; Condon et al., 2008a,b). Moreover, endophytic insects can be focal points for complex multi-trophic interactions which may strongly shape their anagenetic and cladogenetic evolution (Price et al., 1987; Stone and Schonrogge, 2003). Because there have been repeated and often dramatic radiations of endophytic insects in terrestrial ecosystems (Price, 2005), they offer excellent opportunities to compare the 'ecological architecture' underlying the similarities and differences in macroevolutionary patterns (Price, 2005; Nyman et al., 2007).

Of all the major radiations of insects with endophytic lifestyles, the Cecidomyiidae, or gall midges, are arguably the most poorly understood. They represent the most extensive radiation of gall-forming insects, with approximately 5400 extant described species (and many more undescribed species; Gagné, 2004), the overwhelming majority of which form galls on plants. The causes of this diversity, and even its major features, remain largely mysterious (Dorchin et al., 2004; Yukawa and Rohfritsch, 2005; Yukawa et al., 2005; Joy and Crespi, 2007). What is known is that, unlike many of the other major gall-forming lineages of insects, which tend to be restricted to one or a few plant families (e.g., Cynipidae [gall wasps] on Fagaceae and Rosaceae), gall midges have radiated onto an enormous array of plant taxa from ferns to grasses (Gagné, 1989, 1994). In North America at least 89 plant families are known to host one or more species of gall midge (Gagné, 1989; Price, 2005), and in Japan at least 90 families are known to be used (Yukawa and Rohfritsch, 2005). Despite the great range of host-plants used by the family as a whole, most individual species tend to be highly specific, feeding on only one or a few related host-plant species (Gagné, 1989), and often on particular plant organs (e.g., leaf, stem, flower; Jones et al., 1983; Waring and Price, 1989; Joy and Crespi, 2007).

Few studies have analyzed the evolution of gall midge-host-plant associations with modern phylogenetic and comparative

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methods. Here, we seek to provide some insight into the evolution of these associations by reconstructing the phylogeny and the evolution of key traits and associations for members of one small cecidomyiid genus, *Asteromyia*. We employ DNA sequence-based phylogenetic reconstructions and data on ecological associations to examine the evolutionary history of host-plant use and its consequences for diversification. We take a community perspective in examining the evolutionary history of this genus, considering not only the associations with particular host-plant taxa and host-plant structures, but also associations with fungal symbionts that may mediate interactions with the host-plant, and with natural enemies that may encourage ecological diversification. We explore how each of these interacting partners may have influenced the evolutionary diversification of *Asteromyia* gall midges, utilizing this system as a model to assess and generate hypotheses and predictions concerning the evolution of insect-plant interactions.

1.1. The genus Asteromyia

1.1.1. Taxonomy

The genus *Asteromyia* currently consists of eight recognized species, primarily occurring in North America, but with one species, *A. modesta*, extending into South America (Table 1; (Gagné, 1968, 1969, 1989, 1994). Thirty-three species of *Asteromyia* were originally described in the early 20th century (primarily by Felt, e.g., Felt, 1910), however due to the lack of consistent morphological differences, this multitude of species was distilled down to seven morphologically distinguishable species by Gagné (1968) in his careful revision of the genus, with an additional species recognized one year later (Gagné, 1969). In his recent catalogue of Cecidomyiidae, (Gagné, 2004) lists an additional species, *A. grindeliae*, which he had previously synonymized with *A. modesta* (Gagné, 1968); however, we could find no published justification or official reinstatement of this species. Several additional species occurring on *Baccharis* were recognized by Gagné and Boldt (1995); these taxa were not described, however, due to lack of adult material.

1.1.2. Host-plant associations

All *Asteromyia* species are restricted to host-plants in the composite tribe Astereae (Asteraceae), with most being restricted to a single genus or a set of closely-related genera (Table 1). Each of the described species of *Asteromyia* is known from multiple, often many, species of host-plants. For example, *A. carbonifera* attacks at least 50 species of *Solidago* (Gagné, 1968; TGC, JOS pers. obs.). All species of *Asteromyia* form galls, which are distinctive nutritive deformations of plant tissue (Stone and Schonrogge, 2003). Close relatives of *Asteromyia* in the tribe Alycaulini are primarily stem gallers, often on woody plants (e.g., *Neolasioptera*), yet only a single known *Asteromyia* species galls stems. The rest form ‘‘blister” galls on leaves, which have a round bi-convex swollen appearance (Fig. 1).

1.1.3. Fungal associations

In addition to the developing midge larvae, the galls of most *Asteromyia* species house fungi. Such associations are not uncommon among galling Cecidomyiidae, as two major clades are known to have fungal associations, the tribe Asphondyliini (Supertribe Cecidomyiidi) and the (likely sister) tribes Alycauliini and Lasiopterini (Supertribe Lasiopteridi; Gagné, 1989; Roskam, 2005; Yukawa and Rohfritsch, 2005). Like Scolytinae and Platypodinae ‘‘ambrosia” beetles, which have nutritional symbioses with fungi, these cecidomyiids are sometimes referred to as the ambrosia gall midges (e.g., (Bisset and Borkent, 1988). The symbiosis with fungi by *Asteromyia* midges is particularly unusual. The galls cause only minor deformation of plant tissue itself. The bulk of the gall structure is formed by an associated hemibiotrophic fungus in the genus *Botryosphaeria* (Camp, 1981; Janson pers. comm.). The function of the symbiosis with fungi is not entirely clear, in *Asteromyia* or in cecidomyiids in general. There has been debate about whether *Asteromyia* is a plant or fungus feeder (Gagné, 1968, 1989; Bisset and Borkent, 1988), although recent data support the latter (Janson, pers. comm.). While most of the ambrosia gall midges are likewise thought to be fungus-feeders (Bisset and Borkent, 1988), the debate is fueled by the degree of dietary variation between closely-related species. *A. modesta*, for example, is apparently herbivorous: fungal mycelia are sparse or absent in the simple inconspicuous ‘‘pocket” like blister galls (Fig. 1), which resemble circular ‘‘blotch” mines of some leaf-mining insects. How this variation is maintained is uncertain, because the basal lineages of the Cecidomyiidae, subfamilies Porricondylinae and Lestremiinae, are not associated with plants at all, but rather feed exclusively on fungus, suggesting that the fungal associations in the ambrosia gall midges may reflect ancestral associations (Bisset and Borkent, 1988). At the moment, there have been no rigorous phylogenetic analyses of the evolution of fungal associations in cecidomyiids (though see Roskam, 2005 for an overview of the alternative arguments).

1.1.4. Natural enemies

All *Asteromyia* species are subject to parasitism by small hymenopteran parasitoids, primarily Platygastridae, Eulophidae, and Torymidae (Gagné, 1968; Weis, 1982a). Weis and colleagues (Weis, 1982a; Weis et al., 1983), analyzed the parasitoid community of one species, *A. carbonifera*, on a single host species (*Solidago altissima*), where they documented a complex of five parasitoid species that varied widely in their timing of attack relative to host development. Together, these species typically inflict high to moderate mortality, ranging from ca. 20% to nearly 100% in local *A. carbonifera* populations (Weis, 1982a; Stireman et al., 2008; Stireman unpublished data). Weis (1982b) provided compelling evidence that the fungus in *Asteromyia* galls, which forms a crusty black internal stroma layer in mature galls, serves a protective function, limiting access of *A. carbonifera* larvae to parasitoid ovipositors. Outside the body of work by Weis, no other studies have been conducted on the parasitoid complexes associated with *Asteromyia* galls.

Table 1
Asteromyia species included in this study along with the plant organ galled (location), the incorporation of fungi in gall structure, and the host genera typically used.

Species	Location	Fungus?	Hosts
A. carbonifera	Leaf	Y	*Solidago*
A. modesta	Leaf (stem?)	N	*Solidago*, *Symphyotrichum*, *Erigeron*, *Erigeron* (*Conyza*), *Grindelia*[a]
A. laeviana	Leaf	Y	*Symphyotrichum* & *Eurybia* (*Aster*)
A. clarkeae	Leaf	Y	*Eurybia* (*Aster*)
A. chrysothamni	Leaf	Y	*Chrysothamnus*, *Ericameria* (*Chrysothamnus*), *Stenotus* (*Haplopappus*)
A. gutierreziae	Leaf/stem	Y	*Gutierrezia*, *Ericameria* (*Isocoma*), *Baccharis*, *Gymnosperma*
A. euthamiae	Leaf	Y	*Euthamia*
A. tumifica	Stem	N	*Solidago*

[a] Specimens from this host were included in *A. modesta* by Gagné (1968), but are listed as *A. grindeliae* in Gagné, 2004.

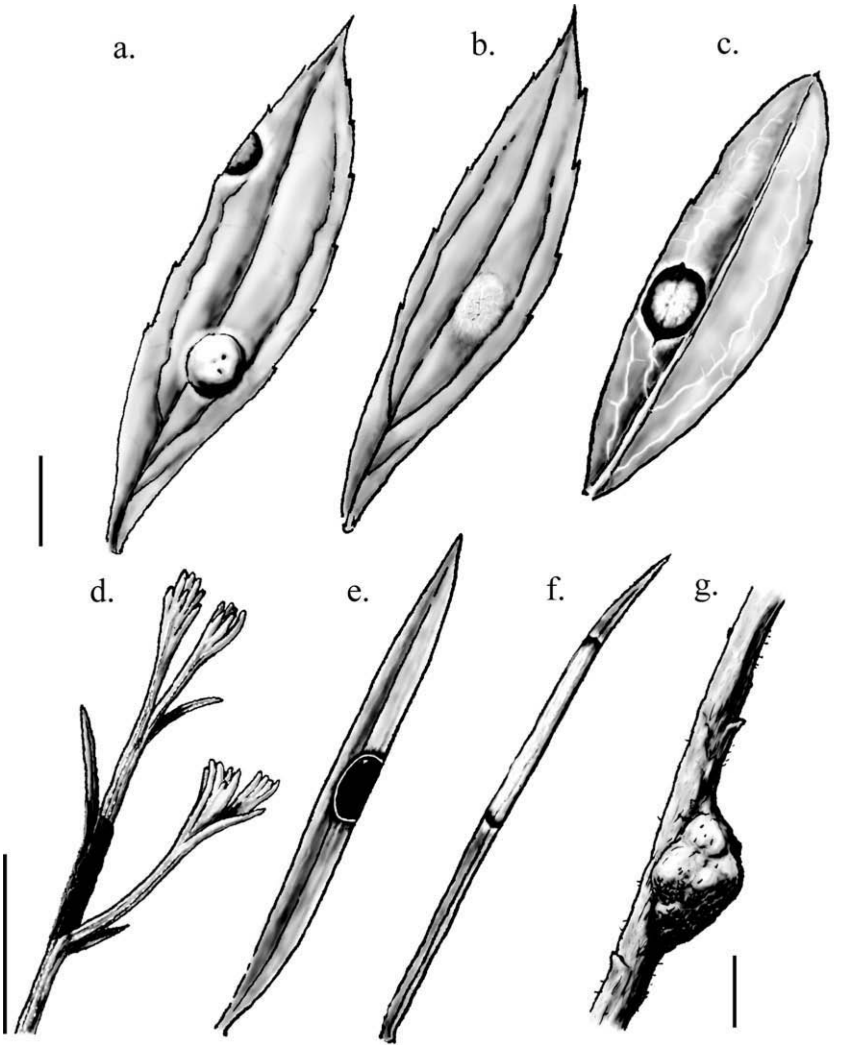

Fig. 1. Galls of *Asteromyia* species: (a) Cushion (below) and crescent (above) gall morphs of *A. carbonifera* on *Solidago altissima*, (b) *A. modesta* on *S. altissima*, (c) *A. laeviana* on *Symphyotrichum* spp. (*A. clarkei* galls are similar), (d) *A. euthamiae on Gutierrezia sarothae*, (e) *A. euthamiae* on *Euthamia graminifolia*, (f) *A. chrysothamni* on *Ericameria nauseosa*, and (g) *A. tumifica* stem gall on *S. altissima*. Vertical lines are equal to ca. 1 cm.

1.2. Questions and hypothesis concerning Asteromyia evolution

We employ phylogenetic and comparative analyses to address six primary questions concerning the evolutionary history and associations of the genus *Asteromyia*:

(1) Are the named species resolved by Gagné (1968) monophyletic? The monophyly of these taxa must first be established if we are to treat them as specific entities and consider their evolutionary relationships.

(2) Is there evidence for additional cryptic differentiation within these named taxa? If selective pressures vary among host species, we might expect to observe evidence of host-associated genetic differentiation among populations and perhaps morphologically cryptic, host-associated species.

(3) Are stem galling lineages basal or derived? Is stem galling plesiomorphic for the genus, or did it evolve secondarily from leaf-galling (and if so, why)? The evolution of stem and leaf-galling may reflect ecological niche shifts ('adaptive zones'), occurring both within and between host-plants (Price, 2005; Nyman et al., 2007).

(4) What is the evolutionary history of host-plant associations? Given that *Asteromyia* species are restricted to hosts belonging to a North American radiation of Astereae (Gagné, 1968; Noyes and Rieseberg, 1999), they may have co-diversified with these plant lineages, a phenomenon rarely documented in insects (though see Farrell and Mitter, 1998). Even if such co-diversification is not indicated, reconstructing host associations can provide insight into the origins and direction of host-plant colonization by *Asteromyia* and the consequences of host-plants use for evolutionary diversification.

(5) What is the evolutionary history of association with fungi and how may the fungus have influenced diversification? Associating with fungi may have important consequences for *Asteromyia* ecology and evolution. If a trophic relationship exists, the fungus could act as a buffer between midge and plant, facilitating shifts to plants with novel defenses or suboptimal nutritional quality. Alternatively, such a symbiotic association may constrain host use if the fungus has narrower ecological or biochemical tolerances than the midge (e.g., see Janson et al., 2008). In addition, if the fungus serves a protective function (Weis, 1982b), it may mediate interactions with parasitoids, which themselves can influence the frequency and direction of host-plant shifts via "enemy-free space" (Jeffries and Lawton, 1984).

(6) Finally, we ask whether *Asteromyia* species vary in their overall parasitoid load, and if so, might this be related to other aspects of their ecology (e.g., host-plant use, fungal associations)? Because parasitoids can exert strong selective pressures on galling insects (Stone and Schonrogge, 2003), understanding how parasitism rates vary among these host taxa may provide clues to the multitrophic causes behind observed evolutionary transitions.

2. Methods

2.1. Collection of Asteromyia samples

Asteromyia galls were collected from a broad array of Astereae species from across much of the United States. Collections focusing on the Northeast United States were made by TGC from 1999 to 2004 and those by JOS, focusing primarily in the Midwest US were made in 2006–2008. Collections were generally made from July to October, when host-plants were at or near their mature size. When a patch of host-plants bearing *Asteromyia* galls was discovered, 5–50 galls were haphazardly sampled. We attempted to spread the sampling across available individual plants to maximize diversity. Sampling locations were recorded and ramets of the host-plant were collected and pressed for later identification (see Appendix A. for a list of all *Asteromyia* samples used in the current analyses, their hosts, and their collection location). *Asteromyia* species were identified using characters of mature larval and pupa (particularly the shape of the sternal spatula) using Gagné (1968) and with reference to gall morphology and host-plant affiliation (Gagné, 1968, 1994). All galls were categorized as to whether a thick mat of fungal hyphae or hyphal stroma was present or absent. Plants sampled by TGC were identified with reference to specimens in the herbarium of the L.H. Bailey Hortorium of Cornell University and United States National Museum. Plants sampled by JOS were primarily identified by Semple (University of Waterloo), although some specimens were identified using Semple et al. (1999, 2002) and generic pages of the Flora of North America (Semple and Cook, 2006; Urbatsch et al., 2006a,b). In a few cases, ramets were not collected from the host-plant, and only the plant genus was recorded. Vouchers of host-plants collected by JOS are currently housed in the Wright State University herbarium collection.

Galls from a particular host-plant and collection site were placed in individual plastic bags and stored in an ice-filled cooler or refrigerator (4 °C) until they could be dissected under a microscope. For each gall, host-plant, number of larvae, developmental stage and parasitism status were recorded. A midge was considered to be parasitized if we observed parasitoid eggs, larvae or pupae in the galls, the presence of bloated moribund midge larvae housing endoparasitoids, or, in cases where eclosion had occurred from galls, the presence of parasitoid pupal exuviae or characteristic circular exit holes. We did not attempt to identify all parasitoids, however many of them could be assigned to family on the basis of life history and pupal/adult characters (using Gibson et al., 1997). Because previous studies have indicated that multiple gall "morphs" may exist on particular host-plant species (Crego et al., 1990; Stireman et al., 2008), the basic morphological form (e.g., general shape, position, and thickness) was also recorded. Larvae and pupae dissected from a subset of these galls were stored in a (−20 °C) freezer, without preservative or in 95% ethanol until DNA was extracted. These preserved midges were further subsampled for DNA extraction to maximize geographic and host-plant diversity.

Outgroup taxa included *Calamomyia phragmites* (ex. *Phragmites* sp.), *Protaplonyx sarcobati* (ex. *Sarcobatus vermiculatus),* and *Meuneriella aquilonia* (ex. *Gleditisia triacanthos),* all members of the tribe Alycaulini, to which *Asteromyia* belongs (see Appendix A). These taxa were collected from the field, identified from their gall structure and host-plant using Gagné (1989) and dissected as above.

2.2. Taxon sampling

Because we sought to characterize the diversity of lineages and associations in *Asteromyia*, we focused on sampling coverage rather than pursuing additional loci to more powerfully assess relationships among species. This approach was taken because this genus has not been previously analyzed with modern phylogenetic methods and we felt it was important to provide a broad initial sampling of taxa. However, the Astereae is the largest tribe of Asteraceae, with approximately 1100 known species in North America (North of Mexico; Noyes and Rieseberg, 1999), and the number of potential *Asteromyia*/host-plant/geography combinations is immense. Ideally, our taxon sampling would accomplish three hierarchical goals: to obtain samples from each of the described *Asteromyia* species, to obtain samples from a broad diversity of the host-plants of those species, and to obtain samples of the same species on the same host-plant from disparate geographic regions. While only the first of these goals was entirely realized, each of the three served as a rough guide for sampling. Finally, we wished to maximize detection of genetic structure related to host-plant use and/or geography, justifying our choice of mtDNA genes COI and COII, which are among the most rapidly evolving and useful genes for animal phylogeny and phylogeography (e.g., Caterino et al., 2000).

2.3. Collection of DNA data

DNA was extracted from *Asteromyia* larvae or pupae using Puregene DNA Purification System, Cell and Tissue Kit (Gentra Systems Inc., Minneapolis, MN). Standard polymerase chain reactions (PCRs) were conducted following conditions outlined in Stireman et al. (2008). Primers Barb1 and S1718 (Simon et al., 1994) were used to amplify an approximately 1800 bp fragment spanning much of COI and COII, as well as the intervening Leucine tRNA gene.

PCRs were purified using an EXO/SAP enzymatic cleanup (Dugan et al., 2002). Sequencing reactions were carried out using ABI Prism Big Dye 3.1 using standard procedures and run on a 3730 DNA Analyzer from Applied Biosystems, Inc. Both primers (forward and reverse) were used for sequencing, but due to the large fragment length there was frequently little or no overlap among forward and reverse sequences. Sequences were inspected for miscalls and edited using CodonCode Aligner. Alignment was performed using Clustal W and further manual alignment in Mega4 (Tamura et al., 2007). The resulting alignment, after truncation of ambiguous ends, was 1812 bp. A total of 212 samples were sequenced, however, a number of samples of the same species from the same host-plant and collection site exhibited identical sequences and many of these were omitted from phylogenetic analyses resulting in 167 operational taxonomic units (OTUs).

In order to test for co-diversification between *Asteromyia* and their Astereae hosts at a broad level, we gathered ITS sequences from NCBI Genbank of Astereae genera representing the North American clade that hosts *Asteromyia*. We aligned 48 sequences (665 bp; see Appendix B for accession numbers) using ClustalW and manually edited them in Mega4 (Tamura et al., 2007). These sequences spanned most of the major North American radiation ("North American Clade") of the tribe Astereae and several related outgroups in the Asteraceae, and were derived largely from the studies of Noyes and Rieseberg (1999) and Beck et al. (2004). A subset of these sequences (15 taxa) from which we had corresponding *Asteromyia* sequences were used in analyses of co-radiation (see below).

2.4. Phylogenetic analysis

The Maximum Likelihood (ML) phylogenetic analysis was conducted using PHYML (Guindon and Gascuel, 2003). In this analysis, a General Time-Reversible (GTR) model of substitution was employed with parameters estimated based on a starting tree inferred by neighbor joining (using the BIONJ algorithm) (see Appendix C) with gamma shape parameter of 0.617 (Ncat = 4) and an estimated proportion of invariant sites of 0.393. Branch support was evaluated with 1000 bootstrap samples of the data set (with nucleotide models as above).

Bayesian phylogenetic analysis was conducted in MrBayes (Ronquist and Huelsenbeck, 2003). In these analyses, the data set was partitioned by gene (COI, tRNA, COII) and a GTR model was employed (as above) with gamma shape parameter and proportion of invariant sites estimated for each partition. All priors were set to zero. Two Markov-chain Monte Carlo runs (MCMC) were conducted simultaneously with six chains (five heated) for 200,000 generations, sampled every 100 generations. Convergence of likelihoods was assessed using Tracer 1.4 (Rambaut and Drummond, 2007) with a burn-in of 500 samples and the posterior probability of all nodes was summarized (also with a burn-in of 500 samples). The two MCMC runs resulted in very similar consensus trees and posterior probabilities and we show results for only the first run.

We used Mesquite 2.5 (Maddison and Maddison, 2008) to reconstruct the evolutionary history of gall location (stem versus leaf), host-plant use (host genus), and the presence or absence of a thick mat of fungal hyphae in galls. We used parsimony and ML methods implemented in the Stochar package (Maddison and Maddison, 2006) to assess ancestral states and the direction of transitions for each of these characters. For the ML analyses we used a Markov k-state 1 parameter model of character evolution (Mk1; Lewis, 2001; Maddison and Maddison, 2006). For clarity, reconstructions of characters are displayed on a tree with a reduced number of terminal taxa (54) representing the major groups that were reconstructed.

Galls on *Gutierrezia* spp. were found on both stems and leaves, often simultaneously. However, because these galls are similar in structure to the blister galls of other taxa occurring on leaves (Fig. 1) and they occurred on green photosynthetic stems, these galls were considered "leaf" galls for reconstruction purposes.

To test whether diversification (rate of speciation) is associated with fungal association, we used the DIVERSE package (Midford and Maddison, 2008); in Mesquite 2.5. For a skeletal tree of 29 OTUs representing the major *Asteromyia* lineages (see below), we estimated a six parameter model of speciation (binary state speciation and extinction [BiSSE] model including speciation and extinction rates for the two groups [fungus, no fungus], and rates of forward and backward character change; see Maddison et al., 2007) via maximum likelihood. We then compared the estimated speciation rates to a model in which speciation rate of the two groups (fungus and no fungus) were constrained to be equivalent and other parameters were free to vary. Significance was assessed with a likelihood ratio test with one degree of freedom.

In order to assess whether *Asteromyia* species have co-diversified with their host-plants, we estimated divergence times of several major nodes for both midges and plants using Bayesian analyses implemented in BEAST v1.4.8 (Drummond and Rambaut, 2007). These included the entire *Asteromyia* clade and the smallest clade of Astereae including all hosts, the *A. carbonifera* clade and *Solidago*, and the *A. laeviana* clade and "*Aster*" (i.e., *Symphyotrichum* and relatives). We were unable to establish calibration dates for either group due to an apparent lack of studied fossilized material (P.D. Wilf, pers. comm.). For *Asteromyia*, we assumed a rate of 1% per million years, which has been frequently used in studies arthropod COI mtDNA divergence (Desalle et al., 1987; Brower, 1994). In order to compare estimated divergence times of *Asteromyia* to their hosts, we estimated that typical herbaceous plants diversify at ITS loci at approximately 0.30% per million years. This estimate is based on a recent study by Smith and Donoghue (2008), that compared rates of molecular evolution in woody versus herbaceous plants, and are derived from the mean estimated rate of the last 10 My period for the Apiales. It also accords well with rates estimates of rbcL in Poaceae of ca. 0.29%/My (Wolfe et al., 1989; Kim et al., 2005). These rates and the estimates of divergence dependent on them are rough, and we are cautious about their interpretation. Our goal was not to establish definitive dates for the clades, but rather to assess the overall likelihood that *Asteromyia* and their Astereae host-plants diversified over similar time frames.

To estimate divergence times in *Asteromyia*, we used an uncorrelated log-normal relaxed clock model to estimate divergence times, which assumes that rates vary among branches according to a lognormal distribution (Drummond et al., 2006). However, the standard deviations of the rate estimates (ucld.stdev) were relatively low (<1) in all analyses (e.g., 0.616), indicating that a strict molecular clock model cannot be rejected (Drummond et al., 2006). In the Bayesian analysis, 10,000,000 MCMC cycles were employed, with rates sampled every 1000 generations and a "burn-in" of 1,000,000 cycles. We used a GTR+I+G model of molecular evolution with a UPGMA starting tree employing a Birth/Death Process speciation model in the Bayesian analysis. Convergence of the chain to a stationary distribution was confirmed visually using the program Tracer version 1.4 (Rambaut and Drummond, 2007). Analysis of divergence in the Astereae host-plants were conducted as above except that a GTR+I model of molecular evolution was used (with parameters estimated via ML).

Phylogenetic associations of *Asteromyia* lineages with their asteraceous host taxa were analyzed using PARAFIT (Legendre et al., 2002). In order to define OTUs, a NJ tree was first computed (using model parameters estimated via ML above) and clades subtended by a branch length of greater than 0.005 were considered to be evolutionarily distinct populations. Host associations for each of these clades based on collection data were noted and one OTU from each clade was then selected to comprise a "skeletal" data set of 29 taxa for which a matrix of pairwise distances was calculated employing the maximum composite likelihood option in Mega 4.0 with gamma shape parameter estimated from ML analyses (above) and allowing rate variation among lineages. A similar procedure was performed on ITS sequences of 15 Astereae host taxa acquired from NCBI Genbank (see Appendix B for accession numbers), to create a second matrix of pairwise distances among hosts. Model parameters for Astereae distance calculations were estimated by maximum likelihood using PHYML. Phylogenetic distance matrices were then converted to principal coordinates using DISTPCOA (Legendre and Anderson, 1999). These matrices, along with a third matrix of host associations, were used by PARAFIT to calculate a fourth matrix of host-midge associations from which a global statistic was calculated to assess the independence of the phylogenetic histories of *Asteromyia* and Astereae (see Legendre et al., 2002). Significance was evaluated based on 9999 permutations. The significance of particular host-plant-midge links (i.e., their contribution to the global statistic) were evaluated using the ParaFitLink1 statistic (Legendre et al., 2002) and also assessed by 9999 random permutations.

2.5. Parasitism rates

Parasitism rates were calculated for each *Asteromyia* species in three ways. First, the fraction of parasitized galls was estimated from all collections across all host-plants by dividing the number of parasitized galls by the total. Second, parasitism frequency was calculated as above for each host-plant species from which a species of *Asteromyia* was reared (from at least 10 galls), and then averaged across host-plant species. Finally, given that mean values are highly sensitive to outliers, the median parasitism frequency per host-plant species was calculated for each named *Asteromyia* species. In all calculations of parasitism frequency, galls that were empty, destroyed, and those that bore only deteriorated insect remains were excluded. However, galls from which adults had eclosed, and the identity (midge or parasitoid) of the ecloser was indicated by pupal remains or characteristic eclosion holes, were included in analyses.

We assessed the significance of variation in total parasitism rate by using two χ^2 tests in *R* (R Development Core Team, 2008): one including all species and another including taxa where we had dissected greater than 50 individuals to minimize problems with low expected cell counts. *P*-values were assessed with 1000 Monte Carlo simulations. Given the relatively low numbers of host-plant collections with sample size greater than ten for most species, we were not able to effectively test for differences in mean parasitism across host-plants.

3. Results

3.1. Phylogenetic reconstructions

The ML analysis resulted in a single tree of $-\ln L = 14175.52$ (Fig. 2). Both runs in the Bayesian analysis conducted in Mr. Bayes (Ronquist and Huelsenbeck, 2003) resulted in a similar distribution of trees (harmonic means of marginal Likelihoods: −14502.82 and −14508.45, respectively). The consensus tree over both runs (50% cutoff of posterior probability) is highly similar to the tree recovered from ML analysis, although it is somewhat less resolved (the ML tree is shown in Fig. 2, with corresponding support values from the Bayesian analysis indicated).

The monophyly of all named species, with the exception of *A. modesta*, is supported, usually by relatively high bootstrap and clade credibility values (>90%), however relationships between these taxa were poorly resolved, with basal nodes generally characterized by low (<50%) bootstrap and clade credibility values (Fig. 2). Support for the monophyly of the species *A. euthamiae* and *A. gutierreziae* is relatively weak, but there is strong support for a sister relationship among these species. The ML analysis suggests that this *A. euthamiae* + *A. gutierreziae* clade is sister to the remaining species, but this relationship is not strongly supported. *A. laeviana* consists of two distinct and robust clades that are weakly supported as sister groups. *Asteromyia modesta* consists of two distinct clades that may be polyphyletic, but low boostrap support (and clade credibility) of interior nodes makes this difficult to ascertain. In addition, *A. tumifica* is reconstructed as arising from within *A. modesta* with high confidence (>90% bootstrap support). A single sequence from a midge collected from *Gutierrezia californica*, indicates a highly divergent phylogenetic position from *A. gutierreziae* and it is subtended by a relatively long branch (ca. 5% divergent from its closest neighbors). This result, along with a unique morphology of the sternal spatula (Carr, pers. obs.), indicates that this collection represents a distinct species.

In several clades, most notably *A. carbonifera*, *A. modesta*, and *A. laeviana*, there is a clear indication of additional morphologically cryptic lineages that do not appear to be geographically defined. Some of these clades, such as the two distinct *A. laeviana* groups, overlap broadly in the use of host species, but many other distinct lineages are defined by the use of particular host species or genera. Examples include clades of *A. carbonifera* on *Solidago rugosa, S. juncea/missouriensis*, *A. modesta* on *S. ohioensis, Erigeron annuus*, and *Erigeron* (*Conyza*) *canadensis*, *A. laeviana* on *Symphyotrichum laeve* and perhaps *S. pilosus*, and *A. chrysothamni* on *Lorandersonia* versus *Chrysothamnus* spp. (Fig. 2).

3.2. Character evolution and diversification

Character reconstructions using parsimony indicate that the stem galling habit of *A. tumifica* is secondarily derived and does not reflect a basal position of this species within the genus. In fact, *A. tumifica* is reconstructed as arising from within the *A. modesta* clade, rendering it paraphyletic (Figs. 2 and 3). Reconstruction of fungal association using both parsimony and ML suggests that the use of *Botryosphaeria* fungus in gall construction appears to have arisen early in the genus, and has most likely been lost in *A. modesta* (Fig. 3). Given, that *A. modesta* consists of two distinct and possibly polyphyletic clades, the fungal association may have been lost multiply. Due to the shallow branch lengths and uncertainty in relationships among major host-associated lineages (i.e., “species”), ML character reconstruction has limited ability to resolve the evolutionary history of host-plant associations (Fig. 4). Parsimony reconstructions of host-plant use provide no more resolution than ML, with basal nodes indicating equal probability of associations with *Euthamia*, *Chrysothamnus* grp., *Symphyotrichum*, and *Solidago*.

We found no significant evidence that association with the fungus resulted in increased or decreased rates of diversification in *Asteromyia*. The difference in (Ln) likelihood between the diversification model allowing rates of speciation and extinction to vary with the presence or absence of fungus and the model constraining all taxa to the same rates was −1.52 ($P = 0.0812$). However, estimated rates of speciation and extinction in the unconstrained model were dramatically different for lineages with and without fungal associates (Table 2). Consistent with our previous inferences that fungal associations arose early in the genus and were lost from some taxa, the rate of gain of fungal associations was estimated to be far lower than the estimated rate of loss (Table 1). Given that sampling intensity is expected to strongly influence estimated speciation rates and that we have sampled only a fraction of the potential populations (or host-plants) for any group of *Asteromyia*, these results must be interpreted with caution.

3.3. Coevolution

Despite our inability to reconstruct a clear view of the evolution of host-plant associations, analyses of phylogenetic matching of *Asteromyia* and their Astereae hosts indicate significant evidence of concordance between their phylogenetic histories ($P = 0.0008$). However, this departure from random associations is driven by a relatively few host–parasite links (Fig. 5), and is likely due in large part to the strong conservatism of host genus within named “species” clades.

Bayesian estimates of divergence times for the *Asteromyia* clades based on substitution rate of 2% divergence per my (i.e., 0.01) were: 6.54 Mya for the genus *Asteromyia*, 3.59 Mya for the *A. carbonifera* clade, and 3.745 Mya for the *A. laeviana* clade (Fig. 6). The North American clade of Astereae that minimally includes all host genera from which *Asteromyia* were reared was estimated to have diversified 26.35 Mya. The estimated diver-

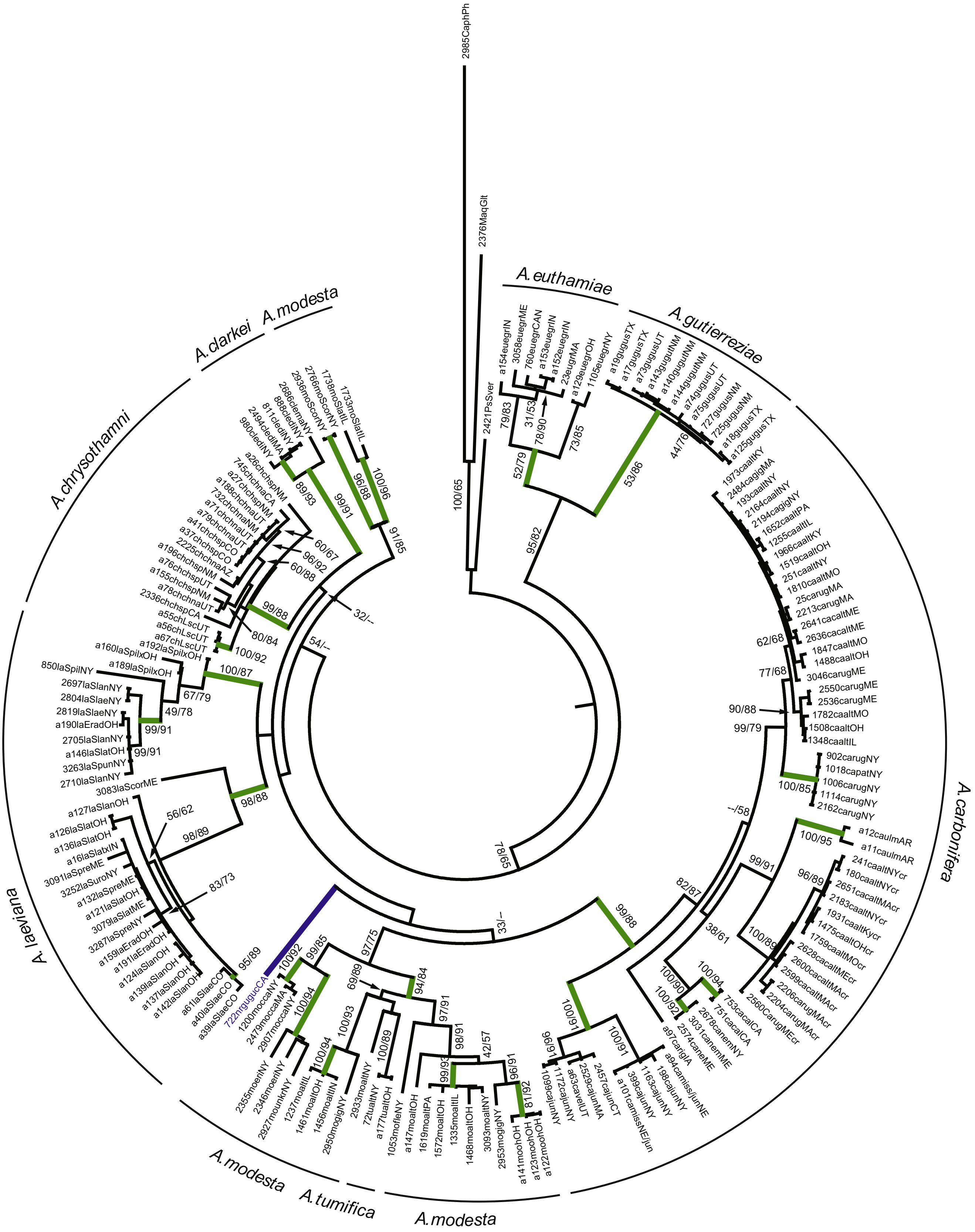

Fig. 2. The phylogenetic reconstruction of *Asteromyia* gall midges of highest likelihood in ML analysis. Species groupings are given in the surrounding circle. Numbers along branches indicate the bootstrap support (ML) followed by clade credibility values (Bayesian) for major nodes. See Appendix A for explanations of the names. Bold green branches indicate strongly supported clades that appear to be associated with host-plant use (although geographic causes cannot be ruled out in some cases). The blue branch indicates an undescribed taxon from *Gutierrezia californica*. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

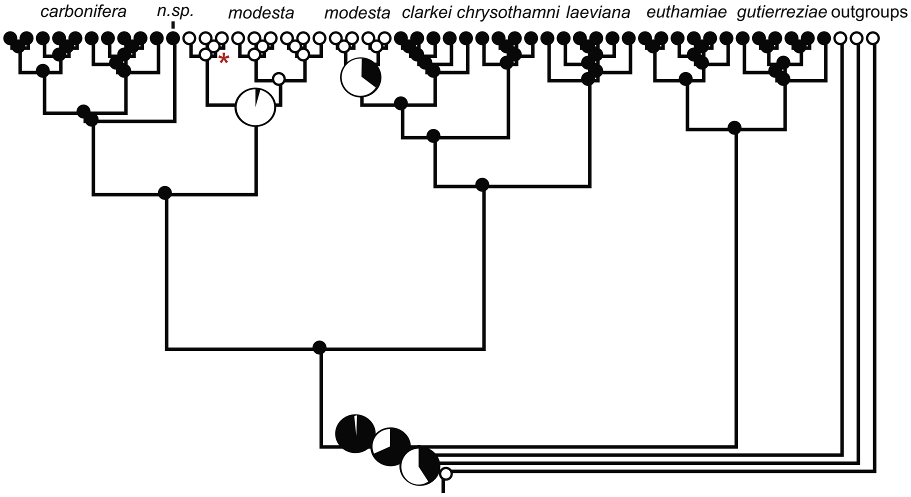

Fig. 3. A ML reconstruction of the evolution of fungal associations (i.e., use of fungus in gall construction). Pie charts indicate the relative likelihood of the absence of fungus in gall structure (white) and presence (black) at particular nodes. Some internal pie charts are enlarged for clarity. The red asterisk indicates the transition to stem galling. For simplicity only a representative fraction of terminal taxa are shown. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

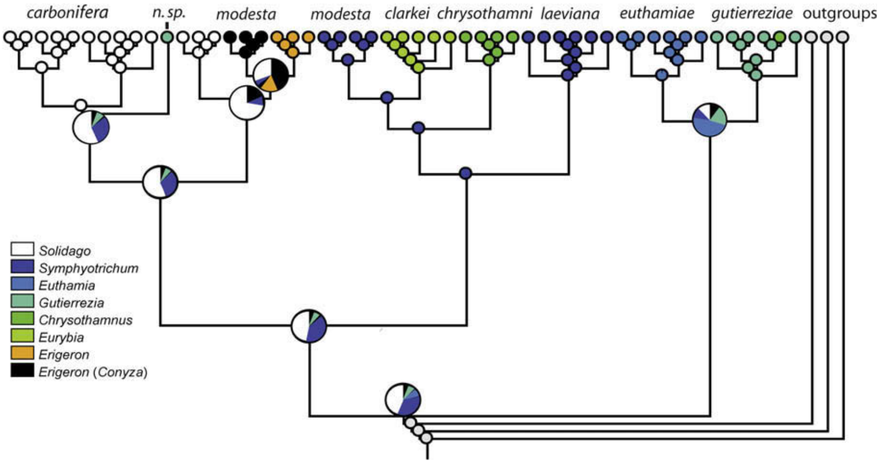

Fig. 4. A ML reconstruction of the evolution of host-plant associations. Pie charts indicate the likelihood of the use of particular genera of Astereae as hosts (see key). Some internal pie charts are enlarged for clarity. For simplicity only a representative fraction of terminal taxa are shown.

Table 2

ML estimates of model parameters in BiSSE diversification models of lineages with and without fungal associates. In the unconstrained model all parameters are free to vary among lineages with and without fungal associates. In the constrained model speciation rates are constrained to be the same. λ = speciation rate, μ = extinction rate, q_{01} = rate of gain, q_{10} = rate of loss of fungal association. Larger values signify higher estimated rates.

Model: parameter	Unconstrained		Constrained	
	No fungus	Fungus	No fungus	Fungus
λ	207.6	51.6	221.9	221.9
μ	35.7	172.1	31.42	186.7
q_{01}	4.4×10^{-4}		8.9×10^{-7}	
q_{10}	1.94		1.76	

gence times of *Solidago* and *Symphyotrichum* (based on the sequences available) were 5.23 Mya and 4.09 Mya respectively (Fig. 6).

3.4. Parasitism

Parasitoids dissected from galls primarily belonged to the hymenopteran families Torymidae, Eulophidae, and Platygastridae. Average and median parasitism rates among host-plants varied among the named *Asteromyia* species (Table 3). Most species experienced total parasitism frequencies of 20–40% (Table 3), but *A. tumifica*, *A. clarkei*, *A.* n. sp. and *A. euthamiae* were subject to relatively low rates of parasitism (0–16%), while *A. modesta* experienced relatively high rates. This resulted in a highly significant effect of species on overall parasitism rate ($\chi^2 = 112.17$, $P < 0.001$; Fig. 7). Results were similar for analyses of all taxa and the reduced taxon set (>50 individuals). Mean and median parasitism rates per host-plant species exhibited a similar pattern, although the differences in parasitism were less pronounced and rates for *A. clarkei* were considerably higher.

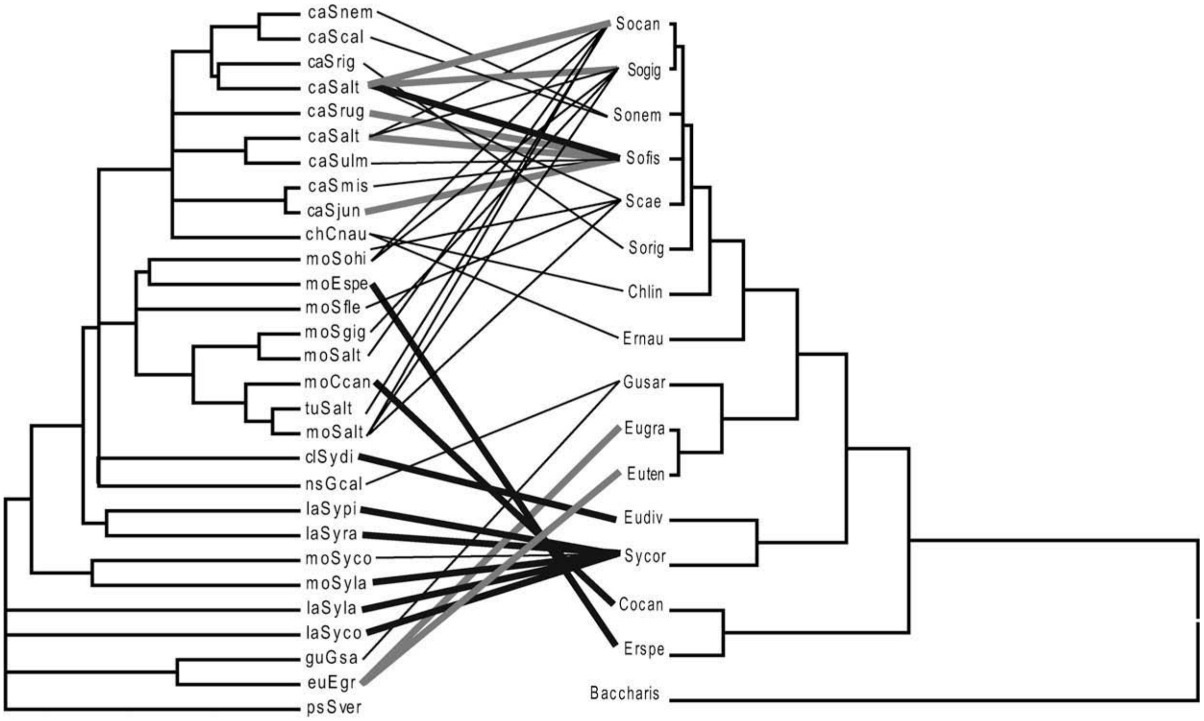

Fig. 5. Linearized skeletal cladograms of *Asteromyia* lineages (left) and major Astereae lineages that host *Asteromyia* (right) inferred by Neighbor Joining. Associations between these taxa are indicated by connecting lines. Bold black lines indicate links that contribute significantly to midge-host phylogenetic associations ($P < 0.05$), and bold gray lines indicate marginally non-significant links ($0.05 > P > 0.10$). See Appendices A and B for abbreviations of host-plants and *Asteromyia* lineages.

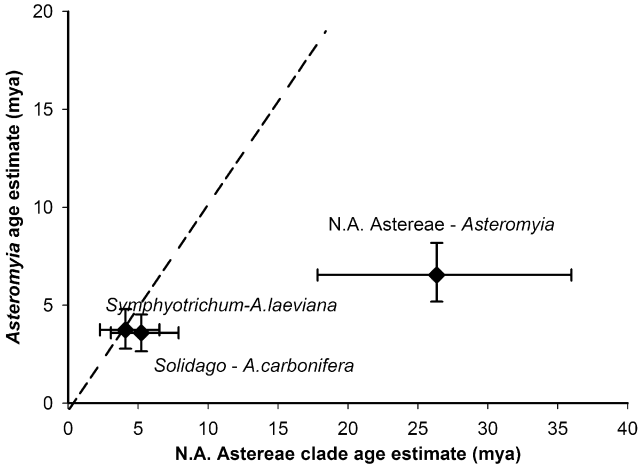

Fig. 6. Estimated ages of *Asteromyia* and Astereae lineages. Points correspond to the lineage ages of a particular clade of *Asteromyia* and the host clade that lineage feeds upon. Bars indicate 95% Bayesian confidence intervals calculated in BEAST (see Section 2.5 *Phylogenetic Analysis* for details). The dashed line indicates dates expected if midge and plant clades corresponded in age.

Table 3
Total, mean, (plus standard error) and median frequencies of parasitism of populations of *Asteromyia* species across host-plants. N_{total} is the total number of galls surveyed across all hosts and N_{hosts} indicates the number of different host-plant species from which collections were made for each midge species. Numbers in parentheses indicate the number of host from which 10 or more galls were reared.

Species	Total *P*	Mean *P*	±SE	Median *P*	N_{total}	N_{hosts}
A. carbonifera	0.342	0.353	0.038	0.294	3816	24 (19)
A. chrysothamni	0.306	0.211	0.095	0.280	314	5 (1)
A. clarkei	0.143	0.371	0.208	0.371	63	3 (2)
A. euthamiae	0.158	0.136	0.034	0.152	101	3 (1)
A. gutierreziae	0.226	0.232	0.101	0.232	93	5 (2)
A. laeviana	0.327	0.374	0.094	0.349	272	12 (7)
A. modesta	0.501	0.397	0.091	0.408	690	19 (7)
A. n. sp.	0.160	–	–	–	35	1
A. tumifica	0.0	–	–	–	7	1
Mean (spp.)	0.240	0.296		0.298		

4. Discussion

Gall midges represent one of the most species rich family of flies and, with nearly 4000 species in a single gall-forming subfamily (Cecidomyiinae), one of the more dramatic associations between the gall-forming habit and diversification (Price, 2005). However, as a group they remain poorly understood, and little is known about the ecological and evolutionary factors that underlie their remarkable diversification. Previous work has shown that midge diversity is strongly associated with host-plant diversity, implying that a principle explanation for gall midge diversity is the host-plants they parasitize (Price, 2005). However, many gall midges have specialized relationships between fungal symbionts that either directly or indirectly mediate the nutritional interaction with their host-plants (Borkent and Bisset, 1985; Janson et al., 2008). Moreover, galling midges, like many other gall-forming insects, are attacked by a rich assemblage of parasitoids. Evidence indicates that fungal symbionts even mediate these interactions as well (Weis, 1982b; Stireman et al., 2008). There is evidence as well that some species groups are radiating within single host-plant species, either onto different modular structures (Joy and Crespi, 2007) or in the absence of any obvious plant-associated niches (Stireman et al., 2008). Thus, the role of host-plants in midge diversity is complex, and

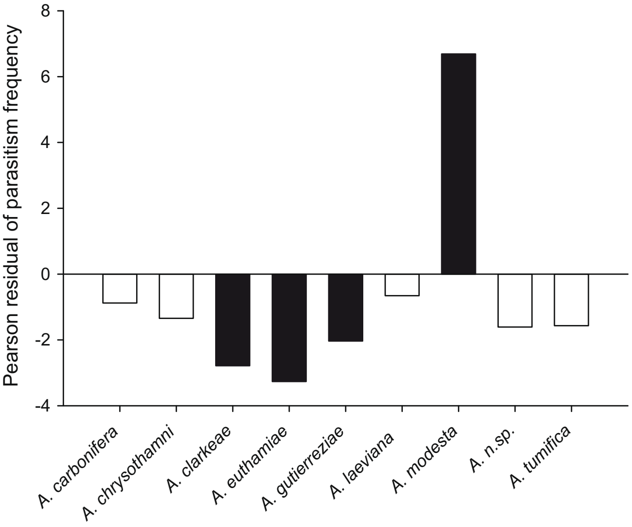

Fig. 7. Residual deviation in total parasitism among *Asteromyia* species. Black bars indicate species that have significantly greater or lower parasitism than expected ($P < 0.05$).

at the moment, the ecological processes at work remain essentially unknown.

Studying an exemplar North American group of gall midges in the genus *Asteromyia*, we used mitochondrial gene trees to describe species relationships, and then examined patterns of association with host-plants, fungal mutualists, and parasitoid assemblages. We confirmed the monophyly of *Asteromyia* (*sensu* Gagné, 1968), and of most of the specific taxa. Diversification has been rapid and recent (postdating the divergence of their host-plants) and, judging by the degree of phylogenetic structure in mitochondrial tree, much diversity remains hidden and undescribed. There is considerable interspecific variation in trophic interactions: fungal associations have waxed and waned repeatedly in the genus, and rates of parasitoid attack vary widely. Notably, *Asteromyia modesta* a species that appears to have secondarily lost the extensive and integrated fungal symbiosis for which anti-predator adaptive functions have been proposed in other species (Weis, 1982b), generally exhibited the highest rates of parasitoid attack.

4.1. Monophyly of Asteromyia species and cryptic diversity

Up until Gagné's 1968 revision, 33 species of *Asteromyia* had been described, primarily by Felt. Some of these species were delineated along host-plant lines (e.g., *A. squarrosae* from *Solidago squarrosa*), but several species overlapped considerably in host-plant use (e.g., three species described from *S. rugosa*, Gagné, 1968), and some were described as using widely divergent host-plants (e.g., *A. albomaculata* associated with both *Solidago* and "*Aster*"; Gagné, 1968). Gagné synonymized most of these species names due to the lack of consistent morphological differences in adult and immature stages. The present ML and Bayesian analyses indicate that most of the species recognized by Gagné are indeed monophyletic. The only clear exception is *A. modesta*, which is rendered paraphyletic by virtue of the placement of *A. tumifica* (Fig. 2). *A. modesta* appears unusual in general, because a separate non-sister clade exists of what appears to be *A. modesta* based on larval and gall morphology. The highly derived placement of *A. tumifica* arising within *A. modesta* is strongly supported, and thus clearly rejects the hypothesis that these stem galling lineages represent the basal condition in *Asteromyia*. Rather, this bulbous stem gall form, which houses many larvae (Gagné, 1968), is derived from the simple solitary leaf-pocket gall form.

Overall, the reconstruction corresponds surprisingly well to a "hypothetical evolutionary scheme" by Gagné (1968) based on larval and adult morphology, but also suggests that there are likely many more reproductively isolated species than currently recognized. The extensive phylogenetic structure within several of the named species of *Asteromyia*, suggests rampant diversity of morphologically cryptic, but genetically, and likely ecologically distinct lineages.

It was previously shown using allozymes and AFLP markers that four different gall forms of *A. carbonifera* on a single host-plant, *Solidago altissima*, represent genetically distinct "races" (Crego et al., 1990; Stireman et al., 2008). However, only one of these lineages (the "crescent" clade, Fig. 2), was clearly resolved as monophyletic in analyses of COI mtDNA sequence data. Extrapolating our results to all the known and potential host-plants not yet surveyed for *Asteromyia*, the genus contains perhaps dozens of species and hundreds of populations with varying degrees of reproductive isolation, representing a recent, rapid, and quite extensive radiation onto their Astereae host-plants. Given cecidomyiids, in which species to genus ratios can be exceedingly large, such a large number of cryptic and distinct lineages would not be at all unusual (Price, 2005). For example, *Dasineura*, a genus closely-related to *Asteromyia*, currently includes 448 named species (Gagné, 2004).

4.2. Asteromyia host-plant coevolution

Our estimates of the ages for *Asteromyia* as a whole and for *A. carbonifera* and *A. laeviana* are rough approximations, but they are far shallower than estimates for the corresponding host-plants. Even so, these divergence times for *Asteromyia* are probably overestimated. Recent studies have shown that commonly used estimates of substitution rates are unreliable when examining relatively shallow divergence times due to the long residence times of slightly deleterious mutations (e.g., (Ho et al., 2005, 2007). Estimates of divergence times in our analysis of the North American clade of Astereae may also be overestimated, as similar biases are likely operating. However, independent estimates by Kim et al. (2005) for a mid-Eocene origin for the Asteraceae as a whole (42–48 Mya), and a divergence of major tribes of Asteraceae occurring in the Oligocene (24–38 Mya), suggest that our age estimates for this clade (26.3 Mya) is reasonable. The Astereae is considered to exhibit among the fastest diversification rates in the flowering plants—and is itself viewed as having undergone a tremendous recent adaptive radiation (Panero and Funk, 2008; Schilling et al., 2008). This is supported by our analyses. For example, the genus *Solidago*, which is represented by a broad range of species in our ITS sequence data set, contains approximately 100 described species (Semple and Cook, 2006). Given our rough estimate of divergence time and ignoring extinction, the rate of new species formation would be an average of almost 20 new species per million years and a lineage diversification rate D (=ln N/T; Good-Avila et al., 2006) of 0.88 species/my; a rate comparable with some of the most rapid adaptive radiations of plants known (Baldwin and Sanderson, 1998; Good-Avila et al., 2006).

Although the North American Astereae group is likely older than the genus *Asteromyia*, there is potential overlap in the estimated ages of the genera *Solidago* and *Symphyotrichum* and the *Asteromyia* clades *A. carbonifera* and *A. laeviana* that gall them (Fig. 6). This, along with the significant phylogenetic associations recovered from PARAFIT, suggest a likely role of coevolution with their host-plants in *Asteromyia* diversification. The overall significant phylogenetic association is due to a small number of significant links (Fig. 5), and may reflect phylogenetically restricted colonization of hosts rather than true co-cladogenesis. However, cecidomyiids are potentially strong dispersers (Pendleton and Teetes, 1994), and also generally monophagous. Thus, like other endophytic insects (gall-forming aphids, for example), it is difficult to reconstruct the sequence of range limitations, dispersal events and host-switches to discriminate cospeciation from alternative hypotheses (Hoberg and Brooks, 2008). We are pursuing these alternative scenarios with finer resolution markers. Although ML and parsimony character reconstructions do not provide much resolution as to the evolutionary history of host associations, there is some suggestion of a relatively early colonization of *Symphyotrichum* with subsequent expansions to *Eurybia* and *Chrysothamnus* (and relatives; Fig. 4).

It is worth noting that *Asteromyia* species are relatively good taxonomists. Each of the named species groups, aside from *A. modesta*, is restricted to a monophyletic clade of Astereae. In fact, they generally appear to recognize the same genera as we do (e.g., *Gutierrezia*, *Euthamia*, *Solidago*, *Symphyotrichum*). This suggests that these genera are not arbitrary systematic constructs, but that there is something biologically and evolutionarily "real" about them and they might be comparable as equivalent evolutionary entities. *A. modesta* distinguishes these genera as well, as this "species" consists of several distinct lineages associated with particular Astereae genera; a radiation within the radiation. In some ways, *Asteromyia* gall midges are ahead of human systematists as they "recognized" the North American radiation of Astereae before we did (failing to exploit, for example, members of the genus *Aster* sensu stricto; Noyes and Rieseberg, 1999), and they "recognized" a close affinity between *Gutierrezia* and *Euthamia*, something that has only been recently confirmed by modern researchers (e.g., Nesom et al., 1990; Beck et al., 2004). This astute taxonomy by *Asteromyia* suggests that species are strongly limited by physiological and biochemical characteristics that are closely correlated with host phylogeny.

4.3. The fungal mutualism

Fungal associations of gall-forming Cecidomyiidae probably arose from opportunistic colonization of pathogenic fungi in galls during oviposition or the subsequent larval invasion of plant tissues. Cecidomyiidae have a long history of fungal associations, and are closely allied with the fungus-inhabiting Sciaridae and Mycetophilidae (the fungus gnats, with which the most basal cecidomyiid lineages Porricondylinae and Lestremiinae share mycophilic lifestyles). In fact, cecidomyiids include the only known true gallers of fungi (Larew et al., 1987). It is likely that ancestral interactions with fungi were associated with midge traits subsequently co-opted in plant-associated midge lineages for exploiting endophytic fungi.

In this study, parsimony and ML character reconstruction methods both support a scenario in which the use of fungi in gall construction was gained early in the history of the genus *Asteromyia*, and subsequently lost, perhaps multiply, in the *A. modesta* lineages (which converged in "leaf-pocket" gall forms). If fungal symbiosis is dynamic in midges, symbiotically-transitional midge lineages are likely to exist, at different stages of acquisition, integration or loss of fungal symbionts. In accord with this, our observations of *A. modesta* galls indicate that this species (or complex of species) retains a diminished or perhaps even relic fungal associate. We frequently observed thin strands of fungal hyphae in mature *A. modesta* galls on *Solidago* hosts, and, although no detailed microscopic studies have yet been conducted, it may well be that all galls of this species contain fungi that contribute to larval nutrition. Our observations of another species, *A. tumifica*, thus far reveal no evidence of associated fungus, but adults of both of these species have been reported to bear fungal conidia (Borkent and Bisset, 1985). Nevertheless, short of more detailed microscopy and molecular analyses, the phylogenetic history of fungal associations in midges remains largely mysterious.

Frequent and biologically-significant changes in the extent of integration between fungal symbionts and midges is somewhat surprising, because in addition to affecting gall traits itself, fungal structures in the gall are likely to affect larval and pupal traits associated with eclosion, such as the larval spatulae. It is unclear what evolutionary forces favor reversals in the extensive use of fungi in gall construction in the *A. modesta* lineages, for example, if indeed this condition is derived. Previous work has demonstrated protective benefits of fungi from parasitoids and other enemies, indicating selective advantages of fungal symbiosis (Weis, 1982b). One possibility is that locating and collecting the fungal conidia may be costly for ephemeral adults. Fungal symbiosis may imply a complex phenological orchestration of fungal conidiation and host-plant tissue suitability, all of which could vary with environmental conditions or host-plant or fungal density. In addition, the benefits of the fungus for protection could be narrow and sensitive to the community context, easily outweighed in some circumstances by the increased visual (and perhaps olfactory) conspicuousness fungi provide for searching parasitoids or predators.

4.4. Parasitism

Parasitism rates were generally high, ca. 30% for dissected galls, across species of *Asteromyia*. These rates should be considered minima given that the galls were removed from potential additional parasitism and many young endoparasitoids were likely overlooked. Within individual collections, parasitism rates sometimes ranged far higher, approaching 100% in some cases. Parasitism frequencies of particular *Asteromyia* species varied widely among host-plants, although many of these collections were composed of small samples and may not accurately reflect overall levels. In addition, parasitism frequencies of *Asteromyia* on a single host-plant can vary appreciably among populations and over time (Stireman et al., 2008), making it difficult to estimate a robust overall value for a population or species.

Given these caveats, we found that three species, *A. euthamiae, gutierreziae* and *clarkei*, experienced significantly lower rates of parasitism relative to the other taxa (Fig. 7). The former two species, which comprise a distinct clade on related host-plant genera, form black galls composed primarily of the hardened carbonaceous fungal stroma, with little external evidence of proliferating white hyphae (Fig. 1). This could limit their accessibility to some parasitoid taxa.

Asteromyia modesta stands out in exhibiting high frequencies of parasitism (50% overall; Fig. 7) relative to the other taxa. Although these parasitism rates varied widely among host-plants, mean and median parasitism frequencies among plant species were also highest for this species. This result is consistent with the hypothesis that the incorporation of fungus in gall morphology serves a defensive function (Weis, 1982b; Stireman et al., 2008). *A. modesta* larvae are protected only by the leaf tissue, with their galls thus resembling mines of leaf-miners, which as a group experience the highest mortality from parasitoids of any herbivorous insect guild (Hawkins, 1994). As discussed, this finding begs the question of why *A. modesta* should have lost this association with fungi in gall development given the apparent advantages of fungal symbiosis. It is interesting that the only stem-galling "species" *A. tumifica*, arose within the *A. modesta* clade. This transition to stem galling may have been selected for by high parasitism. *Asteromyia tumifica* larvae are concealed relatively deeply in the plant tissue, potentially protecting them from parasitoid attack. We have only collected a small number of galls of *A. tumifica* (7 galls, 35 larvae), but have yet to observe parasitism in this species.

We are in the process of identifying the parasitoid species that we encountered to assess how community structure varies among *Asteromyia* species and populations. However, in light of the extensive cryptic diversification apparent in *Asteromyia* populations and increasing evidence for high host-related cryptic species diversity in parasitoids (Stireman et al., 2006; Smith et al., 2007, 2008), we are hesitant to assume that morphological similar parasitoids from different *Asteromyia* populations or species represent genetically continuous populations. This system appears to hold great potential for understanding the process of host associated differentiation across trophic levels (Stireman et al., 2006).

5. Conclusions

Flies in the family Cecidomyiidae are extremely diverse, and are characterized by complex trophic interactions with plants, fungal symbionts, and predators. We characterized the phylogenetic history and trophic relations of the North American midge genus *Asteromyia*, which is engaged in a complex and perhaps co-evolving community of interactions with host-plants, fungi, and parasitoids. Mitochondrial gene trees generally support canonical classifications, but reveal a great deal of cryptic diversity within the eight named species. *Asteromyia* likely radiated after their associated host-plants in the Astereae. The evolutionary patterns are dynamic, however, and suggest rapid and perhaps coordinated changes across trophic levels.

Acknowledgments

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Appendix A

Samples used mtDNA analyses with host-plants, collection site, and Genbank accession number.

Sample name	Midge species	host-plant	Collection location	Genbank Accession No.
1255caaltIL	*A. carbonifera*	*Solidago altissima*	DeKalb, DeKalb Co., IL	EU439784
1348caaltIL	*A. carbonifera*	*Solidago altissima*	Champaign-Urbana, Champaign Co., IL	EU439787
1475caaltOHcr	*A. carbonifera*	*Solidago altissima*	Ohio/Indiana Border, Preble Co, OH	EU439788
1488caaltOH	*A. carbonifera*	*Solidago altissima*	Ohio/Indiana Border, Preble Co, OH	EU439789
1508caaltOH	*A. carbonifera*	*Solidago altissima*	Ohio/Indiana Border, Preble Co, OH	EU439790
1519caaltOH	*A. carbonifera*	*Solidago altissima*	Ohio/Indiana Border, Preble Co, OH	EU439791
1652caaltPA	*A. carbonifera*	*Solidago altissima*	Claysville, Washington Co., PA	EU439792
1759caaltMOcr	*A. carbonifera*	*Solidago altissima*	Weston, Platte Co., MO	EU439793
1782caaltMO	*A. carbonifera*	*Solidago altissima*	Weston, Platte Co., MO	EU439794
180caaltNYcr	*A. carbonifera*	*Solidago altissima*	Enfield, Tompkins Co., NY	EU439795
1810caaltMO	*A. carbonifera*	*Solidago altissima*	Weston, Platte Co., MO	EU439796
1847caaltMO	*A. carbonifera*	*Solidago altissima*	Weston, Platte Co., MO	EU439797
1931caaltKYcr	*A. carbonifera*	*Solidago altissima*	Red River Gorge, Powell Co., KY	EU439798
193caaltNY	*A. carbonifera*	*Solidago altissima*	Enfield, Tompkins Co., NY	EU439799
1966caaltKY	*A. carbonifera*	*Solidago altissima*	Red River Gorge, Powell Co., KY	EU439800
1973caaltKY	*A. carbonifera*	*Solidago altissima*	Red River Gorge, Powell Co., KY	EU439801
2164caaltNY	*A. carbonifera*	*Solidago altissima*	Ithaca, Tompkins Co., NY	EU439803
2183caaltNYcr	*A. carbonifera*	*Solidago altissima*	Ithaca, Tompkins Co., NY	EU439805
241caaltNYcr	*A. carbonifera*	*Solidago altissima*	Caroline, Tompkins Co., NY	FJ803255
251caaltNY	*A. carbonifera*	*Solidago altissima*	Caroline, Tompkins Co., NY	FJ803260
2599cacaltMAcr	*A. carbonifera*	*Solidago altissima*	Marshfield, Plymouth Co., MA	EU439832
2600cacaltMAcr	*A. carbonifera*	*Solidago altissima*	Marshfield, Plymouth Co., MA	EU439829
2628cacaltMEcr	*A. carbonifera*	*Solidago altissima*	Thomaston, Knox Co., ME	EU439831
2636caaltME	*A. carbonifera*	*Solidago altissima*	Thomaston, Knox Co., ME	EU439826
2641cacaltME	*A. carbonifera*	*Solidago altissima*	Thomaston, Knox Co., ME	EU439827
2651cacaltMAcr	*A. carbonifera*	*Solidago altissima*	Blandford, Hampden Co, MA	EU439836
751cacalCA	*A. carbonifera*	*Solidago velutina* ssp. *californica*	Mt. Hamilton, Santa Clara Co., CA	FJ803294
753cacalCA	*A. carbonifera*	*Solidago velutina* ssp. *californica*	Mt. Hamilton, Santa Clara Co., CA	FJ803295
2194cagigNY	*A. carbonifera*	*Solidago gigantea*	Ithaca, Tompkins Co., NY	EU439806
2484cagigMA	*A. carbonifera*	*Solidago gigantea*	Marshfield, Plymouth Co., MA	EU439812
1099cajunNY	*A. carbonifera*	*Solidago juncea*	Caroline, Tompkins Co., NY	FJ803236
1163cajunNY	*A. carbonifera*	*Solidago juncea*	Ithaca, Tompkins Co., NY	FJ803237
1172cajunNY	*A. carbonifera*	*Solidago juncea*	Ithaca, Tompkins Co., NY	FJ803238
198cajunNY	*A. carbonifera*	*Solidago juncea*	Enfield, Tompkins Co., NY	FJ803249
2457cajunCT	*A. carbonifera*	*Solidago juncea*	Middletown, Middlesex Co., CT	FJ803257
2529cajunMA	*A. carbonifera*	*Solidago juncea*	Ludlow, Hampden Co., MA	FJ803261
399cajunNY	*A. carbonifera*	*Solidago juncea*	Ithaca, Tompkins Co., NY	FJ803287
a101camissNE/jun	*A. carbonifera*	*Solidago missouriensis* or *juncea*	Almeria, Loup Co., NE	FJ803301
a94camiss/junNE	*A. carbonifera*	*Solidago missouriensis* or *juncea*	Almeria, Loup Co., NE	FJ803358

(*continued on next page*)

Appendix A (*continued*)

Sample name	Midge species	host-plant	Collection location	Genbank Accession No.
2574caneME	*A. carbonifera*	*Solidago nemoralis*	Freeport, Cumberland Co., ME	FJ803262
2678canemNY	*A. carbonifera*	*Solidago nemoralis*	Ithaca, Tompkins Co., NY	FJ803263
3031canemME	*A. carbonifera*	*Solidago nemoralis*	Brownville Jct., Piscataquis Co., ME	FJ803278
1018capatNY	*A. carbonifera*	*Solidago patula*	Caroline, Tompkins Co., NY	FJ803234
a97carigIA	*A. carbonifera*	*Solidago rigida*	Don Williams Park, Boone Co., IA	FJ803359
1006carugNY	*A. carbonifera*	*Solidago rugosa*	Caroline, Tompkins Co., NY	EU439780
1114carugNY	*A. carbonifera*	*Solidago rugosa*	Ithaca, Tompkins Co., NY	EU439783
2162carugNY	*A. carbonifera*	*Solidago rugosa*	Caroline, Tompkins Co., NY	EU439802
2204carugMAcr	*A. carbonifera*	*Solidago rugosa*	Marshfield, Plymouth Co., MA	EU439807
2206carugMAcr	*A. carbonifera*	*Solidago rugosa*	Marshfield, Plymouth Co., MA	EU439808
2213carugMA	*A. carbonifera*	*Solidago rugosa*	Marshfield, Plymouth Co., MA	EU439809
2536carugME	*A. carbonifera*	*Solidago rugosa*	Clark Island, Knox Co., ME	EU439821
2550carugME	*A. carbonifera*	*Solidago rugosa*	Freeport, Cumberland Co., ME	EU439823
2560carugMEcr	*A. carbonifera*	*Solidago rugosa*	Freeport, Cumberland Co., ME	EU439814
25carugMA	*A. carbonifera*	*Solidago rugosa*	Marshfield, Plymouth Co., MA	EU439837
3046carugME	*A. carbonifera*	*Solidago rugosa*	Brownville Jct., Piscataquis Co., ME	EU439819
902carugNY	*A. carbonifera*	*Solidago rugosa*	Ithaca, Tompkins Co., NY	EU439817
a11caulmAR	*A. carbonifera*	*Solidago ulmifolia*	Ozark NF, Franklin Co, AR	FJ803302
a12caulmAR	*A. carbonifera*	*Solidago ulmifolia*	Ozark NF, Franklin Co, AR	FJ803311
a63cavelUT	*A. carbonifera*	*Solidago velutina*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803349
2225chchnaAZ	*A. chrysothamni*	*Chrysothamnus nauseosus*	Flagstaff, Coconino Co., AZ	FJ803250
732chchnaNM	*A. chrysothamni*	*Chrysothamnus nauseosus*	Albuquerque, Bernalillo Co., NM	FJ803292
745chchnaCA	*A. chrysothamni*	*Chrysothamnus nauseosus*	Mt. Hamilton, Santa Clara Co., CA	FJ803293
a188chchnaUT	*A. chrysothamni*	*Chrysothamnus nauseosus*	Lower Indian Creek, San Juan Co., UT	FJ803332
2336chchspCA	*A. chrysothamni*	*Chrysothamnus* sp.	Layton State Park, Placer Co. CA	FJ803251
a155chchspNM	*A. chrysothamni*	*Chrysothamnus* sp.	Cimmarron Canyon SP, Colfax Co., NM	FJ803326
a196chchspNM	*A. chrysothamni*	*Chrysothamnus* sp.	Cimmarron Canyon SP, Colfax Co., NM	FJ803338
a26chchspNM	*A. chrysothamni*	*Chrysothamnus* sp.	Carson NF, Taos Co., NM	FJ803340
a27chchspNM	*A. chrysothamni*	*Chrysothamnus* sp.	Carson NF, Taos Co., NM	FJ803341
a37chchspCO	*A. chrysothamni*	*Chrysothamnus* sp.	Montezuma Co., CO	FJ803342
a41chchspCO	*A. chrysothamni*	*Chrysothamnus* sp.	Lower Piedra River, Archuleta Co., CO	FJ803345
a76chchspUT	*A. chrysothamni*	*Chrysothamnus* sp.	Emery Co., UT	FJ803355
a71chchnaUT	*A. chrysothamni*	*Chrysothamnus nauseosus*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803351
a78chchnaUT	*A. chrysothamni*	*Chrysothamnus nauseosus*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803356
a79chchnaUT	*A. chrysothamni*	*Chrysothamnus nauseosus*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803357
a55chLscUT	*A. chrysothamni*	*Lorandersonia scopulorum*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803346
a56chLscUT	*A. chrysothamni*	*Lorandersonia scopulorum*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803347
a67chLscUT	*A. chrysothamni*	*Lorandersonia scopulorum*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803350
2494clediMA	*A. clarkei*	*Eurybia divericata*	Marshfield, Plymouth Co., MA	FJ803259
811clediNY	*A. clarkei*	*Eurybia divericata*	Ithaca, Tompkins Co., NY	FJ803297
888clediNY	*A. clarkei*	*Eurybia divericata*	Ithaca, Tompkins Co., NY	FJ803299
980clediNY	*A. clarkei*	*Eurybia divericata*	Ithaca, Tompkins Co., NY	FJ803300
2686clemaNY	*A. clarkei*	*Eurybia macrophylla*	Ithaca, Tompkins Co., NY	FJ803264
1105euegrNY	*A. euthamiae*	*Euthamia graminifolia*	Caroline, Tompkins Co., NY	EU439782
23euegrMA	*A. euthamiae*	*Euthamia graminifolia*	Marshfield, Plymouth Co., MA	EU439810
3058euegrME	*A. euthamiae*	*Euthamia graminifolia*	Brownville Jct., Piscataquis Co., ME	FJ803279
760euegrCAN	*A. euthamiae*	*Euthamia graminifolia*	Ottawa, CANADA	FJ803296
a129euegrOH	*A. euthamiae*	*Euthamia graminifolia*	Beavercreek, Greene Co., OH	FJ803310
a152euegrIN	*A. euthamiae*	*Euthamia graminifolia*	Shades State Park, Parke Co., IN	FJ803323
a153euegrIN	*A. euthamiae*	*Euthamia graminifolia*	Shades State Park, Parke Co., IN	FJ803324
a154euegrIN	*A. euthamiae*	*Euthamia graminifolia*	Shades State Park, Parke Co., IN	FJ803325
725gugusNM	*A. gutierrezia*	*Gutierrezia sarothrae*	Albuquerque, Bernalillo Co., NM	FJ803289
727gugusNM	*A. gutierrezia*	*Gutierrezia sarothrae*	Albuquerque, Bernalillo Co., NM	FJ803290
a17gugusTX	*A. gutierrezia*	*Gutierrezia sarothrae*	Palo Duro Canyon, Randall Co., TX	FJ803331
a18gugusTX	*A. gutierreziae*	*Gutierrezia sarothrae*	Palo Duro Canyon, Randall Co., TX	FJ803334
a19gugusTX	*A. gutierreziae*	*Gutierrezia sarothrae*	Palo Duro Canyon, Randall Co., TX	FJ803339
a74gugusUT	*A. gutierreziae*	*Gutierrezia sarothrae*	Emery Co., UT	FJ803353
a75gugusUT	*A. gutierreziae*	*Gutierrezia sarothrae*	Emery Co., UT	FJ803354
a125gugutNm	*A. gutierreziae*	*Gutierrezia* sp.	Big Burro Mts., Grant Co., NM	FJ803307
a140gugutNM	*A. gutierreziae*	*Gutierrezia* sp.	Big Burro Mts., Grant Co., NM	FJ803316
a143gugutNm	*A. gutierreziae*	*Gutierrezia* sp.	Big Burro Mts., Grant Co., NM	FJ803319
a144gugutNm	*A. gutierreziae*	*Gutierrezia* sp.	Big Burro Mts., Grant Co., NM	FJ803320
a73gugusUT	*A. gutierreziae*	*Gutierrezia sarothrae*	Indian Creek-Newspaper Rock SP, San Juan Co., UT	FJ803352
722nrgugucCA	Near *A. gutierreziae*	*Gutierrezia californica*	Livermore, Alameda Co., CA	FJ803288
a159laEradOH	*A. laeviana*	*Eurybia radula*	Beavercreek, Greene Co., OH	FJ803327
a190laEradOH	*A. laeviana*	*Eurybia radula*	Beavercreek, Greene Co., OH	FJ803335
a191laEradOH	*A. laeviana*	*Eurybia radula*	Beavercreek, Greene Co., OH	FJ803336

Appendix A (*continued*)

Sample name	Midge species	host-plant	Collection location	Genbank Accession No.
3263laSpunNY	*A. laeviana*	*Symphotrichum puniceum*	Ithaca, Tompkins Co., NY	FJ803285
3083laScorME	*A. laeviana*	*Symphyotrichum cordifolium*	Farmington, Franklin Co., ME	FJ803281
a39laSlaeCO	*A. laeviana*	*Symphyotrichum laeve*	Lower Piedra River, Archuleta Co., CO	FJ803343
a40laSlaeCO	*A. laeviana*	*Symphyotrichum laeve*	Lower Piedra River, Archuleta Co., CO	FJ803344
a61laSlaeCO	*A. laeviana*	*Symphyotrichum laeve*	Lower Piedra River, Archuleta Co., CO	FJ803348
2705laSlanNY	*A. laeviana*	*Symphyotrichum lanceolatum*	Ithaca, Tompkins Co., NY	FJ803266
2710laSlanNY	*A. laeviana*	*Symphyotrichum lanceolatum*	Ithaca, Tompkins Co., NY	FJ803267
a124laSlanOH	*A. laeviana*	*Symphyotrichum lanceolatum*	Cowan Lake SP, Clinton Co., OH	FJ803306
a127laSlanOH	*A. laeviana*	*Symphyotrichum lanceolatum*	Yellow Springs, Greene Co., OH	FJ803309
a137laSlanOH	*A. laeviana*	*Symphyotrichum lanceolatum*	Cowan Lake SP, Clinton Co., OH	FJ803314
a139laSlanOH	*A. laeviana*	*Symphyotrichum lanceolatum*	Cowan Lake SP, Clinton Co., OH	FJ803315
a142laSlanOH	*A. laeviana*	*Symphyotrichum lanceolatum*	Beavercreek, Greene Co., OH	FJ803318
a146laSlanOH	*A. laeviana*	*Symphyotrichum lanceolatum*	Beavercreek, Greene Co., OH	FJ803321
3079laSlatME	*A. laeviana*	*Symphyotrichum lateriflorum*	Farmington, Franklin Co., ME	FJ803280
a121laSlatOH	*A. laeviana*	*Symphyotrichum lateriflorum*	Yellow Springs, Greene Co., OH	FJ803303
a126laSlatOH	*A. laeviana*	*Symphyotrichum lateriflorum*	Yellow Springs, Greene Co., OH	FJ803308
a136laSlatOH	*A. laeviana*	*Symphyotrichum lateriflorum*	Yellow Springs, Greene Co., OH	FJ803313
2697laSlaeNY	*A. laeviana*	*Symphyotrichum laeve*	Ithaca, Tompkins Co., NY	FJ803265
2804laSlaeNY	*A. laeviana*	*Symphyotrichum laeve*	Ithaca, Tompkins Co., NY	FJ803269
2819laSlaeNY	*A. laeviana*	*Symphyotrichum laeve*	Ithaca, Tompkins Co., NY	FJ803270
850laSpilNY	*A. laeviana*	*Symphyotrichum pilosum*	Ithaca, Tompkins Co., NY	FJ803298
a160laSpil?OH	*A. laeviana*	*Symphyotrichum pilosum*	Fairborn, Greene Co., OH	FJ803328
a16laSlat?IN	*A. laeviana*	*Symphyotrichum pilosum*	Beavercreek, Greene Co., OH	FJ803329
a189laSpil?OH	*A. laeviana*	*Symphyotrichum pilosum*	Fairborn, Greene Co., OH	FJ803333
a192laSpil?OH	*A. laeviana*	*Symphyotrichum pilosum*	Fairborn, Greene Co., OH	FJ803337
3091laSpreME	*A. laeviana*	*Symphyotrichum prenanthoides*	Farmington, Franklin Co., ME	FJ803282
3287laSpreNY	*A. laeviana*	*Symphyotrichum prenanthoides*	Ithaca, Tompkins Co., NY	FJ803286
a132laSpreME	*A. laeviana*	*Symphyotrichum prenanthoides*	Farmington, Franklin Co., ME	FJ803312
3252laSuroNY	*A. laeviana*	*Symphyotrichum urophyllum*	Ithaca, Tompkins Co., NY	FJ803284
1200moccaNY	*A. modesta*	*Erigeron* (*Conyza*) *canadensis*	Ithaca, Tompkins Co., NY	FJ803239
2479moccaMA	*A. modesta*	*Erigeron* (*Conyza*) *canadensis*	Marshfield, Plymouth Co., MA	FJ803258
2907moccaNY	*A. modesta*	*Erigeron* (*Conyza*) *canadensis*	Ithaca, Tompkins Co., NY	FJ803271
2346moeriNY	*A. modesta*	*Erigeron annus*	Ithaca, Tompkins Co., NY	FJ803252
2355moeriNY	*A. modesta*	*Erigeron annus*	Ithaca, Tompkins Co., NY	FJ803253
1237moaltIL	*A. modesta*	*Solidago altissima*	DeKalb, DeKalb Co., IL	FJ803240
1335moaltIL	*A. modesta*	*Solidago altissima*	Batavia, Kane Co., IL	FJ803241
1456moaltIN	*A. modesta*	*Solidago altissima*	Waynetown, Montgomery Co., IN	FJ803242
1461moaltOH	*A. modesta*	*Solidago altissima*	Ohio/Indiana Border, Preble Co, OH	FJ803243
1468moaltOH	*A. modesta*	*Solidago altissima*	Ohio/Indiana Border, Preble Co, OH	FJ803244
1572moaltOH	*A. modesta*	*Solidago altissima*	Licking Co., OH	FJ803245
1619moaltPA	*A. modesta*	*Solidago altissima*	Claysville, Washington Co., PA	FJ803246
2933moaltNY	*A. modesta*	*Solidago altissima*	Montezuma NWR, Wayne Co., NY	FJ803273
3093moaltNY	*A. modesta*	*Solidago altissima*	Ithaca, Tompkins Co., NY	FJ803283
a147moaltOH	*A. modesta*	*Solidago altissima*	Beavercreek, Greene Co., OH	FJ803322
1053mofleNY	*A. modesta*	*Solidago flexicaulis*	Ithaca, Tompkins Co., NY	FJ803235
2950mogigNY	*A. modesta*	*Solidago gigantea*	Ithaca, Tompkins Co., NY	FJ803275
2953mogigNY	*A. modesta*	*Solidago gigantea*	Ithaca, Tompkins Co., NY	FJ803276
a122moohOH	*A. modesta*	*Solidago ohioensis*	Beavercreek, Greene Co., OH	FJ803304
a123moohOH	*A. modesta*	*Solidago ohioensis*	Beavercreek, Greene Co., OH	FJ803305
a141moohOH	*A. modesta*	*Solidago ohioensis*	Beavercreek, Greene Co., OH	FJ803317
2766moScorNY	*A. modesta*	*Symphyotrichum cordifolium*	Ithaca, Tompkins Co., NY	FJ803268
2936moScorNY	*A. modesta*	*Symphyotrichum cordifolium*	Ithaca, Tompkins Co., NY	FJ803274
1733moSlatIL	*A. modesta*	*Symphyotrichum lateriflorum*	Batavia, Kane Co., IL	FJ803247
1738moSlatIL	*A. modesta*	*Symphyotrichum lateriflorum*	Batavia, Kane Co., IL	FJ803248
2927mounkrNY	*A. modesta*	*Symphyotrichum* sp.	Ithaca, Tompkins Co., NY	FJ803272
72tualtNY	*A. tumifica*	*Solidago altissima*	Ithaca, Tompkins Co., NY	FJ803291
a177tualtOH	*A. tumifica*	*Solidago altissima*	Beavercreek, Greene Co., OH	FJ803330
2985caphPH	*Calamomyia phragmites*	*Phragmites australis*	"Northeast US"	FJ803277
2376MaqGlt	*Meuneriella aqulonia*	*Gleditsia triacanthos*	Ithaca, Tompkins Co., NY	FJ803254
2421PsSver	*Protaplonyx sarcobati*	*Sarcobatus vermiculatus*	Mono Lake, Mono Co., CA	FJ803256

Appendix B

Table of accession numbers for plants in the Astereae used in analyses of divergence times and coevolution (bold). The names in this list are those associated with the sequence and do not reflect subsequent nomenclatural changes (e.g., *Oligoneron rigidum* is called *Solidago rigida*, Semple et al., 1999).

Host-plant name	Abbrev.	Accession No.
Baccharis dracunculifolia	Baccharis	AF046958
Boltonia asteroides		AF046975
Chrysothamnus depressus		AF477638
Chrysothamnus greenei		AY171016
Chrysothamnus linifolius	Chlin	AY170940
Chrysothamnus viscidiflorus		AF046967
Conyza canadensis	Cocan	AF046987
Doellingeria umbellata		AF046966
Ericameria cooperi		AF046973
Ericameria discoidea var. linearis		AY171013
Ericameria laricifolia		AY171018
Ericameria linearifolia		AY171020
Ericameria ***nauseosa***	Ernau	AY171023
Ericameria parryi		AY171029
Ericameria suffruticosa		AY171032
Erigeron byei		AF046974
Erigeron speciosus	Erspe	AF118479
Erigeron uniflorus		AF046988
Eurybia divaricata	Eudiv	AY772423
Euthamia graminifolia	Eugra	AF046982
Euthamia tenuifolia	Euten	AF477652
Grindelia lanceolata		AF046976
Gutierrezia sarothrae	Gusar	AF477657
Gutierrezia texana		AF477658
Gymnosperma glutinosum		AF477659
Laennecia sophiifolia		AF046964
Machaeranthera tanacetifolia		EU200218
Oligoneuron rigidum	Sorig	AY292528
Oreochrysum parryi		AY170958
Solidago arguta		EU125354
Solidago caesia	Socae	EU125356
Solidago canadensis	Socan	EU125361
Solidago fistulosa	Sofis	AF477667
Solidago gigantea	Sogig	EU125362
Solidago nemoralis	Sonem	EU125357
Solidago nitida		EU125368
Solidago ohioensis		EU125369
Solidago patula		EU125355
Solidago petiolaris		AF046968
Solidago ptarmicoides		EU125365
Solidago sempervirens		AF477668
Solidago speciosa		EU125359
Symphyotrichum concolor var. cocolor		EU200224
Symphyotrichum cordifolium	Sycor	EU200225
Symphyotrichum ericoides var. ericoides		EU200227
Symphyotrichum novae-angliae		EU200229
Symphyotrichum oblongifolium		AF046979
Symphyotrichum patens var. patens		EU200230

Appendix C

ML instantaneous rate matrix and base frequencies estimated using PhyML.

	A	C	G	T	ML est. freq.
A	−0.74285	0.07699	0.30021	0.36565	0.37118
C	0.24527	−2.08856	0.03666	1.80662	0.11652
G	1.07509	0.04122	−1.20792	0.09162	0.10365
T	0.33212	0.51511	0.02324	−0.87047	0.40865

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