

## Full Issue

Source: The Journal of the Lepidopterists' Society, 67(1)

Published By: The Lepidopterists' Society

URL: <https://doi.org/10.18473/lepi.v67i1.a15>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Volume 67 Number 1  
2013  
ISSN 0024-0966

# Journal of the Lepidopterists' Society

---



---

Published quarterly by The Lepidopterists' Society

---

## THE LEPIDOPTERISTS' SOCIETY

### EXECUTIVE COUNCIL

ANDREW WARREN, President  
JOHN SHUEY, Immediate Past President  
CURTIS CALLAGHAN, Vice President  
ADAM COTTON, Vice President

DANIEL JANZEN, Vice President  
MICHAEL E. TOLIVER, Secretary  
KELLY M. RICHERS, Treasurer

TODD GILLIGAN  
PETER JUMP  
BRUCE WALSH

*Members at large:*  
MICHELLE DeCOSTA  
DAVID JAMES  
HARRY PAVULAAN

JOHN CALHOUN  
ROBERT DIRIG  
WAYNE WEHLING

### EDITORIAL BOARD

JOHN W. BROWN (*Chair*)  
MICHAEL E. TOLIVER (*Member, at large*)  
KEITH SUMMERVILLE (*Journal*)  
LAWRENCE F. GALL (*Memoirs*)  
JAMES ADAMS (*News*)  
JOHN A. SNYDER (*Website*)

### HONORARY LIFE MEMBERS OF THE SOCIETY

LINCOLN P. BROWER (1990), FREDERICK H. RINDGE (1997), RONALD W. HODGES (2004)  
CHARLES V. COVELL, JR. (2009), DON R. DAVIS (2009), JACKIE MILLER (2010), JERRY POWELL (2010)  
CARL COOK (2011), PAUL R. EHRlich (2011), RUDI MATTONI (2011),  
MOGENS C. ("MO") NIELSEN (2011), PAUL OPLER (2012)

The object of The Lepidopterists' Society, which was formed in May 1947 and formally constituted in December 1950, is "to promote the science of lepidopterology in all its branches, . . . to issue a periodical and other publications on Lepidoptera, to facilitate the exchange of specimens and ideas by both the professional worker and the amateur in the field; to secure cooperation in all measures" directed towards these aims.

Membership in the Society is open to all persons interested in the study of Lepidoptera. All members receive the *Journal* and the *News of The Lepidopterists' Society*. Prospective members should send to the Assistant Treasurer full dues for the current year, together with their full name, address, and special lepidopterological interests. In alternate years a list of members of the Society is issued, with addresses and special interests.

Active members—annual dues \$45.00 within the U.S., \$50.00 outside the U.S.  
Affiliated members—annual dues \$10.00 within the U.S., \$15.00 outside the U.S.  
Student members—annual dues \$20.00 within the U.S., \$25.00 outside the U.S.  
Sustaining members—annual dues \$60.00 within the U.S., \$65.00 outside the U.S.  
Life members—single sum \$1,800.00  
Institutional subscriptions—annual \$60.00, \$65.00 outside the U.S.  
Airmail postage for the *News* \$15.00, \$30.00 outside North America (U.S., Canada, Mexico)

Send remittances, payable to *The Lepidopterists' Society*, to: Kelly M. Richers, Treasurer, 9417 Carvalho Court, Bakersfield, CA 93311; and address changes to: Julian P. Donahue, Natural History Museum, 900 Exposition Blvd., Los Angeles, CA 90007-4057. For information about the Society, contact: Ernest H. Williams, Department of Biology, Hamilton College, Clinton, NY 13323. To order back issues of the *Memoirs*, write for availability and prices to Kenneth R. Bliss, 28 DuPont Avenue, Piscataway, NJ 08854.

---

The additional cost for members outside the U.S. is to cover mailing costs.

---

*Journal of The Lepidopterists' Society* (ISSN 0024-0966) is published quarterly by The Lepidopterists' Society, % Los Angeles County Museum of Natural History, 900 Exposition Blvd., Los Angeles, CA 90007-4057. Periodicals postage paid at Los Angeles, CA and at additional mailing offices. POSTMASTER: Send address changes to The Lepidopterists' Society, % Natural History Museum, 900 Exposition Blvd., Los Angeles, CA 90007-4057.

---

**Cover Illustration:** Adult male of the rare Riordinidae *Joiceya praeclarus* perching on leaves, in Foz do Iguaçu, south Brazil. Photo by Roberto R. Greve. See note on page 56.

**OXYNETRA: FACIES AND DNA BARCODES POINT TO A NEW SPECIES FROM COSTA RICA  
(HESPERIIDAE: PYRGINAE: PYRRHOPYGINI)**

NICK V. GRISHIN

Howard Hughes Medical Institute and Departments of Biophysics and Biochemistry, University of Texas Southwestern, Dallas, TX 75390;  
email: grishin@chop.swmed.edu

JOHN M. BURNS

Department of Entomology, National Museum of Natural History, Smithsonian Institution, Box 37012, Washington, DC 20013-7012;  
email: burnsj@si.edu

DANIEL H. JANZEN

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104; email: djanzen@sas.upenn.edu

WINNIE HALLWACHS

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104; email: whallwac@sas.upenn.edu

AND

MEHRDAD HAJIBABAEI

Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G2W1, Canada;  
email: mhajibab@uoguelph.ca

**ABSTRACT.** *Oxyetra stangelandi* Grishin & Burns, new species, from high elevations of Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica, is most similar to *Oxyetra hopfferi* Staudinger, known from mountains of central and southern Costa Rica and western Panama. These hesperiid species differ mainly in body color pattern and in DNA barcodes. We compare their barcodes, nucleotide by nucleotide, together with barcodes of a congener and a species of the related genus *Olafia*, and use the barcode data to show phylogenetic relationships. We describe the new species, its discovery, its male and female genitalia, and its life history as a cloud forest herbivore of *Prunus annularis* (Rosaceae). In ACG, no other skippers feed on this plant species, and no other skippers of the tribe Pyrrhopygini feed on plants in the family Rosaceae. Various stages of *O. stangelandi* belong to mimicry complexes. Although our adults, which are reared from wild-caught caterpillars, are split between the sexes (4 males, 6 females), there are scarcely any females of *Oxyetra* in the world's museums.

**Additional key words:** cryptic species, sexual dimorphism, genitalia (male and female), mimicry, *Prunus annularis*.

Some butterflies depart from the usual lepidopteran show of opaque scales by evolving wings that are partly to fully transparent. Among skipper butterflies of the tribe Pyrrhopygini, many of which are gaudy, certain members of the genus *Oxyetra* caught our attention owing not only to extensive alar transparency but also to a troubling lack of taxonomic transparency.

The neotropical genus *Oxyetra* C. & R. Felder, 1862—proposed for *O. semihyalina* C. & R. Felder, 1862—currently comprises three species: *O. semihyalina*, *O. confusa* Staudinger, 1888, and *O. hopfferi* Staudinger, 1888. Both sexes of *O. semihyalina*, and especially of *O. confusa*, have extensively transparent wings, with forewings whose hyalinity is primarily medial but also subapical. In males of *O. hopfferi*, the medial hyalinity is reduced and the subapical hyalinity is lacking; but in females, there is

none at all—the wings are entirely black. Given such extreme sexual dimorphism, it is not surprising that the female was originally described as a distinct species in its own genus, *Dis annulatus* Mabille, 1889. However, Godman and Salvin (1893) correctly synonymized genus *Dis* with *Oxyetra* and argued that the female of *O. annulatus* might prove to be that of *O. hopfferi*. Evans (1951) formally synonymized *O. annulatus* with *O. hopfferi*, an action that prevails (Mielke 2005).

Unlike its congeners, *O. hopfferi* is rare in collections, as are females of *Oxyetra* generally (e.g., for BMNH, Evans [1951] records 99 males of *O. semihyalina* and 44 males of *O. confusa*, mainly from Bolivia and especially Peru, but no females of either species). After 22 years of intensive continuous collecting and rearing in Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica (Burns & Janzen 2001, Janzen et al. 2009), the first

*O. hopfferi*-like specimen surfaced in July 2001, too late to appear in the detailed account (then in press) of pyrrophygine skippers of ACG (Burns & Janzen 2001). This unforeseen species adds a new larval foodplant, *Prunus annularis* Koehne, and a new family, Rosaceae, to those eaten by Pyrrophygini in ACG (Janzen & Hallwachs 2012).

That 2001 specimen, a male (voucher code 01-SRNP-6995), has—in contrast to the multi-orange-banded abdominal pattern of male *O. hopfferi*—a single orange band on abdominal tergum III. However, one (02-SRNP-23283) of two males reared in 2002, has, in addition to the bold orange band on tergum III, some sparse orange scaling on tergum IV; and the male (03-SRNP-3638) reared in 2003 has a scattering of 23 orange scales there. Despite such individual variation, the general effect is one band.

Multiple rearings of phenotypically similar wild-caught caterpillars unequivocally associate the dimorphic sexes, and DNA barcoding confirms the association. Barcodes of all 10 ACG specimens are identical (Fig. S1 of Janzen et al. 2011).

In early 2011, when Mike Stangeland began preparing photographs of reared *Oxyntera* males for the Butterflies of America (BoA), website <http://butterfliesofamerica.com> (Warren et al. 2012), he noticed that they differed from photographs of *O. hopfferi* males in other collections and raised the possibility of a new taxon. Subsequent investigation, assisted by many collaborators, indicates that the northwestern Costa Rica specimens represent an undescribed species, closely related to, but distinct from, *O. hopfferi*.

### *Oxyntera stangelandi* Grishin & Burns, new species (Figs. 1a, b, 2a–h, 3a, 5, 7, 8)

**Description.** *Size:* Male smaller than female. Forewing length (mm) of four males: 17.1, 18.1, 18.2, 18.3; of six females: 20.2, 20.7, 21.1, 21.5, 22.2, 22.6. (These measurements will average less than those of wild-caught adults, because conditions and duration of rearing often stunt growth.)

*Nudum of antennal club:* Fewer segments in male than in female. Number of segments in left nudum/right nudum of four males: 20/20, 20/20, ?/20, 22/22; of five females: 24/23, 23/25, 25/25, ?/25, 26/25. (Number of nudum segments not correlated with wing length.)

*Facies:* Pronounced sexual dimorphism:

*Facies, male:* Hindwing (HW) narrow and elongate; forewing (FW) extending well beyond it. Outer wing margin very slightly concave at cell  $CuA_2-1A+2A$  of FW and at cells between veins  $M_1$  and  $M_3$  of HW. Dorsal and ventral FW (including fringe) brownish-black with blue/purple sheen (except for some whitish fringe scales at cell  $CuA_2-1A+2A$  in three of four males), and with bold, median, wide, straight-edged, hyaline band from anterior edge of discal cell to vein  $1A+2A$ ; band divided into three large, aligned parts by dark-scaled veins  $CuA_1$  and  $CuA_2$ ; its outer edge evened by tiny, triangular, hyaline wedge at very base of cell  $M_3-CuA_1$ . Dorsal HW concolorous with FW (except fringe around tornus white), with two median, large, roughly rectangular, aligned, hyaline spots (in cell  $Sc+R_1-R_2$  and discal cell), separated by vein  $R_2$ , and suggesting continuation of FW band; three

of four males with small, postmedian pair of hyaline spots in proximal ends of cells  $M_3-CuA_1$  and  $CuA_2-CuA_3$  (these spots expressed only ventrally in fourth male). Ventral HW similar to dorsal, but with wing base white and with two small, opaque, white spots in cell  $CuA_2-1A+2A$ , one median and one submarginal (the latter spot looking more or less dual). Scaled antenna black; nudum medium brown. Head and body primarily brownish-black with aqua to blue/purple sheen, but marked as follows: two tiny white spots at base of antenna and one small spot at dorsoposterior margin of eye; first and second segments of palpus ventrally white, with white continuing, without interruption, in midventral strip as far as base of foreleg, and thereafter in separate midventral patches between bases of midlegs, of hindlegs, and at posterior margin of each sternite (some abdominal patches resembling transverse dashes and progressively decreasing in size). Large orange spot on anterior 40% of tegula. Orange band (with slight middorsal notch in anterior margin) across tergum III.

*Facies, female:* Remarkably different from male. HW relatively broad and rounded. Both wings uniformly brownish-black, above and below, with strong, purplish, metallic sheen (imparting “greasy” appearance). HW fringe narrowly white, FW fringe variably so (never at apex). White head and body markings of male greatly reduced or not expressed: spot at dorsoposterior margin of eye present, but only one white spot at base of antenna (on anterior side); ventral white of basal segments of palpus narrow, and not continued posteriorly, except for short, vestigial, midventral dashes at posterior margin of abdominal segments. Tegula all black. Conspicuous orange band across tergum III, as in male, except for narrow middorsal break.

*Genitalia, male* (Fig. 3a): Tegumen about twice as wide as high and about twice as long as uncus; supported ventrally, on each side, about halfway along its length; distal end with small middorsal knob, flanked by pair of distally directed, blunt, rudimentary projections. Uncus narrower than tegumen and tapering slightly toward distal end; distal end with medial V-shaped notch; short fat prong on either side of notch curved ventrad, terminating in blunt point. Valva with body prominently humped and appearing triangular in lateral view; distal division narrowed, tapered, curved dorsad, and dentate along anterior edge; ventral inner side of valva giving rise to two dorsal projections: proximal one low, less obvious, and wide, with uneven, finely dentate dorsal edge; distal one long, narrow, curved mediad, dentate, and sharply pointed. Saccus very short. Penis more or less uniform in diameter and conspicuously humped near distal end; no cornuti.

*Genitalia, female* (Fig. 5): Lamella postvaginalis—lying just below, and extending well distad of, ovipositor lobes—shaped like wide flat paddle, expanding somewhat in thickness and width along its length; distal end rounded, with slight medial notch. Lamella antevaginalis narrowly extending dorsad, from dorsolateral rim of ostium bursae, almost as far as dorsal edge of ovipositor lobes; its surface indented and appearing wavy at some angles. Ostium bursae remarkably large, elliptical, and well-sclerotized, forming posteriorly directed cup. Ductus bursae membranous, initially about half diameter of ostium bursae, then abruptly decreasing in diameter, changing direction, and leaving blind pocket at anterior end of large-diameter portion of ductus bursae. Ductus seminalis joining ductus bursae middorsally, slightly anterior of sclerotized ostium bursae cup. Corpus bursae membranous.

*Holotype* (Fig. 1a): Male, voucher code 02-SRNP-23284, Sendero Derrumbe, 1220 m, Sector Cacao, Area de Conservacion Guanacaste, Costa Rica, lat 10.92918, long -85.46426, collected in penultimate larval instar by Harry Ramirez [deposited in USNM]. Yellow label reads “LEGS AWAY/FOR DNA.”

*DNA barcode* (633 bp) of holotype:

```
AACTTTATATTTTATATTTTGGAAATTTGACGAGGAATAATTGGAA
CTTCATTAAGATTACTAATTCGAACTGAATTAAGTACCCCCGG
ATCTTTAATTGGAAATGACCAAATTTATAACACTATTGTAACAG
CTCATGCAATTTATTATAATTTTTTTTATAGTTATACCTATTATAA
TTGGAGGATTTGGAAATTCATTAGTTCCCTTTTAATATTAGGAGC
TCCAGATATAGCTTTCCCTCGAATAAATAATATAAGATTTTGAT
TATTACCTCCATCTTTAACTCTTTTAATTTCAAGAAGAATTGTA
GAAACCGGTGCTGGAAGTGGATGAACACTTTCCCCCTCT
TTCTTCTAATATTGCCCATCAAGGAACCTTCGTTGATTAGCT
```



ATTTTCTCTTCATTAGCTGGAATTTCTTCAATTTAGGGG  
 CTATTAATTTTATTACAACAATTATTAAATATACGAATTAATAAATT  
 TATCTTTTACCAAAATACCTCTTTTGTGTGAGCCGTAGGAAT  
 TACTGCATTATTATTATTATTATCTTTACCTGTATTAGCAGGTG  
 CTATTACTATACTTTTAAACAGACCGAAATATTAATACTTCCTTT  
 TTTGATCCTGCAGGAGGAGGAGA

*Paratypes* (Figs. 1b, 2a–h): 3 males (voucher codes 01-SRNP-6995, 02-SRNP-23283, 03-SRNP-3638), 6 females (02-SRNP-23109, -23110, -23285, -23286, -23540, -24529) from Sendero Toma Agua, 1140 m; Estacion Cacao, 1150 m; and Sendero Derrumbe, 1220 m—all in Sector Cacao, Area de Conservacion Guanacaste, Costa Rica [deposited in USNM]. For further details, see Janzen & Hallwachs 2012.

*Larval habitat; elevation range:* Cloud forest. Type specimens collected at 1140, 1150, and 1220 m.

*Foodplant:* *Prunus annularis* (Rosaceae).

*Etymology:* Named in honor of Mike Stangeland—a research associate of the McGuire Center for Lepidoptera and Biodiversity at the Florida Museum of Natural History, University of Florida, and the photo editor and administrator of BoA—who has prepared >100,000 butterfly images for online display.

**Diagnosis.** Males of *O. stangelandi*, with one orange band across tergum III, differ from males of sister species *O. hopfferi*, with their five-banded abdomen (and even from hesperiids generally [except for *O. semihyalina*, which has a single, pale orange band]). In both sexes, the orange of the abdominal bands is redder in *O. stangelandi* than it is in *O. hopfferi*. Males of *O. stangelandi* lack *O. hopfferi*'s narrow streak of whitish hairlike scales on the dorsal HW near the middle of cell 1A+2A–A3 and parallel to its bounding veins. The number of nudum segments in *O. stangelandi* (ranging in four males from 20 to 22) may exceed that of *O. hopfferi* (whose three inspected males have only 17, 18, and 19 segments).

In females of *O. stangelandi*, as opposed to those of *O. hopfferi*, the ventral white on palpal segments one and two is relatively restricted and narrow, and the adjacent thoracic venter is black instead of white. The black middorsal break in the orange band across tergum III appears slightly narrower in *O. stangelandi* than in *O. hopfferi*. Wing fringes are predominantly white in *O. stangelandi* but, with one exception, not in *O. hopfferi*.

The mitochondrial nucleotide sequence in the C-terminal segment of COI (i.e., the DNA barcode) of *O. stangelandi* is unique; and it differs from those of *O. hopfferi* by 2.6% to 3.2%. This critical distinction is analyzed in detail below, where it is also treated in a broader context.

## DISCUSSION

**Genitalia.** Do male genitalia distinguish *O. stangelandi* from *O. hopfferi*? Close, side by side comparison of the dissections that appear in Fig. 3, as well as another, reveals various small differences. These include a larger middorsal knob at the distal end of the tegumen, a more massive, more dentate distal division of the valva, a longer, more dentate pointed projection

from the inner side of the valva, and a longer penile hump in *O. hopfferi* than in *O. stangelandi*. Judging from over half a century of perusing countless skipper genitalia, Burns cautions that these apparent differences may reflect nothing more than individual variation. No matter how simple their form, genitalia vary appreciably within a population; and individual variation generally becomes more evident as genitalic complexity increases. Given our minimal genitalia samples—two complete dissections of *O. stangelandi* and one of *O. hopfferi*, plus photographs of valval exteriors (in situ, and partly exposed by dusting) of two other specimens of *O. hopfferi*—we cannot identify interspecific differences with certainty. A difference we once considered potentially valuable in telling *O. stangelandi* and *O. hopfferi* apart involves the dorsal margin of the valval body, distal to its pronounced hump: in lateral view, this margin makes an even, concave curve down to a slightly upturned, rounded, protruding base in *O. hopfferi* (Fig. 3b) but a straight line to a sharp, wide, basal angle in *O. stangelandi* (Fig. 3a). No such difference marks the second example of *O. stangelandi*.

However, relatively minor genitalic differences may mean more in *Oxyetra* than they do in many other genera, because the genitalia of all four species are conservative variations on a single complex theme. Even though the facies of *O. semihyalina* and *O. confusa* (Fig. 6) differ considerably from those of *O. hopfferi* and *O. stangelandi* (Figs. 1, 2), their genitalia (Fig. 4) do not. Features showing interspecific variation between *O. semihyalina* and *O. confusa* include the distal division of the valva and the penile hump, which are features noted above that may distinguish *O. stangelandi* from *O. hopfferi*. The differences in the distal division of their valvae (compare the oblique views of genitalia in Figs. 3a and 3b) look especially promising for species discrimination. (For an example of intraspecific variation in genitalic form, compare photos of the male genitalia of *O. confusa* in Orellana 2008:fig. 212 with Fig. 4b.)

### Barcode differences and intrageneric phylogeny.

We obtained barcode sequences for the entire type series of *O. stangelandi* and for three *O. hopfferi* specimens. All 10 of our *O. stangelandi* sequences are identical within their sequenced length, and all specimens are from the same locality. Sequences of the three *O. hopfferi* specimens differ from each other by 2 or 4 nucleotides, which amounts to 0.3% and 0.6% intraspecific differences (Fig. 7a); and these three specimens are from different localities within Costa Rica. The *O. stangelandi* sequence differs from those of *O. hopfferi* by 17, 19, and 21 nucleotides (2.6%, 2.9%, 3.2% differences). Differences of this magnitude are



FIG. 1. *Oxyntera* type specimens. **a**, *O. stangelandi* holotype, male, ACG, Costa Rica, voucher code 02-SRNP-23284. **b**, paratype, female, ACG, Costa Rica, 02-SRNP-23286. **c**, *O. hopfferi* holotype, male, Chiriqui, Panama, leg. Ribbe, Staudinger Collection [ZMHB]; with specimen labels. **d**, *Dis annulatus* holotype, female, Chiriqui, Panama, leg. Trötsch, Staudinger Collection [ZMHB]; with specimen labels. Each specimen in dorsal (above) and ventral (below) view.





FIG. 2. *Oxynetra* adults. **a-h**, *O. stangelandi*. **i-q**, *O. hopfferi*. **f-l**, **n-o**, **q**, males (banded wings). **a-e**, **m**, **p**, females (unmarked wings). **Subscripts**: **v**, ventral view; **l**, live individual; **a**, magnified portion of dorsal hindwing near anal fold. **i-k**, from Puntarenas, COSTA RICA. **l-q**, from Chiriqui, PANAMA. **n**, illustration of *O. hopfferi* holotype from original description (Staudinger 1888). **o-p**, illustrations of *O. hopfferi* and *Dis annulatus* holotypes from Godman & Salvin (1893). **q**, illustration of *O. hopfferi* holotype from Draudt (1921), apparently copied from Staudinger illustration (n) instead of drawn from specimen. Voucher codes of Costa Rican paratypes: **a**, 02-SRNP-23540; **b**, 02-SRNP-23109; **c**, 02-SRNP-23285; **d**, 02-SRNP-23110; **e**, 02-SRNP-24529; **f**, 03-SRNP-3638; **g**, 01-SRNP-6995; **h**, 02-SRNP-23283. **i**, Coto Brus, Sabalito, Cotoncito, 1500 m, 8.943, -82.787, Puntarenas, COSTA RICA, 14 September 2006, R. Gonzalez [INBio]; **j**, Coto Brus, Sabalito, Estacion Biologica Las Alturas, 1500 m, 8.949, -82.836, Puntarenas, COSTA RICA, 31 January 1992, M. Zumbado [INBio]; **k**, 97-ZFuentes-055: Peñas Blancas, Monteverde, Puntarenas, COSTA RICA, 1997, Z. Fuentes [USNM].



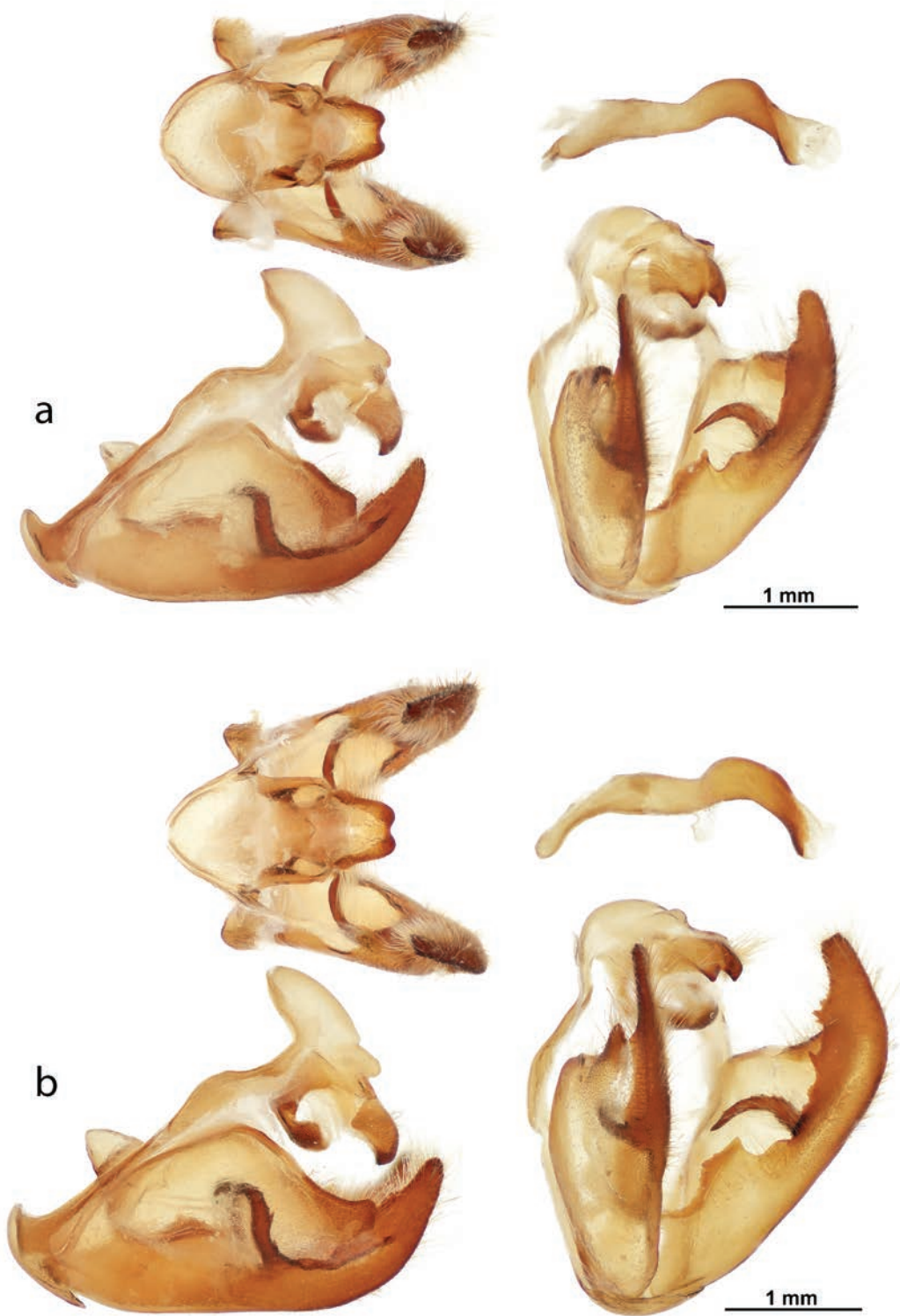


FIG. 3. *Oxyetra* male genitalia. **a**, *O. stangelandi*, dissection code X-6958. ACG, COSTA RICA, voucher code 02-SRNP-23283 [USNM]. **b**, *O. hopfferi*, dissection code X-6959. Peñas Blancas, Monteverde, Puntarenas, COSTA RICA, 1997, Z. Fuentes, voucher code 97-ZFuentes-055 [USNM]. Left side: genitalia (minus penis) in dorsal and left lateral views. Right side: penis in left lateral view, genitalia (minus penis) in oblique view.

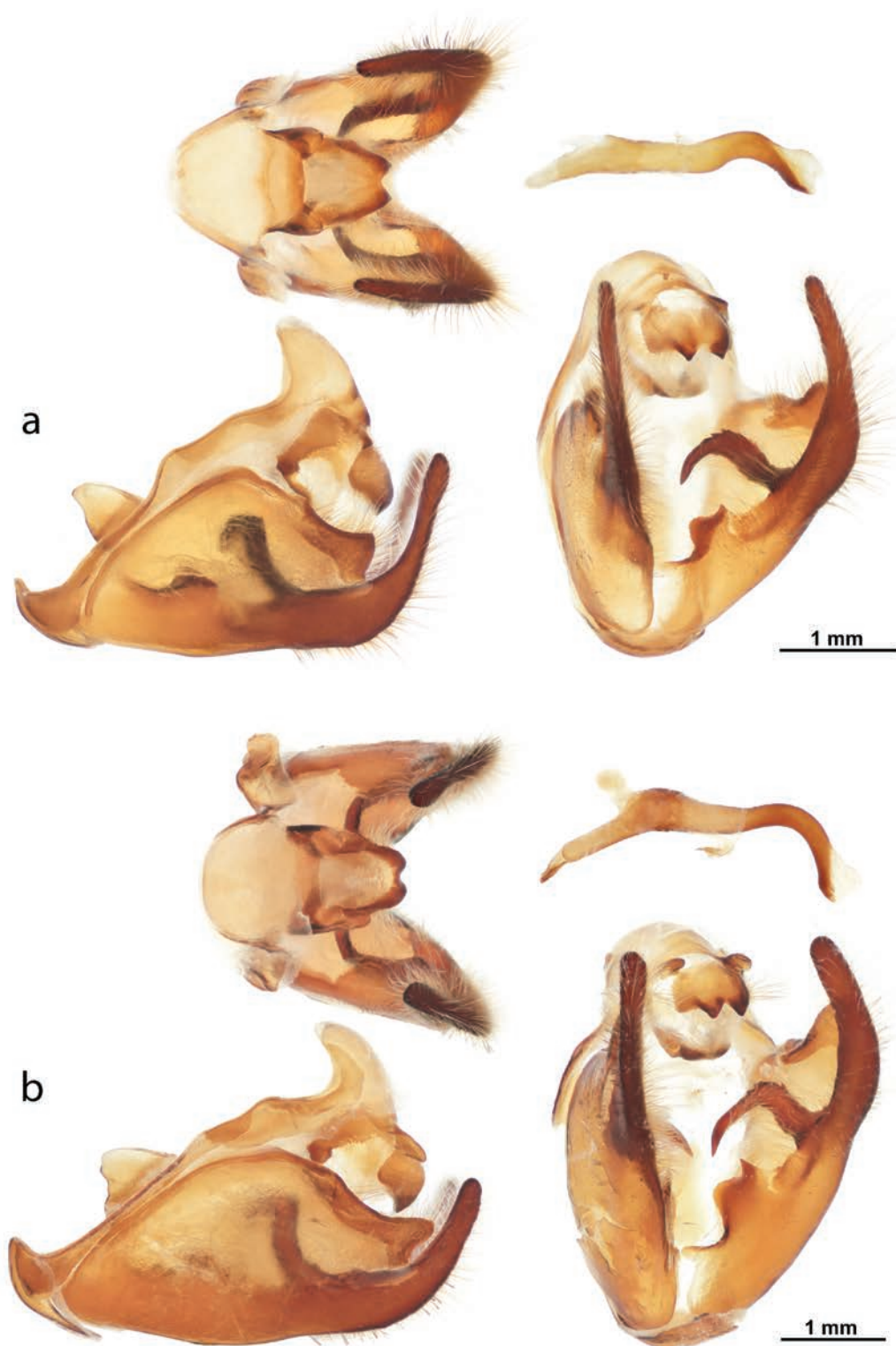


FIG. 4. *Oxyetra* male genitalia. **a**, *O. semihyalina*, dissection code X-6976. Tingo Maria, 300 m, Huanuco, PERU, 24 June 1982, S. S. Nicolay [USNM]. **b**, *O. confusa*, dissection code X-6977. Carpish, 3000 m, 9° 42' S, 76° 04' W, Huanuco Dept., PERU, February 1992 [USNM]. Left side: genitalia (minus penis) in dorsal and left lateral views. Right side: penis in left lateral view, genitalia (minus penis) in oblique view.

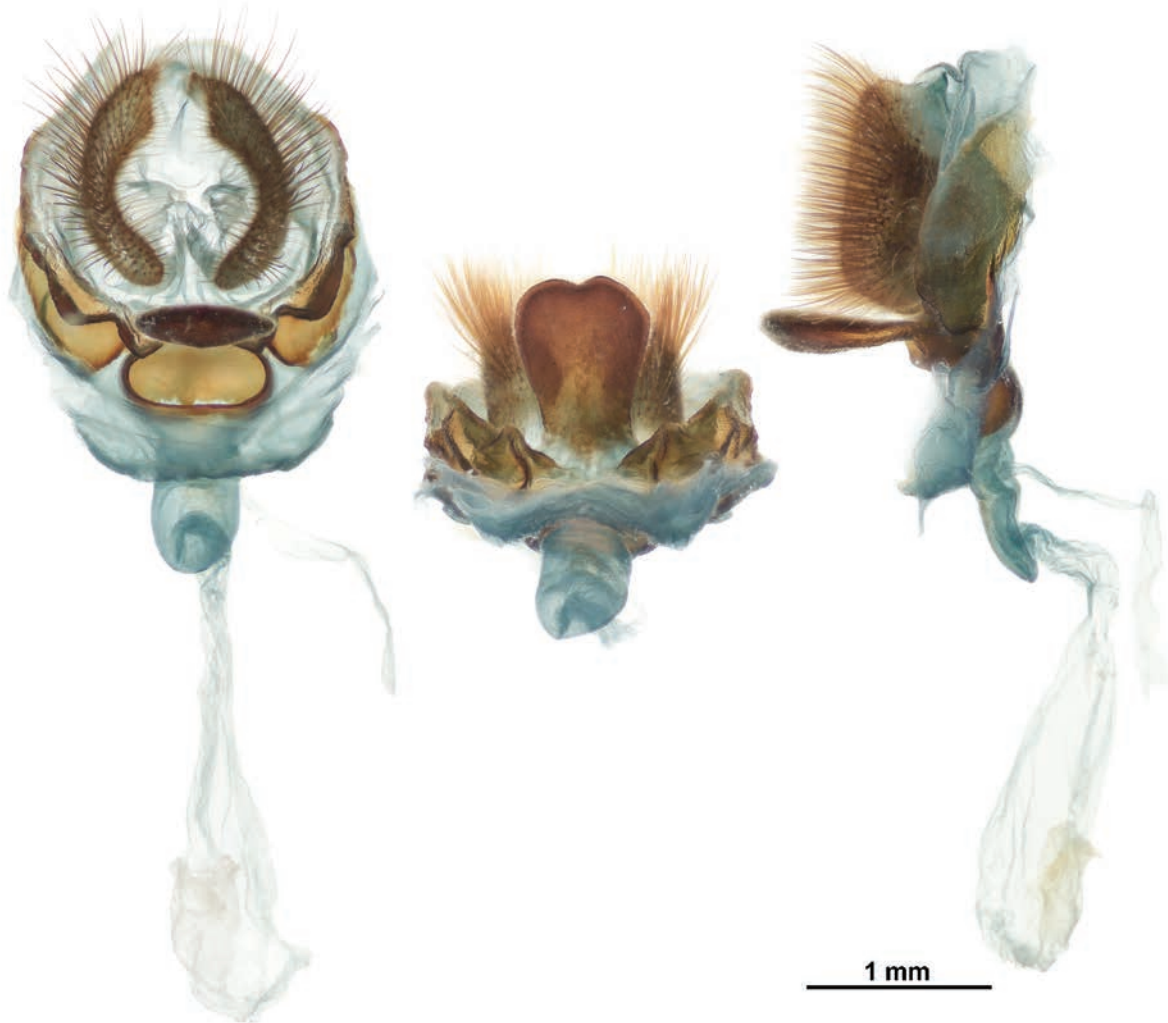


FIG. 5. *Oxynetra stangelandi* female genitalia. Dissection code X-6960. ACG, COSTA RICA, voucher code 02-SRNP-23285 [USNM]. Left: posterior view. Middle: ventral view. Right: right lateral view.

consistent with interspecific distances found in other similar sets of hesperiid species. Moreover, in some unquestionably valid cryptic species, interspecific distances are much smaller: in large ACG samples of *Perichares*, for example, *P. adela* (Hewitson) differs from *P. prestoeaphaga* Burns by about 0.6%, from *P. geomorphaga* Burns by about 0.8%, and *P. prestoeaphaga* from *P. geomorphaga* by about 0.7% (Burns et al. 2008). Indeed, some closely related skipper species may differ by only 1 to 3 nucleotides (Burns et al. 2007).

To compare the differences between *O. stangelandi* and *O. hopfferi* to differences between them and

species that morphologically appear related but more distant, we queried BLAST search <<http://www.ncbi.nlm.nih.gov/blast>> for publically available sequences most similar to that of *O. stangelandi* and obtained barcodes of *O. confusa* and *Olafia rosci* (Hopffer, 1874) (Fig. 7a). *Olafia* is the genus most similar to *Oxynetra*; and its sole species, *Ol. rosci*, was previously treated as *Oxynetra* (Evans 1951). The *O. stangelandi* sequence differs from sequences of *O. confusa* and *Ol. rosci* by 35 and 36 nucleotides, respectively (around 5.5%). Though smaller, the barcode distances between *O. stangelandi* and *O. hopfferi* are still comparable to those between *O.*



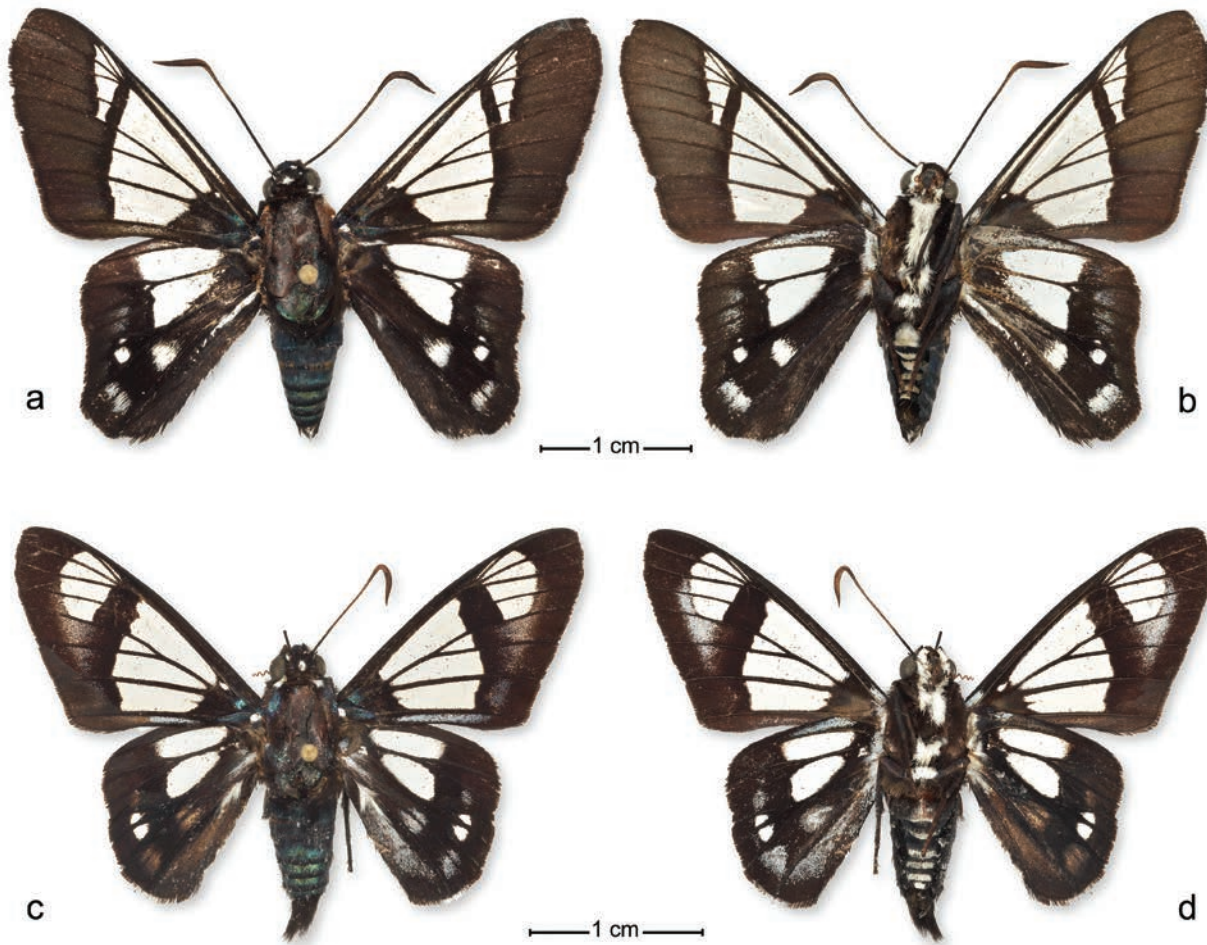


FIG. 6. *Oxynetra* adult males. **a–b**, *O. semihyalina*, Carpish, PERU, May 1992 [USNM]. **c–d**, *O. confusa*, Carpish, 3000 m, 9° 42' S, 76° 04' W, Huanuco Dept., PERU, February 1992 [USNM]. **a, c**, dorsal view. **b, d**, ventral view.

*stangelandi* and *O. confusa*, and even to those between *Oxynetra* and *Olafia*.

Although the 654 base pair sequence is generally too short for reliable phylogenetic inference, we applied four standard phylogenetic reconstruction programs offered at <http://www.phylogeny.fr> to our genetic data. The resulting trees (Fig. 7b–e) are topologically identical and show *O. stangelandi* and *O. hopfferi* as sister species, in agreement with the phenotypic data. Branch lengths in the trees in Fig. 7b, c, and d are to scale and graphically show the extent of intraspecific variation in *O. hopfferi* and how it scales to interspecific differences.

**Distribution, ecology, life history, behavior, and mimicry.** So far, *O. stangelandi* is known only from the 1,000–1,500 m zone of cloud forest covering the top of Volcan Cacao (centered by latitude 10.93328, longitude -85.45729) in central ACG. The enormous rearing project in ACG, which is often coupled with mass barcoding of the reared specimens, has led to the description of many new insect species solely from ACG material; but this does not mean that these species are extremely limited in their geographic distribution.

The shrub-treelet foodplant, *Prunus annularis* (Rosaceae), occurs throughout the understory and edges of this dense forest and reaches a height of 5 m.







FIG. 8. *Oxynetra stangelandi* immatures. a-d, f-m, caterpillars. e, n-z, pupae. Voucher codes: a-d, f-h, n-r, 02-SRNP-23109; e, x, 02-SRNP-23283; i-m, 02-SRNP-23110; s-w, y-z, 02-SRNP-23284 (holotype).



Caterpillars of *O. stangelandi* have been found feeding only on this species; and since almost all other species of plants in this habitat have been intensively searched for over 20 years (Janzen & Hallwachs 2012) for any and all caterpillars (>30,000 records), we assume that *O. stangelandi* is monophagous. Because it is not known to share this foodplant with other species of Hesperidae, we infer that empty hesperiid larval shelters on *P. annularis* belong to this species.

The first individual of *O. stangelandi* (01-SRNP-6995) was found as a pupa (2 July 2001; eclosed 26 July 2001) in a leaf shelter it had made in the top of an unknown 60 cm tall understory sapling below a 3–4 m tall *P. annularis*. Skipper caterpillars often construct pupal shelters on plants adjacent to, but different from, their foodplant. A year later, various instars of *O. stangelandi* were found on *P. annularis* in the general vicinity (Janzen & Hallwachs 2012), although at least 10 years of search of this foodplant prior to 2001 had not turned up any hesperiid larval shelters. Continued explicit and haphazard search of *P. annularis* foliage in the same general area from 2003–2011 has yielded only one more caterpillar (03-SRNP-3638), plus one parasitized pupa on a non-foodplant. *Oxyntera stangelandi* is clearly a very low density species in the 1–3 m height range easily searched by parataxonomists; however, the specimens encountered may represent only the bottom of a higher height distribution of the caterpillars. Dates of adult eclosion range from late July to late December, but the sample is too small to estimate annual phenology. A female caterpillar (02-SRNP-23285) found as second instar on 19 July 2002 and reared in a protective net placed over the foodplant, pupated 30 August 2002 and eclosed 19 September 2002 (at 1220 m elevation).

*Oxyntera stangelandi* caterpillars have the hairy, strikingly yellow- and black-banded body and hairy head (Fig. 8a–d, f–m) characteristic of many pyrhopygine skipper caterpillars (Burns & Janzen 2001; Janzen et al. 2009). They are no doubt part of a generally aposematic mimicry system involving hundreds of other ACG caterpillars. Likewise, orange eyespots on the black head of *O. stangelandi* caterpillars place them in a mimicry complex that comprises hundreds of species of ACG caterpillars exhibiting a great diversity of false eyespot patterns (Janzen et al. 2010). Moreover, the mature pupa is basically white (with small orange and black spots, Fig. 8e, s–z), resembling the white pupae of many other shelter-inhabiting ACG skipper species that mimic pupae attacked by fungi. Pupae that are fungal victims usually become white, highly inedible, and even gustatorially dangerous (see images in Janzen & Hallwachs 2012). In the newly molted *O. stangelandi*

pupa, the head, pronotum, and appendages, including wings, look more or less orange and contrast with dull maroon dorsolateral bands on the cream to tan ground color of the rest of the body (Fig. 8n–r). After about two days, a white waxy layer is exuded over the surface, giving the pupa the white appearance that remains until eclosion.

Although no adult *O. stangelandi* has been observed in the field, its behavior, when newly eclosed in a large rearing container, is unusual. Newly eclosed ACG hesperiids, in many thousands of cases and across more than 400 species, hold their wings together over their back during hardening, and then usually keep them in that position while walking and perching in the rearing containers. However, both sexes of *O. stangelandi* harden their wings in the position shown in Fig. 2h<sub>1</sub> and hold them that way while walking with the characteristic gait and slight up-and-down wing motion of a large ctenuchine arctiid moth (many species of which are diurnal, brightly colored, transparent-winged, and widely believed to be chemistry-based aposematic or Hymenoptera mimics). Though aware that the male in Fig. 2h<sub>1</sub> must be a hesperid that he had photographed repeatedly as a developing larva and pupa, Janzen had to look twice to reassure himself that it was not a contaminating arctiid whose cocoon had inadvertently come in with food foliage. While the striking colors of the male may serve in courtship and the black color of the female in heat absorption in cold cloud forest, these colors also function in at least two mimicry rings.

**History of discovery.** We initially addressed Stangeland's suggestion that the reared ACG material might not be *O. hopfferi* by procuring a specimen whose facies closely match those of the holotype (male) of *O. hopfferi* (Fig. 1c). The new specimen (voucher code 97-ZFuentes-055 [deposited in USNM]) is a male that eclosed from a pupa collected in 1997 in Peñas Blancas, Monteverde, Puntarenas Province, Costa Rica, which is about 100 km southeast of ACG and considerably closer to the *O. hopfferi* type locality (Chiriqui). This male has the characteristic *O. hopfferi* abdomen with five orange bands, as well as a hindwing with a short, narrow white streak in cell 1A+2A–3A. One of its legs, sacrificed to DNA sequencing, yielded a barcode that differed by 2.6% from the uniform barcodes previously obtained for all ten ACG specimens. Subsequent comparison of genitalia gave the uncertain result discussed above.

Our need of a larger sample of *O. hopfferi* to assess variation was frustrated by the rarity of this species in collections. In addition to the Monteverde specimen (Fig. 2k) and the holotypes of *O. hopfferi* (Fig. 1c) and *D. annulatus* (Fig. 1d), we inspected four male

specimens, all from Chiriqui, Panama (1, BMNH; 2, ZMHB; 1, MTD). With the help of Kim Garwood and Isidro Chacón, we obtained photographs of four more specimens: three males (from Chiriqui, Panama [OM-DZUP], Fig. 2l; and Puntarenas, Costa Rica [INBio], Fig. 2i–j) and one female (from Chiriqui, Panama [OM-DZUP], Fig. 2m). The facies of our sample of nine males and two females of *O. hopfferi* from southern Costa Rica and western Panama do not vary significantly (except that fringe color is dark in the *Dis annulatus* holotype and largely white in the other female).

*Oxyntera annulatus*, originally described (in genus *Dis*) from an almost black female with one orange band on her abdomen, has been synonymized with *O. hopfferi*. Were *O. annulatus* the female of the ACG entity, the latter would assume that name. The holotype (Fig. 1d) exhibits a white prothoracic venter devoid of dark scales. The females of *O. stangelandi* have a fully black thoracic venter, concolorous with the rest of the body (Figs. 1b, 2a<sub>v</sub>–e<sub>v</sub>). Moreover, they have narrower white areas on the palpi below and a narrower dark middorsal break in the abdominal orange band, which is redder than in the *O. annulatus* holotype; and they have white wing fringes, in contrast to the mostly dark fringes of the *O. annulatus* holotype. Holotypes of both *O. hopfferi* and *O. annulatus* are from Chiriqui, Panama. We agree that *O. annulatus* is indeed a female of *O. hopfferi*. Even without knowing the locality, it is possible to associate the *O. annulatus* female with the *O. hopfferi* male, as they both exhibit prominently developed white areas on the venter, contrasting to more restricted distribution of white in both sexes of *O. stangelandi*; and orange bands on the abdomen, differing from redder bands in both sexes of *O. stangelandi*. These differences hold up well in available samples of the two species: 9 males, 2 females of *O. hopfferi*, and 4 males, 6 females of *O. stangelandi*.

*Oxyntera* illustrations in historic works are illuminating. Illustrations in Godman and Salvin (1879–1901) are usually more accurate than are those in Draudt (1921). However, for *O. hopfferi*, the white streak near the anal fold of the hindwing of the holotype does not appear in Godman and Salvin (vol. 3, pl. 74, fig. 18, reproduced here, Fig. 2o). Although the Draudt illustration (1921: pl. 165, row b, reproduced here, Fig. 2q) of apparently the same specimen depicts this streak clearly, it lacks the small, transparent, triangular wedge at the base of forewing cell  $M_3$ – $CuA_1$  and shows 7 orange bands (6 major and 1 minor) instead of “5 bright orange-red transverse band[s] on the dorsum of the abdomen” as specified in the text (Draudt 1921:849). The Draudt illustration was probably drawn from the

illustration in the original description (Staudinger 1888: pl. 99, as *felderi*, reproduced here, Fig. 2n), because the Staudinger illustration also shows 7 orange bands.

Collaboration among researchers from five countries has resulted in rearing and recognition of a new species of *Oxyntera* that differs from its sister species, *O. hopfferi*, in body pattern and perhaps in number of antennal nudum segments and in male genitalia. Barcode differences (2.6% to 3.2%) clearly separate the two species. The male and female associations made through rearing and DNA sequencing of *O. stangelandi*, and similar sexual dimorphism in the facies of both *O. stangelandi* and *O. hopfferi*, indirectly support the synonymy of *O. annulatus* (described from a female) with *O. hopfferi* (described from a male).

#### ACRONYMS OF MUSEUMS

BMNH The Natural History Museum, London, England  
 INBio Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica  
 MTD Senckenberg Museum für Tierkunde, Dresden, Germany  
 OM-DZUP O. H. H. Mielke, Curitiba, Paraná, Brazil, together with the collection of Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil  
 USNM National Museum of Natural History, Smithsonian Institution, Washington, DC, USA  
 ZMHB Zoologisches Museum, Humboldt Universität, Berlin, Germany

#### ACKNOWLEDGEMENTS

We are grateful to Mike Stangeland for bringing phenotypic differences of the new species to our attention; Blanca Huertas and David Lees (BMNH), Wolfram Mey (ZMHB), and Matthias Nuss (MTD) for providing unrestricted access to specimens under their care and help in the collections; Gerardo Lamas and Bernard Hermier for sharing images of *Oxyntera* type specimens; Kim Garwood and Isidro A. Chacón for providing photographs of *Oxyntera hopfferi* specimens; Ernst Brockmann for barcode sequences of *Oxyntera confusa* and *Olafia roscius*; Donald Harvey for dissecting genitalia; Karie Darrow for photographing genitalia of four *Oxyntera* species, plus adults of the two South American species; Zobeida Fuentes and ACG parataxonomists Harry Ramirez, Mariano Pereira, Dunia Garcia, Manuel Pereira, and Freddy Quesada for finding and rearing *Oxyntera* caterpillars and for identifying their foodplant; Isidro A. Chacón for a specimen of *O. hopfferi*; many of the above as well as Andy Warren for discussing various aspects of this study; and two anonymous reviewers for helpful comments. The study has been supported by U.S. National Science Foundation grants BSR 9024770 and DEB 9306296, 9400829, 9705072, 0072730, 0515699, and grants from the Wege Foundation, International Conservation Fund of Canada, Jessie B. Cox Charitable Trust, Blue Moon Fund, Guanacaste Dry Forest Conservation Fund, Area de Conservacion Guanacaste, and University of Pennsylvania (DHJ); by the Government of Canada through Genome Canada and the Ontario Genomics Institute, and by NSERC Canada (MH).

## LITERATURE CITED

- BURNS, J. M. & D. H. JANZEN. 2001. Biodiversity of pyrrhopygine skipper butterflies (Hesperiidae) in the Area de Conservación Guanacaste, Costa Rica. *J. Lepid. Soc.* 55:15–43.
- BURNS, J. M., D. H. JANZEN, M. HAJIBABAEI, W. HALLWACHS & P. D. N. HEBERT. 2007. DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper butterflies (Hesperiidae) can differ by only one to three nucleotides. *J. Lepid. Soc.* 61:138–153.
- . 2008. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. *Proc. Natl. Acad. Sci. USA* 105:6350–6355. doi:10.1073/pnas.0712181105
- DRAUDT, M. 1921. Family: Hesperidae, Skippers, pp. 836–1011, pls. 160–193. *In* Seitz, A. (ed.), *The Macrolepidoptera of the world*, vol. 5, *The American Rhopalocera*. Alfred Kernen Verlag, Stuttgart.
- EVANS, W. H. 1951. A catalogue of the American Hesperioidea indicating the classification and nomenclature adopted in the British Museum (Natural History). Part I. Introduction and Group A, Pyrrhopyginae. *British Museum (Natural History)*, London. x + 92 pp., pls. 1–9.
- GODMAN, F. D. & O. SALVIN. 1879–1901. *Biologia Centrali-Americana. Insecta. Lepidoptera-Rhopalocera*. Vol. 2, 782 pp. Vol. 3, 113 pls.
- JANZEN, D. H. & W. HALLWACHS. 2012. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservación Guanacaste (ACG), northwestern Costa Rica <<http://janzen.sas.upenn.edu>>.
- JANZEN, D. H., W. HALLWACHS, P. BLANDIN, J. M. BURNS, J. CADIOU, I. CHACON, T. DAPKEY, A. R. DEANS, M. E. EPSTEIN, B. ESPINOZA, J. G. FRANCLEMONT, W. A. HABER, M. HAJIBABAEI, J. P. W. HALL, P. D. N. HEBERT, I. D. GAULD, D. J. HARVEY, A. HAUSMANN, I. KITCHING, D. LAFONTAINE, J. LANDRY, C. LEMAIRE, J. Y. MILLER, J. S. MILLER, L. MILLER, S. E. MILLER, J. MONTERO, E. MUNROE, S. RAB GREEN, S. RATNASINGHAM, J. E. RAWLINS, R. K. ROBBINS, J. J. RODRIGUEZ, R. ROUGERIE, M. J. SHARKEY, M. A. SMITH, M. A. SOLIS, J. B. SULLIVAN, P. THIAUCOURT, D. B. WAHL, S. J. WELLER, J. B. WHITFIELD, K. R. WILLMOTT, D. M. WOOD, N. E. WOODLEY & J. J. WILSON. 2009. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecol. Resources* 9 (Supplement 1):1–26. doi:10.1111/j.1755-0998.2009.02628.x
- JANZEN, D. H., W. HALLWACHS & J. M. BURNS. 2010. A tropical horde of counterfeit predator eyes. *Proc. Natl. Acad. Sci. USA* 107:11659–11665. doi:10.1073/pnas.0912122107
- JANZEN, D. H., W. HALLWACHS, J. M. BURNS, M. HAJIBABAEI, C. BERTRAND & P. D. N. HEBERT. 2011. Reading the complex skipper butterfly fauna of one tropical place. *PLoS ONE* 6(8): e19874. doi:10.1371/journal.pone.0019874
- MIELKE, O. H. H. 2005. Catalogue of the American Hesperioidea: Hesperioidea (Lepidoptera). Vol. 1. Complementary and supplementary parts to the checklist of the neotropical region. Hesperioidea: Hesperioidea: Pyrrhopyginae. *Sociedade Brasileira de Zoologia, Curitiba, Paraná, Brazil*. XIII + 125 pp.
- ORELLANA, A. 2008. Pyrrhopyginae de Venezuela (Lepidoptera: Hesperioidea: Hesperioidea). *Entomotropica* 23:177–291.
- STAUDINGER, O. 1888. I. Theil. Exotische Tagfalter in Systematischer Reihenfolge mit Berücksichtigung neuer Arten. *In* Staudinger, O. & E. Schatz, 1884–1892, *Exotische Schmetterlinge*. Fürth, G. Löwensohn. (21): 1–2, 271–333 + [10] pp., pls. 99–100.
- WARREN, A. D., K. J. DAVIS, E. M. STANGELAND, J. P. PELHAM & N. V. GRISHIN. 2012. Illustrated lists of American Butterflies. [17–VIII–12] <<http://www.butterfliesofamerica.com>>

*Received for publication 18 May 2012; revised and accepted 24 August 2012.*



NATURAL HISTORY AND CONSERVATION STATUS OF THE ENDANGERED MITCHELL'S SATYR BUTTERFLY: SYNTHESIS AND EXPANSION OF OUR KNOWLEDGE REGARDING *NEONYMPHA MITCHELLII MITCHELLII* FRENCH 1889

CHRISTOPHER A. HAMM

Michigan State University, Department of Entomology, Program in Ecology, Evolutionary Biology and Behavior, 204 Center for Integrated Plant Systems East Lansing, Michigan 48864 USA email: chamm@msu.edu, Present address: Department of Evolution and Ecology, University of California at Davis, One Shields Avenue, Davis, California 95616 Corresponding author, email: cahamm@ucdavis.edu

BARRY L. WILLIAMS

Michigan State University, Department of Zoology, Program in Ecology, Evolutionary Biology and Behavior, 41 Giltner Hall, East Lansing, Michigan 48864 USA

AND

DOUGLAS A. LANDIS

Michigan State University, Department of Entomology, Program in Ecology, Evolutionary Biology and Behavior, 204 Center for Integrated Plant Systems East Lansing, Michigan 48864 USA

**ABSTRACT.** The Mitchell's satyr butterfly, *Neonympha mitchellii mitchellii* French 1889 is a federally-listed endangered species found in parts of the eastern United States of America. Because of its endangered status, considerable research efforts have been devoted to understanding its biology, ecology, and its conservation. Despite these efforts, information about *N. m. mitchellii* has not been summarized for more than a decade. Here we summarize and expand upon the work conducted by governmental and not-for-profit agencies that have produced reports that are not easily accessible to researchers or interested lepidopterists. In addition to summarizing the literature, we present data from feeding trials and also demonstrate that microclimates exist that may be exploited by larvae. We conclude by identifying key areas of needed research and describe steps imperative to the recovery the Mitchell's satyr.

**Additional key words:** endangered species, Satyrinae, Lepidoptera

The Mitchell's satyr butterfly, *Neonympha mitchellii mitchellii* French 1889 (Nymphalidae: Satyrinae) is a federally-listed endangered species found in parts of the eastern United States of America. As one of only 60 endangered insects, and one of only 20 federally endangered Lepidoptera in the US, it is of particular interest to conservation organizations and butterfly enthusiasts alike (US Fish and Wildlife Service 2011). As a result, a considerable amount of research effort has been devoted to understanding its biology and ecology, as well as studies that inform its conservation. Adding to the uniqueness of *N. m. mitchellii* as a target of conservation, is the fact that its sister taxon, the Saint Francis' satyr, *N. m. francisi*, is also endangered in the US. Morphologically similar to Mitchell's satyr, *N. m. francisi* is known from only one small region of North Carolina on the Fort Bragg Military Reservation. While the Saint Francis Satyr has recently received a thorough treatment of its natural history and population biology by Kuefler et al. (2008), information about *N. m. mitchellii* has not been summarized for more than a decade (Shuey 1997). In that time, considerable new findings have emerged that fundamentally shift our understanding of

the species and the prospects for its long-term conservation.

One key discovery that has changed the way we perceive Mitchell's Satyr is the discovery of new populations that greatly expand its known geographic range and habitat use patterns. Discovered in Virginia, Alabama and Mississippi in 1998–2004 (Roble et al. 2001, Hart 2004), these new populations are designated as *N. mitchellii* but are extended the same federally endangered status of *N. m. mitchellii* populations found in Michigan and Indiana [hereafter we use *N. mitchellii* to refer to the species in the broad sense, i.e. including both the northern, and southern populations but excluding the Saint Francis' satyr, and *N. m. mitchellii* to refer specifically to the protected northern populations]. These new populations offer unique opportunities to explore the natural history of the Mitchell's satyr throughout a larger portion of its historic range and to conduct new investigations that may inform its conservation more broadly. While the new populations may increase options for recovery, within Michigan and Indiana there is evidence that *N. m. mitchellii* populations are being lost at an alarming rate,

emphasizing the pressing need for effective recovery plans based on sound conservation science (Landis et al. 2011).

Given its protected status, governmental and not-for-profit conservation organizations have spent thousands of person hours investigating aspects of *N. mitchellii* biology. Unfortunately, many of the reports generated by these efforts enter the so-called “grey literature,” and are not easily accessible to academic researchers or lepidopterists. During many conversations with both researchers and butterfly enthusiasts it has become clear that much confusion surrounds the biology of Mitchell’s satyr, sometimes with little distinction between lore and published data. As such, one goal of this paper is to synthesize and update the literature surrounding *N. mitchellii*. In addition, by pointing to critical gaps in our knowledge, we hope to prioritize future research needs for effective conservation of this endangered species.

**Physical Description.** The following physical descriptions represent those typical of *N. mitchellii* and are not absolute descriptions. All traits are variable and when there are major deviations from the typical forms they are noted as such. The eggs of *N. mitchellii* appear light to pale lime green (see McAlpine et al. 1960 for detailed line drawings of all immature stages) with their color imparted by the developing embryo, since the chorion itself is transparent (C. Hamm pers. obs). The egg is spherical in shape with a diameter between 0.7 and 1.0 mm and covered with an alveolate sculpturing (Harris 1979). Within two days before hatching, the developing head capsule is visible as a dark spot within the egg (McAlpine et al. 1960, Legge & Rabe 1996, C. Hamm pers. obs.).

First instar larvae have a conspicuous dark brown head capsule and bilobed projections that are common to satyrine larvae (Wagner 2005). First instars range in length from 3 to 4 mm (McAlpine et al. 1960, Szymanski 1999) and are cylindrical in shape, with the tip of the abdomen terminating abruptly. All subsequent instars (total of 5) have a green head capsule and retain the bilobed shape, with the abdomen terminating in a bifurcated process. These later instars, which are 6–12 mm in length, also possess two raised white ridges on the dorsum that traverse the antero-posterior axis from the prothoracic segment to the tip of the abdomen. Additionally, later instars are covered with irregular white papillae. Larvae are cryptic and extremely difficult to locate in the field (Darlow 2000). Observations on the size of *N. mitchellii* larvae may be upwardly biased since they were based on individuals reared in captivity under conditions that may not approximate those in nature (McAlpine et al. 1960, Wilsman & Schweitzer 1991,

Legge & Rabe 1996, Darlow 2000; B. Bergman pers. comm., M. Nielsen pers. comm.). The pale green chrysalis is suspended from the cremaster in the head down orientation typical of many satyrine butterflies (Mosher 1916, DeVries 1987), and is between 10 and 15 mm in length. As with the egg, it is the developing imago that imparts color to the pupa, the actual integument being translucent and smoky in color (McAlpine et al. 1960, C. Hamm pers. comm.). Approximately 48 hrs prior to eclosion the chrysalis begins to transition its color from light green to medium brown.

The adult Mitchell’s satyr butterfly was described based on a series of six males and four females collected by J.N. Mitchell, a professor at the University of Michigan (French 1889). The type series was collected in Cass County, Michigan from an “upland dry meadow,” but these butterflies likely originated from the nearby prairie fen (French 1889, McAlpine et al. 1960, Shuey 1997). Over the years *Neonympha* Hübner has been synonymized into *Euptychia* Hübner and *Cissia* Doubleday, but is currently recognized as valid (Dyer 1902, Hemming 1937, Lewis 1974, Hamm 2007, Pelham 2008).

Imagos of *N. mitchellii* are medium-sized brown butterflies that resemble many of the other members of the Satyrinae. Male *N. mitchellii* have a wingspan of roughly 2.5 cm while females are larger, with a wingspan of approximately 3.0 cm (Hamm et al. 2010). The Mitchell’s satyr was originally described with a medium brown dorsal wing surface and lighter brown ventral wing surface, with females darker than males (French 1889). Subsequent research has noted that both sexes are darker when they first emerge from the chrysalis and may even have a ‘sheen’ to them, which wears off within hours of eclosion (Barton & Bach 2005). In addition, *N. mitchellii* color appears to vary throughout its range, may be polyphenic (from tan to a dark brown) and associated with the hydrology of sites (Brakefield 1996, Hamm 2009). We have observed that, in general, sites with high levels of water are associated with darker butterflies (Hamm 2009), although this observation remains to be quantified. Similar observations have been made for other butterflies, including satyrs (Brakefield 1996). Color polyphenism is thought to provide an advantage by correlating the color of the butterfly more closely with its habitat (Brakefield 1996). High water levels support more lush plant growth, against which a light colored butterfly would stand out. By being darker when there are higher levels of ground water, the butterfly is presumably able to blend in more effectively. Adult *N. m. mitchellii* are rather short lived, with the average male living between two to five days



FIG. 1. Dorsal (left) and ventral (right) wing pattern from the right wing of a male *N. mitchellii* specimen collected at the Kellogg Biological Station in 1953. This population was extirpated in the 1960s.

and the average female two to four days (Szymanski et al. 2004).

One of the most conspicuous characters noted in the descriptions of *N. mitchellii* is the prominent border ocelli on the ventral surface of the wings. Border ocelli, sometimes mistakenly referred to as eye spots (Nijhout 1991), are situated in cells between wing veins in the postmedial area of the wings (Fig 1). Females have the same number of border ocelli as males but they tend to be larger (C. Hamm unpub. data). On the forewing, border ocelli may be found in the cells  $M_1$ ,  $M_2$ ,  $M_3$  and  $Cu_1$ , and on the hindwing the border ocelli may be found in the cells  $R_5$ ,  $M_1$ ,  $M_2$ ,  $M_3$ ,  $Cu_1$  and  $Cu_2$ . Based on preliminary data from over 300 museum specimens, each *N. mitchellii* male forewing usually has three and each hindwing has six ocelli (Fig. 1).

Each ocellus appears as two concentric rings of pigment, with an outer ring of buff yellow and an inner ring of black, centered on a silver focus (Fig. 1). In contrast to the original description, which holds that all border ocelli are circular (French 1889), ocelli actually range in shape from circular to oval (C. Hamm unpub. data). A pair of bands surrounding the border ocelli often converge at the leading and trailing edges of the wings. These bands correspond to the proximal and distal bands of the central symmetry system (Nijhout 1991) and range in color from light orange to brown. The thorax and walking legs of *N. mitchellii* are densely covered with setae and scales similar in color to that of the wings, though the setae projecting off of the prolegs are often a dark brown.

**Distribution.** Our understanding of the range of Mitchell's satyr has continued to evolve over time. After French's description was published, the Mitchell's satyr

was subsequently found in fens throughout the Battle Creek-Kalamazoo and Jackson glacial interlobate regions (areas where ice sheets were in contact) of Michigan (Fig 2) (Wolcott 1893, Siepmann 1936, Moore 1939; 1960, Landis et al. 2011). The influential Butterfly Book (Holland 1898) also noted the Mitchell's satyr in Morris and/or Sussex Counties of northern New Jersey. The Mitchell's satyr was next confirmed in Portage County in eastern Ohio (Pallister 1927) and LaGrange County of northern Indiana (Badger 1958).

Several subsequent reports of *N. mitchellii* have been called into question for various reasons. One such report is that of *N. mitchellii* from Anne Arundel County, Maryland. During World War II two brothers collected a butterfly from a "military marsh" in the vicinity of Fort Meade and shipped the specimens home, where they were subsequently lost (Opler & Malikul 1998, P. Opler pers. comm.). The lack of a voucher specimen should warrant skepticism, but in this case some authors are convinced that the sighting was accurate (P. Opler pers. comm.). Arnett (2000) referenced Mitchell's satyr from Pennsylvania but no details were given beyond the state level reference. Rutkowski (1966) stated that it was highly likely the butterfly existed in Pennsylvania and he encouraged lepidopterists to search for it, no specimen of Mitchell's satyr from Pennsylvania is known to exist.

In 1983, a single population of butterflies, which appeared phenotypically similar to the Mitchell's satyr, was discovered on the Fort Bragg Military Reservation in North Carolina. Further exploration uncovered a



FIG. 2. Map highlighting the locations of *N. mitchellii* including both extant and extirpated populations. Extirpated populations are found in Wisconsin, Ohio, and New Jersey.



number of additional occupied sites, all were restricted to Fort Bragg (Parshall & Kral 1989; Kuefler et al. 2008). Citing phenotypic differences, such as the shape of the male valvae, and ecological differences, such as voltinism (these populations are bi-voltine), the *N. mitchellii* in North Carolina were described as a new sub-species, *Neonympha mitchellii francisi*, the Saint Francis' satyr (Parshall & Kral 1989).

In 1998, during a 4th of July Butterfly Count, observers discovered a population of what appeared to be *N. mitchellii* in Floyd County, Virginia, approximately 200 km from Fort Bragg, North Carolina (Roble et al. 2001). Subsequent searching revealed additional sites that harbored *N. mitchellii* populations within Virginia, although only within Floyd County. In June of 2000, a population of *N. mitchellii* was discovered in the Oakmulgee Ranger District of the Talladega National Forest in central Alabama (Glassberg 2000; 2001). Since this discovery, researchers have identified approximately 20 sites within the Oakmulgee Ranger district as well as sites along the Natchez Trace Parkway in northeastern Mississippi that contained *N. mitchellii* (Hart 2004, Hamm 2008). As noted earlier, the recently discovered populations (Virginia, Alabama and Mississippi) are treated as *N. mitchellii* and not as either the Mitchell's (*N. m. mitchellii*) or the Saint Francis' satyr (*N. m. francisi*), hence they have no subspecies designation. Research is underway to determine the taxonomic status of these recently discovered populations.

A number of *N. m. mitchellii* populations have apparently been extirpated leading to the elimination of the species in parts of its former range. The Mitchell's satyr was extirpated from Ohio sometime in the 1950's and it was last seen in New Jersey in 1988 (Shuey 1997, Hamm 2008). High collecting pressure has been implicated in the extirpation of at least one New Jersey population due to a collector returning daily over successive seasons to the site (Glassberg 1999). While examining the Strecker collection in the Field Museum of Natural History, a part of the entomology collection not accessioned with the rest of the material, the first author found *N. mitchellii* with collection labels indicating they were taken from Wisconsin. These specimens were donated by E.T. Owen, who removed Strecker's original labels and replaced them with his own (J. Boone pers. comm.); any date or locality information have apparently been lost, though southeastern Wisconsin has a number of the prairie fens that may provide suitable habitat. We are unaware of any surveys in Wisconsin that have searched for *N. m. mitchellii*, but we suspect that it may be extirpated from Wisconsin and the surrounding region.

**Habitats.** With the discovery of these new populations of Mitchell's satyr, our understanding of its habitat usage patterns has also expanded. *Neonympha mitchellii* was first described from specimens collected near a "bog" (French 1889), although we now know that this habitat was a prairie fen (Speiles et al. 1999, Kost et al. 2007). Prairie fens are groundwater fed, sedge-dominated wetlands, whereas a "bog" is a basin that has no net outflow of water (Pielou 1991). Conditions leading to the formation of fens were a result of the Pleistocene glaciation (Pielou 1991) and prairie fens are concentrated in the interlobate regions of the Laurentide ice sheet (Landis et al. 2011). All *N. m. mitchellii* sites in Michigan and Indiana were subsequently determined to be prairie fens (Shuey 1997). Previous workers have suggested that these wetlands provide microhabitat which allows Mitchell's satyr to escape the high heat that characterizes these sites during the summer (Darlow 2000). Indeed, recent evidence suggests that there are significant differences between the ground level and air temperatures (Fig 3; C. Hamm unpub. data). During the winter (Fig. 3a) the ground is significantly warmer than the air (t-test,  $P < 0.01$ ), likely due to insolation of the sedge tussocks. In the early spring there is no significant difference

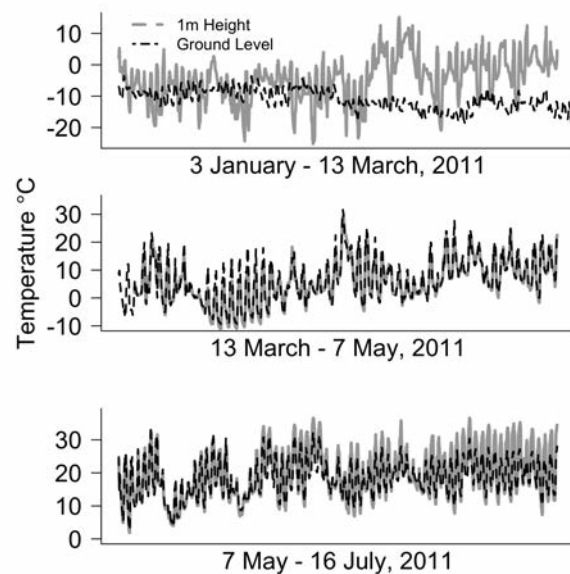


FIG. 3. Temperature data depicting ground level and air temperature in a Michigan prairie fen. A: during the winter (top plot) the ground (black dotted line) was significantly colder than the air (grey dotted line) (t-test,  $P < 0.01$ ). B: during the spring (middle plot) the temperatures were not significantly different (t-test,  $P = 0.28$ ). C: during the early summer (bottom plot) the air was significantly warmer than the ground (t-test,  $P < 0.01$ ).

between the ground and air temperatures Fig. 3b) (t-test,  $P = 0.28$ ), while during the early summer the ground is significantly cooler than the air Fig. 3c) (t-test,  $P < 0.01$ ).

*Neonympha mitchellii* populations located south of the glacial maximum are not found in prairie fens, but rather in other sedge dominated wetlands such as the edges of beaver ponds and groundwater seepage slopes (Roble 2001, Hart 2004). Sites with *N. mitchellii* in Alabama and Mississippi tend to occur on the periphery of beaver ponds, on the edge of pocosin swamps, in areas where roads culvert create a buildup of water, or in proximity to seepage slopes (Hart 2004). In these habitats, it appears that hydrological disturbance creates the necessary conditions for a sedge-wetland to exist, if even for a short time (Hart 2004, Bartel 2010). The Alabama and Mississippi sites were initially surveyed for *N. mitchellii* in 2002 and 2003, during which time sites on the periphery of beaver ponds had high numbers of *N. mitchellii* (Hart 2004). However, when revisited in 2008 and 2009 *N. mitchellii* was absent from all such sites (Hamm 2008, Hamm and Hart, unpub. data). After the beavers abscond the pond filled with silt, which allowed shrubs to encroach on the banks, and left few sedges (Hamm 2008). Immediately upstream from these ponds (approx. 800 m) were recently constructed beaver ponds (approx. 2–3 y.o.; C. Ragland pers. comm.) that had a high number of *N. mitchellii*. This scenario of site loss and colonization was observed at five sites in Alabama and one site in Mississippi (C. Hamm and B. Hart; pers. obs.). This pattern fits into the metapopulation model of Hanski (1994) and suggests that, in Alabama and Mississippi, *N. mitchellii* historically existed in a metapopulation structure with regular movement along riparian corridors. This pattern of utilizing temporally available habitats has also been suggested as the population structure that describes Saint Francis' satyr populations (Hall 1993, Shuey 1997, Kuefler et al. 2008, Barel et al. 2010).

A number of Alabama sites were also found on the edges of seepage slopes or along the margins of impoundments created by road culverts (Hart 2004). Unlike the populations associated with beaver ponds, these sites had maintained *N. mitchellii* populations when surveyed six and seven years later (Hamm 2008; Hamm and Hart unpub. data). It appears that these sites avoid shrub encroachment through hydrological disturbance, though again, we have only observational data to support these postulations. The culverts and seepage slopes were imbedded within a matrix of fire dependent habitat, often a considerable (3 km) distance from the nearest actively populated beaver pond, which indicates the possibility that these sites may experience

other forms of disturbance. Sites with *N. mitchellii* in Virginia are all found in close proximity to groundwater seepage slopes. These sites are very open compared with other *N. mitchellii* sites and are often used as pasture for cattle and other livestock (Roble et al. 2001). Management of the sites for cattle (i.e. the removal of shrubs and prevention of overgrazing) appears to simultaneously manage for *N. mitchellii* as these sites had high population density estimates (C. Hamm, unpub. data).

While there are many apparent differences among sites with *N. mitchellii* there are a number of commonalities that unite these habitats. All habitats, whether beaver pond, seepage slope, pasture or prairie fen, are sedge-dominated, early successional wetlands. Another commonality is that changes in hydrology and shrub encroachment are commonly associated with population extinctions, although the process by which this occurs remains unclear. The postglacial radiation of sedge wetlands northward from what is now the southern US following the Pleistocene glaciation provides a plausible explanation for the current distribution of *N. mitchellii* (Landis et al. 2011). Initially postulated based on distribution maps (Shapiro 1970; 1977, Metzler et al.) researchers have only recently begun to test these hypotheses (Emerson et al. 2010).

**Vagility and Dispersal.** *Neonympha mitchellii* exhibits low vagility relative to many other butterflies. As has commonly been reported for most Satyrinae, *N. mitchellii* has a low and jerky flight with an up and down bobbing motion for each wingbeat (Scott 1986). Males tend to fly through the habitat (between sedges and grasses) rather than over it and they generally fly below the height of the dominant vegetation, perhaps to avoid predators (see below). Individual male flights are short, lasting an average of ten seconds (range: 1 sec to 1 min) (Sferra & Aguiar 1993). Female flight is even shorter, averaging five seconds though this distribution is extremely skewed (range: 1 sec to 19 min). When ovipositing, females approach potential sites and hover a few seconds before alighting (Sferra & Aguiar 1993). Males appear to spend the majority of time (~70%) patrolling, whereas females spend much of their time resting (~60%) early in the flight period, but later females spent 70% of their time flying in search of oviposition sites (Sferra & Aguiar 1993, Barton & Bach 2005).

As with most butterflies, male *N. m. mitchellii* fly with high frequency they appear to have small home ranges (Brussard et al. 1974). After examining two sites in southwest Michigan, Szymanski et al. (2004) reported that *N. m. mitchellii* did not disperse long distances. The mean daily distance moved for males was 18 m and

for females was 11 m (Szymanski et al. 2004). Concordant with these observations, the mean minimum home range for the butterflies were small, with males occupying ~0.04 ha and females occupying ~0.01 ha (Szymanski et al. 2004). However, the sites where these data were recorded were relatively small (2.3 ha and 1.6 ha) and suffered from shrub encroachment, which may have biased the estimates. The size of surveyed habitats can produce a downward bias because habitat size may constrain movement. Using similar protocols at a larger site (12 ha), Barton & Bach (2005) reported larger home ranges for males (0.22 ha) and females (0.07 ha) and higher means for the daily distance moved (males: 35 m; females: 33 m). Overall, the data from both Szymanski et al. (2004) and Barton & Bach (2005) suggest that *N. m. mitchellii* does not disperse very far and thus falls into the sedentary mobility class of Pollard & Yates (1994). Sedentary butterflies are categorized by a movement rate between 10 and 200 m per day with colonization occurring up to 1 km away from natal habitat (Thomas 2000). At present there are no data on the vagility of *N. mitchellii* populations in Virginia, Alabama and Mississippi and studies are needed to examine vagility among these populations.

Individual dispersal events for individual *N. mitchellii* are not well characterized. Habitat corridors of 200 m and 400 m length have been created to connect prairie fens at two sites in Michigan and *N. m. mitchellii* have been observed in both. However, without mark-release-recapture (MRR) studies it is not clear if these individuals were transiting or were resident in the corridor. The longest distance recorded between subsequent captures in MRR studies was recorded by Barton & Bach (2005) and was 510 m for a male and 344 m for a female. A male in Virginia was observed at two different sites along a creek that were 1 km apart (S. Roble, pers. comm.), and this stands as the longest recorded distance for *N. mitchellii* dispersal.

**Population Structure.** The population structure of Mitchell's satyr is influenced by habitat isolation, flight phenology and within-habitat spatial preferences. In Michigan and Indiana today, prairie fens are typically highly isolated from one another and there is no evidence for *N. m. mitchellii* dispersal among them. In contrast, analysis of historic data on the distribution of prairie fens indicated that these habitats may once have been contiguous along geologic formations and would have allowed for increased dispersal among sites (MacKinnon & Albert 1996, Landis et al. 2011), but see Andreas (1985) and Swinehart & Parker (2002). Mitchell's satyr occupied sites in Virginia, Alabama, and Mississippi are typically much closer together, and

dispersal among sites in these states has been observed (Roble 2002; 2003, Hart 2004, Hamm unpub. data).

Flight phenology and patterns of within-patch habitat preference may also contribute to population structure. Overall, *N. mitchellii* is protandrous with males emerging one to two days before the first female. As a result, during the first week of flight the sex ratio is male biased, after which there is a three to four day period of approximately equal sex ratio followed by a female biased sex ratio as the flight progresses (Barton & Bach 2005). This pattern of shifting sex ratios within the flight season is often used to infer the progress of the flight period. The detection probability of males is generally higher than that of females and is probably due to patrolling behavior making males more conspicuous (Szymanski et al. 2004, Barton & Bach 2005). Within sites, *N. m. mitchellii* are not uniformly distributed throughout the available habitat but the location of these aggregations fluctuates from year to year (Szymanski et al. 2004). For an as yet unknown reason, *N. m. mitchellii* are often found near habitat margins, especially at the interface of prairie fen and upland areas (Barton & Bach 2005, Hamm unpub. data).

Several techniques have been used to attempt to estimate the population size of *N. m. mitchellii*. In 1997, Pollard walks were conducted at three sites in southern central Michigan, but the data generated from these walks were not analyzed and may not have had enough samples to generate parameter estimates (Summerville & Clappitt 1997). Mark release recapture (MRR) methods have also been used in several instances. In all cases, the pattern of *N. mitchellii* adult distribution within habitats is complex, which complicates population size estimates. For two sites in southwestern Michigan, Szymanski et al. (2004) used MRR techniques to estimate population size in 1997 and 1998. They found that each site contained no more than 80 *N. m. mitchellii* per day and had a total population of no more than 380 individuals. MRR studies were also conducted at one site in southern central Michigan during the 2003, 2005, 2007 flight periods of *N. m. mitchellii* (Barton 2008). During the 2003 survey, the maximum daily population estimate was approximately 1100 individuals and was approximately 3000 during 2007. That population estimates varied a great deal from year to year is indicative of the stochasticity inherent with insect populations (Hamm 2013, Brown & Boyce 1998). In addition, short-lived study organisms complicate the use of MRR based methods for population estimation and may have influenced the results. In Michigan, the total population of Mitchell's satyr is informally estimated to be less than 10,000 individuals (Barton & Bach 2005; D. Cuthrell and D.



Hyde pers. comm.) but the uncertainty around this estimate reveals the need for standardized methods to more accurately assess the size of Mitchell's satyr populations (Hamm 2013).

Population size estimates are also poorly known for the southern populations of *N. mitchellii*. Sites with *N. mitchellii* outside of Michigan and Indiana have only been the focus of attention since 2000 (Roble et al. 2001, Hart 2004) with approximately 20 known *N. mitchellii* sites in Virginia, 15 in Alabama, and four in Mississippi. The estimates of total population size have been compiled from governmental reports, biological surveys, and our own experience. No statewide survey has been conducted on Mitchell's satyr or *N. mitchellii* in Virginia, Alabama or Mississippi (but see Haddad et al. 2008). Estimates of the total population size of Virginia *N. mitchellii* are roughly 8,000 individuals (Roble 2005). Researchers in Virginia have irregularly visited sites with *N. mitchellii* in Alabama and Mississippi since Hart (2004), but in that time many of these sites have become overgrown by shrubs since they were first surveyed and no butterflies have subsequently been observed (Hamm 2008). At least 15 sites are extant in the Talladega National Forest and are estimated to contain 1,500 individuals total (B. Hart pers. comm.). The three sites in the Natchez Trace Parkway of Mississippi have been surveyed for *N. mitchellii*, and were only found at one of these sites (the others having been overgrown by shrubs). We estimate fewer than 100 individuals occur at this occupied site (C. Hamm and B. Hart pers. obs.). Recently, additional populated sites have been discovered in the same area of Mississippi and there are unconfirmed reports of additional sites in Alabama (S. Surette and P. Hartfield pers. comm., Turner 2007).

The populations of *N. mitchellii* in Alabama and Mississippi are bivoltine. The first flight begins in early June and the second flight in mid August, and all flights last approximately three weeks. In contrast, all populations of *N. m. mitchellii* are univoltine and begin flying in late June in Michigan and Indiana. The *N. mitchellii* in Virginia are also univoltine and their flight begins in late July. Voltinism in *N. mitchellii* appears to be controlled by accumulated degree-days as a second generation can be induced in Michigan and Indiana populations by rearing them at higher temperatures (Shuey 1997, P. Tolson & C. Ellsworth pers. comm., C. Hamm unpub. data). Similarly, a single generation can be induced in Alabama populations of *N. mitchellii* by rearing them under cool conditions (C. Hamm unpub. data).

**Host Plants.** A variety of host plants have been associated with the Mitchell's satyr, but there are

surprisingly few records of observed larval feeding. Based largely on the work of McAlpine et al. (1960) the sedge *Carex stricta* Lam. (Cyperaceae) was assumed to be the host of *N. mitchellii* because it was found at all Michigan and Indiana sites. Further observations, in both the field and artificial conditions, have demonstrated that *N. mitchellii* feeds on Cyperaceae and some graminoids as well (Table 1). It also appears that *N. mitchellii* rarely oviposits onto its sedge host plants (Table 2). One common observation among all oviposition reports is that female *N. mitchellii* generally deposit eggs near to ground level (Hyde et al. 2000; Darlow et al. 2000). We have observed eggs that were deposited singly and in groups of up to six (C. Hamm, unpub. data), which contradicts the commonly accepted theme that satyrs only lay eggs singly (Opler & Krizek 1984).

To address questions of host plant specialization an experiment was conducted using sedges from different regions of the *N. mitchellii* distribution. *Carex mitchelliana* M.A. Curtis, *C. lurida* Wahlenb., and *C. stricta* were collected from North Carolina, Alabama, and Michigan, respectively. These species were selected because they were endogenous to one or two sites but not present at all three. After collection, plants were grown in a 90:10 mixture Fafard 3B soilless potting medium (Conrad Fafard Inc., Agawam, MA) and calcined clay (Diamond Pro, Dallas, TX) in three quart pots. Sedges were initially grown under greenhouse conditions at Michigan State University and were watered *ad libitum* using a 19-4-23-2 Ca fertilizer (Greencare Fertilizers, Chicago, IL) with H<sub>2</sub>SO<sub>4</sub> added to counteract the high alkalinity of the well. Plants were then transferred to environmental growth chambers (Percival I-35LLVL) to simulate environmental conditions in Michigan and Alabama. Two females from Alabama and two from Michigan were collected for oviposition. The females were moved to 0.5 m<sup>3</sup> mesh cages with potted sedge (*C. lurida* for AL females and *C. stricta* for MI females) and allowed to oviposit for 48 hrs, each female laid 30–35 eggs. Eggs were then placed into treatment groups based on the experimental design outlined in Table 3.

Environmental conditions in growth chambers were set to simulate those encountered at *N. mitchellii* sites when the eggs were collected. Temperature, humidity and photoperiod were adjusted weekly based on data acquired from weather stations nearest the appropriate collection sites. Plants were placed in environmental chambers one week before the addition of *N. mitchellii* larvae. Once larvae were added, the plants were enclosed in mesh cages to prevent escape. Individuals were moved by hand to new plants as needed and

TABLE 1. Plant species fed upon by *Neonympha mitchellii* with literature reference and type of observation (field or artificial conditions).

Food plant	Family	Reference	Field
<i>Carex alopecoidea</i>	Cyperaceae	1	N
<i>Carex atlantica</i>	Cyperaceae	5	Y
<i>Carex cephalophora</i>	Cyperaceae	1	N
<i>Carex lasiocarpa</i>	Cyperaceae	2	Y
<i>Carex leptalea</i>	Cyperaceae	7	N
<i>Carex lurida</i>	Cyperaceae	6	Y
<i>Carex mitchellii</i>	Cyperaceae	8	N
<i>Carex stricta</i>	Cyperaceae	2, 3, 4, 5, 7, 9	Y
<i>Carex tetanica</i>	Cyperaceae	3, 7	Y
<i>Cyperus esculentus</i>	Cyperaceae	8	N
<i>Rhynchospora capillaceae</i>	Cyperaceae	7	N
<i>Scripus atrovirens</i>	Cyperaceae	1	N
<i>Poa pratensis</i>	Poaceae	8	N

References: <sup>1</sup>McAlpine et al. 1960; <sup>2</sup>Legge and Rabe 1996; <sup>3</sup>Szymanski and Shuey 2002; <sup>4</sup>Roble 2005 <sup>5</sup>Roble 2006; <sup>6</sup>Hart 2006; <sup>7</sup>Tolson 2008; <sup>8</sup>B. Bergman, unpub. data;

<sup>9</sup>Hamm, unpub. data.

TABLE 2. Plants on which *Neonympha mitchellii* oviposited, listed by family and reference (nomenclature follows Reznicek et al. 2011).

Plant species	Family	Reference
<i>Eupatorium maculatum</i>	Asteraceae	2
<i>Solidago spp.</i>	Asteraceae	2
<i>Symphotrichum ontarionis</i>	Asteraceae	1
<i>Carex bromoides</i>	Cyperaceae	3
<i>Scripus expansus</i>	Cyperaceae	4
<i>Juncus effusus</i>	Cyperaceae	3, 5
<i>Pycnanthemum virginianum</i>	Lamiaceae	1
<i>Thalictrum dasycarpum</i>	Ranunculaceae	1, 2
<i>Galium boreale</i>	Rubiaceae	1
<i>Thelypteris palustris</i>	Thelypteridaceae	2
<i>Viola nephrophylla</i>	Violaceae	1, 5

References: <sup>1</sup>Legge and Rabe 1996; <sup>2</sup>Darlow 2000; <sup>3</sup>Hart 2004; <sup>4</sup>Roble, 2005; <sup>5</sup>Hamm unpub. data.

TABLE 3. Experimental design for larval rearing experiment. All treatments began with 10 larvae, the data presented here indicate the number of survivors for each treatment. Treatments are listed by environmental conditions and the state of origin for *Carex* (L to R): *C. mitchelliana* (NC), *C. lurida* (AL), and *C. stricta* (MI).

		Environmental Conditions					
		Alabama Conditions Sedges from:			Michigan Conditions Sedges from:		
		NC	AL	MI	NC	AL	MI
Larvae from:	AL	5	4	4	5	5	3
	MI	3	5	4	4	4	5

TABLE 4. Observed predators of *Neonympha mitchellii*

Taxon	Common Name	Family	Notes
<i>Erythemis simplicicollis</i>	Eastern Pondhawk	Odonata :Libellulidae	Aerial predation
<i>Asilus sericeus</i>	Robber Fly	Diptera: Asilidae	Aerial predation
	Ambush bug	Hemiptera: Reduviidae	Nymph on <i>Rudbeckia</i>
<i>Formica</i> spp	Wood ant	Hymenoptera: Formicidae	Landed on aphid tended plant
<i>Bombycilla cedorum</i>	Cedar Waxwing	Aves: Bombycillidae	Attempted aerial predation
<i>Tyrannus tyrannus</i>	Eastern Kingbird	Aves: Tyrannidae	Aerial predation

mortality noted daily. The total number of survivors to pupation, by treatment, was noted. Survivors from Michigan grown under Alabama conditions were allowed to mate and produce a second generation while the remaining individuals were sampled for DNA extraction. Voucher specimens were deposited in the Albert J. Cook Arthropod collection at Michigan State University. Logistic regression was used to compare all survival against all two-way interactions in the statistical program R (R Core Development Team 2011) against a significance value of  $\alpha = 0.05$ .

Adults emerged after approximately 2000 degree days (base 50) accumulated. Michigan collected individuals reared under Alabama conditions went through a second generation after an additional 900 degree days accumulated post eclosion. The photoperiod in Alabama was shorter than that of Michigan, which suggests that photoperiod does not play a role in voltinism for *N. mitchellii*. Logistic regression revealed no difference among treatment for survival. Due to permitting restrictions, only two females were sampled. As a result, this experiment did not have high genotypic diversity among treatments. Lastly, this study did not quantify growth rates among treatments, though the final size of adults is not significantly different from other wild caught specimens (C. Hamm unpub. data). These results, while preliminary, indicate no difference in host plant performance, and serve as proof of concept that such rearing experiments can be successfully undertaken.

**Predators.** An eclectic group of predators has been observed to prey on Mitchell's satyr. In the course of various oviposition studies researchers have reported numerous accounts of larval predation by spiders (Arachnida: Araneae) (C. Ellesworth and B. Barton, pers. comm.). During an experiment to test the effects of fire on larval survival, a group of researchers collected gravid females and placed them in enclosures that covered *C. stricta* tussocks. The experiment was quickly abandoned due to high levels of predation by spiders (Barton 2008). Additionally, we have observed a number

of predators attack adult *N. mitchellii* (Table 4) in the course of research. When a male *N. mitchellii* patrols an area he tends to fly through the sedges rather than over them. All aerial predation events (birds and insects such as flies and dragonflies) we observed occurred when a male flew over sedges and was thus exposed.

**Other factors affecting conservation.** Effective conservation of *N. mitchellii* into the future depends on a combination of biological, ecological and social factors. For example, the taxonomic uncertainty of *N. mitchellii* in Virginia, Alabama, and Mississippi will impact the federal conservation status of *N. m. mitchellii* more broadly. Currently, these populations are not included in the endangered species listing but they are protected by other measures. The State of Virginia considers their populations of *N. mitchellii* to be endangered at the state level and many of the sites are protected by conservation easements (S. Roble, pers. comm.). Many of the *N. mitchellii* sites in Alabama and Mississippi are located on U.S. Forest Service and National Park Service lands, thus affording them some level of protection.

The future of the northern protected populations of *N. m. mitchellii* and prairie fens on which they depend is also uncertain (reviewed in Landis et al. 2011). Preliminary data based on the study of >100 sites suggest that the water feeding these sites may enter the aquifer many kilometers away from the fen decades ago (H. Abbas unpub. thesis). For example, the water coming out of the ground today may have entered the aquifer 50 years ago. We do not know the impact that contemporary levels of water consumption and groundwater extraction will have on the future of these sites. Field observations have noted that when fens dry out shrubs move in and as a result the biodiversity is apparently reduced (C Hamm pers. obs.).

The reproductive parasite *Wolbachia* in both *N. m. francisi* and *N. m. mitchellii* raises serious issues for conservation (Hamm et al. in review). *Wolbachia* is a common intracellular bacterium that is found in 20% of arthropods and 66% of insects (Hilgenboecker et al.



2008). This bacterial endosymbiont manipulates its host's reproduction to facilitate its own and can be of major importance for the management of insects (Nice et al. 2009). *Wolbachia* can feminize males, kill male embryos, induce parthenogenesis or, in its most common form, induces cytoplasmic incompatibility (Werren et al. 2008). Cytoplasmic incompatibility only results in successful mating between the same strains of *Wolbachia*, of which there are currently over 200 known strains (Baldo et al. 2006, Stahlhut et al. 2010). *Wolbachia* imparts a reproductive advantage to infected individuals and is spread through maternal transmission, so when a population becomes infected it will pass through a bottleneck until infection rates are high (Werren et al. 2008, Nice et al. 2009, Hamm et al. in review). While the identity of a strain may be deduced from molecular sequence data, the induced phenotype can only be determined by controlled breeding experiments. Demographic models suggest that if differently infected individuals are mixed the consequences for small populations will be catastrophic (Nice et al. 2009, Hamm et al. in review).

**Federal Actions.** The Mitchell's satyr was first petitioned for listing under the endangered species act in November of 1974 by a private citizen; however in May of 1975 the USFWS judged that listing was not warranted due to insufficient data (49 FR 2485). In 1984 the USFWS listed *N. mitchellii* within category 3C in their Animal Notice of Review (49 FR 21664), indicating that it was considered too abundant to be considered for protected status. However, in 1989, the USFWS upgraded the species to category 2 and thus made *N. mitchellii* a candidate for listing under the Endangered Species Act (ESA) (54 FR 554). In 1989 a new subspecies was recognized that altered the taxonomic status of *N. mitchellii*. The newly discovered Saint Francis' satyr was found on the Fort Bragg military reservation in North Carolina and given the trinomial *Neonympha mitchellii francisi* (Parshall & Krall 1989). With this split, the Mitchell's satyr became the nominate subspecies *Neonympha mitchellii mitchellii* (Parshall & Krall 1989).

A 1991 report issued to the USFWS described the rangewide status of *N. m. mitchellii* (Wilsmann & Schweitzer 1991). The authors noted that the Mitchell's satyr was once known from approximately 30 sites in four states (Michigan, Indiana, Ohio, and New Jersey) but at the time of the report, was known from only 15 sites in two states (Michigan and Indiana) (Wilsmann & Schweitzer 1991). This report recommended that the USFWS list *N. m. mitchellii* as endangered, which led to an emergency listing on 25 June 1991 (56 FR 28825). The emergency listing provided 240 days of protection

and on 11 September 1992, the USFWS formally proposed a rule to fully protect the Mitchell's satyr under the ESA (56 FR 46273). The final ruling that listed the Mitchell's satyr as an endangered species was published in May 1992 (57 FR 21564). Note that while the ESA considers a "species" to be any taxonomically recognized subspecies, this does not apply to insects (section 4.(15) of the ESA). Cited among the reasons that the Mitchell's satyr deserved protection were: destruction and modification of its habitat, overutilization for commercial purposes, inadequacy of existing regulatory mechanisms, and other man-made factors affecting its continued existence (i.e. habitat loss due to anthropogenic forces).

The Mitchell's satyr received additional attention in the early 1990's as preparations to extend the US-31 freeway in southern Michigan through a fen were being put into motion. The original 1981 Final Environmental Impact Statement (FEIS) identified Blue Creek fen as a site where the Mitchell's satyr was present, but a 1991 report by the Michigan Department of Transportation (MDOT) mistakenly reported that the site contained the *Lycaeides melissa samuelis*, the Karner Blue butterfly (Lepidoptera: Lycaenidae) and not the Mitchell's satyr (MDOT 1981; MDOT 2004). With the 1981 FEIS no longer accurate, the USFWS required MDOT to revise the path for the freeway. Negotiations between the USFWS and MDOT, ultimately resulted in the freeway being rerouted around the wetland complex and today an easement has been negotiated that allows biologists access to survey for the Mitchell's satyr.

In 1998 the Mitchell's satyr Recovery Team, a group of key stakeholders representing various state and federal governmental agencies and conservation organizations, submitted a recovery plan to the USFWS. This plan described the sites where extant and historical populations of *N. m. mitchellii* were found and noted that many of the original descriptions of those habitats were inaccurate (USFWS 1998). Additionally, the report outlined conservation measures that should be taken to aid in the recovery of the Mitchell's satyr. These included; range-wide surveys for the satyr, host plant identification and general study of the life history and ecology of the satyr, land acquisition and the development of habitat management plans, and the securing of easements with private property owners. The report also outlined the criteria that must be met for the Mitchell's satyr to be have its status changed (USFWS 1998):

"1. For reclassification from endangered to threatened a total of 16 geographically distinct and viable populations or metapopulations must exist and these populations may be extant, established via

translocation, or discovered. 12 of these sites must occur in Michigan, two in Indiana, one in Ohio and one in New Jersey and at least half of these sites must be protected in some form (i.e. conservation easement or under the ownership of a conservation organization).

2. For **delisting** to occur a total of 25 distinct and viable populations must exist and remain viable for five years after delisting. At least 15 of these sites must have legal protection and we should note that the recovery team may modify or change the recovery criteria if new information becomes available.”

In March of 2009 the USFWS began a five-year review of the Mitchell's satyr to determine if the species was still in need of protection (74 FR 11600). In April of 2009 the, the Mitchell's satyr was identified by the USFWS as a “Spotlight Species” and an action plan was instituted that brought additional resources to bear on the butterfly's recovery.

#### DISCUSSION

While much is already known about *N. mitchellii*, this manuscript highlights the need for prioritized research in key areas. One critical need is for the development and use of standardized methods to estimate demographic parameters such as population size. While the currently used method of timed meander surveys is reasonably standardized, it is not quantitative with respect to area and thus does not yield a population density. Such density estimates are critically needed before any management practice can be tested robustly. Without such baseline data we cannot compare treatments let alone determine if populations are in decline. Methods that do not require handling the butterflies, such as distance methods, may be ideal for *N. mitchellii* work and have already been used with butterflies (Brown & Boyce 1998, Isaac et al. 2011).

Natural history forms the foundation for all biological work and without the data contained herein, any inferences based on molecular data could be out of context. Knowledge of the evolutionary history of *N. mitchellii* can aid in the recovery of the species by informing us about the relationship among populations at the regional and state level. Determining if and how the Virginia, Alabama, and Mississippi populations of *N. mitchellii* are related to the northern populations of *N. m. mitchellii* will have an impact on the recovery criteria of the species. These inferences can be made both with morphology and with DNA-based evidence. For example, Parhsal & Krall (1989) cited morphological character differences between the Saint Francis' satyr and Mitchell's satyr. Using methods such

as geometric morphometrics we can test if these differences are robust to statistical testing and may serve to distinguish taxa. The use of DNA technology will allow us to directly compare populations when the time since divergence is not great enough to allow morphological characters to diverge. Using two mitochondrial DNA markers, Goldstein et al. (2004) surveyed a number of *N. mitchellii* from throughout its range. Their findings suggested that the Saint Francis' satyr was distinguishable from other *N. mitchellii*, however, the populations from Michigan, Virginia, and Alabama could not be resolved as unique (Goldstein et al. 2004). These results, while interesting, may be compromised by the presence of the reproductive endosymbiont *Wolbachia*, which is transmitted maternally in the same manner as mitochondria (Nice et al. 2009, Hamm et al. in review). Once *Wolbachia* is corrected for, molecular methods will allow us to test proposed routes of post-glacial radiation that these butterflies undertook (Shapiro 1977, Metzler et al. 2005).

New research is also needed to determine the full implications of the recently discovered infections of the reproductive parasite *Wolbachia* (Hamm et al. in review). Examination of the prevalence and strain type of *Wolbachia* should be conducted before any individuals are moved among populations. This is perhaps the single most pressing need for research because the introduction of a new *Wolbachia* strain into a population could result in population extinction. Once the strain is “typed” its effects must be determined experimentally so that any future introductions can be monitored for the effects of *Wolbachia*.

We must also continue to quantify aspects of *N. mitchellii* biology and habitat ecology. Replicated experiments to compare host plant performance among populations could reveal local adaptation, which if found, may counterindicate the movement of individuals among populations. Finally, understanding the hydrology of prairie fen habitat will better allow us to manage these sites by telling us where the groundwater is coming from and thus prevent the loss of these habitats.

The goal of the Endangered Species Act is to recover species that were placed in peril by anthropogenic forces. No insect has ever been removed from the endangered species list due to recovery; rather they have been removed due to extinction. If sustained recovery is the goal, then quantifiable research must be conducted to address the major obstacles that face *N. mitchellii* conservation. Conservation organizations must partner with academic researchers to design critical experiments and research thrusts that will

directly benefit *N. mitchellii*. The Mitchell's satyr is at a critical juncture, this butterfly presents an amazing opportunity to successfully recover the first insect species if steps are taken immediately. If these steps are not taken immediately, populations will likely continue to decline and surveys will no longer be necessary.

#### ACKNOWLEDGEMENTS

The authors wish to thank: J. M. Scriber, A. Cognato, I. Dworkin, S. Prajzner, J. Conner, M. Nielsen, G. Parsons and R. Priest from Michigan State University; T. Dandridge and C. Tansey from the US Fish and Wildlife Service; C. Hoving and T. Hogrefe from the Michigan Department of Natural Resources; D. Hyde, B. Barton, and D. Cuthrell from the Michigan Natural Features Inventory; C. Ragland and D. Thurmond from the US Forest Service; S. Roble from the Virginia Department of Conservation and Recreation; N. Haddad and L. Vogel from North Carolina State University; D. Lam and J. Lopez-Bautista from the University of Alabama; B. Hart from the US Department of Agriculture; R. Brown and T. Scheifer from Mississippi State University; N. Fuller from the Southwest Michigan Land Conservancy; L. Casebere from the Indiana Department of Natural Resources; J. Boone from the Field Museum of Natural History; M. O'Brien from the University of Michigan; A. Shapiro of the University of California at Davis; and J. Shuey and P. Doran from the Nature Conservancy. All research reported here was conducted under USFWS endangered species permit TE 175852-1.

#### LITERATURE CITED

- ANDREAS, B. K. 1985. The relationship between Ohio peatland distribution and buried river valleys. *Ohio J. Sci.* 85:116–125.
- ARNETT, R. H. 2000. *American Insects, A Handbook of the Insects of America North of Mexico (2e)*. CRC Press.
- BADGER, F. S. 1958. *Euptypchia mitchellii* (Satyridae) in Michigan and Indiana Tamarack Bogs. *Lepid. News* 12: 41–46.
- BALDO, L., J. C. DUNNING HOTOPE, K. A. JOLLEY, S. R. BORDENSTEIN, S. A. BIBER, R. R. CHOUDHURY, C. HAYASHI, M. C. J. MAIDEN, H. TETTELIN, AND J. H. WERREN. 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl. Environ. Micro.* 72: 7098–7110.
- BARTEL, R. A., N. M. HADDAD, AND J. P. WRIGHT. 2010. Ecosystem engineers maintain a rare species of butterfly and increase plant diversity. *Oikos* 119: 883–890.
- BARTON, B. AND C. BACH. 2005. Habitat Use by the Federally Endangered Mitchell's Satyr Butterfly (*Neonympha mitchellii mitchellii*) in a Michigan Prairie Fen. *Am. Mid. Nat.* 153: 41–51.
- BARTON, B. J. 2008. 2007 Mitchell's Satyr (*Neonympha mitchellii mitchellii*) Research Report. Report for US Fish and Wildlife Service: 28 pp.
- BRAKEFIELD, P. M. 1996. Seasonal polyphenism in butterflies and natural selection. *Tr. Ecol. Evol.* 11: 275–277.
- BROWN, J. A. AND M. S. BOYCE. 1998. Line transect sampling of Karner blue butterflies (*Lycæides melissa samuelis*). *Environ. Ecol. Stat.* 5: 81–91.
- BRUSSARD, P. F., P. R. EHRLICH, AND M. C. SINGER. 1974. Adult Movements and Population Structure in *Euphydryas editha*. *Evolution* 28: 408–415.
- BUCKLAND, S. T., D. R. ANDERSON, K. P. BURNHAM, J. L. LAAKE, D. L. BORCHERS, AND L. THOMAS. 2001. *Introduction to Distance Sampling: Estimating Abundance of Biological Populations*. Oxford Univ. Press.
- CABLK, L. D. AND D. R. PEARSALL. 1999. Results of an Assessment of Change in Wetland Community Mosaics and Rare Plant Surveys in Portfolio Sites of the North Central Tillplain. Report to The Nature Conservancy 17 pp.
- CLAMPITT, C. A. 2001. Mitchell's Satyr Monitoring and Management. Report to the U.S. Fish and Wildlife Service 15pp.
- CLAMPITT, C. A. AND J. BEDICK. 1994. Results of 1994 Surveys for Mitchell's satyr (*Neonympha mitchellii mitchellii* French) in southwestern Michigan. Report to The Nature Conservancy 8 pp.
- DARLOW, N. 2000. Behavior, Habitat Usage and Oviposition of the Mitchell's Satyr Butterfly, *Neonympha mitchellii mitchellii*. Report to the U.S. Fish and Wildlife Service 43 pp.
- DEVRIES, P. J. 1987. *The Butterflies of Costa Rica and their Natural History: Papilionidae, Pieridae, Nymphalidae*. Princeton Univ. Press.
- DYER, H. G. 1902. List of North American Lepidoptera and Key to the Literature of this Order of Insects. *Bulletin of the United States National Museum* 52 pp.
- EMERSON, K. J., C. R. MERZ, J. M. CATCHEN, P. A. HOHENLOHE, W. A. CRESKO, W. E. BRADSHAW, AND C. M. HOLZAPFEL. 2010. Resolving postglacial phylogeography using high-throughput sequencing. *Proc. Nat. Acad. Sci.* 107: 16196–16200.
- ENGELHARDT, G. P. 1936. *Cissia mitchellii* in New Jersey. *Bull. Brooklyn Entomol. Soc.* 31: 110.
- FRENCH, G. H. 1889. A New Species of *Neonympha*. *Canad. Entomol.* 21: 25–27.
- GLASSBERG, J. 1999. *Butterflies through Binoculars: The East*. Oxford University Press, Oxford.
- GLASSBERG, J. 2000. Mitchell's Satyricon. *Amer. Butterflies Winter*: 30–33.
- GLASSBERG, J. 2001. Mitchell's Satyr Rides Again. *Amer. Butterflies Fall*: 16–20.
- GOLDSTEIN, P. Z., S. HALL, B. HART, S. M. ROBLE, AND J. SHUEY. 2004. Evaluation of relationships and conservation status within the *Neonympha mitchellii* complex (Lepidoptera: Nymphalidae). Report for US Fish and Wildlife Service, 42 pp.
- HADDAD, N. M., B. HUDGENS, C. DAMIANI, K. GROSS, D. KUEFLER, AND K. POLLOCK. 2008. Determining Optimal Population Monitoring for Rare Butterflies. *Conserv. Biol.* 22: 929–940.
- HAMM, C., D. AGGARWAL, AND D. A. LANDIS. 2010. Evaluating the impact of non-lethal DNA sampling on two butterflies, *Vanessa cardui* and *Satyroides eurydice*. *J. Insect Conserv.* 14: 11–18.
- HAMM, C. A. 2007. Designation of a neotype for Mitchell's satyr, *Neonympha mitchellii* (Lepidoptera: Nymphalidae). *Great Lakes Entomol.* 40: 201–202.
- HAMM, C. A. 2008. 2008 *Neonympha mitchellii mitchellii* (Mitchell's satyr) research report. Annual Report to the US Fish and Wildlife Service.
- HAMM, C. A. 2009. 2009 *Neonympha mitchellii mitchellii* (Mitchell's satyr) Research Report. Annual report to the US Fish and Wildlife Service.
- HAMM, C. A. 2013. Estimating the abundance of the federally endangered Mitchell's satyr butterfly using hierarchical distance sampling. *Insect Conservation and Diversity*. doi: 10.1111/icad.12017
- HANSKI, I. 1994. A Practical Model of Metapopulation Dynamics. *J. Animal Ecol.* 63:151–162.
- HARRIS, R. A. 1979. A glossary of surface sculpturing. *Occasional Papers*. California Department of Food and Agriculture, Laboratory Services, Entomology. Occasional Papers No. 28, 31 pp.
- HART, B. 2004. A Survey for the Mitchell's Satyr (*Neonympha mitchellii* French) in the National Forests in Alabama. Report for US Fish and Wildlife Service: 222 pp.
- HEMMING, F. 1937. Changes in the genotypes of, or in the priority to be accorded to, eleven genera of Lepidoptera, Rhopalocera, consequent upon the determination of the dates of publication of the entomological works of Jacob Hübner. *Proc. Royal Entomol. Soc. London (B)* 6:149 – 153.
- HILGENBOECKER, K., P. HAMMERSTEIN, P. SCHLATTMANN, A. TELSCHOW, AND J. H. WERREN. 2008. How many species are infected with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiol. Letters* 281:215–220.
- HOLLAND, W. J. 1898. *The Butterfly Book; a popular guide to a knowledge of butterflies of North America*. Doubleday & McClure, New York.
- HYDE, D. A., M. L. RABE, D. L. CUTHRELL, AND M. A. KOST. 2000.



- Surveys for the recovery of Mitchell's satyr butterfly (*Neonympha m. mitchellii*) in Michigan. Final report to the US Fish and Wildlife Service.
- ISAAC, N. J. B., K. L. CRUICKSHANKS, A. M. WEDDLE, J. M. ROW-CLIFFE, T. M. BRERETON, R. L. H. DENNIS, A. D. M. SHUKER, AND C. D. THOMAS. 2011. Distance sampling and the challenge of monitoring butterfly populations. *Meth. Ecol. Evol.* 2:585–594.
- KOST, M. A., D. A. ALBERT, J. G. COHEN, B. S. SLAUGHTER, R. K. SCHILLO, C. R. WEBER, AND K. A. CHAPMAN. 2007. Natural Communities of Michigan: Classification and Description. Report 2007–21 to the Michigan Natural Features Inventory.
- KUEFLER, D., N. HADDAD, S. HALL, B. HUDGENS, B. BARTEL, AND E. HOFFMAN. 2008. Distribution, population structure and habitat use of the endangered Saint Francis Satyr butterfly, *Neonympha mitchellii francisci*. *Am. Mid. Nat.* 159:298–320.
- LANDIS, D. A., A. K. FIEDLER, C. A. HAMM, D. L. CUTHRELL, E. H. SCHOOLS, D. R. PEARSALL, M. E. HERBERT, AND P. J. DORAN. 2011. Insect conservation in Michigan prairie fen: addressing the challenge of global change. *J. Insect Conserv.* 16:131–142.
- LEGGE, J. T. AND M. RABE. 1996. Observation of Oviposition and Larval Ecology in Caged Mitchell's Satyr Butterflies (*Neonympha mitchellii mitchellii*) (Lepidoptera: Nymphalidae). Report to the U.S. Fish and Wildlife Service, 17 pp.
- LEWIS, H. L. 1974. Butterflies of the World. Harrison House.
- MACKINNON, W. A. AND D. A. ALBERT. 1996. Mitchell's Satyr Historical Habitat Analysis. Report to the U.S. Fish and Wildlife Service, 21 pp.
- MCALPINE, W. S., S. P. HUBBELL, AND T. E. PLISKE. 1960. The Distribution, Habits, and Life History of *Euptychia mitchellii* (Satyridae). *J. Lepid. Soc.* 14:209–223.
- MDOT. 1981. Final Environmental Impact Statement for the US-31 Freeway in Berrien County. Michigan Department of Transportation.
- MDOT. 2004. Final Environmental Impact Statement for the US-31 Freeway in Berrien County. Michigan Department of Transportation.
- METZLER, E. H., J. A. SHUEY, L. A. FERGE, R. A. HENDERSON, AND P. Z. GOLDSTEIN. 2005. Contributions to the understanding of tall-grass prairie-dependent butterflies and moths (Lepidoptera) and their biogeography in the United States. Ohio Biological Survey, Ohio Department of Natural Resources, Division of Wildlife.
- MOORE, S. 1938. A list of the Butterflies of Michigan. Occasional Papers of the Museum of Zoology University of Michigan 41:1–24.
- MOORE, S. 1960. A Revised Annotated List of the Butterflies of Michigan. Occasional Papers of the Museum of Zoology University of Michigan 617:1–42.
- MOSHER, E. 1916. A Classification of the Lepidoptera Based on Characters of the Pupa. *Bull. Illinois State Lab. Nat. Hist.* 12:323 pp.
- NICE, C. C., Z. GOMPERT, M. L. FORISTER, AND J. A. FORDYCE. 2009. An unseen foe in arthropod conservation efforts: The case of *Wolbachia* infections in the Karner blue butterfly. *Biol. Conserv.* 142:3137–3146.
- NIJHOUT, H. F. 1991. The Development and Evolution of Butterfly Wing Patterns. Smithsonian Institution Press, Washington D.C.
- OPLER, P. A. AND G. O. KRIZEK. 1985. Butterflies East of the Great Plains. The Johns Hopkins University Press, Baltimore, MD.
- OPLER, P. A. AND V. MALIKUL. 1998. A Field Guide to Eastern Butterflies. Houghton Mifflin Harcourt, New York.
- PALLISTER, J. C. 1927. *Cisia mitchellii* (French) found in Ohio with notes on its own habits. *Lepidoptera: Satyridae. Ohio J. Sci.* 27:203–204.
- PARSHALL, D. K. AND T. W. KRAL. 1989. A New Subspecies of *Neonympha mitchellii* (French)(Satyridae) from North Carolina. *J. Lepid. Soc.* 43: 114–119.
- PELHAM, J. 2008. Catalogue of the Butterflies of the United States and Canada. *J. Research Lepid.* 40: xiv + 658 pp.
- PIELOU, E. C. 1992. After the Ice Age: The Return of Life to Glaciated North America. Univ. Chicago Pr., Chicago, IL.
- POLLARD, E. AND T. J. YATES. 1994. Monitoring Butterflies for Ecology and Conservation—The British Monitoring Scheme. Springer.
- RABE, M. L. 1998. Surveys for the Recovery of Mitchell's satyr butterfly (*Neonympha mitchellii mitchellii*) in Michigan. Pp. Report to The Nature Conservancy, 16 pp.
- ROBLE, S. M. 2002. Status survey for and population monitoring of Saint Francis' Satyr (*Neonympha mitchellii francisci*) in Virginia. Virginia Department of Conservation and Recreation, Division of Natural Heritage, 16 pp.
- ROBLE, S. M. 2003. Status Survey for and Population Monitoring of Saint Francis' Satyr (*Neonympha mitchellii francisci*) in Virginia, 2003. Natural Heritage Technical Report 03–20. Virginia Department of Conservation and Recreation, 24 pp.
- ROBLE, S. M. 2005. Population Monitoring of Mitchell's Satyr (*Neonympha mitchellii*) in Virginia, 2004. Pp. Natural Heritage Technical Report 05-05. Virginia Department of Conservation and Recreation, 15 pp.
- ROBLE, S. M. 2006. Results of Larval, Pupal, and Adult Surveys for Mitchell's Satyr (*Neonympha mitchellii*) in Virginia, 2005. Natural Heritage Technical Report 06-04. Virginia Department of Conservation and Recreation, 15 pp.
- ROBLE, S. M., C. T. KESSLER, B. GRIMES, C. S. HOBSON, AND A. C. CHAZAL. 2001. Biology and Conservation Status of *Neonympha mitchellii*, a Globally Rare Butterfly New to the Virginia Fauna. *Banisteria* 18:3–23.
- RUTKOWSKI, F. 1966. Rediscovery of *Euptychia mitchellii* (Satyridae) in New Jersey. *J. Lepid. Soc.* 20:43–44.
- SCOTT, J. A. 1986. The Butterflies of North America, A Natural History and Field Guide. Stanford University Press, Stanford.
- SFERRA, N. J. AND J. M. AGUIAR. 1993. Behavior and Habitat Use by *Neonympha mitchellii mitchellii* (Mitchell's Satyr: Nymphalidae) at Tamarack Swamo, Cass County, Michigan. Pp. 8 pp. Report to The Nature Conservancy.
- SHAPIRO, A. M. 1970. Postglacial Biogeography and the Distribution of *Poanes viator* (Hesperiidae) and other Marsh Butterflies. *J. Research Lepid.* 9:125–155.
- SHAPIRO, A. M. 1977. Evidence for 2 routes of post-pleistocene dispersal in *Poanes viator* (Hesperiidae). *J. Research Lepid.* 16: 173–175.
- SHUEY, J., E. METZLER, D. IFTNER, AND J. CALHOUN. 1987A. Status and habitats of potentially endangered Lepidoptera in Ohio. *J. Lepid. Soc.* 41: 1–12.
- SHUEY, J. A. 1985. Habitat Associations of Wetland Butterflies Near the Glacial Maxima in Ohio, Indiana, and Michigan. *J. Research Lepid.* 24: 176–186.
- SHUEY, J. A. 1997. Conservation status and natural history of Mitchell's satyr, *Neonympha mitchellii mitchellii* French (Insecta: Lepidoptera: Nymphalidae). *Nat. Areas J.* 17: 153–163.
- SHUEY, J. A., J. V. CALHOUN, AND D. C. IFTNER. 1987B. Butterflies that are endangered, threatened, and of special concern in Ohio. *Ohio J. Sci.* 87: 98–106.
- SHUEY, J. A., METZLER, E.H., IFTNER, D.C., CALHOUN, J.V., PEACOCK, J.W., WATKINS, R.A., HOOPER, J.D., & BABCOCK, W.F. 1987. Status and habitats of potentially endangered Lepidoptera in Ohio. *J. Lepid. Soc.* 41:1–12.
- SHUEY, J. A., A. A. REZNICEK, AND E. I. ROGERS. 1994. Mitchell's Satyr Life History and Habitat Studies at Blue Creek Fen, Berrien County, Michigan. Report for the Michigan Department of Transportation, 24 pp.
- SIEPMANN, C. G. 1936. Meeting Notes. *Bull. Brooklyn Entomol. Soc.* 31:221.
- SPIELER, J. B., P. J. COMER, D. A. ALBERT, AND M. A. KOST. 1999. Natural community abstract for prairie fen. Michigan Natural Features Inventory.
- STAHLHUT, J. K., C. A. DESJARDINS, M. E. CLARK, L. BALDO, J. A. RUSSELL, J. H. WERREN, AND J. JAENIKE. 2010. The mushroom habitat as an ecological arena for global exchange of *Wolbachia*. *Mol. Ecol.* 19: 1940–1952.

- SUMMERVILLE, K. S. 1997. Monitoring of Mitchell's Satyr Populations at Rudy Road, Mill Creek, and Thompson Lake Sites. Pp. 10 pp. Report to The Nature Conservancy.
- SUMMERVILLE, K. S. AND C. A. CLAMPITT. 1999. Habitat Characterization of Five Rare Insects in Michigan (Lepidoptera: Hesperidae, Riodinidae, Satyridae, Homoptera: Cercopidae). *Great Lakes Entomol.* 32 :225 – 238.
- SWINEHART, A. L. AND G. R. PARKER. 2002. The relationship between glacial geologic processes and peatland distribution in Indiana. *Proc. Indiana Acad. Sci.* 1: 21–31
- SZYMANSKI, J. AND J. SHUEY. 2002. Conservation Strategy for Mitchell's Satyr Butterfly at Blue Creek Fen, Berrien County, Michigan. Report to the Michigan Department of Transportation, 70 pp.
- SZYMANSKI, J., SHUEY, J.A., OBERHAUSER, K. 2004. Population Structure of the Endangered Mitchell's Satyr, *Neonympha mitchellii mitchellii* (French): Implications for Conservation. *Am. Mid. Nat.* 15: 304–322.
- SZYMANSKI, J. A. 1999. Population and Spatial ecology of the Mitchell's Satyr Butterfly, *Neonympha m. mitchellii* French, in Southwestern Michigan. Pp. 78. University of Minnesota.
- THOMAS, C. D. 2000. Dispersal and extinction in fragmented landscapes. *Proc. Royal Soc. B – Biol. Sci.* 267: 139–145.
- TOLSON, P. 2008. Rearing Mitchell's satyr at the Toldeo Zoo—a first step towards eventual re-introduction in secure habitats. *News Lepid. Soc.* 50: 42–43.
- TURNER, J. D. 2007. Mitchell's satyr revisited. *Southern Lepid. News* 29:98.
- [USFWS] United States Fish & Wildlife Service. 2011. Endangered Species Webpage. Available from: <http://www.fws.gov/endangered/> (1 September 2011).
- [USFWS] UNITED STATES FISH & WILDLIFE SERVICE. 1984. Endangered and Threatened Wildlife and Plants; Findings on Pending Petitions and Description of Progress on Listing Actions. *Federal Register* 2485–22488.
- [USFWS] UNITED STATES FISH & WILDLIFE SERVICE. 1998. Mitchell's Satyr Butterfly *Neonympha mitchellii mitchellii* Recovery Plan. Report to the U.S. Fish and Wildlife Service, 71 pp.
- WAGNER, D. L. 2005. Caterpillars of Eastern North America. Princeton University Press, Princeton, New Jersey.
- WERREN, J. H., L. BALDO, AND M. E. CLARK. 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nature Rev. Microbiol.* 6: 741–751.
- WILSMANN, L. A. AND D. F. SCHWEITZER. 1991. A Rangewide Status Survey of Mitchell's Satyr, *Neonympha mitchellii mitchellii* (Lepidoptera: Nymphalidae). Report to the U.S. Fish and Wildlife Service, 25 pp.
- WOLCOTT, R. H. 1893. The Butterflies of Grand Rapids, Michigan. *Can. Entomol.* 25: 98–107.

*Submitted for publication 16 December 2011; revised and accepted 13 October 2012.*

POPULATION BIOLOGY OF THE ENDANGERED FLUMINENSE SWALLOWTAIL BUTTERFLY  
*PARIDES ASCANIUS* (PAPILIONIDAE: PAPILIONINAE: TROIDINI)

ELISA VIEIRA HERKENHOFF, RICARDO FERREIRA MONTEIRO, ALEXANDRE PIMENTA ESPERANÇO

Laboratório de Ecologia de Insetos, Departamento de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ). CP 68020; CEP 21941-590, Ilha do Fundão, Rio de Janeiro, RJ, Brazil; e-mails: elisabio@gmail.com, monteiro@biologia.ufrj.br, pimentas@yahoo.com

AND

ANDRÉ VICTOR LUCCI FREITAS

Departamento de Biologia Animal and Museu de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109, Campinas, SP, 13083-970, Brazil; email: baku@unicamp.br (corresponding author)

**ABSTRACT.** A population of *Parides ascanius* butterflies was studied for 12 months in Rio de Janeiro, SE Brazil. The population size was approximately 10–20 individuals with a maximum of 50 individuals. Sex ratio was biased, with males dominating in most months. The residence time was  $12.7 \pm 10.1$  days for males, with a maximum of 28 days. Males travel distances of up to 400 m, but most individuals were recaptured within 150 to 250 m of their original capture site. Our results show that *P. ascanius* has a relatively long adult life span and maintains high population numbers, suggesting that habitat loss rather than population attributes explains its rarity and endangered status.

**Additional key words:** Papilionidae, *Parides*, Population biology, Troidini, mark–release–recapture

The genus *Parides* Hübner, 1819 (Papilionidae: Troidini) is exclusively Neotropical, with 34 species occurring from Mexico to Argentina (Tyler et al. 1994; Rachelli 2006). The larvae feed exclusively on *Aristolochia* (*Aristolochiaceae*), a group of plants known for containing toxic secondary compounds (Tyler et al. 1994). *Parides* adults are unpalatable and are involved in mimicry rings with several other species of butterflies and moths (Brown et al. 1981; Tyler et al. 1994; Rachelli 2006). Most *Parides* are associated with forests and other moist habitats (Tyler et al. 1994; Rachelli 2006), and it has been shown that several species are sensitive to anthropic disturbance, making them potentially useful organisms for environmental monitoring in the Neotropics (Tyler et al. 1994).

The fluminense swallowtail butterfly, *Parides ascanius* (Cramer, 1775) (Figure 1A), is a threatened butterfly endemic to the coastal lowland swamps of Rio de Janeiro (Otero & Brown 1986). The species is monophagous, using only *Aristolochia macroura* Gomes (*Aristolochiaceae*) (Fig. 1B) as its larval host (Otero & Brown 1986). D'Almeida (1966) suggested that *Parides ascanius* was potentially endangered, and in 1973 this species became the first Brazilian butterfly officially recognized as being threatened with extinction (Otero & Brown 1986). The current situation for *P. ascanius* is delicate; most known populations live outside the boundaries of protected reserves. The species, under constant pressure from habitat loss due to increased urbanization of all coastal plains in Rio de Janeiro, is considered to be

endangered in Brazil (Otero et al. 2000; Uehara-Prado & Fonseca 2007; Brown & Freitas 2008). In addition, the Biological Reserve of Poço das Antas, a major conservation area that harbors large colonies of this butterfly (Otero & Brown 1986), has suffered severe impacts in the 1970's and 1980's due to drainage projects involved with straightening the “São João” river basin, as well as construction of the Juturnaiba dam to supply water to coastal cities. These actions have produced deep environmental changes in the region, with large areas being flooded by the lake and other areas becoming dry, resulting in frequent vegetation fires.

Because of its importance as a conservation icon, *P. ascanius* has been heavily studied over the past several decades, with research having been done on the butterfly's behavior, natural history, early stages, chromosomes, chemical ecology and conservation (D'Almeida 1922, 1966; Brown et al. 1981; Otero 1984; Otero & Brown 1986; Tyler et al. 1994; Brown et al. 1995; Emmel et al. 1995; Tavares et al. 2006; Uehara-Prado & Fonseca 2007; Silva-Brandão et al. 2008). However, there have been no studies on the population ecology of *P. ascanius*; the few published accounts on this topic have resulted from studies carried out more than 20 years ago (Brown et al. 1995; Tyler et al. 1994).

Given the fragile situation of *P. ascanius*, the goal of the present study is to present detailed population data for this species, gathered in a protected area, in order to provide information that could aid in future management of this endangered butterfly species.



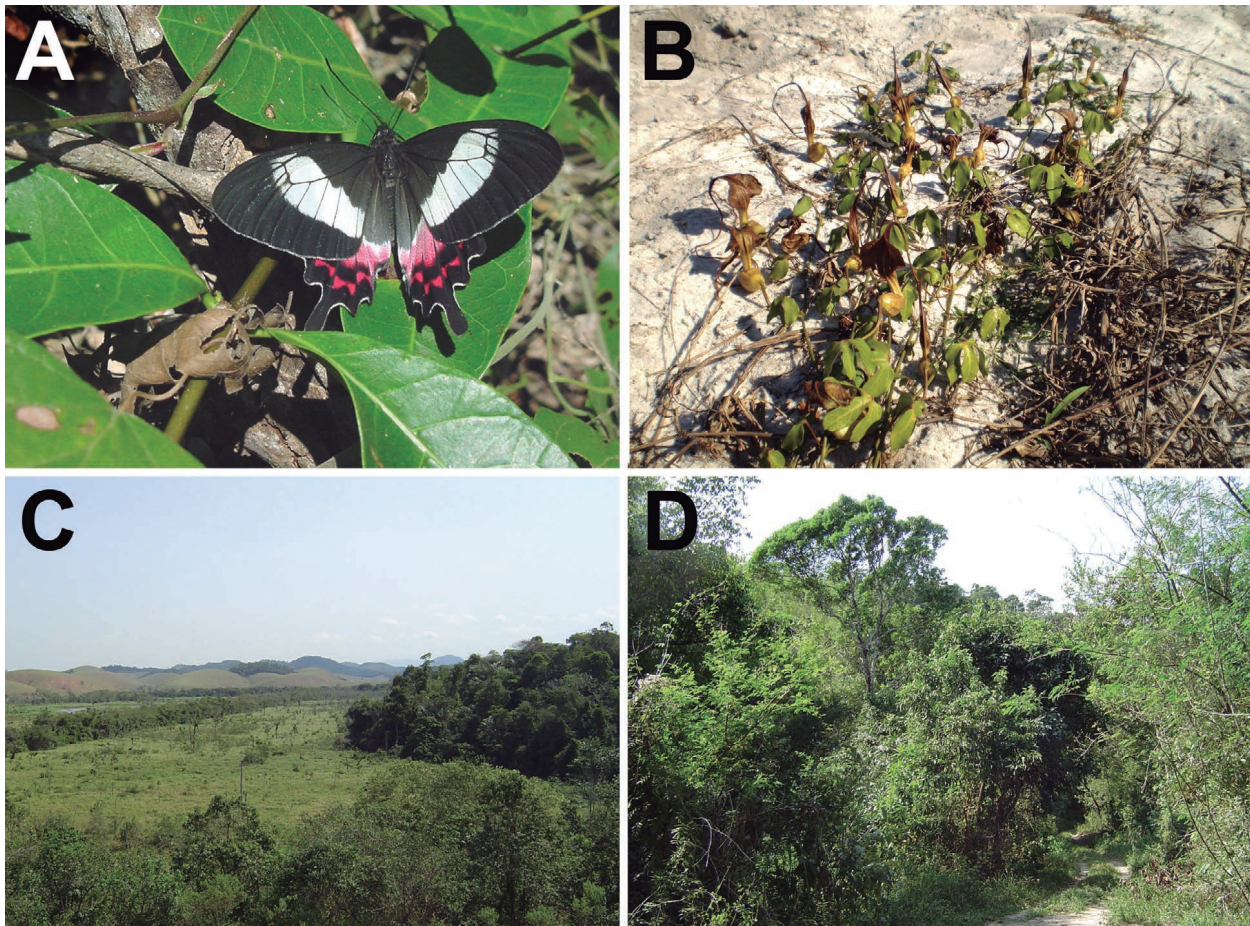


FIG. 1. **A.** A female of *Parides ascanius*; **B.** The host plant, *Aristolochia macroura*, growing in sand; **C.** General view of *P. ascanius* habitat in Poço das Antas Biological Reserve, Silva Jardim, RJ; **D.** Close-up of *P. ascanius* habitat.

#### STUDY SITES AND METHODS

A mark-release-recapture (MRR) study was carried out in the Poço das Antas Biological Reserve (22°30'–22°33'S, 42°15'–42°19'W; ca. 100 m a.s.l.), in the city of Silva Jardim, Rio de Janeiro State, Southeastern Brazil (Fig. 1C, D). The reserve comprises 5,500 ha of lowland swamps and sand forests. Annual rainfall reaches 2000 mm and the average annual temperature is 24°C (DNMET) (a climagram for the study area is presented in Fig. 2). Butterflies were marked and recaptured in a trail (800 m long, divided into 5 m sectors, Fig. 1D) along the São João river during 12 months, from October 10, 2004 to September 7, 2005, for a total of 18 days (approximately 5 hours/day). Butterflies were net-captured, individually numbered on the underside of both forewings with a black permanent felt-tipped pen, and released. Characteristics of each individual (age, wing damage, point of capture, sex and food sources) were recorded for later analysis (as in Ramos & Freitas; 1999 and Beirão et al. 2012). Wing wear, based on three

categories (new, intermediate and old) was used as an additional age measure for individual butterflies (as in Ramos & Freitas 1999). Age structure was calculated for both sexes together, through the monthly means of daily proportions of each category. Wing damage was also measured in three categories: intact, low damage (one to three wing cuts) and high damage (more than 3 wing cuts). Monthly wing damage was calculated for both sexes together, through the monthly means of daily proportions of each category.

The MRR data were analyzed using the Lincoln-Petersen-Bailey method for estimating population parameters (Francini 2010a, b). Only males were analyzed because of the low number of females. To estimate the number of individuals present per day, recaptured individuals were considered to be present on all previous days since the first capture (= marked animals at risk, following Freitas & Ramos 2001). Time of permanence in population (= minimum permanence, an indirect measure of longevity) was calculated as days elapsed between marking and last recapture (following

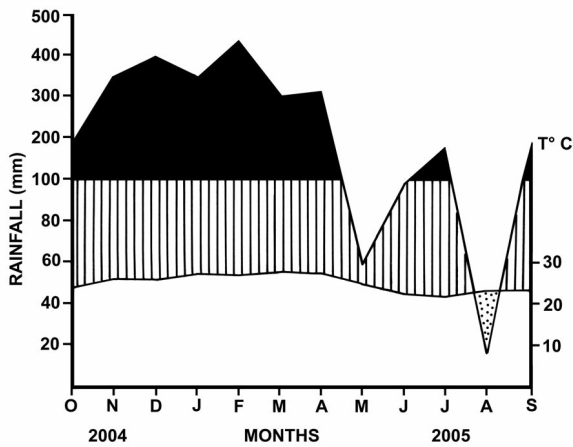


FIG. 2. Climatic diagram of the study site (see methods) during the study period (format following Walter 1985). Dotted = dry periods, hatched = humid periods, black = superhumid periods.

Brussard et al. 1974). The sex ratio was calculated through the monthly means of daily proportions in number of individuals captured per day.

RESULTS

**Population dynamics:** The number of individuals captured per day varied from zero to 38 for males (mean = 15.9; SD = 12.7; n = 17 days), and from 1 to 16 for females (mean = 4.4; SD = 4.0; n = 18 days). The population size (based on number of individuals present per day) was not stable throughout the year, with a marked peak in number of individuals during the spring (Fig. 3). The population analyses showed that population size varied between 16 and 240 males (Table 1). Considering the maximum values (number estimated plus one standard error), the population could reach values of approximately 200 males, with up

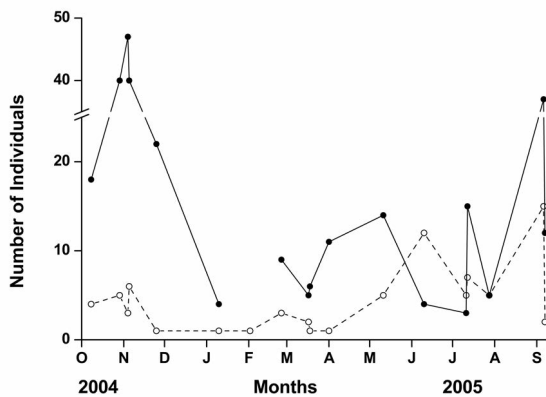


FIG. 3. Number of *Parides ascanius* individuals present per day in the study site within Poço das Antas Biological Reserve (Silva Jardim, RJ) from October 2004 to September 2005. Solid circles = males, open circles = females.

TABLE 1. Population data of *Parides ascanius* in the Poço das Antas Biological reserve. F = marked females, M = marked males, M+R = marked plus recaptured males, NIPD = number of males present per day, N = estimated number of males, SE = standard error, LP = Lincoln-Petersen-Bailey (males only).

Date	F	M	M + R	NIPD	LP
					N ± SE
8.X.2004	4	18	18	18	---
29.X.2004	5	38	38	40	156 ± 84
4.XI.2004	3	27	36	47	111 ± 51
5.XI.2004	6	18	33	40	126 ± 80
25.XI.2004	1	10	22	22	110 ± 136
10.I.2005	1	4	4	4	40 ± 52
2.II.2005	1	0	---	---	---
25.II.2005	3	9	9	9	54 ± 68
17.III.2005	2	5	5	5	30 ± 37
18.III.2005	1	5	5	6	30 ± 30
1.IV.2005	1	9	11	11	165 ± 220
11.V.2005	5	14	14	14	70 ± 86
10.VI.2005	12	4	4	4	16 ± 19
11.VII.2005	5	3	3	3	16 ± 14
12.VII.2005	6	13	15	15	90 ± 113
28.VII.2005	4	5	5	5	190 ± 259
6.IX.2005	16	37	37	37	240 ± 250
7.IX.2005	1	11	12	12	---

to 500 males on some days (Table 1). However, due to the low number of recaptures, these should be considered as rough estimates.

**Residence Time:** The residence time (based on recaptured individuals) varied from one to 28 days for males (mean = 12.7 days; SD = 10.08; n = 36). For the two females recaptured, residence times were of one and 17 days respectively. Table 2 shows male permanence in the population. Male life expectancy (following Cook et al., 1967) was calculated as 12.3 days.

**Sex Ratio:** The sex ratio of individuals captured and marked was male biased, with 230 males and 77 females marked ( $X^2 = 76.2$ ;  $df = 1$ ;  $P < 0.0001$ ), or nearly 3 males : 1 female, with males being dominant in all but one month (Fig. 4). Both sexes had low recapture rates; 36 males (15.6%) and only 2 females (2.3%) were recaptured at least once, and the maximum number of recaptures for a single individual was two.

**Age structure:** During the 12 months of this study, age structure was quite stable. The increase of “new”



TABLE 2. Permanence of marked *Parides ascanius* males in the Poço das Antas Biological reserve. Days elapsed between marking and last recapture represent the minimum permanence (MP) for each individual.

MP	Males	P (%)
1-6	12	33.3
7-12	8	22.2
13-18	2	5.6
19-24	6	16.7
25-30	8	22.2
Total	36	100.0

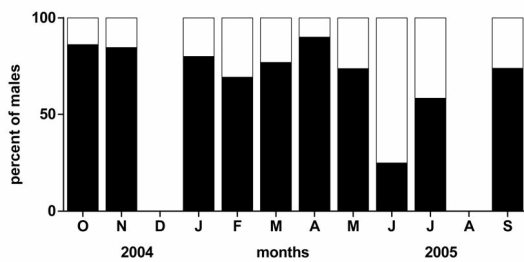


FIG. 4. Sex ratio of *P. ascanius* from October 2004 to September 2005. Data presented as percent of males (in black) by month (based on means of each day's captures).

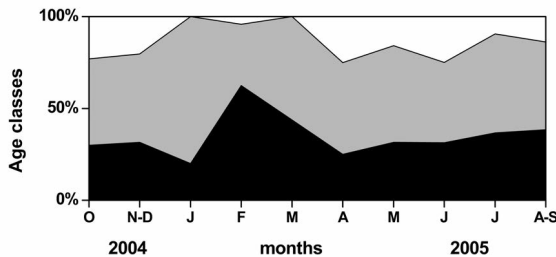


FIG. 5. Age structure for *P. ascanius* (both sexes) from October 2004 to September 2005 within the study site (black = new individuals, gray = intermediate, white = old).

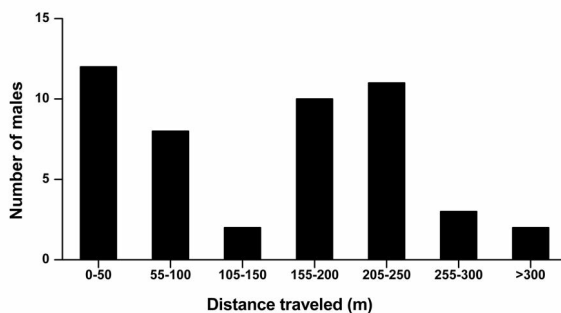


FIG. 6. Maximum distances traveled by males of *P. ascanius*, using data from all recaptured individuals.

individuals in February-March, 2005 is an artifact, since the population size during this period was small (Fig. 5).

**Vagility:** On average, maximum vagility of males (mean = 142.9 m, SD = 95.50, n = 48) was higher than that of females (mean = 65.0 m, SD = 86.31, n = 4), but no comparisons could be done due to the low amount of data for females. The maximum travel distance recorded for a male was 400 m, roughly twice the maximum distance recorded for a female (190 m). More than half of all males (54%) traveled 150 m or more during the study (Fig. 6).

**Wing damage:** The proportion of different damage classes was stable throughout the 12 months of study, with most individuals presenting no substantial wing damage and only a few individuals showing high levels of wing damage (Fig. 7).

**Adult natural history and behavior:** Within the study area, *Parides ascanius* is a relatively common species found along forest edges and in open areas, especially near patches of its larval host plant, *Aristolochia macroura*. Other five species of Troidini are sympatric with *P. ascanius* in the study area: *Battus crassus crassus* (Cramer), *Battus polydistictus galenus* (Fruhstorfer), *Battus polydamas polydamas* (Linnaeus), *Parides zacyanthus zacyanthus* (Fabricius) and *Parides anchises nephalion* (Godart). All but *B. polydistictus* are known to use *A. macroura* as a larval host plant (Brown et al. 1981, 1995, Tyler et al. 1994). Adults of *P. ascanius* became active at approximately 0800 h, but this varied within a season according to weather. They characteristically flew low (0.5 to 2.0 m above the ground) and were seldom observed flying higher than three meters.

## DISCUSSION

The population parameters of *P. ascanius*, including residence time, a male-biased sex ratio, and pronounced fluctuation in numbers throughout the year, are similar to those reported for other *Parides* species (Brown et al. 1981; Tyler et al. 1994; Freitas & Ramos 2001). However, population size was larger than that reported for most species of *Parides* (Cook et al. 1971; Freitas & Ramos 2001; Tyler et al. 1994: 60), being comparable to those of *P. burchellanus* (Westwood) in Central Brazil (Beirão et al. 2012) and *Parides neophilus* (Geyer) and *Parides proneus* (Hübner) in a semi-deciduous forest in southeastern Brazil (Brown et al. 1981; Tyler et al. 1994: 60). Even taking into account that sampling intervals in the present study were not the ideal, the results of population size can be considered trustworthy. The spring population peak of *P. ascanius* (September–November), for example, concurs with previous reports (D'Almeida 1966; Tyler et al. 1994: 67).



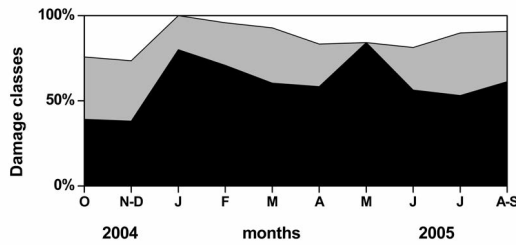


FIG. 7. Percentage of wing damage in *P. ascanius* (both sexes) (black = intact, gray = low damage, white = high damage).

Average and maximum residence times for *P. ascanius* males are equivalent to those reported for most *Parides* species; in Southeastern Brazil, Brown et al. (1981, 1995) reported maximum life spans of 33 to 56 days depending on the species, and Freitas & Ramos (2001) reported a maximum of 30 days and an average of roughly 14 days for males of *Parides anchises nephalion* in coastal Brazil. Life expectancy for males of *P. ascanius* (12.3 days) was high compared to that reported for *P. neophilus* (5.6 days, Cook et al. 1971) and *P. anchises nephalion* (5.7 days, Freitas & Ramos 2001), but was lower than the value obtained for males of *P. burchellanus* (18 days; Beirão et al. 2012).

The sex ratio of *P. ascanius* was male-biased, with a proportion near 3:1, somewhat above the 2:1 ratio reported for most natural populations of *Parides*, including a previous study on *P. ascanius* (Tyler et al. 1994; Brown et al. 1995; Freitas & Ramos 2001; Beirão et al. 2012). Male biased sex ratios are usually observed in butterflies in the field even if laboratory broods are 1:1, and are usually related to behavioral differences between sexes (Brussard & Ehrlich 1970; Freitas 1996; Ramos & Freitas 1999 and references therein). However, sex ratios measured for reared broods of *P. ascanius* will be required before we can confirm that the male-biased sex ratio results from differential capture rates between sexes, as has been proposed for other butterfly species.

Based on our results, especially with regard to the surprisingly large size of our study population, we suggest that the threatened status of *P. ascanius* cannot be explained by its biological traits, such as population size, vagility and life expectancy, all which are very similar to those described for several unthreatened species of *Parides* (see above), but are instead a result of severe habitat loss. This theory, first anticipated by D'Almeida (1966: 65), has been strengthened by over two decades of research on *P. ascanius*; numerous studies have documented the destruction of coastal swamps where this species occurs, and the subsequent

replacement by residential areas (Otero & Brown 1986; Otero & Marigo 1990; Tyler et al. 1994; Uehara-Prado & Fonseca 2007; Brown & Freitas 2008).

The future of *P. ascanius* will depend on a combination of factors, including: the discovery and preservation of additional populations; establishment of additional protected habitats; and management of captive butterflies for reinforcement and reestablishment of viable populations in potentially suitable areas (Otero et al. 2000; Uehara-Prado & Fonseca 2007; Brown & Freitas 2008; Freitas & Marini-Filho 2011). Future molecular studies should focus on investigating how genetically isolated the remaining populations of *P. ascanius* are, and should establish the constraints that limit its distribution to the coastal lowlands of Rio de Janeiro and southern Espírito Santo (but see Uehara-Prado & Fonseca 2007). *Parides ascanius* stands as a conservation symbol for Rio de Janeiro. The actions outlined above, combined with educational programs and an increase in habitat connectivity in the region where *P. ascanius* occurs, will potentially prevent loss of the few remaining populations of this spectacular butterfly.

#### ACKNOWLEDGEMENTS

We thank Jim Miller for critical reading of the manuscript. This study was funded by National Research Council of Brazil (CNPq). Thanks to CNPq for scholarship of EVH (Graduate Program in Ecology, UFRJ) and RFM (research productivity program, number 304348/2010-4) and "Instituto Chico Mendes de Conservação da biodiversidade (ICMBio)" for license to work in Poço das Antas Biological Reserve. AVLF was supported by the "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP, 04/05269-9), and the Brazilian CNPq (302585/2011-7). This publication is part of the RedLep "Rede Nacional de Pesquisa e Conservação de Lepidópteros" SIS-BIOTA-Brasil/CNPq (563332/2010-7), and of the BIOTA-FAPESP Program (2011/50225-3).

#### LITERATURE CITED

- BEIRÃO, M. V., F. C. CAMPOS-NETO, I. A. PIMENTA & A. V. L. FREITAS. 2012. Population biology and natural history of *Parides burchellanus* (Papilionidae: Papilioninae: Troidini), an endangered Brazilian butterfly. *Ann. Entomol. Soc. Amer.* 105:36–43
- BROWN, K. S. JR. & A. V. L. FREITAS. 2008. *Parides ascanius* (Cramer, 1775). In *Livro vermelho da fauna brasileira ameaçada de extinção* (A.B.M. Machado, G.M.M. Drummond & A.P. Paglia, ed.). MMA, Brasília, DF, Fundação Biodiversitas, Belo Horizonte, MG, p.433.
- BROWN, K. S. JR., A. J. DAMMAN & P. P. FEENY. 1981. Troidine swallowtails (Lepidoptera: Papilionidae) in Southeastern Brazil: Natural history and foodplant relationships. *J. Res. Lepid.* 19:199–226.
- BROWN, K. S. JR., C. F. KLITZKE, C. BERLINGERI & P. E. R. DOS SANTOS. 1995. Neotropical swallowtails: Chemistry of food plant relationships, population ecology, and biosystematics, pp. 405–445. In J. M. Scriber, Y. Tsubaki, and R. C. Lederhouse [eds.], *Swallowtail butterflies: Their ecology and evolutionary biology*. Gainesville, Scientific Publishers, FL.
- BRUSSARD, P. & P. R. EHRLICH. 1970. The population structure of *Erebia epistodea* (Lepidoptera: Satyrinae). *Ecology* 51:119–129

- BRUSSARD, P. F., P. R. EHRLICH & M. C. SINGER. 1974. Adult movements and population structure in *Euphydryas editha*. *Evolution* 28:408–415.
- COOK, L. M., L. P. BROWER & H. J. CROZE. 1967. The accuracy of a population estimation from multiple recapture data. *J. Animal Ecol.* 36:57–60.
- COOK, L. M., K. FRANK & L. P. BROWER. 1971. Experiments on the demography of tropical butterflies. I. Survival rate and density in two species of *Parides*. *Biotropica* 3:17–20.
- D'ALMEIDA, R. F. 1966. Catálogo dos Papilionidae americanos. Sociedade Brasileira de Entomologia, São Paulo, Brazil.
- D'Almeida, R. F. 1922. Mélanges lépidoptérologiques. Etudes sur les lépidoptères du Brésil. Berlin, R. Friedländer & Sohn. viii + 226 pp.
- DNMET. 1992. Normais climatológicas (1961–1990). Departamento Nacional de Meteorologia, Ministério da Agricultura. Brasília, Brasil.
- EMMEL, T. C., P. J. ALIAZAR, K. S. BROWN JR. & E. SUOMALAINEN. 1995. Chromosome evolution in the Papilionidae, pp. 283–298. In J. M. Scriber, Y. Tsubaki, and R. C. Lederhouse [eds.], Swallowtail butterflies: Their ecology and evolutionary biology. Gainesville, Scientific Publishers, FL.
- FRANCINI, R. B. 2010A. Métodos para estudar ecologia de populações de borboletas. 201pp. <http://www.archive.org/details/MtdosParaEstudarEcologiaDePopulaesDeBorboletas> (accessed June 2011).
- FRANCINI, R. B. 2010B. CMLR\_2010. Programa para estimativas populacionais (available free by the e-mail rb.francini@yahoo.com).
- FREITAS, A. V. L. 1996. Population biology of *Heterosais edessa* (Nymphalidae) and its associated Atlantic Forest Ithomiinae community. *J. Lepid. Soc.* 50:273–289.
- FREITAS, A. V. L. & R. R. RAMOS. 2001. Population biology of *Parides anchises nephalion* (Papilionidae) in a coastal site in Southeast Brazil. *Braz. J. Biol.* 61: 623–630.
- FREITAS, A. V. L. & O. J. MARINI-FILHO. 2011. Plano de Ação Nacional para Conservação dos Lepidópteros Ameaçados de Extinção. Instituto Chico Mendes de Conservação da Biodiversidade, Brasília, DF.
- OTERO, L. S. 1984. *Parides ascanius* (Cramer, 1775), borboleta ameaçada de extinção. In: Lacerda, L. D., D. S. D. Araujo, R. Cerqueira, & B. Turcq (Orgs.). Restingas: Origem, Estrutura e Processos. Niterói, CEUFF. p. 369–371.
- OTERO, L. S. & K. S. BROWN JR. 1986. Biology and ecology of *Parides ascanius* (Cramer, 1775) (Lep., Papilionidae), a primitive butterfly threatened with extinction. *Atala* 10/12: 2–16.
- OTERO, L. S. & L. C. MARIGO. 1990. Butterflies—beauty and behavior of Brazilian species. Marigo comunicação visual, 127 pp., Rio de Janeiro, RJ, Brazil.
- OTERO, L. S., K. S. BROWN JR, O. H. H. MIELKE, R. F. MONTEIRO, J. COSTA, M. V. MACEDO, J. BECKER, N. D. SALGADO, S. SANTOS & G. E. MOYA. Invertebrados terrestres. In H. G. Bergallo, C. F. D. Rocha, M. A. S. Alves. and M.V. Sluys. (Eds.) A fauna ameaçada de extinção do Estado do Rio de Janeiro. 1 ed. Rio de Janeiro: Editora da Universidade do Estado do Rio de Janeiro, 2000, p. 53–62.
- RACHELI, T. 2006. The genus *Parides*: an unended quest. p. 116. Goecke & Evers, Keltern, Germany.
- RAMOS, R. R. & A. V. L. FREITAS. 1999. Population biology, wing color variation and ecological plasticity in *Heliconius erato phyllis* (Nymphalidae). *J. Lepid. Soc.* 53:11–21.
- SILVA-BRANDÃO, K. L., A. M. L. AZEREDO-ESPIN & A. V. L. FREITAS. 2008. New evidence on the systematic and phylogenetic position of *Parides burchellanus* (Lepidoptera: Papilionidae). *Mol. Ecol. Res.* 8:502–511.
- TAVARES, M. T., A. B. NAVARO-TAVARES & G. DE S. S. ALMEIDA. 2006. The species of Chalcididae (Hymenoptera) parasitoids of *Parides ascanius* (Cramer), an endangered Papilionidae (Lepidoptera) from restingas of Southeastern Brazil. *Zootaxa* 1197:55–63.
- TYLER, H., K. S. BROWN JR. & K. WILSON. 1994. Swallowtail Butterflies of the Americas: A Study in Biological Dynamics, Ecological Diversity, Biosystematics, and Conservation. Scientific Publishers, Gainesville, Florida.
- UEHARA-PRADO, M. & R. L. FONSECA. 2007. Urbanization and mismatch with protected areas place the conservation of a threatened species at risk. *Biotropica* 39: 264–268.

Received for publication 3 June 2012; revised and accepted 20 August 2012.

SYSTEMATICS AND BIOLOGY OF *CREMASTOBOMBYCIA CHROMOLAENAE*, NEW SPECIES  
(GRACILLARIIDAE), A NATURAL ENEMY OF *CHROMOLAENA ODORATA* (L.) KING AND  
H. ROBINSON (ASTERACEAE)

DONALD R. DAVIS

Department of Entomology, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, MRC 105,  
Washington, D.C. 20013-7012, U.S.A., email: davisd@si.edu

RODRIGO DIAZ

Biological Control Research and Containment Laboratory, University of Florida, 2199 S. Rock Rd., Fort Pierce, FL 34945, U.S.A.  
email: rrdg@ufl.edu

AND

WILLIAM A. OVERHOLT

Biological Control Research and Containment Laboratory, University of Florida, 2199 S. Rock Rd., Fort Pierce, FL 34945, U.S.A.  
email: billover@ufl.edu

**ABSTRACT.** A new species, *Cremastobombycia chromolaenae*, is described from Florida and Texas, USA. The larva is a leaf miner on *Chromolaena odorata* (L.) King and H. Robinson, a 2–3 m tall perennial shrub of the family Asteraceae, whose native range extends from southern Florida south to northern Argentina (Fig. 1). The plant is also known to occur in Africa, Asia, and Australia where it is considered exotic and in some countries has become a major weed. The larvae of *C. chromolaenae* are hypometamorphic and possess two distinct larval body forms and feeding behaviors—an early stage sap-feeding form with a flattened body and prognathous mouthparts and a later stage tissue-feeding form with a more cylindrical body and possessing hypognathous mouthparts. Based on head capsule counts and measurements, the larvae undergo five instars, with the first three instars being of the sap-feeding form and the last two tissue-feeding instars. The larval mine begins as a short, serpentine track which enlarges to a rounded, whitish, and eventually tentiform blotch. *Cremastobombycia chromolaenae* may have value as a biocontrol agent in those areas where the host plant has become a major problem.

**Additional key words:** biocontrol agent, biogeography, genital morphology, invasive plant species, larval biology.

The predominantly North American genus *Cremastobombycia* was originally proposed as a subgenus of *Lithocolletis* (= *Phyllonorycter*) by A. Braun (1908). Currently eight species are recognized in *Cremastobombycia* (Davis 1983, Davis and Miller 1984, De Prins and De Prins 2012). Although no new taxa have been reported in this genus in more than a century, at least seven new North American species are known in addition to *C. chromolaenae*, and these will be proposed in a future monograph of the North American Gracillariidae. Five of the named species are restricted to the continental United States, with *C. lantanella* Busck reported from Mexico and later introduced into Hawaii. Two new species of *Cremastobombycia* have recently been described from Africa (J. De Prins and A. Kawahara 2012b). Larvae of all species, except *lantanella* which feeds on *Lantana camara* L. (Verbenaceae), are leaf miners on various genera of Asteraceae (De Prins and De Prins 2012a).

The genus *Cremastobombycia* is most closely related to the genera *Phyllonorycter* and *Cameraria*, based on both molecular (Kawahara et al. 2011) and

morphological data. Morphologically, *Cremastobombycia* differs from these two genera in retaining vein  $M_2$  in the forewing and  $CuA_1$  in the hindwing (Fig. 5), which are lost in *Phyllonorycter* and *Cameraria*. The labial palpi of all three genera are similar in being greatly reduced to two short segments (Fig. 4). The elongate saccus in the male genitalia of *Cremastobombycia* is usually characteristic for this genus and is seldom developed to that extent in other gracillariid genera (Davis and De Prins 2011). Late instar, tissue-feeding larvae of *Cremastobombycia* are distinct in possessing a specialization of the hypopharynx unlike that examined in any other gracillariid genus. In addition to the dense covering of small, dorsal spines, which are present in most genera of tissue-feeding gracillariid larvae, the anterior margin of the hypopharynx of larval *Cremastobombycia* bears a transverse series of six relatively large, spinose, digitate lobes. The spines of these lobes increase in length toward the apex of each lobe. The hypopharynx of *C. chromolaenae* is identical to that of *C. ignota* (Figs. 13–15) and also to another undescribed species of



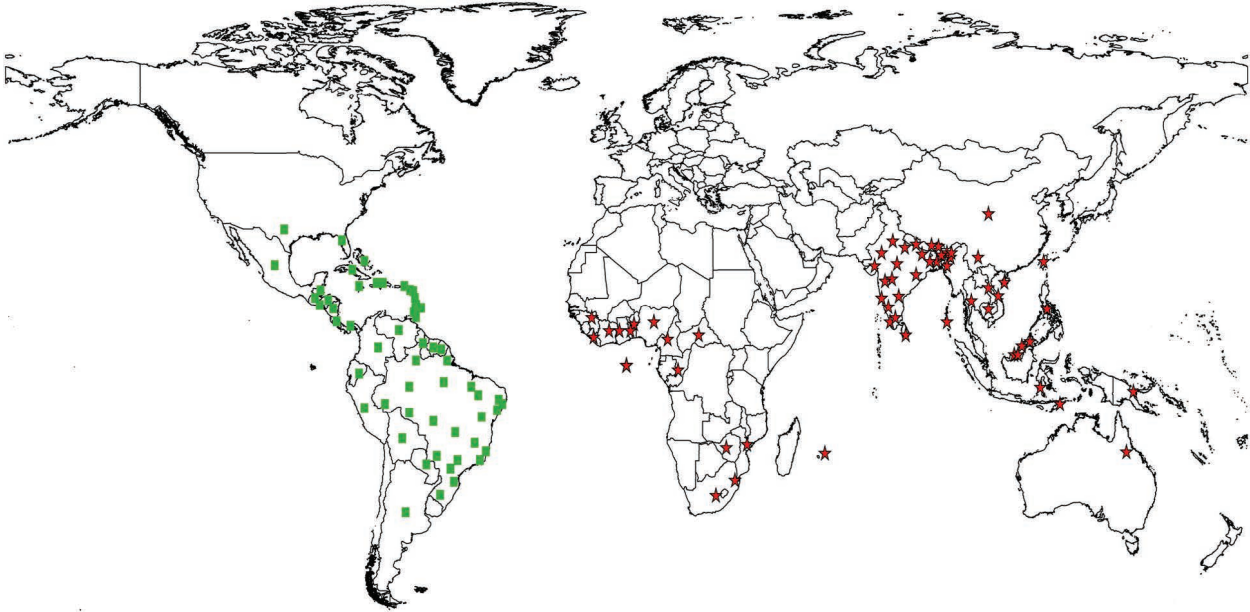
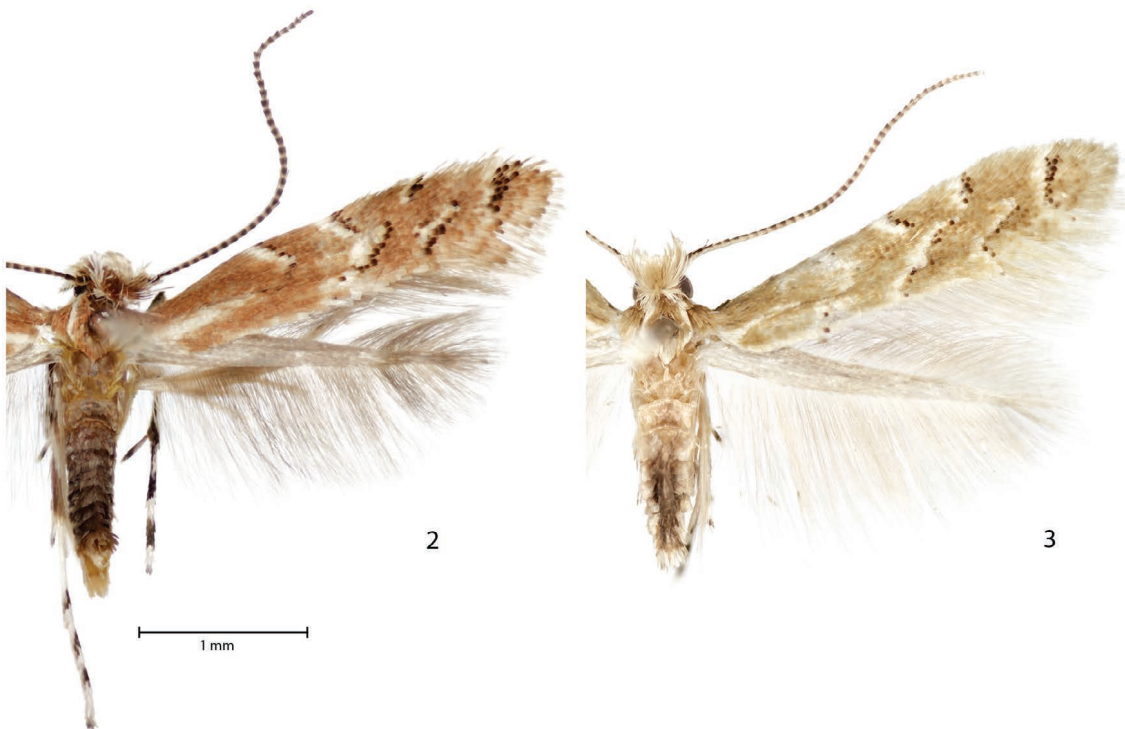


FIG. 1. Native (green squares) and exotic (red stars) distribution of *Chromolaena odorata*. Data obtained from the Global Biodiversity Information Facility ([www.gbif.org](http://www.gbif.org)).



FIGS. 2–3. Adults, *Cremastobombycia chromolaenae*. 2. Holotype ♂, St. Lucie Co: Fort Pierce, Florida; Host: *Chromolaena odorata*. 3. Paratype ♂, Cameron Co: Southmost, Audubon Sabal Palm Refuge, Texas; Host: *Chromolaena odorata* “*Eupatorium odoratum*”.

*Cremastobombycia* examined. In lieu of these prominent hypopharyngeal lobes, the anterior margin of the hypopharynx of tissue-feeding *Phyllonorycter* larvae possess a series of over 20 much smaller, curved, smooth spines (Davis and Deschka 2001). The last two larval instars of *Cameraria* are non-feeding, silk-spinning instars with vestigial mandibles. The dorsal surface of the hypopharynx in *Cameraria* is mostly smooth and without anterior spines.

*Chromolaena odorata* (L.) King and H. Robinson, commonly referred to as Siam weed or Christmas bush, is a 2–3 m tall perennial shrub with white to purple inflorescences (Holm et al. 1977). It has a broad geographic distribution from the southern United States to northern Argentina (Fig. 1, Kriticos et al. 2005). This plant is also present in Africa, Asia and Australia where it is considered exotic and in some countries has become a major weed (Zachariades et al. 2011). In the exotic range, *C. odorata* invades a wide range of habitats including grasslands, open woodland and forest margins and gaps (reviewed by Zachariades et al. 2009). The plant often forms dense stands which prevent establishment of other species, both due to competition and allelopathic effects. Areas invaded by *C. odorata* are considered a fire hazard because of the large biomass accumulation and presence of oils in the stems and leaves (McFadyen 2004). Due to the widespread negative impact to human livelihood and natural areas, several countries have implemented classical biological control as a management strategy against *C. odorata* (Siebert 1989, McFadyen 2002, Zachariades et al. 2011). Herein, we describe a new potential biological control agent of *C. odorata* discovered in southern Florida and Texas.

#### MATERIAL AND METHODS

**Specimen preparation.** Genitalic dissections were cleared by heating in hot 10% KOH for ~30 minutes, and subsequently cleaned and stained with either 2% chlorazol black E or mercurochrome solutions. All genitalic illustrations were drawn from dissections temporarily stored in glycerine, which were later permanently embedded in Canada balsam. Genitalic terminology follows Klots (1970). The lengths of eggs, larvae and pupae were measured with calibrated digital images, and the cocoons and mature mines were measured using a caliper.

Specimens examined in this study are deposited in the following institutions:  
DLW Collection of David L. Wagner, Storrs, CT, USA.  
MGCL McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA.

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

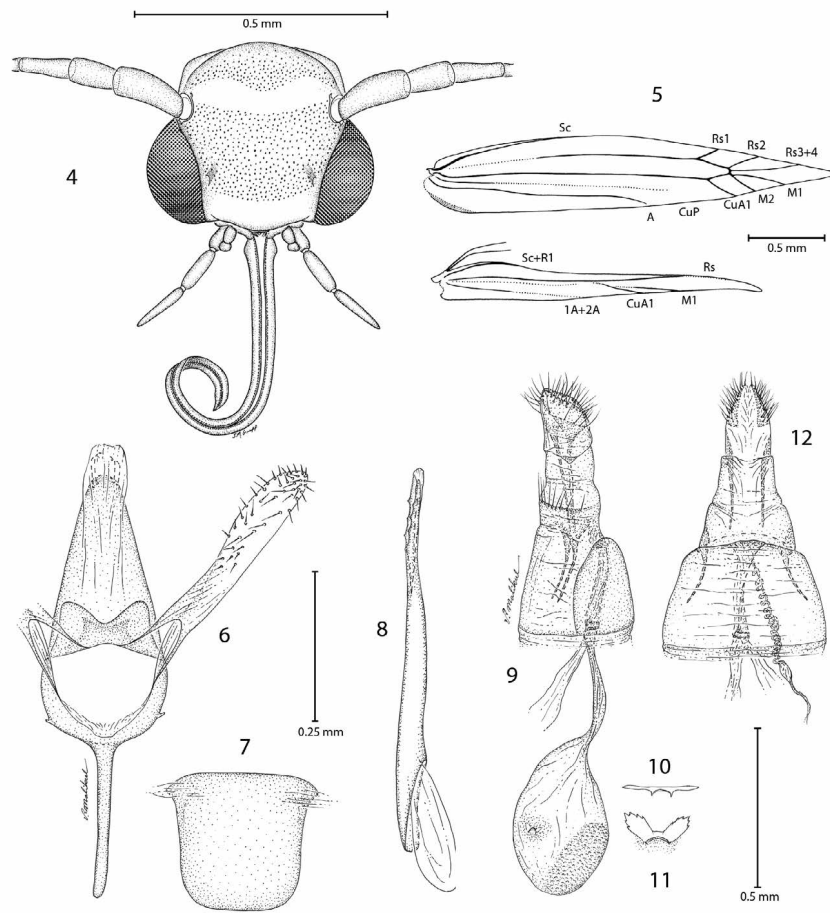
#### *Cremastobombycia chromolaenae* Davis, new species

(Figs. 2–3, 6–12, 17–24)

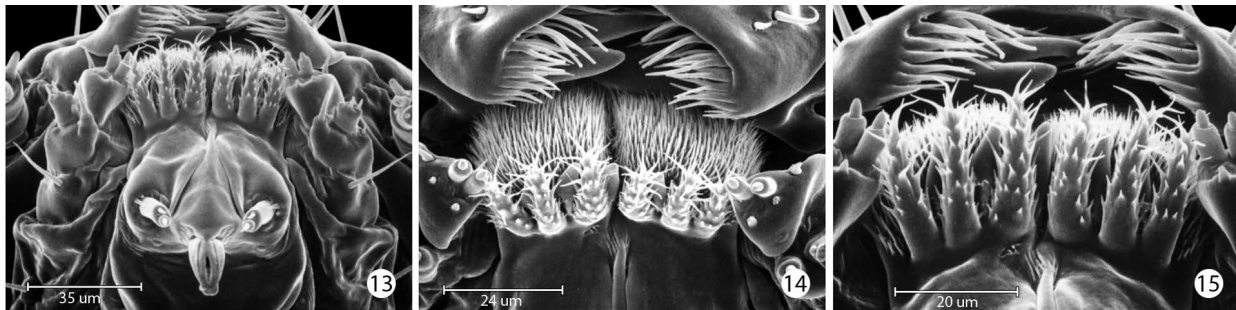
**Diagnosis.** The forewing pattern of *C. chromolaenae* most closely resembles that of *C. solidaginis* (Frey and Boll) in possessing four mostly white costal and two white dorsal strigulae, but differs slightly in having the basal first and second costal strigulae more developed in *chromolaenae*. In particular, the second costal strigula typically continues with the first dorsal one to form a complete fascia across the forewing of the latter. The valva of the male genitalia of *chromolaenae* terminates in a more rounded apex than does that of *solidaginis*. The signum of the female genitalia differs from that of all other *Cremastobombycia* in possessing a pair of minute, serrated alate lobes, with a melanized, rugose, oval disk opposite the signum. The caudal margin of the male eighth abdominal sternum of *chromolaenae* (Fig. 7) is distinct in being more truncate, compared to sharply triangular in *solidaginis*.

**Description.** Adult (Figs. 2–3). Forewing length 2.5–3.0 mm.

**Head:** Frons silvery white, becoming slightly suffused with pale brown toward vertex; vertex brownish-orange with prominent, raised tufts of elongate, piliform setae along occiput; anterior and medial occipital setae brownish-orange; lateral occipital setae white. Labial palpus straight, directed ventrally, silvery white. Antenna ~ 0.9× the length of forewing; flagellomeres uniformly silvery white ventrally, dorsally with basal half of entire antenna dark fuscous, with basal half of each flagellomere gradually becoming more gray over distal half of antenna. **Thorax:** Most of pronotum and tegulae brownish orange, with a lateral pair of white streaks extending from white occipital tufts of head to mesonotum; venter of thorax white. **Forewing:** brownish orange with a conspicuous white streak along basal third of anal vein and four mostly white costal and two white dorsal strigulae, each partially bordered distally by a few black scales; second costal and first dorsal striae usually contiguous near middle of wing, forming a sharp angle; fringe white along dorsal margin, becoming pale brown toward apex. **Hindwing:** uniformly gray. Foreleg with femur black dorsally and silvery white ventrally; tibia black dorsally with two silvery bands ventrally; tarsomeres black, banded with silvery white; midleg with coxa pale brownish dorsally and streaked diagonally with black and white ventrally; tibia paler, brownish dorsally, mostly white ventrally; tarsomeres white with more narrow black bands; hindleg pale brownish with whitish diagonal stripes dorsally, mostly white ventrally with narrow black bands around tarsomeres. **Abdomen:** Gray dorsally, dull white ventrally with lateral diagonal streak of pale brown. Eighth abdominal sternite (Fig. 7) well developed; caudal margin broadly rounded to subtruncate. **Male genitalia** (Figs. 6, 8): Tegumen triangular, elongate, ~ 0.8 × length of valva. Valva elongate, slender with smoothly rounded apex. Transtilla broad, with anterior margin (caudal margin when reversed as in Fig. 6) prominently bilobed. Vinculum a narrow ventral ring terminating in an elongate, slender saccus ~ 0.6 × length of valva. Aedeagus a relatively straight, slender cylinder, gradually tapering to caudal apex, ~ 1.5 × length of valva; apex narrowly divided with 3–4 minute dentations along each border of cleft. **Female genitalia** (Figs. 9–12): Anterior apophyses ~ 0.55 × length of caudal apophyses. Ostium a simple, slightly enlarged

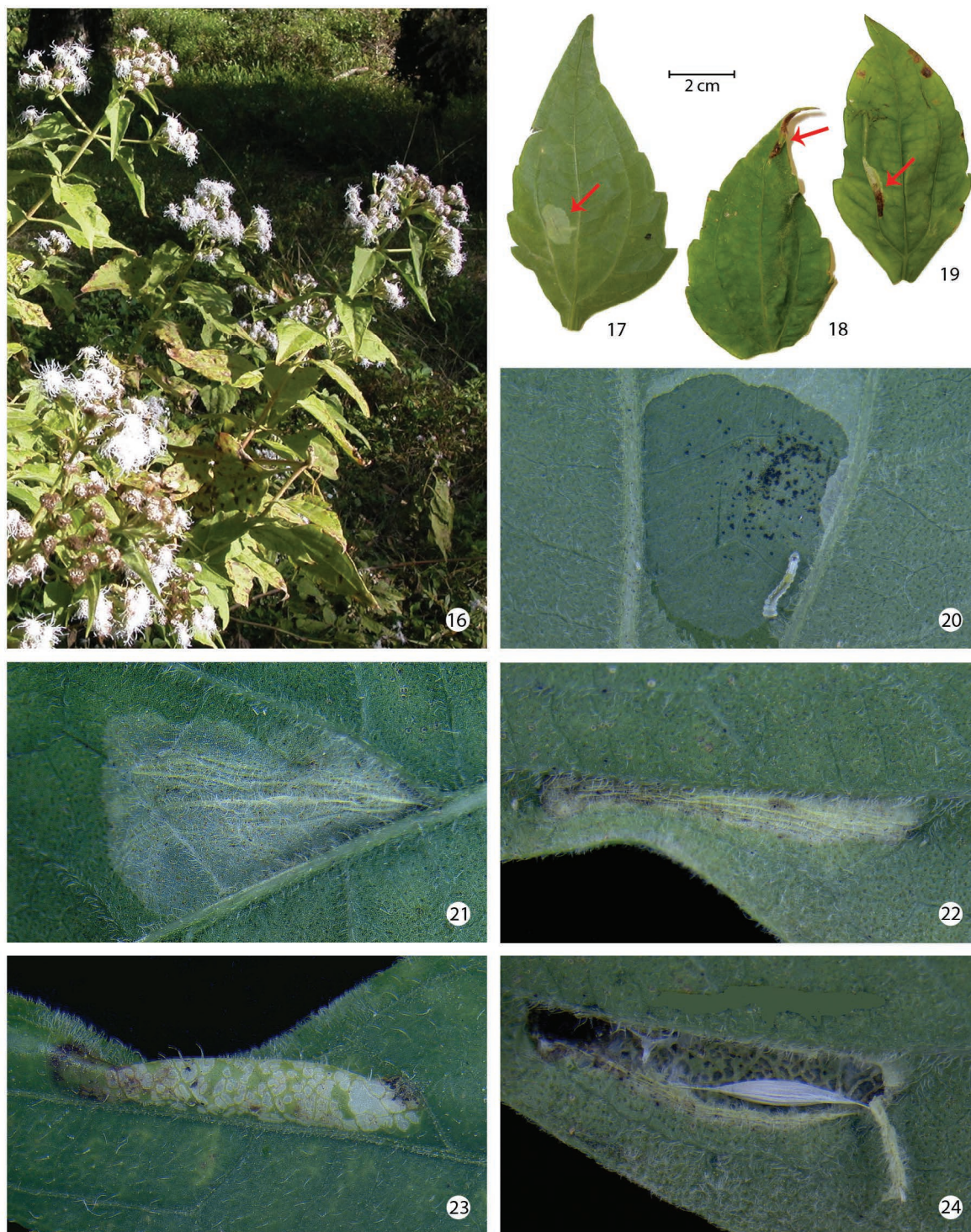


FIGS. 4–12. Adult morphology. **4.** *Cremastobombycia solidaginis*, head, anterior view. **5.** *C. solidaginis*, wing venation. **6–12.** *C. chromolaenae*, genitalia. **6.** Male genitalia, ventral view. **7.** Eighth abdominal sternite. **8.** Aedeagus. **9.** Female genitalia, lateral view. **10.** Signum, enlarged anterior view. **11.** Signum, enlarged lateral view. **12.** Female genitalia, ventral view.



FIGS 13–15. *Cremastobombycia ignota*, head morphology of last instar larva. **13.** Mouthparts, ventral view. **14.** Labrum and hypopharynx, anterior view. **15.** Hypopharynx (enlarged from Fig. 13), ventral view.





FIGS. 16–24. Leaf mines on *Chromolaena odorata* and larval biology of *Cremastobombycia chromolaenae*. **16.** *Chromolaena odorata*, photographed October 2011, Ft. Pierce, Florida. **17.** Early instar abaxial blotch mine (arrow). **18.** Adaxial leaf mine near center of leaf (arrow). **19.** Subapical adaxial leaf mine (arrow). **20.** Opened blotch mine with sapfeeding larva. **21.** Abaxial leaf mine showing the beginning of cuticle contraction (tentiform). **22.** Abaxial tentiform mine. **23.** Adaxial view of Fig. 22. **24.** Opened mine with single cocoon.



opening at caudal edge of eighth sternum. Ductus bursae ~ 1.75 × length of caudal apophyses; duct of accessory bursa arising midway along ductus bursae; corpus bursae with a small, strongly bilobed signum about midway along length of corpus bursae; apical margins of lobes minutely serrate; a single, relatively large, melanized, rugose disk midway along opposite wall of corpus. *Larval mine* (Figs. 17–24): Length: 12.9 ± 2.0 mm, width: 3.6 ± 0.8 mm (mean ± S.D., n = 81). The mine begins as a short, serpentine track which enlarges to a rounded, whitish blotch visible from the abaxial side of the leaf (Figs. 17, 20). Eventually the abaxial surface of the blotch contracts (Figs. 21–22) and the mine becomes narrow and tentiform (Figs. 22–23).

*Holotype*: ♂, UNITED STATES: FLORIDA: St. Lucie Co: Fort Pierce, near University of Florida Campus, 27.430° N, 80.410° W: 5 Dec 2011, R. Diaz, J. McClurg, Host: DRD 2710, *Chromolaena odorata* (L.), em. 12–19 Dec 2011. (USNM).

*Paratypes*: FLORIDA: Monroe Co: Key Largo: J Pennecamp St Pk, 10 ♂, 14–16 Nov 1986, DLW Lot 86L38, Host composite (*Eupatorium*?), em. 17 Nov 1986, 1 ♂, em. 20 Nov 1986, 1 ♂, 1 ♀, 1 ♀ digital image captured, em. 22 Nov 1986, 1 ♀, em. 24 Nov 1986, 2 ♂, 1 ♀, em. 30 Nov 1986, 1 ♂, 1 ♀, genitalia slide 34375, em. 1 Dec 1986, 1 ♀, em. 3 Dec 1986, 1 ♂, 1 ♂, genitalia slide 34374, 4 ♀, em. 8 Dec 1986, 1 ♀, em. 12 Dec 1986, 2 ♂, 1 ♀, em. 20 Dec 1986, 1 ♀, em. 3 Jan 1987, 1 ♂, em. 23 Jan 1987. St. Lucie Co: Fort Pierce: Same data as holotype; 16 ♂, 15 ♀; 5 ♂, 6 ♀; Fort Pierce: Selvitz Road: 27.404° N, 80.368° W: 3 ♂, 2 ♀, 11 Aug 2011, J. McClurg, K. Jordan, Host: *Chromolaena odorata* (L.), slide 34352; Fort Pierce: Weatherbee Road: 27.382° N, 80.330° W, 6 ♂, 6 ♀, 24 Oct 2011, Host: DRD 2708, *Chromolaena odorata* (L.), em. 28 Oct 2011, 2 ♂, 1 ♀, DOA 7 Nov 2011, R. Diaz, J. McClurg, K. Jordan, slides USNM 34344, 34347–34349, 34351, R. Diaz, J. McClurg, K. Jordan. TEXAS: Cameron Co: Southmost: Audubon Sabal Palm Refuge, 1 ♂, 1 ♀, 27–29 Dec 2000, em. 20 Jan 2001, DLW Lot 2000M12, Host *Eupatorium odoratum*. Hidalgo Co: Santa Ana Nat. Wildlife Refuge, vicinity Visitor's Center, 2 ♀, 30 Dec 1986, D. L. Wagner No: 86M39, 2 ♀, 11–14 Jan 1987, Host *Eupatorium odoratum*, slide USNM 34383. (Paratypes deposited in DLW, MGCL, USNM).

**Distribution.** Currently known from the general type locality, Fort Pierce, St. Lucie County, Florida, USA, south to Key Largo, Monroe County, Florida; also occurring in Cameron and Hidalgo Counties in southern Texas. Considering the broad, native range of its host, the actual distribution of the moth may be much greater than currently documented.

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

**Host.** Asteraceae: *Chromolaena odorata* (L.) King and H. Robins. Leaf mines containing *Cremastobombycia chromolaenae* were collected from three suburban sites (27.3816°N, -80.3297°W; 27.4036°N, -80.3672°W; 27.3913°N, -80.3495°W) in the city of Fort Pierce, St. Lucie County, Florida. All collection sites were highly disturbed areas located in open or partially wooded lots. Leaf mines on *C. odorata* were also found common by D. W. Wagner at J Pennecamp State Park, Key Largo, Florida and less so at two sites in southern Texas. Field observations taken at the same time that *C. chromolaenae* were collected in central (St. Lucie Co.) and south Florida (Miami-Dade Co.) revealed that it does not form mines on *Mikania scandens* (L.) Willd., *Mikania micrantha* Kunth or *Mikania cordifolia* (L. f.) Willd. (R. Diaz personal observations). Because *Mikania* is included within the same tribe (Eupatorieae) as *C. odorata*, these

observations suggest that *C. chromolaenae* may have a narrow host range.

**Life history.** Eggs of *C. chromolaenae* (diameter: 0.13mm, n = 2) are laid externally on the abaxial leaf surface near the mid vein. Larvae are hypometamorphic and possess two distinct larval body forms and feeding behaviors—an early stage sap-feeding form with a flattened body and prognathous mouthparts and a later stage tissue-feeding form with a more cylindrical body and possessing hypognathous mouthparts (Davis, 1987). As is typical of other *Cremastobombycia* studied, five larval instars were observed for *C. chromolaenae*, consisting of three sap-feeding instars and two later tissue-feeding instars. Head capsule widths were 0.16 ± 0.01, 0.18 ± 0.01, 0.24 ± 0.04, 0.30 ± 0.01 and 0.34 ± 0.01mm for larval instars 1–5, respectively (mean ± S.D.). First instar larvae initiate a narrow, short serpentine mine that in the later instars becomes visible from both sides of the leaf. Eventually the mine is expanded into an oval, whitish blotch (Fig. 17) which is formed under the abaxial cuticle. Observations suggest that the total leaf area of the mine is formed by the sap-feeding larval stages (Fig. 20). The tissue-feeding instars remove large amounts of the mesophyll layer of the leaf and the resulting injury becomes visible from the adaxial leaf surface as contiguous, whitish blotches (Fig. 23). As the tissue-feeding larvae mature, they lay down silken strands on the inner surface of the mine. Contraction of the blotch mine by the combination of this silk and the empty mesophyll cells creates the strongly cylindrical, tentiform leaf mines observed in the late instars (Figs. 21–23). Pupation occurs inside an elongate, spindle-shaped, silken cocoon (length: 6.1 ± 0.7 mm, n = 31) which is attached to the inner surface of the mine at both ends (Fig. 24). Before adult eclosion, the mature pupa (length: 3.9 ± 0.2 mm, n = 9) pierces the cocoon and leaf cuticle using the pointed frontal process of its head. Empty pupal cases were often found attached to the leaf cuticle. During the first two weeks of October 2011, leaves of *C. odorata* containing *C. chromolaenae* mines were collected from the field sites mentioned above. Leaves were placed in plastic bags, and subsequently examined in the laboratory to assess *C. chromolaenae* survival and sources of mortality. Upon arrival to the laboratory, each mine was inspected under the microscope for signs of holes. Mines with holes were dissected and mines without holes were placed individually in plastic containers and monitored for three weeks. The following criteria were used to assign sources of mortality; 1) death by parasitism: indicated by the presence of live immatures or pupal cases of parasitoids inside the mine or the emergence of parasitoid adults, 2) death by predation of sap-feeding

larva: indicated by a large entry hole on the leaf cuticle and the absence of mesophyll feeding damage in the inner surface of the mine, 3) death by predation of tissue-feeding larva or pupa: indicated by a large entry hole on the leaf cuticle and the presence of mesophyll feeding damage in the inner surface of the mine, 4) emergence of *C. chromolaenae* adult: indicated by the presence of a pupal case or the emergence of an adult, 5) unknown larval mortality: indicated by the presence of a decayed larva inside the mine. A total of 106 *C. chromolaenae* mines were collected. The fate of individuals were characterized as follows: 29% were parasitized, 2% were predated in the sap-feeding larval stage, 26% were predated in the tissue-feeding larval or pupal stages, 6% were dead due to unknown causes, and moths emerged from 29%. These results indicate that biotic mortality plays a major role in the population dynamics of *C. chromolaenae* within its native range.

**Natural enemies.** Braconidae: Microgastinae (unidentified to genus). Eulophidae: *Elasmus albicoxa* Howard; *Baryscapus* species. Approximately four species of parasitoid Hymenoptera were recovered from *Cremastobombycia chromolaenae* leaf mines and deposited in the USNM. Dissections of the leaf mines revealed that all parasitoids emerged from larvae of *C. chromolaenae*.

#### ACKNOWLEDGEMENTS

We are indebted to Vichai Malikul, Donald Harvey, and Carolyn Darrow of the Department of Entomology, Smithsonian Institution, for the illustrations, graphics, and the preparation of plates used in this publication. Harold Robinson, Department of Botany, Smithsonian Institution, provided information on the systematics of the host plant, *Chromolaena odorata*. Michael Gates and Robert Kula, Systematic Entomology Laboratory, USDA, ARS, PSI, identified respectively eulophid and braconid parasitoids. We are grateful to David L. Wagner, Department of Ecology and Evolutionary Biology, University of Connecticut, for the loan of specimens essential to this study. We also thank Julie McClurg and Katie Jordan of the Weed Biological Control Laboratory, University of Florida, for field collections and maintenance of insect colonies.

#### LITERATURE CITED

- BRAUN, A. F. 1908. Revision of the North American species of the genus *Lithocolletis* Hübner. Trans. Amer. Ent. Soc. 34:269-357, plates 20-24.
- DAVIS, D. R. 1983. Gracillariidae pages 2-11. In R.W. Hodges et al, Check List of the Lepidoptera of America North of Mexico. E.W. Classey Ltd. and the Wedge Entomological Research Foundation. London, 9-11.
- DAVIS, D. R. 1987. Gracillariidae, p.372-374, 376-378. In F.W. Stehr (editor), Immature insects, Vol. 1. Kendall/Hunt Publ. Co., Dubuque, Iowa.
- DAVIS, D.R. & G. DESCHKA. 2001. Biology and systematics of the North American *Phyllonorycter* leafminers on Salicaceae, with a synoptic catalogue of the Palearctic species (Lepidoptera: Gracillariidae). Smithsonian Contrib. Zool 614:1-89, figs. 1-451.
- DAVIS, D. R. & J. DE PRINS. 2011. Systematics and biology of the new genus *Macrosaccus* including descriptions of two new species (Lepidoptera: Gracillariidae). ZooKeys 98: 29-82.
- DAVIS, D. R. & S. MILLER. 1984. Gracillariidae and Lyonetiidae; pages XX, 6-7, 25-27. In J.B. Heppner (editor), Atlas of Neotropical Lepidoptera, Checklist: Part I, Micropterigoidea-Immoidea. Dr. W. Junk Publ., The Hague, Boston, Lancaster.
- DE PRINS, J. & W. DE PRINS. 2012a. Global Taxonomic Database of Gracillariidae (Lepidoptera). <http://www.gracillariidae.net> [accessed 11 January 2012].
- DE PRINS, J. AND A. K. KAWAHARA. 2012b. Systematics, revisionary taxonomy, and biodiversity of Afrotropical Lithocolletinae (Lepidoptera: Gracillariidae). Zootaxa 3594, pp. 1-283
- HOLM, L. G., D. L. PLUCKNETT, J. V. PANCHO & P. D. HERBERGER. 1977. The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu, HI. 400pp.
- KAWAHARA, A. Y., I. OHSHIMA, A. KAWAKITA, J. C. REGIER, C. MITTER, M. P. CUMMINGS, D. R. DAVIS, D. L. WAGNER, J. DE PRINS, & C. LOPEZ-VAAMONDE. 2011. Increased gene sampling provides stronger support for higher-level groups within gracillariid leaf mining moths and relatives (Lepidoptera: Gracillariidae). BMC Evolutionary Biology 11:182. doi: 10.1186/1471-2148-11-182.
- KRITICOS, D. J., T. YONOW, & R. E. MCFADYEN. 2005. The potential distribution of *Chromolaena odorata* (Siam weed) in relation to climate. Weed Res. 45: 246-254.
- MCFADYEN, R. C. 2002. *Chromolaena* in Asia and the Pacific: spread continues but control prospects improve. In Zachariades C, Muniappan R, Strathie LW (Eds.), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*. ARC-PPRI, Durban, South Africa, 23-25 October 2000. pp. 13-18
- MCFADYEN, R. C. 2004. *Chromolaena* in East Timor: history, extent and control. In Day, M. D. and McFadyen, R. E. (Eds.), Proceedings of the Sixth International Workshop on Biological Control and Management of *Chromolaena*. ACIAR Technical Reports 55. Canberra, Australia. pp. 8-10.
- SIEBERT, T. 1989. Biological control of the weed *Chromolaena odorata* (Asteraceae) by *Pareuchaetes pseudoinsulata* (Lep.: Arctiidae) on Guam and the Northern Mariana Islands. Entomophaga, 34:531-39
- ZACHARIADES C., M. DAY, R. MUNIAPPAN & G. V. P. REDDY. 2009. *Chromolaena odorata* (L.) King and Robinson (Asteraceae). In Muniappan R, Reddy GVP, Raman A (Eds.). Biological Control of Tropical Weeds using Arthropods. Cambridge Univer. Press. pp 130-162.
- ZACHARIADES, C., L. W. STRATHIE, E. RETIEF & N. DUBE. 2011. Progress towards the biological control of *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae) in South Africa. African Entomol. 19: 282-302.

Received for publication 17 February 2012; revised and accepted 20 August 2012.



TWO NEW SPECIES OF *ANTAEOTRICHA* ZELLER FROM SOUTHEASTERN ARIZONA  
(GELECHIOIDEA: ELACHISTIDAE: STENOMATINAE)

CLIFFORD D. FERRIS

5405 Bill Nye Avenue, R.R.#3, Laramie, WY 82070. Research Associate: McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL; C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Ft. Collins, CO; Florida State Collection of Arthropods, Gainesville, FL, cdferris@uwoyo.edu

**ABSTRACT.** . Two new species, *Antaeotricha baboquivariensis* and *Antaeotricha duckworthi*, are described from southeastern Arizona. Photographs are included of the type imagoes and their associated genitalia. Habitat images and a distribution map for the two new taxa are provided.

**Additional key words:** *Antaeotricha baboquivariensis*, *Antaeotricha demotica*, *Antaeotricha duckworthi*, *Antaeotricha zelotes*, Arizona, Elachistidae, Gelechioidea, North America, Stenomatinae, Taxonomy.

For the past several seasons, I have collected specimens of various *Antaeotricha* species from the southwestern United States using black light. Several undescribed species have been collected during this sampling. Duckworth (1964) reviewed the North American Stenomatinae and described two new species of *Antaeotricha*, expanding the North American species total to fifteen. These same fifteen species were listed by Hodges (1983). I subsequently have described the strongly maculated gray *Antaeotricha arizonensis* (Ferris, 2010), and the glossy white *A. utahensis* from New Mexico and Utah (Ferris, 2012), which brings the total to seventeen for described species from North America. During a recent examination of uncurated *Antaeotricha* specimens in a storage drawer, I discovered two additional species from Arizona that do not match any of the currently described North American or extralimital species.

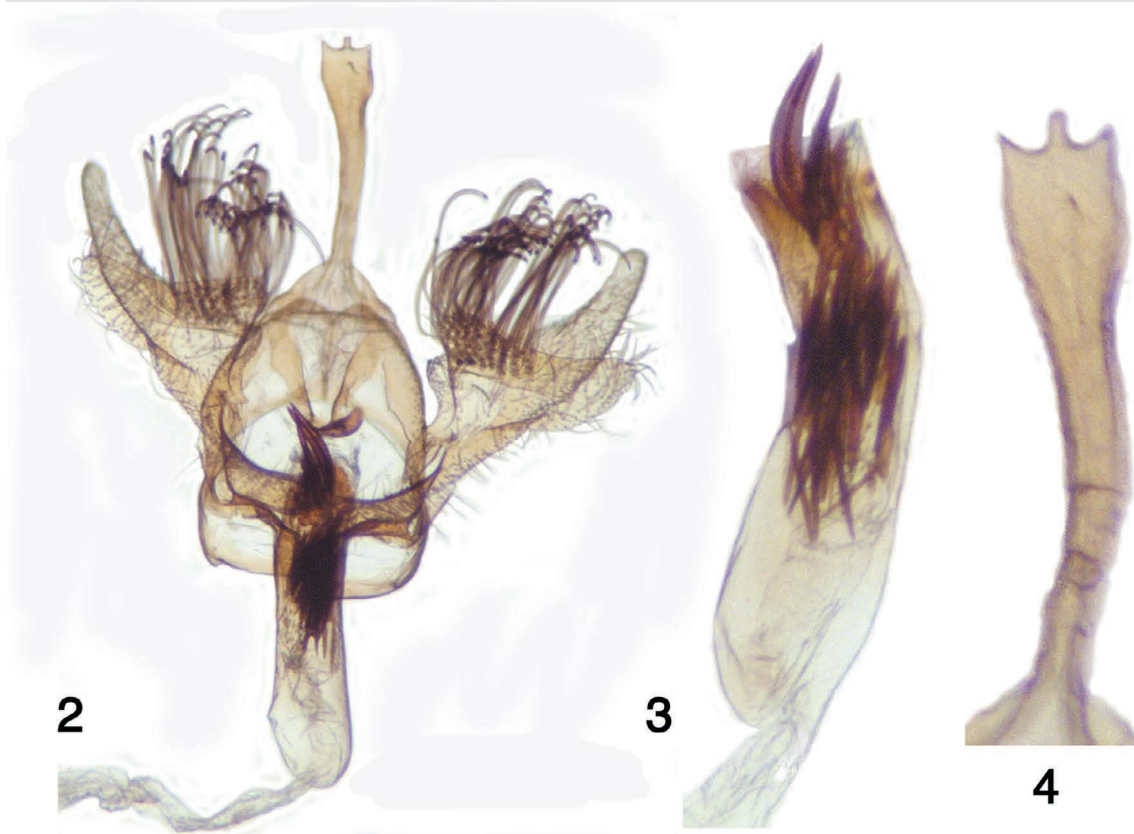
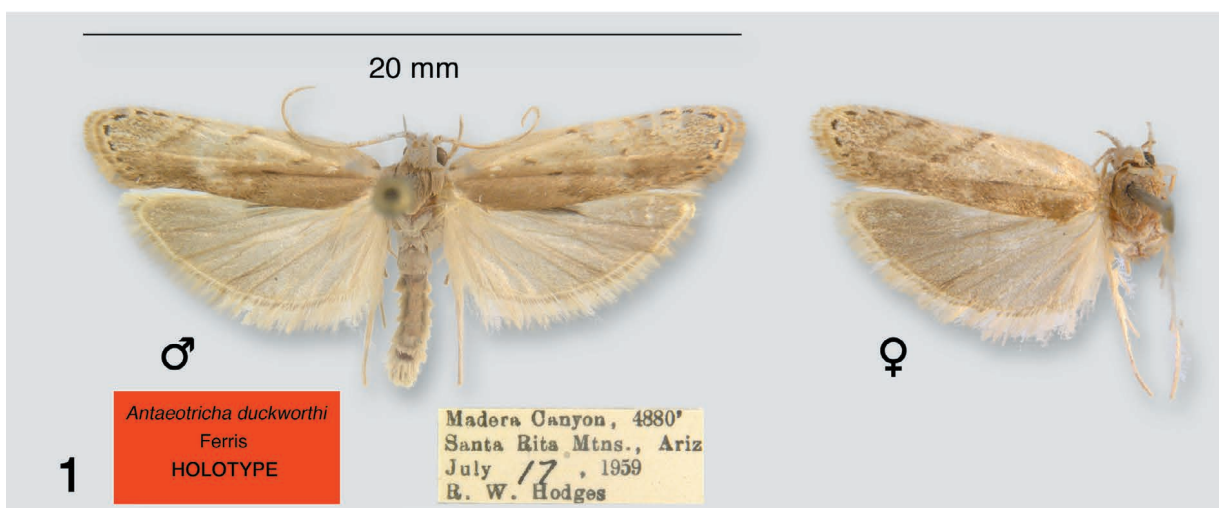
Genus *Antaeotricha* is restricted to the New World with the majority of the species occurring in Central and South America. Lee and Brown (2009) listed 382 species in the Neotropical region, five of which also occur in North America. The total number of described species is thus 394 with the addition of those that occur in North America only. The vast majority of the species were originally described by Meyrick (1914) and Walsingham (1909–15). Meyrick did not provide illustrations, while Walsingham illustrated adults of a number of species, but oddly enough not some that he described in the same publication. Clarke (1955) published a catalog of the Meyrick type specimens in The Natural History Museum [formerly British Museum (Natural History)] with photos of the adults and genitalia. The interested reader is directed to the bibliography provided by Duckworth (1964) for additional author citations relating to species descriptions and some larval host plants.

The larval host plants of many of the North American *Antaeotricha* are unknown. Several species use *Quercus* (Duckworth, 1964), and the Southwestern moths occur in oak habitat with oak as the presumed larval host. Aiello (1981) published some life history information for a species in Panama. Janzen and Hallwachs (2009) document numerous larval hosts used by *Antaeotricha* in Costa Rica.

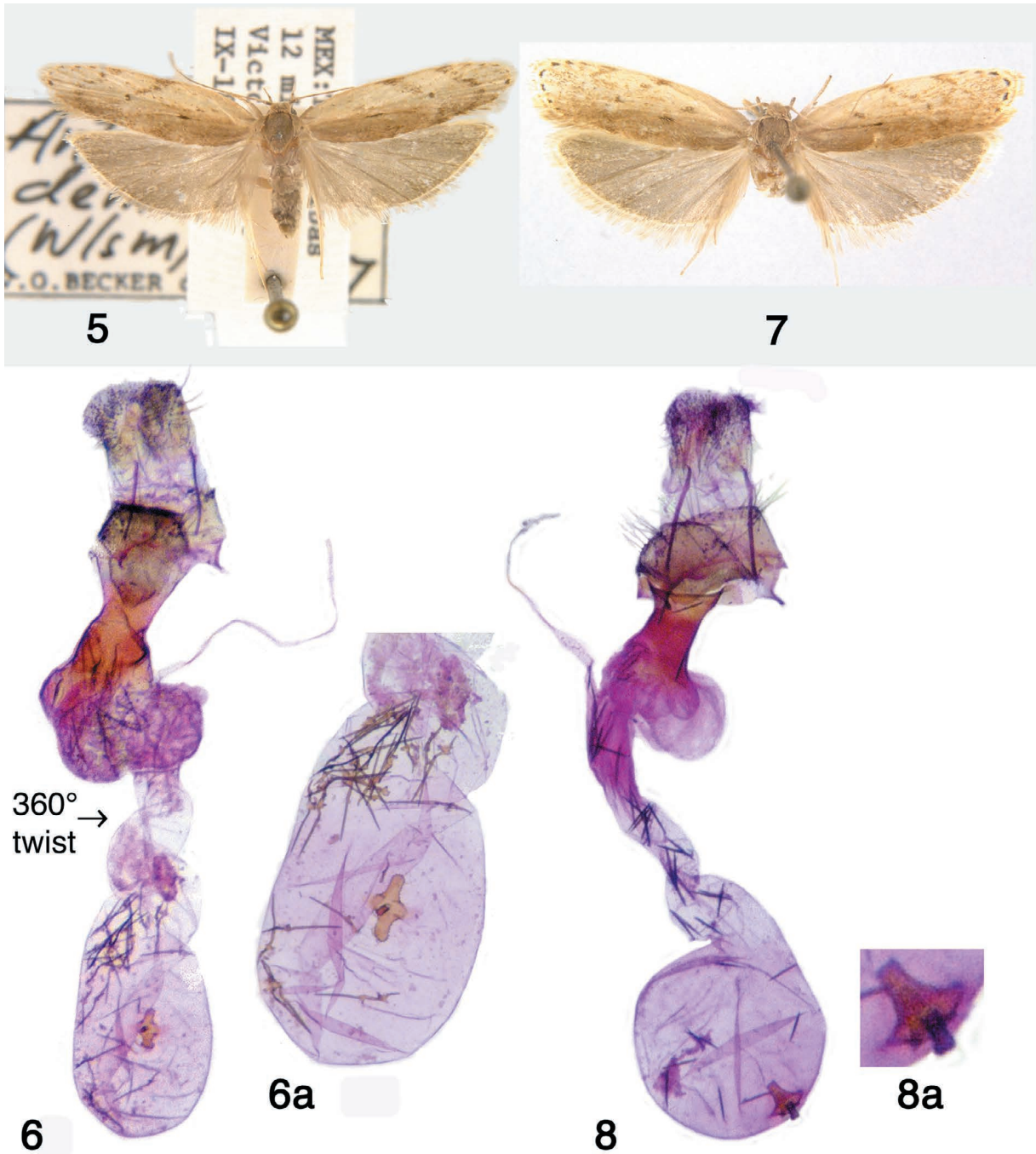
Members of the genus *Antaeotricha* are moderately large for Microlepidoptera, with forewing lengths in the North American species varying from about 7–15 mm, depending upon species. Females tend to be larger than the males, and are frequently less heavily maculated. With the exception of the gray *Antaeotricha arizonensis*, the dorsal forewing color is white. When present, maculation may be gray, brown, fuscous, or pale orange-brown. There is frequently a dark bar along the inner margin basally. Other regions of the forewing may exhibit small spots and/or narrow transverse bands. In some species, such as *A. furcata* (Wlsm.), the forewings of the males are strongly maculated, while the females may be nearly immaculate. Neotropical species display many additional wing patterns.

The genus *Antaeotricha* is most easily recognized by the anatomy of the male genitalia. The lightly sclerotized valves are narrow tapering to a rounded tip. The prominent harpe has a thumb-like costal projection bearing long, bifurcate, recurved setae (Figs. 2, 10–11). The form of the uncus is diagnostic for many species, and can usually be viewed under magnification while gently brushing away the abdominal terminal scales.

In the two new species, the tip of the uncus is broadly spatulate with three sharp projections along the apical margin, producing a trident-like aspect (Figs. 2, 4) in the first species (*duckworthi*) collected from multiple sites in southeastern Arizona, however, in the second species (*baboquivariensis*) from the Baboquivari Mts.,

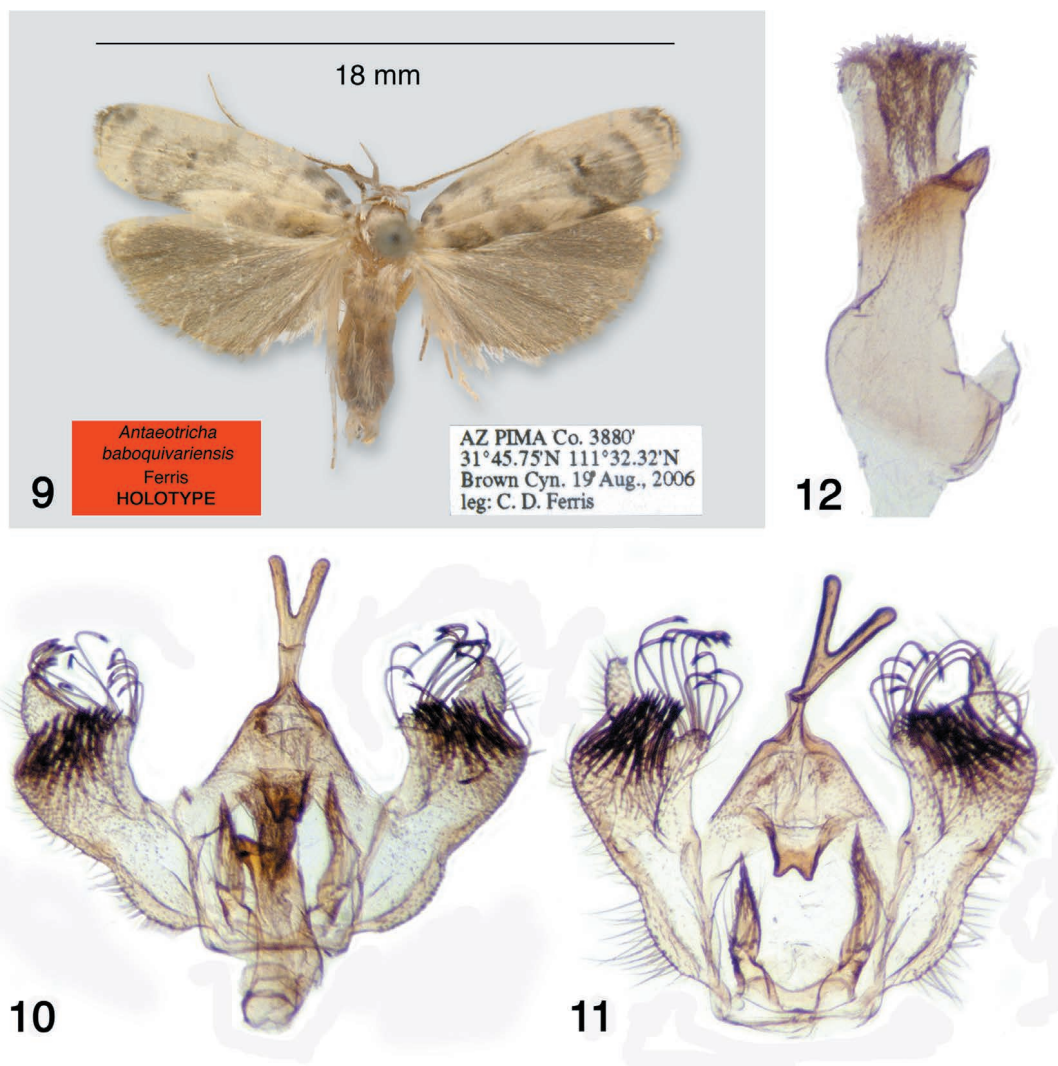


FIGS. 1–4. *Antaeotricha duckworthi*. 1, adult male holotype and female paratype (right wings and genitalia of female paratype mounted on USNM slide #76386). 2–4, male genitalia. 2, genital capsule, aedeagus *in situ*. 3, aedeagus (enlarged). 4, uncus.



FIGS. 5–8. 5–6, *Antaeotricha demotica* (Victoria, Mexico). 5, adult female. 6, female genitalia. 6a, enlarged view of corpus bursae and signum. 7–8, *Antaeotricha duckworthi* (Cochise Co., Arizona). 7, adult female. 8, female genitalia. 8a, enlarged view of signum. Note the presence of male cornuti in the ductus bursae of both species.





FIGS. 9–12. *Antaeotricha baboquivariensis*. 9, adult male holotype. 10–12, male genitalia. 10, genital capsule, aedeagus *in situ*. 11, genital capsule from another specimen, aedeagus removed. 12, enlarged view of aedeagus, vesica partially everted.



FIGS. 13–14. 13, Carr Canyon, Huachuca Mts. habitat. 14, Brown Canyon, Baboquivari Mts. habitat.

Pima Co., Arizona, it is deeply incised forming a long bifurcation (Figs. 10–11). No other known North American species have these configurations, nor could I find any similar extralimital species

In the possibility that one or both species might have been described from Mexico, or Central and South America, I examined available literature (especially Clarke, 1955 and Walsingham, 1909–1915) and contacted several museums and individual collectors requesting the loan of any look-alike material. No matches were found during the literature search. No specimens from Mexico or elsewhere were found to be matched to either species, although some additional specimens from Arizona were found of *Antaeotricha duckworthi* [Essig Museum (EME), University of California–Berkeley and Smithsonian Institution (USNM), Washington, DC]. *Antaeotricha zelotes* (Wlsm.) [Mexican states of Oaxaca, Sinaloa, Veracruz] looks rather similar to the latter, but the genitalia in both sexes are very different in structure, as described in the associated “Diagnosis.” A closer look-alike is *A. demotica* (Wlsm.) originally described from Guerrero, Mexico and Guatemala. The female type in the British Museum (NH) was examined by photograph. It has not been dissected. A series of additional female specimens (males are apparently unknown) in the EME from Tamaulipas, Mexico determined as *A. demotica* by V. O. Becker was examined and a dissection made. Again no match was found as described in the associated “Diagnosis.” The only other species found in which the male has a deeply incised uncus is *A. segmentata* Meyrick [TL British Guiana] (Clarke, 1955, pl. 58, figs 2–2b), but the other genital structures and the imago bear no resemblance to the moth from the Baboquivari Mts.

#### MATERIALS AND METHODS

Specimens were collected in bucket traps of my design using 8 watt BL fluorescent tubes operated from electronic power converters connected to 12 volt motorcycle batteries. Genitalia were dissected after macerating the abdomens in hot 10% KOH for fifteen minutes. Temporary slides were prepared using glycerin as the suspension medium. The genitalia preparations by the author are stored in glycerin in polyethylene genitalia vials attached to the specimen pins. Prepared balsam slides of wing venation and genitalia accompanied the USNM loan material.

#### *Antaeotricha duckworthi* Ferris, new species

(Figs. 1–4, 7–8)

**Diagnosis.** The combination of dorsal forewing markings separates *Antaeotricha duckworthi* from other

North America species: two narrow postmedian parallel transverse golden-brown lines; a series of small dark brown spots extending from the apical region of the costa along the outer margin to the tornus; a wide golden-brown diffuse longitudinal band extending along the inner margin from the base and tapering toward the tornus. The broad trident form of the uncus apex in males is unique among known North American species (Figs. 2, 4). In most specimens, the uncus can be seen clearly by gently brushing away the abdominal terminal scales. The moth can be confused with the Mexican *A. zelotes* and *A. demotica*. From *A. zelotes*, it can be distinguished immediately by genital characters. In males of *A. zelotes*, the basal 0.5 of the uncus is rather uniform in width, then broadens gradually over the next 0.4, and then attenuates to a weakly pointed apex in the distal 0.1, as opposed to the trident-like apex found in *A. duckworthi*. While the forewing maculation of *A. demotica* is similar to that of *A. duckworthi*, the forewings taper markedly toward the outer margin (Fig. 5), whereas in *A. duckworthi* the forewing width remains essentially unchanged (Figs. 1, 7). In the female genitalia of *A. demotica* (Fig. 6) there is a 360° twist in the ductus bursae, the corpus bursae is oval, the ductus seminalis originates from the base of a bulge at the top of the ductus bursae, and the signum has serrated edges, whereas in *A. duckworthi* there is no twist in the ductus bursae, the corpus bursae is spherical, the ductus seminalis originates from the tubular portion of the ductus bursae, and the signum has smooth edges.

**Description.** *Imago:* Glossy, sexes similar except antenna and genitalia. Except wings, remaining body parts (*thorax, legs, abdomen*) are covered by yellowish-white scales; a very few widely scattered individual brown scales occur on the legs and lateral abdomen. *Head.* Antenna (flagellum and scape) creamy white; ciliated ventrally in male, length of curved cilia about twice width of flagellomere; cilia in female. Haustellum tan, basal portion with creamy-white scales. Labial palpus upcurved extending well above vertex. *Tarsi.* Yellowish white blending to pale tan apically; pulvilli white, claws brown. *Wings.* Forewing length: males (n = 8) 9–10.5 mm, ave. = 9.5 mm; females (n = 26) 9–11.5 mm, ave. = 10.4 mm; elongate (length-to-width ratio = 4) with rounded apical margin. Yellowish white, anterior surface of wing very sparsely intermixed with minute individual brown scales; posterior surface with pale golden-brown scales forming a diffuse horizontal band; two narrow postmedian parallel transverse lines of conjoined pale golden-brown spots extending from costa to inner margin; both curving basad towards margin; area between distad line and outer margin darker than central pale region; small dark brown spots extend from apical section of costa along outer margin to tornus. Fringe pale yellowish white. Ventral surface of forewing dark golden brown shading to paler along costa and outer margin. Fringe pale yellowish white. Dorsal hindwing pale fuscous, darkening toward outer margin. Ventral hindwing centrally brownish fuscous shading to yellowish white along outer margin. *Male genitalia* (Figs. 2–4; 4 dissections). Uncus decurved with long narrow basal portion expanding to a squamiform trident-like tripartite apical process. Gnathos upcurved (flattened in Fig. 2 by slide preparation) at midpoint with distal portion tapering to a rounded tip. Vinculum broadly U-shaped. Anellus without distinct lobes. Valva basally broad



gradually narrowing to a rounded tip; harpe thumblike bearing many long recurved bifurcate setae. Aedeagus (phallus) long with bend near middle (length about 5 times diameter); vesica with multiple apparently deciduous (see Fig. 8) robust rod-like cornuti with pointed ends; three large curved cornuti with sharp tips extend from tip of aedeagus. *Female genitalia* (Fig. 8; 6 dissections). Papilla analis basally broad and straight with rounded apex, sparsely covered with short fine hairs. Apophyses posteriores well developed; anterior apophyses vestigial. Sterigma broad. Ductus bursae broad and lightly sclerotized immediately below sterigma to auxiliary spherical sac, then narrowing to long membranous tube expanding as it opens into spherical corpus bursae; ductus seminalis arises from ductus bursae just below and opposite to spherical sac. Signum near fundus a large cross with central outwardly projecting oblong plate perpendicular to base; plate nearly rectangular with rounded edges and corners.

**Types.** Holotype male (Fig. 1): ARIZONA, [Pima/Santa Cruz Co. line], Santa Rita Mts., Madera Canyon, 4880' (1488m), 17.vii.1959, R. W. Hodges. Deposited in National Museum of Natural History (USNM), Washington DC. Six paratypes deposited in USNM; same locality and collector as holotype: 1♂, 9.vii.59, USNM slide #76385; 1♂, 17.vii.59, USNM slide #76461; 1♂, 29.vii.59, USNM slide #76462; 1♀, 24.vii.59, USNM slide #76386; 1♀, 30.vii.59, USNM slide #76464; 1♀, 4.viii.59, USNM slide #76463. Slides contain genitalia and right wings cleared to show veins. Two additional paratypes in USNM: ARIZONA, [Santa Cruz Co.], Santa Rita Mts., Madera Canyon, 5000' (1530m), 25.viii.79, 1♀, R. Leuschner & R. Crandall; Santa Cruz Co., 5000' (1530m), 9.viii.87, 1♂, R. Leuschner. In Essig Museum of Entomology, University of California, Berkeley, CA: ARIZONA, Cochise Co., Huachuca Mts., 5800' (1769m), 3.viii.86, 1♂, J. Brown & J. Powell. In C. D. Ferris collection: ARIZONA, Cochise Co., Huachuca Mts., Carr Canyon, 5615' (1713m), 21.viii.04, 1♂ (dissected), 27♀ (4 dissected); 27.vi.05, 1♀.

**Biology.** Unknown; adults from late June to late August. Collection localities are moderately dry forest with oak and mixed conifer species (Fig. 13).

**Distribution.** To date, known only from southeastern Arizona (Fig. 15).

**Etymology.** This species is named in honor of W. Donald Duckworth, whose revision of the North American Stenomidae initiated my interest in the group.

**Remarks.** As previously noted, the cornuti attached to the vesica in males of *A. duckworthi* are apparently deciduous, as indicated by their presence in the female genitalia (Fig. 8). Although the males of *A. demotica* are apparently unknown, it can be inferred from Fig. 6 that they too have long, slender, needle-like cornuti (longer and thinner than those of *A. duckworthi*).

***Antaeotricha baboquivariensis* Ferris, new species**  
(Figs. 9–11)

**Diagnosis.** Females unknown. In habitus, *Antaeotricha baboquivariensis* most closely resembles *A. schlaegeri* (Zeller) and *A. leucillana* (Zeller). Visual separation of these species is not reliable. In *A. baboquivariensis*, there is a dark gray broken bar extending along the costa from the base to approximately 0.2 costal length. In the other two species, this region is either unmarked, or there are two small gray spots. The dorsal hindwings of *A. schlaegeri* are pale, in *A. leucillana* they are fuscous, and in *A.*

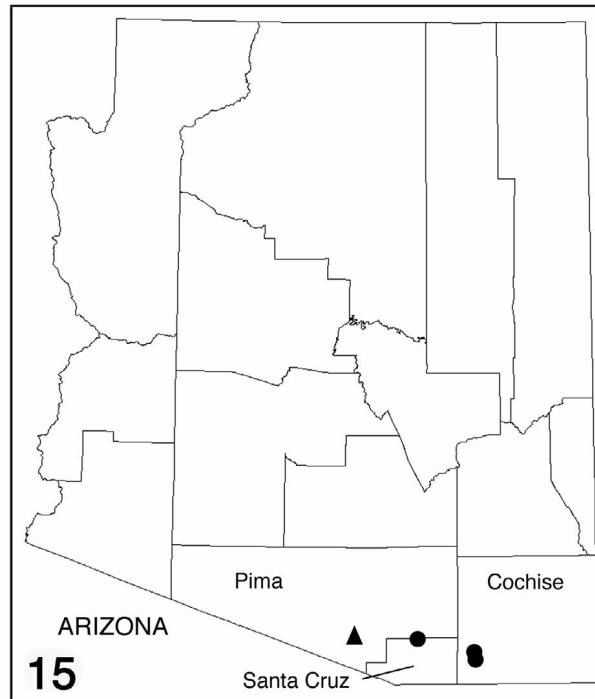


FIG. 15. Distribution map. Circles = *Antaeotricha duckworthi*; triangle = *A. baboquivariensis*.

*baboquivariensis* they are dark fuscous. *A. schlaegeri* has been recorded from the Huachuca Mts. of Arizona, but there are no Arizona records for *A. leucillana*. The deeply incised bifurcate uncus (Figs. 10–11) of *A. baboquivariensis* provides unequivocal separation from all other known North American species. The uncus of *A. schlaegeri* is uniformly slender with rounded apex (Duckworth, 1964, fig. 1); the uncus of *A. leucillana* has a widely expanded bifid tip (Duckworth, 1964, fig. 4).

**Description.** *Head.* Antenna scape brown, flagellum brown with narrow light brown rings at junction of each flagellomere, ciliated ventrally, cilia curved with length about equal to width of flagellomere. Haustellum pale tan, basal portion with white scales. Labial palpus upcurved extending well above vertex, segments 1–2 dark tan on outer surface, light tan on inner surface, segment 3 very light tan. Frons and vertex creamy white. *Thorax.* Creamy white. *Legs:* foreleg brown dorsally, shading to whitish ventrally and in tarsi; midleg similar to foreleg, but paler; hindleg whitish tan speckled with a few isolated brown scales; tarsal claws brown, pulvilli white. *Abdomen:* Pale tan with a few widely spaced single dark brown scales. *Wings.* Forewing length: (n = 5) 7–9 mm, ave. = 8.3 mm; length-to-width ratio about 2.6 with a slightly rounded distal margin. Dorsal forewing white with five transverse zones/bands with diagonal margins: 1. basal third of wing with mottled gray wide irregular bar along inner margin, white central area and narrow gray irregular bar along costa.; 2. white band; 3. gray band with irregular distal border and two central black conjoined irregular spots; 4. white band with convex distal margin and central narrow pale gray band; 5. gray roughly triangular apical patch with narrow white submarginal band. Fringe scales white with those near apex gray tipped. Ventral forewing pale fuscous centrally becoming very pale along margins; fringe scales nearly white. Dorsal hindwing dark fuscous, pale along inner margin, fringe scales nearly white. Ventral hindwing centrally pale fuscous becoming very pale along margins and fringe. *Genitalia* (Figs. 10–12); 2 dissections). Uncus



decurved, divergently bifurcated along apical half from basal stem; bifurcation with two narrow equal tines, each rounded apically. (Figs. 10–11). Gnathos widened basally, gradually tapering to a slightly upcurved [flattened by slide preparation] deeply notched tip (Fig. 11). Vinculum U-shaped. Anellus with two large robust slightly subequal lobes projecting upward to level of gnathos. About one-third length from base, each lobe begins to bow outward and is partially encircled by row of robust nearly transparent, pointed setae of length about one-third the remaining distance to the tip. Above base of these setae, each lobe tapers to a sharp point and has a scobinate surface. Valva basally broad tapering to narrow rounded tip; harpe thumblike bearing many long recurved bifurcate setae. Aedeagus short with bulbous base (length about 2 times diameter) bearing a triangular heavily-sclerotized projection on apicolateral margin. Vesica without cornuti, but membrane outer surface densely covered with small close-set tubercles.

**Types.** Holotype male (Fig. 9): ARIZONA, Pima Co., Baboquivari Mts., Brown Canyon, 3880' (1183m), 19.viii.2006, C. D. Ferris. Deposited in Carnegie Museum, Pittsburgh, PA. Paratypes: 4 males (2 dissected), same data as holotype, in C. D. Ferris collection.

**Biology.** Unknown; adults in late August. Collection locality is a riparian desert mountain canyon with a wide range of vegetation that includes oak (Fig. 14).

**Distribution.** To date, known only from the type locality (Fig. 15).

**Etymology.** The species name (adjective) denotes the geographic locality where the type series was collected.

#### ACKNOWLEDGEMENTS

For providing general information, loan material, specimen photos, and checking museum holdings, my thanks go to: John W. Brown, Smithsonian Institution, National Museum of Natural History, Washington, DC, Jerry Powell and Peter T Oboyski, Essig Museum of Entomology, University of California, Berkeley, CA for the loan of examination material; to Martin Honey (BMNH), London, UK for supplying a photo of the holotype of *Antaeotricha demotica*; to Julian Donahue who kindly searched (unsuccessfully) the collection at the Natural History Museum of Los Angeles County for specimens of the two new species; to Eugenie Phillips-Rodriguez, Guanaaste, Costa Rica and Victor O. Becker, Camacan, Brazil who examined photographs of the two new species and confirmed that they were unknown to them; to Bruce Walsh, Tucson, AZ for ar-

ranging access into Brown Canyon; to an anonymous reviewer who provided useful comments; to David Adamski, Smithsonian Institution, National Museum of Natural History, Washington, DC for providing useful comments on the manuscript and checking specimens in the collection. Special thanks to Ralph and Rosemary Snapp and Noel and Dienne McFarland for allowing me to camp and collect on their respective properties in the Huachuca Mts.

#### LITERATURE CITED

- AIELLO, A. 1981. Life History of *Antaeotricha* sp. (Lepidoptera: Oecophoridae: Stenommatinae) in Panama. *Psyche* 88:163–168.
- CLARKE, J. F. G. 1955. Catalogue of the type specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick. 2:11–531, plates 1–263.
- DUCKWORTH, W. D. 1964. North American Stenommatidae (Lepidoptera: Gelechioidea). *Proc. United States Nat. Mus.* 116:23–72.
- FERRIS, C. D. 2010. A new *Antaeotricha* species from southeastern Arizona (Gelechioidea: Elachistidae: Stenommatinae). *ZooKeys* 57:59–62.
- . 2012. A new *Antaeotricha* species from Utah and New Mexico (Gelechioidea: Elachistidae: Stenommatinae). *Journal of the Lepidopterists' Society* 66(3):168–170.
- HODGES, R. W., et al. 1983. Check List of the Lepidoptera of American North of Mexico. E. W. Classey Ltd. and the Wedge Entomological Foundation. xxiv + 284 pp.
- JANZEN, D. H. AND HALLWACHS, W. 2009. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservacion Guanacaste (ACG), north-western Costa Rica (nn-SRNP-nnnnn voucher codes) <<http://janzen.sas.upenn.edu>>.
- LEE, S. AND BROWN, R. L. 2009. Revised checklist of Elachistidae in Neotropical region. <http://mississippientomologicalmuseum.org/misstate.edu/Researchtaxapages/Lepidoptera/Elachistidae/Checklists/Neotropical-Elachistidae.html>.
- MEYRICK, E. 1915. Exotic Microlepidoptera, vol. 1. Taylor and Francis, London, 640 pp.
- WALSINGHAM, L. 1909–1915. In Godman, E.D. & Salvin, O. *Biologia Centrali-Americana. Insecta. Lepidoptera–Heterocera*, vol. IV. Taylor and Francis, London, 404 pp., plates 1–10.

*Received for publication 12 March 2012; revised and accepted 30 June 2012.*

INTERPRETATION OF WING PATTERN ELEMENTS IN RELATION TO BIRD PREDATION ON  
ADULT *HYALOPHORA* (SATURNIIDAE)

MICHAEL M. COLLINS

Research Associate, Section of Invertebrate Zoology, Carnegie Museum of Natural History, 215 Prospect St., Nevada City, California 95959  
e-mail: michaelmerlecollins@comcast.net

**ABSTRACT.** A large sample of trapped adult males of *Hyalophora euryalus*, *H. columbia gloveri*, and intergrades from a hybrid zone between these taxa were scored for seven categories of wing damage. A total of 6.1% of 471 adults showed wing damage consistent with bird strikes. Among categories of wing damage, a significantly higher frequency of strikes involved the HW discal spot and the apical eye spot. Patterns of wing damage and behavior of adults when threatened were evaluated in the context of formal models of wing markings as anti-predator mechanisms. Discal spots may act to intimidate predators, while apical eye spots may deflect attacks away from the body. Certain markings are highly conserved and concordant with DNA-based saturniid phylogeny.

**Additional key words:** anti-predator mechanism, defensive behavior, defense mechanism, discal spot, eye spot, silk moth.

Biologists historically have been fascinated with interpreting the adaptive significance of wing patterns in Lepidoptera, and have categorized them as playing a role in mating behavior, crypsis and camouflage, mimicry, or as anti-predator mechanisms (Bates 1863, Blest 1957, Brakefield & Monteiro 2003, Carpenter 1941, Cott 1940, Darwin 1859, Poulton 1890).

Birds are considered important predators of butterflies and of moths at rest during daytime. Observations of bird predation on Lepidoptera in the wild, outside of staged situations, are understandably rare. Experimental evidence is provided in Bowers et al. (1985), the classic work by Kettlewell (1958), Majerus (2009), and Sargent (1973; 1976; 1990). Shapiro (1974; pers. corres.) states, based on extensive field experience, that birds are more likely to attack resting butterflies, especially just after daybreak, than they are likely to take them on the wing.

The adults of many saturniids display conspicuous patterns and markings, including discal spots and apical markings, some of which closely resemble vertebrate eyes. Recently, Howse & Wolfe (2012) illustrated many types of saturniid wing patterns and, as a stimulus for research on the role of wing patterns in defense, offer their interpretation of what natural objects a given marking may resemble in the eyes of a predator. Experimentally demonstrating how a predator perceives a specific wing marking and establishing the true adaptive role of such markings in saturniids and other Lepidoptera is difficult. A scientifically rigorous study requires understanding the physiology of vision in a predator (in contrast to human perception), as well as conducting experiments that reveal the behavior of both predator and prey in the wild (Stevens 2005). This is not a controlled study of this type. Rather, I used an extant series of trapped males to analyze types of wing damage

in order to estimate the relative frequency of predator attacks directed toward specific wing pattern elements. I discuss pattern features, such as discal spots and the apical eye spot, in terms of current models of the adaptive role wing pattern elements may play as anti-predator mechanisms.

## METHODS AND MATERIALS

Long series of adult *Hyalophora euryalus* and *H. columbia gloveri* (hereafter *gloveri*) from various geographic populations, including a hybrid zone between them, were amassed over many years in connection with phylogeographic studies (e.g. Collins 1984; 1997). Those males collected in funnel traps, baited with virgin females as a pheromone source, were segregated and examined for wing damage characteristic of bird attacks. The studies of Sargent (1973; 1976; 1990) depict patterns of wing damage, primarily in underwing moths (*Catocala*: Noctuidae), based on observations of bird attacks in the field and with captive birds. Wing damage due to avian predation on butterflies has been characterized by Bowers et al. (1985) and Bowers & Wiernasz (1979). Bird strikes tend to produce characteristic damage, often with straight edges and in some cases V-shaped marks or tears on the wings, typically cutting through one or more wing veins. Underwing moths rest with their wings flattened, the fore wing drawn back over the hind wing, while *Hyalophora* rest butterfly-like, with the wings held vertically. Thus, in comparing the damage seen in *Catocala* to the putative bird strikes on *Hyalophora*, the shape of a bird strike could be expected to be similar in the two moth families but the position of the strike is likely to be different. Birds are hypothesized to be the most likely predator responsible for the characteristic damage I observed in my sample set. I attempted to use

a method of scoring and analysis that is not biased by this prediction, but rather seeks to evaluate if there is a non-random association of damage with respect to wing pattern elements. Damage due to encounters with inanimate objects is unlikely to be disproportionately associated with specific wing markings.

I surveyed all damaged specimens and scored them for seven categories of wing damage: **a** - a missing portion of the fore wing (FW) apex; **b** - a more-or-less rectangular piece torn from the inner margin of the FW and the adjacent costa of the hind wing (HW); **c** - a U- or V-shaped segment torn from the HW outer margin, directed toward, and in some cases touching, the HW discal spot; **d** - a U- or V-shaped segment of the FW outer margin similarly directed toward the FW discal spot; **e** - a hole in, or encompassing, the FW discal spot; **f** - a corresponding hole in the HW discal spot; **g** - a more-or-less rectangular section missing from the HW inner margin including the anal angle (Fig. 1).

Wing damage, often in the form of nearly uniform frayed edges on all four wings, occurs due to wear and tear as a consequence of males seeking "calling" females. This damage is cumulative, so that one can distinguish newly enclosed adult males from those several days old. In captivity males live from 5 to 8 days but judging the exact age of wild-caught males is difficult. Predator strikes may be superimposed on this kind of damage, but in most cases the two can be distinguished, in part from the characteristic shape of bird strikes and also, although deep tears may occur between wing veins in worn specimens, wing veins are usually nearly intact in worn specimens or broken only near their terminus (as typically seen in captive, ovipositing females). Wing damage scores were totaled by category and by taxon and compared for significant difference in frequency by means of  $\chi^2$  tests. A null hypothesis of equal probability of a predator strike was assigned to each wing damage category.

## RESULTS

The results of the wing damage survey are presented in Table 1. Representative specimens illustrating wing damage by category are shown in Fig. 2. An average of 6.1% of the total of 471 adult males for all species and populations showed wing damage judged to be likely due to bird strikes. Twenty one specimens displayed multiple strikes, with an average of 1.9 strikes per damaged moth (56 total strikes / 29 damaged individuals). Eight moths showed single strikes and of these six were damaged at the FW apex. Predation rates were higher in *euryalus* (8.46%) compared to *gloveri* (4.52%), a suggestive but not significant difference ( $\chi^2 = 2.56$ ,  $P > 0.10$ ). By its very nature, the hybrid zone

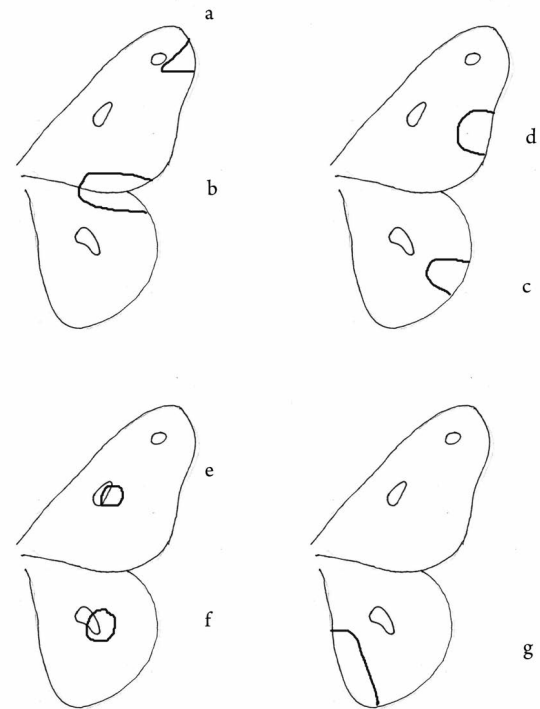


FIG. 1. Wing Damage Categories. Letter designations for categories follows Methods section.

population (Monitor Pass, Alpine and Mono Cos., CA) displays a great deal of variation in many wing pattern characters, but the relatively small sample (3/71) could not be statistically analyzed for individual wing damage categories in comparison to the parental taxa.

The frequency with which different categories of wing damage were observed was highly significant, based on an assumption of equal probability of a strike for all categories ( $\chi^2 = 19.76$ ,  $P < 0.005$ ). To address the potential problem of small sample size, especially for the important discal spot categories, the  $\chi^2$  was recalculated combining categories **e** and **f**:  $\chi^2 = 11.53$ ,  $P < 0.05$ , still a significant difference among categories of wing damage.

Categories **a** (the FW apex where the small "vertebrate eye" spot occurs) and **c** (near the HW discal spot) each comprised 25% of strikes, category **b** (at the junction of the margins of the FW and HW) made up 21.4%. The size and shape of the elongated, comma-shaped, HW discal spot of *euryalus* is significantly different compared to the smaller, kidney-shaped spot in *gloveri* (Collins 1984). However, the rate of attack on the HW discal spot does not differ significantly between the two species, using the combined scores for all categories associated with the HW discal spot (**c** + **f** + **g**) ( $\chi^2 = 3.67$ ,  $P > 0.90$ ). In considering the role the HW





FIG. 2. Sample specimens scored for wing damage by category (a–g; l = left side, r = right side). (2-1) *euryalus* a, b–l. CA Alpine Co., Crystal Sp. Cmpgd. 4 June 1978. (2-2) *gloveri* b–l,r; d–r. MT Madison/Gallatin Co. line. hwy 287, 15 June 1983. (2-3) *euryalus* e–l, f–l,r. CA Alpine Co., Carson Cn. 3 June 1979. (2-4) *intergrade* d–l, c–l,r. CA Mono Co., e Sonora Pass, 10 June 1979. (2-5) *gloveri* e–l, c–l,r, b–r. CA Inyo Co. Death Valley NM, Thorndike Cmpgd. 30 May 1981. (2-6) *gloveri* g–l. CA Mono Co., Bridgeport. 28 May 1966.

discal spot may play in affecting predator behavior, one damaged *euryalus* specimen is particularly noteworthy; this male (Fig. 2–3; scored for both e and f categories) displayed neat holes in three of the four discal spots, precisely centered in the spot, presumably from bird pecks and likely the result of a single attack. Such multiple occurrences of damage, of a shape unlikely to be caused by wear and tear and in such close association with specific wing pattern features, strongly supports the interpretation of avian predation directed toward such features.

#### DISCUSSION

An inherent complication in a forensic study of this kind is that predation on the two test subjects—*euryalus* and *gloveri*—is occurring not in a single controlled environment but in separate biological realms (essentially Sierra/Cascade vs. Great Basin and Rocky Mt.), which differ in many key elements, such as plant community types, species of predator, their abundance, and their predatory behavior. In considering the higher predation rate for *euryalus* compared to *gloveri*, and especially should future work find that

TABLE 1. Wing damage scores of trapped *Hyalophora* males by damage category.

Taxon	Locality	Wing Damage Category							# ♂♂	Total ♂♂	%
		A	B	C	D	E	F	G	Damaged	Scored	Damaged
<i>H. c. gloveri</i>	Rocky Mts.	2	4	1	1	1	1	0	5	130	3.85
<i>H. c. gloveri</i>	CA e slope										
	Sierra Nevada	3	0	1	1	0	0	1	4	69	5.8
	Subtotal	5	4	2	2	1	1	1	9	199	4.52
<i>Intergrades</i>	CA Monitor Pass										
	Alpine/Mono Co.	2	2	2	1	0	0	1	3	71	4.23
<i>H. euryalus</i>	CA Sierra Nevada	2	1	3	1	0	0	0	5	82	6.10
	CA Alpine Co. Ebbett's Pass- Markleeville	5	5	7	2	2	3	1	12	119	10.10
	Subtotal	7	6	10	3	2	3	1	17	201	8.46
Total		14	12	14	6	3	4	3	29	471	6.1
Expected		8	8	8	8	8	8	8			
% of 56 total, all categories		25.0	21.4	25.0	10.7	5.4	7.1	5.4			

difference to be statistically significant, it is important to remember that the frequency of recovery of wing-damaged moths cannot be extrapolated directly to survival rates. Either *gloveri* or *euryalus* might suffer significantly more attacks, but better survive such encounters compared to the other species.

Quantifying wing damage due to predation is inevitably somewhat subjective and subject to several sources of error, including: (1) missed scores due to extensively frayed wings, (2) frayed wings mistakenly scored as damaged by predator attack, (3) damage attributed to birds but actually due to other predators, such as bats, rodents, or lizards, and (4) missing certain strikes that were obscured by subsequent damage suffered during multiple strikes. Nevertheless, the results demonstrate that predators (probably birds) appear to direct their attacks primarily toward the discal spots and the apical eyespot, even though we cannot know for sure how a given bird species perceives and discriminates a given element as part of the entire wing pattern in *Hyalophora*.

**Behavior of *Hyalophora* with respect to predation.** Adults of *Hyalophora* rest with their wings folded dorsally, with the HW largely obscuring the FW. While brightly colored on the upper surface, the undersurface wing color is drab–dull reddish brown to

dark brown in *euryalus* and dull grey to nearly black in *gloveri*, with white scales giving a salt-and-pepper effect, especially in *gloveri* (Collins 2007, pl. 12; Tuskes et al. 1996). The “ecological image” of resting *Hyalophora* is much more similar among all taxa than that seen in the upper wing surfaces. The upper surface color in *euryalus* ranges from a bright red to reddish brown; that in *gloveri* is a bright wine-red to dark red or burgundy. The small subspecies *H. columbia columbia* from the Great Lakes region is significantly darker, in some specimens the upper surfaces are nearly black.

My observations of western *Hyalophora* closely parallel that of Evans (1978) for *H. cecropia*. If disturbed during the day adults quickly open their wings and slowly fan them rhythmically open and closed. This response may be triggered even in response to nearby motion. Resting adults cannot fly immediately but must first vibrate their wings at high frequency but small amplitude in a “shivering” behavior. Such behavior raises wing muscle temperature to a minimum required for flight, the duration of shivering dependent on ambient temperature (Hannegan & Heath 1970, Heinrich 1981, Tuskes et al. 1996). Males may disperse at dusk, but their mating flight occurs from about 0300 until dawn. Females remain inactive until mated and then begin their oviposition flight the following night.

As is typical of most saturniids, *Hyalophora* populations occur at low density, widely dispersed, and usually over a range of plant communities (Ferguson 1971, Tuskes et al. 1996). This population structure, in conjunction with their relatively short life spans, would appear to minimize the likelihood that birds or other predators could form a search image or otherwise concentrate their search for prey on saturniids such as *Hyalophora*. The behavior of *Hyalophora* adults in relation to predation can thus be summarized as follows. Adults resting during the day are more-or-less cryptic or at least inconspicuous. If disturbed during the day they first display their upper wing surfaces; both the large discal spots and the apical eyespot are presented, the former set off against a reddish background and the latter set maximally apart at the wing tips. Display behavior and wing markings are thus closely integrated aspects of an anti-predator defense mechanism. If attacked by a bird it seems likely that an adult would be able to fly only if the attack was somehow delayed or prolonged and ineffective. Presumably the majority of trapped adults bearing what appear to be bird strikes were discovered during the day while at rest and survived by either deflecting the attack away from their bodies or by intimidating their attacker.

Little information is available to judge the ability of adults taking flight during the day to survive bird attacks. I have several times released adult *Hyalophora euryalus* and *Antheraea polyphemus* (which possess very large, eye-like HW discal spots) and provoked them into flight during the day. Invariably, they flew some distance until out of sight, and the *polyphemus* adults especially flew well up into the crowns of large oaks (in an oak-mixed conifer forest of the western Sierra Nevada). Although I did not keep exact records, about half of perhaps ten releases for both species resulted in vigorous attacks by Steller's jays (*Cyanocitta stelleri*), which in all cases quickly killed and consumed the moths after perching on a nearby branch.

Nocturnal flight in *Hyalophora* must minimize exposure of both sexes to bird predation, and the early morning flight of males may avoid the period of peak activity of bats (Collins 2007). Predation by rodents or other mammalian predators on resting saturniids at night, outside periods of mate-seeking or oviposition, is largely unstudied. The defensive value of wing patterns at night must be ineffective or at least greatly reduced.

**Interpretation of the defensive role of wing pattern elements.** The defensive value of wing markings in *Hyalophora* can be discussed in the context of two general and formal models of anti-predator mechanism in Lepidoptera: intimidation of the potential predator and deflection of attack away from the body

(Stevens 2005). Intimidation by wing markings in Lepidoptera is most often discussed in connection with markings that resemble vertebrate eyes. In this study, the apical marking near the FW apex resembling a vertebrate eye (category **a**) shared first place with the HW discal spot (category **c**) as the wing pattern element most often attacked. In the intimidation model a predator perceives specific markings, usually displayed in pairs, as belonging to an enemy, which causes the predator to either flee or delay attack. Markings other than eye-like features may also intimidate a predator because they are conspicuous, either in their novel appearance (inducing neophobia) or because they are suddenly displayed, eliciting a startle response. These two qualities are only subtly different, but the former may be represented by the oval discal spots in *Hyalophora*, displayed against a brightly-colored, contrasting background, and the latter has been exemplified by the flash of HW color in underwing moths, normally camouflaged at rest (Sargent 1973).

The apical markings in saturniids, including those in *Hyalophora*, closely resemble vertebrate eyes and appear to fit the criteria of a signal that produces deflection in attack. They occur at a maximum distance from body, and a strike directed at the apical spot would deflect the attack away from the vulnerable body region. These markings could also be seen as a threat to the predator, especially in conjunction with waving motion of the wings. Discal spots in *Hyalophora* are conspicuous, novel features highlighted by the brightly colored upper wing surface. Endler (1992) and Blest (1957) discuss the effect of background in highlighting eyespots and other such markings; either reflective light-colored areas or dark, brightly-colored patches surrounding the spot amplify its intimidation or startling effect. In this regard, it is interesting to note that the small but detailed discal eye spots on *Saturnia mendocino* and *S. walterorum* fit this model. Those on the dark brown FW (cryptic against the bark of their host plant manzanita (*Arctostaphylos* spp.)) are often surrounded by white patches, while those on the HW are set in a yellow background. The HWs are covered at rest, and revealed when the moths are disturbed. Discal spots in *Hyalophora* may function either to deflect an attack (they somewhat resemble vertebrate eyes), or as novel, conspicuous images, set against a bright background, they may intimidate a predator. Again, the motion of the wings may amplify the defensive effect of discal spots.

Category **b** attacks, directed toward the inner margin of the FW and the adjacent costa of the HW, are not consistent with an attack on a moth at rest. An attack on a resting moth, with wings held together vertically



would tend to produce characteristic paired symmetrical wing damage. Interpretation of this category of damage is uncertain; it could represent an attack generally directed toward the discal spots, or as a somewhat random strike, or as a strike missing an intended target due to the motion of the wings during displaying behavior or while the moth was in flight.

The damage seen in *Hyalophora*—often with somewhat ragged edges—differs from the neatly defined cuts, and V-shaped bill imprints typically seen in other Lepidoptera (e.g. Sargent 1973, Shapiro 1974, Stevens 2005). Could this be due partly to the large size and therefore perhaps tougher wings of this and similar saturniids? Large size in itself might intimidate predators, and it would be worthwhile to assay the family to determine if a correlation exists between types of wing markings (e.g. discal spots, eyespots, bright coloration) and size of adult. Evans (1978) reported that captive catbirds refused to eat live *H. cecropia*, although these and other bird species readily ate the smaller *Callosamia promethea*. Since Waldbauer and Sternburg, in their extensive ecological studies of *H. cecropia* at the University of Illinois, often observed bird predation on *cecropia* (in litt., pers. com.), it seems likely that large size in itself may have intimidated the catbirds. I have often witnessed jays, robins and other bird species feeding at dawn on *Hyalophora* spp. attracted to lights. Such attacks cannot be directly extrapolated into speculation on predation in nature (the moths are in an unnatural setting, and birds quickly learn to exploit such situations), but there is no evidence, either from formal studies (Evans 1978) or from anecdotal observation, that adult *Hyalophora* are in any way distasteful or toxic.

Tough wings, resistant to tearing during bird strikes, might allow escape following attack. On the other hand, a weaker wing in the region of the apical spot might minimize damage to the apex during deflected attacks. Hill and Vaca (2004) found that the wings of *Pierella astyoche* (Erichson, 1849), which possesses wing margin marks thought to produce deflection in bird attacks, had thinner, more easily torn wings in comparison to congeners lacking such markings. Wing strength in saturniids should be measured in a comparison of species with or without conspicuous eye spots, and for different regions of the wings.

**Ability of wing-damaged males to respond to calling females.** No quantitative mark-release-recapture studies were conducted, but examination of the males bearing evidence of bird strikes reveals several that were severely damaged but still able to locate and fly into traps baited with calling females. In order to be trapped a male must follow a pheromone trail upwind, locate the general location of the trap, and

then precisely locate the caged, calling female, and upon touching the metal funnel fall into the trap. Of the total of 29 males scored as attacked, 6 (including *euryalus*, *gloveri* and hybrids) either had at least 50% of one wing removed, or about 50% of total wing area (FW and HW) removed from one side. Of these, two were missing nearly the entire HW on one side (as in Fig. 2–5).

**Brief remarks regarding wing pattern elements and the phylogeny of attacine Saturniidae.** The resting posture of *Hyalophora*, with the wings held vertically, also occurs in the closely related genus *Callosamia*, whose species—although more cryptically colored, especially in females—share with *Hyalophora* the small vertebrate-like eye spot at the wing apex and also fan their wings to display wing markings. The other New World attacine genera, *Rothschildia* and *Eupackardia*, rest with their wings opened horizontally, and their apical spots do not as closely resemble a vertebrate eye. In the Old World, the adults of *Samia* and *Epiphora* possess an apical eyespot nearly identical to that in *Hyalophora*. In a phylogenetic study of the Saturniidae, Regier et al. (2002) place *Epiphora* as ancestral to the clade containing *Samia*, *Callosamia*, and *Hyalophora*, indicating that the expression of a vertebrate eye-like apical spot may be concordant with a DNA-based phylogeny, and that the apical spot appears evolutionarily conservative (although its role in defense may be more labile).

#### CONCLUSION

In conclusion, wing damage consistent with that hypothesized to be caused by bird strikes occurred more often in *H. euryalus* (8.46%) than in *H. columbia gloveri* (4.52%), averaging 6.1% in a total of 471 specimens (collected with funnel traps). Damage differed significantly in frequency among seven standardized categories of wing damage. Damage to the FW apex, HW discal spot area, and the area of overlap of the FW inner margin and HW costa occurred in approximately equal frequencies, comprising together about 71% of total attacks. The location and appearance of the apical spot and wing-waving display behavior of threatened moths, all support the hypothesis that the vertebrate eye-like apical spot functions to deflect attacks away from the body. The HW discal spot, set in a brightly-colored background, may function as an intimidation or startle image. The relatively large size of *Hyalophora* adults, in keeping with a trend for large size among Saturniidae, may bolster the effectiveness of specific wing markings in defense. This hypothesis should be investigated, as well as the tear strength of saturniid wings as a measure of their ability to withstand

attack and retain flight capability. The apical eye spot and certain other saturniid wing pattern elements appear to be evolutionarily conservative and concordant with DNA-based phylogenies, suggesting that they are of significant adaptive value, probably as anti-predator mechanisms.

## ACKNOWLEDGEMENTS

I thank Lincoln Brower, Patricia Hamilton, John Rawlins (Carnegie Museum of Natural History-Invertebrates), Arthur Shapiro (University of California, Davis), and Chris Wemmer (Smithsonian National Zoological Park-Emeritus), who read all or parts of the manuscript and offered many helpful suggestions.

## LITERATURE CITED

- BATES, H.W. 1863. *The Naturalist on the River Amazons*. 2 vols. John Murray, London.
- BLEST, A.D. 1957. The function of eyespot patterns in the Lepidoptera. *Behaviour* 11:209–256.
- BOWERS, M.D. & D.C. WIERNASZ. 1979. Avian predation on a palatable butterfly, *Cercyonis pegala* (Satyridae). *Ecol. Entomol.* 4:205–209.
- BOWERS, M.D., I.L. BROWN & D. WHEYE. 1985. Bird predation as a selective agent in a butterfly population. *Evolution* 39:93–103.
- BRAKEFIELD, P.M. & A. MONTIERO. 2003. The evolution of butterfly eyespot patterns. Pp. 243–258. *In* Boggs, C.L., W.B. Watt & P.R. Ehrlich (eds.), *Ecology and Evolution Taking Flight*. University of Chicago Press.
- CARPENTER, G.D.H. 1941. The relative frequency of bird marks on butterflies of different edibility to birds. *Proc. Zool. Soc. London*. III (A): 223–331.
- COLLINS, M. M. 1984. Genetics and ecology of a hybrid zone in *Hyalophora* (Lepidoptera: Saturniidae). *Univer. California Pr., series Entomol.* 104, 93 pp.
- . 1997. Hybridization and speciation in *Hyalophora* (Insecta: Lepidoptera: Saturniidae): A Reappraisal of W.R. Sweadner's classic study of a hybrid zone. *Annals Carnegie Museum* 66:411–456.
- . 2007. *Moth Catcher: An Evolutionist's Journey through Canyon and Pass*. Univer. Nevada Pr., Reno. 176 pp.
- COTT, H.B. 1940. *Adaptive Coloration in Animals*. Methuen, Ltd., London.
- DARWIN, C. 1859. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London. 315 p.
- ENDLER, J.A. 1992. Signals, signal conditions, and the direction of evolution. *Amer. Natur.* 139:S125–S153.
- EVANS, D.L. 1978. Defensive behavior in *Callosamia promethea* and *Hyalophora cecropia* (Lepidoptera: Saturniidae). *Amer. Mid. Natur.* 100:475–479.
- FERGUSON, D. C. 1971. Pp. 1–154. *In* *The Moths of North America North of Mexico*, Fascicle 20A, Bombycoidea (in part). Dominick, R.B. (ed.), E. W. Classey Ltd. London.
- HILL, R.I. & J.F. VACA. 2004. Differential wing strength in *Pierella* butterflies (Nymphalidae, Satyrinae) supports the deflection hypothesis. *Biotropica* 36:362–370.
- HANEGAN, J.L. & HEATH. 1970. Mechanisms for the control of body temperature in the moth, *Hyalophora cecropia*. *Journal of Experimental Biology* 53:349–362.
- HEINRICH, B. 1981. *Insect Thermoregulation*. John Wiley, N.Y.
- HOWSE, P. & K. WOLFE. 2012. *Giant Silkmoths: Colour, Mimicry and Camouflage*. Papadakis, Winterbourne, England. 192 p.
- POULTON, E.B. 1890. *The Colors of Animals: Their Meaning and Use. Especially Considered in the Case of Insects*. 2nd Ed. The International Scientific Series. Vol. LXVIII. Paul, Trench & Trübner Ltd. London. 389 p.
- KETTELWELL, H.B.D. 1958. Industrial melanism in the Lepidoptera and its contribution to our knowledge of evolution. *Proc. 10th International Congr. Entomol.* (1956) 2:831–841.
- MAJERUS, M.E.N. 2009. Industrial melanism in the peppered moth, *Biston betularia*: an excellent teaching example of Darwinian evolution in action. *Evo. Edu. Outreach*. 2:63–74.
- REGIER, J.C., C. MITTER, R.S. PEIGLER, T.P. FRIEDLANDER. 2002. Monophyly, composition, and relationships within Saturniinae (Lepidoptera: Saturniidae): Evidence from two nuclear genes. *Insect System. Evol.* 33:9–21.
- SARGENT, T.D. 1973. Studies on the *Catocala* (Noctuidae) of southern New England. IV. A preliminary analysis of beak-damaged specimens, with discussion of anomaly as a potential anti-predator function of hindwing diversity. *J. Lepid. Soc.* 27:175–192.
- . 1976. *Legion of Night: The Underwing Moths*. University of Massachusetts Press, Amherst. 280 p.
- . 1990. Startle as an anti-predator mechanism, with special reference to the underwing moths, (*Catocala*). Pp 229–249. *In* Evans, D.L. & J.O. Schmidt (eds.), *Insect Defenses*. State University of New York Press, Albany.
- SHAPIRO, A.M. 1974. Beak-mark frequency as an index of seasonal predation intensity on common butterflies. *Amer. Nat.* 108:229–232.
- STEVENS, M. 2005. The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biol. Rev.* 80:573–588.
- TUSKES, P. M., J. P. TUTTLE, M. M. COLLINS. 1996. *The Wild Silk Moths of North America*. Cornell Univer. Pr., Ithaca. 250 p.

*Received for publication 26 March 2012; revised and accepted 3 July 2012.*

THE REDISCOVERY OF *JOICEYA PRAECLARUS* TALBOT 1928 (LEPIDOPTERA: RIODINIDAE),  
MORE THAN 80 YEARS AFTER ITS DESCRIPTION

**Additional key words:** Atlantic Forest, Brazil, cerrado, endangered species, Nymphidiini, Parque Nacional do Iguaçu

The family Riodinidae is the third most speciose butterfly group in the Neotropics, with more than 1,300 described species in this region (Callaghan & Lamas 2004), and shows an extraordinary diversity of morphological, ecological and life history traits (reviewed in Brown 1993a; DeVries 1997). Although in the past 30 years there have been advances in riodinid taxonomy (see comments in Hall 2005: 3–5), there have been few studies on the ecology and natural history of most riodinid species. Our poor understanding of riodinid biology is partly a result of the apparent rarity of many species, which may be a consequence of them occurring in small, localized populations and/or that some inhabit the forest canopy, are rarely attracted to baits, making them difficult to sample (Callaghan 1978; Brown 1993a; DeVries et al. 1994; Hall & Willmott 2010). Some highly endemic species may therefore be vulnerable to habitat destruction, but the difficulty in observing or sampling them complicates assessing their natural population densities. Consequently, any biological observations of rare riodinid species are potentially important.

*Joiceya praeclarus* Talbot, 1928 (Fig. 1) is a good example of the above situation. This monotypic genus was described from two males collected in two cerrado areas in Tombador and Cuiabá, in Mato Grosso, central Brazil (Talbot 1928), and despite intensive efforts, it has not been observed for more than 80 years following its description, and its biology and natural history remain unknown (Brown 1993b). Because of its apparent rarity and restricted geographical distribution, *J. praeclarus* was the first Riodinidae to appear on a red list of endangered species (Bernardes et al. 1989), and has been evaluated as “Endangered” by the IUCN (IUCN 2011).

On 9 September 2011, at 11:20 hs, a single male of *J. praeclarus* was observed and collected at Foz do Iguaçu, Paraná, Brazil (25°33'S 54°31'W, 205 m a.s.l.), about 1,200 km from its type locality in Mato Grosso. The site consisted of a small patch of secondary riparian forest along the Köhlenberger stream, inside a matrix of old abandoned pastures and country houses, about 8.4 km from Iguaçu National Park. The male was flying about 1 m high, and landing upside down beneath leaves with wings closed over the body. The individual has been collected for photographs (Fig. 1) and was deposited at the Museu de Zoologia “Adão José Cardoso” (ZUEC),

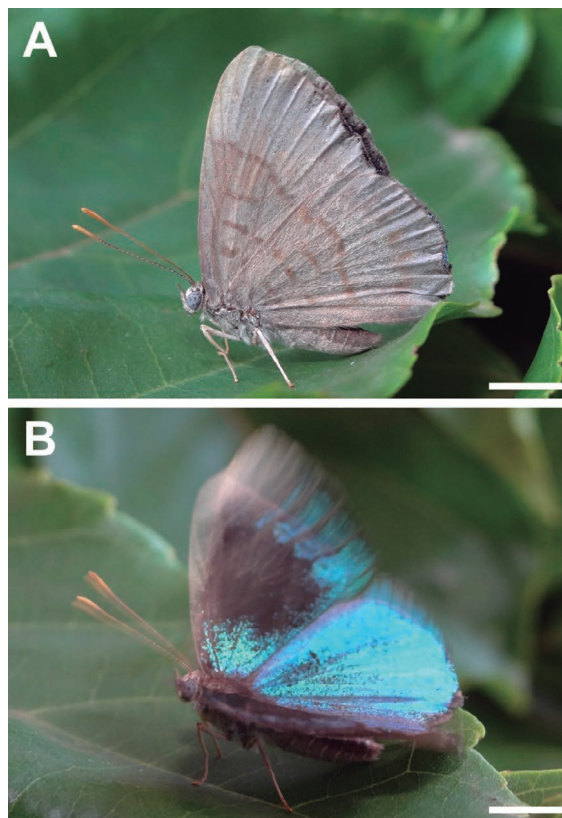


FIG. 1. Adult male of *Joiceya praeclarus*. **A**, perched above leaves; **B**, about to take off—note the dorsal blue metallic coloration. Scale bars = 0.5 mm.

Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Despite intensive subsequent searches, no other individuals were observed on following days at the site. The observation of *J. praeclarus* in Foz do Iguaçu is of obvious importance for its conservation assessment, given the paucity of data previously available for the species. Since the conservation status of the species is based on the assumption of its occurrence at a single locality, this status should be revised from “Endangered” to “Data deficient”. It now seems possible that *J. praeclarus* may occur in similar habitats from Mato Grosso to Paraná, and obviously more observations are needed to assess its habitat requirements and true distribution. The fact that the individual *J. praeclarus* was observed in a small, secondary forest fragment suggests it may be a much more tolerant species than formerly assumed, with populations potentially



persisting in a variety of different habitats in the region of Foz do Iguaçu. In this case the rarity of this species might be explained by its persistence in small localized populations, making adults seldom observed (additionally, adults can fly in restricted periods of the day or of the year, as known for several riodinids), or it is simply a canopy species which is very rarely collected in the understory, as is the case with several rare riodinids which usually fly very high in the forest (DeVries 1997; Hall & Willmott 2010).

#### ACKNOWLEDGEMENTS

We thank Keith Willmott and Phil DeVries for critical reading of the manuscript. RRG thanks to his wife Márcia dos Anjos, who helped in field work, and also to the volunteers of CEAEC (Center for Higher Studies of Conscientiology), who allowed field work in its property. LAK thanks Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 10/51340-8). AVLF thanks FAPESP (grant 04/05269-9) and CNPq (fellowship 302585/2011-7). This publication is part of the RedeLep “Rede Nacional de Pesquisa e Conservação de Lepidópteros” SIS-BIOTA-Brasil/CNPq (563332/2010-7) and BIOTA-FAPESP program (11/50225-3).

#### LITERATURE CITED

- BERNARDES, A.T., A.B.M. MACHADO, & A.B. RYLANDS. 1990. Fauna Brasileira Ameaçada de Extinção. Fundação Biodiversitas / IBAMA, Belo Horizonte, Brazil. 65 pp.
- BROWN JR. K.S. 1993A. Neotropical Lycaenidae—an overview, pp. 45–61. *In*: New, T.R. (ed.). Conservation biology of Lycaenidae (Butterflies). IUCN, Gland, Switzerland.
- . 1993B. Selected Neotropical species, pp. 146–149. *In*: New, T.R. (ed.). Conservation biology of Lycaenidae (Butterflies). IUCN, Gland, Switzerland.
- CALLAGHAN, C.J. 1978. Studies on restinga butterflies. II. Notes on the population structure of *Menander felsina* (Riodininae). *J. Lepid. Soc.* 32: 87–94.
- CALLAGHAN, C.J. & G. LAMAS. 2004. Riodinidae, pp. 141–170. *In*: Lamas, G. (ed.), Checklist: Part 4A. Hesperioidea—Papilionoidea. *In*: Heppner, J.B. (ed.), Atlas of Neotropical Lepidoptera. Scientific Publishers, Gainesville, FL.
- DEVRIES, P.J. 1997. The butterflies of Costa Rica, Vol. 2, Riodinidae. Princeton University Press, Princeton, NJ, 288 pp.
- DEVRIES, P.J., I.A. CHACON & D. MURRAY. 1994. Toward a better understanding of host use biodiversity in riodinid butterflies (Lepidoptera). *J. Res. Lepid.* 31: 103–126.
- IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 21 February 2012.
- HALL, J.P.W. 2005. A phylogenetic revision of the Napaeina (Lepidoptera: Riodinidae: Mesosemiini). The Entomological Society of Washington, Washington, DC. vi + 235 pp.
- HALL, J.P.W. & K.R. WILLMOTT. 2010. Description of a new *Lucillella* species Riodinidae: Symmachiini discovered in the eastern Andes of Ecuador using the single rope canopy access technique. *J. Lepid. Soc.* 64: 139–146.
- TALBOT, G. 1928. List of Rhopalocera collected by Mr. C.L. Collette in Matto Grosso, Brazil. *Bull. Hill Mus.* 2: 192–220.
- ROBERTO R. GREVE, *Rua da Cosmoética, 1847 casa 32 - Bairro Cognópolis Foz do Iguaçu, CEP 85853-755, Paraná, Brazil*, CURTIS CALLAGHAN, *Casa Pica Pau, Floresta de la Sabana, Cra. 7 137-04, Bogotá, Colombia*, LUCAS A. KAMINSKI & ANDRÉ V. L. FREITAS, *Departamento de Biologia Animal e Museu de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, C.P. 6109, CEP 13083-970, Campinas, São Paulo, Brazil; email: baku@unicamp.br*

*Received for publication 15 March 2012; revised and accepted 11 April 2012.*

REPEATED PREDATION OF MEADOW FRITILLARIES (*BOLORIA BELLONA*) BY  
AMBUSH BUGS (*PHYMATA* SPP.)**Additional key words:** *Eupatorium perfoliatum*, fen

The meadow fritillary (*Boloria bellona* Fabricius, 1775) is a common species of Nymphalidae found throughout Canada and the north-central and northeastern United States where it lives in open wet-grassy areas such as prairies, meadows, pastures and fields (Opler 1998; Brock & Kaufmann 2006; Schlicht et al. 2007). In the state of Iowa it is also known to inhabit fens (Schlicht et al. 2007), which are diverse open wetlands characterized by continuous surface to near surface saturation by groundwater, organic soil and non-emergent graminoid vegetation (Thompson et al. 1992; Amon et al. 2002). Nectar habits include many species of flowers, with a preference for members of the Asteraceae (Opler & Krizek 1984).

Ambush bugs (*Phymata* spp., Heteroptera: Phymatidae) are generalist sit-and-wait predators that feed on multiple species of florivorous insects, including members of the Nymphalidae (Balduf 1939). Preferred habitat is on vegetation in open areas with direct exposure to sunlight and wind (Balduf 1941). Seventeen species of *Phymata* are recognized in North America with a distribution that includes all of Canada and the eastern and southern United States (Arnett 2000).

On 8 July 2010 at approximately 2:30 p.m. Central Standard Time a group mortality of *B. bellona* was observed in a small (0.3 hectare) privately owned remnant fen in Muscatine County, Iowa, (41° 28' 6.59" N, 91° 3' 11.44" W). Six dead *B. bellona* were noted on the inflorescence of a common boneset plant (*Eupatorium perfoliatum* L.), whereas no other dead arthropods were observed. An ambush bug was then observed, partially exposed from the inflorescence of the common boneset, feeding on a recently captured seventh *B. bellona*. Closer inspection of the inflorescence revealed at least 3 ambush bugs concealed among the flowers. A more intrusive inspection of the inflorescence was not completed in order to limit disturbance of the *Phymata*, however no other predators were discovered.

Conditions at the time of observation were warm (30° C) and clear (0% cloud cover) with wind speeds of 13.0 kph (gentle breeze on the Beaufort Wind Scale). Although there was no direct evidence of predation by *Phymata* for the 6 dead *B. bellona*, the lack of other predatory species and their position on the inflorescence of the common boneset supported the conclusion that

they were discarded *Phymata* prey. Balduf (1939) noted that under similar circumstances (i.e., warm sunny days with little to no wind) discarded *Phymata* prey were readily visible in the axils and flowers of plants used as ambush sites (i.e., ambush plants). Mortality was not observed for any other Lepidopteran species at the site.

A review of the literature found no published record of this phenomenon being described previously. Balduf (1939) found discarded remains of numerous species preyed on by *Phymata* on flowers, axils, and below ambush plants, but never described significant numbers of a single species. More recent unpublished data of *Phymata* predation on Lepidoptera as well have no records of group predation of a single species (David M. Wright, personal communication). An additional peculiarity of this observation was that pollinators typically avoid individual plants, specific species, and even entire patches as predation risk increases (Jones 2009), and the presence of *Phymata* specifically can deter flower visitation by nectivorous insects (Elliott & Elliott 1991, 1994). Despite the presence of other flowering Asteraceae in the fen (e.g., *Helianthus grosseserratus* M. Martens) no such avoidance by *B. bellona* was observed.

Also, the use of *Eupatorium* as an ambush plant by *Phymata* is infrequent (Balduf 1939; Punzalan et al. 2008). Balduf (1939) did not observe *Phymata* capturing any prey while utilizing *Eupatorium*. However, McAtee and Walton (1918) did observe *Phymata* successfully capturing flies (Diptera: Tabanidae) on *Eupatorium* and in Wisconsin Graenicher (1909) noted that *E. perfoliatum* was visited by as many as 113 different species, including both *Phymata* and *B. bellona*. This observation indicated that, contrary to previously published reports, *Phymata* utilized *E. perfoliatum* as an ambush plant quite effectively.

Although there is no direct evidence that the repeated mortality was the result of predation by *Phymata*, alternative hypotheses (e.g., disease, other predators, etc.) are unlikely. While only a single *Phymata* feeding on a *B. bellona* was observed, *Phymata* are not known to take dead insects as a food source (Balduf 1943), which would suggest that all of the dead *B. bellona* on the boneset were the result of *Phymata* predation since no other predators were present. Predation on Lepidoptera by ambush bugs is believed to be quite common (Fales

1976; Wright 1981); however, the literature is lacking on observations of such behavior (Pyle 1973; Wright 1981). The impact of predation on native populations of pollinators is crudely understood and regarded as a minor problem, though additional research is necessary to test this axiom (Kevan 1999). We concur with Wright (1981) and Kevan (1999) and suggest that additional observations of Lepidoptera predation by *Phymata* would aid in furthering the understanding of Lepidopteran and pollinator ecology.

#### ACKNOWLEDGEMENTS

We thank David M. Wright for his insight on *Phymata* predation on Lepidoptera.

#### LITERATURE CITED

- AMON, J. P., C. A. THOMPSON, Q. J. CARPENTER, & J. MINER. 2002. Temperate zone fens of the glaciated Midwestern USA. *Wetlands* 22:310-317.
- ARNETT, R. H. 2000. American insects: a handbook of the insects of America north of Mexico. CRC Press, Boca Raton. 1003 pp.
- BALDUF, W. V. 1939. Food habits of *Phymata pennsylvanica americana* Melin (Hemip.). *Can. Entomol.* 71:66-74.
- . 1941. Life history of *Phymata pennsylvanica americana* Melin (Phymatidae, Hemiptera). *Ann. Entomol. Soc. Am.* 34:204-214.
- . 1943. Third annotated list of *Phymata* prey records (Phymatidae, Hemiptera). *Ohio J. Sci.* 43:74-78.
- BROCK, J. P. & K. KAUFMANN. 2006. Kaufmann field guide to butterflies of North America. Houghton Mifflin Harcourt, New York. 392 pp.
- ELLIOTT, N. B. & W. M. ELLIOTT. 1991. Effect of an ambush predator, *Phymata americana* Melin, on behavior of insects visiting *Daucus carota*. *Am. Midl. Nat.* 126:198-202.
- . 1994. Recognition and avoidance of the predator *Phymata americana* Melin on *Solidago odora* Ait. by late seasonal floral visitors. *Am. Midl. Nat.* 13:378-380.
- FALES, J. H. 1976. More records of butterflies as prey for ambush bugs (Heteroptera). *J. Lepid. Soc.* 30:147-149.
- GRAENICHER, S. 1909. Wisconsin flowers and their pollination - Compositae, part I. *Bull. Wis. Nat. Hist. Soc.* 7:19-77.
- JONES, E. L. 2009. Optimal foraging when predation risk increases with patch resources: an analysis of pollinators and ambush predators. *Oikos* 119:835-840.
- KEVAN, P. G. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agr. Ecosyst. Environ.* 74:373-393.
- MCATEE, W. L. & W. R. WALTON. 1918. District of Columbia Diptera: Tabanidae. *P. Entomol. Soc. Wash.* 20:188-206.
- OPLER, P. A. 1998. A field guide to eastern butterflies. Houghton Mifflin Company, New York. 486 pp.
- OPLER, P. A. & G. O. KRIZEK. 1984. Butterflies east of the Great Plains: an illustrated natural history. John Hopkins University Press, Baltimore. 294 pp.
- PUNZALAN, D., F. H. RODD, & L. ROWE. 2008. Contemporary sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*. *Behav. Ecol.* 19:860-870.
- PYLE, R. M. 1973. *Boloria selene* (Nymphalidae) ambushed by a true bug (Heteroptera). *J. Lepid. Soc.* 27:305-307.
- SCHLICHT, D. W., J. C. DOWNEY, & J. C. NEKOLA. 2007. The butterflies of Iowa. University of Iowa Press, Iowa City. 233 pp.
- THOMPSON, C. A., E. A. BETTIS, & R. G. BAKER. 1992. Geology of Iowa fens. *J. Iowa Acad. Sci.* 99:53-59.
- WRIGHT, D. M. 1981. Historical and biological observations of Lepidoptera captured by ambush bugs (Hemiptera). *J. Lepid. Soc.* 35:120-123.

JOSEPH W. DIXON, *United States Department of Agriculture, Natural Resources Conservation Service, 3500 Oakview Drive, Suite A, Muscatine, Iowa 52761; email: joe.dixon@ia.usda.gov* and RYAN D. RASMUSSEN, *19200 Lilac Ave., Bloomfield, Iowa 52537; email: rd\_rass@hotmail.com*

*Received for publication 12 December 2012; revised and accepted 5 July 2012.*



**WALLENGRENIA EGEREMET (HESPERIIDAE): A NEW POPULATION FOR WESTERN CANADA.****Additional key words:** tall grass prairie, range extension

The Northern Broken-Dash, *Wallengrenia egeremet* (Scudder, 1864) is a species of skipper (Hesperiidae) found in open grassy meadows throughout most of the eastern United States (Layberry et al. 1998). The current range of *W. egeremet* extends from the Gulf Coast through Florida and south-eastern Texas, continuing north to central North Dakota and southern Maine (Burns 1985, Opler et al. 2012). The northern periphery of the range extends into eastern Canada, including southern Quebec, south-central Ontario (MacNeill 1975, Layberry et al. 1998), and southern New Brunswick (Macy and Shepard 1941, Duffy and Garland 1978 as cited in Burns 1985). Recently the northern range of *W. egeremet* has extended into western Canada.

Two male *W. egeremet* were first recorded within the Tall Grass Prairie Preserve, located in the Rural Municipality of Stuartburn, Manitoba, Canada (49° 08' N, 96° 40' W) in late June 2006 by Bates (2007). The two males were collected by sweep net at 49° 05' 17.34 N, 96° 45' 31.75 W. Between 17 July and 6 August, 2009, eleven male and six female *W. egeremet* adults were collected within the Tall Grass Prairie Preserve by S. Semmler using pollinator pan traps. Four males were observed between 10 July and 14 July, 2010 and three males and two females were observed between 11 July and 18 July, 2011 by R. Westwood.

Previous reports of *W. egeremet* in Manitoba were based on misidentified specimens, with four female Dunn Skipper (*Euphyes vestris* Boisduval) identified as *W. egeremet* in 1955, and an inaccurate record from 1974 (Burns 1985, Klassen et al. 1989). *Wallengrenia egeremet* was absent in comprehensive annual butterfly surveys in southern Manitoba between 1976 and 1988 (P. Klassen and R. Westwood unpublished) in preparation for the publication of the Butterflies of Manitoba (Klassen et al. 1989). *Wallengrenia egeremet* was also absent in extensive surveys for the Poweshiek Skipperling (*Oarisma poweshiek* Parker) and Dakota Skipper (*Hesperia dacotae* Skinner) in the Tall Grass Prairie Preserve between 1996 and 2006 (R. Westwood unpublished, Webster 2003). A record from the Royal Ontario Museum lists at least one specimen of *W. egeremet* being collected from Echo Valley Provincial Park, Saskatchewan, in 1969, although there have been no subsequent reports of the skipper from Saskatchewan.

The Tall Grass Prairie Preserve is the largest remaining tall grass prairie remnant in Canada, with approximately 5000 ha of native prairie owned and managed by several organizations including Nature Manitoba, The Nature Conservancy of Canada and Manitoba Conservation. The Tall Grass Prairie Preserve is separated into northern and southern blocks of similar size, with blocks being approximately 5 km apart. The southern block is characterized by upland tall grass prairie habitat composed of big bluestem (*Andropogon gerardii* Vitman) and little bluestem (*Schizachyrium scoparium* (Michx.) Nash), while the northern block consists of wetter, low lying areas of sedges (*Carex* spp.) and rushes (*Juncus* spp.) (Henne and Diehl 2002 as cited in Westwood and Borkowsky, 2004, Hamel et al. 2006). Prairie meadows within both blocks are often separated by marshes or stands of oak (*Quercus* spp.), aspen (*Populus* spp.), and willow (*Salix* spp.) (Henne and Diehl 2002 as cited in Westwood and Borkowsky 2004, Hamel et al. 2006). The climate in the Tall Grass Prairie Preserve is continental, with an average of 579.1 mm of precipitation annually, a mean summer temperature of 19.8 °C and a mean winter temperature of -17.1 °C (Environment Canada 2004). The soil is a grey-wooded podzol, having a sandy-loam to clay-loam texture with frequent rock outcrops (Canada Soil Inventory 1989). The shallow slope of the landscape (1–3%), poor drainage and high water table (within 3 m of the surface) generally inhibit agricultural productivity and potential within the Tall Grass Prairie Preserve (Westwood et al. 2011).

The 2009 specimens were collected during pan trapping for the Canadian Pollination Initiative (NSERC-CANPOLIN), a Canadian National Science and Engineering Research Council Strategic Network organizing nationwide surveys of insect pollinators (NSERC-CANPOLIN 2009). Pan traps were set at 10 day intervals along 90 m transects in open tall grass prairie meadows to collect pollinating insects. An alternating colour pattern of yellow, blue, and white pans mimicked the reflectance of various floral species. The majority of *W. egeremet* specimens were collected from blue pans (13 of 17 individuals), with three collected from yellow pans and one individual collected from a white pan. Collection records will be incorporated into the NSERC-CANPOLIN pollinator database, and the

specimens have been deposited at the University of Winnipeg, Manitoba, Canada.

In the 2009 pan trapping surveys *W. egeremet* was found in two locations within the Tall Grass Prairie Preserve. The first location in the south block (49° 04' 28.7 N, 96° 43' 06.1 W) was within upland prairie habitat consisting of dense patches of goldenrod (*Solidago canadensis* L., *Solidago rigida* L.), big bluestem, and shrubby cinquefoil (*Dasiphora fruticosa* (L.) Rydb.). Co-flying skippers in this location included Dun Skipper, Peck's Skipper (*Polites peckius* W. Kirby), Long Dash Skipper (*Polites mystic* W.H. Edwards), and European Skipper (*Thymelicus lineola* Ochsenheimer). The second location in the north block (49° 08' 30.3 N, 96° 40' 21.4 W) consisted of wetter sedge meadow dominated by sedges, rushes, and shrubby cinquefoil. *Polites mystic* was also collected from the second location. In 2009 *W. egeremet* males were trapped approximately two weeks earlier than females, and females were most frequently associated with sedge meadow habitat. Records from 2006, 2010 and 2011 came from similar upland tall grass prairie meadows within several km of the pan trapping sites in the southern block only.

*Wallengrenia egeremet* is generally described as a brownish skipper with a wingspan of 24–29 mm (Layberry et al. 1998). A primary diagnostic feature of *W. egeremet* is the broken stigma surrounded by pale orange/brown markings on the male forewings (Layberry et al. 1998). Forewings of the female have prominent cream coloured markings that are also visible on the underside of the wing (Layberry et al. 1998, Opler et al. 2012). The undersides of the hindwings are marked by a faint medial crescent in both sexes (Layberry et al. 1998, Opler et al. 2012). A similar species, *E. vestris*, shares comparable habitat and adult food plants (Layberry et al. 1998, Opler et al. 2012a). Females of *E. vestris* lack the medial crescent under the hind wings, but can be difficult to differentiate from *W. egeremet*, requiring examination of scaled spurs on the tibia of the midlegs (MacNeill 1975, Layberry et al. 1998). However, male *E. vestris* are distinct in that they lack the broken stigma (Layberry et al. 1988).

*Wallengrenia egeremet* is univoltine in the northern areas of its range, with adults reported to be on the wing from June or July through August (MacNeill 1975, Burns 1985, Layberry et al. 1998, Wagner 2005, Opler et al. 2012). In the United States larvae overwinter in protective cases of silk and grass blades or leaves, emerging in early spring to pupate (MacNeill 1975, Wagner 2005).

Larval food plants include western needlegrass (*Achnatherum occidentale* Thurb. ex S. Wats.) Barkworth (MacNeill 1975), deertongue or panic grass

(*Dichantheium clandestinum* (L.) Gould, and *Dichantheium dichotomum* (L.) Gould) and crab grass (*Digitaria sanguinalis* (L.) Scop.) (Layberry et al. 1998, Wagner 2005, Opler et al. 2012). Adults are reported to nectar on a variety of flower species; examples from the United States include red clover (*Trifolium pretense* L.), dogbane (*Apocynum* spp.), New Jersey tea (*Ceanothus americanus* L.) and sweet pepperbush (*Clethra alnifolia* L.) (Opler et al. 2012). The Tall Grass Prairie Preserve supports similar larval food species such as porcupine grass (*Hesperostipa spartea* (Trin.) Barkworth) and panic grass (*Dichantheium acuminatum* (Sw.) Gould & C. A. Clark), and *Dichantheium leibergii* (Vasey) Freckmann), as well as a variety of nectar sources within Asteraceae, Fabaceae, and Apocynaceae.

The most recent published records from North Dakota include the Grand Forks Air Force Base and south-east of Arthur (Opler et al. 2012), North Dakota, approximately 150 and 200 km south of the Tall Grass Prairie Preserve, respectively. Minnesota is the probable source of skippers to Manitoba, with the nearest recent published observations south-east of Crookston and north-west of Fertile (Opler et al. 2012), Minnesota (approximately 180 km from the Tall Grass Prairie Preserve).

There are older reports of *W. egeremet* in Minnesota from Roseau (Flagstad Jr. 1970) and Minnesota Hill (Cuthrell 1991), Roseau Co., approximately 70 km south east of the Tall Grass Prairie Preserve (R. Huber unpublished, R. Dana, pers. comm.). There are 1991 Minnesota records from Kittson Co. which include Devil's Playground Wildlife Management Area, Norway 8, and the Caribou Wildlife Management Area, with the Caribou WMA being approximately 15 to 20 km south of the Tall Grass Prairie Preserve (Cuthrell 1991, R. Dana, pers. comm.). Between 1996 and 2011 there are only three reports of *W. egeremet* in Minnesota in the Season Summary Reports (Lepidopterists Society 1996–2011). In 1998 *W. egeremet* was reported from Roseau and Bemidji, Minnesota, approximately 75 to 120 km southeast of the Tall Grass Prairie Preserve, respectively, and in 2006 in Hubbard, Minnesota, over 200 km southeast of the Tall Grass Prairie Preserve.

Although the records from Minnesota suggest that populations of *W. egeremet* have existed in proximity to the Canadian/U.S. border for several decades, *W. egeremet* had not been observed in southern Manitoba until 2006. Extensive monitoring of butterfly populations in the region leave little possibility that this species was present in Manitoba prior to 2006 thus the appearance of this species in the region appears to be fairly recent. The Tall Grass Prairie Preserve provides habitat suitable for the propagation of *W. egeremet* and the population

appears to be well established. Unlike several other butterfly species which have sporadically been reported in southern Manitoba (Taylor et al. 2008), there are no known records of *W. egeremet* entering Manitoba during the last 75 years. It is unknown if *W. egeremet* will remain confined to the Tall Grass Prairie Preserve in Canada or if it will continue to move northward into central Manitoba and eastern Saskatchewan.

Taylor et al. (2008), Taylor and Westwood (2010) and Westwood and Blair (2010) have recently reported other species of butterflies that have extended their ranges into Manitoba from the north central United States over the past decade. Kerr (2001) reported expanded ranges of several butterflies in eastern Canada and others have reported North American butterfly range extensions in relation to climate warming (Crozier 2003, Hellmann et al. 2008). The recent expansion of the range of *W. egeremet* into Manitoba may be part of a trend northward for butterfly species previously absent from the region.

#### ACKNOWLEDGEMENTS

We would like to thank Robert Dana for providing additional records of occurrence and review of this manuscript, Christie Borkowsky and Laura Reeves for access to preserve flowering species information and an anonymous reviewer. We also thank Jarrod Sumlak, Jaimee Dupont, Melissa Pearn, and Sara Halwas for their assistance during the collection of pan trapped pollinators. Funding was provided by NSERC-CANPOLIN, the Nature Conservancy of Canada, the Manitoba Sustainable Development Fund, and the Manitoba Hydro Research Fund. This is publication number 17 from the Canadian Pollination Initiative (NSERC-CANPOLIN).

#### LITERATURE CITED

- BATES, L. M. 2007. The influence of prescribed burning and grazing on the Dakota Skipper, *Hesperia dacotae*, habitat in South-Eastern Manitoba. Undergraduate Thesis. The University of Winnipeg, Manitoba, Canada.
- Biodiversity occurrence data published by: Royal Ontario Museum: Entomology (Accessed through GBIF Data Portal, data.gbif.org, 02/13/2012).
- BURNS, J. M. 1985. *Wallengrenia otho* and *W. egeremet* in Eastern North America (Lepidoptera: Hesperidae: Hesperinae). Smithsonian Contributions to Zoology Number 423. Smithsonian Institution Press, Washington. 48 pp.
- CANADA SOIL INVENTORY. 1989. Soil landscapes of Canada – Manitoba. Land Resource Research Centre, Agriculture Canada, Ottawa, Ont. Agric. Can. Publ. 524216.
- CROZIER, L. 2003. Winter warming facilitates range expansion: cold tolerance of the butterfly *Atalopedes campestris*. *Oecologia* 135:648–656.
- CUTHRELL, D.L. 1991. The butterflies of Kittson and Roseau counties, MN, with special emphasis on the dakota skipper (*Hesperia dacotae*) [Skinner]. Final report submitted to Nongame Wildlife Program, Minnesota Department of Natural Resources. 48+ pp.
- ENVIRONMENT CANADA. 2004. Canadian Climate Normals 1971–2000. [http://www.climate.weatheroffice.ec.gc.ca/climate\\_normals/index\\_e.html](http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html). Data for Emerson, Manitoba. Accessed December, 2008.
- HAMEL, C., R. REISZ, G. FORTNEY, R. JONES, & D. PIETRUSZEWSKI. 2006. Conservation area plan for the Tallgrass Aspen Parkland. Nature Conservancy of Canada, Manitoba Region, Winnipeg, Manitoba/The Nature Conservancy, Karlstad Field Office, Karlstad, Minnesota. 70 pp. + appendices.
- HELLMAN, J. J., S. I. PELINI, K. M. PRIOR & J. D. K. DZURISIN. 2008. The response of two butterfly species to climatic variation at the edge of their range and the implications for poleward range shifts. *Oecologia* 157:583–592.
- KERR, J. K. 2001. Butterfly species richness patterns in Canada: energy, heterogeneity, and the potential consequences of climate change. *Conserv. Ecol.* 5:10.
- KLASSEN, P., A. R. WESTWOOD, W. B. PRESTON, & W. B. MCKILLOP. 1989. The butterflies of Manitoba. Manitoba Museum of Man and Nature, Winnipeg. 290 pp.
- LAYBERRY, R. A., P. W. HALL L., & J. D. LAFONTAINE. 1998. The butterflies of Canada. Univ. Toronto Pr., Toronto. 280 pp.
- LEPIDOPTERISTS SOCIETY 1996–2011. Seasonal Summaries. *J. Lepid. Soc.* Volumes 39 to 51 Supplements and 2009–2011 Seasonal Summaries.
- MACNEILL, C. D. 1975. Family Hesperidae: the Skippers. In: Howe, W.H. (ed.) 1975. The butterflies of North America. Doubleday and Company, Inc. Garden City, NY. 633 pp.
- NSERC-CANPOLIN. 2009. Background PDF. <http://www.uoguelph.ca/canpolin/Publications/CANPOLIN%20background.pdf> Accessed: 02/13/2012.
- OPLER, P. A., K. LOTT, & T. NABERHAUS, coordinators. 2012. Butterflies and moths of North America. <http://www.butterfliesandmoths.org/species/Wallengrenia-egeremet> Accessed: 02/13/2012.
- . 2012a. Butterflies and moths of North America. <http://www.butterfliesandmoths.org/species/Euphyes-vestris> Accessed: 02/13/2012.
- TAYLOR, P. L., DE MARCH, & A. R. WESTWOOD. 2008. Extended range and flight period of the common Buckeye butterfly in southern Manitoba. *Blue Jay* 66:107–111.
- TAYLOR P. & A. R. WESTWOOD. 2010. New records of the Baltimore Checkerspot butterfly in Manitoba. *Blue Jay* 68: 87–90.
- WAGNER, D. 2005. Caterpillars of Eastern North America. Princeton University Press. Princeton, NJ. 496 pp.
- WEBSTER, R. P. 2003. 2002 Survey of the Dakota Skipper, *Hesperia dacotae* (Skinner). Committee on the Status of Endangered Wildlife in Canada, Ottawa. Unpublished report. 14 pp.
- WESTWOOD, A. R. & D. L. BLAIR. 2010. Effect of regional climate warming on the phenology of butterflies in boreal forests in Manitoba, Canada. *Environ. Entomol.* 39:1122–1133.
- WESTWOOD, A. R., & C. L. BORKOWSKY. 2004. Sphinx moth pollinators for the endangered western prairie fringed orchid, *Platanthera praeclara* in Manitoba, Canada. *J. Lepid. Soc.* 58:13–20.
- WESTWOOD, A. R., C. L. BORKOWSKY & K. E. BUDNICK. 2011. Seasonal variation in the nectar sugar concentration and nectar quantity in the Western Prairie Fringed Orchid, *Platanthera praeclara* (Orchidaceae). *Rhodora* 113:201–121.

SARAH J. SEMMLER *Dept. of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, Canada R3T 2N2; email: semmler.sj@gmail.com* and A. RICHARD WESTWOOD<sup>1</sup> *Dept. of Biology, University of Winnipeg, 515 Portage Ave, Winnipeg, Manitoba, Canada R3B 2E9; email: r.westwood@uwinnipeg.ca*

*Received for publication 17 February 2012; revised and accepted 10 June 2012.*

<sup>1</sup> Author for correspondence



## ON THE FINDING OF DEAD ANTS ATTACHED TO SATURNIID CATERPILLARS: EVIDENCE OF SUCCESSFUL DETERRENT CHEMISTRY?

**Additional key words:** ant predation, anti-predator mechanism, chemical defense

*Hyalophora cecropia* (Saturniidae) is one of the most prominent natural history icons of North America, widely used to teach insect life history in schools and collected and reared by amateur naturalists for generation upon generation. The larvae are as striking as the colorful adults, and display a vivid array of scoli colors, which change with instar. Yet, until very recently, no reported evidence has appeared that the larvae are in any way toxic. As any breeder knows, larvae of all ages appear all too vulnerable to a host of predators and parasitoids (Collins & Weast 1961, p. 84; Tuskes et al. 1996).

Deml & Dettner (2003) discovered an unexpected chemical defense in cecropia larvae. Each scoli is associated with a secretory gland, the complex chemistry of which predictably varies by scoli color and instar. Their conclusion, based also on previous work (Deml & Dettner 1990, 1993, 1997), is that these chemicals are defensive, are released when small spines are broken off a scoli during an attack, and are directed against a sequence of instar-specific predators.

Their work also showed that the chemistry of the body haemolymph in *Hyalophora* does not contain these characteristic chemicals. Human skin seems unaffected by the larval chemistry of scoli, unlike the effect of urticating spines in other saturniid larvae such as the Hemileucinae. There is one obscure record of a robin apparently killed when attempting to eat a mature *H. columbia gloveri* larva (Duncan 1941).

On August 15, 2011 I collected a near-mature *Hyalophora* larva on coyote willow (*Salix exigua*) at 1700

m near a creek flowing into a small canyon along highway 89, just west of US 395. This area is at the eastern edge of a hybrid zone, across Monitor Pass, between *H. c. gloveri* and *H. euryalus*. The larval phenotype was intermediate between these two species; it possessed the spiny scoli typical of *gloveri*, although somewhat reduced in size. Upon close inspection I noticed a dead ant attached to the second thoracic segment, right side, near scoli L<sub>1</sub> (Fig. 1). The ant's abdomen was shrunken, indicating that the ant had remained attached for some days (the fifth instar in *Hyalophora* typically lasts for 7–12 days in the wild). After photographing the larva in the field, I left the ant attached to monitor the health and growth of the larva. It fed normally and spun a cocoon in 5 days. The head of the ant remained attached, but unfortunately the body of the ant became dislodged and lost before I could bring the larva into my lab. Under the microscope I noticed a single spine broken off each of two nearby scoli. From my photographs ant experts Phil Ward (Dept. Entomology, Univ. Calif. Davis) and James Trager (Shaw Nature Res., Gray Summit MO) identified it as belonging to the *Formica rufi* species group, probably either *F. ravida* or *F. moki*.

Surrounding the ant and along that side of the body of the larva were the dried remnants of what appeared to be regurgitated gut contents. *Hyalophora* larvae are not known to regurgitate gut contents unless severely attacked, but when this does occur the larva will curl its body toward the attacker and thrash about. My interpretation is that this action may have killed the ant by



FIG. 1. *Formica* ant (dead) attached to *Hyalophora* larva, Monitor Pass, Mono Co. CA. Note brown stains of dried regurgitant surrounding ant.



FIG. 2. Dead *Formica* (white arrows) attached to *Hemileuca eglanterina*, Monitor Pass, Mono. Co. CA. A similar photograph appears in Tuskes et al., 1996, p.20.

piercing its body with a scoli spine, which broke off and released a toxin. There is no evidence that the gut contents of *Hyalophora* are unpalatable.

In July 1973, coincidentally also on Monitor Pass, near the summit at 2500 m, I photographed a mature larva of *Hemileuca eglanterina* on snowberry (Fig. 2). Attached to the larva were two dead ants, appearing to be the same species of mound-building *Formica* ant as that found on the *Hyalophora*. Again, the simplest interpretation is that the ants died as a result of encountering defensive chemistry, in this case produced by a stinging larva.

Certainly, more careful work needs to be done on the subject of ant predation on lepidopterous larvae. The research on the specialized association of ants with lycaenid butterflies is a fascinating exception, but given the paramount ecological role of ants in biotic communities (Hölldobler & Wilson 1994), the importance of predatory ants in regulating the abundance and distribution of Lepidoptera is poorly understood. Michael Singer (Dept. Biology, Wesleyan U.) is currently researching these topics in temperate regions, and in our discussions pointed out to me two interesting studies: Karhu (1998) on ant exclusion experiments in a boreal forest, and Dyer (1995) on assaying ant predation on protected vs. unprotected Lepidoptera larva in the tropics. Even simple observations of ant predation on captive larvae placed in the field—'staking out lambs in lion country'—would be worthwhile, as suggested for *Eupackardia calleta* (Collins, 2007, p. 41ff), based on its blood chemistry and aposematic coloration. I hope this short note will stimulate further work.

#### ACKNOWLEDGEMENTS

I thank Phil Ward, University of California, Davis, Entomology and James Trager, Shaw Nature Reserve, Gray Summit, MO for

their efforts to identify the ant species from photographs. Mike Singer, Wesleyan University, Biology Dept., suggested certain references on ant predation and both he and Dave Wagner encouraged the publication of this note. John Rawlins, Carnegie Museum of Natural History – Invertebrate Section, kindly made a digital file from my 1973 35mm slide.

#### LITERATURE CITED

- COLLINS, M. M. 2007. *Moth Catcher*. University of Nevada Press, Reno. 200pp.
- COLLINS, M.M. AND R. D. WEAST. 1961. *The Wild Silk Moths of the United States*. 138pp. Privately published.
- DEML, R. AND K. DETTNER. 1990. Chemical defense of *Eudia (Saturnia) pavonia* caterpillars. *Naturwissenschaften* 77:588-590.
- DEML, R. AND K. DETTNER. 1993. Biogenic amines and phenolics characterize the defensive secretion of saturniid caterpillars (Lepidoptera: Saturniidae): a comparative study. *J. Comp. Physiol.* 163:123-132.
- DEML, R. AND K. DETTNER. 1997. Chemical defense of emperor moths and tussock moths (Lepidoptera: Saturniidae, Lymantriidae). *Entomol. Gen.* 21:225-251.
- DEML, R. AND K. DETTNER. 2003. Comparative morphology and secretion chemistry of the scoli in caterpillars of *Hyalophora cecropia*. *Naturwissenschaften* 90:460-463.
- DUNCAN, J. B. 1941. The biological control of *Platysamia gloveri* (Str.) with special reference to enemies of the pupal stage (Saturniidae, Lepidoptera). Master's thesis, Univ. Utah, Salt Lake City.
- DYER, L. A. 1995. Tasty generalists and nasty specialists? Antipredator mechanisms in tropical Lepidopteran larvae. *Ecology* 75:1483-1496.
- HÖLLDOBLER, B. AND E. O. WILSON. 1994. *Journey to the Ants: A Story of Scientific Exploration*. Harvard University Press, Cambridge.
- KARHU, K. J. 1998. Effects of ant exclusion during outbreaks of a defoliator and a sap-sucker on birch. *Ecol. Entomol.* 23:185-194.
- TUSKES, P. M., J. P. TUTTLE, AND M. M. COLLINS. 1996. *The Wild Silk Moths of North America*. Cornell University Press. 250pp.

MICHAEL M. COLLINS, *Research Associate, Carnegie Museum of Natural History, Section of Invertebrates, Pittsburgh PA. Mailing address: 215 Prospect St., Nevada City, CA 95959. email: michaelmerlecollins@comcast.net*

*Submitted for publication 16 September 2011; revised and accepted 5 May 2012.*

THE FRUIT-FEEDING BUTTERFLY *PAREUPTYCHIA OCIRRHOE* (NYMPHALIDAE: SATYRINAE)  
FEEDING ON EXTRAFLORAL NECTARIES

**Additional key words:** Neotropical region, opportunistic behavior, sandy forest, Euphorbiaceae

Tropical butterflies can be divided in two feeding guilds: nectar-feeders and fruit-feeders. Nectar-feeding species gather most of their nutritional requirements from flower nectar, while fruit-feeding butterflies feed on rotting fruits, feces, tree sap and some other decaying organic matter (Young 1975, DeVries 1987). In the Neotropics, fruit-feeding butterflies include members of the subfamilies Biblidinae, Charaxinae, Satyrinae (tribes Satyrini, Brassolini and Morphini) and Nymphalinae (tribe Coeini). Due to their attraction to rotting fruits, these butterflies are easily sampled with bait traps (Shuey 1997), and have been extensively used as models to assess levels of anthropogenic disturbance to the environment (e.g. Ribeiro et al. 2008; 2012, Bonebrake et al. 2010).

The fruit-feeding habit evolved several times in nymphalid butterflies probably as an escape route from periods of low flower abundance (Krenn et al. 2001). Most species have a proboscis adapted to suck fluids from moist surfaces, which is morphologically different from that of nectar-feeding butterflies (Krenn 2010).

On 24 July 2011, in a sandy forest vegetation (“restinga”) of Ubatuba (São Paulo State, SE Brazil), an individual of *Pareuptychia ocirrhoe* (Fabricius, 1776) (Satyrinae: Satyrini) was observed at 1338 h feeding on extrafloral nectaries (EFNs)—nectary glands not directly related to pollination (see Koptur 1992)—of a passion vine (Passifloraceae) at the edge of a trail of the sandy forest (Fig. 1A).

The butterfly was flying 40–50 cm above ground along the trail, moving from one edge to the other, sometimes landing on the vegetation. At some point it started to flutter around the plants for a few seconds before landing on a leaf of the liana. Then it moved toward the petiole and started to feed on the EFNs (Fig. 1B). This behavior was repeated a few times and each time it fed for a few seconds before flying off the plant. When disturbed the butterfly flew to higher foliage along the trail, returning to the liana after a few minutes.

Feeding on EFNs by adult butterflies is commonly observed in specialized ant-tended lineages of Lycaenidae and Riodinidae, but is rare in other families

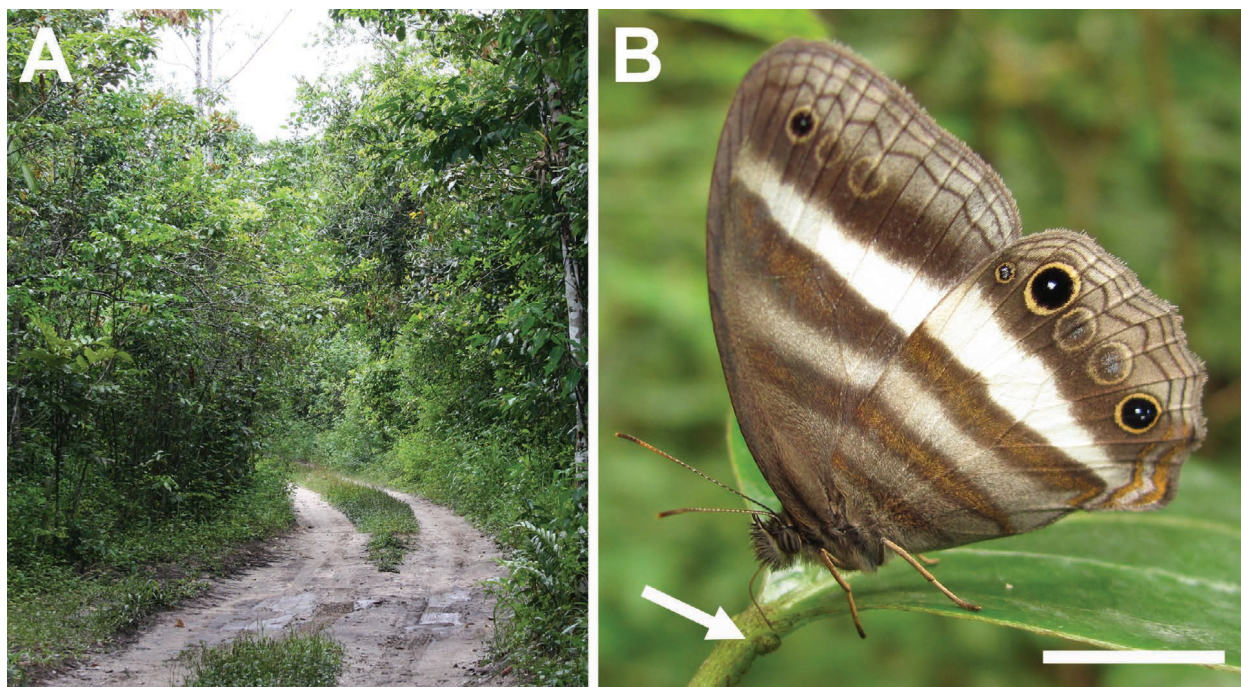


FIG. 1. **A** – Trail along the sandy forest where the *Pareuptychia ocirrhoe* was observed (Ubatuba, state of São Paulo, southeastern Brazil); **B** – Feeding by *Pareuptychia ocirrhoe* on the extrafloral nectary (arrow) of a Passifloraceae plant. Scale bar = 8mm.



(Vila & Eastwood 2006). Thus it appears that feeding on EFNs by *P. ocirrhoe* is an opportunistic behavior.

Fruit-feeding butterflies are usually attracted to the volatiles produced by the fermentation process of their food sources, which differs from nectar-feeding butterflies that are attracted mainly by color displays (Sourakov et al. 2012). So the opportunistic behavior showed by *P. ocirrhoe* is possibly related to volatiles released by sugar fermentation around the gland.

This behavioral record of *P. ocirrhoe* is important because it shows that fruit-feeding butterflies are able to explore alternative food sources. This opportunistic behavior could be especially important in periods when their natural food sources are scarce.

#### ACKNOWLEDGEMENTS

I thank the Núcleo Picinguaba of the Parque Estadual da Serra do Mar and the Programa de Pós-Graduação em Ecologia of UNICAMP for logistic support during fieldwork, Jorge Y. Tamashiro for the identification of the plant family and L.A. Kaminski, A.V.L. Freitas, and P.S. Oliveira for reading the manuscript. The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported the study through a Master fellowship. This publication is part of the RedeLep "Rede Nacional de Pesquisa e Conservação de Lepidópteros" SISBIOTA-Brasil/CNPq (563332/2010-7), and BIOTA-FAPESP Program (11/50225-3).

#### LITERATURE CITED

- BONEBRAKE, T. C., L. C. PONISIO, C. L. BOGGS & P. R. EHRLICH. 2010. More than just indicators: A review of tropical butterfly ecology and conservation. *Biol. Conserv.* 143:1831–1841.
- DEVRIES, P. J. 1987. The butterflies of Costa Rica and their natural history 1. Papilionidae, Pieridae and Nymphalidae. Princeton, NJ, Princeton University Press. xxii + 327 pp.
- KOPTUR, S. 1992. Extrafloral nectary-mediated interactions between insects and plants. pp 81–129. *In* Bernays, E. (ed.), *Insect-Plant Interactions*. CRC Press, Boca Raton.
- KRENN, H. W. 2010. Feeding Mechanisms of Adult Lepidoptera: Structure, Function, and Evolution of the Mouthparts. *Annu. Rev. Entomol.* 55:307–327.
- KRENN, H. W., K. P. ZULKA & T. GATSCHEGG. 2001. Proboscis morphology and food preferences in Nymphalidae (Lepidoptera, Papilionoidea). *J. Zool.* 253:17–26.
- RIBEIRO, D. B., P. I. PRADO, K. B. BROWN JR. & A. V. L. FREITAS. 2008. Additive partitioning of butterfly diversity in a fragmented landscape: importance of scale and implications for conservation. *Divers. Distrib.* 14:961–968.
- RIBEIRO, D. B., R. BATISTA, P. I. PRADO, K. B. BROWN JR. & A. V. L. FREITAS. 2012. The importance of small scales to the fruit-feeding butterfly assemblages in a fragmented landscape. *Biodivers. Conserv.* Published online. doi: 10.1007/s10531-011-0222-x.
- SHUEY, J. A. 1997. An optimized portable bait trap for quantitative sampling of butterflies. *Trop. Lepid.* 8:1–4.
- SOURAKOV, A., A. DUEHL & A. SOURAKOV. 2012. Foraging Behavior of the Blue *Morpho* and Other Tropical Butterflies: the Chemical and Electrophysiological Basis of Olfactory Preferences and the Role of Color. *Psyche* 2012:1–10. doi:10.1155/2012/378050.
- Vila, R. & E. Eastwood. 2006. Extrafloral nectar feeding by *Strymon jacqueline* Nicolay & Robbins, 2005 (Lepidoptera: Lycaenidae: Eumaeini). *Rev. Peru. Biol.* 13(1):125–128.
- YOUNG, A. M. 1975. Feeding behaviour of *Morpho* butterflies (Lepidoptera: Nymphalidae: Morphinae) in a seasonal tropical environment. *Rev. Biol. Trop.* 23:101–123.

EDUARDO P. BARBOSA, PPG-Ecologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil, e-mail: ed\_1482@yahoo.com.br.

Received for publication 3 April 2012; revised and accepted 28 June 2012.

LYCAENIDAE LARVAE FEEDING ON *PEIXOTOA PARVIFLORA* (MALPIGHIACEAE) IN A SEMI-DECIDUOUS FOREST IN SOUTHEASTERN BRAZIL**Additional key words:** *Allosmaitia strophius*, *Strymon mulucha*, *Panthiades hebraeus*, *Parrhasius polibetes*

The knowledge of the host range used by Lycaenidae is a major element in ecological studies as it may be correlated with several characteristics of population dynamics, life histories and interactions with other insects (see Robbins & Aiello 1982, Fiedler 1991, Pierce et al. 2002). In Brazil studies regarding lycaenid and host plant range have increased substantially in the last few years (Schmid et al. 2010, Silva et al. 2011, Kaminski et al. 2012). However in many natural areas, principally in biodiversity hotspots, lycaenid life histories, occurrence and host plant are poorly described when compared to those used by other butterfly groups (Brown Jr. & Freitas 2002, Dessuy & Morais 2007).

Knowledge of lycaenid-plant relationships is important for two reasons. First Lycaenidae is very important in tropical trophic cascades and multi-trophic interactions because larvae have ant-organs and associated behaviors and anatomical structures related to myrmecophily (Pierce et al. 2002, Kaminski et al. 2010). Second, butterflies are highly sensitive to environmental perturbations; departures from their ideal habitat conditions may have negative impacts on their occurrence and performance (Brown Jr. 1993, Brown Jr. & Freitas 2002, Rabasa et al. 2008). In Brazil Malpighiaceae is a host plant family of major interest for the study of lycaenids because it was shown to support a diverse florivorous lycaenid assemblage, principally in Brazilian tropical savanna (Kaminski & Freitas 2010, Silva et al. 2011). In other Brazilian biomes, however, the Malpighiaceae-lycaenid interactions remain to be studied. Thus in this study we provide the first record of the lycaenid community associated to *Peixotoa parviflora* A. Juss. (Malpighiaceae) in a Brazilian Atlantic Forest, and discuss aspects of lycaenid ecology and host association.

Samplings occurred in February 2012 at the Serra do Japi Ecological Reserve, located in Jundiá city, Brazil (46°53'–47°05' W, 23°13'–23°19' S). The area is composed of semi-deciduous forest (Atlantic Forest) and presents two well established seasons, a dry/cold season from April to September and a wet/warm season from October to March (Morellato 1992). *Peixotoa parviflora* is a climbing vine common at the edges along the Observatory trail at Japi, receiving direct sunlight all day long. Its flowers are pentamerous and yellow; flower buds contain oil glands at the base and leaves bear a pair of extrafloral nectaries at the base near the petiole. We were

able to sample a total of 40 *P. parviflora* inflorescences. Because *P. parviflora* is a climbing vine delineating differences between individuals was difficult to accomplish. Thus, the inflorescences were sampled within a more restricted range of 200 meters along the forest edge. All Lycaenidae individuals (eggs and larvae) were collected and reared in transparent covered plastic pots (200 ml) and fed whenever necessary with flowers of *Banisteriopsis malifolia* (Nees & Mart.) B. Gates (Malpighiaceae). *Banisteriopsis malifolia* was used as alternative food source because of the lack of *P. parviflora*. Moreover *B. malifolia* is consumed by several florivore lycaenid species. During samplings we observed all the ant species co-occurring with lycaenids on the plants and whether ants performed tending behavior or not, which is indicative of myrmecophily (positive association with ants - Fiedler 1991). Voucher specimens of the immature stages were deposited at the Museu de Zoologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

We found 16 Lycaenidae larvae belonging to three species. The most abundant was *Allosmaitia strophius* (Godart, 1924) (n = 9), followed by *Strymon mulucha* (Stoll, 1780) (n = 5) and *Panthiades hebraeus* (Hewitson, 1867) (n = 2). All larvae were found feeding on flower buds of *P. parviflora*. Twelve lycaenid eggs were found attached to flower buds, but 10 were already hatched. Eggs belonged to *Strymon* spp. (n = 8) (species level identification from eggs is not possible in *Strymon*), *A. strophius* (n = 3) and *Parrhasius polibetes* (Stoll 1781) (n = 1). No lycaenid larvae found in *P. parviflora* was parasitized. However two eggs (one belonging to *A. strophius* and one belonging to *P. polibetes*) were already parasitized by an unidentified microhymenopteran species. No pupa was found on the plant. *Allosmaitia strophius* larvae were polychromatic, individuals became yellow during their larval stage, camouflaging *P. parviflora* flower color. In field we noticed the presence of four ant species on *P. parviflora*: *Cephalotes pusillus* (Klug, 1824) (Myrmicinae), *Pseudomyrmex flavipes* (Lund, 1831) (Pseudomyrmecinae), and two *Crematogaster* (Lund, 1831) (Myrmicinae) species. Except for *P. flavipes*, all ant species were collecting nectar from the EFNs. We did not observe any type of interaction, neither positive (attendance) nor negative (attack) between ants and lycaenid larvae.

In this study we showed that *P. parviflora* is a new host plant for *A. strophius*, *S. mulucha*, *P. hebraeus* and *P. polibetes*. For *A. strophius*, a common species at Japi (Brown Jr. 1992), *P. parviflora* flowers are an important food resource, because larvae are specialist in feeding on Malpighiaceae flowers (Kaminski & Freitas 2010). *Panhiades* and *Strymon* are scarcely studied in regards to host range. *Panhiades hebraeus* is a neo-tropical butterfly occurring in Argentina, Brazil and Paraguay (Robbins & Lamas 2004) and up to date it was found only on a few Leguminosae and Rosaceae (Beccaloni et al. 2008). *Strymon mulucha* is an uncommon species at Japi (Brown Jr. 1992). According to the records, this lycaenid has five host plant species in different families, none being Malpighiaceae (Beccaloni et al. 2008, Silva et al. 2011). In this sense *P. parviflora* represents not only a new host for *P. hebraeus* and *S. mulucha*, but this is also the first register of these lycaenids in Malpighiaceae. According to *P. parviflora* geographical distribution (covering most of southeast Brazil), this species has a great potential of supplying resources for these florivore lycaenids, notably *A. strophius*. In neotropics there is a general lack of information about butterflies, especially Lycaenidae. Thus information about their host plants as well as the interactions with the associated insect fauna are important to understand the general patterns of host plant use within this butterfly family.

#### ACKNOWLEDGEMENTS

We thank Lucas Kaminski for valuable comments on the manuscript; Rafael F. Almeida for plant identification; John Bagnall for English review; Fapesp (AB grant), Capes (EAS grant) and CNPq (KDC grant) for financial support.

#### LITERATURE CITED

- BECCALONI, G. W., S. K. HALL, A. L. VILORIA, & G. S. ROBINSON. 2008. Catalogue of the host plants of the Neotropical butterflies. Monografias Tercer Milenio. Vol. 8. Zaragoza, Spain. 536 pp.
- BROWN JR., K. S. 1992. Borboletas da Serra do Japi: diversidade, hábitos, recursos alimentares e variação temporal, pp. 142-187. In Morellato, L. P. C. (ed.), História Natural da Serra do Japi: ecologia e preservação de uma área florestal no sudeste do Brasil. Unicamp Press, Campinas, São Paulo.
- . 1993. Neotropical Lycaenidae: an overview, pp.45-61. In New, T.R. (ed.). Conservation Biology of Lycaenidae (butterflies). IUCN Press, Gland, Switzerland.
- & A. V. L. FREITAS. 2002. Butterfly communities of urban forest fragments in Campinas, São Paulo, Brazil: Structure, instability, environmental correlates, and conservation. J. Insect Cons. 6:217-231.
- DESSUY, M. B. & A. B. B. MORAIS. 2007. Diversidade de borboletas (Lepidoptera, Papilionoidea e Hesperioidea) em fragmentos Floresta Estacional Decidua em Santa Maria, Rio Grande do Sul, Brasil. Rev. Bras. Zool. 24:108-120.
- FIEDLER, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonn. Zool. Monogr. 31:1-210.
- KAMINSKI, L. A. & A. V. L. FREITAS. 2010. Natural history and morphology of immature stages of the butterfly *Allosmaitia strophius* (Godart) (Lepidoptera: Lycaenidae) on flower buds of Malpighiaceae. Stud. Neotrop. Fauna E. 45:11-19.
- , A. V. L. FREITAS & P. S. OLIVEIRA. 2010. Interaction between mutualisms: ant-tended butterflies exploit enemy-free space provided by ant-treehopper associations. Am. Nat. 176:322-334.
- , D. RODRIGUES & A. V. L. FREITAS. 2012. Immature stages of *Parthasius polibetes* (Lepidoptera: Lycaenidae): host plants, tending ants, natural enemies, and morphology. J. Nat. Hist. 46:645-667.
- MORELLATO, L. P. C. 1992. Sazonalidade e dinâmica de ecossistemas florestais na Serra do Japi, pp. 98-109. In Morellato, L. P. C. (ed.), História natural da Serra do Japi: ecologia e preservação de uma área florestal no Sudeste do Brasil. Unicamp Press, Campinas, São Paulo.
- PIERCE, N. E., M. F. BRABY, A. HEATH, D. J. LOHMAN, J. MATHEW, D. B. RAND, & M. A. TRAVASSOS. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu. Rev. Entomol. 47:733-771.
- RABASA, S. G., D. GUTIÉRREZ, & A. ESCUDERO. 2008. Relative importance of host plant patch geometry and habitat quality on the patterns of occupancy, extinction and density of the monophagous butterfly *Iolana iolas*. Oecologia 156:491-503.
- ROBBINS, R. K. & A. AIELLO. 1982. Foodplant and oviposition records for panamanian Lycaenidae and Riodinidae. J. Lepid. Soc. 36:65-75.
- & G. LAMAS. 2004. Lycaenidae. Theclinae. Eumaeini. In Lamas, G. (ed.), Checklist: Part 4A. Hesperioidea—Papilionoidea. In Heppner JB, editor. Atlas of Neotropical Lepidoptera. pp.118-137. Volume 5A. Gainesville (IL): Association for Tropical Lepidoptera, Scientific Publishers.
- SCHMID, S., V. K. SCHMID, R. KAMKE, J. STEINER, & A. ZILLIKENS. 2010. Association of three species of *Strymon* Hübner (Lycaenidae: Theclinae: Eumaeini) with bromeliads in southern Brazil. J. Res. Lepid. 42:50-55.
- SILVA, N. A. P., M. DUARTE, I. R. DINIZ, & H. C. MORAIS. 2011. Host plants of Lycaenidae on inflorescences in the central Brazilian cerrado. J. Res. Lepid. 44:95-105.
- ALEXANDRA BÄCHTOLD. Programa de Pós Graduação em Entomologia. FFCLRP, Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes n° 3900, CEP. 14040-901, Ribeirão Preto, São Paulo, Brasil. [cuca\\_bachtold@yahoo.com.br](mailto:cuca_bachtold@yahoo.com.br); ESTEVAO ALVES-SILVA. Programa de Pós Graduação em Ecologia e Conservação de Recursos Naturais, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. [estevaokienzan@yahoo.com.br](mailto:estevaokienzan@yahoo.com.br); KLEBER DEL-CLARO. Instituto de Biologia, Universidade Federal de Uberlândia, Rua Ceará, s/n. Bloco 2D - Campus Umarama, CEP 38400-902, Uberlândia, Minas Gerais, Brasil; [delclaro@ufu.br](mailto:delclaro@ufu.br).

Received for publication 10 May 2012; revised and accepted 21 July 2012.



SMALLNESS AND BIGNESS: RELATION OF UNDERLYING CELL SIZE AND NUMBER TO  
LEPIDOPTERAN BODY SIZE**Additional key words:** developmental biology, hyperplasia, hypertrophy, *Malacosoma disstria*.

Adult body size is a key trait in Lepidoptera as in other insects because it governs or interacts with many physiological, life history, and ecological processes (Chown & Gaston 2010). Variation in adult body size, within or among species, normal or extreme, invites a fundamental developmental question, namely, how are differences in body size manifested—by different numbers of cells (**hyperplasia**), by different sized cells (**hypertrophy**), or by a combination of these? Counting and measuring all adult body cells is difficult if not impossible. An alternative is to compare samples of representative body cells among different sized individuals. Such cells in Lepidoptera include wing scale follicles, facets of the compound eye, fat body, epidermis, and doubtless other as yet unexplored tissues. Although hyperplasia and hypertrophy usually have been studied independently of one another, and not necessarily in the context of body size, previous work on each is informative. Some of the available data are intraspecific, each data point referring to one individual in a one-species dataset; most, however, are interspecific, each point referring to one individual in a multi-species dataset. Previously reported investigations mostly used wing length or span as a body-size surrogate, and surrogates and body size are used interchangeably in what follows.

**Hyperplasia.** Density of wing scales or their follicles reflects number of cells in wing surfaces. Köhler (1940) examined intraspecific scale-follicle density in a precisely defined forewing area of different sized adults of a strain of *Ephestia kuehniella* Zeller (Pyrilidae). The differing body sizes were laboratory-induced by varying larval food availability. Using forewing length as a surrogate for body size, Köhler reported a statistically significant positive relation between follicle density and forewing length. In a sample of 1,700 individuals of many species, genera, and families, Schilder (1950) examined scale density as number of scale rows in a defined forewing area. He found scale density and forewing length positively related in this unusually large interspecific sample.

In passing, egg number—fecundity—is also positively related to female body size, most notably in capital breeding Lepidoptera (Miller 2005). Reproductive cells may or may not be equivalent to body cells in the sense

of the present discussion, but in any case fecundity illustrates an important life history effect of body size.

Facets of the compound eye represent cells nearer the body core than those of the outlying wings. Facets typically appear as hexagonal imprints in the hard surface of the eye (Yagi & Koyama 1963). In data of Yagi and Koyama analyzed and discussed further on here, facet numbers proved highly positively associated with forewing span interspecifically across 10 families.

**Hypertrophy.** Köhler (1940) examined cell size as well as cell number in his strain of *E. kuehniella* and found it likewise positively related to forewing length. Goldschmidt (1932) similarly found scale size positively associated with forewing length within as well as among populations of *Lymantria dispar* (L.) (Erebidae: Lymantriinae) that differed naturally in body size. Finck (1938) reported a similar finding among several different strains of *Ephestia kuehniella*. Interestingly, in parallel with fecundity, Goldschmidt, using a novel measuring method, reported that size of *Lymantria dispar* spermatocytes was positively correlated with male body size. In a 150-species sample, Yagi and Koyama (1963) showed that facet diameter was positively correlated with forewing span. Remarkably, Simonsen and Kristensen (2003) found scale size to be positively correlated with forewing length inter-specifically in small- to large-bodied species across more than 20 families.

Wyatt and Linzen (1965) measured cell size of fat body and abdominal epidermis in different sized pupae of *Hyalophora cecropia* (L.) (Saturniidae). The use of pupae afforded a glimpse of cellular body-size development. Size of fat-body cells was positively associated with body size, but size of epidermal cells proved independent of body size. The authors concluded that such results were consistent with an existing hypothesis that cells destined mainly to fuel growth and development—a likely role of fat body—are correlated with body size, implying hypertrophy, whereas cells destined to persist to adult eclosion, such as epidermal cells, tend to be fixed in number, implying hyperplasia. A further generalization holds that number of body cells is fixed until their size reaches a certain limit, at which point their number increases (Yagi & Koyama 1963, Wyatt & Linzen 1965). This

generalization is plausible in that cell size does not increase indefinitely, but the idea needs further research.

Two original analyses are presented in this report. The first uses scattered data gleaned from Yagi and Koyama (1963) concerning facet numbers and forewing spans (Appendix). The second uses original measurements of facet size relative to fresh female mass in *Malacosoma disstria* (Hbn.) (Lasiocampidae).

**Methods and Results.** The analysis of Yagi and Koyama data here uses the wingspan surrogate. Mass, or weight, is a more direct and accurate measure of body size (Miller 1977 and references in Yagi & Koyama 1963), and its use for that purpose here in *Malacosoma disstria* is a rare departure.

In both analyses, straight lines through data points were fitted to minimize the sum of squares of the vertical differences between the lines and data points, as in typical regressions, but no regressions are implied here. The lines merely describe association. In this report the main interest is whether two variables are associated, as when both co-vary in response to other factors. Olmstead and Tukey's corner test (Sokal & Rohlf 1995), a simple correlation-like graphical method, was used to evaluate association. It does not measure magnitude of association, only probability of association. The method produces an algebraic sum, *S*, which is compared with tabulated values to ascertain statistical significance.

Before analysis, the Yagi and Koyama data were transformed to natural logarithms (ln) to tighten point scatter. The resulting least squares line is positive and described as  $(\ln \text{ No. facets}) = 3.64 + 1.236 \times (\ln \text{ wing span (mm) (15 n)})$  (graph not shown). The resulting *S*-value, 13, indicates the association is significant at the 0.02 level, and that it is unlikely due to sampling error. Thus facet numbers and body size in this sample are associated inter-specifically across 10 families. Interspecific associations in particular show that cell and body-size variables are generally similar for lepidopterans irrespective of taxa or body size.

In the original examination of facet diameter relative to female adult body mass in *Malacosoma disstria*, study insects were collected as pupae in Ontario. Upon eclosion, females were freeze-killed and weighed. The length of a row of 10 contiguous facets near the center of the eye of each female was measured and divided by 10 for an estimate of single-facet diameter. With an *S*-value of 13, coincidentally the same as in the preceding analysis, and significant at 0.02, facet diameter and body mass are clearly and positively associated (Fig. 1). This intraspecific outcome is hardly surprising.

In contrast to linear associations in both of the foregoing analyses, curvilinear relations were the rule in published sources cited earlier, as would be expected for

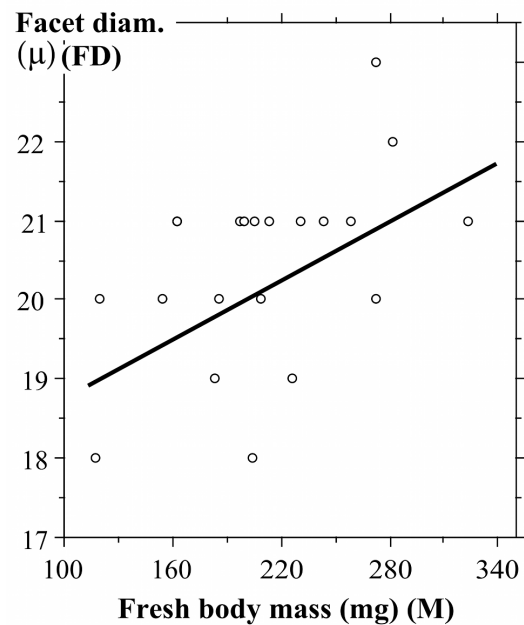


FIG. 1. Eye facet diameter (FD) as related to fresh body mass (M) in female *Malacosoma disstria* ( $FD = 18.07 + 0.0112 M$ , 21 n, *S*-value = 13, association of FD and M significant at 0.02 level).

allometry, and mixed relations of volumetric and one- or two-dimensional body-size variables like wing length. In any case, such curvilinearity has little importance here where the main interest is association.

Finally, all data sources were surveyed to count the number of species in which both hyperplasia and hypertrophy have been documented. Only three were found, but this paucity detracts little from implications of so much other evidence.

In conclusion, the cellular structure of all lepidopteran body sizes appears to consist of both hyperplasia and hypertrophy. Greater understanding of lepidopteran body-size differences will be advanced if future research examines additional body tissues, considers interrelations of cell size and number during ontogeny, and employs strong inference.

#### ACKNOWLEDGEMENTS

I thank Uli Munderloh, Josef Settele, and an unnamed reader for useful manuscript reviews, also Chris Muggli-Miller for computer assistance.

#### LITERATURE CITED

- CHOWN, S. L. & K. J. GASTON. 2010. Body size variation in insects: a macroecological perspective. *Biol. Rev.* 85: 139-169.
- FINCK, E. VON. 1938. Genetische Untersuchungen über die Schuppengröße und Schuppenform bei der Mehlmotte *Ephesia kuehniella* Z. Zeitschrift für Induktive Abstammungs- und Vererbungslehre 74: 161-201.
- GOLDSCHMIDT, R. 1932. Untersuchungen zur Genetik der geographischen Variation. 4. Cytologisches. Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen 126: 591-612.

- KÖHLER, W. 1940. Der Einfluss verschiedenen Ernährungsgrades auf äussere Körpermerkmale, auf die Entwicklungsgeschwindigkeit, Lebensdauer und Fortpflanzungsfähigkeit von *Ephestia kuehniella* Zeller. Biologisches Zentralblatt 60: 34-69.
- MILLER, W. E. 1977. Wing measure as a size index in Lepidoptera: the family Olethreutidae. Ann. Entomol. Soc. Am. 70: 253-256.
- MILLER, W. E. 2005. Extrinsic effects on fecundity-maternal weight relations in capital-breeding Lepidoptera. J. Lepid. Soc. 59: 143-160.
- SCHILDER, F. A. 1950. Körpergrösse und Organzahl der Organismen. Hallische Monographien 18: 1-58.
- SIMONSEN, T. J. & N. P. KRISTENSEN. 2003. Scale length/wing length correlation in Lepidoptera (Insecta). J. Nat. Hist. 37: 673-679.
- SOKAL, R. R. & F. J. ROHLF. 1995. Biometry, ed. 3. Freeman, New York. 887 pp.
- WYATT, G. R. & B. LINZEN. 1965. Relations of cell size to body size in fat body and epidermis of cecropia silkmoth pupae. J. Insect Physiol. 11: 259-262.
- YAGI, N. & N. KOYAMA. 1963. The compound eye of Lepidoptera: approach from organic evolution. Maruzen, Tokyo. 319 pp.

WILLIAM E. MILLER, *Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, Minnesota 55108; e-mail: mille014@umn.edu.*

*Submitted for publication 27 June 2012; revised and accepted 31 October 2012.*

---

Appendix. Species from Yagi and Koyama (1963) with sufficient data for analysis here. Ten families are represented.

---

<i>P. xuthus</i>	<i>A. preyeri</i>	<i>P. glacialis</i>
<i>C. e. poliographus</i>	<i>H. convolvuli</i>	<i>O. cerodelta</i>
<i>P. rapae</i>	<i>D. japonica</i>	<i>S. sp.</i>
<i>L. p. daimio</i>	<i>B. mori</i>	<i>L. ringoliella</i>
<i>T. hamada</i>	<i>T. a. kaguya</i>	<i>P. semifasciella</i>

---



## BOOK REVIEW

*Journal of the Lepidopterists' Society*  
67(1), 2013, 70

**FLIGHT BEHAVIOR.** By Barbara Kingsolver. 437 pp. Hardbound. ISBN: 978-0-06-212426-5. Harper, New York. 2012. \$28.99.

The last best-selling novel in which butterflies played a significant role was *The Collector* by John Fowles (1963). The eponymous Lepidopterist, Frederick Clegg, is a socially-inept, confused man who, having “collected” —kidnapped—a young woman with whom he is obsessed, has no real idea what to do with her. It ends badly. *Flight Behavior* is a very different kind of novel. It is, very briefly, the story of one woman’s epiphany, triggered by *Danaus plexippus*. The local name of the Monarch in Dellarobbia Turnbow’s part of Appalachia is “King Billy” (after the colors of William of Orange), but it has never been a major factor in the local culture until the day Dellarobbia stumbles into a nearby valley with its own “internal flame” like the “inside of joy”: an overwintering roost of millions of Monarchs where no such thing ever existed before. Its presence is an epiphenomenon of climate change. Somehow the Monarch’s internal compass has been reset: yesterday Valle de Bravo, Mexico; today the mountains of Tennessee. Suddenly the point of intersection between Christian belief and science is right here in this valley. Suddenly the politics of global warming is at everyone’s doorstep. Suddenly all the givens of life in this remote place are up for grabs.

There is a Lincoln Brower-esque figure, Ovid Byron. And indeed Brower helped to tutor Kingsolver, who has always been an environmentally-attuned writer, in Monarch biology. However, she needed less tutoring than most novelists would, because she trained as a biologist in her youth.

Many readers of this Journal will be asking themselves whether Barbara Kingsolver, biologist-turned-novelist, is related to Joel Kingsolver, evolutionary insect physiological ecologist, who has published many seminal butterfly studies. After all, there aren’t that many Kingsolvers out there! If one does an on-line search, one will find yes, they are brother and sister. But one learns to be skeptical of what one reads on-line, so I asked Joel. And no, they are not, though they have met and, he says, “decided our most recent common ancestor was in the 1790s in western Virginia.” So now you know.

This is a novel of personal transformation and of the conflict and integration of ways of knowing—of faith and reason, feeling and studying, the head and the heart. It is a great “read” whether you are into Monarch biology or not. Unlike *The Collector*, it does not end badly, insofar as it ends on a note of hope.

ARTHUR SHAPIRO, *Center for Population Biology, University of California, Davis, CA 95616; e-mail: amshapiro@ucdavis.edu*

## EDITORIAL STAFF OF THE *JOURNAL*

KEITH S. SUMMERVILLE, Editor  
Department of Environmental Science and Policy  
Drake University  
2507 University Ave., 131 Olin Hall  
Des Moines, Iowa 50311-4505  
ksummerville@drake.edu

PEG TOLIVER, Layout Editor  
Natural Imprints  
706 Lake Road  
Eureka, Illinois 61530  
naturimp@mtco.com

PHIL DeVRIES, Book Review Editor  
Department of Biological Sciences  
University of New Orleans, 2000 Lakeshore Dr.  
New Orleans, Louisiana 70148-0001 USA  
pdevries@uno.edu

### Associate Editors:

GERARDO LAMAS (*Peru*), KENELM W. PHILIP (*USA*), ROBERT K. ROBBINS (*USA*), FELIX SPERLING (*Canada*),  
DAVID L. WAGNER (*USA*), CHRISTER WIKLUND (*Sweden*), CARLA PENZ (*USA*), ANDREW WARREN (*USA*)

## NOTICE TO CONTRIBUTORS

Contributions to the *Journal* may deal with any aspect of Lepidoptera study. Categories are Articles, Profiles, General Notes, Technical Comments, Book Reviews, Obituaries, Feature Photographs, and Cover Illustrations. Obituaries must be authorized by the president of the society. Requirements for Feature Photographs and Cover Illustrations are stated in Volume 44(2):111 and on the Society's web site at <http://www.lepsoc.org/>.

Send *Journal* submissions electronically to: [ksummerville@drake.edu](mailto:ksummerville@drake.edu) or to the editor at the above address. Contributors should feel free to recommend one or two reviewers upon submission of their manuscript. Send requests to review books for the *Journal* and book review manuscripts to Phil DeVries at the above address (or electronically to: [pdevries@uno.edu](mailto:pdevries@uno.edu)). Short manuscripts concerning new state records, current events, and notices should be sent to the *News*, James Adams, School of Sciences and Math, 650 College Dr., Dalton State College, Dalton, GA 30720, e-mail: [jadams@daltonstate.edu](mailto:jadams@daltonstate.edu).

**Before submitting manuscripts or electronic files**, consult instructions on the Society's web site at <http://www.lepsoc.org/>.

Authors should provide an electronic copy of the manuscript in a standard PC or Macintosh-based word processing format.

**Abstract:** Include an informative abstract for Articles, Profiles, and Technical Comments.

**Additional key words:** Up to five key words or terms not in the title should accompany Articles, Profiles, General Notes, and Technical Comments.

**Text:** Write with precision, clarity and economy, and use the active voice and the first person whenever appropriate. Make title explicit, descriptive, and as short as possible. The first mention of a plant or animal in the text should include the *full scientific name* with *author*, and *family*. Use metric units for measurements, and a 24-h clock (0930 h, not 9:30 AM) to express time. Underline only where *italics* are intended.

**Literature cited:** References to Articles, Profiles, General Notes, and Technical Comments should be given as Sheppard (1959) or (Sheppard 1959, 1961a,b) and listed alphabetically under the heading Literature Cited, in plain text (do not capitalize author names) and without underlining as follows:

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

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

**Illustrations:** Electronic submissions are expected. Initial submissions may be low-resolution \*.jpg format, but final illustrations should be \*.tif format with image layers flattened, saved at a resolution of at least 350 ppi (137 pixels per cm), sent on a CD. Illustrate only half of symmetrical objects such as adults with wings spread, unless whole illustration is crucial. Bear in mind that your illustrations will be sized to fit a *Journal* page (plan to make lettering sufficiently large) and that the figure legend will appear at the bottom of the page (*Journal* page: 16.5 cm width, 22.5 cm height). One-column illustrations should be 1000 pixels wide; two-column illustrations should be 2200 pixels wide. Figures, both line drawings and photographs, should be numbered consecutively in Arabic numerals; do not use "plate." Type figure legends double-spaced at the end of the manuscript following Literature Cited. Use a separate paragraph for each legend. Color illustrations are encouraged; contact editor or consult our web site for submission requirements and cost.

**Tables:** Number tables consecutively in Arabic numerals. Label them in plain text (e.g., Table 1.) and use a concise and informative heading. Type each table on a separate sheet and place after the Literature Cited section, with the approximate desired position indicated in the text. Avoid vertical lines and vertical writing.

**Voucher specimens:** When appropriate, manuscripts must name a public repository where specimens documenting the identity of organisms can be found. Kinds of reports that require vouchering include descriptions of new taxa, life histories, host associations, immature morphology, and some experimental studies.

**Proofs:** The edited manuscript and galley proofs will be mailed to the author for correction of printer's errors. Excessive author's changes will be charged to authors at the rate of \$3.00 per line. A purchase order for reprints will accompany proofs.

**Page charges:** For authors affiliated with institutions, page charges are \$50 per *Journal* page. For authors without institutional support, page charges are \$25 per *Journal* page. For authors who are not members of the Society, page charges are \$75 per *Journal* page. Authors unable to pay page charges for any reason should apply to the editor at the time of submission for a reduced rate. Authors of Book Reviews and Obituaries are exempt from page charges.

**Correspondence:** Address all matters relating to the *Journal* to the editor. Address book reviews directly to the book review editor.

## CONTENTS

<p>OXYNETRA: FACIES AND DNA BARCODES POINT TO A NEW SPECIES FROM COSTA RICA (HESPERIIDAE: PYRGINAE: PYRRHOPYGINI) <i>Nick V. Grishin, John M. Burns, Daniel H. Janzen, Winnie Hallwachs and Mehrdad Hajibabaei</i> -----</p>	1
<p>NATURAL HISTORY AND CONSERVATION STATUS OF THE ENDANGERED MITCHELL'S SATYR BUTTERFLY: SYNTHESIS AND EXPANSION OF OUR KNOWLEDGE REGARDING <i>NEONYMPHA MITCHELLII MITCHELLII</i> FRENCH 1889 <i>Christopher A. Hamm, Barry L. Williams and Douglas A. Landis</i> -----</p>	15
<p>POPULATION BIOLOGY OF THE ENDANGERED FLUMINENSE SWALLOWTAIL BUTTERFLY <i>PARIDES ASCANIUS</i> (PAPILIONIDAE: PAPILIONINAE: TROIDINI) <i>Elisa Vieira Herkenhoff, Ricardo Ferreira Monteiro, Alexandre Pimenta Esperanço, and André Victor Lucci Freitas</i> -----</p>	29
<p>SYSTEMATICS AND BIOLOGY OF <i>CREMASTOBOMBYCIA CHROMOLAENAE</i>, NEW SPECIES (GRACILLARIIDAE), A NATURAL ENEMY OF <i>CHROMOLAENA ODORATA</i> (L.) KING AND H. ROBINSON (ASTERACEAE) <i>Donald R. Davis, Rodrigo Diaz, and William A. Overholt</i> -----</p>	35
<p>TWO NEW SPECIES OF <i>ANTAEOTRICHA</i> ZELLER FROM SOUTHEASTERN ARIZONA (GELECHIOIDEA: ELACHISTIDAE: STENOMATINAE) <i>Clifford D. Ferris</i> -----</p>	45
<p>INTERPRETATION OF WING PATTERN ELEMENTS IN RELATION TO BIRD PREDATION ON ADULT <i>HYALOPHORA</i> (SATURNIIDAE) <i>Michael M. Collins</i> -----</p>	52
GENERAL NOTES	
<p>THE REDISCOVERY OF <i>JOICEYA PRAECLARUS</i> TALBOT 1928 (LEPIDOPTERA: RIODINIDAE), MORE THAN 80 YEARS AFTER ITS DESCRIPTION <i>Roberto R. Greve, Curtis Callaghan, Lucas A. Kaminski and André V. L. Freitas</i> -----</p>	56
<p>REPEATED PREDATION OF MEADOW FRITILLARIES (<i>BOLORIA BELLONA</i>) BY AMBUSH BUGS (<i>PHYMATA</i> SPP.) <i>Joseph W. Dixon and Ryan D. Rasmussen</i>.-----</p>	57
<p><i>WALLENGRENIA EGEREMET</i> (HESPERIIDAE): A NEW POPULATION FOR WESTERN CANADA <i>Sarah J. Semmler and A. Richard Westwood</i>-----</p>	59
<p>ON THE FINDING OF DEAD ANTS ATTACHED TO SATURNIID CATERPILLARS: EVIDENCE OF SUCCESSFUL DETERRENT CHEMISTRY? <i>Michael M. Collins</i> -----</p>	62
<p>THE FRUIT-FEEDING BUTTERFLY <i>PAEUPTYCHIA OCIRRHOE</i> (NYMPHALIDAE: SATYRINAE) FEEDING ON EXTRAFLORAL NECTARIES <i>Eduardo P. Barbosa</i> -----</p>	64
<p>LYCAENIDAE LARVAE FEEDING ON <i>PEIXOTOA PARVIFLORA</i> (MALPIGHIACEAE) IN A SEMI-DECIDUOUS FOREST IN SOUTHEASTERN BRAZIL <i>Alexandra Bächtold, Estevao Alves-Silva and Kleber Del-Claro</i> -----</p>	65
<p>SMALLNESS AND BIGNESS: RELATION OF UNDERLYING CELL SIZE AND NUMBER TO LEPIDOPTERAN BODY SIZE <i>William E. Miller</i> -----</p>	67
BOOK REVIEWS	
<p>FLIGHT BEHAVIOR BY Barbara Kingsolver. <i>Arthur M. Shapiro</i> -----</p>	70
<p>LIST OF REVIEWERS FOR VOLUME 66 (2012) -----</p>	71