Insects are known for their biological diversity, ecological importance, and economic impact (Scudder 2009), making accurate and timely taxonomic classification of insect species imperative (Wheeler 2009). Ideally, an integrative approach that incorporates many sources of evidence should be used to achieve this taxonomic goal. Integrative taxonomy combines morphological, molecular, behavioral, and ecological data to improve identification, discover new species, delimit species boundaries, and reconstruct phylogenetic relationships (Dayrat 2005, Will et al. 2005, Sperling and Roe 2009, Padial et al. 2010, Schlick-Steiner et al. 2010). Use of diverse data sources is invaluable in all aspects of insect taxonomy but is particularly important when examining closely related species (Roe and Sperling 2007, Sperling and Roe 2009, Roe et al. 2010). Evolutionary processes such as introgression and incomplete lineage sorting lead to fuzzy species boundaries, particularly between closely related species where insufficient evolutionary time has passed for diagnostic characters to become fully fixed. As such, incongruence may exist between species limits and diagnostic traits, which could be undetected when a single character set is examined (Rubinoff et al. 2006, Elias et al. 2007, Roe and Sperling 2007, Twewick 2007, Roe et al. 2010).

_Dioryctria_ (Zeller 1846) (Lepidoptera: Pyralidae) is a large, distinct genus of phycitine moths that requires the use of integrative taxonomy for accurate species delimitation (Roe and Sperling 2007). Currently, there are 79 recognized _Dioryctria_ species (Nuss et al. 2010) and at least several undescribed species (Du et al. 2005, Knöllke 2007, Powell and Opler 2009). Although 12 species groups were erected to help clarify morphological variation within _Dioryctria_ (Muto and Munroe 1972, 1974; Wang and Sung 1982; Neunzig 2003; Knöllke 2007), accurate identification of species is still problematic (Roe and Sperling 2007, Roux-Morabito et al. 2008). Many species show interspecific overlap of molecular, morphological, or behavioral traits, thereby impeding species delimitation and identification (Roe et al. 2006, Roe and Sperling 2007, Roux-Morabito et al. 2008). Larvae of all _Dioryctria_ species feed on conifers, many on or in the cones of economically important species (Pinaceae and Cupressaceae) (Neunzig 2003, Roux-Morabito et al. 2008, Whitehouse et al. 2011). As such, several _Dioryctria_ species are considered economically important pests and require...
Table 1. Members of the *D. zimmermani* species group

| Species                                  | Wing Color | Larval Host                                                                 | Pheromonea | Referenceb |
|------------------------------------------|------------|-----------------------------------------------------------------------------|------------|------------|
| *D. albocittella* (Hulst)*               | L          | P. monophylla Torrey & Frémont, *P. cembroides* Zuccarini *F. edulis* Engelmann | Z11–16:Ac + C25-p | Heinrich (1956), Cibrian-Tovar et al. (1986) |
| *D. amatella* (Hulst)*                   | D          | P. palustris Miller, *P. taedivorella* Zahlbruck, P. contorta Douglas ex Loudon, P. coulteri D. Don | Cones, shoots cambium, flowers, rust cankers | Heinrich (1956), Coulson and Franklin (1970), Heldin et al. (1980), Meyer et al. (1986), Miller et al. (2010) |
| *D. banksiella* Mutuura, Munroe & Ross  | D          | F. banksiana Lambert                                                       | Rust cankers | Mutuura (1982), Mutuura et al (1969) |
| *D. cambiocola* (Dyar)*                  | D          | F. ponderosa Douglas ex C. Lawson, P. contorta Douglas ex Loudon, P. coulteri D. Don | Cambium, rust cankers, shoots, cones | Heinrich (1956), Mutuura et al. (1969), Mutuura (1982) |
| *D. contortella* Mutuura, Munroe & Ross* | D          | F. contorta                                                               | Cambium, rust cankers | Mutuura et al. (1969) |
| *D. cuiticeensis* Neunzig                | D          | Unknown                                                                   |            |            |
| *D. delectella* (Hulst)                  | D          | Unknown                                                                   |            |            |
| *D. fordi* Donahue & Neunzig (Hulst)     | L          | P. salicinaus Douglas ex D. Don                                             |            |            |
| *D. merkeli* Mutuura & Munroe*           | D          | F. eliotii Engelmann, P. palustris                                        | Flowers, shoots, cones | Mutuura and Munroe (1979), Hanula et al. (1984), Meyer et al. (1985), Miller et al. (2010) |
| *D. monticolella* Mutuura, Munroe & Ross | D          | F. monticola Douglas ex D. Don                                             | Cambium    | Mutuura et al. (1969) |
| *D. mutuurai* Neunzig                    | L          | Unknown                                                                   |            |            |
| *D. resinosella* Mutuura*                | D          | F. resinosa Aiton                                                         | Shoots, cones | Mutuura (1982), Grant et al. (1993) |
| *D. taelae* Schaber & Wood               | D          | F. taela L., P. echinata Miller                                            | Cones, shoots | Schaber and Wood (1971) |
| *D. taedecorella* Neunzig & Leidy*       | D          | F. taela                                                                  | Cones      | Neunzig and Leidy (1989) |
| *D. tunicoilella* Mutuura, Munroe & Ross*| D          | F. ponderosa                                                             | Rust cankers | Mutuura et al. (1969); G. Grant, unpublished |
| *D. xasterlandi* Donahue & Neunzig       | L          | [P. jeffreyi Balfour]*                                                   |            |            |
| *D. yatesi* Mutuura & Munroe*            | D          | F. pungens Lambert                                                       | Cones      | Donahue and Neunzig (2002) |
| *D. zimmermani* (Grote)*                 | D          | F. strobus L., F. resinosa, P. syylestis L., P. nigra                   | Cambium, shoots | Heinrich (1956); Munroe (1959); Mutuura (1982); G. Grant, unpublished |

Species examined in this study are indicated by an asterisk (*). Host plant information is summarized from Neunzig (2003) and Whitehouse et al. (2011), with additional host plant and pheromone references included.

- Z11–16:Ac, (Z)-11-hexadecenyl acetate; C25-p, (3Z,6Z,9Z,12Z,15Z)-pentacosapentaene; Z9–14:Ac, (Z)-9-tetradecenyl acetate; E9–14:Ac, (E)-9-tetradecenyl acetate; Z9–14:OH, (Z)-9-tetradecen-1-ol; Z9–16:Ac, (Z)-9-hexadecenyl acetate; Z11–16:Ac, (Z)-11-hexadecenyl acetate.
- Includes both pheromone and larval host literature.
- Hypothesized.

Targeted management (Whitehouse et al. 2011, and references therein), necessitating accurate species identification.

Difficulties with species delimitation are common among members of the *zimmermani* species group and typify the taxonomic difficulties commonly found within *Dioryctria*. The *zimmermani* species group is one of the largest groups of *Dioryctria*, containing 18 described species (Table 1), all of which are exclusively Nearctic (Mutuura et al. 1969; Mutuura and Munroe 1979; Neunzig 1990, 2003). Species are characterized by distinctive genitalic structures and prominent forewing scale ridges (Fig. 1), and recent phylogenetic analyses support the monophyly of this group (Du et al. 2005, Roe et al. 2006, Knölke 2007). Although the majority of species have darkly colored forewings, several distinctive pale colored species occur in the western United States (Fig. 2; Table 1). The majority of species in the *zimmermani* group feed almost exclusively on *Pinus* (Munroe 1959, Neunzig 2003, Roe et al. 2006). Larvae feed internally on cambium, shoots, cones, wounds, and rust cankers, causing extensive economic damage, particularly in commercial pine seed orchards (Whitehouse et al. 2011), and
host plant may be an important diagnostic character (Neunzig 2003). Pheromone lures, designed to improve management and control of Dioryctria pests, show distinct species differences (Table 1) (Meyer et al. 1986, Grant et al. 1993, Miller et al. 2010), although cross-species attraction does occur (Hanula et al. 1984).

Despite pheromone and host plant differences among species (Table 1), accurate species identification remains elusive and species limits in this group need further examination. Identification of species relies primarily on minor forewing differences, geographic distribution, and larval host plant associations (Neunzig 2003), although these traits show considerable interspecific overlap (Sopow et al. 1996, Roe et al. 2006), complicating species diagnostics. Furthermore, previous molecular work on Dioryctria has found low levels of molecular variation separating members of the zimmermani group, particularly among dark-scaled species (Richmond and Page 1995, Du et al. 2005).

The objectives of this study were to examine the genetic diversity found within species in the zimmermani species group and relate molecular variation to larval host plant association, geographic distribution, and pheromone attraction. Using this integrated taxonomic approach, we hope to clarify species boundaries within this difficult group.

Materials and Methods

Specimen Collection. Adult and larval specimens in the D. zimmermani group were sampled from sites across North America by using a variety of methods,
including light trapping, pheromone lures, and larval rearing (Table 2). Pheromone trapping was conducted in the southeastern United States as described by Miller et al. (2010). Identification of specimens was based on forewing morphology, host association, and geographic range, based on species descriptions in Neunzig (2003). All specimens are deposited in the Strickland Museum frozen tissue collection at the University of Alberta.

Molecular Methods. Total genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue kit (QIAGEN, Valencia, CA) using manufacturer’s instructions. Two independent molecular markers were sequenced from all samples. Mitochondrial (mtDNA) and cytochrome c oxidase I and II gene regions (COI-COII) were obtained by PCR amplification and cycle sequencing protocols as in Roe et al. (2006) (5′ CGGACACGTGCTAAGTCCG 3′) to rcM44.9 (5′ CTTCTAAATCYCTGTCCTCC 3′) and M44–1 (5′ GCTGACGGYARCTACATC 3′) to E600rc (5′ TCCTTAGCTCAACTGCC 3′) (Cho et al. 1995, Reed and Sperling 1999). For specimens with DNA voucher numbers from AR25 to AR332, mtDNA polymerase chain reaction (PCR) amplification, purification, and cycle sequencing protocols are as in Roe et al. (2006). Protocols for all EF1α amplification and the remaining mtDNA sequences were as follows. PCR amplification was performed in 50 μl reactions using Takara Taq and supplied reagents (R001T, Takara, Otsu, Shiga, Japan). The reaction mix contained 0.25 μl of Takara Taq (5 U/μl), 5 μl of 10× PCR buffer, 4 μl of dNTP mixture (2.5 mM each), and 2 μl of extracted genomic DNA, 2 μl per primer (5 μM each). PCR products were purified with EXO-SAP ( exonuclease I and shrimp alkaline phosphatase, 70073Z and 70092Y, USB Corp., Cleveland OH) according to manufacturer’s instructions. Bidirectional sequencing of purified PCR products with ABI BigDye Terminator version 3.1 on an ABI 3730×l (Applied Biosystems, Foster City, CA) was performed at the DNA Sequencing and Analysis Facility in the University of Minnesota Biomedical Genomics Center. Sequence data were analyzed with Seqencher version 4.8 (Gene Codes Corp., Ann Arbor, MI). All sequence data were submitted to GenBank as follows: mtDNA, JN162706–JN162761; and EF1α, JN162704, JN162705.

Phylogenetic Analyses. Previously published Dioctria sequences also were included in this study (Du et al. 2005, Roe et al. 2006): D. cambicola (DQ295183, DQ296169, DQ296170), D. fordi (DQ295184, DQ296173, DQ296174), D. taedivorella (DQ247731), D. tumicolella (DQ247729), and D. zimmermani (DQ247730) (Table 2). All sequences were initially aligned in Seqencher version 4.8, followed with manual adjustments made by eye. Sequence fragment lengths were not equal and treated as missing data. Alignments of mtDNA and EF1α data sets were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:SI1682).

Parsimony haplotype networks for mtDNA and EF1α data sets were calculated using TCS 1.21 (Clement et al. 2000). Haplotype networks were inferred using a statistical parsimony framework (Templeton 1998), with gaps treated as missing data and a connection limit of 95%. During network inference identical sequences were collapsed, leaving a unique haplotype set (Table 2).

Given the low EF1α variability, genetic diversity indices (nucleotide and haplotype diversity), uncorrected pairwise distances, and a maximum likelihood (ML) tree were calculated for only the mtDNA data set. Haplotype diversity and nucleotide diversity (Nei 1987) were calculated in DNAsp version 5.10.00 (Rozas et al. 2003). Uncorrected pairwise distances were estimated with PAUP* version 4.0b10. ML trees were calculated using only unique haplotypes under a maximum likelihood framework implemented in RaxML version 7.0.4 (Stamatakis 2006) by using the CIPRES portal version 1.0 (Cyberinfrastructure for Phylogenetic Research, http://www.phylo.org/portal/Home.do). Before ML analysis, two additional species were included as outgroup taxa: D. okanaganaella Mutuura, Munroe & Ross (DQ295178, in the D. ponderosa group) and D. penticornella Mutuura, Munroe & Ross (DQ295150, in the D. baumhoferi group) (Roe et al. 2006). These species represent the two additional species groups characterized by raised forewing scales which have been shown to form a “raised scale” clade with the zimmermani group (Whitehouse et al. 2011).

Results

In total, 56 specimens were collected for this study through rearing, light, or pheromone trapping. When combined with previously published data, the total data set includes 66 specimens from 11 species (Table 2). Specimens were collected from across Canada and the United States, and represent half of the described species in the zimmermani group (Table 1). Identifications were based on previously published descriptions of forewing morphology, host plant associations, pheromone attraction, and geographic location (Neunzig 2003, and references therein).

Phylogenetic relationships and genetic diversity of the zimmermani group species were assessed with two independent loci, COI-COII (mtDNA) and EF1α (nuclear) (Figs. 3 and 4). mtDNA sequence length ranged from 450 bp of COI to the full 2.3 kb of COI-COII (Table 2). The zimmermani group formed a monophyletic clade, although the bootstrap support for this clade was low (Fig. 4). Morphologically, the zimmermani group can be circumscribed into two groups of species: dark-scaled and light-scaled species. The presence of a “dark-scaled” group is further supported in the ML tree, where it forms a well-supported monophyletic clade, whereas a “light-scaled” group was paraphyletic with respect to the “dark-scaled” clade (Fig. 4).

mtDNA gene tree topologies within the dark- and light-scaled groups contrasted sharply. For the light-scaled species, D. fordi and D. albocicatella, mtDNA was
Table 2. Specimen collection data and haplotype information

| Species                | Locality                                      | Latitude  | Longitude | Date        | Collector | Collecting information | mtDNA | EF1a | DNA no. | GenBank |
|------------------------|-----------------------------------------------|-----------|-----------|-------------|-----------|------------------------|-------|------|---------|---------|
| *D. zimmermani* gr.    |                                               |           |           |             |           |                        |       |      |         |         |
| *D. albovittella*      | USA, NV, White Pine Co., Baker                | 39.013    | −114.123  | 14 June 2003| A. Cognato| Pin cone               | 33    | E1   | AR307   | JN162706 |
|                        | USA, AZ, Coconino Co., Coconino N.F. near Happy Jack | 34.743    | −111.407  | 25 Aug. 2007| S. Shank  |                        | 34    | AR493| JN162704| JN162707 |
| *D. albovittella*      | USA, AZ, Yavapai Co., Mingus Mts.            | 34.698    | −112.122  | 11 Aug. 2007| S. Shank  |                        | 35    | AR495| JN162708 |         |
| *D. albovittella*      | USA, AZ, Yavapai Co., Mingus Mts.            | 34.698    | −112.122  | 11 Aug. 2007| S. Shank  |                        | 36    | AR496| JN162709 |         |
| *D. amatella*          | USA, SC, Berkeley Co., Francis Marion Ntl Forest | 33.167    | −79.667   | 11 June 2002| A. Roe    | MV-light               | 04    | AR220| JN162722 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 28 Sept. 2006| D. Miller | Z11–16:Ac              | 04    | AR379| JN162723 |         |
| *D. amatella*          | USA, AL, Greene Co., Flatwood seed orchard   | 33.125    | −87.867   | Dec. 1995   | Pt cone   |                        | 05    | AR331| JN162724 |         |
| *D. amatella*          | USA, AL, Greene Co., Flatwood seed orchard   | 33.125    | −87.867   | Dec. 1995   | Pt cone   |                        | 06    | E2   | AR332   | JN162725 |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 28 Sept. 2006| D. Miller | Z11–16:Ac + C25-p     | 11    | AR380| JN162710 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 28 Sept. 2006| D. Miller | Z11–16:Ac + C25-p     | 12    | AR381| JN162711 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 28 Sept. 2006| D. Miller | Z11–16:Ac             | 13    | E2   | AR382   | JN162712 |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 4 June 2007  | D. Miller | Z11–16:Ac + C25-p     | 14    | AR474| JN162713 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 17 April 2007 | D. Miller | Z11–16:Ac + C25-p     | 14    | AR468| JN162714 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 4 June 2007  | D. Miller | Z11–16:Ac + C25-p     | 19    | AR465| JN162715 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 17 April 2007 | D. Miller | Z11–16:Ac + C25-p     | 20    | AR466| JN162716 |         |
| *D. amatella*          | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999    | −83.209   | 17 April 2007 | D. Miller | Z11–16:Ac + C25-p     | 21    | AR467| JN162717 |         |
| Species          | Locality                                    | Latitude | Longitude | Date       | Collector | mtDNA       | EFla | DNA no. | GenBank |
|------------------|---------------------------------------------|----------|-----------|------------|-----------|-------------|------|---------|---------|
| *D. amatella*    | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 4 June 2007 | D. Miller | Z11–16:Ac + C25-p | 22   | AR49    | JN162718 |
| *D. amatella*    | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 4 June 2007 | D. Miller | Z11–16:Ac + C25-p | 23   | AR471   | JN162719 |
| *D. amatella*    | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 4 June 2007 | D. Miller | Z11–16:Ac + C25-p | 24   | AR472   | JN162720 |
| *D. amatella*    | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 4 June 2007 | D. Miller | Z11–16:Ac + C25-p | 25   | AR473   | JN162721 |
| *D. cambiicola*  | CAN, BC, Prince George                      | 53.917   | -122.750  | 19 July 2001 | A. Roe   | UV-light    | 01   | E2      | AR78    | DQ95183\(^a\), JN162705 |
| *D. cambiicola*  | USA, OR, Jackson Co., Medford               | 42.327   | -122.576  |            | C. Masters | *P. cambium* | 01   | AR89    | DQ96169\(^a\) |
| *D. cambiicola*  | CAN, BC, Cl.62                             | 53.917   | -122.750  | 21 July 2005 | A. Roe   | *P. cambium* | 01   | AR390   | JN162726 |
| *D. cambiicola*  | CAN, BC, Prince George                     | 53.917   | -122.750  | 19 July 2001 | A. Roe   | UV-light    | 02   | AR79    | DQ96170\(^a\) |
| *D. cambiicola*  | CAN, BC, Cl.62                             | 53.917   | -122.750  | 21 July 2005 | A. Roe   | *P. cambium* | 03   | AR389   | JN162728 |
| *D. cambiicola*  | CAN, BC, Prince George                     | 53.917   | -122.750  | 19 July 2001 | A. Roe   | *P. cambium* | 01   | E2      | JN162729 |
| *D. cambiicola*  | CAN, BC, Prince George                     | 53.917   | -122.750  | 19 July 2001 | A. Roe   | *P. cambium* | 01   | E2      | JN162730 |
| *D. cambiicola*  | CAN, BC, Pritchard                        | 50.599   | -119.892  | 5 Aug. 2003 | A. Roe   | MV-light    | 02   | E2      | JN162731 |
| *D. fordi*       | USA, CA, Butte Co., Chico                  | 39.728   | -121.837  | 12 June 2001 | A. Roe   | UV-light    | 37   | E1      | AR157   | DQ95184\(^a\), JN162705 |
| *D. fordi*       | USA, CA, Butte Co., Chico                  | 39.728   | -121.837  | 4-6 Oct. 2002 | A. Roe   | MV-light    | 38   | AR285   | JN162706 |
| *D. fordi*       | USA, CA, Butte Co., Chico                  | 39.728   | -121.837  | 4-6 Oct. 2002 | A. Roe   | MV-light    | 38   | AR285   | JN162707 |
| *D. fordi*       | USA, CA, Bakersfield, Kern Canyon          | 35.533   | -118.619  | 13 July 2007 | A. Roe   | MV-light    | 40   | AR434   | JN162731 |
| *D. merkeli*     | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 28 Sept. 2006 | D. Miller | Z9–14:Ac + C25-p | 07   | E2      | JN162732 |
| *D. merkeli*     | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 28 Sept. 2006 | D. Miller | Z9–14:Ac + C25-p | 08   | AR374   | JN162734 |
| *D. merkeli*     | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.209   | 28 Sept. 2006 | D. Miller | Z9–14:Ac + C25-p | 09   | E2      | JN162735 |

\(^a\) Additional Note: Additional information not shown in the table.
| Species         | Locality                                           | Latitude | Longitude | Date       | Collector | Collecting information | mtDNA | EFla | DNA no. | GenBank |
|-----------------|----------------------------------------------------|----------|-----------|------------|-----------|------------------------|-------|------|--------|---------|
| D. merkeli      | USA, GA, Baldwin Co., Milledgeville, Baldwin seed orchard | 32.999   | -83.309   | 28 Sept. 2006 | D. Miller | Z0-14:Ac + C25-p       | 10    |      | AR377  | JN162732 |
| D. resinosella  | USA, MI, Emmet Co., UMBS Stn Stream Lab          | 45.560   | -84.674   | 2006       | B. Scholtens | MV-light             | 14    | E2   | AR386  | JN162730; JN162705 |
| D. resinosella  | USA, MI, Cheboygon Co., Wildwood Rd.             | 45.365   | -84.652   | 15 July 2006 | B. Scholtens |                   | 14    |      | AR413  | JN162737 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR480  | JN162740 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR481  | JN162739 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR482  | JN162740 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR483  | JN162741 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR484  | JN162742 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR485  | JN162743 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR486  | JN162744 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR487  | JN162745 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 14    |      | AR488  | JN162746 |
| D. resinosella  | USA, MN, Chanhassen Co., MN Landscape Arboretum  | 44.663   | -93.617   | 1 June 2007 | A. Roe    | Pe cambium            | 14    |      | AR491  | JN162745 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 23    |      | AR486  | JN162746 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 26    |      | AR485  | JN162747 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 27    |      | AR487  | JN162748 |
| D. resinosella  | USA, MN, Itasca Co., North Star Camper.          | 47.557   | -93.654   | 1 Aug. 2007 | A. Roe    | UV-light              | 28    |      | AR490  | JN162749 |
| D. resinosella  | USA, MN, Chanhassen Co., MN Landscape Arboretum  | 44.663   | -93.617   | 1 June 2007 | A. Roe    | Ps cambium            | 29    |      | AR492  | JN162750 |
| D. taedivorella | USA, MD, Queen Anne's Co., Grasonville           | 38.958   | -76.210   | 1986       | D.C. Fergeson |                   | 32    |      | Du119  | DQ47731b |
| D. tumicolella  | USA, NE                                            |          |           |            |          |                       |       |      | AR25   | JN162731d |
| D. yatesi       | USA, KS, Crawford Co.                             | 37.517   | -94.850   | Aug. 1990  | G. Grant  | Pp                    | 01    |      | AR28   | JN162731d |
| D. yatesi       | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton| 34.596   | -83.362   | 1 Aug. 2004 | J. Hanula | Pu cone               | 07    |      | AR451  | JN162731f |
| D. yatesi       | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton| 34.596   | -83.362   | 1 Aug. 2004 | J. Hanula | Pu cone               | 07    |      | AR454  | JN162735f |
| Species       | Locality                                      | Latitude | Longitude | Date   | Collector | Collecting information | mtDNA | EFla | DNA no. | GenBank   |
|---------------|-----------------------------------------------|----------|-----------|--------|-----------|------------------------|-------|------|---------|-----------|
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 07    |      |         | AR455     | JN162757e |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 07    |      |         | AR456     | JN162758e |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 07    |      |         | AR457     | JN162759f |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 07    |      |         | AR459     | JN162761f |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 07    |      |         | AR450     | JN162752f |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 15    |      |         | AR451     | JN162753f |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 16    |      |         | E2 AR452  | JN162754g, JN162765 |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 17    |      |         | AR453     | JN162756f |
| *D. yatesi*   | USA, GA, Rabun Co., Chattahoochee N.F. nr. Clayton | 34.896   | −83.362   | 1 Aug 2004 | J. Hanula | Pu cone               | 18    |      |         | AR458     | JN162760f |
| *D. zimmermani* | USA, MS, Hinds Co.                            | 32.292   | −90.417   | 1994   | M.E. Poshere |                        | 31    |      |         | Du118     | DQ247730b |
| Outgroup      | *Diorityria ponderosae* |                        |          |         |          |                        |       |      |         |           |          |
|               | *D. okanaganella* |                        |          |         |          |                        |       |      |         |           |          |
|               | *D. baumhoferi* |                        |          |         |          |                        |       |      |         |           |          |
|               | *D. pentictonella* |                        |          |         |          |                        |       |      |         |           |          |

* Pheromone abbreviations as in Table 1; Host plant abbreviations as follows: Pc, Pinus contorta; Pm, Pinus monophylla; Pj, Pinus ponderosa; Ps, Pinus strobus; Pt, Pinus taeda; Pu, Pinus pungens; Pm, Pseudotsuga menziesii.

b Full 2.3-kb COI-COII sequence (TY-J-1460–C2-N-3752).
c Partial 800-bp COI-COII sequence fragment (CI-J-2183–TL2-N-3013). 
d Partial 450-bp COI sequence fragment (C1-J-2183–C1-N-2659).
e Partial 1.5-kb COI-COII sequence fragment (TY-J-1460–C2-N-3389).
congruent with previously described species limits (Figs. 3 and 4). These species had low levels of intraspecific variation and high, nonoverlapping levels of interspecific variation (Fig. 5), with no evidence of shared mtDNA haplotypes (Figs. 3 and 4). Both species formed strongly supported, monophyletic clades in the parsimony haplotype network and ML tree (Figs. 3 and 4). Haplotype diversity was also very high for both species, despite low levels of nucleotide diversity (Table 3).

In contrast to the light-scaled species, mtDNA diversity in the dark-scaled group was not congruent with previously described species limits, host plant association, pheromone attraction, or geographic location (Figs. 3A and 4). Often individuals from different host plants or pheromone blends were more closely related than individuals with similar ecological traits. All dark-scaled species had overlapping intraspecific and interspecific variation (Fig. 5), and several haplotypes were shared among species (Figs. 3A and 4). Five of the 32 dark-scaled haplotypes were shared between species, even when separated by large geographic distances (Fig. 4, e.g., mtDNA haplotype 14). There was little phylogenetic structuring among species (Figs. 3A and 4), and relationships among haplotypes were characterized by short internal branches with little to no bootstrap support (Fig. 4). For species with multiple individuals, nucleotide diversity was low, ranging from 0.00121 (D. contortella) to 0.0484 (D. amatella). Despite the low nucleotide diversity and shared haplotypes among species, overall haplotype diversity within species was high, above 0.900 in several species (Table 3), indicating that nearly all mtDNA sequences were unique.

The second locus, EF1a, was sequenced for a subset of individuals (n = 11), representing eight of the 11 species examined in this study (Table 2). In total, 584 bp were obtained which represented two unique haplotypes. A parsimony network (Fig. 3B) shows that these two haplotypes (E1 and E2) differ by a single mutation and coincide with the light-scaled and dark-scaled groups, which was congruent with the mtDNA results.

**Discussion**

Species limits among the dark-scaled members of the zimmermani group have always been considered problematic. Previous work on a Dioryctria species complex demonstrated that the examination of multiple molecular markers (COI and EF1a) and dense taxon sampling successfully clarified species limits...
between two sympatric species (Roe and Sperling 2007). By applying a similar technique, we sought to clarify species limits, estimate the genetic diversity within species, and clarify the phylogenetic relationships among species within the *zimmermani* group.

Congruence of the mtDNA gene tree with established species limits in the *zimmermani* group was variable. Species limits and the mtDNA gene tree were clearly congruent for the light-scaled species. The two light-scaled species (*D. albovittella* and *D. fordi*) were characterized by high interspecific pairwise variation and low intraspecific variation (Fig. 5), and each species was well supported as monophyletic (Fig. 4).

Discordance between molecular variation and species limits is not unusual. In a recent survey, Funk and Omland (2003) estimate that at $\sim 23\%$ of taxa (26.5% of arthropods) show some species-level polyphyly (considered broadly to represent non-

Gene tree congruence in the light-scaled species contrasts with the broad gene tree—species tree incongruence in the dark-scaled clade of the *zimmermani* group. The nine dark-scaled species showed little phylogenetic resolution (Fig. 4) and had overlapping interspecific pairwise variation (Fig. 5). The nuclear locus (EF1a) lacked species-level variation, despite diagnostic success in other *Dioryctria* species (Roe and Sperling 2007).

Figure 4. Maximum likelihood phylogram ($-\ln -3380.800$) of mtDNA (COI-COII) for the *D. zimmermani* species group. ML model information as follows: GTR+G: $A = 0.299$, $C = 0.140$, $G = 0.135$, $T = 0.456$; $G = 0.0000170$, $A-C = 0.0000170$, $A-G = 11.904$, $A-T = 2.474$, $C-G = 0.0000170$, $C-T = 29.935$, $G-T = 1.000$. Thickened branches indicate clade support $\geq 70\%$. For each haplotype, sample size, haplotype number, sampling locality, host plant, and pheromone association are shown. Host plant abbreviations are as given in Table 2. Pheromone abbreviations are as given in Table 1. (Online figure in color.)
monophyly). Several processes can lead to this phenomenon of gene tree–species tree incongruence.

First, it is possible that the currently recognized species limits are incorrect (i.e., imperfect taxonomy) and the mtDNA gene tree accurately represents the species tree. In the case of the dark-scaled species, the clade would be considered “overspilt” with all the taxa belonging to a single, widely distributed, highly polymorphic species, rather than multiple distinct species. Historically, these taxa have been separated based on minor forewing variation and larval host plant associations, although many authors acknowledge that complex species problems continue to exist within the group (Maktuura et al. 1969, Schaber and Wood 1971, Mutuura and Munroe 1979, Hedlin et al. 1980, Mutuura 1982, Sopow et al. 1996, Neunzig 2003). In fact, although Heinrich (1956) tentatively recognized D. cambicola as a species, he postulated that it might actually represent a western race of D. zimmermani, rather than a distinct species, a sentiment later supported by Munroe (1959). Furthermore, many species have sympatric or parapatric distributions, as well as extensive overlap of diagnostic characters (Sopow et al. 1996), supporting the hypothesis of a single dark-scaled species.

Widely distributed, highly polymorphic species are not unusual in Dioryctria. Dioryctria abieticorella (Grote), an important cone pest throughout North America, has broad larval host associations and a transcontinental distribution. This level of ecological and geographic variation would be comparable to the variation exhibited among the dark-scaled members of the zimmermani group. Morphological variability, particularly in forewing coloration, is also well known for other Dioryctria species (Roe et al. 2006, Roe and Sperling 2007). For example, Dioryctria pentcticontella (Mutuura, Munroe, & Ross), another raised scale species, has highly plastic forewing coloration, ranging from nearly black to red to white, which all occur within a single season at a single collection locality (Roe et al. 2006). Again, forewing variability among the dark-scaled species is within the intraspecific range of variability previously documented in D. pentcticontella.

The second possibility is that the current species limits in the dark-scaled clade are accurate and that the mtDNA gene tree fails to accurately reflect the evolutionary relationships among these species. Although many species show interspecific overlap of larval host associations, other species do not (Table 1). As well, distinct pheromone sex attractants have been described for several dark-scaled species, particularly for dark-scaled species in the southeastern United States (Miller et al. 2010). Although cross species attraction occurs (Hanula et al. 1984), recent work has shown that Dioryctria pheromones are complex (Millar et al. 2005, 2010) and pheromone races exist within Dioryctria species (Grant et al. 2009), although it is uncertain whether these races represent distinct species or show reduced inter-race gene flow.

If individuals in the dark-scaled clade represent a single species, we would expect to observe some phylogeographic structuring among the mtDNA haplotypes. Instead, haplotypes are shared across broad geographic ranges (e.g., mtDNA haplotype 14), with individuals collected in the same location more closely related to individuals from distant locations than to each other (Figs. 3A and 4). The lack of phylogeographic structuring and ecological variation among species suggests that more complex evolutionary processes may be responsible for the observed incongruence (Schmidt and Sperling 2008).

Gene tree–species tree discordance is a common issue when seeking to delimit species boundaries and

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**Table 3. Genetic diversity estimates for members of the D. zimmermani species group**

| Species          | n | H  | Hid (±SD) | Pi (±SD) |
|------------------|---|----|-----------|----------|
| Dark scaled      |   |    |           |          |
| D. amatella      | 16| 14 | 0.983 (0.0250) | 0.0484 (0.000510) |
| D. cambicola     | 6 | 3  | 0.733 (0.155)  | 0.00323 (0.00133)  |
| D. contortella   | 2 | 2  | 1.000 (0.500)  | 0.00121 (0.000610) |
| D. merkeli       | 4 | 4  | 1.000 (0.177)  | 0.00233 (0.000780) |
| D. resinosella   | 15| 6  | 0.571 (0.140)  | 0.00162 (0.000530) |
| D. taedivorella  | 1 | 1  | N.A. a         | N.A. a        |
| D. tunicolella   | 2 | 2  | 1.000 (0.500)  | 0.00842 (0.001421) |
| D. yatesi        | 10| 5  | 0.775 (0.137)  | 0.00365 (0.000900) |
| D. zimmermani    | 1 | 1  | N.A. a         | N.A. a        |
| Light scaled     |   |    |           |          |
| D. albivittella  | 4 | 4  | 1.000 (0.177)  | 0.00905 (0.00209)  |
| D. fordi         | 5 | 4  | 0.900 (0.161)  | 0.00295 (0.000670) |

n, number of specimens; H, number of haplotypes; Hid, haplotype diversity; and Pi, nucleotide diversity.

a N.A., not applicable.
can be caused by several evolutionary processes (e.g., Maddison 1997, Funk and Omland 2003), such as incomplete lineage sorting or introgression. Incomplete lineage sorting results when gene lineages of closely related species have not had sufficient time to coalesce and achieve reciprocal monophony. Generally, mtDNA is considered more robust to incomplete lineage sorting than nuclear genes (Hudson and Turelli 2003) but has been shown to fail among rapidly radiating clades (Funk and Omland 2003), particularly among groups experiencing ecological race formation (Dres and Mallet 2002, Scheffer and Hawthorne 2007). If dark-scaled Dioryctria species are undergoing rapid ecological divergence based on larval host association and pheromone attraction, then the species barriers separating these recently diverged species may be maintained by a small region of the genome (Matsubayashi et al. 2009), whereas other regions of the genome (e.g., mtDNA) will not have had sufficient time for purifying selection to produce reciprocally monophyletic clades (Funk and Omland 2003).

Conversely, interspecific hybridization and subsequent introgression is the movement of foreign genetic material into a conspecific genome. This process leads to reticulate evolutionary relationships and gene tree–species tree discordance, clouding genealogical species boundaries (Maddison 1997). Interspecific hybridization is surprisingly common (Mallet 2005), with hybridization rates ranging from 6 to 29% among species of Lepidoptera (Sperling 1990, Mallet et al. 2007). mtDNA introgression may occur without nuclear introgression (Ballard and Whitlock 2004, Petit and Excoffier 2009), particularly if mtDNA is impacted by direct or indirect selection (Ballard and Whitlock 2004, Hurst and Jiggins 2005). For hybridization to occur, species must be sympatric/parapatric, synchronous, and be capable of interbreeding (Schmidt and Sperling 2008). Dark-scaled zimmermani species have sympatric and parapatric distributions, overlapping flight times, and have shown evidence for cross-species pheromone attraction (Hanula et al. 1984, Whitehouse et al. 2011), all conditions necessary for hybridization to occur.

As stated previously in many studies, we must acknowledge that difficult species problems continue to exist in the zimmermani species group. Despite our dense taxon sampling and inclusion of multiple lines of evidence, we were unable to fully resolve species limits among dark zimmermani species group members. Many of the dark-scaled taxa are considered “good” species, with extensive information available on their behavioral and ecological differences, as well as their economic impacts (Whitehouse et al. 2011). Although mtDNA has been used extensively as a diagnostic marker in Lepidoptera (e.g., DNA barcoding: Hebert et al. 2003, 2004), and is successful in other species of Dioryctria (Roe and Sperling 2007), including light-scaled members of the zimmermani group, studies have shown that a single marker is prone to failure, particularly when differentiating closely related species (Roe and Sperling 2007, Schmidt and Sperling 2008, Roe et al. 2010). Given the economic importance of these dark-scaled species and in the interest of nomenclatural stability, we choose not to recommend any taxonomic changes to this group based on a single molecular marker.

Based on the currently available data, we are unable to differentiate among the alternative hypothesis for the cause of the gene tree–species tree discordance detected among the dark-scaled zimmermani species. Effective evaluation of these hypotheses requires data from multiple regions of the genome (Maddison 1997) and analytical means for resolving gene tree discordance (Degnan and Rosenberg 2009). Highly variable molecular markers, such as microsatellites or single-nucleotide polymorphisms from regions throughout the genome, in addition to behavioral, ecological, and morphological characters will be required to provide clarity to the dark-scaled zimmermani species complex.

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References Cited

Ballard, J.W.O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13: 729–744.
Cho, S., A. Mitchell, J. C. Regier, C. Mitter, R. W. Poole, T. P. Friedlander, and S. Zhan. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1a recovers morphology-based tree for heliothine moths. Mol. Biol. Evol. 12: 650–656.
Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657–1659.
Dayrat, B. 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 87: 407–415.
Degnan, J. H., and N. A. Rosenberg. 2000. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24: 332–340.
Dres, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357: 471–492.
Du, Y., A. D. Roe, and F.A.H. Sperling. 2005. Phylogenetic framework for Dioryctria (Lepidoptera: Pyralidae: Pyraustinae) based on combined analysis of mitochondrial DNA and morphology. Can. Entomol. 137: 685–711.
Elias, M., R. I. Hill, K. R. Willmott, K. K. Dasmahapatra, A. V. Brower, J. Mallet, and C. D. Jiggins. 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. Proc. Biol. Sci. 274: 2881–2889.
Funk, D. J., and K. E. Omland. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34: 397–423.

Grant, G. G., J. G. Millar, and R. Trudel. 2009. Pheromone identification of Dioryctria abieticolella (Lepidoptera: Pyralidae) from an eastern North American population: geographic variation in pheromone response. Can. Entomol. 141: 129–135.

Grant, G. G., A. Kato, T. J. Hall, D. A. Lombardo, and K. N. Slessor. 1993. Sex-pheromone identification and trapping of Dioryctria resinosella (Lepidoptera, Pyralidae). Environ. Entomol. 22: 154–161.

Hanula, J. L., C. W. Berisford, and G. L. DeBarr. 1984. Pheromone cross-attraction and inhibition among four coneworns, Dioryctria spp. (Lepidoptera: Pyralidae) in a loblolly pine seed orchard. Environ. Entomol. 13: 1298–1310.

Hebert, P. D. N., A. Cywinska, S. L. Ball, J. R. deWaard. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B 270: 313–321.

Hebert PDN, EH Penton, J Burns, DH Janzen, and W Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly, Astraptes fulgerator. Proc. Natl. Acad. Sci. U.S.A. 101: 14812–14817.

Hedlin, A. F., H. O. Yates III, B. H. Ebel, T. W. Koerber, and E. P. Merkel. 1980. Cone and seed insects of North American conifers, vol. Canadian Forestry Service, Environment Canada, Ottawa ON, United States Forest Service, Washington, D.C., and Secretaría de Agricultura y Recursos Hidráulicos, Mexico.

Heinrich, C. 1956. American moths of the subfamily Phycitinae, vol. 207. Smithsonian Institution, Washington, DC.

Hudson, R. R., and M. Turelli. 2003. Stochasticity overrules the “three-times rules”: genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution 57: 182–190.

Hurst, G. D., and F. M. Jiggins. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. Biol. Sci. 272: 1525–1534.

Knöllke, S. 2007. A revision of the European representatives of the microlepidopteran genus Dioryctria Zeller, 1846 (Insecta: Lepidoptera: Pyralidae: Phycitinae). Ludwig-Maximilians-Universität München, München, Germany.

Maddison, W. P. 1997. Gene trees in species trees. Syst. Biol. 46: 523–536.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20: 229–237.

Mallet, J., M. Beltran, W. Neukirchen, and M. Linares. 2007. Natural hybridization in heliconiine butterflies: the species boundary as a continuum. BMC Evol. Biol. 7: 28.

Matsumiyashiki, K., I. Ohshima, and P. Nosil. 2009. Ecological speciation in phytophagous insects. Entomol. Exp. Appl. 134: 1–27.

Meyer, W. L., G. L. DeBarr, J. L. Hanula, B. Kovalev, R. S. Cameron, C. W. Berisford, and W. L. Roeufs. 1986. (Z)-11-hexadecenyl acetate, a sex pheromone component for the southern pine coneworm, Dioryctria amatella (Lepidoptera: Pyralidae). Environ. Entomol. 15: 316–320.

Millar, J. G., G. G. Grant, J. S. McElfresh, W. Strong, C. Rudolph, J. D. Stein, and J. A. Mallet. 2005. (3Z,6Z,9Z,12Z,15Z)-Pentacosapentaene, a key pheromone component of the fir coneworm moth, Dioryctria abieticolella. J. Chem. Ecol. 31: 1229–1234.

Miller, D. R., J. G. Millar, A. Mangini, C. M. Crowe, and G. G. Grant. 2010. (3Z,6Z,9Z,12Z,15Z)-Pentacosapentaene and (Z)-11-hexadecenyl acetate: sex attractant blend for Dioryctria amatella (Lepidoptera: Pyralidae). J. Econ. Entomol. 103: 1216–1221.

Monroo, E. 1959. Canadian species of Dioryctria Zeller (Lepidoptera: Pyralidae). Can. Entomol. 91: 65–72.

Mutuura, A. 1982. American species of Dioryctria (Lepidoptera: Pyralidae) VI. A new species of Dioryctria from eastern Canada and north-eastern United States. Can. Entomol. 114: 1069–1076.

Mutuura, A., and E. Munroo. 1972. American species of Dioryctria (Lepidoptera: Pyralidae) III. Grouping of species: species of the auranticella group, including the Asian species, with the description of a new species. Can. Entomol. 104: 609–625.

Mutuura, A., and E. Munroo. 1974. A new genus related to Dioryctria Zeller (Lepidoptera: Pyralidae: Phycitinae), with definition of an additional species group in Dioryctria. Can. Entomol. 106: 937–940.

Mutuura, A., and E. Munroo. 1979. American species of Dioryctria (Lepidoptera: Pyralidae) V. Three new cone-feeding species from the southeastern United States. J. Ga. Entomol. Soc. 14: 290–304.

Mutuura, A., E. Munroo, and D. A. Ross. 1969. American species of Dioryctria (Lepidoptera: Pyralidae) I. Western Canadian species of the zimmermani Group. Can. Entomol. 101: 1009–1023.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Neunzig, H. H. 1990. A new species of Dioryctria (Pyralidae): Pyralidae) from Mexico. Proc. Entomol. Soc. Wash. 92: 493–496.

Neunzig, H. H. 2003. Pyraloidea, Pyralidae (part), Phycitinae (part). The moths of America North of Mexico. Fasc. 15. Allen Press Inc., Lawrence, KS.

Nuss, M., B. Landry, F. Vogliante, A. Tränkner, R. Malley, J. Hayden, A. H. Segerer, H. Li, R. Schouten, M. A. Solis, et al. 2010. Global information system in Pyraloidea. (http://www.pyraloidea.org).

Padijal, M. J., A. Miralles, I. De la Riva, and M. Vences. 2010. The integrative future of taxonomy. Front. Zool. 7: 16.

Petit, R. J., and L. Excoffier. 2000. Gene flow and species delimitation. Trends Ecol. Evol. 24: 386–393.

Powell, J. A., and P. A. Oppler. 2009. Moths of western North America. University of California Press, Berkeley, CA.

Reed, R. D., and F.A.H. Sperling. 1999. Interaction of process partitions in phylogenetic analysis: an example from the swallowtail butterfly genus Papilio. Mol. Biol. Evol. 16: 286–297.

Richmond, J. A., and M. Page. 1995. Genetic and biochemical similarities among four species of pine coneworns (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 88: 271–280.

Roe, A. D., and F.A.H. Sperling. 2007. Population structure and species boundary delimitation of cryptic Dioryctria moths: an integrative approach. Mol. Ecol. 16: 3617–3633.

Roe, A. D., J. D. Stein, N. E. Gillette, and F.A.H. Sperling. 2006. Identification of Dioryctria (Lepidoptera: Pyralidae) in a seed orchard at Chico, California. Ann. Entomol. Soc. Am. 99: 433–448.

Roe, A. D., A. V. Rice, S. E. Bromilow, J.E.K. Cooke, and F.A.H. Sperling. 2010. Multilocus species identification and fungal DNA barcoding: insights from blue stain fungal symbionts of the mountain pine beetle. Mol. Ecol. Resour. 10: 946–959.

Roux-Morabito, G., N. E. Gillette, A. Roques, L. Dormont, J. D. Stein, and F.A.H. Sperling. 2008. Systematics of the
Dioryctria abietella species group (Lepidoptera: Pyralidae) based on mitochondrial DNA. Ann. Entomol. Soc. Am. 101: 845–859.

Rozas, J., J. C. Sanchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP. DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497.

Rubinoff, D., S. Cameron, and K. Will. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. J. Hered. 97: 581–594.

Schaber, B. D., and F. E. Wood. 1971. A new species of Dioryctria infesting loblolly pine. Proc. Entomol. Soc. Wash. 73: 215–223.

Schaffer, S. J., and D. J. Hawthorne. 2007. Molecular evidence of host-associated genetic divergence in holly leafminer Phytomyza glabricola (Diptera: Agromyzidae): apparent discordance among marker systems. Mol. Ecol. 16: 2627–2637.

Schlick-Steiner, B. C., F. M. Steiner, B. Seifert, C. Stauffer, E. Christian, and R. H. Crozier. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu. Rev. Entomol. 55: 421–438.

Schmidt, B. C., and F.A.H. Sperling. 2008. Widespread decoupling of mtDNA variation and species integrity in Grammia tiger moths (Lepidoptera: Noctuidae). Syst. Entomol. 33: 613–634.

Scudder, G.G.E. 2009. The importance of insects, pp. 7–32. In R. Footitt and P. Adler (eds.), Insect biodiversity: science and society. Wiley-Blackwell, Chichester, United Kingdom.

Sopow, S. L., R. G. Bennet, J.-F. Landry, and B. Landry. 1996. Identification of the ‘grey’ Dioryctria species of British Columbia (Lepidoptera: Pyralidae). J. Entomol. Soc. Br. Columbia 93: 73–91.

Sperling, F.A.H. 1990. Natural hybrids of Papilio (Insecta: Lepidoptera): poor taxonomy or interesting evolutionary problem? Can. J. Zool. 68: 1790–1799.

Sperling, F.A.H., and A. D. Roe. 2009. Molecular dimensions of insect taxonomy, pp. 397–415. In R. Footitt and P. Adler (eds.). Insect biodiversity: science and society. Wiley-Blackwell, Chichester, United Kingdom.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7: 381–397.

Twewick, S. 2007. DNA barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). Cladistics 23: 1–15.

Wang, P.-Y., and S.-M. Sung. 1982. Description of a new species of Dioryctria Zeller on Pinus silvestris var. Mongolica from north-east China, with establishment of a new species group. Acta Entomol. Sin. 25: 324–327.

Wheeler, Q. D. 2009. The science of insect taxonomy: prospects and needs, pp. 359–380. In R. Footitt and P. Adler (eds.). Insect biodiversity: science and society. Wiley-Blackwell, Chichester, United Kingdom.

Whitehouse, C., A. D. Roe, W. Strong, M. Evenden, and F.A.H. Sperling. 2011. The biology and management of North American cone-feeding Dioryctria species. Can Entomol. 143: 1–34.

Will, K. W., B. D. Mishler, and Q. D. Wheeler. 2005. The perils of DNA barcoding and need for integrative taxonomy. Syst. Biol. 54: 844–851.

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