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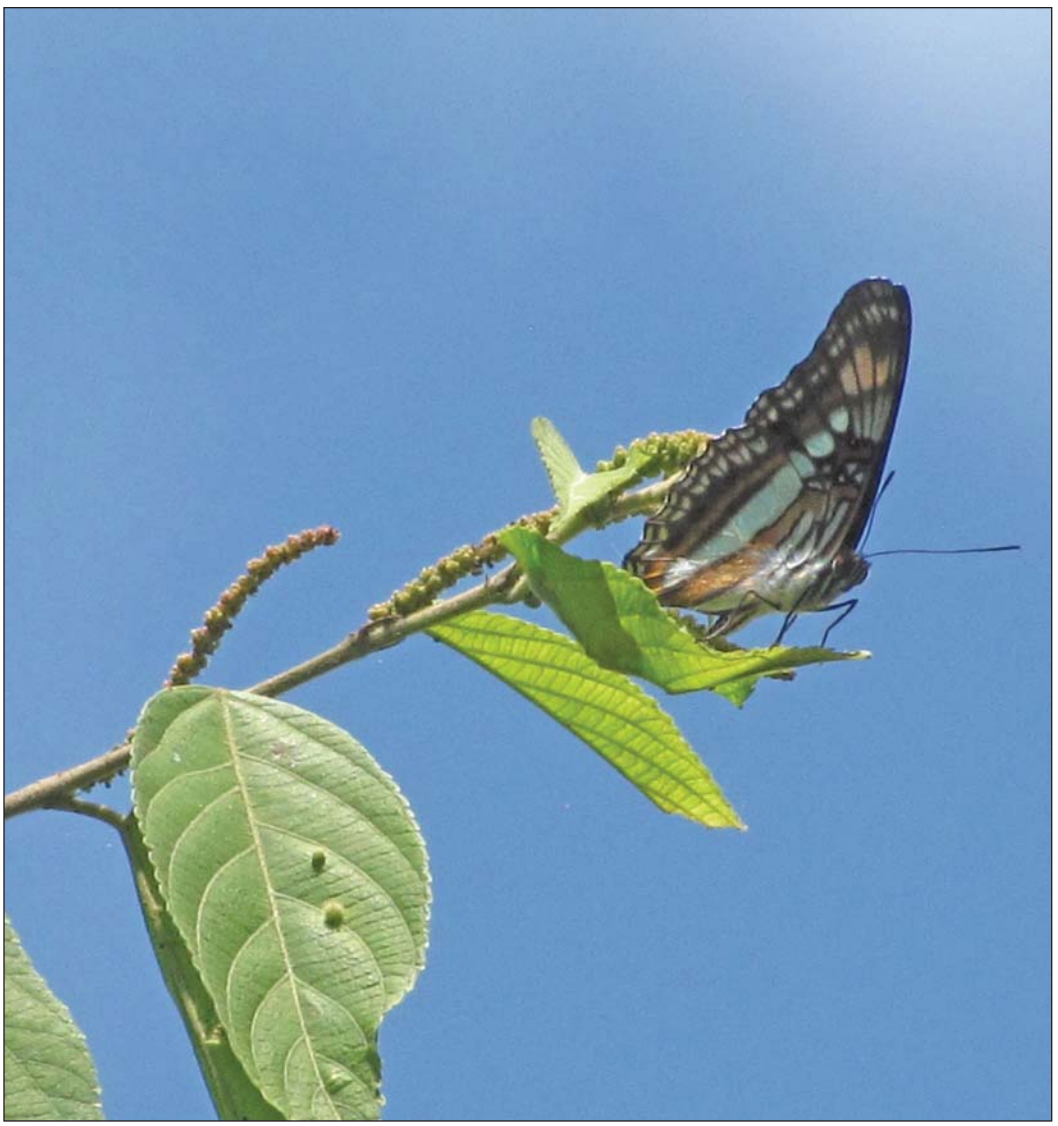

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Cover Illustration: *Adelpha godmani,* western Ecuador, photograph by Thierry Garcia. Study of museum specimens and DNA sequence data resulted in the recognition of this former subspecies as a distinct species. See article on page 241 by Willmott & Hall.

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A NEW SPECIES AND TWO NEW SUBSPECIES OF *ADELPHA* HÜBNER, [1819] FROM THE TROPICAL ANDES (NYMPHALIDAE: LIMENITIDINAE)

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ABSTRACT. A new species, *Adelpha margarita* Willmott & Hall, **new species**, is described from Andean cloud forest habitats from southern Ecuador to Bolivia. *Adelpha margarita garleppi* Willmott, **new subspecies**, is described for southern Peruvian and Bolivian individuals, which differ from the nominate subspecies in having complete orange postdiscal bands on the dorsal surface. The new species differs from related species in the *Adelpha serpa* group in wing pattern, DNA sequence data and habitat. A lectotype is designated for *Adelpha seriphia therasia* Fruhstorfer, because the type series of this name contains individuals of both *A. seriphia* and *A. margarita*. Neighbor-joining and maximum parsimony analyses of 579 bp of the mitochondrial *cytochrome oxidase I* (COI) 'barcode' region, for 27 *Adelpha* specimens representing 9 species and 13 taxa, suggest that the closest relative to *A. margarita* is the Central American to west Andean taxon *A. seriphia godmani* Fruhstorfer. The DNA sequence data, coupled with a re-analysis of museum specimens, suggest that *Adelpha godmani* should be treated as a distinct species (**revised status**). Finally, a new subspecies, *Adelpha justina pichincha* Willmott & Hall, **new subspecies**, is described from Pichincha province in western Ecuador.

Additional key words: Ecuador, Peru, Bolivia, DNA barcode, COI

Like other better known mimetic butterfly genera, the diverse nymphalid genus *Adelpha* Hübner, [1819], is notable for its marked geographic wing pattern variation within species. In addition, the genus displays a frustrating simplicity and homogeneity in genital and other morphological characters that typically are used to define butterfly species. These two traits have conspired to cause much historical taxonomic confusion, and Willmott's (2003a) revision of the genus represents just the first step towards developing a firm species classification. In particular, intensive field work coupled with newly available DNA sequence data are likely to result in refinement of the taxonomy. For example, Prudic et al. (2008) provided convincing data to support recognition of the three taxa formerly placed within *Adelpha bredowii* Geyer, 1837, as distinct species. New field work demonstrated micro-sympatry of some phenotypes, and DNA sequence data show that the three taxa are reciprocally monophyletic and therefore most likely reproductively isolated. *Adelpha bredowii* is a member of the *Adelpha serpa* (Boisduval, 1836) group, one of the most taxonomically challenging in the genus, characterized elsewhere by the often extreme rarity of its species. For example, *Adelpha radiata explicator* Willmott & Hall, 1999, remains known from the entire Amazon basin by just the holotype specimen. Here, we use data from DNA sequences, new field work and a re-examination of museum specimen wing patterns to clarify relationships among tropical Andean members of the *A. serpa* group, and describe a hitherto overlooked species of *Adelpha*, comprising two subspecies, that ranges from southern Ecuador to Bolivia. We also take the opportunity to describe an additional new subspecies of *Adelpha justina* (C. & R. Felder, 1861) from central western Ecuador.

MATERIALS AND METHODS

Adelpha specimens were examined in major public and private collections in Europe, North and South America, as listed in Willmott (2003a), to record distributional data, study morphological variation, assess taxonomic diversity and locate type specimens. In addition, we made particular efforts to obtain tissue samples for DNA analysis from the most recently collected specimens of taxonomically important *Adelpha*. Such specimens include, especially, those that can be assigned reliably to a taxon by virtue of having been collected near type localities. Unprepared, papered specimens were an especially valuable resource because the rehydration needed to spread and pin such specimens appears to degrade DNA significantly. Acronyms used here include: BMNH: Natural History Museum, London, UK; FLMNH: Florida Museum of Natural History, Gainesville, USA; HAWA: Haydon Warren-Gash collection, Pressac, France; KWJH: Keith R. Willmott & Jason P. W. Hall collection, Gainesville, FL, USA; MECN: Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador; MUSM: Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; PIBO: Pierre Boyer collection, Le Puy,

France; USNM: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ZMHU: Zoologisches Museum, Humboldt Universität, Berlin, Germany.

Morphology was studied using standard techniques, with adult abdomens being soaked in hot 10% KOH for 10–15 minutes, dissected and subsequently stored in glycerin. Body morphology and dissections were studied using a binocular microscope at 50× magnification. The terminology for male genital and abdominal structures largely follows Klots (1956), nomenclature for venation follows Comstock and Needham (1918), and wing pattern elements are named as in Willmott (2003a). We use the abbreviations DFW, VFW, DHW and VHW for dorsal and ventral forewing and hind wing.

Specimens for tissue samples, distributional data and field observations for *Adelpha* were gathered by the authors during more than 650 days of field work in Ecuador between 1991 and 2011, representing 385 sites in 20 provinces, ranging from sea level to 4000 m on both Andean slopes.

We extracted genomic DNA from two legs removed from dried, papered specimens of the new species of *Adelpha* and close relatives (some spread specimens of the latter were sampled) using Qiagen's DNeasy Blood & Tissue Kit following the manufacturer's protocol, incubating samples overnight (20–24 hr) and using a final elution volume of 100 µl (50 µl for specimens older than 20 yrs). We amplified the first half of the mitochondrial gene *cytochrome oxidase I* (COI), also known as the barcode region for animals (Hebert et al. 2003), using the primers LepF1 (forward, ATTCAACCAATCATAAAGATAT) and LepR1(reverse, TAAACTTCTGGATGTCCAAAAA) (Hebert et al. 2004), or LCO (forward, GGTCAACAAATCATAAAGATATTGG and HCO (reverse, TAAACTTCAGGGTGACCAAAA AATCA) (Folmer et al. 1994). For samples that failed to amplify with these primers, we amplified two shorter, partially overlapping fragments of the barcode region using primer pairs LCO and K699 (reverse, WGGGGGGTAAACTGTTCATCC), and Ron (forward, GGATCACCTGATATAGCATTCCC) and Nancy (reverse, CCTGGTAAAATTAAAATATAAACTTC) (Monteiro & Pierce 2001, Elias et al. 2007). All PCR reactions were conducted in a 20 µl volume comprising 2 µl DNA, 0.8 µl MgCl2 (50 mM), 13.4 µl ddH2O, 2 µl buffer (10X), 0.8 µl dNTPs (10mM), 0.4 µl of each primer (10 µM), and 0.2 µl Platinum® Taq DNA Polymerase (5 U/µl). Reaction conditions were as follows: 1 min at 94°C followed by 5 cycles of 30 s at 94°C, 40 s at 45°C, 1 min at 72°C, followed by 35 cycles of 30 s at 94°C, 40 s at 51°C, 1 min at 72°C, followed by 5 min at 72°C. Single strands of PCR products were sequenced by University of Florida's Interdisciplinary Center for Biotechnology Research Sanger Sequencing Group using the same primers as in the PCR. Sequences were aligned using BioEdit v. 7.1.3 (Hall, 1999) and by eye, and fragments were assembled into composite sequences where necessary. To test hypotheses of species limits based on morphology and distribution, we conducted both a neighbor-joining (NJ) analysis and a maximum parsimony (MP) analysis on the DNA sequence data. Despite criticism of NJ analyses (Goldstein & DeSalle 2011), they remain a simple way to graphically represent similarity among taxa and hence decide the most appropriate taxonomic rank, still a necessarily subjective step in almost all studies. MP analysis complements NJ by allowing the identification of putative nucleotide autapomorphies for species, which may be tested by inclusion of additional individuals. All analyses were conducted using MEGA 5.05 (Tamura et al. 2011), with the following options selected: for NJ, we used the Kimura 2-parameter substitution model and other default settings, while for MP analysis we used the Close-Neighbor-Interchange heuristic search with 100 random addition starting trees, evaluating branch support with 100 bootstrap replicates. Trees were rooted with *Adelpha californica* (Butler, 1865), which is part of a small clade sister to all other Central and South American *A. serpa* group species (Willmott 2003b). All new sequences are deposited in GenBank.

RESULTS AND DISCUSSION

DNA sequence data and the taxonomy of *Adelpha seriphia*

We amplified part of the COI barcode region for 26 *Adelpha* specimens representing 8 species and 12 taxa, and obtained sequence data for *Adelpha californica* from GenBank (Appendix 1). The final aligned dataset, after trimming to remove poor quality sequence (a problem in particular with older museum specimens), was 579 bp. Of these, 106 sites were variable and 67 were parsimony informative. The NJ analysis showed that individuals of all putative species clustered together except for *A. seriphia godmani* Fruhstorfer, 1913, which formed a paraphyletic cluster separate from other *A. seriphia* (C. & R. Felder, 1867) (Fig. 1). The three Ecuadorian *A. s. godmani* formed a cluster, even though KW-080510-01, from southwestern Ecuador, was markedly divergent from the other two northwest Ecuadorian specimens. This cluster was sister to *A. margarita* **new species**, while LEP-04017 from El Águila in west Colombia was sister to both of these clusters. The MP analysis found 2206 trees of length 326 steps, which were topologically similar to the NJ tree and also contained the clade with *A. seriphia*, *A.*

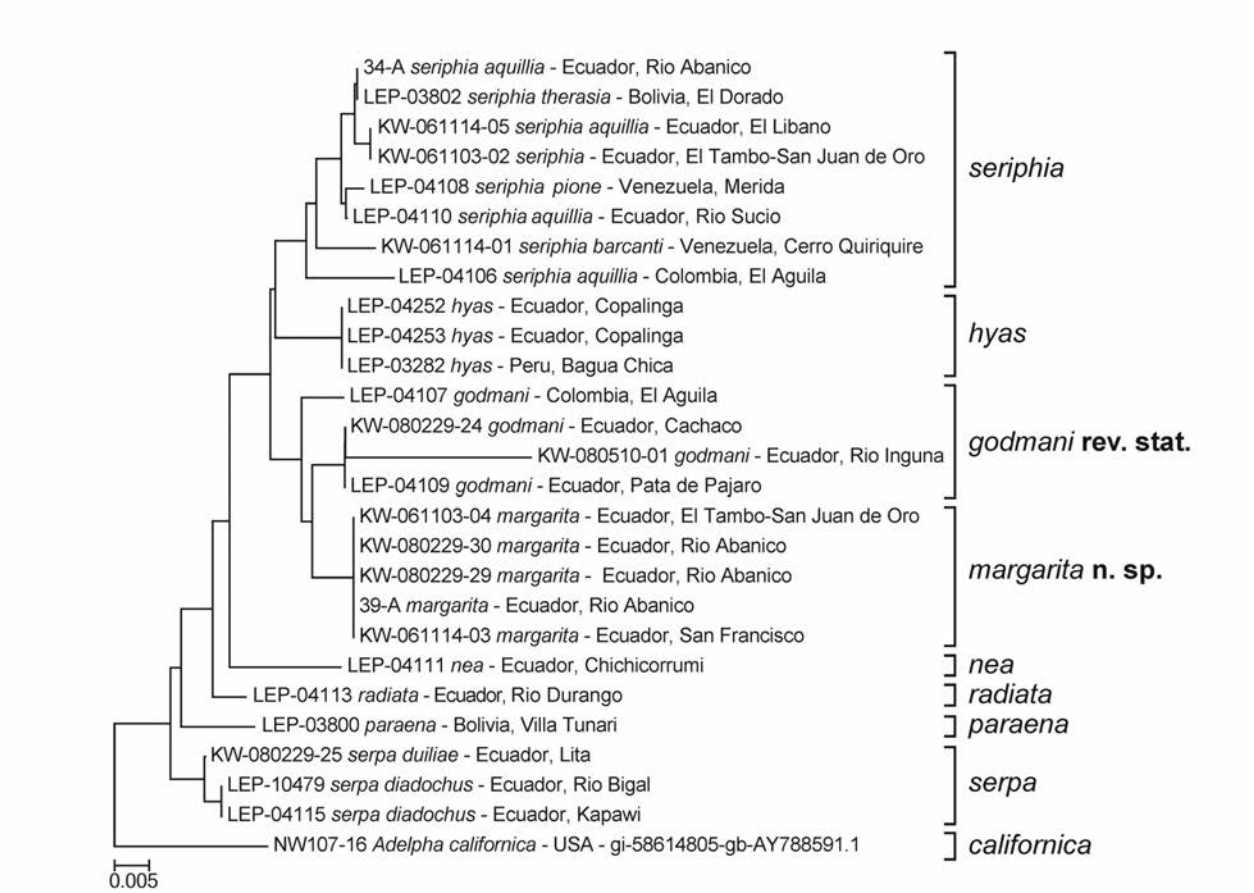

FIG. 1. Neighbor-joining tree (Kimura 2-parameter) for *Adelpha serpa* group based on 579 bp of COI (barcode region).

hyas, *A. s. godmani* and *A. margarita*. Within this clade the only conflicting result was the placement of LEP-04107 as sister to *A. margarita* in the MP analysis, rather than to *A. margarita* + *A. s. godmani* as in the NJ analysis. Bootstrap support for *A. seriphia* excluding *A. s. godmani* was 63%, for west Ecuadorian *A. s. godmani* was 98%, and for all other species represented by multiple individuals was > 90%. Reasonable bootstrap support (82%) was found for a sister relationship between *A. s. godmani* and *A. margarita*.

The DNA sequence data, coupled with a re-analysis of museum specimens, suggest that *Adelpha godmani* should be treated as a distinct species (**revised status**). Specimens of *A. godmani* were, on average, 3.1% divergent from remaining *A. seriphia*, and both NJ and MP analyses suggest that *A. hyas hewitsoni* Willmott & Hall, 1999, which occurs on the east Andean slopes with *A. seriphia* but at lower elevations, is more closely related to *A. seriphia* than is *A. godmani*. Furthermore, there is evidence that *A. godmani* and *A. seriphia* are sympatric in west Colombia. Two specimens in the FLMNH, one male and one female, from Colombia, Caldas, El Águila, 1700 m, appear to represent *A. seriphia aquillia* Fruhstorfer, 1915, and *A. godmani*, respectively. In common with other, east Andean *A. seriphia*, the male has dark orange interneural stripes intruding into the pale orange subapical marking on the VFW, the white submarginal series on the VFW in cells Cu_1–M_1 are reduced, and the FW postdiscal band is narrow with dislocated spots. The female resembles west Ecuadorian *A. godmani* in having a clean pale orange subapical marking on the VFW, the white VFW submarginal series expressed throughout the wing, and the FW postdiscal spots less dislocated. The female sequence (LEP-04107) did not cluster conclusively with remaining *A. godmani*, but given the unusually high variation within west Ecuadorian *A. godmani* (1.8%), it is premature to speculate whether this result is

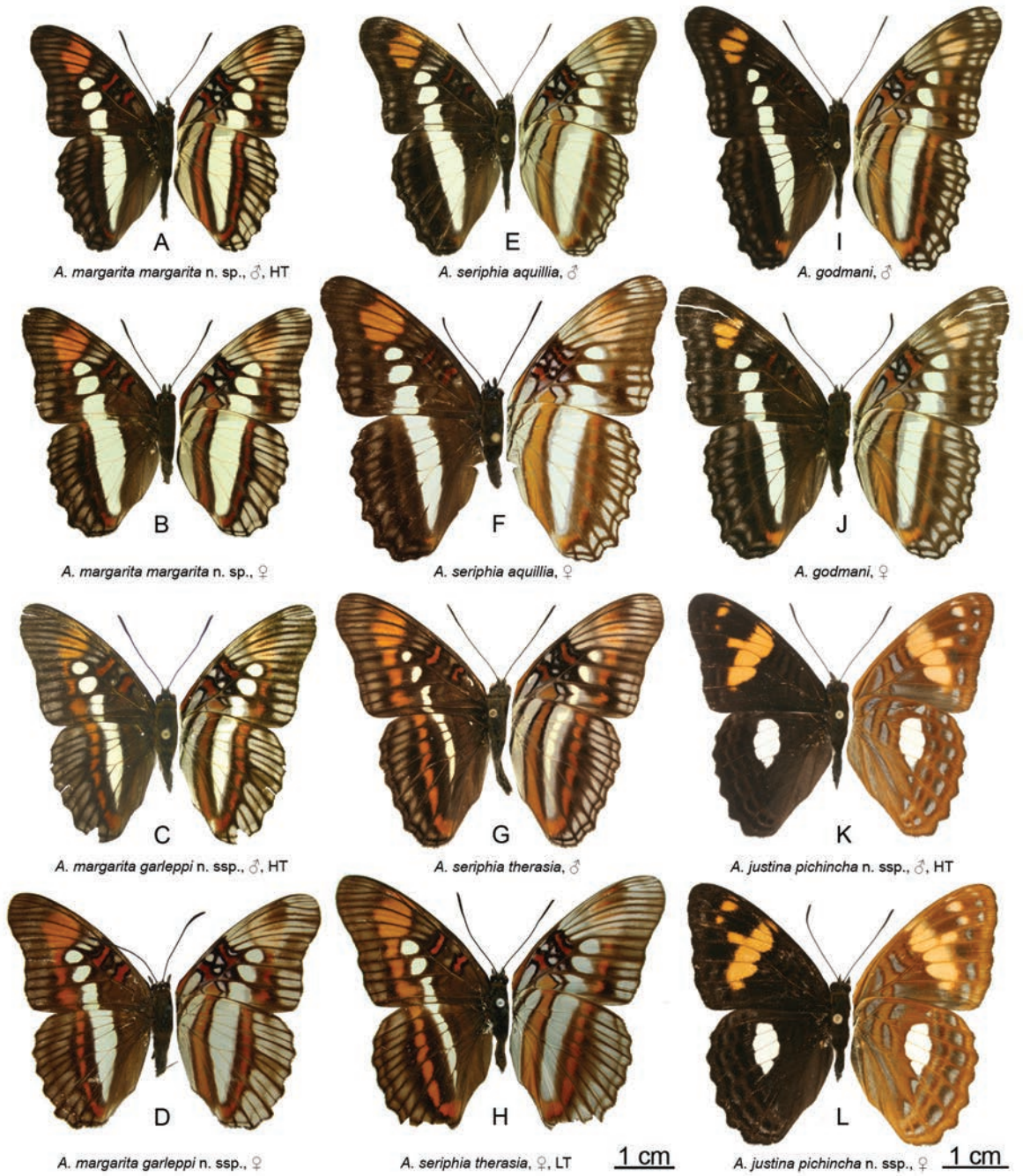

FIG. 2. *Adelpha* specimens, left half dorsal surface, right half ventral surface. **A**) *A. margarita margarita* **new species, holotype** ♂, Ecuador, MECN; **B**) *A. margarita margarita* **new species,** paratype ♀, Ecuador, FLMNH; **C**) *A. margarita garleppi* **new subspecies,** holotype ♂, Peru, MUSM; **D**) *A. margarita garleppi* **new subspecies,** paratype ♀, Peru, BMNH (mirror image of specimen); **E**) *A. seriphia aquillia*, ♂, Ecuador, FLMNH; **F**) *A. seriphia aquillia*, ♀, Ecuador, PIBO; **G**) *A. seriphia therasia*, ♂, Peru, MUSM (mirror image of specimen); **H**) *A. seriphia therasia*, **lectotype** ♀, ZMHU; **I**) *A. godmani*, ♂, Ecuador, FLMNH; **J**) *A. godmani*, ♀, Ecuador, FLMNH; **K**) *A. justina pichincha* **new species, holotype** ♂, Ecuador, MECN; **L**) *A. justina pichincha* **new subspecies,** paratype ♀, Ecuador, FLMNH. Scale bar near middle at bottom of figure applies to all specimens **except K**, **L**, which are represented by scale bar at bottom right.

meaningful; more sequences of *A. godmani* are clearly needed from west Colombia, and throughout Central America, to test the current hypothesis. In addition to wing pattern (see Willmott 2003a) and DNA sequence differences, in Ecuador *A. godmani* occurs at significantly lower elevations (750–2020 m, mean 1339 m, n=14 localities) than *A. seriphia aquillia* (1350–2500 m, mean 1755 m, n=15 localities) (*t*-test, $p<0.01$) (Willmott & Hall unpublished). The DNA sequence data otherwise confirm the existing classification, showing remaining *A. seriphia* taxa to form a single cluster with specimens from northern Venezuela to Bolivia. Given that there have been a number of changes to the classification of the *Adelpha serpa* group, based on findings of Prudic et al. (2008) and this paper, we present an updated classification for these species in Appendix 2.

Descriptions of new taxa

Adelpha margarita Willmott & Hall, **new species**

Figs. 1, 2A–D, 3, 4C

Diagnosis and identification: Numerous wing pattern and male genital characters (see Willmott 2003b), especially the 'V'-shaped 3rd discal cell bar on the VFW and lack of a clunicula (spinose process on inner basal edge of valva), place *Adelpha margarita* **new species** within the *A. serpa* group, within a clade of species including *A. zea* (Hewitson, 1850), *A. paroeca* (Bates, 1864), *A. nea* (Hewitson, 1847), *A. paraena* (Bates, 1865), *A. serpa*, *A. radiata* Fruhstorfer, 1915, *A. seriphia*, *A. godmani* (**revised status**) and *A. hyas* (Doyère, [1840]). The new species is distinguished from all of these species by the paired markings of the white VHW inner submarginal series in cell M_3–M_2 being faded in comparison with the markings in adjacent cells, with this part of the cell thus appearing blackish. The species is further distinguished from *A. zea*, *A. paroeca*, *A. nea* and *A. paraena* by having the white VHW submarginal series spots divided in each cell. In comparison with the most phenotypically similar remaining species, *A. hyas*, *A. seriphia* and *A. godmani*, it is further distinguished by the white HW postdiscal band being slightly constricted in cells M_2–Rs and the reddish orange postdiscal band on the VHW being correspondingly displaced basally in these cells. The VHW reddish orange postdiscal band is parallel and close to the inner edge of the white submarginal series, without a broadened, blackish space visible in cells Cu_2–M_1 as in *A. seriphia* (Fig. 2E–H) and *A. godmani* (Fig. 2I, J). In comparison with other members of the *A. serpa* group and based on the limited data available, the following five DNA nucleotides appear to be autapomorphies for *A. margarita* (position from alignment with *Bombyx mori* (Linnaeus, 1758) mitochondrial genome, NCBI Reference Sequence NC_002355.1): position 11986: C (not T); position 12157: C (not T); position 12298: G (not A); position 12397: C (not T); position 12463: C (not T).

Description: MALE: (Fig. 2A): Forewing length 27-29mm (mean 27.4 mm, n=15). *Wing shape*: triangular, slightly produced at apex. *Dorsal surface*: Ground color dark grayish brown. Forewing discal cell with dark reddish, narrow dash at base, a dark reddish band in middle between thinner black first and second discal cell bars, and a dark reddish band covering discocellulars, dirty whitish marking between second and third discal cell bar; white postdiscal band with faint turquoise tinge present as dislocated spots between anal margin and vein M_3, decreasing in width anteriorly; postdiscal band present anterior of vein M_3 as dashes of paler ground color; subapical orange spot formed of fused postdiscal series extending and tapering from costa to anterior half cell Cu_1–M_3. Hind wing with postdiscal band similar to forewing extending from costa into cell 2A–Cu_2, slightly constricted in cells M_1–Rs, broadest in discal cell and tapering to rounded point towards tornus; dark, curved, orange dash (representing postdiscal series) in cell 2A–Cu_2; submarginal series visible as dashes of paler ground color. Ventral surface: Ground color dark blackish brown. Forewing with orange stripe along costa from base to mid-discal cell; discal cell silvery white with four black cell bars, first curved basally at posterior edge cell, second slightly bent in middle, third 'V'-shaped and touching second in middle, fourth straight, dark orange between first and second bar and forming band over discocellulars; base cell 2A–Cu_2 with orange spot ringed in white, then thin black bar, white bar, black bar, basal to pale postdiscal band, base cell Cu_2–Cu_1 with white spot split by black line; postdiscal band as on dorsal surface except continuing as clear white dashes to costa anterior of vein M_3; pale orange subapical spot formed of fused postdiscal series as on FW but with indistinct edges, especially in cell M_1–R_5, with spot in each cell split by interneural darker orange stripe; fused postdiscal series continue as three dark orange dashes in cells Cu_1–M_3, Cu_2–Cu_1 and 2A–Cu_2; submarginal series silvery white, inner twice width of outer, divided in each cell by black interneural stripe, almost absent in cells Cu_1–M_2, inner series basal edge fused with subapical orange spot in cells M_3–M_1, dark division between inner and outer series indistinct. Hind wing basal area white except for black stripe from wing base to tip subcostal vein, distal edge anal margin lined with black, black discal stripe from Sc+R_1 extending across cell then down through 3A–2A just anterior of and parallel to vein 3A; black discal stripe bordered distally by dark orange band, this bordered distally by black stripe extending from costa through discocellulars and along vein 2A to tornus; pale postdiscal band as on dorsal surface; postdiscal series fused forming a dark orange line from costa to tornus, convex except indented basally in cell M_1–Rs; submarginal series silvery white, inner two to three times width of outer, divided in each cell by black interneural stripe, white scaling much reduced in cell M_3–M_2, dark division between inner and outer series indistinct. *Head*: eyes dark brown with short setae; dense white scales at ventral base of eyes; antennae black, head with white scales at ventral base of antennae; labial palpi white with broad black inner and outer lateral stripe and dorsal surface, latter also with black hairs; top of head with chestnut brown scales, frons with brown hairs. *Thorax*: dorsal surface black with short dark brown hairs, ventral surface white with black stripes where legs rest against thorax, forelegs white, mid and hind legs black except for white ventral femur. *Abdomen*: dorsal surface black with short dark brown hairs, ventral surface white, then with dark gray lateral stripe, then white lateral stripe. *Genitalia* (Fig. 3A): saccus broad, similar in length to tegumen; valva triangular in lateral view tapering smoothly to posterior tip, with short spines at tip extending about one quarter distance along ventral edge; clunicula (spinose process on inner basal edge of valva) absent; aedeagus short, approximately straight except for slight 'spoon'-shaped curve at posterior tip, small spinose pad inside vesica near tip.

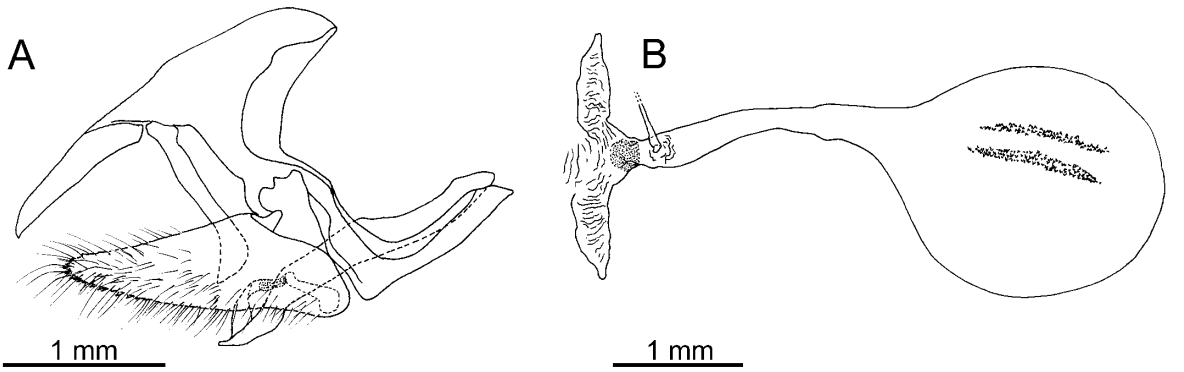

FIG. 3. Genitalia of *Adelpha margarita* **new species** **A**) Paratype ♂, lateral view, Ecuador, FLMNH; **B**) Paratype ♀, dorsal view, Ecuador, FLMNH.

FEMALE: (Fig. 2B): *Forewing length* 28–29 mm (mean 28.5 mm, n=2). *Wing shape*: similar to male except slightly broader. *Dorsal surface*: similar to male except slightly paler ground color, postdiscal band slightly broader, pale dashes of upper postdiscal band more distinct. *Ventral surface*: similar to male except for broader postdiscal band, and submarginal series more strongly fused. *Head, thorax, abdomen*: similar to male. *Genitalia* (Fig. 3B): lamella postvaginalis a narrow, laterally elongated, weakly sclerotized, wrinkled plate; ostium bursae a sclerotized simple tube incomplete dorsally; ductus seminalis origin on dorsal surface of ductus bursae, near ostium bursae; ductus bursae a simple tube; corpus bursae round, of similar length to ductus bursae, with two parallel bands of small, teeth-like signae in middle of dorsal surface.

Types: HOLOTYPE ♂: **ECUADOR**: *Zamora-Chinchipe*: km 3.5 El Tambo-San Juan del Oro, [3°57'9"S,79°3'33"W], 1900m, (K.R. Willmott, R. Aldaz), 3 November, [FLMNH-MGCL-111890], (FLMNH, to be deposited in MECN).

PARATYPES (19 ♂, 2 ♀): **ECUADOR**: *Loja*: 'above Yangana' [=La Entrada], [4°25'6"S,79°9'18"W], 2500m, (I. Aldas), October, 2 ♂, (PIBO), (I. Aldas), Oct-Nov, 5 ♂, (KWJH); *Morona-Santiago*: Condor Mirador, [3°37'42"S,78°23'40"W], 1972m, (J. Radford), 22 August, 1 ♂ [CON45], (FLMNH) (CULEPEX Expedition); Condor Mirador, [3°37'42"S,78°23'41"W], 1972m, (J. Radford), 23 August, 1 ♂ [CON111], 1 ♂ [CON113], (FLMNH) (CULEPEX Expedition), 1 ♂ [CON112], (MECN); Condor Mirador, [3°37'43"S,78°23'40"W], 1972m, (J. Radford), 27 August, 1 ♂ [CON288], (FLMNH) (CULEPEX Expedition); Condor Mirador, [3°38'44"S,78°23'44"W], 1973m, (E. Hartley), 27 August, 1 ♂ [CON276], (FLMNH) (CULEPEX Expedition), (K. Buckland), 27 August, 1 ♂ [CON262], (FLMNH) (CULEPEX Expedition); km 19 Macas-Nueve de Octubre rd., Río Abanico, [2°15'18"S,78°12'0"W], 1600m, (I. Aldas), September, 1 ♂, (KWJH); km 19 Macas-Nueve de Octubre rd., Río Abanico, [2°15'18"S,78°12'0"W], 2200m, (I. Aldas), September, 2 ♂, (KWJH); Zamora-Chinchipe: km 24 Loja-Zamora rd., San Francisco, casa de Arcoiris, [3°59'18"S,79°5'42"W], 2000-2100m, (K. Willmott), 3 December, 1 ♀ [FLMNH-MGCL-111893], (FLMNH), (K. Willmott, R. Aldaz), 14 October, 1 ♀ [FLMNH-MGCL-111892], (FLMNH), 15 October, 1 ♂ [FLMNH-MGCL-111891], (FLMNH); km 3.5 El Tambo-San Juan del Oro, [3°57'9"S,79°3'33"W], 1900m, (K. Willmott, R. Aldaz), 3 November, 1 ♂ [FLMNH-MGCL-111889], (FLMNH).

Other examined specimens: PERU: *San Martín*: Jorge Chávez, [5°41.0'S,77°40.0'W], 1450m, (Calderón, B.), May, 1 ♂, (PIBO).

Etymology: This species is named for Margaret Robinson, in gratitude for all her friendship and support. The name is formed from the feminine Latin noun '*margarita*', meaning 'pearl', and thus also alludes to the variegated black and silvery white submarginal markings on the ventral surface which are such a distinctive feature of this species.

Taxonomy and variation: The only specimens examined by Willmott (2003a) of this species were two of the type specimens of *A. m. garleppi* **new subspecies**, described here, which were treated as representing a form of *A. seriphia therasia* Fruhstorfer, 1915. Subsequently, however, we and others have collected series of specimens of both *A. margarita margarita* and *A. seriphia aquillia* at five sites in central and southern Ecuador, from Morona-Santiago to Loja (Río Abanico, San Francisco, El Tambo-San Juan del Oro rd., Cóndor Mirador, La Entrada above Yangana) (Fig. 4B, C). These specimens show consistent wing pattern differences as described above under the Diagnosis, and COI sequence data confirm the hypothesis of *A. margarita* as a species distinct from *A. seriphia aquillia*. In fact, the data suggest that the closest relative of *A. margarita* is the allopatric west Andean-Central American *A. godmani* (Fig. 4A). Mean DNA sequence divergence between *A. margarita* and *A. godmani* is 2 %, similar to differences between *A. nea* and *A. radiata* (2.4 %), *A. serpa* and *A. radiata* (1.5 %) and *A. seriphia* and *A. hyas* (2.3%), all sympatric or elevationally parapatric species pairs. *Adelpha margarita* and *A. godmani* both occur on the west Andean slopes in southern Ecuador (Fig. 4A) but at different elevations, with the former known only from 2500 m and the latter from below 1400 m (Willmott & Hall unpublished).

Adelpha margarita margarita shows variation in the width of the postdiscal band and DFW orange marking, which may be slightly narrower than in the holotype, and in the intensity of white scaling in the ventral submarginal series, which may appear more blackish than the holotype in some specimens. Some specimens show a trace of orange scaling in the DFW postdiscal series.

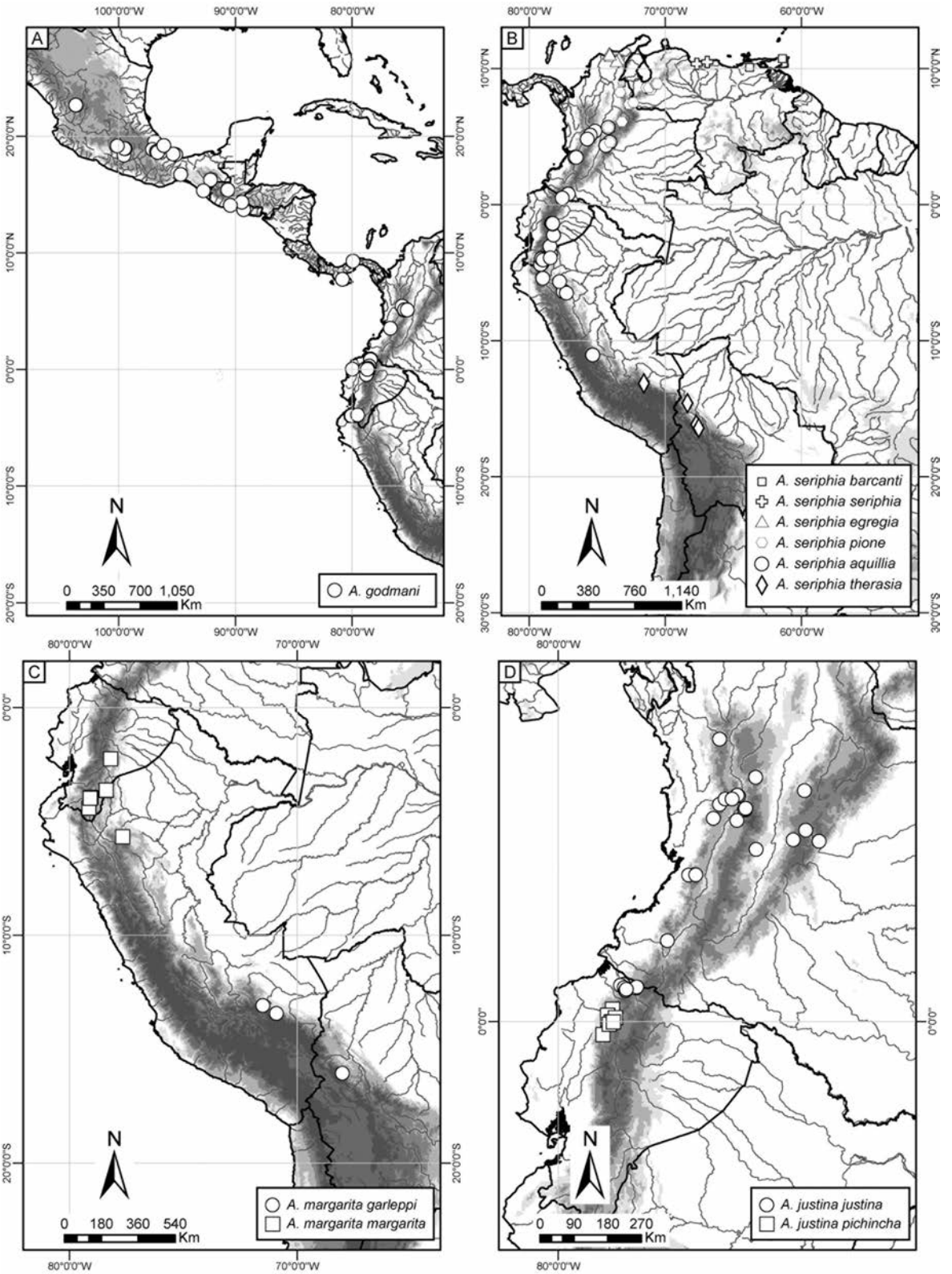

FIG. 4. Distribution of *Adelpha* taxa. **A**) *A. godmani*; **B**) *A. seriphia*; **C**) *A. margarita* **new species**; **D**) *A. justina justina* and *A. justina pichincha* **new subspecies**.

Distribution: *Adelpha margarita margarita* is known from the eastern Andes from central Ecuador (Morona-Santiago, Río Abanico) to northern Peru (San Martín, Jorge Chávez) (Fig. 4C). It has also been collected on the west Andean slopes in southern Ecuador, on the road from Yangana to Valladolid.

Habitat and adult ecology: This species is known from 7 localities, most of which are hilltops or ridge tops in cloud forest, with reliable elevation data ranging from 1900–2500 m. At the lower limit of its elevation *A. margarita* occurs at the same sites as *A. seriphia*, but otherwise the two species appear to replace one another in elevation. We found males perching on the tops of small trees 8 m above the ground at 11:00 hrs in bright sun. During approximately 6 months of trapping we also collected one male and the two known females in canopy traps baited with rotting fish, c. 15 m above the ground, in undisturbed cloud forest on a hillside above the Río San Francisco.

Adelpha margarita garleppi Willmott, **new subspecies**

Figs. 2C,D, 4C

Description and Diagnosis: This subspecies differs from the nominate subspecies in having the fused postdiscal series on the DFW visible as a broad orange band extending from the subapical orange marking on the FW to the tornal orange spot on the DHW. The postdiscal band is also slightly narrower in *A. m. garleppi*. This taxon is also very similar to the sympatric *A.* s. *therasia* (Fig. 2G,H), but may be distinguished by characters discussed under the Diagnosis of *A. margarita margarita*. Most obviously, *A. m. garleppi* has very broad, almost fused silvery white submarginal series on the VHW which closely border the orange postdiscal band, without the broad black area which separates these two pattern elements in the middle of the wing in *A.* s. *therasia*.

Types: HOLOTYPE ♂: **PERU**: *Cuzco*: [Valle de Cosñipata], El Mirador, 1720m, 13º 04'S, 71º 33'W, 11 February 2011, M[ichael] McInnis (MUSM).

PARATYPES (1 ♀): **PERU**: *Cuzco*: Marcapata, [13°26.0'S,70°55.0'W], 4500 ft, 1 ♀, (BMNH). **BOLIVIA**: *La Paz*: Río Zongo, [16°3.40'S,68°1.2'W], 1200m, (Garlepp), 1896, 1 ♂ (ZMHU).

Etymology: This subspecies is named for Otto Garlepp, who collected the Bolivian paratype, in addition to many other important specimens of Bolivian butterflies, including the lectotype of *A.* s. *therasia*.

Taxonomy and variation: Willmott (2003a) included the BMNH and ZMHU type specimens of *A. m. garleppi* under *A. seriphia therasia*, and illustrated the male under this name (Willmott 2003a: Fig. 41i,j). However, all of the wing pattern characters discussed above to distinguish *A. margarita margarita* from other *Adelpha* also occur in the three type specimens of *A. m. garleppi*, and suggest that these two taxa are conspecific. Willmott (2003a) listed two Bolivian specimens in the ZMHU as possible syntypes of *A.* s. *therasia*, a female from Coroico labeled as a syntype by G. Lamas (Fig. 2H), and a male from Río Zongo (Fig. 2C), both collected by Garlepp. The former represents *A. seriphia*, the latter *A. margarita*. Neither specimen was labeled as a type by Fruhstorfer, but that is also true of other ZMHU Fruhstorfer *Adelpha* types, such as the Ocaña paralectotype of *A. seriphia aquillia* referred to specifically by Fruhstorfer (1915: 531) as being in the "coll. Staudinger in the Berlin Museum" (Willmott 2003a). Fruhstorfer clearly, therefore, had examined the Staudinger material in the ZMHU, and it seems reasonable to conclude that this also included the putative syntype specimens of *A.* s. *therasia* collected by Garlepp. Although Fruhstorfer (1915) stated that the *A.* s. *therasia* type specimens were collected by Fassl, it seems most likely that this was a mistake, because neither we ourselves nor G. Lamas (pers. comm.) have located any other similar Bolivian specimens collected by Fassl in world collections. The female syntype (Fig. 2H), in particular, closely matches the original description in having very narrow postdiscal bands and the "white zone of the hind wings besides more pregnantly bordered in black" (Fruhstorfer, 1915: 531). To fix the identity of *A.* s. *therasia*, while minimizing nomenclatural change, we therefore designate this female specimen in the ZMHU, with the following labels, as the lectotype of *Adelpha seriphia therasia* Fruhstorfer (Fig. 2H): "Corvico [sic] ca. 2000 m. Bolivia (Yungas) 1895 (2-5) Garlepp", "SYNTYPE ♀ *Adelpha seriphia therasia* Fruhstorfer G. Lamas det. '87", "SYNTYPE".

Distribution: This subspecies is currently known only from the three type specimens from southern Peru (Cuzco) and northern Bolivia (La Paz) (Fig. 4C).

Habitat and adult ecology: According to G. Lamas and M. McInnis (in litt.), the holotype specimen was collected on a bend of the road from Acjanaco to Pilcopata which overlooks a cliff. The specimen was feeding on flowers of *Miconia* (Melastomataceae) trees, along with a number of other rarely collected Lycaenidae and Riodinidae. The rarity of the taxon otherwise suggests that it flies at similarly poorly collected elevations and heights above the ground as the nominate subspecies. The similarity of the dorsal wing patterns of *A. m. garleppi* and *A.* s. *therasia* and other presumably sympatric *Adelpha*, such as *A. alala* (Hewitson, 1847) and *A. aricia* (Hewitson, 1847), suggests that these species may be involved in mimicry.

Adelpha justina pichincha Willmott & Hall, **new subspecies**

Figs. 2K,L, 4D

Description and Diagnosis: This subspecies (Fig. 2K,L) is most similar to the nominate subspecies (see Willmott, 2003a, Fig. 114a,b), but distinguished from it by the narrower DFW orange postdiscal

band. The ratio of the width of the band measured along vein Cu_1 to the forewing length varied from 0.046 to 0.106 in 7 specimens (mean 0.084), while in the most phenotypically similar subspecies, *A. justina justina*, this ratio varied from 0.139 to 0.201 in 11 specimens from Ecuador and Colombia (mean 0.170). A *t*-test indicates this difference to be significant ($p<0.01$).

Types: HOLOTYPE ♂: **ECUADOR**: *Imbabura*: km 26 Chontal Bajo-Chontal Alto, Chontal Alto, [0°17'48"N,78°42'3"W], 1550–1650m, (K. Willmott & J. Hall), 10 August, [FLMNH-MGCL-149747], (FLMNH, to be deposited in MECN).

PARATYPES (15 ♂, 3 ♀): **ECUADOR**: *Imbabura*: km 26 Chontal Bajo-Chontal Alto, Chontal Alto, [0°17'48"N,78°42'3"W], 1550–1650m, (K. Willmott & J. Hall), 10 August, 1 ♂ [FLMNH-MGCL-149745], 1 ♂ [FLMNH-MGCL-149746], 1 ♂ [FLMNH-MGCL-149748], 1 ♂ [FLMNH-MGCL-149749], (FLMNH); *Pichincha*: 7 km SW Las Tolas, [0°3'26"N,78°49'6"W], 1350m, (K. Willmott & J. Hall), 3 August, 1 ♂ [FLMNH-MGCL-149735], 1 ♂ [FLMNH-MGCL-149736], 1 ♂ [FLMNH-MGCL-149737], (FLMNH); c. 1 km S Nanegalito, Hostería El Rosal, [0°3'27"N,78°40'53"W], 2000m, (Warren-Gash, H.), 5 September, 1 ♂, (HAWA); km 9 Pacto-Guayabillas rd., [0°9'18"N,78°49'14"W], 1630m, (K. Willmott & J. Hall), 5,6 August, 1 ♂ [FLMNH-MGCL-149743], 1 ♂ [FLMNH-MGCL-149744], (FLMNH); Mindo, Río Napombillo, [0°3'49"S,78°47'0"W], 1200m, (K. Willmott), 13 November, 1 ♂, (KWJH); old Quito-Nono rd., Tandayapa, [0°0'24"S,78°41'2"W], 1600-1800m, (Boyer, P.), 30 January, 1 ♀, (PIBO); Quito-Sto. Domingo old rd., Hacienda Santa Isabel, [0°18'48"S,78°56'0"W], 1200m, (K. Willmott & J. Hall), 2 September, 1 ♂, (KWJH); Reserva Las Gralarias, [0°0'39"S,78°43'50"W], (T. Kell), 25 February, 1 ♂ [FLMNH-MGCL-151113], (FLMNH); Reserva Las Gralarias, Quebrada Santa Rosa, [0°0'28"S,78°43'27"W], 1800m, (K. Willmott & J. Hall), 4 August, 1 ♂ [FLMNH-MGCL-149738], 1 ♀ [FLMNH-MGCL-149739], 1 ♀ [FLMNH-MGCL-149740], 1 ♀ [FLMNH-MGCL-149741], (FLMNH); Reserva Las Gralarias, Quebrada Santa Rosa, [0°0'28"S,78°43'27"W], 1950m, (K. Willmott & J. Hall), 4 August, 1 ♂ [FLMNH-MGCL-149742], (FLMNH).

Etymology: This subspecies is named for the province of Pichincha, itself named for Volcán Pichincha, whence comes the majority of specimens of this subspecies.

Taxonomy and variation: Willmott (2003a: Fig. 114c,d) figured a specimen of this subspecies under *A. justina justina* and discussed the possibility that it might represent a distinct subspecies, but refrained from describing it because only a single specimen was then known. Specimens are now known from 9 sites up to 72 km apart, from southern Imbabura, just north of the Río Guayllabamba, to southern Pichincha, along the Alóag-Santo Domingo road (Fig. 4D). There is some variation within populations in the width of the orange DFW postdiscal band, but this band is always considerably narrower than in the nominate subspecies, especially in comparison with neighboring northwest Ecuadorian specimens which have the broadest bands (Willmott 2003a). The extent of the white DHW postdiscal spot is also variable, and in fact most specimens have more extensive white than the geographically closest specimens of *A. justina justina*, from northwestern Ecuador, but show little difference in this character in comparison with Colombian specimens of the nominate subspecies.

Distribution: This subspecies is known only from the western Andes of Ecuador, from southern Imbabura to southern Pichincha province (Fig. 4D). In addition to the localities listed above, Raguso and Gloster (1996) also reported '*Adelpha justina*' from Reserva Maquipucuna (Ecuador, Pichincha, Río Alambi, c. 0°5'42"N,78°38'0"W, 1600 m), presumably based on specimens of this subspecies.

Habitat and adult ecology: *Adelpha justina pichincha* occurs in relatively undisturbed cloud forest from 1200–2000 m. We have collected males in traps baited with rotting fish from 1–15 m above the ground along ridge tops. The majority of males that we have encountered have been perching along ridge tops from 3–15 m above the ground, in small to large light gaps, from 10:00 hrs to 13:00 hrs. We also observed a single male perching and patrolling a small area above a river 5–8 m above the water from 13:15–13:30 hrs. At the same site and time, three females were encountered gliding downstream, 1–2 m above the water in a slightly shaded area. The latter observation is particularly unusual given the extreme rarity of females of this species; out of 211 specimens of *A. justina* located by Willmott (2003a) in collections, only 2 were females, and we have yet to observe a female of the nominate subspecies.

Use of DNA sequence data in Neotropical butterfly taxonomy

One of the first studies to use DNA sequence data to support the recognition and description of a new butterfly species was Brower (1996), and since then there has been an exponential increase in publications using such data in systematics. Nevertheless, although a number of generic-level studies have inadvertently revealed problems in species taxonomy (e.g., Mallarino et al. 2005, Elias et al. 2009, and Mullen et al. 2010), surprisingly few publications have deliberately employed DNA sequence data to solve species-level taxonomic issues. DNA sequence data are clearly of value in revealing potential cryptic species within intensive inventories of particular regions (e.g., Janzen et al. 2011), as illustrated by Chacón et al.'s (2012) recent description of a new species of *Opsiphanes* Doubleday, [1849], from Costa Rica. However, delimiting sympatric morpho-species is usually just the first step in species taxonomy, with the second, critical, issue being the association of allopatric taxa (e.g., Silva-Brandão et al. 2008). Not only is this second step necessary for a sound understanding of species distributions and diversity, but it is also essential for the stable application of taxonomic names. In the case of our study, the separation between *A. seriphia* and *A. margarita* was reasonably clear, but the relationships of

A. margarita to other *Adelpha* taxa were not. Broad geographic sampling is therefore essential in most studies, as illustrated by Prudic et al. (2008), Sourakov and Zakharov (2011), and Bonebrake et al. (2011). Such sampling is especially challenging in large, highly diverse and biologically complex regions such as the tropical Andes, and the lack of recently collected specimens amenable to molecular study is no doubt a major factor hindering the incorporation of molecular data into species-level taxonomic research. The acquisition of new material and appropriately preserved tissue samples from geographically diverse regions, including those that have been historically well-collected, should therefore remain a top priority for museums.

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APPENDIX 1. Voucher specimen information for DNA sequence data.

Taxon	Locality (decimal latitude and longitude)	DNA Voucher number	GenBank number
Adelpha californica	USA: Oregon: Benton Co. (44.467, -123.483)	NW107-16	AY788591.1
Adelpha godmani	Colombia: Caldas: El Águila, Neira (5.105, -75.506)	LEP-04107	KC681843
Adelpha godmani	Ecuador: Imbabura: Cachaco, ridge to south (0.813, -78.417)	KW-080229-24	KC681845
Adelpha godmani	Ecuador: Loja: Río Inguna, km 5 Zambi-Piñas rd. (-3.907, -79.532)	KW-080510-01	KC681846
Adelpha godmani	Ecuador: Manabí: Cerro Pata de Pájaro (0.011, -79.968)	LEP-04109	KC681844
Adelpha hyas hewitsoni	Ecuador: Zamora-Chinchipe: Cabañas Ecológicas Copalinga, Río Bombuscaro (-4.091, -78.959)	LEP-04252	KC681827
Adelpha hyas hewitsoni	Ecuador: Zamora-Chinchipe: Cabañas Ecológicas Copalinga, Río Bombuscaro (-4.091, -78.959)	LEP-04253	KC681828
Adelpha hyas hewitsoni	Peru: Amazonas: Bagua Chica (-5.633, -78.533)	LEP-03282	KC681829
Adelpha m. margarita	Ecuador: Morona-Santiago: Río Abanico (-2.255, -78.2)	39-A	KC681840
Adelpha m. margarita	Ecuador: Morona-Santiago: Río Abanico (-2.255, -78.2)	KW-080229-29	KC681839
Adelpha m. margarita	Ecuador: Morona-Santiago: Río Abanico (-2.255, -78.2)	KW-080229-30	KC681838
Adelpha m. margarita	Ecuador: Zamora-Chinchipe: km 3.5 El Tambo-San Juan del Oro (-3.952, -79.059)	KW-061103-04	KC681842
Adelpha m. margarita	Ecuador: Zamora-Chinchipe: San Francisco, casa de Arcoiris (-3.988, -79.095)	KW-061114-03	KC681841
Adelpha nea nea	Ecuador: Napo: Chichicorrumi (-1.07, -77.629)	LEP-04111	KC681821
Adelpha paraena paraena	Bolivia: Cochabamba: Villa Tunari (-16.95, -65.418)	LEP-03800	KC681822
Adelpha radiata aiellae	Ecuador: Esmeraldas: Río Durango (1.038, -78.617)	LEP-04113	KC681823
Adelpha seriphia aquillia	Colombia: Caldas: El Águila, Neira (5.105, -75.506)	LEP-04106	KC681832
Adelpha seriphia aquillia	Ecuador: Morona-Santiago: Río Abanico (-2.255, -78.2)	34-A	KC681834
Adelpha seriphia aquillia	Ecuador: Sucumbíos: Río Sucio (0.475, -77.555)	LEP-04110	KC681833
Adelpha seriphia aquillia	Ecuador: Zamora-Chinchipe: El Líbano (-4.08, -78.969)	KW-061114-05	KC681835
Adelpha seriphia aquillia	Ecuador: Zamora-Chinchipe: km 3.5 El Tambo-San Juan del Oro (-3.952, -79.059)	KW-061103-02	KC681836
Adelpha seriphia barcanti	Venezuela: Monagas: Cerro Quiriquire (10.102, -63.809)	KW-061114-01	KC681830
Adelpha seriphia pione	Venezuela: Mérida: Mérida (8.6, -71.133)	LEP-04108	KC681831
Adelpha seriphia therasia	Bolivia: Cochabamba: El Dorado (-17.158, -65.767)	LEP-03802	KC681837
Adelpha serpa diadochus	Ecuador: Orellana: Reserva Biológica del Río Bigal, main campsite (-0.525, -77.418)	LEP-10479	KC681825
Adelpha serpa diadochus	Ecuador: Pastaza: Kapawi village (-2.538, -76.836)	LEP-04115	KC681826
Adelpha serpa duiliae	Ecuador: Carchi: Lita, ridge east of Río Baboso (0.888, -78.438)	KW-080229-25	KC681824

APPENDIX 2. Updated classification for members of the *Adelpha serpa* group following Prudic et al. (2008) and this paper. A single dash before a name indicates a valid subspecific name, two dashes indicates a synonym, and three dashes indicates an unavailable name.

bredowii Geyer, 1837
eulalia (Doubleday, [1848]) (raised to species rank by Prudic et al. (2008))
--*guatemalensis* (Carpenter & Hobby, 1944)
californica (Butler, 1865) (raised to species rank by Prudic et al. (2008))
diocles Godman & Salvin, 1878
- *creton* Godman, 1901
herbita Weymer, 1907
zea (Hewitson, 1850)
--*serpentina* Fruhstorfer, 1915
--*tarpeia* Fruhstorfer, 1915
paroeca (Bates, 1864)
--*emathia* (R. Felder, 1869)
- *pseudodonysa* Salazar, 2000
nea (Hewitson, 1847)
--*campeda* Fruhstorfer, 1915
- *sentia* Godman & Salvin, 1884
paraena (Bates, 1865)
- *massilia* (C. Felder & R. Felder, 1867)
- *reyi* Neild, 1996
- *lecromi* Willmott, 2003
radiata Fruhstorfer, 1915
- *myrlea* Fruhstorfer, 1915
- *gilletella* Brévignon, 1995
- *aiellae* Willmott & Hall, 1999
- *explicator* Willmott & Hall, 1999
- *romeroi* Willmott & Neild, 2003
serpa (Boisduval, 1836)
--*damon* Fruhstorfer, 1913
--*ornamenta* Fruhstorfer, 1915
- *celerio* (Bates, 1864)
--*diademeta* Fruhstorfer, 1913
--*phintias* Fruhstorfer, 1913
- *duiliae* Fruhstorfer, 1913
- *diadochus* Fruhstorfer, 1915
---*timehri* Hall, 1938
--*florea* Brévignon, 1995
seriphia (C. Felder & R. Felder, 1867)
- *pione* Godman & Salvin, 1884
- *aquillia* Fruhstorfer, 1915
--*naryce* Fruhstorfer, 1915
- *therasia* Fruhstorfer, 1915
- *egregia* Röber, 1927
- *barcanti* Willmott, 2003
godmani Fruhstorfer, 1913, **revised status**
--*syrna* Steinhauser, 1974
---*syrna* Fruhstorfer, 1915
margarita Willmott & Hall, **new species**
- *garleppi* Willmott, **new subspecies**
hyas (Doyère, [1840])
- *viracocha* Hall, 1938
- *hewitsoni* Willmott & Hall, 1999

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DELIMITATION OF *PHANETA TARANDANA* (MÖSCHLER 1874) AND *P. MONTANANA* (WALSINGHAM 1884) (TORTRICIDAE: OLETHREUTINAE) IN WESTERN CANADA USING MORPHOLOGY AND DNA

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ABSTRACT. *Phaneta* comprises a large genus of Nearctic tortricids with several species that are difficult to delimit using morphological characters. We examined *Phaneta tarandana* (Möschler 1874) and *P. montanana* (Walsingham 1884), using morphology and DNA. Our results upheld the distinction between these two species, even though the wing coloration and male genital characters traditionally used to diagnose them were unreliable due to intraspecific variation. Nonetheless, a combination of forewing fringe scale detail, wing streaks, mitochondrial, and nuclear DNA delimited these moth species in a region of overlap in western Canada.

Additional key words: ITS2, COI, wing fringe, cryptic species

Phaneta Stephens was proposed as a genus in 1852 and the delimitation of its species has challenged taxonomists ever since. In North America, *Phaneta* is a speciose group closely related to *Eucosma*, separable only by the absence of a costal fold in males, a distinction of convenience with no phylogenetic justification (Heinrich 1923; Razowski 2003; Gilligan et al. 2). Currently 260 species of these two genera are recognized in North America, of which 39 *Phaneta* are found in Alberta, Canada (Pohl et al. 2010; Gilligan et al. 2008). Two *Artemisia*-feeding species (Robinson et al. 2002; Brown et al. 2008), *Phaneta tarandana* (Möschler 1874) and *Phaneta montanana* (Walsingham 1884), provide an example of the taxonomic difficulties in the genus. They are part of a species complex that may include other *Phaneta* in the western and south-central United States, such as *Phaneta transversa* (Walsingham 1895), *Phaneta benjamini* (Heinrich 1923), and *Phaneta clarkei* Blanchard & Knudson (1983).

Möschler (1874) described *P. tarandana* based on 3 males from Labrador. The fate of those specimens is unknown. Heinrich (1923) suggested that the "type" might be a specimen from the Fernald Collection, presently in the USNM, that was collected in Labrador and "bears Möschler's label". However Brown (2005) reported the *P. tarandana* holotype as lost. Möschler's description relied on forewing characters but not genitalia. He distinguished this species from other *Phaneta* through its "noticeable size", recording its wingspan as 25-27 mm (Möschler 1874). Walsingham (1884) based his description of *P. montanana* on the forewing characters of one male specimen from Montana. *Phaneta montanana* and *P. tarandana* have not been rigorously reevaluated since their original descriptions.

Recent collections from western Canada have produced an abundance of material putatively representing *P. montanana*, *P. tarandana*, and what appear to be various intermediate forms. This region constitutes a potential zone of interaction between montane and boreal species, whether by overlap or hybridization (e.g. Lumley and Sperling 2010). In this study, we reexamine these two putative *Phaneta* moth species using multiple character types, with the aim of determining how many species they represent and how they may be identified.

MATERIALS AND METHODS

Samples and species criteria. We examined 130 specimens, mainly from Alberta but ranging from Kamloops BC to Prince Albert SK (locations mapped in Fig. 1). Wing pattern, color, and size were examined in all specimens, while a subset of specimens was sequenced for one mitochondrial and one nuclear gene. Outgroups for molecular analyses were selected from GenBank sequence data available for other *Phaneta* species. The species status of *P. montanana* and *P.*

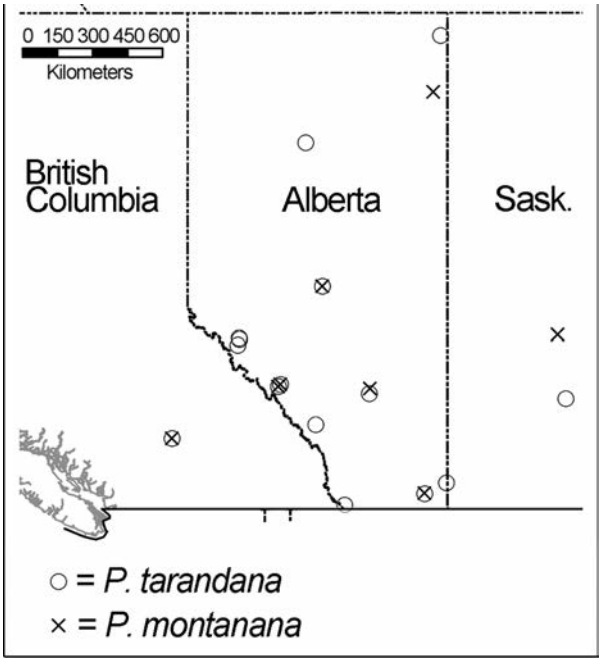

FIG. 1. Geographic locations of sample sites for *P. tarandana* (open circles) and *P. montanana* (crosses) in western Canada. Identifications are based on DNA sequences, forewing ground color, wing fringe and wing streaks, as described in the text.

tarandana was examined under the genomic integrity (Sperling 2003) and general lineage concepts (De Queiroz 1998, e.g. Dombroskie and Sperling 2012), which emphasize the use of multiple stable and consistent criteria to evaluate the distinctness of populations.

Morphology. Wing character terms used here are consistent with those of Razowski (2003). For wing morphometric analyses, the angles of the tornus and apex of the forewing were measured in the same specimens used in molecular analyses (12 male *P. montanana* and 25 *P. tarandana*), using digital images and the measure tool in Photoshop CS4 version 11.0.2 (Adobe Systems Incorporated, CA, USA). Other wing characters examined included ground color, fascia color, longitudinal wing streaks, and coloration of individual scales (See Fig. 2) of the forewing fringe (see Fig. 3 for general wing coloration, and Fig. 4 for forewing fringe scale character states). Ground color is that of the inter-fascia regions and strigulae. We define fascia color as the color present in the dorso-postbasal blotch, median fascia, terminal fascia, and divisions between strigulae.

Ground color and fascia color were examined because they traditionally have been used to delimit tortricid species (e.g. Heinrich 1923). Wing streaks were chosen because we found the character to be variable between specimens and clearly present in the adult drawing of Walsingham's illustration of the holotype of *P. montanana*, but lacking in the description of *P. tarandana*. Patterning on the forewing fringe scales was chosen because it was observed to be variable and is a non-traditional character for which taxonomic utility had not been previously evaluated in *Phaneta*. Heinrich (1923) used forewing fringe colouration to delimit *P. tarandana* from *P. transversa*; however the taxonomic utility of this character has not been tested since Heinrich (1923). We describe and illustrate this character here due to our resurrection of forewing fringe coloration for species delimitation. Wing characters were examined in all specimens, but were only scored in specimens with genetic information. Pearson's Chi-squared test (Naiman et al. 1972) was used to evaluate the statistical significance of associations between wing characters and DNA sequences.

Dissection methods follow Brown et al. (2009) except as follows. Dissections were performed under an M5-72711 Wild dissecting microscope. Genitalia were stained with chlorozol black. Female genitalia were removed using iris scissors to cut the intersegmental membrane between the 7th and 8th abdominal segments. Once open, the genitalia were placed on a pre-cleaned Gold Seal® microscope slide No. 3010, then one drop of glycerol was placed above the genitalia and a square glass cover slip was placed over the genitalia. Genitalia were photographed using a Nikon Coolpix 8400 attached to an Olympus S2X16 microscope, with illumination provided by an Olympus LG-PS3 fiber optic light source. After photography, genitalia were placed in vials along with the abdomen and pinned with the specimen. Genitalic terminology follows Gilligan et al. (2008).

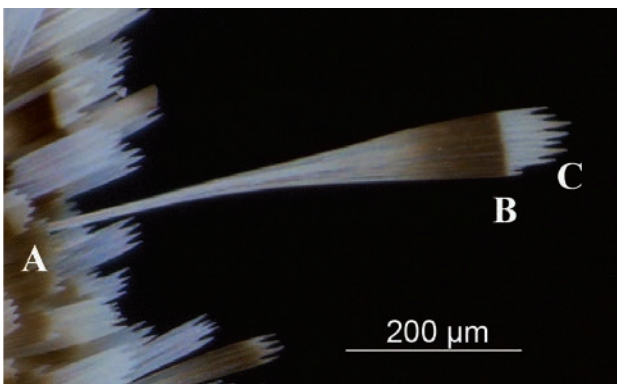

FIG 2. Individual scale along the termen of a *Phaneta tarandana* showing the root (**a**) and gradient origin (**b**) at the base of the apical teeth (c).

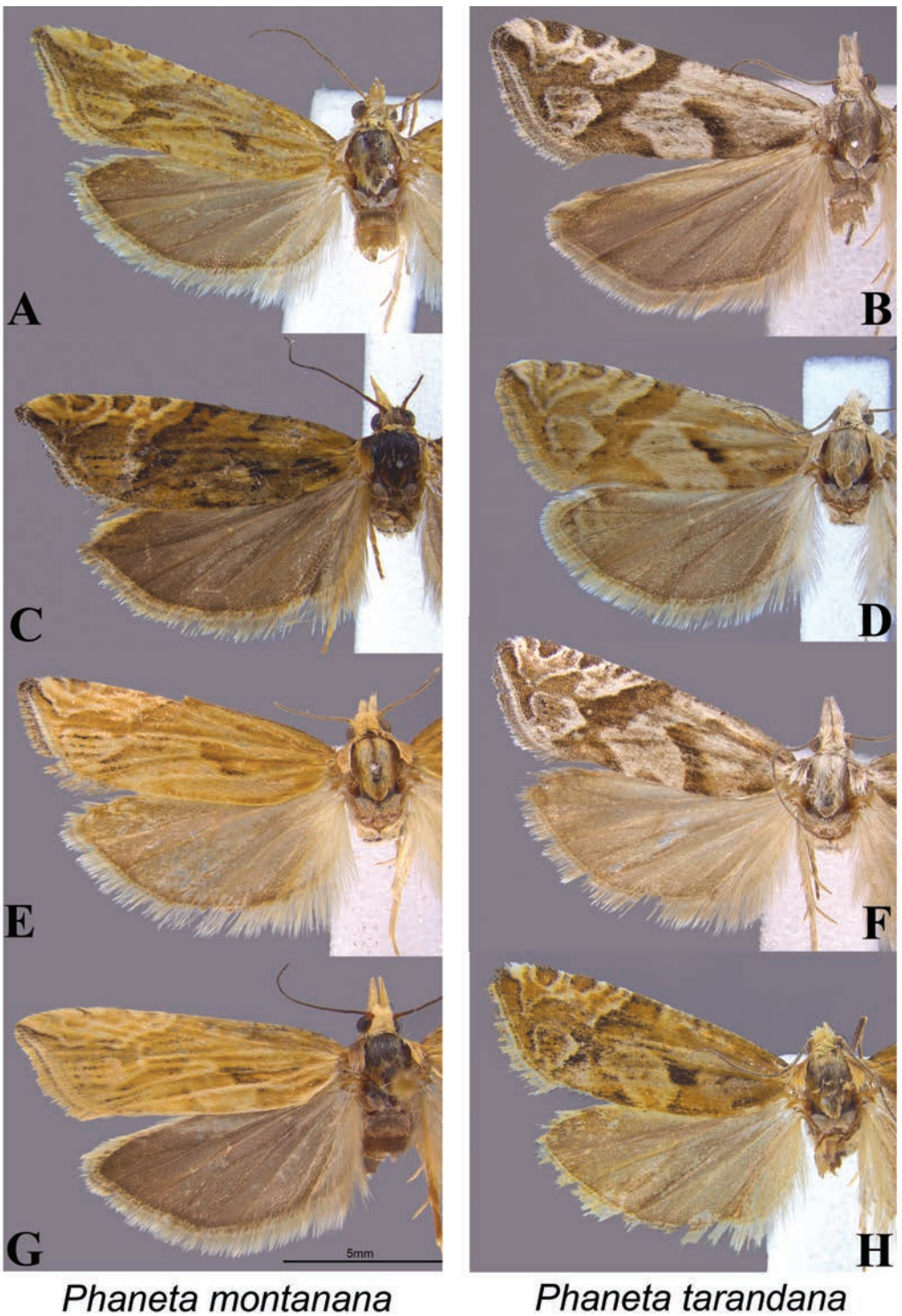

FIG 3. Wing coloration variation of *P. montanana* (right) and *P. tarandana* (left)

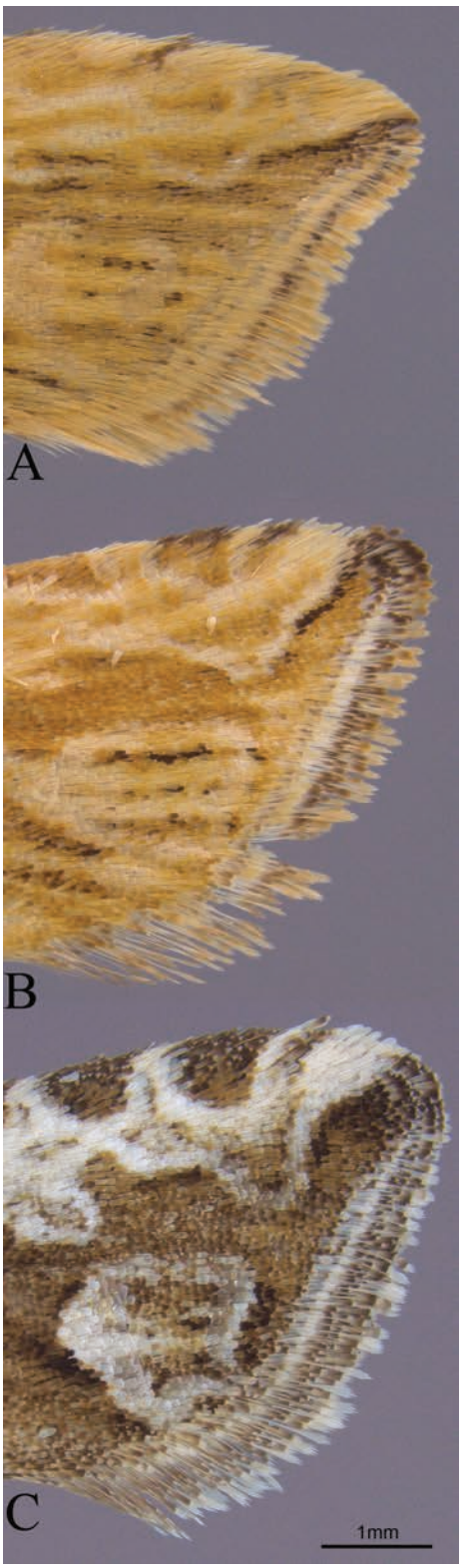

FIG 4. Forewing fringe of a typical *P. montanana* (**a**), *P. montanana* with darker scales (**b**) and typical *P. tarandana* (**c**).

Selection of samples for DNA analysis. Specimens were chosen for DNA analyses to represent a wide range of specimen age and wing coloration. We selected recently collected moths that varied in wing pattern from apparently typical (i.e. matching the species description) *P. tarandana* to typical *P. montanana* as well as intermediates and strongly marked specimens agreeing with the description of *P. transversa* (Walsingham 1895). The main wing character used to choose specimens was streaked coloration on the forewings (Fig. 3). Typical *P. montanana* exhibit strong streaks from the base of the wing to the apex, while *P. tarandana*, according to the original description, has distinct fasciate markings that are not smeared across the wing. The only characters that distinguish *P. transversa* from *P. tarandana*, based on Walsingham's (1895) description, are the presence of more distinct markings in *P. transversa* and a darker patch at the base of the forewing.

DNA extraction, amplification and sequencing. We removed 3 legs from the left side of each specimen; legs were stored in 95% ethanol at -20°C until DNA was extracted. Extractions followed the QIAamp Mini Kit (Qiagen, Canada) protocol with a modification of the final elution steps. To further concentrate DNA we eluted 50µl three times to a final volume of 150µl. PCR protocols followed Lumley & Sperling (2010) for COI and Dombroskie and Sperling (2012) for ITS2. Primers for COI were LCO (GGTCAACAAATCATAAAGATATTGG) and HCO (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994); those for ITS2 were ITS2F (TGTGAACTGCAGGACACATGAA) and ITS2R (ATGCTTAAATTTAGGGGGTAGTC) (White et al. 1990). PCR product was purified using ExoSAP-IT (USB corporation, Cleveland, OH). BigDye Terminator version 3.1 cycle sequencing (Applied Biosystems, Foster City, CA) was used for the sequencing reaction. Dye-labeled DNA was further purified using ethanol precipitation, then run on an ABI Prism 3730 DNA analyzer (Molecular Biology Service Unit, University of Alberta, Canada).

Analysis of DNA sequences. SeqMan Pro version 7.2.0 (DNASTAR) was used to examine chromatograms and assemble contigs; sequences were then aligned manually using Mesquite version 2.73 (Maddison & Maddison 2010). Parsimony and bootstrap analyses were performed using PAUP 4.0 (Swofford 2003) with 1000 replicates each. Likelihood bootstrapping (100 replicates) used Garli version 2.0 (Zwickl 2006) under settings consistent with the GTR + I + G model, as determined by Modeltest (Posada & Crandell 1998). Posterior probabilities were calculated using Bayesian analyses in MrBayes v3.1.2 (Ronquist & Huelsenbeck

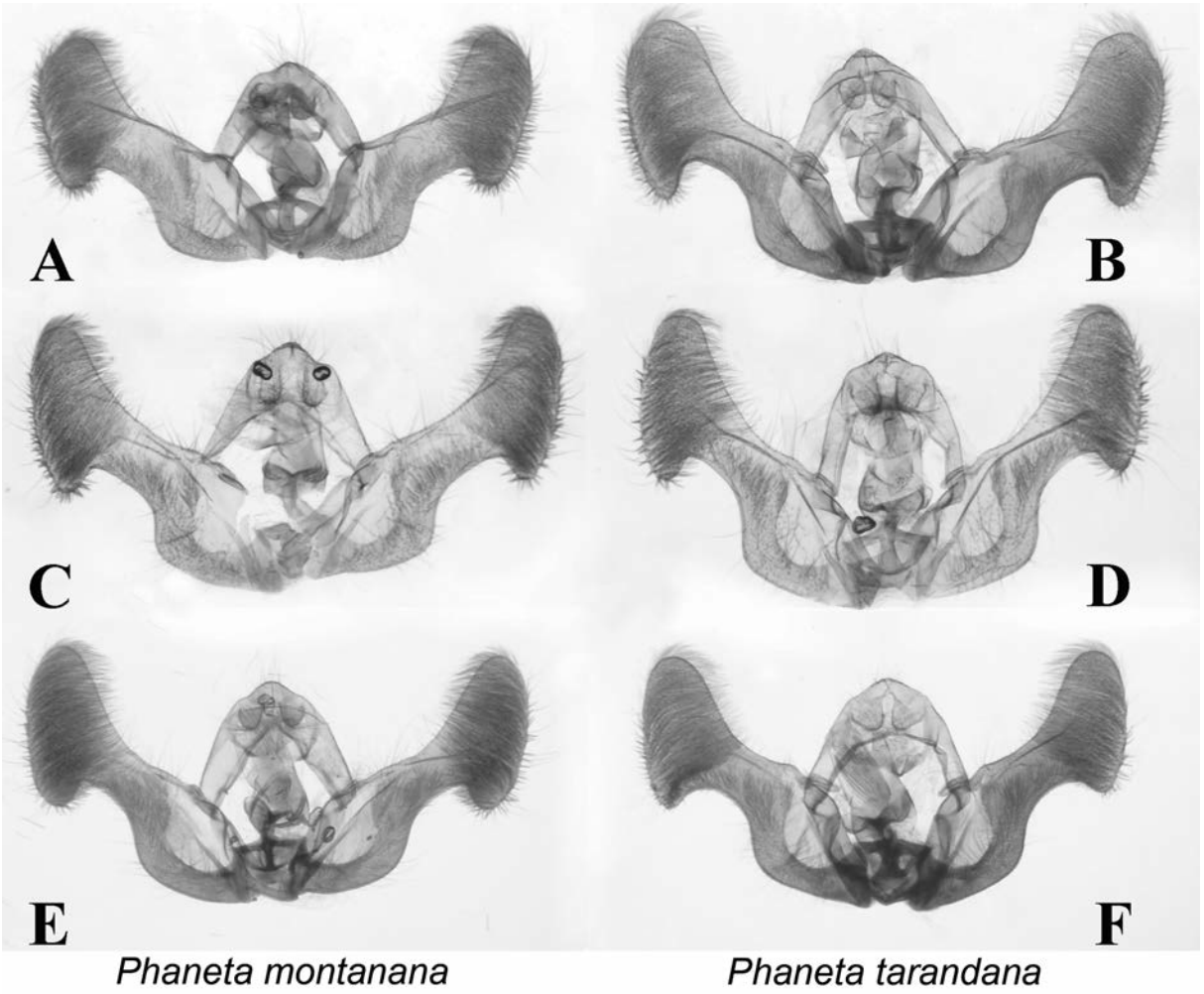

FIG. 5. Male genitalia of representative specimens of *P. tarandana* (right) and *P. montanana* (left).

2003) with default settings and two sets of 120,000 generations. Outgroups for COI were downloaded from GenBank as follows: *P. striatana* (Clemens 1860), GU088462.1; *P. elongana* (Walsingham 1879), HM863962.1; *P. corculana* (Zeller 1874); HQ683323.2, and *P. autumnana* (McDunnough 1942), HQ964424.1.

RESULTS

Morphological variation. For the following, descriptors for individual scales include "root", referring to the tapered base of each scale that attaches to the wing membrane, and "apical teeth", referring to the widened jagged edge of the distal portion of scales. For wing character variation, we identified two character states for fascia color: (1) dark brown to grayish brown (Fig. 3 b,c,f), and (2) yellowish tan to light brown (Fig. 3 a,d,e,g); three character states for ground color (interfascial coloration): (1) yellowish to light tan (Fig. 3 a,d,e,g,h), (2) white (Fig. 3 b,f), and (3) brown (Fig. 3 c); and three for wing streaks: (1) wing streaks strongly present (Fig. 3 a,c,e,g), (2) absent (Fig. 3 b,d) and (3) faintly present (Fig. 3 f,h). Wing fringe scale coloration exhibited three character states: (1) wing fringe scales on termen with a distinct dark band (median band) below the apical teeth fading into the root (Fig. 4 c, also present in Fig. 3 b,c,d,f,h); (2) Cilia concolorous throughout, amelanistic, therefore lacking gradient entirely; and (3) dark median band present on wing fringe scales, but without a distinct origin of the gradient (Fig. 4 b, also seen in Fig. 3 a,e,g). The *P. tarandana* specimen in Fig. 3 "d" does not exhibit a strong dark brown row of scales along the termen, however, the scales do exhibit a distinct gradient at the base of the apical teeth of each scale. They appear superficially like

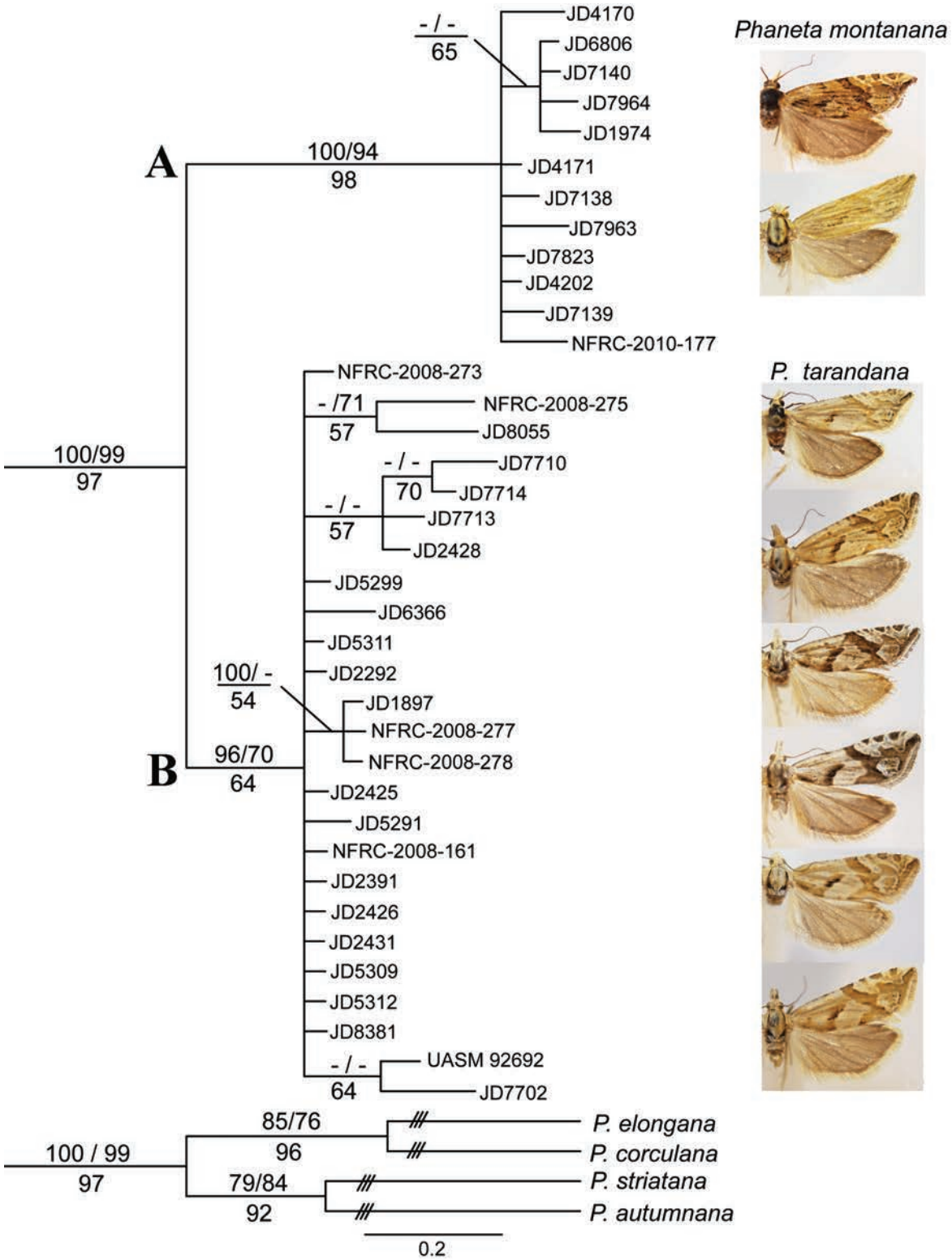

FIG 6. 50% majority rule consensus of 2402 trees generated via Bayesian analysis (120,000 generations) of 631 bp COI for 41 *Phaneta* samples. Numerical values represent parsimony bootstrap support (1000 replicates), and likelihood bootstrap support (100 replicates) above and Bayesian posterior probabilities below. "-" = <50% support. TreeLength=160, CI=0.77, RI= 0.88, HI=0.23.

P. montanana until examined under a microscope since the color of the apical teeth and the stem of the scale are not heavily contrasted as in other *P. tarandana* (Fig. 3 b,f)

Of the 130 specimens examined, 37 fit the description of *P. montanana*, 51 of *P. tarandana*, and 34 appeared to be intermediates, exhibiting diagnostic characters mentioned in both descriptions. An additional eight specimens that were intermediate between *P. montanana* and *P. tarandana* were exceptionally darker brown (dark ground and fascia color) and exhibited some wing streaks.

Variation in genitalic characters was so continuous that characters could not be sorted reliably into distinct character states. Consequently we did not relate genitalic variation to wing pattern variation. In Fig. 5, we show specimens from both *P. tarandana* and *P. montanana* to demonstrate the similarity among genitalia of these species.

DNA sequences. Parsimony and Bayesian trees for 631 nucleotide sites in COI showed two distinct lineages (Fig. 6). COI clade A contained moths that generally resembled the description of *P. montanana*, while those in COI clade B were closer to the description of *P. tarandana*. Additionally, of the 631 sites, 31 sites were parsimony informative. The optimum likelihood tree was partially congruent with this arrangement; COI clade A was monophyletic but placed within a paraphyletic COI clade B. Bootstrapping of the likelihood analysis (100 replicates) supported the monophyly of two distinct clades. Average uncorrected COI sequence divergence between the two major COI lineages (Fig. 6) was 3.12% (min= 2.37% max= 4.18%). Average divergence was 0.56% among the 25 moths in clade B, and 0.36% among the 12 moths in clade A.

Examination of the 521 nucleotide positions in ITS2 sequences revealed only one phylogenetically informative character, a 1 bp indel at ITS2 position 203. This nucleotide position was a cytosine in all 12 specimens characterized as having COI clade A and was coded as a gap in all 25 specimens with COI clade B. Chi-squared analysis showed a statistically significant correlation between COI and ITS2 (X-squared = 23.8138, df = 1, p-value = <0.001). Because the distinction between mitochondrial COI clades A and B is congruent with variation in nuclear ITS2 sequences, we hereafter refer to DNA clades A and B without restricting the names to particular loci.

Integration of morphology and DNA variation. All but one (NFRC-2010-000177, Ft. Assiniboine, AB) of the moths that were resolved in clade A could be identified as *P. montanana* based on wing pattern; all were yellowish-tan and possessed wing streaks, NFRC-2010-000177 was exceptionally darker brown. Moths resolved in clade B ranged in appearance from typical *P. tarandana*, a light form of *P. tarandana*, as well as moths that are intermediate between the two species descriptions. In particular, pale wing coloration, considered to be diagnostic of *P. montanana*, was also represented in clade B in seven specimens. Several specimens of *P. tarandana* exhibited weak wing streaking (See Table 1.0). Values in table 1.0 are based on moths that we had both ITS2 and COI data for (10 *P. montanana*, 18 *P. tarandana*); Moths that had sequence data for only one locus were not included.

The four wing characters that were scored in all specimens showed varying degrees of conformance to the DNA clades. Fascial color character states showed no significant relationship to the two DNA clades (χ^2= 1.61, df = 1, p-value = 0.20). Ground color did correlate significantly with DNA clades (χ^2= 11.10, df = 2, p-value = <0.001) (See Table 1.0)

Wing streaks occurred in members of both DNA clades (Fig. 6). Although present in all members of clade A, wing streaks were also present to some degree in 6 of the 25 members of clade B. Chi-squared analysis showed a significant association between moths with wing streaks and those with clade A DNA (χ^2= 22.16, df = 2, p-value = <0.001).

There was also a significant association between wing fringe scale detail and DNA clades (χ^2= 18.56, df = 3, p-value = <0.001). However, members of DNA clade A predominantly expressed this character as state 3, which lacks a distinct gradient in individual terminal scales. Members of clade B expressed this character as either a distinct band (state 1) or lighter forms that lacked the median band completely (state 2).

In summary, although uncommon, some specimens of *P. tarandana* have lighter wing forms, and in these cases their wing features are less distinct and the fringe is light with no visible dark gradient on individual scales of the termen. *Phaneta montanana* differs from *P. tarandana* in that it lacks the distinct gradient on individual scales on the wing fringe except in one exceptionally darker moth. *Phaneta montanana* wings are also always streaked, a character that is also present in some specimens of *P. tarandana*.

Since the preponderance of morphological evidence supported the existence of two evolutionarily distinct lineages, as initially defined by clades A and B in the molecular analyses, from this point forward we refer to these lineages as *P. montanana* (clade A) and *P. tarandana* (clade B).

Diagnostic characters. The relationship between morphological characters and COI+ITS2 lineages was used to evaluate which character states were most

TABLE 1. Utility of characters in delimiting *P. tarandana* and *P. montanana*. Morphological characters were only assessed in specimens where COI and ITS2 data was available (10 *P. montanana* and 18 *P. tarandana*)

Character	Description of character states		Utility
	P. montanana	*P. tarandana*	
Wing ground color	**9/10** yellowish to light tan **1/10** brown	**11/18** white **5/18** yellowish to light tan **2/18** brown.	Sometimes
Wing fascia color	**6/10** yellowish to tan **4/10** dark brown to grayish	**5/18** yellowish to tan **13/18** dark brown to grayish	No
Forewing fringe scale detail	**8/10** lack distinct band before apical teeth on fringe scales; rather the dark band blends into the apical teeth. **1/10** distinct dark band present just below apical teeth in one exceptionally dark specimen **1/10** too worn to assess	**10/18** possess distinct dark band on the forewing fringe scales, not extending into the apical teeth. **7/18** lack dark pigments on fringe scales, but distinct band on scale still present with less contrast between apical teeth and band **1/18** too worn to assess	Yes
Forewing Streaking	**10/10** wings streaked	**1/18** with streaked wings **6/18** wing slightly streaked, but noticeably less than *P. montanana* **9/18** wing streaks absent **2/18** could not assess, wings greased.	Sometimes
Male Genitalia	*P. montanana* and *P. tarandana* indistinguishable. Angle of sacculus variable, simple rounded "C" shaped cucullus, no modified spines present, phallus simple, short fingerlike socii. Cornuti present in virgin males.		No
Habitat and location	*P. tarandana* and *P. montanana* found across Alberta in sagebrush habitats.		No
Flight time	May 19– Sept. 2	May 31–Aug. 20	No

useful to identify specimens in the absence of molecular information. Apical wing scale coloration, wing streaks, and fascia color were all significantly correlated with the two DNA lineages in the subset of 37 specimens for which DNA was sequenced. When only morphological characters were considered in all available specimens, there were 84 putative *P. tarandana*, and 38 putative *P. montanana*.

Excluding the light forms that do not possess dark scales along the termen, all *P. tarandana* exhibited terminal scales that had a definite origin of the dark gradient at the base of the apical teeth (Fig. 2). All but one *P. montanana* (NFRC-2010-000177) possessed dark scales along the termen that lacked a definite origin of the dark gradient as seen in *P. tarandana*. Evaluation of the angle of the tornus and apex showed no significant difference between the two species.

All specimens were male, excluding two individuals of *P. montanana*. Genitalia showed extensive overlap in variation between *P. tarandana* and *P. montanana*, including in valve shape and length, cucullus shape, and the angle of the sacculus (Fig. 5). Therefore no consistent genitalic characters distinguished putative *P. montanana* from putative *P. tarandana*. In addition, genitalia of either species were indistinguishable from those of *P. transversa* (sensu Heinrich 1923).

For the following two sections, species were determined as above, using fascia color, apical wing scale detail, streaking on the forewings and, when available, COI+ITS2 DNA sequences.

Flight time and habitat association. Flight times overlap extensively between *P. tarandana* and *P. montanana* (Table 1). Both species have two flight peaks each year, one in late spring and one later in summer.

Gilligan et al. (2008) also suggests that *P. montanana* may have 2 broods per year.

Phaneta montanana tends to occur in lowland dune habitat; however, the species has been sampled from higher elevation sagebrush habitats together with *P. tarandana*. There are fewer *P. montanana* specimens from the Rocky Mountain suture zone (Remington 1968) but the two species cannot be reliably delimited based on locality (Fig. 1). In two cases, both species were sampled from the same location on the same night. This occurred in Tranquille Ecological Reserve, British Columbia in September and again in Kootenay Plains Ecological Reserve, Alberta, in August. The seven light form specimens of *P. tarandana* completely lacked dark scales along the termen (unlike any *P. montanana*). This character combination was also seen in four *P. tarandana* from Cypress Hills, one of which appeared to be completely amelanistic, as well as one specimen from Pakowki dunes and two from Waterton Lakes National Park (Crandell campground and the Wishbone trailhead).

Some of our specimens fit Wasingham's (1895) description and illustration of *P. transversa* in that they express markings much more distinctly than typical *P. tarandana*. However, these specimens lack the complete dark subbasal patch illustrated by Walsingham (1895) for *P. transversa*. Neither forewing fringe coloration nor DNA support these specimens as representing a lineage distinct from *P. tarandana*.

Discussion

The *P. tarandana* complex remains a challenging group of moths due to poorly defined species boundaries. Both species treated here presumably feed on sagebrush (Brown 2005) and overlap in distributions. Genitalia, flight time and geographic location have low utility in separating these species. Nonetheless, COI and ITS2 sequences demonstrate that *P. tarandana* and *P. montanana* are distinct but closely related. Most specimens can be diagnosed using wing fringe coloration, as corroborated using molecular markers. Forewing fringe scales on most *P. tarandana* have a distinct dark band below the apical teeth that suffuses into the root of the scale, but light forms of this species have a wing fringe band that is less contrasted with the apical teeth. *Phaneta montanana* generally do not possess a distinct band on the fringe scales.

Walsingham (1895) did not have enough material to draw conclusions about the range of *P. transversa* but he implied that *P. transversa* was a southern form of *P. tarandana*. This could be resolved by examining specimens from nearer to the type locality of *P. transversa* in Colorado.

Our data supports the current taxonomy of *P. tarandana* and *P. montanana* and justifies the continued treatment of both as separate species. The geographical and temporal overlap of *P. tarandana* and *P. montanana* may still allow some gene flow between them, explaining the presence of intermediate moths. However, the fact that most specimens are clearly one species or the other suggests that members of both lineages are able to maintain their genomic integrity, satisfying both the genomic integrity and general lineage species concepts (Sperling 2003; De Quieroz 1998).

Acknowledgements

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HYBRIDIZATION STUDIES OF GENOMIC COMPATIBILITY AND PHENOTYPIC EXPRESSION IN THE GREATER FRITILLARY BUTTERFLIES (NYMPHALIDAE: ARGYNNINI)

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ABSTRACT. The genetic compatibility of the greater fritillary butterflies was tested through hybrid crosses made between the North American genus *Speyeria* and the Eurasian genera *Argynnis* and *Mesoacidalia*. Numerous hybrid crosses made between *A. paphia* and *Speyeria* failed to produce any viable progeny, but a hybrid male was successfully produced between *M. aglaja* and *S. nokomis*. The genetic compatibility and divergence of *Speyeria* species was tested through hybrid crosses and back-crosses among the various species. All species of *Speyeria* appear to be inter-fertile in hybrid crosses with the exceptions of the two most divergent species, *S. idalia* and *S. diana*, that produce non-viable hybrid females and sterile hybrid males. As a consequence, it is postulated that inter-species gene flow through hybridization accidents in nature has been important in the past evolutionary history of this genus, and is partly responsible for the absence of consistent, non-overlapping diagnostic taxonomic characters among most species of the genus.

Additional key words: *Argynnis, Fabriciana, Mesoacidalia, Speyeria,* Mendelian traits

The greater fritillary butterflies belong to the tribe Argynnini, and consist of approximately 41 species that are widely distributed in temperate regions of Eurasia and North America, extending south to Mexico, Australia, India, and North Africa. Larvae feed almost exclusively on violets in the genus *Viola* (Violales: Violaceae). The adaptive radiation of these butterflies is entirely based upon this single larval foodplant. In the present paper, we report on the results of extensive inter-species hybridization studies with these butterflies, both within the genus *Speyeria* of North America and between *Speyeria* and the Eurasian genera *Argynnis* and *Mesoacidalia*.

Taxonomy

The taxonomy of these butterflies is controversial regarding the delineation of both genera and species. Simonsen et al. (2006) have prepared the most recent cladistic analysis of this group based on a combination of both morphological and DNA sequence data that shows four major clades within the group. These clades may be classified into four genera or subgenera (Tuzov 2003) including *Argynnis*, *Fabriciana*, *Mesoacidalia*, and *Speyeria* based on a combination of wing pattern and genitalic characters. The first three genera are endemic to Eurasia and North Africa, while the latter genus is endemic to North America. These genera are defined as follows.

The genus *Fabriciana* is characterized by having black crescent-shaped or indented submarginal spots on the dorsal wings. On the ventral hindwing, the median spots are broad and square in shape, forming a somewhat continuous median band, and distinct dark postmedian spots are present. The valve of the male genitalia has a slender, finger-like process that is free above the valve. The uncus has a prominent dorsal tooth. This genus is comprised of 11 species as outlined by Tuzov (2003), with *F. kamala* (Moore) transitional in wing pattern towards *Argynnis*. *Fabriciana niobe* (Linnaeus) exhibits the most primitive characters in wing pattern and genitalic structure among the greater fritillaries that are shared with the lesser fritillaries of the genera *Issoria, Brenthis,* and *Boloria* (see cladogram in Simonsen et al. 2006). In general, species of *Fabriciana* are relatively conservative in these characters with the greatest divergence seen in such species as *F. argyrospilata* (Kotzsch) and *F. nerippe* (Felder & Felder).

The genus *Argynnis* is characterized by having round black submarginal spots on the dorsal wings. On the ventral hindwing, the median band is reduced to a thin median stripe, and dark postmedian spots are present. The male genitalia are similar to those of *Fabriciana*, but are highly variable among different species in ornamentation of the process and valve. The uncus is

also highly variable, and may have multiple dorsal teeth or no teeth. This genus is comprised of 10 species as outlined by Tuzov (2003), except that we recognize *A. castetsi* Oberthur on the Indian subcontinent as a species fully distinct from the more common and widespread *A. hyperbius* (Linnaeus). The species of *Argynnis* are all extremely divergent in wing color pattern and genitalic structure. On the basis of this divergence, the genus has been taxonomically divided into a number of small or monotypic genera in the past, including *Pandoriana, Damora, Nephargynnis, Argyronome, Childrena,* and *Argyreus* (Tuzov 2003). Despite this great divergence among the various species, they appear to be closely related in a phylogenetic sense as shown by the cladogram of Simonsen et al. (2006).

The genus *Mesoacidalia* has a wing pattern identical to that of *Fabriciana* on the dorsal wings. However on the ventral hindwing, the median band is broken into discrete, separate median spots, and dark postmedian spots are mostly absent except for faint shadows. In the male genitalia, the dorsal process of the valve is pressed down on top of the valve, instead of being free above the valve as in *Fabriciana* and *Argynnis.* In addition, the uncus lacks the dorsal tooth of *Fabriciana. Mesoacidalia* is comprised of 4 species in Eurasia including *M. vitatha* (Moore), *M. clara* (Blanchard), *M. aglaja* (Linnaeus), and *M. alexandra* (Menetries). Of these species, *M. vitatha* has thin or narrow black median bars on the forewings, and elongate or pointed median spots and sharp, triangular submarginal spots on the ventral hindwing. By contrast, *M. aglaja* has variable narrow to wide black median bars, short or rounded median spots, and rounded or flat submarginal spots. *Mesoacidalia clara* has a more extreme wing pattern similar to that of *M. vitatha*, while *M. alexandra* has a more extreme wing pattern similar to *M. aglaja.*

The genus *Speyeria* has a wing pattern virtually identical to that of *Mesoacidalia.* However, the process of the male valve is relocated along the side of the valve, and is broadly expanded into a club-like structure. This contrasts with the narrow, dorsal process of *Mesoacidalia.* Of these, *S. mormonia* (Boisduval) of the Rocky Mountains has a wing color pattern virtually identical to that of *M. vitatha*, and only differs in smaller size. However, *S. mormonia* has the *Speyeria*-type of male genitalia, while *M. vitatha* retains the more primitive *Mesoacidalia*-type of genitalia. As shown in the cladogram of Simonsen et al. (2006), *Mesoacidalia* and *Speyeria* are closely related sister genera. On this combined basis of genitalia and wing color pattern, the genus *Speyeria* in North America appears to be a monophyletic group that was probably derived from the Eurasian *M. vitatha*.

In sharp contrast to the species of *Argynnis*, most species of *Speyeria* are extremely conservative in both wing pattern and genitalic structure, and there are often no consistent diagnostic characters for separating the various species. Only two species exhibit highly divergent wing color patterns that allow for easy taxonomic identification, *S. idalia* and *S. diana.* All other species have overlapping characters of phenotype at least to some extent, and most exhibit a tremendous amount of geographic variation in wing color pattern (Hammond 1991). This is why Grey (1961) was not able to provide a diagnostic key for the identification of most species in the genus.

We presently recognize 16 reproductively isolated species in the genus *Speyeria* that maintain strongly distinct identities in sympatry, and exhibit strong ecological segregation in habitat and violet resource partitioning (Hammond 1981). These may be divided into two species groups based upon differences in the male and female genitalia as outlined by Grey (1961). The *callippe* group including *S. mormonia* has a slender claw-like uncus in the male and a single bursal chamber in the female. Other species of this group include *S. atlantis* (Edwards), *S. hollandi* (Chermock & Chermock), *S. sorocko* Scott, Kondla, & Spomer, *S. callippe* (Boisduval), *S. edwardsii* (Reakirt), *S. egleis* (Behr), *S. zerene* (Boisduval), *S. coronis* (Behr), *S. hydaspe* (Boisduval), and *S. adiaste* (Edwards). By contrast, the *cybele* group has a thick hook-like uncus in the male and a double bursal chamber in the female, although *S. nokomis* does retain the more primitive single bursal chamber. Species of this group include *S. aphrodite* (Fabricius), *S. cybele* (Fabricius), *S. nokomis* (Edwards), *S. idalia* (Drury), and *S. diana* (Cramer).

We should note that a major taxonomic disagreement presently exists regarding the delineation of species boundaries among taxa closely related to *S. atlantis.* Scott, Kondla, & Spomer (1998) separated the western subspecies of *S. atlantis* as a distinct species, *S. hesperis* (Edwards), an approach recently followed by Dunford (2009). However, Grey (1951) pointed out that a broad zone of clinal intergradation exists across Canada between the eastern *S. atlantis atlantis* and western members of the *hesperis* group, beginning in the Great Lakes region and extending westward across central Manitoba and northern Saskatchewan to the Rocky Mountains of Alberta, and then south to Colorado and New Mexico. Within these regions, both *S. hollandi* and *S. sorocko* maintain separate identities as distinct, reproductively isolated species. Based upon the reproductive relationships, we suggest that the western *hesperis* group must be treated as geographic subspecies of *S. atlantis* as originally classified by dos

Passos & Grey (1947), despite the superficial similarities of *S. hollandi* and *S. sorocko* to the eastern *S. a. atlantis*.

Hybridization Studies

Over the past ten years, we have conducted extensive hybridization experiments with most of the species within the genus *Speyeria*. An earlier unpublished study attempted hybrid crosses between *Speyeria* and some of the European species, and we report on the results of this work in the present paper. Compatibility between the genomes of different taxa may be measured at six stages of divergence as discussed by Robinson (1971).

Stage 1 shows no genetic incompatibility with completely normal male and female hybrids.

Stage 2 shows some minor Haldane effects where the heterogametic sex (females) shows some minor abnormalities due to incompatible sex chromosomes and autosomes.

Stage 3 shows major Haldane effects where the hybrid females show major abnormalities or are completely non-viable, resulting in unequal sex ratios.

Stage 4 shows normal hybrid male development, but the males are completely sterile due to a failure of disparate chromosomes to pair up during the process of meiosis.

Stage 5 shows major abnormalities in male hybrids.

Stage 6 shows no hybrid viability at all. Either fertilized eggs die in early embryonic development or no fertilization takes place.

Reproductive isolation among the various species of *Speyeria* appears to result from species-specific sex pheromones in both males and females that are used during courtship and mating (personal observations). Interspecies courtship is frequently observed in the field, but this almost never results in hybrid matings. In the laboratory, we have by-passed this isolating mechanism by using the hand-pairing technique of Clarke (1952) for swallowtail butterflies. Male and female genitalia are mechanically joined together, forcing the butterflies to mate whether they are inclined to do so or not. In this way, every species of *Speyeria* can be hybridized with every other species no matter how divergent or distantly related they might be.

Hybridization of *Speyeria* with European Genera. An earlier study was designed to conduct hybridization experiments between *Speyeria* and the three European genera, and these results are discussed as follows. For this work, three European species were selected to represent each of these genera including *Fabriciana adippe* (Denis & Schiffermuller), *Argynnis paphia* (Linnaeus), and *Mesoacidalia aglaja* (Linnaeus). Unfortunately, the breeding stock of *F. adippe* was not particularly viable and no hybrid crosses could be made. However, an extensive effort was made to hybridize *A. paphia* with various *Speyeria* species. A total of six hybrid crosses were successfully completed with *A. paphia*, but none of these crosses resulted in any viable progeny. This suggests a Stage 6 level of genomic divergence between *Argynnis* and *Speyeria* with virtually no genetic compatibility between these genera. This also corroborates the wide divergence between these genera shown in the cladistic study of Simonsen et al. (2006).

By contrast, two hybrid crosses were made between *Speyeria* and *M. aglaja*, and one of these made with *S. nokomis nitocris* (Edwards) from Arizona successfully produced a few viable male hybrids. These results indicate that some genetic compatibility still remains between the genomes of *Speyeria* and *Mesoacidalia*, and corroborates the cladistic study of Simonsen et al. (2006) that shows these genera as closely related sister genera. Unfortunately, it was not possible to backcross this hybrid to test for other measures of genetic compatibility such as fertility. The hybrid is shown in Figure 1 (1–2), and is intermediate in size and color pattern phenotype between the two parental species. On the ventral hindwing, it shows the green disc of the *M. aglaja* parent, and the wide yellow submarginal band and small silver median and submarginal spots of both parents. Thus in this hybrid, the green disc color of the *M. aglaja* parent was strongly dominant in phenotypic expression over the red-brown disc color of the *S. nokomis* parent.

Hybridization of *Speyeria mormonia*. As noted above, *S. mormonia* is the one North American species that is most similar to the Eurasian genus *Mesoacidalia*, and may be considered the most primitive for the genus *Speyeria*. We tested the genomic divergence of *S. mormonia* by crossing it with *S. aphrodite*. Specifically, we mated a male of *S. m. erinna* (Edwards) from the Oregon Cascades with a female of *S. a. alcestis* (Edwards) from Michigan. This hybridization combines a small species with a large species that are divergent in both male and female genitalic structure, although the adult color patterns are relatively similar.

Figure 1 (3-4) shows the phenotype of the hybrid male obtained from this cross. It was relatively small in size, only slightly larger than a normal *S. mormonia*, and it retained the narrow yellow submarginal band typical of *S. m. erinna*. Female hybrids showed a major Haldane effect and died as eggs in early embryonic development. However, male hybrids proved to be fertile in back-crosses, indicating a Stage 3 level of genomic divergence between *S. mormonia* and *S. aphrodite*.

The male hybrid was back-crossed to *S. a. alcestis*, and the genetic recombinants are illustrated in Figure 1

FIG. 1. Hybrid crosses of *Mesoacidalia* and *Speyeria* species. Scale-bar equals 20 mm. (**1**) *M. aglaja* × *S. nokomis* male dorsal, (**2**) *M. aglaja* × *S. nokomis* male ventral, (**3**) *S. mormonia erinna* × *S. aphrodite alcestis* male dorsal, (**4**) *S. m. erinna* × *S. a. alcestis* male ventral, (**5**) back-cross (*S. m. erinna* × *S. a. alcestis*) × *S. a. alcestis* male dorsal, (**6**) back-cross (*S. m. erinna* × *S. a. alcestis*) × *S. a. alcestis* male ventral, (**7**) back-cross (*S. m. erinna* × *S. a. alcestis*) × *S. a. alcestis* male ventral, (**8**) back-cross (*S. m. erinna* × *S. a. alcestis*) × *S. a. alcestis* female dorsal, (**9**) *S. aphrodite* × *S. c. cybele* male dorsal, (**10**) *S. aphrodite* × *S. c. cybele* male ventral, (**11**) *S. aphrodite* × *S. c. cybele* female dorsal, (**12**) back-cross (*S. zerene picta* × *S. c. cybele*) × *S. c. cybele* male ventral, (**13**) *S. z. picta* × *S. c. cybele* male dorsal, (**14**) *S. z. picta* × *S. c. cybele* male ventral, (**15**) *S. z. picta* × *S. c. cybele* female dorsal, (**16**) back-cross (*S. z. picta* × *S. cybele*) × *S. c. cybele* female ventral, (**17**) *S. edwardsii* × *S. aphrodite ethne* male dorsal, (**18**) *S. edwardsii* × *S. a. ethne* male ventral, (**19**) *S. edwardsii* × *S. a. ethne* female dorsal, (20) back-cross (*S. z. picta* × *S. c. cybele*) × *S. c. cybele* female ventral.

FIG. 2. Hybrid crosses of *Speyeria* species. Scale-bar equals 20mm. (**1**) *S. zerene picta* × *S. callippe semivirida* male dorsal, (**2**) *S. z. picta* × *S. c. semivirida* male ventral, (**3**) *S. a. atlantis* × *S. nokomis nitocris* male dorsal, (**4**) *S. a. atlantis* × *S. n. nitocris* male ventral, (**5**) *S. c. cybele* × *S. n. nitocris* male dorsal, (**6**) *S. c. cybele* × *S. n. nitocris* male ventral, (**7**) *S. c. cybele* × *S. n. nitocris* female dorsal, (**8**) *S. c. cybele* × *S. n. nitocris* female ventral, (**9**) *S. c. cybele* × *S. n. apacheana* male dorsal, (**10**) *S. c. cybele* × *S. n. apacheana* male ventral, (**11**) *S. c. cybele* × *S. n. apacheana* female dorsal, (**12**) *S. c. cybele* × *S. n. apacheana* female ventral, (**13**) *S. n. nitocris* × *S. idalia* male dorsal, (**14**) *S. n. nitocris* × *S. idalia* male ventral, (**15**) *S. edwardsii* × *S. idalia* male dorsal, (**16**) *S. edwardsii* × *S. idalia* male ventral, (**17**) *S. a. atlantis* × *S. idalia* male dorsal, (**18**) *S. a. atlantis* × *S. idalia* male ventral, (**19**) *S. z. picta* × *S. idalia* male dorsal, (**20**) *S. z. picta* × *S. idalia* male ventral.

(5–8). Normal female hybrids were obtained in this back-cross (8), and butterflies segregated in phenotype between large individuals that exhibited red suffusion over the submarginal band on the ventral hindwing as in the parental *S. a. alcestis* (5–6), and small individuals that retained a narrow yellow submarginal band as in the parental *S. m. erinna* (7). Thus, the small size and yellow submarginal band of *S. mormonia* was somewhat dominant in phenotypic expression over the large size and red suffused submarginal band of *S. aphrodite*.

Hybridization within the Central Radiation of *Speyeria*

Most species within the *callippe* species group have no constant diagnostic characters in either wing color pattern or genitalic structure as noted by Grey (1961), while *S. cybele* and *S. aphrodite* are slightly more divergent with genitalic differences in both males and females as discussed above. We conducted many hybrid crosses between various species, both within the *callippe* group and with both *S. cybele* and *S. aphrodite*. No adverse Haldane effects were observed in hybrid females, and all hybrids appeared to be fertile. Thus, the species of the *callippe* group plus *S. cybele* and *S. aphrodite* exhibited a Stage 1 level of genomic divergence among the various species with no indications of any genetic incompatibility.

These hybrid crosses provided an indication of the Mendelian phenotypic expression of the diagnostic characters that distinguish the various *Speyeria* taxa. A cross between a male of *S. zerene picta* (McDunnough) and a female of *S. callippe semivirida* (McDunnough) from the Washington Cascades was particularly informative as shown in Figure 2 (1–2). On the forewings, *S. callippe* usually has narrow black median bars, while *S. zerene* usually has wide median bars. On the ventral hindwing, *S. callippe* has a greenish brown disc, sharp pointed or elongate median spots, and sharp triangular submarginal spots. By contrast, *S. zerene* has a reddish brown disc, and rounded median and submarginal spots. In the hybrid, the wide median bars of *S. zerene* exhibited a strong dominant expression over the narrow median bars of *S. callippe*. However, the sharp, pointed median and submarginal spots of *S. callippe* showed a strong dominant expression over the rounded spots of *S. zerene*. Incomplete expression was exhibited by the disc colors, with some individuals having a reddish brown disc and others having green and red colors mixed together.

Figure 1 illustrates the phenotypic expression of these characters among various hybrid crosses. The primary cross between a male *S. zerene picta* from the Oregon Cascades with a female *S. c. cybele* from Michigan showed the dominance of the wide black median bars of *S. zerene* over the narrow median bars of *S. cybele* (Fig. 1, 13–15). On the ventral hindwing, the large median spots and narrow submarginal band of *S. zerene* were dominant over the small median spots and wide submarginal band of *S. cybele*. However, the pointed submarginal spots of *S. cybele* showed a dominant phenotypic expression over the rounded submarginal spots of *S. zerene*. When the primary hybrid was back-crossed to *S. cybele*, these characters showed recombination that indicated an independent segregation and inheritance of such traits. Thus, Figure 1 (12) shows a back-cross male with the small median spots and wide submarginal band of *S. cybele*, (16) shows a back-cross female with the large median spots and narrow submarginal band of *S. zerene*, and (20) shows a back-cross female with the large median spots of *S. zerene* combined with the wide submarginal band of *S. cybele*.

Figure 1 (9–11) shows a hybrid cross between *S. aphrodite* and *S. cybele* both from Michigan. The former species has little dark basal suffusion and thin veins in the male dorsal forewing, while the latter species has heavy dark basal suffusion and veins strongly thickened with androconial scales. On the ventral hindwing, *S. aphrodite* has large median spots combined with a narrow submarginal band, while *S. cybele* has small median spots and a wide submarginal band. The dorsal characters of the hybrid were intermediate between those of the parental species, indicating an incomplete dominance or polygenic inheritance of these traits. On the ventral hindwing of the hybrid, the larger median spots and narrow submarginal band of *S. aphrodite* were mostly dominant in expression over those of *S. cybele*. When the primary hybrid was back-crossed with *S. c. cybele*, these characters recombined independently in different individuals. This correlates with the dominance of similar *S. zerene* characters over those of *S. cybele* in the hybrid crosses previously discussed.

Figure 1 (17–19) shows a hybrid cross between *S. edwardsii* and *S. aphrodite ethne* (Hemming) both from Nebraska. The former species has a yellow-orange ground color on the dorsal wings, and a greenish brown disc and very large spots on the ventral hindwing. By contrast, the latter species has a reddish orange dorsal color, a red-brown disc, and smaller spots. In the hybrid, the reddish orange dorsal color of *S. aphrodite* and the very large spots of *S. edwardsii* showed a dominant expression. However, the expression of disc color was variable and intermediate, with some hybrid individuals having a pure dark brown disc and others showing a greenish brown disc (18). Similar results with mixed green and brown disc colors were seen in hybrid crosses

FIG. 3. Hybrid crosses of *Speyeria* species. Scale-bar equals 20 mm. (**1**) *S. c. cybele* × *S. idalia* male dorsal, (**2**) *S. c. cybele* × *S. idalia* male ventral, (**3**) *S. c. cybele* × *S. idalia* female dorsal, (**4**) superhybrid ((*S. cybele pugetensis* × *S. c. charlottii*) × *S. nokomis nitocris*) × *S. idalia* male ventral, (**5**) *S. idalia* × *S. c. cybele* male dorsal, (**6**) *S. idalia* × *S. c. cybele* male ventral, (**7**) *S. diana* × *S. idalia* male dorsal, (**8**) *S. diana* × *S. idalia* male ventral, (**9**) *S. diana* × *S. n. nitocris* male dorsal, (**10**) *S. diana* × *S. n. nitocris* male ventral, (**11**) *S. diana* × *S. n. apacheana* male dorsal, (**12**) *S. diana* × *S. n. apacheana* male ventral, (**13**) *S. c. cybele* × *S. diana* male dorsal, (**14**) *S. c. cybele* × *S. diana* male ventral, (**15**) *S. aphrodite* × *S. diana* male dorsal, (**16**) *S. aphrodite* × *S. diana* male ventral, (**17**) *S. a. atlantis* × *S. diana* male dorsal, (**18**) *S. a. atlantis* × *S. diana* male ventral, (**19**) *S. z. picta* × *S. diana* male dorsal, (**20**) *S. z. picta* × *S. diana* male ventral.

between *S. callippe* and *S. zerene*, and between *S. coronis* and *S. zerene*. This suggests an incomplete dominance in the expression of green and brown disc colors in *Speyeria*. Pure green and brown colors appear to represent homozygous expressions, while greenish brown color is probably a heterozygous expression. By contrast, the green disc color of *Mesoacidalia aglaja* was strongly dominant over the red-brown color of *S. nokomis nitocris* as previously discussed.

We should also mention the hybrid expression of sexual dimorphism within *S. cybele*. The eastern *S. c. cybele* and the southern Colorado *S. c. carpenterii* (Edwards) both lack sexual dimorphism in having orange males and females in dorsal colors. However, subspecies of the western *leto* complex including both *S. c. pugetensis* Chermock & Frechin from the Pacific Northwest and *S. c. charlottii* (Barnes) from northwestern Colorado show a very strong sexual dimorphism in which the females have a pale yellow dorsal ground color. A hybrid cross between *S. c. pugetensis* from Oregon and *S. c. cybele* from Michigan produced intermediate yellow-orange females. A backcross of this hybrid to *S. c. cybele* produced a wide range of variation from pure orange to yellow-orange female phenotypes. These laboratory results are closely similar to the natural phenotypic variation in female coloration seen in populations of *S. cybele* from western Colorado and eastern Utah that are intermediate between *S. c. carpenterii* and *S. c. charlottii* in a broad clinal blend zone.

Hybridization of *Speyeria nokomis*

Speyeria nokomis is slightly more divergent in wing pattern and large size compared to the various species in the central radiation of *Speyeria* discussed above. Figure 2 (3–12) shows the results of hybrid crosses made between *S. nokomis*, *S. cybele*, and *S. a. atlantis*. Crosses between *S. nokomis* and both *S. cybele* and *S. aphrodite* showed a Stage 2 level of genomic divergence. Minor Haldane effects were evident in that female hybrids were somewhat smaller than normal. Male hybrids were fertile. However, crosses between *S. nokomis* and both *S. atlantis* and *S. adiaste* showed much stronger Haldane effects where few or no female hybrids survived. Thus, a Stage 3 level of genomic divergence appears to exist between *S. nokomis* and members of the *callippe* species group.

A hybrid cross between *S. n. nitocris* (Edwards) from Arizona and *S. aphrodite* from Michigan produced similar results to those seen in the *aphrodite* × *cybele* cross shown in Figure 1 (9–11), except the dorsal dark basal suffusion of the hybrid male was greatly reduced and the forewing veins were thin without androconial scales as in *S. aphrodite*. On the ventral hindwing, the larger median spots and narrow submarginal band of *S. aphrodite* were dominant over the small spots and wide submarginal band of *S. nokomis* just as in the *aphrodite* × *cybele* cross. A hybrid cross between *S. n. nitocris* and *S. a. atlantis* from Minnesota produced almost identical results as shown in Figure 2 (3–4), except the veins of the male dorsal forewing were thickened with androconial scales as in the *S. atlantis* parent.

In addition, *S. nokomis* exhibits a strong sexual dimorphism similar to that in the *leto* subspecies group of *S. cybele* in which the female dorsal ground color is yellow instead of orange. As with the hybrid cross between *S. c. cybele* and *S. c. pugetensis*, female hybrids of *S. nokomis* crossed with either *S. aphrodite* or *S. c. cybele* showed an intermediate yellow-orange ground color as shown in Figure 2 (7, 11).

Two subspecies of *S. nokomis* differ in the color of the disc on the ventral hindwing. In Arizona, males of *S. n. nitocris* have a dark reddish brown disc, while females have a reddish black disc. In Nevada and California, males of *S. n. apacheana* (Skinner) have a pure yellow disc and females have a green disc. The hybrid between these subspecies was intermediate with a light reddish brown disc in the male and a reddish green disc in the female. A hybrid cross between *S. n. nitocris* and *S. c. cybele* produced a dark reddish brown disc in both the male and female as shown in Figure 2 (6, 8), while a hybrid cross between *S. n. apacheana* and *S. c. cybele* produced a light reddish brown disc in the male and a dark greenish brown disc in the female as shown in Figure 2 (10, 12). The dorsal forewing veins of the hybrid males were thickened with androconial scales as in *S. cybele* as shown in Figure 2 (5, 9).

A hybrid cross between *S. adiaste* from the Coast Range of California and *S. n. nitocris* from Arizona combined a small butterfly with a pure yellow disc and unsilvered spots on the ventral hindwing and a large butterfly with a dark reddish brown disc and silver spots. The hybrid was intermediate in size and disc color between the two parental species, again showing the polygenic inheritance of size and disc color. As with the *aphrodite* and *atlantis* hybrid crosses described above, the large median spots and narrow submarginal band of *S. adiaste* were completely dominant over the small spots and wide band of *S. nokomis*. In addition, the unsilvered spots of the *S. adiaste* parent were completely dominant over the silver spots of the *S. nokomis* parent. As a result, the hybrid more closely resembled *S. adiaste* than *S. nokomis*. In populations of *Speyeria* that are polymorphic for both silver and unsilvered spots, it is not unusual to see intermediate phenotypes with partially silvered or unsilvered spots. These may represent heterozygotes with modifier genes

that allow partial expression or incomplete dominance of these traits, but this was not seen in the *adiaste* × *nokomis* hybrid.

Hybridization of *Speyeria idalia*

Multiple hybrid crosses were made between *S. idalia* from Nebraska and many other species of *Speyeria.* This species and *S. diana* are both highly divergent in wing color pattern from all other species in the genus, and show completely unique diagnostic characters not found in any other species. For *S. idalia*, these characters include (1.) a black dorsal hindwing with white median spots and white submarginal spots in the female, (2.) a black or reddish black disc on the ventral hindwing with solid black suffusion over a narrow submarginal band, and (3.) very large pointed or elongate median spots and pointed submarginal spots on the ventral hindwing.

In the various hybrid crosses with *S. idalia*, major Haldane effects occurred where hybrid females were severely stunted. They usually died in the pupal stage or were unable to expand wings upon eclosion, and were rarely able to develop into a deformed adult (Fig. 3-3). In addition, male hybrids appeared to be completely sterile, and we were never able to produce a hybrid back-cross with any other species. Thus, *S. idalia* exhibits a Stage 4 level of genomic divergence from other species.

Figure 2 shows the results of hybrid crosses made with *S. nokomis nitocris* from Arizona (13-14), *S. edwardsii* from Nebraska (15-16), *S. a. atlantis* from Minnesota (17-18), and *S. zerene picta* from Oregon (19-20). Likewise, Figure 3 (1-3) shows a hybrid cross with a male *S. c. cybele* from South Dakota. Figure 3 (5–6) shows the reciprocal cross using a male *S. idalia* and a female *S. c. cybele* to check for any sex-linked or sex-limited traits. None were found. A hybrid cross with *S. coronis* from Oregon produced similar results to those shown with *S. zerene.* Figure 3 (4) shows a super-hybrid cross where the primary *S. cybele pugetensis* × *S. c. charlottii* hybrid was crossed with *S. nokomis nitocris*, and then this secondary hybrid was crossed with *S. idalia* to produce a tertiary hybrid.

In all of these various hybrids, the black dorsal hindwing and white spots of *S. idalia* were completely recessive in phenotypic expression to the normal dorsal orange colors of other *Speyeria* species. On the ventral hindwing, the sharp, pointed median and submarginal spots were strongly dominant over the rounded spots of such species as *S. zerene*, *S. coronis*, *S. cybele*, and *S. nokomis.* Likewise, the narrow submarginal band covered with black to reddish suffusion of *S. idalia* was completely dominant over the wide yellow submarginal band of *S. cybele* and *S. nokomis*, although a few of the *cybele* hybrids showed a slight trace of the yellow band. However, the size of the median spots in hybrids was intermediate between the large spots of *S. idalia* and the small spots of *S. cybele* and *S. nokomis.* Also, the hybrid ground color of the disc on the ventral hindwing was usually a dark reddish brown as seen in the *zerene*, *atlantis*, and *cybele* crosses, but ranged between bright red-brown in the *nokomis* cross and pure black in the *edwardsii* cross.

Hybrids did show some unique phenotypic expressions depending on the parental species crossed with *S. idalia.* The cross with *S. nokomis* shown in Figure 2 (13–14) produced gigantic males with exceptional hybrid vigor, but they were often deformed and had difficulty with eclosion. This suggests a Stage 4 to Stage 5 level of genomic divergence between these two species. In the *zerene* × *idalia* cross (19–20), the wide black median bars of the forewing in *S. zerene* were strongly dominant over the narrow bars of *S. idalia*, duplicating similar results in other hybrid crosses made with *S. zerene.* In the *edwardsii* × *idalia* cross (15–16), the pale yellow-orange dorsal ground color of *S. edwardsii* was strangely dominant over the dark orange color of *S. idalia* on the forewing, which was the reverse of the *edwardsii* × *aphrodite* cross shown in Figure 1 (17–19). However, the greenish brown color of the disc on the ventral hindwing of *S. edwardsii* was strongly suppressed by the black color of *S. idalia* in the hybrid.

Hybridization of *Speyeria diana*

Multiple hybrid crosses were also made between *S. diana* from Virginia and many other species. Unique diagnostic characters of *S. diana* include (1.) very large wing size, (2.) intense solid black basal suffusion that extends to the median bars of the dorsal wings, (3.) extreme suppression of the spots on the ventral hindwing, and (4.) an extreme sexual dimorphism with orange-black males and blue-black females.

Major Haldane effects were evident in these hybrid crosses where females died as eggs in early embryonic development and never produced viable larvae. Hybrid males appeared to be completely sterile and no hybrid back-crosses were possible. Thus, *S. diana* exhibits a Stage 4 level of genomic divergence from most other species.

Figure 3 shows the results of hybrid crosses made with *S. idalia* from Nebraska (7-8), *S. nokomis nitocris* from Arizona (9-10), *S. n. apacheana* from Nevada (11–12), *S. cybele* from Michigan (13-14), *S. aphrodite* from Michigan (15-16), *S. atlantis atlantis* from New York (17-18), and *S. zerene picta* from Washington (19–20). A cross made with *S. coronis* from Oregon produced similar results to those shown with *S. zerene.*

The expression of dark black basal suffusion on the dorsal wings of hybrids was intermediate between that of *S. diana* and the various parental species. Thus, hybrids made with *S. n. nitocris*, *S. cybele*, and *S. atlantis* still showed very intense, dark suffusion. However, *S. n. apacheana* and *S. aphrodite* both have greatly reduced suffusion in the male, and their hybrids made with *S. diana* showed greatly reduced suffusion as well. Therefore, the intense solid black basal suffusion of *S. diana* appears to be the result of polygenic expression that has become fixated at one extreme end of a continuum.

On the ventral hindwing of hybrids, the suppression of the silver basal and submarginal spots was less extreme and intermediate compared with the parental *S. diana*. However, the suppression of the silver median spots in hybrids was nearly as great as in *S. diana* for virtually all crosses, even the cross made with *S. idalia*. This unique character of *S. diana* shows a strong dominant phenotypic expression over the normal median spots of other *Speyeria* species. In wing size, hybrids were usually intermediate between *S. diana* and the respective parental species, indicating a polygenic expression for size. We could not assess the inheritance of blue and orange colors in the hybrid females because they were completely non-viable.

The hybrid cross between *S. idalia* and *S. diana* exhibited major abnormalities in the males. Larvae showed a major defect in feeding behavior, and chewed randomly on leaf surfaces instead of feeding efficiently by chewing at the leaf margins. As a result, larvae grew very slowly and were quite stunted at pupation. Most individuals died as pupae or could not expand wings upon eclosion. Although several hundred larvae were reared, only a single individual was able to expand its wings as shown in Figure 3 (7–8). These two species display a Stage 5 level of genomic compatibility and divergence as a consequence, which is concordant with their extreme morphological divergence.

Phenotypic Expression of Larval Color Patterns in Hybrid *Speyeria*

Most species of *Speyeria* have a larval color pattern that is mottled gray, black, brown, or yellow with two parallel mid-dorsal gray, yellow, or cream stripes. Such species include *S. mormonia*, *S. hollandi, S. sorocko*, *S. callippe*, *S. edwardsii*, *S. egleis*, *S. zerene*, *S. coronis*, *S. nokomis*, and *S. idalia*. Other species have pure black larvae that lack the pale body mottling and the pale mid-dorsal stripes. These include *S. hydaspe*, *S. cybele*, and *S. diana*. Still other species are polymorphic in larval color pattern with either pale striped morphs or pure black morphs including *S. atlantis*, *S. adiaste*, and *S. aphrodite*. These polymorphisms represent geographic variations to a considerable extent in these species.

When we hybridized a pure black larval species such as *S. cybele* with a yellow-striped larval species such as *S. idalia*, an intermediate dusky striped and mottled phenotype was produced in the hybrid. Thus, hybrids display an incomplete dominance of larval color pattern expression in the heterozygous condition. Similar results were produced when *S. cybele* or black larval forms of *S. aphrodite* were crossed with various pale striped larval forms such as *S. nokomis*, *S. zerene*, *S. coronis*, and *S. edwardsii*. These intermediate dusky striped morphs are common in the wild within polymorphic populations of such species as *S. atlantis* and *S. aphrodite*.

DISCUSSION

In general, there appears to be a close correlation between divergence in wing color pattern and genomic compatibility in the greater fritillary butterflies. Thus, the wing patterns of *Speyeria* and *Argynnis* are highly divergent, and there appears to be virtually no genetic compatibility between these genera. By contrast, *Speyeria* and *Mesoacidalia* both share the same basic wing color pattern, and still retain some genetic compatibility as evidenced by the production of viable hybrids. These results corroborate the results from the cladistic study of the fritillary butterflies by Simonsen et al. (2006). Likewise within *Speyeria*, all of the species within the central radiation of the genus are extremely conservative in sharing the same basic wing color pattern, and exhibit virtually no genetic incompatibility with fully normal, fertile hybrid males and females. These include the various species of the *callippe* group plus *S. aphrodite* and *S. cybele*.

In sharp contrast, both *S. idalia* and *S. diana* are extremely divergent in wing color pattern, both from each other and all of the other species as well. Our hybridization studies show that these two species of *Speyeria* have experienced a large amount of genetic divergence, and produce non-viable hybrid females and infertile hybrid males when crossed with all other species of the genus. Indeed, the divergence between *S. idalia* and *S. diana* is so great that even the hybrid males are only weakly viable. Two other species show minor genetic incompatibility with other species resulting in adverse Haldane effects in hybrid females, but hybrid males are fertile. These are *S. mormonia* and *S. nokomis*.

Although the pheromone mating system is probably at least 99% effective in promoting reproductive isolation among *Speyeria* species, hybridization accidents still occur at a low frequency in nature. For example, Scott (1981) observed a natural hybrid between *S. nokomis* and *S. cybele* in California. The

hybrid cross between *S. cybele* and *S. idalia* shown in Figure 3 (1–3) is not an artificial laboratory cross, but occurred naturally along the White River in Mellette Co. South Dakota. In this area, *S. cybele* occupies riparian forest habitats along the river, while *S. idalia* occupies the adjacent upland prairie. We collected the parental *S. idalia* female for breeding stock, but all of her progeny proved to be hybrids, showing that a male *S. cybele* had accidentally mated with the female in the wild.

Hybrid crosses that result in sterility as with *S. idalia* and *S. diana* are clearly an evolutionary dead end. However, we have found that most other interspecies hybrid crosses in *Speyeria* are fertile. Even if accidental hybridization in nature is a very rare event, it still provides a conduit for inter-species gene flow. As previously discussed, there are no constant diagnostic characters that consistently distinguish the various species of the *callippe* group due to overlapping character traits. In particular, *S. atlantis, S. hydaspe, S. callippe, S. egleis, S. zerene*, and *S. coronis* broadly overlap in characters across many geographic regions, which may result from low rates of hybridization taking place constantly through time. An earlier study of allozymes (Brittnacher et al. 1978) and several unpublished studies of the COI-COII sequence of mitochondrial DNA show similar overlapping genetic polymorphisms both within and between species of *Speyeria* at the molecular level. Proshek & Houghton (2012) have recently made similar observations with the COI sequence in the genus *Phyciodes* (Nymphalidae). Low rates of hybridization in nature would not be expected to affect the fundamental reproductive isolating mechanisms among the various species or to impact their fundamental identities as distinct biological species.

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HESPERIA ILLINOIS DODGE (HESPERIIDAE): AN INVALID NEOTYPE AND THE DESIGNATION OF A LECTOTYPE

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ABSTRACT. The nominal taxon *Hesperia illinois* Dodge, 1872 (now recognized as *Euphyes bimacula illinois*) was described from over 40 specimens collected in Bureau County, Illinois, by the brothers Edgar and George Dodge. Since 1999, *H. illinois* has been represented by a neotype from Grundy County, Illinois, but this designation is nomenclaturally invalid for technical reasons. In addition, at least three syntypes of *H. illinois* exist. A lectotype is designated, thereby returning the type locality to Bureau County, Illinois.

Additional key words: Edgar A. Dodge, George M. Dodge, *Hesperia acanootus* Scudder, *Hesperia bimacula* Grote & Robinson.

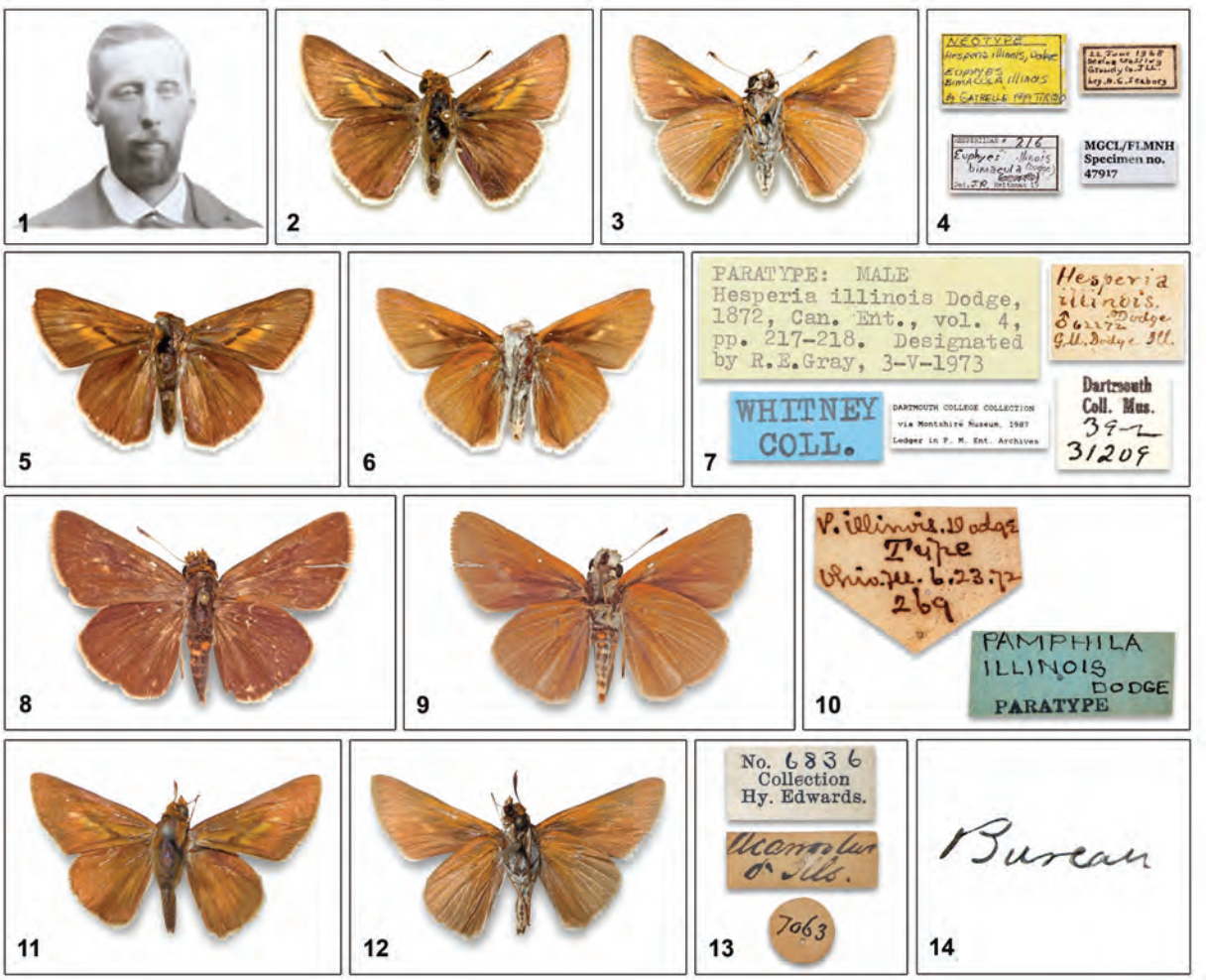
On 20 June 1872, Edgar A. Dodge (1853–1933) discovered some unfamiliar skipper butterflies in Bureau County, Illinois. He and his older brother, George M. Dodge (1846–1912), collected over 40 specimens that season, only nine of which were females. George (Fig. 1) soon after described the species as *Hesperia illinois*, either to honor the native Americans recognized by that name (as in the tradition involving many other skipper butterflies) or the state in which the Dodge family had resided since 1854 (Dodge 1872; Calhoun 2013). Dodge (1872) stated that the species was abundant "upon grassy slopes on the high rolling prairie that forms the divide between the Illinois and Rock Rivers." Such high rolling prairie occurs in northern Bureau County and is particularly noticeable within the townships of Bureau, Walnut, and Ohio (Matson 1872). The Dodges lived on 32 ha (80 ac) of land, which comprised the northeastern one-eighth of Section 19 of Ohio Township, 4.4 km (2.74 mi) southwest of the town of Ohio in north-central Bureau County (Calhoun 2013). This is located about 160 km (99.4 mi) southwest of present-day downtown Chicago. The specimens of illinois were most likely collected in the vicinity of their property. A typesetter for the Canadian Entomologist misread G. M. Dodge's handwriting (Fig. 14), resulting in an erroneous type locality of "Burcan" County, Illinois, in Dodge (1872). In exchange for other North American butterflies, Dodge (1872) offered syntypes of *H. illinois*, as well as specimens of *Oarisma poweshiek* (Parker), which he and his brother found abundantly at the same locality. Their specimens of *O. poweshiek* remain the only known examples of this species from Illinois (Bouseman et al. 2006). The nominal taxon *Hesperia illinois* is currently represented by a neotype (Gatrelle 1999), but this designation is nomenclaturally invalid for technical reasons and disregards the existence of syntypes.

METHODS

The original description of *Hesperia illinois* by Dodge (1872) and the subsequent neotype designation by Gatrelle (1999) were reviewed. The relevant provisions of the Code (ITZN 1985; ICZN 1999) were consulted to determine the validity of the neotype. With the kind assistance of staff members, I searched for specimens in the following institutions: The Academy of Natural Sciences (Philadelphia, Pennsylvania; ANSP), the American Museum of Natural History (New York, New York; AMNH), Boston University (Boston, Massachusetts; BU), the California Academy of Natural Sciences (San Francisco, California; CAS), the Carnegie Museum of Natural History (Pittsburgh, Pennsylvania; CMNH), the Field Museum of Natural History (Chicago, Illinois; FMNH), the Illinois Natural History Survey (Champaign, Illinois; INHS), the McGuire Center for Lepidoptera and Biodiversity (Florida Museum of Natural History, Gainesville, Florida; MGCL), the Museum of Comparative Zoology (Harvard University, Cambridge, Massachusetts; MCZ), the National Museum of Natural History (Smithsonian Institution, Washington, D. C.; USNM), The Natural History Museum (London, UK; BMNH), the New York State Museum (Albany, New York, NYSM), and the Peabody Museum of Natural History (Yale University, New Haven, Connecticut; PMNH). Also consulted were letters and manuscripts preserved in the library of FMNH, archives of MGCL, Archives and Special Collections, Olin Library, Rollins College (Winter Park, Florida; RC), and the Charles C. Wise, Jr. Library (West Virginia State Archives, West Virginia University, Morgantown; WVSA; copies at MGCL).

RESULTS

Soon after the description of *H. illinois* was published, the young New York naturalist Theodore L.



FIGS. 1–14 *Hesperia illinois*. **1,** George M. Dodge, 1892. **2,** invalid neotype of *H. illinois* (dorsal; MGCL). **3,** invalid neotype (ventral). **4,** labels of invalid neotype. **5,** male lectotype (dorsal; PMNH). **6,** lectotype (ventral). **7,** labels of lectotype. **8,** female paralectotype (dorsal; ANSP). **9,** female paralectotype (ventral). **10,** labels of female paralectotype. **11,** male paralectotype (dorsal; AMNH). **12,** male paralectotype (ventral). **13,** labels of male paralectotype. **14,** "Bureau" as written by G. M. Dodge, resulting in the typesetting error of "Burcan County" in Dodge (1872) (from letter to T. L. Mead, RC).

Mead (1852–1936) notified G. M. Dodge that this species had been described four years earlier as *Hesperia acanootus* Scudder, 1868, which in turn was a synonym of *Hesperia bimacula* Grote & Robinson, 1867 (copy letter, 29.xii.1872, RC). This species is currently recognized as *Euphyes bimacula*. Although the original description of *bimacula* by Grote & Robinson (1867) was detailed, it characterized only the female, did not include figures, and was published in a somewhat obscure journal. Several years after its description, entomologists still struggled to understand its true identity. Scudder (1872) was the first to publish the connection between *acanootus* and *bimacula*. Minot (1872) offered the first detailed description of the male of *bimacula*, which was published just before G. M. Dodge described *H. illinois*. Prior to describing illinois, Dodge had seen neither Scudder's (1872) updated synonymy nor his earlier description of *acanootus*.

After reviewing the description of *H. illinois*, Mead informed Dodge, "*Acanootus* is a synonym of *Bimacula* G&R of which there is a single ♀ specimen in the Robinson collection of the Amer[ican]. Mus[eum]. Accordingly I made a closer examination of that specimen, probably the original type, and I am afraid they are identical with *Illinois*" (copy letter, 29.xii.1872, RC). As Mead surmised, the specimen of *bimacula* in AMNH was likely the holotype and this represents the last known reference to its existence. The insect

collection of Coleman T. Robinson (1838–1872), who coauthored the description of *H. bimacula*, was donated in early 1870 to the newly founded AMNH. Among Robinson's specimens were the name-bearing types of taxa that he had co-described with Augustus R. Grote (Grote 1873). At that time, the museum was temporarily housed on the second and third floors of the Central Park arsenal building, then known as the "Central Park Museum, Observatory, and Menagerie" (Robinson 1870; Sweeny et al. 1871; Grote 1876). Robinson's donations of 10,000 Lepidoptera, 4,000 shells, and 100 volumes of books were among the earliest to the fledgling museum, where Robinson served without pay as Curator of Entomology from 1870 until his death (Osborn 1911). Construction on the current AMNH building was begun in 1874 and it opened to the public three years later. Mead transferred the Robinson collection into "insect-proof" boxes during late 1872 (Stuart 1872), thus explaining his familiarity with the holotype of *bimacula.* Mead was 20 years old and living with his parents at 596 Madison Avenue, just north of E. 61st Street in New York City (the building address changed on 1 May 1873 to 674 Madison Ave.). His home was located less than three blocks southeast of the arsenal building, which still stands in the southeast corner of Central Park, at the intersection of Fifth Avenue and 64th Street.

The holotype of *bimacula* was evidently collected in the vicinity of Philadelphia (Grote & Robinson 1867), possibly by Charles A. Blake (1834–1903), a local entomologist whose specimens were cited in several publications by Grote and Robinson. Miller and Brown (1981) and Pelham (2008) suggested that this specimen may be deposited in AMNH or NYSM. In his review of *E. bimacula*, Gatrelle (1999) did not attempt to find the holotype. I could not locate it at AMNH or NYSM, nor was it found in the collections of BMNH, NYSM, FMNH, or USNM. On at least one occasion, Mead borrowed type material from AMNH on behalf of Grote. The holotype of *bimacula* was perhaps loaned out and never returned.

I likewise was unable to find any recognizable types of H. *acanootus* at MCZ, where the insect collection of Samuel H. Scudder is deposited. According to Scudder (1868), the type (or types) of *acanootus* came from the fellow Massachusetts entomologist Francis G. Sanborn (1838–1884), who collected the species in the vicinity of Lexington, Massachusetts, during the month of August (unusually late for *E. bimacula*). Sanborn was then working as an assistant in the museum of the Boston Society of Natural History (BSNH) (Dickinson 1885), thus it is possible that his specimens of *acanootus* were deposited in the collection of BSNH, whose insects were given to Boston University during the first half of the 20th century (Boston, Massachusetts; BU) (Johnson 2004). Although specimens from Sanborn exist in the BU collection, no *E. bimacula* from Lexington, Massachusetts, were found.

Because Mead recognized that *acanootus* and *illinois* were synonymous with *bimacula*, he encouraged Dodge to immediately publish a retraction to "correct the error" before anyone else found out (copy letter, 29.xii.1872, RC). Mead reminded a disheartened Dodge that even S. H. Scudder, "the highest authority in America," had made the very same mistake in redescribing an existing species. In his follow-up note, Dodge (1873) confessed that he had not seen any specimens of *acanootus* and was misled by Minot's (1872) description of the male of *bimacula.* Although Dodge blamed himself for re-describing an established species "with injudicious haste," Mead felt responsible: "I should have been more careful in comparing the specimens." Mead later examined a male specimen of *bimacula* from New York and confirmed to Dodge that *illinois* was indeed synonymous (copy letter, 23.iii.1873, RC).

Dodge (1873) observed that the majority of his female specimens of *H. illinois* differed from *H. acanootus* as described by Scudder (1868). Such minor differences largely went unnoticed until Stanford (1981) tentatively recognized *illinois* as a western subspecies of *E. bimacula*, "pending examination of the type." Subsequent authors (e.g. Ferris 1989; Miller 1992; Orwig 1992; Scott 1992) followed this treatment. Citing Stanford (1981), Gatrelle (1999) also accepted *illinois* as a subspecies of *E. bimacula* that occurs from Illinois westward. Evidently in response to Stanford's (1981) call for an examination of type material, Gatrelle conducted a casual search for syntypes of *H. illinois.* "The best lead I had was that some of these specimens may have been deposited in the Field Museum in Chicago and from there to the Allyn Museum," he explained, adding, "I received no reply from my inquiry to the Allyn Museum about the possibility of any of Dodge's specimens being there" (Gatrelle 1999).

Gatrelle's inquiry to the former Allyn Museum of Entomology (Sarasota, Florida) was based on a hunch that G. M. Dodge had sent specimens of *illinois* to the lepidopterist F. H. Herman Strecker, whose collection was acquired by FMNH in 1908 (Gerhard 1909; González et al. 2010). In 1976, the Allyn Museum received on semi-permanent loan all of Strecker's butterflies and sphingid moths. In his letter to the Allyn Museum, dated 22 October 1999, Gatrelle asked Curator Lee D. Miller if he could borrow any of Dodge's specimens of *E. b. illinois* "in the Field

Museum's holdings" (archives, MGCL). Six years earlier, however, Miller had informed Gatrelle that the Strecker material was returned to FMNH in 1987. More important, Gatrelle overlooked a reference by Brown (1974) to a "paratype" of *H. illinois* at ANSP. Obviously in response to the failure of Miller and Brown (1981) to mention this "paratype," Bridges (1984, 1994) emphatically cited type material at "ANSP!"

Neotype. Instead of searching elsewhere for syntypes, Gatrelle (1999) designated a neotype of *H. illinois* using a male specimen of *E. bimacula* from "Denine Crossing," Grundy County, Illinois (Gatrelle 1999) (Figs. 2–4). He included the caveat, "If any syntypes are found, I withdraw this specimen as neotype only on condition that such syntype is designated as lectotype." Despite this proviso, Gatrelle had no control over the fate of the neotype in the event that any other name-bearing types were found. According to the third edition of the Code then in force, only the International Commission on Zoological Nomenclature could rule whether the neotype would be retained (ITZN 1985, Art. 75(h)). The current edition of the Code, which took effect one week after Gatrelle (1999) was published, stipulates that unless the Commission rules to retain the neotype following an application, the neotype is automatically set aside upon the publication of the discovery of any other name-bearing types (ICZN 1999, Art. 75.8). Accordingly, Pelham (2008) asserted that the neotype would be considered invalid if the specimen reported by Brown (1974) in ANSP is confirmed to be a syntype.

The neotype of *H. illinois* (Figs. 2–4) was collected on 22 June 1968 by the late Norman G. Seaborg (1935–2010) of Lockport, Illinois. Gatrelle (1999) interpreted Seaborg's handwritten label to read "Denine Crossing," but I was unable to find any town or other feature in Illinois by this name. Although the first word of the locality is somewhat illegible, I could not accept Gatrelle's interpretation based on Seaborg's writing style. A clue to unraveling this mystery was found in Heitzman (1969), who reported that *E. bimacula* was collected on 23 June 1968 at Goose Lake Prairie in Grundy County, Illinois. Just north of this prairie, where Collins Road intersects a railway line, was the former community of Divine, also known as Devine. Moreover, there are two specimens of *E. bimacula* in the collection of INHS that are labeled "Divine, Goose Lake Twp. Grundy Co. Ill." These specimens were collected on the same day as the neotype by the Illinois lepidopterist Roderick R. Irwin. Based on this evidence, Seaborg's label undoubtedly reads "Devine Crossing," in reference to the settlement found closest to the collection site. Seaborg and Irwin presumably visited the locality together. The neotype, currently deposited at MGCL, was captured about 102 km (63.4 mi) southeast of the Dodge's home in Bureau County, and 76 km (47.3 mi) southwest of present-day downtown Chicago.

From a technical standpoint, there are fundamental problems with the neotype designation for *H. illinois*. There existed no exceptional need for this action in the interest of nomenclatural stability or to resolve a complex zoological problem. The identity of *illinois* has not been questioned since Dodge (1873), thus the designation was unnecessary (ITZN 1985, Art. 75(c); ICZN 1999, Art. 75.2). In addition, Gatrelle did not conduct a reasonable search for existing syntypes or indicate why he believed them to be lost or destroyed, thereby failing to satisfy one of the qualifying conditions of such a designation (ITZN 1985, Art. 75(d)(3); ICZN 1999, Art. 75.3.4). Not only was the designation nomenclaturally invalid for these reasons, any argument to the contrary is now rendered moot by the rediscovery of three syntypes of *H. illinois*.

Syntypes. Two obvious syntypes of *H. illinois* were located during my research on the entomological contributions of the Dodge brothers (Calhoun 2013). A male (Figs. 5–7) and a female (Figs. 8–10) are deposited at PMNH and ANSP, respectively. A second male (Figs. 11–13), at AMNH, is provisionally recognized as a syntype. Additional museum collections (AMNH, BMNH, CMNH, FMNH, MGCL, and USNM) were searched for potential syntypes, but none were found. The remainder of the Dodge brothers' insect collection was deposited in CAS after the death of Edgar in 1933 (Calhoun 2013). Although CAS possesses four *E. bimacula* from Bureau County, Illinois, all are topotypes that were collected after *H. illinois* was described. Because *H. illinois* was described from a large type series (over 40 specimens), it seems likely that additional syntypes exist.

The male syntype of *H. illinois* in PMNH (YPM ENT 746877) (Figs. 5–7) was collected by G. M. Dodge in Illinois on 22 June 1872, just two days after his brother first found the species in that area. The specimen is from the collection of Charles P. Whitney (1838–1928), a druggist and naturalist from New Hampshire who was a correspondent of the Dodge brothers (Calhoun 2013). Details about the provenance of this specimen were found in unpublished documents at PMNH, including Furth (1987). After his death, a portion of Whitney's insect collection was evidently acquired by his long-time friend, Herbert S. Hutchinson (1849–1942), a physician who had a general interest in natural history and sometimes collected butterflies with Whitney (Whitney 1874; Rotch 1906). In 1939, Hutchinson donated

Whitney's specimens to Dartmouth College (Hanover, New Hampshire), whose natural history museum closed during the mid-1970s (Baas 1985). Dartmouth's insects were conveyed in 1976 to the newly founded Montshire Museum of Science (Norwich, Vermont), where they remained until 1987 when they were transferred to PMNH. Whitney's collection of horseflies (his primary interest) was donated to BSNH by his housekeeper in 1932 (some of the types were later transferred to MCZ) (Bequaert 1933).

Affixed to the male syntype at PMNH is a small rectangular label, written in Whitney's hand (Fig. 7). Data on the label were undoubtedly transcribed from Dodge's original label. A large typescript unit tray label indicates that Richard E. Gray, former Associate Curator of Biology at the Dartmouth College Museum, identified this specimen in 1973 as a "paratype" of *H. illinois*. Other labels denote the specimen's prior ownership by Dartmouth College and the Montshire Museum. An entry in a ledger from the Dartmouth College Museum (at PMNH) corresponds to a label on the syntype, identifying it as specimen no. 31209 from the Whitney collection.

The female syntype (Figs. 8–10) was collected by the Dodges in the vicinity of Ohio, Illinois, on 23 June 1872, one day after the male syntype at PMNH was captured. Handwriting and label comparisons reveal that the pentagonal label affixed to this specimen (Fig. 10) was created by the entomologist Herbert K. Morrison, who exchanged and sold insects for many years. This label is consistent with those that Morrison affixed to type specimens in his collection (see Wilterding 1997, fig. 4). Morrison corresponded with G. M. Dodge as early as 1873 (letter to T. L. Mead, 26.xii.1873, RC) and he described several species of moths based on Dodge's specimens, including *Mamestra dodgei* (=*Lacinipolia lorea* (Guenée, 1852)). Although they exchanged numerous specimens, these transactions usually benefited Morrison. Regarding his dealings with Morrison, Dodge wrote, "So far as my experience goes I have never been able to send him anything that he didn't 'growl' about" (letter to T. L. Mead, l9.iv.1874, RC). Morrison likely received the female syntype of *H. illinois* directly from Dodge and copied the collection data from the original label. The abbreviated genus, "*P.*" on Morrison's label refers to *Pamphila* [Fabricius], a once commonly-used junior objective synonym of the genus *Hesperia* Fabricius as employed by Dodge (1872). Other Morrison specimens are deposited in ANSP, some of which came to the museum in 1908 with the collection of the lepidopterist Henry Skinner, a former curator of ANSP. However, the female syntype of *H. illinois* was not listed by Skinner and Williams (1924) as among the specimens of *E. bimacula* at ANSP It possibly was overlooked or was acquired sometime after early 1924. The blue "paratype" label probably was prepared by Roswell C. Williams, Jr., an electrical engineer and lepidopterist who was active at ANSP for many years prior to his death in 1946 (Bell 1946). Williams collaborated on the study of Hesperiidae with several other entomologists, including Skinner. Similar labels, apparently written by Williams in the same upper case block letters, are affixed to other name-bearing types at ANSP, as well as some specimens at CMNH that were transferred from ANSP in 1963.

Although Brown (1974) indicated that the "paratype" in ANSP is a male, no male syntype was found in that collection. The female bears a "paratype" label, thus Brown (1974) possibly erred in reference to the gender of the specimen. In addition, there is no type numbered "1872" as identified by Brown (1974), nor are there any Lepidoptera type labels at ANSP that use such a numerical format. Brown likely derived this number from the collection date as given on the specimen's label. Many of the manuscripts of F. Martin Brown are preserved at MGCL, but this collection does not include his notes for Brown (1974). It is possible that Brown's more extensive (but largely unprocessed) archives at AMNH offer an explanation.

The female syntype bears very small postmedian forewing spots, which are consistent with Dodge's (1872) description of *H. illinois*. Concerning these spots, Dodge noted that the one closer to the apex is "so small as to be indistinct," while the other is "a little larger." They are entirely wanting in some specimens of *E. b. illinois*, a phenotype described by Leussler (1933) as aberration '*contradicta*.' Dodge (1872) also described a secondary form of female *H. illinios*, "Variety A," in which "the two spots in the centre of the primaries are much larger." Based on my examination of over 100 females of *E. b. bimacula* and *E. b. illinois* in MGCL, the reduced-spot form is somewhat more prevalent in the latter subspecies. Gatrelle (1999) also mentioned this difference in his diagnosis of *illinois*. This tendency explains Dodge's (1873) observation that the majority of his females of *illinois* differed from the description of *acanootus* (=*E. b. bimacula*) "in the spots on the primaries." When comparing females of *illinois* and *bimacula*, T. L. Mead did not initially make the connection between these taxa, as the holotype of *bimacula* "at first sight seemed something quite distinct" from female specimens of *illinois*, "in which the yellow spots are very faint" (copy letter, 29.xii.1872, RC). Mead ultimately suggested that the name *illinois* could be used to denote the reduced-spot form of *bimacula* (copy letter, 23.iii.1873, RC).

The second male syntype of *H. illinois* (AMNH; Figs. 11–13) bears a white rectangular label denoting that it is from the collection of the 19th century lepidopterist Henry Edwards (HE), whose 60,000 Lepidoptera specimens were acquired by AMNH in 1892 (Osborn 1911). This label was likely placed by William Beutenmüller, who served as Curator of Entomology at AMNH when the HE collection was accessioned. This specimen is identified as "*acanootus* / ♂ Ills. [Illinois]" in the distinctive hand of the lepidopterist William H. Edwards (WHE) (Fig. 13). A small circular label, reading "7063" in HE's hand, corresponds to an entry in his collection catalog (at AMNH) which identifies the specimen as "*bimacula*" from Illinois, received from W. H. Edwards.

For many years HE and WHE exchanged Lepidoptera, including Hesperiidae, but I found no specific references to this male specimen within HE's correspondence at AMNH (copies in MGCL archives). WHE also corresponded directly with the Dodge brothers (Calhoun 2013), who are the only known early source of this species from Illinois. The personal journals of WHE (WVSA; copies at MGCL) reveal that he first recorded the address of George Dodge in 1872; the same year in which Dodge described *H. illinois*. In the hesperiid section of his *Synopsis of North American Butterflies*, issued in September of 1872, WHE gave only "New England" as the range of *bimacula* (Edwards [1872]). Dodge's description of *illinois*, in which he offered syntypes for exchange, was published two months later. William H. Edwards' use of the name *acanootus* for the male specimen at AMNH implies that he was reconsidering the nomenclature of the species following Dodge (1873), which was published three months before adults of this skipper would emerge in Illinois in 1873. Edwards was good friends with T. L. Mead, who confirmed in early 1873 that *bimacula*, *acanootus*, and *illinois* were synonymous. It seems likely that Mead would have shared this conclusion with Edwards. This evidence suggests that this specimen was collected by the Dodges in 1872 and sent to WHE no later than early 1873. I therefore provisionally recognize it as a syntype of *H. illinois*.

Discussion

I designate the male syntype at PMNH as the **lectotype** of *Hesperia illinois* Dodge, 1872 in accordance with ICZN (1999) Art. 74.7, thereby fixing the status of this specimen as the sole name-bearing type of this nominal taxon. Although headless and lacking most of its legs, the specimen (Figs. 5, 6) otherwise is in good condition and readily identifiable. It represents the primary gender of the original description and was previously recognized as a name-bearing type when it was deposited at Dartmouth College. In addition, it includes full collection data and ostensibly passed directly from Dodge to Whitney. The lectotype bears 1) a rectangular label in the hand of C. P. Whitney [Hesperia / illinois. / Dodge / ♂ 62272 / G. M. Dodge. Ill.], 2) a blue printed rectangular label [WHITNEY / COLL.], 3) a small printed rectangular label [DARTMOUTH COLLEGE COLLECTION / via Montshire Museum, 1987 / Ledger in P. M. Ent. Archives], and 4) a printed and handwritten label [Dartmouth / Coll. Mus. / 39-2 / 31209]. Also associated with the specimen is a large typescript unit tray label [PARATYPE: MALE / *Hesperia illinois* Dodge, / 1872, Can. Ent., vol. 4, / pp. 217–218. Designated / by R.E.Gray, 3-V-1973] (Fig. 7). A red printed lectotype label [LECTOTYPE / *Hesperia illinois* / Dodge 1872 / Designated by / John V. Calhoun] has been affixed to the specimen. Through this action the type locality is returned to Bureau County, Illinois.

The female specimen at ANSP (Figs. 8, 9) is considered to be paralectotype and labeled accordingly. It bears 1) a pentagonal label in H. K. Morrison's hand [P. illinois. Dodge / Type / Ohio.Ill. 6.23.72 / 269] and 2) a blue handwritten and stamped label, possibly in the hand of R. C. Williams [PAMPHILA / ILLINOIS / DODGE / PARATYPE] (Fig. 10).

The male specimen at AMNH (Figs. 11–13) is likewise recognized as a paralectotype and labeled as such. Although this specimen is in better condition than the male at PMNH, its lack of complete data precludes it as the most appropriate choice as the lectotype of *H. illinois* (ICZN 1999, Recommendation 74E). It bears 1) a rectangular label in the hand of W. H. Edwards [*acanootus* / ♂ Ills.], 2) a small circular label in the hand of H. Edwards [7063] and 3) a rectangular printed and handwritten label [No. 6836 / Collection / Hy. Edwards] (Fig. 13).

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SYSTEMATICS AND BIOLOGY OF *CALOPTILIA TRIADICAE* (LEPIDOPTERA: GRACILLARIIDAE), A NEW SPECIES OF LEAF-MINING MOTH OF THE INVASIVE CHINESE TALLOW TREE(*TRIADICA SEBIFERA* (L.) EUPHORBIACEAE)

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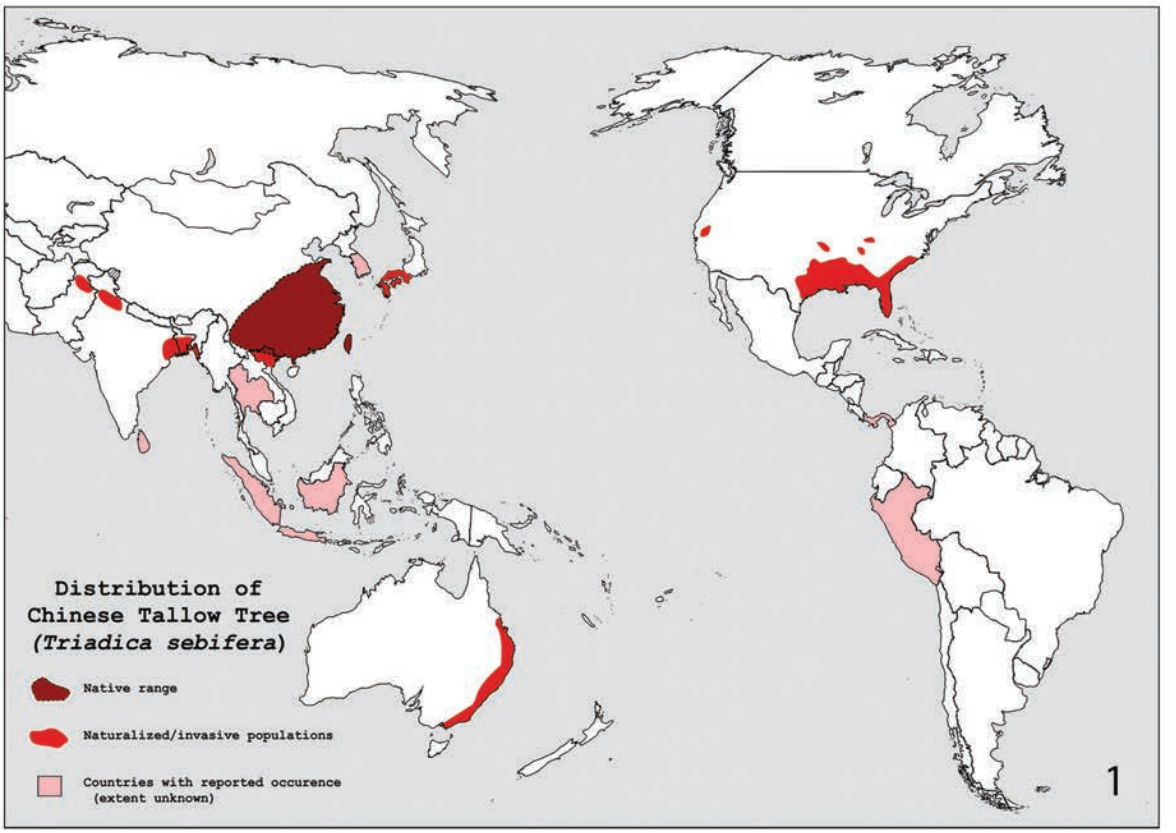
ABSTRACT. A new species of leaf-mining moth, *Caloptilia triadicae*, is described from the southern United States from Florida to eastern Texas. Larvae of this moth are known to feed preferentially and at high densities on the Chinese Tallow tree, *Triadica* (= *Sapium*) *sebifera* (L.) Roxb. (Euphorbiaceae), a tree first introduced into Georgia in 1772 from Asia, which has since become an invasive plant species of grave concern over much of the southeastern United States and California. *Caloptilia triadicae* is also known to feed rarely on *Gymnanthes lucida* Sw. (Euphorbiaceae), a tree not known to occur in the Old World but native to Florida, the Bahamas, the Caribbean, and Central America. Because of the origin of the preferred host and the morphological affinities of the moth to the Chinese species, *Caloptilia hamulifera* Liu and Yuan, it appears likely that *C. triadicae* also originated from Asia. The larvae of *Caloptilia* are hypometamorphic and possess two distinct larval body forms and feeding behaviors—an early stage sap-feeding form with a flattened body and prognathous mouthparts and a later stage tissue-feeding form with a more cylindrical body and possessing hypognathous mouthparts. The sap-feeding larvae initially construct long, serpentine, subepidermal mines on the upper (adaxial) leaf surface. After developing to the tissue-feeding form, the larva of *C. triadicae* leaves the mine and crawls to the edge of the leaf and cuts a narrow strip of leaf which is rolled into a tight coil. It continues feeding externally on the leaf inside the roll in which it eventually forms a silken cocoon for pupation.

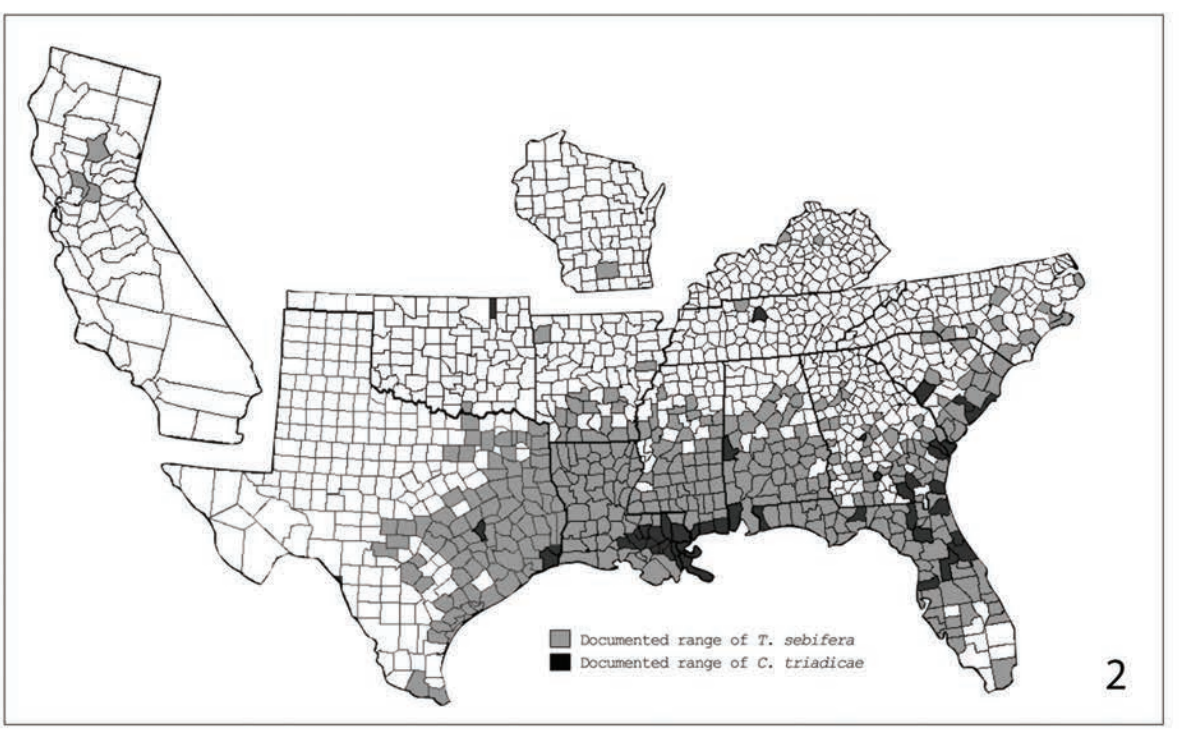
Additional key words: Biocontrol, biogeography, genital morphology, hypermetamorphic larva, invasive plant species, larval biology.

Chinese tallow tree (*Triadica sebifera* (L.) Roxb., Euphorbiaceae) is native to south-southeastern China, Taiwan, and northern Vietnam (Pattison & Mack 2008), is cultivated in Japan and may have naturalized there (Huang et al. 2010), and is either naturalized or invasive in northern India, northern Pakistan, Bangladesh, Java, Indonesia, and southeastern Australia (Figure 1). Tallow tree is reported from Thailand (Sangsawang et al. 2009), and from Panama and Peru (Pattison & Mack, 2008), though the status of the tree populations in these countries is unknown. The species is presently cultivated in South Korea, where there is interest in its capacity to support honey production (Ryu et al. 2008).

In the United States, *Triadica sebifera* is a noxious and highly invasive species that reportedly was introduced into Georgia in 1772, by Benjamin Franklin (Bell 1966). Recent studies on the genetic variation of *T. sebifera* (DeWalt, et al 2011; Boyd 2011) have revealed that the genetic strain that Franklin imported (and now confined to coastal areas of southern South Carolina and northern Georgia) is genetically distinct from the more invasive, widespread strain which was introduced by federal biologists around 1905 and has since spread primarily from Florida to East Texas. DeWalt et al, (2011) also concluded that the different phenotypic traits and relative invasiveness evident between the different strains of *T. sebifera* probably resulted from their different origins within the native range in Asia as well as geographic differences in selective pressures within their introduced ranges. The tree generally is chemically well-defended (although this varies between the different genetic strains), and few native herbivores consume Chinese tallow, though the species has been in the United States for more than 240 years (Siemann & Rogers, 2001). *T. sebifera* has been documented in 12 states in the southeastern United States, as well as three counties in California and one county in Wisconsin (Figure 2). Tallow tree grows well in riparian or swampy areas where soil is consistently damp or frequently inundated. It is also highly successful in disturbed habitats and open fields where sunlight is plentiful, and thrives in urban areas including Baton Rouge and New Orleans, LA, and Houston, TX.

The earliest known records of the proposed new species, *Caloptilia triadicae*, are from Baton Rouge, Louisiana and Sumter County, Alabama, both based on diagnostic leaf mine damage from July (photograph) and October (herbarium specimen) of 2004, respectively (Fox et al. 2012). One year later, June 7, 2005, a single male was collected by G. T. Austin in Gainesville, Florida. Larvae of *C. triadicae* were first discovered on September 16, 2008 and reared by Jason





FIGS. 1–2. Distributions of Chinese Tallow tree (*Triadica sebifera*) and *Caloptilia triadicae*. **1.** World distribution of *Triadica sebifera*. **2.** Distributions of *Caloptilia triadicae* and its host, *Triadica sebifera*, in the United States.

Sharp from *Triadica sebifera* at Branchton Park in Tampa, Hillsborough County, Florida and from Gainesville, Florida on September 20, 2008 by Susan Wright and James Lollis (Heppner 2008). These adults were sent eventually to DRD who identified them as a probably undescribed species of *Caloptilia*, not closely allied to any known North American *Caloptilia*.

Because of the morphological characteristics observed, as well as the origin of its host, DRD suspected that *C. triadicae* may have originated from Asia. A search for all *Caloptilia* known to feed on *Triadica* (= *Sapium*) revealed three Old World species: *Caloptilia octopunctata* Turner (= *C. cirrhocrotala* (Meyrick), *C. tetratype* (Meyrick)), reported from Australia and India; *C. sapina* Vári, from South Africa; and *C. sapiivora* Kumata, from Japan (De Prins & De Prins 2012). *Caloptilia sapina* resembles *C. octopunctata* in morphology and the two may be conspecific (Kumata 1981). The male genitalia of all three species are distinct from that of *C. triadicae*, and none possess the relatively large spine from the ventral angle of the cucullus which partally characterizes *triadicae*. Images of *C. triadicae* also were sent to colleagues T. Kumata (Japan) and D. Yuan (China), as well as to the late G. Robinson at the Natural History Museum, London, for comparison with other possibly similar Old World species. Nothing identical was reported.

The biology, morphology, and synonymy of *Caloptilia* (*Sphyrophora*) *octopunctata* (Turner) were treated in detail by Kumata (1981). Kumata followed Vári (1961) in recognizing *Sphyrophora* as a valid subgenus, characterized primarily by the strongly constricted valvae and presence of only a single, short pair of coremata on the male abdomen. Although these characters clearly distinguish *C. triadicae* (without constricted valvae and with two pairs of long coremata) from this subgenus, it is interesting to note that the late instar larval biology of *C. triadicae* resembles that of *octopunctata* with the larva cutting a strip from the leaf margin of its host and rolling this to form a cone on the abaxial (underside) of the leaf, within which it eventually pupates. *Caloptilia triadicae* closely agrees in wing venation with *C. octopunctata*, particularly with regard to their extremely narrow hindwings and separation of M_2 and M_3 in the forewing (Fig. 9). The forewing pattern of *C. octopunctata* also is similar to that of *C. triadicae* in possessing four evenly spaced, whitish strigulae along the wing margins, compared to three pairs in the latter. All subgenera previously recognized in *Caloptilia* were treated as synonyms of *Caloptilia* by W. and J. De Prins (2005). Adults of the four species of *Caloptilia* currently known to feed on *Triadica sebifera* can be distinguished using the following key.

1. Forewing with four white marginal spots, two on the costa and two on the hind margin; male valva constricted at apical third to form relatively large, lobate cucullus.....2
   Forewing either lacking spots or with only 3 white spots; male valva not constricted; cucullus connected smoothly to base of valva.....3
2. Male valva with sacculus bearing prominent lobe (ampulla) arising near base of cucullus; dorsal margin of cucullus rounded; distribution Australia, India..... *octopunctata*
   Ampulla indistinct; dorsal margin of cucullus angulate; distribution South Africa..... *sapina*
3. Forewing with 3 large white spots, two on costa and one on hind margin; fascia absent; male valva with short spine present from lower apical margin of cucullus (Fig.); distribution United States.....*triadicae*
   Forewing without large white spots, instead with 3 narrow, oblique, lemon-yellow fascia; spine absent from lower apical margin of cucullus; distribution Japan..... *sapiivora*

Materials and Methods

Specimens examined in this study are deposited in the following institutions:

FSCA Florida State Collections of Arthropods, Gainesville, FL, USA.

USNM Collections of the former United States National Museum, now deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

Specimen preparation. Genitalic dissections were cleared by heating in hot 10% KOH for ~30 minutes, and subsequently cleaned and stained with either 2% chlorazol black E or mercurochrome solutions. All genitalic illustrations were drawn from dissections temporarily stored in glycerine, which were later permanently embedded in Canada balsam. Genitalic terminology follows Klots (1970) and Kristensen (1984b).

The COI mitochondrial gene region was sequenced for *C. triadicae* recovered from Florida (n = 18) and Louisiana (n = 20) (Table 1). Specimens were field collected in 2008, curated in 95% EtOH, and were destructively sampled using whole larvae. DNA extraction was performed on each specimen using a Chelex extraction protocol. Each mtDNA PCR was carried out with Stratagene © Paq polymerase (0.1 µl), with primers LCOI-2198 and HCOI-1490 (Folmer et al. 1994) (1.5 µl) and additional MgCl2 (0.5 µl). Samples were thermocycled on MBS Satellite 0.2 G © units at an annealing temperature of 49° C for 35 cycles. PCR products were cleaned using Exo-SAP IT (Affymetrix) and then prepared in separate forward and reverse PCR reactions with Dyenamic dye-terminator chemistry (GE Health Care) for sequencing. Resulting

TABLE 1. Sample information for specimens submitted for COI barcoding.

Sequence ID	Locality	GenBank Accession number
F_1	USA: FL: Gainesville	KF061052
F_2	USA: FL: Gainesville	KF061061
F_3	USA: FL: Gainesville	KF061048
F_4	USA: FL: Gainesville	KF061047
F_5	USA: FL: Gainesville	KF061046
F_6	USA: FL: Gainesville	KF061060
F_7	USA: FL: Gainesville	KF061045
F_8	USA: FL: Gainesville	KF061059
F_9	USA: FL: Gainesville	KF061053
F_10	USA: FL: Gainesville	KF061054
F_11	USA: FL: Gainesville	KF061055
F_12	USA: FL: Gainesville	KF061062
F_13	USA: FL: Gainesville	KF061051
F_14	USA: FL: Gainesville	KF061056
F_15	USA: FL: Gainesville	KF061057
F_16	USA: FL: Gainesville	KF061050
F_17	USA: FL: Gainesville	KF061049
F_18	USA: FL: Gainesville	KF061058
h1	USA: LA: Honey Island Swamp	KF061067
h2	USA: LA: Honey Island Swamp	KF061073
h4	USA: LA: Honey Island Swamp	KF061074
h5	USA: LA: Honey Island Swamp	KF061066
h7	USA: LA: Honey Island Swamp	KF061078
h8	USA: LA: Honey Island Swamp	KF061079
h9	USA: LA: Honey Island Swamp	KF061076
h10	USA: LA: Honey Island Swamp	KF061063
h11	USA: LA: Honey Island Swamp	KF061075
h12	USA: LA: Honey Island Swamp	KF061072
h13	USA: LA: Honey Island Swamp	KF061065
h14	USA: LA: Honey Island Swamp	KF061064
h15	USA: LA: Honey Island Swamp	KF061080
h16	USA: LA: Honey Island Swamp	KF061071
h17	USA: LA: Honey Island Swamp	KF061070
h18	USA: LA: Honey Island Swamp	KF061068
h19	USA: LA: Honey Island Swamp	KF061069
h20	USA: LA: Honey Island Swamp	KF061077

products were cleaned with Sephadex columns. Sequences were then electrophoresed on an ABI 3730xl Genetic Analyzer ©.

RESULTS

Caloptilia triadicae Davis, **new species**
(Figs. 3 – 15)

Diagnosis. The forewing pattern of *Caloptilia triadicae*, consisting of two, white, slightly oblique, costal stigulae and a single white dorsal strigula, is distinct among the North American species of *Caloptilia*. In addition, the presence of a relatively large, curved spine from the ventral angle of the male cucullus is not known to occur in any other American species. A similar spine is present on the cucullus of *Caloptilia hamulifera* Liu and Yuan, an unreared species described from two males from Hunan and Sichuan Provinces, China. The distal half of the valva of *C. hamulifera* differs significantly from that of *triadicae* in being curved more sharply dorsad. The forewing pattern of *hamulifera* also differs from that of *triadicae* in being "brown and scattered with yellow spots."

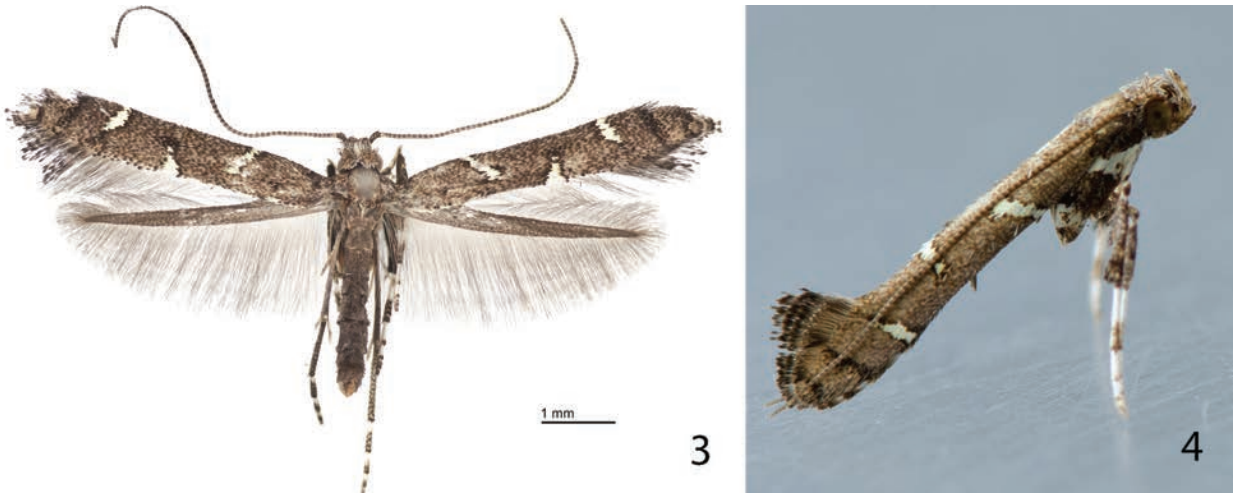
Description. Adult (Figs. 3 – 4). Forewing length 4.3 –4.7 mm.

Head: Vestiture mostly smooth, covered with moderately broad, round-tipped scales; a pair of raised, broad scale tufts from either side of occiput; all scales with pale grayish-white apices, dark fuscous subapically, and with more slender bases pale grayish white. Antenna ~ equal to forewing in length; scape mostly fuscous dorsally, minutely irrorated with pale cream; mostly pale cream ventrally; pedicel mostly pale cream with dark fuscous base; flagellomeres with single annulus of slender, dark fuscous-tipped scales with pale brownish bases. Maxillary and labial palpi mostly dark fuscous laterally and ventrally, pale cream mesally.

Thorax: Smoothly scaled, concolorous with head and base of forewings; scales moderately broad with pale grayish-white apices, dark fuscous subapically, with more slender bases pale grayish white. Vestiture along caudal margin of mesothorax white on lateral-ventral surfaces. Forewing mostly dark fuscous (fuscous scales with pale gray bases), with two white, slightly diagonal strigulae located at basal ¼ and distal ¾ of costa; a single median strigula near middle of dorsal (hind) margin; margins of strigulae irregular, lined with black scales; a small white spot often present along anal hind margin; cilia fuscous, with a subterminal row of pale gray scales. Hindwing immaculate, with similar ground color as forewing. Foreleg mostly dark fuscous, white at base of coxa; tarsomeres mostly white, banded with dark fuscous. Midleg similar in color to foreleg but with less white at base of coxa. Hindleg similar to other legs in color but with white annuli on tarsomeres more reduced in width.

Abdomen: Dark fuscous dorsally, mostly pale cream irorrated with fuscous scales ventrally. Seventh abdominal segment of male mostly membranous, with a narrow, transverse sternum and a pair of large, dense coremata with piliform scales as long as segment VI; eighth segment also membranous with a much smaller, slender "T"-shaped sternum and a pair of slightly shorter coremata ~ 2/3 the length of previous pair. Female abdomen unspecialized.

Male genitalia (Figs. 7–8): Tegumen simple, smoothly rounded, weakly sclerotized, sparsely setose. Vinculum U-shaped, slightly narrowing anteriorly. Valva becoming gradually broader from base to nearly truncate apex of cucullus; subapical region of cucullus densely covered with long setae with apical margin of cucullus nearly devoid of setae; costal margin of valva smoothly curved ventrally; subapical margin below apex of valva curved inwards (dorsally); a short, slightly



FIGS. 3–4. *Caloptilia triadicae*, adults. **3.** Holotype ♂, Gainesville, Florida. **4.** Adult in resting posture, Bartlesville, Oklahoma.

curved spine present at lower angle of cucullus. Transtilla with a slender process directed anteriorly. Aedeagus ~ 1.5 x length of valva, consisting of a slender, subacute cylinder, without cornuti; hood of phallobase relatively slender, elongate, ~ 1.3 x length of aedeagus.

Female genitalia (Figs. 5– 6): Anterior apophyses short, ~ 0.8 x length of posterior apophyses. Eighth abdominal segment ~ equal in length to papilla analis. Ostium bursae a small opening in membrane between abdominal sterna VII and VIII; ventral rim of ostium (antrum) with more sclerotization than dorsal rim; ductus bursae slender, walls of anterior 2/3 finely wrinkled; corpus bursae pyriform, walls minutely wrinkled, with a pair of cresentic signa; one signum ~ ¼ x larger than other; arms of both signa minutely serrated, but more densely serrated along inner margins

Types. Holotype - ♂; UNITED STATES: FLORIDA: Alachua Co: Gainesville, 29 Oct 2008, S. Wright & J. Lollis 08-6506, ex. *Triadica* (= *Sapium*) *sebifera*, digital image captured. (FSCA). Paratypes. UNITED STATES: FLORIDA: Alachua Co: Gainesville: 2004 SE 41st Avenue: 29°36.95'N, 82°17.91'W: 1 ♂, 7 Jun 2005, G. T. Austin. Gainesville: 5 ♂, 7 ♀, 25 Aug – 2 Sep 2008, S. Wright & J. Lollis 08-6506, Host: *Triadica sebifera*, slide USNM 34067, (USNM). Gainesville: lot 0811971, 2 ♂, 2 ♀, 2 Sep 2008, J. A. Wright, Host: *Triadica sebifera*, slide USNM 34065, (USNM). Gainesville: 1 ♂, 1 ♀, 29 Oct 2008, S. Wright & J. Lollis 08-6506, Host: *Triadica sebifera*, slide USNM 34066, (USNM). Gainesville: N 29° 38.114' W 082° 22.245': 1 ♂, 1 ♀, 23 Sep 2011, em. 6, 17, and 31 October 2011, J. G. Duncan, Host: *Gymnanthes lucida*, collected from live plant cage, (USNM). Broward Co: USDA ARS PRL: 1 ♀, 27 Jun 2012, Wheeler and Dyer, reared from *Triadica sebifera*; 2 ♂, 2 ♀, 27 Jun 2012, Wheeler and Dyer, reared from leaves *Triadica sebifera*, (USNM). Miami/Dade Co: Alice Wainwrite Park: 1 ♂, 27 Jun 2012, Wheeler and Duncan, reared from leaves *Gymnanthes lucida*, slide USNM 34520, (USNM). LOUISIANA: St. Tammany Par: near Pearl River, PRWMA, Honey Island Swamp: 6 ♂, 4 ♀, 22 May 2009, R. Hazen & M. Fox. PRWMA 30'23.442"N/89'43.297"W Plot 240: 8 ♂, 4 ♀, 22 May 2009, Rebecca Hazen, Host: *Triadica sebifera*. (Paratypes deposited in FSCA, USNM).

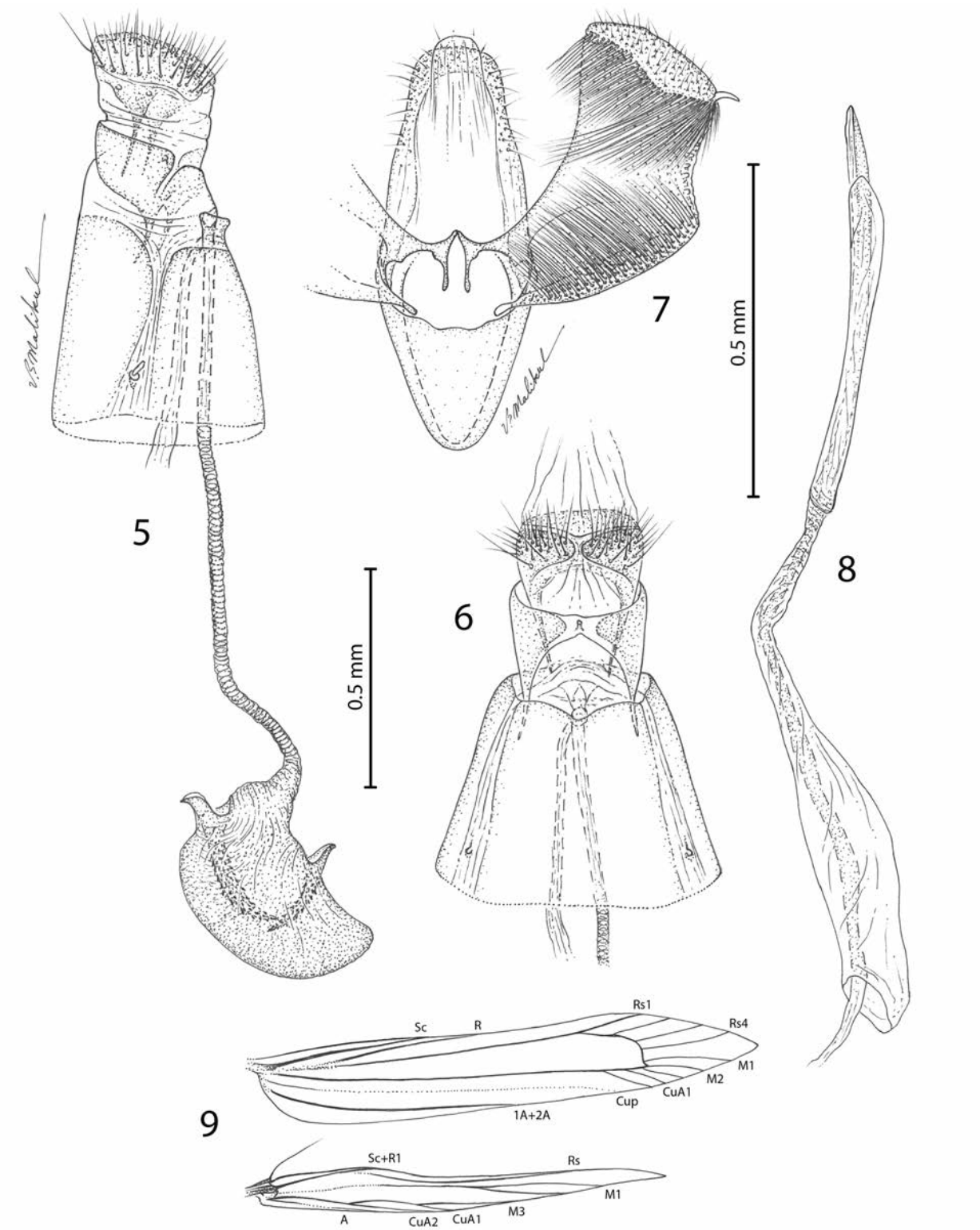
Distribution (Fig. 2). Though it has not yet been collected from any other country, *Caloptilia triadicae* is presumed to be adventitious to the United States. This presumption is partly based on the close association with its host plant, the Chinese tallow tree (*Triadica sebifera*), which is native to southeastern China (Fig. 1). *Caloptilia triadicae* has only been collected on or in the vicinity of its host plant, accordingly the known distribution of the moth is entirely encompassed by the distribution of the Chinese tallow tree (Fig. 2). The earliest U.S. records of *C. triadicae* are from 2004, in coastal Louisiana and Alabama. The species has since been found in Texas, Oklahoma, Mississippi, Florida, Georgia, South Carolina and Tennessee. In the U.S. it appears that *C. triadicae* is an effective disperser and that there may be little or no temporal lag between the invasion of new territory by the host tree and by the moth. The first photographic records of the moth in Davidson County, TN (S. Bren, 2009) and Washington County, OK (Fig. 4; M. Dreiling, 2012) actually preceded the first documentation of Chinese tallow tree in those counties.

Etymology. The species name is derived from the generic name of its primary plant host, *Triadica*, and is considered an adjective in the nominative singular.

Hosts. Euphorbiaceae: *Triadica* (= *Sapium*) *sebifera* (L.) Roxb.; *Gymnanthes lucida* Sw.

DISCUSSION

Biology. Eggs are laid on either the lower or upper leaf surface of young, tender leaves (Hazen and Fox 2011), often adjacent to a major vein or at the junction of two veins (Fig. 12). The eggs are flat and ~ 0.3 mm in diameter with a white, reflective surface; they are pressed to the leaf surface, resembling a small blemish on the leaf epidermis. First instar larvae emerge from the egg and immediately chew into the cuticle of the leaf. The initial subepidermal, serpentine mine is ~ the same width as the diameter of the egg (~ 0.3 mm). The sapfeeding larva tends to form a rather meandering mine, except upon encountering a vein or the margin of the leaf it typically follows that boundary (Figs. 10, 13). The mine is often enlarged two- or threefold into a chamber at two or three locations along its course. If such a chamber occurs at the leaf margin, the entire leaf may be slightly folded over giving the superficial appearance of an external shelter, but the epidermis of the leaf is not broken and the larva continues to feed internally. Sapfeeding larvae do not consume all the tissue between the upper and lower leaf surfaces and on heavily infested trees there may be distinct mines on



FIGS. 5–9. *Caloptilia triadicae*, adult morphology. **5.** Female genitalia, lateral view, USNM 34067. **6.** Female genitalia, ventral view. **7.** Male genitalia, ventral view, USNM 34065. **8.** Male aedeagus. **9.** Wing venation, USNM 34065, male.

FIGS. 10–15. *Triadica sebifera*, biology and larval feeding by *Caloptilia triadicae*. **10.** Foliage with initial evidence of larval feeding (New Orleans, Orleans Parish, Louisiana. 22 June 2012). **11.** Infested tree with detail insets showing various stages of larval feeding (along Hwy 90, Lake St. Catherine, Orleans Parish, Louisiana. 14 May 2012.) **12.** Oviposition sites of *Caloptilia triadicae* (indicated by arrows) (same collection site as 11). **13.** Serpentine mines of early stage sapfeeding larvae (New Orleans, Orleans Parish, Louisiana. 10 October 2012). **14.** Characteristic leaf cones rolled by later stage tissue feeding larva (same collection site as 13). **15.** Larval predation within cones by ant, Pseudomyrmex gracilis (Bell Chasse, Plaquemines Parish, Louisiana. 19 August 2011).

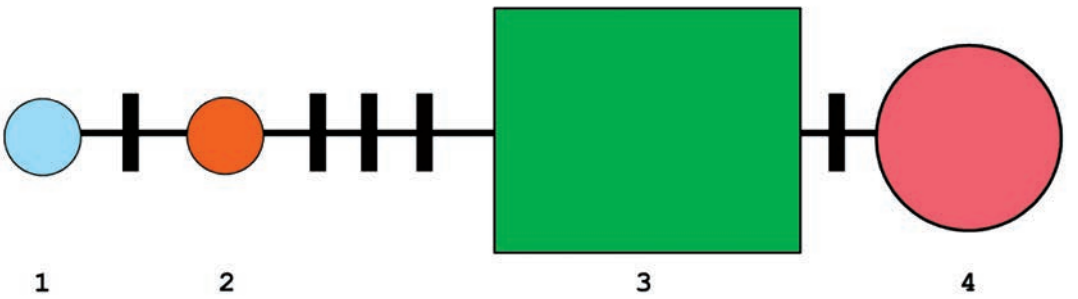


FIG. 16. *C. triadicae* haplotype tree generated for four haplotypes exhibited by study populations in North America. Bars on connecting lines indicate single basepair changes that differentiate lineages. The size of the numbered shapes, representing different haplotypes, corresponds with the abundance of each haplotype relative to the overall sample size for North America. The colors of the numbered shapes correspond to the pie charts in Figure 17, illustrating haplotype distribution by geography).

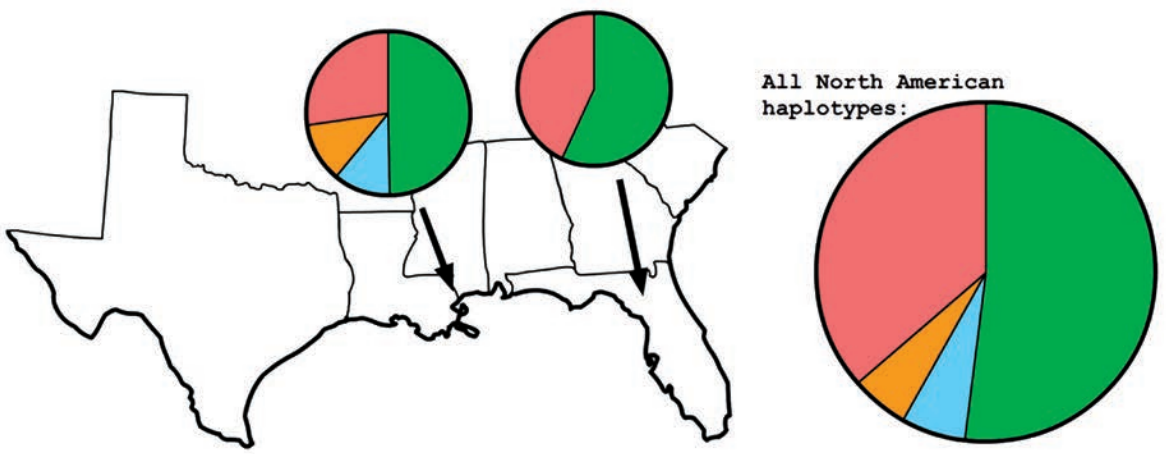


FIG. 17. Distribution of North American *C.triadicae* haplotype diversity from initial collections in the southeastern U.S. in Louisiana and Florida.

both upper and lower leaf surfaces which overlap without interfering with each other. The later externally feeding larval stages (tissue-feeding instars) construct a coiled, rosette-shaped leaf shelter (Figs. 10, 11, 14, 15). An incision is chewed across the leaf, perpendicular to the leaf margin, and the larva uses silk to coil the resulting flap of leaf (usually the flap closer to the apex of the leaf) into the shape of a cone. The dimensions of the coiled leaf shelter vary with the size of the larva and the size of the leaf, but it is always approximately cone-shaped, with the smaller base of the cone (diameter 4–9 mm) toward the midline of the leaf, and the apex of the cone (diameter 7–16 mm, or typically ~1.75 × diameter of base) at the leaf margin. Pupation takes place within the same shelter, and following the emergence of the adult moth the empty pupal exuvium remains protruding from the center of the leaf coil.

In New Orleans, LA, both pupae and external feeding larvae of *C. triadicae* were collected in February, 2012 by RH and MF; adults were photographed by R. L. Zimlich in Mobile, AL, in January, November, and December of 2012. These observations suggest that there is no extended overwintering stage for *C. triadicae*, at least for the populations in the southeastern United States. In the vicinity of New Orleans, in a typical year, the authors

commonly begin observing damaged leaves of *T. sebifera* in April or May, with adult *C. triadicae* emerging shortly thereafter. A complete generation takes only a few weeks, and abundance increases through the growing season, typically reaching a nearly total infestation of host trees by July or August. However, it seems that abundance of the moth early in the growing season and its subsequent rate of population growth are associated with the severity of the preceding winter: following several sustained hard freezes in winter of 2009-10, MF did not observe any evidence of *C. triadicae* in New Orleans until August, 2010. The population of *C. triadicae* plummets in winter when the temperature first approaches freezing and the host trees shed their leaves. Winter temperatures may determine the eventual northern boundary of the Chinese tallow tree's distribution, and therefore should present a similar boundary for *C. triadicae*.

Three species of *Triadica*, all native to Eastern and southeastern Asia, are currently recognized (Esser 2002). All are trees growing to maximum heights between 10–25 meters. *Triadica cochinchinensis* Lour. ranges widely from India to China and the Philippines. *Triadica rotundifolia* (Hemsl.) Esser is the least widespread of the species and occurs from southern China into Vietnam. The native range of *Triadica sebifera* (L.) Small includes the more southern provinces of Japan and China and is widespread in Taiwan. Because this species can grow within a wide range of dry or wet conditions and is frost hardy, its potential as an invasive species is the greatest. It is likely that larvae of *Caloptilia triadicae* are capable of feeding on all three species of *Triadica*.

Adults of *Caloptilia triadicae* have also been reared in Florida from a secondary host, *Gymnanthes lucida* Sw. (Euphorbiaceae). The genital morphology of males from this host was found to be identical with those reared from *T. sebifera*. *Gymnanthes lucida* is native to Florida, the Bahamas, the Caribbean, and Central America, and is not known to occur in the Old World (Miroslav 2005). Although possessing dissimilar native distributions and are not considered sister taxa, *Gymnanthes* and *Triadica* are closely related and belong to the same subclade within the tribe Hippomaneae of the Euphoriaceae (Wurdack et al 2005). Both genera have latex and appear relatively pest free except for specialist herbivores. Consequently, host switching between these two genera by certain herbivores may be possible. Because *Caloptilia triadicae* is most similar mophologically to other Asian species of *Caloptilia*, particularly *Caloptilia hamulifera* Liu and Yuan, and not to any North American *Caloptilia*, its utililization of New World *Gymnanthes* as a host is believed to be a later, secondary adaptation.

Natural enemies. The authors (RH & MF) have commonly seen polistine wasps investigating leaf rolls on tallow trees heavily infested with *C. triadicae*, though actual predation by these wasps has not been observed. Ants, most commonly *Pseudomyrmex gracilis* (Fabricius 1804), have been observed marauding leaf rolls and removing larvae of *C. triadicae* (Figure 15). Arachnids are often found residing in leaf rolls formerly inhabited by *C. triadicae*, but it is unclear whether the spiders consumed the resident larva or pupa or were opportunistically using empty shelters. Several species of parasitoid wasp have also been reared from *C. triadicae* in the U.S., but have not yet been identified (Hazen and Fox, unpublished data, Greg Wheeler, personal communication).

COI Sequence data. To date, sequence data for the mtDNA COI gene region of *C. triadicae* from two North American locations (Florida and Louisiana), suggest that populations exhibit multiple mtDNA haplotypes (Fig. 16) and appear to comprise a single species. Analysis of mtDNA sequences yielded four total haplotypes, all of which were found in Louisiana while only two were found in Florida (Fig. 17). This degree of genetic diversity is consistent with field observations (by MF and RH) of outbreak-level infestations of *C. triadicae* larvae on *T. sebifera* trees.

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LARVAE OF IO MOTH, *AUTOMERIS IO*, ON THE CORAL BEAN, *ERYTHRINA HERBACEA*, IN FLORIDA—THE LIMITATIONS OF POLYPHAGY

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ABSTRACT. The coral bean, *Erythrina herbacea*, is reported as a new host for *Automeris io* in north-central Florida, based on a single batch of larvae found on this plant in nature and reared through on it in the laboratory. However, the consequent laboratory rearing showed a high cost associated with using this host plant. The mortality of young larvae of the F-2 generation reared on *E. herbacea* was very high (over 90%), and much higher than that of control larvae, which were reared on mature leaves of *Quercus nigra* (14–38%). The leaves of *E. herbacea*, known for their toxic alkaloids, were extracted with methanol, made into a water solution, applied on the mature leaves of *Q. nigra*, and fed to 1st instars of *A. io*. This produced no negative effect on the caterpillars. While the mature leaves of *Q. nigra* produced low mortality in young caterpillars, the young terminal leaves of this plant were as lethal to *A. io* as leaves of *E. herbacea*. Additionally, it was noted that rearing larvae on *E. herbacea* (a diet with a higher N2 content) led to faster larval development and smaller adult moths. The *A. io* larvae in this study developed during 63-100 days and underwent seven larval instars, which contradicts many popular accounts of the fifth instar being the final in *A. io*. Finally, *Prunus angustifolia* proved to be an unsuitable hostplant for *A. io*, as feeding on this species led to the arrested development of larvae and their eventual death.

Additional key words: alkaloids, caterpillar, community ecology, nutrition, plant and herbivore defense

On 13 September 2011 in Gainesville, Florida, I found a cluster of neonate caterpillars of *Automeris io* Fab. (Saturniidae) feeding on leaves of the coral bean, *Erythrina herbacea* L. (Fig. 4A, B). *Automeris io* is a highly polyphagous species, with over 100 host plants listed just for the state of Florida (Heppner 2003). Among them are plants such as *Citrus, Solanum, Salix*, and *Prunus* that contain toxic compounds in their leaves. Therefore, it is not surprising to find yet another host plant to be palatable to the *A. io* larvae, even if this host plant is laced with alkaloids (e. g., Powell & Westley 1993). However, considering how few Lepidoptera are able to utilize this widely available plant as their host, this discovery seemed to constitute a notable expansion in the diet of *A. io*.

Indeed, in Florida, where ca. 3,000 Lepidoptera species are found, the only ones that were recorded to feed on *Erythrina herbacea* are a psychid, *Cryptothelea gloverii* (Packard), a tineid, *Opogona sacchari* (Bojer), lycaenids, *Celastrina ladon* Cramer and *C. neglecta* Edwards, a pyralid, *Trachylepidia fructicassiella* Ragonot, three crambids, *Epicorsia oedipodalis* Guenée, *Terastia meticulosalis* Guenée and *Agathodes designalis* Guenée (Heppner 2003), and a lyonetiid, *Leucoptera erythrinella* Busck. During my extensive field work in north central Florida that involved searching multiple *E. herbacea* bushes for caterpillars, I observed only the latter three species feeding on this plant (Sourakov 2011; 2012).

Genus *Automeris* has been known to utilize various other species of *Erythrina* in Central America. For instance, *Automeris metzli* (Sallé) was found to feed on *Erythrina costaricensis* Micheli; *Automeris banus* Boisd. and *A. zugana* Druce - on *Erythrina lanceolata* Standl. (Janzen & Hallwachs 2012); *Automeris illustris* (Walker) - on *Erythrina cysta-gali* L. and *E. falcata* Bentham (Specht et al. 2006). Hence, the genes responsible for the ability to detoxify *Erythrina* alkaloids are not novel in *Automeris*, but whether they have migrated to the *A. io* population in Florida from more tropical populations remains to be shown. Alternatively, of course, *A. io* may have always occasionally utilized *Erythrina* as one of its host plants, and this has simply not been heretofore discovered.

Closely related species and even populations within species can vary in detoxification abilities, as was shown in *Papilio glaucus* L./*Papilio canadensis* Rothschild & Jordan species complex and the luna moth (e. g., Lindroth et al. 1986, Lindroth 1989), and this can even become a driving force for speciation (e. g., Mercader et al. 2009, Zvereva et al. 2010). Some plant compounds can have metabolic repercussions for the insect in the form of delayed larval development (e. g., Lindroth et al. 1988a). Such compounds can be present in different quantities in different, even closely related, species of plants, and can determine the growth pattern and ability to complete metamorphosis in generalist moth species (e. g., Ruuhola et al. 2001). Herbivores frequently develop adaptations to plant-specific combinations of defensive compounds and these compounds can determine their choice of food (e. g., Tahvanainen et al. 1985). Other types of plant compounds, such as lectins, were shown to negatively affect larval growth, survival, and pupation, as well as adult emergence and fecundity

(Machuka et al. 1999, Fitches et al. 1997, Fitches et al. 2001, Shukle & Murdock 1983). Lectins are also known to be a deterrent of oviposition behavior (e. g., Michiels & Smagghe 2010). Plant lectins were shown to have a species-specific effect on insects, causing high mortality in some herbivores, while simultaneously being completely neutral to others (e. g., Machuka et al. 2000).

Larval growth rate can also be positively affected by an increased nitrogen contents in plants (e. g., Manuwoto & Scriber 1985). Leaves of different plant species can seasonally differ in value as host plants to the same species of herbivore, and, remarkably, such variation is not universal, but unique for each host plant considered (e. g., Lindroth et al. 1988b, Finke & Scriber 1988). In the latter study, the low water content of ingested leaves was found to be responsible for decreasing the growth rate of larvae, even when the nitrogen levels in leaves remained adequate. The nitrogen and water in poplar trees, for example, as well as glycosides responsible for suppressing herbivores, decline in the fall, but such fluctuations vary between individual trees (Lindroth et al. 1987).

If *Erythrina herbacea* is not among the normal host plants for *Automeris io*, the question is: How and why did the *A. io* female choose to oviposit on it? Studies on butterflies suggest that contact chemoreception can guide oviposition choices (e. g., Frankfater & Scriber 2003). Oviposition preference can also be on a genotypic level, not only of insects but of plants as well (Bossart & Scriber 1995). It is not the nutritional value, but rather presence of repellent compounds that may be responsible for a host plant not being regularly utilized by polyphagous herbivores. Frankfater and Scriber (1999) showed, for example, that red bay leaf extracts deter oviposition by *Papilio glaucus*, even though the host plant might otherwise be suitable for larval development. Larvae of many other Lepidoptera species can survive on host plants on which they would never oviposit in the wild (e. g., Dowell et al. 1990). Studies (e. g., Sétamou et al. 2002; Hogervorst et al. 2008; Bell et al. 2004) suggest that the detrimental effect of insecticidal plant compounds can be passed on to the next trophic level—the parasitoids. Thus, it is possible that the generalist parasitoids avoid searching *E. herbacea* plants for hosts, because there are few potential hosts that feed on it and because those that do might reduce the fitness of the parasitoid's offspring as a result of the ingested toxins. During my previous studies, which involved collecting and rearing numerous caterpillars of crambid moths off of *E. herbacea* (Sourakov 2011; 2012), not a single larva of these moths was found to be parasitized. Hence in Florida, where the larval parasitism by generalist tachinid flies is frequent and can reach as high as 90% (e. g., Sourakov 2009), oviposition on a toxic plant, such as *E. herbacea* by a polyphagous species, such as *A. io*, while possibly a complete "accident", could also prove to be an example of exploiting an enemy-free space, in which a tradeoff exists between high larval mortality and low parasitism rates.

In the present study, following the initial discovery of *Automeris io* larvae feeding on *Erythrina herbacea* in the wild, I examined the relative suitability of this host plant as a host of *A. io* by comparing its costs and benefits to those of other diets in controlled settings and attempted to provide an explanation to the resulting observations.

MATERIALS AND METHODS

Experiment 1: Rearing a brood of field collected *A. io* larvae on different hostplants

Sibling first instar larvae of *Automeris io* (N=14) were found in the wild feeding as a group near their empty egg shells on *E. herbacea* foliage on 13 September 2011(Fig. 4A, B). They were maintained as a group inside a plastic bag feeding on *E. herbacea* until the end of the 3rd instar (3 October 2011), when they were split into five groups and from this point raised in separate plastic bags. Two groups of three larvae were continued on *E. herbacea*, the third group of three larvae was switched to the black oak (*Quercus nigra* L.), the fourth group of three larvae to the chicksaw plum (*Prunus angustifolia* Marsh.), and the remaining two larvae to the blackberry (*Rubus flagellaris* Willd.). Bags and host plant cuttings were replaced three times a week, and an effort was made to use conspecific plants and leaves of similar age. Fresh brown tissue paper was placed in each bag to absorb excessive moisture. Towards the end of larval development, larvae that reached the final (7th) instar were maintained individually and pupated inside cocoons spun among tissue paper and hostplant leaves. Bags with pupae were kept on an ambient light cycle at ca. 75°F until mid-December, when they were transferred to a non-heated garage, where conditions reflected ambient variations in temperature and humidity. In February 2012, the bags with pupae were brought back into the laboratory, where they were kept inside the plastic bags until emergence in May. Drops of water were periodically added to keep the pupae from desiccating. Live adults were weighed upon emergence and their right forewing was measured using calipers.

Experiment 2: Rearing a lab-obtained brood 1 of *A. io* on *E. herbacea* and *Q. nigra*

A female and a male raised on *Erythrina herbacea* during Experiment 1 emerged on 6 May and 8 May

2012 respectively, and were allowed to mate in a cage. The resulting 332 eggs proved to be fertile and the first instar larvae were offered pieces of *Quercus nigra* and *E. herbacea* onto which they crawled at will. These pieces of host plants were then transferred into separate plastic cups where clippings of respective host plants were added and larvae were raised using the feeding protocol outlined in Experiment 1. A total of 18 such cups with first instar larvae ranging in number from 14 to 30 were initially set up, and were treated as separate replications in the statistical analysis of the data. Ten cups contained *Erythrina herbacea* (mean number of larvae per cup =20 ±5 (SD); N (total)=199), and eight cups contained *Q. nigra* (mean number of larvae per cup =18 ±3 (SD); N(total)=133). During this experiment, only older foliage was provided as food, corresponding to the type of foliage on which larvae were found in the field and maintained during Experiment 1. Larval mortality was assessed three times a week during the change of host plant clippings and cups.

Experiment 3: Rearing a lab-obtained brood 2 of *A. io* on *E. herbacea* and *Q. nigra* (leaves with variable water contents)

By crossing a male raised on oak during the Experiment 1 and female raised on blackberry (both emerged on 6 June 2012), a brood of 278 larvae was obtained. These larvae hatched on 9 July, were split into groups, and reared using protocol similar to Experiment 2. This time however, several types of foliage were used: very young tender leaves of the terminal leaf growth, firm but still immature leaves of slightly older terminal leaves (light green in color), and old firm and dark green leaves of *Quercus nigra* were placed in separate cups. Similarly, older (dark green) leaves of *Erythrina herbacea* were placed in one of the cups, while other cups contained tender terminal leaves (light green). There were a total of 14 cofee cups: 7 with larvae feeding on young *E. herbacea*, 7 with larvae feeding on *Q. nigra* (two of which contained terminal tender leaves, four terminal firm leaves, and one mature leaves). Larval mortality was assessed during the change of hostplant material.

Experiment 4: Determining the relative toxicity of *E. herbacea* leaf compounds to young *A. io* larvae

The mature leaves of *E. herbacea* and *Q. nigra* were collected in June, dried at room temperature, and ground up separately using a coffee maker. Three grams of the leaf powder resulting from each of the species were separately extracted using 20 ml of cold methanol in an ice-bath for 25 minutes. The mixture was filtered, resulting in a bright green solution of leaf chemicals, which was allowed to evaporate under the hood. The glass beakers with the resulting residue of dry plant compounds were then stored at -23°C until use. The residue was diluted with 10 ml of water and the leaves of *Q. nigra* were dipped into the solutions and air dried prior to feeding to *A. io* caterpillars. Fourteen first instar *A. io* larvae in separate plastic cups were fed on leaves dipped into each of the solutions. Leaves dipped in tap water were used as the control. Mortality was assessed over the span of one week, during which caterpillars fed continuously on the leaves.

The water contents of leaves was measured by clipping them at the base from the plant, weighing them fresh, and then drying them in the lab at room temperature and appx. 50% humidity prior to re-weighing. A sample of host plant leaves was dried, ground-up into powder, and analyzed for nitrogen content using an Eager 200 CHN analyzer.

RESULTS

Experiment 1: Rearing a brood of field collected *A. io* larvae on different hostplants

On *Erythrina herbacea:* All six larvae of *Automeris io* continued developing normally; one male pupated in 46 days; five females pupated simultaneously after 63 days of development. In *A. io*, males are smaller, hence faster development and smaller larva/pupa in males is to be expected. On *Quercus nigra*: two larvae (one male, one female) pupated after 65 days, and one female pupated after 70 days. Despite the small sample size, time to pupation of *A. io* females raised on *E. herbacea* (= 63±0 days (N=5)) differed significantly ($P < 0.05$) from that of those raised on *Q. nigra* (= 67.5±3.5 days; (N=2)). *Automeris io* females raised on *E. herbacea* were

TABLE 1. Water and nitrogen contents in leaves of *Erythrina herbacea* and *Quercus nigra* and the corresponding larval mortality of *Automeris io*.

	Erythrina herbacea-young leaves	*Quercus nigra*-young leaves	*Erythrina herbacea*-old leaves	*Quercus nigra*-old leaves
Nitrogen (%)	N/A	N/A	2.6–2.8%(Jun); 2.0–2.1%(Sep)	1.2–1.3%
Water (%)	78.3%	57.9-67.5%	59.2–62.4%	40.1%
Mortality	High	High	High	Low

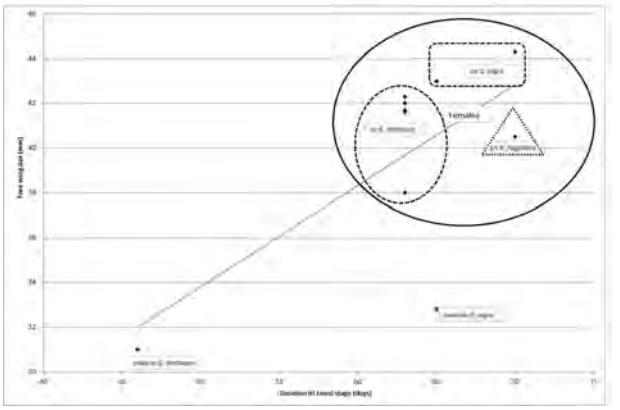

FIG. 1a. Experiment 1: Correlation between larval stage duration and size of resulting adult *Automeris io* moths found on *Erythrina herbacea* in the wild as 1st instar and subsequently raised on *E. herbacea*, *Quercus nigra* and *Rubus flagellaris*.

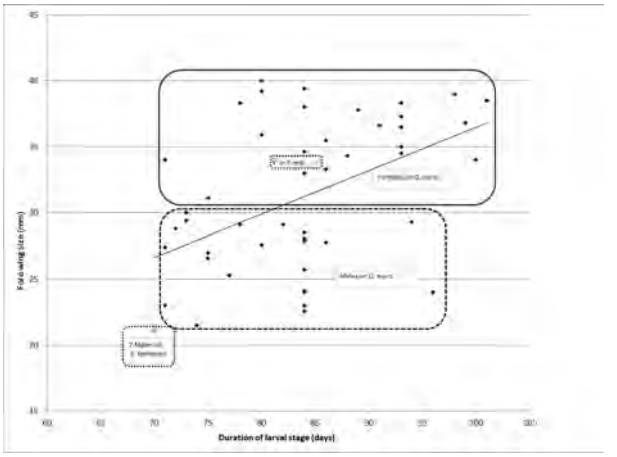

FIG. 1b. Experiment 2: Correlation between larval stage duration and size of resulting adult *Automeris io* moths raised on *Erythrina herbacea* vs. *Quercus nigra*.

smaller than those raised on *Q. nigra* (FW length=40.8±1.9mm; N=4 vs. FW length=43.7±0.7mm; N=2). Correlation between size of the resulting adults and length of larval development can be observed in Figure 1a. On *Rubus flagellaris*: One larva developed at a normal rate, but died after 30 days as an early fifth instar; the other (female) pupated after 70 days. It appears that development on this host plant occurred at a similar rate to that on *Q. nigra*, but the resulting female was smaller. On *Prunus angustifolia*: One larva continued feeding after transfer from *Erythrina*, but showed completely arrested development, and died 71 days later at the 4th instar without visibly gaining in size. The other two larvae died at the 6th instar after 85 and 110 days of feeding. Their growth was noticeably slower than the growth of those that fed on other host plants.

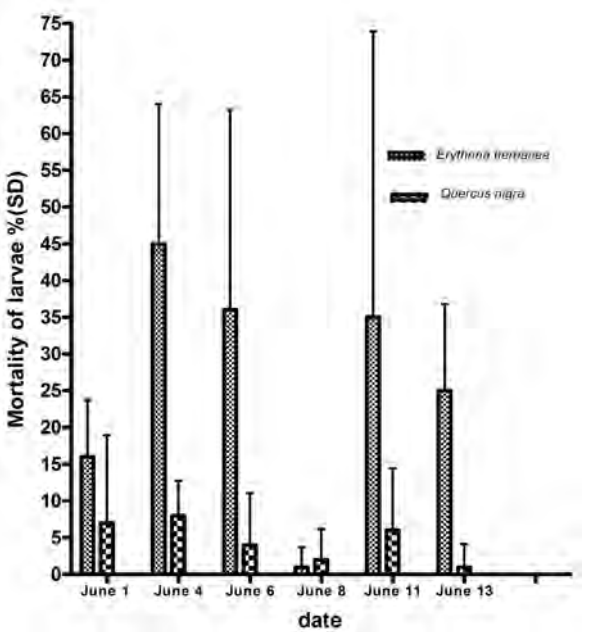

FIG. 2. Experiment 2: Differences in average mortality (±St. Dev.) of young *Automeris io* larvae raised on *Erythrina herbacea* vs. *Quercus nigra*.

Seven larval instars: The present study allowed, among other things, for a detailed assessment of *Automeris io* biology. The larvae underwent seven larval instars, which contradicts many popular accounts of the fifth instar being the final (e. g., Wikipedia 2013). Such discrepancy is quite common, partly because it is frequently erroneously assumed that the final instar is the fifth one. Only by thoroughly observing and collecting head capsules at every molting (Fig. 4D) is it possible to determine the true number of instars in Lepidoptera.

Experiment 2: Rearing a lab-obtained brood 1 of *A. io* on *E. herbacea* and *Q. nigra*

Figures 2 and 3a illustrate the difference in survival dynamics of larvae fed on *Erythrina herbacea* and *Quercus nigra*. Young *Automeris io* larvae survived much better when raised on *Q. nigra* than on *E. herbacea*. After the first three days, the average mortality on *E. herbacea* was twice as high, =16±8% (±SD), versus 7±12% on *Q. nigra* (P<0.05). More importantly, dead larvae were found in every one of the 10 cups containing *E. herbacea*, while larvae experienced mortality in only three of the cups containing *Q. nigra*. Between 1 June and 4 June, the trend continued, with mortality on *E. herbacea* being much higher at an average of 45±19%, vs. only 8±5% when feeding on *Q. nigra* (P<0.0006). Between 4 June–6 June, after nine days of feeding, at which point some larvae reached the 2nd instar, larvae feeding on *E.*

herbacea experienced 36±27% average mortality vs. only 4±7% on *Q. nigra* (P<0.004). This occurred in every cup containing *E. herbacea*, while 100% survival occurred in 5 out of 8 cups containing *Q. nigra*. Relative mortality was calculated only for cups which still retained six larvae or more, and hence was not assessed following June 13, by which date all *E. herbacea* test cups contained fewer than six larvae. The initial mortality on *Q. nigra* plateaued, while on *E. herbacea* larvae continued to die off throughout the first two instars. Larvae developed to pupal stage faster on *E. herbacea* (70.3±0.6 days (N=3)) than on *Q. nigra* (86±7.5 days (N=41)) (P<0.001). The 51 adults that emerged in 2012–13 showed the same trend as in Experiment 1: adults raised on *E. herbacea* were smaller than those raised on *Q. nigra* (Figure 1b).

Experiment 3: Rearing a lab-obtained brood 2 of *A. io* on *E. herbacea* and *Q. nigra* (leaves with variable water content)

Figure 3b illustrates the difference in survival dynamics of larvae fed on leaves of *E. herbacea* and on young and old leaves of *Q. nigra*. At the end of July, the water content in mature *Erythrina herbacea* was 59.2%, while the water content in mature *Quercus nigra* was only 40.1%. However the firm terminal young leaves of *Q. nigra* had a water content of 57.9%, while the terminal young immature (tender) leaves were 67.5% water. Leaves of *E. herbacea* collected at the end of September contained 62.4% of water, while *Q. nigra* leaves contained just 41.4%. In mid-June, the nitrogen content in *E. herbacea* was 2.8%, in contrast to the nitrogen content of *Q. nigra*—1.2%.Towards the end of September, nitrogen in *E. herbacea* leaves dropped to 2.0%, while it remained the same (1.2%) in *Q. nigra* (Table 1).

Larvae started feeding successfully and evenly on *Q. nigra* in all 15 cups. With the exception of the cup containing old *Q. nigra* leaves where larvae reached second instar three days earlier, the larvae developed at similar rates. Mortality was first observed in cups containing tender *Q. nigra* leaves, with all larvae in these cups dying off. During molting leading to the second instar, high mortality was observed in all cups, except for the cup with old *Q. nigra* leaves (Figure 3b). This mortality correlated with an apparent bloating of the larvae (Fig. 4H) when compared to the larvae fed on old oak leaves (Fig. 4G). Also, the normal ability of the larvae to stay in tight-knit groups when migrating from leaf to leaf, feeding, or molting (Figs. 4 B, C, F, I) was lost, with larvae spreading out to feed solitarily or in small groups of 2–3.

Experiment 4: Testing the relative toxicity of *E. herbacea* leaf compounds to young *A. io* larvae

Larvae developed normally in all three cups and 0% mortality was observed in cups containing *Q. nigra* leaves treated with *E. herbacea* extract, *Q. nigra* extract, and the control leaves.

DISCUSSION

It appears that the principals postulated by Scriber and Feeny (1979) concerning individual leaf qualities and those proposed by Lindroth (1989) concerning individual broods/populations being the key to the survival of larvae apply to the usability of *Erythrina herbacea* as a host plant of *Automeris io*. The same applies to *Quercus nigra*: successful as I was in Experiments 1 and 2 when raising *A. io* on that plant, this success vanished when leaves with a higher water

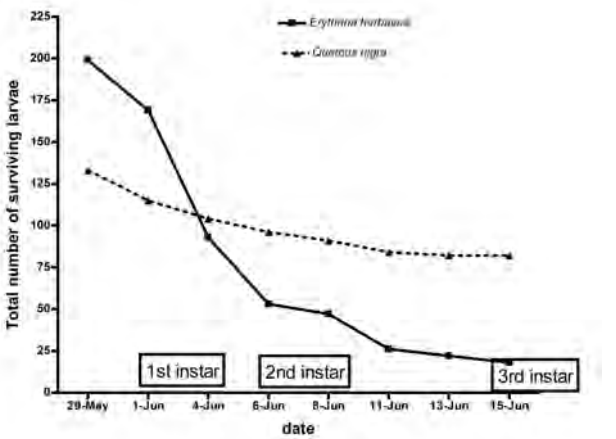

FIG. 3a. Experiment 2: Survival of young *Automeris io* larvae when raised on *Erythrina herbacea* and *Quercus nigra*.

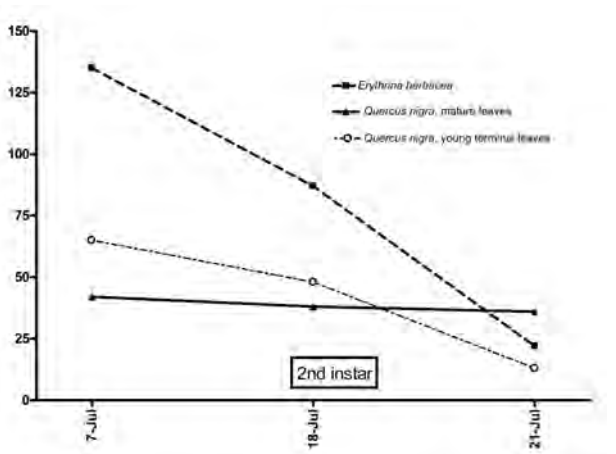

FIG. 3b. Experiment 3: Survival of young *Automeris io* larvae when raised on old leaves of *Erythrina herbacea* (water contents 59%), young *Quercus nigra* leaves (water contents 58%) and old *Q. nigra* leaves (water contents 40%).

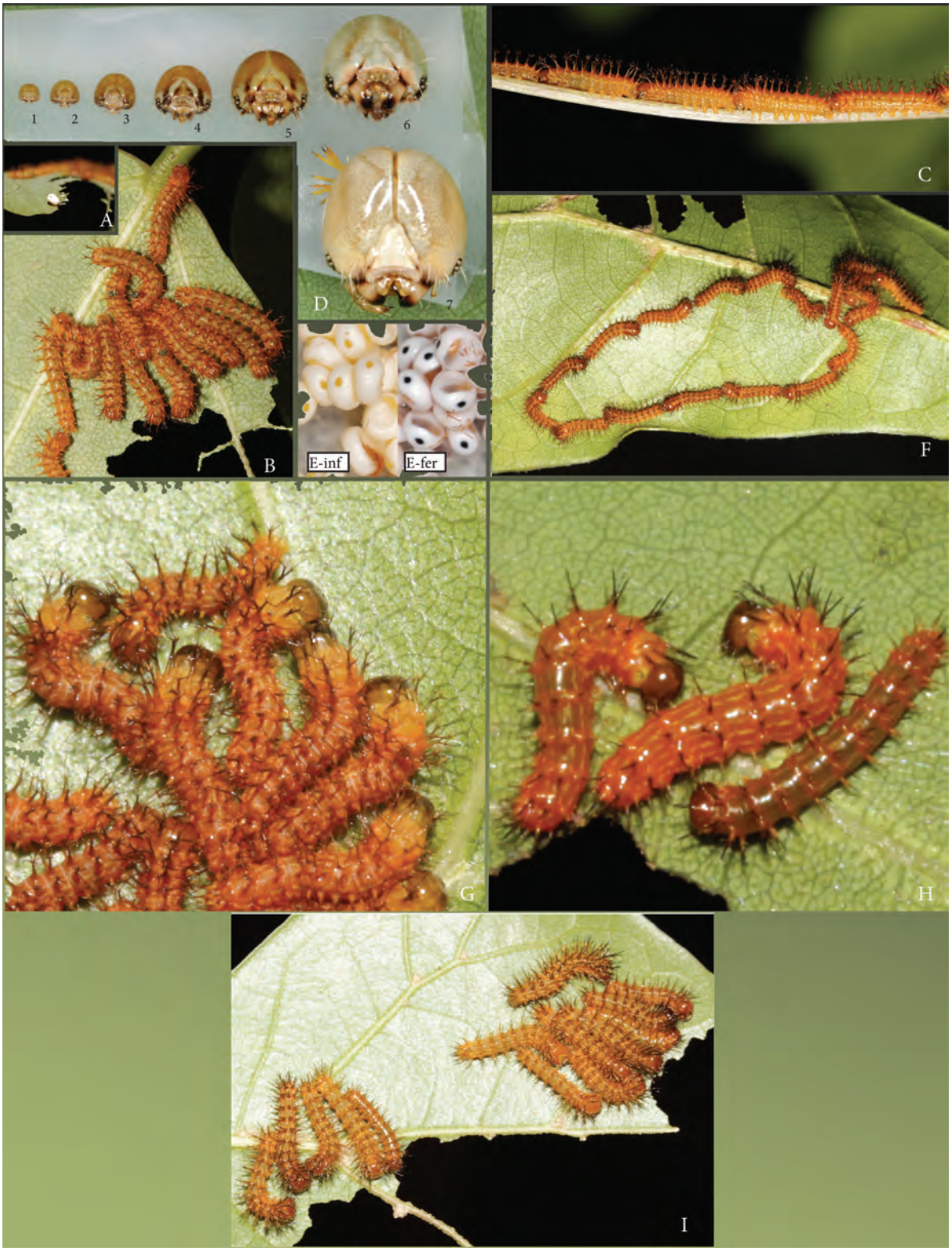

FIG. 4. Early stages of *Automeris io*: (A-C) A batch of 1st instar larvae found feeding on the Coral Bean, *Erythrina herbacea*, Gainesville, FL, 23 Sept 2011; (D) Head capsules of the seven instars that these larvae underwent prior to pupation; (E) Fertile and infertile eggs; (F) 1st instar of F-2 generation feeding on the Water Oak, *Quercus nigra*; (G) same as (F) molting into 2nd instar; (H) larvae fed on *E. herbacea* showed a characteristic bloating when molting into 2nd instar (see text for details); (I) late 1st and early 2nd instars feeding together on *Q. nigra*.

content were introduced. The dynamics of larval survival then became very similar to those seen on *E. herbacea* leaves. This, combined with the fact that extracts of *E. herbacea* leaves applied to *Q. nigra* leaves did not cause larval mortality and larvae fed on high-H_2O containing leaves displayed a bloated appearance, leads me to conclude that the high water content of the leaves may have been detrimental to the larvae. This conclusion can appear contradictory to accounts of rearing *A. io* on young leaves of other plants, specifically the black cherry, *Prunus serotina* Ehrh. (e. g., Eric Anderson, pers. com.; Manley 1990 & 1993), and perhaps individual properties of leaves and plants are important for larval survival. As a side note, the larvae of *Agathodes designalis*—the only large larvae that specialize on *E. herbacea* leaves—have a thin, transparent cuticle, perhaps designed to deal with the hostplants' high water content by allowing evaporation. The observed acceleration in development of *A. io* larvae on *E. herbacea* is most likely due to the nitrogen content, which was shown to be twice as high in this hostplant as in *Q. nigra*.

The present study showed that, although *Automeris io* has a rather remarkable ability to survive feeding on *E. herbacea*, the cost (in the form of larval mortality) of ovipositing on this hostplant can be high, which probably leads to selective pressure against the adaptation of this hostplant as a norm by *A. io*. The mechanism by which *A. io* deals with the alkaloids of this plant (via sequestration, or elimination straight from the gut, etc.) is yet to be determined. It is clear from this study, however, that the polyphagous nature of *A. io* has its limitations, even though this species has an incredible ability to adapt to feeding on a wide variety of, sometimes toxic, host plants.

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ADDENDUM

Following the acceptance of this manuscript, I conducted two additional experiments involving *Erythrina herbacea* and *A. io*. The eggs from cage-mated *A. io* were obtained in June 2013. When a cluster of ca. 70 neonate larvae were simultaneously offered blackberry and *E. herbacea* leaves, they quickly dispersed and started feeding on both hosts. A day later, the ca. 35 larvae that had been feeding on *E. herbacea* were observed to fall into a temporary "comatose" state: they interrupted their feeding, were not moving or responding to external stimuli, and were in unnatural positions as if in a spasm. They were presumed to have been poisoned, but the following day, they resumed normal feeding and continued growing. However, while the control batch of larvae that were feeding on blackberry underwent normal development, the larvae feeding on *E. herbacea* mostly died at molting, with only four larvae surviving past the first instar, and two surviving past the second instar. These two larvae developed faster (in 72 and 79 days) than their ten siblings raised on blackberry, the average development time of which was 89 ± 8 days.

From a different egg batch, 20 late-penultimate and early-last-instars raised on blackberry were switched to *E. herbacea*. These larvae also readily accepted the new hostplant, but underwent high mortality: only eight larvae (40 %) reached the pupal stage. Death was preceded by an interruption in feeding followed by a discharge of clear fluids, but otherwise the larvae appeared normal. The symptoms began after the larvae had been feeding on *E. herbacea* for several days and up to 3 weeks, so it is unclear if the mortality was due to the cumulative effect of gradually ingested toxins or to the ingestion of a lethal dose contained within particular leaves. These trials confirm the experiments described in the main body of the manuscript: while there may be a limited benefit to feeding on *E. herbacea* in the form of faster development, very few *A. io* larvae are able to take advantage of it.

During the summer of 2013, while searching various plants for caterpillars in the Gainesville, FL, area, I found four additional batches of *A. io* eggs (16-20 eggs each). One, found on *Prunus serotina*, was parasitized by *Trichogramma*. Three others were found on *Crotalaria pumila* and *Crotalaria pallida*, which are, like *E. herbacea*, legumes high in nitrogen and rich in alkaloids that very few larvae can detoxify. The neonates fed on leaves of *C. pumila* and *C. pallida*, if offered an alternative (cherry or oak) immediately switched, and if not offered an alternative, died before reaching the second instar. These findings, in addition to my earlier finding of *A. io* on *E. herbacea* begin to form a pattern that requires an explanation other than "accidental" or "mistaken" oviposition. The first possible reason is that *A. io* females, while searching for hostplants, lay small batches of eggs on nitrogen-rich plants regardless of their toxicity and, shall it be unsuitable, rely on larvae to disperse to a different hostplant. So far all five batches found in nature have been 14–20 eggs, so the risk to an individual female resulting from losing a batch or two is relatively small. The second, more farfetched yet interesting hypothesis is that *A. io* females favor toxic plants for oviposition to avoid egg parasitoids and to offer potential enemy and competition-free space to their progeny and added protection to caterpillars via secondary plant chemicals. More fieldwork will be required to prove either of these hypotheses.

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USE OF TWO OVIPOSITION PLANTS IN POPULATIONS OF *EUPHYDRYAS PHAETON* DRURY (NYMPHALIDAE)

Additional key words: Baltimore Checkerspot, iridoid glycosides, Vermont

The Baltimore Checkerspot, *Euphydryas phaeton* Drury (Nymphalidae: Melitaeini), is a univoltine species that ranges from Georgia in the south, north to Maine and southern Canada and west to Kansas (Scudder 1889; Masters 1968; Harris 1972). Its native host plant is White Turtlehead, *Chelone glabra* L. (Plantaginaceae), a denizen of marshy, wetland habitats. In the Ozarks, another subspecies, *E. phaeton ozarkae* Masters, was described as somewhat different in appearance and using the oviposition plant *Aureolaria flava* (L.) Farw. (Orobanchaceae) (Masters 1968). Just over 30 years ago, a third oviposition plant was described for *E. phaeton*, the introduced weed, Ribwort or Narrow-leaved Plantain, *Plantago lanceolata* L. (Plantaginaceae) (Stamp 1979). *Plantago lanceolata* was introduced into North America about 200 years ago (Cavers et al. 1980) and has been incorporated into the diets of many native North American herbivores (Robinson et al. 2002). These three different species of oviposition plants are united by the presence of a particular group of plant chemical compounds, the iridoid glycosides (Bowers et al. 1992; Belofsky et al. 1989). Indeed all host plants of *E. phaeton* contain iridoid glycosides (Bowers 1980; Bowers et al. 1992).

Euphydryas phaeton has been declining in numbers in many areas such as Maryland and Rhode Island (Durkin 2009); however, in Vermont, there are many healthy colonies. Specifically, the recent Vermont Butterfly Atlas Project has documented *E. phaeton* populations at nearly 200 sites (McFarland and Zahendra 2010). Furthermore, the use of *Plantago lanceolata* has allowed some populations to get extremely large; for example, a recent survey of adults from a population on June 19, 2010, in Bristol, Rhode Island, in a field of approximately seven acres, revealed a population estimate of over 3,200 individuals of *E. phaeton* (4th of July butterfly count Rhode Island, 2010). A careful search of this site and surrounding areas revealed no evidence of *C. glabra*. More recently, during the 2012 butterfly count in Rhode Island, another large population (over 1000 individuals counted) was located on private land near Little Compton (2012 4th of July butterfly count Rhode Island, 2012).

Typically, only a single plant species is used as an oviposition plant by a single population of Baltimore Checkerspots, although post-diapause larvae may feed on a variety of plant species, including *Penstemon* (Plantaginaceae), Ash (*Fraxinus*, Oleaceae), *Viburnum* (Adoxaceae), false foxglove (*Aureolaria*) and honeysuckle (*Lonicera*, Caprifoliaceae). For example, in New York (Stamp 1979) and Rhode Island (Bowers and Schmitt 2013), populations of *E. phaeton* use solely *P. lanceolata* for both oviposition and larval feeding. Other populations using exclusively *P. lanceolata* also likely occur. Most populations, however, still use *C. glabra* as the sole oviposition plant.

Here we report the occurrence of two populations in Vermont that use both *C. glabra* and *P. lanceolata* as oviposition plants at the same sites (Figures 1 and 2). At both sites, ovipositing *E. phaeton* females were observed using both plant species on the same day (although we did not follow individual females) in 2011 and 2012 (Figure 1 and 2). The two sites were 1) Clark, Washington County (Figure 1); 2) Connor, Washington County (Figure 2). There are likely to be other *E. phaeton* populations that use both species for oviposition as well because many Vermont wetlands where *C. glabra* occurs are located in or near agricultural landscapes, where *P. lanceolata* is a very common weed of old fields, hay fields and roadsides. Recent studies of *E. phaeton* in Massachusetts indicate that populations using both host plant species as oviposition sites may be relatively common (G. Breed, E. Crone, personal communication; http://www.butterfliesofmassachusetts.net/baltimore-checkerspot.htm).

Use of these two host plant species for oviposition by a single *E. phaeton* population may have important consequences for those populations. For example, in the two populations we studied, there is likely to be strong selection against oviposition on *P. lanceolata*: haying operations destroyed all egg masses we detected on *P. lanceolata*. The *P. lanceolata* plants we observed occurred almost exclusively in hayfields and these fields may be cut two or more times in a single summer, depending on grass growth. Early season (i.e., June) haying kills post-diapause late instar larvae and pupae, when *Plantago* is a common food plant; while later season haying could kill adults, egg masses, and prediapause larvae. Because the native host plant, *C. glabra*, often occurs near farm fields, use of this non-native, alternative oviposition host by checkerspots may

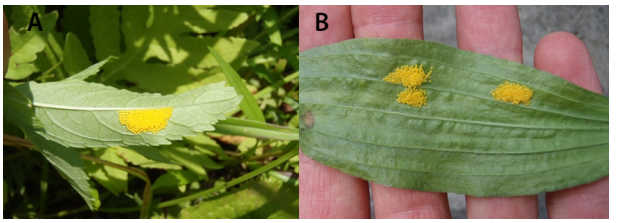

FIG. 1. Egg masses from the Clark site (East Montpelier County, Vermont). **A)** Egg mass on *C. glabra* from this site; **B)** egg mass on *P. lanceolata* from this site. Photographs taken on July 11, 2011.

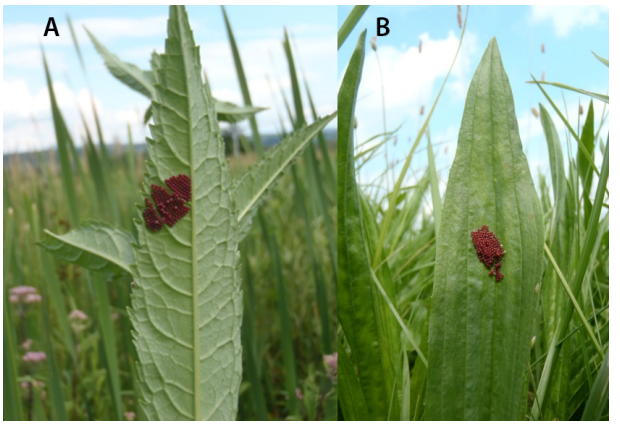

FIG. 2. Egg masses from the Connor site (East Montpelier County, Vermont). **A)** egg mass on *C. glabra* from this site; **B)** egg mass on *P. lanceolata* from this site. Photographs taken on July 18, 2011.

be relatively common. There may be other effects on *E. phaeton* populations as well. For example, specialist parasitoids such as *Cotesia euphydryidis* (Muesebeck) (Braconidae) and *Benjaminia euphydryadis* Vierick (Ichneumonidae) often search for hosts on larval webs of *E. phaeton* (Stamp 1982). These webs may be much less conspicuous when they occur on *P. lanceolata* because of the low stature of this species compared to *C. glabra*, making them more difficult for parasitoids to find. Thus larvae from egg masses on *P. lanceolata* may better escape parasitoids. Furthermore, larval feeding on these two different host plant species may also affect palatability of both larvae and adults. When reared on *P. lanceolata*, larvae and adults contain two iridoid glycosides, aucubin and catalpol, whereas those reared on *C. glabra* contain almost exclusively catalpol (Bowers et al. 1992). Feeding experiments with birds showed that the *C. glabra*-reared individuals are much less palatable than those reared on *P. lanceolata* (Bowers 1980); thus use of *P. lanceolata* may affect this important chemical defense in this species.

In conclusion, use of both the native *C. glabra* and the introduced *P. lanceolata* in individual populations of *E. phaeton* may have important consequences for these insects. As wetlands where *C. glabra* is found become less common and agriculture and disturbance become more common, use of *P. lanceolata* may increase in this butterfly, with multiple and potentially long-term effects on its populations.

Thanks to E. Crone and G. Breed for their observations on Massachusetts *E. phaeton* populations.

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GNORIMOSCHEMA BRACKENRIDGIELLA (BUSCK, 1903), A VALID SPECIES (LEPIDOPTERA: GELECHIIDAE)

Additional key words: *Gelechia detersella*, Gelechiinae, North America, taxonomy, nomenclature.

"*G.*[*elechia*]? *detersella*" was described by James Brackenridge Clemens in 1860, with no indication of the type locality. Hodges (1986:36) suggested that unless otherwise stated, Clemens' home and environs in Easton, Pennsylvania should be considered as the locality for his material. The type specimen of *detersella*, together with the rest of Clemens' collection now reside in the Academy of Natural Sciences, Philadelphia (ANSP, type #7341).

Clemens' original description reads as follows: "*G.? detersella. Head and face grayish fuscous. Labial palpi pale yellowish-white, with two fuscous patches on the middle joint, a very narrow fuscous ring at the base of terminal joint, a broad one near the tip, with the extreme apex whitish. Antennae grayish fuscous, annulated with dark fuscous. Fore wings grayish, very profusely dusted with dark fuscous, with a dark fuscous spot on the disk; cilia ochreous gray. Hind wings pale ochreous-gray; cilia pale ochreous. Feet annulated with whitish.*"

Clemens also sent two specimens from his original series to Henry Stainton in the Natural History Museum (BMNH), where they still reside. Acknowledging their receipt, Stainton (1872) expressed doubts about their identity and thought that they were affiliated with the European *Gelechia affinis* Haworth, 1828 (now in *Bryotropha*). He also realized that Clemens' name *detersella* was preoccupied by *Gelechia detersella* Zeller, 1847, and was in need of a replacement name. This was later accomplished by Busck (1903a) who proposed the name *Gelechia brackenridgiella* as a replacement for *detersella* Clemens. At the time of his publication, Busck was unaware of the existence of Clemens's material which was already in ANSP. He wrote: "… no types exist in this country [United States] of this species … Co-types of this species should be examined in British Museum, where the two specimens sent to Stainton by Clemens in 1860 presumably are found" (1903a). But soon after, in a supplement to the same paper (Busck 1903b), he clarified: "A very unexpected source of information has come to light in the discovery of the types of the late Brackenridge Clemens, in the Academy of Natural Sciences in Philadelphia. These types had been given up as lost, but were found a short time ago in an old-fashioned box, which had been put away in some out-of-the-way corner and forgotten. My delight in unearthing this gold mine for the student of American *Tineina* quite overshadowed my first very natural chagrin over the changes necessitated in my work". Here, under the new combination "*Gnorimoschema brackenridgiella*", Busck wrote: "The type of *Gelechia detersella*, Clemens' No. 75, was found in good condition, though lacking the left wings. It proves the species to belong to the genus *Gnorimoschema* Busck, and is very close to but distinct from *scutellariella* Chambers." He went on to describe the differences between *detersella* and *scutellariella*, and concluded, "The removal of this species to *Gnorimoschema* may make the change of specific name questionable, but for the present I shall retain the new name".

Over the years, the name *brackenridgiella* was used as a valid name under *Gnorimoschema* (Smith 1903, Busck 1939), although Barnes and McDunnough (1917) and McDunnough (1939) listed *detersella* as the valid name and placed *brackenridgella* [sic] under it as a synonym. Forbes (1923) incorrectly identified a gall-making species on Aster from Magnolia, Massachusetts, as *Gnorimoschema brackenridgella* [sic] and compared it to several other gall-making species; these are a group of much larger moths with a completely different habitus.

In 1929 Meyrick examined and identified a series of 20 specimens collected by Herbert Simpson Parish from "Toronto, Muskoka, May–August" in the British Museum as *brackenridgella* [sic]. It seems that Meyrick's concept of the *brackenridgiella* was based solely on Clemens' short description of *detersella*, because there is no evidence that he ever saw Clemens' specimens in the BMNH. At the time, Stainton's world collection was maintained separately from the main world collection at the BMNH, and unless Meyrick specifically checked the former, he would have missed them. Meyrick re-described and transferred *brackenridgiella* (as well as *batanella* Busck) under *Phthorimaea*, stating "… I believe this to be *detersella* Clem[ens]; as it seems to be little known, and published descriptions are very imperfect, I redescribe it".

Nearly 40 years later, after examining a male specimen from Parish's series identified by Meyrick in BMNH, Povolný (1967) wrote, "The specimen of *Phthorimaea brackenridgella* [sic] is conspecific with

FIG. 1. Adults, labels and genitalia dissections of specimens of *Gelechia detersella* Clemens. **Top row:** Lectotype (ANSP), dissection RWH2940; **mid row:** Paralectotype specimen 2/2 (BMNH), dissection BMNH33489; **bottom row:** Paralectotype specimen 1/2 (BMNH) [=*Scrobipalpula henshawiella* (Busck), original misidentification by Clemens], dissection BMNH33488. The scale bar on genitalia images represents 100 µm.

Scrobipalpa atriplicella (F.v.R.) which fact [sic] suggests the possible synonymy of the former". Povolný also never examined Clemens' specimens of *detersella* and his statement on the synonymy of *brackenridgiella* with *atriplicella* was solely based on one of Parish's specimens from Canada identified as such by Meyrick. Since then, Hodges (1983), Lee et al (2010) and Huemer & Karsholt (2010) have all followed Povolný in accepting *brackenridgiella* (=*detersella*) as a synonym of *Scrobipalpa atriplicella*.

The two specimens of G. *detersella* in the BMNH were at some later point moved into the main world collection, given additional labels to indicate their provenance and labeled as paralectotypes. This was perhaps accomplished by Brian Ridout, an assistant in the 1970s (K. Tuck, pers. comm.). These specimens are now in drawer M10-189 of BMNH main world collection of Gelechiidae under *S. atriplicella*.

As part of an ongoing study of the North American Gnorimoschemini, I borrowed the type and genitalia dissection of *G. detersella* from ANSP, as well as Clemens' two specimens in the BMNH. In order to verify Povolný's identification of Meyrick's *brackenridgiella* from Canada (on which the synonymy is based), I also borrowed a male specimen from Parish's series in BMNH. From the original 20, only 16 specimens remain today in BMNH, none of which bear an identification label by Povolný. It seems likely that Povolný kept part of the material loaned to him back in 1960s (Tuck, pers. comm.).

I dissolved, unrolled and remounted the male genitalia of the *detersella* type (Hodges prep. RWH2940), and made new dissections of the three BMNH specimens. One of Clemens' specimens was a very close match with the ANSP type, and a genuine *Gnorimoschema* that is unlike any of the other described species within this genus that are known to me. The taxon therefore merits recognition as a valid species. I hereby designate Clemens' specimen of *Gelechia detersella* in ANSP as the Lectotype (**here designated**), and reinstate the replacement name *Gnorimoschema brackenridgiella* (Busck) as a valid species (**stat. rev.**). No syntypes of *detersella* exist in ANSP (Weintraub, pers. comm.), and the two paralectotypes in BMNH (**here designated**) seem to be the only other known specimens. One of these two (labeled specimen 2/2; dissection BMNH33489) is a genuine *detersella*; it carries an inverted red "Type" label as well as a hand-written label by Stainton that reads: "*Gelechia* ? *detersella*, Clemens / Proc. n. S. Phil. 1860 p.164 / perhaps allied to *G. affinis*". The second specimen (labeled 1/2; dissection BMNH33488) however proved to be a *Scrobipalpula henshawiella* (Busck, 1903), a common North American species with an external appearance that is somewhat similar to *G. brackenridgiella*. This was an original misidentification by Clemens. A new label is added to this specimen to rectify the error. And finally, dissection of the male specimen from Parish's Canadian series (not shown) confirmed that it is indeed a *Scrobipalpa atriplicella* (F.v.R.). Povolný may be vindicated for a correct identification, but he was responsible for an incorrect synonymy that persisted for nearly half a century.

Revised nomenclature

Gnorimoschema brackenridgiella (Busck, 1903) (stat. rev.)

G.[*elechia*]? *detersella* Clemens, 1860: 40, 116.
Preoccupied by *Gelechia detersella* Zeller, 1847: 846.

Gelechia brackenridgiella Busck, 1903a: 894.
Replacement name for *Gelechia detersella* Clemens, 1860: 164. — Huemer & Karsholt 2010: 128 [as a synonym of *Scrobipalpa atriplicella* (F. v. Röslerstamm].

Gnorimoschema brackenridgiella; Busck, 1903b: 934.

Gnorimoschema brackenridgella; Smith 1903: 110 (as a synonym of *detersella*). — Barnes & McDunnough 1917: 155. — Forbes 1923: 274. — Povolný 1967: 125 [as a synonym of *Scrobipalpa atriplicella* (F. v. Röslerstamm)].— Hodges 1983: 22.— Lee et al 2010: 26 [under *S. atriplicella* (F. v. Röslerstamm)].
Misspelling of *brackenridgiella*.

Phthorimaea brackenridgella; Meyrick 1929: 493.
Misspelling of *brackenridgiella*.

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A NOTE ON OVERWINTERING OF POLITES MARDON (HESPERIINAE) IN THE WILD

Additional key words: overwintering stage, larval shelters

Polites mardon W.H. Edwards (Washington State endangered) is a grass skipper endemic to the Pacific Northwest of the United States. Currently *P. mardon* occurs in four disjunct populations from northern California to the Puget lowland of Washington (Fig. 1, Mattoon et al. 1998). The butterfly's historic distribution in each geographic region is unknown but has likely contracted over the last 100 years along with the loss of grassland and montane meadow habitats due to development, forest succession, fire suppression, and spread of invasive species (Potter et al. 1999). Because little habitat remains throughout the butterfly's range, active habitat restoration and management such as prescribed burning, invasive species control, and conifer removal is ongoing (Beyer & Schultz 2010, Henry & Schultz 2013). Until recently, much of this management occurred with very little knowledge of the habitat requirements and life history of the butterfly.

Early knowledge of the life history of *P. mardon* is based on captive rearing work by Newcomer (1966). He found the larval period of captive individuals to last approximately three months and diapause to occur in the pupal stage. More recently, James and Nunnallee (2011) also found captive individuals to overwinter as pupae. Our field observations of wild populations contradict these observations of captive individuals and provide insights into the biology of the butterfly that can inform conservation and habitat restoration planning. In this note we document *P. mardon* overwintering as larvae in the wild and describe larval shelters observed in the field. The data presented herein were collected as part of larger studies of the oviposition habitat requirements of *P. mardon* in Washington State (Beyer & Schultz 2010, Henry & Schultz 2013).

To determine the overwintering stage of *P. mardon* in the wild, we followed larval development in two disjunct areas of the butterfly's range in Washington State. In 2006/2007, we worked in the Bunny Hill Meadow, a 164 m^2 alpine meadow (1097m elevation) on the Gifford Pinchot National Forest, Cascade Mountains, hereafter referred to as the Cascades (Beyer & Black 2006). In 2009/2010 our focus was Scatter Creek Wildlife Area (60m elevation), a remnant glacial outwash prairie in the south Puget Lowland containing 250 hectares of prairie, hereafter referred to as Puget prairie (Henry & Schultz 2013).

In the wild, female *P. mardon* lay eggs singly in the grass without affixing them to the hostplant (unlike in captivity where James and Nunallee (2011) observed females lightly gluing eggs to hosts). Therefore, to establish egg locations, we performed extensive oviposition surveys in the Cascade Mountains and the Puget prairie during the 2006 and 2009 flight seasons, respectively (see Beyer & Schultz 2010 and Henry & Schultz 2013 for detailed methods). We marked all observed oviposition locations with a wooden skewer as close to the egg as possible and re-visited egg locations every few weeks to determine developmental stages of butterfly larvae.

Fig. 1. *P. mardon* range. Stars indicate locations of known extant *P. mardon* populations, each of which consists of multiple occupied sites. **1**) Puget Lowland, **2**) Southern Washington Cascade mountains, **3**) Southern Oregon Cascade mountains, **4**) Del Norte, California.

In 2006 we documented *P. mardon* larvae in the wild for the first time during surveys in the Cascades. During the summer and fall of 2006 we searched 32 oviposition locations on 6 days (July 31, August 14, August 26, September 9, September 30, and October 21), locating a total of seven individual larvae. We located the first larva on July 31st in its second instar (instar stages determined from descriptions in James & Nunnallee 2011; Fig 2). Three individuals were located more than once over consecutive survey efforts, four individuals were located only once. On October 21, two days prior to the first snow of the season, we located two fourth instar larvae; one of which we found on our previous visit, the other we discovered for the first time on this visit.

Larvae observed in summer and early fall were actively feeding from vertical larval shelters with their heads upward. These larvae responded to disturbance by becoming still or crawling away from their shelters. By the last week of October, larvae were sedentary, curled up in sealed larval shelters, and frass was absent or old suggesting that larvae were no longer feeding. During these final observations, larvae became active only after they were physically removed from their larval shelters, indicating they were partially dormant. It is possible that disturbing larvae and shelters during our previous observations may have slowed their development. However, given that both larvae observed on October 21 were fourth instars and neither larva showed any signs of pupation, including the individual that had not been previously disturbed, we do not think our work had negative impacts on development rates. These observations strongly suggest that the *P. mardon* populations in the Cascades overwinter as larvae. When we returned to egg locations post-snowmelt the following spring we were unable to find evidence of either larvae or pupae.

We followed *P. mardon* development again over the winter of 2009/2010, but this time worked in the Puget prairies where the sites remain snow-free all winter. On September 11, 2009, we searched 88 known oviposition locations and located six third instar larvae (8–10 mm, all instar classifications based on data from James & Nunnallee 2011; Fig 3). We returned to all six larval locations on October 5 and December 23 2009, and February 21, March 24, April 8, and April 19 2010 to document development through the year. On October 5 and December 23 we detected all six larvae in their shelters but did not disturb them. On February 21 we found four of the six initial larvae, thus establishing that wild *P. mardon* populations overwinter as larvae in the Puget prairies. These larvae were fourth instar larvae (12–13mm; Fig 4). On March 24 we were still able to locate one full-grown fifth instar larva (24mm; Fig 5). We located, but did not disturb this larva again on April 8. On our next visit, April 19, the larva could not be found. Although this was the only larva we were able to find in late March and early April, this observation suggests that *P. mardon* do not pupate until late spring, 4-6 weeks before the adult flight season (mid-May–mid-June).

In both the Cascades and the Puget prairies, when we initially found larvae, they were actively feeding and occupied tubular larval shelters made of grass blades and/or litter fragments stitched together with silk. Larval shelters were nearly vertical, surrounded by frass, and located at the base of the grass just above the soil (Fig. 2 & 3). All larvae were found in the initial egg location, adjacent to the wooden skewer marker. In the fall of 2006, larvae were disturbed every 2–3 weeks. When removed from their shelters they would rebuild shelters by the next visit, moving no more than three inches from the initial egg location. This behavior suggests *P. mardon* are highly sedentary in their larval state, likely only moving in response to disturbance or potentially when they have outgrown shelters or exhausted local food resources as observed in other Hesperiinae species (MacNeill 1964, Dana 1991). During all winter visits (late October–late February; including October visit in Cascades) the larval shelters consisted of silk lined chambers of dry grass blades, moss, litter, and dried up frass perfectly camouflaging with the detritus within the grass tufts (Fig 4). These chambers were horizontal in the vegetation, and located at the base of the grass, near the soil surface. Inside these shelters, larvae rested curled in a U-shape with their back toward the shelter opening, if present. The shelter of the fifth instar larva we found on March 24, 2010 was a horizontal tunnel made of dried grass blades (Fig 5).

While little is known about the larval habits of most Hesperiinae species, our findings of *P. mardon* overwintering as larvae are consistent with previous ideas about the general habits of the Hesperiinae subfamily (Scott 1986, 1992). Other Hesperiinae species known to overwinter as larvae in the wild include *Atalopedes campestris* (Crozier 2003, 2004), *Hesperia dacotae* (Dana 1991), *H. ottoe* (Dana 1991), and a number of other western *Hesperia* species (MacNeill 1964). Additionally, our observations of the larval shelters of *P. mardon* are comparable to detailed observations of the larval shelters of Hesperiinae skippers made by Dana (1991), MacNeill (1964), and James and Nunnallee (2011).

Understanding the basic biology of rare species is fundamental to their successful conservation. For

FIGS. 2–5. Examples of *P. mardon* larvae and their corresponding grass shelters. **2)** 2nd instar larva at Bunny Hill meadow on 26 August 2006, **3)** 3rd instar larva at Scatter Creek on 11 September 2009, **4)** 4th instar larva at Scatter Creek on 21 February 2010, **5)** 5th instar larva at Scatter Creek on 24 March 2010. Larvae and shelter locations are circled.

species whose persistence depends on habitat management and/or restoration, such as *P. mardon*, this is especially important. Individual insect species may respond differently to management treatments based on their life histories (Stark et al. 2004). Knowledge of the timing of a rare butterfly's life cycle allows land managers to time events such as prescribed burning, mowing or herbicide application when they are likely to have minimal impacts on butterfly species of concern (Dana 1991, Konvicka et al. 2008, Johst et al. 2006). Captive rearing and reintroduction are suggested conservation strategies for many rare butterflies (Schultz et al. 2008) and have been suggested for *P. mardon* (A. Potter, pers. com.). In the context of reintroduction, knowledge of the timing of the species' life cycle is of utmost importance to ensure that the life stage and timing of release are matched appropriately.

By determining that *P. mardon* overwinters as a mid-late instar larva, we have added to the body of knowledge of an understudied butterfly family as well as provided new life history information important for conservation of this rare and threatened butterfly.

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Sheppard, P. M. 1959. Natural selection and heredity. 2nd ed. Hutchinson, London. 209 pp.

—— 1961a. Some contributions to population genetics resulting from the study of the Lepidoptera. Adv. Genet. 10:165–216.

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