Taxonomy and phylogeny of the Asphondylia species (Diptera: Cecidomyiidae) of North American goldenrods: challenging morphology, complex host associations, and cryptic speciation

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Reproductive isolation and speciation in herbivorous insects may be accomplished via shifts between host-plant resources: either plant species or plant organs. The intimate association between gall-inducing insects and their host plants makes them particularly useful models in the study of speciation. North American goldenrods (Asteraceae: Solidago and Euthamia) support a rich fauna of gall-inducing insects. Although several of these insects have been the subject of studies focusing on speciation and tritrophic interactions, others remain unstudied and undescribed. Among the latter are at least seven species of the large, cosmopolitan gall midge genus Asphondylia Loew (Diptera: Cecidomyiidae), the taxonomy and biology of which are elucidated here for the first time using morphological, molecular, and life-history data. We describe Asphondylia pseudorosa sp. nov., Asphondylia rosulata sp. nov., and Asphondylia silva sp. nov., and redescribe Asphondylia monacha Osten Sacken, 1869 and Asphondylia solidaginis Beutenmüller, 1907, using morphological characters of adults, immature stages, and galls, as well as sequence data from both nuclear and mitochondrial genes. A neotype is designated for A. solidaginis, the type series of which is considered lost. We also provide information on the life history of all species, including a description of two inquilinous cecidomyiids commonly found in the galls, Clinodiplosis comitis sp. nov. and Youngomyia podophyllae (Felt, 1907), and on parasitoid wasps associated with the gall midges. Asphondylia johnsoni Felt, 1908, which was described from an unknown gall on an unknown Solidago host, is assigned to nomina dubia. Our phylogenetic analyses show that some of the Asphondylia species associated with goldenrods induce two different types of galls during their life cycle, some exhibit host alterations, and some do both. In the absence of reliable morphological differences, recognising species boundaries and deciphering host associations of species must rely heavily on molecular data. Our analysis suggests that radiation in this group has been recent and occurred through shifts among host plants.


INTRODUCTION

Phytophagous insects are well-known models for studying diversification and speciation (Futuyma & Agrawal, 2009; Nosil, 2012). The evolution of host associations among phytophagous insects has been shown to be caused by both spatial and temporal shifts between host-plant species and within host-plant species (to different plant organs or different time periods of plant growth; Joy & Crespi, 2012); however, the relative importance and the context of between- versus within-host shifts remains unknown. Gall midges (Diptera: Cecidomyiidae) are excellent models for studying the importance and context of such shifts because of the intimate nature of their relationships with their host plants, and because closely related groups of gall midges are known to diversify through shifts between hosts and through shifts between organs within a host-plant species (e.g. Jones, Gagné & Barr, 1983; Hawkins, Goeden & Gagné, 1986; Joy & Crespi, 2007; Stireman, Devlin & Abbot, 2012).

Asphondylia Loew, 1850 belongs to the tribe Asphondylini, currently with 505 species, and within Asphondylini to the subtribe Asphondylina, which is most diverse in the Neotropics (Gagné & Jaschhof, 2014). The subtribe contains 19 genera that share clear morphological apomorphies, but no cladistic or other phylogenetic analysis has been conducted on it to date. All genera included in this group other than Asphondylia are small (with between one and 11 species), and most are restricted to South America. By contrast, Asphondylia is one of the largest genera in the family Cecidomyiidae, with 320 described species that feed on a great diversity of plant families worldwide (Gagné & Jaschhof, 2014), and the number of undescribed species in this genus is probably far greater. Despite descriptions of numerous new Asphondylia species in recent years from South America and Australia (Veenstra-Quah, Milne & Kolesik, 2007; Kolesik & Veenstra-Quah, 2008; Maia et al., 2008; Maia, Santos & Fernandes, 2009; Kolesik, Adair & Eick, 2010), knowledge about the fauna of the southern hemisphere is greatly lacking, in particular that of the Afrotropical region, and there is little doubt that it includes hundreds of undescribed species (ND, unpublished data). The North American fauna of plant-feeding gall midges is relatively well known (Gagné, 1989), but undescribed species are still continuously discovered, and many of these belong to Asphondylia.

All Asphondylia species are gall inducers, and the galls are almost always associated with a fungus that lines the inside walls of the gall. Similar associations are known in other cecidomyiid genera, and although several studies suggested that the larvae of such ‘ambrosia’ gall midges feed on the fungus rather than on the plant tissue (Bisset & Borkent, 1988; Rohfritsch, 2008; Adair et al., 2009), this has been demonstrated only in the genus Asteromyia Felt, 1910 (Heath & Stireman, 2010; Janson et al., 2010). In general, gall midge genera with such known associations with fungi have a broader host range and are more diverse than gall midge genera that do not exhibit fungal associations, suggesting that the fungus is important in mediating relationships with the host plant (Joy, 2013). The hypothesis that females actively collect conidia in specialized structures on their ovipositors (Borkent & Bisset, 1985; Rohfritsch, 1997, 2008) remains untested, and the mechanistic role of the fungus in Asphondylia galls is still unknown.

The life histories of Asphondylia species vary considerably, depending largely on zoogeographical region and host-plant phenology (Yukawa, 1987; Uechi & Yukawa, 2006a, b; Tokuda, 2012). Many species are univoltine and enter extended diapause, whereas others are bi- or multivoltine and present complex host associations (reviewed in Tokuda, 2012). The great majority of gall-inducing cecidomyiids are considered monophagous or narrowly oligophagous, but systematic and molecular studies have revealed some Asphondylia species to be polyphagous, as they develop on several host plants from two or more unrelated plant families. Documented examples of polyphagous species include Asphondylia gennadii Marchal, 1904 (Gagné & Orphanides, 1992), Asphondylia yushimai Yukawa & Uechi, 2003 (Yukawa et al., 2003), and Asphondylia websteri Felt, 1917 (Gagné & Jaschhof, 2014). Some of these species are multivoltine and alternate between hosts that are available at different times of the year (Tokuda, 2012), whereas others alternate between different organs on the same host plant (Parnell, 1964; Plakidas, 1988). On the other end of the scale are groups of strictly monophagous species that are plant-organ specific and have apparently radiated, or are in the process of radiating, in situ on a single plant genus (Hawkins et al., 1986; Gagné & Waring, 1990; Joy & Crespi, 2007; Stokes et al., 2012).

Asphondylia taxonomy is challenging because adult morphology differs little among species. All species share the neckless, cylindrical antennal flagellomeres in both sexes, the needle-like ovipositor and conspicuously enlarged seventh sternite in the female, and the compact male genitalia with the spherical gonostyli positioned dorsally rather than apically (Gagné, 1994). The third-instar larvae possess a well-developed, usually four-toothed spatula on the first thoracic segment. Because pupation in all Asphondylia species takes place inside the gall rather than in the soil, the larvae do not need the spatula for digging; thus the function of the spatula in this genus is unknown. The pupae are characterized by well-developed horn-like antennal bases, a varying number of facial horns, and transverse dorsal rows of spines on the abdominal segments (Gagné, 1989,
that assist in breaking out of the galls just before adult egression. While larvae and pupae sometimes offer useful taxonomic characters – for example in the species groups associated with *Larrea Cavanilles*, 1800 (*Zygophyllaceae*) and *Atriplex Linnaeus*, 1753 (*Chenopodiaceae*) in North America (Hawkins et al., 1986; Gagné & Waring, 1990) – often the only way to verify species identity is by conducting molecular analyses (Yukawa et al., 2003; Uechi, Yukawa & Yamaguchi, 2004; Kolesik et al., 2010).

Here, the *Asphondylia* fauna of North American goldenrods was studied as part of a general review of gall-inducing cecidomyiids on these plants. Goldenrods (the genera *Solidago Linnaeus*, 1753 and *Euthamia Nuttal ex Cassini*, 1825; *Asteraceae*), which include many common herbs throughout eastern USA, support a remarkably diverse community of insects (McEvoy, 1988; Gagné, 1989; Maddox & Root, 1990; Root & Cappuccino, 1992; Fontes, Habeck & Slansky, 1994). Because they are easy to manipulate in field and greenhouse experiments, goldenrods have served as model systems for numerous studies on evolutionary and ecological aspects of speciation and tritrophic interactions (e.g. Abrahamson & Weis, 1997; Stireman et al., 2005, 2006, 2012; Crutsinger, Cadotte & Sanders, 2009; Dixon, Craig & Itami, 2009; Wise, Cole & Carr, 2010; Craig et al., 2011; Hakes & Cronin, 2012). Of the 50 or so insect species known to induce galls on goldenrods, about 30 species are cecidomyiids. Of these, 16 belong to the genus *Rhopalomyia* Rübsamen, 1892 (Dorchin et al., 2009), three belong to the genus *Asteromyia* (Stireman et al., 2010), and two species belong to the genus *Dasineura* Rondani, 1840 (Dorchin et al., 2007). To date, only two *Asphondylia* species have been described from goldenrods: one in the 19th and the other in the early 20th century (Gagné, 1989); however, in the course of our work on goldenrod-galling cecidomyiids, we regularly encountered numerous types of *Asphondylia* galls that have not been previously recorded or attributed to species.

In the present study, we provide a systematic review of *Asphondylia* species from goldenrods using a combination of morphological, ecological, and molecular data. We describe three new species, re-describe the two known species, add numerous host records, and provide detailed information about species life histories. We also describe two common inquisitive cecidomyiids and provide information on parasitic Hymenoptera that are found regularly in the galls. Furthermore, we conducted a molecular phylogenetic analysis based on both mitochondrial and nuclear genes, which assisted with taxonomic decisions and provided insight into the role of host use in the radiation of species in this group. A revision of *Asphondylia* via extensive sampling across the genus is a major task that is beyond the scope of this paper; however, the distinct morphological attributes of the goldenrod- and *Aster*-associated species suggest that they are a monophyletic group.

### MATERIAL AND METHODS

#### COLLECTING AND REARING OF INSECTS

In this work, the term ‘goldenrods’ refers to *Solidago* and *Euthamia*, which were historically treated as a single genus (*Solidago*) but are currently recognized as distinct genera (Semple & Cook, 2006). Goldenrods were extensively surveyed for galls in numerous localities in central Pennsylvania in 2005–2007, and occasionally thereafter. Other occasional collecting was performed in New York (Lake District) and Virginia (Roanoke area), and material has also been received from Maine (Perry and Sipp Bay) and Massachusetts (Nantucket). Goldenrod species that were surveyed on a regular basis were *Solidago altissima* Linnaeus, 1753, *S. gigantea* Aiton, 1789, *S. rugosa* Miller, 1768, *S. juncea* Aiton, 1789, *S. caesia* Linnaeus, 1753, and *Euthamia graminifolia* (Linnaeus) Nuttall, 1840. Species that were sampled intermittently included *Solidago bicolor* Linnaeus, 1767, *S. canadensis* Linnaeus, 1753, *S. erecta* Banks ex Pursh, 1813, *S. flexicaulis* Linnaeus, 1753, *S. nemoralis* Aiton, 1789, *S. sempervirens* Linnaeus, 1753, and *S. ulmifolia* Muhlenberg ex Willdenow, 1803. Additionally, *Asphondylia* species from several other Asteraceae were reared and studied for comparison, including *Asphondylia recondita* Osten Sacken, 1875 (on *Aster novae-angliae* Linnaeus, 1753), *Asphondylia rudbeckiae conspicua* Osten Sacken, 1870 (on *Rudbeckia laciniata* Linnaeus, 1753), and *Asphondylia eupatorii* Felt, 1911 (on *Ageratina altissima* (L.) King & Robinson, 1970).

Galls were either bagged in the field or collected and brought to the laboratory, where they were kept at room temperature (20–25°C) in ventilated rearing cages until adult emergence. Some of the galls were dissected under a stereomicroscope to obtain the immature stages of the gall midges as well as those of parasitoids and inquilines.

### TAXONOMY

Immature stages and emerging adults of the gall midges, their parasitoids, and inquilines were preserved in 70% ethyl alcohol for morphological study, and the gall midges were later mounted on permanent microscope slides in euparal, according to the method outlined in Gagné (1989). Illustrations were made with the aid of a drawing tube mounted on a Leica DM1000 LED compound microscope. Pupae were also studied under a scanning electron microscope. Some adults and immature stages were preserved in 96% ethanol for molecular study, which limited the number of
specimens that were available for the morphological study in some of the species. Relevant material deposited in the National Museum of Natural History (USNM), Smithsonian Institution, Washington, DC, USA, was also examined. The terminology for adult morphology follows McAlpine et al. (1981), and the terminology for immature morphology follows Gagné (1989). Taxonomy and nomenclature of the host plants follow Semple & Cook (2006); thus the name *S. canadensis* used by Felt is referred to here as *S. altissima*. All taxonomic decisions were made by the first author. The material examined in this work is deposited in the National Collection of Insects, Zoological Museum, Tel Aviv University (TAUI), unless otherwise indicated. All types are mounted on permanent microscope slides. Holotypes of newly described species are deposited in TAUI. Other types are deposited as indicated in the relevant section of the species descriptions.

**Molecular methods**

Genomic DNA was extracted from whole adult or immature midges using a BioSprint96 magnetic bead extractor and corresponding extraction kits by Qiagen Inc. (Valencia, CA, USA and Hilden, Germany). We PCR-amplified one mitochondrial and one nuclear gene using the following procedures: a fragment of \( \sim 680 \) bp of the mitochondrial cytochrome oxidase *c* subunit I (COI) was amplified using the primers LCO1490 (F) and HCO2198 (R) (Folmer et al., 1994); and a fragment of \( \sim 570 \) bp of the elongation factor 1 alpha gene (*EF-1\( \alpha \)) was amplified using the primers EF1aF and EF1aR (Joy & Crespi, 2007).

Following enzymatic clean up (Exo/SAP), double-stranded sequencing was conducted on an automated ABI 3730XL sequencer (Applied Biosystems) at the Pennsylvania State University nucleic acid facility or at the Macrogen facility, Amsterdam, the Netherlands. Sequences were initially inspected for sequencing errors using CodonCode Aligner 1.5.2 (CodonCode Corporation, Dedham, MA, USA).

**Phylogenetic analyses**

The phylogenetic analysis included 54 individuals from goldenrods (*Solidago* and *Euthamia*), one from *Aster*, two from other Asteraceae (*Chrysothamnus Nuttall*, 1840 sp. and *Gutierrezia Lagasca*, 1807 sp.), and one from *Larrea* (*Zygophyllaceae*). The last three were used as out-group taxa. Two additional species, *A. rudbeckiaeconspicua* and *A. conglomerata* De Stefani, 1900, yielded problematic sequences that could not be aligned with the rest of the data set, and were therefore eliminated from the analysis. Data on all specimens that were included in the analysis are provided in Table 1. Sequences were aligned using the local alignment tool MAFFT (Katoh, Asimenos & Toh, 2009), and then inspected and adapted by eye using Se-Al (Rambaut, 1996). Models of molecular evolution for each gene were assessed using jModeltest (Posada, 2008). The codon position (CP) model (Shapiro, Rambaut & Drummond, 2006) was employed for each gene. Bayesian phylogenetic analyses were performed on the resulting data partitions under an uncorrelated lognormal relaxed molecular clock model (Drummond et al., 2006), with a birth–death speciation tree prior (Gernhard, 2008), as implemented in BEAST 1.7.1 (Drummond et al., 2012). Four independent chains were run for 50 million generations. Convergence was assessed graphically using TRACER 1.5 (Rambaut & Drummond, 2007), and through evaluation of the effective sample size (ESS) values (Drummond et al., 2006). Following burn-in and a combination of the four runs, ESS values of greater than 200 for all parameters were taken as evidence of parameter stability and an adequate run time. The maximum clade credibility (MCC) phylogenetic tree was produced from the resulting distribution of trees using TreeAnnotator 1.7.1 (Drummond et al., 2012). We reconstructed the evolution of host use as an unordered multistate character on our phylogenetic tree using the maximum parsimony approach, as implemented in MESQUITE (Maddison & Maddison, 2011).

**RESULTS**

**Morphology**

As the *Asphondylia* species associated with goldenrods are very similar, we could not differentiate among them based on morphology alone. Although larval and pupal characters often offer better morphological characters for this purpose in *Asphondylia* than adult characters, this is generally not the case in the goldenrod-associated species. Larvae are robust, light to dark orange, and have a greatly reduced terminal segment that is typical of the genus. The most conspicuous larval character is the strong, four-toothed sternal spatula, which is accompanied by five setose lateral papillae on each side (Fig. 31); however, in the species associated with goldenrods, the intraspecific variation in the shape of the spatula is often so great that it is of no taxonomic value.

Pupae of all species in this group have well-developed antennal bases that form the ‘antennal horns’, two upper and one lower facial horns, long and pointed prothoracic spiracles, and minute cephalic setae (e.g., Fig. 65). On each side of the lower facial horn there are two papillae, one of which has a short seta. This arrangement may not be unique to the species from goldenrods, but it differs at least from some *Asphondylia* species that develop on other North American Asteraceae. For
Table 1. Samples used for DNA analysis with GenBank accession numbers

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<th>Collection date</th>
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<td>USA, PA, Lewisburg, Furnace Rd.</td>
<td>4 July 2007</td>
<td>N. Dorchin &amp; D. Ryan</td>
<td>KP208520 KP208531</td>
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<td>Asphondylia solidaginis</td>
<td>sn3 Euthamia graminifolia</td>
<td>USA, PA, Lewisburg, Furnace Rd.</td>
<td>4 July 2007</td>
<td>N. Dorchin &amp; D. Ryan</td>
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<td>Asphondylia solidaginis</td>
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<td>USA, PA, Lewisburg, Furnace Rd.</td>
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<td>Asphondylia solidaginis</td>
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<td>USA, PA, Lewisburg, Furnace Rd.</td>
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<td>N. Dorchin &amp; D. Ryan</td>
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<td>Asphondylia solidaginis</td>
<td>bud1 Solidago altissima</td>
<td>USA, PA, Lewisburg, Furnace Rd.</td>
<td>4 July 2007</td>
<td>N. Dorchin &amp; D. Ryan</td>
<td>KP208524 KP208537</td>
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<td>Asphondylia solidaginis</td>
<td>bud2 Solidago altissima</td>
<td>USA, PA, Lewisburg, Furnace Rd.</td>
<td>4 July 2007</td>
<td>N. Dorchin &amp; D. Ryan</td>
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<tr>
<td>Asphondylia solidaginis</td>
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<td>USA, PA, Lewisburg, Furnace Rd.</td>
<td>4 July 2007</td>
<td>N. Dorchin &amp; D. Ryan</td>
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example, the pupae of *A. rudbeckiaeconspicua* and *A. eupatori* have two upper and three lower facial horns. Each of the abdominal terga bears one straight row and a couple of less ordered rows of pointed spines (Fig. 76), except for the first abdominal segment, which is bare. The only useful taxonomic character we found for distinguishing among pupae of the goldenrod-associated species is the shape of the antennal horns. The species fall into one of two groups in this respect: those with relatively short and wide-based horns (*Asphondylia solidaginis* Osten Sacken, 1869), and those with longer and more slender horns (*Asphondylia rosulata* sp. nov., *Asphondylia silva* sp. nov., and *Asphondylia pseudorosa* sp. nov.). An exception is the morphology of *A. pseudorosa* sp. nov. pupae that develop in inflorescences, which have very short and blunt horns and thus differ markedly from the pupae of the same species that develop in buds. We consider this to be an intraspecific variation as a result of adaptation to the galled organ.

Adults of all species from goldenrods (as well as species from *Aster*) are basically black, with black-and-white banded legs due to a thick covering of black scales and patches of white scales on the tibiae and first tarsomeres (Fig. 18). Wings are dark grey and densely covered by black hairs. Tarsal claws are untoothed on all legs. As in all members of the genus and tribe, there are 12 antennal flagellomeres in both sexes, which are all cylindrical except for the successively shorter and rounder flagellomeres 10–12 in the female. Male flagellomeres bear twisting circumfila, whereas circumfila on female flagellomeres consist of only two horizontal bands connected by two longitudinal strands (Figs 23, 24). The first antennal flagellomere in females of all species from *Solidago* is much longer than the more distal flagellomeres, whereas in the species from *Euthamia* these flagellomeres are equal or subequal in length (compare Figs 46 and 56). To compare this character among species, the length of antennomere 1 relative to the length of flagellomere 5 is given for each species in the following descriptions (referred to as ‘flagellomere 1/flagellomere 5 ratio’). The palpal segments of all species from *Solidago* are successively longer, whereas in the species from *Euthamia*, the third segment is not appreciably longer than the second segment (compare Figs 45 and 46 with Figs 55 and 56). Species may otherwise differ in overall size (e.g. *A. monacha* is a big species, whereas *A. silva* sp. nov. and *A. pseudorosa* sp. nov. are relatively small species), and in the length of the ovipositor relative to overall size (larger species have relatively longer ovipositors). As in all members of the genus, females have a pair of cerci-like appendages at the end of the eighth abdominal segment, a sternite 7 that is much longer than the preceding sternites, and a needle-like, retractable ovipositor (Fig. 58). The male terminalia are round and compact, with gonocoxites that are short and wide, extending into a short mediastral lobe (Fig. 29). The gonostyli are round–ovoid, situated dorsally on the gonocoxites, and bear a crescent-shaped tooth. Male genitalia are very similar in all species from goldenrods, and the only variation we found was in the shape of the hypoproct, which is notch to a varying degree in different species (in *A. silva* sp. nov. it is virtually entire). Given the general lack of reliable morphological differences, identification of species in this group must rely heavily on their galls and on molecular markers. In the species descriptions that follow, the two previously described species, *A. monacha* and *A. solidaginis*, are re-described first, followed by descriptions of the new species. Characters that are common to all species are not mentioned again.

**Life history**

The *Asphondylia* galls on goldenrods can be found from early spring to late autumn (April–October). They develop either in buds or leaves, and *A. pseudorosa* sp. nov. also galls inflorescences. Although bud galls are diverse in size and shape, they are similar in basic structure: they contain a rigid central chamber made of shortened leaves and surrounded by one or more whorls of short leaves at shoot tips. When such a gall develops singly and is accompanied by a small number of leaves, it is hardly noticeable (as in *A. silva* sp. nov., Fig. 19), but when it is surrounded by numerous leaves, the gall is much more conspicuous, as in the summer-generation galls of *A. solidaginis* and *A. rosulata* sp. nov. (Figs 13, 14). When several individual galls develop in aggregation, they form complex, spherical structures, as in the summer generation of *A. monacha* (Fig. 3). In all of these cases, the larval chamber is rigid to the touch and contains white fungal mycelium that sometimes spills out of the chamber. Although the leaf galls on goldenrods are simple blisters, they are unusual because they join together two or more leaves in a snap, in which one leaf usually forms the bottom part of the gall and the other forms the upper part. These ‘snap galls’ also contain a thick layer of white mycelium.

Host associations in goldenrod *Asphondylia* species are complex in that several species use more than one host and/or induce different types of galls during the season. In such cases, we were able to associate the different generations of an individual species only with the aid of molecular data. For example, *A. monacha* is bivoltine and induces two types of galls on different host plants in spring and late summer. The solitary, inconspicuous spring gall of this species (Figs 1, 2) develops quickly in young sprouts of *Solidago altissima*, whereas the late-summer generation takes several
Figures 1–6. *Asphondylia* bud galls: 1, 2, *Asphondylia monacha* spring galls on *Solidago altissima* sprouts; 3, *Asphondylia monacha* summer-generation gall on *Solidago juncea*; 4, *Asphondylia monacha* summer-generation gall on *Solidago erecta*; 5, *Asphondylia* sp. gall on *Solidago sempervirens* (photo: Charley Eiseman); 6, *Asphondylia* sp. gall on *Solidago bicolor*. 

months to develop in the aggregated galls on S. juncea, S. erecta, and S. uliginosa (Figs 3, 4). Females of the late-summer generation probably lay their eggs in plant tissue close to the ground, and the first-instar larvae that will develop in S. altissima sprouts in the following spring must overwinter in dormant buds on rhizomes. The life history of the undescribed species from S. bicolor and S. sempervirens is probably similar. The remaining species on goldenrods are multivoltine, and some of them induce two types of galls at different times during the season. Interestingly, the snap galls that are all structurally similar on S. altissima, S. rugosa, and Euthamia graminifolia belong to three different species, each of which also induces bud galls on the same host. Two of these species use S. gigantea as a supplementary host. Larvae of the last generation of all multivoltine species must overwinter in dormant plant tissues underground and complete their development in inconspicuous galls in early spring, but this has not been confirmed in the present study.

Several other gall midges and the tephritid fly Procecidochares atra (Loew, 1862) develop in bud galls on goldenrods, but their galls can be easily distinguished from those of the Asphondylia species. The very common rosette galls of Rhopalomyia solidaginis Loew, 1862 on S. altissima contain white, soft-walled larval chambers that are hidden among the rosette leaves. By contrast, the rosette gall of A. solidaginis on the same host is smaller and flatter, and the single, rigid larval chamber is visible in the middle of the gall (Fig. 13). The compact, artichoke-like galls of P. atra contain a large, semi-open larval chamber, and are almost always found in aggregations. The very common bud galls of A. pseudorosa sp. nov. on E. graminifolia (Fig. 17) cannot be mistaken for the only other bud gall on this host, Rhopalomyia lobata Felt, 1908, the galls of which are usually much bigger and contain a large mass of spongy tissue in which the larval chambers are embedded.

**INQUILINES AND PARASITOIDS**

Asphondylia galls on goldenrods suffer remarkable parasitism and inquilinism rates, which often make it extremely difficult to find a single gall with an intact larva or pupa of the original gall inducer, let alone rear its damage to the gall or to the Asphondylia larva. More than ten species of parasitic Hymenoptera were reared from Asphondylia galls on goldenrods, the most common of which by far is Galeopsomyia haemon. This species forms ‘a gall within a gall’ during its development. Larvae of G. haemon induce the development of numerous dark, spherical structures inside the gall that are clearly made of plant tissues (Fig. 22). Each of these ball-like structures contains a single larval chamber with a single wasp larva. As the balls grow larger, they appear to physically crush the larva of the gall inducer, similar to what has been described for Asphondylia atriplicis (Townsend, 1893) galls on saltbush in Southern California (Hawkins & Goeden, 1982). Because they do not feed on the midge larvae, the wasps can be considered inquilines rather than parasitoids. By contrast, in galls of Asphondylia borrichiae Rossi & Strong, 1990 on Borrichia frutescens (Linneaeus) de Candolle, 1836 (Asteraceae), G. haemon seems to behave like a gregarious parasitoid (Stiling et al., 1992). In the galls of Asphondylia floccosa Gagné, 1986 on Atriplex polycarpa (Torrey) Watson, 1874 in Arizona, it has not been determined whether larvae of G. haemon feed on the midge larvae or on the gall tissue (Dixon et al., 1998). When we took the wasp ‘internal galls’ out of the ecidomyiid gall on goldenrods and isolated them in the lab, the wasps did not complete their development; however, when we left them inside the ecidomyiid gall, we were able to rear them in great numbers. This wasp was very common in all snap galls and to a lesser extent in the rosette galls of A. solidaginis, A. rosulata sp. nov., and A. pseudorosa sp. nov.

Additional hymenopteran parasites that were reared from the goldenrod galls in much smaller numbers included the following: Rileya insularis Ashmead, 1894; Tenuipetiolius teredon (Walker, 1843) and Tenuipetiolius Bugbee, 1951 sp. (Eurytomidae); Torymus advenus (Osten Sacken, 1870) and Torymus Dalman, 1820 sp. (Torymidae); Aprostocetus Westwood, 1833 sp.; Neochrystoscharis Kurdjumov, 1912 sp. (Eulophidae); and
KEY TO ASPHONDYLIA GALLS ON NORTH AMERICAN GOLDENRODS

1. Bud gall..................................................................................................................2
   – Leaf or inflorescence gall..................................................................................2
2. Gall in young Solidago altissima sprouts (in April–May; Figs 1, 2)..........................9
   – Gall in apical (usually) or lateral bud of mature goldenrod spp..........................3
3. Inconspicuous gall, up to 1 cm long, composed of two or three leaves that form a single chamber in shoot tips (Fig. 19).................................................................4
   – Gall composed of many leaves that form a rosette, at least 4 cm wide...............5
4. Gall on Solidago caesia..................................................................................5
   – Gall on Solidago nemoralis............................................................................5
5. Gall forms spherical structure, 5–8 cm in diameter, composed of many individual units, each with a central larval chamber (Figs 3–6).........................................................6
   – Gall not spherical, containing single, central chamber........................................7
6. On Solidago juncea, Solidago erecta, and Solidago uliginosa................................6
   – On Solidago sempervirens and Solidago bicolor....................................................8
   – Gall dorsoventrally flat, up to 5 cm wide; on Solidago spp. (Figs 13, 14)............8
   – Gall narrower, resembling a rosebud when young; on Euthamia graminifolia (Fig. 17).........................................................................................................................8
7. Gall forms dorsoventrally flat, up to 5 cm wide; on Solidago spp (Figs 13, 14).......8
   – Gall not spherical, containing single, central chamber........................................7
8. Gall on Solidago altissima.............................................................................11
   – Gall on Solidago rugosa..................................................................................11
9. Leaf gall, joining two or more adjacent leaves on various goldenrods................10
   – Gall on Euthamia graminifolia........................................................................10
10. Gall on Solidago spp......................................................................................10
   – Gall on Euthamia graminifolia........................................................................10
11. Gall on Solidago altissima.............................................................................11
   – Gall on Solidago rugosa..................................................................................11

Lyric Walker, 1842 sp. (Pteromalidae). Some of these parasitoids have been recorded from other Asphondylia galls on Asteraceae (e.g. Rossi et al., 2006), and appear to have specialized on this cecidomyiid group.

Species descriptions
In the following descriptions, the identity of the host plants and the description of the galls appear first, because these are the clearest and most obvious manifestations of the species. One is more likely to recognize species in this group (as in many other cecidomyiids) by the appearance and structure of their galls than by the morphology of adults or immature stages.

Asphondylia monacha Osten Sacken, 1869
Asphondylia monacha Osten Sacken, 1869: 299.

Hosts plants
Solidago juncea, S. erecta, S. uliginosa (summer generation), and S. altissima (spring generation).

Gall and biology
This species has two generations a year that induce distinct bud galls on different Solidago species. The early-spring generation was found only on S. altissima in April–May. Galls were discovered accidentally in early April while digging out rhizomes, as they developed in buds that grew from the rhizomes and were barely visible above ground (Fig. 1). Galled buds were wider and felt harder to the touch than normal buds, were 5 cm long and 2 cm wide, and contained a single chamber, the internal walls of which were lined by a thick layer of white mycelium. Each gall contained a single larva or pupa. In May, some galls were found in much longer sprouts (~15 cm long) that still appeared stunted and somewhat thicker than normal sprouts (Fig. 2). The larval chamber in these galls was situated at the very tip of the shoot. Adults of the spring generation emerged in May. The much more conspicuous summer-generation gall of this species on S. juncea (the host was incorrectly identified as S. canadensis in the original description) is a rosette bud gall that is found in great numbers (Fig. 3). The galls become apparent in mid-June and reach their final size while the larvae inside them are still tiny first instars. They are usually composed of 15–30 individual units, each with a single larval chamber that is surrounded by shortened leaves and lined internally by white mycelium. These units form a spherical structure on shoot tips that is 4–7 cm in diameter and can be spotted from
Figures 7–14. Galls: 7, 8, Asphondylia solidaginis snap galls on Solidago altissima; 9, Asphondylia solidaginis snap galls on Solidago gigantea; 10, Asphondylia solidaginis pupa in snap gall on Solidago altissima – gall was cut open to show pupa in fungus-lined chamber; 11, 12, Asphondylia rosulata sp. nov. snap galls on Solidago rugosa; 13, Asphondylia solidaginis rosette gall on Solidago altissima; 14, Asphondylia rosulata sp. nov. rosette gall on Solidago rugosa.
Figures 15–22. Galls: 15, *Asphondylia pseudorosa* sp. nov. inflorescence gall on *Euthamia graminifolia*; 16, *Asphondylia pseudorosa* sp. nov. snap gall on *Euthamia graminifolia*; 17, *Asphondylia pseudorosa* sp. nov. rosette galls on *Euthamia graminifolia*; 18, *Asphondylia* sp. from *Solidago* sp. (photo: Tom Murray); 19, 20, *Asphondylia silva* sp. nov. galls on *Solidago caesia*; 21, *Clinodiplosis comitis* sp. nov. larvae around *Asphondylia pseudorosa* sp. nov. bud gall; 22, *Galeopsomia haemon* ‘internal galls’ inside *Asphondylia pseudorosa* sp. nov. bud gall.

a distance. Adults of the summer generation emerged in late August to mid-September. Although it was not observed, we assume that adults of the autumn generation lay their eggs in plant tissue close to the ground and the hatching first-instar larvae overwinter next to dormant buds in the rhizomes. Old galls turn black and may remain on dry shoots of *S. juncea* throughout winter and into the next spring. Galls of similar structure were found on *S. erecta* (Fig. 4) and *S. uliginosa*, and our molecular analysis indicates that they are all induced by *A. monacha*. No morphological differences were found among adults from these three host plants. *Asphondyliia monacha* galls superficially resemble those of *Rhopalomyia solidaginis* on *S. altissima*, but they are not as wide and flat as the *Rhopalomyia* galls, are never found on *S. altissima*, and their structure is different, as indicated by Osten Sacken (1869) in his original description of *A. monacha*.

**Adult**

**General colour black.**

**Head:** Eye facets round. Palpus three-segmented, segments successively longer, with several strong setae and otherwise covered by microtrichia. Labella slightly pointed, with numerous strong setae on lateral surface.

**Antenna:** Scape and pedicel with long, dark setae. Male flagellomeres cylindrical, flagellomere 1 slightly longer than succeeding flagellomere, apical flagellomere slightly shorter than preceding flagellomere, all covered by anastomosing loops of circumfila, numerous strong setae, and microtrichia (Fig. 23); flagellomere 1/flagellomere 5 ratio = 1.13–1.34 (N = 23). Female flagellomeres 1–9 cylindrical with well-developed circumfila, with two transverse connections, numerous strong setae, and otherwise covered by microtrichia (Fig. 24); flagellomere 1 conspicuously longer than succeeding flagellomere, flagellomere 1/flagellomere 5 ratio = 1.41–1.62 (N = 38); flagellomeres 7 and onwards successively shorter; flagellomeres 10–12 with two whorls of circumfila and several longitudinal connections, numerous strong setae, and otherwise covered by microtrichia (Fig. 25); flagellomere 10 slightly longer than wide; flagellomere 11 slightly wider than long; flagellomere 12 rudimentary.

**Thorax:** Legs: densely covered by black scales other than a patch of white scales from apical part of femur to base of tibia, and from base of tarsomere 1 to first third of tarsomere 2; ventral part with silvery hair-like scales, coxae with long black setae. Tarsal claws thick, evenly curved; empodia longer than bend in claw. Wing: dark grey, densely covered by dark hair-like microtrichia (Fig. 18); length 2.00–2.80 mm in males (N = 41) and 2.01–3.30 mm in females (N = 57) of summer generation, 2.91–3.11 mm in males (N = 2) and 3.29–3.59 mm in females (N = 5) of spring generation; R1 joins C proximal to mid-length of wing, R5 joins C posterior to wing apex, M weak, CuA forked into CuA1 and CuA2.

**Female abdomen (Fig. 26):** Dorsum covered by black scales, pleuron and venter with silvery hair-like scales. Tergites 1–7 rectangular, with posterior one or two rows of strong setae and otherwise evenly covered by scales; tergite 8 narrower than preceding tergite, saddle-like, without setae. Stermites 2–6 with posterior row of setae and several setae on mid part; sternite 7 much longer than preceding sternite, narrowed posteriorly, with group of strong setae on posterior half. Ovipositor relatively long; sclerotized part 1.96–3.04 times as long as sternite 7 (N = 55) in summer-generation females, 2.62–3.25 times as long in spring-generation females (N = 6).

**Male abdomen (Fig. 27):** Colour pattern as in female. Tergite 1 narrow, band-like, without setae; tergites 2–7 rectangular, with posterior row of strong setae, few setae on basal area, and evenly scattered scales; tergite 7 more setose than preceding tergite; tergite 8 narrow, band-like, without setae. Stermites 2–6 rectangular, with posterior row of strong setae and several strong setae medially, otherwise evenly covered by scales. Sternite 7 more setose than preceding sternite. Sternite 8 with small but strongly setose sclerotized area.

**Male terminalia (Figs 28–30):** Gonocoxite compact, wide, and short, with short apical projection extending medially, bearing numerous strong setae and evenly setulose. Gonocoxal apodeme extending on both sides of aedeagus to form complex, strongly sclerotized structure (Figs 28, 30). Gonostylus round–ovoid, with numerous strong setae and otherwise evenly setulose, bearing crescent-shaped apical tooth. Aedeagus wide at base, tapered towards rounded apex, curved anteriorly in lateral view (Fig. 30). Hypoproct wide at base, deeply divided into two lobes apically, setose and setulose, with two longer setae apically on each lobe. Cerci completely or almost completely separated, bulbous, strongly setose and setulose throughout.

**Larva (third instar) (Fig. 31)**

Orange; integument covered by spicules. Length 2.06–2.84 mm (N = 6). Antennae about 1.5 times as long as wide; cephalic apodeme as long as head capsule. Spatula shape variable (Figs 32–37): lateral teeth slightly or conspicuously longer and more pointed than median teeth, gap between median teeth slightly or clearly deeper than gaps between lateral and median teeth, shaft thick and well-sclerotized in summer-generation.
larvae (Figs 35–37), thinner and less sclerotized in spring-generation larvae (Figs 32–34).

Pupa (Figs 62–65)
The pupae of the summer and spring generations differ from each other in the shape of the antennal horns. In summer-generation pupae antennal horns are robust, wide at base, slightly arched (Fig. 65), with apices flat and finely serrated in frontal view (Fig. 64); in spring-generation pupae antennal horns are longer, more slender (Fig. 63), and are tapered at apex in frontal view (Fig. 62). Other attributes are similar in pupae of both generations, as follows. Cephalic seta minute. Upper facial horn divided into two apices separated by shallow, curved notch. Lower facial horn curved dorsally at apex, on each side with two papillae, one bearing a relatively long seta. Frons on each side with three lateral papillae: one setose and two asetose. Prothoracic spiracle long and slender, with widened base; trachea ends at apex. Abdominal segments, except for first, each

Figures 23–27. Asphondylia monacha: 23, male flagellomere 5; 24, female flagellomere 5; 25, female flagellomeres 10–12; 26, female abdomen; 27, male abdomen. Scale bars: 0.1 mm.

with posterior straight row and two or three anterior less ordered rows of spikes.

Notes
We could not find any substantial morphological differences among populations from *S. juncea*, *S. erecta*, and *S. uliginosa*, and our molecular analysis indicates that all belong to *A. monacha*. The galls on *S. uliginosa* are somewhat smaller than those on the other two host plants, and were also found in lateral buds, whereas galls on *S. juncea* and *S. erecta* almost always develop in apical buds. Adults reared from galls on *S. uliginosa* were likewise smaller than those from the two other hosts. Additional molecular work on the *S. uliginosa* population may show that it represents a separate species.

Adults of the spring generation that develop on *S. altissima* are clearly bigger than those of the summer generation. Based on the collection date, one individual from the Felt collection represents the spring generation of *A. monacha*, but the host from which it was reared is not indicated. It is possible that the spring generation of *A. monacha* induces galls on other *Solidago* species in areas where *S. altissima* is uncommon, but such galls have not been found in the present study. Aggregated bud galls that are very similar to those of *A. monacha* are also found on *S. sempervirens* (Fig. 5) and *S. bicolor* (Fig. 6), and although we could not find morphological differences between *A. monacha* and individuals from these populations, our molecular analysis indicates that the latter belong to one or more undescribed species. There are additional *Solidago* species on which similar composite galls have been observed, and further molecular study will probably be necessary to determine whether they belong to *A. monacha* or to undescribed species. Felt (1908, 1916) attributed rosette and inflorescence galls on *Euthamia lanceolata*, as well as leaf galls on *S. gigantea* or *S. Canadensis*, to *A. monacha*, but these galls belong to different species discussed in the present paper.

Material examined
*Spring generation (from *S. altissima* bud galls)*: 4♀, 1 exuviae, 3 larvae, USA, PA, Route 642 (40°59.114′N

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76°38.567′W), 28 April 2005, N. Dorchin; 3♀, 2 exuviae, USA, PA, Montour Environmental Preserve, 25 May 2007, N. Dorchin; 1 larva, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 1 May 2005, N. Dorchin; 1♂, 2 exuviae, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 13 May 2005, N. Dorchin; 5 pupae, USA, PA, multiple localities, May 2005–2007, N. Dorchin (on SEM stubs).

Material from Felt collection with no indication of host:
1♀, USA, NJ, Orange Mountain, May 1907? (Felt no. 813; USNM).

From S. juncea: Gall (syntype), USA, NY, near Brooklyn, 1867 (USNM); 2 larvae, USA, MD, Wheaton Park, 22 August 1976, R.J. Gagné (USNM); 1 larva, USA, PA, Pittsburgh, 13 August 1991, J. Plakidas (USNM); 1♂, USA, PA, Warrendale, 30 August 1991, J. Plakidas (USNM); 1♀, 1♂, 3 larvae, USA, PA, Lewisburg, 22 August 2005, N. Dorchin; 1♂, 2♀, USA, PA, Route 642 (40°59.114′N 76°38.567′W), 16 September 2005, N. Dorchin; 1♂, USA, PA, Mauses Creek, 16 September 2005, N. Dorchin; 3♂, 12♀, 7 exuviae, USA, PA, Lewisburg, 23 August 2007, N. Dorchin; 1♂, USA, PA, Lewisburg, 30 August 2007, N. Dorchin; 12♂, 11♀, USA, VA, Bedford, Sharp-top, 15 September 2012, M.J. Wise; 9 pupae, USA, PA, multiple localities, August–September 2005–2007, N. Dorchin (on SEM stubs).


From S. uliginosa: 7♂, 6♀, USA, ME, Winter Harbor, 7 September 2007, R.J. Gagné (USNM).

Material from Felt collection with no indication of host and collector (recognized by Felt as A. monacha; all in

USNM): 1♀, USA, NY, Albany, 11 June 1906 (Felt no. 208); 1♂, USA, NY, Albany, 20 July 1906 (Felt no. 650a); 1♂, 1♂♀, USA, NY, Karner, 5 August 1906 (Felt no. 1583); 1 exuviae, USA, NY, Albany, 4 September 1906 (Felt no. 1200); 1♂, USA, NY, Albany, 21 August 1906 (Felt no. 761); 1♂♀, USA, NY, Nassau, 17 September 1906 (Felt no. 1336); 1♀, USA NY, Albany, 20 July 1907 (Felt no. 1568a); 1♂♀, USA, NY, Bath, 24 July 1907 (Felt no. 1568a); 1 larva, USA, NY, Albany, 24 July 1907 (Felt no. 1583); 1♂♀, USA, NY, Bath, 16 July 1907 (Felt no. 1568a); 1♂♀, USA, NY, Nassau, 7 August 1907 (Felt no. 1583a); 1♀, 1 exuviae, USA NY, Bath, 18 July 1907 (Felt no. 1268); 1♂♀, USA, MA, Magnolia, 11 August 1908 (Felt no. 1879).

**Asphondylia solidaginis** Beutenmüller, 1907

Asphondylia solidaginis Beutenmüller, 1907: 305.

**Host plants**

* Solidago altissima and *S. gigantea.*

**Gall and biology**

This species has several generations a year and it forms two very different types of galls. The galls that are formed in spring and early summer are blister leaf galls that join two (and sometimes three or four) leaves together like a snap (Figs 7–10). When made of two leaves, one leaf contributes the bottom part of the gall and the other contributes the upper part to form a single-chambered gall that is thickly lined by white mycelium on the inside. An individual leaf can participate in multiple galls, forming the upper part of some and the bottom part of others. These ‘bifoliate’ galls (termed snap galls in this paper) are very abundant on *S. altissima* and can sometimes also be found on *S. gigantea.* Adults emerge from the galls in June and July. From June to August, a rosette gall also appears in the apical or axillary buds of *S. altissima.* These rosette galls are small (3–5 cm in diameter), composed of shortened leaves, and contain a single chamber at their centre (Fig. 13). The chamber is formed by several very short leaves that are attached together to form a small, rigid cone, and is lined internally by white mycelium. Adults emerge from these rosette galls from late June to late August, when the leaf snap galls become less abundant. Although the snap galls dominate in early summer and the rosette galls dominate in late summer, the two types overlap in June–July, and the type of gall that will result from an oviposition event is apparently determined by the location in the plant where the egg is laid. As is nicely described by Beutenmüller (1907), the snap galls are formed in immature leaves in young, rapidly growing buds, so that the leaves that form the gall remain attached as they grow. The later-developing rosette galls are probably induced in more mature buds that develop more slowly.

**Adult**

Characters as described in *A. monacha* except for the following.

**Head:** Flagellomere 1/flagellomere 5 ratio = 1.19–1.35 in male (*N* = 7), 1.53–1.72 in female (*N* = 5).

**Thorax:** Wing length 2.15–2.62 mm in males (*N* = 7), 2.26–2.80 mm in females (*N* = 5).

**Female abdomen:** Sclerotized part of ovipositor 2.44–3.20 times as long as sternite 7 (*N* = 6).

**Male terminalia:** Aedeagus cylindrical, same width throughout length, tapered at apex.

**Larva (third instar)**

Orange; integument covered by round, flat bumps. Length 2.66–3.59 mm (*N* = 7). Antennae about 1.5 times as long as wide; cephalic apodeme as long as head capsule. Spatula shape relatively uniform among larvae from *S. altissima* (Figs 38, 39), with lateral teeth the same length or slightly longer than median teeth, and equal gaps between all teeth. In larvae from *S. gigantea,* median teeth considerably smaller than lateral teeth, and gap between median teeth much deeper than gap between lateral and median teeth (Figs 42–44). Shaft well sclerotized in larvae from both host plants.

**Pupa (Figs 66, 67)**

Characters as described in *A. monacha,* except for the following: antennal horns long and slender, almost straight (Fig. 67), apices splayed, pointed and finely serrated in frontal view (Fig. 66); cephalic seta minute; upper facial horn divided into two apices separated by deep notch.

**Notes**

We did not find morphological differences among adults or immature stages from the two types of galls induced by this species, and our molecular analysis suggests that individuals from these galls belong to the same species, as the respective haplotypes are intermingled. Although adult morphology is virtually identical between individuals from *A. solidaginis* and *A. monacha,* their pupae differ in the shape of the antennal horns, which are shorter and wider in *A. monacha* than in *A. solidaginis* (as well as than those of all other *Asphondylia* species from *Solidago* described in this paper). Our molecular analysis shows that *A. solidaginis* uses *S. gigantea* as a complementary host on which it induces snap galls (but never bud galls), whereas snap galls on *S. rugosa* and *E. graminifolia* belong to different species.
Asphondylia solidaginis snap galls on *S. altissima* cannot be mistaken for any other gall on this host, but the bud galls induced by this species later in the season may superficially resemble bud galls of other Diptera on this plant. The differences among these galls were summarized above.

**Material examined**
The type series of *A. solidaginis*, consisting of several male and female specimens from New York, New Jersey, and North Carolina, could not be located in the American Museum of Natural History in New York, where it was originally deposited, and is presumed to be lost (D. Grimaldi, pers. comm.). We therefore designate a neotype for this species as follows.

**Neotype:** Pupal exuviae, USA, PA, Dale’s Ridge, 18 June 2005, N. Dorchin; taken from leaf snap gall on *S. altissima*. The neotype is mounted on a permanent microscope slide in Euparal and is deposited in the USNM.

Other material examined in this study includes the following.

**From *S. altissima* snap galls:** 4 exuviae, USA, MD, Beltsville, July 1979, R.J. Gagné; 1 larva, USA, PA, Pittsburgh, Weible Rd., 22 June 1991, J. Plakidas (USNM); 1 larva, USA, PA, Dale’s Ridge, 19 June 2006, N. Dorchin; 1 larva, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 21 June 2006; 1 larva, USA, PA, Montour Environmental Preserve, 30 June 2006, N. Dorchin; 1 larva, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 7 June 2007, N. Dorchin; 1♂, 2♀, USA, PA Montour Environmental Preserve, 12 June 2007, N. Dorchin; 1♀, 3♀, USA, PA, Mifflinburg, 14 June 2007, N. Dorchin; 1 larva, USA, PA, Dale’s Ridge, 17 June 2007, N. Dorchin; 3♀, 1♀, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 24 June 2007, N. Dorchin; 8 pupae, USA, PA, multiple localities, June–July 2006, 2007, N. Dorchin (on SEM stubs).

**From *S. altissima* rosette galls:** 2 larvae, USA, PA, Lairdsville, 19 July 2007, N. Dorchin; 2 exuviae, 1♂, 1♀, USA, PA, Route 642 (40°59.114′N 76°38.567′W), 20 July 2007, N. Dorchin and D. Ryan; 1♂, 1♀, USA, PA, Lewisburg, Furnace Road, 26 July 2007, D. Ryan; 1♀, USA, PA, Lewisburg, Stein Lane, 15 August 2007, N. Dorchin; 2 pupae, USA, PA, Lairdsville, July 2007, N. Dorchin (on SEM stubs).

**From *S. gigantea* snap galls:** 1 larva, USA, PA, Montour Environmental Preserve, 20 June 2006, N. Dorchin; 1 larva, USA, PA, Dale’s Ridge, 19 June 2006, N. Dorchin; 1 larva, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 21 June 2006; 1 larva, USA, PA, Montour Environmental Preserve, 30 June 2006, N. Dorchin; 1 larva, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 7 June 2007, N. Dorchin; 1♂, 2♀, USA, PA Montour Environmental Preserve, 12 June 2007, N. Dorchin; 1♂, 3♀, USA, PA, Mifflinburg, 14 June 2007, N. Dorchin; 1 larva, USA, PA, Dale’s Ridge, 17 June 2007, N. Dorchin; 3♀, 1♀, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 24 June 2007, N. Dorchin; 8 pupae, USA, PA, multiple localities, June–July 2006, 2007, N. Dorchin (on SEM stubs).

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**Figures 38–44.** *Asphondylia* larval spatulae: 38, 39, *Asphondylia solidaginis*; 40, 41, *Asphondylia rosulata* sp. nov.; 42–44, spatulae of larvae taken from *Solidago gigantea* snap galls. Scale bars: 0.1 mm.

**Asphondylia pseudorosa** Dorchin sp. nov.

*Host plants*

*Euthamia graminifolia*.

**Gall and biology**

This species has several generations between late June and mid-September, during which it forms two types of galls in buds and inflorescences. The bud galls are single-chambered and contain only one larva; they are composed of a cluster of leaves that are wider and shorter in the outer part of the gall, and thinner and longer in its inner part, resembling a rosebud (Fig. 17). Some of the wider leaves have a very wide central longitudinal vein that is lighter in colour than the remainder of the leaf. Galls are 40–200 mm wide at the base, and most often found in the apical bud but sometimes also in lateral buds. The base of the galled bud is wide and somewhat rigid. The innermost leaves are joined to form a conical, apically tapered chamber, the internal walls of which are lined by white mycelium. As the gall matures, the innermost leaves turn black. The galls are very common from late June to mid-August, and become scarcer thereafter. Adults were reared from them from late June to early September. Beginning from August, when inflorescences start developing on the host plants, these are also galled by *A. pseudorosa* sp. nov. Infested inflorescence buds do not usually show any external signs of infestation, although they may appear somewhat wider than normal buds (Fig. 15). The larva feeds on the developing inflorescence within the closed bud, and the gall is accompanied by a white fungal mycelium that lines its internal walls. Larvae and pupae are found in the inflorescences during August, and adults emerge from mid-August to mid-September. The bud galls of this species are heavily attacked by the predator/inquiline *Youngomyia podophyllae*, the large orange larvae of which usually occupy the central chamber of the gall, but are occasionally found among its outer leaves. Between two and ten inquiline larvae can be found in a single gall, usually with no signs of the larva of the gall inducer, but it is not clear whether they actually prey on it before taking over the gall. The inquiline larvae are very active and squirm out of the gall when disturbed. When they are present in the gall, the fungus lining the central chamber is not as apparent as when the *Asphondylia* larva is present. Some inflorescence galls were also found to contain inquilinous larvae, usually with one found per gall. The larvae of the gall inducer and the inquiline are both orange, but the latter are much bigger, longer, and more active, and the two species are easily distinguished by their spatula shape. Larvae of a second species of gall midge inquiline, *Clinodiplosis comitis* sp. nov., were often found among the outer leaves of the bud gall. These are much smaller than both the *Asphondylia* and the *Youngomyia* larvae, and are pale yellow. Other natural enemies found in the galls are two species of parasitic wasps: one that induces dark, spherical endo-galls, such as those found in *Asphondylia* snap galls, and the other that pupates freely inside the gall. Both are usually found in groups of between four and eight individuals. Parasitism rates in galls may reach 90%.

**Adult**

Colour pattern as in *A. monacha*. Individuals from bud galls are notably larger than those from inflorescence galls.

**Head** (Figs 45, 46): Eye facets round. Frons with two groups of seven to ten setae. Palpus three-segmented, segment 3 about as long as segment 2, all segments with several strong setae and otherwise covered by microtrichia. Labella slightly pointed, with numerous strong setae on lateral surface.

**Antenna**: Scape and pedicel with long, dark setae. Male flagellomere 1 about as long as succeeding flagellomere, apical flagellomere slightly shorter than preceding flagellomere, all flagellomeres covered by anastomosing loops of circumfila, numerous strong setae, and microtrichia; flagellomere 1/flagellomere 5 ratio = 1.04–1.27 (N = 14). Female flagellomeres 1–9 cylindrical, with two circumfila whorls, numerous strong setae, and otherwise covered by microtrichia; flagellomere 1 only slightly longer than succeeding flagellomere (flagellomere 1/flagellomere 5 ratio = 1.31–1.42, N = 7); flagellomeres 7–12 successively shorter; flagellomeres 10 and 11 with two whorls of circumfila and one or two longitudinal connections, numerous strong setae, and otherwise covered by microtrichia; flagellomere 12 spherical, with a single loop of circumfila (Fig. 47).

**Thorax**: Legs: densely covered by black scales other than a patch of white scales from base of tarsomere 1 to first third of tarsomere 2; ventral part with silvery hair-like scales, coxae with long black setae. Tarsal claws thick, evenly curved; empodia longer than bend in claw. Wing: dark grey, densely covered by dark hair-like microtrichia; wing length 2.38–2.56 mm in males (N = 7), 2.33–2.98 mm in females (N = 3) from bud galls; 1.57–1.92 mm in males (N = 9), 1.62–1.75 mm in females.
\( N = 5 \) from inflorescence galls; R1 joins C before mid-length of wing, R5 joins C behind wing apex, M very weak, CuA forked.

**Female abdomen:** Dorsum covered by black scales, pleuron and venter with silvery hair-like scales. Tergites 1–7 rectangular, with posterior row of strong setae and otherwise evenly covered by scales; tergite 8 narrower than preceding tergite, without setae. Sternites 2–6 with posterior row of setae and several setae on mid-dorsal part; sternite 7 much longer than preceding sternite, narrowed posteriorly, with group of strong setae on posterior half. Sclerotized part of ovipositor 2.10–2.61 as long as sternite 7 \( (N = 2) \) in individuals from bud galls; 2.23–2.53 times as long as sternite 7 \( (N = 5) \) in individuals from inflorescence galls.

**Male abdomen:** Colour pattern as in female. Tergite 1 narrow, band-like without setae; tergites 2–7 rectangular, with posterior row of setae and evenly scattered scales; tergite 7 more setose than preceding tergite; tergite 8 narrow, band-like dorsally, widened ventrally, sometimes completely unsclerotized, without setae. Sternites 2–6 rectangular, with posterior row of setae and several setae medially, otherwise evenly covered by scales. Sternite 7 more setose than preceding sternite. Sternite 8 with small but strongly setose sclerotized area.

Figures 45–49. *Asphondylia pseudorosa* sp. nov.: 45, male head; 46, female head; 47, female flagellomeres 10–12; 48, male terminalia, dorsal; 49, male hypoprocts showing intraspecific morphological diversity. Scale bars: 0.1 mm.
Male terminalia (Fig. 48): Gonocoxite compact, wide, and short, with short apical projection extending medially; bearing numerous strong setae and evenly setulose. Gonocoxal apodeme extending on both sides of aedeagus to form strongly sclerotized structure. Gonostylus ovoid, with numerous strong setae and otherwise evenly setulose, bearing crescent-shaped apical tooth. Aedeagus cylindrical, same width throughout length, tapered at apex. Hypoproct wide at base, divided into two lobes apically by notch of variable size: deep in some individuals, very shallow in others (Fig. 49), setose and setulose, with one long seta apically on each lobe. Cerci separated to or almost to base, bulbous, strongly setose and setulose throughout.

Larva (third instar)
Orange; integument covered by tiny, shallow bumps. Length 1.44–3.26 mm (N = 18). Spatula (Figs 50–54): variable in shape; median teeth shorter than lateral teeth, sometimes rudimentary (Fig. 52); gap between median teeth clearly deeper than gaps between lateral and median teeth, shaft well-sclerotized, especially along mid-section.

Pupa (Figs 70–73)
Antennal horns clearly different between pupae from buds and pupae from inflorescences. In pupae from buds, antennal horns long, almost straight, apices pointed and serrated in frontal view; in pupae from inflorescences, antennal horns very short, wide and blunt in frontal view. Cephalic setae minute. Upper facial horn divided into two apices separated by shallow, curved notch. Lower facial horn curved dorsally at apex, on each side with two papillae, one bearing relatively long seta. Frons on each side with three lateral papillae, one setose, two asetose. Prothoracic spiracle long and slender, with widened base; trachea ending at apex. Abdominal segments, except for first, each with posterior straight row and two or three anterior, less ordered, rows of spines.

Diagnosis
*Asphondylia pseudorosa* sp. nov. can be easily distinguished from other *Asphondylia* spp. on goldenrods by association with its host plant and galls: the bud gall is single-chambered, resembling a small rosebud, and is not as flat as the galls of *A. rosulata* sp. nov. and
A. solidaginis on Solidago rugosa and S. altissima, respectively. Pupae developing in buds are very similar morphologically to those of A. rosulata sp. nov., A. solidaginis, and A. silva sp. nov., which all differ from pupae of A. monacha in having more slender, tapered antennal horns. Individuals of A. pseudorosa sp. nov. that develop in inflorescences are the smallest of the Asphondylia species on goldenrods, and are the only ones known to use this plant organ. Their pupa differs from that of all other goldenrod-associated Asphondylia in having short antennal horns that are distally truncate rather than tapered.

Etymology
The name pseudorosa refers to the typical appearance of the galls, which resemble rosebuds.

Notes
Smaller bud galls of similar structure to those described above commonly develop in lateral buds on this host, and at this point it is unclear whether they represent a separate species. The adults and immature stages described here were taken from the larger bud galls. Some pupae taken from small bud galls have shorter, blunter antennal horns than those of pupae from large galls, representing a transitional condition between pupae from large buds and from inflorescences. More detailed morphological and molecular work will be needed in order to determine whether the different types of bud galls on Euthamia graminifolia represent different species. Our molecular analysis failed to separate between individuals from bud and inflorescence galls, hence they are described here as belonging to the same species.

Leaf snap galls are uncommonly found on E. graminifolia (Fig. 16), and we were unable to rear adults from them. We attribute these galls to A. pseudorosa based on the pupal morphology and on the similar situation observed in A. solidaginis and A. rosulata sp. nov., where the same species induces both snap and bud galls on its host plant.

Type material
Holotype: ♀, USA, PA, RB Winter State Park, 10 September 2006, N. Dorchin, ex. bud gall on Euthamia graminifolia (TAUI).


Other material examined
Ex bud galls: 1♀, USA, PA, RB Winter State Park, 22 July 2007, N. Dorchin; 1♀, USA, PA, Pine Creek, Hampton, 8 August 2010, J. Plakidas (Plakidas, private collection); 1♂, USA, PA, Dorseyville Rd. Fox Chapel, 8 August 2010, J. Plakidas (Plakidas, private collection); 7 pupae, USA, PA, various localities, June–August 2006–2007, N. Dorchin (on SEM stubs). Ex inflorescence galls: 1♂, USA, PA, Route 642 (40°59.114′N 76°38.567′W), 18 August 2006, N. Dorchin. 2 pupae, USA, PA, Mauses Creek, 8 August 2006, N. Dorchin (on SEM stub).

ASPHONDYLIA ROSULATA DORCHIN sp. nov.

Host plants
Solidago rugosa, S. gigantea.

Gall and biology
This species has several generations a year and forms snap and bud galls (Figs 11, 12, 14), similar to the situation in A. solidaginis. Snap galls join two or more leaves together, are formed in spring and early summer, and are usually found on leaves very close to the apical bud, rather than on more mature leaves farther down the shoot. In this respect, the galls of A. rosulata sp. nov. constitute intermediate steps between snap and rosette galls, and the distinction between these two types is not as clear as in A. solidaginis (Figs 7, 8, 13). As in A. solidaginis, this species appears to use S. gigantea as a complementary host, as indicated by our molecular analysis, but the bud galls are found only on the primary host: S. rugosa. The small rosette galls develop only in apical buds and can be locally very common. These galls are composed of several shortened leaves that surround a single central, rigid chamber made of closely attached leaves (Fig. 14), and are lined by white mycelium on the inside. Adults emerge from these galls from late June to late August.

Adult
Characters as in A. monacha, except for the following.

Head: Flagellomere 1/flagellomere 5 ratio = 1.11–1.18 in male (N = 5), 1.38–1.48 in female (N = 5).

Thorax: Wing length 2.62–2.85 mm in males (N = 6), 2.61–2.78 mm in females (N = 6).

Female abdomen: Sclerotized part of ovipositor 2.31–2.56 as long as sternite 7 (N = 5).

Male terminalia: Aedeagus cylindrical, same width throughout length, tapered at apex.

Larva (third instar)
Orange; integument covered by round, flat bumps. Length 2.18–3.46 mm (N = 3). Antennae about as long as wide; cephalic apodeme slightly longer than head capsule. Spatula (Figs 40, 41) with lateral teeth longer than median teeth, and gap between median teeth much deeper than between lateral and median teeth.

Pupa (Figs 68, 69)
Characters as in A. monacha, except for the following: antennal horns long and slender, almost straight, apices tapered and finely serrated along median margins in frontal view.

Diagnosis
Asphondylia rosulata sp. nov. can be distinguished from other Asphondylia spp. on goldenrods by its host plant and gall structure. The bud gall resembles that of A. solidaginis on S. altissima, and is flatter than that of A. pseudorosa sp. nov. Pupae are morphologically similar to those of A. solidaginis, A. pseudorosa sp. nov., and A. silva sp. nov., all having antennal horns that are more slender than those of A. monacha.

Etymology
The species is named after its gall, which forms a small rosette in apical buds.

Notes
We did not find morphological differences among adults from the two types of galls induced by this species, and our molecular analysis indicated that they belong to the same species. The small rosette galls of this species may be mistaken for the superficially similar galls of Rhopalomyia solidaginis on S. rugosa but differ from them in being flatter rather than spherical, composed of a smaller number of leaves, and containing a central, rigid larval chamber that is lined by white mycelium, similar to the rosette galls of A. solidaginis.

Paratypes: 1 exuviae, USA, MD, Silver Spring, unspecified date, R.J. Gagné (USNM); 1 larva, USA, PA, Pittsburgh, Weible Road, 15 August 1991, J. Plakidas (USNM); 1 ♀, USA, PA, Lairdsville, 9 August 2006, N. Dorchin; 4 exuviae, 4♂, 4 ♀, USA, PA, Black Moshannon State Forest, 8 July 2007, N. Dorchin and M.J. Wise (1♂, 1 ♀ USNM, others TAUI), 2 larvae, USA, PA, Lairdsville, 19 July 2007. N. Dorchin & D. Ryan.

Other material examined
1♂, 1 ♀, 1 exuviae, USA, PA, Fox Chapel, Squaw Run Road, 25 June 2010, J. Plakidas (Plakidas, private collection).

Asphondylia silva Dorchin sp. nov.

Host plants
Solidago caesia.

Gall and biology
This species induces very small, single-chambered galls in apical shoot tips (Figs 19, 20). The gall is composed of several very short leaves that are pressed together to form a conical chamber, the internal walls of which are lined by a white layer of mycelium. Each gall contains a single larva. Galls are 4.5–7.5 mm long and 1.5–3.0 mm wide, and are barely noticeable. They may be very common in some localities, but absent in others. The species has at least two generations between June and September; galls were first apparent in early June and adults emerged from them in early July. In early September, galls were found among flower buds on the shoot tips, and all were already empty, some with pupal skins stuck in them (Fig. 20). This species is heavily parasitized, and thus very few galls yielded adult midges in the laboratory. Out of six regularly surveyed localities in central PA, galls were found in only two (Shikellamy State Park and Dale’s Ridge), where they were consistently abundant.

Adult
Characters as in A. monacha, except for the following.

Head: Flagellomere 1/flagellomere 5 ratio = 1.09–1.23 in male (N = 3), 1.41–1.56 in female (N = 4).

Thorax: Wing length 2.37–2.63 mm in males (N = 3), 2.15–2.51 in females (N = 4).

Female abdomen (Fig. 58): Sclerotized part of ovipositor 2.15–2.24 times as long as sternite 7 (N = 4).

Male terminalia (Fig. 59): Aedeagus about same width throughout length, slightly tapered towards rounded apex. Hypoproct with very shallow notch apically.

Type material
Holotype: ♀, USA, PA, Lewisburg, 26 June 2007, G. Lee and D. Ryan, from Solidago rugosa leaf snap gall (TAUI).
Figures 55–61. *Asphondylia silva* sp. nov.: 55, male head; 56, female head; 57, female flagellomere 5; 58, female abdomen; 59, male terminalia, dorsal; 60, 61, larval spatulae. Scale bars: 0.1 mm.

Larva (third instar)
Orange; integument covered by small bumps. Length 2.02–3.26 mm (N = 6). Antennae about 1.0–1.5 times as long as wide; cephalic apodeme as long as head capsule. Spatula shape variable (Figs 60, 61), lateral teeth longer than median teeth, gap between median teeth as deep as, or clearly deeper than, gaps between lateral and median teeth, shaft thick and well sclerotized.

Pupa (Figs 74, 75)
Characters as in A. monacha, except for the following. Antennal horns long and slender, only slightly curved, apices pointed and finely serrated in frontal view.

Diagnosis
This is the second smallest Asphondylia species from goldenrods and the only one that is found on S. caesia, hence it can be easily recognized from its host and tiny bud gall. Other than their size and the slightly different shape of the male hypoproct, adults of this species do not differ morphologically from those of A. solidaginis, A. rosulata sp. nov., and A. pseudorosa sp. nov., but molecular data consistently indicate that this is a distinct species most closely related to A. rosulata sp. nov.

Etymology
The name silva (Latin for forest) refers to the typical habitat in which this species is found.

Type material


Other Asphondylia species on goldenrods
Aggregate bud galls that resemble those of A. monacha on S. juncea and S. erecta are known from several other goldenrod species, including S. patula, S. odorata, and an undetermined Solidago species from Florida, based on material in the National Museum of Natural History, Washington, D.C. (USNM). Populations that are associated with S. sempervirens in Maine and Massachusetts (Fig. 5), and with S. bicolor in Virginia (Fig. 6), were studied in detail in the present work, and although we did not find morphological differences between them and A. monacha, our molecular analysis suggests that they belong to at least two undescribed species. These species are not described here because additional molecular data are needed in order to determine their boundaries and host associations. Their galls are locally common and appear similar in structure to those of A. monacha, although some galls on S. sempervirens develop in lateral rather than apical buds (C. Eiseman, pers. comm.). Galls are composed of numerous individual units, each with a single central chamber surrounded by several short leaves that are aggregated together to form a spherical structure on apical buds. Adult midges emerged from S. sempervirens from mid-August to mid-September, and from S. bicolor from early September to mid-October. Like other Asphondylia galls from goldenrods, these are also commonly attacked by the inquiline Yougomyia podophyllae and by several parasitoid Hymenoptera species. One individual from a rosette gall on S. uliginosa is also included in the sempervirens/bicolor clade, whereas another individual from this host grouped with A. monacha, suggesting that S. uliginosa is used by two different species.

Leaf snap galls were observed once during this study on S. nemoralis, but we were unable to rear adults from them or to characterize them with molecular tools. Similar galls may be found on other Solidago hosts but the identity of the species that induce them requires further study. Similarly, small rosette galls that resemble those of A. rosulata sp. nov. were observed by R.J. Gagné (pers. comm.) on Solidago tortifolia Elliott, 1823 in Maryland in late October, but the identity of the gall inducer is currently unknown.

Asphondylia johnsoni Felt (1908) has been described from an unknown species of Solidago and an unknown gall collected in Lansdowne, PA, USA, and is separated from A. monacha based on differences in adult colour, which is hardly a reliable character. The type we examined and Felt’s more detailed description of the male and pupa do not offer conclusive taxonomic information (Felt, 1916). Without a known host and gall, it is impossible to determine whether A. johnsoni is a distinct species or to verify its identity through future collections. We therefore assign this species to nomina dubia.

Inquilines in Asphondylia galls on goldenrods

Clinodiplosis comititis Dorchin sp. nov.

Hosts and biology
This species lives as an inquiline in Asphondylia monacha and A. pseudorosa sp. nov. galls. Dozens of tiny, yellowish larvae were found frequently among the rosette leaves (Fig. 21), but we never observed them actually interacting with larvae of the gall inducer. They
Figures 70–77. Pupal heads: 70, 71, *Asphondylia pseudorosa* sp. nov. pupae from inflorescence galls; 72, 73, *Asphondylia pseudorosa* sp. nov. pupae from bud galls; 74, 75, *Asphondylia silva* sp. nov.; 76, *Asphondylia silva* sp. nov., dorsal spines on pupal abdominal segments; 77, *Clinodiplosis comitis*, female cerci. Scale bars: 200 μm, except 80 μm in Fig. 76.

leave the gall to pupate in the ground and adults emerge 10–14 days later. This is an extremely delicate cecidomyiid whose dark eyes stand out on the background of an otherwise whitish body. Several consecutive generations appear to develop during spring and summer, and larvae of the last generation most probably overwinter in the ground.

**Adult**

Tiny whitish-hyaline gall midge with black eyes.

**Head** (Fig. 78): Small dorsal projection at top of vertex present just behind eyes, bearing two setae. Eye facets round. Palpus four-segmented; segment 1 about as long as wide, segments 2 and 3 about 2.5 times as long as segment 1, segment 4 about 4.0 times as long as segment 1; all segments with fine, long setae and otherwise covered by microtrichia. Face with four or five short setae on each side. Labrum and labella pointed, strongly setose. Antenna: 12 flagellomeres in both sexes; first two flagellomeres fused. Male flagellomeres 1–11 trinodal (Fig. 79): first node setulose, with basal whorl of strong setae, distal whorl of long-looped circumfila, followed by long, bare neck; second node setulose, with one median circumfilar whorl, followed by third setulose node, with basal whorl of strong setae and whorl of long-looped circumfila, followed by long, mostly bare neck. Flagellomere 12 with third node followed by long and thin vestigial, setulose appendage, 0.2–0.3 times as long as flagellomere. Both proximal and distal necks

Figures 78–83. Clinodiplosis comitis sp. nov.: 78, head; 79, male flagellomere 3; 80, female flagellomere 9; 81, female flagellomere 12; 82, wing; 83, acropod. Scale bars: 0.1 mm.
longer in distal flagellomeres than in proximal flagellomeres: neck 1 to node 1 ratio 0.83–1.37 for flagellomere 3 (N = 8), 1.10–1.47 for flagellomere 7 (N = 7); neck 2 to nodes 2 + 3 ratio 0.66–0.90 for flagellomere 3 (N = 8), 0.89–1.14 for flagellomere 7 (N = 7). Female flagellomeres cylindrical, setose and setulose, with simple circumfila and long bare necks of same relative length throughout antenna (Fig. 80); neck to node ratio for flagellomere 7, 0.61–0.80 (N = 12). Flagellomere 12 with long vestigial appendage, about 0.3 times as long as flagellomere, setose and setulose, rounded apically (Fig. 81).

Thorax: Legs: tarsal claws bent beyond mid-length, untoothed (Fig. 83); empodia almost reaching bend in claw. Wing (Fig. 82): completely transparent, with sparse, delicate hairs; length 1.60–2.00 mm in male (N = 11), 1.40–2.20 mm in female (N = 10). R1 joins C at third of wing length, R5 joins C far beyond wing apex, Rs incomplete, situated around midlength of R1; M weak, CuA forked, with CuA2 strongly curved posteriad.

Female abdomen (Fig. 84): Sclerites rectangular, virtually undifferentiated from surrounding membrane; tergites with posterior row and one or two median rows of setae; sternites with uniformly scattered setae; no discernible trichoid sensilla. Ovipositor protractible, about 3.5 as long as sternite 7. Cerci large, setose and setulose, ventral and apical areas with numerous thicker, blunt sensory setae ending in apical pore (Fig. 77).

Male abdomen: Segments and setation as described in female.

Male terminalia (Fig. 86): Gonocoxites slender, with strong setae on mid-distal half. Gonostylus long and slender, only slightly arched, about same width throughout length, with numerous evenly spread short setae, bearing small apical tooth. Aedeagus wide, conical, extending far beyond hypoproct, with two pairs of pits on apical third; slightly constricted at level of apical pits towards rounded apex. Hypoproct widest distally, with wide, deeply concave notch apically, evenly setulose dorsally except for bare, widened sections along apicolateral margins. Cerci trapezoid, separated almost to base by a deep notch, narrowing abruptly at half-length to form triangular lobes; evenly setulose, with several long setae apically and one strong median seta at base of triangular lobe.

Figures 84–87. Clinodiplosis comitis sp. nov.: 84, female post-abdomen; 85, larva head and prothorax; 86, male terminalia, dorsal; 87, larva terminal segment with associated papillae. Scale bars: 0.1 mm.
Pupa
Unknown.

Larva (third instar) (Figs 85, 87)
Pale yellow, slender. Length: 1.36–2.19 mm (N = 12). Integument covered by shallow, acute bumps. Antennae about twice as long as wide; cephalic apodeme as long as head capsule. Spatula with two triangular teeth and long, slender shaft, on each side with two groups of three tiny lateral papillae with no perceptible setae (Fig. 85). Terminal segment with one large and two smaller pairs of coniform papillae, and fourth pair bearing long setae (Fig. 87).

Diagnosis
This species is unique among all 45 described species of Clinodipsis in North America for the shape and setation of the male hypoproct and the large female cerci with their ventral group of blunt sensory setae.

Etymology
The species name is derived from the Latin word for ‘companion’, with reference to the fact that it accompanies Asphondyliinae galls without causing apparent damage to the gall inducer.

Notes
Clinodipsis is a large cosmopolitan genus represented in North America by 45 described species and many undescribed species (Gagné, 1994; Gagné & Jaschhof, 2014). Whereas most European species appear to be mycophagous, many New World species are inquilines, gall inducers, or even predators (Gagné & Jaschhof, 2014), but the life history of many is unknown because they were caught in flight. Morphological attributes of the male genitalia, and in particular of the cerci, are the best taxonomic characters in the genus, although in some cases there may be considerable intraspecific variability that renders these characters unusable (Skuhravá, 1973). The shape and setation of the male hypoproct and the large female cerci, with their blunt sensory setae, in C. comitis make this species unique among all described North American species (R. Gagné, pers. comm.). At present we do not know how specific its association with Asphondyliinae galls on goldenrods is, but we never reared it from galls of other cecidomyiids on these plants.

Type material
Holotype: ♂, USA, PA, Millersburg, 29 June 2007, N. Dorchin and M.J. Wise, ex Asphondyliinae pseudorosa sp. nov. bud gall on Euthamia graminifolia (TAUI).

Paratypes: 8 larvae, USA, PA, Lewisburg, 8 August 2005, N. Dorchin, ex Asphondyliinae pseudorosa sp. nov. bud galls on Euthamia graminifolia (4 USNM, 4 TAUI); 7 larvae, USA, PA, Rt. 487 (41°21.2‘N, 76°17.8‘W), 31 July 2006, N. Dorchin, ex Asphondyliinae monacha bud galls on Solidago juncea; 3♂, 6♀, same data as holotype (1♂ & 1♀ USNM, others TAUI); 4♂, 3♀, USA, PA, White Deer Creek, 4 July 2007, N. Dorchin and D. Ryan, ex Asphondyliinae pseudorosa sp. nov. bud galls on Euthamia graminifolia (TAUI).

Other material examined

Youngomyia podophyllae Felt, 1907
Dicrodipsis podophyllae Felt, 1907: 30.

Hosts and biology
The six species in this genus are inquilines, or possibly predators, in galls of various cecidomyiids in the Nearctic, Neotropical, and Oriental regions (Gagné, 1989; Gagné & Jaschhof, 2014). Two species are currently known from North America, Youngomyia quercina Felt, 1911 from Quercus in California and Y. podophyllae, which is particularly associated with Asphondyliinae galls on Asteraceae host plants in the north-eastern US. In the present study, the large and very active larvae of Y. podophyllae were regularly found in groups of between three and five in galls of all the surveyed Asphondyliinae species, but were especially common in A. pseudorosa sp. nov. and A. monacha galls. It is unclear whether they actually prey on the gall inducer’s larva, or kill it indirectly by feeding on gall tissue, because feeding behaviour has not been observed directly. Youngomyia podophyllae larvae were abundant in galls from June to September, and the species probably completes at least two generations during this time. The larvae leave the gall to pupate in the ground, and those of the last (fall) generation most probably overwinter in the soil as third instars.

Adult
General colour brownish orange.

Head (Fig. 88): Eye facets round–hexagonal. Palpus four–segmented, with distinct palpiger; segment 1 slightly longer than wide, segment 2 about 3.5 times as long as segment 1, segments 3–4 about 0.75 times as long as segment 2; all segments with several strong setae and otherwise covered by microtrichia. Face with two or three short setae on each side. Labrum and labella elongate, pointed, and strongly setose. Antenna: 12 flagellomeres in both sexes; first two flagellomeres fused. Male flagellomeres 1–11 trinodal (Figs 93–95): first node bearing long setae (Fig. 87).
neck; second node setulose, with one median circumfilar whorl, followed by short setulose neck; third node setulose, with basal whorl of strong setae and whorl of long-looped circumfila, followed by long, bare neck. Flagellomere 12 (Fig. 95) with third node followed by smaller, vestigial setulose appendage bearing few setae, ending in short, apically rounded neck; both proximal and distal flagellomere necks longer in distal flagellomeres than in proximal ones (Figs 93, 94): neck 1 to node 1 ratio 0.67–1.34 for flagellomere 3 (N = 17), 1.13–1.98 for flagellomere 10 (N = 10); neck 2 to nodes 2 + 3 ratio 0.44–0.54 for flagellomere 3 (N = 17), 0.54–0.79 for flagellomere 10 (N = 10). Female flagellomeres cylindrical, setose and setulose, with simple circumfila and short, setulose necks of same relative length throughout antenna (Fig. 89): neck to node ratio

Figures 88–95. Youngomyia podophyllae: 88, head; 89, female flagellomere 3; 90, female flagellomere 12; 91, acropod (second tiny tooth on claw not shown); 92, wing; 93, male flagellomere 3; 94, male flagellomere 8; 95, male flagellomere 12. Scale bars: 0.1 mm, except 1 mm for wing.

for flagellomere 5, 0.24–0.28 (N = 5). Flagellomere 12 with short vestigial appendage, setose and setulose, rounded apically (Fig. 90).

Thorax: Legs: very long and slender. Tarsal claws curved close to base, with long, thin tooth and a tiny, barely visible proximal second tooth (Fig. 91); empodia much shorter than bend in claw. Wing (Fig. 92): transparent, covered by hair-like microtrichia; length 2.47–3.70 mm in male (N = 19), 2.49–4.22 in female (N = 17). R1 joins C at third of wing length, R5 joins C far beyond wing apex, Rs incomplete, situated slightly beyond midlength of R1; M weak, CuA forked.

Female abdomen (Fig. 98): Sclerites rectangular, weakly sclerotized, with posterior row of setae, several scat-
tered setae on mid-proximal part, and pair of proximal trichoid sensilla. Abdomen not protractible. Cerci large and bulbous, bearing evenly scattered setae and densely covered by short, peg-like setulae.

Male abdomen: Tergites 1–7 rectangular, with posterior row of setae, a group of setae at mid-proximal part, and pair of proximal trichoid sensilla; tergite 8 completely undifferentiated from surrounding membrane. Sternites 2–7 rectangular, with posterior row of setae, numerous setae on proximal half, and a pair of closely adjacent trichoid sensilla. Sternite 8 smaller and more setose than preceding sternite.

Male terminalia (Figs 96, 97): Gonocoxites slender, widely splayed, with numerous very strong setae on distal half, a prominent angular lobe basoventrally, and conspicuous setose lobe basodorsally. Gonostylus long, slender, evenly arched, with numerous setae on distal two-thirds, bearing small apical tooth. Aedeagus wide, almost heart-shaped at apex, extending far beyond hypoproct. Hypoproct conspicuously and densely covered by short, blunt setae, rounded at apex. Cerci thin, deeply separated and splayed, with several strong setae along posterior margin, setulose throughout.

Larva (third instar) (Fig. 100)
Bright orange, very long and slender. Length 2.19–5.14 mm (N = 19). Integument covered by tiny spicules, spiracles conspicuously protruding above body surface. Antennae about 1.5 times as long as wide; cephalic apodeme much longer than head capsule. Spatula (Figs 99, 100) long and robust, with two large triangular teeth and long shaft, on each side with two groups of three tiny lateral papillae; two setose, one asetose in each group. Terminal segment on each side with four setiform papillae on slightly elevated bases.

Pupa (Fig. 101)
Small pointed antennal horns; no facial horns. Face on each side with pair of papillae medially, one setose, one asetose, and a group of three asetose papillae laterally. Prothoracic spiracle conspicuously long, pointed and strongly sclerotized; trachea ends at apex. Abdominal segments, except for first and last, each with one proximal row of long and slender barbed spikes, and otherwise covered by tiny spicules.

Notes
Several larvae of this species were found in Felt’s slide-mounted collection, in which they were attributed to Asphondyliia because they were taken from Asphondyliia galls; however, larvae of the two genera can be easily distinguished from each other and the labels on the relevant specimens were therefore amended.

Material examined
1 larva, USA, NY, Albany, 26 August 1907, EP. Felt, ex gall on Solidago patula (USNM); 1 larva, USA, NY, Albany, 26 August 1907, E.P. Felt, ex gall on Solidago odora (USNM); 1 larva, USA, MA, Magnolia, 26 August 1908, E.P. Felt, ex gall on Solidago altissima (USNM); 2 larvae, USA, MD, Wheaton Pk., 15 July 1968, R.J Gagné, ex. gall on Solidago altissima (USNM); 2 larvae, USA, MD, Wheaton Pk., 22 August 1970, R.J. Gagné, ex Asphondyliia monacha gall on Solidago juncea (USNM); 12 larvae, USA, PA, Lewisburg, 8 August 2005, N. Dorchin, ex Asphondyliia monacha galls on S. juncea; 10♀, 4♂, USA, PA, White Deer Creek, 4 July 2007, N. Dorchin and D. Ryan, ex Asphondyliia pseudorosa sp. nov. galls on Euthamia graminifolia; 5♂, 7♀, USA, PA, Mauses Creek, 4 July 2007, N. Dorchin and D. Ryan, ex Asphondyliia pseudorosa sp. nov. galls on Euthamia graminifolia; 3♂, 2♀, USA, PA, Millersburg, 4 July 2007, N. Dorchin and D. Ryan, ex Asphondyliia pseudorosa sp. nov. galls on Euthamia graminifolia; 5 exuviae, 1♂, 5♀, USA, PA, Bucknell University Chillisquaque Creek Natural Area, 15 July 2012, N. Dorchin, ex Asphondyliia pseudorosa sp. nov. galls on Euthamia graminifolia.


PHYLOGENETIC ANALYSIS
The complete molecular data set of cytochrome c oxidase subunit I (COI, 53 sequences) and elongation factor 1 alpha (EF-1α, 33 sequences) consisted of 1047 positions (690 COI and 357 EF-1α). All sequences are deposited in GenBank (http://www.ncbi.nlm.nih.gov/) and accession numbers are provided in Table 1. Tree topologies inferred from the mitochondrial COI and from the nuclear EF-1α genes are largely compatible (Figs 102, 103). The combined phylogenetic analysis yielded five well-supported clades representing at least six species of Asphondyliia on goldenords (Fig. 104). The previously described species A. monacha and A. solidaginis, and the newly described species A. pseudorosa sp. nov., A. rosulata sp. nov., and A. silva sp. nov., are well supported in this analysis, corroborating inferences drawn from morphological and life-history data. A fifth, early branching clade includes individuals from three different Solidago hosts as well as Asphondyliia recondita from Aster novae-angliae. This clade requires further morphological and molecular sampling before systematic conclusions can be reached; the taxa represented by this clade are therefore not described in the present paper.

Analysis of EF-1α (Fig. 102) provides enhanced resolution and support at deeper nodes in the tree, but does not differentiate between A. silva sp. nov. and A. rosulata sp. nov., which form distinct species in the COI tree (Fig. 103), where shallower nodes are
strongly supported. Other discrepancies between the two gene trees include the division in the EF-1α tree to *Euthamia*-associated and *Solidago*-associated clades (Fig. 102), whereas in the COI tree the *Euthamia* clade is nested within the *Solidago + Aster* clade (Fig. 103). In both trees, *A. monacha* is most closely related to *A. roslata* sp. nov. and *A. silva* sp. nov., and it is clearly separated from *A. solidaginis*.

Reconstruction of the evolution of host-plant preferences among *Asphondylia* spp. on goldenrods (Fig. 105) reveals a complex history of host-plant use. Maximum-parsimony analyses suggest substantial uncertainty in host-plant usage at deeper nodes in the phylogeny. Our analyses suggest that *S. rugosa* is the ancestral host for the *A. roslata* sp. nov. clade with subsequent shifts to *S. gigantea*, and that *A. silva* sp. nov. shifted onto and retained *S. caesia* as its host. The *Euthamia*-feeding clade shifted onto *E. graminifolia* early and retained this host, inducing galls in both buds and inflorescences. The history of host use within the *A. monacha* clade is uncertain, with four different hosts used by the same gall midge species.

Our phylogenetic analyses yielded novel insights into contexts of host shifts and the evolution of *Asphondylia* host–plant relationships in the goldenrod species complex. Further, finer scale patterns of host-plant use (plant-part and gall-type associations) within individual species are revealed with greater granularity (Fig. 104). *Asphondylia solidaginis* is shown to include individuals from two very different types of galls – a rosette bud gall and a leaf snap – that represent the overlapping spring and summer generations of this species on *S. altissima*. This species appears to use *S. gigantea* occasionally as a supplementary host for the leaf snap galls. *Asphondylia roslata* sp. nov.

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**Figure 102.** Phylogenetic tree of *Asphondylia* species associated with goldenrods based on Bayesian analysis of partial sequence of the elongation factor 1α (*EF-1α*) gene. Support values are shown next to nodes.
exhibits a very similar pattern but uses *S. rugosa* rather than *S. altissima* as its primary host. Despite the morphological and biological similarity between these two *Asphondylia* species, they are not closely related. Instead, the sister species to *A. rosulata* sp. nov. is *A. silva* sp. nov., which is found exclusively on *Solidago caesia*, on which it completes multiple generations in tiny bud galls throughout the season. The *A. monacha* clade comprises individuals from complex rosette galls on *S. juncea*, *S. erecta*, and *S. uliginosa* that represent the summer generation of this species. Also included in this clade are individuals from simple

**Figure 103.** Phylogenetic tree of *Asphondylia* species associated with goldenrods based on Bayesian analysis of partial sequence of the cytochrome *c* oxidase subunit I (COI) mitochondrial gene. Support values are shown next to nodes.
Figure 104. Phylogenetic tree of *Asphondylia* species associated with goldenrods based on combined Bayesian analysis of the cytochrome *c* oxidase subunit I (COI) mitochondrial and elongation factor 1α (EF-1α) genes. Support values are shown next to nodes. Colours refer to host-plant species; icons on branches refer to galled plant organ. Rectangular brackets on right indicate species boundaries.
bud galls on *S. altissima* that represent the previously unknown spring generation of *A. monacha*.

**DISCUSSION**

The systematics and taxonomy of *Asphondylia* is challenging because of the general morphological uniformity among species and the complexities of their host and gall-type associations. The ability of certain species in this genus to exploit numerous unrelated host plants is unusual in the Cecidomyiidae, and it is not known why this phenomenon occurs in *Asphondylia* more often than in other genera in the family (Tokuda, 2012). This situation makes it very difficult to circumscribe host ranges and associate populations of the same species that develop in different plant species and/or gall types at different times of the year (e.g. Plakidas, 1988; Yukawa et al., 2003; Uechi et al., 2004). In such cases, recognizing species and deciphering their host associations must rely on the morphology of immature stages and on molecular data.

In the present study, we observed only minor differences between the results of the analyses based on the mitochondrial and nuclear genes (Figs 102, 103),
which lends strong support to our taxonomic decisions and conclusions about evolutionary trends in the studied group. Discordance between mitochondrial and nuclear markers may result from limited phylogenetic signal or from introgression resulting from continued gene flow in a recently radiating group (Fontenot, Makowsky & Chippindale, 2011). Alternatively, discordance could arise from the retention of ancestral polymorphism (Petit & Excoffier, 2009) or incomplete lineage sorting among nuclear genes, which typically have longer coalescent times relative to mitochondrial genes (Stelkens & Seehausen, 2009). The topology of our combined tree is influenced to a greater extent by the mitochondrial gene, in accordance with the morphological and life-history data. Consequently, we currently recognize five species of Asphondylia on goldenrods, and another one or two species that induce complex bud galls on *S. bicolor* and *S. sempervirens*, which require further work. Unlike other species complexes in the genus (e.g. Hawkins *et al.*, 1986; Gagné & Waring, 1990; Kolesik *et al.*, 2010), immature stages in this group do not offer reliable morphological characters that can be used to distinguish among them, and many of the taxonomic decisions in this paper rely on molecular evidence. Any work that is done on this group as additional morpho-species are found will likewise warrant the use of molecular markers to verify species identities and host associations.

**HOST ASSOCIATIONS**

The radiation of the *Asphondylia* species associated with goldenrods apparently occurred through shifts to new host species, which are likely to have driven the evolution of reproductive isolation. This may be a continuing process that will eventually lead to the establishment of distinct species of the host-associated populations within *A. monacha*. Assuming that the clade as a whole is monophyletic, ancestral state reconstruction reveals a complex history of host associations in this group; however, such analysis is complicated in the present case by the large number of states (11 hosts) and small number of tips (51), yielding low statistical power to make inferences about deeper nodes in the phylogeny.

Although the systematic relations within *Solidago* have not been sufficiently resolved, the currently available phylogeny of the genus (Zhang, 1996) allows us to make some assumptions about the evolution of associations between the gall midges and their goldenrod hosts. An emerging pattern from the present study and previous ones (e.g. McEvoy, 1988; Gagné, 1989; Dorchin *et al.*, 2007, 2009) is that the gall midges are most numerous and diverse on the most common goldenrods. The five *Asphondylia* species discussed in this paper develop on some of the most common goldenrod species in the north-eastern USA: *S. altissima*, *S. gigantea*, *S. rugosa*, *S. juncea*, *S. caesia*, and *E. graminifolia*. Although different habitat preferences and growth forms among these plant species result in differences in small-scale distribution patterns (Abrahamson *et al.*, 2005), they are largely sympatric in most places where we sampled them. Therefore, the pattern of host use by gall midges that we revealed here cannot be attributed to the availability or absence of certain goldenrod species in a given area.

It is noteworthy that some of the *Asphondylia* species use several host plants that are not closely related: for example, *A. monacha* develops in four *Solidago* species, each belonging to a different subsection (of Zhang 1996 and Semple & Cook, 2006). Similarly, *A. rosulata* sp. nov. uses *S. rugosa* (subsection Venosae) and *S. gigantea* (subsection Triplinerviae). It therefore appears that the dominant factor for gall midges colonizing new host plants in this system is not the taxonomic relatedness of the plants but local abundance and physical proximity. This could also account for the position of the *Euthamia*-associated clade, which is more closely related to species that are associated with abundant goldenrod hosts than to those from plants with more restricted distributions. Had taxonomic relatedness of the host plants played a more important role in the speciation of the gall midges in this group, the *Euthamia*-associated clade could be expected to form a sister group to the *Solidago*-associated clade, rather than being nested within it.

**INTRASPECIFIC MORPHOLOGICAL DIFFERENCES IN RELATION TO GALLED ORGAN**

Superimposing the type of gall on the phylogenetic tree (Fig. 104) suggests that bud galling is the ancestral habit among goldenrod *Asphondylia*, with one switch to inflorescence galls (by *A. pseudorosa* sp. nov.) and three switches to leaf galls (by *A. solidaginis*, *A. rosulata* sp. nov., and *A. pseudorosa* sp. nov.). Each of the leaf-galling species also induces bud galls, and the relative proportions of the two types in the population depend on the season. The ability to cause these two types of galls is less surprising once the structure of the galls is examined closely. Some snap galls – most often those of *A. rosulata* sp. nov. – are found in small apical leaves near the shoot apex, and appear to constitute an intermediate stage between a ‘true’ snap gall and an apical bud gall. In all cases, snap galls are more common early in the season, whereas rosette galls of the same species dominate later on. A possible explanation for this phenomenon may be that the plant’s growth rate is much faster early in the season, so that fully expanded leaves remain attached around the larva after an oviposition event to
form a snap gall far below the shoot tip. Later in the season, oviposition on slower growing leaves around the apical bud stunts their growth and causes a rosette gall with a central larval chamber.

It is remarkable that the snap-like structure is so similar on all host plants on which these galls are found, and yet they unequivocally belong to three different species. The same is true for the bud galls induced by these species. Together, the similarity in these snap- and bud-gall structures induced by different species suggests convergent evolution of these structures as a result of the same selection pressures on different plant species (probably from a combination of natural enemies and host plant-mediated selection). Alternatively, if this galling strategy evolved only once among goldenrod Asphondylia, then A. silva sp. nov. and A. monacha must have lost the ability to induce leaf galls and thus develop only in bud galls throughout the season; however, A. monacha does cause a different type of bud gall in early spring in young shoots of S. altissima, which is the only Solidago species for which sprouts are available for galling in the field at that time. The structure of this solitary bud gall generally resembles that of the individual units that compose the large spherical gall of this species much later in the season on other hosts. Asphondylia pseudorosa sp. nov. is the only species in this complex that develops also in inflorescences, and the differences in size and pupal morphology between individuals from buds and inflorescences in this species probably arise from the type of plant tissue they use, as they do not appear to have a genetic basis. The shift to inflorescence buds occurs in summer, once these become available, and probably offers superior nutritional conditions for the larvae than the now slower-developing vegetative buds (Abrahamson & McCrea, 1985). One wonders why the ability to gall inflorescences did not develop in Asphondylia species on other goldenrods that flower around the same time. The ability of many Asphondylia species to use different host plants and host tissues according to their availability at different times is probably a key factor in the successful radiation of this genus worldwide.

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