Phylogenetic relationships within the leaf-mining flies (Diptera: Agromyzidae) inferred from sequence data from multiple genes

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Abstract

The leaf-mining flies (Diptera: Agromyzidae) are a diverse group whose larvae feed internally in leaves, stems, flowers, seeds, and roots of a wide variety of plant hosts. The systematics of agromyzids has remained poorly known due to their small size and morphological homogeneity. We investigated the phylogenetic relationships among genera within the Agromyzidae using parsimony and Bayesian analyses of 2965 bp of DNA sequence data from the mitochondrial COI gene, the nuclear ribosomal 28S gene, and the single copy nuclear CAD gene. We included 86 species in 21 genera, including all but a few small genera, and spanning the diversity within the family. The results from parsimony and Bayesian analyses were largely similar, with major groupings of genera in common. Specifically, both analyses recovered a monophyletic Phytomyzinae and a monophyletic Agromyzinae. Within the subfamilies, genera found to be monophyletic given our sampling include *Agromyza*, *Amauromyza*, *Calycomyza*, *Cerodontha*, *Liriomyza*, *Melanagromyza*, *Metopomyza*, *Nemorimyza*, *Phytobia*, and *Pseudonapomyza*. Several genera were found to be polyphyletic or paraphyletic including *Aulagromyza*, *Chromatomyia*, *Phytoliriomyza*, *Phytomyza*, and *Ophiomyia*. We evaluate our findings and discuss host-use evolution in light of current agromyzid taxonomy and two recent hypotheses of relationships based on morphological data.
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1. Introduction

The leaf-mining flies (Diptera: Agromyzidae) are small flies having phytophagous larvae that feed within leaves, stems, roots, flowers, or seeds. Agromyzids attack a broad diversity of plant hosts, including more than 140 plant families representing all major terrestrial plant groups, with the exception of mosses and most gymnosperms (Spencer, 1990; Benavent-Corai et al., 2005). These flies are distributed worldwide with their greatest diversity in northern temperate regions (Spencer, 1977). Despite the broad diet breadth exhibited by agromyzids as a group, most agromyzid species are remarkably specialized, feeding on only

one or a few closely related plant species (Spencer, 1990; Scheffer and Wiegmann, 2000). Host-use evolution within the Agromyzidae is a subject of great interest because diversification in this group appears to be largely associated with host shifts and dietary specialization (Spencer, 1990; Kulp, 1968; Scheffer and Wiegmann, 2000). However, modern phylogenetic analyses necessary for elaborating the history of agromyzid/host associations have only recently been applied to studies of these flies (Scheffer and Wiegmann, 2000; Dempewolf, 2001).

Despite their near ubiquity and interesting larval habits, the systematics of agromyzids has remained rather poorly understood due to their small size and morphological homogeneity. Species identification generally requires dissection of the male genitalia, and it is difficult to identify some female specimens even to genus using only morphological characters. Currently, the Agromyzidae contains

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two subfamilies, the Agromyzinae and the Phytomyzinae, based on one larval and one adult character (Spencer and Steyskal, 1986) although these characters are not always consistent (von Tschirnhaus, 1971). Twenty-eight genera are recognized containing approximately 2860 described species (Table 1), although it is clear from the number of new species found during regional surveys and detailed study of species groups (e.g., Scheffer and Wiegmann, 2000; Zlobin, 1994a, 2002) that this represents only a fraction of agromyzid diversity (see also Spencer, 1977; Spencer and Steyskal, 1986). Nearly a third of the known genera have fewer than 10 species (Table 1), while many of the other genera are large, containing many groups of morphologically similar species that often feed on closely related host plants (Spencer, 1990; also e.g., Griffiths, 1974, 1976, 1977; Scheffer and Wiegmann, 2000; Zlobin, 2000).

Modern hypotheses of relationships among agromyzid genera come primarily from two sources: a verbal "phyletic lines" scheme by Spencer (1990) in his consummate treatise on agromyzids and their host plants (Table 2), and a quantitative morphological phylogeny based on 83 larval and adult characters (Fig. 1) in a dissertation by Dempewolf (2001). Spencer envisioned four phyletic lines within the family with the "*Penetagromyza* group" corresponding to the Agromyzinae, and the traditional Phytomyzinae broken into the "*Phytobia* group," the "*Phytoliriomyza* group," and the "*Napomyza* group." Relationships among the latter three groups are unspecified. Most important in Spencer's scheme is the assertion, first suggested by Nowakowski (1962), that *Phytobia*, a widespread genus of large, cambium-mining species, is of ancient origin and represents the "primitive" condition within the family. This continues a long-held belief among agromyzid workers that groups of small leaf-mining species are derived from larger stem-feeding species (Nowakowski, 1962; Spencer, 1990; von Tschirnhaus, 1991). In fact, within each of Spencer's four phyletic lines the genera are arranged from a "primitive" stem-mining genus to "more advanced" leaf-mining groups.

Dempewolf's (2001, 2005) phylogenetic analysis of 83 larval and adult characters resulted in 90 most parsimonious trees obtained by successive weighting using the consistency index. The strict consensus of these trees is shown in Fig. 1 (redrawn from Dempewolf, 2005). In this analysis, the two subfamilies are found to be monophyletic, although support values for these and all other relationships are not available. Relationships differ from those suggested by Spencer in several ways, most notably in finding *Aulagromyza* and *Gymnophytomyza* to be associated with the *Napomyza* group of Spencer. Additionally, *Amauromyza* is found to be sister to *Cerodontha* and well inside a clade also including *Liriomyza*, *Galiomyza*, *Phytoliriomyza*, *Metopomyza*, and *Calycomyza*. Most importantly, reconstruction of larval feeding mode suggests that the ancestral feeding mode within the Agromyzidae was leaf-mining rather than stem- or cambium-mining (Dempewolf, 2005).

Here, we present the first comprehensive molecular phylogenetic analysis of the entire family Agromyzidae. A major goal is to provide new evidence from nuclear and mitochondrial genes on higher-level relationships within the family. With these new phylogenetic results, we investigate patterns of diversification within leafminers, evaluate relative support from genes and morphology for currently recognised genera as well as for key areas of agromyzid classification, and establish an initial framework for future investigations of agromyzid relationships and host-use evolution.

2. Materials and methods

Agromyzid specimens representing 86 species in 21 genera were obtained from a variety of locations around the world (Appendix A). We attempted to include congeners from different subgenera or different species groups whenever possible, particularly for the larger genera. For outgroups, we chose two *Fergusonina* species (Fergusoninidae). Several authors have suggested that the Agromyzidae and the Fergusoninidae are closely related, and at one time fergusoninids were even placed within the Agromyzidae (Tonnoir, 1937; Colless and McAlpine, 1970). Several morphological characters suggest that the Fergusoninidae may be the sister group to the Agromyzidae (McAlpine, 1989), although recent molecular work does not corroborate this (Rung, Winkler, and Scheffer, unpublished data). The Odiniidae has also been suggested to be the sister group to the Agromyzidae (Hennig, 1958 as reported by McAlpine, 1989; Spencer, 1969; Dempewolf, 2005). We included a species of *Odinia* as an additional outgroup in our analyses.

Specimens were preserved for study in 95% ethanol and stored at −80 °C prior to DNA extraction. For most species, a male specimen was chosen for DNA extraction because of the importance of male genitalia for species identification. Preliminary identification of specimens based on external morphology was performed by SJS and ISW using keys by Spencer (1969, 1972, 1976, 1977), Spencer and Stegmaier (1973), and Spencer and Steyskal (1986). Each male specimen was dissected by removing the posterior half of the abdomen and clearing it by submerging it in a hot 10% KOH solution for 5 min. Cleared genitalia were then used for final identifications and retained as voucher specimens. These genitalia and, in some cases, additional intact specimens have been deposited as vouchers in the National Museum of Natural History in Washington, DC.

Total nucleic acids were extracted from single dissected specimens by grinding the specimen in PBS solution and following the insect protocol B of the DNeasy DNA extraction kit (Qiagen Inc., Valencia, CA). A few specimens, extracted most recently, had the dissected abdomens subjected to the DNeasy extraction protocol without grinding. The integument containing the genitalia was retrieved after the completion of the DNeasy

Table 1
Currently recognized genera within the Agromyzidae

	# Species described[a]	Larval habits	# Species with known hosts/host plant families (# specialist species)
Subfamily Agromyzinae			
Agromyza Fallén	200 (5)	Leafminers	98/Monocots: Poaceae (36); Rosids: Betulaceae (3), Cannabaceae (4), Fabaceae (20), Geraniaceae (2), Malvaceae (3), Moraceae (1), Oxalidaceae, Rosaceae (6), Salicaceae (3), Ulmaceae (1), Urticaceae (4); Asterids: Asteraceae (3), Boraginaceae (6), Dipsaceae (2), Lamiaceae (1), Loasaceae; Other Eudicots: Grossulariaceae, Polygonaceae (2)
Cecidomyiaceltis Patton[b]	1 (–)	Petiole galler	1/Dicot: Cannabaceae
Hexomyza Enderlein	16 (1)	Twig gallers	15/Monocots: Asparagaceae (1); Rosids: Celastraceae (1), Fabaceae (4), Malvaceae (1), Salicaceae (5); Asterids: Asteraceae (1), Rubiaceae (2)
Japanagromyza Sasakawa	74 (1)	Leafminers	26/Monocots: Orchidaceae (1); Rosids: Elaeagnaceae (1), Euphorbiaceae (2), Fabaceae (15), Fagaceae (2), Myrtaceae (1), Rosaceae (1), Salicaceae (1); other Eudicots: Polygonaceae (1), Loranthaceae (1)
Kleinschmidtimyia Spencer	7 (–)	Leafminers	4/Monocots: Hemerocallidaceae (1); Rosids: Fabaceae (2), Thymelaeaceae (1)
Melanagromyza Hendel	358 (5)	In stems, flower heads and roots	134/Monocots: Bromeliaceae (1), Orchidaceae (5), Poaceae (1); Rosids: Brassicaceae (1), Euphorbiaceae (1), Fabaceae (23), Malvaceae (2), Rosaceae (1), Urticaceae (5); Asterids: Acanthaceae (5), Apiaceae (12), Araliaceae (2), Asteraceae (50), Boraginaceae (3), Convolvulaceae (6), Lamiaceae (3), Pedaliaceae, Polemoniaceae (1), Scrophulariaceae (2), Solanaceae (4), Verbenaceae (2); other Eudicots: Amaranthaceae (4), Crassulaceae (1), Ranunculaceae (1)
Ophiomyia Braschnikov	262 (7)	Stemminers, and some leafminers	96/Monocots: Asparagaceae (1), Hemerocallidaceae (1); Rosids: Brassicaceae (2), Elaeagnaceae (1), Euphorbiaceae (3), Fabaceae (15), Malvaceae (3), Moraceae (1); Asterids: Acanthaceae (1), Apiaceae (1), Asteraceae (29), Campanulaceae (4), Dipsacaceae (1), Gentianaceae (1), Goodeniaceae (2), Lamiaceae (5), Orobanchaceae (1), Rubiaceae (1), Scrophulariaceae (2), Solanaceae (3), Valerianaceae (1), Verbenaceae (7); other Eudicots: Amaranthaceae (4), Caryophyllaceae (2), Ranunculaceae (6)
Penetagromyza Spencer[c]	3 (–)	Mining inside thick leaves	2/Asphodelaceae (2)
Tropicomyia Spencer[d]	40 (1)	Leafminers	29/Nonang: Gnetaceae, Marratiaceae (1), Oleandraceae; Magnoliids: Monimiaceae, Piperaceae (1); Monocots: Agavaceae, Amaryllidaceae (2), Araceae (1), Colchicaceae (1), Dioscoreaceae, Orchidaceae (1), Philesiaceae, Smilacaceae; Rosids: Achariaceae (1), Celastraceae (2), Combretaceae, Cucurbitaceae (1), Euphorbiaceae (3), Fabaceae (4), Malpighiaceae, Malvaceae, Meliaceae, Moraceae, Myrsinaceae, Myrtaceae, Passifloraceae, Rhamnaceae, Rutaceae, Salicaceae; Asterids: Acanthaceae (1), Apocynaceae, Aquifoliaceae, Caprifoliaceae, Convolvulaceae, Cornaceae, Gesneriaceae, Hydrangeaceae, Lecythidaceae, Loganiaceae, Oleaceae, Pittosporaceae, Rubiaceae, Styracaceae, Symplocaceae, Theaceae, Verbenaceae; other Eudicots: Crassulaceae (1), Menispermaceae, Nyctaginaceae
Subfamily Phytomyzinae			
Amauromyza Hendel	57 (3)	Leafminers	24/Monocots: Iridaceae (1); Rosids: Elaeagnaceae (2), Fabaceae (2); Asterids: Asteraceae (1), Bignoniaceae (1), Campanulaceae (1), Lamiaceae (8), Oleaceae (1), Scrophulariaceae (1), Verbenaceae (1); other Eudicots: Amaranthaceae (4), Caryophyllaceae (1), Polygonaceae (1)
Aulagromyza Enderlein[e]	50 (5)	Leafminers	32/Rosids: Brassicaceae (2), Fabaceae (1), Rosaceae (1), Salicaceae (5); Asterids: Apocynaceae (1), Asteraceae (1), Caprifoliaceae (10), Dipsacaceae (1), Oleaceae (3), Rubiaceae (7)
Calycomyza Hendel	87 (6)	Leafminers	53/Rosids: Fabaceae (2), Malvaceae (5); Asterids: Apiaceae (1), Asteraceae (25), Bignoniaceae (1), Boraginaceae (1), Convolvulaceae (7), Lamiaceae (4), Verbenaceae (5); other Eudicots: Polygonaceae (1), Ranunculaceae (1)
Cerodontha Rondani	270 (8)	Leafminers	81/Monocots: Cyperaceae (23), Iridaceae (5), Juncaceae (9), Poaceae (44)
Chromatomyia Hardy	112 (6)	Leafminers	85/Nonang: Adiantaceae (1), Aspleniaceae (3), Dryopteridaceae (1), Polypodiaceae (1); Monocots: Alliaceae, Cyperaceae, Juncaceae (3), Poaceae (12); Rosids: Anacardiaceae, Brassicaceae, Cannabaceae, Cucurbitaceae, Elaeagnaceae (3), Euphorbiaceae, Fabaceae, Linaceae, Malvaceae, Onagraceae, Resedaceae, Rutaceae, Tropaeolaceae, Violaceae; Asterids: Acanthaceae, Actinidiaceae (1), Apiaceae, Asteraceae (16), Balsaminaceae, Boraginaceae (3), Campanulaceae, Caprifoliaceae (15), Convolvulaceae, Dipsacaceae (5), Gentianaceae (11), Lamiaceae (1), Loasaceae, Orobanchaceae (1), Phrymaceae (1), Polemoniaceae, Primulaceae (2), Solanaceae, Valerianaceae (1), Verbenaceae; other Eudicots: Amaranthaceae, Caryophyllaceae, Papaveraceae, Plumbaginaceae, Polygonaceae, Ranunculaceae (2), Saxifragaceae (5)
Galiomyza Spencer[f]	12 (1)	Leafminers	8/Rosids: Violaceae (4); Asterids: Rubiaceae (4)
Gymnophytomyza Hendel	2 (1)	Seed feeders	1/Eudicots: Rubiaceae (1)
Haplopeodes Steyskal	15 (–)	Leafminers	13/Asterids: Solanaceae (9); other Eudicots: Amaranthaceae (3), Portulacaceae (1)

Table 1 (*continued*)

	# Species described[a]	Larval habits	# Species with known hosts/host plant families (# specialist species)
Liriomyza Mik	383 (8)	Leafminers	182/Nonang: Equisetaceae (3); Magnoliids: <u>Aristolochiaceae</u>, <u>Piperaceae;</u> Monocots: Alismataceae, Alliaceae (3), <u>Alstroemeriaceae</u>, Asphodelaceae (1), Commelinaceae (2), <u>Dioscoreaceae</u>, Hemerocallidaceae (1), <u>Iridaceae</u>, Juncaginaceae (2), Liliaceae (1), Melanthiaceae (1), Poaceae (12), Ruscaceae (1), <u>Smilacaceae</u>, <u>Typhaceae;</u> Rosids: Anacardiaceae, Brassicaceae (5), Cannabaceae (1), <u>Celastraceae</u>, <u>Cucurbitaceae</u>, Euphorbiaceae (5), Fabaceae (15), <u>Geraniaceae</u>, <u>Linaceae</u>, <u>Malvaceae</u>, <u>Moringaceae</u>, Onagraceae, Oxalidaceae, <u>Passifloraceae</u>, Polygalaceae (1), <u>Resedaceae</u>, <u>Rosaceae</u>, Sapindaceae, Thymelaeaceae (1), Tropeolaceae (2), <u>Turneraceae</u>, Urticaceae (1), Violaceae (2), <u>Zygophyllaceae;</u> Asterids: Acanthaceae (1), Adoxaceae (2), Apiaceae (3), Apocynaceae (2), Asteraceae (70), <u>Bignoniaceae</u>, <u>Boraginaceae</u>, Campanulaceae (2), <u>Convolvulaceae</u>, <u>Dipsacaceae</u>, Gentianaceae (1), Goodeniaceae (1), Hydrangeaceae (2), Lamiaceae (2), <u>Loganiaceae</u>, <u>Loasaceae</u>, <u>Oleaceae</u>, <u>Pedaliaceae</u>, Pittosporaceae (1), Plantaginaceae (8), Polemoniaceae (2), Primulaceae (1), <u>Rubiaceae</u>, Scrophulariaceae (1), Solanaceae (5), Valerianaceae (2), <u>Verbenaceae;</u> other Eudicots: <u>Aizoaceae</u>, Amaranthaceae (2), <u>Basellaceae</u>, Caryophyllaceae (3), Crassulaceae (1), Menispermaceae (1), <u>Nyctaginaceae</u>, <u>Papaveraceae</u>, <u>Phytolaccaceae</u>, <u>Plumbaginaceae</u>, <u>Polygonaceae</u>, Portulacaceae (1), Ranunculaceae (2), Santalaceae (1), Saxifragaceae (1)
Metopomyza Enderlein	22 (2)	Leafminers	4/Monocots: Cyperaceae (2), Juncaceae (1), Poaceae (1)
Napomyza	56 (4)	Stemminers, flower head feeders	13/Rosids: Fabaceae, <u>Linaceae</u>; Asterids: Apiaceae (1), Asteraceae (9), Campanulaceae (1), Lamiaceae (1)
Nemorimyza	4 (2)	Leafminers	2/Asterids: Asteraceae (2)
Phytobia Lioy	85 (3)	Cambium miners	11/Rosids: Betulaceae (2), Fagaceae (1), Rosaceae (6), Salicaceae (1), Sapindaceae (2)
Phytoliriomyza	111 (5)	Leafminers, stemminers	23/Nonang: Aspleniaceae (1), Cyatheaceae (3), Dennstaedtiaceae (4), Grammitidaceae (1), Polypodiaceae (1), Ricciaceae (1), Woodsiaceae (1); Monocots: Butomaceae (1); Rosids: Fabaceae (3); Asterids: Asteraceae (3), Balsaminaceae (1), Bignoniaceae (1), Pittosporaceae (2), Solanaceae (1)
Phytomyza Fallén[g]	532 (10)	Leafminers, stemminers, flower head feeders	353/Basal Angiosperms: Illiciaceae (1); Monocots: Alliaceae (1); Rosids: Brassicaceae (2), Fabaceae (6), Rhamnaceae (1), Rosaceae (3), Urticaceae (1); Asterids: Apiaceae (64), Aquifoliaceae (9), Araliaceae (8), Asteraceae (81), Balsaminaceae (1), Boraginaceae (11), Campanulaceae (2), Cornaceae (2), Gelsemiaceae (1), Hydrangeaceae (2), Lamiaceae (11), Oleaceae (1), Orobanchaceae (15), Plantaginaceae (11), Solanaceae (1), Styracaceae (1); other Eudicots: Crassulaceae (3), Lardizabalaceae (1), Papaveraceae (1), Ranunculaceae (113)
Pseudoliriomyza Spencer	1 (–)	Leafminer	1/Asterids: Boraginaceae (1)
Pseudonapomyza Hendel	95 (2)	Leafminers	19/Monocots: Poaceae (10); Asterids: Acanthaceae (7), Asteraceae (1); other Eudicots: Amaranthaceae (1)
Ptochomyza Hering	4 (–)	Stemminers	4/Monocots: Asparagaceae (2); Asterids: Apiaceae (1); other Eudicots: Ranunculaceae (1)
Selachops Wahlberg	3 (–)	Stemminers	1/Monocots: Cyperaceae (1)
Xeniomyza Hering	2 (–)	Leafminer	1/other Eudicots: Amaranthaceae (1)

The approximate number of described species[a] is given (with the number of species included in the present study in parentheses), along with the dominant larval habits found within each genus. The number of described species with known hosts and the host plant families are listed, along with the number of specialist agromyzids feeding on each family in parentheses (compiled from Spencer (1990) and Benavent-Corai et al. (2005); plant classification following Angiosperm Phylogeny Group (2003)). Plant families that are attacked only by a polyphagous species within a particular genus or which have been recorded as exceptional hosts of species normally restricted to another host family are underlined.

[a] Total species numbers for each genus were obtained from a working version of the Biosystematic Database of World Diptera (Thompson, F.C., (Ed.), 2005. http://www.sel.barc.usda.gov/diptera/biosys.htm, supplemented by the Zoological Record (Zoological Society of London, Thomson Scientific), Spencer and Martinez (1987) and several other references.

[b] The species comprising this genus was described from empty galls on *Celtis* (see Spencer, K.A., 1988. Entomol. Mon. Mag. 124, 63–64), and may correctly belong in *Agromyza* (M. von Tschirnhaus, cited by Dempewolf, 2001).

[c] Synonymised by Dempewolf (2001) with *Melanagromyza* in an unpublished dissertation.

[d] Totals include species transferred from *Tropicomyia* and *Melanagromyza* to the newly erected genus *Epidermomyia* (Ipe and Ipe, 2004). We were not able at the time of publication to obtain a copy of this paper.

[e] *Paraphytomyza* of earlier authors (see von Tschirnhaus, 1991).

[f] One species feeding on Rubiaceae was found by M. von Tschirnhaus to correctly belong to *Liriomyza* (cited in Spencer and Martinez, 1987). Other species lack the stridulation mechanism diagnostic of *Liriomyza*, but their generic status requires clarification (Spencer, 1990).

[g] The monotypic genus *Indonapomyza* Singh and Ipe was not considered valid by Sasakawa (1977, In: Delfinado, M.D., Hardy, D.E. (Eds.), A Catalog of the Diptera of the Oriental Region, vol. III, Honolulu, HI, pp. 243–269). The description and illustration given by Singh and Ipe (1973, Memoirs of the School of Entomology No. 1, St. John's College, Agra, India) suggest *I. vanga* is in most aspects a typical *Phytomyza* species.

extraction procedure. This allowed us to retain both the genitalia and the dissected specimen as voucher material, and seemed not to affect subsequent amplification of either mitochondrial or nuclear genes.

Nucleotide sequences from fragments of three different genes, 28S ribosomal DNA, CAD (or rudimentary), and mitochondrial cytochrome oxidase 1 were obtained from each specimen. 28S rDNA is widely used for phylogenetics

Table 2
Spencer's phyletic lines hypothesis (Spencer, 1990)

Phyletic line	Genera
Phytobia	**Phytobia**, **Amauromyza**, **Nemorimyza**
Penetagromyza	*Penetagromyza*, **Hexomyza**, **Melanagromyza**, **Ophiomyia**, Kleinschmidtimyia, **Tropicomyia**, **Japanagromyza**, **Agromyza**
Phytoliriomyza	**Phytoliriomyza**, **Metopomyza**, Selachops, Pseudoliriomyza, **Liriomyza**, **Cerodontha**, **Galiomyza**, Xeniomyza, **Calycomyza**, **Aulagromyza**, **Gymnophytomyza**, Haplopeodes
Napomyza	**Napomyza**, **Phytomyza**, **Chromatomyia**, **Pseudonapomyza**, Ptochomyza

Genera included in the present study indicated in bold.

and contains both highly conserved and highly variable regions within the well-known stem-loop containing secondary structure of the RNA product (Dixon and Hillis, 1993). CAD is a nuclear protein coding gene that was recently characterized for use as a phylogenetic marker in higher Diptera (Moulton and Wiegmann, 2004). The mitochondrial protein coding gene, cytochrome oxidase I is also used extensively in phylogenetic analyses at the species and population levels (Caterino et al., 2000; Farrell, 2001; Scheffer and Wiegmann, 2000), but its amino acid encoding sites have also been successfully applied at somewhat older divergences within and among insect families (Lunt et al., 1996; Farrell, 1998).

For the 28S rDNA gene, an approximately 1200 bp region from the 5′-end of the 28S rDNA molecule was amplified and sequenced. This region contains the large D2 divergent domain, the fastest evolving region of the molecule (Gillespie et al., 2005). The 28S rDNA primers were based on Hamby et al. (1988) and modified to match the published 28S rRNA sequence for *Drosophila melanogaster* (M21017; Tautz et al., 1988). The CAD region used corresponds to the first 820 bp of the 5′-end of the carbamoyl phosphate synthetase domain used by Moulton and Wiegmann (2004) to infer relationships among eremoneuran Diptera. This region appears to be the fastest region of CAD. The 1500 bp of COI sampled from agromyzid taxa comprises nearly the entire coding region of the gene.

PCR amplifications were carried out using a Mastercycler Gradient thermocycler (Eppendorf Scientific, Inc., Westbury, NY, USA) with a touchdown amplification program: initial denaturation at 92 °C for 2 min, followed by 2 touchdown cycles from 58 to 46 °C (10 s at 92 °C, 10 s at 58–46 °C, 2 min at 72 °C), 29 cycles of 10 s at 92 °C, 10 s at 45 °C, 2 min at 72 °C, and a final extension step for 10 min at 72 °C. A combination of published primers and those designed specifically for agromyzids were used for both PCR amplification and sequencing (Table 3). Amplification products were purified using the Qiaquick PCR Purification Kit or the Qiaquick Gel Extraction Kit in the rare case of multiple bands. Sequencing reactions were carried out using BigDye Sequencing kits (Applied Biosystems, Foster City, CA) and the products were fractionated using an ABI-377 Automated DNA Sequencer (Applied Biosys-
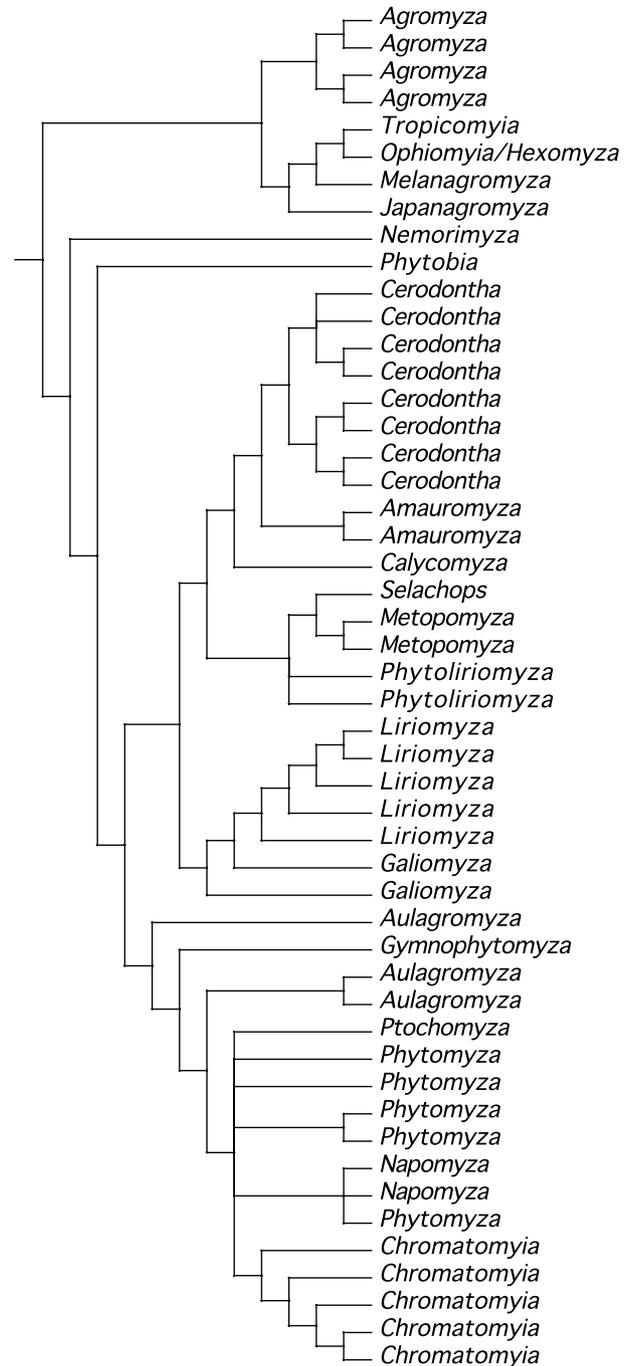
Fig. 1. Dempewolf's agromyzid phylogeny. Strict consensus of 90 equally parsimonious trees obtained by successive weighting of 83 morphological characters by the consistency index. Redrawn after Dempewolf (2005).

tems). Assembly of contigs for each gene region for each specimen was accomplished using the software package Sequencher (Gene Codes, Ann Arbor, MI). All final sequences have been deposited in GenBank with the accession numbers listed in Appendix A.

2.1. Nucleotide alignment

Alignment of the 28S rDNA data partition was carried out manually. Regions of high length or primary sequence

Table 3
Primers used for PCR amplification (indicated with an asterisk) and DNA sequencing

Gene	Primer	Primer sequence 5′–3′	Source
COI	C1-J-1535*	ATTGGAACTTTATATTTTATATTTGG	Scheffer et al. (2004)
	C1-N-2191	CCGGTAAAATTAAAATATAAACTTC	Shao et al. (2001)
	C1-J-2183	CAACATTTATTTTGATTTTTTGG	Sperling and Hickey (1994)
	C1-N-2508	CTCCAGTTAATCCTCCAACTGTAAAT	Simon et al. (1994)
	C1-J-2441	CCTACAGGAATTAAAATTTTTAG TTGATTAGC	Simon et al. (1994)
	TL-N-3017*	CTTAAATCCATTGCACTAATCTGCCATA	Scheffer et al. (2004)
28S	rc28A*	AGCGGAGGAAAAGAAAC	Modified from Hamby et al. (1988)
	28B	GGTCCGTGTTTCAAGACGGG	Modified from Hamby et al. (1988)
	rc28B	CCCGTCTTGAAACACGGACC	Modified from Hamby et al. (1988)
	28C*	GCTATCCTGAGGGAAACTTCGG	Modified from Hamby et al. (1988)
CAD	54F*	GTNGTNTTYCARACNGGNATGGT	Moulton and Wiegmann (2004)
	AG-360AR	CCATGATTYTGTGARGTCAT	
	AG-360BR	CCRTGRTTYTGTGAYGTCAT	
	405R*	GCNGTRTGYTCNGGRTGRAAYTG	Moulton and Wiegmann (2004)

variability, for which ad hoc assignment of positional homology would affect the phylogenetic outcome, were excluded from analyses. CAD and COI sequence partitions were aligned with reference to the translated amino acid sequence of the protein. Intron sequences inserted in the CAD fragment of some agromyzid species were excluded from the analysis. Alignments and data sets are archived in TreeBase (www.treebase.org: acc #[SN3150]) and are available by request from the authors.

2.2. Phylogenetic analyses

The aligned gene fragments were concatenated into a single phylogenetic data set using the program MacClade 4.0 (Maddison and Maddison, 2000). Phylogenetic trees were reconstructed using parsimony and Bayesian methods. Parsimony analyses were carried out in PAUP* 4.0b10 (Swofford, 2001) and minimum-length trees were found by heuristic search with tree bisection reconnection (TBR) branch swapping and 100 random taxon addition sequences. Reliability of clades found in parsimony analyses was assessed using 1000 replicate bootstrap searches conducted in PAUP*. Bayesian tree search was carried out using the program Mr.Bayes v. 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Prior model parameters were chosen using pairwise likelihood ratio tests and the Akaike Information Coefficient (AIC) option in MrModeltest 2.2 (Nylander, 2004), a modified version of Modeltest 3.6 (Posada and Crandall, 1998). Bayesian Markov chain Monte Carlo tree search was conducted using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) incorporating the models and parameters suggested for each data matrix by MrModeltest. Each Markov chain in the Bayesian search was started from a random tree and run for 1×10^6 to 3×10^6 cycles, sampling every 1000th cycle from the chain. Four chains were run simultaneously, three hot and one cold. Each simulation was run twice. We used the default settings for the priors on the rate matrix (0–100), branch lengths (0–10), and proportion of invariant sites

(0–1). Stationarity was evaluated by monitoring likelihood values graphically. The initial 20% of trees from each run were discarded as burn-in samples. The remaining trees were used to construct majority rule consensus trees. Bayesian posterior probabilities for each clade were derived from a majority-rule consensus of the trees remaining after discarding the burn-in samples (Huelsenbeck and Ronquist, 2001).

3. Results

Our final aligned concatenated data set for the three genes contains 2965 positions of which 1463 are variable and 1269 parsimony informative. The three gene partitions are 824 (28S), 710 (CAD), 1431 (mt COI) nucleotides in length, respectively. Uncorrected pairwise distances for all three genes are shown in Table 4, across the range of agromyzid divergences included in the current sample. Because of the high variability, and thus high potential for homoplasy, found in 3rd codon position sites of both CAD and mtCOI, 3rd positions were excluded from the parsimony analyses reported here. All three nucleotide positions were included in Bayesian analyses that incorporated specific molecular evolutionary model parameters that account for differences in rates among sites.

Parsimony analysis of the combined data with third positions removed from COI and CAD resulted in six equally parsimonious trees. The strict consensus of these six trees recovered two monophyletic groups that correspond to the traditionally recognized subfamilies, the Agromyzinae and the Phytomyzinae (Fig. 2), although only the Phytomyzinae was supported by a moderate bootstrap value (80%) (Fig. 2). Within the Agromyzinae, a clade containing *Melanagromyza*, *Ophiomyia*, *Hexomyza*, and *Tropicomyia* was supported by a bootstrap value of 93%. *Agromyza* and *Melanagromyza* were each found to be monophyletic with 93% and 99% bootstrap support, respectively. The genus *Ophiomyia* was found to be polyphyletic with five species (*Ophiomyia* group A) forming a strongly supported (96%

Table 4
Uncorrected pairwise distances between exemplar study taxa

Gene(s)[a]	bp	All	Nt1 + Nt2	Nt3
28S + COI + CAD	2965			
Agromyza ambrosivora × Agromyza frontella[b]		0.1151	N/A	N/A
Agromyza ambrosivora × Melanagromyza obtusa[c]		0.1688		
Agromyza ambrosivora × Phytomyza ilicicola[d]		0.1587		
Agromyza ambrosivora × Odinia sp.[e]		0.1632		
28S	824			
Agromyza ambrosivora × Agromyza frontella		0.0245	N/A	N/A
Agromyza ambrosivora × Melanagromyza obtusa		0.0527		
Agromyza ambrosivora × Phytomyza ilicicola		0.0539		
Agromyza ambrosivora × Odinia sp.		0.0823		
COI	1431			
Agromyza ambrosivora × Agromyza frontella		0.1327	0.0394	0.3198
Agromyza ambrosivora × Melanagromyza obtusa		0.1648	0.0436	0.4048
Agromyza ambrosivora × Phytomyza ilicicola		0.1556	0.0594	0.3484
Agromyza ambrosivora × Odinia sp.		0.1336	0.0392	0.3224
CAD	710			
Agromyza ambrosivora × Agromyza frontella		0.1877	0.0550	0.4646
Agromyza ambrosivora × Melanagromyza obtusa		0.3138	0.1631	0.6164
Agromyza ambrosivora × Phytomyza ilicicola		0.2885	0.1388	0.5888
Agromyza ambrosivora × Odinia sp.		0.3171	0.1824	0.5894

Nt1, Nt2, and Nt3 refer to first, second, and third codon positions, respectively.
[a]Each included comparison with *A. ambrosivora* is included to represent a typical pairwise distance value calculated between study species: [b]within a genus; [c]in different agromyzid genera within an agromyzid subfamily; [d]in different agromyzid subfamilies; and [e]between agromyzid and outgroup odiniid species.

bootstrap support) monophyletic group related to *Tropicomyia* and *Hexomyza*, and two species (*Ophiomyia* group B) forming a sister group to *Melanagromyza*.

Within the Phytomyzinae, a number of genera represented by at least two species were found to be monophyletic. All seven subgenera of *Cerodontha* were included in the study, and this genus was found to be monophyletic and strongly supported (94% bootstrap support). *Cerodontha muscina* and *Cerodontha incisa*, the only *Cerodontha* species from the same subgenus (*Poemyza*), group together with 100% bootstrap support. *Calycomyza*, *Napomyza s.s.* (Zlobin, 1994b), *Nemorimyza*, *Pseudonapomyza*, and *Phytobia* were each found to be monophyletic with 100% bootstrap support, while *Amauromyza*, *Liriomyza*, and *Metopomyza* were found to be monophyletic with moderate to high bootstrap support values of 89%, 79%, and 98%, respectively (Fig. 2). Several genera within the Phytomyzinae were found to be polyphyletic including *Phytoliriomyza, Aulagromyza, Chromatomyia*, and *Phytomyza*. Only single representatives were obtained for *Galiomyza* and *Gymnophytomyza*.

Within the Phytomyzinae, there was little or no support for relationships among genera. *Cerodontha* was found to be the sister taxon to the rest of the Phytomyzinae. This was broken into two main clades: one containing *Calycomyza, Liriomyza, Galiomyza, Metopomyza* and most of *Phytoliriomyza*; and a second clade containing the remaining genera including the *Napomyza/Phytomyza/Chromatomyia* group.

Calculation of pairwise likelihood ratio tests to examine the fit of alternative models of molecular evolution calculated in MrModel Test for each of the three gene partitions found that the general time reversible model with gamma and invariant sites (GTR + I + G) was an adequate choice for each of the individual partitions. For this reason Bayesian tree searches were carried out on the full combined dataset with third positions of COI and CAD included. The Bayesian analysis yielded a consensus tree that is highly similar to the parsimony tree (Fig. 3). The majority rule phylogram from the Bayesian analysis is shown (Fig. 4) to indicate branch lengths. The larval feeding location for each of the species with known larval habits is indicated on the Bayesian phylogram (Fig. 4). Relationships supported by moderate to high bootstrap values in the parsimony analysis were also supported by high posterior probabilities on the Bayesian tree (Figs. 2 and 3). The Bayesian tree differed from the strict consensus of the most parsimonious trees in placing *Japanagromyza viridula* as sister to a clade containing *Ophiomyia, Melanagromyza, Hexomyza*, and *Tropicomyia* rather than as a sister to *Agromyza*. Another striking difference is in the placement of *Amauromyza* and *Phytobia*: in the Bayesian analysis these were found to be sister genera, while in the parsimony analysis they formed separate groups (Figs. 2 and 3). Additionally, although neither analysis recovered a monophyletic *Aulagromyza*, in the parsimony analysis *Aulagromyza tridentata* clustered with several other *Aulagromyza*, while in the Bayesian analysis it came out as sister to the *Phytomyza/Chromatomyia* clade.

4. Discussion

A major means of assessing the utility of a character system for phylogenetics is to investigate whether phylogenetic analysis recovers groups that are strongly supported by
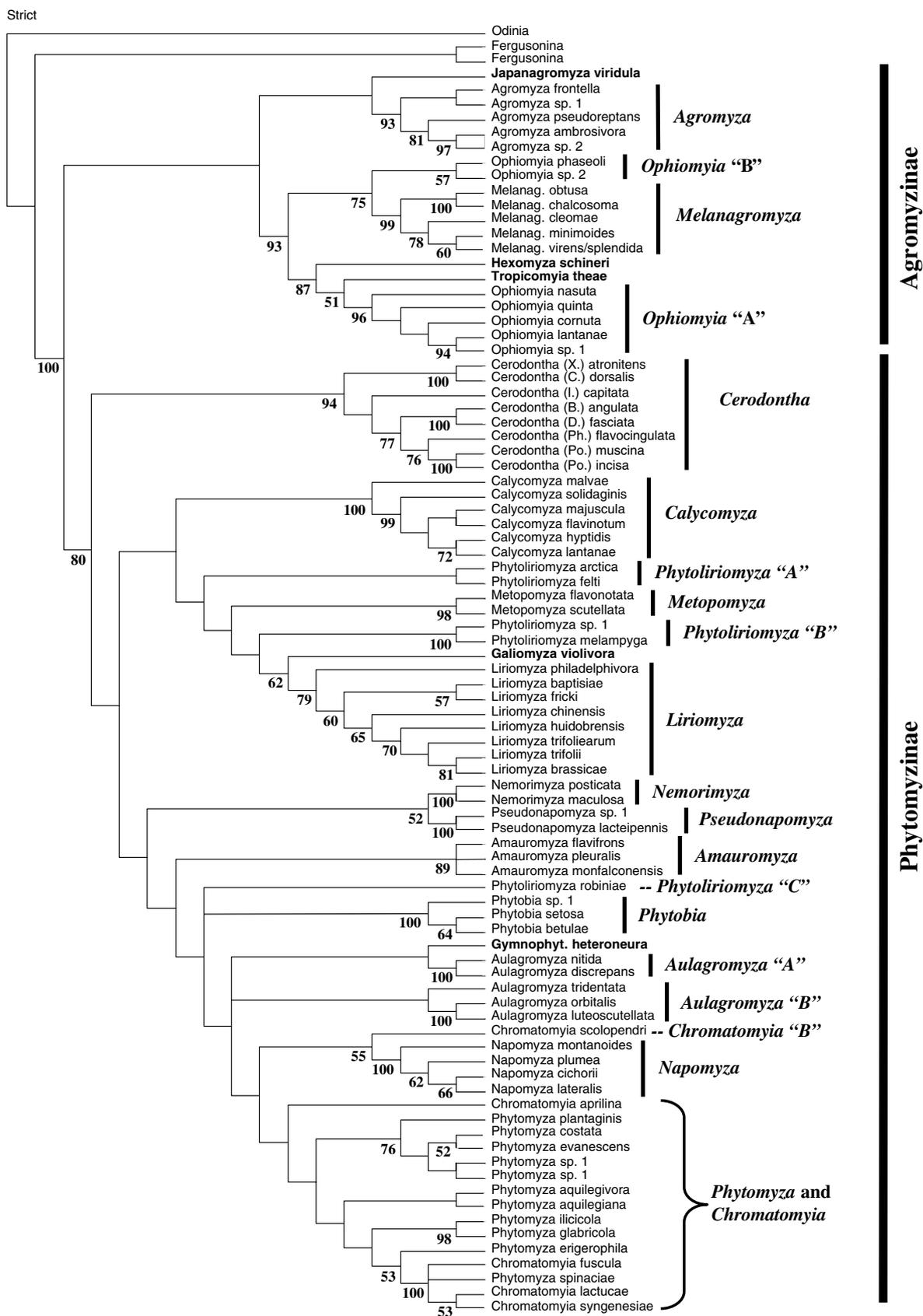
Fig. 2. Strict consensus of six equally parsimonious trees obtained from analysis of DNA sequence data from COI, 28S, and CAD (with 3rd positions removed from COI and CAD). Bootstrap support values shown beneath branches. Single representatives of a genus shown in bold.

Majority rule

Fig. 3. Bayesian consensus tree resulting from analysis of DNA sequence data from COI, 28S, and CAD (3rd positions retained). Posterior probabilities shown above branches.
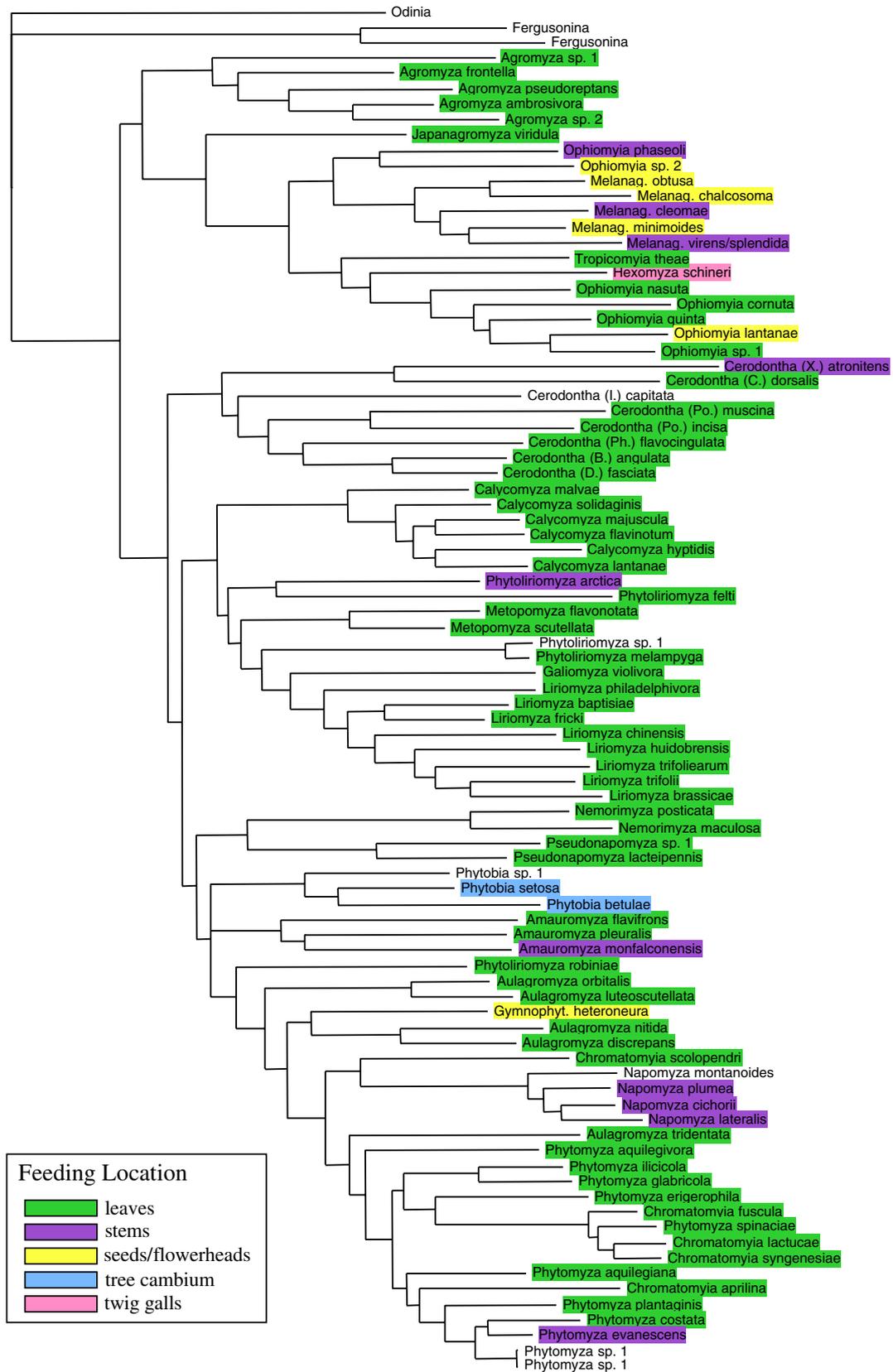
Fig. 4. Bayesian phylogram indicating branch lengths and feeding location of larval stage for species with known larval habits.

other sources of data (Kluge, 1989; Miyamoto and Cracraft, 1991; Friedlander et al., 1994). The Agromyzidae has only recently been the subject of quantitative phylogenetic

analysis, leaving us with little previous work on agromyzid relationships for strong comparisons with the results of this study. However, for this purpose we can use traditional

agromyzid taxonomy that is based primarily on morphological characters, Spencer's intuitive phyletic lines scheme based on his 50-year study of the family (Spencer, 1990), and Dempewolf's recent phylogeny based on 83 larval and adult characters (Dempewolf, 2001, 2005).

4.1. Subfamilies

In the present study, the subfamilies Agromyzinae and Phytomyzinae are reciprocally monophyletic in both the parsimony and the Bayesian analyses with only low support values for the Agromyzinae and moderate to high support values for the Phytomyzinae. The two subfamilies are supported by one wing and one larval character and have been recognized by most modern agromyzid workers. Spencer's phyletic lines scheme recognizes the Agromyzinae as the "*Penetagromyza* group," but does not specify the relationships among groups comprising the Phytomyzinae. In Dempewolf's analysis, the subfamilies are reciprocally monophyletic (Fig. 1). Our molecular data confirm the basic generic composition of the two subfamilies corroborating the morphological evidence for the two major lineages of leafminers.

4.2. Monophyly of genera

Within each subfamily, our analysis found many of the genera to be monophyletic, particularly those genera supported by at least one unique, apomorphic character. Specifically, monophyletic genera included *Agromyza* (possessing a stridulatory organ on the first abdominal tergite (von Tschirnhaus, 1971), (*Cerodontha* (possessing an L-shaped sclerite within the male aedeagal complex (Nowakowski, 1962)), and *Liriomyza* (possessing a male stridulatory organ in the membrane between the abdominal tergites and sternites (von Tschirnhaus, 1971) and additional characters (Zlobin, 1999a)). We found that the *Ophiomyia* species that possess a prominent facial keel and a well developed facial carina in the male, characters typical of the genus, form a monophyletic group (*Ophiomyia* group A), while specimens from two species lacking these characters instead form a monophyletic sister group (*Ophiomyia* group B) to the *Melanagromyza* clade. One of these species is undescribed; while the other, *Ophiomyia phaseoli*, is one of several species that was once placed in *Melanagromyza* based on external characters (in particular, the lack of external *Ophiomyia* characters) but was later moved to *Ophiomyia* based on larval and genitalic characters (Spencer, 1966). There are a number of species that have been moved to *Ophiomyia* even though they lack the external characters typical of this genus (Spencer, 1966), and revisionary work on this group using morphological and molecular characters is greatly needed to determine their correct placement. From the current analysis of nucleotide evidence, it appears that the two species comprising *Ophiomyia* group 2 may represent a new genus, but we advocate a cautious approach to taxonomic changes until more data are available.

Other genera traditionally recognized by a combination of characters were also found to be monophyletic including *Calycomyza, Melanagromyza, Napomyza, Phytobia, Amauromyza, Nemorimyza* (as redefined by Zlobin, 1996), *Metopomyza*, and *Pseudonapomyza*. Our taxon sampling within these genera is varied, such that the analyses reported here do not test the monophyly of these genera equally. The six included species of the relatively small and uniform genus *Calycomyza* represent much of the genitalic variation present in the genus, making our finding of monophyly a fairly robust test for this group. Five species from the large genus *Melanagromyza* were included, but certainly additional investigation of this group and its relatives, as discussed above, is warranted. The four included *Napomyza* are monophyletic and all represent the large *lateralis* group, one of three retained by Zlobin (1994b) within *Napomyza* when he moved species in the *albipennis, nigritula*, and *ranunculella* groups from *Napomyza* to *Phytomyza*. The species moved include *Phytomyza evanescens* and *Phytomyza costata*, which our molecular evidence places well within the *Phytomyza/Chromatomyia* group.

The three *Phytobia* species included in this study are all north temperate representatives of this genus, and sampling of some of the diverse tropical *Phytobia* noted by Spencer (1990), Sasakawa (1992, 1996), von Tschirnhaus (1991) and others would be desirable to test monophyly with the northern *Phytobia*. The *Amauromyza* species included here represent subgenera *Catalpomyza* (*Amauromyza pleuralis*) and *Cephalomyza* (*Amauromyza flavifrons* and *Amauromyza monfalconensis*); some species in the now disbanded subgenus *Annimyzella* were moved to the previously monotypic genus *Nemorimyza* by Zlobin (1996), a move corroborated by the finding of monophyly of *Nemorimyza posticata* and *Nemorimyza maculosa* (previously in *Amauromyza*) in both analyses presented here (Figs. 2 and 3). Two species each of the genera *Metopomyza* and *Psuedonapomyza* were included in our analyses. In the case of the *Pseudonapomyza*, both species represent the group referred to by Spencer (1977; Spencer and Steyskal 1986) as the "third antennal segment angulate" group; we were unable to obtain species representing the "third antennal segment round" group within this genus.

Several genera were found to be either polyphyletic or paraphyletic, including *Phytoliriomyza, Aulagromyza, Chromatomyia*, and *Phytomyza* (Figs. 2 and 3). *Phytoliriomyza* is a morphologically variable genus of small flies resembling *Liriomyza* in several characteristics, but lacking the stridulatory organ in the male (Spencer and Steyskal, 1986; see Zlobin (1996, 1999a) for genitalic characters differentiating *Phytoliriomyza* from *Liriomyza*). It has been suggested that *Phytoliriomyza* is not monophyletic (Spencer, 1990), and this has been confirmed by the present study. The five *Phytoliriomyza* species included in this study came out in three places in the trees produced from both parsimony and Bayesian analyses, four species (in two groups) within the *Calycomyza/Liriomyza* clade and the fifth species, *Phytoliriomyza robineae*, being near the base

of the *Aulagromyza*/*Napomyza*/*Chromatomyia*/*Phytomyza* clade. Spencer (1990) comments that *P. robineae* is an isolated species with unclear affiliations, which is consistent with its placement here by itself and well removed from other *Phytoliriomyza*. Examination of the relationships of species currently placed in *Phytoliriomyza* clearly warrants more study, especially as the relatively diverse species assemblages of the Australian and Neotropical regions were not represented in this study.

Aulagromyza (= *Paraphytomyza* of earlier authors (von Tschirnhaus, 1991)) is another genus strongly suspected to be polyphyletic (Spencer and Steyskal, 1986; Zlobin, 1999b). The five species included here are placed slightly differently depending on the method of analysis. Both analyses found *Gymnophytomyza heteroneura* to be sister to *Aulagromyza nitida* + *Aulagromyza discrepans*, consistent with the suggestion that this species, which feeds in seeds of *Galium*, is related to *Galium* feeders in *Aulagromyza* (Spencer, 1990; von Tschirnhaus, 1991; but see Zlobin, 1999b). The parsimony analysis found the five *Aulagromyza* species (plus *G. heteroneura*) to be comprised of two groups having undefined relationships with the *Napomyza*/*Chromatomyia*/*Phytomyza* clade. The Bayesian analysis found *A. tridentata* to be placed separate from the others within the clade containing *Napomyza*, *Chromatomyia*, and *Phytomyza* (Fig. 3). Spencer and Steyskal (1986) and Spencer (1990) suggest that the group of species that *A. tridentata* belongs to is not congeneric with the other *Aulagromyza*, but the differing placement of this species depending on method of analysis limits what we can conclude at this time.

Chromatomyia and *Phytomyza* are notoriously difficult groups to differentiate and cannot be diagnosed by external features; in fact, some female specimens cannot be determined unambiguously to genus. *Phytomyza* is a very large genus (>500 species) primarily defined by the presence of proclinate orbital setae, the costa extending only to vein R4 + 5, and the absence of the outer crossvein (Spencer and Steyskal, 1986). *Chromatomyia* was originally erected for those *Phytomyza* having slipper-shaped rather than barrel-shaped pupae and pupating in the leaf, although now it refers to those species having a characteristic form of reduced male genitalia (with a simple rather than bifid section of the ejaculatory duct lying below a dorsal lobe) (Griffiths, 1974; Spencer and Steyskal, 1986). Spencer (1990) points out that although most species with *Chromatomyia*-like genitalia also have slipper-shaped pupae that pupate in the leaf, many *Phytomyza* with differing genitalia also have *Chromatomyia*-like pupal characteristics. He considers the boundaries of *Chromatomyia* uncertain and worthy of additional study.

Neither of our analyses of the molecular dataset recovered a monophyletic *Chromatomyia* or a monophyletic *Phytomyza*. Both parsimony and Bayesian methods found that four species, *Chromatomyia syngenesiae*, *Chromatomyia lactucae*, *Phytomyza spinacea* (never formally transferred to *Chromatomyia*), and *Chromatomyia fuscula*, form a monophyletic group with moderate to good support. The

two species *Chromatomyia scolopendri* and *Chromatomyia aprilina* come out in two different places, the former associated with the *Napomyza* group and the latter either sister to the *Phytomyza*/*Chromatomyia* clade or within *Phytomyza*. Spencer (1990, pp. 403, 405) transferred *C. scolopendri* (and related species) from *Phytomyza* to *Chromatomyia* based solely on pupal characters. *C. aprilina* is considered by Spencer to represent an isolated species (Spencer, 1990, pg. 240), but the genitalia are similar to other *Chromatomyia* feeding on Caprifoliaceae. It appears to be fairly clear from our data that *Chromatomyia* species should be included in *Phytomyza*; this synonymy has already been implicitly followed by some (Papp, 1984; but see Spencer and Martinez, 1987), and furthermore, Spencer (1990, p. 406) points out that the name *Chromatomyia* is technically not valid. A more thorough molecular study of >100 species of *Phytomya* and *Chromatomyia* (Winkler et al., unpublished data) is underway and should finally resolve this question.

Several genera were only represented by a single species in our analysis, including *Japanagromyza, Hexomyza, Tropicomyia, Galiomyza*, and *Gymnophytomyza* (previously discussed). A more thorough study of these genera is needed; the monophyly of *Hexomyza* (Zlobin, 1998), *Tropicomyia* (Ipe and Ipe, 2004) and *Galiomyza* (Spencer, 1990) has been recently questioned.

4.3. Relationships among genera

Within the Agromyzinae, both methods of analysis found *Melanagromyza* + *Ophiomyia* group B to be sister to *Ophiomyia* group A + *Tropicomyia* and *Hexomyza*, although the two analyses varied in which taxon was placed as the sister to *Ophiomyia* group A. Both *Tropicomyia* and *Hexomyza* are more typically associated with *Melanagromyza* by Spencer (Spencer and Steyskal, 1986; Spencer, 1990), while Dempewolf's (2001) analysis does not differentiate *Ophiomyia* from *Hexomyza* (Fig. 1). The placement of *Japanagromyza* is also not consistent across the two analyses, with parsimony finding it to be sister to *Agromyza* and Bayesian analysis finding it to be sister to all remaining Agromyzinae. We were unable to include any representatives of the small Australian genus *Kleinschmidtimyia*, the members of which were once included in *Melanagromyza* and later *Tropicomyia*. Additional investigation of the Agromyzinae will be necessary to more fully understand relationships within this subfamily.

Within the Phytomyzinae, both of our analyses found the same three major lineages, although support values for many of the relationships within this subfamily are often low (Figs. 2 and 3). The placement of *Cerodontha* as the sister to the rest of the Phytomyzinae has not previously been suggested by agromyzid workers but was consistent, although poorly supported, across analyses. The grouping of *Liriomyza, Galiomyza, Metopomyza*, and most *Phytoliriomyza* is consistent with Spencer's general view that these are closely related (especially with *Galiomyza* as sister to *Liriomyza* (Spencer, 1990)), but his phyletic lines hypothesis

would include a number of additional genera with this group (Table 2). Likewise, Dempewolf's tree places the *Galiomyza*/*Liriomyza* lineage as the sister to a group including *Amauromyza*, *Cerodontha*, *Calycomyza*, *Metopomyza*, and *Phytoliriomyza* (Fig. 1), implying several relationships not supported by our analyses.

Probably the most unexpected and least well-supported placements in our analyses involve *Phytobia*, *Amauromyza*, *Pseudonapomyza*, and *Nemorimyza*. The placement of these taxa was largely consistent in our analyses as a grade (more or less) at the base of the *Phytomyza*/*Chromatomyia*/*Napomyza* group along with *Aulagromyza*, *Phytoliriomyza robiniae* and *G. heteroneura*, but with little or no support (Figs. 2 and 3). In fact, in the parsimony analysis, no basal relationships within the Phytomyzinae are well supported, indicating that the placement of these lineages could easily change with additional data. *Napomyza*, along with *C. scolopendri*, was found to be the sister taxon to the *Phytomyza*/*Chromatomyia* clade in both analyses, and this placement of *Napomyza* is consistent with both Spencer's and Dempewolf's views.

4.4. Host-use evolution

Although larval feeding mode within the Agromyzidae is somewhat conserved at the level of genera (Table 1 and Fig. 4), in agreement with Dempewolf's recent analysis (2005) we found no evidence for any sort of obvious progression from stem-feeding to leaf-mining. Predominantly stem-feeding genera such as *Ophiomyia* and *Napomyza* were found to be embedded within predominantly leaf-mining groups. Most importantly, the cambium-mining *Phytobia* and other stem-feeders were not found to comprise a basal grade as might be expected if all Agromyzidae evolved from *Phytobia*-like and stem-feeding ancestors, as has been repeatedly suggested (Nowakowski, 1962; Spencer, 1990). Although many agromyzid genera exhibit a single predominant larval feeding mode, most genera also contain species exhibiting alternative feeding modes (Table 1 and Fig. 4). For example, only *Hexomyza* is exclusively gall-forming (plus one stemminer (Dempewolf, 2005)), but isolated or small groups of species of gallers can be found in *Agromyza*, *Japanagromyza*, *Melanagromyza*, *Ophiomyia*, *Phytoliriomyza* and *Phytomyza* (Spencer and Steyskal, 1986; Dempewolf, 2005). It remains to be seen whether there are general patterns governing evolutionary transitions between feeding modes within genera.

Consistent with the conclusions of Spencer (1990), we found no evidence for an overall pattern of codiversification of agromyzids with their host plants as envisioned by Ehrlich and Raven (1964). Instead, host-use at the level of genus appears to be quite labile, with most genera feeding on many plant families, and at least 10 genera feeding on both monocots and eudicots (Table 1). The few agromyzid genera with species feeding on non-angiosperm hosts (Table 1) are nested within otherwise strictly angiosperm-feeding clades, also suggesting secondary coloniza-

tion. Major radiations on grasses and herbaceous asterid (sensu Angiosperm Phylogeny Group, 2003) hosts have occurred in both subfamilies, accounting for over 10% and 40%, respectively, of agromyzid diversity. Despite this high degree of evolutionary lability, the major clades of agromyzid genera do differ in their predominant host plants. For example, about a third of species in the Agromyzinae feed on woody or herbaceous rosid hosts, plants which are hosts to relatively few species of phytomyzine genera. Hosts of *Cerodontha* are almost entirely of the order Poales, while the other two clades within Phytomyzinae are extremely varied in their host use, but contain a high percentage (>50%) of species feeding on herbaceous asterid hosts. Further exploration of evolutionary patterns of host use will require more detailed phylogenetic study of individual genera.

The Agromyzidae are extremely specialized, with about 95% of species feeding on only a single plant family (Ward and Spaulding, 1993). Broad polyphagy (defined here as routinely feeding on plants in different orders (Spencer, 1990)) is very rare, occurring in only 14 described species of the more than 1190 species with known hosts (Table 92 in Spencer, 1990). These polyphagous species do not appear to form a single monophyletic cluster as such species occur in three genera (*Liriomyza* (8 spp.), *Tropicomyia* (5 spp.), and *Chromatomyia* (1 spp.)) and both subfamilies. The host range of the polyphagous species can be exceptionally broad, spanning several plant classes and including as many as 35 families (Spencer, 1990). How such broad polyphagy evolves in a family of specialists is an interesting and important question, as the evolution of generalism/specialization is a major topic within evolutionary biology, and also because broadly polyphagous species are also likely to be agricultural pests (Spencer, 1973; Dempewolf, 2004). A plot of the relationship between the number of species in a genus with known hosts and the number of plant families fed upon by that genus indicates that the three genera containing polyphagous species feed on a disproportionately large number of hosts (plot not shown). Because much of this effect is due to the inclusion of hosts only fed on by the polyphagous species themselves we removed these families (underlined in Table 1) from the analysis. The graph of the modified data appears to show a pattern of a general increase in the number of host families fed upon as the number of species with known hosts within a genus increases, as might be expected (Fig. 5).

The three genera *Cerodontha*, *Liriomyza*, and *Phytomyza* appear to stand out from the main cluster of points formed by the other genera (Fig. 5). *Cerodontha* feeds on many fewer host families than might be expected for its size; in fact, members of *Cerodontha* only feed on four families of monocots even though there has been considerable speciation in this group as evidenced by the fairly large size of this genus (270 described species, 81 with known hosts). In the cases of *Liriomyza* and *Phytomyza*, the interpretation of their placement on the graph is difficult as interpolation of the relationship
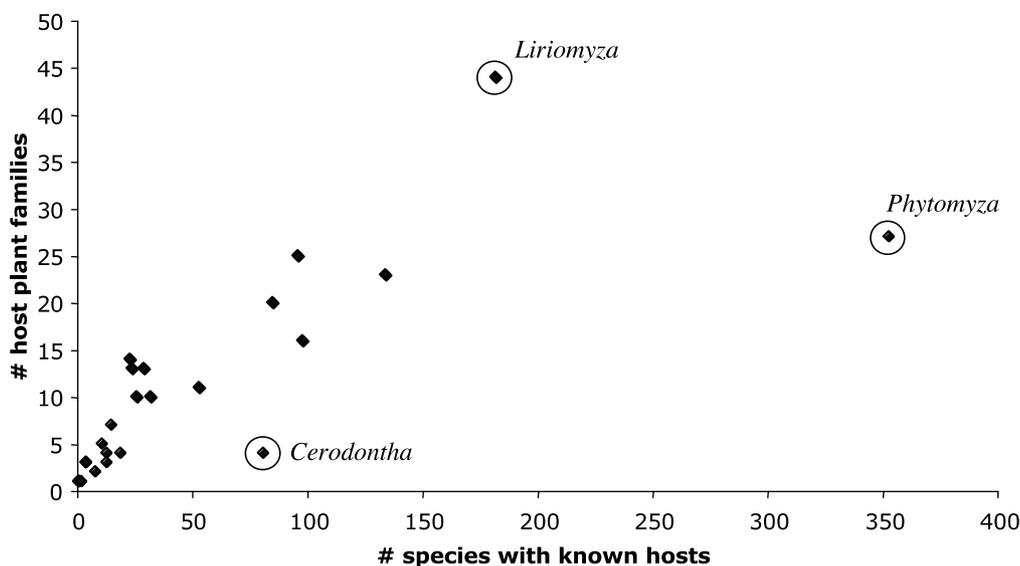
Fig. 5. Plot of the number of plant host families fed upon by a genus by the number of species within each genus with known host plants (see Table 1). Host families used by only polyphagous members of a genus (underlined in Table 1) were not included.

between the number of species and diet breadth is not straightforward past 150 species or so (Fig. 5). If the relationship remains a more or less straight line, then the large number of hosts fed on by *Liriomyza* simply reflects a linear accumulation of hosts as diversity increases. If, in contrast, the relationship reaches a plateau, as is suggested by the placement of *Phytomyza*, then the implication is that a substantial amount of diversification is associated with shifts onto previously used or related plant taxa rather than colonization of new plant groups. While this is certainly true of *Phytomyza*, it remains to be seen whether this pattern predominates within other genera that have fewer species with known hosts. Of particular interest are the host-use patterns of the stem, root, and seed/flowerhead feeders, of which there are many within the Agromyzidae, but whose host relationships and feeding modes are unknown because they feed relatively inconspicuously and have been only poorly sampled.

Even with the data corrected for host records of polyphagous species, *Liriomyza* remains the genus with the greatest number of host families utilized (Fig. 5). This could suggest that even non-polyphagous species within *Liriomyza* are likely to colonize new hosts that are phylogenetically distant from current hosts. Such a tendency or ability may predispose species towards the evolution of polyphagy. Alternatively, if oscillations in host range (Janz et al., 2006) have been significant in generating the observed diversity of *Liriomyza* species, then ancestral polyphagy at some level may help explain the varied host use in this genus. A key question may be—What is driving most *Liriomyza* species (and, in fact, most agromyzids) towards host specialization? Recent work with the broadly polyphagous *Liriomyza trifolii* (which feeds on at least 25 plant families) has suggested that a pepper-restricted population has recently evolved from within this polyphagous species (Morgan et al., 2000; Reitz and Trumble, 2002; Scheffer and Lewis, 2006), possibly providing an unusual opportunity to investigate the genetic, environmental, and behavioral features leading to host specialization.

5. Conclusion

This project is the first to use molecular data to investigate higher-level relationships within the Agromyzidae. Many of our findings are consistent with what is known concerning agromyzid morphology and taxonomy, although several areas requiring additional study have been revealed. Most importantly, there is no evidence that cambium-mining or stem-feeding is the ancestral condition within the family or the general precursor to leaf-mining. Further work on higher-level relationships as well as on relationships within genera will be necessary to fully explore the evolutionary history of host-use patterns in these phytophagous flies.

Acknowledgments

Appendix A

Specimen, voucher, and GenBank information for included taxa

Species	Voucher code	GenBank #			Specimen collection information	Records of larval feeding mode and host(s)
		COI	CAD	28S		
Agromyzinae						
Agromyza ambrosivora Spencer	Aamb	EF104649	EF104735	EF104822	MD: Prince George's Co., Candy Hill Rd., swept, September 2000, coll. S.J. Scheffer	Leafminer of *Ambrosia* and possibly *Artemesia* (Asteraceae)
Agromyza frontella (Rondani)	Afrn	EF104650	EF104736	EF104823	MN: Ramsey Co., Shoreview, swept, May 1998, coll. S.J. Scheffer	Leafminer of *Medicago* and *Melilotus* (Fabaceae)
Agromyza pseudoreptans Nowakowski	Apsr	EF104651	EF104737	EF104824	MN: Ramsey Co., Shoreview, swept, May 1998, coll. S.J. Scheffer	Leafminer of *Urtica* (Urticaceae)
Agromyza sp. 1	Agpa	EF104652	EF104738	EF104825	MD: Calvert Co., Huntingtown, ex. leafmine on *Panicum*, June 1998, coll. S.J. Scheffer 98–55	Not previously known
Agromyza sp. 2	Agvt	EF104653	EF104739	EF104826	VT: Rutland Co., Rutland, ex. leafmine on yellow composite, July 2002, coll. S.J. Scheffer	Not previously known
Hexomyza schineri (Giraud)	Hxsh-3	EF104654	EF104740	EF104827	UT: Utah Co., Provo, larva ex. gall on *Populus tremuloides*, November 2004, coll. C. R. Nelson	Stem-galler of *Populus* (Salicaceae)
Japanagromyza viridula (Coquillett)	Javi-2	EF104655		EF104828	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	Leafminer of *Quercus*, possible *Castanea* (Fagaceae)
Melanagromyza chalcosoma Spencer	Mech	EF104656	EF104741	EF104829	Kenya, Nairobi, Kabete, ex. *Cajanus cajan*, August 2000, coll. E. M. Minja	In pods of *Cajanus*, *Vigna*, and *Flemingia* (Fabaceae)
Melanagromyza cleomae Spencer	Mecl-2	EF104657	EF104742	EF104830	Sri Lanka, Gannoruwa, ex. "cabbage", August 1997, coll. A. Wijesekara	In stems of *Cleome* and *Brassica* (Brassicaceae)
Melanagromyza minimoides Spencer	Msu-1	EF104658	EF104743	EF104831	Bolivia, ex. seeds of *Helianthus*, September 2000	In seeds of various Asteraceae
Melanagromyza obtusa (Malloch)	Mob-2	EF104659	EF104744	EF104832	Puerto Rico, APHIS PPQ interception on pods of *Cajanus*, February 2000	In pods of *Cajanus*, *Vigna*, and *Phaseolus* (Fabaceae)
Melanagromyza virens (Loew)/ *splendida* Frick	Mevi	EF104660	EF104745	EF104833	MD: Prince George's Co., Candy Hill Rd., swept September 2000, coll. S.J. Scheffer C-60	In stems of Asteraceae
Ophiomyia nasuta (Melander)	Ona	EF104661	EF104746	EF104834	OH: Hamilton Co., Cincinnati, Cedar Pt. Rd., malaise trap, August 1996, coll. S.J. Scheffer	In midribs and stalks of *Taraxacum* (Asteraceae)
Ophiomyia cornuta de Meijere	Opco-2	EF104662	EF104747	EF104835	Sri Lanka, Ussangoda, ex. *Scaevola taccado*, September 1998, coll. A. Wijesekara	Leafminer of *Scaevola* and *Goodenia* (Goodeniaceae)
Ophiomyia lantanae (Froggatt)	Opla	EF104663	EF104748	EF104836	Sri Lanka, Gannoruwa, ex. *Lantana camara*, September 1998, coll. A. Wijesekara	In fruits of *Lantana* (Verbenaceae)
Ophiomyza phaseoli (Tryon)	Opph	EF104664	EF104749	EF104837	Philippines, Mountain Prov., Tadian, Bantey, ex. sitao, April 2001, coll. R. C. Joshi PH-243	In stems and roots of various Fabaceae
Ophiomyia quinta Spencer	Oqu	EF104665	EF104750	EF104838	NY: Suffolk Co., Gnarled Hollow Rd., ex. *Aster*, June 1994, coll. S.J. Scheffer 94–46	Leafminer of *Aster* and *Solidago* (Asteraceae)
Ophiomyia sp. 1	Opfl	EF10466	EF104751	EF104839	FL: Gadsden Co., Quincy, ex. leafmine on *Lantana*, October 1999, coll. S.J. Scheffer	Not previously known
Ophiomyia sp. 2	Opcam-1	EF104667	EF104752	EF104840	Cambodia, 19 km sw of Phnom Penh, ex. pods of *Tephrosia purpurea*, October 2003, coll. D. Coutinot, K. Chen	Not previously known
Tropicomyia theae (Green)	Trpc-2	EF104668	EF104753	EF104841	Sri Lanka, Galpalama, ex. leafmine on *Camelia*, September 1998, coll. A. Wijesekara	Miner in leaf epidermis, polyphagous
Phytomyzinae						
Amauromyza flavifrons (Meigen)	Amfl	EF104669	EF104754	EF104842	MN: Anoka Co., Cedar Creek Nat. Hist. Area, ex. *Silene*, Sep. 1997, coll. S.J. Scheffer 97–62	Leafminer of various Caryophyllaceae, also *Beta* and *Spinacea* (Amaranthaceae)

Amauromyza monofalconensis (Strobl)	Ammf	EF104670	EF104755	EF104843	UK: Oxfordshire, Whitehorse Hill, swept, June 2004, coll. I.S. Winkler	In stems of *Rumex* (Polygonaceae)
Amauromyza pleuralis (Malloch)	Ampl	EF104671	EF104756	EF104844	MD: Prince Georges Co., College Park, pupa ex. *Catalpa*, May 2004, coll. I.S. Winkler	Leafminer of *Catalpa* (Bignonicaceae)
Aulagromyza discrepans (van der Wulp)	Aldc	EF104672	EF104757	EF104845	UK: Kent, Folkstone, swept June 2004, coll. I.S. Winkler	In stems of *Galium* (Rubiaceae)
Aulagromyza luteoscutellata (de Meijere)	Palu	EF104673	EF104758	EF104846	NY: Albany Co., E. N. Huyck Preserve, ex. "bush honeysuckle", June 1996, coll. S.J. Scheffer 96–69	Leafminer of *Lonicera* and *Symphoricarpos* (Caprifoliaceae)
Aulagromyza nitida (Malloch)	Pani	EF104674	EF104759	EF104847	MD: Prince George's Co., Candy Hill Rd., malaise trap, April 2000, coll. S.J. Scheffer	Unknown
Aulagromyza orbitalis (Melander)	Paor	EF104675	EF104760	EF104848	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #1, May 2001, coll. B.M. Wiegmann	Leafminer of *Lonicera* (Caprifoliaceae)
Aulagromyza tridentate (Loew)	Alsa	EF104676	EF104761	EF104849	CO: Jefferson Co., Wheatridge, ex. *Salix*, June 2003, coll. I.S. Winkler	Leafminer of *Salix* (Salicaceae)
Calycomyza flavinotum (Frick)	Cafl	EF104677	EF104762	EF104850	MN: Ramsey Co., Shoreview, ex. leafmine on *Arctium*, August 1997, coll. S.J. Scheffer 97–47	Leafminer of *Arctium, Eupatorium, Ageratina* (Asteraceae)
Calycomyza hyptidis Spencer	Cahy	EF104678	EF104763	EF104851	FL: Gadsden Co., Quincy, ex. "mint," October 1999, coll. S.J. Scheffer	Leafminer of *Hyptis* (Lamiaceae)
Calycomyza lantanae (Frick)	Cala	EF104679	EF104764	EF104852	FL: Collier Co., Marco Island, ex. *Lantana camara*, Jan. 1998, coll. S. J. Scheffer	Leafminer of *Lantana, Lippia, Verbena* (Verbenaceae)
Calycomyza majuscula (Frick)	Camj	EF104680	EF104765	EF104853	MT: Park Co., Suce Creek Trail, malaise trap, August 2001, coll. R. Wharton, J. Wooley	Leafminer of *Senecio, Petasites* (Asteraceae)
Calycomyza malvae (Burgess)	Cama	EF104681	EF104766	EF104854	MD: Prince George's Co., Candy Hill Rd., swept, September 2000, coll. S.J. Scheffer	Leafminer of *Althea, Malva, Abutilon, Malvastrum, Sida* (Malvaceae)
Calycomyza solidaginis (Kaltenbach)	Caso	EF104682	EF104767	EF104855	MD: Prince George's Co., Candy Hill Rd, swept, September 1999, coll. S.J. Scheffer	Leafminer of *Solidago* (Asteraceae)
Cerodontha (Butomyza) angulata (Loew)	Cean-3	EF104683	EF104768	EF104856	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	Leafminer of *Carex* (Cyperaceae)
Cerodontha (Xenophytomyza) atronitens (Hendel)	Ceat	EF104684	EF104769	EF104857	UK: Kent, Folkstone, swept, June 2004, coll. I.S. Winkler	In stems of *Poa* (Poaceae)
Cerodontha (Icteromyza) capitata (Zetterstedt)	Cecp	EF104685	EF104770	EF104858	UK: Hampshire, New Forest, swept, June 2004, coll. I. S. Winkler	Probably *Juncus* (Juncaceae) (Spencer and Steyskal, 1986)
Cerodontha (Cerodontha) dorsalis (Loew)	Cedo-2	EF104686	EF104771	EF104859	CO: Gunnison Co., Irwin, swept, June 1997, coll. S.J. Scheffer	Leafminer of many Poaceae
Cerodontha (Dizygomyza) fasciata (Strobl)	Cefa	EF104687	EF104772	EF104860	MN: Ramsey Co., Shoreview, swept, May 1998, coll. S. J. Scheffer	Leafminer of *Poa* (Poaceae)
Cerodontha (Phytagromyza) flavocingulata (Strobl)	Cfcg	EF104688	EF104773	EF104861	UK: Powys, Talybont Reservoir, swept, June 2004, coll. I.S. Winkler	Leafminer of several Poaceae
Cerodontha (Poemyza) incisa (Meigen)	Cein	EF104689	EF104774	EF104862	MN: Ramsey Co, Shoreview, swept, May 1998, coll. S.J. Scheffer	Leafminer of many Poaceae
Cerodontha (Poemyza) muscina (Meigen)	Cemu	EF104690	EF104775	EF104863	MN: Ramsey Co., Shoreview, swept, May 1998, coll. S.J. Scheffer	Leafminer of many Poaceae
Chromatomyia aprilina (Goureau)	Capr	EF104691	EF104776	EF104864	UK: Hampshire, New Forest, swept, June 2004, coll. I.S. Winkler	Leafminer of *Lonicera* (Caprifoliaceae)
Chromatomyia fuscula (Zetterstedt)	Chfu-2	EF104692	EF104777	EF104865	Norway, Kvithamar, swept, August 1998, coll. A. Andersen	Leafminer of many Poaceae

Appendix A (*continued*)

Species	Voucher code	GenBank #			Specimen collection information	Records of larval feeding mode and host(s)
		COI	CAD	28S		
Chromatomyia lactucae (Frost)	Chlc	EF104693	EF104778	EF104866	NC: Wake Co., Raleigh, ex. leafmine on *Lactuca*, coll. S.J. Scheffer	Leafminer of *Lactuca* (Asteraceae)
Chromatomyia scolopendri (Goureau)	Csco	EF104695	EF104780	EF104868	UK: Kent, Folkstone, swept, June 2004, coll. I.S. Winkler	Leafminer of *Asplenium* (Aspleniaceae) and *Polypodium* (Polypodiaceae)
Chromatomyia syngenesiae Hardy	Chsy-2	EF104694	EF104779	EF104867	CA: Monterey Co., Salinas, ex. sowthistle, May 1998, coll. W. E. Chaney	Leafminer of many Asteraceae
Galiomyza violivora Spencer	Gavi	EF104697	EF104782	EF104870	NC: Wake Co., Raleigh, ex. leafmine on *Viola*, coll. S.J. Scheffer 96–79	Leafminer of *Viola* (Violaceae)
Gymnophytomyza heteroneura (Hendel)	Ghet	EF104698	EF104783	EF104871	UK: Oxfordshire, Whitehorse Hill, swept, June 2004, coll. I.S. Winkler	In seeds of *Galium* (Rubiaceae)
Liriomyza baptisiae (Frost)	Lbap	EF104699	EF104784	EF104872	CO: Park Co., Bailey, June 1997, coll. S.J. Scheffer	Leafminer of *Baptisia, Lupinus* (Fabaceae)
Liriomyza brassicae (Riley)	Lbra	EF104700	EF104785	EF104873	Sri Lanka, Gannoruwa, ex. leafmine on *Brassica*, March 1998, coll. A. Wie	Leafminer of various Brassicaceae
Liriomyza chinensis (Kato)	Lch-1	EF104701	EF104786	EF104874	Philippines, Benguet Prov., Wangal, LT, ex. leafmine on onion, August 2000, coll. R. Joshi, N. Baucas, G. Sacla	Leafminer of *Allium* (Alliaceae)
Liriomyza fricki Spencer	Lfri	EF104702	EF104787	EF104875	MD: Calvert Co. Huntingtown, ex. clover, June 1998, coll. S.J. Scheffer 98–59	Leafminer of *Lathyrus, Medicago, Trifolium, Vicia* (Fabaceae)
Liriomyza huidobrensis (Blanchard)	Tlh-114	EF104703	EF104788	EF104876	South Africa, Western Cape Province, Lambert's Bay, ex. leafmine on potato, November 1999, coll. D. Visser	Leafminer, polyphagous
Liriomyza philadelphivora Spencer	Liph	EF104704	EF104789	EF104877	NY: Suffolk Co., Smithtown, ex. *Philadelphus*, June 1994, coll. S.J. Scheffer 94–18	Leafminer of *Philadelphus* (Hydrangeaceae)
Liriomyza trifoliearum Spencer	Ltfm	EF104705	EF104790	EF104878	MD: Prince George's Co., Candy Hill Rd., ex. clover, May 1999, coll. S.J. Scheffer 99–21	Leafminer of *Medicago, Coronilla, Pisum, Trifolium* (Fabaceae)
Liriomyza trifolii (Burgess)	Ltr-45	DQ516575	EF104791	EF104879	CA: colony in John Trumble's lab from southern California, 1999	Leafminer, polyphagous
Metopomyza scutellata Sehgal	Mtgr	EF104706	EF104792	EF104880	MN: Anoka Co., Carlos Avery, swept, May 1998, coll. S.J. Scheffer C-64	Probably leafminer of *Carex* (Cyperaceae); (Spencer and Steyskal, 1986)
Metopomyza flavonotata (Haliday)	Mtfl	EF104707	EF104793	EF104881	UK: Kent, Folkstone, swept, June 2004, coll. I.S. Winkler	Leafminer of *Alopecurus, Deschampsia* (Poaceae)
Napomyza cichorii Spencer	Nach-2	EF104708	EF104794	EF104882	France, ex. *Lactuca sativae*, coll. H. Heinz [FF 235]	Leaf- or stem- or rootminer in *Cichorium* (Asteraceae)
Napomyza lateralis (Fallén)	Nap-17	EF104710	EF104796	EF104884	Switzerland, Delmont, ex. seeds of *T. perforatum*, 2000, coll. U. Schaffner	In seeds of various Asteraceae
Napomyza montanoides Spencer	Namo	EF104711	EF104797	EF104885	CO: Chaffee Co., swept, June 1997, coll. S.J. Scheffer	Unknown
Napomyza plumea Spencer	Nplu	EF104712	EF104798	EF104886	CO: Clear Creek Co., Guanella Pass, swept, August 2003, coll. I.S. Winkler	In stems of *Achillea* (and perhaps other Asteraceae; Spencer and Steyskal, 1986)
Nemorimyza posticata (Meigen)	Nepo-5	EF104714	EF104800	EF104888	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	Leafminer of *Aster, Baccharis, Erechtites, Solidago* (Asteraceae)

Nemorimyza maculosa (Malloch)	Amma	EF104715	EF104801	EF104889	NC: Wake Co., Raleigh, NCSU, ex. *Erichtites*, June 1996, coll. S.J. Scheffer 96–63	Leafminer of various Asteraceae
Phytobia betulae (Kangas)	Ptbe-2	EF104716	EF104802	EF104890	Finland, Laukansaari, Punkaharju, pupa coll. September–October 1996, coll. T. Ylioja	Cambium miner of various Betulaceae
Phytobia setosa (Loew)	Ptse	EF104717	EF104803	EF104891	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	Cambium miner of *Acer*
Phytobia sp. 1	Ptb-2	EF104718	EF104804	EF104892	MT: Park Co., Suce Creek Trail, malaise trap, August 2001, coll. R. Wharton, J. Wooley	Unknown
Phytoliriomyza arctica (Lundbeck)	Plar	EF104719	EF104805	EF104893	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	In stems of *Sonchus*, *Solidago*, perhaps other Asteraceae
Phytoliriomyza felti (Malloch)	Plft	EF104720	EF104806	EF104894	TN: Davidson Co., March 2002, ex. leafmine on fern, coll. D.J. Funk	Leafminer of *Asplenium*, *Camptosorus* (Aspleniaceae)
Phytoliriomyza melampyga (Loew)	Pme	EF104721	EF104807	EF104895	VA: Albermarle Co., female coll. on *Impatiens*, July 1996, coll. S.J. Scheffer	Leafminer of *Impatiens* (Balsaminaceae)
Phytoliriomyza robiniae (Valley)	Plro	EF104722	EF104808	EF104896	MD: Prince George's Co., Candy Hill Rd., malaise trap, April 2000, coll. S.J. Scheffer	Leafminer of *Robinia* (Fabaceae)
Phytoliriomyza sp. 1	Plnc	EF104723	EF104809	EF104897	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	Unknown
Phytomyza aquilegiana Frost	Phag-2	EF104724	EF104810	EF104898	MD: Montgomery Co., Takoma Park, ex. leafmine on *Aquilegia*, August 2003, coll. S.J. Scheffer 03-05	Leafminer of *Aquilegia* (Ranunculaceae)
Phytomyza aquilegivora Spencer	Paq	EF104725	EF104811	EF104899	NC: Wake Co., Raleigh, NCSU, ex. *Aquilegia*, April 1996, coll. B.M. Wiegmann	Leafminer of *Aquilegia* (Ranunculaceae)
Phytomyza costata Harrison	Ncos	EF104709	EF104795	EF104883	New Zealand, Christchurch, Styx Mill wetlands, swept, October 2002, coll. W. Mathis	Leafminer of *Ranunculus* (Ranunculaceae)
Phytomyza erigerophila Hering	Per	EF104726	EF104812	EF104900	NC: Swain Co., GSMNP, Noland Creek, ex. leafmine on *Erigeron*, May 1997, coll. S.J. Scheffer	Leafminer of *Erigeron* (Asteraceae)
Phytomyza evanescens Hendel	Naev	EF104730	EF104816	EF104904	NC: Wake Co., Apex, Zeno Rd., swept, April 1995, coll. S.J. Scheffer	In stems of *Ranunculus* (Ranunculaceae)
Phytomyza glabricola Kulp	Pgl-54	EF104727	EF104813	EF104901	NY: Suffolk Co., Swan Pond, ex. leafmine on *Ilex glabra*, August 1996, coll. S.J. Scheffer	Leafminer of *Ilex glabra* (Aquifoliaceae)
Phytomyza ilicicola Loew	Pil-6	EF104728	EF104814	EF104902	MD: Charles Co., Cedarville, ex. leafmine on *Ilex opaca*, March 1999, coll. S.J. Scheffer 99–17	Leafminer of *Ilex opaca* (Aquifoliaceae)
Phytomyza plantaginis Robineau-Desvoidy	Ppl	EF104729	EF104815	EF104903	NC: Wake Co., Raleigh, NCSU, ex. leafmine on *Plantago lanceolata*, May 1996, coll. S.J. Scheffer 96–45	Leafminer of *Plantago* (Plantaginaceae)
Phytomyza spinaciae Hendel	Cspi	EF104696	EF104781	EF104869	UK: Oxfordshire, Whitehorse Hill, swept, June 2004, coll. I.S. Winkler	Leafminer of *Carduus*, *Cnicus*, and *Serratula* (Asteraceae)
Phytomyza sp. 1	Nanc	EF104713	EF104799	EF104887	NC: Swain Co., GSMNP, Big Cove Rd., malaise trap #4, May 2001, coll. B.M. Wiegmann	Unknown
Pseudonapomyza sp. 1	Pnp	EF104731	EF104817	EF104905	Australia	Unknown
Pseudonapomyza lacteipennis (Malloch)	Psla	EF104732	EF104818	EF104906	MT, Carbon Co., SW of Red Lodge, swept, July 2003, coll. I.S. Winkler	Probably Poaceae

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