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History, Distribution, and Identification Of *Exoteleia dodecella* (L.) (Lepidoptera: Gelechiidae) in North America, With Insights into the Systematics of Exoteleia Wallengren using Characters of the Adult, Immatures, Bionomics, and DNA Barcodes

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HISTORY, DISTRIBUTION, AND IDENTIFICATION OF *EXOTELEIA DODECELLA* (L.) (LEPIDOPTERA: GELECHIIDAE) IN NORTH AMERICA, WITH INSIGHTS INTO THE SYSTEMATICS OF *EXOTELEIA* WALLENGREN USING CHARACTERS OF THE ADULT, IMMATURES, BIONOMICS, AND DNA BARCODES

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Abstract.—*Exoteleia dodecella* (L.) (Lepidoptera: Gelechiidae), a native of Europe, was first documented from North America at several locations in eastern Canada. Additional records indicate this moth has now spread throughout New England and west to northern Pennsylvania, New York, and possibly into Michigan in the United States. A second introduction of *E. dodecella* has occurred near the Vancouver area of British Columbia in Canada. To help with the identification of *E. dodecella*, morphological, biological, and molecular evidence are presented. Key features of the adult, larval, and pupal morphology are compared to other species of *Exoteleia* and illustrated with line drawings or scanning electron micrographs. The high sequence divergence (>7%) of *E. dodecella* compared to samples of related native North American species demonstrates that DNA barcodes are a useful identification tool for this pest. A summary of the biology of *E. dodecella*, including 12 species of larval and pupal parasitoids (most representing new host records), is also included.

Key Words: bud-miner, chaetotaxy, DNA barcode, Europe, *Exoteleia pinifoliella*, invasive species, life cycle, morphology, needle-miner, North America, parasitoids, pine

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The pine bud moth, *Exoteleia dodecella* (L.) (Lepidoptera: Gelechiidae), the type species of *Exoteleia* Wallengren, was first recorded in North America as an established population on the Canadian side of the Niagara Peninsula (Ontario) in 1928 (Sheppard 1930, Martin 1959). Attempts to erad-

* Accepted by Robert R. Kula

icate this pest failed, and it spread to at least Quebec (Handfield 1992) in eastern Canada. *Exoteleia dodecella* is also known from the Vancouver area of British Columbia (specimens in the CNC), which likely represents a second introduction to North America.

New World records for *E. dodecella* usually cite only Ontario (USDA 1986, Mattson et al. 1994) or North America (Hodges 1983, 1986). The history of this species in the United States is poorly documented. *Exoteleia dodecella* was first collected from New York State in 1934 (see Material Examined). These records apparently were never published, or perhaps the population never established. Tracy collected *E. dodecella* from Maine in 1978 and sent voucher specimens to R. W. Hodges (formerly of the Systematic Entomology Laboratory, Washington, DC) for confirmation. His discovery became the first published United States record (Tracy 1980). The New York or Maine records were never entered into any of the older databases documenting the Nearctic immigrant arthropod fauna (USDA/ARS/BBII 1986, Knutson et al. 1990, Kim and Wheeler 1991). Additionally, USDA-APHIS-PPQ has no record of *E. dodecella* in the United States (J. Cavey pers. comm.), this pest is absent from their most current database (NAPIS pest tracker), and no New Pest Advisory Group Report ever documented this introduction.

Exoteleia dodecella is a serious pest of pine in Europe (Martin 1959, Bland et al. 2002) and more recently in Canada, where up to 60% of the buds of Scots (or Scotch) pine, *Pinus sylvestris* L. (Pinaceae), were destroyed in Ontario (Martin 1959). Although the preferred hosts are Scots and Mugo pines (*Pinus mugo* Turra), *E. dodecella* occasionally lays eggs on larch (*Larix*) (Pinaceae) (Bland et al. 2002) and feeds on other pine species under certain conditions (Martin 1959). Both *P. sylvestris* and *P. mugo* have been introduced into the United States; the former species during colonial times for lumber (Elias 1989) and both species more recently for ornamental use or in tree plantations on state forests (Braun 1961, White and Hosie 1980).

In Europe, Lemarie (1958a, b, 1959a, b, 1961) reared 50 species of parasitoids that have been associated with the life cycle of *E. dodecella*, while Martin (1959) reared seven species of parasitoids of *E. dodecella* in Canada.

In addition to *E. dodecella*, there are currently seven nominal species of native *Exoteleia* recorded from North America (if not described in *Exoteleia*, original combinations are in parentheses): *E. anomala* Hodges, 1985; *E. burkei* Keifer, 1932; *E. californica* (Busck, 1907) (*Paralechia*); *E. chillcotti* Freeman, 1963; *E. graphicella* (Busck, 1903) (*Gnorimoschema*); *E. nepheos* Freeman, 1967; and *E. pinifoliella* (Chambers, 1880) (*Gelechia*).

Exoteleia chillcotti has been erroneously listed under *Coleotechnites* (Hodges 1983, Lee and Brown 2008). Examination of its holotype (CNC) shows that it is properly placed in *Exoteleia*. Hodges (1985) correctly indicated that *E. californica* and *E. graphicella* are misplaced in *Exoteleia* without giving a proper generic placement. The genitalia of *E. graphicella* show that it is not a *Gnorimoschema* and suggests that it may belong in *Recurvaria*, although we are not certain. The genitalia of *E. californica* confirm its misplacement, but we cannot make a statement as to its positive generic placement although the wing pattern is similar to *Coleotechnites*. After removing two possible misplaced species (see details in Results section), five native nominal species of North American *Exoteleia* remain: *E. anomala*, *E. bur-*

kei, *E. chillcotti*, *E. nepheos*, and *E. pinifoliella*.

There is no single work for the identification of North American *Exoteleia*. All existing publications present individual species descriptions. Only two show a black-and-white photo of an adult (Hain and Wallner 1973, Hodges 1985). Genitalia have been illustrated only for the following three species: *E. chillcotti* Freeman, 1963; *E. nepheos* Freeman, 1967; and *E. anomala* Hodges, 1985, but they show no reliable differences.

In discussing the taxonomy of *Exoteleia*, Hodges (1985) expressed frustration when attempting to delineate species. Four taxa were recognized (*E. anomala*, *E. pinifoliella*, *E. dodecella*, and one undescribed entity from the eastern U.S.), but he was unable to define three nominal species (*burkei*, *chillcotti*, and *nepheos*). He also felt that there were possibly two additional undescribed "entities based on adult characters," and that pupal characters might be useful to define species if a larger series of immatures could be associated with known adults. His conclusion was that it was not possible "to resolve the question of separation of species in Nearctic *Exoteleia*." He provided a discussion of how characters vary within the groups as he perceived them but did not formally attach names to those groups or illustrate any of the variation he observed.

Two species of *Exoteleia*, *E. dodecella* and *E. succinctella* (Zeller), are recognized in Europe (Huemer and Karsholt 1999). *Exoteleia dodecella* is variable with respect to wing pattern and size; smaller and darker specimens occur in central Europe. The smallest specimens of *E. dodecella* are quite similar in size and pattern compared to some of the North American "species." Minor genital differences are given to separate *E. dodecella* and *E. succinctella* with the caveat that too few female specimens were available to assess variability and reliability of the characters (Huemer and Karsholt 1999). Interestingly, upon describing *E. nepheos*, Freeman (1967) indicated that the initial discovery and distribution near Lake Erie suggested that it could be an introduced species. Specimens of *E. nepheos* are similar in pattern to the smaller and dark brown "variants" of *E. dodecella* from central Europe.

Taxonomic problems concerning *Exoteleia* raise the following two questions. Is weak morphological differentiation evidence of distinct species, or is it simply intraspecific variation? Can the existing nominal species concepts in *Exoteleia*, based on morphological characters, be applied with any degree of confidence to any or all of the clusters delineated by DNA barcodes?

The purpose of this study is to (1) document that *E. dodecella* is established in the United States and Canada, (2) provide new records of its present distribution in North America, (3) make available a modern description and diagnosis of *E. dodecella*, including the adult and immature stages, for the accurate identification of this species where it is discovered, and (4) assess the usefulness of classical morphology and DNA barcodes as identification tools for *E. dodecella* and other North American species of *Exoteleia*.

Materials and Methods

Late-instar larvae were field collected by removing twigs about a centimeter from the infested buds. One-half of the infested buds were dissected to obtain larvae, and the remaining buds were placed in empty plastic artificial diet cups closed with paper lids. Moth pupae were dissected from infested buds at a later time. Larvae were preserved by placing live specimens in boiling H_2O for less than a minute before transfer-

ring them to 70% ethyl alcohol. Pupae were preserved by placing live specimens directly into 70% ethyl alcohol.

For SEM study, larvae and pupae were cleaned in a full-strength solution of Formula 409 ™ detergent, rinsed in distilled water, and subsequently dehydrated in increasing concentrations of alcohol, ending with absolute alcohol. After dehydration, specimens were critical point dried using a Tousimis critical point dryer, mounted on SEM stubs using carbon paste, and coated with gold-palladium (40/60%) using a Cressington sputter coater. Forewings were detached from pinned specimens and mounted on stubs using carbon adhesive tabs. The finestructure of the larva, pupa, and the male sex scales on the undersurface of the forewing was studied with an Amray 1810 scanning electron microscope at an accelerating voltage of 10 kV.

Morphological observations and measurements of the adults, larvae, and pupae were made using a Leitz RS dissecting microscope with a calibrated micrometer. Genitalia were dissected as described by Clarke (1941) except mercurochrome and chlorazol black were used as stains. The Methuen Handbook of Colour (Kornerup and Wanscher 1978) was used as a color standard. Voucher specimens of adults and immature stages of *E. dodecella* and its parasitoids from this study are deposited in Smithsonian Institution National Museum of Natural History, Washington, D.C. (USNM), the Insect Collection, Department of Entomology, University of Maine at Orono, Orono, Maine (UMDE), and the S. Passoa collection, Columbus, Ohio. Larval nomenclature follows Stehr (1987), and pupal nomenclature follows Mosher (1916). Plant taxonomy, including nomenclature and authorship, follows GRIN (2008). Because voucher specimens to associate adults with the immature stages were not available, we have placed quotations around species identifications of larval and pupal *Exoteleia* taken from the literature and collections to acknowledge that these names are tentative. The only exception is of *E. dodecella* because our field-collected immature specimens are associated with reared adults.

For molecular analysis specimens were collected live and killed using ammonium hydroxide or cyanide prior to mounting and spreading. Specimens were labeled with individual voucher codes (Specimen IDs), databased, and photographed. All collateral data, images, sequences, and trace files were uploaded to the project entitled, "Gelechiidae of North America 02 (GONAB)" and "Lepidoptera of Eastern North America" in the Barcode of Life Database (BOLD) (www.barcodinglife.org, Ratnasingham and Hebert 2007). Barcode sequences (658 bp segment from the 5′ end of mitochondrial cytochrome c oxidase I—or COI—gene) have been submitted to GenBank and have the following accession numbers: FJ412321 - FJ412323, FJ412931 - FJ412939, GU088331 - GU088335, GU089773 - GU089774, GU092285, GU095766 - GU095772, and GU358079 - GU358181.

One or two legs were removed from each specimen and stored in individual tubes within a 96-tube sample box obtained from Matrix Technologies. Analysis of DNA sequences followed DNA barcoding methods of Hajibabaei et al. (2005) and deWaard et al. (2008).

Barcode sequences were obtained at the Biodiversity Institute of Ontario at the University of Guelph. Tissue was placed in a 96-well plate of proteinase K lysis buffer and incubated for about 18 hours. The lysate was then processed following the glass fibre-protocol of Ivanova et al. (2006) on a Beckman Coulter Biomek^{Fxp} liquid handler. For

PCR amplification, 2 μl of DNA extract was added to each well of a premade PCR plate stored at 20°C and containing 2 μl of H_20, 6.25 μl of 10% trehalose, 1.25 μl of 10× buffer, 0.625 μl of 50 mM M_gCl_2, 0.0625 μl of 10 mM dNTPs, 0.06 μl of Platinum Taq polymerase (Invitrogen), and 0.125 μl of each of the 10 μM primers LepF1 and LepR1 (Hebert et al. 2004). Thermocycling conditions consisted of an initial denaturation at 94°C for 1 min, five cycles of 94°C for 30 sec, annealing at 45°C for 40 sec, and extension at 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 51°C for 40 s, and 72°C for 10 min. The PCR reactions were visualized with an Introgen E-Gel 96 agarose electrophoresis system before performing the sequencing reactions, again in premade and frozen plates. Both the forward and reverse direction plates contained 0.25 μl of Applied Biosystems Dye terminator mix v3.1, 1.875 μl of 5× sequencing buffer, 5 μl of 10% trehalose, and 1 μl of 10 μM PCR primer. Sequencing reactions were run at an initial denaturation at 96°C for 2 min, followed by 30 cycles of 96°C for 30 s, annealing at 55°C for 15 s, and extension at 60°C for 4 min. The reactions were purified using an Agencourt Bioscience CleanSEQ system on a Biomek ^{Fxp} liquid handler before being run on an Applied Biosystems 3730XL DNA Analyzer. Electropherograms were edited and manually aligned in an Applied Biosystems Seqscape v.2.5, and the resultant sequences were uploaded into BOLD.

Genetic distances were analyzed with the BOLD analysis tool (Ratnasingham and Hebert 2007) and with MEGA4 (Tamura et al. 2007) using the Kimura 2-parameter model of base substitution and to produce neighbor-joining (NJ) similarity trees. For the MEGA analysis, the default 'complete deletion' option was used whereby positions containing gaps and missing data were omitted from the analysis. Maximum parsimony analysis was performed with PAUP* 4.0b10 (Swofford 2003) set to heuristic search, stepwise addition with 100 random addition sequence replicates (max = 500 trees). All other options were set to default. Prior to performing parsimony analysis, redundant sequences were merged with MacClade (Maddison and Maddison 2002). Bootstrap values were estimated using the stepwise algorithm and 1000 repetitions. For comparison, barcode sequences for three species of *Coleotechnites* and one species of *Recurvaria* were included as outgroups in the analysis based upon Lee and Brown (2008) who suggested that these two genera are closely related to *Exoteleia*.

Results and Discussion

Exoteleia dodecella (Linnaeus, 1758)

(Figs. 1–32)

Adult diagnosis and remarks.—In addition to the grayish ground color with darkly pigmented scale tufts on the forewings, *E. dodecella* can be distinguished from native North American *Exoteleia* by having the male genitalia with the costal part of the valva extending at least 1/5 its length beyond the saccular part and a reduced base of the saccular part of the valva. In females, the inception of the ductus seminalis is anterior to the seventh segment, and an invaginated bulla is near to the posterior end of seventh segment.

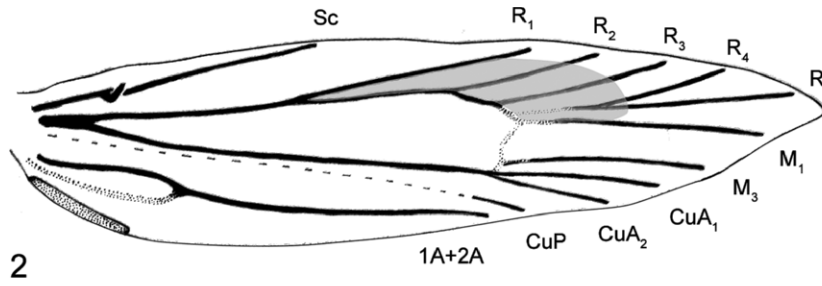
The male sex scales located on the undersurface of the forewing are present in *E. dodecella*, *E. anomala*, *E. burkei*, *E. chillcotti*, *E. nepheos*, and *E. pinifoliella* but are absent in "*californica*" or "*graphicella*." We found that there are some differences in the lengths of the scale cluster and that these differences may help to discriminate

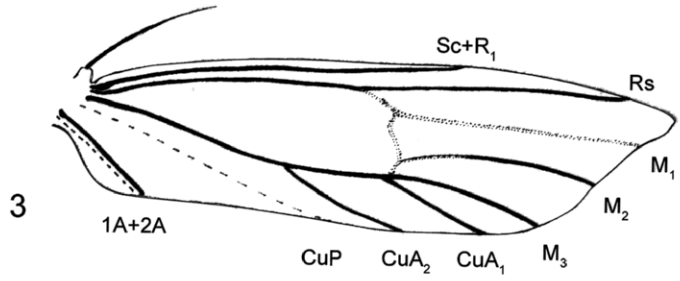
Fig. 1. Adult of *Exoteleia dodecella*.

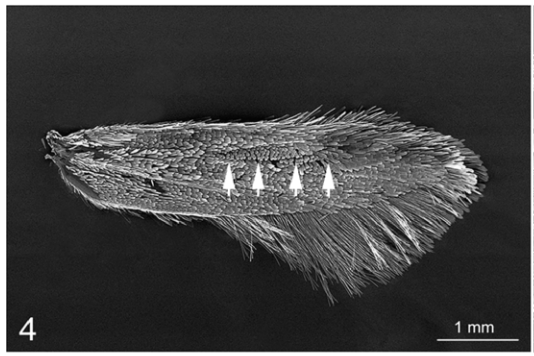
species. Furthermore, the finestructure of these sex scales has not been examined, and it too shows promise for species recognition within *Exoteleia*. The absence of the male sex scales on the undersurface of the forewing may indicate monophyly within *Exoteleia* and adds to the evidence of the misplacement of "*californica*" or "*graphicella*."

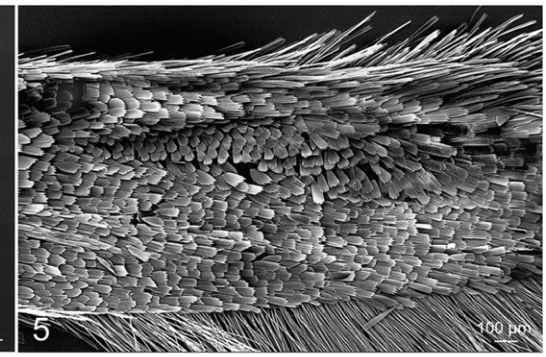
The adult of *E. dodecella* was described and illustrated by Freeman (1960), Piskunov (1989), Huemer and Karsholt (1999), Bland et al. (2002), Gómez de Aizpúrua (2003), and Lee and Brown (2008). Freeman (1960), Piskunov (1989), and Bland et al. (2002) included *Exoteleia* in their keys emphasizing forewing pattern and the male genitalia. With regard to the Holarctic Teleiodini, Lee and Brown (2008) diagnosed *Exoteleia* by having an ocellus and forewing scale tufts in addition to several other characters depending on the sex of the individual. They noted that males have black scent scales on the underside of the forewing between R_1 and R_5 but lack hair pencils at the base of the hind wing. Female specimens of *Exoteleia* lack a signum in the corpus bursae, a very unusual character for the tribe. Species of *Coleotechnites* feed on the same hosts as *Exoteleia* but differ in having asymmetrical male genitalia and a rhomboid signum in females.

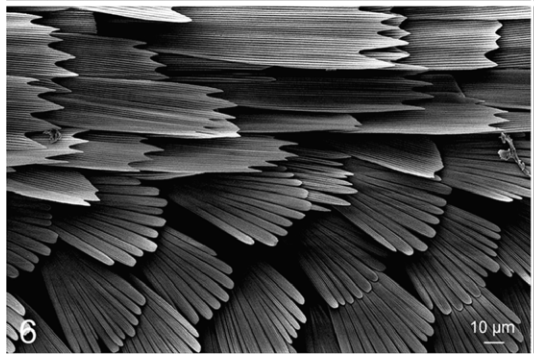
Redescription.—Adult. *Head:* Scales of vertex agouti patterned (transversely tri-banded from base to apex); basal 2/3 pale gray, distal 1/3 gray or dark gray with narrow pale-gray margin; fronto-clypeus pale gray to white; outer surface of labial palpus with scales patterned as above; basal article dark gray; 2nd article dark gray with paler scales along distal margin, or scales on distal half paler with white band of scales along distal margin; distal article with three irregularly-shaped alternating white bands and two dark-gray

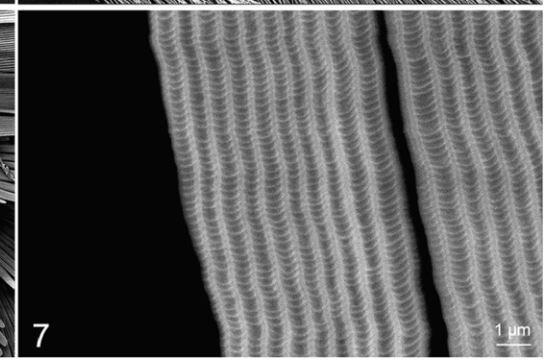




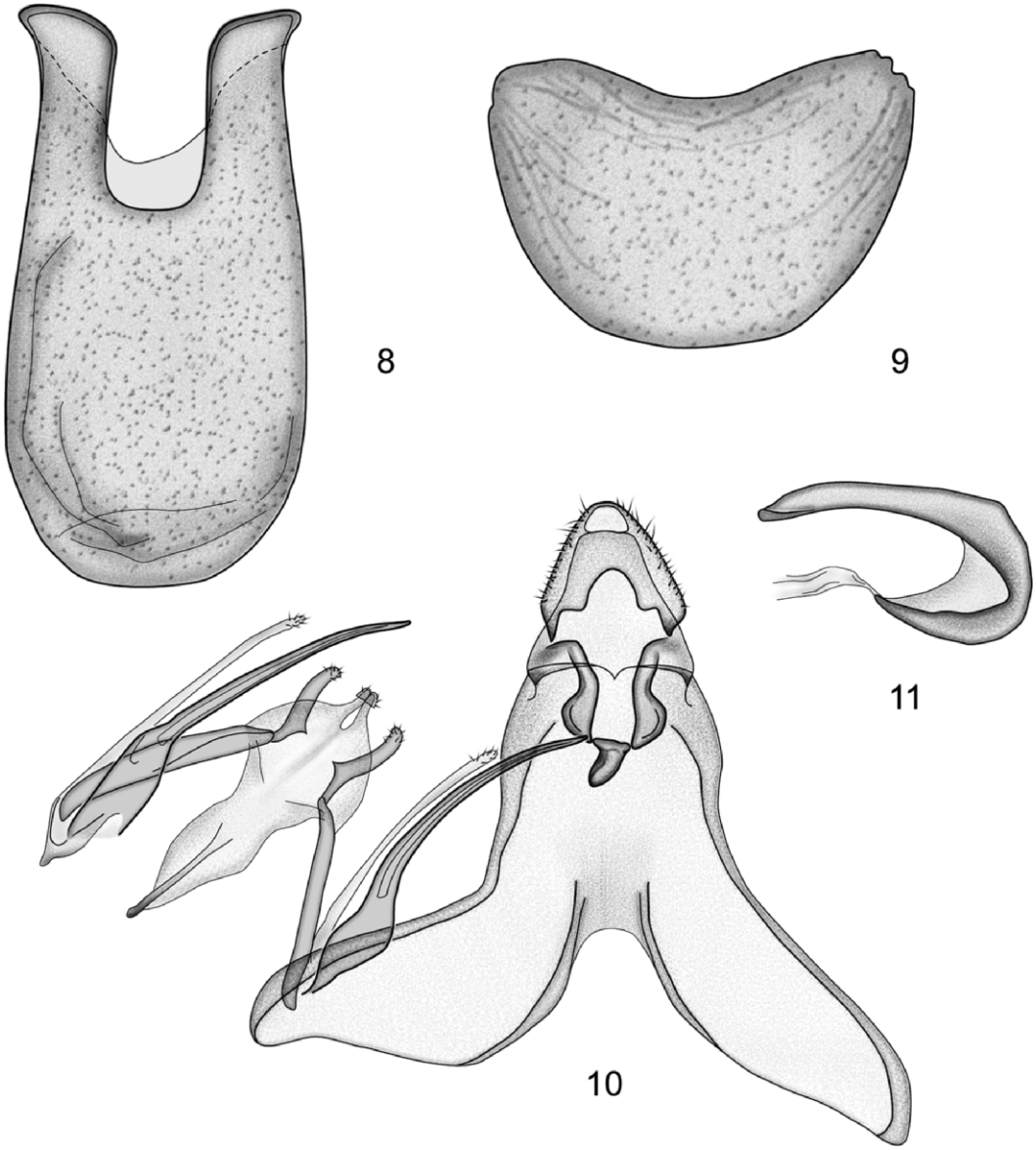








Figs. 2–7. Wing venation and scanning electron micrographs of male sex scales on undersurface of forewing of *E. dodecella*. 2, Venation of forewing with shaded area indicating location of male sex scales; 3, Venation of hind wing; 4, Undersurface of forewing of male, arrows = positional area of sex scales, scale bar = 1 mm; 5, Male sex scales, scale bar = 100 µm; 6, Comparison of male sex scales and other wing scales, scale bar = 10 µm; 7, Finestructure of male sex scales, scale bar = 1 µm.



Figs. 8–11. Male genitalia and 8th sternal and tergal plates of *Exoteleia dodecella*. 8, Eighth sternum; 9, Eighth tergum; 10, Genital capsule (sternal elements detached on one side and folded to opposite side); 11, Aedeagus.

bands; inner surface as above except 2nd article with more white; scape of antenna dark gray with narrow band of white along distal margin, each flagellomere of antenna dark gray basally, pale gray distally. Ocellus absent. Proboscis with pale-gray scales.

Thorax: Scales of tegula and mesonotum agouti patterned; basal 1/2 pale gray, distal 1/2 gray with narrow pale-gray margin; mesonotum with dark gray to black scale tuft (one or two scales wide) on posterolateral surface near margin. Scales of legs as above; foreleg

and midleg dark gray with narrow white bands on distal margin of tibia and tarsomeres; hind leg similarly patterned with more white scales. Forewing (Fig. 1): Length 5.9–7.8 mm (n = 30) scales agouti patterned; basal, median, and postmedian fasciae distally demarcated with paler scales; one black scale tuft near middle of base (three scales wide); two on opposite sides of CuP near end of basal fascia (anterior scale tuft two scales wide, posterior scale tuft five scales wide); two on opposite sides of CuP near end of median fascia (anterior scale tuft two scales wide, posterior scale tuft four to five scales wide); and two scale tufts on proximal margin of post median fascia above CuP (anterior scale tuft two scales wide, posterior scale tuft five scales wide); four or five marginal black spots present or absent; apex pale gray or white. Venation (Fig. 2) with all veins not reaching margin; R_4 and R_5 stalked near basal 1/4; M_2 absent. Undersurface gray, contrasted by darkly pigmented male sex scales within shaded area (Fig. 2); scanning electron micrographs (Figs. 4–7) of male sex scales indicate palmate structure with 9–12 digitate processes, in contrast to other wing scales (Figs. 5–6) having only irregularly-serrated distal margins; finestructure of palmate sex scales with latticelike pattern of crossbraces between scutes (Fig. 7). Hind wing pale gray. Venation (Fig. 3) with M_1 weak; cubitus 4-branched with M_2 originating closer to M_3 than to M_1; frenulum with one acanthus in male, three acanthae in female.

Abdomen (Figs. 8–9): Dorsal surface pale gray; ventral surface pale gray, gradually darkening to distal end. Eighth sternum (Fig. 8) elongate, broadly rounded distally, with 2 short basal arms; 8th tergum (Fig. 9) crescent-shaped, wider than long, with broadened posterolateral margins.

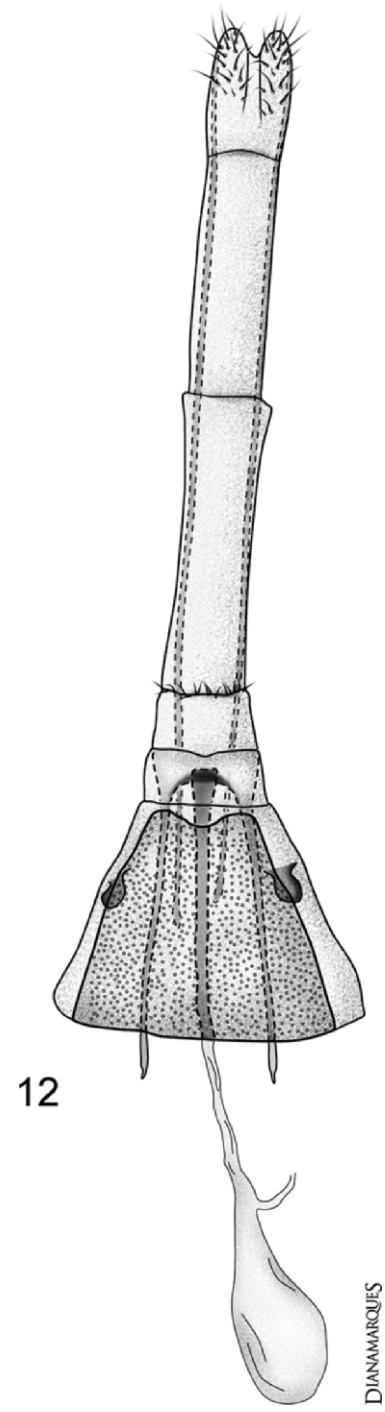
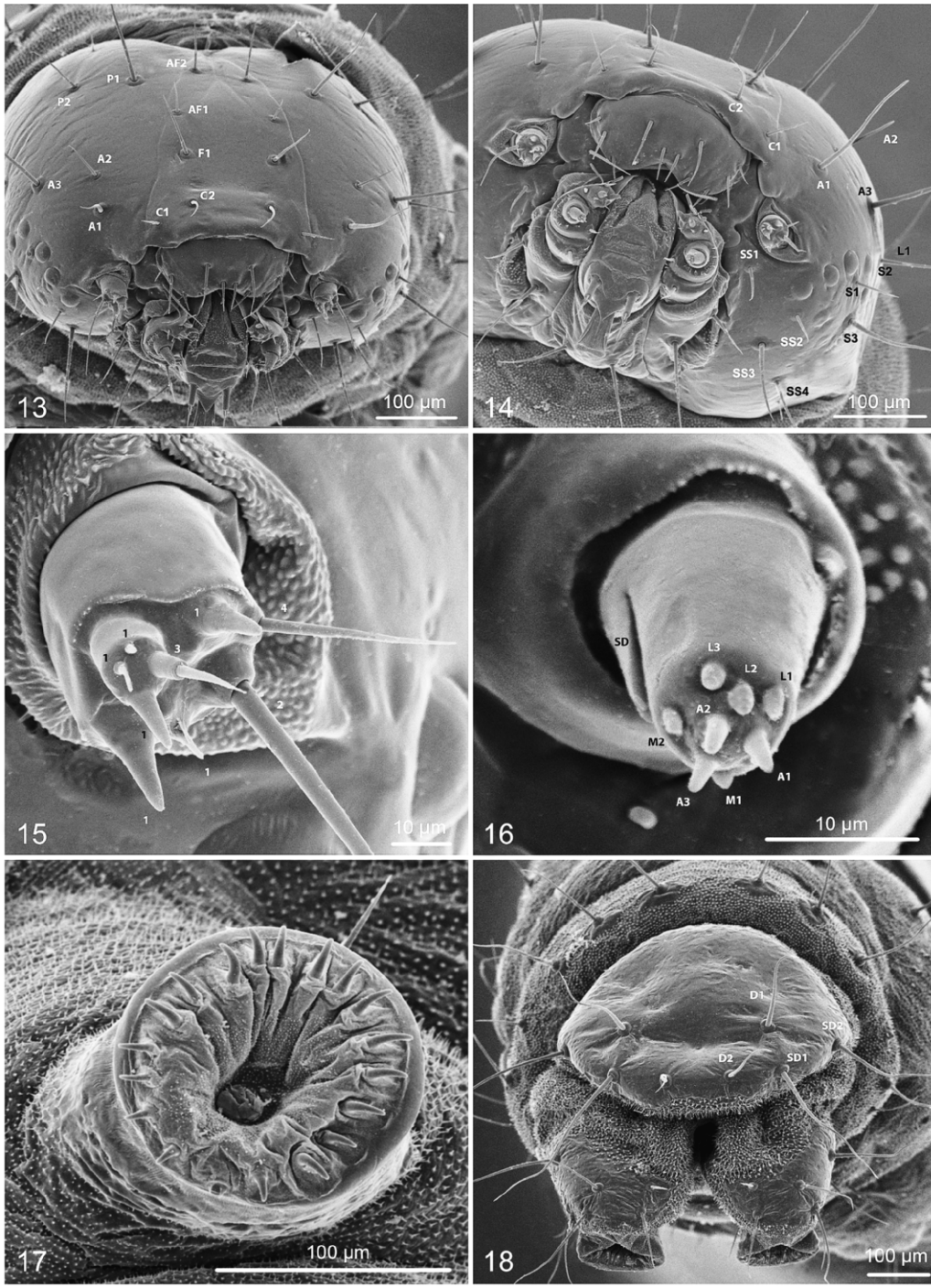
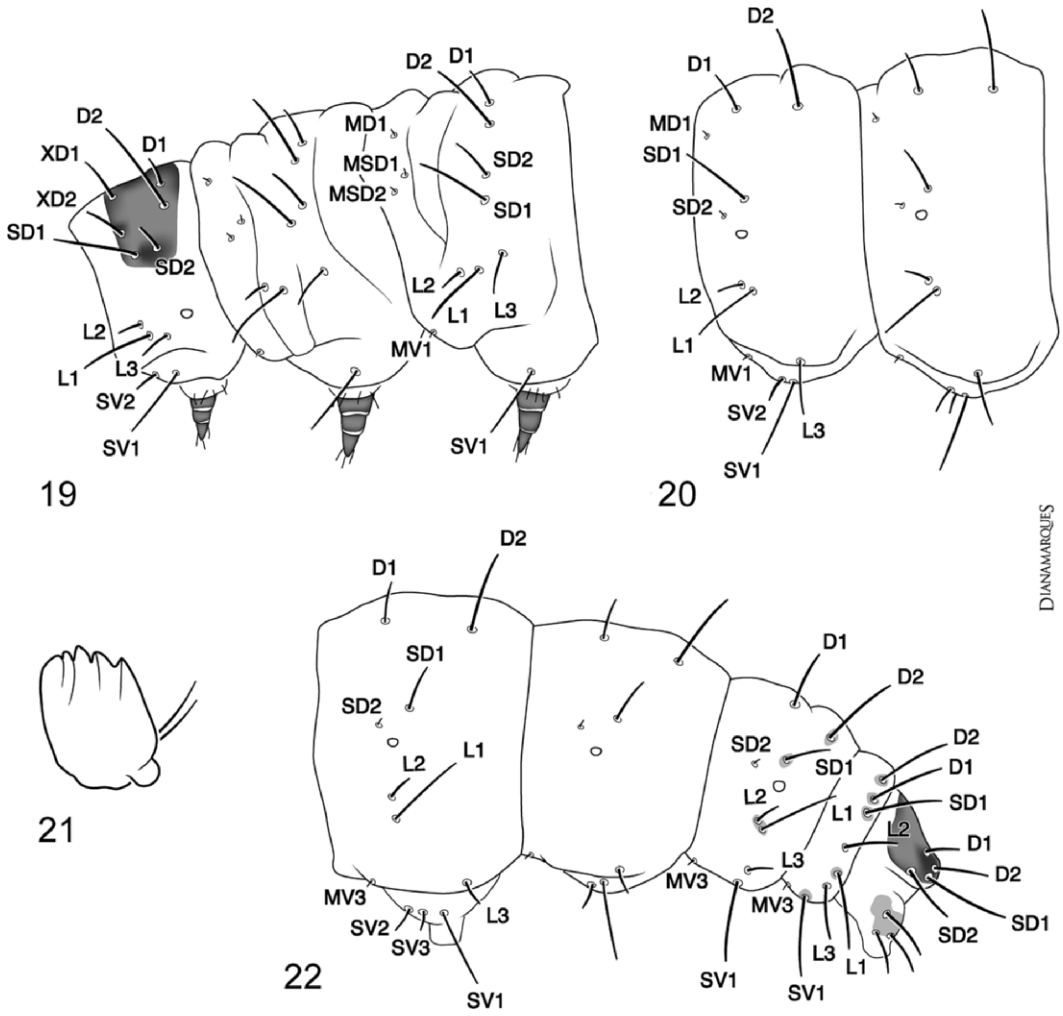


Fig. 12. Female genitalia of *Exoteleia dodecella*.



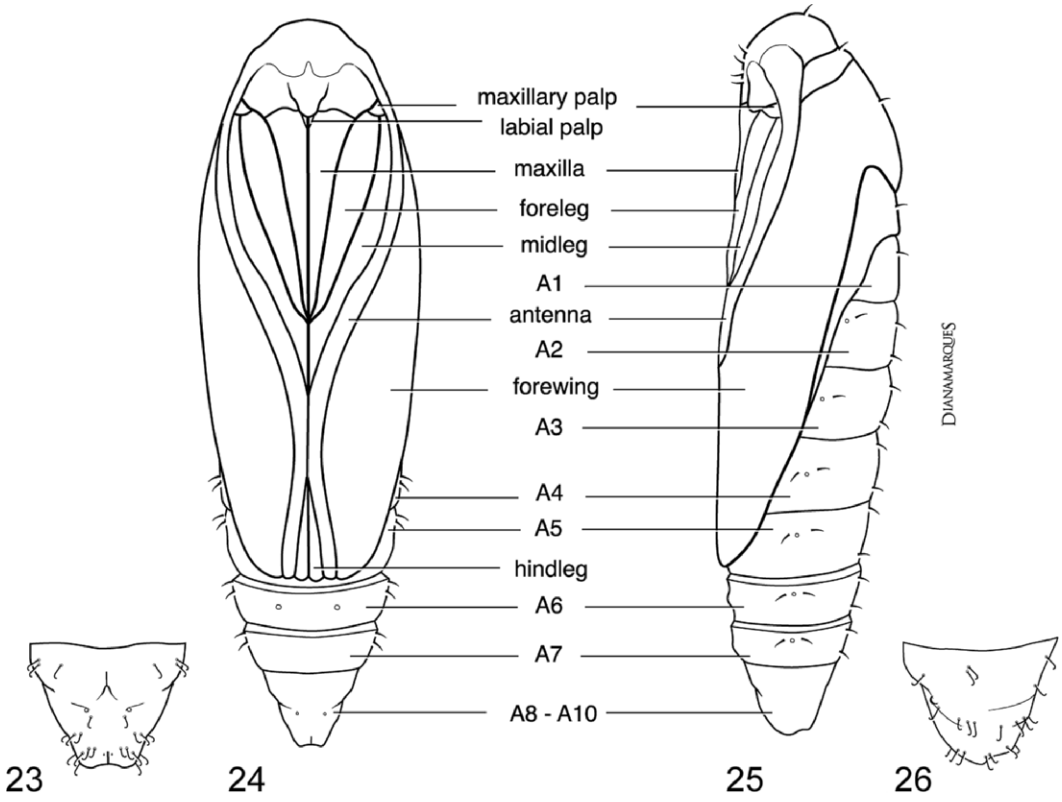
Figs. 13–18. Scanning electron micrographs of *Exoteleia dodecella*. 13, Head, frontal view, scale bar = 100 µm; 14, Head, ventral view, scale bar = 100 µm; 15, Apical sensilla of left antenna, 1 = sensilla basiconica, 2 = sensilla chaetica, 3 = sensillum styloconicum, 4 = sensillum trichodeum, scale bar = 10 µm; 16, Apical sensilla of left maxillary palpus, A2 = sensillum styloconicum, A1, A3, M1, M2, L1, L2, L3 = sensilla basiconica, SD = sensillum digitiform, scale bar = 10 µm; 17, Left proleg on A5, dorsolateral view, scale bar = 100 µm; 18, Setal arrangement of anal plate on A10, scale bar = 100 µm.



Figs. 19–22. Setal maps and mandible of *Exoteleia dodecella*. 19, T1–T3, lateral view; 20, A1–A2, lateral view; 21, Right mandible, view of inner surface; 22, A6 – 10, lateral view.

Male genitalia (Figs. 10–11): Uncus hoodlike, setose; gnathos with shortened lateral arms extending inwards, acutely curved anteriorly and dilated distally, each arm fusing with a median conical process; tegumen gradually widened basally forming two broad, anteriorly divergent arms; vinculum an elongate median process widened posteriorly and narrowed anteriorly; vinculum with two short, convergent, digitate lobes on posterior end and one elongate process originating from 2/3 length, extending slightly beyond anterior end; an elongate lateral lobe fused with vinculum near 1/3 length, extending posteriorly, forming slightly curved digitate lobe; lateral lobe fused at base with anterolateral arm of tegumen ventral to an elongate, inwardly curved spinelike valva and a slightly shorter digitate lobe; aedeagus acutely bent near 1/3 length from base, without cornuti.

Female genitalia (Fig. 12): Ovipositor telescopic, with three membranous



Figs. 23–26. Pupa of *Exoteleia dodecella*. 23, A8 – 10, ventral view; 24, Pupa, ventral view; 25, Pupa, lateral view; 26, A8 – 10, lateral view.

subdivisions posterior to 8th segment; papillae anales lobate and setose; apophyses posteriores about 2.5× longer than apophyses anteriores; part of ductus bursae from ostium to anterior margin of 7th sternum about 1.5× longer than part of ductus bursae from posterior margin of 7th sternum to inception of ductus seminalis; corpus bursae longer than wide; medioposterior margin of 7th sternum broadly emarginate; an opening within pleural membrane demarcates an invaginated bulla near posterior end of 7th segment.

Specimens examined: Four specimens from Howland, Penobscot County, Maine and three specimens from Medford, Piscataquis County, Maine, reared from *P. sylvestris* by R. Tracy, (USNM Coll.), serve as voucher specimens for Tracy (1980). In addition, these specimens also serve as voucher specimens for the first documentation of the introduction of this species into the United States. Two specimens collected from Croton Dam, New York, June 9, 1934, and one specimen from Valhalla, New York, June 12, 1934, reared from *Pinus resinosa* Aiton are in the USNM, suggesting that *E. dodecella* may have been established in the United States prior to its documented introduction into Canada in 1928 (Sheppard 1930).

Other specimens examined: United States: Howland, Maine, 24 ex; Royalton, Vermont, 5 ex; Meriden, Connecticut, 5 ex; and Great Bend, Pennsylvania, 1 ex. All adult specimens were reared from *P. sylvestris* [USNM]; Europe: 22 ex [USNM]. Canada: Ontar-

io, Grimsby, reared from *P. sylvestris*, 1938, 1 ex; Ontario, Lorraine, 1932, 11 ex; Ottawa, reared from *Pinus mugo* and *P. sylvestris*, various dates 1934–1971, 7 ex; Ridgeville, reared from *P. sylvestris*, 1932, 1 ex; Simcoe, reared from *P. sylvestris*, 1962, 8 ex; Vernon, reared from *P. sylvestris*, 1955, 30 ex; Port Franks, collected at light, 4 ex; British Columbia: Port Coquitlam, collected at blacklight, 2006, 15 ex [all Canada specimens in CNC].

Larval diagnosis and remarks.—*Exoteleia dodecella* can be distinguished from native species of North American *Exoteleia* by having a bisetose SV-group on A1 and a single row of 8–14 crochets on the anal prologs. Biological characters also provide important clues; the needle-mine of *E. dodecella* usually contains frass, and any specimen of *Exoteleia* on Scots or Mugo pine is most likely *E. dodecella* because species of *Exoteleia* that occur in the U.S. tend to avoid those trees.

The larva of *E. dodecella* was partially described or illustrated by Martin (1959), Lindquist and Trinnell (1967), and Gómez de Aizpúrua (2003). A combination of biology, host plants, and morphology are all useful for identifying *E. dodecella*.

According to Freeman (1960), *Exoteleia* "*pinifoliella*," *E. dodecella*, and *Coleotechnites ardas* (Freeman 1960) (Gelechiidae) are the only North American pine leaf-miners to retain frass in their mines. *Coleotechnites ardas*, known only from Montana, has a silk ramp near a large exit hole on the middle of the mine. This differs from the above two species of *Exoteleia*, which have an exit hole at the base of the mine without a silken ramp (Freeman 1960: fig. 64). *Exoteleia* "*pinifoliella*" pupates in the mine, whereas *E. dodecella* pupates in the bud. Interestingly, the North American population of *E. dodecella* produces only a single hole in the last instar mine instead of two holes that is characteristic of their biology in Europe. Another complicating factor is that *E. dodecella* may expel frass from the last mine of the season, which is used as an overwintering shelter. Thus, biological characteristics alone cannot be used to identify this species.

Host plant data can aid in identification. Scots and Mugo pine are readily colonized by *E. dodecella*. *Exoteleia* "*pinifoliella*" from New York preferred jack pine (*Pinus banksiana* Lamb.) and pitch pine (*Pinus rigida* Mill.) but did not survive well on Scots pine (Bennett 1954a). Thus, miners on Scots pine are more likely to be *E. dodecella* than *E.* "*pinifoliella*," at least in New York State. Unfortunately, Mugo pine was not tested by Bennett (1954a).

Because *E. dodecella* will opportunistically feed on many species of pines if they are growing near their preferred hosts (Martin 1959), species of *Exoteleia* on native pines are unlikely to be *E. dodecella* unless they are near Scots or Mugo pine. *Exoteleia nepheos* is found mostly on red pine, *P. resinosa*, less frequently on Scots pine, and rarely on Mugo pine (Lindquist and Trinnell 1967) again suggesting that *E. dodecella* is the most likely species on Scots or Mugo pine.

Morphological characters used to separate pine-needle miners in Ontario were given by Lindquist (1963) and Lindquist and Trinnell (1967). Both "*E. nepheos*" and "*E. pinifoliella*" have the anal crochets reduced in number and divided into two groups. The anal proleg of *E. dodecella* has a single row of 8–14 crochets (Lindquist and Trinnell 1967). In addition, Lindquist (1963) found that *E.* "*pinifoliella*" has a unisetose SV group on A1. This is unusual because *E. dodecella* and *E.* "*nepheos*" (Lindquist and Trinnell 1967), *E.* "*burkei*" from the western

U.S. (Burdick and Powell 1960), and most Gelechiidae (Hodges 1998) have the SV group of A1 bisetose.

Exoteleia dodecella can sometimes be confused with two unrelated species, *Rhyacionia bouliana* (Tortricidae) and *Recourvaria resinosae* (Freeman 1960) (Gelechiidae). *Exoteleia dodecella* can be separated from *R. bouliana* by examination of the cuticular texture. *Exoteleia dodecella* has a smooth texture, whereas *R. bouliana* is covered with course microspinules (Martin 1959). In addition, larvae of Tortricidae and Gelechiidae can be separated by the chaetotaxy of A9 (Stehr 1987). As a leaf-miner, *E. dodecella* and *R. resinosae* can be confused because both lack an anal comb. *Exoteleia dodecella* has 8–14 crochets on the anal proleg, and the SV setae of A3 – A6 are on separate pinacula (Lindquist 1963). These contrast with *R. resinosae*, in which there are 16 crochets on the anal proleg, and the SV setae of A3 – A6 are on a single pinaculum. In addition, *R. resinosae* feeds on *P. resinosa* and occasionally on *P. banksiana* (Lindquist 1963), two hosts rarely colonized by *E. dodecella*.

As pointed out by Lindquist (1963), a large number of taxa are leaf-miners only in the early instars. Our diagnosis is for the most common associates of *E. dodecella* that are leaf miners throughout their entire life. Identification of early instar pine leaf miners is more complicated.

Redescription.—Larva (Figs. 13–22). Length 5.5–9.6 mm (n = 10 preserved larvae). Body pale gray, with dense covering of microspinnules; most pinacula less than twice diameter of setal socket of A1 or absent, pinacula on A8 – 10 slightly darker than pinacula on other segments of body, and pinacula on A8 – 10 greater than twice diameter of setal sockets on A1; head capsule, prothoracic shield, thoracic legs, anal plate, and large pinaculum on anal proleg of A10 dark brown.

Head (Figs. 13–16, 21): Hypognathous; adfrontal area extends to epicranial notch, frontal area extends about 3/4 that distance; integument shallowly wrinkled; AF2 slightly above apex of frons, about equal in length to F1; AF1 shorter than and closer to F1 than to AF2; C2 about 1/4–1/3 longer than C1; P1 in line with A1, about twice as long as P2; P2 slightly posterodorsal of P1; L1 dorsal to stemma 2 and dorsolateral to A3; A3 and A1 about equal in length, about twice the length of A2; A2 slightly lateral to line connecting P1 and A1; six stemmata in an irregular C-shaped pattern, with stemma 3 and 4 approximate, and stemma 6 slightly below stemma 4; S-group setae in an arclike pattern below stemmata; S3 posteroventral to stemma 6; S2 posterior to stemma 1; S1ventroposterior to stemma 3; SS1 beneath and between base of antenna and condyle of mandible; SS2 ventral to and between stemma 5 and 6; SS3 in near vertical line with antenna and ventral to SS2; SS4 ventroposterior to SS3; labrum with six pairs of setae, two equal median pairs, two subequal frontomarginal pairs, and two subequal lateromarginal pairs; hypopharyngeal complex with broad spinneret, gradually widening from base, longer than labial palpus; proximomedial region spinulate; sensilla of antenna as figured (Fig. 15); sensilla of maxillary palpus as figured (Fig. 16); mandible with four teeth, middle two larger than outer two, two subequal mandibular setae present above condyle (Fig. 21).

Thorax (Fig. 19): T1 with L1 2.0–2.5× longer than L2 and L3; L1 in horizontal line or ventral to L3, both setae beneath spiracle, L2 equal in length or slightly shorter than L3, in horizontal line to L1, or slightly anterodorsal to L1 at level of center of

spiracle; SV-group bisetose, SV1 about 2.5–3.0× longer than SV2; prothoracic shield with SD1 slightly posterior to and about 1 1/2 longer than XD1 and XD2; XD2 about 2.0× farther from XD1 than SD1; SD2 slightly anterior to D2 and D1, about same length as D1, both setae slightly shorter than XD1 and XD2; D1 in line with XD1; D2 about same length as SD1, equidistant to XD1 and XD2, slightly posterior to D1. T2 - 3 (Fig. 19): D2 about twice length of D1, both slightly posterior to SD-group setae; SD1 about twice length of SD2; L1 about same length as D2 and SD1, about 3.0–4.0× longer than L2, both setae slightly anterior to SD setae; L3 slightly longer than L2, posterodorsal to L1; SV1 in vertical line with or slightly posterior to L3; MD, MSD, and MV setae along anterior margin of segments; MD1 in line with or slightly ventral to D2; MSD1, in line with or slightly dorsal to SD2, MSD2 anteroventral to MSD1; MV1 beneath L setae; V1 setae on T3 slightly farther apart than V1 setae on T2, each pair at least twice as far apart as V1 setae on T1; MV3 (not shown) farther apart than V1setae; MV2 absent.

Abdomen: A1 – A2 (Figs. 17–18, 20, 22): D1 about 2.0–2.5× length of D2; SD1 about as long as D1, in vertical line with or slightly posterior to D2 on A1, directly above or posterodorsal to spiracle on A2; SD2 minute, anterodorsal to spiracle (need high magnification to see); L1 about 2.0–2.5× length of L2, both setae approximate, L2 in vertical line with or slightly posterior to spiracle; L3 slightly anterior of D1 and posterior of SV group; SV group bisetose on A1 (in transverse line), trisetose (in triangular pattern) on A2; MD1 and MV1 as above; V1 setae equidistant to or slightly closer than V1setae on T2 – 3; MV3 (not shown) farther apart than V1 setae, MV2 absent. A3 – 10 (Figs. 17–18, 22): setae as in A1 – 3 except A7 with SV group bisetose, approximate, near parallel with median longitudinal axis; crochets of A3 – 6 in uniordinal mesal pennellipse with small gap or circle closed by a few small crochets. A8 with SV group unisetose; D1 and SD1 each on slightly widened pinaculum, L1 and L2 usually on same, slightly widened pinaculum; L3, SV1, and V1 almost aligned vertically. A9 with all setae approximating a straight vertical line; D2, D1, the hairlike SD1, L1, and SV1 about equal in length, each on slightly widened pinaculum, about twice length of L2, L3, and V1. A10 (Figs. 18, 22): anal plate with SD1 slightly longer than SD2 and D2, D1 slightly shorter than SD1 and D2; anal comb absent; anal prolegs bearing 8–14 crochets in uninterrupted arc.

Specimens examined: 28 Specimens from Howland, Penobscot County, Maine from *P. sylvestris* by R. Tracy (USNM Coll.).

Pupal diagnosis and remarks.—The pupa of *E. dodecella* was described or illustrated by Martin (1959), Patočka (1987), Gómez de Aizprua (2003), and Patočka and Turčáni (2005). It can be distinguished from native species of *Exoteleia* by having a vertex that lacks a cutting plate, a body that is widest medially and not parallel-sided, a single pair of proleg scars on A6, and a notched terminal abdominal segment. The pupation site, in the bud of the host, is unusual, however, *E. nepheos* is rarely known to pupate within buds of its hosts.

Compared to the few North American genera studied by Mosher (1916), *E. dodecella* is most similar to *Coleotechnites* (listed in her key as *Recurvaria* group B). Both genera lack modified abdominal setose knobs, dense body setae, a fringe of setae around the posterior margin of A7, and stout spines on the end of the abdomen. *Exoteleia*

and *Coleotechnites* are similar in that the antennae reach the caudal margin of the wings, and apically hooked setae are present on the terminal segments.

Exoteleia differs from other known Holarctic gelechiids in the relative length of the maxillae and prothoracic legs. In particular, the maxillae are equal to or shorter than the prothoracic legs in *E. dodecella* and other species of *Exoteleia* (Bennett 1954b, 1966; Lindquist and Trinnell 1967). This diagnosis of *E. dodecella* was used by Patočka and Turčáni (2005) and formed the basics for Lee and Brown's (2008) placement of *E. dodecella* as the sole member of their group I in the tribe. Undoubtedly, other species of *Exoteleia* will be added to this group when their pupal morphology is confirmed.

Hodges (1985) noted that pupal morphology may help define species of *Exoteleia*. We found substantial differences between our specimens of *E. dodecella* and those of an *Exoteleia* labeled as "*pinifoliella*" from North Carolina. In agreement with the warnings by Hodges (1985) that *pinifoliella* may be a complex of three sibling species, our unassociated pupal specimens of *E. pinifoliella* are listed as *Exoteleia* "*pinifoliella* or near" in the following.

Characters that help separate *E. dodecella* from related species include the shape of the body and vertex, number of proleg scars, and the terminal abdominal segments. In particular, the body of *E. dodecella* is widest at the middle (Fig. 24) compared to *E.* "*pinifoliella* or near," which is cylindrical and more parallel-sided (similar to Bennett 1954b). There is only one pair of proleg scars in *E. dodecella* visible on A6 (Fig. 24); the scars on A5 are hidden by the overlying wings. The pupa of *E.* "*pinifoliella* or near" has two exposed proleg scars on A5 and A6. In addition, A8–A10 of *E. dodecella* has scattered and apically hooked setae (Figs. 24, 26) on segments A8–A10, whereas in *E.* "*pinifoliella* or near" the apically hooked setae are restricted to a small clump on A10 (similar to Bennett 1954b).

Bennett (1954b, 1966) described the pupa of *E.* "*pinifoliella*" and *E.* "*chillcotti*." He illustrated *E.* "*pinifoliella*" with a spined vertex called the "cutting plate." The vertex of *E. dodecella* is rounded without a spine. The cylindrical body shape of *E.* "*chillcotti*" is more similar to *E.* "*pinifoliella*" than to *E. dodecella*. Hodges (1985) noted that it was unclear which species of *Exoteleia* was illustrated by Bennett (1954b, 1966), but they cannot be of *E. dodecella*.

Lindquist and Trinnell (1967) mentioned characters to separate three species of *Exoteleia* found on pine in Ontario. They noted that *E. dodecella* has a dark reddish brown pupa with the anal end tending to be broadly notched inwardly (see Fig. 23). The pupa of *E.* "*nepheos*" is yellow brown, and the anal end is not notched. Larval exuvium will show the characteristic crochet arrangement that readily separates these two species. Details and references on mounting microlepidopteran larvae on microscope slides are listed in Passoa (2008: 305–306).

These examples show that pupal morphology is useful in separating *E. dodecella* from related species. Future problems will remain in assigning the correct species name to the various phenotypes of *Exoteleia* pupae that can be recognized in collections and in the literature, especially for the rarer species.

Redescription.—Pupa (Figs. 23–26). Length 5.1–5.7 (n = 5): Brownish orange, smooth, slightly flattened dorsoventrally; vertex rounded, with fine punctures almost forming longitudinal rows; frontoclypeus bilobed, suture not

Figs. 27–30. Larval damage of *Exoteleia dodecella* to Scots pine in Maine. 27, "Bunched growth" resulting from repeated infestations; 28, Needle mine of third instar. Arrow pointing to entrance; 29, Bud attacked by fourth instar. Arrow indicates white, pitch-impregnated silken tube at base; 30, Shoots attacked after beginning of spring growth. Arrow indicates silken tube at base.

straight; labrum U-shaped, labial palpi hidden or minutely exposed; maxillary palpi exposed, not extending beyond anterior margin of eye; maxillae slightly shorter than or equal to the length of prothoracic legs; mesothoracic legs shorter than antennae; antennae meet mesially; apical parts of metathoracic legs exposed; pair of proleg scars present on A6; thoracic spiracle a small tubercle; abdominal spiracles small and circular; dorsum of abdomen shagreened; A8–10 with apically hooked setae on dorsal and ventral surfaces (Figs. 23, 26).

Specimens examined: Four specimens of *E. dodecella* from Howland, Penobscot County, Maine from *P.*

Table 1. Percent mitochondrial cytochrome c oxidase I (COI) sequence divergence among species of *Exoteleia*, *Coleotechnites*, and *Recurvaria*. Uncorrected average pairwise distances are shown. Cells below diagonal = mean between-species distances in %. Cells above diagonal = mean number of base pair changes between species. Diagonal (shaded) cells = mean within-species distances in %, with mean number of base pair changes in parentheses. Species abbreviations are as follows: dod = *Exoteleia dodecella*, ex1 = "*E. pinifoliella* complex" group1, ex2 = "*E. pinifoliella* complex" group2, ex3 = "*E. pinifoliella* complex" group3, ex4 = "*E. pinifoliella* complex" group4, atr = *Coleotechnites atrupictella*, flo = *Coleotechnites florae*, que = *Coleotechnites quercivorella*, nan = *Recurvaria nanella*.

	dod	ex1	ex2	ex3	ex4	atr	flo	que	nan
Exoteleia dodecella	0% (0.0)	35	37	36	41	55	61	50	60
Exoteleia group1	7.02	0.05% (0.3)	9	13	14	58	57	48	54
Exoteleia group2	7.47	1.66	0.16% (0.9)	13	14	59	60	48	54
Exoteleia group3	7.21	2	2.48	0.42% (2.2)	15	55	57	48	55
Exoteleia group4	8.22	3.03	2.73	2.87	0.19% (1.0)	61	58	51	58
Coleotechnites atrupictella	11.36	12.03	12.2	11.44	12.66	0.26% (1.3)	39	27	58
Coleotechnites florae	12.64	11.71	12.51	11.72	12.66	7.99	0.38% (2.0)	26	61
Coleotechnites quercivorella	10.15	9.74	9.86	9.69	10.46	5.35	5.08	0.19% (1.0)	51
Recurvaria nanella	12.48	11.12	11.13	11.35	11.92	12.02	7.99	5.35	0% (0.0)

sylvestris, (USNM); 12 specimens of "*Exoteleia pinifoliella*" from Lunenburg from *P. rigida* needles, Massachusetts, (USNM); six specimens from Bent Creek Experimental Forest, North Carolina, from *P. resinosa* needles (USNM).

Biology (Figs. 27–30).—The life history of *E. dodecella* was studied in detail by Lemarie (1958a) in Slovakia, Martin (1959) and Freeman (1960) in Ontario (Canada), and Tracy (1980) in Maine (United States). Larvae of *E. dodecella* cause extensive damage to buds of Scots pine. Infested trees show a bunched growth (Fig. 27). Severe infestations can cause a witches' broom effect as these trees suffer repeated attacks.

The elliptical eggs are laid on the current year's growth or sometimes on one-year-old stems. The first instar enters a needle tip in July. Second and third instar larvae continue to feed on needles (Fig. 28) until later in the summer when the caterpillar seals the mine after ejecting most of the frass. This shelter becomes the overwintering site. Feeding continues the following spring, and in early May the larva molts to the fourth instar and enters a bud before spring growth begins (Fig. 29). The larva constructs a silken tube at the base of the bud, which becomes impregnated with pitch. If the bud is not large enough for the larva to complete its development, it will leave the bud and enter into an elongated shoot (Fig. 30). The presence of dead buds and shoots with a tube of silk and pitch at the base is unique for *E. dodecella*. Other species that feed on Scots and Mugo pine, such as *E. nepheos* and *R. bouliana*, enter the stem after growth has begun and the buds have elongated into shoots.

Pupation occurs in the bud or shoot from late May to early June. Adults emerge from mid June to early July.

Distribution.—In North America, *E. dodecella* is known from the Niagara Peninsula of Ontario, Canada south into the northeastern United States from Maine, Vermont, Connecticut, and west into New York and northeastern Pennsylvania. We also have unconfirmed reports that *E. dodecella* occurs in Michigan (R. Tracy pers. obs.), but the records could not be confirmed by voucher specimens. In the West, it is

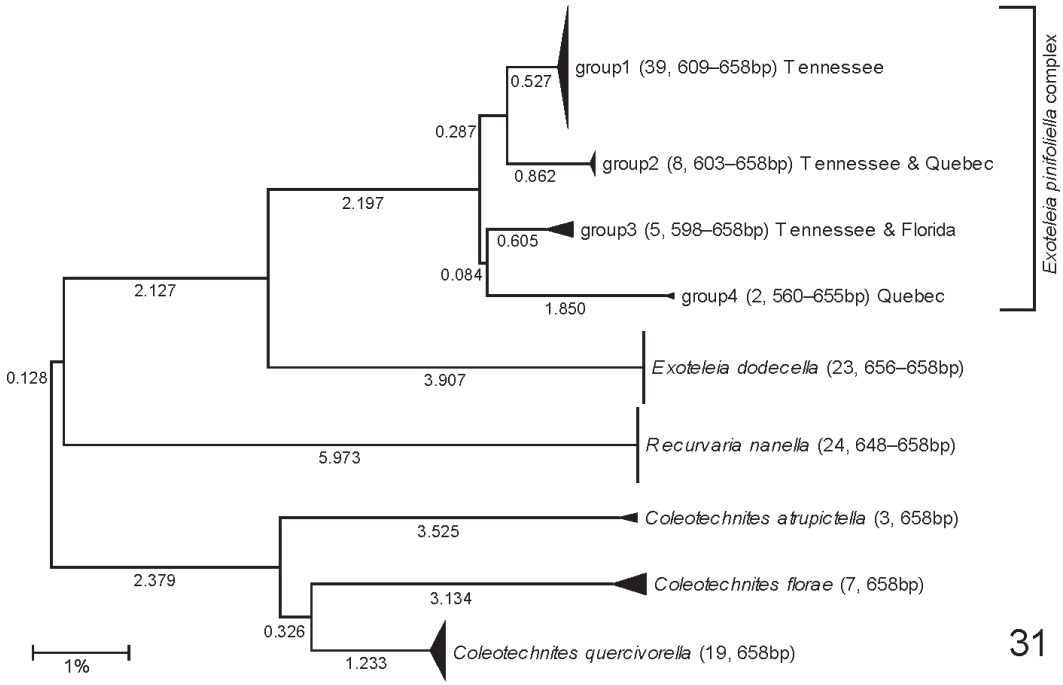


Fig. 31. Neighbor-joining tree based on Kimura-2-Parameter distances for 658 bp of cytochrome c oxidase I (COI) in species of *Exoteleia*, *Coleotechnites*, and *Recurvaria* (total = 130 specimens). Numbers below branches indicate % of sequence divergence. Numbers in parentheses after species names indicate the number of specimens analyzed and the range of sequence lengths obtained. Individual species branch clusters were collapsed.

also present in the Vancouver area of British Columbia, Canada.

The citation for *E. dodecella* in China may represent another introduction of this species to an Old World locality (Zhang 1994). China is outside the normal range of this species (Bland et al. 2002).

Parasitoids.—Tracy (1980) reared the following species of parasitoids from *E. dodecella* in Maine: Diptera. *Phytomyptera usitata* (Coquillett) (Tachinidae). Hymenoptera. *Goniozus* sp. (Bethylidae); *Chelonus recurvariae* McComb, *Orgilus* sp. (Braconidae); *Elachertus* sp., *Sympiesis stigmatipennis* Girault, *Peckekachertus* sp. (Eulophidae); *Copidosoma geniculatum* (Dalman) (Encyrtidae); *Scambus* sp., *Phaeogenes* sp., *Exeristes comstockii* (Cresson) (Ichneumonidae); *Telenomus* sp. (Scelionidae).

Results from DNA barcodes.—There were 523 positions in the final dataset; 135 positions were omitted in the analysis due to gaps or missing data. This was due primarily to four specimens in the "*Exoteleia pinifoliella* complex" with shorter barcode sequences (ca. 560–600 bp).

Samples of *E. dodecella* from the United States exhibited more than 7% sequence divergence from the closest samples that represented native North American species of *Exoteleia*, indicating strong evidence for a distinct species. While these findings are congruent with morphological differences in the adult and immature stages, it is notable that this high amount of divergence translates into a relatively

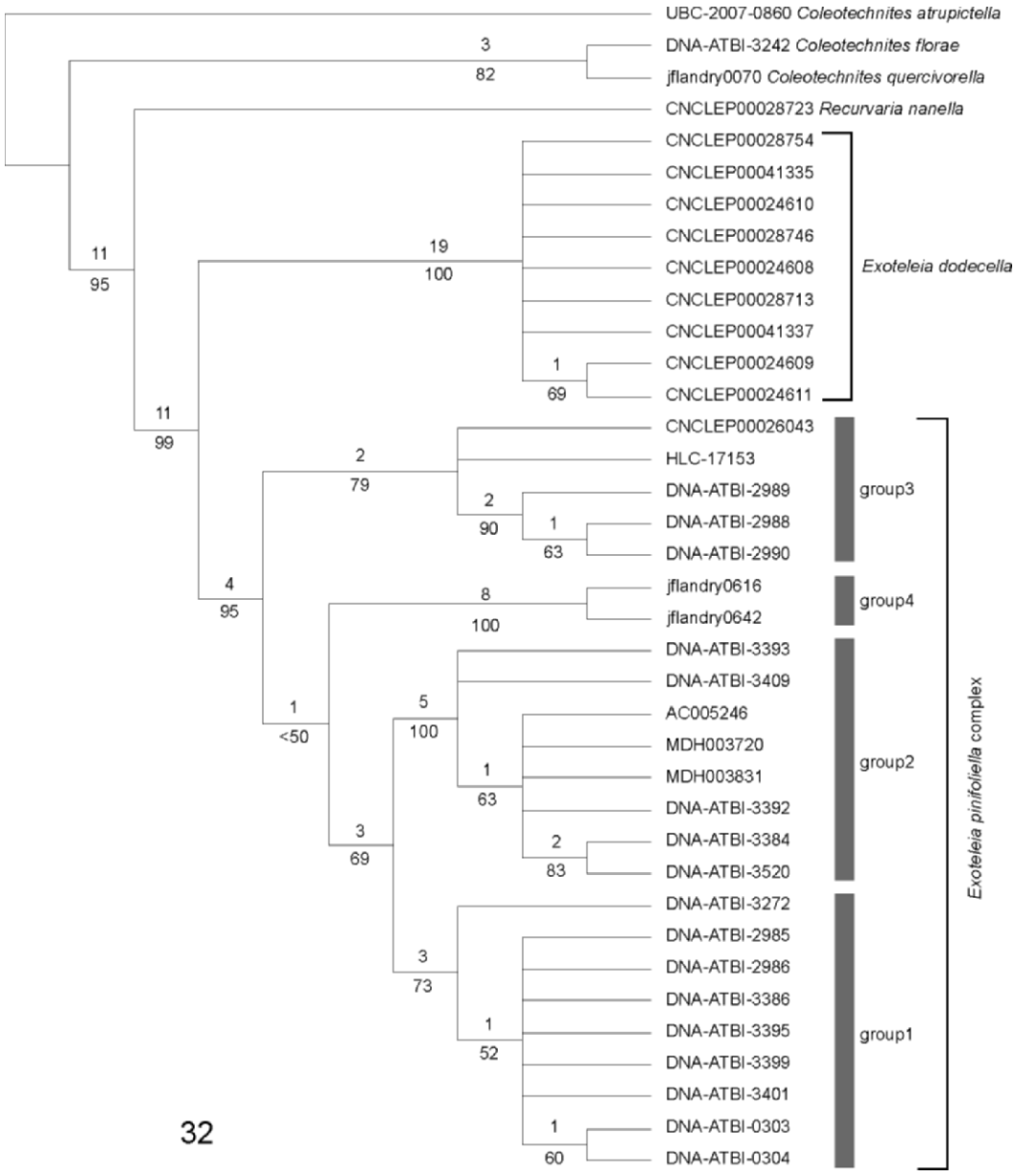


Fig. 32. Strict consensus tree of 42 most parsimonious trees (length = 224, CI = 0.763, RI = 0.918) based on 658 bp of cytochrome c oxidase I (COI) in species of *Exoteleia*, *Coleotechnites*, and *Recurvaria* (total = 130 specimens). Bremer support indices are shown above branches; bootstrap support values are below branches. Branch terminal labels indicate unique specimen identifiers (Specimen IDs in BOLD) and taxonomic assignment.

small amount of morphological difference.

In contrast, samples of native North American taxa revealed a comparatively low level of sequence divergence (<3%). Geographic representation of the samples was uneven with only eastern specimens and a stronger rep-

resentation from the southeastern region in Tennessee and Florida. Four weak clusters can be detected (Table 1, Fig. 31) with distances ranging from 0.04–3.03%. The clusters are moderately to strongly corroborated by parsimony analysis (Fig. 32), which lends to support the contention based on morphology that there are several weakly differentiated species. An alternative interpretation would suggest that native populations of *Exoteleia* in North America represent a single variable species with different haplotypes that reflect its phylogeographic history through ancestral polymorphism, host races, or some other biological pattern. Available evidence is insufficient to make a convincing argument either way. However, we prefer to retain the hypothesis of multiple species at this time because it agrees with the traditional classification (although it lacks clarity) and is consistent with interspecific barcode divergences observed in other genera of Lepidoptera with well defined species (Hebert et al. 2009). Intra-cluster divergences are consistently lower than inter-cluster divergences (Table 1).

DNA barcodes can reliably distinguish *E. dodecella* from native species of *Exoteleia* sampled from eastern North America. However, the inadequate state of current taxonomy precludes the unambiguous naming of the barcode clusters of native species, so they have been informally labeled "group 1" and "group 2." For convenience, and to distinguish them from the *E. dodecella* samples, we use the "*E. pinifoliella* complex" based upon the oldest name. This is the name that would apply if additional data led to the conclusion that they represent but a single and variable species.

All other attributes as reported in the literature (i.e., adult coloration, genitalia, larval hosts, immatures, geographic distribution) are either insufficient or not congruent with each other and do not support species separation. Hodges' (1985) "entities" based on color pattern and genitalia appear incongruent with our sequence data. For example, specimen CNCLEP00026043 from Florida is a very pale moth whose barcode clusters tightly with very dark specimens from Tennessee and Florida. All "*E. pinifoliella* complex" sensu lato barcodes obtained in the present study were obtained from specimens collected at light; consequently, there is no host information for these samples. All reared specimens of *E. pinifoliella*, as well as the other nominal species, had degraded DNA that is not suitable for sequencing. Consequently, additional data will be necessary to achieve better taxonomic resolution of *Exoteleia*, notably preservation of the immature stages associated with reared adults, careful host and biological observations, and preservation of material for barcode data from samples with known hosts and detailed biological data.

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the fine computer generated illustrations of the male and female genitalia and produced the plates. We also thank J. R. deWaard, D. Handfield, P. D. N. Hebert, and D. Holden for supplying some barcoded specimens. Support for DNA barcoding was provided by the Canadian Barcode of Life Network from Genome Canada through the Ontario Genomics Institute, NSERC (to JFL), and other sponsors listed at www.BOLDNET.ca.

Literature Cited

Bennett, W. H. 1954a. The effect of needle structure upon the susceptibility of hosts to the pine need miner *Exoteleia pinifoliella* (Chamb.) (Lepidoptera: Gelechiidae). The Canadian Entomologist 86: 49–54.

———. 1954b. The pupal morphology of the pine needle miner. Proceedings of the Entomological Society of Washington 56(1): 41–42.

———. 1966. Pupal morphology of *Exoteleia chillcotti* Freeman. Proceedings of the Entomological Society of Washington 68(3): 181–183.

Bland, K. P., M. F. V. Corley, A. M. Emmet, R. J. Heckford, P. Huemer, J. R. Langmaid, S. M. Palmer, M. S. Parsons, L. M. Pitkin, T. Rutten, K. Sattler, A. N. B. Simpson, and P. H. Sterling. 2002. Gelechiidae. The Moths and Butterflies of Great Britain and Ireland. Vol. 4 (part 2). Harley Books, England. 277 pp.

Braun, E. L. 1961. The Woody Plants of Ohio. Ohio State University Press, Columbus. 362 pp.

Burdick, D. J. and J. A. Powell. 1960. Studies on the early stages of two California moths which feed in the staminate cones of digger pine (Lepidoptera: Gelechiidae). The Canadian Entomologist 92: 310–320.

Clarke, J. F. G. 1941. The preparation of slides of the genitalia of Lepidoptera. Bulletin of the Brooklyn Entomological Society 36: 149–161.

deWaard, J. R., N. V. Ivanova, M. Hajibabaei, and P. D. N. Hebert. 2008. Assembling DNA Barcodes: Analytical Protocols, pp. 275–293. *In* C. Martin, ed. Methods in Molecular Biology: Environmental Genetics. Humana Press Inc., Totowa.

Elias, T. S. 1989. Field Guide to North American Trees. Grolier Book Clubs, Danbury. 51 pp.

Freeman, T. N. 1960. Needle-mining Lepidoptera of pine in North America. The Canadian Entomologist, Supplement 16, 51 pp.

———. 1963. Two new species of coniferous needle miners from Louisiana and the description of a new genus (Lepidoptera: Gelechiidae). The Canadian Entomologist 95: 727–730.

———. 1967. A new Nearctic species of *Exoteleia* Wallengren (Gelechiidae) on Pine. Journal of the Lepidopterists' Society 21: 9–11.

Germplasm Resources Information Network (GRIN). 2008. United States Department of Agriculture, Agricultural Research Service, Beltsville Area. Website: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?401686. Accessed 2 February 2008.

Gómez de Aizpúrua, C. 2003. Orugas y Mariposas de Europa. I. Ministerio de Medio Ambiente. 352 pp.

Hain, F. P. and W. E. Wallner. 1973. The life history, biology, and parasites of the pine candle moth, *Exoteleia nepheos* (Lepidoptera: Gelechiidae), on Scotch pine in Michigan. The Canadian Entomologist 105: 157–164.

Hajibabaei, M., J. R. deWaard, N. V. Ivanova, S. Ratnasingham, R. Dooh, S. L. Kirk, P. M. Mackie, and P. D. N. Hebert. 2005. Critical factors for the high volume assembly of DNA barcodes. Philosophical Transactions: Biological Sciences 360: 1959–1967.

Handfield, L. 1992. Liste des Lépidoptères du Québec et du Labrador. Fabreries. Supplement 7, 155 pp.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of USA 101: 14812–14817.

Hebert, P. D. N., J. R. deWaard, and J.-F. Landry. 2009. DNA barcodes for 1/1000 of the animal kingdom. Biology Letters doi: 10.1098/rsbl.2009.0848.

Hodges, R. W. 1983. Gelechiidae, pp. 19–25. *In* R.W. Hodges, ed. Check List of the Lepidoptera of America North of Mexico. E. W. Classey Ltd., Faringdon, Oxfordshire, England, and The Wedge Entomological Research Foundation, Washington, DC, USA, xxiv + 284 pp.

———. 1985. A new species of *Exoteleia* (Gelechiidae) reared from Ponderosa Pine. Journal of the Lepidopterists' Society 39: 139–144.

———. 1986. Gelechioidea, Gelechiidae (part). Moths of America North of Mexico. E.W. Classey Ltd., and the Wedge Entomological Research Foundation. Fascicle 7.1, 195 pp.

———. 1998. Gelechioidea, pp. 131–158. *In* N. P. Kristensen, ed. Handbuch der Zoologie, Lepidoptera, Part 1, Volume 35, Walter de Gruyter & Co, Berlin, New York. 495 pp.

Huemer, P. and O. Karsholt. 1999. Microlepidoptera of Europe. Vol. 3, Gelechiidae. Apollo Books, Stenstrup. 356 pp.

Ivanova, N. V., J. R. de Waard, and P. D. N. Hebert. 2006. An inexpensive, automation-friendly protocol for recovering high quality DNA. Molecular Ecology Notes. doi: 10.111/j.1471–8286.2006.01471.x.

Kim, K. C. and A. G. Wheeler, Jr. 1991. Pathways and Consequences of the Introduction of Non-indigenous Insects and Arachnids in the United States. Report to the U.S. Congress Office of Technology Assessment. 74 pp.

Knutson, L., R. I. Sailer, W. L. Murphy, R. W. Carlson, and J. R. Dogger. 1990. Computerized data base on immigrant arthropods. Annals of the Entomological Society of America 83(1): 1–8.

Kornerup, A. and J. H. Wanscher. 1978. Methuen Handbook of Colour. 2nd ed., Methuen and Co., Ltd., London. 243 pp.

Lee, S. and R. L. Brown. 2008. Revision of Holarctic Teleiodini (Lepidoptera: Gelechiidae). Zootaxa 1818: 1–55.

Lemarie, J. 1958a. Druhý příspěvek k poznání bionomie makadlovky borové *Exoteleia* (*Heringia*) *dodecella* L. [Second investigation on the bionomics of the pine bud moth, *Exoteleia dodecella* L.] Zoologicke Listy 7: 208–220.

———. 1958b. Příspěvek k poznání cizopasníků makadlovky borové *Exoteleia* (*Heringia*) *dodecella* L. Část I. Nadčeled' Chalcidky, Chalcidoidea; Vejřitky, Proctotrupoidea; Hbitěnky, Bethyloidea. [Contribution to knowledge of the parasites of the pine bud-moth *Exoteleia* (*Heringia*) *dodecella* L. Part I. Chalcidoidea, Proctotrupoidea, Bethyloidea.] Zoologicke Listy 21: 221–230.

———. 1959a. Erster Beitrag zur Kenntnis der Parasiten von *Exoteleia dodecella* L. (Hymenoptera: Chalcidoidea; Proctotrupoidea; Bethyloidea). [First contribution to the knowledge of the parasites of *Exoteleia dodecella* L.]. Beiträge Zur Entomologie 9: 805–809.

———. 1959b. Příspěvek k poznání cizopasníků makadlovky borové *Exoteleia* (*Heringia*) *dodecella* L. Část II. Celedi Ichneumonidae, Braconidae, Larvaevoridae. [Contribution to the knowledge of parasites of the pine-bud wandering moth *Exoteleia* (*Heringia*) *dodecella* L. 2. Ichneumonidae, Braconidae, and Larvaevoridae.]. Zoologicke Listy 22: 309–314.

———. 1961. Příspěvek k poznání cizopasníků makadlovky borové *Exoteleia* (*Heringia*) *dodecella* L. Část IV. Ichneumonidae, Braconidae, Chalcidoidea, Bethyloidea. [Contribution to knowledge of the parasites of the pine bud-moth *Exoteleia* (*Heringia*) *dodecella* L. Part 4. Ichneumonidae, Braconidae, Chalcidoidea, Bethyloidea.]. Zoologicke Listy 24: 119–126.

Lindquist, O. H. 1963. Larvae of pine needle miners in Ontario. The Canadian Entomologist 95(5): 517–521.

Lindquist, O. H. and J. R. Trinnell. 1967. Biology and description of immature stages of *Exoteleia nepheos* (Gelechiidae) on pine in Ontario. Journal of the Lepidopterists' Society 21(1): 15–21.

Maddison, W. P. and D. R. Maddison. 2002. MacClade 4.05 OSX [computer program]. Sinauer Associates, Sunderland, Massachusetts.

Martin, J. L. 1959. The bionomics of the pine bud moth, *Exoteleia dodecella* L. (Lepidoptera: Gelechiidae), in Ontario. The Canadian Entomologist 91: 5–14.

Mattson, W. J., P. Niemela, I. Millers, and Y. Inguanzo. 1994. Immigrant phytophagous insects on woody plants in the United States and Canada: an annotated list. United States Department of Agriculture, Forest Service, North Central Forest Experiment Station General Technical Report NC-169. 27 pp.

Mosher, E. 1916. A classification of the Lepidoptera based on characters of the pupa. Bulletin of the Illinois State Laboratory of Natural History 12: 17–159, pls. 19–27.

National Agricultural Pest Information System (NAPIS) Pest Tracker. http://pest.ceris.perdue.edu/searchnews.php. Accessed January 2009.

Passoa, S. 2008. Part III. Immature Stages, pp. 295–314. *In* T. M. Gilligan, D. J. Wright, and L. D. Gibson, eds. Olethreutine Moths of the Midwestern United States, An Identification

Guide. Ohio Biological Survey Bulletin, New Series, Vol. XVI, No. 2, The Ohio Biological Survey, Columbus. vii + 334 pp.

Patočka, J. 1987. Uber die Puppen der Mitteleuropaischen Gelechiide (Lepidoptera). 3. Teil Tribus Teleiodini und benachbarte Gattungen. Vestnik-Ceskoslovenske-Spolecnosti-Zoologicke 51(4): 286–299.

Patočka, J. and M. Turčáni. 2005. Lepidoptera Pupae. Central European Species. Apollo Books, Stentrup. 863 pp.

Piskunov, V. I. 1989. Gelechiidae, pp. 889–1,024. *In* G. S. Medvedev, ed. Keys to the Insects of the European Part of the USSR. Lepidoptera. Vol. 4, part 2. Oxonian Press Ltd., New Delhi. 1,092 pp. [English translation].

Ratnasingham, S. and P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7: 355–364.

Sheppard, R. W. 1930. Notes on the occurrence of the pine bud moth, *Exoteleia dodecella* L. in Welland County, Ontario. Annual Report of the Entomological Society of Ontario 61: 79–81.

Stehr, F. W. 1987. Immature Insects. Kendall/Hunt Pub. Co., Dubuque. xiv + 754 pp.

Swofford, D. L. 2003. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b.10. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 10.1093/molbev/msm092.

Tracy, R. A. 1980. Distribution, abundance, damage and parasites of *Exoteleia dodecella* L., *Dioryctria* sp. and *Petrova albicapitana* (Busck) on pines in Maine. Masters Thesis. Department of Entomology, University of Maine at Orono, Orono, Maine. vii + 67 pp.

United States Department of Agriculture Research Service, Biosystematics and Beneficial Insects Institute. 1986. Western Hemisphere Immigrant Arthropod Database (WHIAD). Unpublished printout. Doc 0402M. 95 pp.

White, J. H. and R. C. Hosie. 1980. The Forest Trees of Ontario. Ontario Ministry of Natural Resources, Toronto, Canada 7th edition. 114 pp.

Zhang, B. C. 1994. Index of Economically Important Lepidoptera. CAB International, United Kingdom. 599 pp.