A NEW SPECIES OF LESTODIPLOSIS (DIPTERA: CECIDOMYIIDAE) PREYING ON PINEUS (HEMIPTERA: ADELGIDAE), WITH A REDESCRIPTION OF LESTODIPLOSIS JUNIPERINA (FELT)

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Abstract.—A new species, Lestodiplosis pinei Gagné (Diptera: Cecidomyiidae), from the United States is described from males, females, pupae and larvae. Larvae are egg predators of Pineus strobi Hartig, Pineus pini Goeze and Pineus cembrae (Cholodkovsky) (Hemiptera: Adelgidae). This is the first North American record for P. cembrae, which was found feeding on Pinus cembra L. planted at Arnold Arboretum in Massachusetts. Lestodiplosis juniperina (Felt) is redescribed as similar to L. pinei and for having once been considered to include specimens now identified as L. pinei. Lestodiplosis cerasi Felt and Lestodiplosis novangliae Felt are new synoynms of L. juniperina.

Key Words: Cecidomyiinae, Cecidomyiidi, predaceous gall midges, pine bark adelgid, pine woolly aphid, Swiss stone pine adelgid.

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Predaceous flies (Diptera) have long been considered biological control agents of pest Adelgidae (Hemiptera) (e.g., Pschorn-Walcher and Zwo¨lfer 1958, Ross et al. 2011). Currently, two silver fly species (Diptera: Chamaemyiidae) from western North America are being evaluated for potential biological control of hemlock woolly adelgid in eastern North America (Havill et al. 2018). Prior to their release, efforts are being made to understand better the community of dipteran predators that feed on other adelgids. In the course of this work, to be described in a subsequent publication by the second author, samples of Pineus spp. (Adelgidae: Hemiptera) and their predators were collected throughout the United States. Among the predators found feeding on Pineus strobi Hartig, Pineus pini Goeze, and Pineus cembrae (Cholodkovsky), was a species of Lestodiplosis (Diptera: Cecidomyiidae) that is described here as new to science.

Lestodiplosis is a cosmopolitan genus of predaceous Cecidomyiidae with 185 described species (Gagné and Jaschhof 2017). Larvae are predators of insects, mites and a millipede. Most are presumed to be specialists, and many quite probably are, but little information beyond the initial description is available for the majority of species (Gagné and Jaschhof 2017). The genus contains
many apparent synonyms that only a proper revision will discover.

The new species was long ago (Felt 1933) mentioned as a predator of *Rhyacionia buoliana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae; pine shoot moth), but misidentified as *Lestodiplosis novangliae* Felt [now *Lestodiplosis juniperina* (Felt), this paper]. The present rediscovery of the new species actually feeding on *Pineus* may indicate that the earlier association was incorrect.

The first author has already done preliminary revisionary work on the Nearctic species of *Lestodiplosis*, so was aware of the misidentification in Felt (1933). The new species, *L. pinei* Gagné, is described and *L. juniperina* (Felt), the most similar congener in both adult and larval appearance, is redescribed for comparative purposes. Males, females and larvae of both species can readily be separated anatomically. By contrast with the apparent near monophagy of the new species, *L. juniperina* is shown here to have a wide variety of prey.

**Materials and Methods**

Larvae and pupae were collected by examination of *Pineus* spp. colonies on *Pinus strobus* L. bark and branch tips under a dissecting microscope. Adults emerged in a cage holding infested bark strips.

Larvae, pupae and adults were mounted for study in Canada balsam using techniques outlined in Gagné (1989). A glossary of adult morphological terms can be found in Gagné (2018). Anatomical terminology of the larval stage follows Gagné (1989). Drawings were made with the use of a camera lucida attached to a Wild phase contrast microscope. In Cecidomyiinae most scales and setae become lost in the mounting process, but enough remain to show that the setae leave larger sockets than do scales. The line illustrations show these sockets in their actual placement and number. Figure 4 was made with a Zeiss EVO Ma15. Figures 1–3 were taken with a Canon EF 200 mm Telefoto fixed lens attachment, then compiled into a single image using Zerene Stacker. The holotype of the new species is deposited in the National Museum of Natural History, Smithsonian Institution, Washington DC (USNM). Paratypes are divided between the USNM and the Yale Peabody Museum (YPM). Additional *L. pinei* specimens are deposited in the Cornell University Insect Collection (CUIC). Specimens of *L. juniperina* are deposited in the USNM and the Collections Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario (CCBG). All specimens are from Canada and USA and arranged in alphabetical order by province and state. Specimens are deposited in the USNM unless otherwise indicated.

The standard DNA barcoding region of the mitochondrial cytochrome c oxidase subunit I (COI) gene of the new species was amplified using primers LepF1 and LepR1 (Hebert et al. 2004). Sequencing was performed at the DNA Analysis Facility on Science Hill, Yale University, and chromatograms were edited with Geneious v7 (Kearse et al. 2012). All sequences generated for this study were deposited in GenBank with accession numbers MT139462–MT139593. Immature specimens of the new species from localities other than the type locality were initially identified by DNA barcode matches, and later examined morphologically for confirmation.

The first author determined the taxonomy, made the pencil illustrations, and co-wrote the manuscript. The second author conducted field collections and received adelgid samples for laboratory examination, conducted DNA analysis, and co-wrote the manuscript.
RESULTS AND DISCUSSION

The two species treated below are described in a way comparable to other recent descriptions of *Lestodiplosis* (Gagné and Lill 1999, Gagné and Etienne 2009). Whenever possible, one should illustrate male terminalia in lateral view as well as dorsoventral to show the profile of the aedeagus and any ventral lobes of the hypoproct. For other examples of morphological diversity in *Lestodiplosis* larvae, see Baylac (1987) and Harris (1982). Major differences between the two species treated here are emphasized with italics in the descriptions.

*Lestodiplosis pinei* Gagné, new species

http://zoobank.org/948B7721-840A-4030-BFA1-CE657CB61606
Figs. 1, 3–7, 9–11, 15–16, 19–22

Description.—*Adult. Head:* Eyes large, connate, 8 facets long at vertex; facets circular, closely juxtaposed, less so laterally. *Occipital protuberance short, obtuse; barely protruding above level of eyes,* with pair of large apical setae. Antenna of uniform hue, with 12 flagellomeres; scape with a few ventrolateral setae and 1 mesal seta; pedicel with several ventral and lateral setae; male flagellomeres (Figs. 6–7) binodal, first node spheroid with one circumfilum, second node obpyriform, with 2 circumfila, all circumfila comparable in length, loops short, not reaching next distal node or circumfilum, *internode and neck no longer than preceding node;* female flagellomeres cylindrical, circumfila closely appressed except for short loops at apex of node, *neck of third flagellomere less than 2/3 of nodal length.* Frons with 6–8 setae per side, without scales. Labella hemispheroid with several uniform, slightly thickened setae. Palpus 4-segmented, first segment spheroid, remaining segments elongate-ovoid, each with several scattered, pointed setae of uniform length, without scales.

*Thorax:* Wing: length in male, 1.2–1.3 mm (n = 4), in female 1.4 mm (n = 2); hyaline except with dark shading adjacent to branches of M₄ and CuA fork on female only and not always visible on slide mounts; R₅ straight and joining C near wing apex; Rs evanescent; wing fold not evident; M₄ and CuA forming a fork. Scutum with 4 longitudinal groups of setae mixed with scales, the central group terminating some distance from scutellum. Scutellum with 8–10 setae along width. Anepimeron with 3–4 setae, remaining pleura bare. Legs: claws untoothed, strongly curved beyond midlength; empodia nearly as long as claws; pulvilli diminutive.

*Male abdomen:* Tergites 1–7 with single row of posterior setae, a few lateral setae, an anterior pair of trichoid sensilla and elsewhere with scattered scales; tergite 8 membranous, with 0–2 posterior setae and pair of trichoid sensilla. Sternites 2–6 with single row of sparse posterior setae, a group of lateral setae and scales near midlength and anterior pair of closely approximated trichoid sensilla; sternite 7 similar except lateral groups of setae merged medially and closely approaching posterior row of setae; sternite 8 triangular, pointed anteriorly with each of the 2 well-separated trichoid sensilla situated in the membranous area on each side of the point, with full posterior row of setae and a few scales elsewhere. Terminalia (Figs. 9–11): cerci broadly rounded apically with sparse ventrolateral and apical setae; hypoproct convex laterally and at apex, dorsoventrally flattened along entire length, *with pair of subapical setae longer than width of hypoproct,* and pair of diminutive apical setae, entire surface uniformly short-setulose; aedeagus elongate, longer than hypoproct and *slightly longer than gonocoxite,* evenly recurved.
dorsally, **narrowest at midlength, widened near apex**, the tip rounded; gonocoxite cylindrical, **with ovoid, apically rounded, short-spinose mesobasal lobe**; gonostylus slender beyond bulbous base, especially slender in caudal view, with a few setae, microtrichose only at base, carinate beyond.

**Female abdomen:** Tergites 1–7 as for male but lateral setae and scales more numerous; tergite 8 membranous with anterior pair of trichoid sensilla and a few short setae or none on posterior margin. Sternites 2–7 as for male but setae and scales at midlength more numerous and not prominently divided into separate groups; sternite 8 membranous, with a few setae and scales posteriorly and a widely separated anterior pair of trichoid sensilla. Ovipositor (Figs. 15–16) short, extendable part not much longer than tergite 7, bare dorsally and laterally, with mixed long and short setae ventrally; cerci elongate-ovoid, bilaterally flattened, dorsally with widely spaced setae, those closer to apex longer,

none thickened and blunt tipped, ventrally with a few short setae, longer near apex, with dense apicoventral field of more than 50 short setae, the setae barely longer than width of their sockets; hypoproct acutely tapered to end, with 1–2 apical setae.

**Pupa** (Fig. 19): Exuviae hyaline. Vertex bearing two papillae on each side, one blank, the other with long seta. Antennal bases blunt anteriorly. Face smooth, frons with papilla on each side, without seta, face with papilla anteriad of each palpal base, without seta. Prothoracic spiracles elongate, cylindrical, tracheae reaching to apex. Abdominal spiracles on segment 1 not raised above surface, several times longer than basal width, tapered and stiff on segments 2–6, and barely longer than basal width on segments 7 and 8. Abdominal segment 1 with short spicules only on pleura, remainder smooth; segments 2–8 covered with short spicules of uniform length except anterior third of terga mostly covered with larger, spinose spicules; terminal segment covered with short spicules.

**Larval third instar** (Figs. 3–4, 20–22): Head shaped as in Fig. 20. Antenna long, evenly cylindrical, obtuse apically. Spatula absent. Integument mostly smooth, ventrally with elongate, cylindrical, flexible pseudopods with circular, concave apices (Fig. 4), 2 each on meso- and metathorax and 3 each on first through seventh abdominal segments, and with 3 low, smooth, convexities on anal segment (Fig. 3). Anus dorsal. Papillae as follows: 2 collar papillae dorsally and ventrally, 6 dorsal papillae with setae on each thoracic segment (occasionally one papilla missing on prothorax) and each of abdominal segments 1–7, the first and sixth setae of each row longest, the second and fifth setae somewhat shorter than remainder, and 2 dorsals on segment 8, the setae longer than on previous segments; 6 long terminal papillae bearing setae with rounded apices on anal segment; two pleural papillae with setae present on each side of thoracic and abdominal segments 1–8; lateral papillae in triplets on each side of midline of thoracic segments, one of each triplet with seta, remainder without; a ventral papilla with seta on each side of venter on thoracic and abdominal segments 1–7 and 2 ventrals without setae on abdominal segment 8. Rows of spicules present ventrally at midlength surrounding pseudopods and on much of anal segment ventrally, and with a few rows on each side of anus.

**Type material.**—Holotype, male, West Lafayette, Tippecanoe Co., Indiana., Lat: 40.4425, Long: -86.8995, 31 August 2018, coll: Holly Wantuch, with *Pineus strobi* on *Pinus strobus*, [molecular voucher], Sample 18-667-02, deposited in USNM. Paratypes, same data as holotype [molecular vouchers], all deposited in USNM except YPM (Yale Peabody Museum) as noted: male, Sample 18-667-04, YPM ENT960508; male, Sample 18-667-07; female, Sample 16-667-08, YPM ENT960510; female, Sample 18-667-17; 3 males, female, Samples 18-629; larva, Sample 18-613-01; larva, Sample 18-613-02; larva, Sample 18-613-04; pupa, Sample 18-613-05, YPM ENT960500; larva, Sample 18-613-07, YPM ENT960501; larva, Sample 18-613-08; pupa, Sample 18-613-11; larva, Sample 18-613-12, YPM Ent960504; larva, Sample 18-613-16.


**Etymology.**—*Lestodiplosis pinei* is named after *Pineus*, the main prey of this species. The name is to be pronounced as three syllables with a hard ‘e’ and hard final ‘i’.

Natural history notes.—Larvae are egg predators of *Pineus* spp. Pupation evidently occurs on bark as adults were reared from caged bark strips. Full-grown predaceous larvae are known to make cocoons in the vicinity of where they feed (Gagné & Lill 1999; Gagné & Stein 1982), but cocoons were not observed during the collections of *L. pinei*.

Remarks.—Among *Lestodiplosis* the new species is generally similar to *L. juniperina*, which is redescribed below. The occipital prominence of the adult is short in both species, although a little longer in *L. juniperina*. Males have similarly disposed antennae with circumfilar loops of regular length, a flat hypopygium, gonocoxites with spinose mesobasal lobes, and a dorsally recurved aedeagus with rounded apex. Third instars share the loss of a spatula and have asymmetric setae on the dorsal papillae. Aside from these similarities the two species are readily distinguishable. Males of *L. pinei* differ in details of the terminalia with rounded, less strongly spinose gonocoxal lobes that are situated closer to the gonostylar base, much longer subapical setae on the hypopygium, and a longer aedeagus; females have many more and shorter setae in the api-coventral field of sensoria of the cerci; and larvae a broader head and shorter and more evenly cylindrical antennae. *L. pinei* is distributed from New England, west to Indiana, and south to Virginia. The record of *Pineus cembrae* as prey is also the first North American record for this Eurasian adelgid species, which was found feeding on European stone pine, *Pinus cembra* L., planted in the Arnold Arboretum in Massachusetts. Additionally, there are disjunct populations in Washington state feeding on the non-native, European species, *P. pini*, and in Colorado feeding on *P. strobi*, which is not native to western United States. All DNA barcode sequences generated for this study were deposited in GenBank with accession numbers MT139462–MT139593. The pairwise sequence divergence between *L. pinei* specimens ranged from 0.0 to 4.0% with a mean of 0.5%.

**Lestodiplosis juniperina** (Felt)
Figs. 2, 8, 12–14, 17–18, 23

*Cecidomyia juniperina* Felt 1907a: 44;
*Lestodiplosis juniperina* 1908: 408.

**New synonym.**
*Lestodiplosis cerasi* Felt 1908b: 407.

**New synonym.**

**Lestodiplosis novangliae** Felt 1933: 114.

**New synonym.**

Description.—Adult. Head (Fig. 2): Eyes large, connate, 8 facets long at vertex; *facets circular*, closely juxtaposed. Occipital protuberance short, acute; barely protruding above level of eyes. Antenna of uniform hue, with 12 flagellomeres; scape with a few ventral setae and 1 mesal seta; pedicel with
several ventral and lateral setae; male flagellomeres (Fig. 8) binodal, first node spheroid with one circumfilum, second node obpyriform, with 2 circumfila, all circumfila comparable in length, loops short, not reaching next distal node or circumfilum, internode longer than preceding node, and neck as long or longer than preceding node; female flagellomeres cylindrical, neck of third flagellomere more than \( \frac{3}{4} \) length node, circumfilum closely appressed except for short loops at apex of node. Frons with 6–8 setae per side, without scales. Labella hemispheroid with several uniform, slightly thickened setae. Palpus 4-segmented, first segment spheroid, remaining segments elongate ovoid, each with several scattered, pointed setae of uniform length, without scales.

**Thorax:** Wing: length in male, 1.1–1.4 mm (n = 10), in female 1.4–1.5 mm. (n = 4); hyaline except with dark shading adjacent to branches of M and CuA fork on female only; R\(_5\) straight and joining C near wing apex; Rs evanescent; wing fold not evident; M and CuA forming a fork. Scutum with 4 longitudinal groups of setae mixed with scales, the central group terminating some distance from scutellum. Scutellum with 8–10 setae along width. Aneplomerion with 3–4 setae, remaining pleura bare. Legs: claws untoothed, curved beyond midlength; empodia nearly as long as claws; pulvilli diminutive.

**Male abdomen:** Tergites 1–7 with single row of posterior setae, a few lateral setae, an anterior pair of trichoid sensilla and elsewhere with scattered scales; tergite 8 membranous, with 0–2 posterior setae and pair of trichoid sensilla. Sternites 2–6 with single row of sparse posterior setae, a group of lateral setae and scales near midlength and anterior pair of closely approximated trichoid sensilla; sternite 7 similar except lateral groups of setae merged medially and closely approach posterior row of setae, sternite 8 triangular, pointed anteriorly with each of the 2 well-separated trichoid sensilla situated in the membranous area on each side of the point, with full posterior row of setae and a few scales elsewhere. Terminalia (Figs. 12–14): cerci ovoid, broadly rounded apically with sparse ventrolateral and apical setae; hypoproct parallel-sided, convex apically, dorsoventrally flattened along entire length, with pair of subapical setae no longer than width of hypoproct and pair of diminutive apical setae, entire surface uniformly short-setulose; aedeagus elongate, much longer than hypoproct and nearly as long as gonocoxite, cylindrical, not noticeably widened at apex, evenly recurved ventrally; gonocoxite cylindrical, with angular, spinose mesobasal lobe situated near midlength of gonocoxite; gonostyly slender beyond bulbous base, with a few setae, microtrichose only at base.

**Female abdomen:** Tergites 1–7 as for male but lateral setae and scales more numerous; tergite 8 membranous with anterior pair of trichoid sensilla and a few short setae or none on posterior margin. Sternites 2–7 as for male but setae and scales at midlength more numerous and not prominently divided into separate groups; sternite 8 membranous, with a few setae and scales posteriorly and a widely separated anterior pair of trichoid sensilla. Ovipositor (Figs. 17–18) short, protrusible part not much longer than tergite 7, bare dorsally and laterally, with mixed long and short setae ventrally; cerci elongate-ovoid, bilaterally flattened, dorsally with widely spaced setae, those closer to apex longer, none thickened or blunt tipped, ventrally with a few short setae, longer near apex, with sparse apicoventral field of 15–20 short setae, somewhat longer than their
bases, near apex; hypoproct obtusely tapered to end, with 1–2 apical setae.

_Pupa:_ Unknown.

**Third and last instar:** Head shaped as in Fig. 23. Antennae long, tapered from base to pointed apex. Spatula absent. Integument mostly smooth, ventrally with elongate, cylindrical, flexible pseudopods with circular, concave apices, 2 each on meso- and metathorax and 3 each on first through seventh abdominal segments, and with 3 low, smooth, convexities on anal segment. Anus dorsal. Papillae as follows: 2 collar papillae dorsally and ventrally, 6 dorsal papillae with setae on each of thoracic segments and each of first through seventh abdominal segments, the first and sixth setae of each row longest, the second and fifth setae somewhat shorter than remainder, and 2 dorsals on eighth, the setae longer than on previous segments; 6 long terminal papillae bearing setae with rounded apices on anal segment; two pleural papillae with setae present on each side of thoracic and first through seventh abdominal segments; lateral papillae in 2 triplets on each side of midline of thoracic segments, one of each triplet with seta, remainder without; a ventral papilla with seta on each side on thoracic and first through seventh abdominal segments and 2 ventrals without setae on eighth abdominal segment. Rows of spicules present ventrally at midlength surrounding pseudopods, on much of anal segment ventrally and with a few rows on each side of anus.

Type material of names included in this taxon.—

_Cecidomyia juniperina:_ Holotype, male, deposited in the Felt Collection. Type data: Nassau, New York, USA, 7 August 1906, Felt type 746. The choice of the name _juniperina_ was not meant to imply that the species had any connection to juniper other than perhaps having been swept from its branches (see Felt 1907b in response to criticism in Beutenmüller 1907).

_Lestodiplosis cerasi:_ Holotype, male, deposited in the Felt Collection. Type data: West Nyack, New York, Felt type a1593a, from twig swellings of _Prunus serotina_ (Rosaceae).

_Lestodiplosis novangliae:_ Lectotype designated here, male, deposited in the USNM. Type data: associated with _Epinotia nanana_ (Treitschke) (Lepidoptera: Tortricidae; European spruce leafminer) from spruce twigs (prey not further specified), South Bristol, Maine, USA, 26 January 1932, A.B. Proper, Gypsy Moth Lab. No. 9590c43, USNM No. 44883. Paralectotype, female, same data as lectotype with addition of collector Imms. Associated with the type series are two larvae with identical data as lectotype but not originally described. The type locality given above was not in Felt (1933) but is copied here from the slide mounts. The only locality given in Felt (1933) is “Melrose Highlands, Mass.” (now part of Melrose), Which was the origin of the shipment and at that time the site of the Gypsy Moth (also Gipsy-Moth) Laboratory, U.S. Bureau of Entomology, USDA. When describing this species, Felt (1933) studied other specimens from Maine and Massachusetts that he had received simultaneously from the Gypsy Moth Laboratory. These specimens, listed below under Material Examined, had different Gypsy Moth Lab numbers and collection dates not specifically stated in the paper, so are not considered here as part of the newly designated lectotype series. Felt (1933) wrote that the specimens he had were reared from larvae found near or upon the dead larvae of _E. nanana_, so the cecidomyiid was not absolutely tied to that prey. The record is nevertheless credible from what we now know of _Lestodiplosis_.


Remarks.—Lestodiplosis juniperina is a widely distributed species found from coast to coast in North America. It preys on at least two species of Lepidoptera and one of Hemiptera and has been reared in association with a variety of niches, including small grains, strawberries, stem swellings of wild black cherry and a gall made by a cynipid (Hymenoptera).

Among Lestodiplosis, L. juniperina is generally similar to the new species, but is distinct and easily differentiated by male, female and larval characters as listed under L. pinei above. The type males of the three species names synonymized here have identical male terminalia, as do all the other male specimens examined. Felt (1933) stated that his
L. novangliae, one of the three species names in this taxon, approached closely Lestodiplosis scrophulariae Felt. The latter, known only from the holotype male, is distinct in the following ways. Its head completely lacks an occipital protuberance. The two large setae that usually surmount that structure in all other species of Lestodiplosis known to RJG are in this species placed below the dorsal margin of the occiput. Additionally, the aedeagus of L. scrophulariae is only two-thirds as long as the gonocoxites and is flattened at its apex.

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