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Cover illustration: *Papaipema aweme* adult resting on dead leaf of larval host Buckbean (*Menyanthes trifoliata*) in lab (August 5, 2016), reared from larva collected at the Pine Creek Peatland, Roseau Co., Minnesota. Photo by Kyle Johnson.

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NATURAL HISTORY AND DISTRIBUTION OF *PAPAIPEMA AWEME* (NOCTUIDAE)

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ABSTRACT. *Papaipema aweme* (Lyman) was previously known only from seven specimens and five localities globally, and was thought to be a critically imperiled species of dunes, alvars, and other dry habitats. Life history information was lacking. We documented fifty-nine specimens from 2009–2016 at six new locations spanning 1,555 km from the Upper Peninsula of Michigan to eastern Saskatchewan. Larvae were found boring in the stems and rhizomes of *Menyanthes trifoliata* L. (Menyanthaceae) in open graminoid rich fen habitats at two of these localities. All localities are rich fens with abundant *M. trifoliata*; such habitats are or were present in the vicinity of all five historical records (1905–2005). These discoveries suggest *P. aweme* is a peatland specialist and is likely much more common and widespread than present records indicate.

Additional key words: peatland, fen, host plants, conservation, *Menyanthes*

DEDICATION

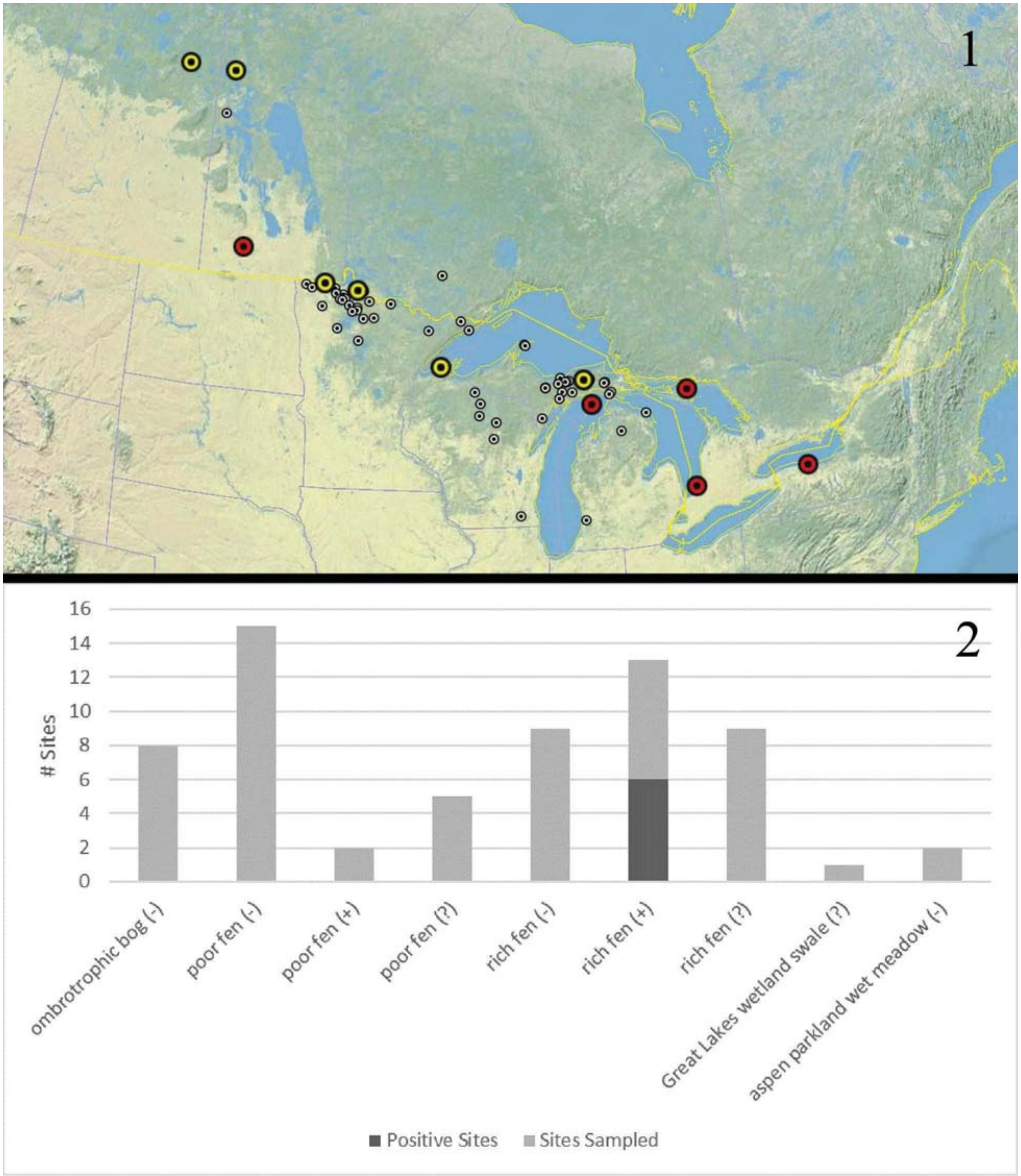
We dedicate this paper to the late Mogens C. "Mo" Nielsen, honorary life member of the Lepidopterists' Society, charter member of the Michigan Entomological Society, and an inspiration for anyone studying Lepidoptera of the Northern Great Lakes. *Papaipema aweme* was a "holy grail" to Mo, and he spent many a night chasing this phantom across the Michigan landscape. Although he never encountered one himself, Mo's encouragement of the authors' studies helped lead to the successes presented in this paper.

Papaipema aweme (Lyman) is an enigmatic species within a relatively popular and well-studied genus of moths. It was known from only seven adult specimens taken at light from 1905–2005 (COSEWIC 2006). From August 24–26, 1905, Norman Criddle collected single specimens on three successive nights in the vicinity of Aweme, Manitoba. The female specimen collected on August 24th would subsequently serve as the holotype (Shepard and Vickery 1975). On August 13, 1925, Sherman Moore took a single specimen on a boat anchored off Beaver Island, Michigan. On August 7, 1932, A. Richards took a single specimen in Rochester, New York. On August 15, 1936, a single specimen was taken (collector unknown) in Grand Bend, Ontario. The moth then went unreported for 69 years until August 19, 2005, when John Morton took a single specimen near Pike Lake on Manitoulin Island, Ontario.

The geography of these records suggested a relationship with shorelines of the Great Lakes, or relict shorelines of Glacial Lake Agassiz in the case of Aweme, Manitoba. These records also led to the belief that *P. aweme* was a denizen of dry habitats such as dunes, sand prairie, oak savannah, or alvar (COSEWIC 2006, Michigan Natural Features Inventory 2007, NatureServe 2017). Extensive surveys to re-document the moth at Aweme and Grand Bend (blacklighting), and Manitoulin Island (larval searches) all focused on dry habitats but without success. The small number of localities, presumed restriction to imperiled habitats, and failed re-documentation at historical localities led to listing *P. aweme* as an endangered species in Canada in 2006 (COSEWIC 2006).

On September 10, 2009 DRB chanced upon a single fresh female *P. aweme* at the Wanamaker Lake Peatlands in Michigan. The moth was taken in a UV light trap placed on a sandy conifer woodland low ridge within a large peatland complex. The dry ridge best fit previous habitat hypotheses, but the common and widespread flora growing there could not explain the moth's rarity. The adjacent peatlands, however, contained an assortment of fen types with diverse and interesting flora.

Peatland specialists of comparatively well-known groups such as butterflies have often eluded



FIGS. 1–2. **1**. *Papaipema aweme* historical records (large red circles), new localities (large yellow circles), and negative sampling results (small white circles). **2**. *Papaipema aweme* sampling effort vs. positive results (adults and larvae) across different habitat types. For sites with multiple types, only the predominant type in the sampling vicinity is counted. *Menyanthes trifoliata* presence is ranked as absent/sporadic (–), common (+), or unknown (?).

entomologists until surprisingly recent times. *Boloria freija* (Thunberg) went undocumented from the northern Great Lakes states until May 22, 1965 when Ron Huber collected examples from a raised bog in northern Minnesota; better understanding of its habitats and phenology has since led to widespread documentation across the northern counties of Minnesota, Wisconsin, and Michigan (Johnson 2011). *Boloria frigga* (Thunberg) went undocumented from the region until June 25, 1956 when Steve Hubbell collected a specimen in a peatland near Manistique, Michigan (Hubbell 1957); likewise, there has since been widespread documentation across the northern counties of Michigan, Wisconsin, and Minnesota (Johnson 2011) as well as the first discovery in New England (Maine) in 2002 (Maynadier and Webster 2009).

Similarly, since a cryptic, nocturnal, late-season moth with similar or greater peatland habitat specificity could also be poorly known, we hypothesized *P. aweme* was originating from peatlands or other wetland habitats.

MATERIALS AND METHODS

Peatland (bog and fen) terminology herein largely follows Wright et al. 1992 and differs from that used in older literature. Plant nomenclature follows USDA, NRCS 2017.

Peatlands are wetlands with an accumulation of poorly decomposed organic matter (peat). They are divided into three major classes based on dissolved mineral content and acidity: rich fen (high mineral content and alkaline to weakly acidic), poor fen (low mineral content and moderately to strongly acidic), and bog sensu stricto (living plant layer isolated from groundwater minerals and strongly acidic). They can be separated with floral indicators. Bogs sensu stricto are always dominated by hummocky *Sphagnum* moss (Sphagnaceae) carpets, low ericaceous shrubs (Ericaceae), a limited suite of sedges (e.g. *Carex oligosperma* Michx., *Eriophorum vaginatum* L. (Cyperaceae)), and variable cover of stunted conifers (usually *Picea mariana* (Mill.) Britton, Sterns & Poggenb. (Pinaceae)). They lack minerotrophic indicators. Poor fens may support any of the flora present in bogs, but also contain weak minerotrophic indicators (e.g. *Betula pumila* L. (Betulaceae), *Carex chordorrhiza* Ehrh. ex L. f. (Cyperaceae)). Many habitats referred to as "bogs" in older literature are actually poor fens in this classification. Rich fens may support both bog and poor fen flora (but lack acidophiles such as *Eriophorum vaginatum*) and also include strong minerotrophic indicators (e.g. *Dasiphora fruticosa* (L.) Rydb. (Rosaceae), *Thuja occidentalis* L. (Cupressaceae), *Trichophorum alpinum* (L.) Pers. (Cyperaceae)). Open sedge dominated rich fens usually support brown mosses (Amblystegiaceae) instead of *Sphagnum*. Forested rich fens and poor fens are often called rich swamps and poor swamps, respectively.

We sampled 62 sites with peatlands or peatland elements across Michigan (24), Wisconsin (6), Minnesota (25), Ontario (3), Manitoba (3; 1 of these also a Minnesota site), and Saskatchewan (1) from 2005–2016 (Fig. 1). We searched for larvae at six of these sites across Michigan (2), Wisconsin (1), Minnesota (3), and Manitoba (1; also a Minnesota site) from 2011–2016.

We sampled ombrotrophic bogs, poor fens (including poor swamps), rich fens (including rich swamps), Great Lakes shoreline interdunal wetlands, and aspen parkland sedge meadows with peatland floral elements (Fig. 2). Some of these habitats were within patterned peatland complexes (peatlands in which the vegetation forms distinctive patterns in aerial view; see Wright et al. 1992. for discussion of the various types) and included raised bogs, internal water tracks of raised bogs, featureless water tracks, ribbed fens, ribbed fens with tear-drop tree islands, and spring fen channels.

We sampled numerous other sites and habitats during this period, but these are not included since many (e.g. dunes, alvar) had already received considerable sampling effort during the appropriate *Papaipema aweme* flight period (COSEWIC 2006, pers. obs.).

We sampled each site at least once during the documented *P. aweme* flight period extremes (August 7–September 10), though we had no way of knowing whether our efforts (particularly those near the date extremes) coincided with the peak flight period for a particular site in a given year. Some sites were visited multiple times; the Wanamaker Lake Peatlands in Michigan (the 2009 *P. aweme* capture site) received exceptional coverage with 145+ trap nights spread over 18 nights (each light trap or sheet used on a given night was considered a trap night) plus larval searches in 2011 and 2016.

We relied predominately on UV (ultraviolet) light traps (8 and 15 watt) and UV sheets (15 watt) for sampling adults (total of 368 trap nights spread over 132 nights). Other techniques employed sporadically included MV (mercury vapor) sheets, propane lantern sheet, fermenting fruit bait trails and traps, netting adults at flowers, and netting free flying adults.

We searched for larvae between June 14 and August 1, 2010–2016. Timing for larval searches was based on adult flight times and knowledge of larval phenology across the genus. To find larvae we scanned vegetation in various peatland habitats looking for wilted, discolored, or otherwise compromised plants. Any such plants were carefully dissected to reveal larvae or signs of larval presence such as burrows and frass. Our efforts were

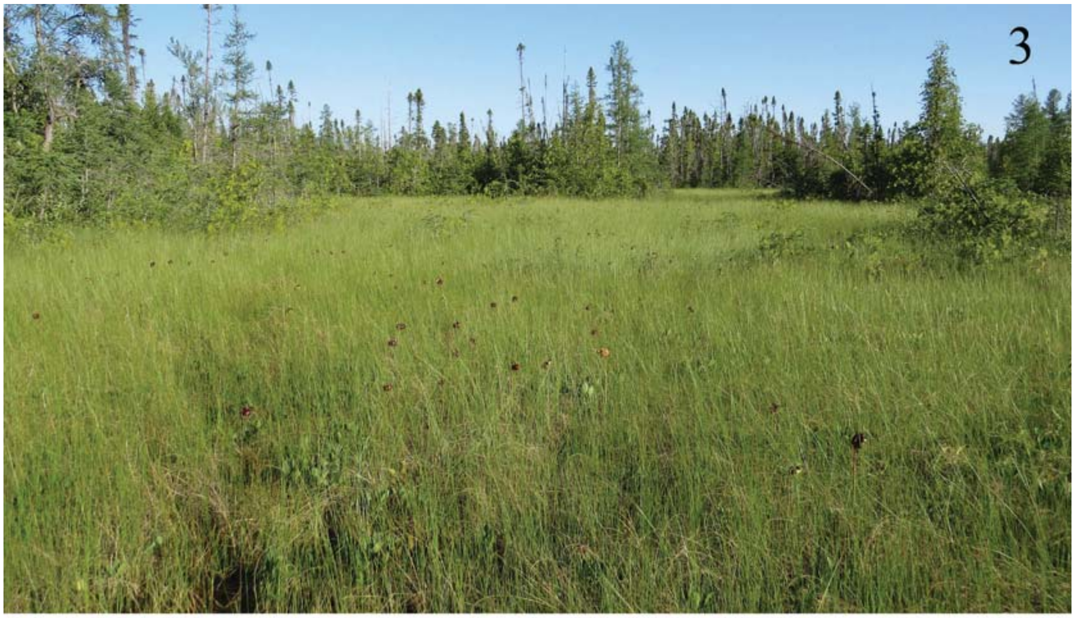


FIG. 3-4. **3**. *Papaipema aweme* habitat, Pine Creek Peatland, Roseau Co., Minnesota (June 23, 2016). Spring fen channel open graminoid rich fen; this is bordered by *Picea mariana* (*Thuja occidentalis-Larix laricina*) rich swamp. *Menyanthes trifoliata* is abundant. **4.** *Papaipema aweme* habitat, First Central Lake, Northern Region, Manitoba (August 21, 2016). Graminoid reticulate ribbed rich fen with *Picea mariana-Larix laricina-Sphagnum*-ericad tree islands; *Menyanthes trifoliata* is abundant.

biased to areas where adults were captured, and toward host plants with distributions that fit the general range of historical captures of the moth. We paid particular attention to the following plants: *Cladium mariscoides* (Muhl.) Torr. (Cyperaceae), *Menyanthes trifoliata* L. (Menyanthaceae), *Pedicularis lanceolata* Michx. (Scrophulariaceae), native genotype of *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae), *Sarracenia purpurea* L. (Sarraceniaceae), *Triglochin maritima* L. (Juncaginaceae), and various orchids (Orchidaceae).

Specimens collected by the authors were deposited in the University of Wisconsin-Madison Insect Research Collection (WIRC), Canadian National Collection (CNC), University of Minnesota Entomology Collection (UMSP) and the research collections of DRB and AM. Photographs were taken by KEJ with a Canon Powershot SX50 HS digital camera. Maps were created with Google Earth and Earth Point.

FIGS. 5–6. **5**. *Papaipema aweme* habitat, Deschambault Lake, Division No. 18, Saskatchewan (August 22, 2016). Narrow open graminoid rich fen water track through semi-treed *Picea mariana*-*Larix laricina*-*Sphagnum*-ericad rich to poor fen. *Menyanthes trifoliata* is abundant. **6.** *Papaipema aweme* habitat, Agassiz Peatland, Rainy River District, Ontario (August 29, 2016). Sparsely treed *Larix laricina*-graminoid-*Sphagnum*-ericad rich fen along featureless margin of patterned water track. *Menyanthes trifoliata* is abundant.

RESULTS

Fifty-nine specimens of *P. aweme* were documented from 2009–2016 at six new locations spanning 1,555 km from the Upper Peninsula of Michigan to eastern Saskatchewan (Fig. 1, Tables 1, 2). We collected 51 specimens from five sites (Michigan, Minnesota, Ontario, Manitoba, and Saskatchewan) which include 18 found as larvae (16 reared to adult, 2 preserved as larvae). Additional larvae were found but not successfully reared or preserved. Les Ferge collected 7 adults from the Lake Superior shoreline in Wisconsin and Jim Vargo took 1 adult at our Minnesota site.

Habitat. Sites where *Papaipema aweme* were sampled were mosaics of different peatland habitats, but all contained areas of open to sparsely treed graminoid rich fen (Tables 1, 2; Figs 3–6). These areas were sedge (Cyperaceae) dominated and very wet (quaking or at least with shallow standing water). *Carex lasiocarpa*

TABLE 1. *Papaipema aweme* positive adult survey results 2005–2016.

State/Prov.	Site (County/District)	Date	Collector	Method	Weather (Time)	#	Habitat
MI	Wanamaker Lake Peatlands (Luce)	2009 Sept. 10	DRB	8 watt UV trap		1 ♀	sandy conifer woodland raise bordering peatland (semi-treed poor to rich fen)
MN	Pine Creek Peatland (Roseau)	2014 Sept. 8	KEJ	15 watt UV trap	15—9°C; mostly cloudy night/light wind	1 ♂	wide graminoid-herb path (*Menyanthes trifoliata* common) through *Larix laricina* rich swamp (trees 7-10m); *Picea mariana* common in understory, few *Thuja occidentalis*; hummocky *Sphagnum*-feathermoss-ericad-herb-graminoid carpet; *Sarracenia purpurea*, *Betula pumila*, *Salix*, *Cornus* common
MN	Pine Creek Peatland (Roseau)	2015 Aug. 21	KEJ	15 watt UV trap	16–14°C; mostly clear/calm night	1 ♂	wide graminoid-herb path (*Menyanthes trifoliata* common) through *Larix laricina* rich swamp (trees 7-10m); *Picea mariana* common in understory, few *Thuja occidentalis*; hummocky *Sphagnum*-feathermoss-ericad-herb-graminoid carpet; *Sarracenia purpurea*, *Betula pumila*, *Salix*, *Cornus* common
MN	Pine Creek Peatland (Roseau)	2015 Aug. 21	KEJ	15 watt UV sheet	16–14°C; mostly clear/ calm night (0130 h)	1 ♂	spring fen forest; wide path through *Picea mariana* rich swamp (trees 6-10m) with hummocky *Sphagnum*-feathermoss-ericad-herb-graminoid carpet; few *Larix laricina*, *Thuja occidentalis*; near spring fen channels
MN	Pine Creek Peatland (Roseau)	2015 Aug. 21	KEJ	15 watt UV trap	16—14°C; mostly clear/calm night; scattered strong storms the following morning	9 ♂	spring fen channel; quaking graminoid rich fen (*Carex lasiocarpa* dominant, *Menyanthes trifoliata* abundant) bordering *Picea mariana* rich swamp (*Sphagnum*-feathermoss-ericad-herb-graminoid carpet) and border path; *Thuja occidentalis*, *Larix laricina*, *Dasiphora fruticosa*, *Betula pumila* common along margin
MN	Pine Creek Peatland (Roseau)	2015 Aug. 29	KEJ	net	18°C; mostly clear /calm night (2159 h)	1 ♂	weakly patterned ribbed fen; sparsely treed *Larix laricina*-graminoid-mixed moss-ericad-*Betula pumila*-*Dasiphora fruticosa* rich fen; *Picea mariana*, *Thuja occidentalis Menyanthes trifoliata*, *Sarracenia purpurea*, *Salix* common; near spring fen forest/channels
MN	Pine Creek Peatland (Roseau)	2015 Aug. 29	Jim Vargo	UV trap	19–12°C; mostly clear/calm night	1 ♂	spring fen forest/channel; *Picea mariana*-*Thuja occidentalis*-*Sphagnum*-mixed moss-ericad-graminoid rich swamp (trees 2-8m) bordering quaking graminoid rich fen; *Larix laricina*, *Dasiphora fruticosa* common
MB	First Central Lake (Northern Reg.)	2016 Aug. 21	KEJ	15 watt UV trap	13–7°C; partly cloudy/ mostly calm night	1 ♀	reticulate ribbed fen with tree islands; wet quaking graminoid rich fen with *Sphagnum*-ericad-*Picea mariana*-*Larix laricina* tree islands; *Menyanthes trifoliata*, *Sarracenia purpurea* common; scattered *Betula pumila*, *Salix*
SK	Deschambault Lake (Div. No. 18)	2016 Aug. 22	KEJ	15 watt UV trap	17–16°C; partly cloudy night/light wind; T-storms around 0200 h	14 ♂ 1 ♀	semi-quaking graminoid rich fen narrow water track through semi-treed *Larix laricina*-graminoid-mixed moss-ericad-herb rich fen; abundant *Menyanthes trifoliata*; *Betula pumila*, *Salix*, *Sarracenia purpurea* common
SK	Deschambault Lake (Div. No. 18)	2016 Aug. 22	KEJ	8 watt UV trap	17–16°C; partly cloudy night/light wind; T-storms around 0200 h	2 ♂	semi-quaking graminoid-*Menyanthes trifoliata* rich fen narrow water track through semi-treed *Larix laricina*-graminoid-mixed moss-ericad rich fen; *Betula pumila*, *Salix*, *Sarracenia purpurea* common
WI	Lake Superior shoreline (Bayfield)	2016 Aug. 28	Les Ferge	15 watt UV trap	18–15°C; mostly clear/calm night, T-storms around sunrise	7 ♂	Lake Superior coastal peatland; shrubby margin of wet graminoid rich fen (*Carex lasiocarpa* dominant); sparse *Larix laricina*
ON	Agassiz Peatland (Rainy River)	2016 Aug. 29	KEJ	8 watt UV trap	13–7°C; clear/ mostly calm night	1 ♂	wet sparsely treed *Larix laricina*-graminoid-*Sphagnum*-ericad rich fen; *Betula pumila*, *Menyanthes trifoliata*, *Sarracenia purpurea*, *Phragmites australis* common

Ehrh. was usually dominant, with numerous other sedges present including *Carex chordorrhiza*, *C. limosa* L., *C. livida* (Wahlenb.) Willd., *C. tenuiflora* Wahlenb., *Cladium mariscoides*, *Eleocharis compressa* Sull., *Rhynchospora alba* (L.) Vahl, and *Trichophorum alpinum*. Forbs were prominent and included *Menyanthes trifoliata*, *Sarracenia purpurea*, and *Drosera* spp. (Droseraceae). Brown mosses including *Scorpidium scorpioides* (Hedw.) Limpr. (Amblystegiaceae) were sometimes prominent. Raised areas of *Sphagnum* moss hummocks, adjacent to (or sometimes intermixed with) the graminoid rich fen habitats, were dominated by various ericaceous shrubs, stunted trees of *Larix laricina* (Du Roi) K. Koch (Pinaceae), *Picea mariana*, and/or *Thuja occidentalis*, and additional shrubs including *Betula pumila*, *Salix* spp. (Salicaceae), *Myrica gale* L. (Myricaceae), and *Dasiphora fruticosa*. Raised *Sphagnum* areas included well-developed strings and tear-drop tree islands in ribbed fens and transitions between open fen and rich swamp forests dominated by *Picea mariana* and/or *Larix laricina*. The raised *Sphagnum* areas graded into poor fen or poor swamp at some sites.

Two sites had distinct patterned peatland features, and three others had either subtle patterns or were near patterned features. The Pine Creek Peatland of Minnesota-Manitoba had both a well-developed complex of spring fen channels (Fig. 3; where most specimens were taken) and a ribbed fen (one specimen taken). First Central Lake in Manitoba featured a reticulate ribbed fen with tree islands (Fig. 4). Captures at Deschambault Lake in Saskatchewan were within a featureless water track (Fig. 5) which connected to a well-developed ribbed fen within 1 km. The Agassiz Peatland in Ontario (Fig. 6) was along the non-patterned margin of a large water track dominated by a well-developed ribbed fen with tear-drop tree islands. The larval collections at Wanamaker Lake Peatlands in Michigan were within a predominately featureless water track with subtle elements of strings and flarks.

Adults. Of the 41 specimens taken as adults (August 21–September 10), 34 were taken with 15 watt UV light traps, 5 were taken with 8 watt UV light traps, 1 was taken at UV sheet, and 1 was netted (Table 1). The netted and UV sheet specimens were taken at 2159 h and 0130 h, respectively, indicating *P. aweme* flies both before and after midnight, though our data does not establish ideal nightly temporal parameters for survey work. Temperatures ranged from 19 to 7°C. The three most productive nights (11 specimens on August 21, 2015; 16 specimens August 22, 2016; 7 specimens on August 28, 2016) were unseasonably warm (17–14°C) and featured strong storms during or the morning after the sampling night.

Larvae. We found 30 larvae (plus the burrow and parasitized remains of 1 larva) at two sites (Pine Creek Peatland in Minnesota-Manitoba; Wanamaker Lake Peatlands in Michigan) from June 23–July 24, 2016. Larvae were found boring in the lower stems and rhizomes of *Menyanthes trifoliata* in open graminoid rich fen habitats (Table 2, Figs 7–11). Plants harboring larvae were typically wilted and sometimes discolored (Fig. 8), though plants containing earlier instars were not always so obviously affected. Plants exhibiting these symptoms often did not harbor *P. aweme* but rather a variety of other invertebrates including sawfly larvae (Hymenoptera: Symphyta), snails feeding externally, and an unidentified small lepidopteran boring in the stems (burrows approx. 1–3cm long, much smaller than those of *P. aweme*).

The plants bored by *P. aweme* were relatively large (stem length approximately 20–30 cm, Fig. 8) and always rooted in a wet (quaking or at least with shallow standing water) sedge mat typically dominated by *Carex lasiocarpa*. Brown mosses (e.g., *Scorpidium scorpioides*) were sometimes prominent. We failed to find any sign of larvae in *M. trifoliata* rooted in other microhabitats such as atop *Sphagnum* hummocks. Both peatland complexes where we found larvae were very large (over 15 km2) but larvae were localized in relatively small areas of habitat (Fig. 12).

Larvae of *P. aweme* (Fig. 9) had markings that were consistent with the genus, with pale dorsal and subdorsal longitudinal stripes. These stripes were unbroken on abdominal segments 1 –4 and helped differentiate larvae of this species from those of congeners also present at the sites (McBride and Wiker 2017).

Larvae were reared in the lab on *Menyanthes trifoliata* stems and rhizomes. AM initially tried transferring larvae to potatoes, as is a common practice for rearing other species in the genus, but most fed very little after a few days and some ceased feeding entirely. These were transferred back to *M. trifoliata* where they resumed feeding activity. Pupation (Fig. 10) occurred within the stem (AM recorded pupation dates from July 7–31 with a mean pupal period of 17.5 days; n=9) and adults (Fig. 11) emerged July 24–September 3 (all rearings).

Companion species. We documented over 139 species in 18 families of Lepidoptera on the evenings and at the sites where *P. aweme* was taken 2009–2016. The most frequent associates are shown in Table 3. *Oligia minuscula* (Morrison) was the only species co-collected with every *P. aweme* capture. However, it is

TABLE 2. *Papaipema aweme* positive larval survey results 2010–2016. Numbers include failed rearings lacking vouchers.

State/ Prov.	Site (County/District)	Date	#	Habitat	Microhabitat of host ***Menyanthes trifoliata***
MB	Pine Creek Peatland (Eastman Reg.)	2016 June 23	2	spring fen channel; quaking graminoid rich fen (*Carex lasiocarpa* dominant) bordering *Picea mariana* rich swamp; *Thuja occidentalis*, *Larix laricina*, *Dasiphora fruticosa*, *Betula pumila* common along margin	plant growing in shallow water of quaking *Carex lasiocarpa-Menyanthes trifoliata-Sarracenia purpurea*-mixed sedge mat; brown mosses present
MN	Pine Creek Peatland (Roseau)	2016 June 23	8	as above	as above
MN	Pine Creek Peatland (Roseau)	2016 June 24	18	as above	as above
MI	Wanamaker Lake Peatlands (Luce)	2016 July 24	3	wet sparsely treed *Larix laricina*-graminoid rich fen; scattered *Sphagnum*-ericad hummocks; *Menyanthes trifoliata*, *Betula pumila*, *Dasiphora fruticosa*, *Viburnum cassinoides*, *Sarracenia purpurea* common; few *Picea mariana* plant growing in shallow water of flark-like *Carex lasiocarpa-Rhynchospora alba*-mixed sedge-*Menyanthes trifoliata* mat; brown mosses present	

nearly ubiquitous in northern Great Lakes peatlands and has a relatively long flight period (KEJ data July 6–October 5; n=124) and thus is not a useful indicator of either habitat or flight period of *P. aweme*. Better flight period indicators included *Papaipema appassionata* (Harvey) (KEJ data August 12–September 18; n=113) and *Hypocoena basistriga* (McDunnough) (KEJ data August 21–September 7; n=63). We did not find any conspicuously helpful habitat indicator moths, but combinations of the above species as well as others with specialized peatland niches (e.g., *Crambus awemellus* McDunnough) might prove useful, although this would certainly vary by region.

DISCUSSION

Historical localities. All five historical localities have (or had) *Menyanthes trifoliata* and rich fen habitats in the general vicinity. KEJ examined an extensive graminoid rich fen (part of a spring fen channel complex) with abundant *M. trifoliata* near Aweme, Manitoba (Fig. 13). The habitat there appeared ideal for *P. aweme*, but weather conditions were not optimal during sampling attempts in 2016. Judith Jones (pers. comm.) examined a fen with abundant *M. trifoliata* 7 km east of John Morton's capture site on Manitoulin Island. The fact that Morton only took one specimen in 20 years of sampling the area, and that strong winds gusted out of the east on the night of capture (Jones 2015) suggest this fen was the source population. Ms. Jones and Chris Schmidt sampled this fen and two others in 2016 without success, but sampling conditions were not ideal. Satellite imagery (Google Earth) reveals numerous fens on Beaver Island, Michigan that could have been the source of the dispersing individual captured offshore by Sherman Moore, and *M. trifoliata* has been recorded from the island (Reznicek et al. 2011). Most fens in the vicinity of Rochester, New York are largely degraded but some still include localized, high quality areas with *Menyanthes trifoliata* (Steven Daniel pers. comm.). Grand Bend, Ontario had a potentially suitable fen in the vicinity but this was drained sometime after the moth's capture there (Dale Schweitzer pers. comm.). Extensive surveying at this locality over several decades has failed to turn up any specimens (COSEWIC 2006). Thus, Grand Bend is the only historical locality where a persisting *P. aweme* population is doubtful.

Larval food plants. Although more effort is needed to search alternative food plants, a hypothesis that *P. aweme* larvae are monophagous on *Menyanthes trifoliata* is supported by our preliminary data. All recorded sites were in the vicinity of rich fen habitats that support or could have supported *M. trifoliata*. A

FIGS. 7–11. **7**. White frass visible on lower stem of *Menyanthes trifoliata* inhabited by *Papaipema aweme* larva; Pine Creek Peatland, Eastman Region, Manitoba (June 23, 2016); inset, a detailed view of frass and larval boring hole. **8**. Wilted and browned central petiole of *Menyanthes trifoliata* induced by *Papaipema aweme* larval boring; Pine Creek Peatland, Roseau Co., Minnesota (June 24, 2016). **9.** *Papaipema aweme* larva inside lower stem of *Menyanthes trifoliata*; Pine Creek Peatland, Eastman Region, Manitoba (June 23, 2016). **10**. *Papaipema aweme* pupa in lab, removed from burrow in stem of *Menyanthes trifoliata* (July 13, 2016), reared from larva collected at the Pine Creek Peatland, Roseau Co., Minnesota. **11**. *Papaipema aweme* adult resting on dead leaf of *Menyanthes trifoliata* in lab (August 5, 2016), reared from larva collected at the Pine Creek Peatland, Roseau Co., Minnesota.

FIGS. 12–13. **12**. Distribution of *Papaipema aweme* larvae (yellow circles) and adults (red squares n = 1-2; red star n = 9) at the Pine Creek Peatland on the Minnesota-Manitoba border. Habitats range from open rich fen to rich swamp; patterned types include spring fen channels (left) and ribbed fen (lower center). The points span 1.9 km. **13.** Potential *Papaipema aweme* habitat near Aweme, Manitoba (August 24, 2016). Spring fen channel open graminoid rich fen bordering *Larix laricina* (*Picea mariana*) rich swamp groves. *Menyanthes trifoliata* is abundant. A UV light trap is visible in the photo center.

restriction to this host plant, growing in this particular rich fen microhabitat, would explain the moth's rarity in collections.

At Michigan's Wanamaker Lake Peatlands, DRB and others attempted to re-document *P. aweme* 2010–2015 with over 143 trap nights targeting adults, and seven days searching for larvae. None was found despite targeting a wide variety of peatland habitats with diverse flora, including most of the targeted potential larval food plants mentioned above. Attempts prior to 2016, however, were isolated from habitats with plentiful *Menyanthes trifoliata* by 0.8 km or more. By contrast, once *M. trifoliata* was known as a host in 2016 (based on the Pine Creek Peatland discoveries) DRB and KEJ found larvae on the first attempt at the Wanamaker Lake Peatlands, and KEJ collected 19 adult specimens with only 22 trap nights/9 calendar nights of effort across Manitoba, Ontario, and Saskatchewan (of these, 12 trap nights/5 calendar nights were probably not suitable for proper survey given phenology and weather). The extensive negatives of 2010–2015 versus successes of 2016 could be readily explained if *P. aweme* was restricted to *Menyanthes trifoliata*.

Additional effort is also needed to search for larvae in *Menyanthes trifoliata* growing in other microhabitats such as among *Sphagnum* mosses (both in rich and poor fen habitats), in open water on the edge of peatland pools, or in partial shade of *Picea mariana* and *Larix laricina* rich swamps. Larval presence of *P. aweme* in these situations would expand the possibilities for new localities.

Negative sites. The 57 sites where we failed to document *P. aweme* give a misleading impression of rarity since prior to 2016, most of our sampling sites appear to lack suitable habitat (Fig. 2), at least in the vicinity of searches. In addition, many sites were sampled only once, and some of these under marginal survey conditions. We expect that additional effort at our "negative" sites with abundant *Menyanthes trifoliata* will disclose additional *P. aweme* localities.

Phenology. Our adult capture dates (August 21–September 10) averaged later than historical records (August 7–26) but this could be an artifact of insufficient sampling during advanced seasons. *Papaipema appassionata* specimens co-collected with *P. aweme* exhibited similar wear. We found relatively fresh *P. appassionata* as early as August 12 and as late as September 8, but appropriate *P. aweme* habitats were not sampled on early dates during advanced seasons. Thus surveys should adjust for advanced or delayed seasons and not rely solely on calendar dates.

Our larval captures were split between earlier instars (June 23–24) and mature larvae (July 24). Although *P. aweme* larval damage was not always obvious during the early dates, finding larvae was relatively easy since damage from other invertebrates was not yet widespread among the *M. trifoliata*. By contrast, the few larvae found on the latter date had all dramatically disfigured their hosts (brown and wilted), but the majority of plants in their vicinity had been sufficiently damaged by other invertebrates to make finding *P. aweme* larvae considerably more difficult. Pupae could conceivably be located in the host stems as was observed

TABLE 3. Other Lepidoptera co-collected with *Papaipema aweme* adults 2009–2016 on 3 or more nights (out of 7 total).

Species	# nights co-documented
Oligia minuscula (Morrison)	7
Nepytia canosaria (Walker)	6
Plusia magnimacula Handfield & Handfield	6
Endothenia hebesana (Walker)	5
Epinotia septemberana Kearfott	5
Hypenodes sombrus Ferguson	5
Lithomoia germana (Morrison)	5
Papaipema appassionata (Harvey)	5
Scoparia biplagialis Walker	5
Sutyna privata (Walker)	5
Xestia dilucida (Morrison)	5
Archips alberta (McDunnough)	4
Crambus bidens Zeller	4
Epiglaea apiata (Grote)	4
Eulithis testata (Linnaeus)	4
Helotropha reniformis (Grote)	4
Lambdina fiscellaria (Guenée)	4
Sympistis dentata (Grote)	4
Tolype laricis (Fitch)	4
Xestia smithii (Snellen)	4
Acleris variana (Fernald)	3
Amphipyra pyramidoides Guenée, 1852	3
Capsula subflava (Grote)	3
Cingilia catenaria (Drury, 1773)	3
Coenophila opacifrons (Grote, 1878)	3
Crambus albellus Clemens	3
Enargia decolor (Walker, 1858)	3
Eremobina claudens (Walker)	3
Eurois occulta (Linnaeus, 1758)	3
Fishia illocata (Walker, 1857)	3
Hypenodes palustris Ferguson	3
Prochoerodes lineola (Goeze)	3
Sparganothis sulfureana (Clemens)	3
Syngrapha epigaea (Grote)	3
Syngrapha octoscripta (Grote)	3

in captivity but this has yet to be confirmed in the wild. As with the adult surveys, larval searches should adjust for advanced or delayed seasons.

Biogeography. The new *P. aweme* localities and refined habitat associations suggest a much broader range of occupancy than is currently documented. The habitats where we found the moth, particularly the patterned peatland examples, are typical of the boreal forest region from Newfoundland to Alaska, and *Menyanthes trifoliata* is similarly distributed (Crum 1988; Wright et al. 1992; USDA, NRCS 2017, pers. obs.). *Papaipema aweme* localities south of the 50th parallel occur along the southern range edge of boreal peatlands, whereas those north of the 54th parallel lie within the habitat range core. Similarly, the Saskatchewan and northern Manitoba localities may lie within the core range of *P. aweme*, and are not necessarily at the northwestern range extreme as suggested by Figure 1. This is supported by the relative ease of the moth's discovery there, since both sites sampled north of the 54th parallel yielded positive results on the first attempt. The site in Manitoba sampled between the 54th and 53rd parallels had negative results but this was on a single night with poor weather (rainy and windy, with only 4 species of moths documented). Further exploration of peatlands at northerly latitudes should be productive both for the discovery of new populations and for range extensions. Promising areas for future surveys include the James Bay Highway in northwestern Quebec, Sundance (along the southern edge of the Hudson Bay Lowlands) in northern Manitoba, and the vicinity of Cold Lake in eastern Alberta.

Papaipema aweme is currently the most northerly known *Papaipema* (Eric Quinter, pers. com.), and given its habitat associations and potential for range extensions it may be the most widespread *Papaipema* in boreal North America.

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EXPLORING DNA BARCODES OF NEOTROPICAL AND AFROTROPICAL SPECIES OF *ECCOPSIS* ZELLER (LEPIDOPTERA: TORTRICIDAE)

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ABSTRACT. Originally described from Africa, the genus *Eccopsis* Zeller (Lepidoptera: Tortricidae) currently includes 25 Afrotropical and five Neotropical species. Adult morphological characters suggest that the Afrotropical and Neotropical species might not be congeneric. Here we present the first DNA sequences for Neotropical *Eccopsis* and use these data in a maximum likelihood (ML) analysis to evaluate the monophyly of the genus, and to examine the utility of DNA barcodes in separating the South American *E. galapagana* Razowski & Landry, 2008 and *E. razowskii* Vargas, 2011. Intraspecific and interspecific pairwise distances (K2P) were 0–0.5% and 4.9–5.2%, respectively, and each species was recovered as a distinct, well supported group of sequences (i.e., species) in the ML analysis. An analysis including barcodes of Afrotropical *Eccopsis* (four species), Afrotropical *Paraeccopsis* (one species), and Neotropical *Eccopsis* (two species) failed to recover *Eccopsis* as monophyletic. Consistent with previous suggestions based on adult morphology, this study highlights the necessity to reassess the congeneric status of Afrotropical and Neotropical species of *Eccopsis*.

Additional key words: *Acacia macracantha*, *Eccopsis galapagana*, *Eccopsis razowskii*, Fabaceae, *Prosopis alba*

As currently defined, the genus *Eccopsis* Zeller, 1852 (Lepidoptera: Tortricidae: Olethreutinae: Olethreutini) is represented in both the Afrotropical and Neotropical regions (Brown 2014, Gilligan et al. 2014a). The Afrotropical fauna includes 25 described species, including the type species, *Eccopsis* wahlbergiana Zeller, 1852, from South Africa (Gilligan et al. 2014a). The genus was first reported from the Neotropics by Razowski and Wojtusiak (2008), who described a species of *Eccopsis* from the mountains of Ecuador, which subsequently was transferred to *Megalota* Diakonoff, 1966 by Brown (2014). Later, three additional Neotropical species were described in *Eccopsis*, two from the Galapagos (Razowski et al. 2008) and one from Chile (Vargas 2011). Brown (2014) transferred two other species to the genus, resulting in a total of five described *Eccopsis* in the Neotropics. All five appear to be closely related to each other and very similar to Afrotropical *Eccopsis*. However, the generic assignment of the Neotropical species recently was questioned based on adult morphological characters (Brown 2014).

Two species of *Eccopsis* are known from the Atacama Desert of northern Chile: *E. galapagana* Razowski & Landry, 2008 and *E. razowskii* Vargas, 2011. *Eccopsis galapagana* was described from the Galapagos Islands, Ecuador, and it was subsequently discovered in Chile (Vargas 2011) and Colombia (Gallego et al. 2012). It is apparently widespread in western South America where its larvae feed on Fabaceae (Vargas 2011, Gallego et al. 2012). In Colombia, *E. galapagana* can be a serious pest in silvopastoral systems of *Prosopis juliflora* under outbreak conditions (Gallego et al. 2012). In contrast, *E. razowskii* appears to have a more restricted geographic range; it is known only from the coastal valleys of northern Chile where its larvae feed on *Acacia macracantha* Willd. (Vargas 2011).

DNA barcodes are useful for exploring biodiversity and taxonomy, especially in concert with other character sources (Brown et al. 2014a, Gilligan et al. 2016, Escobar-Suárez et al. 2017, Razowski et al. 2017). Barcodes also can be used to identify immature stages of insects, including Lepidoptera, providing knowledge of their trophic interactions when rearing larvae to obtain the adults is difficult or impossible (Gossner & Hausmann 2009, Hausmann & Parra 2009, Frye & Robbins 2015). This application is important in studying trophic ecology (Hrcek et al. 2011) or species of economic concern (Shashank et al. 2015), such as *E. galapagana* (Gallego et al. 2012).

The objectives of this study are to provide the first DNA barcode sequences of *Eccopsis* for the Neotropical Region and to provide a preliminary study of the relationships between Afrotropical and Neotropical *Eccopsis*. We also assess the usefulness of DNA barcodes in separating and identifying *E. galapagana* and *E. razowskii*.

MATERIALS AND METHODS

Specimens

Larvae of *E. galapagana* and *E. razowskii* were collected on *Prosopis alba* in the city of Arica, and on *Acacia macracantha* in the Azapa and Chaca valleys, in the Atacama Desert of northern Chile, from March 2014 to January 2015 (Table 1). The larvae were brought into the laboratory in plastics vials, where they were kept at room temperature. New leaves were added periodically until larvae were ready to pupate. Some pupae were preserved in ethanol 95% at -20°C for DNA extraction; others were kept in plastic vials to obtain adults, which were deposited as vouchers in the Colección Entomológica de la Universidad de Tarapacá (IDEA), Arica, Chile. In addition, two specimens of *E. galapagana* reared from *Prosopis pallida* from the Tumbes Region in northwestern Peru were included (vouchers in the T. M. Gilligan collection, Colorado, USA).

DNA extraction and sequencing

For Chilean specimens, genomic DNA was extracted from pupae following procedures described in Huanca-Mamani et al. (2015). PCR amplification and sequencing of the DNA barcode fragment of the COI gene were performed by a commercial facility (Macrogen, South Korea) using the primers LEP-F1 (5′-ATTCAACCAATCATAAAGATAT-3′) and LEP-R1 (5′-TAAACTTCTGGATGTCCAAAAA-3′) developed by Hebert et al. (2004). PCR conditions were those described in Vargas et al. (2014). For Peruvian specimens, genomic DNA was extracted from legs, amplified using the Hebert et al. (2004) primers, and sequenced according to procedures described in Gilligan et al. (2014b).

Data analysis

The software MEGA6 (Tamura et al. 2013) was used to align the sequences with MUSCLE (Edgar 2004), to calculate the mean nucleotide composition, and to calculate pairwise distances among sequences according the Kimura 2-parameter (K2P) model (Kimura 1980). The number of haplotypes, the number of variable sites, and the number of parsimony informative sites were determined using the software DnaSP (Librado & Rozas 2009).

A maximum likelihood (ML) analysis (Felsenstein 1981) was performed to assess the evolutionary relationships of the two Neotropical *Eccopsis* with four Afrotropical representatives of the genus. Additional DNA barcode sequences (658 bp) were downloaded from GenBank (Benson et al. 2013) and BOLD (Ratnasingham & Hebert 2007), including those of congeneric species and some of species belonging to the closely related genera *Cosmorrhyncha* Meyrick, 1913, and *Paraeccopsis* Aarvik, 2004; also, one sequence of a representative Tortricinae, *Eugnosta percnoptila* (Meyrick, 1933), was used to root the tree (Regier et al. 2012). The ML analysis and the selection of the best model to describe the substitution pattern were performed in MEGA6 following the procedures described by Hall (2013). The General Time Reversible model with invariable sites (GTR + I) was selected previous to ML analysis according the Bayesian Information Criterion (BIC). The bootstrap method (Felsenstein 1985) was used with 1,000 replicates to assess the statistical support of the clades.

RESULTS

DNA barcodes

Eighteen sequences of DNA barcodes (658 bp) were obtained for *E. galapagana* (n = 7 from Chile; n = 2 from Peru) and *E. razowskii* (n = 9), the mean nucleotide composition of which was 39.14% (T), 15.73% (C), 30.54% (A) and 14.59% (G). Pairwise distances (K2P) among the 18 sequences of the two South American *Eccopsis* were between 0.0–0.5% at the intraspecific level, and 4.9–5.2% at the interspecific level. Three haplotypes were detected in the *E. galapagana* sample, which were differentiated by three mutations: two transitions (T-C, site 238; and G-A, site 493) and one transversion (G-C, site 620). Three haplotypes were found in the *E. razowskii* sample, which were defined by two mutations: one transversion (A-T, site 281) and one transition (A-G, site 424) (Table 2).

ML analysis

The alignment for the ML analysis was composed of 33 DNA barcode sequences (658 bp), including the 18 newly reported here and 15 downloaded from GenBank and BOLD (Table 1). The mean nucleotide composition of all these sequences was 38.9% (T), 15.5% (C), 30.9%

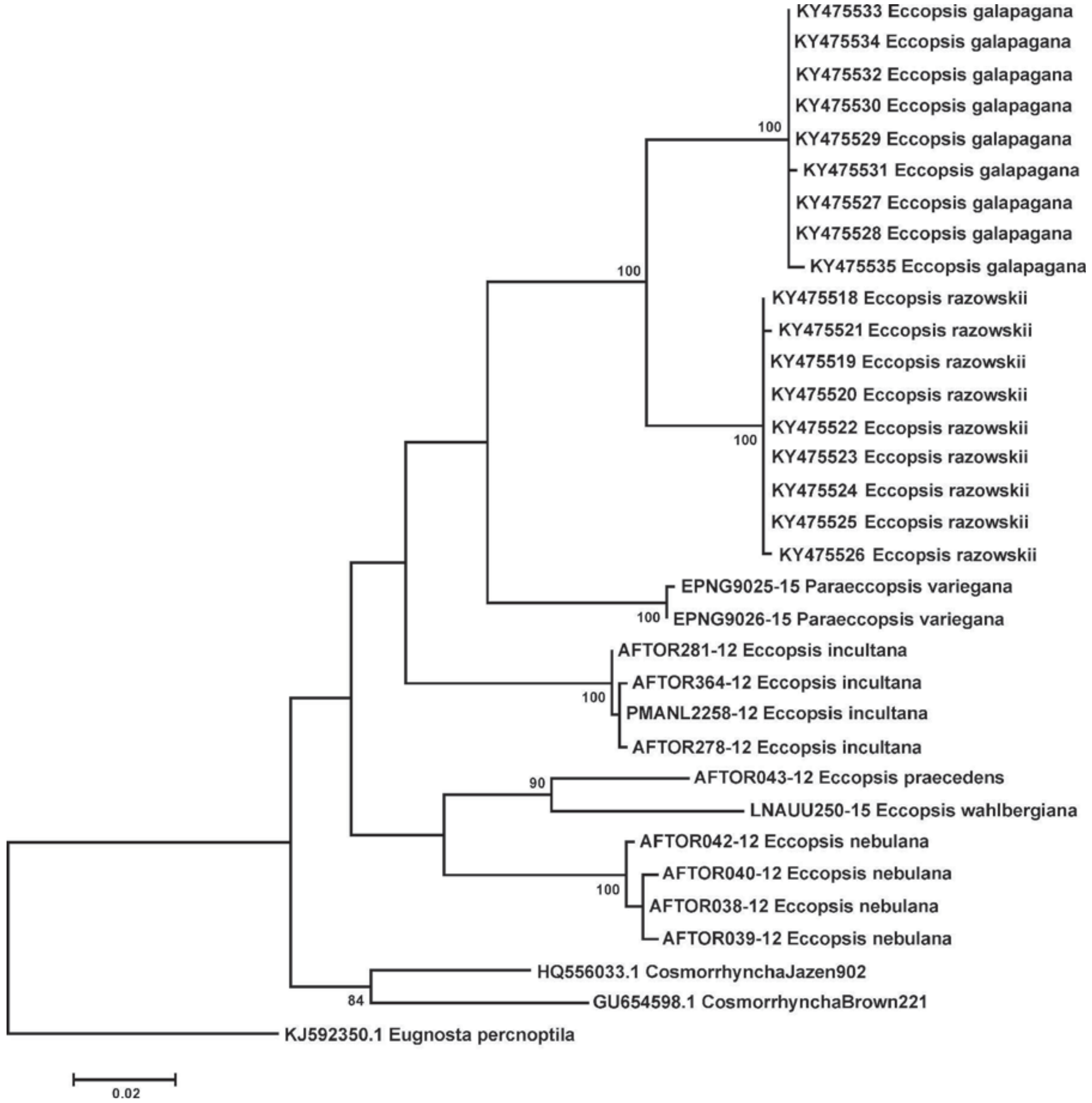

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FIG. 1. Maximum likelihood tree of *Eccopsis* and *Paraeccopsis* species based on sequences of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene. Bootstrap values >70 are shown at nodes.

(A) and 14.7% (G). Overall, the alignment had 186 variable sites, 148 of which were parsimony informative; no insertions or deletions were found. The lowest pairwise distances (K2P) among the Neotropical *Eccopsis* and *Paraeccopsis* were 9.3% with *E. galapagana* and 8.5% with *E. razowskii*.

The nodes representing each of the two Neotropical species of *Eccopsis* were strongly supported, and the cluster formed by these two species was also strongly supported (Fig. 1). However, an *Eccopsis* group including both Afrotropical and Neotropical representatives was not supported; instead, the genus was found to be polyphyletic. Although single gene trees are no substitute for rigorous phylogenetic analyses, the results do provide limited insight into relationships, or at least similarity, among the species. The type species of *Eccopsis* (*E. wahlbergiana*) clustered with two other Afrotropical representatives: *E. praecedens* Walsingham, 1897 and *E. nebulana* Walsingham, 1891. Although *E. wahlbergiana* clustered with *E. praecedens* with high support, the association of this clade with *E. nebulana* was only weakly supported (67% bootstrap). *E. incultana*

TABLE 1. Voucher data of the DNA barcode sequences (658 bp) used in the analyses of this study.

Species	Country	Locality	Voucher	Accession	Reference
Eccopsis razowskii	Chile	Azapa	IDEA003-02-01	KY475518	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-24	KY475519	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-25	KY475520	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-26	KY475521	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-27	KY475522	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-28	KY475523	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-29	KY475524	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-30	KY475525	This study
Eccopsis razowskii	Chile	Chaca	IDEA003-02-31	KY475526	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-01	KY475527	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-02	KY475528	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-03	KY475529	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-04	KY475530	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-05	KY475531	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-06	KY475532	This study
Eccopsis galapagana	Chile	Arica	IDEA003-03-07	KY475533	This study
Eccopsis galapagana	Peru	Tumbes Region	TMG-802	KY475534	This study
Eccopsis galapagana	Peru	Tumbes Region	TMG-803	KY475535	This study
Paraeccopsis variegana	Kenya	Mpala Ranch		EPNG9025-15	BOLD
Paraeccopsis variegana	Kenya	Mpala Ranch		EPNG9026-15	BOLD
Eccopsis incultana	Nigeria	Int. Inst. Trop. Ag.		AFTOR278-12	BOLD
Eccopsis incultana	Nigeria	Int. Inst. Trop. Ag.		AFTOR281-12	BOLD
Eccopsis incultana	Kenya	Mpala Res. Centre		AFTOR364-12	BOLD
Eccopsis incultana	Nigeria	Int. Inst. Trop. Ag.		PMANL2258-12	BOLD
Eccopsis nebulana	Kenya	Ololua Forest		AFTOR038-12	BOLD
Eccopsis nebulana	Kenya	Muhaka Forest		AFTOR039-12	BOLD
Eccopsis nebulana	Kenya	Arabuko-Sokoke Forest		AFTOR040-12	BOLD
Eccopsis nebulana	Kenya	Muhaka Forest		AFTOR042-12	BOLD
Eccopsis praecedens	Kenya	Kamwana Forest		AFTOR043-12	BOLD
Eccopsis wahlbergiana	Somalia	Baidoa		LNAUU250-15	BOLD
Cosmorrhyncha sp. (Brown221)	Costa Rica	Area de Conservacion Guanacaste		GU654598.1	GenBank
Cosmorrhyncha sp. (Janzen902)	Costa Rica	Area de Conservacion Guanacaste		HQ556033.1	GenBank
Eugnosta percnoptila	Kenya	Kereta Forest		KJ592350.1	GenBank

(Walker, 1863) was not clustered with any other Afrotropical *Eccopsis*. Although *P. variegana* Agassiz & Aarvik, 2014 was placed as a sister group of the Neotropical group of *Eccopsis*, the statistical support of this relationship was low.

DISCUSSION

These are the first sequence data for Neotropical *Eccopsis* (i.e., *E. galapagana* and *E. razowskii*) and the first study to examine relationships between the Neotropical and Afrotropical members of the genus using DNA sequences. Although a number of barcodes of Afrotropical *Eccopsis* are available on BOLD and in GenBank, the only previously published DNA barcodes for *Eccopsis* are two Afrotropical species (Brown et al. 2014b). The sequences of *E. razowskii* are particularly useful because eight of the specimens used for DNA extraction were collected at the type locality (Chaca Valley). The barcode data for *E. galapagana* may have slightly less fidelity to that species because it was described from the Galapagos Islands.

The high genetic divergence at the interspecific level compared with the low divergence at the intraspecific level suggests that DNA barcodes can be successfully used to separate *E. galapagana* and *E. razowskii*. Furthermore, the sequences of each of the two species were recovered as groups reciprocally monophyletic with high statistical support in the ML analysis (Fig. 1). As already reported for other study systems involving immature stages of Lepidoptera (Gossner & Hausmann 2009, Vargas et al. 2014, Rivera-Cabello et al. 2015, Frye & Robbins 2015, Shashank et al. 2015), DNA barcodes could be useful in studies focused on the identification of immature stages of the Chilean species of *Eccopsis*, such as surveys of larvae on additional Fabaceae to determine host breadth.

The high statistical support found in the ML analysis for the clade formed by *E. galapagana* and *E. razowskii* is an interesting result in favor of the probable monophyly of the Neotropical species currently included in *Eccopsis* (Brown 2014). However, additional Neotropical representatives should be included in future analyses. Furthermore, it is probable that a number of Neotropical species are waiting to be discovered and studied; thus, additional work on the alpha taxonomy of the South American fauna and additional taxon sampling are required to reach more meaningful phylogenetic conclusions.

Although sequences of only six species of *Eccopsis* were included in the ML analysis, the preliminary results argue against the monophyly of the genus as currently defined, and this corroborates the conclusions of Brown (2014) based on morphology. There is little doubt that Afrotropical and New World members of *Eccopsis* are closely related. However, New World *Eccopsis* appears to be the sister-group to the African *Paraeccopsis* based on forewing shape and maculation (i.e., the forewing is longer and narrower in both genera than in Afrotropical *Eccopsis*, with a somewhat *Lobesia*-like forewing pattern). Also, polymorphism or extreme individual variation in forewing markings is the norm in New World *Eccopsis* and *Paraeccopsis*, whereas it is absent in Afrotropical *Eccopsis*. New World *Eccopsis* and *Paraeccopsis* also share the absence of the hindwing anal roll characteristic of many Olethreutini including *Eccopsis*. In the male genitalia of *Eccopsis*, *Cosmorryncha*, and other Neopotamiae group genera (sensu Aarvik 2004), the apex of the uncus is usually conspicuously bilobed with each lobe bearing a patch of ventrally-projecting setae, whereas it is blunt, rounded, or only slightly emarginate apically in New World *Eccopsis* and *Paraeccopsis*, often with a "crown" of setae rather than two patches. And whereas species of many genera of the Neopotamiae group have a digitate process from the costa of the valva (e.g., *Eccopsis*, *Cosmorryncha*, *Megalota*, *Metendothenia*), this process is entirely absent in New World *Eccopsis* and *Paraeccopsis*.

Despite the low statistical support (52%), the African *P. variegana* was recovered as the nearest species to the

TABLE 2. Nucleotide variation among haplotypes of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Eccopsis galapagana* (n = 9) and *E. razowskii* (n = 9) from Peru and Chile. ([a]) "-" indicates nucleotide identity to the first haplotype of the respective species. ([b]) Ts: transition, Tv: transversion.

Haplotype	Variable sites([a,b])			n
	Eccopsis galapagana			
	238	493	620	
	Ts	Ts	Tv	
H1EG	T	G	G	7
H2EG	-	-	C	1
H3EG	C	A	-	1
	Eccopsis razowskii			
	281	424		
	Tv	Tv		
H1ER	A	A	7	
H2ER	-	G	1	
H3ER	T	-	1	

two Neotropical *Eccopsis* based on the lowest K2P divergence. Although our results did not capture the monophyly of Afrotropical *Eccopsis*, because *E. incultana* did not clustered with any "congeneric" species, Afrotropical *Eccopsis* appear to be monophyletic based on morphology. The lack of support along the "backbone" of the phylogenetic tree is not unusual given the relatively short length of COI and its relatively rapid rate of evolution (McDonagh et al. 2016). Other data also support the conclusion that *Eccopsis*, as currently defined, is not monophyletic. Gallego et al. (2012) indicated a biological difference, as the Neotropical species appear to be restricted to Fabaceae, whereas many African representatives are known to be polyphagous (Diakonoff 1977, Aarvik 2004, Gallego et al. 2012, Brown et al. 2014b). Recently, Vargas-Ortiz et al. (2017) highlighted the potential usefulness of the external morphology of the immature stages to help solve taxonomic problems in *Eccopsis*.

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LIFE CYCLE AND ETHOLOGICAL NOTES ON *BURCA BRACO BRACO* (HERRICH-SCHÄFFER, 1865) (HESPERIIDAE: PYRGINAE)

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ABSTRACT. In this study, we describe for the first time the immature stages of a *Burca* species: eggs, five larval instars, prepupa, and pupa. Morphometric measurements for each stage are given. Based on the measures of head width and growth rates, we recognize the possible presence of non-typical individuals with more than five instars. We describe four larval shelters types that are associated with specific instars. Each individual constructs three to four shelters during its larval development. We also report notes on feeding patterns and oviposition.

Additional keywords: *Burca*, *Croton*, Hesperiidae, skippers, larval shelters, immature stages

The genus *Burca* (Hesperiidae: Pyrginae: Carcharodini) was described by Bell & Comstock (1948). These butterflies are dark brown to blackish brown with the forewing rounded along the outer margin and with the hindwing margin angular. Male secondary characters are variable and palpal coloration is a useful character for species determination (Smith et al. 1994). *Burca* is closely related to *Nisoniades*, *Noctuana* and *Staphyllus* (Warren et al. 2008). *Burca* is restricted to Cuba, the Bahamas, and Hispaniola, where it is represented by five species endemic to the West Indies (Bell & Comstock 1948, Smith et al. 1994).

Prior knowledge concerning the natural history of *Burca* species is limited to data on geographic distribution and nectar sources (Alayo & Hernández 1987, Smith et al. 1994). Recently published were the first records of host plants; *Burca b. braco* caterpillars feed on *Croton lucidus* (Euphorbiaceae) (Núñez 2001) and *C. glabellus* (Lauranzón et al. 2013), while *Burca c. concolor* uses *C. sagraeanus* and *C. origanifolius* (Fernández 2004). Immature stages and their behavior, however remain undescribed. Knowledge of the natural history in lepidopterans is necessary for ecological studies (Young 1972). In this paper, we describe *Burca b. braco* immature stages and provide ethological notes related to larval shelter construction, oviposition behavior, and additional nectar sources.

MATERIALS AND METHODS

Field observations of immature specimens were conducted at Piedra Alta (23010' N and 81059' W) and Boca de Canasí (23009' N, 81046' W). Both places are close to Cuba's northern coastline and are located at 45 and 65 km E from Havana City respectively. Habitats at Piedra Alta were a characteristic dry coastal scrubland, while a microphyllous evergreen forest predominated at Boca de Canasí (Capote & Berazaín 1984). Both habitats supported abundant *Croton lucidus*. Field observations were taken between 09:00 and 16:00 hours.

One hundred and fifty-eight eggs were collected from host plants, 39 from September to November 2000, and 119 from March 2006 to April 2007. Each egg was placed alone in a Petri plate. We measured the perpendicular base diameters (d1 and d2) and height (h) with an ocular micrometer coupled to a stereoscope (error=50 μm). To describe the array of the egg's vertical ridges were placed into three categories: (i) complete ridges (CR), with one extreme on base and the other on micropyle, (ii) incomplete ridges with one extreme on basis and the other interrupted (IRB), and (iii) incomplete ridges with one extreme on micropyle and the other interrupted (IRM).

Ninety-seven caterpillars were maintained in captivity, 54 reared from eggs and the others collected as larvae in nature. Larvae were provided with fresh *C. lucidus* leaves and cleaned every day to remove fecal pellets, leftovers and head capsules. Head width (HW) was measured for every larva and in every instar, while body length (L) was measured only for first and second instars. Both measurements were taken with the micrometer described above. For chaetotaxy of first instar, five individuals were sacrificed and photographed

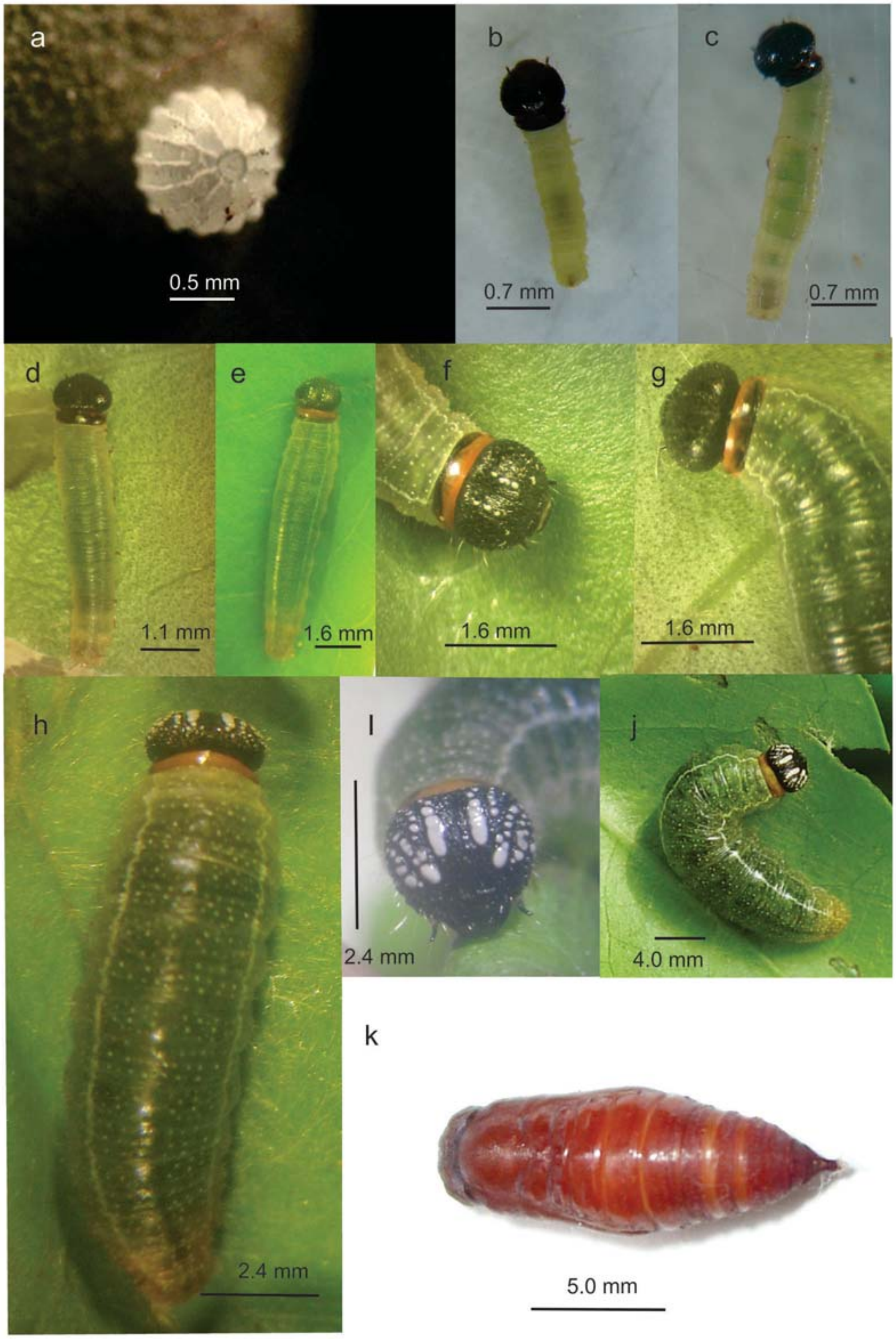

FIG. 1. Immature stages of *Burca braco braco*. (**a**) Egg in upper view, (**b**) upper view of first instar just after eclosion, (**c**) same as (**b**) but after eating, (**d**) upper view of second instar, (**e**) upper view of third instar, (**f**) detail of the head of third instar with typical white spots, (**g**) same as (**f**) but without white spots , (**h**) upper view of fourth instar, (**i**) detail of the head of fourth instar, (**j**) upper view of fifth instar, (**k**) pupa.

by a scanning electron microscope and by a Zeiss AxioCam MRc 5 video camera attached to a Carl Zeiss Discovery stereomicroscope. We follow Hinton's (1946) and Stehr's (1987) terminology for setae. Descriptions of larval shelters were referenced by collection time in nature, after Greeney (2009).

Pupae were obtained from larvae reared in captivity. They were maintained in a 5-liter container to assure that adults could extend their wings after emergence. We measured the major thoracic width of pupa in dorsal view (MTW) and the body length of pupa (LP) from vertex to cremaster. Both measurements were taken with a slide gauge (error=0.05 mm). We also measured the weight of pupae with a scale (error=0.001 g).

RESULTS

Life cycle and description of immature stages

Eggs: Eggs are light green when laid, and then change to light gray or light orange. Infertile eggs are recognizable because they are transparent white. Vertical ridges are white. Eggs are vertical with flattened base and micropyle (Fig. 1a). Base diameters are very similar and larger than height (Table 1), so the egg form is hemispherical. There is a large variation in number of ridges, and also in the three categories CR, IRB and IRM (Table 2). Egg duration was recorded in two cases: 5 and 6 days.

We could not rear any larva from the first to the last instar. However, we did determine that *Burca b. braco* larvae have five instars based on all measurements of HW (Fig 2). The head is rounded, robust, heart shaped and wider than the prothorax in all instars (Figs. 1b–j). The head is widest at the mid region in a frontal view. The body is tapering and slim in the first instar. In subsequent instars, the body becomes larger and more robust, changing proportions between the head and body.

First instar (Figs. 1b, c): At birth L=2.31 ± 0.27 mm (min 1.85, max 3.00 mm, N=49). HW=0.70 ± 0.05 mm (min 0.60, max 0.80 mm, N=56). The body is yellowish-white and the head is glossy black (Fig. 1b). Prothorax is dorsally reddish-brown and ventrolaterally light red. The mouth parts are beige. The first pair of legs are light red, while the second and third pair of legs and prolegs have the same coloration as the body. The tarsi are black. Primary setae are tiny, hair-like or Y shape (Fig. 3). None of our first instar larvae would feed in captivity so we were unable to note any changes in coloration following feeding. However, we collected two first instar larvae in nature with dull green bodies and that were slightly larger: 3.50 and 4.25 mm, respectively (Fig. 1c).

Second instar (Fig. 1d): Similar to the two individuals mentioned above but with the coloration

TABLE 1. Eggs morphometric means of *Burca braco braco* (d1 and d2=base diameters and h=height)

	N	X	SD	Min	Max
d1 (mm)	158	1.08	0.06	0.90	1.20
d2 (mm)	158	1.06	0.07	0.85	1.20
h (mm)	117	0.89	0.08	0.75	1.05

TABLE 2. Number of vertical ridges and their categories (CR=complete, IRB=incomplete with one extreme on base, IRM= incomplete with one extreme on micropyle) arraying eggs of *Burca braco braco* . Number of eggs analyzed = 70.

	Mode	Min	Max
CR	6	3	10
IRB	11	6	16
IRM	5	1	6
Total	21	17	25

TABLE 3. Substrate surfaces used in shelters constructed by different instars of *Burca braco braco* .

Surface/Instars	1st	2nd & 3rd	4th & 5th
Upper-leaf	1	11	6
Under-leaf	15	14	6
Two leaves joined	0	0	11

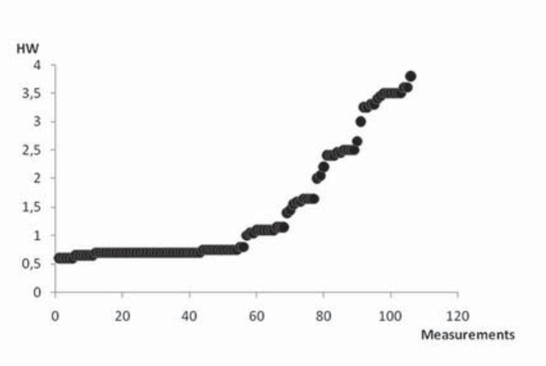

FIG. 2. Dispersion of measurements of head width of *Burca braco braco* caterpillars.

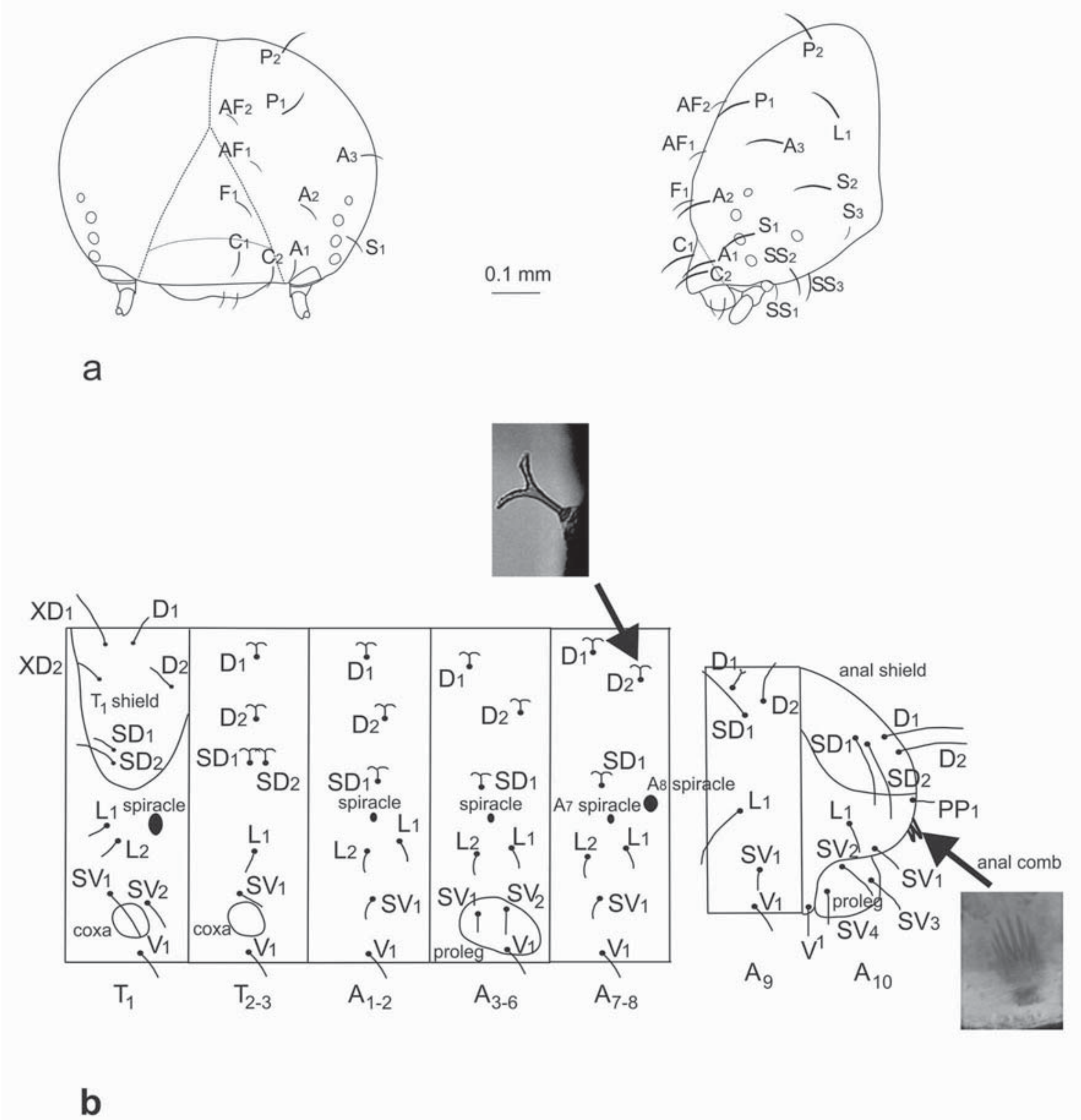

FIG. 3. Chaetotaxy of first instar of *Burca braco braco*. (**a**) Head scheme in frontal view (left) and lateral view (right), and (**b**) segments of the body scheme with details of "Y" shape setae and anal comb: T=Thoraxic segment, A=Abdominal segment.

darker. There are no changes in coloration of the head and prothorax. L=3.97 ± 0.49 mm (min 3.40, max 4.25, N=3). HW=1.10 ± 0.05 mm (min 1.00, max 1.15 mm, N=12).

Third instar (Figs. 1e–g): The body is olive green with the last three abdominal segments dark orange. The prothorax is orange and the head is black with two lines of tiny white elliptical spots (Figs 1e, f), rarely absent (Fig. 1g). There are two slight white longitudinal lines at both sides of the dorsal region from mesothorax to the end of the body. HW=1.58 ± 0.09 mm (min 1.40, max 1.65 mm, N=9).

Fourth instar (Figs. 1h, i): The head has a great number of elliptical or sub-rectangular white spots on the vertex. These sometimes reach the epicranial notch dorsally and the genae ventrally. These spots are arrayed in five to seven lines. The internal spot lines run parallel to the ecdysial cleavage line, while most external lines are perpendicular to the frontoclypeus. Major spots are placed in the internal lines, and in every line the largest spot is the lowest. The number of points by line differs between individuals. Even in the same individual, the arrays of spots from left and right sides of the head are different (Fig. 1i). The rest of head is black while the

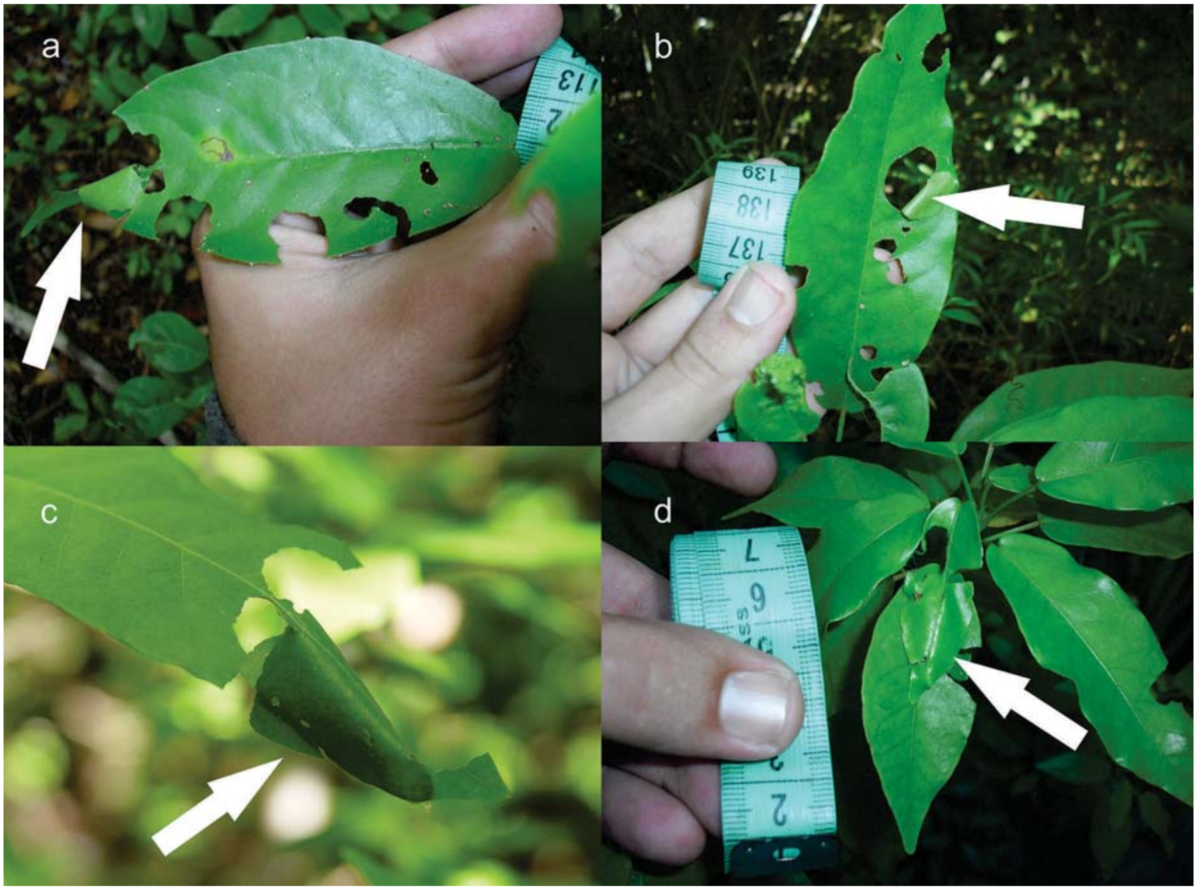

FIG. 4. Larval shelters of *Burca braco braco*. (**a**) Usual shelters type 5 for second and third instars, (**b**) non-usual shelters type 3 of a third instar, (**c**) usual shelters type 5 for fourth and fifth instars, (**d**) usual shelters type 2 for fourth and fifth instars. Shelter classification of Greeney (2009).

prothorax is orange. The color of the body is similar to the third instar but is lighter. In the dorsal region of the three last abdominal segments color changes gradually from orange-green to orange-brown at the end of the body. The two longitudinal white lines are more conspicuous than in third instar. Except for the mid-dorsal line and prothorax, the body is uniformly covered by a large number of short white setae. The base of the setae are also white, giving the body the appearance of being covered with tiny white dots. HW=2.39 ± 0.19 mm (min 2.00, max 2.65 mm, N=13).

Fifth instar (Fig 1j): Not very different from the fourth instar. The body color is lighter. Longitudinal lines and head spots are more conspicuous. The mandibles are black, while the labrum varies from yellowish brown to reddish brown, sometimes both. Antennae are light gray and yellowish on base. Maxillary palpi are light gray too, but with the extremes and setae reddish brown. There are more setae on body than in the fourth instar, but they are so short that the body looks bald. There is a dark green mid dorsal line. Legs are light greenish brown with tarsi reddish brown. Spiracles are elliptical and orange but paler than the last abdominal segments, except the bigger thoracic spiracles, which have the same color of prothorax. Prolegs are very pale, almost transparent. When larvae become prepupa (N=3), they transform to the orange-brown coloration gradually from last abdominal segment toward the mesothorax. At that point, the body clarifies from yellowish green to pale yellow. The prothorax transform the orange coloration to pale orange, while the head changes from black to dull brown. Head spots become more inconspicuous and gain coloration like bronze. HW=3.38 ± 0.18 mm (min 3.00, max 3.80 mm, N=13).

Pupa (Fig. 1k): We never observed pupae in the field. They are greenish brown in the first hours. When the cuticle hardens it becomes bright reddish brown, just prior to ecolsion it becomes dull dark brown. The labrum and the large pronounced edge of the thoracic spiracle are black. Although the body appears bald, the thorax

FIG. 5. Usual way of laying eggs for *Burca braco braco*, singly and in upper-surface of leaves of *Croton lucidus*.

and abdomen are actually covered by tiny white setae. The frontoclypeus and eyes are covered by tiny white setae as well, but a little longer that mentioned above. The only conspicuous setae are the large white ones on the cremaster. The wings pads totally cover the first two thoracic segments, mostly the third one, and the first three abdominal segments. Frontoclypeus and vertex are noticeably wrinkled while the rest of the body is quite smooth. LP=15.37 ± 0.77 mm (min 14.00 mm, max 16.1 mm, N=7). MTW was measured in two individuals: 5.13 and 5.35 mm, respectively. Weight measured in these two individuals was 256 and 199 mg, respectively.

Ethology

After eclosion, the larva eats the upper half of egg shell. All instars of *Burca b. braco* feed on *Croton lucidus*. Larvae construct shelters with sections of a leaf or complete leaves of the host plant modified with silk and cuts. We never observed larvae outside their shelters. These structures have been never damaged by feeding activity. On the other hand, the rest of the substrate leaf (or leaves) of shelter and/or very nearby leaves show some damage attributed to feeding activity. Architecture of shelters varies between instars. First to third instars inhabit type 5 shelters (N=40) with the two cuts in the same margin (Fig. 4a). Unlike second and third instars, first instar shelters are rarely folded to upper-leaf surface (Table 3). Larvae of second and third instars seldom construct circular type 3 shelters (N=1) (Fig. 4b). Fourth and fifth instars construct either type 5 shelters (N=12) with cuts in opposite margins (Fig. 4c) or type 2 (N=11) (two leaves joined) shelters (Fig. 4d). Sometimes we observed close to an occupied shelter, other recently abandoned shelters used by previous instars. We never found shelters with feces accumulations. In captivity, larvae could not construct shelters as they do in nature. When they are disturbed, they react bending laterally their body to cover the head with the abdomen, or vomiting, or attacking with their mandibles.

We observed females laying eggs twice. The vast majority of the eggs observed (167) were laid singly (164) and on the upper-leaf surface (165) (Fig. 5). Adults typically fly no more than 3 m from the ground and move quite rapidly between shrubs. In resting position, they extend their two pairs of wings in the same horizontal plane of the body. We recorded four nectar sources: *Croton lucidus* (Euphorbiaceae), *Duranta erecta* (Verbenaceae), *Morinda royoc* (Rubiaceae) and *Chiococca alba* (Rubiaceae).

DISCUSSION

The typical five instars of larval development of *Burca b. braco* (see Fig. 1) are similar to the records of most Pyrginae (Scudder 1889, Moss 1949, Torres 1998). However, according to our data the presence of non-typical individuals with more than five instars is possible (see Fig. 2). The intra-specific variations in number of instars, even with the existence of a typical number, are quite normal in Lepidoptera (Knopf & Habeck 1976, Otazo et al. 1984, Farr 2002, Holland 2003, Barro 2006). If some of our individuals are non-typical in the number of instars, our estimated HW and especially their variances, are biased. We can expect that bias is larger in major instars, and logically the first instar estimation of HW is not biased.

The descriptions of immature stages of *Burca b. braco* are the first ones for the genus. Immature morphology, color patterns, form and disposition of setae (see Fig. 1 and 3) are similar to other Pyrginae species (Scudder 1889, Moss 1949, Stehr 1987). The complex and irregular design of white spots in the head of fourth and fifth instars is unknown for other Pyrginae in the literature we reviewed (Moss 1949, Janzen 2017) (see Fig. 1i). Most ethological records in this paper are similar to those for other skippers, especially Pyrginae. Several hesperiids eat the upper half of egg shell at birth (Heitzman 1965, Young 1985, Stehr 1987, Torres 1998). Construction of larval shelters is distinctive of Hesperiidae (Moss 1949, Stehr 1987, Scoble 1992), and possibly it is the most diverse family in construction patterns of larval shelters (Greeney & Jones 2003). Pyrginae and Eudaminae may be the subfamilies with more diversity because their larvae change the pattern of construction between instars (Moss 1949, Lind et al. 2001, Greeney & Jones 2003, Greeney 2009). Larvae of *Burca b. braco* construct four to five shelters during their lifetime and of two to four different types (see Table 3 and Fig. 4) based on the classification of Greeney (2009). It seems to be usual that first instar shelters are different from second and third instars because the shelters differ in which leaf surfaces are folded, even if they belong to the same type (see Table 3). Since we never saw larvae outside their shelters, we expect that they are nocturnal or display low rates of activity. Hesperiid caterpillars are considered nocturnal by several authors (Scott 1986, Stehr 1987, Smith et al. 1994). It could be that *Burca b. braco* larvae do not walk far away outside their shelter. It is likely that larvae spend all their lifetime in the same host plant. A similar case was experimentally demonstrated on larvae of *Epargyreus clarus* (Eudaminae) which feed closely to their shelter and spend more than 95% of daytime inside it (Lind et al. 2001).

Except *Croton lucidus*, all nectar sources reported here are new to *Burca b. braco* and also to the genus. *Morinda royoc* and *Chiococca alba* are also the first Rubiaceae nectar sources for the genus (Smith et al. 1994). Other *Croton* species have been also reported as nectar sources for *B. concolor* and *B. stillmani* (Smith et al. 1994). *Burca concolor concolor* known hostplants also belong to *Croton*; *C. sagraeanus* and *C. origanifolius* (Fernández 2004), so it is probable that *Croton*, with a large number of species in the area, is the host genus of all *Burca* species. The inclination in related lepidopterans to use related plants is well known (Gilbert & Singer 1975), so if our suspicion is true, then immature of other species of *Burca* should be described in the near future by looking for them on *Croton*.

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NATIVE PRAIRIE GRAMINOID HOST PLANTS OF MINNESOTA AND ASSOCIATED LEPIDOPTERA: A LITERATURE REVIEW

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ABSTRACT. Native grasses act as host plants, providing food and shelter, for numerous Lepidoptera species during their larval stage. As grassland habitat decreases because of conversion to agriculture and urban areas, prairie specialist butterflies and moths have also declined. Addition of native species to urban and agriculture landscapes has been shown to benefit Lepidoptera communities in various ways. Native grasses have grown in popularity as a landscaping plant due to their low nutrient requirements, drought tolerance, and soil stabilization properties. However, the benefits of native grasses to Lepidoptera are not well known to many entomologists or horticulturists, let alone the average consumer. We reviewed the literature that identified native prairie graminoids as host plants for native Lepidoptera in Minnesota, especially plants widely available in the horticultural trade that could be planted for restoration or landscaping purposes. The context of the Lepidoptera and host plant associations found in the literature are described. In total, we found 36 Lepidoptera species that used 17 prairie graminoids as host plants cited in the literature. Three native grasses, *Schizachyrium scoparium* (Michx.) Nash, *Andropogon gerardii* Vitman and *Panicum virgatum* L. and were found to be used by the most Lepidoptera species, 11, 9, and 8, respectively. Most likely there are additional moth species that use these grasses as host plants because butterfly species tend to be better documented than moth species. The specific larval habits and host plant species were unknown for many species of moths that feed or are suspected to feed on graminoids, showing the need for further research in this area. This information can assist horticulturalists, ecologists, landscape planners, land managers, and homeowners in their decisions to buy and plant native grass species. In general, this knowledge provides increased awareness about the larval life stage of butterflies and moths to concerned citizens and green industry and further supports the importance of conserving native prairie to support and maintain Lepidoptera species.

Additional key words: Butterflies, moths, larval host plants, pollinators, sustainable landscapes

Lepidoptera go through a complete metamorphosis from larva to pupa to adult during their life cycle (Scott 1986). The larval and adult stages have different food requirements. Adults often feed on flower nectar and other liquid substrates, while almost all lepidopteran larva are phytophagous (Scoble 1992). Lepidoptera evolved with the flowering plants, initially eating plants from the family Fabaceae (Ehrlich & Raven 1964, Scott 1986). Since then, certain families and species have evolved to eat monocotyledons such as grasses and sedges (Ehrlich & Raven 1964, Scott 1986). In North America, these include the butterfly subfamilies Satyrinae and Hesperiinae (Scott 1986), and various subfamilies, genera, and species of moths (Powell & Opler 2009, Wagner et al. 2011).

Native grasses provide food sources for numerous species of Lepidoptera (Scott 1986). However, native grassland habitats are some of the most endangered in North America (White et al. 2000). Less than 1% of the original tallgrass prairie remains (Samson & Knopf 1994), putting pressure on prairie endemic species of butterflies and moths. Declining populations of prairie-specialist Lepidoptera have been documented in North America for several decades and they are continuing to decline even on prairie preserves (Orwig 1990, Schlict et al. 2009, Swengel et al. 2011, Swengel & Swengel 2015). In Minnesota, of the 19 Lepidoptera species listed as endangered, threatened, or of special concern, nine are prairie dependent, and two are suspected of being prairie dependent (Metzler 2005, MN DNR 2013).

The loss of grasslands in North America is due to conversion to agriculture and urban areas (White et al. 2000), and has significantly altered native habitat, replacing native plant species with non-native species, such as agronomic crops and exotic landscape ornamentals (Burghardt et al. 2008, Tallamy & Shropshire 2009). Host specificity is common in Lepidoptera; non-native species usually support fewer Lepidoptera species as larval hostplants than native species (Tallamy & Shropshire 2009). Planting native species as ornamentals in urban or semi-urban areas has shown to benefit some Lepidoptera (Vickery 1995, Fontaine et al. 2016). Studies in agricultural and urban landscapes have shown that grasslands with higher cover and richness of native species had higher numbers of uncommon or specialist butterfly species (Collinge et al. 2003) and higher diversity of butterfly and moth larvae (Burghardt et al. 2008).

Native grasses have grown in popularity as landscaping plants due to their low nutrient requirements, drought tolerance, and soil stabilization (Meyer 2012). Although the benefits of native flowers to adult Lepidoptera are well known, the food requirements of the larval forms of these same Lepidoptera are much more obscure and undocumented. Many entomologists and horticulturists know little of the feeding habits or preferences of graminoid-feeding Lepidoptera. The purpose of this review is to compile the information known to date about native graminoids that serve as larval host plants for Lepidoptera in Minnesota.

MATERIALS AND METHODS

The literature was reviewed for Lepidoptera species that use native Minnesota prairie graminoids, especially those widely available in the horticultural trade that could be planted for restoration or landscaping purposes. We included graminoid species from the upland prairie, wet meadow/carr, and wetland prairie system descriptions in the Field Guide to the Native Plant Communities of Minnesota (Minnesota Department of Natural Resources 2005), which listed the dominant, characteristic, and distinguishing graminoids for each community. Nomenclature for plant species followed the PLANTS database (USDA, NRCS, 2017). *Carex pensylvanica* Lam. ssp. *heliophila* (Mack.) W.A. Weber (Cyperaceae) which was cited in Scott (1992) as a host plant, was updated to its current synonym in the PLANTS database (USDA, NRCS, 2017), *Carex inops* L.H. Bailey ssp. *heliophila* (Mack.) Crins. This sedge is a dry prairie species which differentiates it from *Carex pensylvanica* Lam., which is primarily a woodland species (Gleason & Cronquist 1963). Only records that identified the host plant by species (not just genus) were included. We included records for Lepidoptera species that occur in Minnesota. If the host plant record was obtained outside of the region, the information was still included in the review. However, host plant records for an adjacent lepidopteran subspecies that did not occur in Minnesota were not included because host plant preferences can differ between adjacent subspecies (MacNeil 1964). Host plant relationships are determined by evidence that feeding has occurred, observations of larvae on a plant, evidence of larval shelters, or oviposition choice of adult butterflies and moths. The validity of host plant relationships can be difficult to determine because some species oviposit indiscriminately. For this reason, the context of the lepidopteran host plant associations found in the literature were included in the review. Nomenclature for butterflies follows Pelham (2008). Nomenclature for moths follows Hodges et al. (1983), except where modified by Lafontaine and Schmidt (2010), Kaila (1999), Metzler and Brown (2014), and Hodges (1978).

RESULTS

Host plant associations. Seventeen native graminoid species occurring in the upland prairie, wet meadow/carr, and wetland prairie systems of Minnesota were found to serve as food for native Lepidoptera species (Table 1). In total, we found 36 Lepidoptera species cited in the literature that used these native prairie graminoids as host plants in Minnesota (Table 2). *Schizachyrium scoparium* (Michx.) Nash (Poaceae) was found to be used by the most species, 11 (Table 3). *Andropogon gerardii* Vitman (Poaceae) served as a host plant for nine species; *Panicum virgatum* L. (Poaceae) for eight species; *Bouteloua gracilis* Willd. ex Kunth (Poaceae) and *Bouteloua curtipendula* Michx. (Poaceae) served as a host plant for six species; *Carex lacustris* Willd. (Cyperaceae), *Carex stricta* Lam. (Cyperaceae), *Elymus canadensis* L. (Poaceae), and *Sporobolus heterolepis* (A. Gray) A. Gray (Poaceae) for five species; *Koeleria macrantha* (Ledeb.) Schult. (Poaceae) and *Carex inops* subsp. *heliophila* (Cyperaceae) for four species and; *Bouteloua hirsuta* Lag. (Poaceae) and *Elymus trachycaulus* (Link) Gould ex Shinners (Poaceae) for three species; *Spartina pectinata* Bosc. ex Link (Poaceae), *Sorghastrum nutans* (L.) Nash (Poaceae), and *Hesperostipa spartea* (Trin.) Barkworth (Poaceae) for two species and *Hesperostipa comata* (Trin. & Rupr.) Barkworth (Poaceae) for one species (Table 3).

Oviposition. Many Lepidoptera oviposit on their host plant, presumably to increase the chances that the larvae will encounter their host plant after hatching, increasing survival rates (Ehrlich & Raven 1964, Celik et al. 2015). However, oviposition "mistakes" have been observed when females accidentally oviposit on the wrong plant (Scott 1986, Thompson & Pellmyr 1991). Non-specific oviposition, or ovipositing indiscriminately on plant species, has also been observed. This behavior is common among graminoid-feeders, such as those in the Satyrinae subfamily (Scott 1992, Tiitsaar et al. 2016, Wiklund 1984). It is hypothesized that these species oviposit at random because their food plants grow abundantly, and so they do not need to target as precisely as other species that feed on less abundant plants (Wiklund 1984).

Less has been published about the Hesperiinae, the graminoid-feeding subfamily of Hesperiidae. Scott

TABLE 1. Graminoids native to the Upland Prairie, Wet Meadow/Carr, and Wetland Prairie systems of Minnesota as defined by Minnesota DNR (Minnesota Department of Natural Resources 2005) that serve as food for Lepidoptera larvae.

Common name	Scientific Name
big bluestem	*Andropogon gerardii* Vitman
sideoats grama	*Bouteloua curtipendula* Michx.
blue grama	*Bouteloua gracilis* Willd. ex Kunth
hairy grama	*Bouteloua hirsuta* Lag.
sun sedge	*Carex inops* ssp. *heliophila* (Mack.) Crins
hairy sedge, lake sedge	*Carex lacustris* Willd.
tussock sedge, upright sedge	*Carex stricta* Lam.
Canada wildrye	*Elymus canadensis* L.
slender wheatgrass	*Elymus trachycaulus* (Link) Gould ex Shinners
needleandthread	*Hesperostipa comata* (Trin. & Rupr.) Barkworth
porcupine grass	*Hesperostipa spartea* (Trin.) Barkworth
Junegrass	*Koeleria macrantha* (Ledeb.) Schult.
switchgrass	*Panicum virgatum* L.
little bluestem	*Schizachyrium scoparium* (Michx.) Nash
Indiangrass	*Sorghastrum nutans* (L.) Nash
prairie cordgrass	*Spartina pectinata* Bosc. ex Link
prairie dropseed	*Sporobolus heterolepis* (A. Gray) A. Gray

(1973) found that *Notamblyscirtes simius* (W. H. Edwards) oviposited only on its host plant, *Bouteloua gracilis.* However, *Hesperia dactoae* (Skinner) has been observed to oviposit on a variety of plant species (McCabe & Post 1977, Dana 1991).

Shelter building. Some caterpillars construct shelters in which they reside during their larval life stage (Scoble 1992). Various families of Lepidoptera exhibit this behavior, including families with graminoid-feeding species such as Tortricidae, Gelechiidae, Pyralidae, Nymphalidae, and Hesperiidae (Greeney & Jones 1998). The moth families, Tortricidae, Gelechiidae, and Pyralidae are leaf rollers, named for the shelters they make by folding or rolling one leaf or multiple leaves together, using silk as a fastener (Lafontaine et al. 2010). While there are nest builders in the Nymphalidae, none of the graminoid-feeding satyrs in this family exhibit this behavior (Scott 1992). However, almost all species in the Hesperiidae family make shelters (Greeny & Jones 1998).

The Hesperiidae may contain the largest diversity of shelter types (Greeny & Jones 1998). Shelters are built at various heights, often changing during the life of the larvae, using different techniques and on different grass species and substrates (MacNeill 1964, Dana 1991, Lafontaine et al. 2010). *H. dactoae* larvae make shelters near the base of bunch grasses, *Schizachyrium scoparium* and *Sporobolus heterolepis*, by weaving together blades of grass and leaf litter (Dana 1991). *Hesperia assiniboia* (Lyman) make nests by rolling or attaching leaves together or sometimes even using dried cattle feces (McCabe & Post 1977, Scott 1986). Early larval instars of *Hesperia ottoe* W. H. Edwards and *Polites origenes* (Fabricius) make aerial nests, by weaving grass leaves together above the soil surface, using bunch grass species *Andropogon gerardii* (Scott 1992) or *Schizachyrium scoparium* (Dana 1991). *Polites themistocles* (Latreille) larvae are suspected of making silk tunnels in the litter and/or soil (Scott 1992). *Amblyscirtes oslari* (Skinner) larvae make conventional rolled leaf tube nests (Scott 1992). The placement of larval nests determines the vulnerability of larval species to different kinds of land management, such as prescribed burning or haying, throughout the year (Dana 1991).

Feeding behavior. Caterpillars feed on grasses using various techniques. Some caterpillars feed in the open, exposed on the plant on which they are feeding (Scoble 1992), while others are concealed feeders, feeding internally in the plant or hiding themselves in shelters (Lafontaine et al. 2010). Shelter-builders often feed from inside or near their shelter. Dana (1991) observed larvae of *H. dacotae* leaving shelters to forage, cutting off blades of grass, and then returning to their shelter with the blade to eat it. Species that do not build shelters, like those in the Satyrinae, protect themselves by camouflage or hiding at the base of plants during the day and then feeding at night (Scott 1992).

Graminoid-feeding moths exhibit multiple concealed feeding behaviors. Many fall into the borer category. Borers drill into either the stem or roots of plants using specialized mouth parts (Wagner et al. 2011). Graminoid-feeders in the moth family Elachistidae are leaf miners, eating the chlorophyll between the outer layers of the leaf (Braun 1948). Graminoid-feeding moths from the Gelechiidae family are leaf-rollers, feeding from the inside of their shelter (Lafontaine et al. 2010). Like butterflies, moths that are exposed feeders employ camouflage and nocturnal eating behaviors to

TABLE 2. Lepidoptera recorded to use the native prairie graminoids in Table 1, and their native ranges in the Upper Midwest (Minnesota, South Dakota, North Dakota, Iowa, Illinois, Wisconsin, Michigan). Ranges are not listed for some subspecies.

Lepidoptera Species	**MN**	**SD**	**ND**	**IA**	**IL**	**WI**	**MI**	**Reference**
Aethes spartinana (Barnes & McDunnough, 1916)	x	x	x	x	x	x		Ainslie 1917; Prasifika 2012
Amblyscirtes hegon (Scudder 1863)	x			x	x	x	x	Scott 1986
Amblyscirtes vialis (W. H. Edwards 1862)	x	x	x	x	x	x	x	Scott 1986
Anatrytone logan (W. H. Edwards, 1863)	x	x	x	x	x	x	x	Scott 1986
Anatrytone logan logan (W. H. Edwards,1863)								-
Anicla tenuescens (Smith, 1890)	x	x	x	x		x		Lafontaine 2004
Atrytone arogos (Boisduval & Leconte, [1837])	x	x	x	x	x	x		Scott 1986
Atrytone arogos iowa (Scudder, 1868)								-
Atrytonopsis hianna (Scudder, 1868)	x	x	x	x	x	x	x	Scott 1986
Atrytonopsis hianna hianna (Scudder, 1868)								-
Blastobasis repartella (Dietz, 1910)	x	x	x	x	x	x	x	Adamski et al. 2010
Cercyonis pegala (Fabricius, 1775)	x	x	x	x	x	x	x	Scott 1986
Cercyonis pegala nephele (W. Kirby, 1837)								-
Deltote bellicula (Hübner, 1818)	x	x	x	x	x	x	x	Beadle & Leckie 2012
Euphyes conspicua (W. H. Edwards, 1863)	x			x	x	x	x	-
Euphyes dion (W. H. Edwards, 1879)	x	x	x	x	x	x	x	Scott 1986
Euphyes vestris (Boisduval 1852)	x	x	x	x	x	x	x	Scott 1986
Faronta diffusa (Walker, 1856)	x	x	x	x	x	x	x	Beadle & Leckie 2012
Faronta rubripennis (Grote & Robinson, 1870)	x	x		x	x	x	x	Beadle & Leckie 2012
Hesperia assiniboia (Lyman, 1892)	x	x	x					Dana & Huber 1988
Hesperia comma (Linnaeus, 1758)	x	x	x			x	x	Scott 1986
Hesperia dacotae (Skinner, 1911)	x	x	x					Scott 1986
Hesperia leonardus T. Harris, 1862	x	x	x	x	x	x	x	Scott 1986
Hesperia leonardus leonardus T. Harris, 1862	x				x	x	x	Scott 1986
Hesperia leonardus pawnee Dodge, 1874	x	x	x	x				Scott 1986; Metzler et al. 2005
Hesperia metea Scudder, 1863	x			x	x	x	x	Scott 1986
Hesperia ottoe W. H. Edwards, 1866	x	x	x	x	x	x	x	Scott 1986
Hesperia sassacus T. Harris, 1862	x				x	x	x	Scott 1986
Hesperia uncas W. H. Edwards, 1863	x	x	x	x				Scott 1986
Hesperia uncas uncas W. H. Edwards, 1863								-
Lethe appalachia R. Chermock, 1947	x	x		x	x	x	x	Scott 1986
Lethe eurydice (Linnaeus, 1763)	x	x	x	x	x	x	x	Scott 1986
Meropleon ambifusca (Newman, 1948)	x	x	x	x	x	x	x	Wagner et al. 2011; Beadle 2012
Mocis texana (Morrison, 1875)	x			x	x	x	x	Wagner et al. 2011
Oarisma garita (Reakirt, 1866)	x	x	x					Scott 1986
Oarisma poweshiek (Parker, 1870)	x	x	x			x	x	Scott 1986
Papaipema cataphracta (Grote, 1864)	x			x	x	x	x	Wagner et al. 2011
Papaipema nebris (Guenee, 1852)	x	x		x	x	x	x	Beadle & Leckie 2012
Poanes massoit (Scudder, 1863)	x	x	x	x	x	x	x	Scott 1986
Poanes viator (W. H. Edwards, 1865)	x	x	x	x	x	x	x	Scott 1986
Poanes viator viator (W. H. Edwards, 1865)								-
Polites origenes (Fabricius, 1793)	x	x	x	x	x	x	x	Scott 1986
Polites themistocles (Latreille, [1824])	x	x	x	x	x	x	x	Scott 1986
"Resapamea" stipata (Morrison, 1875)	x	x	x	x	x	x	x	Metzler et al. 2005

avoid predators (Lafontaine et al. 2010). Other tactics include physical defenses, like spines, and coloration warning of toxicity (Lafontaine et al. 2010).

Although most Lepidoptera specialize in feeding on just one or few species, others are generalist feeders (Scott 1992, New 1997). Graminoid-feeding butterflies are suspected of being able to eat numerous species of grass, making them graminoid specialists (Scott 1992). The grass skippers (Hesperiinae) range from preferring a certain species, growth-form, or genus of grass to eating grass and sedge species (Scott 1986, Scott 1992). Their limitations to certain species are suspected to be determined by their preference for shelter building and not necessarily nutrition requirements (MacNeil 1964). Butterflies in the Satyrinae subfamily, which do not build above-ground shelters, are polyphagous, feeding on a variety of grass species and sometimes grass and sedge species (Scott 1992). Moth species range from very host-specific, eating only one or two species of grass, to extremely polyphagous, feeding on species from multiple families (Wagner et al. 2011).

Discussion

Additional grass skipper species that feed on native Minnesota grasses were not included here because either their host plants were listed only to genus, or were not listed in the plant community reference that defined the scope of our study. Further research on grass skippers may reveal additional species that feed on the native grasses listed.

In general, butterfly species are better documented than moth species because they are larger, showier, and fly during the day, making them easier to study (Thompson & Pellmyr 1991). Although many moth species are known to eat grass, the specific larval habits and host plants of individual species are often unknown. Forty-six additional native moth species that occur in Minnesota were suspected or confirmed to feed on grasses or sedges but could not be added to the list because their larval food preferences were unknown (Hodges 1978, Lafontaine & Poole 1991, Landry 1995, Lafontaine 2004, Metzler et al. 2005, Mikkola et al. 2009). This large number demonstrates the need for further research and documentation of larval moth habits and host plants.

The host plants and larval habits of some moth species have been documented in detail because they are considered as pests. These tend to be polyphagous species that in addition to eating the native grasses on our list, feed on many additional graminoid, forb, shrub species, and/or cultivated grass species such as corn, wheat, and barley (Decker 1930, Decker 1931, Reddy & Antwi 2016). *Papaipema nebris* (Guenee) (Noctuidae), *Papaipema cataphracta* (Grote) (Noctuidae), *Faronta diffusa* (Walker) (Noctuidae), and *"Resapamea" stipata* (Morrison) (Noctuidae) are all native moth species included in this review that have been documented as occasional pests of agricultural crops (Decker 1930, Decker 1931, Solomon 1995, Reddy & Antwi 2016). *P. nebris* has also been documented as an occasional pest of gardens (Decker 1931).

The information in this review can assist horticulturalists, ecologists, landscape planners, land managers, and homeowners in their decisions to buy and plant native grass species to benefit Lepidoptera. This important attribute of native grasses can be used in garden center promotion and to educate the general public. In general, this knowledge provides increased awareness about the larval life stage of butterflies and moths for both concerned citizens and horticultural and ecological businesses and further supports the importance of conserving native prairie to maintain these Lepidoptera.

As Lepidoptera populations decline, it is important to maximize the ecological benefits of anthropogenic landscapes that are replacing their native habitat. However, native grass use in landscaping should not detract from the importance of conserving native habitat. Rather, the purpose of this literature review is to provide information on the values of native grasses to Lepidoptera.

Additional research is needed to fully understand the benefits of native landscaping in suburban and urban areas to Lepidoptera communities and rare species. While the addition of native nectar flowers into human dominated landscapes has shown to be successful in providing nectar to butterflies (Vickery 1995), there is debate surrounding the efficacy of butterfly gardens as breeding habitat (Di Mauro et. al. 2007; Cutting & Tallamy 2015). The main benefit of residential butterfly gardens may be as stepping stones between larger natural areas, where Lepidoptera can obtain nectar before continuing on to permanent habitat (Vickery 1995; Di Mauro et. al. 2007). Studies differ in their findings on the influence of patch characteristics, such as habitat quality, versus landscape characteristics, such as surrounding matrix, on butterfly diversity (Collinge et al. 2003; Di Mauro et al. 2007; Olivier et al. 2016). The influence of these factors also differs for individual species due to species-specific traits such as habitat preference and mobility (Olivier et al. 2016). Olivier et al. (2016) found a stronger negative correlation between urbanization and habitat specialists than between urbanization and habitat generalists. Considering this information, further research is needed to understand how much the landscape context influences the

TABLE 3. List of native graminoids species and associated Lepidoptera, references for individual associations, and the context of the host plant record in the literature. No notes were added if the species was listed as a host plant or food plant with no additional context.

Larval Plants	Lepidoptera	References	Notes
Andropogon gerardii	*Anatrytone logan*	Layberry et al. 1998; Scott 1986	-
	Anatrytone logan logan	McCabe & Post 1977	-
	Atrytone arogos	Scott 1992	Field observations of shelters in Colorado
		Metzler et al. 2005	Field observations of feeding
		Opler & Krizek 1984	Listed as host plant in Missouri
		Scott 1986; Opler & Malikul 1992	-
		Pyle 1981	Listed as a species used for oviposition
	Atrytone arogos iowa	McCabe & Post 1977	-
	Atrytonopsis hianna	Layberry et al. 1998; Scott 1986; Opler & Krizek 1984	-
	Atrytonopsis hianna hianna	McCabe & Post 1977	-
	Faronta diffusa	Godfrey 1972	Larvae collected from this plant species in the field
		Wagner et al. 2011	Field observation of oviposition
	Hesperia dacotae	Dana 1991	Field observations of feeding
		McCabe 1981	Accepted by confined first instar larvae
		Scott 1986	Listed as a species used for oviposition
	Hesperia metea	Scott 1986; Opler & Krizek 1984	-
	Hesperia ottoe	Scott 1992	Field observations of shelters and oviposition in Colorado
		Dana 1991	Accepted grass during no choice experiment; field observations of shelters
		Scott 1986; Metzler et al. 2005	-
	Hesperia sassacus	Opler & Malikul 1992	-
	Meropleon ambifusca	Wagner et al. 2011	Field observations of feeding
Bouteloua curtipendula	*Atrytone arogos*	Scott 1992	Field observations of shelters and oviposition in Colorado
	Hesperia assiniboia	Scott 1992	Field observations of oviposition in Colorado
	Hesperia dacotae	Dana 1991	Field observations of feeding
	Hesperia leonardus pawnee	Scott 1986	-
	Hesperia ottoe	Scott 1986	-
		Dana 1991	Accepted during a no choice experiment; field observations of shelters in MN
	Oarisma poweshiek	Selby 2005	Field observations of feeding
Bouteloua gracilis	*Hesperia assiniboia*	Layberry et al. 1998	-
		Scott 1992	Field observations of oviposition in Colorado
	Hesperia comma	Scott 1986	-
	Hesperia leonardus	Layberry et al. 1998; Opler & Malikul 1992	-
	Hesperia leonardus pawnee	Scott 1992	Field observations of oviposition in Colorado
	Hesperia ottoe	Scott 1986	-

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TABLE 3. (Continued) List of native graminoids species and associated Lepidoptera, references for individual associations, and the context of the host plant record in the literature. No notes were added if the species was listed as a host plant or food plant with no additional context.

Larval Plants	Lepidoptera	References	Notes
Bouteloua gracilis (continued)	*Hesperia uncas*	Scott 1992	Field observations of oviposition in Colorado
		Scott 1986; Opler & Krizek 1984; Layberry et al. 1998	-
	Hesperia uncas uncas	McCabe & Post 1977	Listed as a food plant, but only observed oviposition
	Oarisma garita	Scott 1992	Field observations of oviposition in Colorado
		Scott 1986	-
Bouteloua hirsuta	*Hesperia uncas*	Dana & Huber 1988	Listed as a host plant in Minnesota
	Hesperia ottoe	Dana 1991	Field observations of shelters
	Hesperia leonardus pawnee	Scott 1986	-
Carex inops ssp. *heliophila*	*Hesperia dacotae*	Dana 1991	Field observations of feeding
	Euphyes vestris	Scott 1986; Layberry et al. 1998	Listed as host plant with no additional context
		Pyle 1981	Listed as a host plant in Colorado
	Hesperia assiniboia	Scott 1992	Field observations of oviposition in Colorado
	Oarisma garita	Scott 1992	Field observation of oviposition in Colorado; larvae readily accepted in lab
Carex lacustris	*Euphyes dion*	Scott 1986; McCabe & Post 1977	-
	Euphyes vestris	Scott 1986	-
	Lethe eurydice	Scott 1986	-
	Lethe appalachia	Scott 1986	-
	Poanes viator	Scott 1986	-
	Poanes viator viator	McCabe & Post 1977	-
Carex stricta	*Deltote bellicula*	Wagner et al. 2011	Raised on plant in lab
	Euphyes conspicua	Scott 1986	-
	Lethe appalachia	Scott 1986	-
	Lethe eurydice	Scott 1986	-
	Poanes masassoit	Scott 1986	-
Elymus canadensis	*Amblyscirtes vialis*	Scott 1992	Field observations of larval shelters in Colorado
	Faronta diffusa	Godfrey 1972	Eggs were found on the plant
	"Resapamea" stipata	Tietz 1972	-
	Papaipema cataphracta	Tietz 1972	-
	Poanes zabulon taxiles	Scott 1986	-
Elymus trachycaulus	*Amblyscirtes vialis*	Scott 1992	Field observations of larval shelters in Colorado
	Poanes zabulon taxiles	Scott 1986	Listed as host plant for this subspecies
	Faronta diffusa	Tietz 1972	-

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TABLE 3. (Continued) List of native graminoids species and associated Lepidoptera, references for individual associations, and the context of the host plant record in the literature. No notes were added if the species was listed as a host plant or food plant with no additional context.

Larval Plants	Lepidoptera	References	Notes
Hesperostipa spartea	*Hesperia dacotae*	McCabe 1981	Accepted by confined larvae
		Dana 1991	Feeding observed in the field, but only by older larvae. Early instars did not feed on this species under captive feeding situations.
	Cercyonis pegala	Scott 1986	-
Hesperostipa comata	*Hesperia leonardus pawnee*	McCabe & Post 1977	-
Koeleria macrantha	*Polites themistocles*	Scott 1992	Field observations of oviposition
	Hesperia assiniboia	Layberry et al. 1998	-
	Hesperia dacotae	McCabe 1981	Accepted by confined larvae. Dana (1991) found that confined early instar larvae did not accept
	Oarisma garita	Scott 1992	Field observations of oviposition in Colorado
Panicum virgatum	*Aethes spartinana*	Adamski et al. 2010	Larvae were collected from the plant in the field
	Anatrytone logan	Layberry et al. 1998; Scott 1986; Opler & Krizek 1984	-
	Anatrytone logan logan	McCabe and Post 1977	Field observations of oviposition
	Blastobasis repartella	Adamski et al. 2010	Field observations of feeding
	Faronta rubripennis	Metzler et al. 2005	Field observations of feeding
		Wagner et al. 2011	Listed as a host plant in New Jersey
	Hesperia leonardus	Layberry et al.1998; Opler & Krizek 1984	-
	Hesperia leonardus leonardus	Scott 1986	-
	Mocis texana	Wagner et al. 2011	Listed as a host plant in New Jersey
	Papaipema nebris	Prasifika et al. 2011	Field observations of feeding within stem
	Polites themistocles	Scott 1986	-
Schizachyrium scoparium	*Atrytone arogos*	Scott 1986	-
		Scott 1992	Field observations of oviposition; Listed as a popular host in Kansas and E. US
	Atrytonopsis hianna	Layberry et al. 1998; Scott 1986; Opler & Krizek 1984; Opler & Malikul 1992	-
	Cercyonis pegala nephele	Scott 1992	Field observations of oviposition; considered rare host plant in Colorado
	Hesperia comma assiniboia	Scott 1992	Field observations of oviposition in Colorado

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TABLE 3. (Continued) List of native graminoids species and associated Lepidoptera, references for individual associations, and the context of the host plant record in the literature. No notes were added if the species was listed as a host plant or food plant with no additional context.

Larval Plants	Lepidoptera	References	Notes
Schizachyrium scoparium (continued)	*Hesperia dacotae*	Opler & Krizek 1984; Opler & Malikul 1992; Pyle 1981	-
		Scott 1986	Field observations of larvae on the plant
		Dana 1991	Field observations of feeding
		Layberry et al. 1998	Field observations of larvae on the plant
	Hesperia leonardus	Opler & Krizek 1984	Associated with stands of little bluestem
		Layberry et al. 1998; Opler & Malikul 1992	-
	Hesperia leonardus pawnee	Scott 1986	-
	Hesperia metea	Scott 1986; Opler & Krizek 1984	-
	Hesperia ottoe	Dana 1991	Accepted during no-choice experiment; Field observations of shelters
		Opler & Malikul 1992; Scott 1986	-
		Layberry et al. 1998; Opler & Krizek 1984	Listed as a species used for oviposition
		McGuire 1982	Field observations of oviposition
	Hesperia sassacus	Layberry et al. 1998; Scott 1986	-
		Scott 1986	-
	Oarisma poweshiek	Metzler et al. 2005; Swengel & Swengel 1999	-
		Selby 2005	Field observations of oviposition
	Polites origenes	Scott 1986; Robinson et al. 2002; Layberry et al. 1998	-
Sorghastrum nutans	*Amblyscirtes hegon*	Opler & Krizek 1984; Scott 1986; Layberry et al. 1998; McCabe & Post 1977	-
	Faronta diffusa	Godfrey 1972; Robinson et al. 2002	Larvae of the species were collected from the plant
Spartina pectinata	*Aethes spartinana*	Barnes & McDunnough 1916; Ainslie 1917; Prasifka et al. 2012.	Field observations of feeding
		Metzler et al. 2005	Listed as a host plant in Ohio
	"Resapamea" stipata	Decker 1930	Field observations of larvae on the plant
		Crumb 1956; Tietz 1972	-
		Metzler et al. 2005	Field observations of larvae on the plant
Sporobolus heterolepis	*Anicla tenuescens*	Lafontaine 2004; Metzler et al. 2005	Field observations of feeding
	Hesperia dacotae	Dana 1991	Field observations of feeding
	Hesperia leonardus pawnee	Scott 1986	-
	Hesperia ottoe	Dana 1991	Accepted in a no choice experiment
	Oarisma poweshiek	Metzler et al. 2005; Swengel & Swengel 1999	-
		Selby 2005	Field observations of oviposition

effectiveness of native plantings in attracting and benefiting specialist species with low mobility such as prairie skippers. General recommendations to maximize the benefits native plantings provide to Lepidoptera include increasing the size of the planting, increasing the number of blooming nectar plants, and strategically positioning the planting to better connect corridors or areas of suitable habitat (Di Mauro et al. 2007).

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THE EFFECT OF AGRICULTURAL MANAGEMENT ON THE DISTRIBUTION AND ABUNDANCE OF *ARSENURA ARMIDA* (LEPIDOPTERA: SATURNIIDAE) IN CHIAPAS, MEXICO

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ABSTRACT. *Arsenura armida* plays an important ecological role in the development of woods and forests throughout Latin America. It is also consumed in several rural communities of Mexico. At present, there is no information on the host preferences of this species of moth, habitat type or the effects of human consumption on population numbers. This paper determines the effect of agricultural management on the distribution, occupation and abundance of larvae of *A. armida* in order to promote strategies for its sustainable exploitation. Six observation plots, each with an area of 4 km^2, were established in three localities of the state of Chiapas, Mexico. Three plots were subject to agricultural management (La Pimienta, La Ilusion and La Loma) and three without management (Carrizal, Huachinadero and Mirador). All host plants together with their diameter at breast height (DBH) were quantified. In order to estimate host preference, occupation, distribution and abundance, the number of larvae per tree were recorded over two consecutive years (between March and October). The occupation and abundance of *A. armida* varied among plots and the six tree species observed. Both variables presented a relationship with the mean DBH of the host species. A preference for the host species *Heliocarpus appendiculatus* and *Rollinia mucosa* was observed. The greatest abundance of larvae and host occupation was found in plots subject to agricultural management during the first year. Abundance decreased during the second year due to tree mortality. The insect presented a clustered distribution that depended on the distribution and development of host trees (highest DBH). It is suggested that host management is considered for the control of populations of *A. armida* in agricultural areas and its sustainable exploitation for human consumption.

Additional key words: Agroecosystem, edible insects, entomophagia, local adaptation

The *Arsenura* genus belongs to the Saturniidae family within the order of Lepidoptera (Peigler 1993, De Camargo et al. 2011). Their larvae attain a length of 11cm. In general, members of the Saturniidae family present a high capacity for flight, thus facilitating greater dispersal. Moth displacement generally takes place during the night to avoid water loss by evaporation and attack by predators (Palanca-Soler 1987, Amarillo 1997).

Arsenura armida (Crammer, 1779) is important for many ethnic groups in Mexico as the edible larvae are highly nutritious; they provide non-saturated fats and are an excellent source of protein (Ramos-Elorduy 1993, 2004, Ramos-Elorduy et al. 2008). They are consumed and commercialized in the Mexican states of Chiapas, Guerrero, Hidalgo, Oaxaca, Quintana Roo, Tabasco, Tlaxcala, Veracruz and Yucatan. In Chiapas, in the southeast of Mexico, this species of moth has been recorded in 31 municipalities but is only consumed in five (Morales 2010, Landero-Torres et al. 2012).

It presents a Neotropical distribution, from central and southern Mexico to southeastern Brazil and demonstrates a gregarious and aposematic behavior (Costa et al. 2004), with two generations within one year observed in some areas. The adults generally emerge between April and June and then mate immediately. One day after mating, the females oviposit between 350 and 500 eggs. The adult female lives for only six days during which it does not feed (Costa et al. 2004). Egg eclosion is associated with the start of the rainy season and maximum foliage production of the host plant with the larvae present between July and August, remaining on the same tree and feeding on its leaves during the night. The larvae can reach a length of 11 cm during the last stage when they descend to ground level to transform into a pupa (Janzen 1970).

Arsenura armida is considered as a polyphagous species (Jermy 1984, Pérez-Contreras 1999), given that they feed on at least 13 species of tree, belonging to three distinct families: *Annona montana* Macfad and *Rollinia membranacea* Triana & Planchon (Annonaceae); *Bombacopsis quinata* Jacq (Bombacaceae); *Ceiba pentandra* L. Gaertn., *Chorisia* sp., *Guazuma ulmifolia* Lam, *Guazuma tomentosa* Kunth, *Heliocarpus appendiculatus* Turcz., *Heliocarpus*

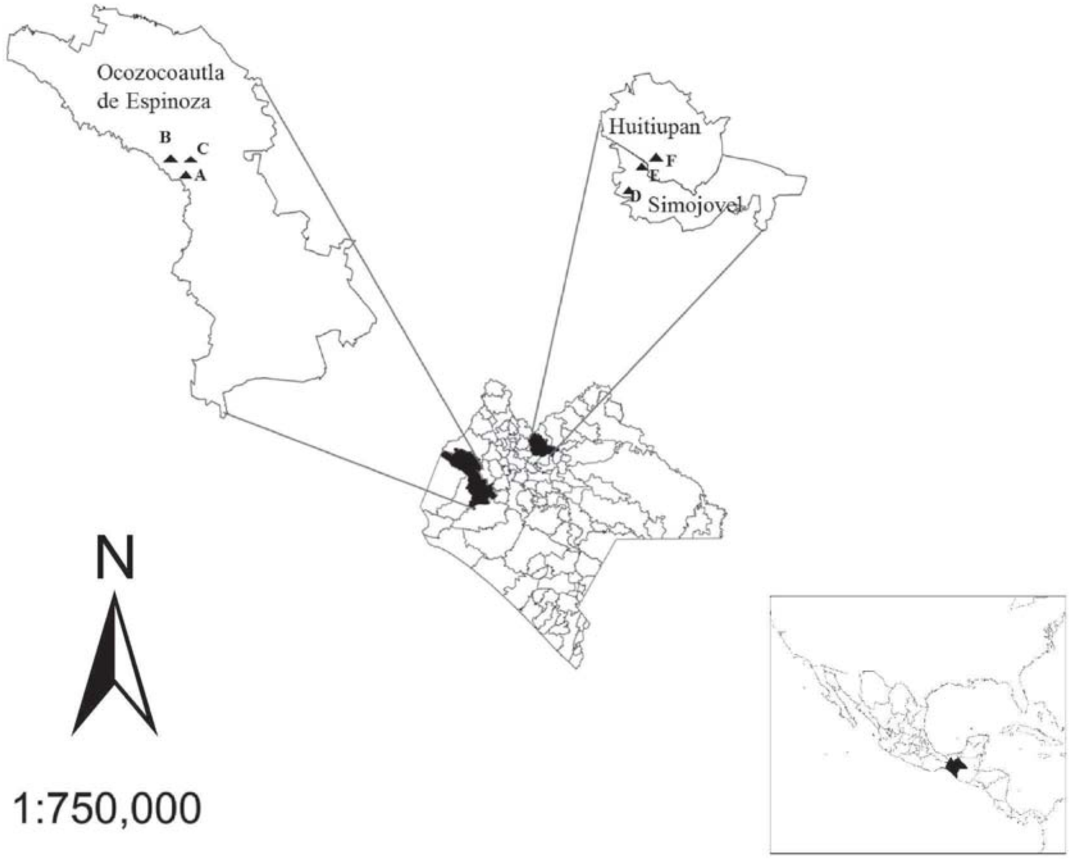


FIG. 1. Location of the study area: **A**) Carrizal; **B**) Huachinadero; **C**) Mirador; **D**) La Pimienta; **E**) La Ilusion; **F**) La Loma.

donnell-smithii Rose ex Donn., *Luehea candida* Moç & Sessé ex DC, *Theobroma cacao* L., *Pachira aquatica* Aubl and *Pseudobombax ellipticum* (Kunth) Dugand (Malvaceae) (Costa et al. 2003, Nielsen et al. 2004, Escamilla-Prado et al. 2012, Landero-Torres et al. 2012).

Most its hosts are distributed throughout the American continent and three species (*C. petandra, P. aquatica* and *T. cacao*) are also found in Europe (GBIF 2014). These species are typical of secondary semi-evergreen rain forest (Vázquez-Sánchez 1988, SEMARNAT-CONANP 2001, Orantes-García et al. 2013) and are frequently found in association with agricultural management (Escamilla-Prado et al. 2012, Landero-Torres 2012). Farmers use the host species of *A. armida* to define the border of their lands, some species are used to feed cows, as firewood and posts, even as medicinal plants. In spite of these uses, farmers do not reproduce these tree species (Escamilla-Prado et al. 2012).

The availability and nutritional quality of the hosts can affect population development, distribution and abundance of guest (Futuyma 1976, Viejo et al. 1997, Novotny et al. 2002, García-Pereira et al. 2003, Molina-Martínez & León-Cortés 2006); however, there is no data on host preference or the availability of host plant in which way has an effect on distribution and abundance of *A. armida*. In addition, the consumption of the larvae of this specie could have an impact on population development, although any potential effects are not yet known. Although highly sensitive to environmental conditions, lepidopterans can withstand changes by means of local adaptation and dispersion (García-Pereira et al. 2003, Muñoz & Amarillo-Suárez 2010, Villanueva & Saldamando 2013).

The study of the interaction between *A. armida* and its host plants is essential for the development of management strategies that promote the growth and sustainable exploitation of this lepidopteran, particularly in areas where as well as providing a source of high-protein food, it is of cultural and economic importance.

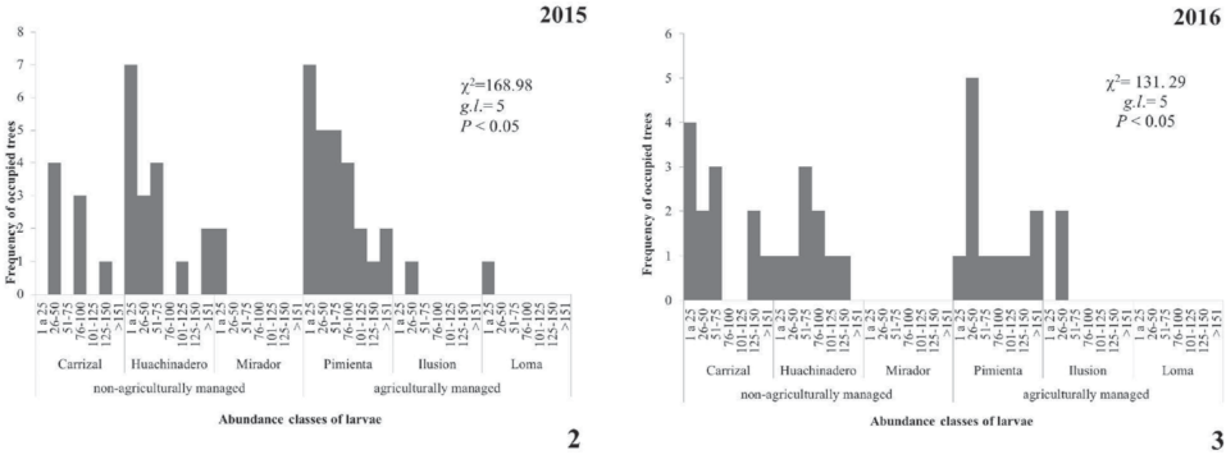
The human consumption of the larvae occurs in the months of July and August. When the larvae are not present in the field, they are stored under refrigeration for its conservation and subsequent preparation. Local and regional demand can be considered high (Escamilla-Prado et al. 2012, Landero-Torres 2012). The retail price in local markets for 2007 was $ 2.50 U.S./kg (Escamilla-Prado et al. 2012) and for 2016 it was $ 25 U.S./kg (Molina-Nery, personal observation). In some places where the insect is consumed, protocultives are carried out to achieve optimum development and commercialization (Ramos-Elorduy et al. 2008). There is evidence that *A. armida* contributes the same or more minerals than white bread, avocado, wheat germinated and menhaden fish (Ramos-Elorduy et al. 1998) and can therefore be considered as an important food source. The human consumption of larvae of *A. armida* does not cause toxicity as has been reported in birds (Janzen 1970) and what is used is the cuticle. The larvae's head and hemolymph are removed, and the body is washed and placed in boiling water for 5 to 15 minutes, thereafter they are prepared in broth or frits (Morales 2010).

In recent years, there has been a decrease in the number of hosts in plots with agricultural use, mainly due to the management of coffee plantations, maize and livestock pastures, which may affect the distribution and abundance of *A. armida*. The purpose of this research is to ascertain the influence of agricultural management on the distribution, occupation and abundance of larvae of *A. armida*, to inform local communities about the conditions that favor the emergence and proliferation of populations of this insect. This was carried out by establishing plots with and without agricultural management in areas where host plants are present. Assuming that *A. armida* tolerates high levels of perturbation (Escamilla-Prado et al. 2012, Landero-Torres et al. 2012), it is expected that this species occupies all available hosts and that abundance and distribution is homogeneous, independent of agricultural management.

MATERIALS AND METHODS

Study sites

Selected plots were either subject or not subject to agricultural management and possessed trees that are hosts for *A. armida*. A total of six sampling plots were established, located in the municipalities of Ocozocoautla de Espinosa, Huitiupan and Simojovel de Allende in Chiapas, Mexico (Fig. 1). In the first municipality, three plots were established within The Selva El Ocote Biosphere Reserve (REBISO, from Spanish name Reserva de la Biosfera Selva El Ocote). The vegetation at this site is semi-evergreen medium forest which includes several host tree species (Vázquez-Sánchez 1988, SEMARNAT-CONANP 2001, Orantes-García et al. 2013); anthropogenic disturbance is practically imperceptible, although high impact natural phenomenon, such as forest fires, can occasionally occur (Maldonado et al. 2009). The most recent forest fires were in 2003 (Table 1); however, those in 1998 resulted in significant damage to some areas of the reserve. Two of these plots are located in the periphery of the polygon of the reserve (Carrizal and Mirador) and one inside the polygon (Huachinadero), the three plots were partially affected by the fire of 1998, reason why they are formed of secondary vegetation, but none has been used for agricultural purposes. In Huitiupan and Simojovel, one and two plots were established respectively; in both municipalities host trees were present and local inhabitants were willing to conduct periodic observations in the field. The farmers harvested the trees



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FIGS. 2, 3. Frequency of occupied trees according to abundance classes (number of larvae), for *Arsenura armida*, in agriculturally managed and non-agriculturally managed plots, for each year of observation (Chiapas, Mexico): **2**, year 2015; **3**, year 2016.

TABLE 1. Geographical location, environmental characteristics and abundance of host species of *Arsenura armida* in agriculturally managed and non-agriculturally managed plots in Chiapas, Mexico. **G**, *Guazuma ulmifolia*; **HD**, *Heliocarpus donnell-smithii*; **HM**, *Heliocarpus americanus*; **HP**, *Heliocarpus appendiculatus*; **P**, *Pseudobombax ellipticum*; **R**, *Rollinia mucosa*.

Plot (municipality)	Latitude-longitude (altitude)	Abundance of hosts per plot (number of individuals)						Mean annual temperature °C[a]	Annual precipitation mm[a]	Natural Vegetation	Consumption of larvae	Agricultural activities
		G	**HD**	**HM**	**HP**	**P**	**R**					
Carrizal (Ocozocoautla de Espinosa)	16° 54' 37.1" 93° 38' 17.7" (824 m)	49	201		5	1	5	23.3	2145.2	High and medium semi evergreen forest	None	None. Recovering vegetation following fires of 1998
Huachinadero (Ocozocoautla de Espinosa)	16° 55' 43.4" 93° 38’ 9.2" (715 m)	7	208	1	21		10	23.3	2145.2	High and medium semi evergreen forest	None	None. Recovering vegetation following fires of 1998
Mirador (Ocozocoautla de Espinosa)	16° 54' 40.1" 93° 36'47.0" (776 m)	91	10	55		23	14	23.3	2145.2	High and medium semi evergreen forest	None	None. Recovering vegetation following fires of 1998
La Pimienta (Simojovel)	17° 8' 41.1" 92° 46' 8.0" (837 m)	33	15				31	22.4	2024.3	Medium forest	High	Cattle farming, coffee groves, cornfields
La Ilusion (Simojovel)	17° 8.0' 56.1"’ 92° 43"49.4" (875 m)	14	12				14	22.4	2024.3	Medium forest	High	Cattle farming, coffee groves, cornfields
La Loma (Huitupán)	17° 13' 18.4" 92° 38' 57.1" (554 m)	124	68	112				24.4	1622.1	High forest	Low	Cattle farming, coffee groves, cornfields

aSource: Servicio Meteorológico Nacional, Estaciones La Selva, Ocozocoautla de Espinosa; Simojovel y Almandro; y Huitiupán, Chiapas.

the natural forest for several years ago (at least 30), in order to have clear areas to do a variety of agricultural activities, some tree were left to delimit property boundaries. The plots with agricultural management change constantly according to the interest of the producers, just as the trees remain or are eliminated according to their usefulness in the main activity.

The three plots without agricultural management were located within the REBISO and those subject to agricultural management in the municipalities of Huitiupan and Simojovel. Each plot covered an area of 4 km^2, separated by a minimum distance of 500 m and maximum of 110 km. All the plots were visited systematically and exhaustively in order to identify the host tree species. The host species were individually georeferenced and DBH was measured during the first year of the study to estimate the development stage of each tree. Botanical samples were collected for reference and deposited in the CH Herbarium of El Colegio de la Frontera Sur (ECOSUR), San Cristobal de Las Casas, Chiapas, Mexico.

To detect the presence of larvae, each host tree was marked to conduct bimonthly observations during March-October on 2015 (year 1) and 2016 (year 2); this annual period encompasses the complete development cycle of *A. armida* (Costa et al. 2004). The location of larvae on each tree was recorded during the day, as this is when larvae are clustered together on the surface of the trunk and other woody parts of the plant. A pair of binoculars was required to record the number of larvae (tasco 8 x 30 mm-15–8x30RB, 131M/1000M) on high parts of the tree. Larvae occupation of each plot was estimated by calculating the proportion of total trees that possessed larvae during the observation period.

The consumption of larvae by inhabitants living close to the sampling plots was confirmed through direct questioning while agricultural activities within the plots were determined by observation.

Data analysis

Each annual cycle was analysed separately. Chi-squared statistical tests were performed to determine whether larvae occupation was associated with tree species and sampling plot. For this test, the presence/absence of larvae on each species of tree was considered; the test consisted of six rows (species or parcels) and two columns (presence or absence).

A Kruskal-Wallis analysis of variance (Snedecor & Cochran 1971) was performed to ascertain if abundance (number of larvae) differed significantly among plots and host species. Similarly, a χ^2 test was implemented to determine whether *A. armida* abundance per tree depended on sampling plot. Several classes of larvae abundance were established and tree frequency per class was determined.

To explore if host species development (DBH) and abundance influences the presence (occupation) and abundance of *A. armida*, a regression analysis was performed between the percentage of occupation (previously transformed by the arcsin $x^{1/2}$ function), mean DBH, and species abundance (number of trees), by plot and by species. In addition, a regression analysis of *A. armida* abundance against mean DBH and the number of trees by plot and species was carried out.

Mann-Whitney U tests were implemented to determine whether *A. armida* abundance differed between plots with and without agricultural management (comparison of two samples). All of the statistical analysis was carried out using the package IBM SPSS v. 21 (IBM Corp. 2012).

The geographical location of each tree was plotted onto a map created using the package Arc Map 10.2.1 (ESRI 2014). The data on *A. armida* occupation, collected over the whole observation period, and tree mortality, recorded during the second year, was added to the map. An analysis of the variance/mean relationship (v/m) was performed and the Morisita Index (Zarco-Espinosa et al. 2010) was obtained in order to determine the distribution of *A. armida* for each plot. The unit of observation was the abundance of larvae on the host trees in each plot. An obtained value close to 0 signified a random distribution, while a value approximate to 1 implied a uniform distribution; values above 1 indicated a clustered distribution (Badii et al. 2011, Ledo et al. 2012, De Sousa et al. 2015).

RESULTS

The host trees observed in the plots belonged to the following species: *Guazuma ulmifolia, Heliocarpus donnell-smithii, Heliocarpus americanus* L. 1753, *H. appendiculatus, Pseudobombax ellipticum* y *Rollinia mucosa* (Jacq. 1764) Baillon 1868 (Table 1). Tree abundance varied between 1 and 208 (number by plot) individuals per species/plot in the first year and between 1 and 193 in the second year. The species *G. ulmifolia* and *H. donnell-smithii* were present in all of the sampling plots, generally with a high level of abundance. The species *R. mucosa* was found in five plots but less abundant, while *H. americanus, H. appendiculatus* and *P. ellipticum* was observed in two or three plots with comparatively low abundance (Table 1). Tree mortality was observed in five plots; this was due to pests in three plots within the REBISO sample, while in the two Huitiupan and Simojovel plots, tree death was induced by local inhabitants making a cut at the base of the trunk. The dead trees in the REBISO corresponded to

TABLE 2. Diameter at breast height (DBH), tree abundance, percentage occupation and abundance of *Arsenura armida* for each year of observation (year 1 and 2), by plot type and host species, in Chiapas, Mexico.

Plot and species	Mean DBH (cm)	Abundance of trees (number of individuals)		Occupation of trees (%)		Abundance (Number of larvae)	
		Year					
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
A. Mean values per plot							
Carrizal (non-agriculturally managed)	22.7	261	253	3.0	4.7	545	728
Huachinadero (non-agriculturally managed)	22.9	247	212	7.0	4.2	1151	653
Mirador (non-agriculturally managed)	11.4	193	192	1.0	0	18	0
La Pimienta (agriculturally managed)	27.8	79	74	33.0	16.2	1730	1259
La Ilusion (agriculturally managed)	24.5	40	40	3.0	5	30	88
La Loma (agriculturally managed)	8.2	304	302	0.3	0	9	0
B. Mean values per species							
Guazuma ulmifolia	13.3	318	317	3.0	0.9	554	102
Heliocarpus donnell-smithii	21.5	514	467	3.0	3.4	793	1220
Heliocarpus americanus	8.2	168	168	1.0	0	9	0
Heliocarpus appendiculatus	23.3	26	26	33.0	15.4	621	449
Pseudobombax ellipticum	8.7	24	24	0.0	0	0	0
Rollinia mucosa	26.5	74	71	28.0	16.9	1506	957

the species *H. donnell-smithii* (44) and *G. ulmifolia* (1), *H. donnell-smithii* (3) and *R. mucosa* (3) in the other plots.

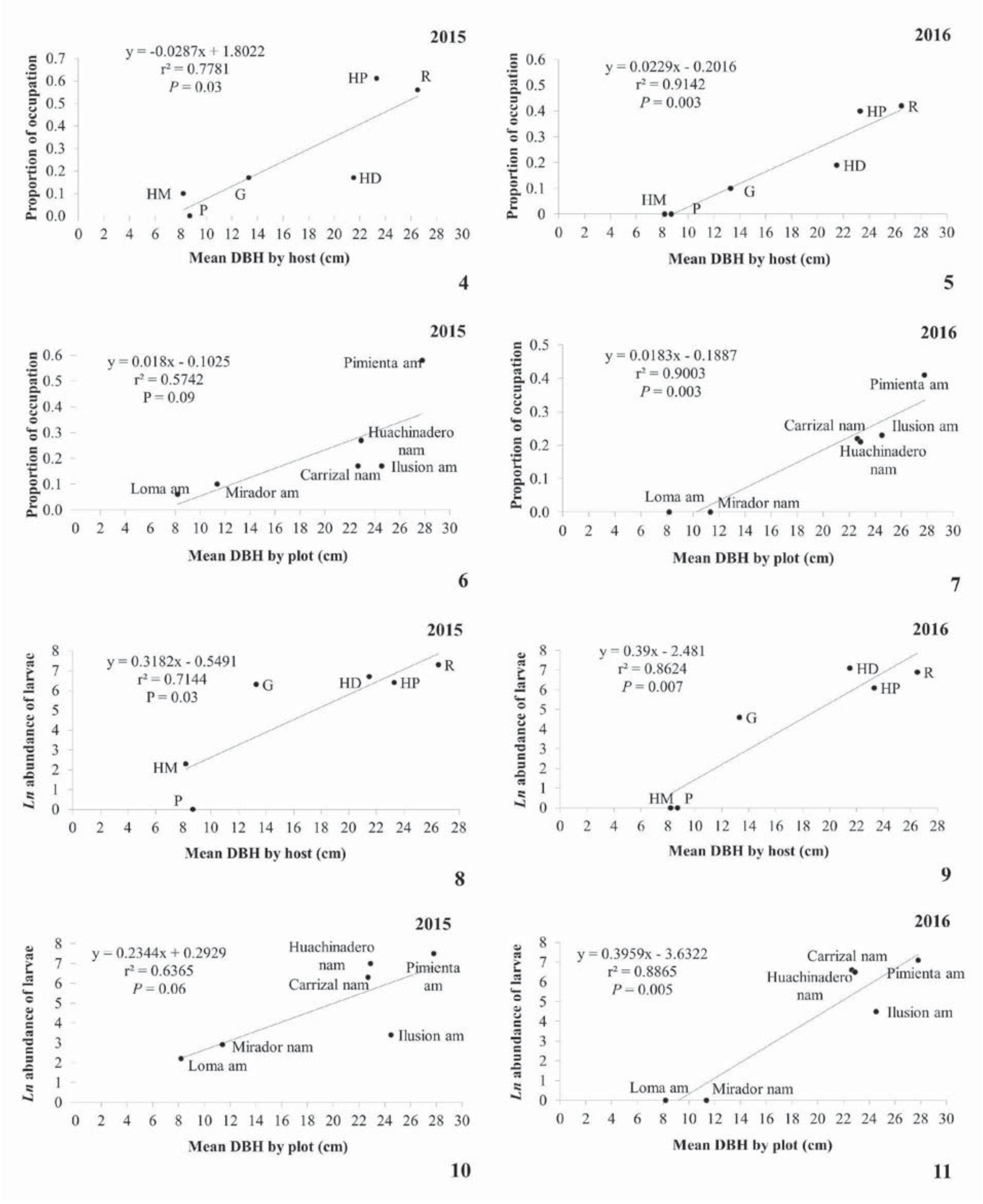
The mean diameter at breast height (DBH cm) was highest in two of the three plots with agricultural management (La Pimienta and La Ilusion) and human consumption of the larvae of *A. armida* (Table 2A). Significant differences in DBH were found between host species (Kruskall-Wallis, E= 405.78, g.l.= 4, $p<0.05$) and plots (Kruskall-Wallis, E= 568.21, g.l.= 5, $P < 0.05$). In one plot without agricultural management (El Mirador) and another with agricultural management (La Loma), mean DBH was lower, 11.4 and 8.2 cm respectively; *R. mucosa* presented a greater DBH than the other species, in particular when compared with the mean DBH of *H. americanus* and *P. ellipticum* (Table 2B).

In the first year of observations, occupation (proportion of trees observed with larvae) per plot fluctuated between 0.3 and 33% (Table 2A), while at the species level, this varied between 0 and 33% (Table 2B). Occupation by *A. armida* was significantly associated with species (year 1: χ^2= 139.42, g.l. = 5, $P < 0.0001$; year 2: χ^2= 65.87, g.l. = 5, $P < 0.0001$) and plot (year 1:χ^2= 157.51, g.l. = 5, $P < 0.0001$; year 2: χ^2= 58.80, g.l. = 5, $P < 0.0001$). In year 1, the host species with the highest level of occupation was *H. appendiculatus* followed by *R. mucosa*; in year 2, occupation was highest in *R. mucosa* followed by *H. appendiculatus* (Table 2B). The plot that presented the highest level of larval occupation, during the whole two year observation period, was La Pimienta (with agricultural management), notably higher than the other plots. A slight increase in the number of larvae was observed in two plots during the second year, one in the REBISO and the other in Simojovel; in contrast, a decrease in numbers was recorded in the remaining plots (Table 2A). *Arsenura armida* occupied three of the five available host species in the Carrizal and Huachinadero plots during year 1, one of five in El Mirador, all three species in La Pimienta and one of three in La Ilusion and La Loma. In year 2, the pattern was identical in Carrizal and Huachinadero, two out of three species were occupied in La Pimienta and La Ilusion and the insect was not present in Mirador and La Loma (Table 3).

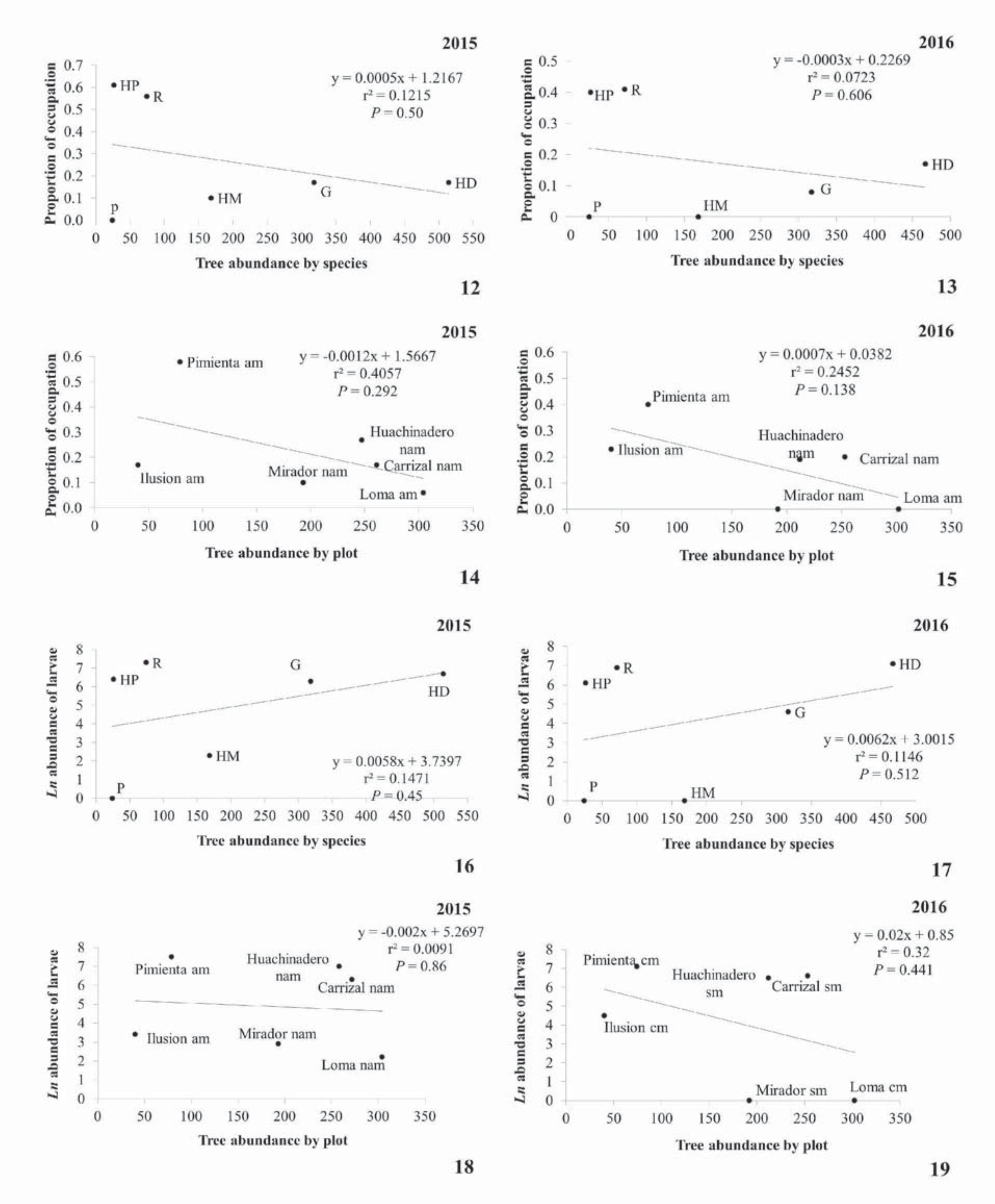
The number of larvae varied between 9 and 1730 per plot and between 0 and 1506 per species during the first

TABLE 3. Abundance of larvae, mean larvae/tree, abundance and occupation of trees by host species of *Arsenura armida* in agriculturally managed and non-agriculturally managed plots in Chiapas, Mexico. **G**, *Guazuma ulmifolia*; **HD**, *Heliocarpus donnell-smithii*; **HM**, *Heliocarpus americanus*; **HP**, *Heliocarpus appendiculatus*; **P**, *Pseudobombax ellipticum*; **R**, *Rollinia mucosa*. **AL**, Abundance of larvae; **ML**, Mean of larvae/tree **AT**, Abundance of trees; **OT**, Occupied trees; -, host species not present.

		Year 2015						Year 2016					
		G	**HD**	**HM**	**HP**	**P**	**R**	**G**	**HD**	**HM**	**HP**	**P**	**R**
Carrizal (non-agriculturally managed)	AL	168.0	157.0	-	0	0	220.0	54.0	505.0	-	169.0	0	0
	ML	3.4	0.8	-	0	0	44.0	1.1	2.6	-	33.8	0	0
	AT	47.0	201.0	-	5.0	1.0	5.0	49.0	193.0	-	5.0	1.0	5.0
	OT	2.0	3.0	-	0	0	3.0	2.0	9.0	-	1.0	0	0
Huachinadero (non-agriculturally managed)	AL	0	235.0	0	621.0	-	295.0	0	227.0	0	280.0	-	146.0
	ML	0	1.1	0	29.6	-	29.5	0	1.3	0	13.3	-	14.6
	AT	7.0	208.0	1.0	21.0	-	10.0	7.0	173.0	1.0	21.0	-	10.0
	OT	0	7.0	0	8.0	-	2.0	0	4.0	0	3.0	-	2.0
Mirador (non-agriculturally managed)	AL	0	0	9.0	-	0	0	0	0	0	-	0	0
	ML	0	0	0.2	-	0	0.6	0	0	0	-	0	0
	AT	91.0	10.0	55.0	-	23.0	14.0	91.0	9.0	55.0	-	23.0	14.0
	OT	0	0	1.0	-	0	1.0	0	0	0	-	0	0
La Pimienta (agriculturally managed)	AL	386.0	362.0	-	-		982.0	0	448.0	-	-		811.0
	ML	11.7	24.1	-	-		31.7	0	34.5	-	-		29.0
	AT	33.0	15.0	-	-		31.0	33.0	13.0	-	-		28.0
	OT	7.0	4.0	-	-		15.0	0	2.0	-	-		10.0
La Ilusion (agriculturally managed)	AL	0	30.0	-	-		0	48.0	40.0	-	-		0
	ML	0	2.5	-	-		0	3.4	3.3	-	-		0
	AT	14.0	12.0	-	-		14.0	14.0	12.0	-	-		14.0
	OT	0	1.0	-	-		0	1.0	1.0	-	-		0
La Loma (agriculturally managed)	AL	0	9.0	0	-		-	0	0	0	-		-
	ML	0	0.1	0	-		-	0	0	0	-		-
	AT	124.0	68.0	112.0	-		-	123.0	67.0	112.0	-		-
	OT	0	1.0	0	-		-	0	0	0	-		-



FIGS. 4–11. Lineal regression analysis of occupation (proportion of trees with larvae) and abundance of *Arsenura armida* against mean DBH by host species and plot type. **4**, Relationship between occupation of trees and mean DBH of host species in year 2015; **5**, year 2016; **6**, Occupation of trees and DBH by plot for year 2015; **7**, year 2016; **8**, Relationship between abundance and mean DBH by host species for year 2015; **9**, year 2016.; **10**, Relationship between abundance and mean DBH by plot for year 2015; **11**, year 2016 . **G**, *Guazuma ulmifolia*; **HD**, *Heliocarpus donnell-smithii*; **HM**, *Heliocarpus americanus*; **HP**, *Heliocarpus appendiculatus*; **P**, *Pseudobombax ellipticum*; **R**, *Rollinia mucosa*. **nam**, non-agriculturally managed; **am**, agriculturally managed.



FIGS. 12–19. Lineal regression analysis of occupation (proportion of trees with larvae) and abundance of *Arsenura armida* vs tree abundance by host species and plot: **12**, relationship between occupation of trees and tree abundance by species for year 2015; **13**, year 2016; **14**, Occupation of trees and tree abundance by plot for year 2015; **15**, year 2016;**16**, Relationship between larvae abundance and tree abundance by species for year 2015; **17**, year 2016; **18**, Relationship between larvae abundance and tree abundance by plot for year 2015; **19**, year 2016. **G**, *Guazuma ulmifolia*; **HD**, *Heliocarpus donnell-smithii*; **HM**, *Heliocarpus americanus*; **HP**, *Heliocarpus appendiculatus*; **P**, *Pseudobombax ellipticum*; **R**, *Rollinia mucosa*. The non- agriculturally managed plots are identified by "**nam**" and agriculturally managed plots by "**am**".

year of observations. In the second year, this varied between 0 and 1259 larvae per plot and 0 to 1220 per species.

Application of the Kruskall-Wallis test to the data from year one produced significant differences in abundance among plots (E = 158.454, g.l. = 5, $P < 0.05$) and host species (E = 140.116, g.l. = 5, $P < 0.05$), as did the same test with data from year 2 (plots: E = 58.973, g.l. = 5, $P < 0.05$; host species: E = 66.359, g.l. = 5, $P < 0.05$).

Throughout the two year observation period, the distribution of larvae per tree varied within and among plots (Figs. 2, 3). In year one, a few trees each with a small number of larvae were found at La Loma (Huituipan) while more trees with larvae were observed in La Pimienta (between 1 and 100 larvae per tree). No larvae were found on trees at El Mirador and La Loma during year 2. A χ^2 test detected a significant association between larvae abundance and plot for the two years' observation period (year 1: χ^2=168.98, g.l.= 30, $P < 0.001$; year 2: χ^2=131.28, g.l.= 30; $P < 0.001$).

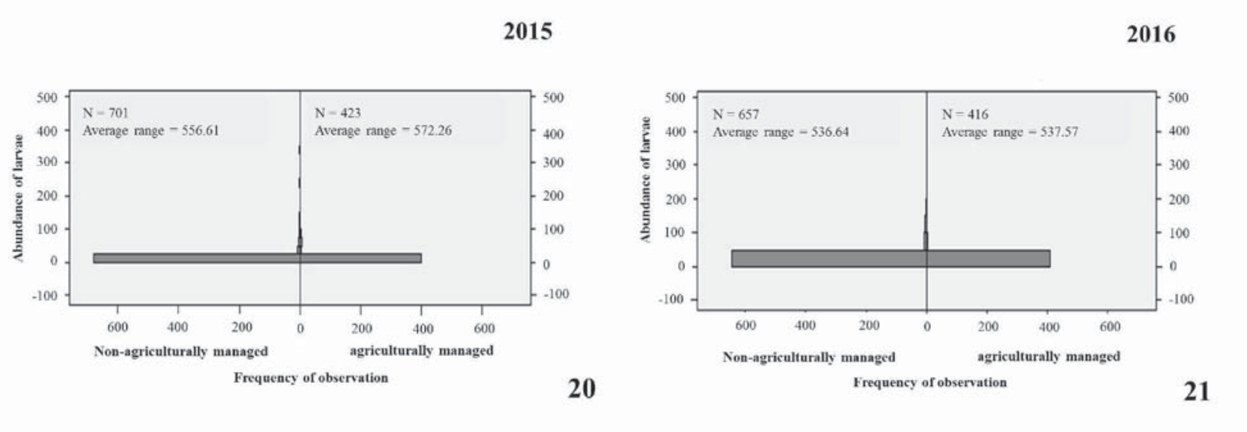
The regression analysis identified a significant relationship between occupation and mean host DBH for the two year period (Fig. 4, 5); however, there was no significant relationship between occupation and DBH per plot for year one (Fig. 6), in contrast with a significant association in year 2 (Fig. 7).

A significant relationship was found between the abundance of *A. armida* and mean DBH of host species for the two year study period (Fig. 8, 9). This relationship was marginally significant for the first year (Fig. 10) and significant for the second (Fig. 11). Occupation by *A. armida* did not demonstrate any significant relationship with tree abundance by species (number of trees) (Fig. 12, 13) or plot (Fig. 14, 15). Similarly, no significant relationship was demonstrated between the abundance of *A. armida* and tree abundance, species (Fig. 16, 17) or plot (Fig. 18, 19).

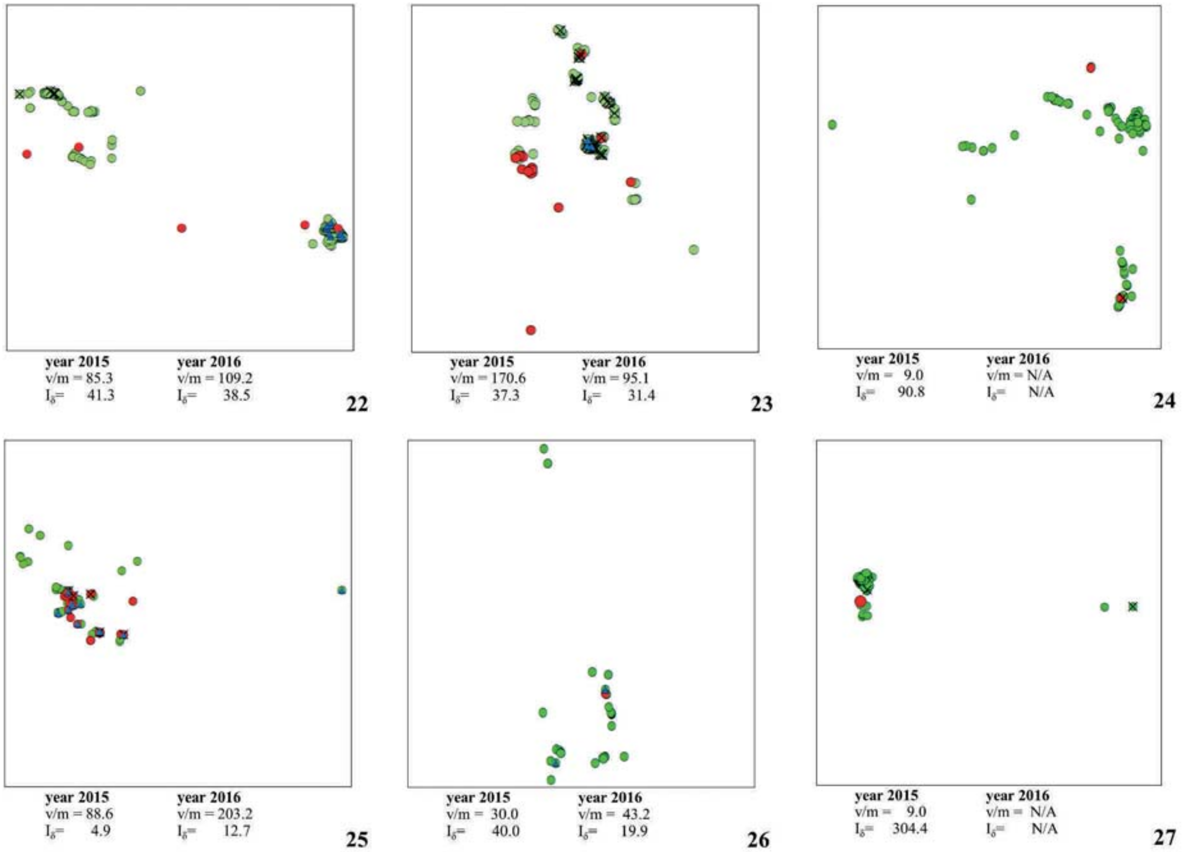
There were significant differences in larvae abundance between plots with or without agricultural management in year one (U = 153,389.5, Z= 2.094, P= 0.036; Fig. 20), but not for year two (U = 136,895.0, Z= 0.157, P= 0.875: Fig. 21). The lowest mean abundance was observed in plots without agricultural management during both years. The variance/mean relationship (v/m) and the Morisita distribution index (I_δ) was greater than one for all the plots, signifying that with respect to its host trees, *A. armida* demonstrated a clustered spatial distribution over the two year period (Figs. 22, 23, 24, 25, 26, 27).

DISCUSSION

We are reporting by first time *H. americanus* y *Rollinia mucosa* as hosts for *A. armida*. The tree species, *Pseudobombax ellipticum* has been recognized as host (Costa et al. 2003, Nielsen et al. 2004, Escamilla-Prado et al. 2012, Landero-Torres et al. 2012), but no larvae were observed on this species. The abundance and occupation of *A. armida* varied considerably among plots, host species and number of years of observation. In the first year, plots subject to agricultural management that presented a greater mean DBH of host trees, demonstrated a higher abundance of larvae and number of occupied trees. The results suggest that agricultural management favors the occupation and abundance of *A. armida*, when the presence and growth of the host trees is not restricted. This is probably because host plants in agroecosystems



FIGS. 20, 21. Results of the Mann-Whitney U test on the abundance of *A. armida* according to plot management: **20**, for year 2015; **21**, year 2016. To interpret the graph, consider that on the y axis is the range of larvae abundance assigned by the test; the x axis represents the frequency of observations of each interval. For example, for year 1, on the non-agriculturally managed for the average range 12.5, there were 682 observations and 402 with agriculturally management.



FIGS. 22–27. Distribution and occupation of host trees of *A. armida* in each plot for years' 2015 and 2016 (Chiapas, Mexico): **22**, Carrizal; **23**, Huachinadero; **24**, Mirador; **25**, La Pimienta; **26**, La Ilusion; **27**, La Loma. Host trees marked in the first year are represented by a green circle; trees occupied by larvae of A. armida in the first year (2015) are represented by a red circle; trees occupied by larvae during the second year (2016) by a blue triangle and dead trees observed in year 2016, by a "×". **22–24** represent non-agriculturally managed plots while **25–27** represent agriculturally managed plots.

are more visible and detectable by insects and can therefore be colonized more easily (Feeney 1976, Stiling 2002).

Tree mortality in plots with and without agricultural management reduced larvae abundance during the second year; this corresponded with a higher abundance of lepidopteran larvae recorded in areas with higher tree density (Vargas-Zapata et al. 2015). In our case, tree mortality not only resulted in a decrease in tree density but also represented a direct loss of *A. armida* as a local food resource. In addition, this could result in the modification of micro-environmental conditions required for larvae development (Muriel et al. 2011). This differs from other studies that have documented that although anthropogenic intervention results in a decrease in host availability, the abundance of polyphagous insects is maintained (Brown 1984, Molina-Martínez and León-Cortes 2006, Vargas & Parra 2009).

Arsenura armida larvae were more abundant on trees with a higher DBH, indicating a potential preference for mature trees probably due to their higher food availability (leaves). However, it may also be because they are more easily located by the female for oviposition or are used for mating (Pérez-Contreras, 1999). Pacheco-Flores et al. (2006) found that larvae of *Acharia extensa* (Schaus 1896) (Lepidoptera: Limacodidae) were more abundant on old trees (DAP > 88 cm), consistent with our observations on *A. armida*. There is a possibility that agricultural management allows trees to grow over a prolonged period of time. Furthermore, an absence of interspecific competition results in larger DBH than in systems subject to a lower degree of human intervention. This is evident in the relatively undisturbed forest plots of REBISO where pioneer plant species such as the host species of *A. armida*, maintain lower diameters as a consequence of the natural successional processes that take place during mature forest regeneration (Ramírez-Marcial et al. 2017).

The larvae of *A. armida* did not occupy all the trees of all the species available in the plots. The highest abundance and occupation was observed on the hosts *H. appendiculatus* and *R. mucosa*, this pattern was observed during both year l and 2. This result can be interpreted as a possible choice of the host, however data of more observation time are necessary, as well as palatability test and response to volatile compound stimuli, assuming that insect response is related to the nutrient and phytochemical content of the plants (Feeney 1976, Coley et al. 1985). Also, genetic studies are required to improve our understanding of this interaction and to discover if the plant hosts are generating a process of differentiation in populations of *A. armida*.

The distribution of trees varied according to sampling plot; highly dispersed in El Carrizal, Huachinadero and Mirador; but clustered in La Pimienta, La Ilusion and La Loma. The larvae presented a clustered, non-uniform distribution with respect to the tree hosts, that is to say the larvae were present on a few trees belonging to only several species. This could have been due to the social habits of the larvae of *A. armida*, as they remain together on the tree bark from the first to the fifth instar, forming groups of 350 to 500 individuals on only one host (Janzen 1970). However, lepidopterans with solitary habits have also displayed this distribution pattern on monoculture farms (Serra et al. 2005). In this study, between one and 329 larvae were observed on one individual tree host during year 1 and between one and 398 in year 2. The mean number of larvae per tree was higher for species *R. mucosa* (year 1: 35, year 2: 22) and *H. appendiculatus* (year 1: 30, year 2: 24).

Human consumption of *A. armida* was practiced in the communities with plots subject to agricultural management. As the majority of larvae found on the host are collected, very few individuals attain adulthood. However, the results of this research suggest that human consumption does not have a negative effect on the abundance of *A. armida*. The individuals that are undetected by collectors, predators and parasitoids are those that will maintain future generations. The surviving larvae descend from the trees, pupate in the soil and emerge the following year. In some parts of Mexico the growth of host trees in coffee plantations is allowed in order to promote the presence of *A. armida* (Landero-Torres et al. 2012) but there are no strategies for the cultivation and sustainable harvesting of *A. armida*. Our findings may eventually contribute to the development of this strategy.

In plots with agricultural management, the appearance of adult insects commenced at the start of May in while this occurred at the end of May and start of June in non-managed plots. Eclosion occurred with 15 days of difference between both types of plot. This differentiation in the biological cycle of *A. armida* could be related to environmental conditions within the plots (García-Pereira et al. 2003, Muñoz and Amarillo-Suárez 2010). In REBISO, the plots are forested and thus the environment is more humid and warmer (23 °C), while in the managed plots conditions are drier and 1°C cooler. The sustainable exploitation of *A. armida* is possible in agricultural plots, maintaining a diversity of host species that are allowed to grow to a large size (DBH approximately 20 cm).

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LIFE HISTORY DESCRIPTIONS OF *ADELPHA NAXIA NAXIA* AND *A. NEA NEA* (NYMPHALIDAE) FROM COSTA RICA

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ABSTRACT. Butterflies in the genus *Adelpha* Hübner (Nymphalidae) are a challenging group to identify owing to their convergence in wing patterns among species. Immature stage biology including morphology and hostplant relationships have helped clarify relationships in this group and will likely continue to do so. Here we report descriptions of the immature stages of two species, *Adelpha naxia naxia* (C. Felder & R. Felder, 1867) and *Adelpha nea nea* (Hewitson, 1847), based on recent fieldwork on the Osa peninsula of Costa Rica. We observed *A. naxia naxia* caterpillars feeding on *Vitex cooperi* Standl. (Lamiaceae), and the immature stage morphology corroborates its position in recent phylogenetic analyses. The food plant of *A. nea nea* is *Micropholis melinoniana* Pierre (Sapotaceae), representing the first record of this host family for *Adelpha* and Neotropical nymphalids. *Adelpha nea nea* also appears unique in always developing through six larval instars. In addition to descriptions and discussion of immature stage biology and food plant interactions, adult observations are also provided.

Additional keywords: immature stages, latex, Limenitidinae, vein cutting, vein trenching

Butterflies in the genus *Adelpha* are widespread and conspicuous throughout the Neotropics, with species found in a diverse range of habitats extending from the United States to Uruguay (Willmott 2003b). Convergence in wing pattern among species (Willmott 2003b, Ebel et al. 2015) makes species delimitations based on adult morphology difficult, and misidentifications have increased the confusion through the description of numerous synonyms. Work by Willmott (2003b) gave a comprehensive summary of the present knowledge of *Adelpha* systematics and clarified the redundant names. In combination with subsequent work (Prudic et al. 2008, Willmott & Hall 2013), the genus contains more than 90 species and more than 120 additional subspecies.

Immature stages have been useful in helping to clarify *Adelpha* species groups and understand taxonomy in the face of mimetic similarity among adults (Aiello 1984; 1991, Otero & Aiello 1996). Using immature stage morphology, Aiello and others (Aiello 1984; 1991, Otero & Aiello 1996) identified seven or more species groups within *Adelpha*. Based on his studies of adults and immatures, Willmott (2003a) rearranged *Adelpha* into six provisional groups, after finding some of the previous groups to be polyphyletic. Recent molecular work on the phylogeny of *Adelpha* corroborates some of these species groups (e.g. *A. alala*, *A. serpa*, *A. phylaca*) (Ebel et al. 2015) and allows further exploration of the traits associated with hypothesized clades.

Despite the series of studies focused on this genus thus far, there remains much to be discovered. Willmott (2003b) and Aiello (2006) called for publishing any information on *Adelpha*, and on life history stages in particular, in order to clarify species boundaries, determine larval host breadth, and elucidate mimicry patterns. During recent fieldwork in Costa Rica we discovered the immature stages and larval food plants for two *Adelpha* species with previously unknown life histories; *A. naxia* (C. Felder & R. Felder, 1867) and *A. nea* (Hewitson, 1847). In this paper we describe the life histories of those two species, and discuss their immature stage characters as related to identification and systematic relationships, as well as larval behavior and food plant interactions, and also provide observations on adult behavior.

MATERIALS & METHODS

Observations were made in the vicinity of Barrio Guadalupe (08.61801,-83.48025), near the town of La Palma, on the Osa Peninsula, from June to August 2016. In general, forest edges, and ridges in primary forest were searched for larvae and adults. *Adelpha naxia naxia* were collected in secondary forest habitat and edges, whereas *Adelpha nea nea* were collected along the sides of a narrow dirt road that follows a ridge in primary forest. Larval food plants were identified by Orlando Vargas and Reinaldo Aguilar, as well as by referencing the Organization for Tropical Studies online plant database.

Larvae were reared individually in plastic bags under ambient conditions. Bags were hung along a line so that leaves would approximate natural positions, and to expose leaf margins for larvae to build frass chains. Larval bags were cleaned daily with toilet paper. Larval and pupa durations were recorded daily. Head capsules

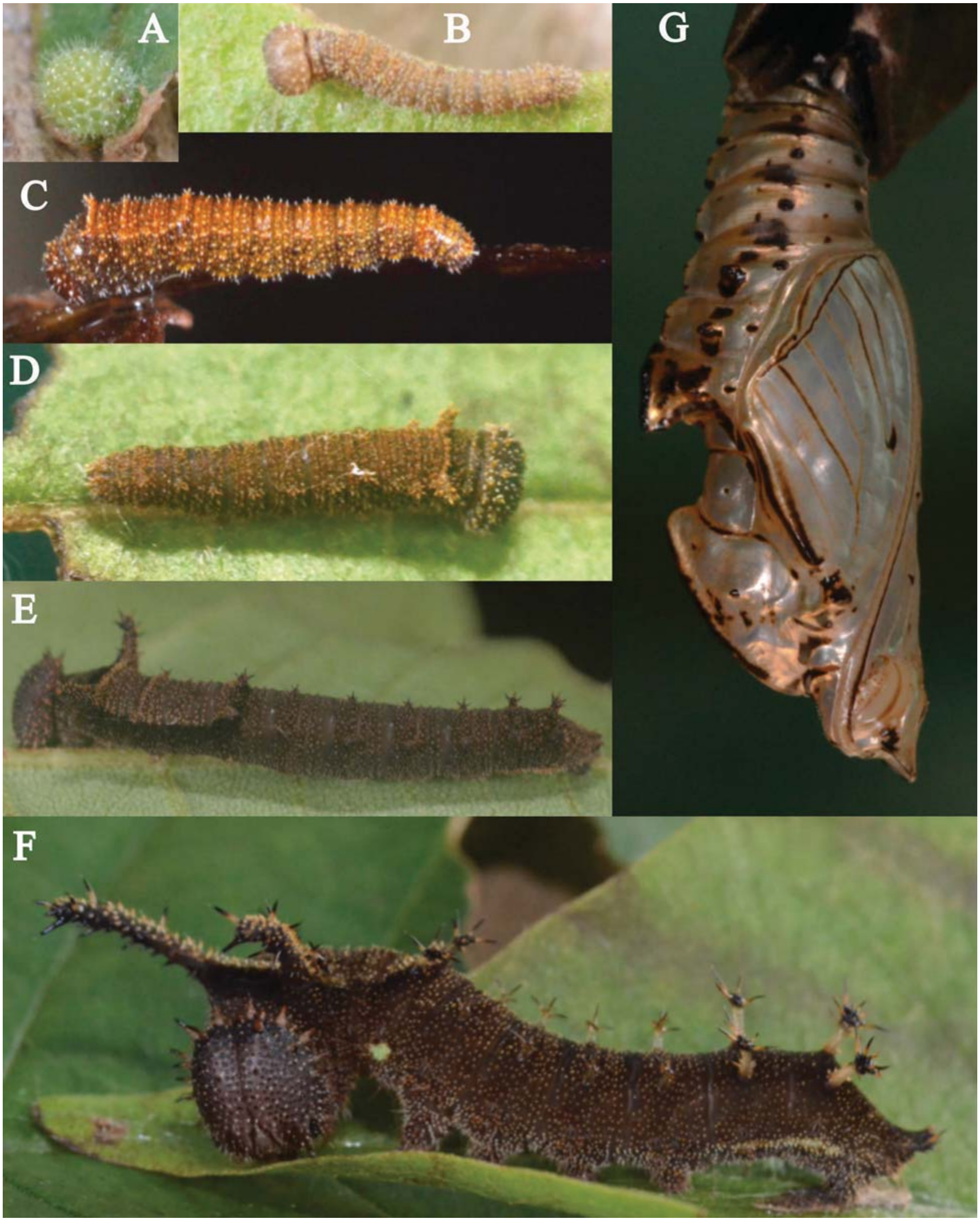

FIG. 1. Immature stages of *Adelpha naxia naxia*. **A.** Egg. **B.** First instar. **C.** Second instar. **D.** Third instar. **E.** Fourth instar. **F.** Fifth instar. **G.** Pupa, lateral view.

and pupal exuviae were collected for later description. Photographs of each stage were taken using a Nikon D7000, and Micro Nikkor 105mm lens fitted with extension tubes (Kenko) and a ring flash (Nikon SB-R200 Speedlight).

Larval descriptions were made based on photos taken during rearing. Morphological terminology follows that described and used by Willmott (2003b) and Aiello (2006). We use the taxonomic arrangement of Willmott (2003b). Reported head size is based on measurements of head capsules. Head capsule drawings and measurements were made with a Leica S8 APO stereomicroscope with an attached Leica DFC295 camera. Head capsule width was measured at the widest part (generally near the most dorsal stemmata) using Leica Application Suite version 3.8.

Results

Adelpha naxia naxia C. Felder & R. Felder, 1867

Food plant and oviposition. The larval food plant of *Adelpha naxia naxia* was found to be *Vitex cooperi* Standl. (family Lamiaceae, formerly placed in Verbenaceae). *Vitex cooperi* is found from sea level to 600m in secondary habitats and along edges in tropical moist to wet forests, and is native from Guatemala to Panama (Croat 1978). The range of *A. naxia* extends beyond that of *V. cooperi*, being found from Mexico to as far south as Bolivia and the Amazon basin of Brazil. Eggs were laid singly on the upper surfaces of leaves at the leaf margin or tip, from one to four meters above the ground. Plants containing eggs were mature trees in open habitats. It should be noted that DeVries' host records for *A. naxia* feeding on *Vitex cooperi* and *Piper arieianum* C.DC. (Piperaceae) reported in Aiello (1984) were errors (see Appendix C of Willmott 2003b), and actually pertain to *A. heraclea* (C. Felder & R. Felder, 1867).

Vitex cooperi is also a host for *Adelpha heraclea heraclea* (C. Felder & R. Felder, 1867) in Costa Rica (CER pers. obs, DeVries 1985(86)), and eggs and larvae of *A. h. heraclea* were found on *V. cooperi* at the study site. Both *A. n. naxia* and *A. h. heraclea* were found intermingled on the same trees, but not on the same leaf. *Adelpha h. heraclea* immatures were found in much higher abundance than *A. n. naxia* when collected haphazardly from the three main trees where immatures were found.

Egg. Fig. 1. Duration unobserved. Like other *Adelpha* the egg is pale greenish, round with hexagonal pits, and with a single seta arising from each angle of the hexagon. Eggs are laid singly, up to several per plant.

First Instar. Fig. 1. Duration: 4 days, n = 1. Head: 0.63 mm, n = 1. Very similar to the first instar of other *Adelpha* species. The head is rounded with short setae, it is pale brown dorsally and laterally, and has a dark frons. The body is pale green after first feeding and takes on a brown hue by the end of the stadium. The body tapers noticeably toward the posterior, is darker in between segments, and is adorned with numerous tubercles and short setae.

Second Instar. Fig. 1. Duration unobserved. Head: 0.86 mm, n = 1. The head is brown dorsally and medially and has a black frons. The body is dark brown with shades of black between segments, and is covered with yellow-white bumps and short setae. Developing subdorsal scoli are most pronounced on T2 and A2.

Third Instar. Fig. 1. Duration: 6 days, n = 1. Head: $\bar{x}$ = 1.19 mm, s.d. = 0.22, n = 3. The head is entirely brown-black with tubercles and developing chalazae that are both light colored. The body is dark brown to black, darkest between segments. Short, dull brown-orange setae cover the body. A pair of pronounced subdorsal scoli are present on T2 with a slightly raised transverse ridge connecting them. Short subdorsal scoli are also present on T3 and abdominal segments A2–A8 and A10.

Fourth Instar. Fig. 1. Duration: $\bar{x}$= 4.4 days, s.d. = 0.60, n = 5. Head: $\bar{x}$ = 2.07 mm, s.d. = 0.10, n = 4). The head is similar to that of the previous instar but the chalazae are better developed and the colors contrast less. The body is dark brown to black, and is textured with yellow-white bumps. Only subdorsal scoli are present. The pair of subdorsal scoli on T2 are most prominent, with spines developing along their length. The scoli on T3 and A3–A6 have spines radiating directly off the body in groups of 3–5. The scoli on A7–A8 are short with radiating spines at their tip, and A10 has short scoli.

Fifth Instar. Fig. 1. Duration: $\bar{x}$ = 7.7 days, s.d. = 1.0, n = 6. Head: $\bar{x}$ = 3.27 mm, s.d. = 0.07, n = 5. The head is dark brown to black and the frons has vertical pale-brown streaks laterally. Some variation is seen in the width of the streaks on the frons, and they also vary from off-white to dark brown. Chalazae on the head are triangular, dark brown with black tips, and arranged in 3 series: 6 posterior, 4 medial, 2 anterior (Fig. 2). The body is dark brown with shades of black and is covered in small pale yellow dots. A subspiracular yellow-green spot is present on A2, and a similarly colored subspiracular streak is present on A7–A8. The body becomes pale brown the day before pupation. The body is adorned with prominent subdorsal scoli and reduced subspiracular scoli. Subdorsal scoli on T2 are relatively long, cylindrical, notably thick and arced anteriorly. The

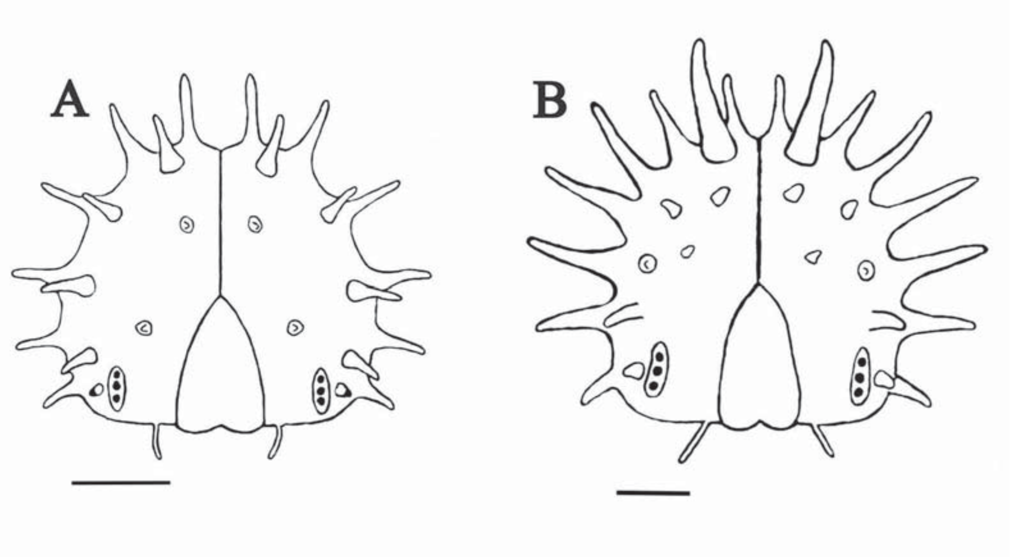

FIG. 2. Ultimate instar head capsule drawings of *A. naxia naxia* (**A**) and A. *nea nea* (**B**). Each scale bar represents one millimeter.

T2 scoli are dark brown, with many spines along their length. These spines are orange-brown on the posterior side and dark brown on the anterior side. A slightly raised transverse ridge connects the T2 scoli at their base and is nearly black on its anterior side. Subdorsal scoli on T3 are conical and pale-tan with a dark brown tip and 3–5 spines along their length. Scoli on A2 are conical and thicker than the T3 scoli, and show variation between individuals. The A2 scoli range from dark brown to tan, and have orange-brown spines along their length and radiating from the tip. Subdorsal scoli on A3–A6 are the shortest present. They are pale tan and conical with 3–5 spines radiating at the tip. The A7–A8 scoli resemble A3–A6 in shape, but are longer and dark brown at the crown where the spines arise. Variation was seen in one individual with the A2 scoli paler, resembling the A7–A8 scoli. All spines arising from the subdorsal scoli are orange-brown and tipped with black, and vary in size and thickness. Subspiracular scoli on A3–A7 are somewhat reduced, consisting of a crown of 1–3 spines.

Larval Behavior. Larvae were observed feeding in a manner typical for the genus. Once hatched, larvae ate the remains of the egg, and began eating at the border of the leaf either at the central vein or at a secondary vein, eating around the vein to expose it. After the vein was exposed the larvae silked together pieces of frass to extend the vein. They then rested on this frass chain when not feeding, either in a straight position or the front-curved position (as described by Aiello 1984) with the head and thorax lifted off the substrate. At the base of the frass chain bits of leaf and frass were silked together, creating a barricade between the leaf and the extended vein. This barricade sat on top of the leaf or hung below. Larvae quickly retreated to their frass chain when the leaf was disturbed. This behavior continued through the fourth instar. During the fifth stadium, the larvae no longer created or used frass chains, and ate the entire leaf. Larvae rested at the base of the leaf on the upper or lower surface in the front-arched-rear-up position (Aiello 1984), in which both the anterior and posterior portions of the body were raised and arched, or in a straight position along the main vein. Pupation did not occur at a specific place on the leaf, and larvae often attached themselves to the side of the plastic rearing bag.

Pupa. Fig. 1 and Fig. 3. Duration: $\bar{x} = 7.1$ days, s.d. = 0.30, n = 9. The pupa is pearly white with copper tints, and has dark sutures, spots, and streaks that are highlighted with orange-brown. The pupa darkens the day before adult eclosion. As is characteristic for *Adelpha*, the head bears a pair of apical projections (appearing as "head horns"). In *A. n. naxia* these projections are widely separated at the base (as in *A. h. heraclea*), and shaped like small laterally pointing triangles. The thorax rises abruptly posterior to the head and forms a keel posteriorly along the dorsal midline. On T2 the dorsal keel leads into a projection that forms a slight hump directed posteriorly. A dorsal projection, curved sharply to the anterior, is also present on A2, and is slightly larger than that on T2. The dorsal projections on T2 and A1 are moderately sized for the genus. Posterior to A2 each abdominal segment has a dorsal keel that is smaller than that of the preceding segment. Segments A3 and A4 have a pair of dorsolateral tubercles that are irregularly shaped and dark in color. These partly create a rough broken line from A2 merging with the dark colored spiracles. The abdomen also has two ventral series of dark spots, one ventrolateral and one ventral, with the ventrolateral series smaller than the ventral series. Segment A9 has a short lateral spine and a pair of curved black ridges ventrally. The cremaster is pale, tipped with black. The wing pad is unmarked except for brown along wing veins. Pupation occurred on the surface of the leaf or on the rearing bag.

Adult. Fig. 4a. Based on our field observations adult male *A. naxia naxia* were found flying from early morning to early afternoon (2–3pm). In the morning males were observed basking very low or on the ground in direct sunlight, and flying lazily compared to their afternoon flight.

Adelpha n. naxia male perching behavior appeared unique compared to other *Adelpha* males in Costa Rica. *Adelpha n. naxia* males perched as low as 1 m and higher, with the highest observed perch being ~8m. In contrast, our observations of *A. serpa celerio* (H. W. Bates, 1864), *A. erymanthis erymanthis* (Godman and Salvin, 1884), *A. salmoneus salmonides* (Hall, 1938) and *A. n. naxia*'s sister species *A. h. heraclea* (Ebel et al. 2015), indicate that these species typically perch high (usually observed 5m and higher) and defend their perches with direct fast flight. Furthermore, the flight of *A. n. naxia* males while defending their perches was especially fast and erratic with seemingly higher accelerations compared to the aforementioned species.

Adelpha nea nea Hewitson, 1847

Food plant and oviposition. Immatures of *Adelpha nea nea* were found eating leaves of *Micropholis melinoniana* Pierre (Sapotaceae). This tree ranges from Brazil to Mexico in moist to wet lowland tropical forests, at low to medium elevations, and the damaged parts dripped a white latex (Condit et al. 2011, Gentry 1993). Immatures were found on a single tree. The host plant grew on a ridge in primary forest at the edge of a road that created a gap through which sun could reach during the middle of the day. Eggs were laid singly on mature trees and on old or damaged leaves. Eggs were placed along the leaf edge or at damaged portions of the leaf.

Egg. Fig.5. Duration unobserved. Like other *Adelpha* eggs, pale green and round with hexagonal pits that have a seta at each angle. *Adelpha nea nea* eggs are relatively large for *Adelpha*, similar to other members of the *serpa* group.

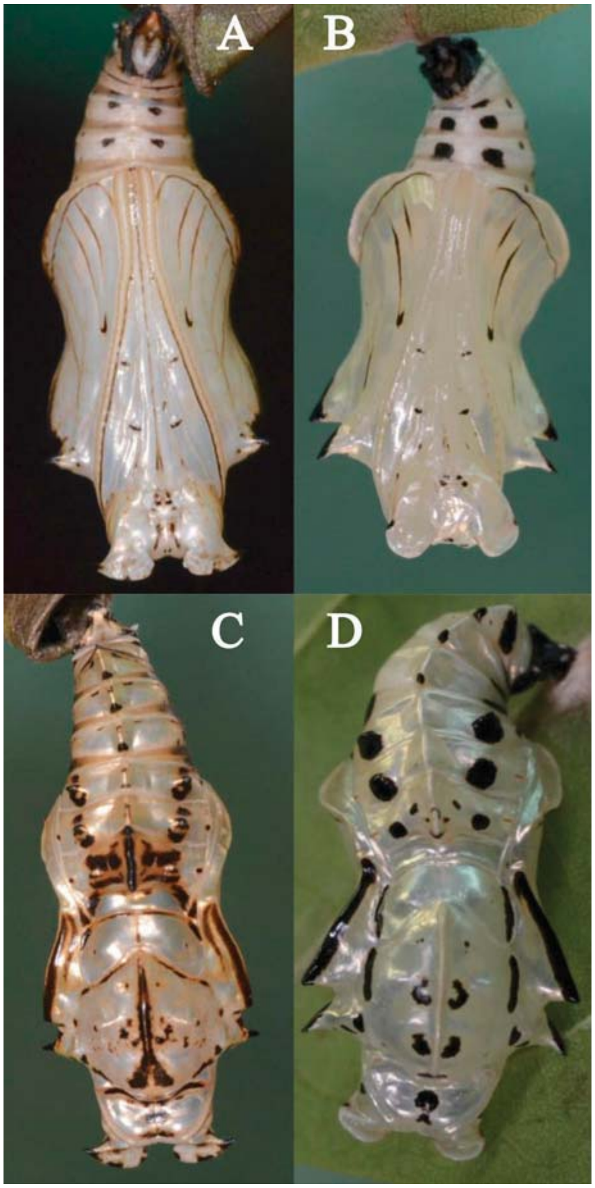

FIG. 3. Comparison of pupae of *A. naxia naxia* (A & C) and *A. heraclea heraclea* (B & D) from the Osa peninsula.

First Instar. Fig.5. Duration: $\bar{x}$ = 4.3 days, s.d. = 0.50, n = 4. Head: $\bar{x}$ = 0.85 mm, s.d. = 0.38, n = 4. Similar to other species of *Adelpha*. After hatching, the larva is green-grey and bears small pale bumps along the body, with short setae on the head. The head is light brown with a black frons. By the end of this instar the body is light brown with small bumps where scoli will be in later instars.

Second Instar. Fig. 5. Duration: $\bar{x}$ = 5.4 days, s.d. = 0.80, n = 7. Head: $\bar{x}$ = 1.12 mm, s.d. = 0.57, n = 3. The larva is brown with shades of black, blending in with the

FIG. 4. Adult images of *A. naxia naxia* (**A**) and *A. nea nea* (**B**) reared in this study. Dorsal view is on the left of each image and ventral view is on the right.

frass and leaf debris, and the body tapers slightly to the posterior. The head is brown with a black frons, with the black extending dorsally to the rudimentary m1 chalazae. The subdorsal scoli begin to show in this instar and are distinct from the other miscellaneous lighter colored bumps by the end of this stage.

Third Instar. Fig.5. Duration: $\bar{x}$ = 5.3 days, s.d. = 0.80, n = 6. Head: $\bar{x}$ = 1.50 mm, s.d. = 0.62, n = 5. This instar is very similar to the previous one, with the following changes. The scoli are more developed this instar, with subdorsal scoli on T2, A2, A4 and A7 most pronounced. Both supra- and subspiracular scoli appear along the body. The larva is brown and contrasts with lighter colored scoli,and there are darker black patches present as well as more apparent black bands between segments.

Fourth Instar. Fig.5. Duration: $\bar{x}$ = 5.2 days, s.d. = 1.0, n = 6. Head: $\bar{x}$ = 2.03 mm, s.d. = 0.83, n = 5. The body is brown with shades of darker brown and light brown mottling on segments, and light brown between segments. The head is the same light brown as the body, but has darker vertical stripes down the frons, and small distinct patches laterally between the first and second rows of developing chalazae. Thick short subdorsal scoli are present on T1, T2, A2, A4, A7, and A8. The subdorsal scoli terminate in an irregular spreading crown of spines. A9 and A10 have a pair of thick dorsal scoli.

Fifth Instar. Fig.5. Duration: $\bar{x}$ = 6 days, s.d. = 0.89, n = 6. Head: $\bar{x}$ = 2.85 mm, s.d. = 1.16, n = 5. The body coloration in this instar is a combination of dark and tan patches with purple-brown showing between segments. The head is patterned similar to the previous instar but with chalazae more developed. The chalazae are relatively pale compared with the head. The subdorsal scoli are brown, and are generally long and cylindrical with spines arising at irregular intervals along their entire length and clustered near the tip. Subdorsal scoli are well developed on all segments except A1. The presence of well-developed subdorsal scoli on A9 in *A. nea nea* is distinct from other *serpa* group species. The prolegs are relatively pale, as are the scoli arising dorsal to them.

Sixth Instar. Fig.5. Duration: $\bar{x}$ = 10.2 days, s.d. = 0.98, n = 6. Head capsule: $\bar{x}$ = 3.94 mm, s.d. = 1.61, n = 4. Distinct in color from the previous instars. This instar is predominantly mottled pale green and dark green with pink to purple bands between segments. The larva changes to yellow-brown the day before pupating. The head is pale tan with distinct brown vertical stripes along the frons reaching from the tip of the m1 chalazae to the bottom of the frons. The lateral part of the head is dark brown near the anterior and medial rows of chalazae. The chalazae are pale brown with m1 having a brown anterior face. The chalazae are elongated and triangular in shape, and arranged in 3 series: 7 posterior, 4 medial, 2 anterior (Fig. 2). Subdorsal scoli are the best developed and present on each segment except T1 and A1. Subdorsal scoli are generally relatively long and cylindrical with intermittent spines, and end in an asymmetric fan of flattened spines. Subdorsal scoli are shortest on A5, A6, A9, and A10. The subdorsal scoli are brown and have dark green patches at the base, except forA9 and A10 which are pale green. Supraspiracular scoli on T1 are cylindrical and pale brown with intermittent spines along the length and curved slightly dorsally. Short supraspiracular scoli are found T3, A2–A7, are pale green and have 2–6 spines radiating at the tip. Supraspiracular scoli on T2 are slightly shorter than the

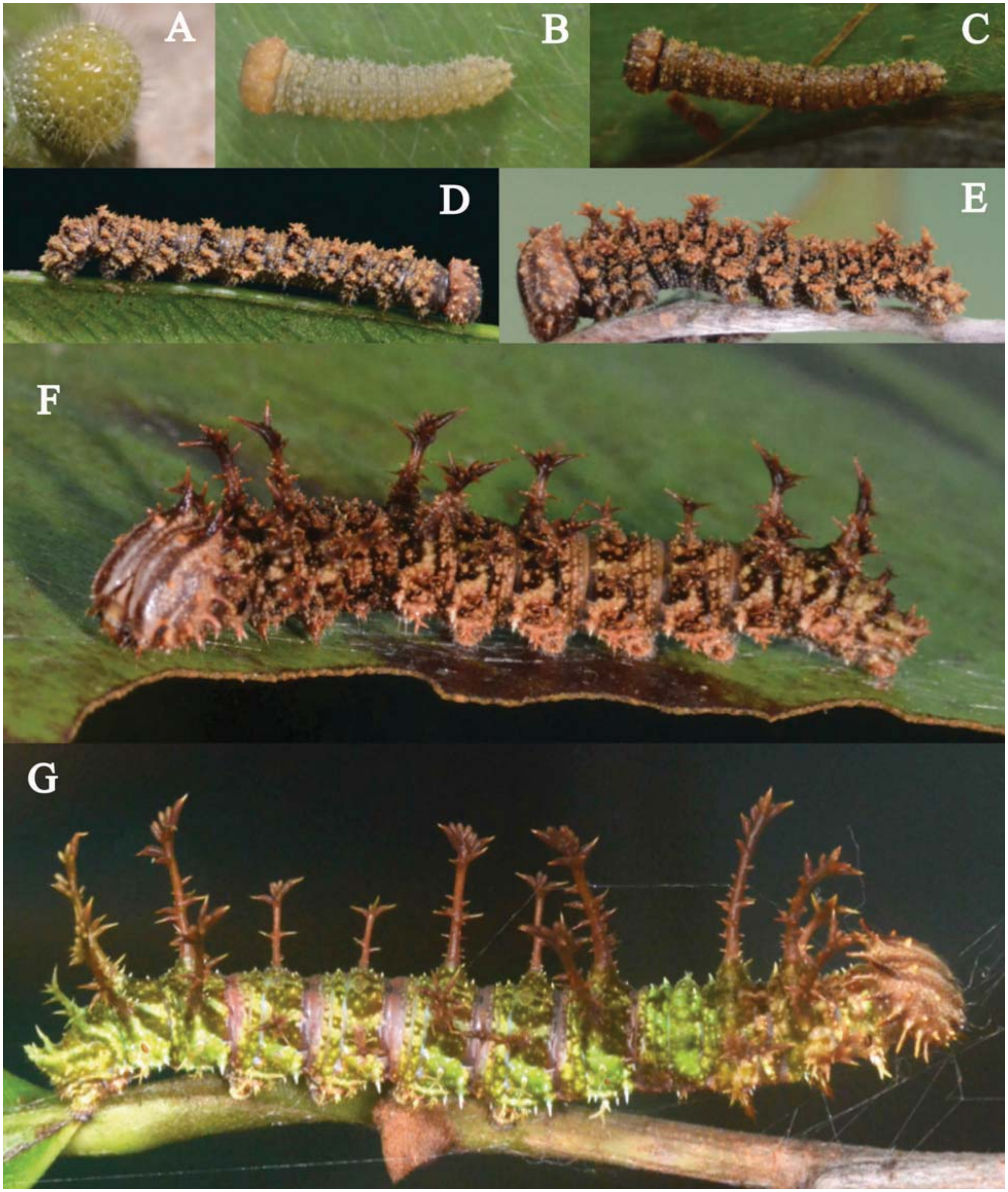

FIG. 5. Immature stages of *Adelpha nea nea*. **A.** Egg. **B.** First instar. **C.** Second instar. **D.** Third instar. **E.** Fourth instar. **F.** Fifth instar. **G.** Sixth instar.

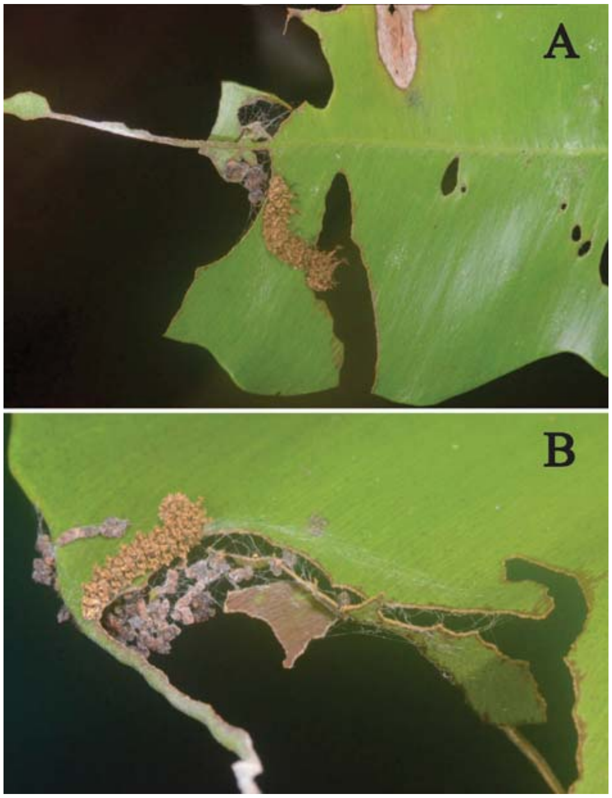

FIG. 6. Feeding damage typical of *A. nea nea*. Images illustrate feeding pattern and structures made by *A. nea nea*. In the top image (**A**) the larva is feeding on the portion of the leaf distal to the cut in the typical basal-to-apical manner, consuming tissue distal to where secondary, intersecondary and tertiary veins have been cut. In the bottom image (**B**) nearly all the leaf distal to the cut has been consumed.

subdorsal scoli, and are pale brown. Reduced subspiracular spines are found on A2–A8, and these pale green spines project directly from the body in groups of 2–4 spines. Prolegs are tan.

Larval behavior. Upon hatching, larvae alternated between eating the remains of the egg and wandering over the leaf. Compared to other newly hatched *Adelpha* (CER pers. obs.), *A. nea nea* wandered over more area and for a longer time before beginning to eat at the border of the leaf, generally at points of leaf damage, where a frass chain was made. To feed, larvae first made an arc or U-shaped cut basal to where the frass chain would be made, effectively making an island of leaf to consume. The cut was generally perpendicular to the secondary, inter-secondary and tertiary veins (which are parallel in this host plant), but did not cross the main vein of the leaf (Fig. 6). Once this cut was made they commenced eating distal to the cut and worked toward the leaf tip or leaf margin in an overall basal-to-apical fashion.

First to fifth instars made frass chains off the main or a secondary vein, and created a mass of silked-together frass and leaf bits. The mass was constructed at the base of the frass chain on top of the leaf, or hanging below the leaf (Fig.6). Early instars rested on the middle or tip of the frass chain, with the body either straight or with the anterior portion raised ("front-curved position" of Aiello 1984). If disturbed while feeding, larvae retreated to the frass chain and either rested in the front-curved position or began adjusting the mass. If further disturbed by touch they would thrash about and head-butt the source of the disturbance. Early instars rested on frass chains during molts, but molting to the sixth larval stadium took place on the surface of the leaf.

During the sixth stadium the larva rested on the top or underside of the leaf with the body straight or in a front-arched-rear up position, and were not observed in the curled-with-spines-out position (in which the larva is curled to the side in a C or J shape with scoli pointing outward all around) typical of other *Adelpha* (Aiello 1984). Pupation occurred on the underside of the leaf or on the rearing bag.

Pupa. Fig.7. Duration: $\bar{x}$ =9 days, s.d. = 0, n=6. Typical of species in the *serpa* group. Pupa is shimmering silver after first day, with dark sutures and orange-brown highlights. The pupa turns black the day before eclosion. The head has short, sickle-shaped lateral projections that are widely separated at the base. The thorax rises abruptly behind the head and has a dorsal keel that merges into the larger dorsal projection on T2. The T2 projection slopes gradually from the head and then drops off abruptly to T3, and has a rounded apex. There is also a dorsal projection on A2 that is somewhat square when viewed laterally. This projection is curved anteriorly at the apex, and arises abruptly on the anterior side. On the posterior side the projection slopes somewhat gradually into A3 where a dorsal keel continues with rounded apices on A3–A6. The keel on A7 is squared off and slopes abruptly into A8. The T2 and A2 projections greatly resemble those of *A. serpa*. There are short dorsolateral projections present on T2, T3, and A2–A4. Those on T2–T3 each make a narrow transverse ridge. The projections on A2 are rounded bumps, and those on A3–A4 are conical tubercles with sharp tips. On the abdomen these tubercles are dark colored, forming the beginning of a dark dorsolateral stripe that runs dorsal to the spiracles along the length of the abdomen. Beginning at A4 there is a dark ventrolateral stripe that runs to the end of the abdomen. The ventral portion of A8/9 has dark curved ridges and the cremaster is dark brown.

Adult. Fig. 4b. The known range of *A. nea nea* is Costa Rica to Venezuela and southern Peru, Amazonian

Brazil and the Guianas. Adult morphology of the *A. nea nea* collected in Northeastern Costa Rica and reared in Southwestern Costa Rica matches descriptions by Willmott (2003b) for the subspecies *A. nea nea* and reflects previous knowledge of range and variation. Individuals from Costa Rica that we have studied do not resemble the distinctive *A. nea sentia* (Godman and Salvin, 1884) to the North (potentially Guatemala and Nicaragua to Mexico, Willmott 2003b) and indicate no gradation into *A. nea sentia*. The subspecies are distinguished by *A. nea nea* being entirely orange in cells Cu_2–Cu_1 and Cu_1–M_3 of the DFW postdiscal (Willmott 2003b), and this is true of all Costa Rica specimens observed so far both in the north (n = 4) and south (n = 4) of Costa Rica. There is variation in the DFW postdiscal band of *A. nea nea*, across its range and in Costa Rica, with cells 2A–Cu_2 being either entirely orange, or orange and white, and the orange sometimes extending to the anal margin.

Adelpha nea nea is a rare species in Costa Rica, and in general throughout its range (Willmott 2003b). Adults were observed only four times in ten months of fieldwork, all instances at La Selva Biological Station in Sarapiqui, never at Guadalupe where immatures were found. Adult males were observed perching from 3–5 m at the edges of secondary forest and at 1m in a gap in primary forest, in both the wet and dry season (June n=2, January n=2). No territorial perching behavior was observed, but possibly it does occur in the canopy (~20 m). Males landed on ripe to over-ripe fruits of fig trees (*Ficus* sp., Moraceae), as well as puddling at water droplets on top of leaves of *Heliconia* L. (Heliconiaceae).

DISCUSSION

Immature stage morphology and systematics. The immature morphology described here agrees with previous systematic hypotheses and strongly corroborates recent phylogenetic analyses. *Adelpha naxia* and *A. heraclea* are hypothesized to be within the same species group (*capucinus* group) based on shared adult characters of the male genitalia and ventral hind wing pattern (Willmott 2003b). However, adult characters in *Adelpha* are known to be misleading (Moss 1933, Aiello 1984), and Aiello (1984) heavily emphasizes the utility of using immatures to determine relatedness among species. A recent molecular analysis (Ebel et al. 2015) recovered *A. naxia* and *A. heraclea* as sister species, and this is supported by several immature stage characters. First, these species both use *Vitex cooperi* as a larval host in Costa Rica, and Aiello (1984) notes that species that feed on *Vitex* seem more specialized in their foodplant choice than are most *Adelpha* species. Second, the pupal morphology is very similar, being pearly white with black spots and streaks and very similar in shape (Fig.3). Across the genus at large both pupal morphology and foodplant use are indicators of close evolutionary relationships (Aiello 1984, Willmott 2003b). In addition, although larval coloration is exceptionally different in the ultimate larval stadium between the two species, both exhibit the longest subdorsal scoli at T2/A2 and A7/8, while noticeably lacking supraspiracular scoli, as do other members of this group, including *A. malea fundania* (Fruhstorfer, 1915) (07-SRNP-58380 & 59259) and *A. zina lacina* (Butler, 1872) (05-SRNP-2674) (Janzen & Hallwachs 2009). Overall, integrating molecular data with information from immature stages provides the best resolution of species relationships in the genus.

Larvae of *A. naxia naxia* and *A. heraclea heraclea* closely resemble each other in early instar morphology, but may be clearly distinguished in later stages. Larvae can be identified to species beginning with the third stadium. At this stage *A. n. naxia* is significantly darker brown, the head becomes uniformly black/brown, the T2 scoli are significantly more pronounced than all other scoli and are connected by a low prominent ridge. In contrast, third instar *A. h. heraclea* are light brown and the scoli on T2,T3, and A2 are equally pronounced. Larvae are clearly different by the ultimate stadium, with *A. n. naxia* being dark brown and black, whereas *A. h. heraclea* is dull green and pink. The pupae are most readily differentiated in that *A. n. naxia* has the lateral head projections more triangular whereas in *A. h. heraclea* they are rounded (Fig. 3) (although described as 'laterally pointing triangles' by Willmott 2003b, this more accurately describes those of *A. n. naxia* when being compared). This is a difference similar to that seen between pupae of *A. phylaca pseudaethalia* (Hall, 1938) and *A. messana messana* (C. & R. Felder, 1867) (Aiello 2006, CER and RIH pers. obs.). Additionally, lateral wing projections on the T2–T3 junction are rounded in *A. n. naxia* and pointed in *A. h. heraclea*, along with differences in placement and shape of black spots and streaks (Fig.3).

Results presented here also corroborate the placement of *A. nea* within the *serpa* group. The *serpa* group is the most strongly supported of the species groups based on characters of the male genitalia, wing pattern, pupal coloration, and by being non-Rubiaceae feeders (Aiello 1984, Willmott 2003b). *Adelpha nea* is included in the *serpa* group based on adult characters (Willmott 2003b). *Adelpha nea*'s adult morphology also makes it unique within the group, with a unique male valva, and the DFW postdiscal band of subspecies *A. nea nea* entirely orange in cells Cu_2–Cu_1 and Cu_1–M_3

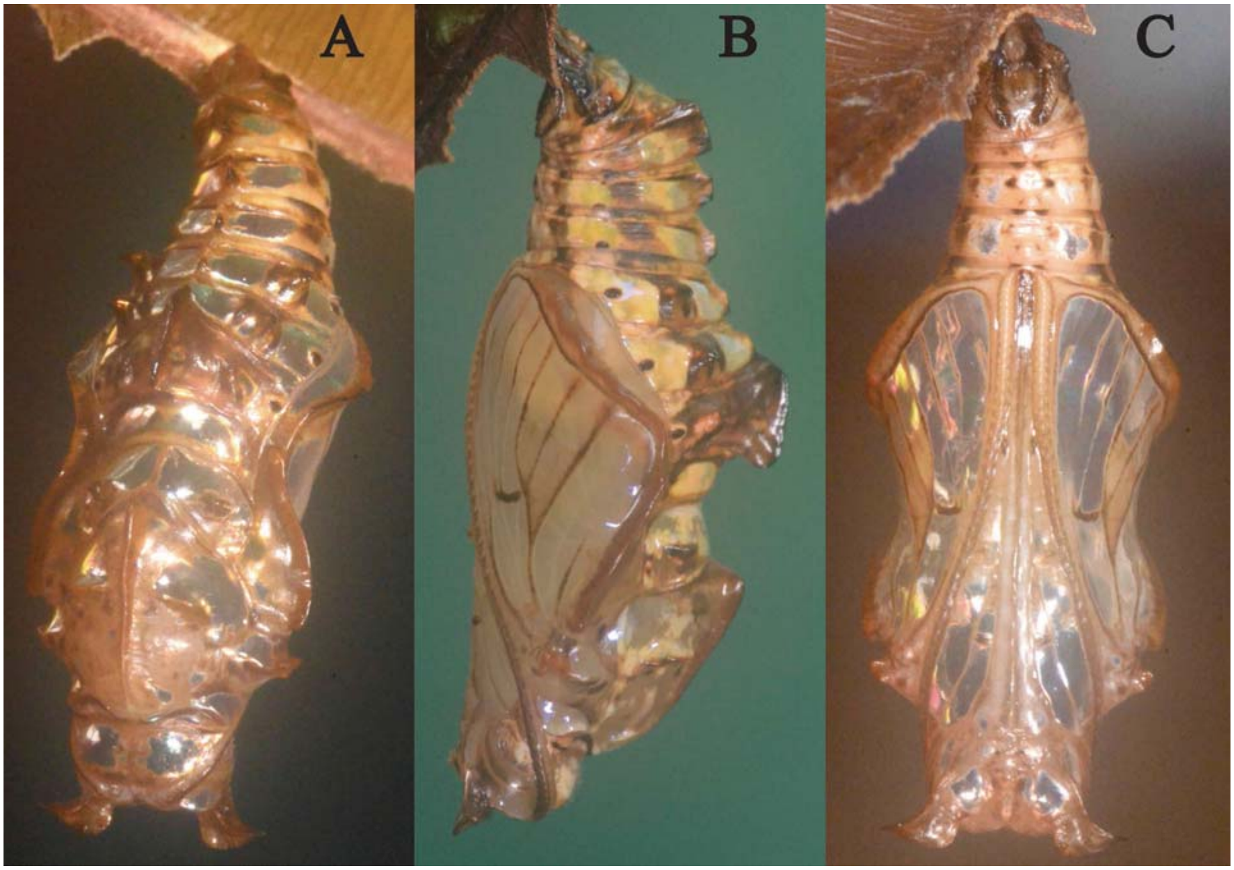

FIG. 7. Pupal images of *Adelpha nea nea*. Dorsal (**A**), lateral (**B**), and ventral (**C**) views are shown. The lateral image was taken the first day of pupation before silver coloration formed.

(Willmott 2003b) (Fig. 4). The immature morphology of *A. n. nea* also strongly corroborates its position in the *serpa* group. The relatively large egg, and the shape and shimmering silver coloration of the pupa (Fig. 7), resemble other *serpa* group species (e.g. *A. serpa celerio*, *A. paraena massilia* (C. Felder & R. Felder, 1867), *A. radiata aiellae* Willmott & Hall, 1999) (CER & RIH pers. obs.). The presence of conical tubercles on T2, T3 and A2–A4 is similar to other derived *serpa* group species (Willmott 2003b). The sixth instar is very similar to the ultimate instar of other *serpa* group species, (e.g. *A. serpa celerio*, *A. paraena massilia, A. radiata aiellae*) in the arrangement of scoli and pattern of flattened spines on the scoli. *Adelpha nea nea*'s ultimate instar is especially similar to that of *A. radiata aiellae* (CER & RIH pers. obs.) being very similar in color pattern and in the asymmetric arrangement of spines at the apex of the scoli. However, *A. nea nea* is clearly distinguished by the prominent subdorsal scoli on A9 which *A. radiata aiellae* lacks.

Implications of Sapotaceae feeding. Much of the natural history of *Adelpha nea nea* observed here appears to be strongly related to its unique sapotaceous food plant. Various species within the plant family Sapotaceae are known to produce latex (Gentry 1993) which functions as a potent defense against herbivores (Agrawal & Konno 2009). This includes the larval food plant of *A. n. nea*, *Micropholis melinoniana*, which produces visible latex from leaves and stems when torn or cut. To our knowledge this is the first record of Sapotaceae being used as a host for the genus *Adelpha*, or any Neotropical member of the family Nymphalidae (Beccaloni et al. 2008, Robinson et al. 2010).

Our observations on the egg placement and feeding pattern in *A. nea nea* indicate that these are likely adaptations to overcome the latex defense of its host *Micropholis melinoniana*. Eggs were placed on older damaged leaves adjacent to fresh undamaged leaves, and young larvae found in the field were on previously damaged leaves. During rearing the larvae had equal access to old damaged leaves and young leaves, and preferentially fed on the older damaged leaves. Field observations also indicated that the older leaves have reduced latex (C.E.R pers. obs.). Thus the observed female oviposition and larval preference for older leaves likely reduced latex ingestion and increases larval survival.

An additional reason for selection of damaged leaves could be the feeding difficulty associated with the durability of this food plant. A healthy third instar *A. nea nea* was given a young undamaged leaf with an entire margin to feed on in place of its original damaged leaf. This individual died the following day with no damage seen to the leaf and no obvious cause of death other than starvation. These observations suggest the small early-instar was unable to chew through the thick border of *M. melinoniana* leaves, and latex was not the only defense. This is important to consider for future rearing of *Adelpha*, as giving larvae leaves with whole margins may result in unwanted fatalities. An alternate interpretation is that the larva rejected the host leaf because of secondary defenses other than latex in the young leaf. However this does not seem to be the case because the leaf was from the same tree as all other leaves fed to the larvae reared in this study.

The trenching feeding pattern we described here for *A. nea nea* (Fig. 6) appears to be unique among *Adelpha*, and likely reduces latex consumption. *Adelpha* species that we have studied, like *A. n. naxia*, feed by removing apical (or marginal) leaf tissue from both sides of the frass chain first, and working their way back toward the base (or midvein) of the leaf. In contrast, *A. n. nea* feeds in a basal-to-apical pattern by trenching, which is likely a mechanism for severing the lateral veins that contain latex, so that the larvae can then feed distal to the cut without encountering latex. Other insects feeding on latex-defended plants, such as milkweeds (*Asclepias* L. Apocynaceae), have evolved similar defenses, where they sever the veins of the leaf to stop the flow of latex before feeding (Dussourd & Eisner 1987, Dussourd 1993).

The presence of six larval stadia and the long development time in *A. nea nea* appear to be unique in *Adelpha* and are likely additional adaptations to its sapotaceous host. All *A. nea nea* larvae reared on *M. melinoniana* passed through six larval stadia. This is in contrast to the five instars typical of other *Adelpha* species, although Aiello (1984) observed *A. basiloides* (Bates, 1865) occasionally molting to sixth instar. Aiello (1984) noted that the sixth instar of *A. basiloides* occurred only in individuals feeding on *Amaioua corymbosa* Kunth (Rubiaceae), and that this occurred in only a portion of individuals. In addition to the number of instars, the total development time of *A. n. nea* appears to be ~10 days longer when compared to another *serpa* group species (*A. serpa celerio*) reared on the Osa Peninsula (C.E.R. pers. obs.). Aiello (1984) observed that *A. basiloides* individuals showed an extended development time when feeding on *Amaioua corymbosa* whether they went through five or six instars. Together these observations suggest variation in development time in *Adelpha* may represent individual responses to food plant quality or defenses. Thus the sixth instar and extended development time in *A. nea nea* may be specific to *M. melinoniana*, and likely help *A. n. nea* to feed on this well-defended food plant.

The discovery of novel larval hostplant families and life histories in a country such as Costa Rica where the butterfly fauna has been intensively studied (DeVries 1987; 1997, Janzen & Hallwachs 2009) indicates that there remains much to be discovered about *Adelpha*. For example while adults of *A. nea nea* were observed at La Selva Biological Station the immatures remain unknown there, and according to botanist Orlando Vargas (pers. com.) the food plant *M. melinoniana* has not been found on the station property. The presence of adult *A. n. nea* could mean that the host remains to be found there or very close by, or that *A. n. nea* uses an additional unknown host, or perhaps it is transient at LSBS, showing up when it is especially abundant elsewhere. However, the fresh condition of individuals observed at LSBS suggests it is resident, making it most likely that it uses a different but as yet unknown host there. It would be typical for an *Adelpha* species to use hosts from multiple species, genera and even families (Aiello 1984; Janzen & Hallwachs 2009; Willmott 2003b). This is especially true for species within the *serpa* group who feed on the greatest variety of plant families (Aiello 1984, Willmott 2003b, Janzen & Hallwachs 2009). With the discovery of this potential additional host we may be able to determine whether six instars is a general trait for *A. n. nea*, or whether it is due to the foodplant. Observations recorded here indicate that we are still just developing our understanding of *Adelpha* host breadth.

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FIRST REPORT OF THE GENUS *TELAMOPTILIA* FROM THE WESTERN HEMISPHERE WITH DESCRIPTIONS OF TWO NEW SPECIES (GRACILLARIIDAE)

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ABSTRACT. The genus *Telamoptilia* (Acrocercopinae) is reported from the Western Hemisphere for the first time. Two new species, *Telamoptilia hibiscivora*, from the eastern United States, and *T. pavoniae* , from western Cuba, are described and illustrated. The larvae initially begin as serpentine leafminers and eventually form mostly full depth blotch leafmines on Malvaceae. *Telamoptilia hibiscivora* mines the leaves of *Hibiscus moscheutos* L. and the lavae of *T. pavoniae* mine *Pavonia fructicosa* (Mill.), Malvaceae.

Additional key words: *Hibiscus moscheutos*, *Pavonia fruticosa*, leafminer, Cuba, North America.

The genus *Telamoptilia*, with *Acrocercops cathedraea* Meyrick as the generotype, was first proposed by Kumata and Kuroko (1988) to include four species from Japan, India, Taiwan, Madagascar, and South Africa. Subsequently, three additional species have been added, although *T. phalarotis* (Lefroy) has been considered an unavailable name (De Prins & De Prins 2005, De Prins & De Prins 2017). Kumata and Kuroko considered *Telamoptilia* to be most related to *Spulerina* Vári, and differing from *Spulerina* by the presence of a minute flap on the antennal scape, by the absence of a palmate pectinifer on the male valvae, and by the different shape of the female signa.

The larvae of all *Telamoptilia* are leafminers, with three plant families having been reported as hosts: Amaranthaceae, Convolvulaceae, and Malvaceae ((De Prins & De Prins 2005, De Prins & De Prins 2017). Vári (1961) described the biology and larvae of *T. geyeri* (Vári) from South Africa, Kumata et al (1988) described the biology of *T. cathedraea* (Meyick), *T. hemistacta* (Meyrick), *T. prosacta* (Meyrick) from Japan, Taiwan, and India, and *T. tiliae* (Kumata and Ermolaev) from Japan and USSR. Liu et al (2015) reported on the biology of *T. grewiae* Liu, Wang, and Li from China, and included a detail discussion of the immature stages.

MATERIALS AND METHODS

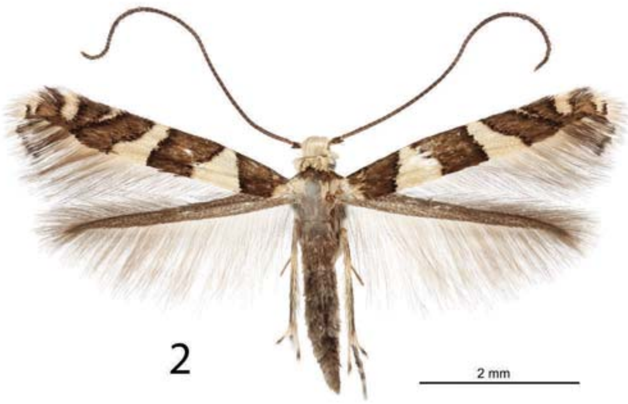
Specimens examined in this study are deposited in the former United States National Museum (USNM), now the National Museum of Natural History,

FIG. 1. Distribution of Western Hemisphere *Telamoptilia*

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. Some 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).

Molecular support for the recognition of the two new species was obtained through sequencing specimens of *Telamoptilia* for the 'barcode region' of the cytochrome oxidase I (COI) gene (Hebert et al. 2003). Legs from the specimens (Figure 14) were used for sequencing. The collection data, COI sequence information, and an image for each specimen are available in the Barcode of Life Data Systems (BOLD, www.boldsystems.org) (Ratnasingham & Hebert 2007) in the public dataset 'Western Hemisphere *Telamoptilia*' (DS-TELA2016).



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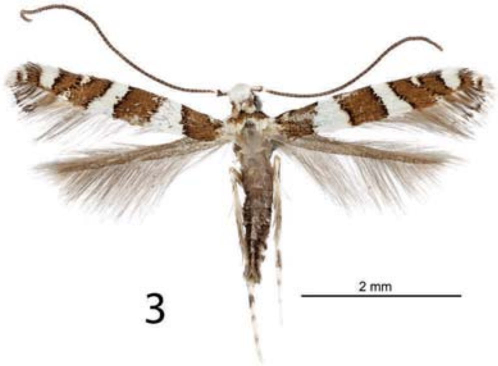

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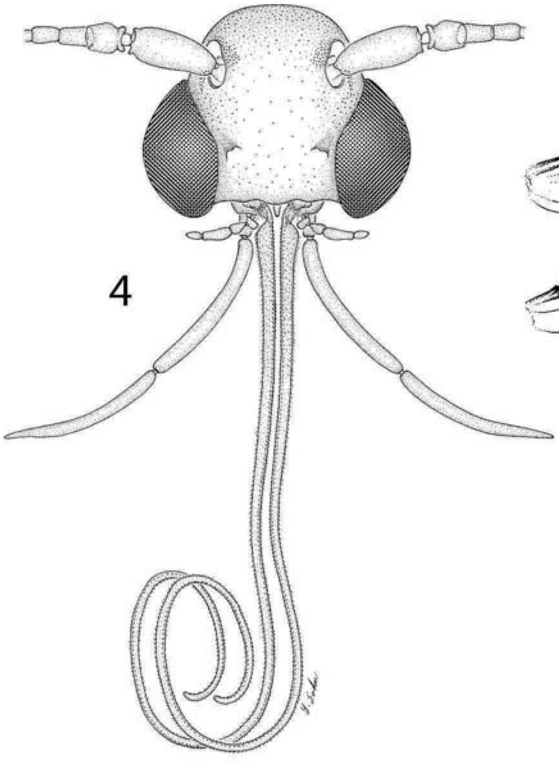

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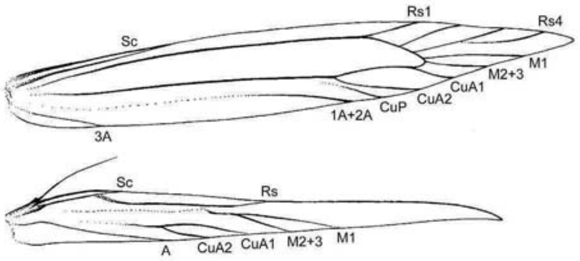

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FIGS. 2–5 (*Telamoptilia*: adult moths, head, and wing venation): **2**. *T. hibiscivora*, holotype ♂. **3**. *T. pavoniae*, holotype ♂. **4**. Head, *T. hibiscivora*. **5**. Wing venation, *T. hibiscivora*.

Sequences have also been deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/) under the accession numbers KX038711-KX038714.

Results

Telamoptilia Kumata and Kuroko

Telamoptilia Kumata and Kuroko, 1988: 57
Type species: *Acrocercops cathedraea* Meyrick, 1908.

ADULT. - *Head* (Fig. 4). Smooth scaled with a small tuft of frontal scales from anterior rim of eye; scales of vertex directed forward, a pair of occipital scale tufts arising near dorsal rim of eye. Ocelli absent. Antenna filiform, 1.0-1.1x length of forewing; scape elongate, ~ 2x the diameter of flagellomeres; pedicel less than half the length of scape but nearly equal in diameter; each flagellomere with a single annulus of slender scales. Haustellum naked, long, ~ 2.5x length of labial palpus. Labial palpus long, ~ 0.4x length of haustellum; slightly upcurved; second segment slightly rough scaled ventrally; apical segment nearly as long as second, entirely smooth scaled. Maxillary palpus 4-segmented, reduced in length, 1-3-1/2 the length of apical segment of labial palpus. *Thorax*: Smoothly scaled. Forewing (Fig. 5) lanceolate; discal cell ~ 0.8x length of wing; R with 4 branches, R_1 absent, Rs with 4 branches and with Rs3 and 4 stalked about half their length; M 2 branched with M2+3; Cu_1 and Cu_2 widely separated. Hindwing extremely slender; cell open between M_{2+3} and Cu_{A1}; frenulum a single stout bristle in both sexes. Legs long and slender; foreleg with slender epiphysis arising from distal third of tibia, accompanied by a scattered row of ~ 12 small bristles immediately distad of epiphysis; midleg with a pair of apical tibial spurs, and hindleg with a 2 pairs of long tibial spurs located at basal third and apex of tibia.

Male genitalia: Tegumen elongate, weakly sclerotized, with an elongate, moderately clustered series of fine setae laterally. Uncus undeveloped. Valva elongate, relatively slender and typically without lobes or processes, with gradually curved costal and hind margins tapering to a variable (according to species), acute to rounded apex. Vinculum relatively broad anteriorly, tapering to broad, V-shaped apex. Anellus a relatively short, membranous tube. Phallus slightly longer than valva, cylindrical and relatively straight, with a single to a few slender, sometimes uncinate cornuti.

Female genitalia: Anal papillae short, triangular when viewed laterally. Anterior and posterior apophyses slender, relatively short and of approximately similar lengths. Ostium bursae a moderately small oval opening located near anterior margin of sternum A8. A short, sclerotized, ring-shaped antrum present, with a length ~ half its width. Ductus bursae sometimes lined with scale-like granules caudally, then enlarging gradually to elongate, membranous corpus bursae. A single signum usually present (absent in *T. tiltiliae* Kumata and Ermolaev); signum elongate, mostly slender except for a pair of uncinate median processes which project from wall of corpus bursae.

Discussion: As mentioned previously, Kumata et al (1988) reported *Telamoptilia* to be most related to the South African genus *Spulerina* Varí, based in part by the absence of the R_1 vein and the stalking of Rs_3 and Rs_4 in the forewings. The male pregenital segments are also similar in possessing a slender anterior apodeme with its median sclerotization extending caudad into the eighth tergite. Their larval chaetotaxy are also similar in having D2 and SD2 positioned on the meso- and metathorax and with setae D1 and D2 arising close together on abdominal segments 1–8.

A recent molecular analysis of the family Gracillariidae (Kawahara et al, 2016) established *Telamoptilia* (based on *T. hibiscivora* D. and M. Davis) as a distinct genus closely associated with *Spulerina* within the newly proposed subfamily Acrocercopinae Kawahara and Ohshima, one of eight subfamilies recognized in this study for the Gracillariidae.

Key to the species of New World *Telamoptilia*

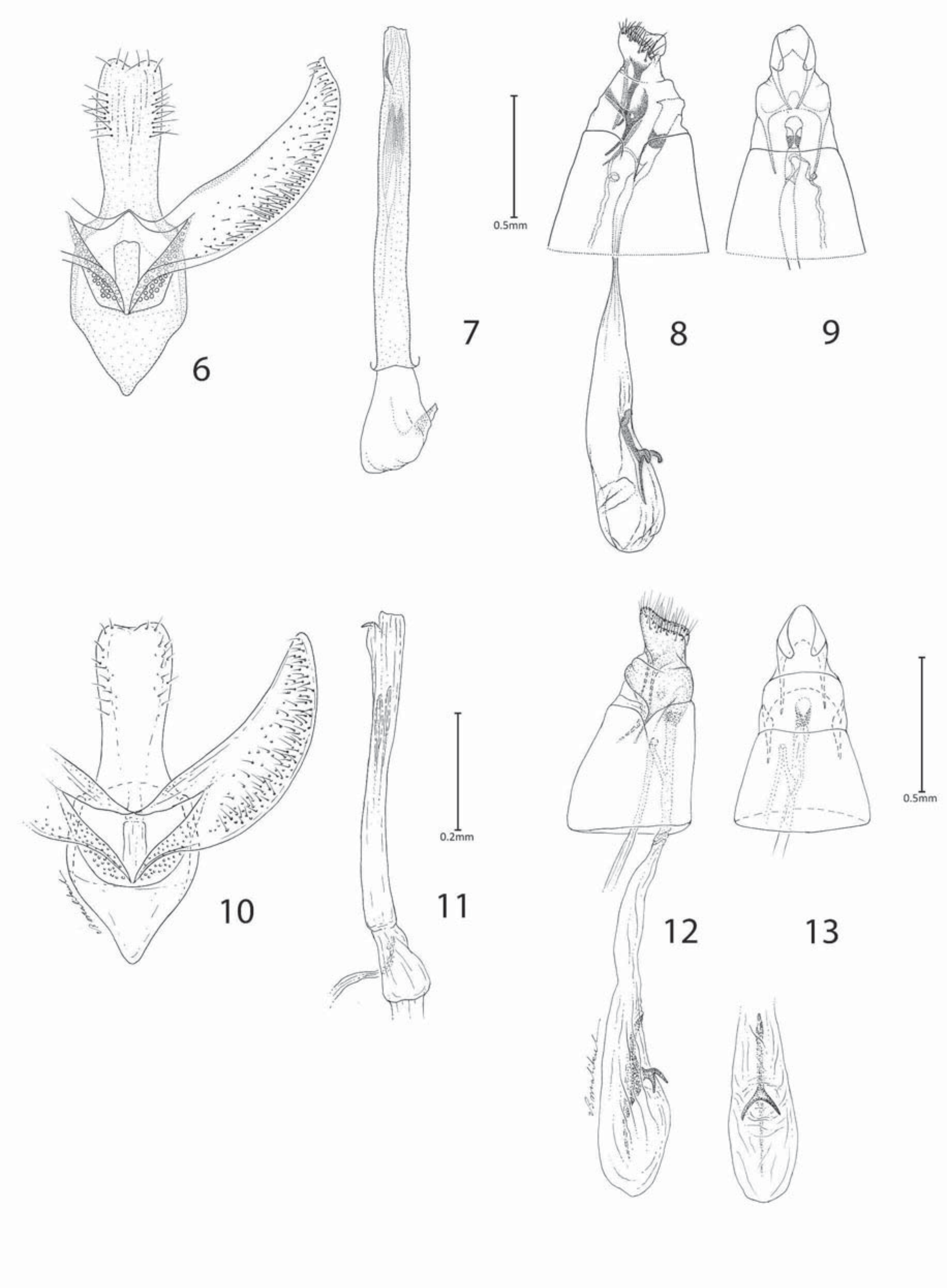
Forewing with very slender, oblique, white costal fascia across ~ half of wing at distal fourth (Fig. 2); male valva more slender with acute apex (Fig. 6); female signum relatively short, ~1.6x the length of posterior apophysis (Fig. 8); distribution (Fig. 1) eastern and southern United States to northern Mexican border......................................*T. hibiscivora*

Forewing with a small, white costal spot at distal fourth and another, smaller white spot obliquely opposite on dorsal margin (Fig. 3); male valva more broad and with more rounded apex (Fig. 10); female signum relatively long, ~ 2.75x the length of posterior apophysis (Fig. 12); distribution Cuba (Fig. 1) .... *T. pavoniae*

Telamoptilia hibiscivora D. and M. Davis, **new species**

Figs. 1–2, 5–9, 14–67

Adult Description (Fig. 2). Forewing length 3.0 – 4.1 mm. *Head* (Fig. 4) – Vertex and frons mostly smooth, appressed; scales broad, light golden brown; frons with a loose tuft of more slender scales projecting beneath scape inwards from anterior margin of eye; scales of vertex directed forward, very broad and flat; occipital tufts extending from dorsal margin of eye and



FIGS. 6–12. *Telamoptilia* Genitalia: **6**. *T. hibiscivora*: male, ventral view. **7**. Phallus. **8–9**. Female genitalia: **8**. lateral view. **9**. Ventral view. **10**. *T. pavoniae*: male, ventral view. **11**, Phallus; **12-13**: Female genitalia: **12**. Lateral view. **13**. Ventral view.

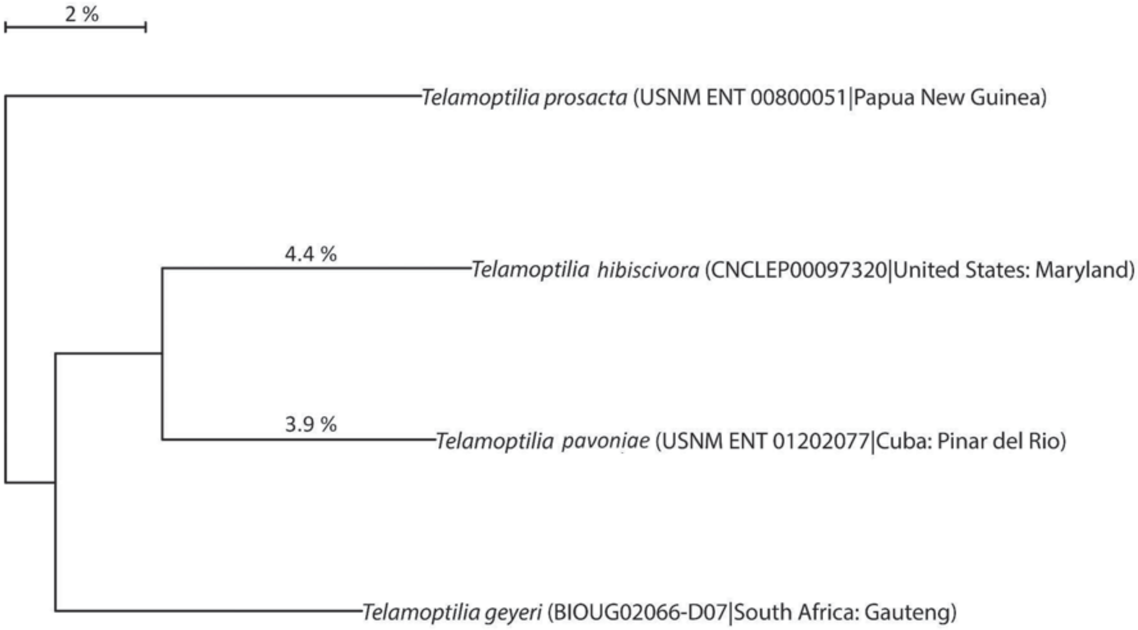


FIG. 14. Neighbor-joining tree of genetic distances (K2P model) for cytochrome c oxidase I (COI) for select *Telamoptilia*, including *T. hibiscivora* D. and M. Davis and *T. pavoniae* D. and M. Davis. End-branch labels are specimen Sample IDs, followed by the geographic origin. GenBank numbers for the specimens sampled are: KX038714 (*T. prosacta*), KX038712 (*T. hibicivora*), KX038711 (*T. pavoniae*), KX038713 (*T. geyeri*).

converging at vertex, and consisting of moderately broad, flat, mostly white scales; anterior occipital scales dark brown. Antenna ~ equal to length of forewing; scape either brown or white dorsally, brown ventrally; flagellomere 2 dark brown; flagellomere 3 usually white, sometimes brown; all other flagellomeres smoothly scaled, uniformly dark brown dorsally, slightly paler brown ventrally. Labial palpus slightly curved, directed ventrad, smooth scaled, and mostly white with apices of second and third segments dark brown.

Thorax – Dorsum white posteriorly; anterior third including pronotum and anterior portion of tegula brown; thorax mostly white to pale brownish white ventrally with suffusion of dark brown beneath wings. Forewing predominantly dark brown with two, broad, somewhat triangular white fasciae traversing basal third and distal third of wing; a moderately large, costal, subapical, white, fasciate spot extending two thirds across the wing; a much smaller, very slender white costal spot located midway between outer fascia and subapical fasciate spot and extending half way across wing; all white fascia and spots bordered by darker brown margins; apical fringe white, largely divided by a narrow, transverse, dark brown band; subapical dorsal fringe brown; most of dorsal fringe pale brown to white. Hindwing and fringe uniformly brown. Legs with femur entirely or predominatly dark brown especially dorsally; tibiae predominatly dark brown, encircled with a white band near middle; tibial spurs dark brown with white scaling near apex; tarsomeres banded with white and dark fuscous of variable widths.

Abdomen – Dark brown dorsally; predominantly white ventrally, with caudal margins of each segment bordered with dark brown.

Male genitalia (Figs. 6–7) – Vinculum broadly V-shape; vinculum-saccus ~ half the length of tegumen. Valva slender, nearly 2x the length of anellus, gradually narrowing to acute apex; dorsal and ventral margins smoothly curved; dorsal margin of valva with numerous, moderately long setae. Anellus membranous, ~ equal to length of vinculum-saccus. Tegumen weakly sclerotized, ~ 0.8x the length of valva, caudal apex mostly truncate with a shallow median cleft. Phallus slender, slightly longer than valva; cornutus indistinct, resembling a narrow, subapical, crescentic, internal ridge.

Female genitalia (Figs. 8–9) – Anal papillae reduced, obliquely transverse in lateral view. Both pairs of apophyses reduced in length and ~ equal is size; base of anterior apophyses slightly broadened. Eighth abdominal segment short. Ostium simple, membranous, with a short, sclerotized ring near middle of antrum. Ductus bursae ~ equal in length to seventh abdominal segment and gradually enlarging to elongate, relatively slender corpus bursae. Signum consisting of a single, elongate (~ 1.6x length of posterior apophysis), slightly curved, darkly sclerotized internal rod, with a pair of short, uncinate processes projecting ~ midway along rod through wall of corpus bursae.

Larva: Sap-feeding instar (Figs. 15 – 26).- Head: Greatly depressed, approximately round (dorsal view). Most setae lost or reduced. Stemma 2 pairs, immediately caudad to antennae;

FIGS. 15–26. *Telamoptilia hibiscivora*. Third instar (sap-feeding) larva. **15**. Head, dorsal view (167 µm). **16**. Labrum (67 µm). **17**. Head, ventral view (167 µm). **18**. Labium (67 µm). **19**. Left antenna, ventral view (20 µm). **20**. Thorax, T 2, ventral view (214 µm). **21**. Thorax, T 2, ventral view (136 µm). **22**. Thorax, T 2, ventral callus (30 µm). **23**. Proleg, A4 (30 µm). **24**. Segments A 9 – 10, venter (67 µm). **25**. Proleg, A10 (27 µm). **26**. Lateral view A10 (67 µm).

Labrum broadly bilobed, almost completely divided into a pair of elongate, slender lobes; labral setae absent. Mandibles a pair of flat, mostly rounded lobes. Labium broadly bilobed, with anterior ventral margin bearing a broad band of dense, spines. Maxillary and labial palpi absent. Antenna reduced in length, 3-segmented, with two moderately large and one minute basiconic sensillae as illustrated (Fig. 19). Body: Maximum length ~ 4.0 mm. Setae generally absent or reduced except laterally along body. Legs absent; prolegs reduced to smooth planta without crochets (Figs. 23).

Last instar, tissue-feeding larva (Figs. 27 – 54). – Head: Maximum length ~ 7.5 mm. Head: Dark reddish brown; maximum width ~ 6 mm. Frons (Fig. 28) elongate, extending almost to epicranial notch. Ecdysial line terminating near epicranial notch. AF2 present. P1 arising slightly laterad to AF1. Setae A1, A2, S2, and SS2 the most elongate; L1 reduced and arising slightly caudad to most posterior stemmata; 4 pairs of stemmata present and of ~ equal size (Fig. 47); labrum with 5 pairs of setae, with M3 absent. Thorax: Pronotum with 4 pairs of setae including D1, D2, XD1 and SD1; SD2 arising close and anterior to SD1; all 3 lateral setae present anterior to spiracle; SV1 and SV 2 arising from reduced pinnaculum; lateral setae bisetose on segments A2 and 3. Bases of thoracic legs well separated; tarsal claw with broad base terminating in slender, strongly curved claw (Fig. 50). Abdomen: D1 and D2 arising close together on segments A1 – 9; lateral setae bisetose on A1 – 8, only L1 present on A9. Prolegs present on A3 – 5 and 9; anterior half of planta smooth; with a single circular row of 5 – 6 crochets present along anterior margin on abdominal prolegs 3 – 5 (Fig. 51); a vestigial proleg present on A6 without crochets; anal prolegs without crochets.

Leafmine (Fig. 66 – 67) – The larva begins forming a narrow, serpentine mine which abruptly enlarges to a mostly full depth, elongate blotch that often obliterates the earlier serpentine mine. The blotch tends to turn pale yellow with age and is usually located between leaf veins and near the leaf edge. The larval frass is typically pushed to the edge and usually to one end of the mine. When mature, the larva exits the mine ventrally and forms an elliptical, white to brownish white cocoon usually on the leaf surface.

Pupa (Figs. 55 – 64). Maximum length ~ 4.0 mm. Vertex with moderately short, broadly rounded and minutely serrated frontal process (cocoon cutter, Figs. 56, 58 – 59). Forewing extending to caudal margin of A5. Dorsum of A2 – 8 densely covered with evenly scattered minute, stout spines, with anterior spines the largest and decreasing in size caudally (Fig. 61). Terminal cremaster consisting of 4 short dorsal spines and 3 short ventral spines (Figs. 62 – 64).

Holotype - ♂, USA: MARYLAND: Anne Arundel Co: South River Marsh: Rt. 450:38°59´ N, 76°36´40" W: ♂, 17 Sep 1989, em.17 Feb 1990, HOST: DRD: 307.4, *Hibiscus moscheutos* L., digital image, BOLD sequence USNMENT 00657240, D. R. Davis, Type No. 013254 15, (USNM).

Paratypes (42 ♂, 47 ♀, 28 larvae, 3 pupae) – USA: MARYLAND: Anne Arundel Co: N. Riv. Hwy. 50: 6 ♂, 2 ♀, 26 Jul – 2 Aug 1941, em. 5-12 Aug 1941, ex. *Hibiscus palustris* L, [= *Hibiscus moscheutos* L]; 1 ♀, em. 16 Sep 1951, J. F. G. Clarke, Div. Ins. Id. No. 33 – 41 L (USNM). Anne Arundel Co: South River Marsh: Rt. 450: 38°59´ N, 76°36´40" W: 6 ♂, 11♀, 28 Jul 1974, DRD 30765 ♀, em.1 ♂, 10 ♀, 19 Jan 1975, 5 ♂, 1 ♀, 29 Jan 1975; HOST: DRD: 307 *Hibiscus moscheutos* L., D. R. Davis, (USNM); HOST: DRD: 307.2 *Hibiscus moscheutos* L, 24 Aug 1975; 1 ♂, em. 15 Nov 1975; Rt. 450, 38°59´ N, 76°36´40" W, 10 ♂, 5 ♀, 17 Sep 1989, D. R. Davis, (USNM); HOST: DRD: 307.4, *Hibiscus moscheutos* L; 6 tissue feeding larvae, larval slides USNM 30473, 30478, 2 ♂, 2 ♀, em. Dec 1989; 1 ♂, 3 ♀, Jan, 2 ♂, BOLD 00657246, 00657247, 1 ♂ BOLD 00657240; digital image, D. R. Davis, (USNM); DRD 307.5, 4 sap feeding larvae, 15 tissue feeding larvae, SEM larval slide USNM 30507, 17 Sep 1989, 4 ♂, em Feb 1990, D. R. Davis, (USNM); 3 pupae SEM pupal slide USNM 31352, 26 Sep 1993, HOST: DRD 1399, *Hibiscus moscheutos* L., D. R, Davis, (USNM). Prince Georges Co: Piscataway Park: 3 tissue feeding larvae, larval slide 30969, 8 Sep 1991, HOST: DRD 996, *Hibiscus moscheutos* L; 1 ♂, 29 Jul 2012; HOST: DRD: 2720 *Hibiscus moscheutos* L, em.13 Aug 2012, D. R. & M. M. Davis, (USNM). LOUISIANA: St. John Par: Edgard: 2 ♀, 28 Jul 1981, DRD 34679 ♀, 1 ♂, 7 Jul 1981, LNAUU4533-15/USNM ENT 01202081; 1 ♂, 5 Aug 1981; 1 ♂, 9 Sep 1981, DRD 23566, at UV light trap, V. A. Brou, (USNM). TEXAS: Brownsville: 10 ♀, 23-28 Jan 1932, DRD 32127, Plant 24, S. W. Frost, (USNM). MEXICO: Tamaulipas: Matamoros: 2 ♂, 2 ♀, 21 Jul 1937; slide DRD ♀ 34678; on *E. cisneros*, #19084 Brownsville, (USNM). USNM 34706

Distribution (Fig. 1). Primarily confined to the coastal wetlands of North America, from Maryland to Brownsville, Texas and adjacent Matamoros, Mexico.

Host plant (Fig. 65). Malvaceae: *Hibiscus moscheutos* L

Etymology. The species name is derived from the generic name of the host plant, *Hibiscus*, and the Latin *voro* (to eat, devour).

Discussion. This species was first discovered and reared from *Hibiscus moscheutos* by J. F. Gates Clarke (deceased 1990) from marsh habitats in Anne Arundel County, Maryland. The host plant ranges widely through marshy riverine systems of the southeastern United States from Texas to the Atlantic states as far north as southern Ontario, Canada. This species normally overwinters in the pupal stage, with the last instar larva not pupating until subjected to cold temperatures.

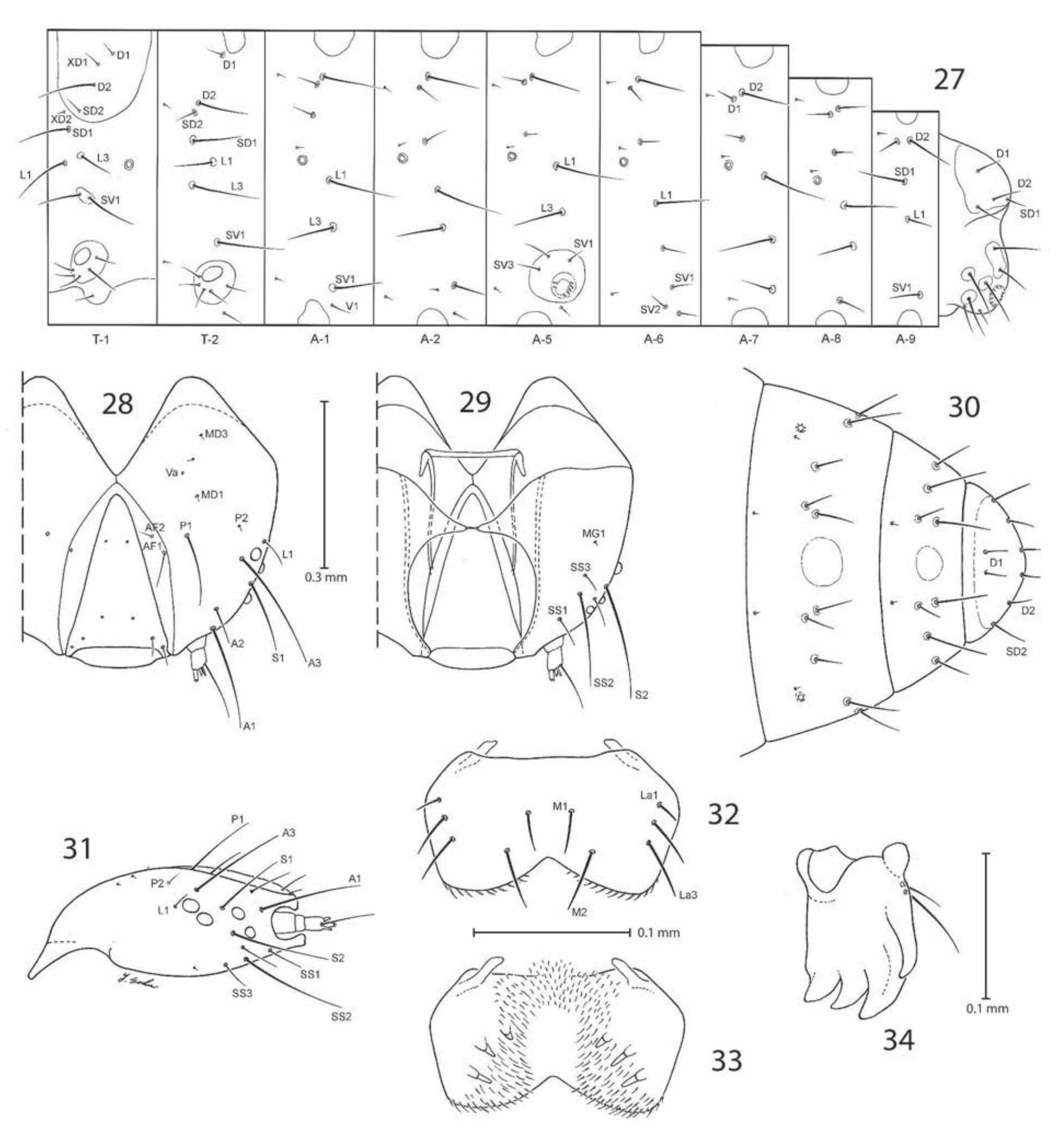
Telamoptilia pavoniae D. and M. Davis, **new species**

Figs. 1, 3, 10–13, 14.

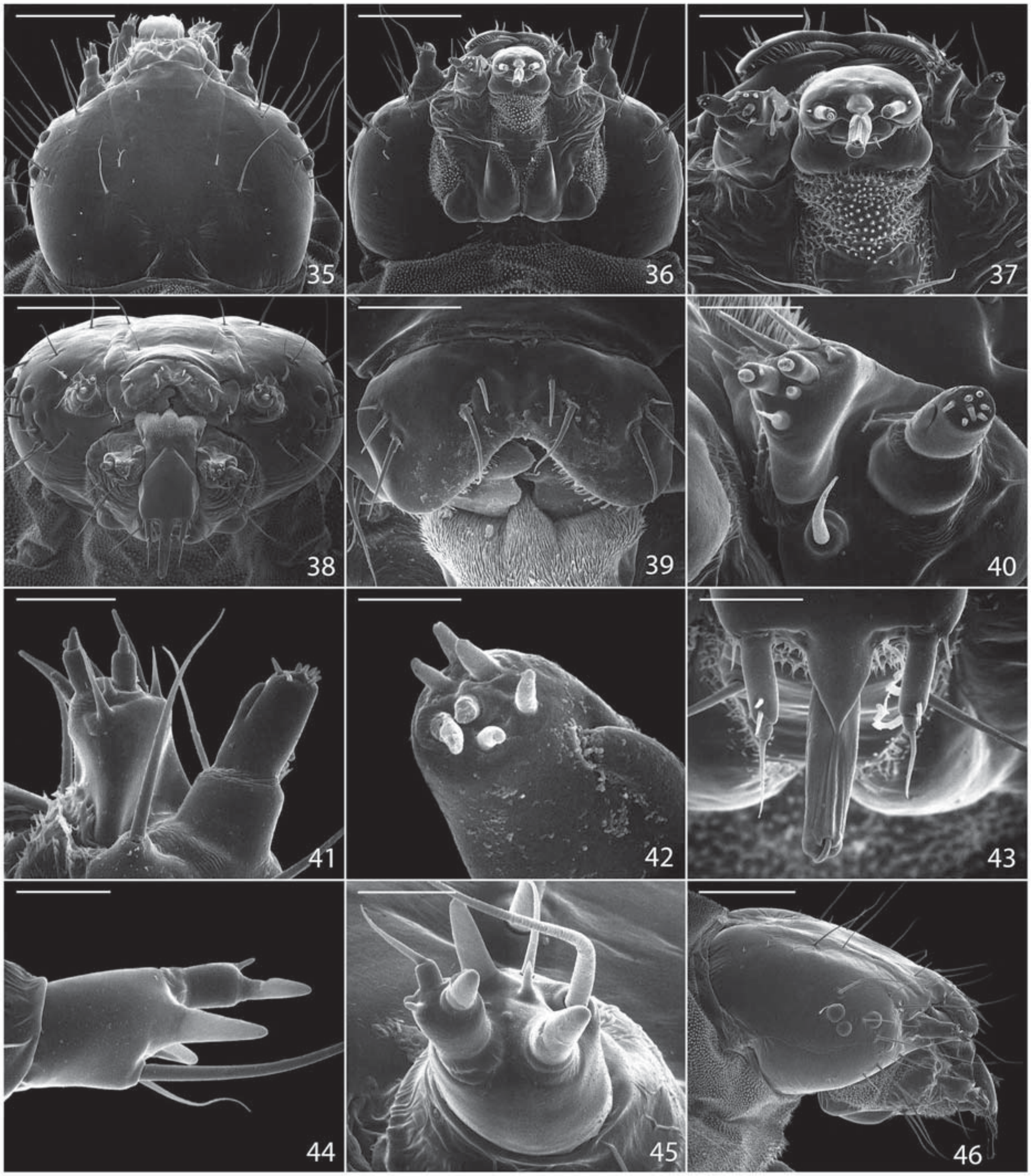
Adult Description. (Fig. 3). Forewing length 2.5–3.0 mm.

Head – Vertex and frons smooth; scales white, moderately broad and directed forward and down; a small tuft of slender, white to brown scales projecting ventrad beneath scape from dorsal - anterior margin of eye; occipital tufts extending from dorsal margin of eye, converging at vertex, and consisting of broad, flat, mostly white scales; anterior occipital scales dark brown. Antenna slightly longer than length of forewing; scape and flagellomere 3 white, flagellomere 2 dark brown; all other flagellomeres smoothly scaled and brown. Labial palpus slightly curved, directed ventrad, smooth scaled, and mostly white with apices of first and second segments dark brown.

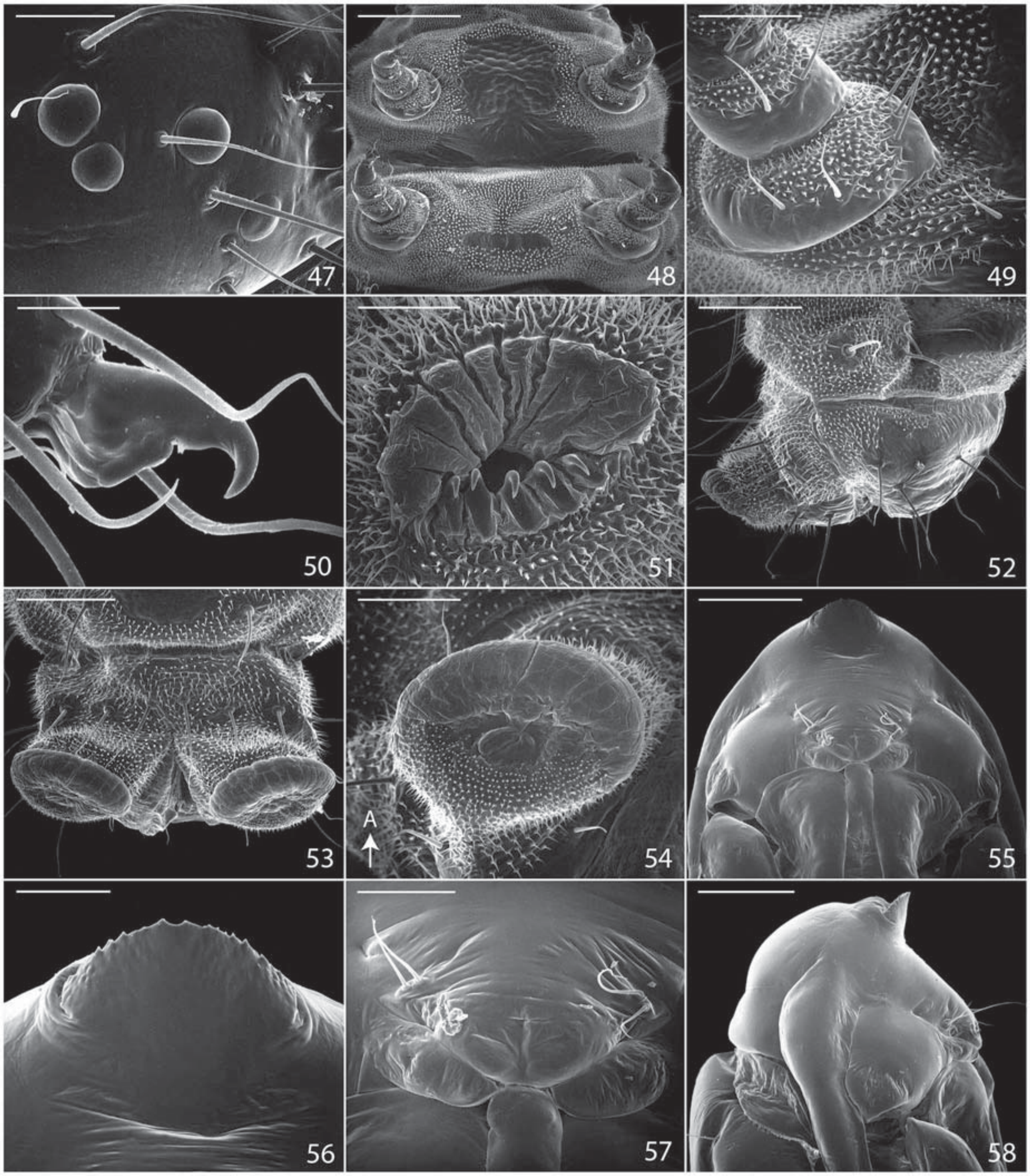
Thorax – Dorsum white posteriorly; anterior third including pronotum and anterior portion of tegula brown; thorax mostly white to pale brownish white ventrally with suffusion of dark brown beneath wings. Forewing predominantly dark brown with two, broad, triangular white fasciae traversing basal third and distal third of wing; a large, costal, subapical, white, fasciate spot (slightly larger than similar spot in *T. hibiscivora*)



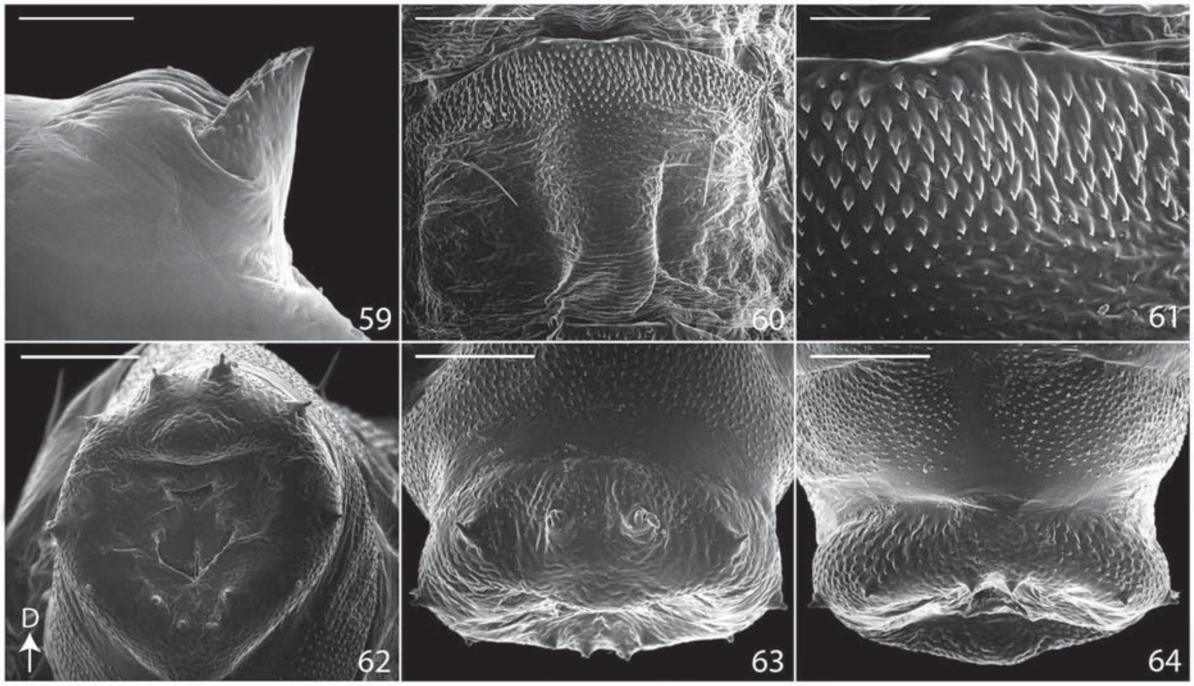
FIGS. 27–34. *Telamoptilia hibiscivora*. Larval chaetotaxy of last instar larva. **27**. Lateral schematic of larval prothorax, mesothorax, and abdominal segments 1–2, 5–10. **28**. Head, dorsal view (0.3 mm). **29**. Head, ventral view. **30**. Dorsal view of abdominal segments 8–10. **31**. Head, lateral view. **32**. Labrum, dorsal view (0.1 mm). **33**. Labrum, ventral view. **34**. Mandible (0.1 mm). (Scale length in parentheses).



FIGS. 35–58. *Telamoptilia hibiscivora*. Last instar (tissue-feeding) larva. **35**. Head, dorsal view (176 µm). **36**. Head, ventral view (150 µm). **37**. Head, ventral view (67 µm). **38**. Head, frontal view (150 µm). **39**. Labrum, dorsal view (50 µm). **40**. Right maxillary lobe and palpus (20 µm). **41**. Right maxillary lobe and palpus, ventral view (23.1 µm). **42**. Maxillary lobe, apical view (4850x). **43**. Spinneret and labial palpi, dorsal view (38 µm). **44**. Antenna, lateral view (25 µm). **45**. Antenna, apical view (17.6 µm). **46**. Head, lateral view (176 µm). (Scale length in parentheses).



FIGS. 47–58. T*elamoptilia hibiscivora*. Last instar (tissue-feeding) larva and pupa. **47**. Head, lateral view of stemmata (50 µm). **48**. Thoracic legs, A2 – 3, ventral (231 µm). **49**. Basal segments of third thoracic leg (75 µm). **50**. Tarsal claw, A3 (13.6 µm). **51**. Proleg on A4 (38 µm). 52. Abdominal segments 9-10, lateral view (136 µm). **53**. Anal prolegs (150 µm). **54**. Anal proleg (75 µm). **55**. Pupa (Figs. 55–58), anterior ventral view of head (176 µm). *56*. Frontal process of pupa (cocoon cutter), ventral view (50 µm). **57**. Frontal area of pupa, ventral view (67 µm). **58**. Pupal head, lateral view (176 µm). (Scale length in parentheses).



FIGS. 59–64. *Telamoptilia hibiscivora*. Pupa. **59**. Lateral view of frontal process (cocoon cutter) (60 µm). **60**. Dorsal view of abdominal tergum 3 (150 µm). **61**. Detail of tergal spines in figure 60 (50 µm). **62**. Caudal view of last abdominal segment (91 µm). **63**. Abdominal segments 9–10, dorsal view (75 µm). **64**. Ventral view of figure 63 (75 µm). (Scale length in parentheses).

extending three quarters across the wing; a much smaller, white costal spot (less elongate than similar spot in *T. hibiscivora*) and a similar white spot on dorsal margin obliquely opposite costal spot, both located midway between outer fascia and large subapical spot; all white fascia and spots bordered by darker brown margins; apical fringe white, largely divided by a narrow, transverse, dark brown band; subapical and dorsal fringes slightly darker and more brown than in *T. hibiscivora*. Hindwing and fringe uniformly dark brown, slightly darker than in *T. hibiscivora*. Legs with femur of fore and midlegs entirely or predominatly dark brown especially dorsally; femur of hindleg mostly white ventrally; tibiae predominatly dark brown, encircled with a white band near middle; tibial spurs dark brown with white scaling near apex; tarsomeres mostly white with fuscous apices.

Abdomen – Color similar to that of *T. hibiscivora*: dark brown dorsally; predominantly white ventrally, with caudal margins of each segment bordered with dark brown.

Male genitalia (Figs. 10 – 11) – Similar to *T. hibiscivora* except valva noticeably broader at midlength and apex of cucullus more rounded. Phallus with a single short, minute, apical cornutus that usually projects laterally.

Female genitalia (Figs. 12 – 13) – Similar to *T. hibiscivora* except signum more elongate, ~ 2.75x length of posterior apophysis.

Leafmine – Similar to mines of *Telamoptilia hibiscivora*, with sapfeeding larvae first imitating a slender serpentine mine that enlarges abruptly into a large, full depth, pale gray blotch mine. The larval frass is usually pushed to the perimeter of the blotch. The mature larva eventually exits the mine to form an elliptical silken cocoon often on the leaf surface.

Holotype - ♂, CUBA: Pinar del Rio La Caridad: Soroa: 22°48´N, 83°01´W: 5 Dec 1994; HOST: DRD 1542 *Pavonia fruticosa* (Mill.), em. 20-22 Dec 1994, digital image captured, D. R. Davis, Type No. 013254 16, (USNM).

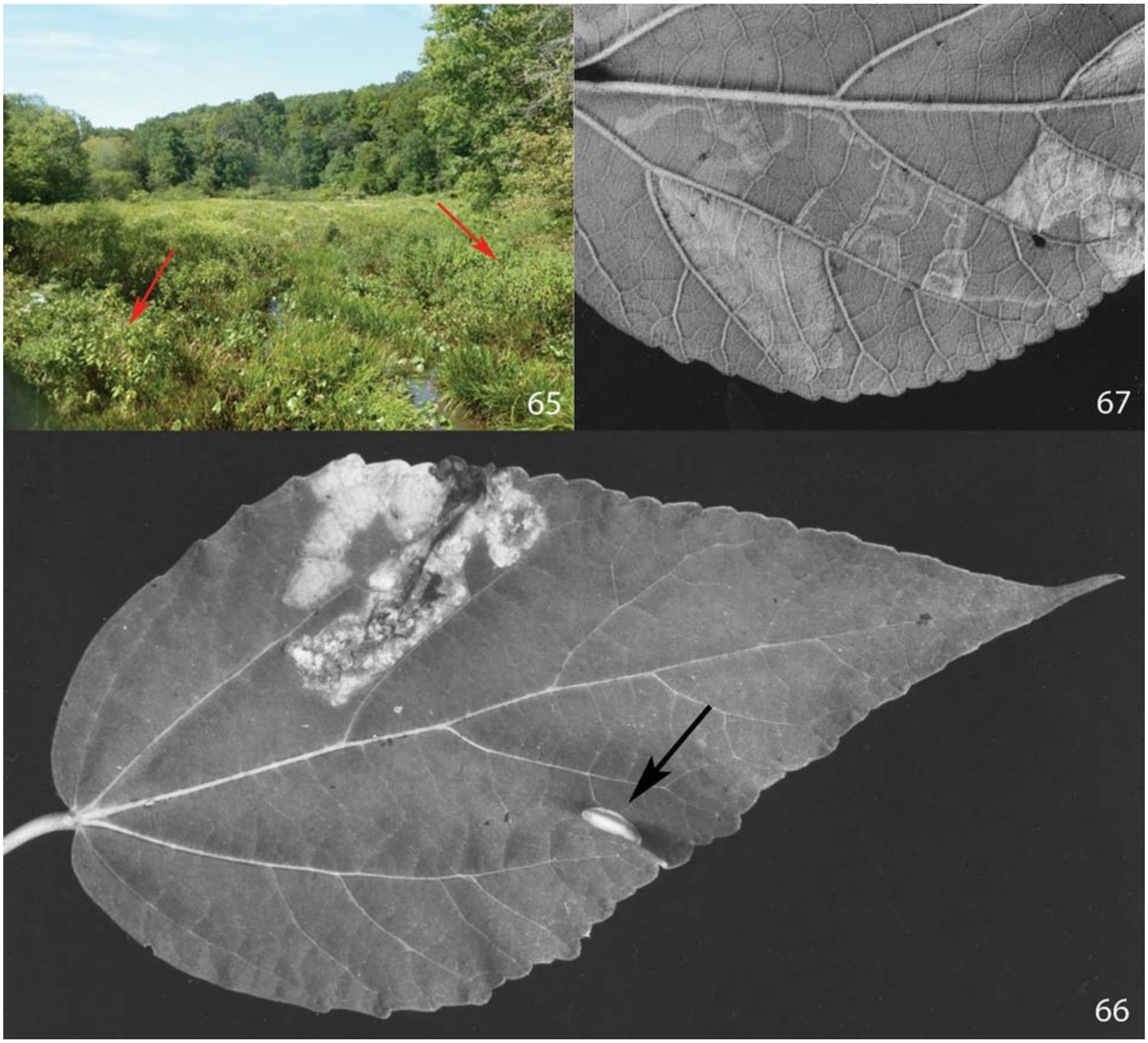
Paratypes (4 ♂, 6 ♀) - Same locality and host data as holotype, slides USNM ♂ 34674, 34676, USNM ♀ 34675; USNM ENT 01202079, D. R. Davis (USNM).

Distribution (Fig. 1). Known only from the type locality, Soroa, located in Pinar del Rio La Caridad, in western Cuba.

Host plant. Malvaceae: *Pavonia fruticosa* (Mill.)

Etymology. The species name is derived from the genitive form of the generic name of the host plant, *Pavonia*.

Discussion. A second species of *Telamoptilia* is known to feed on *Pavonia* in southern Africa. Vári (1961) described *Acrocercops geyeri* from specimens reared from *Pavonia columella* Cav. in the Transvaal, South Africa and Zimbabwe (Rhodesia). The species was later transferred to *Telamoptilia* by De Prins and De Prins, 2005. In addition to their very disjunct distributions, the forewing patterns of *T. pavoniae* and *T.* geyeri differ with the white forewing bands of *geyeri* being narrower than those of *pavoniae*. Most significantly are the distinct CO1 sequence differences between the two species (Fig. 14). A neighbor-joining tree (Fig. 2) constructed with



Figs. 65–67. *Hibiscus moscheutos*: **65**. Marsh habitat (arrows indicate plant clusters of *H. moscheutos*), South River Marsh, Anne Arunndel County, Maryland. **66**. Mature leafmine of *Telamoptilia hibiscivora* on *Hibiscus moscheutos*, with pupal shelter indicated by arrow. **67**. Early serpentine mines of *Telamoptilia hibiscivora* as viewed from ventral side of leaf.

the COI sequences revealed that *Telamoptilia pavoniae* and *T. hibiscivora* are clearly distinct with a divergence of 8.3% (p-distance), and this species pair are a minimum of 9.5% from congeneric species. Analyses of all specimens used the 658 bp fragment of mitochondrial CO1 except for *T. prosacta* KX 038714, for which only the 407 bp fragment was available.

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Journal of the Lepidopterists' Society
71(4), 2017, 274–278

GENETIC DIVERGENCE OF THE PEST MOTH *CHLORIDEA VIRESCENS* (NOCTUIDAE: HELIOTHINAE) FEEDING ON A NEWLY DOCUMENTED HOST PLANT IN THE ATACAMA DESERT OF NORTHERN CHILE

Additional key words: Asteraceae, *Chloridea subflexa*, *Heliothis subflexa*, *Heliothis virescens*, *Trixis cacalioides*

The polyphagous moth *Chloridea virescens* (Fabricius) (Lepidoptera: Noctuidae: Heliothinae) is a widely distributed pest in the New World, ranging from United States to Argentina (Poole et al. 1993). Along the Pacific coast it reaches the Pacific Northwest as its northern limit, including the states of Oregon and Washington, United States (Landolt 2008), while its southern limit is in the northernmost part of Chile (Parra et al. 1986). The wide host range of *C. virescens* includes a great number of plants, many of which are agricultural crops, belonging to many families (Pogue 2013, Ventura et al. 2016).

It is known that characterization of the genetic variation is important to understanding the biology of insect pests, especially in widely distributed species, because these sometimes exhibit genetic differentiation throughout their geographic ranges, which can be especially interesting to develop pest management strategies at the local level (Salinas-Hernandez & Saldamandano-Benjumea 2011, Diaz-Montilla et al. 2013, Maia et al. 2016, Velasco-Cuervo et al. 2016). Albernaz et al. (2012) assessed the genetic variation of *C. virescens* populations feeding on cotton and soybean in Brazil based on sequence analysis of mitochondrial

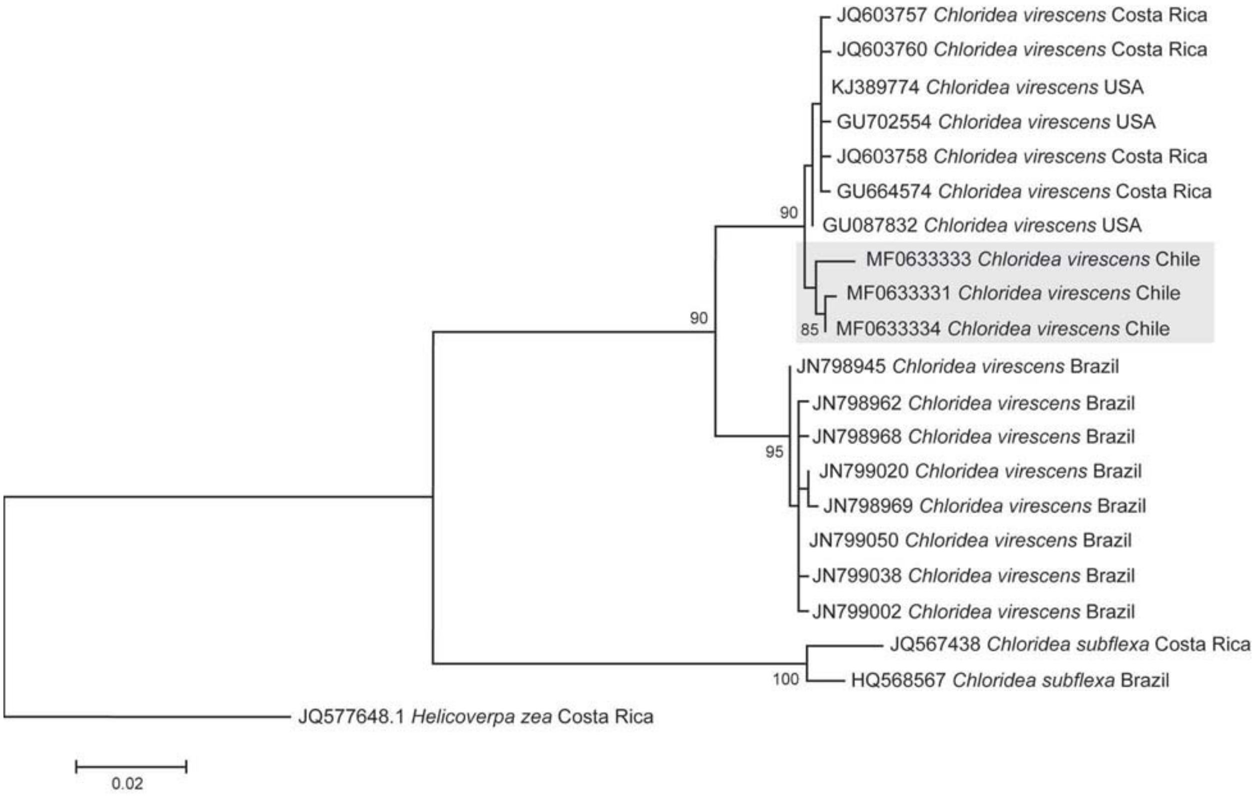

Fig. 1. Maximum likelihood tree of the sequences of the DNA barcode fragment (651 bp) of the cytochrome c oxidase subunit I (COI) gene of *Chloridea virescens* (Lepidoptera. Noctuidae) from different parts of the New World. Gray area indicates the newly reported sequences from the Azapa Valley, Atacama Desert of northern Chile. Bootstrap supports above 70% are shown.

DNA. Interestingly, they found that some haplotypes were exclusive to one geographic region or one host plant, which was interpreted as an initial differentiation of some populations in the Brazilian range of *C. virescens*, a pattern that was mostly corroborated using microsatellites in a later study (Domingues et al. 2013). Subsequently, Mitchell and Gopurenko (2016) analyzed the DNA barcode fragment (sensu Hebert et al. 2003) of the sequences provided by Albernaz et al. (2012) with samples from additional New World sites and found two very distinct clusters, one of which included Central and Northern American haplotypes, while the other only included Brazilian haplotypes.

The narrow Chilean range of *C. virescens* is restricted to the transverse valleys of the Atacama Desert (Parra et al. 1986), where the feeding activity of its larvae is a serious problem for some horticultural crops (Klein-Koch & Waterhouse 2000). We provided the first assessment of the genetic divergence of *C. virescens* of Chile based on analysis of DNA barcode sequences of specimens collected at the larval stage on a newly documented host plant.

Larvae of *C. virescens* were collected on the shrub *Trixis cacalioides* (Kunth) D. Don (Asteraceae) in the Azapa Valley, Arica Province, Atacama Desert of northern Chile in August 2016, and were brought to the laboratory in plastic bags. Some larvae were kept in 95% ethanol at -20 °C for DNA extraction, and the remainder were reared in the laboratory to obtain adults for taxonomic identification based on the examination of the genitalia morphology. Three legs and a bit of thoracic muscles of the adults were removed before mounting and were used for DNA extraction.

Genomic DNA was extracted from larvae and thoracic muscles of the adults following the procedures described in Huanca-Mamani et al. (2015). Amplification and sequencing was performed by Macrogen Inc. (South Korea) using the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). Conditions for the polymerase chain reaction were: 5 min at 94 ºC, 35 cycles of 30 sec at 94 ºC, 30 sec at 47 ºC, 1 min at 72 ºC, and a final elongation step of 10 min at 72 ºC.

The sequences were analyzed in the software MEGA6 (Tamura et al. 2013) following the procedures described by Hall (2013), including sequence alignment by the ClustalW method, estimation of sequence divergence by the Kimura 2-parameter (K2P) method and a Maximum Likelihood (ML) analysis with TN93+G as model of sequence evolution. The bootstrap method (1,000 replicates) was used to assess the statistical support of the nodes. The *C. virescens* sequences analyzed in the study of Mitchell & Gopurenko (2016), which include the Brazilian sequences provided by Albernaz et al. (2012), were downloaded from BOLD (Ratnasingham & Hebert 2007), and the software DnaSp (Librado & Rozas 2009) was subsequently used to select one sequence of each haplotype to be included in the ML analysis. As the Brazilian barcode sequences were 651 base pairs (bp) length, this was the minimal size for sequence selection. Sequences of the only congeneric species available in BOLD (*C. subflexa* Guenée) were also included in the analysis, and one sequence of *Helicoverpa zea* (Boddie) was used to root the tree, as *Helicoverpa* Hardwick is close to *Chloridea* Duncan & Westwood (Pogue 2013).

TABLE 1. Nucleotide variation among haplotypes of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Chloridea virescens* (Lepidoptera: Noctuidae) collected as larvae on *Trixis cacalioides* (Asteraceae) in the Azapa Valley, Atacama Desert of northern Chile.

	Variable sites [(a)]						**n**
	217	427	451	542	616	646	
Haplotype							
H1	G	C	T	C	T	T	2
H2	A	T	C	T	C	C	1
H3	-	-	-	-	C	-	1

(a) "-" indicates nucleotide identity to the H1 haplotype.

Four sequences of 658 bp of the DNA barcode fragment were obtained from the Chilean samples of *C. virescens*, with mean nucleotide composition 40.2% T, 14.7% C, 30.9% A, and 14.2% G. Six variable sites were detected, with all the mutations of the transition type (Table 1). The variation determined the presence of three haplotypes, H1 represented by two individuals (GenBank accession: MF063331, MF063332) and H2 and H3 by one individual each (MF063333 and MF063334, respectively). The genetic distance was 0.2–0.9% (K2P) among the Chilean haplotypes, 0.5–0.9% with *C. virescens* from Central and North America, 2.4–3.0% with *C. virescens* from Brazil, and 5.8–6.6% with the congeneric *C. subflexa*.

The alignment for the ML analysis included 21 DNA barcode sequences (Table 2) of 651 bp with 74 variable sites, 45 of which were parsimony informative. All the *C. virescens* sequences were clustered in a well-supported group (Fig. 1). Furthermore, in accordance with the results reported by Mitchell & Gopurenko (2016), this group was in turn internally divided in two also well-supported subgroups, one of which included only the Brazilian haplotypes, while the three samples from Chile

TABLE 2. DNA barcode sequences used in the maximum likelihood analysis.

Species	BOLD accession	GenBank accession	Length (bp)	Country
Chloridea virescens	---	MF063331	658	Chile
Chloridea virescens	---	MF063333	658	Chile
Chloridea virescens	---	MF063334	658	Chile
Chloridea virescens	GBMIN30031-13	JN799050	651	Brazil
Chloridea virescens	GBMIN30037-13	JN799038	651	Brazil
Chloridea virescens	GBMIN30046-13	JN799020	651	Brazil
Chloridea virescens	GBMIN30055-13	JN799002	651	Brazil
Chloridea virescens	GBMIN30075-13	JN798962	651	Brazil
Chloridea virescens	GBMIN30072-13	JN798968	651	Brazil
Chloridea virescens	GBMIN30128-13	JN798969	651	Brazil
Chloridea virescens	GBMIN30140-13	JN798945	651	Brazil
Chloridea virescens	BBLOD1268-11	KJ389774	658	USA
Chloridea virescens	HELNA527-09	GU702554	658	USA
Chloridea virescens	LOT347-04	GU087832	658	USA
Chloridea virescens	MHMXF822-07	JQ603757	658	Costa Rica
Chloridea virescens	MHMXF823-07	JQ603758	658	Costa Rica
Chloridea virescens	MHMXF825-07	JQ603760	658	Costa Rica
Chloridea virescens	MHMXZ853-09	GU664574	658	Costa Rica
Chloridea subflexa	BLPBH617-07	JQ567438	658	Costa Rica
Chloridea subflexa	INCTA907-10	HQ568567	658	Brazil
Helicoverpa zea	BLPAA705-06	JQ577648.1	658	Costa Rica

were clustered with the Central and North American haplotypes. However, the internal relationships of the two subgroups of *C. virescens* were not well resolved.

The K2P distances and the result of the ML analysis suggest that the Chilean representatives of *C. virescens* are more closely related to Central and North American moths than to those of Brazil. It is probable that despite the recognized high dispersal power of the adults of *C. virescens* (Hernández & Blanco 2010) the Andes Cordillera has been an effective barrier for this moth in South America. As a consequence, gene flow would be possible mostly between populations on the same side of the Andes, enhancing the genetic divergence of the populations belonging to the eastern and western sides of these mountains. Similarly some mitochondrial haplotypes of *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) are restricted to specific geographic areas separated by the Andes (Díaz-Montilla et al. 2013). Indeed, the uplift of the Andes has been also described as an important factor underlying the diversification of other Neotropical groups of Lepidoptera (Brower 1994, Massardo et al. 2015).

As already highlighted by Mitchell & Gopurenko (2016), the divergence level of the Brazilian with Central and North American haplotypes is in the range generally recognized as interspecific for Lepidoptera (Hebert et al. 2003, Hausmann et al. 2011). Indeed Mitchell & Gopurenko (2016) suggested that the Brazilian populations could have been misidentified as *C. virescens*. Although this issue is beyond the scope of our work, the results also show high divergence between the Chilean and Brazilian haplotypes (2.4–3.0% K2P). In contrast, the divergence of the Chilean and Central and North American haplotypes (0.5–0.9% K2P) is in the range generally recognized as intra-specific for Lepidoptera (Hebert et al., 2003; Hausmann et al. 2011). In addition, the high support of the two internal clusters of *C. virescens* and their reciprocally monophyletic status highlight the necessity of further examination of the genetic differentiation of *C. virescens* throughout its range using a multi-locus approach in order to assess cryptic speciation.

Most pest species of Heliothinae are highly polyphagous (Cunningham & Zalucki 2014). Although *C. virescens* is a polyphagous pest using several hosts of the family Asteraceae, this is the first time that *T. cacalioides* is reported as its host plant. This finding has at least two important consequences. First, as the knowledge of alternative host plants is useful for the understanding of the biology of the insect pests at a local scale (Abney et al. 2007, Albernaz et al. 2012, Domingues et al. 2013), this newly recorded interaction should be considered by local farmers for planning pest management practices. It should be interesting, for example, to assess the role of *T. cacalioides* as a reservoir of natural enemies of *C. virescens*. Second, during our field observations the larvae of *C. virescens* were found mostly feeding on flower buds of *T. cacalioides*, but also fed on open flowers and leaves. Thus the feeding activity of the larvae could be affecting both the reproductive success and the vegetative vigor of the shrub.

Trixis cacalioides is native to the coastal valleys of the Atacama Desert of northern Chile (Zöllner 1976, Katinas 1996), where it is also used by some native host-specialist Lepidoptera (Vargas 2011, Méndez-Abarca et al. 2014). Furthermore, the near threatened status has been recently proposed for this shrub in the study site (Gatica-Castro et al. 2015). Obviously, the use of *T. cacaliodes* by the voracious larvae of *C. virescens* can be a threat for both the shrub and the native Lepidoptera species that depend exclusively on this plant for their food. Accordingly, the effect of this polyphagous pest on the populations of *T. cacalioides* and its associated host-specialist Lepidoptera should be assessed in further studies.

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NEW RECORD OF THE THREATENED BUTTERFLY *DREPHALYS MOUREI* (HESPERIIDAE) IN A HEAVILY DISTURBED AREA IN SOUTHEASTERN BRAZIL

Additional key words: Atlantic Forest, conservation, Eudaminae, Red List of Brazilian Fauna, skipper

The genus *Drephalys* E. Watson, 1893 (Hesperiidae: Eudaminae) includes currently 18 species distributed from Mexico to Southern Brazil (Warren et al. 2009, Warren et al. 2016). All known species are diurnal and apparently have very specific life traits, keeping potential collectors apart, making them remarkably rare in collections (Mielke 1968; Burns 1999; Burns & Janzen 1999). Two out of the 13 Brazilian species of *Drephalys* (Casagrande & Duarte 2017) are in the Red List of Brazilian Fauna (Freitas & Marini-Filho 2011, MMA 2014), including *Drephalys mourei* Mielke, 1968, apparently a rare and poorly known butterfly. This species has been recorded in two sites in the Atlantic Forest at nearly 200 m of altitude. Males are apparently territorial, flying fast at one meter from the ground level displaying hilltopping behavior in clearings from 0830 h to 0900 h (Mielke 1968; Mielke & Casagrande 2008). Records of *D. mourei* includes the type locality in Joinville, Santa Catarina state (Mielke 1968), the small hill Morro do Cavalão, Niterói, Rio de Janeiro state (Mielke & Casagrande 2008) and an unconfirmed and dubious record from north of Espírito Santo state between the municipalities of Linhares e Pedro Canário (Brown Jr. 1991; Freitas et al. 2016). Based on the known distribution, Casagrande & Mielke 1992 suggest that *D. mourei* could be present in the state of Paraná. On 3 December 2016, at 1020 h, a single male individual of *D. mourei* has been observed and photographed by Marcos Cesar Campis ("Marquinhos Aventureiro", nature photographer) at Morro Agudo municipality, São Paulo state (20°48'S 48°00'W, 580 m a.s.l.). The male was observed flying fast and landing on flowers of *Coussarea* sp. (Rubiaceae) feeding on nectar (Fig. 1) on the edge of a small forest fragment (14.7 ha) surrounded by agricultural lands (sugar cane and soybean crop rotation). This record is important for four reasons: 1) this is the third confirmed locality for *D. mourei*; 2) this record extends the occurrence of this species to 300 km through the interior; 3) the site presents vegetation (semideciduous forest) and climate (seasonal with a marked dry season) distinct from the other two sites (both covered by ombrophilic forest without a marked dry season); and 4) the high degree of disturbance suggests that the species could occur in a wide variety of habitats, and its rarity could be an artifact of low detectability instead of biological attributes of the species (as suggested by the riodinid *Joiceya praeclarus*, see Greve et al. 2013). In addition,

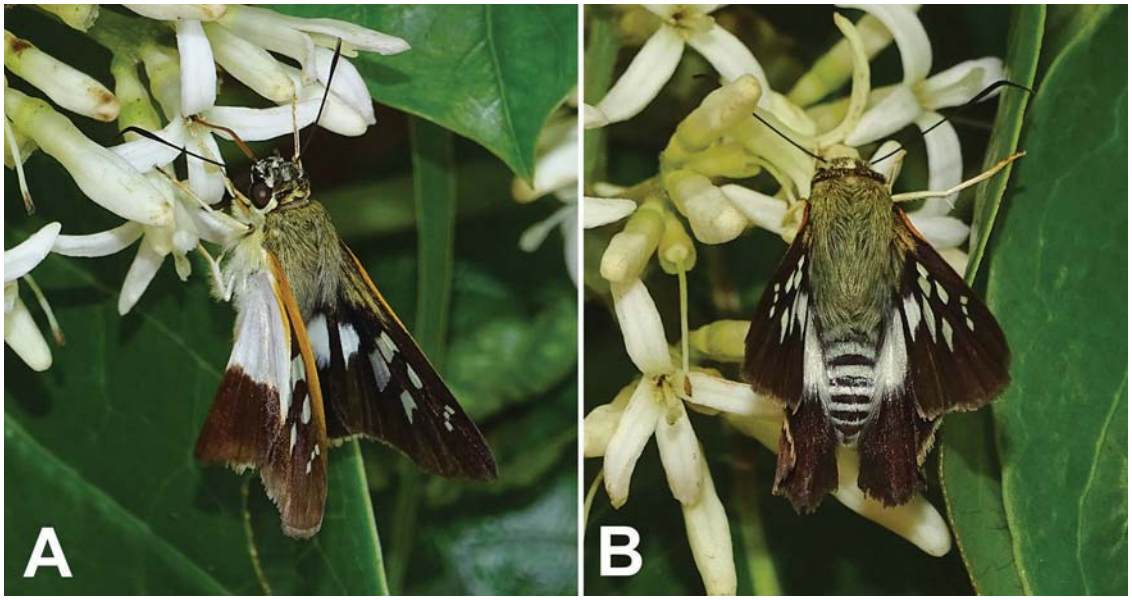

FIG. 1. Two views of *Drephalys mourei* feeding on nectar on *Coussarea* sp. (Rubiaceae), **A**. lateral view showing the ventral wing pattern, **B**. dorsal view. .

the present record emphasizes the importance of citizen science in providing valuable data for biological conservation. As recently shown for other threatened butterfly species (*Rhetus belphegor* (Westwood, 1851), Kaminski et al. 2015), both scientists and amateurs can effectively contribute to the science by revealing new unknown populations of rare or threatened species of animals and plants (Theobald et al. 2015).

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GENERAL NOTES

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