Full Issue

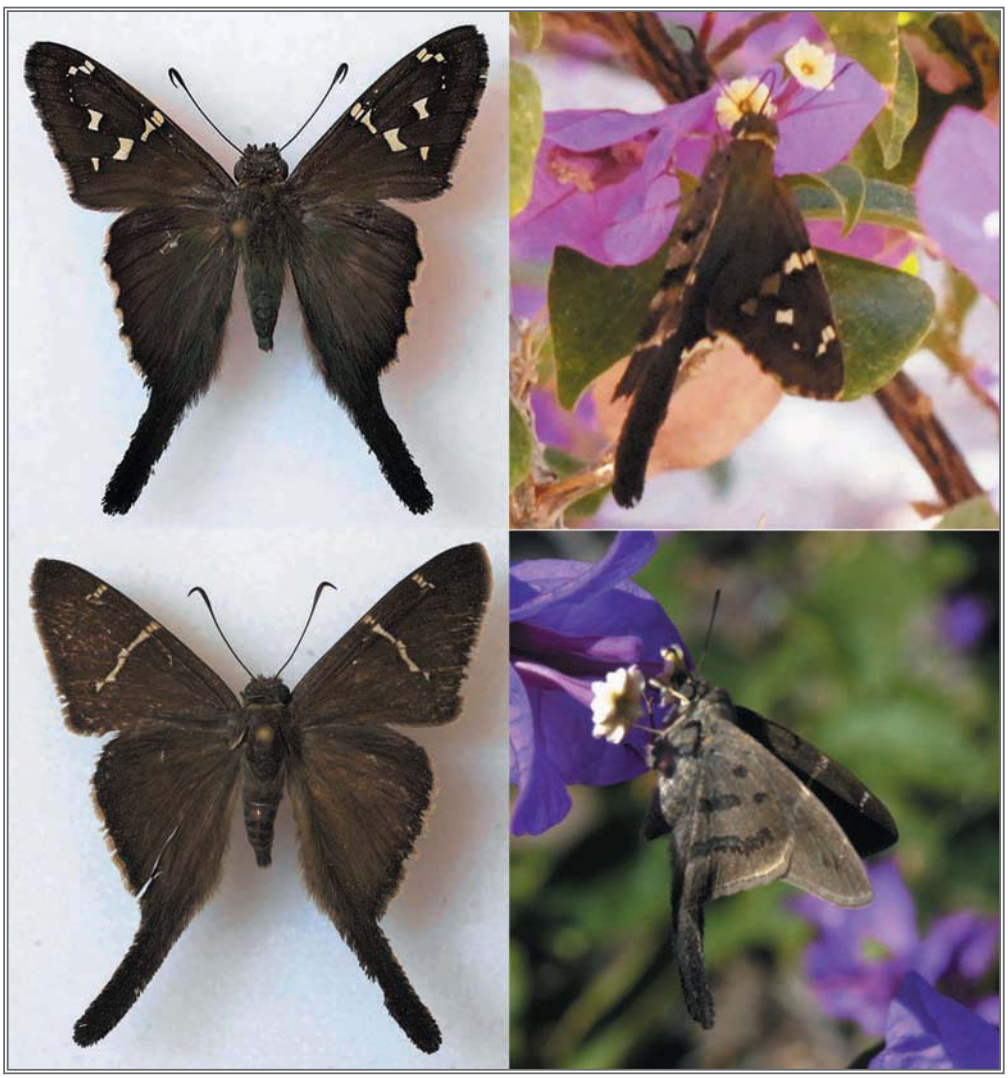
Source: The Journal of the Lepidopterists' Society, 70(2)

Published By: The Lepidopterists' Society

URL: https://doi.org/10.18473/lepi.v70i2.a16

Volume 70 Number 2
2016
ISSN 0024-0966

Journal of the Lepidopterists' Society



Published quarterly by The Lepidopterists' Society

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 (except Affiliate) receive the *Journal* and the *News of The Lepidopterists' Society.* Prospective members should send to the Treasurer full dues for the current year, together with their full name, address, and special lepidopterological interests. The additional cost for members outside the U.S. is to cover mailing costs. In even-numbered years the Society publishes a Membership Directory, with addresses, a geographical index, and a subject index of special interests.

Active members—annual dues $45.00 in U.S. ($50.00 in Canada & Mexico; $55 elsewhere)
Affiliate members—annual dues $10.00 worldwide
Student members—annual dues $20.00 in U.S. ($25.00 in Canada & Mexico; $30 elsewhere)
Sustaining members—annual dues $60.00 in U.S. ($65.00 in Canada & Mexico; $70 elsewhere)
Life members—single sum $1,800.00
Institutional subscriptions—annual $60.00 ($65.00 in Canada & Mexico; $70 elsewhere)
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 93311-1846; and address changes, claims for missing or damaged issues, and back issue orders to: Chris Grinter, Illinois Natural History Survey, 1816 South Oak St., Champaign, IL 61820-0904, e-mail: cgrinter@gmail.com. For information about the Society, contact: Michael E. Toliver, Division of Math & Sciences, Eureka College, 300 E. College Ave., Eureka, IL 61530-1500, e-mail: miketol@eureka.edu. To order back issues of the *Memoirs,* write for availability and prices to Kenneth R. Bliss, 1321 Huntington Trail, Round Rock, TX 78664, e-mail: krbliss@gmail.com.

Journal of The Lepidopterists' Society (ISSN 0024-0966) is published quarterly by The Lepidopterists' Society, *c/o* Chris Grinter, Asst. Secretary, Illinois Natural History Survey, 1816 South Oak St., Champaign, IL 61820-0904. Periodicals postage paid at Champaign, IL and at additional mailing offices. POSTMASTER: Send address changes to THE LEPIDOPTERISTS' SOCIETY, *c/o* Chris Grinter, Illinois Natural History Survey, 1816 South Oak St., Champaign, IL 61820-0904.

Website: http://www.lepsoc.org/

Facebook: https://www.facebook.com/lepsoc | Twitter: @thelepsoc

---

Cover illustration: Representatives of two separate groups of the genus *Urbanus* (Hesperiidae: Eudaminae). **Top Row:** *U. proteus* (Linnaeus) of the "*proteus* group" typically characterized dorsally by blue-green iridescence on the body and wing bases. **Bottom Row:** *U. procne* (Plötz), a member of the so-called "brown-bodied" *Urbanus*. The right column shows individuals of each species nectaring on ornamental *Bougainvillea* sp. (Nyctaginaceae) at San Carlos, Sonora, Mexico (December, 2015). Mounted specimens are from the same location collected on 07-Dec-2015 (*U. proteus*) and 01-Dec-2014 (*U. procne*). **Photograph credits:** Wain Evans, left column; E. Pfeiler, right column. See article on page 85.

Journal of the Lepidopterists' Society
70(2), 2016, 85–95

POLYPHYLY IN *URBANUS* AND *ASTRAPTES* (HESPERIIDAE: EUDAMINAE) ASSESSED USING MITOCHONDRIAL DNA BARCODES, WITH A REINSTATED STATUS PROPOSED FOR *ACHALARUS*

EDWARD PFEILER

Centro de Investigación en Alimentación y Desarrollo, A.C., Unidad Guaymas, Apartado Postal 284, Guaymas, Sonora C.P. 85480, México, *
*Corresponding author e-mail: pfeiler@ciad.mx

MARIANA RAMÍREZ LOUSTALOT LACLETTE

Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), Irapuato, Guanajuato C.P. 36821, México

AND

THERESE ANN MARKOW

Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), Irapuato, Guanajuato C.P. 36821, México
Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA

ABSTRACT. Phylogenetic analysis of mitochondrial DNA (cytochrome c oxidase subunit I; COI) barcode sequences is shown here to be an effective tool to aid in assessing polyphyly in selected genera of the subfamily Eudaminae (Hesperiidae). Results of phylogenetic analyses using both Bayesian inference and the neighbor-joining (NJ) algorithm were largely congruent and confirm and considerably expand on results from previous studies based on morphology and multilocus genetic data suggesting that the genera *Urbanus* and *Astraptes* consist of polyphyletic taxa as currently circumscribed. The genera *Narcosius* and *Ridens*, closely-related to *Urbanus* and *Astraptes*, emerged as monophyletic, although barcodes were available for only several species from each genus. Amino acid composition of the barcode segment in these and other closely allied genera was also useful in assessing polyphyly. Based on an analysis of barcodes, external morphology and the original description, a reinstated status is proposed for *Achalarus jalapus* (Plötz, 1881). We suggest that the phylogenetic barcode approach presented here to identify probable misplaced taxa can be used successfully in other taxa of the Eudaminae as well as in other subfamilies of Lepidoptera.

Additional key words: barcode amino acids; Bayesian inference; cytochrome c oxidase subunit I; neighbor-joining analysis; phylogenetic signal; *Thessia*

Since its inception more than a decade ago, the DNA barcode initiative has resulted in an extensive database of nucleotide sequence data with the expressed goal of providing a "reliable, cost-effective and accessible" means to identify known animal species and aid the discovery of new ones (Hebert et al. 2003, Hebert & Gregory 2005, Ratnasingham & Hebert 2007). The use of DNA barcodes, consisting of an approximately 650 base pair (bp) fragment beginning near the 5' end of the mitochondrial cytochrome c oxidase subunit I gene (COI or *cox1*), for species identification and discovery has also generated much controversy, with numerous authors pointing out its limitations (e.g., Brower 2006, Taylor & Harris 2012, Kwong et al. 2012, Kvist 2013). In particular, specific problems related to molecular taxonomy and phylogeography of the Lepidoptera that arise from using single-locus genetic data, including introgressive hybridization, incomplete lineage sorting, presence of symbiotic bacteria, and limited geographic sampling, have been discussed in detail (Forister et al. 2008, Zakharov et al. 2009, Kodandaramaiah et al. 2013). While caution clearly must be exercised when interpreting results based on COI barcodes, the barcode database is continuously growing (Ratnasingham & Hebert 2007, Zahiri et al. 2014) and thus represents a rich and unprecedented source of genetic information, much of which is publicly available.

Our goal in the present paper is to test whether barcodes could also be used to assess polyphyly in selected taxa of the Hesperiidae. Although higher level phylogenetic relationships are now typically assessed using large multilocus datasets (Lin and Danforth 2004, Regier et al. 2013, Wahlberg et al. 2013, McCormack et al. 2013), the single locus mitochondrial COI barcode segment shows substantial phylogenetic signal in Lepidoptera below the family level (Wilson 2011). Specifically, barcodes have proven their utility in interspecific phylogenetic studies in lasiocampid pine moths, *Dentrolimus* (Dai et al. 2012), noctuid moths, *Lasionycta* (Zahiri et al. 2014), nymphalid butterflies, *Junonia* (Pfeiler et al. 2012a, Gemmel and Marcus 2015) and *Hermeuptychia* (Seraphim et al. 2014), as well as in grasshoppers, Orthoptera (Huang et al. 2013) and caddisflies, Trichoptera (Boyle & Adamowicz 2015).

We focused our analysis on the hesperiid genera *Urbanus* and *Astraptes*, and their close relatives *Achalarus*, *Thessia*, *Ridens* and *Narcosius*, of the subfamily Eudaminae (previously a tribe [Eudamini] of the subfamily Pyrginae, recently elevated to a subfamily [Warren et al. 2009]). We chose this group of genera for two reasons. Firstly, detailed morphological analyses had previously suggested that *Urbanus* and *Astraptes* each consisted of a polyphyletic group of taxa in need of taxonomic revision (Steinhauser 1981, 1986, 1987). In particular, Steinhauser (1981) pointed out that some species of *Astraptes* appear to be more closely related to the so-called *"proteus* group" of *Urbanus* than some of the brown-bodied species assigned to *Urbanus*. The *"proteus* group" currently comprises about 18 species that show little variation in morphology and are characterized by green, blue or turquoise bodies and brown tails (Steinhauser 1981, Bertrand et al. 2014). Overall, the genus *Urbanus* consists of about 37 species as currently circumscribed (Mielke 2005, Bertrand et al. 2014). The genus *Astraptes* consists of about 38 species (Brower 2009), which includes the "ten species in one" proposed for *A. fulgerator* (Hebert et al. 2004, Brower 2010). Secondly, a comprehensive molecular phylogenetic study of the Hesperiidae based on maximum parsimony (MP) analysis of combined mitochondrial and nuclear genes showed that the two species of *Urbanus* analyzed, *U. dorantes* and *U. simplicius*, did not emerge as sister species (Warren et al. 2008), consistent with the earlier views of Steinhauser that the genus is polyphyletic. Most of the approximately 200 genera analyzed by Warren et al (2008), however, were represented by single species, including *Astraptes fulgerator*, *Narcosius colossus*, *Achalarus albociliatus*, *Autochton longipennis* and *Thorybes pylades* which were shown to be closely related to *U. dorantes* and *U. simplicius*. Thus, relationships among most species of *Urbanus* and *Astraptes*, and taxa in the other genera mentioned above, remain unclear. Complete (658 bp) COI barcode data are presently available in GenBank for a variety of nominal species of *Urbanus* (n = 16, nine of which are from the *"proteus* group") and *Astraptes* (n = 15). These sequences, together with those from closely allied genera, allowed us to assess in more detail whether barcodes could aid in assessing polyphyly in the Eudaminae and reveal taxa in need of taxonomic revision.

MATERIALS AND METHODS

Taxon sampling. Most of the barcode sequences examined here were taken from GenBank, primarily from the long-term biodiversity study of the butterfly fauna of Area de Conservación Guanacaste (ACG), Guanacaste Province, Costa Rica (Janzen et al. 2011). We also included new sequences for *Achalarus toxeus*, *A. albociliatus*, *Urbanus procne* and *U. dorantes* collected at coastal sites on the Gulf of California near Guaymas (27.961° N, 110.988° W) (Pfeiler et al. 2012b, 2016) and at Alamos (27.017° N, 108.947° W) in southern Sonora, Mexico. Links to additional information on collection data and adult photographs (where available) for voucher specimens deposited in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007) and the ACG database (Janzen & Hallwachs 2009) can be found in GenBank under the accession numbers provided on Fig. 1. In most cases, we have omitted subspecies designations because of their inconsistent use in GenBank and BOLD.

DNA extraction and data analysis. Methods for extracting DNA from butterfly legs, and for amplifying and sequencing the 658 bp barcode segment are found in Pfeiler et al. (2012a). GenBank accession numbers for the new COI sequences obtained for taxa from Sonora, Mexico are KT290034-KT290038 and KU041804-KU041805.

Alignments of all sequences were easily conducted by eye. Base composition showed little variation among sequences, with CG content averaging 29.5% (n = 69). Aligned sequences were translated in MEGA version 5.0.5 (Tamura et al. 2011). No gaps or stop codons were found. Together these results suggest that the barcode sequences analyzed represent mitochondrial DNA (mtDNA), and are not nuclear mitochondrial pseudogenes (numts) which have been reported for the COI gene in insects (Song et al. 2008). Calculations of Kimura (1980) 2-parameter (K2P) genetic distances (d) and uncorrected p-distances were carried out in MEGA. Although the use of K2P distances in barcode studies has been criticized (Srivathsan & Meier 2012), we found that p-distances and K2P distances were similar, or identical, in most of the taxa examined, consistent with results from previous studies in other closely-related congeneric arthropod taxa reported by us (Pfeiler et al. 2009, 2010) and others (Hamilton et al. 2014).

Phylogenetic relationships among 45 ingroup taxa of the Eudaminae were assessed using Bayesian inference implemented in MrBayes version 3.1 (Huelsenbeck & Ronquist 2001). The model of nucleotide substitution that best fit the data set, determined with Modeltest 3.7 (Posada & Crandall 1998) using the Akaike Information Criterion, was GTR + G. Bayesian analyses were run under the parameters of this model for 1,000,000 generations, sampled every 250th generation (4,000 trees sampled), using the default random tree option to begin the analysis. Clade support for Bayesian trees was estimated utilizing a Markov chain Monte Carlo

(MCMC) algorithm and expressed as posterior probabilities. We also examined relationships using the neighbor-joining (NJ) algorithm of Saitou and Nei (1987) carried out in MEGA using a matrix of K2P distances. The NJ analysis provides a rapid assessment of relationships and is the clustering method used to identify taxa from barcode data deposited in BOLD (Ratnasingham & Hebert 2007). Statistical support for nodes was obtained by bootstrap analyses using 1000 pseudoreplicates (Felsenstein 1985). We chose *Polythrix asine*, a species of Eudaminae which was not closely related to the ingroup taxa of interest (*d* >12.0%; *p*-distance >11.0%), and *Pyrgus communis* (Pyrginae) as outgroups.

Results and Discussion

Phylogenetic analysis of barcodes in selected genera of the Eudaminae yielded five highly-supported clades (labeled clades 1, 2, 3*a*, 3*b*, and 4) in the Bayesian tree (Fig. 1). Clade 3*b* was further subdivided in two moderately supported subclades 3*b** and 3*b***. Of the five main clades, only two were monophyletic, clade 2 (*Narcosius*) and clade 4 (*Ridens*). The genera *Urbanus* and *Astraptes* were shown to be polyphyletic as predicted from morphological studies (Steinhauser 1981, 1986, 1987). *Urbanus* species resolved in three separate clades, 1*a*, 1*b* and 3*b**. *Astraptes* species clustered mainly in clades 3*a* and subclade 3*b***. With the exception of the inclusion of *Astraptes tucuti*, subclade 3*b** was comprised entirely of the *"proteus* group" of *Urbanus.* External morphology of *A. tucuti* is similar to its congeners resolving in clades 3*a* and 3*b*** and its clustering with the *"proteus* group" of *Urbanus* may reflect either limits on the phylogenetic resolution of barcodes, or possibly mitochondrial introgression from a species of *Urbanus.* Phylogenetic analyses, and amino acid composition of barcodes (Table 1), suggest that that subclades 3*b** and 3*b*** are very closely related (net *d* = 2.8%). It is also noteworthy that two additional species of *Astraptes* did not cluster with their congeners in clades 3*a* and 3*b***. *Astraptes phalaecus* resolved in clade 1*a* and *A. egregius* was sister to *U. dorantes* in clade 1*b* (discussed further below).

Clade 3*a*, comprised of *A. aulus, A. janeira* and *A. enotrus*, resolved basally to subclades 3*b** and 3*b*** and was highly supported in both Bayesian (posterior probability = 1.00; Fig. 1) and NJ (96% bootstrap support) phylogenetic trees. The interior branch labeled clade 3 on Fig.1, however, was poorly supported in both trees (<0.75 posterior probability and 50% bootstrap support, respectively). Barcode amino acid composition of the three species of *Astraptes* resolving in clade 3*a* also showed an apparent fixed substitution at position 71 compared with subclades 3*b** and 3*b*** (Table 1; discussed further below).

Brown-bodied *Urbanus* (*U. dorantes, U. procne, U. simplicius, U. teleus, U. tanna, U. doryssus* and *U. albimargo*) clustered in clade 1(1*a* and 1*b*) along with a number of species from other genera, including *Astraptes* (as mentioned above), *Thorybes*, *Achalarus*, *Thessia* and *Autochton.* The same resolution of the five clades found in the Bayesian tree, as well as the partitioning of taxa within clades, also was seen in the NJ tree (not shown).

Clustering of *U. simplicius, U. dorantes, Thorybes pylades, Achalarus albociliatus* and *Autochton longipennis* in clade 85 of the MP tree of Warren et al. (2008) was also recovered in clade 1 of our Bayesian tree and NJ trees based on barcodes alone, although *Autochton zarex* (GenBank JF753725) was substituted for *A. longipennis* because only one partial barcode (387 bp) was available in GenBank. Also consistent with the results of Warren et al. (2008) was that *U. simplicius* and *U. dorantes* were not sister species in our trees, and *Narcosius colossus* and *Astraptes fulgerator* resolved outside of clade 1. In Warren et al. (2008), *N. colossus* and *A. fulgerator* clustered as sister species in a separate clade (clade 81) basal to clade 85. Finding similar results in the two studies is noteworthy given that Warren et al. (2008) used genetic data from nuclear genes together with a segment of the mitochondrial COI gene that did not overlap the 658 bp barcode segment. Overall, our barcode analyses provide clear support for Steinhauser's (1981) view that some species of *Astraptes* appear more closely related to the *"proteus* group" of *Urbanus* than do some of the brown-bodied species of *Urbanus*.

Barcode amino acid composition, based on examination of more than 1880 sequences from 45 species (Table 1), provided additional characters that support the polyphyly of *Urbanus* and *Astraptes.* All nine species of the *"proteus* group" of *Urbanus* resolving in subclade 3*b** (including *A. tucuti*), in addition to the nine species of *Astraptes* in subclade 3*b***, the sister clade to the *"proteus* group", shared the same amino acid substitution at site 71 (methionine) in the 219 amino acid barcode segment, a substitution not seen in any of the other clades, including clade 3*a* which contained three additional species of *Astraptes* (Table 1). All species from the various genera resolving in clades 1, 2, 3*a* and 4, including five species of *Astraptes* and seven species of *Urbanus*, show a leucine at site 71. The five species of *Ridens* shared a threonine at site 118 whereas all other species analyzed possessed a serine at this site, with the only exception being an alanine found in *U. tanna* (Table 1). All four species of *Narcosius* (clade 2) showed an invariant amino acid profile at sites 67, 71, 118 and 169

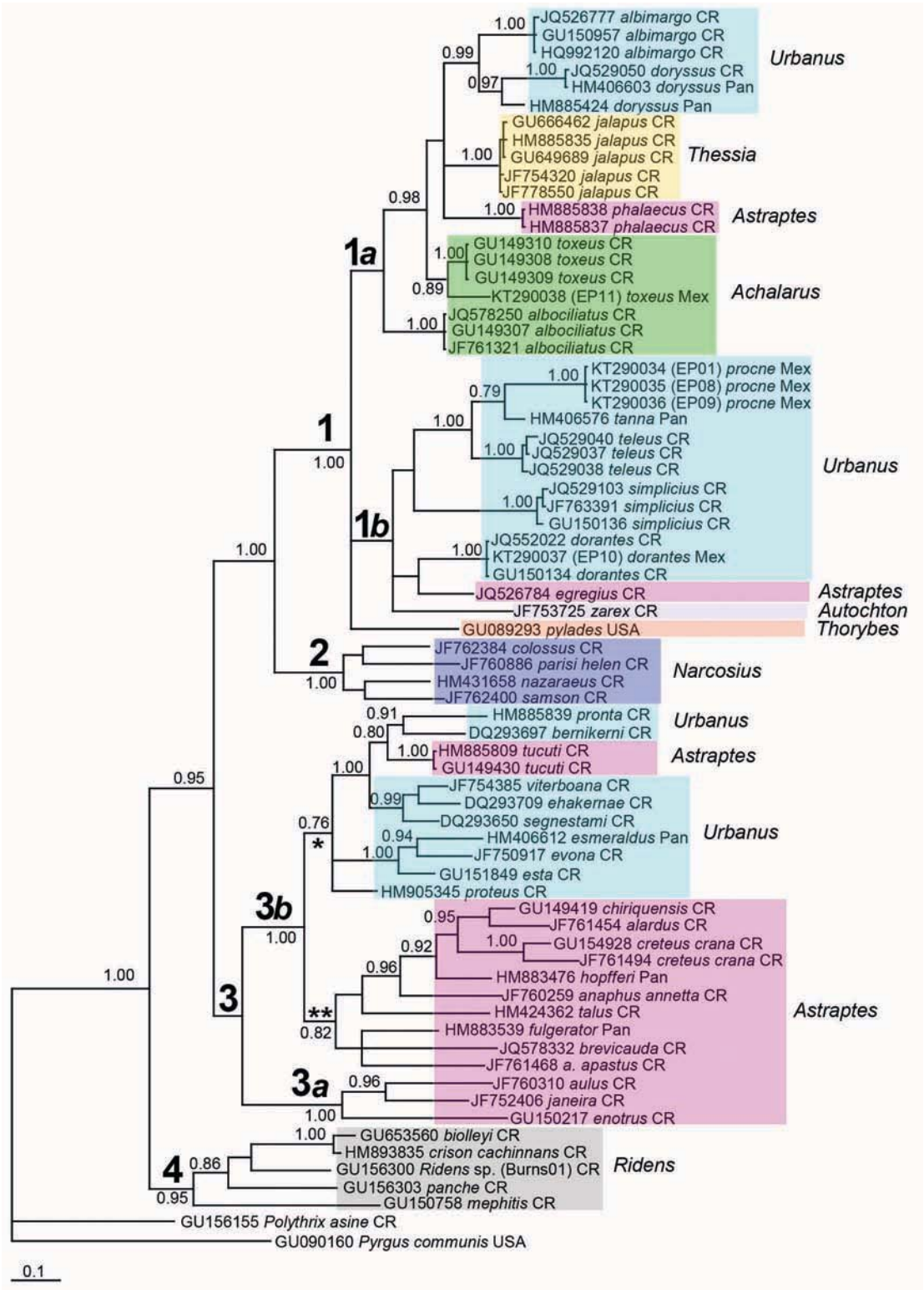


FIG. 1. Bayesian 50% majority rule consensus tree showing relationships among closely–related taxa of the Eudaminae based on COI barcode sequences. GenBank accession numbers are given for each sequence. Locality abbreviations: CR, Costa Rica; Pan, Panama; Mex, Mexico. Clade support values (posterior probabilities) are shown on branches; values <0.75 were omitted. Numbering of the main clades discussed in the text are shown in large bold type. The hesperiids *Pyrgus communis* (Pyrginae) and *Polythrix asine* (also in the Eudaminae but not closely related to the taxa of interest) were used as outgroups. The scale represents expected substitutions per site.

TABLE 1. Informative amino acid characters in the COI barcode protein segment (219 amino acids) in 45 species of the Eudaminae. Clades correspond to those in Fig. 1.

Species/Clade	*N*	Amino Acid Position 67	71	118	169
Clade 1					
Thorybes pylades	1	I	L	S	I
1*a*					
Urbanus albimargo	7	I	L	S	I
Urbanus doryssus	81	V/I	L	S	I
Thessia jalapus	26	I	L	S	I
Astraptes phalaecus	3	I	L	S	I
Achalarus toxeus [a]	6	I	L	S	I
Achalarus albociliatus	10	V	L	S	I
1*b*					
Urbanus procne	3	V	L	S	I
Urbanus tanna	1	V	L	A	I
Urbanus teleus	13	I	L	S	I
Urbanus simplicius	18	V	L	S	I
Urbanus dorantes	20	V	L	S	I
Astraptes egregius	12	V	L	S	I
Autochton zarex	13	V	L	S	I
Clade 2					
Narcosius colossus	32	V	L	S	I
Narcosius parisi helen	8	V	L	S	I
Narcosius nazareus	1	V	L	S	I
Narcosius samson	29	V	L	S	I
Clade 3					
3*a*					
Astraptes aulus	5	V	L	S	I
Astraptes janeira	16	V	L	S	V
Astraptes enotrus	100	V	L	S	V
3*b**					
Urbanus pronta	21	V	M	S	V
Urbanus bernikerni [b]	68	V	M	S	V
Astraptes tucuti	23	V	M	S	V
Urbanus viterboana	16	V	M	S	V
Urbanus ehakernae [b]	31	V	M	S	V
Urbanus segnestami [b]	177	V	M	S	V
Urbanus esmeraldus	134	V	M	S	V
Urbanus evona	18	V	M	S	V
Urbanus esta	78	V	M	S	V
Urbanus proteus	150	V	M	S	V
3*b***					
Astraptes chiriquensis	7	V	M	S	V
Astraptes alardus	30	V	M	S	V
Astraptes creteus crana DHJ01	14	V	M	S	V
Astraptes creteus crana DHJ02	82	V	M	S	I
Astraptes hopfferi	69	V	M	S	V
Astraptes anaphus annetta	122	V	M	S	V
Astraptes talus	25	V	M	S	V
Astraptes fulgerator	243	V	M	S	V
Astraptes brevicauda	22	V	M	S	V
Astraptes a. apastus	3	V	M	S	V
Clade 4					
Ridens biolleyi	11	V	L	T	V
Ridens crison cachinnans	6	V	L	T	V
Ridens panche	55	V	L	T	V
Ridens mephitis	49	V	L	T	I
Ridens sp. (Burns01)	13	V	L	T	I
Total	1872				

N, number of barcodes analyzed; *N* values for *Urbanus proteus* and *Astraptes fulgerator* are each subsamples of >400 available sequences. *Astraptes fulgerator* is now considered a complex of species-level taxa (Hebert et al. 2004; Brower 2010); all members of the complex were examined and included under *A. fulgerator*. Amino acid abbreviations: A, alanine; I, isoleucine; L, leucine; M, methionine; S, serine; T, threonine; V, valine. Partitioning of taxa of *Astraptes* and *Urbanus* among clades, and amino acid composition at sites 67, 71, 118 and 169 of all species, are highlighted.

[a] Includes *Achalarus* sp. cf. *toxeus* (EP11 and EP27);

[b] *Urbanus segnestami*, *U. bernikerni*, and *U. ehakernae*, previously *U. belli* DHJ01, –02, and –03 (Bertrand et al. 2014).

(VLSI) which was identical to that seen in *A. aulus* (clade 3*a*), most of the species in clade 1*b*, *Achalarus albociliatus*, and 30 of the 81 *U. doryssus* from clade 1*a* (labeled *U. doryssus* DHJ01 in Janzen et al. 2011).

It is important to point out that the amino acid compositions at the four sites shown in Table 1 were informative for evaluating relationships of the ingroup taxa analyzed here, but are not diagnostic when considering the entire Eudaminae. For example, we randomly chose several additional taxa outside of our ingroup and found that the amino acid pattern VLSI was also present in *Chioides zilpa* and *Proteides mercurius*. Likewise, VLTV, a pattern characteristic of three species of *Ridens* (clade 4) was also present in *Codatractus imalena, C. melon, C. carlos, C. alcaeus*.

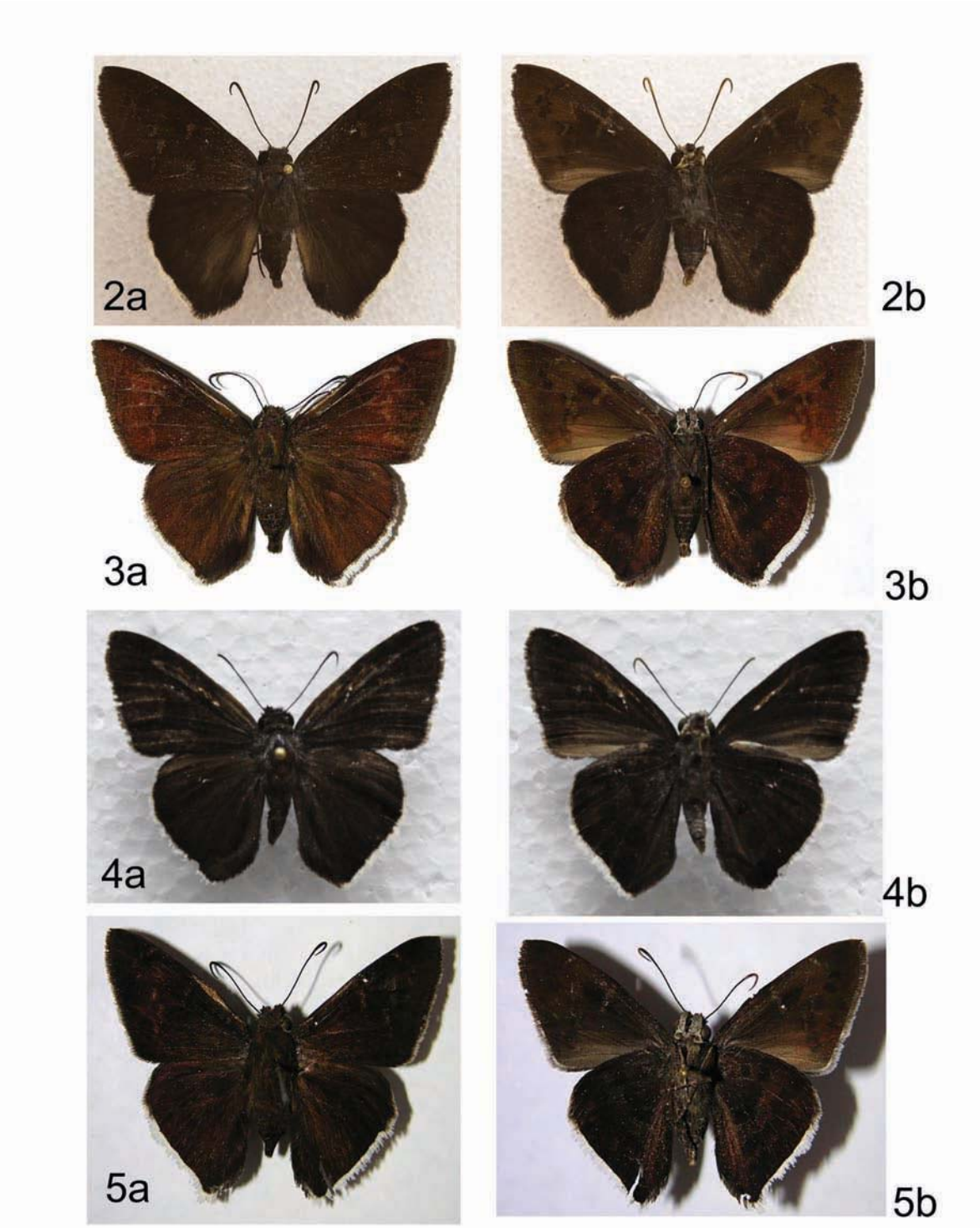
***Narcosius* and *Ridens*.** The genus *Narcosius* was erected by Steinhauser (1986) to include nine similar species of the Eudaminae, five of which had previously been placed in *Astraptes* (Evans 1952). Subsequently, Austin (1996) described three additional species from western Brazil. Of these 12 species, barcodes were available for four, *N. parisi helen, N. colossus, N. nazaraeus* and *N. samson*. Adults of the four species are essentially indistinguishable (see Fig. 4 in Janzen et al. 2011) but can be separated by genitalia, caterpillar facies, foodplant choice and/or DNA barcodes (Steinhauser 1986; Austin 1996; Janzen et al. 2011). Phylogenetic analyses of barcodes (Fig. 1) resolved these four species in a highly supported monophyletic clade (clade 2), supporting the taxonomic arrangement proposed by Steinhauser (1986) based mainly on genitalic morphology. Additionally, our analyses indicated that *N. samson* and *N. nazaraeus* resolved as sister species, also in agreement with Steinhauser (1986). Although *N. samson* and *N. nazaraeus* show the most similarities in genitalic morphology, most of the

remaining species of *Narcosius*, including *N. parisi helen* and *N. colossus* show substantial differences, especially in the male valvae (Steinhauser 1986; Austin 1996). Because we were limited in our analysis to only four of the 12 nominal species of *Narcosius*, and five of the approximately 20 nominal species of the genus *Ridens* (Evans 1952; Steinhauser 1983), additional molecular studies on the missing taxa, which includes the type species *Narcosius narcosius* and *Ridens ridens*, will be required to confirm the monophyly of both genera.

Clade 1 *Urbanus* and *Astraptes*. The observation that *Urbanus proteus*, the type species of the genus *Urbanus*, resolved in clade 3*b** along with its morphologically similar and turquoise-colored congeners, suggests that the name *Urbanus* be confined to this group and that the genetically distinct brown-bodied species of *Urbanus* clustering in clade 1 be transferred to a different genus (or genera). Otherwise, the genus *Urbanus* would remain polyphyletic. But it is clear that additional data, including life history information and molecular data from both mitochondrial and nuclear genes, will be required to sort out and stabilize the taxonomy of clade 1 *Urbanus*. Increased taxon sampling, especially in the genera *Thorybes* and *Autochton*, will also be required. For example, *Thorybes pylades* and *Urbanus dorantes* resolved as sister lineages in the study of Warren et al. (2008), and a close relationship among the two taxa was also seen in our study (d = 5.4%). The genetic relationship between *U. dorantes* and other species currently assigned to *Thorybes*, however, is unclear, as *T. pylades* currently is the only species with publicly available barcodes. Of particular interest was that *Astraptes egregius*, a species not included in the study of Warren et al. (2008), resolved as sister to *U. dorantes* (Fig. 1, clade 1*b*; also see Janzen et al. 2011), although the branch was poorly supported in both Bayesian and NJ (55% bootstrap value) trees. The close relationship between the two taxa (d = 4.4%) seems surprising given that the facies of the turquoise-colored *A. egregius* is typical of its congeners from clade 3, with the notable exception of *A. anaphus annetta*. More than a century ago, however, Godman & Salvin (1887-1901) commented that male genitalia of *Thymele* (=*Astraptes*) *egregius* were similar to *Eudamus* (=*Urbanus*) *simplicius*, again suggesting a closer relationship between *A. egregius* and the brown-bodied *Urbanus* species than superficial tail size and color differences would suggest. Barcode amino acid composition of *A. egregius* also is typical of most brown-bodied *Urbanus* and distinct from most other species of *Astraptes* (Table 1).

In the original description, it was noted that *Thymele* (=*Astraptes*) *phalaecus*, the Yellow-edged flasher, was superficially similar to *Telegonus* (=*Astraptes*) *anaphus anaphus*, the Yellow-tipped flasher, but that *Astraptes phalaecus* could be easily distinguished by the presence of three semihyaline subapical spots near the costa, as well as by the presence of a costal fold in males and differences in the shape of the valvae (Godman & Salvin 1887–1901). Phylogenetic analysis (Fig. 1), genetic distance (d >11.0%; p-distance >10.0%), and three differences in barcode amino acid composition (Table 1), also support the view that, although superficially similar, *A. phalaecus* and *A. anaphus annetta* (sensu Evans 1952) are not closely related and belong in different genera. Although barcode analysis suggests that *A. phalaecus*, as well as *Urbanus doryssus* and *U. albimargo*, are closely related to *Achalarus*, any taxonomic changes in these taxa and other apparently misplaced taxa of *Astraptes* will first require additional data, especially given that publicly available barcodes are currently lacking for *Achalarus lyciades* and *Astraptes aulestes*, the type species of their respective genera. Barcodes, however, are available for *Astraptes janeira* (see Table 1) which was listed by Steinhauser (1987) as a synonym of *Telegonus* (= *Astraptes*) *aulestes* (= *aulestis*) and which resolved in clade 3*a* (Fig. 1). Clearly, much additional work is needed to clear up the taxonomic questions in these genera and the Eudaminae in general. We suggest, however, that at this time there is enough evidence, detailed below, to propose reinstating *Thessia jalapus* to the genus *Achalarus*.

Genetic differentiation in *Achalarus*. In a recent biodiversity study on coastal butterflies of the state of Sonora in northwestern Mexico (Pfeiler et al. 2016), a specimen of what appeared to be *Achalarus toxeus* (Fig. 2a, b) was found, but because *A. toxeus* cannot be confidently distinguished from *Thessia jalapus* on external examination (Janzen et al. 2011) a barcode was obtained (voucher EP11) and queried in the BOLD database. The EP11 barcode showed 99.85% similarity (i.e. a single nucleotide difference) to three sequences of *A. toxeus* from the state of Jalisco, Mexico. The BOLD query of EP11, however, also showed about 3% sequence divergence (d) from *A. toxeus* from Costa Rica (Table 2; also see Figs. 1 and 3a, b) and the states of Sinaloa and Jalisco, Mexico. The EP11 sequence is labeled *A. toxeus* in Fig 1, but we suggest that an undescribed cryptic species, *Achalarus* sp. c.f. *toxeus*, may occur in northwestern Mexico together with nominate *A. toxeus* in Jalisco and possibly adjacent regions. Recently we obtained an additional specimen of *Achalarus* sp. c.f. *toxeus* from Alamos, Sonora (EP27;



FIGS. 2–5. Comparison of dorsal (**a**) and ventral (**b**) images of adult males of *Achalarus* species. Wingspans are given in mm; ACG, Area de Conservación Guanacaste, Guanacaste Province, Costa Rica. (**2**) *Achalarus* sp. cf. *toxeus*, 47 mm, San Carlos, Sonora, Mexico, 8 September 2014 (voucher EP11; GenBank KT290038); (**3**) *Achalarus toxeus*, 50 mm, ACG (voucher 02–SRNP–10179; GenBank GU149309); (**4**) *Achalarus albociliatus*, 43 mm, Alamos, Sonora, Mexico, 4 August 2015 (voucher EP28; GenBank KU041805); (**5**) *Achalarus jalapus* **reinstated status**, 44 mm, ACG (voucher 07–SRNP–21140; GenBank JF763249). Photograph credits: 2, Wain Evans; 3 and 5, Janzen & Hallwachs (2009); 4, E. Pfeiler.

GenBank KU041804) in which the barcode was identical to EP11.

The barcode from a specimen of *A. albociliatus* from Alamos (EP28; Fig. 4a, b) was also queried in BOLD and found to be identical to seven specimens of *A. albociliatus* from Costa Rica, apparently confirming its identity. BOLD results, however, also showed the Alamos sequence to be 100% identical to three sequences labeled *A. toxeus* from Mexico (Jalisco and Sinaloa), which we suggest is the result of misidentification of the Mexican specimens. Interestingly, however, the BOLD query also showed another genetically divergent clade of eight specimens labeled *A. albociliatus albociliatus* from southeastern Mexico (states of Campeche, Quintana Roo and Yucatán; no public records), resolving sister to the clade from Sonora and Costa Rica (d ~3.2%). This value of genetic divergence suggests that a cryptic species of *A. albociliatus* may also occur in southeastern Mexico. Thus, preliminary evidence for substantial genetic differentiation of *A. albociliatus* and *A. toxeus* in Mexico adds to the growing list of potential cryptic species of Lepidoptera revealed by barcode analyses (Dincă et al. 2015).

Thessia. Steinhauser (1989) erected the genus *Thessia* (type species: *Thessia athesis*) to include *Achalarus jalapus* (sensu Evans 1952) and *Urbanus athesis* (sensu Evans 1952), based mainly on similarities in genitalia characters among the two taxa compared with other related species of the Eudaminae. Although *T. jalapus* is generally recognized as a valid name (Opler & Warren 2005; Pelham 2008; ITIS 2015), its placement in *Thessia* rather than *Achalarus* has met with criticism (e.g. Cassie et al. 2001). Based on morphological and molecular evidence presented below we formally propose that *T. jalapus* should be returned to *Achalarus*.

TABLE 2. K2P genetic distances (d) (below the diagonal) and uncorrected p–distances (above the diagonal) among species of *Achalarus* based on COI barcodes. Values for within taxa genetic distances are shown in bold type along the diagonal. (**1**), *Achalarus* sp. c.f. *toxeus* (Sonora, Mexico; n = 2); (**2**), *A. toxeus* (Costa Rica; n = 3); (**3**), *A. albociliatus* (Sonora, Mexico and Costa Rica; n = 8); (**4**), *A. jalapus* **reinstated status** (Costa Rica; n = 5)

	1	**2**	**3**	**4**
1	**0.000**	0.029	0.062	0.050
2	0.030	**0.000**	0.046	0.036
3	0.066	0.047	**0.000**	0.051
4	0.052	0.037	0.054	**0.003**

Achalarus jalapus (Plötz, 1881), **reinstated status** (Fig. 5a, b)

Eudamus jalapus Plötz 1881: 100
Achalarus jalapus: Evans 1952: 128
Thessia jalapus: Steinhauser 1989: 12-13
=*Telegonus xerxes* Bell 1934: 90-92 (synonym)

We suggest that morphology of male genitalia was a poor diagnostic character to be used by Steinhauser (1989) as the main criterion for erecting the genus *Thessia* and was not consistent with his use of genitalia characters in earlier comprehensive taxonomic work on the Eudaminae. Specifically, Steinhauser (1986) had previously shown that diagnostic differences in genitalia morphology, especially in male valvae, were critical for separating species of *Narcosius* in which adults are so similar that they cannot be confidently placed using adult external characters alone (see above section on *Narcosius* and *Ridens*). A comparison of the valvae of *T. jalapus* and *T. athesis* with those of *A. toxeus* and *A. albociliatus* reveals that the interspecific differences among the four taxa are no greater than those seen among the 12 nominal species of *Narcosius* (Steinhauser 1986; Austin 1996). Other than the absence of a costal fold in males of *A. albociliatus*, adults of *A. albociliatus*, *A. toxeus* and *T. jalapus* are so similar (Figs. 2–5) that confident identifications often require additional genetic and/or life history information (Janzen et al. 2011), as mentioned earlier. Mean pairwise genetic distance between *A. toxeus* and *T. jalapus* based on barcodes (d = 3.7%) is less than the value seen between *A. toxeus* and *A. albociliatus* (d = 4.7%) and is similar to that seen between the two genetically distinct populations of both *A. toxeus* (d = 3.0%; Table 2) and *A. albociliatus* (d ~3.2%) in Mexico, providing additional support for the view that *Thessia jalapus* should be returned to the genus *Achalarus*.

We also considered transferring *Thessia athesis* to *Achalarus* and formally suppressing the name *Thessia*. Unfortunately, no barcodes for *T. athesis* are currently available in BOLD or GenBank to assess the genetic relationship of *T. athesis* to *A. jalapus* and the other taxa of the Eudaminae. Because *T. athesis* is the type species of the genus (Steinhauser 1989), further genetic studies will be required before considering any change in its taxonomic status.

Concluding remarks. We have shown that phylogenetic analysis of COI barcodes is an effective tool in screening for polyphyly, revealing taxa that probably require taxonomic revision, in selected genera of the hesperiid subfamily Eudaminae. We suggest that this method could also be successfully used to help

untangle confused taxonomy in other closely related species of the Eudaminae, as well as in other subfamilies of Lepidoptera. We envision this tool as an initial screening to be used mainly in conjunction with multilocus genetic data (Forister et al. 2008, Rubinoff & Holland 2005) and traditional methods for species descriptions that also incorporate ecological, behavioral, and life history data into an integrative taxonomy framework (Dayrat 2005). But as we have also shown here, single locus barcodes, assessed together with external morphological characters and critical analysis of original species descriptions, can provide important information in support of taxonomic rearrangements.

Although an extensive database of barcodes is publicly available for use in phylogenetic studies of the type proposed here, several limitations warrant attention. Species coverage in GenBank for *Urbanus*, *Astraptes*, *Ridens* and *Narcosius* was about 40–50%, sufficient to confirm the polyphyly of *Urbanus* and *Astraptes*, and to hypothesize that *Ridens* and *Narcosius* are monophyletic as currently circumscribed. Additional species of both *Ridens* and *Narcosius* will need to be analyzed, however, to test the hypothesis that both genera are monophyletic. Also, data records in both GenBank and BOLD are known to contain taxonomic misidentifications and other errors that question their reliability (Wilson 2010, Shen et al. 2013). One suspect species identification of *Achalarus* was mentioned above. Possible introgression of mitochondrial DNA from other species also needs to be considered. But of particular concern is the question of the robustness of phylogenetic analyses based on the single locus mitochondrial barcode segment compared with that from large data sets obtained from next generation sequencing (McCormack et al. 2013). By limiting the phylogenetic barcode analyses to taxa at the subfamily level we suggest that potential problems with homoplasy and loss of phylogenetic signal of a single locus mitochondrial marker is probably minimal. The observation that our barcode results on closely related species of the Eudaminae are consistent with those obtained using multilocus genetic data (Warren et al. 2008) supports this suggestion.

ACKNOWLEDGMENTS

We thank James P. Brock, Janitzio Egido Villarreal, Wain Evans, Nick V. Grishin and Trinidad Hernández Mendoza for their help with this project. This study was supported by National Science Foundation (NSF) Grants DEB 00-75312 and OISE-0440648, University of California Institute for Mexico and the United States (UC MEXUS) Grant FA11-75, and Consejo Nacional de Ciencia y Tecnología (CONACYT) Proyecto 180385 (all to T.A.M), and funds from LANGEBIO and the Centro de Investigación en Alimentación y Desarrollo (CIAD).

LITERATURE CITED

AUSTIN, G.T. 1996. Hesperiidae of central Rondônia Brazil: three new species of *Narcosius* (Pyrginae). J. Lepid. Soc. 50: 54–60.

BELL, E.L. 1934. New American Hesperiidae (Lepidoptera, Rhopalocera). Bull. Brooklyn Entomol. Soc. 29: 89–96 (+ pl. VI).

BERTRAND, C., D.H. JANZEN, W. HALLWACHS , J.M. BURNS, J.F. GIBSON, S. SHOKRALLA & M. HAJIBABAEI. 2014. Mitochondrial and nuclear phylogenetic analysis with Sanger and next-generation sequencing shows that, in Área de Conservación Guanacaste, northwestern Costa Rica, the skipper butterfly named *Urbanus belli* (family Hesperiidae) comprises three morphologically cryptic species. BMC Evol. Biol. 14: 153. doi:10.1186/1471-2148-14-153.

BOYLE, E.E. & S.J. ADAMOWICZ. 2015. Community phylogenetics: assessing tree reconstruction m methods and the utility of DNA barcodes. PLoS ONE 10(6): e0126662. doi:10.1371/journal.pone.0126662

BROWER, A.V.Z. 2006. Problems with DNA barcodes for species delimitation: 'ten species' of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperiidae). Syst. Biodivers. 4: 127–132.

BROWER, A.V.Z. 2009. *Astraptes* Hübner 1819. Version 14 June 2009 (under construction). <http://tolweb.org/Astraptes/95276/2009.06.14> *In* The Tree of Life Web Project <http://tolweb.org/> (accessed 5 November 2015).

BROWER, A.V.Z. 2010. Alleviating the taxonomic impediment of DNA barcoding and setting a bad precedent: names for ten species of '*Astraptes fulgerator*' (Lepidoptera: Hesperiidae: Eudaminae) with DNA-based diagnoses. Syst. Biodivers. 8: 485–491.

CASSIE, B., J. GLASSBERG, A. SWENGEL & G. TUDOR. 2001. North American Butterfly Association (NABA) Checklist & English Names of North American Butterflies (2nd edition). North American Butterfly Association, Morristown, NJ. 36 pp.

DAI Q.-Y., Q. GAO, C.-S.WU, D. CHESTERS, C.-D. ZHU & A.-B. ZHANG. 2012. Phylogenetic reconstruction and DNA barcoding for closely related pine moth species (*Dendrolimus*) in China with multiple gene markers. PLoS ONE 7(4): e32544. doi:10.1371/journal.pone.0032544.

DAYRAT, B. 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 85: 407-415.

DINCĂ, V., S. MONTAGUD, G. TALAVERA, J. HERNÁNDEZ-ROLDÁN, M.L. MUNGUIRA, E. GARCÍA-BARROS, P.D.N. HEBERT & R. VILA. 2015. DNA barcode reference library for Iberian butterflies enables a continental-scale preview of potential cryptic diversity. Sci. Rep. 5: 12395. doi:10.1038/srep12395.

EVANS, W.H. 1952. A Catalogue of the American Hesperiidae Indicating the Classification and Nomenclature Adopted in the British Museum (Natural History). Part II. Pyrginae, Section I. British Museum (Natural History), London. 178 pp., pls. 10–25.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

FORISTER, M.L., C.C. NICE, J.A. FORDYCE, Z. GOMPERT & A.M. SHAPIRO. 2008. Considering evolutionary processes in the use of single-locus genetic data for conservation, with examples from the Lepidoptera. J. Insect Conserv. 12: 37–51.

GEMMELL, A.P. & J.M. MARCUS. 2015. A tale of two haplotype groups: evaluating the New World *Junonia* ring species hypothesis using the distribution of divergent *COI* haplotypes. Syst. Entomol. 40: 532–546.

GODMAN, F.D. & O. SALVIN. 1887–1901. Biologia Centrali-Americana. Insecta-Rhopalocera. Vol. II (text). Taylor & Francis, London. 782. pp. (plates in Vol. III, 1879–1901).

HAMILTON, C.A., B.E. HENDRIXSON, M.S. BREWER & J.E. BOND. 2014. An evaluation of sampling effects on multiple DNA barcoding methods leads to an integrative approach for delimiting species: A case study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae, Theraphosidae). Mol. Phylogenet. Evol. 71: 79-93.

HEBERT, P.D.N., A. CYWINSKA, S.L. BALL & J.R. DEWAARD. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B 270: 313–321.

HEBERT, P.D.N. & T.R. GREGORY. 2005. The promise of DNA barcoding for taxonomy. Syst. Biol. 54: 852–859.

HEBERT, P.D.N., E.H. PENTON, J.M. BURNS, D.H. JANZEN & W. HALLWACHS. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA 101: 14812–14817.

HUANG, J., A. ZHANG, S. MAO & Y. HUANG. 2013. DNA barcoding and species boundary delimitation of selected species of Chinese Acridoidea (Orthoptera: Caelifera). PLoS ONE 8(12): e82400. doi:10.1371/journal.pone.0082400.

HUELSENBECK, J.P. & F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754-755. ITIS. 2015. Integrated Taxonomic Information System on-line database. <http://www.itis.gov> (accessed 5 November 2015).

JANZEN, D. H. & W. HALLWACHS. 2009. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservación Guanacaste (ACG), northwestern Costa Rica (SRNP voucher codes). http://janzen.sas.upenn.edu (accessed 5 November 2015).

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.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

KODANDARAMAIAH, U., T.J. SIMONSEN, S. BROMILOW, N. WAHLBERG & F. SPERLING. 2013. Deceptive single-locus taxonomy and phylogeography: *Wolbachia*-associated divergence in mitochondrial DNA is not reflected in morphology and nuclear markers in a butterfly species. Ecol. Evol. 3: 5167–5176.

KVIST, S. 2013. Barcoding in the dark?: A critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. Mol. Phylogenet. Evol. 69: 39–45.

KWONG, S., A. SRIVATHSANA & R. MEIER. 2012. An update on DNA barcoding: low species coverage and numerous unidentified sequences. Cladistics 28: 639–644.

LIN. C.-P. & B.N. DANFORTH. 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. Mol. Phylogenet. Evol. 30: 686–702.

MCCORMACK, J.E., S.M. HIRD, A.J. ZELLMER, B.C. CARSTENS & R.T. BRUMFIELD. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phylogenet. Evol. 66: 526–538.

MIELKE, O.H.H. 2005. Catalogue of the American Hesperioidea: Hesperiidae (Lepidoptera). Vol. 2. Pyrginae 1: Eudamini. Socidedade Brasileira de Zoologia, Curitiba, Paraná, Brazil. pp. 126–410.

OPLER, P.A. & A.D. WARREN. 2005. Lepidoptera of North America. 4. Scientific Names List for Butterfly Species of North America, north of Mexico. Contributions of the C. P. Gillette Museum of Arthropod Diversity, Colorado State University. 79pp. <http://www.biology.ualberta.ca/old_site/uasm//Opler&Warren.pdf> (accessed 8 September 2015).

PELHAM, J.P. 2008. A catalogue of the butterflies of the United States and Canada. J. Res. Lepid. 40: 1–652.

PFEILER, E., B.G. BITLER, S. CASTREZANA, L.M. MATZKIN & T.A. MARKOW. 2009. Genetic diversification and demographic history of the cactophilic pseudoscorpion *Dinocheirus arizonensis* from the Sonoran Desert. Mol. Phylogenet. Evol. 52: 133–141.

PFEILER, E., J.E. VERGARA-QUINTANAR, S. CASTREZANA, M.S. CATERINO & T.A. MARKOW. 2010. Phylogenetic relationships of Sonoran Desert cactus beetles in the tribe Hololeptini (Coleoptera: Histeridae: Histerinae), with comments on the taxonomic status of *Iliotona beyeri*. Mol. Phylogenet. Evol. 56: 474–479.

PFEILER, E., S. JOHNSON & T.A. MARKOW. 2012a. DNA barcodes and insights into the relationships and systematics of buckeye butterflies (Nymphalidae: Nymphalinae: Junonia) from the Americas. J. Lepid. Soc. 66: 185–198.

PFEILER, E., S. JOHNSON & T.A. MARKOW. 2012b. Insights into population origins of Neotropical *Junonia* (Lepidoptera: Nymphalidae: Nymphalinae) based on mitochondrial DNA. Psyche. doi:10.1155/2012/423756.

PFEILER, E., M.R.L. LACLETTE & T.A. MARKOW. 2016. Butterfly biodiversity in a threatened coastal desert ecosystem of northwestern Mexico, with a focus on the life history and ecology of potentially endangered species. J. Lepid. Soc. 70: 47–60.

PLÖTZ, C. 1881. Die Heperiinen-Gattung *Eudamus* und ihre Arten. Stettiner entomologische Zeitung 43: 87–101.

POSADA, D. & K.A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. Bioinform. 14: 817–818.

RATNASINGHAM, S. & P.D.N. HEBERT. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol. Ecol. Notes 7: 355–364.

REGIER, J.C., C. MITTER, A. ZWICK, A.L. BAZINET, M.P. CUMMINGS, A.Y. KAWAHARA, J.-C. SOHN, D.J. ZWICKL, S. CHO, D.R. DAVIS, J. BAIXERAS, J. BROWN, C. PARR, S. WELLER, D.C. LEES & K.T. MITTER. 2013. A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). PLoS ONE 8(3): e58568. doi:10.1371/journal.pone.0058568.

RUBINOFF, D. & B.S. HOLLAND. 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Syst. Biol. 54: 952–961.

SAITOU, N. & M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

SERAPHIM, N., M.A. MARÍN, A.V.L. FREITAS & K.L. SILVA-BRANDÃO. 2014. Morphological and molecular marker contributions to disentangling the cryptic *Hermeuptychia hermes* species complex (Nymphalidae: Satyrinae: Euptychiina). Mol. Ecol. Resour. 14: 39–49.

SHEN, Y.-Y., X. CHEN & R.W. MURPHY. 2013. Assessing DNA barcoding as a tool for species identification and data quality control. PLoS ONE 8(2): e57125. doi:10.1371/journal.pone.0057125.

SONG, H., J.E. BUHAY, M.F. WHITING & K.A. CRANDALL. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proc. Natl Acad. Sci. USA 105: 13486–13491.

SRIVATHSAN, A. & R. MEIER. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics 28: 190–194.

STEINHAUSER, S.R. 1981. A revision of the *proteus* group of the genus *Urbanus* Hübner Lepidoptera: Hesperiidae. Bull. Allyn Mus. 62: 1–41.

STEINHAUSER, S.R. 1983. Notes on *Ridens* Evans, 1952 with description of a new species from Mexico. Bull. Allyn Mus. 79: 1–7.

STEINHAUSER, S.R. 1986. A review of the skippers of the *narcosius* group of species of the genus *Astraptes* Hübner (sensu Evans, 1952) and erection of a new genus. Lepidoptera: Hesperiidae. Bull. Allyn Mus. 104: 1–43.

STEINHAUSER, S.R. 1987. Notes on the identity of the species-group names in the genera *Urbanus* and *Astraptes* (sensu Evans). Bull. Allyn Mus. 111: 1–16.

STEINHAUSER, S.R. 1989. Taxonomic notes and descriptions of new taxa in the Neotropical Hesperiidae. Part I. Pyrginae. Bull. Allyn Mus. 127: 1–70.

TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI & S. KUMAR. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.

TAYLOR, H.R. & W.E. HARRIS. 2012. An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. Mol. Ecol. Resour. 12: 377–388.

WAHLBERG, N., C.W. WHEAT & C. PEÑA. 2013. Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). PLoS ONE 8(11): e80875. doi:10.1371/journal.pone.0080875.

WARREN, A.D., J.R. OGAWA & A.V.Z. BROWER. 2008. Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperiidae (Lepidoptera: Hesperioidea). Cladistics 24: 642–676.

WARREN, A.D., J.R. OGAWA & A.V.Z. BROWER. 2009. Revised classification of the family Hesperiidae (Lepidoptera: Hesperioidea) based on combined molecular and morphological data. Syst. Entomol. 34: 467–523.

WILSON, J.J. 2010. Assessing the value of DNA barcodes and other priority gene regions for molecular phylogenetics of Lepidoptera. PLoS ONE 5(5): e10525. doi:10.1371/journal.pone.0010525.

WILSON, J.J. 2011. Assessing the value of DNA barcodes for molecular phylogenetics: effect of increased taxon sampling in Lepidoptera. PLoS ONE 6(9): e24769. doi:10.1371/journal.pone.0024769.

ZAHIRI, R., J.D. LAFONTAINE, B.C. SCHMIDT, J.R. DEWAARD, E.V. ZAKHAROV, P.D.N. HEBERT. 2014. A transcontinental challenge—A test of DNA barcode performance for 1,541 species of Canadian Noctuoidea (Lepidoptera). PLoS ONE 9(3): e92797. doi:10.1371/journal.pone.0092797.

ZAKHAROV, E.V., N.F. LOBO, C. NOWAK & J.J. HELLMANN. 2009. Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). Heredity 102: 590–599.

Submitted for publication 10 November 2015; revised and accepted 21 January 2016.

Journal of the Lepidopterists' Society
70(2), 2016, 96–98

A SIMPLE METHOD FOR OBSERVING AND MEASURING HEART RATES IN LIVE ADULT MONARCHS (*DANAUS PLEXIPPUS*) AND OTHER LARGE BUTTERFLIES

ANDREW K. DAVIS* AND SHERAYAR ORAKZAI
Odum School of Ecology, University of Georgia, Athens GA 30602
*Corresponding author email: akdavis@uga.edu

ABSTRACT. Heart rates of insects reflect the current level of activity and stress individuals are experiencing, and therefore this information can be useful from a research perspective. In Lepidoptera, as with most insects, the 'heart' consists of a transverse longitudinal tube that runs along the abdomen, just under the abdominal tergites, which pumps or contracts rhythmically to distribute hemolymph. Here, we describe a simple method we developed to observe heart contractions in adult monarch butterflies, *Danaus plexippus*. The procedure involves stabilizing the live specimen in a pre-prepared plastic bag, while positioning the abdomen in such a way so that the beating heart can be seen (with magnification) through the intersegmental membranes. With this procedure, no harm comes to the specimen, making the technique useful in both lab and field studies. The technique also requires little equipment, except for a dissecting microscope (or other magnifier). Moreover, the procedure should be useful for monitoring other similarly-sized Lepidoptera, and we confirmed this with a *Papilio glaucus* specimen. Using this method on 10 male and 10 female monarch specimens from captive-reared stock, we found the resting heart rate was 63 beats/min on average (range: 35–86). This information will be useful for comparative purposes, or as a reference point for future studies of monarch physiology.

Additional key words: heart rate, monarch butterfly, *Danaus plexippus*, physiology

There is a long history of scientific investigation into the physiology of the insect heart, with observations from early scientists dating as far back as the 19th century (Newport 1837). The insect heart consists of a transverse tube (the 'dorsal vessel') that runs along the dorsal section of the abdomen, which pumps or contracts rhythmically to distribute hemolymph from the anterior to the posterior parts of the body. Over the years there have been various methods employed to monitor these heart contractions, including using electro-mechanical transducers (Senff 1971), infra-red sensors (Kuusik et al. 2001), and more recently, electrical resistance measurements (Wasserthal 2012). One of the earliest and simplest procedures came from Newport (1837), who scraped the upper abdominal scales off of a hawk moth (*Sphinx ligustri*) in order to view the pulsations of the dorsal vessel. He noted when the specimen was at rest, the heart rate was 42–49 beats/min, and after the moth had "flown around his sitting-room" for several minutes it was 127–139 beats/min. Other studies where insect heart rates have been monitored have been extensively reviewed by Jones (1974), and from this review it is clear that there is a wide range of heart rates among insect orders and across species within orders. Also notable from this review is that within the Lepidoptera, nearly all research efforts have focused on either larval or pupal stages.

Here, we describe a simple method we developed to observe heart contractions in adult monarch butterflies, *Danaus plexippus*. The procedure allows live monarchs to be monitored without harm (and without scraping off scales), requires minimal equipment (except for a low-power dissection scope), and it requires no formal

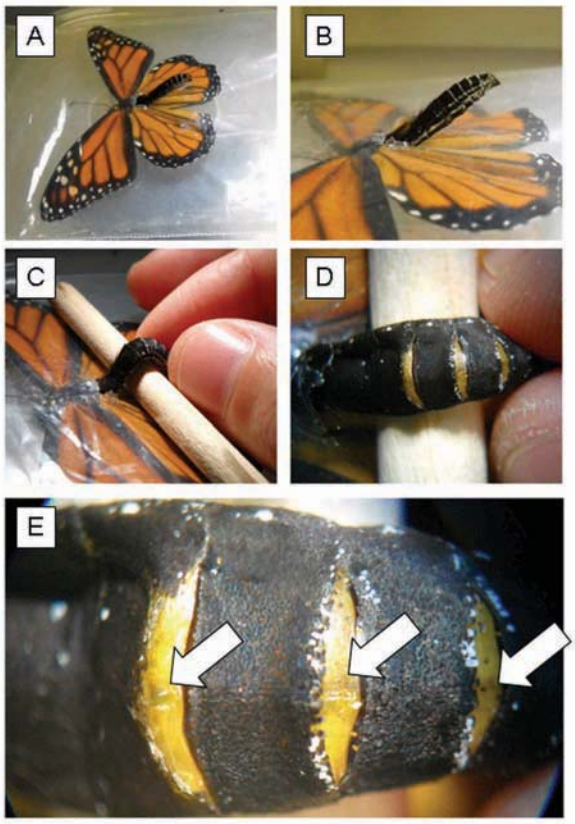


FIG. 1. Visual depiction of the heart observation of monarch butterflies, *Danaus plexippus*. The live monarch is placed in a clear plastic pouch with wings open and abdomen protruding from a small hole (**A, B**). The abdomen is gently pressed down over a round object (a pencil, or probe handle, as in **C**), which stretches the intersegmental membranes (**D**). The dorsal vessel (heart) can be visualized under these membranes (**E**) under a low-power dissection scope.

expertise except in safe handling of butterflies. Note that while we focused on the monarch, this procedure would be equally-suited for use on any similarly-sized or larger butterfly species, such as most swallowtails (genus *Papilio*).

Procedure description. To begin, the live monarch (or other butterfly) is placed in a clear plastic ziplock bag, which has a small (1 cm diameter) hole cut into one side. The butterfly is positioned in the bag with its wings opened horizontally, then moved so that its abdomen extrudes through the hole (Fig. 1A, B). The sides of the bag are then weighed down so that the butterfly remains immobile, which frees up the observer's hands. Next, a round object such as a pencil or a probe handle is placed under the abdomen (Fig. 1C), and with a free hand, the observer gently presses the abdomen over the object (Fig. 1D). In this position, the intersegmental membranes become stretched (Fig. 1E), which allows the dorsal vessel (i.e. the heart) to be observed with a low-power dissection microscope, or other visual aid, such as a visor magnifier. With practice, butterflies can be held in this position for several minutes, while the observer watches and counts the pulses of the heart (although a 1-minute interval is sufficient).

Monarch heart rates. Using this method we evaluated the resting heart rates of 20 live monarchs (10 males, 10 females) that had been reared in captivity as part of other projects during summer 2015 at the University of Georgia. These butterflies were progeny of several generations of captive stock (originating from wild-caught adults from Texas and Georgia), and had been fed cuttings of greenhouse-grown *Ascelpias incarnata* as larvae. As adults, they had been stored in glassine envelopes in an incubator set to 14°C for one month, and removed once a week for feeding, which was a 1:4 mixture of honey-water. For the heartbeat readings (conducted the week of Sept 7–14, 2015), we removed the monarchs from the incubator (but not from their envelopes) 30min prior to assessment. The temperature of the room was constant at 22°C. The average resting heart rate of the 20 monarchs was 63.2 beats/min (16.7 SD), and ranged from 35 to 86 (Table 1). While female heart rates were slightly higher than that of males, the difference was not significant (Student's *t*-test, $df=18$, $t=0.712$, $p=0.485$).

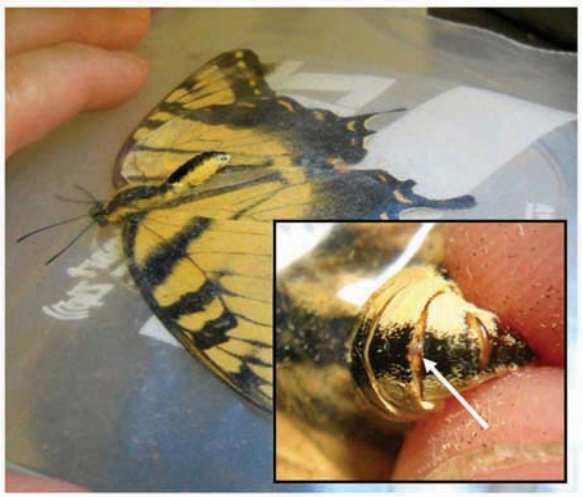


FIG. 2. The technique was evaluated on a *Papilio glaucus* specimen; the same procedure was followed, including positioning of the specimen in the bag, and stretching the intersegmental membranes (inset) to see the dorsal vessel contracting (arrow).

TABLE 1. Summary of heartbeat measurements for male and female monarch butterflies in this project. All heartbeat values are expressed as the number of beats/min. There was no significant difference between sexes in average heart rate.

Sex	N	Mean	SD	Min	Max
Females	10	65.9	17.8	35	85
Males	10	60.5	16.1	37	86
Both Groups	20	63.2	16.7	35	86

As mentioned previously, the lack of comparable data (i.e. from adult, intact specimens, measured visually) from other Lepidoptera species makes it difficult to know if the data above are normal for adult monarch butterflies. Similar visual-based observations from non-manipulated mosquitoes (*Anopheles gambiae*) indicated an average rate of 82 beats/min (Glenn et al. 2010), and heart rates of the adult moth *Mamestra brassicae* were between 60–90 beats/min, measured with electrocardiographs (Queinnec & Campan 1972). Interestingly, other research from our lab has found horned passalus beetles (*Odontotaenius disjunctus*) have an average resting heart rate comparable to monarchs, averaging 63 beats/min (Davis, unpubl. data). From this collective information we surmise that the heart rate data we collected is in line with results from prior work on insects. At the very least, these values for monarchs should be of use as a baseline which future projects could add to.

The procedure outlined here should be useful for observing heartbeat of other similarly-size Lepidoptera. As a test of this, we examined a wild-caught tiger swallowtail (*Papilio glaucus*) specimen, following the same steps outline above, and had no difficulty seeing the heart contractions (Fig. 2). Other strengths of this technique are its ease of use, and the fact that it requires no advanced equipment besides a dissecting

microscope, or some other magnifying tool (such as a visor magnifier). Moreover, this procedure does not injure the butterfly, and does not require removal of abdominal scales, as Newport did 178 years ago (Newport 1837). We note that removing abdominal scales actually may not be effective for observing heart contractions in all Lepidoptera; we attempted to do this with the *P. glaucus* specimen, but the abdominal tergites in this species are dark (i.e. not translucent), and the underlying dorsal vessel was not easily seen. In contrast, the inter-segmental membranes are translucent on most species (compare Fig. 1 and Fig. 2), regardless of the species' integument color.

Potential applications. Given that the procedure does not injure the butterfly, this technique could be used for a wide variety of research applications, both in the lab and in the field. For example, a defining characteristic of the monarch is its long-distance migration in eastern North America (Brower 1995, Howard & Davis 2009), and in theory this procedure would allow migrant monarchs to be captured, assessed in the field, then released to continue their migration. This information could be compared with the heart rates in other behavioral activities to quantify relative energy expenditure in each state. Other questions could be addressed relating to stress and stress susceptibility. Transient increases in heartbeat frequency are a universal response to acute stressors in vertebrates and invertebrates (Beerda et al. 1998, Rushen et al. 2001, Terkelsen et al. 2005, Papaefthimiou & Theophilidis 2011). Thus, knowing what factors cause heart rates to increase in monarchs could help to identify times or conditions under which monarchs become stressed. This may include exposure to agricultural chemicals (Pecenka & Lundgren 2015), and/or being infected with parasites (Altizer & Oberhauser 1999). While research into these issues is ongoing, so far there has been no evaluation of how much physiological stress these factors cause to individual monarchs. Because of its many potential applications, the technique described here should be useful to Lepidopterists, and moreover, it will hopefully help to reinvigorate new investigations into heart rate variations in insects.

ACKNOWLEDGEMENTS.

We thank Ania Majewska, Dara Satterfield and Alexa McKay at the University of Georgia for providing monarch specimens for this project. We also thank Lincoln Brower for providing helpful comments on the manuscript.

LITERATURE CITED

ALTIZER, S. M., AND K. OBERHAUSER. 1999. Effects of the protozoan parasite *Ophryocystis elektroscirrha* on the fitness of monarch butterflies (*Danaus plexippus*). J. Invert. Path. 74: 76–88.

BEERDA, B., M. B. H. SCHILDER, J. VAN HOOFF, H. W. DE VRIES, AND J. A. MOL. 1998. Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. Appl. Anim. Behav. Sci. 58: 365–381.

BROWER, L. P. 1995. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857–1995. J. Lepid. Soc. 49: 304–385.

GLENN, J. D., J. G. KING, AND J. F. HILLYER. 2010. Structural mechanics of the mosquito heart and its function in bidirectional hemolymph transport. J. Exp. Biol. 213: 541–550.

HOWARD, E., AND A. K. DAVIS. 2009. The fall migration flyways of monarch butterflies in eastern North America revealed by citizen scientists. J. Insect Cons. 13: 279–286.

JONES, J. C. 1974. Factors affecting heart rates in insects. pp. 119–167. *In* M. Rockstein (ed.). The Physiology of Insecta. vol IV. Academic Press, New York & London, second edition.

KUUSIK, A., U. TARTES, A. VANATOA, K. HIIESAAR, AND L. METSPALU. 2001. Body movements and their role as triggers of heartbeats in pupae of the Colorado potato beetle *Leptinotarsa decemlineata*. Physiol. Entomol. 26: 158–164.

NEWPORT, G. 1837. On the temperature of insects, and its connection with the functions of respiration and circulation in this class of invertebrate animals. Phil. Trans. Roy. Soc. London 127: 259–339.

PAPAEFTHIMIOU, C., AND G. THEOPHILIDIS. 2011. Octopamine-A single modulator with double action on the heart of two insect species (*Apis mellifera macedonica* and *Bactrocera oleae*): Acceleration vs. inhibition. J. Insect Physiol. 57: 316-325.

PECENKA, J. R., AND J. G. LUNDGREN. 2015. Non-target effects of clothianidin on monarch butterflies. Science of Nature 102: 4.

QUEINNEC, Y., AND R. CAMPAN. 1972. Heart beat frequency variations in moth *Mamestra brassicae* during ontogeny. J. Insect Physiol. 18: 1739-1744.

RUSHEN, J., L. MUNKSGAARD, P. G. MARNET, AND A. M. DEPASSILLE. 2001. Human contact and the effects of acute stress on cows at milking. Appl. Anim. Behav. Sci. 73: 1-14.

SENFF, R. E. 1971. Cardiac acceleration in response to stimulation of segmental nerves in adult cockroach, *Periplaneta americana*. Comp. Biochem. Physiol. 40: 1009-1013.

TERKELSEN, A. J., H. MOLGAARD, J. HANSEN, O. K. ANDERSEN, AND T. S. JENSEN. 2005. Acute pain increases heart rate: Differential mechanisms during rest and mental stress. Autonomic Neuroscience-Basic & Clinical 121: 101-109.

WASSERTHAL, L. T. 2012. Influence of periodic heartbeat reversal and abdominal movements on hemocoelic and tracheal pressure in resting blowflies *Calliphora vicina*. J. Exp. Biol. 215: 362-373.

Submitted for publication 17 September 2015; revised and accepted 26 October 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 99–107

QUANTIFYING WING SHAPE AND SIZE OF SATURNIID MOTHS WITH GEOMETRIC MORPHOMETRICS

MINJIA ZHONG*
Buchholz High School, Gainesville, FL 32606, USA
Stanford University, Stanford, CA 94305, USA

GEENA M. HILL*, JUAN P. GOMEZ, DAVID PLOTKIN
Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA

JESSE R. BARBER
Department of Biology, Boise State University, Boise, ID 83725, USA

AND

AKITO Y. KAWAHARA**
Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA Email: AYK: kawahara@flmnh.ufl.edu
*Equally Contributing Authors **Corresponding Author

ABSTRACT. Butterflies and moths exhibit a spectacular diversity of wing shape and size. The extent of wing variation is particularly evident in wild silk moths (Saturniidae), which have large wing shape and size variation. Some species have jagged wing margins, rounded forewing apical lobes, or narrow hind wings with long tails, while others lack these traits entirely. Surprisingly, very little work has been done to formally quantify wing variation within the family. We analyzed the hind wing shape and size of 76 saturniid species representing 52 genera across five subfamilies using geometric morphometrics. We identified fifteen landmarks that we predict can be applied to families across Lepidoptera. PCA analyses grouped saturniid hind wings into six distinct morphological clusters. These groups did not appear to follow species relatedness—some phylogenetically and genetically distantly related taxa clustered in the same morphological group. We discuss ecological factors that might have led to the extraordinary wing variation within Saturniidae.

Additional key words: Bombycoidea, morphology, Saturniidae, wild silk moth, tail

Butterflies and moths are known for their incredible diversity in wing morphology, which has fascinated naturalists for centuries (e.g., Merian 1705, Bates 1862, Grote 1897, Field 1898, Howse & Wolfe 2012). Some families have wings that resemble plumes (Alucitidae, Pterophoridae), while others are wasp-like (many Sesiidae). Perhaps the greatest degree of wing variation within a single lepidopteran family occurs in the Saturniidae, a cosmopolitan moth family that includes over 2,300 described species (van Nieukerken et al. 2011). Saturniidae contains some of the world's largest insects, with species that have wingspans of up to 250 mm, while others are small, with wingspans of only 30 mm (Imes 1992). Saturniids vary greatly in wing shape; some species have leaf-like jagged wing margins, expanded round forewing apical lobes, or have narrow hind wings with long tails, while others lack them entirely (Ylla et al. 2005, Nath & Devi 2009). Thus far, research on wing variation has focused primarily on venation (e.g., Packard 1905, Michener 1952, Albrecht & Kaila 1997) or on color pattern (e.g., Beldade & Brakefield 2002, Prieto et al. 2009, Monteiro et al. 1994, Mallet & Gilbert 1995). The few studies on moth wing shape have primarily dealt with intraspecific variation and sexual dimorphism (Ricklefs & O'Rourke 1975, Ricklefs 2009, Benitez et al. 2011, Nath & Devi 2009).

In Lepidoptera, wing morphology can be correlated with dispersal (Hill et al. 1999, Altizer & Davis 2010, Sekar 2012), gliding (Cespedes et al. 2015), mating (Wickman et al. 1992), predator evasion (Barber et al. 2015), or possibly nectar searching (Betts & Wootton 1988). However, studies that correlate wing size and wing shape with a species' biology across many taxa are limited, in part because comprehensive wing morphology data and robust phylogenies are often lacking. In this study, we characterized hind wing morphology in 76 distantly related saturniid species using geometric morphometrics and inferred the evolution of wing morphology using genetic similarity and a phylogeny. We focused on the hind wing, as it is an area of the wing that is highly variable and has direct implications for ecology, especially predator-prey interactions (Barber et al. 2015).

MATERIALS AND METHODS

Specimen selection

We sampled a phylogenetically and morphologically diverse set of 76 saturniid species, representing 52 genera in five subfamilies across a broad geographical range (Supplementary Table 1). Specimens were obtained from the dry, pinned collection of the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History (MGCL), Gainesville, Florida, USA. All specimens studied had undamaged right hind wings. In order to avoid bias associated with sexual dimorphism, we examined males. Most species were represented by a single specimen; five species were represented by ≥ 6 specimens as exemplars to assess the degree of intraspecific variation (Supplementary Table 1). Statistically significant low intraspecific hind wing variation (see Results) justified the examination of one specimen per species. All specimens chosen for inclusion in the study were digitally imaged for geometric morphometrics, and these pinned specimens have an associated white label with the printed text, "tail project." Supplementary tables and additional files can be found on the Dryad Data Repository (www.datadryad.org, accession number 10.5061/dryad.gs296).

Digitization

In order to obtain high-quality images for digitization, each saturniid specimen was pinned to an upright, square, 355 × 355 × 3 mm opaque acrylic sheet placed 18 cm above a millimeter ruler, positioned parallel to the focal plane of the right hind wing. The pin on the moth was inserted into a small foam block glued to one side of the sheet. A digital image of each specimen was taken in RAW format (5184 × 3456 pixels) using an EOS Rebel T3i digital camera with EF 35 mm F2.0 lens, mounted on an aluminum tripod 50 cm from the acrylic sheet. Two external Yongnuo YN 560 III flashguns, one placed behind the acrylic sheet, and another in front of it, were positioned to overexpose wings so that the venation could be visualized clearly. Prior to imaging, the dorsal surface of the right hind wing was lightly sprayed with 70% ethanol. Digital images of each specimen were taken in RAW format, converted to TIFF, and labeled in Photoshop CS5® with a species name and a unique identification number (e.g., "LEP-01234").

Tps file compilation and landmark designation in tpsDig

Each TIFF file was imported into tpsUtil ver. 1.58 (Schutz & Krieger 2007) and fifteen points on the dorsal surface of each hind wing were selected as homologous landmarks. Each wing image in the tps file was landmarked with tpsDig 2.12 (Rohlf 2010). Landmarks were chosen based on the approach of Zelditch et al. (2012); they were selected given the following criteria: a point was considered a good landmark if it was 1) easy to identify, 2) homologous and independent from other landmarks, 3) situated on the same plane as other landmarks, and 4) described the overall morphological shape of the wing being measured. Landmarks were located either at a point where a vein met the wing margin or where veins intersected each other (Fig. 1).

In order to accurately characterize wing shape, semilandmarks were also selected to supplement homologous landmarks. One hundred semilandmarks were placed along the perimeter of the right hind wing using tpsDig. The semilandmarks were applied in a clockwise direction from the base of the wing. Because tpsDig requires a consistent number of semilandmarks across species, the number of semilandmarks chosen was determined by the taxon that required the largest number of semilandmarks (i.e., *Copiopteryx semiramis*).

Procrustes Analysis, Principal Component Analysis, and estimating wing size

We used a Procrustes Analysis (PA) on the fifteen homologous landmarks to determine how each landmark varied among species and to determine if landmarks varied in a correlated fashion. In PA, landmarks are superimposed to optimize the squared

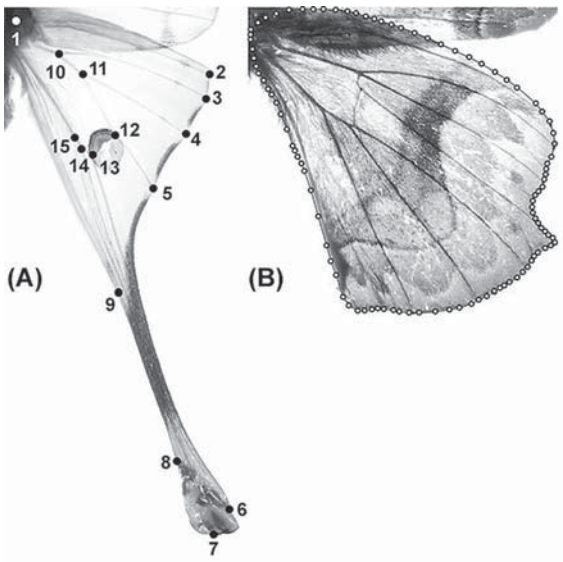


FIG. 1. Digital images of the dorsal surface of the right hind wing of (**A**) *Argema mimosae* (Boisduval) (Saturniinae), and (**B**) *Caio championi* (Druce) (Arsenurinae). Numbers indicate the 15 homologous points chosen as landmarks. Landmark 1 was placed at the wing base, landmarks 2–9 were placed where $Sc+R_1$, R_s, M_1, M_2, M_3, Cu_1, Cu_2, and A1+2 meet the wing margin, and landmarks 10–15 were placed around the periphery of the discal cell.

distances from a common centroid through translation, rotation, and scaling, resulting in new Procrustes shape coordinates (Gower 1975). These coordinates are reduced to a smaller number of variables using a standard Principal Component Analysis (PCA). The Procrustes fit operation corrects for landmark errors by separating size and shape, and also allows shape coordinates to be projected into a Euclidean space tangent to the Procrustes shape space (Viscosi & Cardini 2012). After performing the PA, we retained only the axes with eigenvalues greater than 1 to represent the fifteen original landmarks. These analyses were performed in MorphoJ (Klingenberg 2011) and R (R Core Team 2014), using the R package, Shapes (Dryden 2012). The R script used for this analysis is provided in Appendix 1.

Cluster analysis

Using the results of the PA, we performed a cluster analysis to determine the number of groups that reduced the sum of squares of the data. To determine the most likely number of clusters in which the data could be split, we used the gap statistic as a measurement of goodness of clustering using fifteen as the maximum number of clusters (Tibshirani et al. 2001). Statistical significance was determined by the overlap between the confidence intervals estimated from 1000 bootstrap replicates. The cluster analysis was performed using the R package, Cluster (Maechler et al. 2013).

Estimating wing shape and size with semilandmarks

We performed an additional PA using the 100 semilandmarks in order to compare the results to those from the landmark data PA. Variation in wing shape was assessed using Procrustes ANOVA, using the groups from the landmark data PA as an independent variable. The Procrustes ANOVA calculates the sum of squares of the Procrustes distances between individuals, determines whether this sum is reduced by the introduction of an explanatory variable, and assesses statistical significance by randomizing individuals per group 999 times (Anderson 2001). Centroid size per individual, as estimated by the PA, was used as a proxy for wing size. Centroid size data was log-transformed to meet the assumptions for normality and one-way ANOVA was used to evaluate differences in wing size. All of the semilandmark analyses were performed using the R package, Geomorph (Adams & Otarola-Castillo 2013).

Estimating the evolution of wing morphology: phylogeny and genetic distance

In order to infer the evolution of hind wing morphology, we took two approaches: 1) phylogeny and 2) genetic distance. We used two methods because a comprehensive phylogeny including all 76 species examined in this project did not exist at the time of this study. To acquire a general understanding of how the six morphological groups evolved, we mapped the six groups onto a recently published phylogeny of Saturniidae (Barber et al. 2015). However, because only 12 of the 76 species studied were represented in the Barber et al. tree, we reduced it to a genera-level phylogeny and examined broad patterns of wing morphological evolution. This approach assumes that morphological groups do not vary within genera.

We also applied a genetic distance-based approach because it is conceivable that a genus includes species with different morphological groups. In order to determine if genetic distance correlates with morphological distance, we used the 658 bp, first subunit of the mitochondrial cytochrome oxidase gene (COI). COI is frequently used to delimit species of Lepidoptera, as there is a large reference sequence database of species (Hajibabaei et al. 2006, Hebert et al. 2003, Janzen et al. 2005). We downloaded all publicly available COI sequences from GenBank and the BOLD Taxonomy Browser (Ratnasingham & Hebert 2007) for the taxa in our study (Supplementary Table 1). We aligned the sequences using ClustalW (Larkin et al. 2007), implemented in Geneious v. 7.1 (Biomatters, Auckland, New Zealand) and estimated the best model of evolution using jModelTest v. 2.1.7 (Darriba et al. 2012). W used the R package, ape (Paradis et al. 2004) to estimate the corrected distance among species and performed a Principal Coordinates Analysis (PCoA) to scale the distance matrix into a two-axis ordination. We used the PCoA scores to perform an analysis of similarity (ANOSIM) on the morphological groups, using the R package, Vegan (Oksanen et al. 2013). The ANOSIM enabled us to determine whether the calculated genetic distance between morphological groups is greater than expected by chance.

Finally, because we did not have a complete sampling for molecular data across all species, we calculated the genus to species ratio (Generic coefficient, Jaccard 1922) in each group. Traditionally, this ratio was used originally to represent the ecological diversity in a community, assuming that higher the ratio, the more ecologically diverse the community was (Jaccard 1922, Elton 1946). Even though some statistical sampling issues have been raised against the use of the genus to species ratio (Jarvinen 1982), if coupled with the appropriate null models, these ratios can be useful for the purposes of determining the diversity of a sample (Jarvinen 1982, Gotelli 2001). In

our case, we did not use the genus to species ratio to infer the ecological diversity of a community but rather applied the same approach to represent phylogenetic diversity of morphological groups. Assuming that taxonomy is a fair representation of phylogenetic relationships, this method can be useful to make inferences about trait evolution in taxa with little or no molecular data.

In this approach, the genus to species ratio can vary from 1 to 1/n, where n is the group size. A value of 1 indicates that all species present in the group are representatives of different genera and it takes the value of 1/n when all species in the group belong to a single genus. To estimate the significance of the genus to species ratio, we randomly assigned a morphological group (1–6) to each species, constraining the number of species in each group to match the observed number of species per group in our data set. We then calculated the genus to species ratio for the randomly generated data set. We repeated this procedure 9999 times to create a null distribution of genus to species ratios to which we compared our observed values (Gotelli 2001). Using the null distribution, we calculated the standardized effect size of the genus to species ratio:

$$G/S_{ses} = -1 \times \frac{G/S_{obs} - G/S_{null}}{G/S_{sdnull}}$$

where G/S_{obs} is the observed generic coefficient, G/S_{null} is the mean generic coefficient from the randomly generated groups, and $G/S_{schnull}$ is the standard deviation of the null distribution of generic coefficients. Values greater than 1 indicate that the generic coefficient is larger than expected by chance. Values smaller than 1 indicate that the generic coefficient is smaller than expected by chance. The *P* value for the genus to species ratio and standardized effect size was calculated using the formula:

$$G/S_{p} = \frac{2 \times rank(G/S_{obs})}{9999 + 1}$$

Intraspecific versus interspecific variation

Since most of our sampling included only one individual per species, it was important to determine whether sampling limitation introduced a bias that would skew our results. We used a one-way ANOVA to compare the log-transformed variance of wing shape and size variables between individuals in the same species with the log-transformed variance of individuals belonging to different genera. However, because some morphological groups contained species belonging to different genera, we also compared the intraspecific variation with the intra-group variation. The objective of these comparisons is to demonstrate that the individuals sampled represent the mean value of wing shape and size for that species, and that intraspecific variation does not influence the patterns observed (Harmon & Losos 2005).

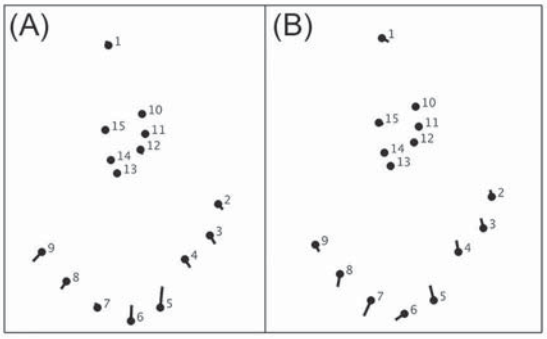


FIG. 2. PCA results from MorphoJ representing (**A**) PC1 and (**B**) PC2. The length and direction of the line corresponding to each point explains the variation found within each landmark and whether it is negatively or positively correlated to that PC. The x and y axes represent arbitrary coordinate values from a centroid in the original specimen image.

RESULTS

Procrustes and Principal Components Analysis

The PA and ensuing PCA produced two PCs with eigenvalues greater than one, which explained 80% of the original variation. The first PC (PC1) was negatively correlated to the y values of landmarks 5 and 6 and positively correlated to the x and y values of 9 (Fig. 2A). The second PC (PC2) was negatively correlated to the y values of landmarks 2, 3, and 4, and positively correlated to the y values of 7 and the x and y values of 9 (Fig. 2B). All other landmarks were related to PCs with eigenvalues less than 1. The cluster analysis suggested that the species evaluated can be separated into six clusters (Supplementary Table 1).

Overall, saturniid hind wing morphology varied from relatively small, rounded wings (Group 1) to large wings with long, thin tails (Group 6, Supplementary Table 1). Species in Groups 1 and 2 had no hind wing tails. Groups 3 and 4 included species that have short, wide hind wings that either have very short tails or nubs. Group 3 (*Arsenura, Caio*) had hind wings that are more rounded than Group 4 (*Dysdaemonia, Paradaemonia, Titaea*). Group 5 was comprised of *Actias* and *Graellsia* that have shorter hind wing tails than those in Group 6. Group 6 consisted entirely of species in *Copiopteryx*.

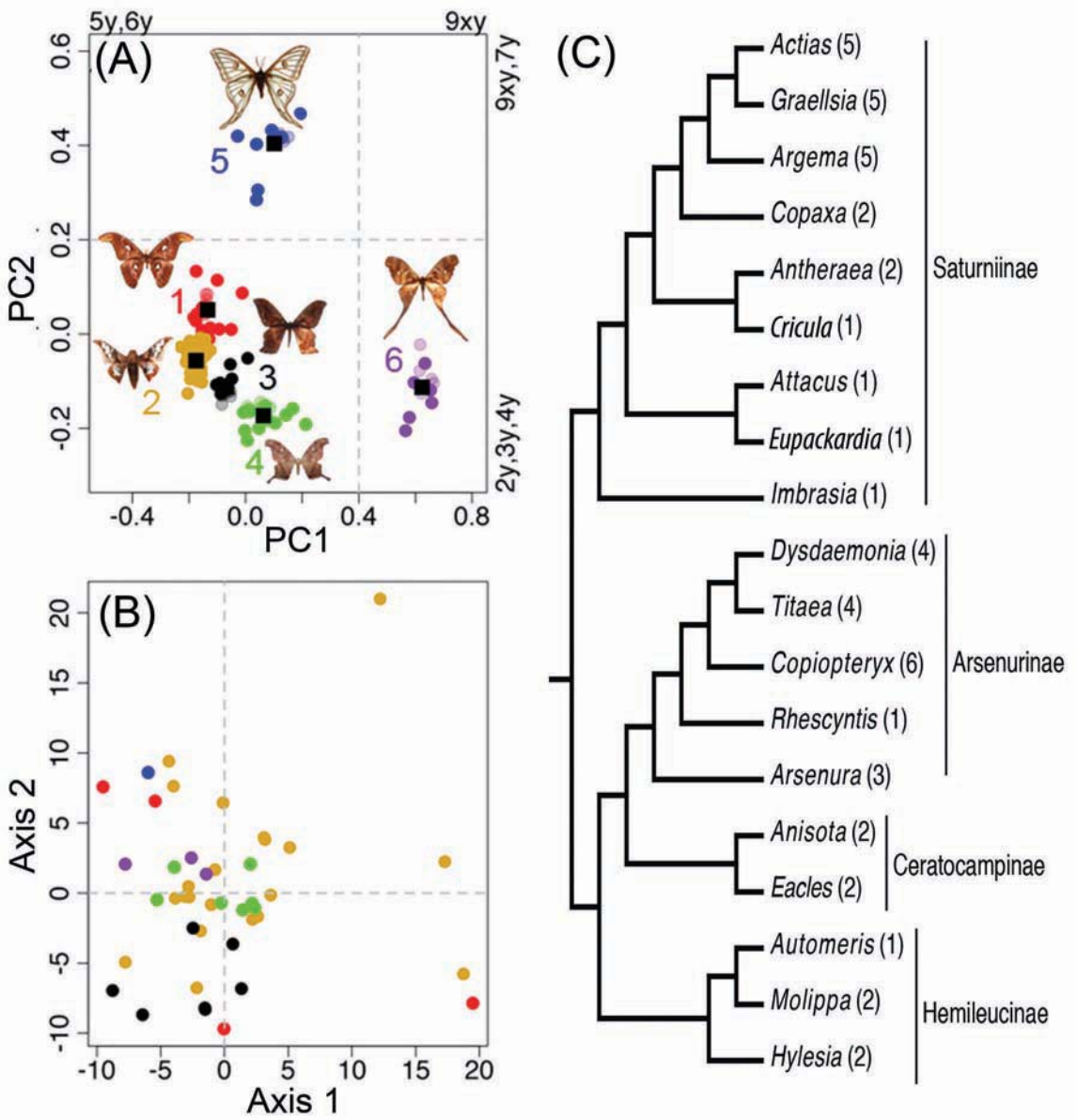


FIG. 3. Variation in hind wing shape across Saturniidae. (**A**) PCA results showing the six morphological groups based on the 15 homologous landmarks. Groups defined in this study are color-coded and the numbers at the top and right margins represent landmarks that are most highly correlated with PC1 and PC2, respectively. Black squares represent the centroid of each morphological group. Semi-transparent circles represent multiple individuals of the same species. (**B**) A comparison of COI genetic distance with the six morphological groups using multidimensional scaling. (**C**) A reduced, genus-level phylogeny, redrawn from Figure 2 of Barber et al. (2015) showing the six morphological groups.

PC1 characterized wing morphology based on tail length, whereas PC2 discriminated mainly on the width of the hind wing. PC1 thus created a gradient in which the tailless or very short tailed groups (Groups 1-4) were relatively close to each other (Fig. 3A). Although Groups 5 and 6 included tailed taxa, their PC groups were relatively distant from each other (Fig. 3A), suggesting that a tail is formed with either 1) having high x values of landmark 9 and intermediate y values of landmarks 7 and 9 (i.e. Group 5), or 2) having high x values of landmark 9 and high y values of landmarks 2, 3, 4, and 9 (i.e. Group 6).

Wing size and shape comparisons

The saturniids examined differed significantly in shape (Procrustes ANOVA: SS.obs = 2.97, df = 5, p = 0.001), with shapes following the pattern described using the PCA on the landmarks. Shape variation occurred on a gradient from hind wings that are

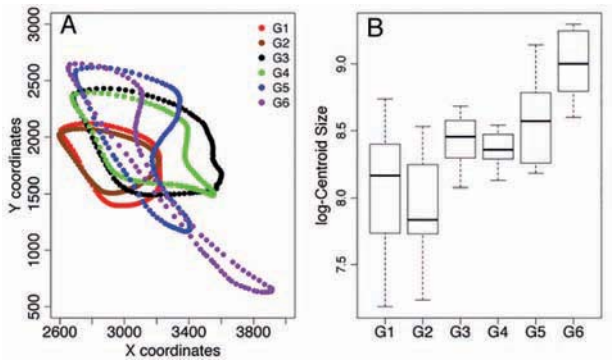


FIG. 4. **A**) Procrustes analysis showing the representation of wing shape in each of the six morphological groups. **B**) Boxplots showing the difference in wing size between the six groups.

rounded and tailless (Group 1) to hind wings that are elongate and tailed (Group 6; Fig. 4A). The species sampled also exhibited significant differences in wing size (ANOVA landmarks: F = 93.89, df = 5, p <0.001; semilandmarks: F = 13.68, df = 5, p<0.001). Size variation occurs on a gradient from small (Group 1) to large (Group 6; Fig. 4B). Interestingly, outlining the wing shape reveals that the hind wing tail is located in a similar position on most saturniids, but different wing veins form tails in unrelated groups.

Genetic distance and phylogeny

The COI genetic distance analysis revealed that the genetic distance within morphological groups is larger than the distance between the six morphological groups (ANOSIM: R statistic = -0.16, p = 0.049; Fig. 3B). Tailless groups were mainly composed of monotypic species, whereas tailed groups contain species that have multiple congeners in the same group (i.e., multiple species per genus; genus (G) to species (S) ratio: Group 1: G/S_{obs} = 1.0, G/S_{ses} = -1.38, p = 0.17; Group 2: G/S_{obs} = 1.0, G/S_{ses} = -3.58, p < 0.01; Group 3: G/S_{obs} = 0.25, G/S_{ses}= 7.1, p <0.01; Group 4: G/S_{obs} = 0.25, G/S_{ses} = 7.05, p < 0.01; Group 5: G/S_{obs} = 0.58, G/S_{ses} = 3.81, p < 0.01; Group 6: G/S_{obs} = 0.17, G/S_{ses} = 8.1 p < 0.01). Mapping the six groups onto the reduced, genus-level tree revealed that two morphological wing groups (Group 1 and 2) are present in multiple saturniid clades (Fig. 3C).

Intraspecific versus interspecific variation

Within each group, there was less intraspecific variation than interspecific variation (ANOVA; PC1: F = 10.75, df = 1, p < 0.01; PC2: t = 9.61, df = 1, p = 0.01). Hind wing variation within a species was equal to the variation within a single genus in PC1, but greater than the variation within a genus in PC2 (ANOVA; PC1: F = 3.7, df = 1, p = 0.08; PC2: t = 5.8, df = 1, p = 0.03), supporting the examination of one individual per species.

DISCUSSION

Saturniid moths have evolved a broad range of hind wing shape and size. We identified fifteen points on the moth hind wing that can be consistently used as landmarks for geometric morphometrics. Of these landmarks, those positioned at the wing margin (landmarks 2–9; Fig. 2) exhibited the greatest interspecific variation. Landmarks located at margins of eyespots and wing marks (e.g., landmarks 12, 13; Fig. 1) were useful for intraspecific comparisons, but our tests for intraspecific variation were limited in sample size, and therefore should not be treated as final or conclusive. While saturniid hind wings were clustered into six distinct morphological groups, these groups did not correspond directly to COI genetic clusters. Mapping morphological groups onto phylogeny showed that wing shape and size might have evolved convergently among subfamilies of Saturniidae.

A question that remains largely unanswered is why unrelated saturniid species of different genera evolved significantly different wing shape and size. Wings of Lepidoptera are complex, serving multiple adaptive functions, such as flight, thermal regulation, mating behavior, and predator avoidance (reviewed in Scoble 1992). Hind wings have been shown to play a limited role in lepidopteran flight (Jantzen & Eisner 2008), but they might be critical under particular circumstances, such as evading deterring, or delaying predation (Collins 2013, Vallin et al. 2010).

Hind wing tails, which appear to have originated independently in several saturniid subfamilies, is thought to be an anti-bat strategy. Janzen (1984) postulated that moths with long tails make them appear larger to echolocating bats. Barber et al. (2015) showed that twisted hind wing tails are acoustic lures that reflect bat sonar and divert bat attack to these appendages. Insectivorous bats present a strong selective force on nocturnal moths (Conner & Corcoran 2012), and if the presence of a tail is an anti-bat defense, one could predict an evolutionary transition from an ancestral condition of no tails to long tails. This trend is seen across Saturniidae (Barber et al. 2015), and also within particular saturniid clades (e.g., the *Actias* group, Ylla et al. 2005), although these transitions are likely influenced by adaptive factors such as aerodynamic drag that could slow or change a moth's movement. A transition from simple anti-bat strategies to more complex ones is seen in other moth groups, such as hawkmoths, in which derived lineages use a combination of ultrasound hearing and jamming

(Kawahara & Barber 2015). While we are beginning to understand behavioral and morphological adaptations of moth wings in light of evolution, what we know about them is still severely limited. Our next step is to examine moth wing traits (wing shape, size, color, pattern) and relate them to ecology and gene function.

ACKNOWLEDGEMENTS

We thank Chris Johns and Lary Reeves for providing digitization equipment and Ivonne Garzon for supplying morphometrics resources. Dean Adams assisted with the Geomorph package. James Adams, Jesse Breinholt, Jon Bremer, Jon Colburn, Michael Collins, Jaret Daniels, Dale Halbritter, Nick Homziak, Peter Houlihan, Simon McClung, Elena Ortiz-Acevedo, Pablo Sebastian Padron, Craig Segebarth, Ian Segebarth, John Snyder, Andrei Sourakov, Matt Standridge, Lisa Taylor, Andrew Warren and Lei Xiao provided support and feedback. This project was funded by the Mu Alpha Theta Summer high school research award to MZ, and in part by National Science Foundation grants DEB-15057007 and IOS-1121739 to AYK, IOS-1121807 to JRB, REU, ROA, and RAHSS supplements to these NSF awards.

LITERATURE CITED

ADAMS, D. C. & E. OTAROLA-CASTILLO. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol. Evol. 4: 393–399.

ALBRECHT, A. & L. KAILA. 1997. Variation of wing venation in Elachistidae (Lepidoptera: Gelechioidea): methodology and implications to systematics. Syst. Entomol. 22: 185–198.

ALTIZER, S. & A. K. DAVIS, 2010. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology. Evolution 64: 1018–1028.

ANDERSON, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26: 32–46.

BARBER, J. R., B. C. LEAVELL, A. L. KEENER, J. W. BREINHOLT, B. A. CHADWELL, C. J. W. MCCLURE, G. M. HILL, & A. Y. KAWAHARA. 2015. Moth tails divert bat attack: Evolution of acoustic deflection. Proc. Natl. Acad. Sci. USA 112:2812–2816.

BATES, H. W. 1862. XXXII. Contributions to an Insect Fauna of the Amazon Valley. Lepidoptera: Heliconidae. Trans. Linn. Soc. Lond. 23: 495–566.

BELDADE, P. & P. BRAKEFIELD. 2002. The genetics of evo-devo of butterfly wing patterns. Nat. Rev. Genet. 3: 442–452.

BENITEZ, H. A., L. E. PARRA, E. SEPULVEDA, & M. J. SANZANA. 2011. Geometric perspectives of sexual dimorphism in the wing shape of Lepidoptera: the case of *Synneuria* sp. (Lepidoptera: Geometridae). J. Entomol. Res. Soc. 13: 53–60.

CESPEDES, A., C. M. PENZ, & P. J. DEVRIES. 2015. Cruising the rain forest floor: butterfly wing shape evolution and gliding in ground effect. J. Anim. Ecol. 84: 808–816.

COLLINS, M. M. 2013. Interpretation of wing pattern elements in relation to bird predation on adult *Hylaphora* (Saturniidae). J. Lepid. Soc. 67: 49–55.

CONNER, W. E. & A. J. CORCORAN. 2012. Sound strategies: the 65-million-year-old battle between bats and insects. Ann. Rev. Ent. 57: 21-39.

DARRIBA D., G. L. TABOADA, R. DOALLO, & D. POSADA. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9: 772.

DRYDEN, I. L. 2012. Shapes package. R Foundation for Statistical Computing, Vienna, Austria. Contributed package. http://www.R-project.org

ELTON, C. S. 1946. Competition and the structure of animal communities. J. Anim. Ecol. 15: 54–68.

FIELD, W. L. 1898. A contribution to the study of individual variation in the wings of Lepidoptera. Proc. Am. Acad. Arts Sci. 33: 389–396.

GOTELLI, N.J. 2001. Research frontiers in null model analysis. Global Ecol. Biogeogr. 10: 337–343.

GOWER, J. C. 1975. Generalized procrustes analysis. Psychometrika 40: 33–51.

GROTE, A. R. 1897. An attempt to classify the holarctic Lepidoptera by means of the specialization of the wings: Part I. The day-butterflies. J. NY Entomol. Soc. 5: 151–160.

HAJIBABAEI, M., D. H. JANZEN, J. M. BURNS, W. HALLWACHS, & P. D. N. HEBERT. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. USA 103: 968–971.

HARMON, L.J. & LOSOS, J.B. 2005. The effect of intraspecific sample size on type I and type II error rates in comparative studies. Evolution 59: 2705–2710.

HEBERT, P. D. N., A. CYWINSKA, & S. L. BALL. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B: Biol. Sci. 270: 313–321.

HILL, J. K., C. D. THOMAS, & D. S. BLAKELEY. 1999. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. Oecologia 121: 165–170.

HOWSE, P. & K. WOLFE. 2012. Giant silkmoths: Colour, mimicry, and camouflage. Papadakis, Winterbourne, England. 192 pp.

IMES, R. 1992. Practical Entomologist. Simon and Schuster.

JACCARD, P. 1922. La chorologie sélective et sa signification pour la sociologie végétale. Mémoires de la Société Vaudoise des Sciences Naturelles 2 : 81–107.

JANTZEN, B. & T. EISNER. 2008. Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. Proc. Natl. Acad. Sci. USA 105: 16636–1664.

JANZEN, D. H. 1984. Two ways to be a tropical big moth: Santa Rosa saturniids and sphingids. Oxford Surveys in Evolutionary Biology 1: 85–139.

JANZEN, D. H., M. HAJIBABAEI, J. M. BURNS, W. HALLWACHS, E. REMIGIO, & P. D. N. HEBERT. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. Philos. Trans. R. Soc. Lond. B. 360: 1835–1845.

JARVINEN, O. 1982. Species-to-genus ratios in biogeography: a historical note. J. Biogeogr. 9: 363–370.

KAWAHARA, A. Y. & J. R. BARBER. 2015. Tempo and mode of antibat ultrasound production and sonar jamming in the diverse hawkmoth radiation. Proc. Natl. Acad. Sci. USA 112: 6407–6412.

KLINGENBERG, C. P. 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11: 353–357.

LARKIN, M. A., G. BLACKSHIELDS, N. P. BROWN, R. CHENNA, P.A. MCGETTIGAN, H. MCWILLIAM, F. VALENTIN, I. M. WALLACE, A. WILM, R. LOPEZ, J. D. THOMPSON, T. J. GIBSON, & D. G. HIGGINS. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

MAECHLER, M., P. ROUSSEEUW, A. STRUYF, M. HUBERT, & K. HORNIK. 2013. cluster: Cluster analysis basics and extensions: version 1.14.4. R package.

MALLET, J. & L. E. GILBERT. 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. Biol. J. Linn. Soc. 55: 159–180.

MERIAN, M. S. 1705. Metamorphosis Insectorum Surinamensium. 2: 1–60.

MICHENER, C. D., 1952. The Saturniidae (Lepidoptera) of the Western Hemisphere: morphology, phylogeny, and classification. Bull. Amer. Mus. Nat. Hist. 98: 335–502.

MONTEIRO, A. F., P. M. BRAKEFIELD, & V. FRENCH. 1994. The evolutionary genetics and developmental basis of wing pattern variation in the butterfly *Bicyclus anynana*. Evolution 48: 1147–1157.

NATH, R. & D. DEVI. 2009. Venation pattern and shape variation in wing of *Antheraea assamensis* (Lepidoptera: Saturniidae) of Assam, India. Int. J. Trop. Insect Sci. 29: 70–78.

VAN NIEUKERKEN, E. J., L. KAILA, I. J. KITCHING, N. P. KRISTENSEN, D. C. LEES, J. MINET, C. MITTER, M. MUTANEN, J. C. REGIER, T. J. SIMONSEN, N. WAHLBERG, S.-H. YEN, R. ZAHIRI, D. ADAMSKI, J. BAIXERAS, D. BARTSCH, B. Å. BENGTSSON, J. W. BROWN, S. R.

BUCHELI, D. R. DAVIS, J. DE PRINS, W. DE PRINS, M. E. EPSTEIN, P. GENTILI-POOLE, C. GIELIS, P. HÄTTENSCHWILER, A. HAUSMANN, J. D. HOLLOWAY, A. KALLIES, O. KARSHOLT, A. Y. KAWAHARA, S. J. C. KOSTER, M. KOZLOV, J. D. LAFONTAINE, G. LAMAS, J. F. LANDRY, S. LEE, M. NUSS, K. T. PARK, C. PENZ, J. ROTA, B. C. SCHMIDT, A. SCHINTLMEISTER, J. C. SOHN, M. A. SOLIS, G. M. TARMANN, A. D. WARREN, S. WELLER, R. V. YAKOVLEV, V. V. ZOLOTUHIN, & A. ZWICK. 2011. Order Lepidoptera Linnaeus, 1758. *In* Zhang, Z.-Q. (Ed.), Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 212–221.

OKSANEN, J., F. G. BLANCHET, R. KINDT, P. LEGENDRE, P. R. MINCHIN, R. B. O'HARA, G. L. SIMPSON, P. SOLYMOS, M. H. H. STEVENS, & H. WAGNER 2013. vegan: Community Ecology Package: version 2.0-8. R package.

PACKARD, A. S. 1905. Monograph of the Bombycine moths of North America, including their transformations and origin of the larval markings and armature. Part II. Natl. Acad. Sci. Vol. IX. 272 pp.

PARADIS E., J. CLAUDE, & K. STRIMMER. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290.

PRIETO, C. G., M. L. MUNGUIRA, & H. ROMO. 2009. Morphometric analysis of genitalia and wing pattern elements in the genus *Cupido* (Lepidoptera, Lycaenidae): are *Cupido minimus* and *C. carswelli* different species? Deut. Entomol. Z. 56: 137–147.

R CORE TEAM. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

RATNASINGHAM, S., & P. D. N. HEBERT. 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Mol. Ecol. Notes 7: 355–364.

RICKLEFS, R. E. 2009. Aspect diversity in moths revisited. Am. Nat. 173: 411–416.

RICKLEFS, R. E., & K. O'ROURKE. 1975. Aspect diversity in moths: a temperate–tropical comparison. Evolution 29: 313–324.

ROHLF, F. J. 2010. tpsDig, version 2.12. Department of Ecology and Evolution, State University of New York at Stony Brook.

SCHUTZ, H. & J. KRIEGER. 2007. Guide to Geometric Morphometrics. www.morpho-tools.net/ (May 2013).

SCOBLE, M. J. 1992. The Lepidoptera: form, function, and diversity. Oxford University Press, Oxford. 404 pp.

SEKAR, S. 2012. A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? J. Anim. Ecol. 81: 174–184.

STRAUSS, R. E. 1990. Patterns of quantitative variation in lepidopteran wing morphology: the convergent groups Heliconiinae and Ithomiinae (Papilionoidea: Nymphalidae). Evolution 44: 86–103.

TIBSHIRANI, R., G. WALTHER, & T. HASTIE. 2001. Estimating the number of clusters in a data set via the gap statistics. J. R. Stat. Soc. B. 63: 411–423.

VALLIN, A, S. JAKOBSSON, & C. WIKLUND. 2010. Constant eyespot display as a primary defense—survival of male and female emperor moths when attacked by blue tits. J. Res. Lepid. 43: 9–17.

VISCOSI, V. & A. CARDINI. 2012. Leaf morphology, taxonomy, and geometric morphometrics: a simplified protocol for beginners. PLoS ONE 7:10.

WICKMAN, P. O. 1992. Sexual selection and butterfly design—a comparative-study. Evolution 46: 1525–1536.

YLLA, J., R. S. PEIGLER, & A. Y. KAWAHARA. 2005. Cladistic analysis of moon moths using morphology, molecules, and behaviour: *Actias* Leach, 1815; *Argema* Wallengren, 1858; *Graellsia* Grote, 1896 (Lepidoptera: Saturniidae). SHILAP Rev. Lepid. 33: 299–317.

ZELDITCH, M. L., D. L. SWIDERSKI, & H. D. SHEETS. 2012. Geometric morphometrics for biologists: A primer. 2nd ed. Elsevier Academic Press, London. 488 pp.

Submitted for publication 21 November 2015; revised and accepted 18 December 2015.

Please see next page for
SUPPLEMENTARY TABLE 1

SUPPLEMENTARY TABLE 1.

List of saturniid species tested for geometric morphometrics. In square brackets: [Number of specimens examined/COI Genbank or BOLD code]

Group 1

Athletes ethra (Westwood, 1849) [1/-]
Attacus atlas (Linnaeus, 1758) [1/-]
Cercophana venusta (Walker, 1856) [1/-]
Cricula trifenestrata (Helfer, 1837) [1/GU702848]
Eupackardia calleta (Westwood, 1853) [1/-]
Gamelia neidhoeferi Lemaire, 1967 [1/-]
Heliconisa pagenstecheri (Geyer, 1835) [1/JX216141]
Hylesia thaumex Draudt, 1929 [1/JN272755]
Pseudimbrasia deyrollei (Thomson, 1858) [1/-]
Psigida walkeri (Grote, 1867) [1/-]
Rhescyntis hippodamia norax Druce, 1879 [1/-]
Rothschildia aurota speculifera (Walker, 1855) [1/JN827810]
Travassosula mulierata Lemaire, 1971 [1/-]

Group 2

Adeloneivaia boisduvalii (Doumet, 1859) [1/JN827448]
Anisota dissimilis (Boisduval, 1872) [1/JX215944]
Antheraea assamensis Helfer, 1837 [1/AY605249]
Arsenura cymonia (W. Rothschild, 1907) [1/KX281953]
Automeris annulata Schaus, 1906 [1/GU663221]
Caio championi (Druce, 1886) [1/GU663240]
Ceratesa hemirhodia (W. Rothschild, 1907) [1/-]
Citheronia phoronea (Cramer, 1779) [1/JN827533]
Citioica anthonilis (Herrich-Schäffer, 1854) [1/KF491637]
Copaxa flavina Draudt, 1929 [1/JX216068]
Dirphiopsis multicolor (Walker, 1855) [1/-]
Eacles imperialis oslari Rothschild, 1907 [1/-]
Eudyaria veneta (Butler, 1871) [1/JX216121]
Grammopelta lineata (Schaus, 1906) [1/HQ581368]
Hirpida gaujoni (Dognin, 1894) [1/KX281946]
Kentroleuca boliviensis Brechlin & Meister, 2002 [1/KX281949]
Leucanella stuarti stuarti (Rothschild & Jordan, 1901) [1/FJ027007]
Lonomia electra Druce, 1886 [1/JX216237]
Meroleuca litura (Walker, 1855) [1/-]
Molippa nibasa Maassen & Weyding, 1885 [1/-]
Neorcarnegia basirei (Schaus, 1892) [1/KX281948]
Ormiscodes cinnamomea (Feisthamel, 1839) [1/-]
Oiticella luteciae (Bouvier, 1924) [1/-]
Periga insidiosa (Lemaire, 1972) [1/HQ972132]
Periphoba arcai (Druce, 1886) [1/JQ552208]
Pseudautomeris irene Irene (Cramer, 1779) [1/-]
Pseudodirphia agis (Cramer, 1775) [1/HM432552]
Rachesa breteuili (Bouvier, 1927) [1/-]
Schausiella polybia (Stoll, 1781) [1/JN264482]
Scolesa viettei Travassos, 1959 [1/JX216445]
Syssphinx gomezi Lemaire, 1984 [1/-]

Group 3

Arsenura albopicta Jordan, 1922 [7/GU663237]
Arsenura batesii (R. Felder & Rogenhofer, 1874) [1/JQ559921]
Arsenura beebei (Fleming, 1945) [1/JX215952]
Arsenura ciocolatina Draudt, 1930 [1/GU663280]
Arsenura ponderosa W. Rothschild, 1895 [1/JN263078]
Arsenura rebeli Gschwander, 1920 [1/KX281951]
Arsenura sylla (Cramer, 1779) [1/JX215958]
Caio richardsoni (Druce, 1890) [1/-]

Group 4

Dysdaemonia boreas (Cramer, 1775) [1/JQ564262]
Dysdaemonia fosteri W. Rothschild, 1906 [1/-]
Paradaemonia andensis (Rothschild, 1907) [1/GU663281]
Paradaemonia mayi (Jordan, 1922) [1/JX216287]
Paradaemonia nycteris (Jordan, 1922) [1/-]
Paradaemonia orsilochus (Maassen, 1869) [1/JX216288]
Paradaemonia platydesmia (W. Rothschild, 1907) [1/HM382419]
Paradaemonia terrena (Jordan, 1922) [1/-]
Paradaemonia thelia (Jordan, 1922) [1/JX216296]
Titaea tamerlan (Maassen, 1869) [7/-]
Titaea timur (Fassl, 1915) [1/GU663150]

Group 5

Actias artemis (Bremer & Gray, 1853) [1/-]
Actias heterogyna Mell, 1914 [1/-]
Actias selene (Hübner, 1806) [10/-]
Actias truncatipennis (Sonthonnax, 1899) [1/KX281952]
Argema mimosae (Boisduval, 1847) [1/-]
Coscinocera anteus Bouvier, 1927 [1/-]
Graellsia isabellae Grote, 1896 [1/-]

Group 6

Copiopteryx derceto (Maassen, 1872) [1/KX281947]
Copiopteryx jehovah (Strecker, 1874) [6/HQ581388]
Copiopteryx semiramis banghaasi Draudt, 1930 [2/-]
Copiopteryx semiramis semiramis (Cramer, 1775) [1/-]
Copiopteryx sonthonnaxi Em. Andre, 1905 [1/KX281950]
Copiopteryx virgo Zikan, 1929 [1/-]

Journal of the Lepidopterists' Society
70(2), 2016, 108–113

FOREST HABITAT REDUCES THE FLIGHT OF *PONTIA OCCIDENTALIS* (REAKIRT) (LEPIDOPTERA: PIERIDAE) RELATIVE TO ALPINE MEADOW HABITAT

STEPHEN F. MATTER

Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006 USA and
Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9 Canada email: mattersf@uc.edu

COREY GARGANO

Environmental Studies Program, University of Cincinnati, Cincinnati, OH 45221-0006 USA email: corey.gargano@gmail.com

KENNETH I. KIM

Department of Biology, Truman State University, Kirksville, MO 63501 USA email: kimki@uci.edu

AND

ANNE WICK

Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006 USA email: Wickak@gmail.com

ABSTRACT. We compared the movement of *Pontia occidentalis* in forest and meadow habitat. We hypothesized that flight distances and overall movement would be greater in forested habitat than in meadow habitat. This hypothesis was based on forging theory predicting that organisms should spend less time in areas where resources are scarce than where they are abundant. Because this species is a generalist in its use of open habitat and regularly encounters forest when dispersing and hilltopping, we also hypothesized that forested habitat would not impose a physiological limitation on their flight. To test this hypothesis we released 68 butterflies in either forest or alpine meadow habitat and followed their movement. Contrary to our hypothesis, the total distance moved, mean flight distance, and rate of flight were all lower in forest relative to meadow habitat. Forest habitat did not exert an edge effect for *Pontia occidentalis* flying in meadow habitat. Contrary to our second hypothesis, differences in movement appeared to be due to differences in the light levels between forest and meadow habitat. *Pontia occidentalis* flew more often and farther distances with increasing light intensity, which was greater in meadow habitat than in forest. Overall, the results indicate that forest may impede the movement of *Pontia occidentalis*, despite it regularly encountering it. The results also indicate that structural and physiological limitations on movement imposed by different habitats may preclude optimal responses to resources.

Additional key words: dispersal, ecotone, light intensity, matrix, migration

The movement of organisms is a fundamental feature of life, affecting processes from foraging, to spatial population dynamics, to speciation (Fretwell & Lucas 1970, Roland & Matter 2007, Claramunt et al. 2012). Many insect species exist in relatively heterogeneous environments and regularly encounter different habitat types, which may affect their movement (Pither & Taylor 1998, Ross et al. 2005, Dover & Settle 2009). Thus, the presence of different types of habitat in a landscape has the potential to affect a range of ecological and evolutionary processes.

Understanding movement in different habitats has been approached from different perspectives. According to foraging (Zollner & Lima 1999) and mating (Gilroy & Lockwood 2012) theory, organisms should minimize time in habitats where resources are lacking. Thus, rates of movement and distances moved are predicted to be greater in habitat that contains few or no resources than in habitats containing an abundance of resources or mates (Turchin 1991, Merckx et al. 2003). From a physiological perspective, different habitats may impose a variety of constraints on dispersal ability. Many ectotherms, and butterflies in particular, rely on the external environment to raise their body temperature to levels where movement or flight are possible. Thus, differences in temperature, light, and the physical structure of different habitats can affect the ability to move as well as movement distances and rates (Merckx et al. 2003, Ross et al. 2005, Dover & Settle, 2009, Schultz et al. 2012).

The effects of habitat type on dispersal have been evaluated most frequently for specialist species using distinct habitats within spatial population networks, where habitat patches are imbedded in an inhospitable matrix. In these studies, the implicit assumption is that habitat patches contain resources and matrix habitat does not (Dennis et al. 2013); dispersal is then compared between or among different habitat types

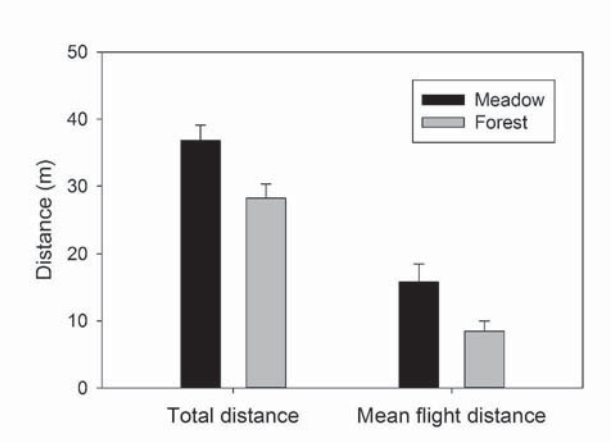


FIG. 1. Total distance moved and mean flight distance of *Pontia occidentalis* in forest and meadow habitat. Error bars represent one standard error. The means shown for total distance moved are adjusted for differences in observation time.

(Dover & Settle 2009). Possibly due to the ability to assess habitat and non-habitat for specialist species (but see Dennis et al. 2013), the effect of different types of habitat on the dispersal of generalist species has received less attention than specialists. For generalists, we might expect more similar movement among different types of habitat because different habitats are more likely to contain resources, albeit at different abundance and quality (Dennis et al. 2013). Similarly from a physiological perspective, generalists likely have to contend with a wider range of environmental conditions. Thus, the dispersal of generalists may not vary as greatly among habitats as for specialists.

Here, we compare the movement of the Western White, *Pontia occidentalis*, between alpine meadow and forested habitat. We were particularly interested in whether forest affects its flight because previous research at the same location has shown that forest habitat greatly reduces the flight and dispersal of the alpine specialist *Parnassius smintheus* Doubleday among meadow habitats (Matter & Roland 2003) largely through a reduction in light levels in forest (Ross et al. 2005). Forest encroachment at this site has reduced non-forested habitat by over 75% over the last 75 years (van Ee et al. 2015). Thus, if forest reduces dispersal among alpine meadows it may reduce persistence in this habitat in addition to any effects from habitat loss.

Based on foraging theory, we predicted that movement distances and rates of *Pontia occidentalis* would be greater in forested than in meadow habitat because resources are very low to non-existent in forested habitat. Additionally, because of *Pontia occidentalis*' generalist nature and regularly encountering forested habitat, we predicted that it would not experience limitation in flight due to lower light levels in forest.

MATERIALS AND METHODS

Study site and species. Experiments were conducted during July and August (2003–05 and 2007–08) in meadows above treeline (~2100 m) on Jumpingpound Ridge, Alberta, Canada (51°57'N, 114°54'W, see Matter et al. (2009) for a depiction of the study site). Vegetation within the meadows consists of grasses, sedges, and wildflowers. Meadows are bordered by forest consisting of *Abies lasiocarpa* (Hook.), *Picea engelmannii* Parry ex Engelm., and *Pinus contorta* Dougl. ex. Loud. (Pinaceae).

Pontia occidentalis (Reakirt) (Lepidoptera: Pieridae) inhabits a variety of open habitats in western North America from alpine meadows, to prairies, to grassy roadsides (Guppy & Shepard 2001). It ranges from Alaska to northern New Mexico. In Alberta, there is one to possibly three generations per year (Bird et al. 1995). Many species of Brassicaceae are host plants (Bird et al. 1995, Guppy & Shepard 2001) and a wide variety of nectar flowers are used in these meadows (Ezzedidne & Matter 2008). Because this butterfly exploits patchily distributed resources, it likely encounters a variety of habitat types and range of environmental conditions. In addition, these butterflies often traverse forested habitat to hilltop and feed on nectar in alpine meadows. At our site, *Pontia occidentalis* is an irruptive species. In most years they are present in moderate numbers, but in 2003 they were extremely numerous with many individuals presumably arriving from lower elevations.

Experimental design. Butterflies were netted by hand and kept at ambient temperature until use (< 1 hr). One trial was conducted per butterfly at independent sites with well-defined forest edges. At each site a butterfly was released onto vegetation at ground level from 5–20 m from a forest edge in either forest or meadow habitat. Butterflies were then observed for up to one hour, or until we lost sight of the butterfly. We placed a marking flag at each alighting point just after the butterfly left and took a reading of light intensity (lux/100) using a portable light meter (Extec). After each trial, we measured the distance and bearing between alighting points and the distance and bearing from each point to the closest forest edge by hand using a meter tape and compass. These data allowed us to evaluate movement in each habitat as well as any edge effects.

All trials were conducted on days suitable for mark-recapture, i.e., sunny and not too windy. Being in an alpine environment, however, weather conditions did

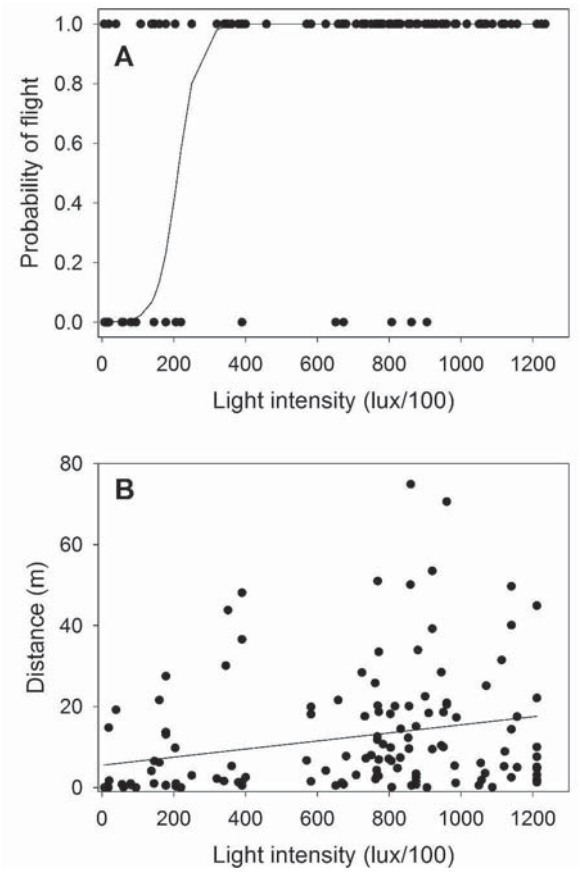


FIG. 2. Effects of light levels on the flight of *Pontia occidentalis*. The top panel (**A**) shows the probability of flight versus crawling or not moving relative to light intensity in lux divided by 100 (the units in which it was measured). The fitted line is the predicted logistic response not accounting for random effects variation among individuals (N=45). The bottom panel (**B**) shows the distance moved by *Pontia occidentalis* relative to light levels (mixed model fixed effect slope and intercept: $\beta = 0.014 \pm 0.004$, $t = 3.40$, $P = 0.08$; $\beta_0 = 4.088 \pm 2.365$). The plotted line does not account for differences among individuals. The model explained significant deviance in distance moved relative to a null containing only the mean and random effect of individuals ($\chi 2 = 18.17$, $df = 3$, $P < 0.01$). Random effects variation due to individuals was low (1.11×10^{-4}, SD = 0.011). Several trials could not be used because light levels were not measured for individual movements.

vary within and among trials. Meteorological conditions (wind speed, temperature) which can affect movement (Merckx et al. 2003) were collected at the start of most, but not all trials. Trials were conducted under wind speeds ranging from 0.0 to 14.2 km/h and temperatures from 10.7° to 30.6° C. We conducted a total of 68 trials (34 each in forest and meadow habitat).

Statistical analyses. To evaluate movement in forest and meadow habitat, we examined the number of flights, total distance moved by flight and/or crawling (the sum of the distance between each alighting point), rate of flight (total distance moved by flight/total observation time), and mean flight distance (total distance moved by flying/number of flights) for butterflies released in each habitat. For these analyses forest or meadow was considered a fixed effect. Number of flights consists of counts, thus it was analyzed using a generalized linear model with a quasipoisson error term to account for overdispersion. Total distance moved, rate of flight, and mean flight distance were analyzed using general linear models with normal error. Because the number of flights and total distance moved may be influenced by the length of observation, observation time was included as a covariate in the analysis of these variables.

We used logistic regression to examine the effect of light levels on flight. At each alighting point (including the point of release) we examined whether a butterfly flew from there or did not (crawled or did not move) relative to the light intensity. Because multiple observations of each butterfly were made within a trial for this analysis, the individual butterfly was included as a random effect in the model. We used a similar analysis, but with normal errors, to examine whether the distance that a butterfly moved varied with light intensity.

To determine whether forest exerts an edge effect, we first classified movements toward or away from a forest edge. We first used a 90° arc; any movement was considered toward the edge if it fell on a bearing ± 45° of the bearing to the nearest forest edge. Because this angle was arbitrary, we also examined a broader 180° arc. For each arc, we constructed a logistic regression with individual as a random effect and distance from the forest edge as an independent variable. We expected that if there is an edge effect, it would diminish with increasing distance from the forest edge. However, movements were tallied as either toward the edge or away from the edge. Thus, the expectation for a logistic model implies "attraction" to the forest edge at increasing distance, where in contrast, we expect no effect of the edge at greater distance. We evaluated this expectation by fitting models with an interaction between distance from the forest edge and a dummy variable. The dummy variable coded for whether a distance was "near" or "far" from the edge, and was scored as either 1 for near (including an effect of distance from the edge) or 0 for far (no effect of distance from the edge). Thus, these models with the interaction tested whether there was repulsion close to the forest edge and no effect, rather than an attraction to the edge farther from the forest edge. Because the choice of how to define near and far distances from treeline was arbitrary, we used a range of distances in 5

TABLE 1. Analysis of edge effects induced by forest habitat for *P. occidentalis* flying in alpine meadows. Results from mixed logistic models are shown assuming a 90° or 180° arc determining whether movements were towards or away from forest expecting that if there is an edge effect it should diminish with increasing distance from the forest edge. A dummy variable coded (1 = near, 0 = far) whether a distance was near or far from a forest edge. The interaction between distance and the dummy variable tested whether there would be repulsion near a forest edge and no effect far from the edge (rather than attraction). For each analysis individual butterflies were considered a random effect.

90° arc				180° arc		
	Estimate(SE)	Z	P	Estimate(SE)	Z	P
>5m "far"						
Intercept	2.12(0.76)	2.80	<0.01	1.40(0.62)	2.26	0.02
Distance	-0.01(0.02)	-0.12	0.91	-0.07(0.02)	-2.85	<0.01
Dummy	-0.14(1.27)	-0.11	0.91	0.14(1.10)	0.13	0.90
Interaction	-0.10(0.25)	-0.38	0.70	-0.23(0.22)	-1.03	0.30
>10m "far"						
Intercept	1.90(0.89)	2.14	0.03	1.09(0.75)	1.45	0.15
Distance	0.01(0.03)	0.10	0.92	-0.06(0.03)	-2.12	0.03
Dummy	-0.42(1.27)	-0.33	0.74	-0.27(1.07)	-0.26	0.80
Interaction	0.07(0.17)	0.43	0.67	0.03(0.13)	0.25	0.80
>15m "far"						
Intercept	2.67(1.02)	2.60	0.01	1.18(0.83)	1.41	0.16
Distance	-0.02(0.03)	-0.53	0.60	-0.06(0.03)	-2.05	0.04
Dummy	-0.50(1.28)	-0.39	0.70	-0.30(1.04)	-0.28	0.77
Interaction	-0.07(0.12)	-0.57	0.57	0.02(0.10)	0.20	0.84
>20m "far"						
Intercept	5.35(1.77)	3.02	<0.01	1.58(1.29)	1.22	0.22
Distance	-0.07(0.04)	-1.85	0.06	-0.07(0.04)	-1.75	0.08
Dummy	-3.50(1.79)	-2.00	0.05	-0.58(1.37)	-0.42	0.67
Interaction	0.06(0.06)	0.91	0.36	0.01(0.05)	0.22	0.83

m intervals encompassing the distance from treeline where we observed butterflies. We tested the interaction using each near and far setting in separate models.

All analyses were conducted using the program R version 3.0.2 (R Core Development Team, 2013).

RESULTS

We were able to follow individual butterflies for a mean of 25.8 ± 2.8 (S.E.) min. Only three butterflies crossed habitat boundaries; two moved into forest from meadow and one moved from forest into meadow.

Contrary to our hypothesis, the movement of *Pontia occidentalis* was reduced in forest habitat relative to meadow habitat (Fig. 1); its total distance moved was significantly farther in meadow than in forest habitat ($F_{1,65} = 8.00$, $P < 0.01$) after accounting for the effects of observation time ($F_{1,66} = 8.81$, $P < 0.01$). Its mean flight distance was also greater in meadow than in forest ($F_{1,66} = 8.16$, $P < 0.01$) as was its rate of flight (8.5 ± 1.8 vs. 1.0 ± 0.4 m/min.; $F_{1,66} = 15.59$, $P < 0.01$). *Pontia occidentalis* also tended to initiate more flights in meadow (3.4 ± 0.5) than in forest habitat (1.4 ± 0.8), although the difference was not significant ($F_{1,65} = 2.82$, $P = 0.09$) after accounting for the marginal effect of observation time ($F_{1,66} = 3.60$, $P = 0.06$).

The flight of *Pontia occidentalis* was affected by light intensity (Fig. 2). They flew more often when light

levels were high than under low light intensity (β = 0.036 ± 0.018, Z = 2.00, P = 0.04; β_0 = -7.627 ± 3.524). The overall model explained significant variation in whether a butterfly flew or not (χ^2 = 48.849, df = 3, P < 0.01); variation attributable to mean differences among individuals was minimal (2.4×10^{-3} , SD = 0.05). Flight distance also increased with increasing light intensity (Fig. 2). Not surprisingly, light levels in meadow habitat were greater than in forested habitat (771900 ± 47600 vs. 421500 ± 48300 lux; t = 6.33, separate variance df = 125.6, P < 0.01).

There was little evidence that *Pontia occidentalis* avoided forest habitat though edge effects. These butterflies were equally likely to move towards or away from forest at any distance based on the more conservative 90° arc (Table 1). Using a 180° arc, we found that *Pontia occidentalis* tended to move away from forest near the edge and towards forest father from the edge as indicated by the significant effect of distance. However, the interaction between distance from the edge and the dummy variable was not significant, implying that there is attraction towards forest when far from an edge, but not repulsion from forest when close to the edge.

Discussion

Our hypothesis that the movement distances and rates of *Pontia occidentalis* would be greater in forest than in meadow habitat was not supported; its movement was reduced in forest habitat relative to alpine meadow habitat. That *Pontia occidentalis* does not move as far or fast in forest habitat, is inconsistent with the hypothesis that movement should be greater in habitats with low resources than in habitats with abundant resources (Zollner & Lima 1999). Our hypothesis that the light environment would not affect the movement *Pontia occidentalis* was somewhat equivocal. Low light intensity may reduce the flight of butterflies adapted to high light environments such as alpine meadows, but we predicted that the generalist nature of *Pontia occidentalis* would allow it to fly in lower light such as that experienced in forested habitat. Dispersal by *Pontia occidentalis* was affected by ambient light intensity. Under high light conditions *Pontia occidentalis* flew almost exclusively and the distance moved increased with increasing light intensity; however, its flight was not particularly limited by low light levels. Thus, shadier forest habitat may not place physiological limits on the flight of *Pontia occidentalis*. That is, there may be enough ambient sunlight to allow them to warm their flight muscles, but they tended to fly less often when light levels were low limiting their ability to respond optimally to available resources, i.e., moving quickly through the low resources in forest habitat. Our results show that movement can differ among habitat types even for generalist insect species regularly encountering these different types of habitat.

The reduced flight in forest relative to alpine meadow for *Pontia occidentalis* was somewhat similar to that seen for the alpine specialist butterfly *Parnassius smintheus* (Ross et al. 2005). For male *P. smintheus* flight was reduced in forest relative to open meadow habitat and the propensity for this butterfly to fly decreased dramatically with light intensity, much more so than for *Pontia occidentalis*. For *P. smintheus* there also was a strong edge effect; however, this was not seen for *Pontia occidentalis*. For both of these species, forest may be a barrier to flight by limiting light, particularly if the butterfly alights in forested habitat. Forest habitat has been shown to reduce the between population dispersal of *P. smintheus* affecting its population growth (Roland & Matter 2007) and genetic structure (Keyghobadi et al. 2005, Caplins et al. 2014). Despite the effects seen for flight, it seems less likely that forest habitat will have similar effects at the population level for *Pontia occidentalis*. First, it is unlikely that individual meadows contain semi-independent populations of *Pontia occidentalis* due to the influx of hilltopping butterflies from lower elevations, particularly during warm, dry summers. Second, *P. smintheus*, like many alpine specialists, generally does not fly high above the ground (Ross et al. 2005). In contrast, *Pontia occidentalis* often flies high above the ground and may simply fly over forested habitat, avoiding shading and any structural effects, but potentially at a cost of encountering buffeting winds avoided by low flying species in alpine regions (Matter, personal observation).

Numerous studies now have shown that dispersal is a function of habitat type (e.g., Pither & Taylor 1998, Haddad 1999, Jonsen et al. 2001) and many studies have been conducted in habitats that differ in resources. These studies generally have found results that are consistent with foraging theory, i.e., that dispersal distances are longer and more directed where resources are lacking. Such dispersal results in less time spent in resource poor habitat than in areas where resources are abundant (Haynes & Cronin 2006, Schtickzelle et al. 2007, Kuefler et al. 2010). However, a growing number of studies indicate that structural differences in habitat can affect dispersal, sometimes with larger effects than resources (Ross et al. 2005, Schultz et al. 2012).

The results of this study indicate that dispersal can vary among habitat types even for the generalist *Pontia occidentalis* which regularly encounters a variety of habitat types. Environmental differences among habitats potentially impose physiological or behavioral

constraints, such as the propensity to fly, which may preclude appropriate response based on foraging theory.

ACKNOWLEDGEMENTS

This research was supported by NSF grants DEB 0918929 and 0326957 to S.F. Matter and an NSERC Discovery grant to Jens Roland. We thank two reviewers for comments that improved the manuscript.

LITERATURE CITED

CAPLINS, S.A., K. J. GILBERT, C. CIOTIR, J. ROLAND, S. F. MATTER & N. KEYGHOBADI. 2014 Landscape structure and the genetic effects of a population collapse. Proc. R. Soc. B 281: 1796-1798.

CLARAMUNT, S., E. P. DERRYBERRY, J. V. REMSEN JR. & R. T. BRUMFIELD. 2012. High dispersal ability inhibits speciation in a continental radiation of passerine birds. Proc. R. Soc. B 279: 1567–1574.

BIRD, C. D., G. J. HILCHIE, N. G. KONDELA, E. M.PIKE & F.A.H. SPERLING. 1995. Alberta butterflies. The Provincial Museum of Alberta, Edmonton. 349 pp.

DOVER, J. & J. SETTELE. 2009. The influences of landscape structure on butterfly distribution and movement: a review. J. Insect Conserv. 13: 3-27.

EZZEDDINE, M. & S. F. MATTER. 2008. Nectar flower use and electivity by butterflies in sub-alpine meadows. J. Lep. Soc. 62: 138-142.

FRETWELL, S. D. & H. L. LUCAS. 1970. On territorial behaviour and other factors influencing habitat distribution in birds. Acta Biotheor. 19: 16-36.

GILROY J. J. & J. L. LOCKWOOD. 2012. Mate-finding as an overlooked critical determinant of dispersal variation in sexually-reproducing animals. PLoS ONE 7: e38091 doi:10.1371/journal.pone.0038091

GUPPY, C. & J. SHEPARD. 2001. Butterflies of British Columbia. University of British Columbia Press, Vancouver. 414 pp.

HADDAD, N. M. 1999. Corridor use predicted from behaviors at habitat boundaries. Am. Nat. 153: 215–227.

HAYNES, K. J. & J. T. CRONIN. 2006. Interpatch movement and edge effects: the role of behavioral responses to the landscape matrix. Oikos 113: 43–54.

KEYGHOBADI, N., J. ROLAND, S. F. MATTER & C. STROBECK. 2005. Among- and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an empirical demonstration. Proc. R. Soc. B 272: 553-560.

KUEFLER, D., B. HUDGENS, N. M. HADDAD, W. F. MORRIS & N. THURGATE. 2010. The conflicting role of matrix habitats as conduits and barriers for dispersal. Ecology 91: 944–950.

MATTER, S.F., M. EZZEDDINE, E. DUERMIT, J. MASHBURN, R. HAMILTON, T. LUCAS & J. ROLAND. 2009. Interactions between habitat quality and connectivity affect immigration but not abundance or population growth of the butterfly, *Parnassius smintheus*. Oikos 118: 1461-1470.

MERCKX, T., H. VAN DYCK, B. KARLSSON O. LEIMAR. 2003. The evolution of movements and behaviour at boundaries in different landscapes: a common arena experiment with butterflies. Proc. R. Soc. B 270: 1815–1821.

PITHER, J. & P. D. TAYLOR. 1998. An experimental assessment of landscape connectivity. Oikos 83: 166-174.

R CORE TEAM. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

ROLAND, J. & S. F. MATTER. 2007. Encroaching forests decouple alpine butterfly population dynamics. PNAS 104: 13702-13704.

ROSS, J. A., S. F. MATTER & J. ROLAND. 2005. Edge avoidance and movement of the butterfly *Parnassius smintheus* in matrix and non-matrix habitat. Land. Ecol. 20: 127-135.

SCHTICKZELLE, N., A. JOIRIS, H. VAN DYCK & M. BAGUETTE. 2007. Quantitative analysis of changes in movement behaviour within and outside habitat in a specialist butterfly. BMC Evo. Bio. 7: doi:10.1186/1471-2148-7-4.

SCHULTZ, C.B., A. M. A. FRANCO & E. E. CRONE. 2012. Response of butterflies to structural and resource boundaries. J. Anim. Ecol. 81: 724-734.

TURCHIN, P. 1991. Translating foraging movements in heterogeneous environments into the spatial distribution of foragers. Ecology 72: 1253–1266.

VAN EE, J., M. WESTBROOK, S. YADAV, J. ROLAND & S.F. MATTER. 2015. Predicting the effects of forest encroachment for *Sedum lanceolatum* and *Rhodiola integrifolia* at a subalpine ecotone. In Press, Alp. Bot. DOI:10.1007/s00035-015-0148-0.

ZOLLNER, P. A. & S. L. LIMA. 1999. Search strategies for landscape-level inter-patch movements. Ecology 80: 1019–1030.

Submitted for publication 11 June 2015; revised and accepted 11 September 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 114–120

REPRODUCTIVE STRATEGIES AND LIFE HISTORY EVOLUTION OF SOME CALIFORNIA *SPEYERIA* (NYMPHALIDAE)

STEVEN R. SIMS
1973 Rule Ave., Maryland Heights, MO 63043; email: steve.sims@blueimago.com

AND

ARTHUR M. SHAPIRO
Center for Population Biology, University of California, Davis, CA 95616; email: amshapiro@ucdavis.edu

ABSTRACT. Egg weights and total eggs produced by nine *Speyeria* spp. (Nymphalidae) in California allowed estimates of per-egg and lifetime reproductive effort. Interpopulation reproductive effort in four species and intrapopulation reproductive effort of two species in different years were documented. Female body weight was uncorrelated with either individual egg weight or total lifetime egg weight. Smallest eggs (mean dry wt/egg = 0.05 – 0.06 ug), and the greatest number of eggs, were from *S. coronis* (Skinner) and *S. zerene* (Boisduval) whose females undergo summer reproductive diapause in dry habitats. Largest eggs (mean dry wt/egg = 0.10 ug) were from *S. nokomis* (Skinner), a wet habitat species. The greatest relative reproductive effort was made by dry habitat species lacking reproductive diapause. Reproductive effort and duration of first instar exposure to summer temperatures were positively correlated. Intrayear variation in total egg weight did not vary significantly among populations of *S. callippe* (Boisduval), *S. hesperis* (Boisduval), *S. nokomis*, or *S. zerene* but mean total egg weights were significantly different in females from the Donner Pass, CA population of *S. mormonia* (Boisduval) in two years. *S. zerene* from high elevations lacked the reproductive diapause characteristic of lower elevation populations. The reproductive strategies of *Speyeria* spp. are adaptive responses to the desiccation stress that their habitats impose on diapausing first instars.

Additional key words: female size, egg numbers, first instars

Comparative analyses of reproductive strategies among closely related species have provided significant contributions to the field of evolutionary biology (Smith & Fretwell 1974, Stearns 1992). One particularly interesting area of research involves determining the possible reasons for differences among related taxa, in offspring size, number, and traits affecting total reproductive effort. Egg size is one adaptive life history response that can be selected upon because it can confer fitness effects on both maternal parent and progeny (Bernardo 1996, Fox & Czesak 2000). For example, larvae from larger eggs may have greater resistance to environmental stresses such as starvation, desiccation, and extreme temperatures (Fox & Czesak 2000, Roff 2002), but high fecundity, and relatively small eggs, may be of selective advantage within habitats, or during growth periods, that have favorable temperature, moisture, and food (Braby 1994, Torres-Vila & Rodriguez-Molina 2002, Seko & Nakasuji 2004). There is a trade-off between egg size and fecundity (Roff 1992, Cummins 1986, Braby 1994, Sinervo & Licht 1991, Heath et al. 1999) and, within species, the selective forces acting upon egg size are not always obvious. In addition, the compromise between egg size and number plays out within the intra- and interspecific variation in female body size which usually sets a limit on the total amount of energy that can be used for reproduction (Boggs 1994, Fox & Czesak 2000).

Speyeria spp. (Nymphalidae) inhabit a range of montane habitats throughout the northern and western portions of North America. All species have one generation per year (univoltine) and utilize native violets (*Viola* spp.) exclusively as larval host plants. Females usually do not lay ova directly on the host plants but often on other vegetation near ground level. First instars immediately undergo an obligate diapause; feeding and resumption of larval development is synchronized with *Viola* regrowth the following spring. Diapausing larvae of *Speyeria* spp. from xeric habitats are exposed to considerable desiccation stress, especially during the summer and fall months. In California, desiccation stress is especially severe among species occurring in summer hot and dry habitats where adults of species such as *S. callippe* (Boisduval) often emerge in May and conclude oviposition before July (Brittnacher et al. 1978). The probability of individual larvae surviving through diapause to successfully complete development the following year is almost certainly very low and life-history strategies should reflect selection for reproductive patterns and larval adaptations that maximize larval survival and overall reproductive success (Zalucki et al. 2002, Sims & Shapiro 2014). Relatively little is known about the comparative reproductive strategies of most *Speyeria* spp. over the range of habitats they occupy. To address this, we studied interspecific differences in egg size,

total numbers of eggs produced, and female body weights and related them to preferred species habitats. We asked: 1) What is the relationship between female body size, egg size, and total reproductive effort? 2) Is there a relationship between reproductive effort and species habitats? and 3) What is the nature of intraspecific variation in the reproductive strategies?

MATERIALS AND METHODS

Insects. Adult *Speyeria* spp. collection locations in California and Nevada (NV) during 1974 and 1975 were as follows: Anthony Peak, Mendocino Co., 2100 M (AP); Boggs Mountain State Forest nr. Cobb, Lake Co., 850 M (BM); Del Puerto Canyon, Stanislaus Co., 600 M (DL); Devils Gate Pass, Mono Co., 2230 M (DG); Donner Pass, Nevada Co., 2100 M (DP); Kings Canyon, Carson City Co., NV 1600 M (KC); Round Valley, Inyo Co., 1400 M (RV); Yuba Pass, Sierra Co., 2000 M (YP), Fales Hot Springs, Mono Co., 2230 M (FS); Sonora Pass, Mono Co., 2900 M (SP). The species and populations studied and collection locations were: *S. callippe* (Boisduval) (AP, BM, DL); *S. coronis snyderi* (Skinner) (YP); *S. cybele leto* (Behr) (KC); *S. egleis* (DP, YP); *S. hesperis irene* (Boisduval) (AP, DP); *S. hydaspe* (Boisduval) (AP); *S. mormonia* (Boisduval) (DP, SP); *S. nokomis apacheana*, (Skinner) (DG, RV); *S. zerene zerene* (Boisduval) (BM), *S. zerene conchyliatus* (Comstock) (YP), *S. zerene* malcolmi (Comstock) (FS).

Egg collection. Field-collected adult females were transported back to the laboratory in glassine envelopes under refrigeration (~4 - 8°C) in insulated coolers. Only females in freshly emerged condition, based on wing wear and scale loss, were used in the experiments. Wing wear is commonly used as a surrogate measure of age in butterflies and judged to be a reasonable index for reproductive comparisons (see Kemp 2006). Females were handled, and ova obtained, as previously described by Sims (1979, 1984). Briefly, adults were fed once daily, until replete, on a 10% honey-water solution and maintained, in 1 liter cardboard containers over leaves of *Viola papilionacea* Pursh, at a photoperiod of 15:9 (L:D) h and 24+/-1ºC within an environmental

TABLE 1. Life span, temporal pattern of oviposition, egg weight, and total egg production in *Speyeria*.

Species, location	Sample size	Life span, days (SD)	Days to first ova, mean (SD)	Total # eggs, mean (SD)	Egg weight, dry, mg, mean (SD)	Total dry wt of eggs per female (mg) (SD)
S. hydaspe-AP	16	24.2 (6.7)	1.6 (1.0)	291 (92)	0.097 (0.007)	28.2 (8.9)
S. coronis-YP	8	47.5 (10.7)	36.3 (10.0)	436 (298)	0.058 (0.003)	27.1 (17.5)
S. callippe-DL	13	12.8 (3.8)	3.6 (2.2)	231 (95)	0.092 (0.005)	21.2 (11.4)
S. callippe-AP	11	23.5 (8.3)	3.2 (2.3)	278 (160)	0.097 (0.004)	27.0 (15.6)
S. callippe-BM	34	17.3 (6.0)	3.0 (2.3)	182 (98)	0.102 (0.007)	17.0 (7.5)
S. nokomis-DG, 1974	22	11.9 (2.4)	2.1 (0.6)	156 (80)	0.103 (0.001)	19.3 (6.8)
S. nokomis-RV, 1974	9	12.7 (3.7)	2.1 (0.8)	190 (59)	0.106 (0.001)	21.5 (11.1)
S. hesperis-DP	34	17.6 (9.2)	2.7 (1.9)	230 (127)	0.087 (0.004)	16.9 (10.8)
S. hesperis-AP	6	18.3 (9.2)	6.7 (3.4)	209 (151)	0.081 (0.002)	17.7 (12.5)
S. cybele leto-KC	11	12.0 (5.7)	3.9 (1.7)	246 (129)	0.069 (0.001)	14.5 (8.8)
S. zerene-YP, 1974	8	38.6 (15.2)	25.6 (11.2)	314 (118)	0.061 (0.001)	20.9 (6.4)
S. zerene-BM, 1975	29	33.2 (7.9)	20.1 (5.4)	286 (187)	0.059 (0.001)	18.3 (8.9)
S. zerene-FS, 1975	8	9.6 (3.8)	5.3 (3.3)	184 (142)	0.050 (0.001)	8.1 (9.1)
S. egleis-YP, 1974	23	19.8 (7.5)	3.4 (3.2)	162 (98)	0.089 (0.004)	14.7 (8.9)
S. egleis-DP, 1974	7	13.6 (4.4)	2.2 (1.4)	131 (43)	0.093 (0.004)	11.9 (3.8)
S. mormonia-DP, 1974	27	10.6 (6.0)	2.8 (3.1)	94 (36)	0.079 (0.001)	7.7 (2.3)
S. mormonia-DP, 1975	19	5.4 (1.6)	2.4 (1.3)	44 (13)	0.071 (0.001)	3.7 (0.9)
S. mormonia-SP, 1974	7	8.4 (6.5)	5.3 (4.2)	69 (20)	0.074 (0.002)	5.5 (1.4)

chamber (Percival Scientific, Perry, IA). Eggs, which were laid singly, were removed from containers and counted daily and this was continued until female death. Measurements were made of the time interval between initial exposure to violet leaves and the date of first oviposition because the length of this period is an indicator of reproductive diapause in females of *S. coronis* and *S. zerene* (Sims 1984). Females of *Speyeria* other than *S. coronis* and *S. zerene* are reproductively mature (i.e. with mature oocytes) when they eclose and showed little or no behavioral delay in the initiation of oviposition within the containers.

Egg and female body weight estimation. Groups of 45 to 50, 3 to 5 d old ova were weighed on a Mettler balance (Mettler-Toledo Inc., Columbus, OH) to determine mean ova weight. There were 3 to 6 replications per species representing the ova from ≥ 10 females. Following live weight determination, eggs were dried for 72 h at 75°C in a vacuum oven and then re-weighed; average dry weight and water loss were calculated. Dry female weights were obtained using field collected females in newly emerged condition from the 1974 and 1975 seasons; females were frozen, dried for 72 h at 75°C in a vacuum oven, and then weighed. These data were supplemented with weights of fresh condition dried museum specimens with the pin weight subtracted. Total mean reproductive effort was estimated by the ratio of the mean total dry egg weight to mean dry female weight.

Relationship between reproductive effort and climate variables. Monthly temperature and precipitation data were obtained for each *Speyeria* collection site or from the nearest weather station. The collection site and associated weather station (WS) were as follows: Anthony Peak (Anthony Peak WS); Boggs Mountain (Clearlake 4 SE WS); Del Puerto Canyon (Newman WS near Patterson); Devils Gate Pass (Bridgeport WS); Donner Pass (Truckee RS WS); Kings Canyon (Carson City, NV WS); Round Valley (Bishop AP WS, Bishop Creek Intake 2 WS); Yuba Pass (Loyalton WS), Fales Hot Springs (Bridgeport WS); Sonora Pass (Coalville 13 E WS). All weather stations were located <25 km from collection locations. For each species-population the mean temperature (°C) of each month during the estimated period comprising the summer first instar diapause (ending October 1 for all species) was determined. The total number of temperature degrees (sum of the mean monthly temperatures) was used as an approximation for the magnitude of desiccation stress on diapause larvae. Precipitation, or lack of it, is another stress component for larvae and therefore the sum and mean precipitation (mm) during these months was also determined. For example, the species-population with the greatest summer diapause stress was *S. callippe*-DL with 4 months (June–September) of mean temperatures >22°C and mean precipitation of only 2.2 mm/month. In contrast, larvae from the DG and RV *S. nokomis* populations experience only two summer months (August and September) of diapause stress during which mean temperatures are 19.6 and 11.4°C and mean precipitation amounts are 18.8 and 12.7 mm, respectively.

Statistics. Because distributions deviated from normality, inter- and intra-population between-year data on female lifespan, days to first egg, total number of eggs laid, dry egg weight, total dry weight of eggs per female, and dry weight per female (Table 1) were analyzed using the Kruskal-Wallis One Way ANOVA on Ranks followed by Dunn's Test to evaluate mean differences (SAS 2001). Correlations between dry weight per female, reproductive effort, and weather parameters were determined using the Product-moment correlation coefficient. Throughout, all means are given as ±1 SD.

RESULTS

Life span, temporal pattern of oviposition, egg weight, and total egg production of *Speyeria* spp. are summarized in Table 1. *S. coronis*-YP, *S. zerene*-YP and *S. zerene*-BM populations had females with the longest mean life spans (47.5 d, 38.6 d, 33.2 d, respectively) and the longest periods prior to production of ova (36.3 d, 25.6 d, 20.2 d, respectively). These values are consistent with female reproductive diapause previously documented in *S. coronis* and *S. zerene* (Sims, 1984; James & Nunnallee, 2011). In contrast, the high altitude *S. zerene*-FS population lacked a reproductive diapause. *S. zerene*-FS also had a significantly shorter life span than *S. zerene*-YP (q=11.711, P<0.001) and *S. zerene*-BM (q=14.688, P<0.001) and had significantly fewer days to first oviposition compared to *S. zerene*-YP (q=9.933, P<0.001) and *S. zerene*-BM (q=8.749, P<0.001). Mean egg weight, total number of eggs, and total dry egg weight per female in the *S. zerene*-FS population were also significantly less (P<0.05) than in the *S. zerene*-BM and *S. zerene*-YP populations.

Among the *S. callippe* populations, the highest elevation population, CA-AP, had a significantly longer life span than CA-DL (q = 3.772, P < 0.05) and CA-BM (q = 3.214; P < 0.05). CA-AP and also produced significantly more eggs than CA-BM (q = 3.936; P < 0.05). The reasons for these differences are unknown.

Speyeria coronis produced the greatest total number of eggs (mean ± SD = 436 ± 298) but there was considerable interfemale variation. One *S. coronis*

female, for example, produced 1042 eggs. *Speyeria coronis* eggs were small (0.058 ± 0.003 mg) and this fell within the range of egg sizes from females of three *S. zerene* populations (0.50–0.061 mg) which were the smallest eggs of the species group (Table 1). The largest species, *S. nokomis*, produced the largest eggs (N-DG = 0.103 ± 0.001 mg and N-RV = 0.106 ± 0.001 mg but these eggs were only slightly larger than eggs from the *S. callippe*-BM population 0.102 ± 0.007) mg.

S. mormonia-DP females studied in 1974 and 1975 differed significantly in mean life span ($q = 4.244$, $P < 0.05$), mean number of eggs laid ($q = 5.904$, $P < 0.05$), and total dry weight of eggs ($q = 5.785$; $P < 0.05$) with 1974 females being greater in these categories (Table 1). Another high elevation population, M-SP, was not significantly different, in any category, from either the 1974 M-DP or the 1975 M-DP populations.

The reproductive effort of *Speyeria* spp. females in relation to body weight is shown in Table 2. The total dry weight of eggs produced as a percentage of mean female dry weight is defined as "relative reproductive effort" (RRE). Among the nine species, there was no correlation between female body size and either individual egg weight ($q = 0.127$, $P > 0.05$) or total weight of eggs produced ($q = 0.258$, $P > 0.05$).

In general, species associated with dry habitats, from low to high elevations had a greater RRE. An exception to this trend was *S. zerene* FS, a high altitude population lacking reproductive diapause. *S. cybele leto* and *S. nokomis*, species from mid to high elevation meadow habitats, had RRE values less than 50% those of dry habitat species such as *S. hydaspe* and *S. hesperis*.

For the nine species, including three samples of *S. mormonia* (two populations, one population with two sample years) and three *S. zerene* populations (two diapause, one non-diapause), the relationship between RRE and total number of temperature degrees accumulated during the months of first instar diapause was significant ($r = 0.660$, $N = 12$, $P = 0.0195$), Figure 1. However, the relationship between RRE and the mean monthly rainfall during the larval diapause period was not significant ($r = -0.301$, $N = 12$, $P = 0.342$).

Discussion

Life history strategies maximizing reproductive success should be selectively favored (Roff 1992, Stearns 1992, Bernardo 1996). Models of optimal reproductive effort often assume a tradeoff between progeny size and number with progeny fitness increasing with size (Roff 1992). Reproductive success will, in theory, be maximized when females, provided with a limited amount of available resources derived

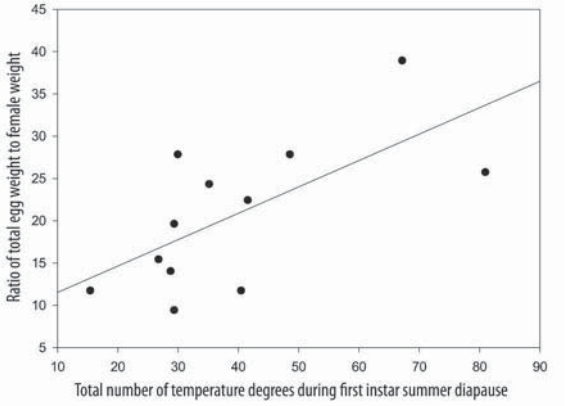


FIG. 1. Relative reproductive effort (RRE = ratio of total egg weight to female weight) of *Speyeria* species related to cumulative summer temperatures experienced by first instars ($y = 0.3121x + 8.3954$, $r^2 = 0.436$, $P = 0.0195$).

from larval feeding (e.g. fat body, proteins) and adult foraging (nectar), partition resources into progeny of optimal size and number. Timing and location of reproduction will also significantly affect reproductive success. *Speyeria* reproductive strategy components include egg size, number, and the total weight of eggs allocated in proportion to female weight (RRE). Reproductive timing appears to follow two general pathways. Females either begin to lay eggs soon after adult emergence or they delay reproduction, via reproductive diapause, until environmental conditions are more favorable for larval survival.

There was no correlation between female body weight and either individual egg weight or total weight of eggs produced. The heaviest eggs produced by the largest species, *S. nokomis*, were only slightly heavier than the intermediate size *S. callippe*; the lightest eggs were produced by two species of intermediate size, *S. coronis* and *S. zerene*. The optimal reproductive strategies of *Speyeria* appear to involve other life history adaptations, less related to egg or larval size, but strongly related to larval habitat. Adaptations of diapausing first instars for surviving heat and drought are a significant component of the reproductive strategies; larvae show significant interspecific variation in desiccation resistance and diapause strength that is positively correlated to the severity of their habitat (Sims & Shapiro 2014).

The *Speyeria* fall into three general categories of reproductive strategy and life history evolution based on the duration of larval exposure to warm temperature - low precipitation conditions. Category 1 includes species such as *S. callippe* and *S. hydaspe* inhabiting xeric habitats at low to mid elevations. Female oviposition begins soon after mating and females have a

high RRE. Larvae possess a strong diapause and are very resistant to desiccation allowing survival during long exposure to warm-dry conditions. Category 2 includes two species, *S. coronis* and *S. zerene*, inhabiting xeric habitats typically at mid-elevations. Females undergo a reproductive diapause and delay oviposition until late summer. They have an intermediate RRE but partition reproductive effort into large numbers of small eggs. Larvae are small, susceptible to desiccation, with a relatively weak diapause. Because of late season oviposition, the duration of larval exposure to desiccating conditions is relatively short. Exceptions to this category are *S. zerene* populations from coastal (McCorkle & Hammond 1988) and high elevations that lack reproductive diapause (this study). Other United States *Speyeria* species, *S. aphrodite, S. diana, S. edwardsii*, and *S. idalia* occur in relatively more mesic habitats but appear to also have a reproductive diapause. Females emerge in early summer but typically delay oviposition until late August or September (Edwards 1897, Scott, 1992, Kopper et al. 2001, Adams & Finkelstein 2006). Category 3 includes species from high elevations and wet meadows. Adult emergence occurs in mid-late summer and females begin oviposition immediately; RRE is relatively low. Larvae have a weak diapause and are very susceptible to desiccation but the duration of larval exposure to desiccating conditions is brief. One species, *S. nokomis*, has larvae that actively move toward high humidity in a gradient (Sims & Shapiro 2014).

The warm, dry summer time lapse between first instar larval emergence and cool/moist fall weather represents a major period of environmental stress, and selection, on diapause first instar *Speyeria* spp. larvae. This period can extend four months or longer for populations of species such as *S. callippe*. Documentation of larval adaptations, and species-specific differences, were presented in a previous report (Sims & Shapiro 2014). Important physiological adaptations of first instars to summer habitats include desiccation resistance and diapause strength. There is a positive relationship between larval diapause strength and desiccation resistance, but neither diapause strength nor desiccation resistance was related to larval size. This suggests that desiccation resistance and larval survival has been enhanced more by physiological adaptations than by increases in size. Karlsson & Wiklund (1985) reported similar results in a group of five Satyridae that showed no correlation between either egg weight and desiccation resistance or first instar weight and starvation resistance. Reavey (1992) found no relationship between first instar size and starvation resistance in 42 species of British Lepidoptera.

Climate, especially the severity and duration of the dry season and available moisture, has been a key selective factor in the evolution of *Speyeria* spp. reproductive strategies and larval adaptations. Among species, there is significant positive relationship between RRE and the sum of summer temperatures experienced by diapause larvae. The relationship

TABLE 2. Reproductive effort of *Speyeria* females in relation to female body weight.

Species, location(s)	n	Total dry egg weight, mg, per female, mean (SD)	Female dry body weight, mg, mean (SD)	Total dry weight of eggs as percentage of dry female weight***
S. hydaspe-AP	16	28.2 (8.9)	72.5 (14.5)	38.9
S. hesperis-DP	34	16.9 (10.6)	60.9 (8.9)	27.8
S. coronis-YP	8	27.1 (17.5)	97.5 (12.7)	27.8
S. callippe-DL, AP, BM	58	19.9 (10.8)	77.3 (16.8)	25.7
S. egleis-YP, DP	30	14.1 (8.0)	58.1 (8.5)	24.3
S. zerene-YP, BM	37	18.9 (8.1)	84.4 (18.2)	22.4
S. zerene-FS	8	12.4 (9.1)*	80.3 (20.6)	15.4
S. mormonia-DP, 1974	27	7.7 (2.3)	39.3 (8.1)	19.6
S. mormonia-DP, 1975	19	3.7 (0.9)**	39.3 (8.1)	9.4
S. mormonia-SP, 1974	7	5.5 (1.4)	38.1 (13.1)	13.3
S. cybele leto-KC	11	14.5 (8.8)	123.7 (8.3)	11.7
S. nokomis-DG, RV	31	20.0 (8.1)	171.1 (22.0)	11.7

*Significantly different ($p<0.05$) from *S. zerene*-YP, BM

**Significantly different ($p<0.05$) from *S. mormonia*-DP, 1974

***Relative reproductive effort (RRE)

between RRE and mean monthly precipitation during the summer periods was not significant, but summer precipitation in many habitats may not have predictable impacts on diapause larvae. Larvae can imbibe water (Sims & Shapiro 2014) but episodes of brief or light precipitation might not reach the diapause larval habitat and thus would not be available for drinking. Species inhabiting dry habitats, from low to middle elevations, had higher RRE than wet meadow and alpine species. Intraspecific differences in reproductive strategies can be significant within *Speyeria* spp. For example, *S. zerene* populations from cool coastal habitats in California and Oregon emerge late in the summer season and probably lack a reproductive diapause (McCorkle & Hammond 1988). Similarly, high altitude *S. zerene* populations (*S. zerene* malcolmi) lack a female reproductive diapause. In contrast, mid-elevation *S. zerene* populations all show a pronounced reproductive diapause. In mid-elevation populations, reproduction is delayed until late in the season when environmental stress on diapause larvae is reduced. This is especially important with *S. zerene* which, along with *S. coronis*, has the smallest eggs and larvae of the species studied.

Boggs (1987) evaluated the population dynamics of Colorado *S. mormonia* over a 4-year period and found between-year variation in several demographic factors. Between-year variation in *S. mormonia* reproduction is documented for the first time in this report. In California *S. mormonia*, between-year variation in life span, total numbers and weight of eggs, and RRE can be significant as demonstrated by the differences between 1974 and 1975 values for the Donner Pass population. Interpopulation differences in fecundity and resource allocation have been documented in Lepidoptera and other insects (Parry et al. 2001, Franzén et al. 2013), but the reasons for between-year *S. mormonia* differences are unclear. They may be related to a severe drought in California that started in 1975 and its effects on the quality and quantity of larval host plants. Alternatively, body size and/or reproductive capacity in insects can vary over the course of a growth season with late season emergers sometimes being smaller and less fecund (Palmer 1984, Corkum et al. 1997). *S. mormonia* female samples from Donner Pass in 1974 were made on Aug. 12 (19), Aug. 20 (5), and Sept. 13 (3) while the 1975 collection was made on Aug. 24 (19). The 1974 samples predominantly represent early emergers whose size/fecundity may have been enhanced by cooler temperatures experienced during larval and pupal development (Atkinson 1994). This level of between-year variation in *S. mormonia* indicates that future studies on *Speyeria* reproductive strategies should include two or more years of data with samples taken throughout the entire emergence period.

Female weight and individual egg weight were uncorrelated and there was no relationship between female weight and either total number or total weight of eggs produced. However, the weights of eggs produced over the lifespan of individual females can vary and generally decline with female age. As females of *S. mormonia*, *S. nokomis* and *S. zerene* age, the weights of eggs they produce declines and the resulting larvae have significantly reduced diapause intensity and probably reduced resistance to desiccation (Sims & Shapiro 2014). The eggs produced at the end of female reproduction and end of the flight season produce larvae that will experience reduced exposure to desiccation stress. Smaller late season larvae might therefore be somewhat buffered from stress-related mortality. A reduction in egg weight with female age has been documented in Colorado *S. mormonia* and in other Lepidoptera (Boggs 1986, Karlsson 1987).

ACKNOWLEDGEMENTS

We thank A. G. Appel, P. C. Hammond, and S. O. Mattoon for comments on the manuscript. In addition, two reviewers made suggestions that improved the text. This research was supported by California Agricultural Experiment Station Project CA-D*-AZO-3994-H, "Climatic Range Limitation of Phytophagous Lepidopterans", AMS, Principal Investigator.

LITERATURE CITED

ADAMS, J. K. & I. L. FINKELSTEIN. 2006. Late season observations on female *Diana* fritillary aggregating behavior. News Lepid. Soc.48:106–107.

ATKINSON, D. 1994. Temperature and organism size—a biological law for ectotherms? Adv. Ecol. Res. 25:1-58.

BERNARDO, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Amer. Zool. 36:216-236.

BOGGS, C. L. 1986. Reproductive strategies of female butterflies: variation in and constraints on fecundity. Ecol. Entomol.:7-15.

BOGGS, C. L. 1987. Within population variation in the demography of *Speyeria mormonia* (Lepidoptera: Nymphalidae). Holarctic Ecol. 10:175-184.

BOGGS, C. L. 1994. The role of resource allocation in understanding reproductive patterns. Pp. 25-33, *In* Leather, S. R., A. D. Watt, N. I. Mills, & K. E. A. Walters (eds.). Individuals, Populations, and Patterns in Ecology, Intercept Press, Andover, Hampshire, UK.

BRABY, M. F. 1994. The significance of egg size variation in butterflies in relation to hostplant quality. Oikos 71:119-129.

BRITTNACHER, J. G., S. R. SIMS & F. J. AYALA. 1978. Genetic differentiation between species of the genus *Speyeria* (Lepidoptera: Nymphalidae). Evolution 32:199-210.

CORKUM, L. D., J. J. H. CIBOROWSKI & R. G. POULIN. 1997. Effects of emergence date and maternal size on egg development and sizes of eggs and first-instar nymphs of a semelparous aquatic insect. Oecologia 111:69-75.

CUMMINS, C. P. 1986. Temporal and spatial variation in egg size and fecundity in *Rana temporaria*. J. An. Ecol.55:303-316.

EDWARDS, W. H. 1868-1897. The Butterflies of North America. Publ by author. Philadelphia, Boston, & New York, 3 vol.

FOX, C. W. & M. E. CZESAK. 2000. Evolutionary ecology of progeny size in arthropods. Ann. Rev. Entomol. 45:341-369.

FRANZÉN, M. S., G. NILSSON, V. JOHANSSON, & T. RANIUS. 2013.

Population fluctuations and synchrony of grassland butterflies in relation to species traits. PLoS ONE 8: e78233.

HEATH, D. D., C. W. FOX & J. W. HEATH. 1999. Maternal effects on offspring size: Variation through early development of Chinook Salmon. Evolution 53:1605-1611.

JAMES, D. G. & D. NUNNALLEE. 2011. Life Histories of Cascadia Butterflies. Oregon State University Press, Corvallis, OR, 447 pp.

KARLSSON, B. 1987. Variation in egg rate, oviposition rate and reproductive reserves with female age in a natural population of the speckled wood butterfly, *Pararge aegeria*. Ecol. Entomol. 12:473-476.

KARLSSON, B. & C. WIKLUND. 1985. Egg weight variation in relation to egg mortality and starvation endurance of newly hatched larvae in some satyrid butterflies. Ecol. Entomol. 10:205–211.

KEMP, D. J. 2006. Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. Evol. Ecol. Res. 8:515–527.

KOPPER, B. J., S. SHU, R. E. CHARLTON, & S. B. RAMASWAMY. 2001. Evidence for reproductive diapause in the fritillary *Speyeria idalia* (Lepidoptera: Nymphalidae). Ann. Entomol. Soc. Am. 94:427-432.

MCCORKLE, D. V. & P. C. HAMMOND. 1988. Biology of *Speyeria zerene hippolyta* (Nymphalidae) in a marine modified environment. J. Lepid. Soc. 42:184-195.

PALMER, J. O. 1984. Environmental determinants of seasonal body size variation in the milkweed leaf beetle, *Labidomera clivicollis* (Kirby) (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Amer. 77:188-192.

PARRY, D., R. A. GOYER & G. J. LENHARD. 2001. Macrogeographic clines in fecundity, reproductive allocation, and offspring size of the forest tent caterpillar *Malacosoma disstria*. Ecol.Entomol. 26:281-291.

REAVEY, D. 1992. Egg size, first instar behaviour and the ecology of Lepidoptera. J. Zool. 227:277–297.

ROFF, D. A. 1992. Evolution of Life Histories: Theory and Analysis. Springer, New York, NY, 535 pp.

SAS INSTITUTE. 2001. JMP v. 4., SAS Institute, Cary, NC.

SCOTT, J. A. 1992. Hostplant records for butterflies and skippers (mostly from Colorado), 1959–1992, with new life histories and notes on oviposition, immatures, and ecology. Papilio (New Series) #6: 1-185.

SEKO, T. & F. NAKASUJI. 2004. Effect of egg size variation on survival rate, development and fecundity of offspring in a migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperiidae). Appl. Entomol. Zool. 39:171–176.

SIMS, S. R. 1979. Aspects of mating frequency and reproductive maturity in *Papilio zelicaon*. Amer. Midland Nat. 102:36-50.

SIMS, S. R. 1984. Reproductive diapause in *Speyeria* (Lepidoptera: Nymphalidae). J. Res. Lepid. 23:211-216.

SIMS, S. R. & A. M. SHAPIRO. 2014. Interspecific variation in size, diapause intensity, and moisture responses of first-instar *Speyeria* (Lepidoptera: Nymphalidae) larvae. Ann. Entomol. Soc. Amer. 107:163-169.

SINERVO, B. & P. LICHT. 1991. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. Science, 252:1300-1302.

SMITH, C. C. & S. D. FRETWELL. 1974. The optimal balance between size and number of offspring. Amer. Nat. 108:499-506.

STEARNS, S. 1992. The Evolution of Life Histories. Oxford University Press, Oxford, U.K. 249 pp.

TORRES-VILA, L.M. & M. C. RODRIGUEZ-MOLINA. 2002. Egg size variation and its relationship with larval performance in the Lepidoptera: the case of the European grapevine moth *Lobesia botrana*. Oikos 99:272-283.

ZALUCKI, M. P., A. R. CLARKE & S. B. MALCOLM. 2002. Ecology and behavior of first instar larval Lepidoptera. Ann. Rev. Entomol. 47:361–93.

Submitted for publication 22 June 2015; revised and accepted 6 September 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 121–129

COMPARATIVE REPRODUCTIVE PERFORMANCE AND DIGESTIVE ENZYMATIC ACTIVITY OF *HELICOVERPA ARMIGERA* (NOCTUIDAE) ON SEVEN BEAN CULTIVARS

ZAHRA GOLPARVAR AND BAHRAM NASERI*

Department of Plant Protection, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil, Iran.
Corresponding author: email: bnaseri@uma.ac.ir

ABSTRACT. The cotton bollworm, *Helicoverpa armigera* (Hübner), is one of the most important pests of many host crops in Iran and worldwide. The effect of different cultivars of bean (*Phaseolus vulgaris* L.) including white kidney bean (cultivars Pak, Daneshkadeh and Shokufa), common bean (cultivar Talash) and red kidney bean (cultivars Akhtar, Sayyad and Naz) was studied on the reproductive parameters of *H. armigera* under laboratory conditions (25±1 °C, 65 ± 5% RH, and photoperiod of 16: 8 (L: D) h) and on the activity of some digestive enzymes of the larvae under field conditions. The highest rates of gross fecundity and gross fertility were on red kidney bean Naz, and the lowest values of these parameters were on white kidney bean Pak and red kidney bean Akhtar, respectively. The highest rates of net fecundity and net fertility were observed on common bean Talash, whereas the lowest values of these parameters were seen on red kidney bean Akhtar. The lowest proteolytic activity of fourth and fifth instar larvae of *H. armigera* was respectively on the leaf of red kidney bean Akhtar and red kidney bean Naz. Among the pods of different bean cultivars, proteolytic activity of fourth and fifth instar larvae was the lowest when they were fed on the green pod of red kidney bean Akhtar. The lowest amylolytic activity of fourth and fifth instar larvae of *H. armigera* was on the leaf of red kidney bean Naz. The fourth and fifth instar larvae reared on the green pod of common bean Talash showed the lowest amylolytic activity. The results indicated that red kidney bean Akhtar was an unsuitable host for *H. armigera* feeding. The findings of this study could be used in designing new strategies to control *H. armigera*.

Additional key words: *Helicoverpa armigera*, reproductive performance, digestive enzyme, bean

Among the agricultural plants, beans (*Phaseolus vulgaris* L.) are important legumes because they have a high percentage of protein, have the capability to fix nitrogen and are rich in mineral nutrients as compared to other agricultural crops (Daliry et al. 2010). The gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the polyphagous and voracious pests of agricultural crops—especially beans, in Iran (Farid, 1986) and many countries of the world (Zalucki et al. 1994, Singh and Mullick 1997, Reddy et al. 2004). The larvae of *H. armigera* attack most plant structures (stems, leaves, flower, heads, and fruits) and create serious economic losses in cultivated crops including cotton, bean, corn, tobacco, and tomato (Liu et al. 2004). Polyphagy, high mobility, high reproductive potential as well as facultative diapause are important traits that make *H. armigera* as an important pest of different host crops (Raheja 1996). Since the application of chemical insecticides has increased the risk of environmental contamination and development of insecticide resistance in *H. armigera* populations (McCaffery 1998, Naseri et al. 2009), several studies have been done to identify control measures that are environmentally-safe and economically acceptable.

For polyphagous insects, the availability of different host plants plays a central role in population outbreaks (Singh and Parithar 1988). Different nutritional values of host plants can influence growth, development and population dynamics of *H. armigera* (van Steenis and El-khawass, 1995; Du et al. 2004). Some proteins in seeds and vegetative organs of host plants may affect the key gut digestive enzymes of insects including amylases and proteinases (Biggs and McGregor 1996). Because the abundance and activity of α-amylases (α-1, 4-glucan-4-glucanohydrolases, EC 3.2.1.1) of the insects are dependent upon food sources, many lepidopteran insects living on a polysaccharide rich diet require digestive α-amylase for starch digestion (Valencia-Jiménez et al. 2008). The ability to synthesize particular digestive enzymes in herbivorous insects can result in successful development and reproductive potential (Ishaaya et al. 1971). Plants with protein inhibitor mechanisms decrease not only insect fecundity and fertility but also are effective on digestive enzymatic activity. Inhibition of an insect's digestive enzyme activity by enzyme-inhibitors of a host plant can result in poor nutrient utilization and developmental retardation (Jongsma and Bolter 1997, Gatehouse and Gatehouse 1999).

Host plant resistance among crop plants is a major part of an integrated pest management program (IPM). Developing cultivars that are resistant to *H. armigera* would supply an effective complementary approach in IPM to reduce the extent of losses caused by this pest (Jallow et al. 2004). Plants with an antibiosis mechanism may decrease directly fecundity of insect pests (Sarfraz et al. 2006). Knowledge about some properties of digestive enzymes is critical to physiologically-based control methods (Fathipour and Naseri 2011). Thus, studying the insects' digestive system is an important tool to find a new control techniques in IPM programs (Lawerence and Koundal 2002). So, we have focused on

two key digestive enzymes (protease and amylase) of *H. armigera* and fecundity in response to feeding on different bean cultivars.

Because the bean cultivars used in this study have different nutritional values for *H. armigera* larvae (Namin et al. 2014), it was hypothesized that the adult females emerging from larvae fed on some bean cultivars will have higher reproductive potential than those reared on any other cultivar. Furthermore, because there were significant differences in protein and starch contents among tested bean cultivars (Namin et al. 2014), we hypothesized that *H. armigera* larvae feeding on bean cultivars poor in protein and starch contents would show lower levels of digestive enzymatic activity that can affect reproductive performance of this insect.

Hitherto, several studies have been done on the effect of different host plants on reproductive performance (Liu et al. 2004, Fathipour and Naseri, 2011, Naseri et al. 2011, Hemati et al. 2013) and digestive enzymes activity of *H. armigera* (Kotkar et al. 2009, Hemati et al. 2012, Namin et al. 2014, Nemati Kalkhoran et al. 2014). However, no published articles are available regarding the effect of different bean cultivars on reproductive performance, as well as on the digestive enzymatic activity (amylases and proteases) of this species under field conditions. Because the insects are ectothermic animals, developmental and physiological processes of them can be affected by environment temperature (Johnson et al. 1992, Na and Ryoo 2000). Our objective here was to compare the effect of different bean cultivars on reproductive parameters (under laboratory conditions) and some digestive enzymatic activity (under field conditions) in *H. armigera* reared on bean cultivars to develop pest management programs for beans in Iran. We hope that the results of this research along with the findings of previous research on life table parameters (Naseri et al. 2014) and nutritional indices (Namin et al. 2014) of *H. armigera* on different bean cultivars would provide useful information to develop a comprehensive pest management program of the pest on beans.

MATERIALS AND METHODS

Plant. Different bean (*P. vulgaris*) cultivars including white kidney bean (WKB) (cultivars Pak, Daneshkadeh and Shokufa), common bean (CB) (cultivar Talash) and red kidney bean (RKB) (cultivars Akhtar, Sayyad and Naz) were acquired from the Seed and Plant Improvement Institute (Khomein, Iran) and were cultivated in the research farm of the University of Mohaghegh Ardabili (Ardabil, Iran) in May 2012. Selected bean cultivars in this research are commercially cultivated legumes in Iran (Namin et al. 2014). The protein content of these cultivars ranged from 0.857 mg mL^{-1} in WKB cultivar Daneshkadeh to 0.737 mg mL^{-1} in CB cultivar Talash. Also, the starch content differed from 2.244 mg mL^{-1} in RKB cultivar Akhtar to 0.438 mg mL^{-1} in WKB cultivar Pak (Namin et al. 2014).

The experiments were started when bean cultivars reached reproductive stage. For this research, the young leaves and green terminal pods (all of the equal size) of bean cultivars were transferred to a growth chamber at 25 ± 1 °C, 65 ± 5% RH, with a photoperiod of 16: 8h (L: D). The leaves of different bean cultivars were used for feeding of first and second *H. armigera* larval instars and the green pods were used for feeding of the third to fifth *H. armigera* larval instars (Green et al. 2002, Naseri et al. 2009, Namin et al. 2014).

Laboratory rearing of *H. armigera*. *H. armigera* eggs used in this research were obtained from a laboratory colony maintained on an artificial diet, as described by Shorey and Hale (1965), from Tarbiat Modares University (Tehran, Iran) and transferred to a growth chamber at 25 ± 1°C, 65 ± 5% RH and a photoperiod of 16:8 h(L:D). Before starting the experiments, *H. armigera* was reared for two generations on different bean cultivars, and data were collected from third generation.

Reproductive parameters. To study the reproductive parameters of *H. armigera* on different bean cultivars, fifty eggs (within 12 h) were collected from adults that were reared on each cultivar and then used for each experiment. After hatching, first instar larvae were transferred individually into plastic Petri dishes (diameter 8 cm, depth 2 cm), containing the detached fresh leaves of each tested cultivar. To ventilate, a hole (diameter 1 cm) covered by a mesh net was made in the Petri dishes. A piece of water-soaked cotton was wrapped around the petioles of detached leaves and green pods to prevent desiccation. The leaves and green pods were changed daily until prepupation. The larvae in each Petri dish were checked daily for the mortality or ecdysis. Prepupae and pupae were kept inside small plastic tubes (diameter 2 cm, depth 5 cm). Duration of pre-pupal and pupal stages and their mortality were also recorded daily.

After emergence of adult moths, a pair of female and male moths were transferred into oviposition containers (diameter 11.5 cm, depth 9.5 cm), which was closed at the top with a fine mesh net for aeration. The internal walls of oviposition containers were covered with the same mesh net as an oviposition substrate. A small cotton wick soaked in 10% honey solution was inserted in each oviposition container for the adult's feeding.

The oviposition containers were checked daily for adult's mortality and number of deposited eggs. The reproductive parameters of *H. armigera* were calculated as follows (Carey 1993):

Gross fecundity rate= $\sum_{x=\alpha}^{\beta} M_x$

Gross fertility rate= $\sum_{x=\alpha}^{\beta} h_x M_x$

Gross hatch rate= $\frac{\sum_{x=\alpha}^{\beta} h_x M_x}{\sum_{x=\alpha}^{\beta} M_x}$

Net fecundity rate= $\sum_{x=\alpha}^{\beta} L_x M_x$

Net fertility rate= $\sum_{x=\alpha}^{\beta} L_x h_x M_x$

where, L_x is the days lived in interval x and x+1, M_x is the average number of offsprings produced by females at age x, and h_x is the hatching rate; a is the age of female at the first oviposition and $^{\beta}$ is the age of female at the last oviposition.

Fitness index. Fitness index (*FI*) of *H. armigera* was calculated on seven tested bean cultivars using the following formula (Itoyama et al. 1999):

$$FI = (P \times P_w)/(L + P_d)$$

where, *P*= percentage of pupation, *Pw*= pupal weight (gr), *L*= larval period (day) and *Pd*= pupal period (day).

Digestive enzymes activity

Chemicals. Azocasein, Bradford reagent, dinitrosalicylic acid (DNS), maltose and starch were obtained from Sigma chemical Co., St Louis, USA, (Sigma, www.sigmaaladrich.com). Bovine serum albumin (BSA) was purchased from Roche Co., Germany, (Roche, www.roche.com).

Preparation of digestive enzymes. Fifty neonate larvae were reared on the leaves and green pods of each bean cultivar until fourth and fifth instars according to the method mentioned at "Laboratory Rearing" section. For assessing the digestive amylolytic and proteolytic activities, the fourth and fifth instar larvae of *H. armigera* were transferred to the research field of the University of Mohaghegh Ardabili (Ardabil, Iran) in August 2012, and they were reared on the leaves and green pods of each related bean cultivar for 24 h. These larvae were collected from the field after 24 h feeding, immediately anesthetized on ice and dissected under a stereoscopic microscope. The midguts were washed in pre-cooled distilled water, cleaned by removal of unwanted tissues; they were then collected into a known volume of distilled water. The homogenates were centrifuged at 16000 × g for 10 min at 4 °C and the resulting supernatants were collected in new micro tubes and stored at -20°C in aliquots for further use (Hosseininaveh et al. 2007).

Proteolytic activity assay. General proteolytic activity in the midgut of fourth and fifth instar *H. armigera* larvae fed on the leaves and green pods of the tested bean cultivars (for 24 h feeding) was assayed using azocasein as a substrate at the optimal pH (Elpidina et al. 2001). To evaluate the azocaseinolytic activity, the reaction mixture containing 80 µL of 1.5% azocasein solution in 50 mM universal buffer (pH 12) and 50 µL of crude enzyme was incubated at 37 °C for 50 min. Proteolysis was finished by the addition of 100 µL 30% trichloroacetic acid (TCA), and continued with cooling at 4 °C for 30 min and centrifugation at 16000 × g for 10 min. An equal quantity of 2 M NaOH was added to the supernatant, and the absorbance was read at 440 nm (using ELIZA-Reader, Anthos 2020, England). Appropriate blanks, to which TCA had been added prior to the substrate, were prepared for each treatment. Unit activity was represented as an increase in optical density per mg protein of the tissue per min due to azocasein proteolysis. All experiments were carried out in triplicate.

Amylolytic activity assay. Digestive amylolytic activity in crude homogenates of midgut extracts from *H. armigera* fourth and fifth instar larvae fed on bean cultivars was determined using the dinitrosalicylic acid (DNS) method, with 1% soluble starch as a substrate at the optimal pH (Bernfeld 1955). A quantity of 50 µL of the midgut enzyme extracts was incubated with 250 µL of universal buffer (pH 9) and 20 µL of soluble starch for 30 min at 37 °C. The reaction was stopped by the addition of 100 µL DNS and heating the tubes in boiling water bath for 10 min. The absorbance was read at 540 nm using spectrophotometer (JENWAY 6705 UV/Vis, USA) after cooling on ice. One unit activity was characterized as the amount of enzyme required to produce 1 mg of maltose in 30 min at 37 °C under the given assay conditions. All experiments were carried out in triplicate.

Protein quantification. Protein concentrations were assayed by the method of Bradford (1976) using BSA as a standard.

Statistical analysis. Reproductive parameters and digestive enzymes activity of *H. armigera* on seven bean cultivars were analyzed with one-way ANOVA using the statistical software Minitab 16 (Minitab, State College, PA, USA). Statistical differences among the means were evaluated using the Tukey test at $\alpha = 0.05$. All data were tested for normality before analysis. A dendrogram of different bean cultivars based on the reproductive parameters and digestive enzymatic activity in fourth and fifth instar larvae of *H. armigera* reared on these cultivars was constructed after cluster analysis by Ward's method (Ward 1963) using SPSS 16.0 statistical software (SPSS, Chicago, IL, USA).

RESULTS

Reproductive parameters. The results of the effect of different bean cultivars on reproductive parameters of *H. armigera* are given in Table 1. According to the results, the highest rate of gross fecundity ($F = 2.48$, df = 6, 66, $P<0.01$) was on RKB cultivar Naz, whereas the lowest value of this parameter was on WKB cultivar Pak. Among different bean cultivars, the gross fertility rate ($F = 1197.60$, df = 6, 66, $P<0.01$) was the highest on RKB cultivar Naz. The gross hatch rate of *H. armigera* on CB cultivar Talash was higher than other tested cultivars. The highest and lowest rates of the net fecundity ($F = 7.47$, df = 6, 71, $P<0.01$) and the net fertility ($F = 7.32$, df = 6, 71, $P<0.01$) were on CB cultivar Talash and RKB cultivar Akhtar, respectively.

Fitness index. Different bean cultivars as larval food had a significant effect ($F = 26.87$; df = 6, 154; $P<0.01$) on fitness index of *H. armigera*, which was the highest on CB cultivar Talash, and lowest on RKB cultivar Akhtar (Fig. 1).

Proteolytic activity. The general proteolytic activity in midgut extracts from *H. armigera* fourth and fifth instar larvae fed on the leaves and green pods of different bean cultivars under field conditions is indicated in Tables 2 and 3. The highest proteolytic activity of the fourth instar *H. armigera* was in the larvae fed on leaves ($F = 5.50$; df = 6, 7; $P<0.05$) of WKB cultivar Pak and green pods ($F = 29.19$; df = 6, 7; $P<0.05$) of RKB cultivar Naz and WKB cultivar Daneshkadeh. Also, the lowest proteolytic activity of the fourth instar was observed when larvae were fed on the leaves of RKB cultivar Akhtar, and green pods of WKB cultivars shokufa and Pak, RKB cultivars Akhtar and Sayyad, and CB cultivar Talash. Proteolytic activity of the fifth instar *H. armigera* was the highest when larvae were fed on the leaves ($F = 5.05$; df = 6, 7; $P<0.05$) and green pods ($F = 6.53$; df = 6, 7; $P<0.05$) of WKB cultivar Pak.

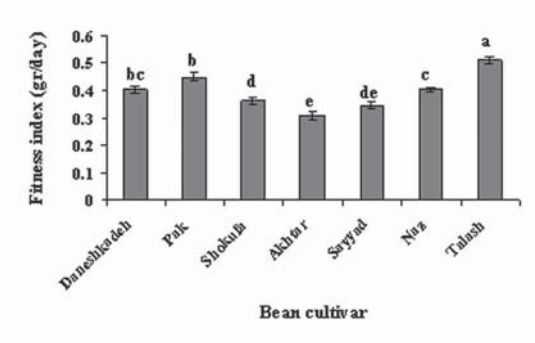


FIG. 1. Mean (±SE) fitness index of *Helicoverpa armigera* fed on different bean cultivars under laboratory conditions. The means followed by different letters are significantly different (Tukey, $P<0.01$)

Amylolytic activity. Tables 2 and 3 indicates amylolytic activity in midgut extracts from *H. armigera* fourth and fifth instar larvae reared on the leaves and green pods of different bean cultivars under field conditions. Regarding the results of this study, amylolytic activity of fourth instar larvae of *H. armigera* was influenced by feeding on the leaves ($F = 3.69$; df = 6, 7; $P<0.05$) and green pods ($F = 25.83$; df = 6, 7; $P<0.05$) of different bean cultivars. The fourth instar larvae fed on the leaves of WKB cultivar Shokufa showed the highest levels of amylolytic activity, whereas the lowest activity was in the larvae reared on the leaves of RKB cultivar Naz. Although the highest amylolytic activity was observed in the fourth instar larvae fed on green pods of WKB cultivar Daneshkadeh, the lowest activity was in the larvae fed on green pods of CB cultivar Talash. The highest amylolytic activity of *H. armigera* fifth instar fed on the leaves ($F = 18.94$; df = 6, 7; $P<0.05$) of different bean cultivars was on WKB cultivar Pak , which was not significantly different from WKB cultivars Shokufa and Daneshkadeh, and CB cultivar Talash. However, the lowest activity was in the larvae reared on RKB cultivar Naz. The fifth instar larvae of *H. armigera* fed on the green pods ($F = 16.18$; df = 6, 7; $P<0.05$) of RKB cultivars Sayyad, Naz and Akhtar showed the highest levels of amylolytic activity. However, amylase activity was the lowest in midgut extracts from fifth instar larvae fed on the green pods of WKB cultivar Pak and CB cultivar Talash.

Cluster analysis. A dendrogram according to the reproductive parameters, proteolytic and amylolytic activities in fourth and fifth instar larvae of *H. armigera* reared on seven bean cultivars is shown in Figure 2. The dendrogram shows two clusters labelled A (including subclusters A1 and A2) and B. Different cultivars of bean were grouped within each cluster according to the comparison of reproductive parameters and digestive enzymatic activity of the pest on these cultivars. Cluster A included subclusters A1 (WKB cultivars Pak, Shokufa, and Daneshkadeh, CB cultivar Talash, and RKB cultivar

TABLE 1. Mean (±SE) reproductive parameters of *Helicoverpa armigera* fed on different bean cultivars under laboratory conditions

Host (cultivar)	Parameter				
	Gross fecundity rate (eggs female-1)	Gross fertility rate (eggs female-1)	Gross hatch rate (%)	Net fecundity rate (eggs female-1)	Net fertility rate (eggs female-1)
white kidney bean (Daneshkadeh)	954.5±179ab	372.3±69.8b	39	353.9±91.9ab	138.0±35.8b
white kidney bean (Pak)	697.5±191b	308.1±83.6b	44	392.8±94.9ab	172.8±41.8ab
white kidney bean (Shokufa)	719.3±117b	309.3±50.3b	43	440.0±62.1ab	189.2±26.7ab
red kidney bean (Akhtar)	894.2±254ab	214.6±60.9b	24	173.5±111.5b	41.6±26.5b
red kidney bean (Sayyad)	1208.4±88ab	338.3±24.8b	28	480.9±76.2ab	134.7±21.3b
red kidney bean (Naz)	1505.0±204a	878.1±53.5a	52	297.3±112.5ab	154.6±58.4ab
common bean (Talash)	744.8±153ab	394.7±80.9b	53	609.5±95.8a	323.0±50.8a

The means followed by different letters in the same column are significantly different ($P<0.01$, Tukey).

Sayyad) and A2 (RKB cultivar Akhtar), and cluster B included RKB cultivar Naz.

DISCUSSION

The results of this study show that various bean cultivars have significant effects on the reproductive performance and digestive physiology of *H. armigera*. The females of *H. armigera* reared on RKB cultivar Akhtar had lower rates of gross fertility, net fecundity and net fertility than the other tested cultivars. Naseri et al. (2014) have previously demonstrated that *H. armigera* reared on RKB cultivar Akhtar showed the lowest values of r_m and R_0, suggesting that this cultivar was less suitable to this pest compared with the others.

Among different bean cultivars, we observed the highest gross fecundity rate of *H. armigera* on RKB cultivar Naz probably due to high nutrient values. This rate approximately was 2-fold lower than that reported by Hemati et al. (2013) for *H. armigera* on chickpea Arman (2947.8 eggs female^{-1}). This variation indicates that RKB cultivar Naz has lower nutrient values than chickpea Arman. The gross fertility rate of *H. armigera* ranged from 878.1 to 214.6 eggs female^{-1} on RKB cultivars Naz and Akhtar, respectively. According to the results of Naseri et al. (2011), the lowest gross fertility rate of *H. armigera* on different soybean cultivars was on Gorgan3 (149 eggs female^{-1}), which was in disagreement with our results, suggesting that the quantity and/or the quality of nutrients in bean are more suitable than those in soybean as a food for *H. armigera* larvae. Among various bean cultivars tested in the current study, the net fecundity and net fertility rates of *H. armigera* on RKB cultivar Akhtar were lower than those reported by Hemati et al. (2013) for *H. armigera* on common bean Khomein (780.4 eggs and 694.5 8 eggs female^{-1}, respectively). Some possible reasons for such disagreement may be due to the genetic differences as a result of laboratory rearing or variations in geographic populations of the pest.

The lowest value of fitness index of *H. armigera* on RKB cultivar Akhtar showed nearly 2-fold lower than that reported for fitness index of this pest on corn hybrid SC700 (0.69 gr/day) (Arghand et al. 2014). Some probable reasons for these variations are due to the physiological differences depending on the type of the host plant and genetic differences in geographic populations of the pest. In this study, we examined the effects of different bean cultivars on physiological responses at the level of activity of two key digestive enzymes (protease and α-amylase) in *H. armigera* fourth and fifth instar larvae as well. The activity of digestive enzymes, including proteases and α-amylases, depends on the nature of food or ingested chemical compounds and enzyme-inhibitors (Mendiola-Olayaet

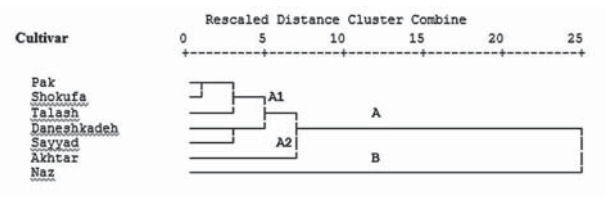


FIG. 2. Dendrogram of different bean cultivars based on reproductive parameters and digestive enzymatic activity in fourth and fifth instar larvae of *Helicoverpa armigera* reared on these cultivars (Ward's method).

TABLE 2. Mean (±SE) proteolytic and amylolytic activity of midgut extracts from *Helicoverpa armigera* fourth instar larvae fed on leaves and pods of different bean cultivars under filed conditions

	Proteolytic activity of fourth instar (U/mg)		Amylolytic activity of fourth instar (mU/mg)	
Host (cultivar)	Reared on leaves	Reared on pods	Reared on leaves	Reared on pods
white kidney bean (Daneshkadeh)	0.244±0.021 ab	3.987±0.253 a	0.466±0.050 b	1.521±0.068 a
white kidney bean (Pak)	0.585±0.137 a	2.090±0.103 b	0.651±0.184 ab	1.114±0.044 ab
white kidney bean (Shokufa)	0.498±0.005 ab	1.653±0.184b	0.868±0.032 a	0.735±0.093 bc
red kidney bean (Akhtar)	0.104±0.005b	1.612±0.033 b	0.495±0.092 ab	1.050±0.076 b
red kidney bean (Sayyad)	0.171±0.135 ab	1.613±0161 b	0.593±0.141 ab	0.555±0.039 c
red kidney bean (Naz)	0.458±0.063 ab	3.641±0.308a	0.205±0.029 b	0.714±0.132 bc
common bean (Talash)	0.271±0.017 ab	2.417±0.084 b	0.630±0.109 ab	0.326±0.054 c

The means followed by different letters in the same column are significantly different ($P<0.05$, Tukey).

et al. 2000). Protein as the primary component of the insect diets is digested into amino acids by proteases. Also, the complex polysaccharides are broke down into simple sugars by amylases. The synthesis of particular enzymes in herbivorous insects ensure appropriate quality and quantity of the reproductive success (Ishaaya et al. 1971); therefore, any interfering in normal food digestion and absorption by enzyme-inhibitors of a host plant can result in population growth retardation, especially intrinsic rate of increase (Jongsma and Bolter 1997, Gatehouse and Gatehouse 1999). Accordingly, study of life table parameters, digestive enzyme activity and reproductive performance of *H. armigera* can play an important role to recognize anti-feeding compounds and using them for designing comprehensive pest management strategies against this pest.

Among seven bean cultivars, the highest proteolytic activity was observed in fourth and fifth instar larvae of *H. armigera* on the leaves of WKB cultivar Pak. However, lower proteolytic activity was in fourth instar larvae of *H. armigera* on the leaves of RKB cultivar Akhtar. Moreover, the fifth instar larvae reared on the green pods of WKB cultivar Pak had the highest proteolytic activity, whereas a low proteolytic activity of this instar was on the green pods of RKB cultivar Akhtar. The results of life table parameters of *H. armigera* reared on different bean cultivars indicated that the highest intrinsic rate of increase was on WKB cultivar Pak and CB cultivar Talash, and the lowest one was on RKB cultivar Akhtar (Naseri et al. 2014).

By combining the results from Naseri et al. (2014) and the findings of the current research it could be suggested that RKB cultivar Akhtar is an unsuitable cultivar against *H. armigera*. The resistance of this cultivar may be due to the absence of primary nutrients necessary for the development of *H. armigera* larvae or the presence of some secondary biochemicals (Naseri et al. 2014). However, according to Namin et al. (2004), various bean cultivars had significant effects on the nutritional performance of *H. armigera*. They suggested that higher protease and amylase activities in *H. armigera* larvae fed on some bean cultivars might be due to the differences in protein and starch contents of the diet. Therefore, since *H. armigera* larvae had the complexity of the digestive enzymes (Brito et al. 2001, Kotkar et al. 2009), their different ability to access these macronutrients via digestive enzymes or nutritional imbalances between protein and carbohydrate in the tested cultivars could explain the differences in the digestive enzymatic activity of this pest on various bean cultivars. Nutrient balance, especially P/C ratio, has been reported as an important factor for growth and development of many insect pests (Lee et al. 2002, Bede et al. 2007).

The results of this study showed that the highest proteolytic activity was in fifth instar larvae fed on leaves of WKB cultivar Pak (3.974 U/mg) and the lowest activity was on RKB cultivar Naz (0.666 U/mg). Nemati Kalkhoran et al. (2014) reported that the highest proteolytic activity of *H. armigera* larvae reared on the leaves of different tomato cultivars, under field conditions, was on Hed riogrande (3.235 U/mg) and the lowest was on Korral (0.940 U/mg), which are different from our results in this study. Some possible reasons for

TABLE 3.Mean (±SE) proteolytic and amylolytic activity of midgut extracts from *Helicoverpa armigera* fifth instar larvae fed on leaves and pods of different bean cultivars under filed conditions

	Proteolytic activity of fifth instar (U/mg)		Amylolytic activity of fifth instar (mU/mg)	
Host (cultivar)	Reared on leaves	Reared on pods	Reared on leaves	Reared on pods
white kidney bean (Daneshkadeh)	0.812±0.329 b	2.569±0.032b	1.150±0.089 a	0.906±0.029 ab
white kidney bean (Pak)	3.974±1.30 a	3.947±0.379a	1.377±0.185 a	0.328±0.209 b
white kidney bean (Shokufa)	1.077±0.195 ab	2.585±0.068b	1.056±0.046 a	0.971±0.004 ab
red kidney bean (Akhtar)	1.281±0.014 ab	2.260±0.267b	0.835±0.066 ab	1.616±0.205 a
red kidney bean (Sayyad)	0.823±0.214 b	3.463±0.325ab	0.342±0.160 bc	1.622±0.115 a
red kidney bean (Naz)	0.666±0.347 b	2.548±0.232b	0.238±0.009 c	1.155±0.105 a
common bean (Talash)	2.450±0.163 ab	2.990±0.082ab	1.342±0.058 a	0.219±0.150 b

The means followed by different letters in the same column are significantly different ($P<0.05$, Tukey).

the discrepancy might be due to either physiological differences of host plants or variations in the examined larval instars of *H. armigera*. The proteolytic activity of fifth instar larvae of *H. armigera* fed on the green pods of cultivar WKB cultivar Pak showed approximately 2.5 fold lower activity than those fed bean Dehghan (Hemati et al. 2012). The proteolytic activity of fifth instar larvae of *H. armigera* fed on the green pods of RKB cultivar Akhtar showed approximately 2 fold higher activity than those fed on WKB cultivar Shokufa (Namin et al. 2014). Some possible reasons for disagreement might be due to the differences in rearing condition or variations in the geographic populations of *H. armigera*.

In the current study, the fourth and fifth instar larvae fed on the leaves of RKB cultivar Naz showed lower amylolytic activity than the others. Namin et al. (2014) reported that the fourth instar larvae of *H. armigera* fed on RKB cultivar Naz showed the highest values of ECI (efficiency of conversion of ingested food) and ECD (efficiency of conversion of digested food). It has been reported that the efficiency of conversion of digested food into larval biomass depends on the activity of digestive enzymes (Lazarevic et al. 2004). As a result, it can be expressed that despite a low digestive amylolytic activity of *H. armigera* on RKB cultivar Naz, the larvae fed on this cultivar had more performance to convert the ingested and digested food to body biomatter.

The current research shows that the fourth and fifth instar larvae of *H. armigera* fed on green pods of CB cultivar Talash had lower proteolytic and amylolytic activity than those fed on the other tested cultivars. Namin et al. (2014) reported that although proteolytic activity of the last instar of *H. armigera* was the lowest on CB cultivar Talash, the highest survival and larval growth index were observed on this cultivar. Furthermore, the highest intrinsic rate of increase (r_m) of *H. armigera* was reported on CB cultivar Talash (Naseri et al. 2014). Therefore, *H. armigera* larvae fed on CB cultivar Talash required less energy to produce digestive enzymes, and stored more energy for growth and reproduction. It could be suggested that CB cultivar Talash, among tested cultivars, was a suitable host for *H. armigera*.

The results of the cluster analysis indicated that grouping within each cluster might be due to a high physiological similarity among different bean cultivars, whereas the separate clusters might indicate significant variability in physiological characteristics between clusters. The comparative reproductive parameters and proteolytic and amylolytic activities in fourth and fifth instar larvae of *H. armigera* on seven bean cultivars revealed that subcluster A2 was the least suitable cultivar, and cluster B was the most suitable cultivar for *H. armigera*. However, cultivars classified in subcluster A1 had an intermediate status.

It is noticeable that the host plant cultivars are different in suitability for herbivorous insects; thus, they can affect life history traits of the insects such as development, survival and reproductive rates (Tsai and Wang 2001, Kim and Lee 2002). The greater total reproduction and shorter developmental time of insects on a host plant indicate greater suitability of that plant (van Lenteren and Noldus 1990). The quality and

quantity of nourishment ingested by an insect can lead to diverse negative effects including reduced intrinsic rate of increase and reproductive parameters, which we found the majority of these effects in *H. armigera* reared on RKB cultivar Akhtar. Also, the lowest value of proteolytic activity was observed on RKB cultivar Akhtar that may be due to the lack of nutritional components. However, our study about reproductive parameters was conducted under laboratory conditions, preventing the evaluation of other ecological factors that can play an important role in the reproductive performance of the moths. So, for a better understanding of the *H. armigera* – bean interaction to control of this pest, more attention should be allocated to study the demographic and reproductive parameters of this pest on different bean cultivars under field conditions.

ACKNOWLEDGEMENTS

This research is financially supported by the University of Mohaghegh Ardabili (Ardabil, Iran), which is appreciated.

LITERATURE CITED

ARGHAND, A., B. NASERI, J. RAZMJOU, M. HASSANPOUR, & F. RAHIMI NAMIN. 2014. Comparative biological parameters of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) on various corn hybrids. J. Entomol. Soc. Iran. 34:33–35.

BERNFELD, P. 1955. Amylases, α and ß. Meth. Enzymol. 1:149–154.

BEDE, J. C., J. N. MCNEIL & S. S. TOBE. 2007. The role of neuropeptides in caterpillar nutritional ecology. Peptides 28:185–196.

BIGGS, D. R. & P. G. MCGREGOR. 1996. Gut pH and amylase and protease activity in larvae of the New Zealand grass grub (*Costelytra zealandica*; Coleoptera: Scarabaeidae) as a basis for selecting inhibitors. Insect Biochem. Molec. 26:69–75.

BRADFORD, M. A. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

BRITO L.O., A. R. LOPES, J. R. PARRA, W. R. TERRA & M. C. SILVA-FILHO. 2001. Adaptation of tobacco budworm *Heliothis virescens* to proteinase inhibitors may be mediated by the synthesis of new proteinases. Comp. Biochem. Physiol. Part B 128:365–375.

CAREY, J. R. 1993. Applied demography for biologists with special emphasis on insects. Oxford University Press, NewYork.

DALIRY, S., R. SIEDSHARIFI & B. ESMAIELPOUR. 2010. Pulse crops. Challos Islamic Azad University Press. 265 pp.

DU, L., F. GE, S. ZHU & M. N. PARAJULEE. 2004. Effect of cotton cultivar on development and reproduction of *Aphis gossypii* (Homoptera: Aphididae) and its predator *Propylaea japonica* (Coleoptera: Coccinellidae). J. Econ. Entomol. 97:1278–1283.

ELPIDINA, E. N., K. S. VINOKUROV, V. A. GROMENKO, Y. A. RUDENSHAYA, Y. E. DUNAEVSKY & D. P. ZHUZHIKOV. 2001. Compartmentalization of proteinases and amylases in *Nauphoeta cinerea* midgut. Arch. Int. Physiol. Biochem. 48:206–216.

FARID, A. 1986. Study of bollworm *Heliothis armigera* (Hübner) on tomato in Jyroft and Kahnuj. Appl. Entomol. Phytopathol. 54:15–24

FATHIPOUR, Y. & B. NASERI. 2011. Soybean cultivars affecting performance of *Helicoverpa armigera*(Lepidoptera: Noctuidae), *In* Ng, T. B. (Ed), Soybean -Biochemistry, Chemistry and Physiology. In Tech, Rijeka, Croatia, pp. 599–630.

GATEHOUSE, J. A. & A. M. R GATEHOUSE. 1999. Genetic engineering of plants for insect resistance, *In* Rechcigl, J. E. & Rechcigl, N. A. (Eds.), Biological and Biotechnological Control of Insect Pests. CRC Press, USA, pp. 211–241.

GREEN, P. W. C., STEVENSON, P. C., SIMMONDS, M. S. J. & SHARMA, H. C. 2002. Can larvae of the pod borer, *Helicoverpa armigera* (Noctuidae: Lepidoptera), select between wild and cultivated pigeon pea Cajanus sp., Fabaceae? Bull. Ent. Res. 92:45–51.

HEMATI, S. A., B. NASERI, G. N. GANBALANI, H. R. DASTJERDI & A. GOLIZADEH. 2012. Digestive proteolytic and amylolytic activities and feeding responses of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different host plants. J. Econ. Entomol.150:1439–1446.

HEMATI, S. A., B. NASERI, & J. RAZMJOU. 2013. Reproductive performance and growth indices of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) on various host plants. J. Crop. Prot. 2:193–208.

HOSSEININAVEH, V., A. BANDANI, P. AZMAYESHFARD, S. HOSSEINKHAN & M. KAZEMI. 2007. Digestive proteolytic and amylolytic activities in *Trogoderma granarium* Everts (Dermestidae: Coleoptera). J. Stored Prod. Res. 43:515–522.

ISHAAYA, I., I. MOOR & D. JOSEPH. 1971. Protease and amylase activity in larvae of the Egyptian cotton worm, *Spodoptera littoralis*. J. Insect. Physiol. 17:945–953.

ITOYAMA, K., Y. KAWAHIRA, M. MURATA, & S. TOJO. 1999. Fluctuations of some characteristics in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) reared under different diets. Appl. Entomol. Zool. 34:315–321.

JALLOW, M. F. A., P. J. CUNNINGHAM & M. P. ZALUCKI. 2004. Intraspecific variation for host plant use in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae): implications for management. J. Crop. Prot. 23:955–964.

JONGSMA, M. A. & C. BOLTER. 1997. The adaptation of insect to plant protease inhibitors. J. Insect Physiol. 43:885–896.

JOHNSON, J. A., P. L. WOFFORD, & L. C. WHITEHAND. 1992. Effect of diet and temperature on development rates, survival, and reproduction of the Indian meal moth (Lepidoptera: Pyralidae). J. Econ. Entomol. 85:561–566.

KIM, D. S. & J. H. LEE. 2002. Egg and larval survivorship of *Carposina sasakii* (Lepidoptera: Carposinidae) in apple and peach and their effects on adult population dynamics on orchards. Environ. Entomol. 31:686–692.

KOTKAR H. M., P. J. SARATE, V. A. TAMHANE, V. S. GUPTA & A. P. GIRI. 2009. Responses of midgut amylases of *Helicoverpa armigera* to feeding on various host plants. J. Insect Physiol. 55:663–670.

LAZAREVIC, J., V. PERIC-MATARUGA, M. VLAHOVICAND & M. MRDAKOVIC. 2004. Effects of rearing density on larval growth and activity of digestive enzymes in *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Folia Biol. 52:1–2.

LAWERENCE, P. K. & K. R. KOUNDAL. 2002. Plant proteinase inhibitors in control of phytophagous insects. Electron. J. Biotechn. 5:93–109.

LIU, Z., D. LI, P. Y. GONG & K. J. WU. 2004. Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), on different host plants. Environ. Entomol. 33:1570–1576.

LEE, K. P., S. T. BEHMER, S. J. SEMPSON & D. A. RAUBENHEIMER. 2002. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). J. Insect Physiol. 48:655–665.

MCCAFFERY, A. R. 1998. Resistance to insecticides in heliothine Lepidoptera: a global view. Philosophical Transactions of the Royal Society of London Series B. Biol. Sci. 353:1735–1750.

MENDIOLA-OLAYA, E., A. VALENCIA-JIMENEZ, S. VALDES-RODRIGUEZ, J. DELANO-FRIER & A. BLANCO-LABRA. 2000. Digestive amylase from the larger grain borer, *Prostephanus truncates* Horn. Comp. Biochem. Physiol Part B. 126:425–433.

NA, J. H. & M. I. RYOO. 2000. The influence of temperature on development of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried vegetable commodities. J. Stored Prod. Res. 36:125–129.

NAMIN, F. R., B. NASERI, & J. RAZMJOU. 2014. Nutritional performance and activity of some digestive enzymes of the cotton bollworm, *Helicoverpa armigera*: response to bean cultivars. J. Insect

Sci. 14:93. Available online: www.insectscience.org/14.93.

NASERI, B., Y. FATHIPOUR, S. MOHARRAMIPOUR & V. HOSSEININAVEH. 2009. Comparative life history and fecundity of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different soybean varieties. Entomol. Sci. 12:147–154.

NASERI, B., Y. FATHIPOUR, S. MOHARRAMIPOUR & V. HOSSEININAVEH. 2011. Comparative reproductive performance of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) reared on thirteen soybean varieties. J. Agr. Sci. Tech. 13:17–26.

NASERI, B., Z. GOLPARVAR, J. RAZMJOU, & A. GOLIZADEH. 2014. Age-stage, two-sex life table of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different bean cultivars. J. Agr. Sci. Tech. 16:19–32.

NEMATI KALKHORAN, M., B. NASERI, F. RAHIMI NAMIN & D. KOUHI. 2014. Life table parameters and digestive enzymes activity of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different tomato cultivars. J. Entomol. Soc. Iran. 33:45–58.

RAHEJA, A. K. 1996. IPM research and development in India: progress and priorities, *In* Lal, O. P. (Ed), Recent Advances in Indian Entomology. APC Publications Pvt. Ltd., New Delhi, pp.115–126.

REDDY, K. S., G. R. RAO, P. A. RAO & P. RAJASEKHAR. 2004. Life table studies of the capitulum borer, *Helicoverpa armigera* (Hubner) infesting sunflower. J. Entomol. Res. 28:13–18.

SARFRAZ, M, L. M. DOSDALL & B. A. KEDDIE. 2006. Diamondback moth-host plant interactions: implications for pest management. Crop Prot. 25: 625–636. 669

SHOREY, H. H. & R. L. HALE. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol. 58: 522–524.

SINGH, A. K. & S. MULLICK. 1997. Effect of leguminous plants on the growth and development of gram pod borer, *Helicoverpa armigera*. Indian J. Entomol. 59:209–214.

SINGH, O. P. & S. B. B. PARIHAR. 1988. Effect of different hosts on the development of *Heliothis armigera*. Hub. Bull. Entomol. Res. 29:168–172.

TSAI, J. H. & J. J. WANG. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera: Aphididae). Environ. Entomol. 30:45–50.

VALENCIA-JIMENEZ, A., J. W. ARBOLEDA, A. LOPEZ AVILA. & M. F. GROSSI-DE-SA. 2008. Digestive αamylases from *Tecia solanivora* larvae (Lepidoptera: Gelechiidae): responseto pH, temperature and plant amylase inhibitors. Bull. Entomol. Res. 98:575–579.

VAN LENTEREN, J. C. & L. P. J. J. NOLDUS. 1990. Whitefly-plant relationship: behavioral and biological aspects. *In* Gerling, D. (Eds), Whitefly: Their Bionomics, Pest Status and Management. Intercept, Andover, U. K. PP. 47–89.

VAN STEENIS, M. J. & K. A. M. H. EL-KHAWASS. 1995. Life history of *Aphis gossypii* on cucumber: influence of temperature, host plant and parasitism. Entomol. Exp. Appl. 76:121–131.

WARD, J. H., JR. (1963). Hierarchical grouping to optimize an objective function". J. Am. Stat. Assoc. 58:236–244.

ZALUCKI, M. P., D. H. A. MURRAY, P. C. GREGG, G. P. FITT, P. H. TWINE, & C. JONES. 1994. Ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) in the Inland of Australia: larval sampling and host plant relationships during winter and spring. Aust. J. Zool. 42:329–346.

Submitted for publication 5 July 2015; revised and accepted 1 December 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 130–138

BUTTERFLY KLEPTOPARASITISM AND FIRST ACCOUNT OF IMMATURE STAGES, MYRMECOPHILY, AND BAMBOO HOST PLANT OF THE METALMARK *ADELOTYPA ANNULIFERA* (RIODINIDAE)

PHILLIP J. TORRES

Department of Ecology and Evolutionary Biology, Rice University, MS-170, 6100 Main Street, Houston, Texas 77005.
Email: phillip.juan.torres@gmail.com

AND

AARON F. POMERANTZ

Department of Entomology and Nematology, University of Florida, Gainesville, FL, 32611. Email: pomerantzaaron@gmail.com

ABSTRACT. This paper describes the life history, host plant use, and myrmecophily of the Neotropical riodinid butterfly *Adelotypa annulifera* (Godman, 1903) in Tambopata, Peru. Eggs of *A. annulifera* are laid at the tips of new growth bamboo culm sheaths bearing extrafloral nectary sites where adult butterflies and ants gather to feed. *Adelotypa annulifera* larval stages are actively tended by multiple species of ants and were observed feeding on the extrafloral nectaries of the bamboo. Pupation of *A. annulifera* occurs on the host plant near the base of the bamboo. We also document the potential kleptoparasitic behavior of adult butterflies on ant species that tend the caterpillars. To our knowledge, this is the first account describing the immature stages and life history of a species belonging to the genus *Adelotypa* and the first account of adult riodinid butterfly kleptoparasitism on ants.

Additional key words: bamboo, extrafloral nectaries, Nymphidiini, Peru, Tambopata

The metalmark butterflies of the family Riodinidae are diverse, small bodied, and primarily confined to the Neotropics, where approximately 1300 described species occur (Heppner 1991, Robbins 1993, Hall & Harvey 2002). Members of this family exhibit substantial phenotypic diversity, utilize a wide array of host plant families, and many immatures of these species engage in relationships with ants (myrmecophily) (DeVries 1997, Pierce et al. 2002, Kaminski et al. 2013). In these myrmecophilous relationships, caterpillars feed and communicate with ants in exchange for the ants' active protection from parasitoids and predators. Among the Lepidoptera, myrmecophily is unique to the family Riodinidae and their larger sister family Lycaenidae (Fiedler 1991, Pierce et al. 2002). Riodinidae are monophyletic, originated in the Neotropics, and are estimated to have split from Lycaenidae around 96 Mya in the mid-Cretaceous (Espeland et al. 2015).

Myrmecophily in riodinid larvae is associated with specialized organs that produce nutritional resources and semiochemicals as well as an organ to communicate acoustically with ants (Ross 1964, Fiedler et al. 1996, DeVries 1988). Myrmecophilous butterfly larvae secrete substances that attract and appease their attendant ants, including sugars and amino acids that the ants harvest from specialized exocrine glands (Pierce 1983). The majority of myrmecophilous larvae feed exclusively on plant tissue, but some feed on insect-derived food sources including ant eggs, larvae, pupae, and ant regurgitation (Cottrell 1984). Myrmecophilous butterflies, their attendant ants, host plants, and natural enemies have become a model for the study of insect-plant interactions, chemical communication, mutualism, biodiversity, conservation, and the evolution of complex life history traits (Pierce 1984). The family Riodinidae is interesting not only for its species diversity but also for its great morphological and ecological diversity. Butterflies in this family exhibit the greatest variation in wing shape, color and pattern relative to any other butterfly family and mimic species belonging to other lepidopteran families (DeVries 1997). Their biology is poorly known relative to other butterfly groups, yet the study of riodinids has the potential to provide insights into several aspects of evolutionary biology, including mimicry-driven phenotypic plasticity and myrmecophily (D'Abrera 1994, DeVries 1991).

The study organism *Adelotypa annulifera* (Godman, 1903) is a Neotropical riodinid butterfly currently placed in the tribe Nymphidiini that ranges from the Guyana Shield to Bolivia. Nymphidiini is the largest of the tribes in the Riodinidae (Hall 1999) with over 300, often rare, species and it is thought to be an entirely myrmecophilous tribe (Hall & Harvey 2002). The majority of riodinid species have unknown life histories (DeVries et al. 1992) and until now, there are no published accounts on the larval biology of members belonging to the genus *Adelotypa* (Penz & DeVries

2006). Here we provide the first detailed description of the biology and behavior of *A. annulifera* immatures and adults.

Methods

Field observations were carried out in proximity of the Tambopata Research Center (TRC, 13°8'1.13" S, 69°36'46.11" W) in the Tambopata National Reserve of Southeastern Peru during May and August 2013, December 2014, January and May 2015. The Tambopata rainforest has five major forest types: terra firme (upland forest, mature floodplain forest), primary successional floodplain forest, swamp forest, and bamboo forest. Mean annual rainfall at TRC is approximately 3,150 mm and greater than 80% of the rainfall in this region occurs during the October–May wet season. The monthly temperature ranges between 21–27° C year-round, and there is a weak seasonal signal in temperature (Brightsmith 2004).

Initial observations of adult *A. annulifera* butterflies feeding on bamboo sap in association with ants were made in May 2013. Bamboo plants were visually scanned for the presence of eggs, larvae and tending ants. During daily inspections of plants containing larvae and ants we documented and photographed larval instars, the adult feeding behavior in association with ants, and the species of ants. Four pupae of unknown age were collected at a bamboo culm sheath in December 2014 and brought to the Tambopata Research Center to be reared. Measurements were taken with a ruler and general aspects of larval morphology observed using a stereomicroscope. Color patterns of *A. annulifera* immature stages and adults in vivo were recorded using a Canon 70D DSLR camera equipped with a 100mm macro lens. Terminology for early stage descriptions follows Downey and Allyn (1980) for eggs, Stehr (1987) for general morphology of larvae, Mosher (1916) for pupae, and DeVries (1988) for ant-organs.

Results

Natural history of *Adelotypa annulifera*. This species occurs in primary rainforest at altitudes between about 400–700m. In this study, six male butterflies and five female butterflies were observed in the field feeding on the extrafloral nectaries at the tips of bamboo shoots, always in association with ants. Up to three *A. annulifera* butterflies were observed feeding at the same time at a nectary and while the males fed throughout the day and would return when disturbed, the females only fed for short periods of time (less than one hour) and did not return to the site when disturbed. The behavior typically started with the butterflies fluttering around various shoots and they were only observed landing and feeding in the presence of ants. Upon landing, the butterflies walked towards the location of the flowing sap while probing with their proboscis. Once the location of the sap flow was located, the butterflies spent up to several hours at the same location. One butterfly, identified by a missing part of its left hind wing, was seen at the same flow three days in a row. Though observations were not continuous throughout each day, the butterfly was seen at the same location as early as 0900 h and as late as 1730 h on the same day. If disturbed, the butterflies would fly away and often land on the underside of a nearby leaf. After several minutes to an hour the butterflies eventually returned to the plant, though not always to the same nectary location, as each bamboo often had multiple areas of ants feeding. Despite seeing males and females share nectaries for extensive periods of time, no mating attempts were observed.

A total of 13 eggs laid in clusters of four to five were observed on early growth bamboo near the culm sheath tips approximately 0.5–1.0 meter above ground. The larvae were solely observed feeding at bamboo extrafloral nectaries and remained present on the bamboo in association with ants throughout all instars. On 8 December 2014, two final instar larvae and two pupae of unknown age were found at the base of a bamboo culm sheath. On 10 December 2014 the two larvae pupated at the same location and the four pupae total were collected and brought to the Tambopata Research Center to be reared. Only one of the pupae eclosed to an adult four days after collection, which was identified as a female *A. annulifera*.

Description of immature stages. **Egg:** Dorso-ventrally flattened, grayish color, general spherical shape, convex, exochorion with hexagonal cells in lateral view, slightly depressed micropylar area centered on the top surface (Fig. 1A). Oviposition occurs at the tips of new growth bamboo culm leaves.

First instar: Head capsule light brown, body dorso-ventrally compressed, body reddish with four longitudinal light bands, total length 2.1 mm (n=2) (Fig. 1B). Prothoracic and anal plate same color as body. Body with short setae in lateral areas and in prothoracic and anal shields. The larvae remain in physical contact with an individual bamboo host plant and feed on the liquid extrafloral nectar produced at the tips of new growth shoots.

Mid instars: Head capsule light brown, body reddish to light brown with four longitudinal light bands, total length ranges from 5.6–6.4 mm (n=3) (Fig. 1C–D, Fig. 2). Prothoracic and anal shields light brown. Ant-organs present, including tentacle nectary organs (TNOs) on

FIG. 1. Immature stages of *Adelotypa annulifera* on bamboo and their association with ants. (**A**) Eggs with *Megalomyrmex balzani*. (**B**) First instar larva with *Ectatomma tuberculatum*. (**C**) Mid-instar larva with *Pheidole* sp. (**D**) Mid-instar larvae with *Paraponera clavata*. (**E**) Final instar larvae with *E. tuberculatum*. (**F**) Pupae.

FIG. 2. Dorsal (left) and lateral (right) views of early instar *Adelotypa annulifera*.

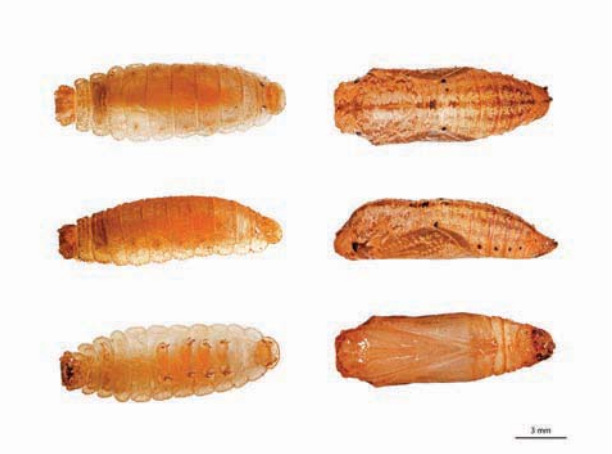


FIG. 3. Final instar larva (left) dorsal, lateral, and ventral view. Presence of tentacle nectary organ (TNO) on abdominal segment 8. Pupa (right) dorsal, lateral, and ventral view.

the eighth abdominal segment which appear similar to those described in *Lemonias caliginea* (in Ross, 1964, as *Anatole rossi*) and *Thisbe irenea* (DeVries 1988) and one pair of vibratory papillae (VPs) located on anterior border of the prothoracic shield, anteriorly directed, similar in overall appearance to those described by Ross (1964, 1966) and DeVries (1988). Later instar body becomes greenish in color (Fig. 2), head capsule lighter yellow color, general aspects of morphology similar to preceding instars'.

Final instar: Head brown, body turns to a light brown and beige color, total length 12.5 mm (n=2) (Fig. 1E, Fig. 3). Prior to pupation, final instars found at base of bamboo host plant under tan colored culm leaf. Prominent tentacle nectary organs on abdominal segment 8.

Pupa: Body variegated coloring with light brown, beige, and dark spots, abdominal segments mobile, total length 12.1 mm (n=2) (Fig. 1F, Fig. 3) Tegument is entirely sculptured with irregular striations and lacking prominent tubercles. Silk girdle crossing the A1 segment near one pair of dark spiracles. Pupation occurs on the same host plant near the base of the bamboo under the culm leaf.

DISCUSSION

Interactions of immature stages with ants on bamboo. All life stages of *A. annulifera* were observed in association with ants on young bamboo shoots. At least four different species of ants were observed in association with *A. annulifera* immatures: *Ectatomma tuberculatum* (Fig. 1B, 1E), *Pheidole* sp. (Fig. 1C), *Megalomyrmex balzani* (Fig. 1A), and *Paraponera clavata* (Fig. 1D). In each case, only one species of ant was present on each bamboo plant. It is possible that the ants claim and defend their bamboo from other ant species for access to the extrafloral nectaries and caterpillar secretions.

Ectatomma tuberculatum (Formicidae) was one of the most prevalent ant species at the study site. The genus *Ectatomma* is unusual in that all species spend large fractions of their life harvesting secretions from extrafloral nectaries (EFNs), sap-feeding hemipterans, and myrmecophilous butterfly larvae (DeVries 1991, Bentley 1977, Wheeler 1986). At one site, *E. tuberculatum* ants were observed feeding at the same extrafloral nectary as both larval and adult *A. annulifera*. This relationship contrasts with the observations by Ross (1964 and 1966) in which the myrmecophilous riodinid larvae of *Lemonias caliginea* (in Ross, 1964, as *Anatole rossi*) are preyed upon by *E. tuberculatum* workers in Mexico. However, DeVries (1988) observed the riodinid larvae of *Thisbe irineae* associating primarily with *E. ruidum* as well as *E. tuberculatum* workers in Panama and devised an experiment to explain this difference in *Ectatomma* behavior. *Thisbe irineae* larvae were exposed to a species of *Azteca* ant and were then offered to *E. ruidum* workers where they were subsequently attacked and killed, presumably because the riodinid larvae had acquired an *Azteca* ant chemical odor (DeVries 1988). Future experiments on *A. annulifera* could be performed to determine if larvae are attacked by workers of one ant species after exposure to another ant species.

At another site, high numbers of *Pheidole* ant workers (n=6-33) were observed surrounding early instar *A. annulifera* larvae (Fig. 1C). When the caterpillars were physically disturbed, *Pheidole* soldiers and more

workers were recruited to defend the caterpillar. *Megalomyrmex balzani* were seen at two different bamboo shoots (May and August 2013) and were only observed in association with adults and eggs. Interestingly, *Paraponera clavata* were observed tending numerous *A. annulifera* larvae in May 2015 and would aggressively fend off invading insects, as well as one of the researchers, on the bamboo stalk (Fig. 1D).

Based on our observations, *A. annulifera* appears to be an ant generalist and future work will help to reveal further details of the butterfly-ant relationships. A satisfying evolutionary explanation is unknown as to why some myrmecophilous riodinids and lycaenids are allied with only a single species of ant whereas others are generalists (Pierce 1984, Pierce et al. 2002).

Bamboo as a host plant. Two common species, *Guadua sarcoparpa* Londono and Peterson and *Guadua weberbaueri* Pilger (Poaceae: Bambuseae) dominate the bamboo forests in southwestern Amazonia (Griscom & Ashton 2006). Bamboo forests cover approximately 180,000 km^2 in southwestern Amazonia, representing the largest bamboo-dominated forest in the Neotropics. These plants are biologically interesting because they primarily occur as mono-dominant forests with a patchy distribution throughout terra firma and floodplain forests (Nelson 1994, Griscom & Ashton 2003). Bamboo forests have been assumed to be a species-poor, weedy habitat, but researchers are discovering that bamboo forests are an important component of the regional ecosystem in southwestern Amazonia (Emmons & Feer 1990, Kratter 1997).

It is common for many species of ants, wasps, beetles, flies, bees, hemipterans, and other insects to forage on the extrafloral nectaries of the bamboo (personal observation). Using their mandibles, ants appear to manipulate the tips of the shoots to improve the flow of nectar and will guard bamboo stalks against other insects. Young bamboo grows rapidly and, considering nectaries were the only observed food source for the *A. annulifera* larvae, the fluids secreted from the nectaries likely contain sugars and amino acids. Future research should investigate the contents of this bamboo extrafloral nectar and potential nutritional benefits for Neotropical arthropod fauna, including the immature stages of *A. annulifera*.

The family Riodinidae contains immature stages with diverse diets, which include live and dead leaves, flower buds, fungi, extrafloral nectar, and cases of entomophagy (DeVries et al. 1997; DeVries & Penz 2000). Bamboo is a relatively unusual host plant choice for Lepidoptera immatures and this appears to be the first record as a host plant for a species belonging to Riodinidae. For Riodinidae, it has been proposed that obligate symbiotic relationships with ants are associated with an expansion in the number of host plants (polyphagy) (DeVries et al. 1992, DeVries 1997, Hall & Harvey 2001). The tendency of female butterflies to oviposit in the presence of ants could lead to 'mistakes' in plant selection and as a result, polyphagy could evolve more easily in myrmecophilous butterflies than in non-myrmecophilous ones (Pierce 1984; Pierce & Elgar 1985). Perhaps the utilization of bamboo as a host plant by *A. annulifera* was initiated by the butterflies ovipositing near bamboo nectary sources in the presence of ants.

As the bamboo grows and develops, portions of the shoots and leaves change color. Interestingly, *A. annulifera* instar coloration changes as well and seems to match the appearance of the host plant. For instance, early instar caterpillars appear to be reddish in color, as are the young bamboo tips they feed under. Later instars become more greenish in color, like the green bamboo they are exposed on, and final instars and pupae become beige colored which coincides with the color of the dead culm leaves.

Adult butterfly-ant interactions: a case of kleptoparasitism. There are few reports of ants interacting with the adult butterflies of myrmecophilous riodinids or lycaenids, or if they do, ants treat the butterflies much as they would any insect prey. To investigate the specificity of the butterfly-ant relationship, one of the authors presented the *M. balzani* ants with three live unidentified species of moth, similar in size to *A. annulifera* adults, which the ants immediately proceeded to attack. While adult *A. annulifera* were feeding, ants would investigate various parts of the butterflies with their antennae and at times crawl over their head, legs, and open wings (Fig. 4A-D). Taken together, these observations support the idea that *A. annulifera* has co-evolved in the presence of ants and the adult butterflies are somehow able to reduce their aggressive behavior.

These ants not only tolerate the presence of the butterflies, but the butterflies appear to display a kleptoparasitic behavior by taking a nectar resource from the ants. Butterflies were seen feeding exclusively from bamboo nectary flows, a resource which the ants were protecting, feeding upon, and maintaining (Fig. 4D–F). The ants attempted to remove the butterflies' proboscises to gain better access to the fluid, but would eventually settle with little to no access and wait. In addition, butterflies were twice observed drinking bamboo fluid directly out of an ant's mandibles, essentially stealing a resource with no consequence (Fig. 4F). Ants were seen antennating the terminal portion of the butterfly's abdomen for extensive periods

FIG. 4. Adult *Adelotypa annulifera* interactions with ants on bamboo. (**A**) Antennation of adult butterfly wings. (**B**) Ant crawling on butterfly wing. (C) Antennation of butterfly abdomen. (**D-E**) Butterflies and ants utilizing extrafloral nectary source on bamboo. (**F**) Butterfly drinking bamboo fluid directly from *Ectatomma* ant mandibles.

FIG. 5. Adult *Adelotypa annulifera* putative wing pattern mimicry. (**A**) Male (left) and female (right) butterflies perched on bamboo shoot in presence of *Megalomyrmex balzani* ants. View of *A. annulifera* wing pattern: (**B**) Ventral (**C**) Dorsal (**D**) Lateral.

of time, not unlike when the ants antennate the caterpillars in return for a nectar reward (Fig. 4C), however, extensive observations revealed that the butterflies did not provide any apparent resource for the ants. Thievery of a food source (kelptoparasitism) occurs in many arthropod groups (Eisner et al. 1991; Sivinsky et al. 1999) and in this case, *A. annulifera* adult butterflies appear to display a kelptoparasitic behavior towards attendant ants by taking a nutritive resource, in this case bamboo sap secretions (Fig. 4D–F).

Several observations in the Lycaenidae suggest that chemical interactions between adult butterflies and ants may be more complex than currently appreciated and some adults may appease ants that would otherwise attack them. In *Curetis regula*, butterfly adults feed on leaf tissue damaged by their larvae alongside the larvae's attendant ants (DeVries 1984). The butterflies could emit a chemical signal which appeases the ants or mimics cuticular hydrocarbons to reduce aggressive behavior. Future studies should investigate the chemical profiles emitted from *A. annulifera* larvae and adults. Overall, this potentially kleptoparasitic interaction between *A. annulifera* and ants is, to our knowledge, the first documented case of this behavior in the family Riodinidae.

Finally, the red markings on the *A. annulifera* butterfly wings, at least to a human observer, are strikingly ant-like in appearance (Fig. 5A–D). For

example, the size and color of the wing spots are similar to the body segment size and color of the *Megalomyrmex* and *Ectatomma* ants that *A. annulifera* adults were observed associating with (Fig. 4, Fig. 5), suggestive of mimicry. Cases of myrmecomorphy (arthropods that mimic ants morphologically and/or behaviorally) have been described in over 2000 arthropods and include groups such as spiders, beetles, and hemipterans (McIver 1993). Some salticid spiders mimic ants to avoid being preyed upon by them and other ant-mimics likely gain protection from all predators that tend to avoid ants (Cushing 2012). In a striking case of a lepidopteran mimicking a predator, the metalmark moth *Brenthia hexaselena* has evolved to mimic jumping spiders with wing markings, wing positioning, posture, and movement (Rota and Wagner 2006). Members of Riodinidae exhibit a wide array of wing shapes and patterns (DeVries et al. 1992; Robbins & Busby 2015) and it is possible that selective pressures by predation have resulted in butterfly wing-patterns resembling noxious ants. While this observation requires more scrutiny, the red wing markings on *A. annulifera* adults could serve as visual mimicry of the ants that the butterflies associate with and could function to ward off would-be visual predators.

Acknowledgements

We thank Rainforest Expeditions and the Tambopata Research Center staff for providing us with the necessary field support to carry out our study. We thank Brendon Boudinot and Alex Wild for assistance with ant identification as well as Jeff Cremer, Frank Pichardo and Katie Mack who helped with field work. Finally, we thank SERNANP for research permits (N°017-2015-SERNANP-JEF) and Gerardo Lamas for support in the Entomology Department at the Museo de Historia Natural, Universidad de San Marcos, Lima, Peru.

Literature Cited

Bentley, B. L. 1977. The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). J. Ecology 65:27–38.

Brightsmith, D. J. 2004. Effects of weather on avian geophagy in Tambopata, Peru. Wilson Bulletin 116:134–145.

Cottrell, C. B. 1984. Aphytophagy in butterflies: its relationship to myrmecophily. Zool. J. Linn. Soc. 79:1–57.

Cushing, P. E. 2012. Spider-ant associations: an updated review of myrmecomorphy, myrmecophily, and myrmecophagy in spiders. Psyche. vol. 2012, Article ID 151989, 23 pages, 2012. doi:10.1155/2012/151989.

D'Abrera, B. 1994. Butterflies of the Neotropical Region, pp. 880–1096. Part VI: Riodinidae. Hill House, Victoria, Australia.

DeVries, P. J. 1984. Of crazy-ants and Curetinae: are *Curetis* butterflies tended by ants? Zool. J. Linn. Soc. 79:59–66.

DeVries, P. J. 1988. The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants. Zool. J. Linn, Soc. 94:379–393.

DeVries, P. J. 1991. Mutualism between *Thisbe irenia* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. Biol. J. Linn. Soc. 43:179–195.

DeVries, P. J. 1997. The butterflies of Costa Rica and their natural history. Vol II. Riodinidae. Princeton University Press. xxv + 288 pp.

DeVries, P. J. 2000. Entomophagy, behavior, and elongated thoracic legs in the myrmecophilous Neotropical butterfly *Alesa amesis* (Riodinidae). Biotropica 32:712–721.

DeVries,, P. J., I. A. Chacon, & D. Murray. 1992. Toward a better understanding of host use and biodiversity in riodinid butterflies (Lepidoptera). J. of Res. Lep. 31:103–126.

Downey, J. C., & A. C. Allyn. 1980. Egg of Riodinidae. J. Lep. Soc. 34:133–145.

Eisner, T., M. Eisner, & M. Deyrup. 1991. Chemical attraction of kleptoparasitic flies to heteropteran insects caught by orb-weaving spiders. Proc. Natl. Acad. Sci. USA. 88:8194–8197.

Emmons, L. H., & F. Feer. 1990. Neotropical rainforest mammals: a field guide. University of Chicago Press, Chicago, IL.

Espeland, M., J. P. W. Hall, P. J. DeVries, D. C. Lees, M. Cornwall, Y. Hsu, L. Wu, D. L. Campbell, D. Talavera, R. Vila, S. Salzman, S. Ruehr, D. J. Lohman, & N. E. Pierce. 2015. Ancient Neotropical origin and recent recolonization: Phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). Mol. Phyl. Evo. 93: 296–306.

Fiedler, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonner Zool. Monogr. 31:1–210.

Fiedler, K., B. Holldobler, & P. Seufert. 1996. Butterflies and ants: the communicative domain. Experentia 52:14–24.

Griscom, B. W., & P. M. S. Ashton. 2003. Bamboo control of forest succession: Guadua sarcocarpa in Southeastern Peru. Forest Ecol. Man. 175:445–454.

Griscom, B. W., & P. M. S. Ashton. 2006. A self-perpetuating bamboo disturbance cycle in a Neotropical forest. J. Trop. Ecol. 22:587–597.

Hall, J. P. W. 1999. The genus *Theope* and relatives: their systematics and biology (Lepidoptera: Riodinidae: Nymphidiini). Ph.D. Dissertation, University of Florida, Gainesville.

Hall, J. P. W., & D. J. Harvey. 2001. A phylogenetic analysis of the Neotropical riodinid butterfly genera *Juditha, Lemonias, Thisbe* and *Uraneis*, with a revision of *Juditha* (Lepidoptera: Riodinidae: Nymphidiini). Syst. Entom. 26:453–490.

Hall, J. P. W., & D. J. Harvey. 2002. Basal subtribes of the Nymphidiini (Lepidoptera: Riodinidae): phylogeny and myrmecophily. Cladistics 18:539–569.

Heppner, J. B. 1991. Faunal regions and the diversity of Lepidoptera. Trop. Lep. 2:1–85.

Kaminski, L. A., L. M. Mota, A. V. L. Freitas, & G. R. P. Moreira. 2013. Two ways to be a myrmecophilous butterfly: natural history and comparative immature-stage morphology of two species of *Theope* (Lepidoptera: Riodinidae). Biol. J. Linn. Soc. 108:844–870.

Kratter, A. W. 1997. Bamboo specialization by Amazonian birds. Biotropica 29: 100–110.

McIver, J. D., & G. Stonedahl. 1993. Myrmecomorphy: morphological and behavioral mimicry of ants. Ann. Rev. Entom. 38:351–379.

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

Nelson, B. W. 1994. Natural forest disturbance and change in the Brazilian Amazon. Remote Sensing Rev. 10:105–125.

Penz, C. M., & P. J. DeVries. 2006. Systematic position of *Apodemia paucipuncta* (Riodinidae), and a critical evaluation of the nymphidiine transtilla. Zootaxa 1190:1–50.

Pierce, N. E. 1983. The ecology and evolution of symbiosis between lycaenid butterflies and ants. Ph.D. dissertation, Harvard University.

Pierce, N. E. 1984. Amplified species diversity: A case study of an Australian lycaenid butterfly and its attendant ants, pp. 197–200. *In* R. I. Vane-Wright, and P. R. Ackery (Eds.). The biology of butterflies, Symposium of the Royal Entomological Society of London Number 11, Academic Press, London, UK.

PIERCE, N. E., & M. A. ELGAR. 1985. The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. Behav. Ecol. Sociobiol. 16:209–222.

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). Ann. Rev. Entom. 47:733–771.

ROBBINS, R. K. 1982. How many butterfly species? News Lepid. Soc. 1982: 40–41.

ROBBINS, R. K. 1993. Comparison of butterfly diversity in the Neotropical and Oriental regions. J. Lepid. Soc. 46:298–300.

ROBBINS, R. K., & R. C. BUSBY. 2015. Evolutionary gain of male secondary sexual structures in the widespread Neotropical montane genus *Lathecla* (Lepidoptera, Lycaenidae, Eumaeini). Ins. Syst. Evol. 46:47–78.

ROSS, G. N. 1964. II. Early stages of *Anatole rossi*, a new myrmecophilous metalmark. J. Res. Lep. 3:81–94.

Ross, G.N. 1966. Life-history studies on Mexican butterflies. IV. The ecology and ethology of *Anatole rossi*, a myrmecophilous metalmark (Lepidoptera: Riodinidae). Ann. Entomo. Soc. Am. 59:985–1004.

ROTA J., & D. L. WAGNER. 2006. Predator mimicry: metalmark moths mimic their jumping spider predators. PLoS One 1:e45 doi:10.1371/journal.pone.0000045.

SIVINSKI J., S. MARSHALL, & E. PETERSSON. 1999. Kleptoparasitism and phoresy in the Diptera. Flor. Entomo. 82:179–197.

STEHR, F. W. 1987. Order Lepidoptera. *In* Immature Insects, pp. 293–294. Vol. I, F. W. Stehr, Ed, Kendall/Hunt Publishing, Dubuque, Iowa.

WHEELER, D. E. 1986. *Ectatomma tuberculatum* foraging biology and association with *Crematogaster* (Hymenoptera Formicidae). Ann. Entom. Soc. Amer. 79:300–303.

Submitted for publication 22 July 2015; revised and accepted 9 October 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 139–144

A NEW GENUS FOR *TORTRIX DRUANA* WALSINGHAM, 1914 AND A NEW SPECIES FROM THE NORTHERN NEOTROPICS (LEPIDOPTERA: TORTRICIDAE: COCHYLINI: EULIINA)

TODD M. GILLIGAN

Identification Technology Program, USDA-APHIS-PPQ-S&T, 2301 Research Blvd., Suite 108, Fort Collins, Colorado 80526, USA; email: todd.m.gilligan@aphis.usda.gov

AND

JOHN W. BROWN

Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA; email: tortricidae.jwb@gmail.com

ABSTRACT. *Durangularia*, **gen.n.**, is described and illustrated from the northern Neotropics. As currently defined, the genus includes two species: *D. druana* (Walsingham, 1914), **comb.n.**, from the southwestern U.S. (Arizona, Texas), Mexico, and Guatemala; and *D. giganteana*, **sp.n.**, from Costa Rica. The new genus is assigned to Cochylini (Euliina) on the basis of the presence of non-deciduous cornuti in the phallus of the male genitalia and the absence of a signum in the female genitalia.

Additional key words: "Durangarchips," *giganteana*, Euliini

The generic name "Durangarchips" first appeared in the Tortricoidea portion of the Atlas of Neotropical Lepidoptera, Checklist: Part 2 (Powell et al. 1995: 148), assigned to the tortricid tribe Archipini. Listed under that genus was the single species *Tortrix druana* Walsingham, 1914, described from Durango, Mexico. Although the authorship of "Durangarchips" is attributed to "Powell 1991," there is no publication in which the name was described.

Even though it can be argued that a type species was designated by monotypy, the genus name is not available per the ICZN (1999) because it lacks a description and/or diagnosis. Brown (2005: 263) retained the generic status of "Durangarchips," recognizing that the species *druana* does not fit into any described genus, but he correctly referred to "Durangarchips" as a nomen nudum. Despite being widely cited in internet resources, such usage is no substitution for a formal description/diagnosis.

This was not the first time that *druana* had been assigned to a new generic manuscript name. Obraztsov had examined many Walsingham types in the 1960s with the intent of revising the Nearctic Tortricidae; unfortunately, he died before publishing much of this work. While examining some of his unpublished manuscripts now on loan to the Mississippi Entomological Museum from the American Museum of Natural History, we discovered a paper titled "Genus *Aztecotortrix*, new genus." The manuscript consists of three typewritten pages and two pages of pencil drawings describing a new genus with *Tortrix druana* Walsingham, 1914 as the type species. Obraztsov did not specifically assign the genus to a tribe, but he stated that wing venation "separates this genus from the remaining known Epitymbiini genera," and he compared the genitalia to those of *Pseudeulia* Obraztsov and *Aphelia* Hübner (both Archipni).

Over the past decade, specimens of *druana* and an undescribed species congeneric with *druana* have accumulated in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, DC, and the Instituto Nacional de Biodiversidad, Santa Domingo de Heredia, Costa Rica. From examining genitalic dissections of these specimens, it is clear that they belong to the tribe Cochylini (subtribe Euliina), not Archipini (or Epitymbiini). Here we describe a new genus for *druana* along with one new species so that these congeners can be assigned to an available genus in the correct tribe.

MATERIALS AND METHODS

We examined 55 adult specimens (48 ♂, 7 ♀) together with 14 associated genitalia preparations deposited in the following collections: Colorado State University, Fort Collins, Colorado, U.S.A. (CSU); Essig Museum of Entomology, University of California, Berkeley, California, U.S.A. (EME); Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica (INBio); National Museum of Natural History, Washington, D.C.,

U.S.A. (USNM); and Vitor Becker Collection, Reserva Serra Bonita, Bahia, Brazil (VBC).

Images of adults were taken with Canon 100 mm and MP-E 65 mm macro lenses attached to a Canon 7D digital SLR (Canon U.S.A., Inc., Melville, NY). Figs. 7–9 are composite stacks of many individual images created with Zerene Stacker (Zerene Systems, Richland, WA). Images of genitalia were taken with a Nikon DS-Fi1 digital microscope camera attached to a Nikon Labophot-2 compound microscope (Nikon Instruments, Inc., Melville, NY). All images were edited using Photoshop CS6 (Adobe Systems, Inc., San Jose, CA). Forewing length (FWL) is defined as the distance from the base to the apex including the fringe, reported to the nearest one-tenth of a millimeter. Measurements were made with a stereomicroscope equipped with an ocular micrometer or a compound microscope using a slide micrometer. The number of observations supporting a particular statistic is indicated by "n =." Other abbreviations are as follows: HTP = holotype, PTP = paratype. Dissection methods follow those presented in Brown and Powell (1991), and morphological nomenclature follows Gilligan et al. (2008).

RESULTS AND DISCUSSION

DURANGULARIA, **gen.n.**

Type species: *Tortrix druana* Walsingham, 1914.

Durangarchips Powell, 1995, in Heppner, Atlas Neotropical Lepid., Checklist 2: 148; nomen nudum.

Durangularia is assigned to Cochylini (Euliina) based on the presence of non-deciduous cornuti in the phallus of the male genitalia and the absence of a signum in the female genitalia. Virtually all Western Hemisphere Archipini have a pair (or more) of deciduous, basally-attached, aciculate cornuti (Anzaldo et al. 2014) and a signum that includes an internal narrow-cresecent-shaped spine and an external capitulum (Horak 1984). Although species of *Durangularia* lack the foreleg hairpencil proposed as a synapomorphy for the Euliina (Brown 1990), many euliines have lost this structure secondarily (Brown 1990), and *Durangularia* is consistent with a suite of other euliine characters: M-stem and chorda absent; forewing costal fold absent in male; M_3 and CuA_1 separate; abdominal dorsal pits absent; uncus well-developed; gnathos present, arms joined distally; papillae anales simple, unmodified; and primarily Neotropical (Brown & Powell 1991).

The most convincing morphological character supporting assignment of the genus to Euliina is the presence of non-deciduous cornuti, which represents a putative synapomorphy for a clade within Tortricinae that includes Tortricini, Cnephasiini, and Cochylini (Cochylina and Euliina) (Regier et al. 2012, Anzaldo et al. 2014). The absence of both a brachiola in the male genitalia and upraised scales on the forewing excludes the genus from Tortricini. The absence of a floricomous ovipositor (i.e., with specialized setae) excludes the genus from Cnephasiini. And the presence of a gnathos excludes the genus from Cochylina.

Diagnosis. Superficially, adults of *Durangularia druana* are somewhat reminiscent of a large *Argyrotaenia* Stephens or *Clepsis* Guenèe (Archipini); owing to its large size, *D. giganteana* is not similar to any described Archipini. *Durangularia* are easily distinguished from all Archipini by features of the male and female genitalia as described above. Obraztsov (in litt.) suggested a similarity with *Aphelia*, probably based on the lateral lobes of the gnathos arms and the emarginated apex of the uncus; however, many euliine genera show a similar modification of the gnathos (e.g., *Ortognathosia* Razowski, *Oregocerata* Razowski, *Ernocornutia* Razowski, and others). Within Euliina, adults of *Durangularia* are somewhat similar in forewing maculation to some species of *Ernocornutia* and *Proeulia* Clarke, with a nearly uniform ground color with faint remnants of a submedian fascia, but the genitalia of *Durangularia*, with a variably emarginated apex of the uncus and a somewhat rectangular valva, are unlike those of any other euliine genus.

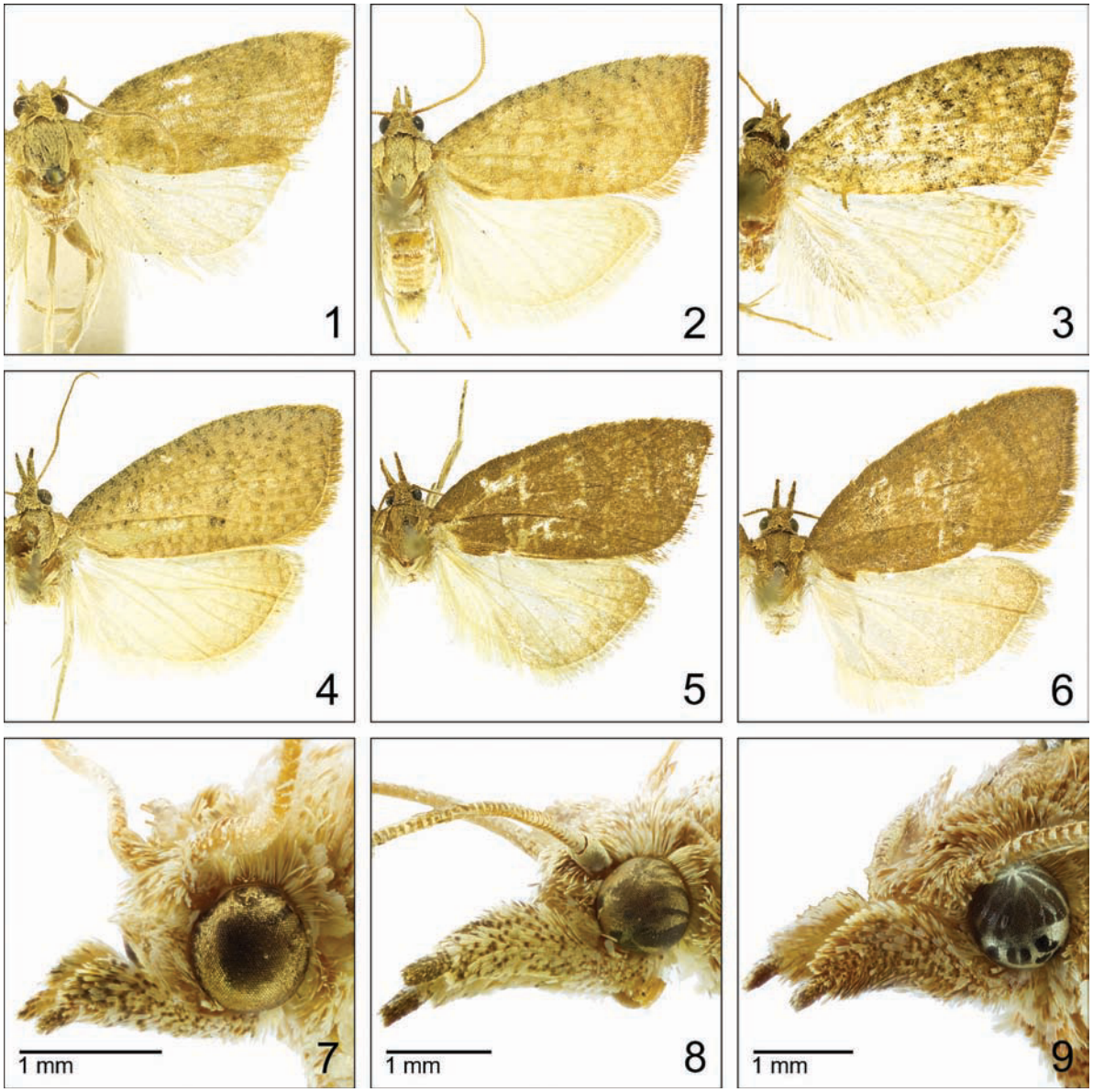
Description. Male. *Head*: Vertex rough scaled, scales projecting anteriorly over frons; frons smooth scaled; labial palpus ca. 1.8–2.2 times horizontal diameter of compound eye, segment II straight or slightly curved, rough scaled, segment III porrect, smooth scaled; antenna fasciculate-ciliated in male, with sensory setae 0.50–0.75 times flagellomere diameter. *Thorax*: Smooth scaled; legs densely scaled; male foreleg hairpencil absent. Forewing length 6.7–15.2 mm; costa convex, apex rounded, slightly acute, termen slightly concave, tornus broadly rounded; all veins separate, m-stem and chorda absent. Hindwing with Sc + R_1 and Rs separate, Rs and M_1 closely approximate. *Abdomen*: Uncus spatulate, ca. 1.5–2.0 as long as wide, tapering to tegumen, apically bifid with variable U-shaped emargination; dorsolateral shoulders of tegumen produced; socius fingerlike, projecting ventrolaterally, setose; gnathos V-shaped, arms joined distally, arms with variable dorsolateral and

ventrolateral lobes; transtilla weakly sclerotized; valva rhomboid or rectangular; cucullus sclerotized along costal margin, setose in distal 0.5, anal angle rounded; sacculus well sclerotized, consisting of a basal setose lobe, a medial subtriangular process, and a free distal lobe; juxta a pentagonal plate; phallus ca. as long as valva, strongly curved in distal 0.5, phallobase with a pair of lateral, flattened, membranous lobes; vesica with two large basal non-deciduous cornuti.

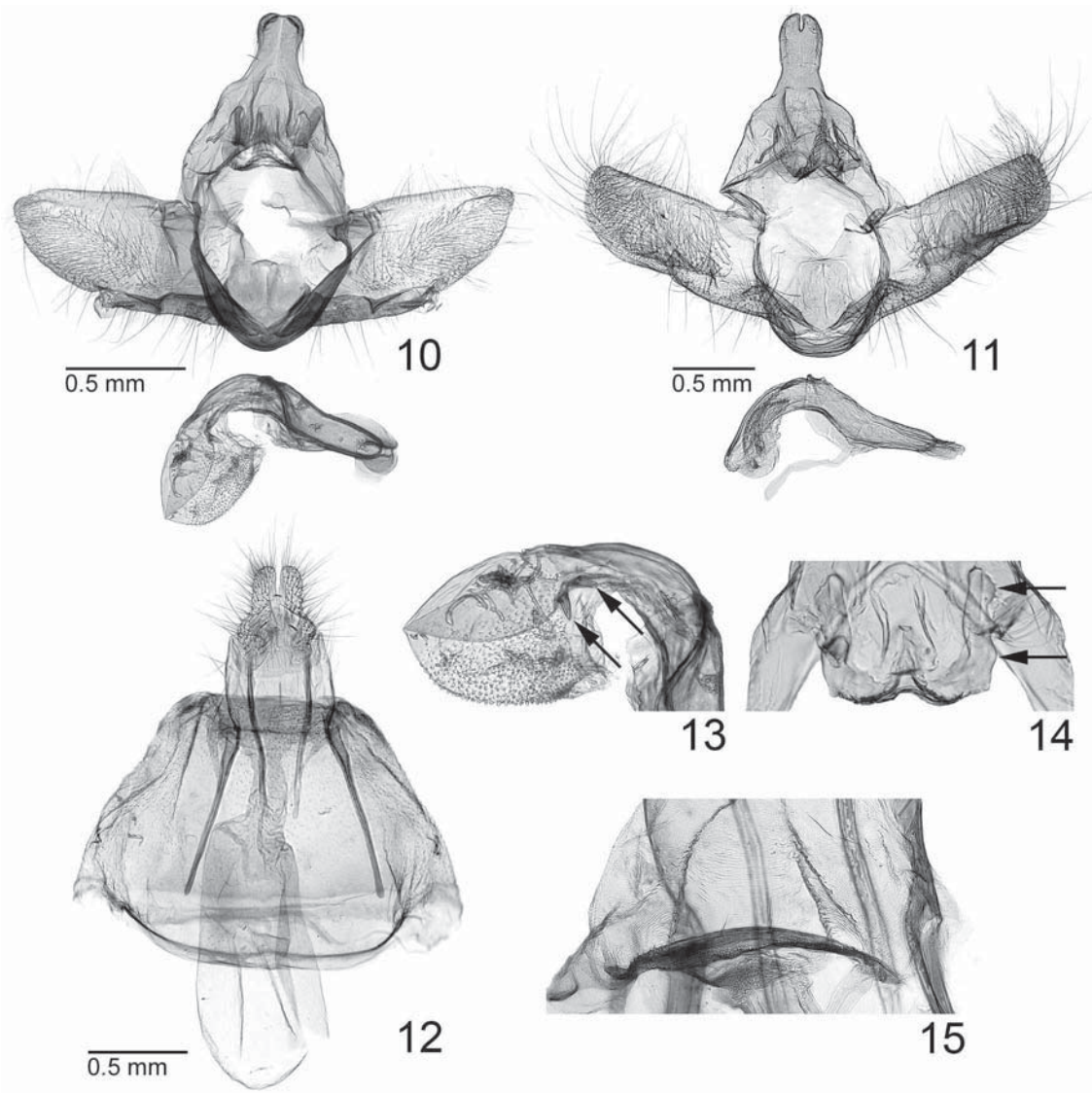
Female. *Head*: Labial palpus ca. 2.7 times horizontal diameter of compound eye; other characters as in male. *Thorax*: As in male. *Abdomen*: Papillae anales simple, unmodified; apophyses anteriores ca. as long as apophyses posteriores; lamella antevaginalis a weakly sclerotized microtrichiate extension of sternum VII covering the ostium; lamella postvaginalis a sclerotized concavity nearly as wide as the apophyses anteriores; ductus bursae ca. 0.5 times length of corpus bursae; corpus bursae elongate, rounded anteriorly; ductus seminalis arises from corpus bursae just anterior to junction with ductus; signum absent.

Distribution and Biology. *Durangularia* has been recorded from the southwestern U.S. (Arizona and Texas), Mexico, Guatemala, and Costa Rica. Collection sites range in elevation from 1,800–3,100 m (5,900–10,170 ft). The type species was reared from cynipid galls on *Quercus* (Fagaceae), an association that subsequently was confirmed by J. Powell (in litt.). Adult captures range from March to November.

Remarks. The apparent shape of the uncus varies in slide-mounted genitalia preparations. The U-shaped emargination of the uncus (Fig. 11) is formed by two dorsolateral projections. If these projections are not flattened on the slide, the uncus will appear as in Fig. 10. There are no significant differences in the structure of the uncus for the two species described here.



FIGS. 1–9. Adults of *Durangularia*. **1.** *Durangularia druana* (male, HTP). **2.** *D. druana* (male). **3.** *D. druana* (male). **4.** *D. druana* (female). **5.** *D. giganteana* (male, HTP). **6.** *D. giganteana* (male, PTP). **7.** *D. druana* (head, male). **8.** *D. druana* (head, female). **9.** *D. giganteana* (head, male, HTP).



FIGS. 10–15. Genitalia of *Durangularia*. **10.** *Durangularia druana* (male, phallus below). **11.** *D. giganteana* (male, HTP, phallus below). **12.** *D. druana* (female). **13.** *D. druana* (male vesica, arrows denote two large basal non-deciduous cornuti). **14.** *D. druana* (male gnathos, arrows denote dorsolateral and ventrolateral lobes). **15.** *D. druana* (female sterigma detail). (Figs. 13–15 not to scale).

Durangularia druana (Walsingham, 1914), **comb.n.**

Figs. 1–4, 7–8, 10, 12–16

Tortrix druana Walsingham 1914: 288.
Durangarchips druana: Powell, 1995, in Heppner: 148; Brown, 2005: 263.

Diagnosis. *Durangularia druana* is separated from *D. giganteana* by its smaller size (FWL 6.2–11.5 mm in *druana*, 13.3–15.2 mm in *giganteana*) and forewing color (tan to light brown in *druana* vs. reddish brown in *giganteana*). Males of the two species can be distinguished by the shape of the valva (rhomboid in *D. druana*, rectangular in *D. giganteana*).

Description. Male (Figs. 1–3, 10, 13–14). *Head* (Fig. 7): Vertex tan to orange-brown, with some scales banded apically with brownish gray; frons scaling tan; labial palpus ca. 1.8 times horizontal diameter of compound eye, segment II straight, enlarged apically, rough scaled, tan to brown, some scales banded with dark brownish gray or orange, segment III smooth scaled with dark brown; antenna brown with tan scales, sensory setae 0.5 times flagellomere diameter. *Thorax*: Dorsum tan, some scales banded with orange-brown; legs tan, densely scaled, pro- and mesothoracic legs mottled with dark orange-brown. Forewing (Figs. 1–3, 16) length 6.7–11.5 mm (mean 8.7 mm; n = 24); ground color tan to light brown, some scales banded apically with orange; wing markings (costal strigulae, fasciae)

expressed with scales banded apically with dark brown to black; costal strigulae well expressed in most individuals from base to apex; media fascia expressed in many individuals from costa along distal margin of the discal cell to dorsum with conspicuous dark patch between CuA_2 and A_{1+2}; postmedian and preterminal fasciae expressed in some individuals as mottling in apical half of wing; fringe scales tipped with grayish brown at apex, becoming solid brown at tornus. Hindwing (Fig. 16) pale yellowish brown, mottled with brown to dark brown apically in some individuals; fringe scales pale yellowish brown, banded with brown near apex, becoming solid pale yellow at anal angle. *Abdomen*: Pale yellowish brown. Genitalia (Figs. 10, 13–14) with uncus spatulate, ca. 1.5 times as long as wide, tapering to tegumen, apically bifid with U-shaped invagination; dorsolateral shoulders of tegumen produced; socius as long as uncus, fingerlike, projecting ventrolaterally, setose along basal 0.75; gnathos V-shaped, arms joined distally with variable dorsolateral and ventrolateral lobes (Fig. 14); transtilla a sinuate band, weakly sclerotized medially; valva rhomboid; cucullus sclerotized along costal margin, apex acute, moderately setose in distal 0.5 and along costal margin, anal angle broadly rounded; sacculus well sclerotized, consisting of a basal setose lobe, a medial subtriangular process, and a free distal lobe; juxta a pentagonal plate; phallus ca. as long as valva, curved in distal 0.5; vesica with two large basal non-deciduous cornuti (Fig. 13), ventral surface microtrichiate.

Female (Figs. 4, 12, 15). *Head* (Fig. 8): Vertex and frons as in male; labial palpus ca. 2.7 times horizontal diameter of compound eye, segment II slightly curved, rough scaled with some scales banded apically with dark brownish gray, segment III dark brown; antenna simple, brown with tan scales. *Thorax*: Dorsum and legs as in male. Forewing (Fig. 4) as in male except length 9.8–12.0 mm (mean 10.7 mm; n = 7); hindwing as in male. *Abdomen*: Pale yellowish brown. Genitalia (Fig. 12, 15) with papillae anales simple, unmodified, moderately setose; apophyses anteriores ca. as long as apophyses posteriores; sterigma (Fig. 15) a sclerotized concavity nearly as wide as the apophyses anteriores covered by a microtrichiate extension of sternum VII; ductus bursae 0.5 times length of corpus bursae; corpus bursae elongate, rounded anteriorly; signum absent.

Holotype (Fig. 1) ♂, "7360, from cynipid gall, on *Quercus* sp., Durango. Mex.[ico], iss[ued] Febr[uary] 11. [18]97; 6002, WLSM. 1908; ♂ genitalia on, slide 18. xi 1958, J. F. G. C. 10748; Genitalia slide, by JFGC ♂, USNM 68309; *Tortrix druidana*, Wlsm., ♂ TYPE desc., figd" (USNM).

Additional specimens examined. GUATEMALA: Chimaltenango, Tecpan, [ca. 2286 m], 14°15'N, 90°58'W, 30 Jul 2000 (5 ♂ VBC). MEXICO: Chihuahua: 5 mi W Buenaventura, 7,200 ft, 5 Jul 1986, P. M. Jump, Acc. #1042 (1 ♂ EME). Chiapas: San Cristobal L. C., 6–8 Aug 1965, Flint & Ortiz (2 ♂ USNM, slide USNM 68498). Durango: 30 mi W Durango, 8,000 ft, 3–7 Aug 1972, J. Powell, D. Veirs & C. D. MacNeill (2 ♂ EME), 8,500 ft, 31 Jul 1964, J. Powell (1 ♂ EME, slide JAP2913); 3 mi E Revolcaderos, 11 Aug 1972, J. Powell (1 ♂ EME, slide JAP3912). Oaxaca: Cerezal, Ixtepeji, 2300 m, 7 Nov 1980, E. Welling (1 ♀ EME, slide JAP5534); Matalan, 2 mi S Oaxaca, 28 Jun 1957, J. A. Chemsak & B. J. Rannells (1 ♂ EME, slide JAP2915). Sinaloa: 8 mi W El Palmito, 6,400 ft, 8–12 Aug 1972, J. Powell, D. Veirs & C. D. MacNeill (1 ♂ EME). Sonora: 20 km NW Yecora, 3200 m, 5 Sep 1998 (3 ♂ VBC). Nuevo Leon: Cerro Potosí, 2800 m, 26 Jun 1997 (1♂ VBC); Santiago, 1760 m, 25 21'N, 100 18'W, 25–30 May 2000 (1♂ VBC). Veracruz: Cofre de Perote, 3300 m, 4 Jun 1997 (12 ♂ VBC). UNITED STATES: Arizona: Cochise County: Copper Canyon, 6,000 ft, 15 Apr 1986, J. Powell, JAP 86D49 (1 ♂ 1 ♀ EME); Huachuca Mountains, Ash Canyon, 5,800 ft, 13 Apr 1986, Powell & Wagner, JAP 86D47, 86D49 (2 ♀ EME, slide JAP6239); Coronado Nat. Forest, Chiricahuas, Upper Pinery Canyon Campground, 30 Jul–2 Aug 1999, P. A. Opler & E. Buckner, BOLD Proc. ID:LNAUS2436-13 (1 ♂ CSU), 6,800 ft, 4 Sep 1992, R. Leuschner (1 ♂ USNM, slide TMG662); Paradise, 16–23 Aug (1 ♂ USNM); Miller Canyon, Huachuca Mountains, 5,800ft, 14 Apr 1986, Powell & Wagner, emgd. 15 Jun 1986, JAP 86D49, reared from cynipid gall on *Quercus* (1 ♂ EME), 12 Apr 1988, J. A. Powell (1 ♂ EME). Texas: Jeff Davis County: Davis Mountains Resort, 5,800 ft, 3 Apr 2004, D. G. Marqua, BOLD Proc. ID:LNAUS2434-13 (1 ♂ USNM); Davis Mountains, Mt. Locke, 6,700 ft, 10 Jun 1969, A. & M. E. Blanchard (2 ♀ USNM, slide USNM 97904), 30 Aug 1969 (1 ♂ 1 ♀ USNM, slide USNM 144866), 21 Oct 1973 (5 ♂ USNM, dissection TMG650, slide USNM 144865); Davis Mountains, 5 mi SE Livermore, 6,000 ft, 4 Oct 1969, A. & M. E. Blanchard (1 ♂ USNM, slide USNM 97905).

Distribution and Biology. This species has been recorded from Guatemala; the states of Chihuahua, Chiapas, Durango, Oaxaca, Sinaloa, Sonora, and Veracruz in Mexico; and Arizona and Texas in the U.S. Most collection sites are between 1,800–3,300 m (5,900–10,826 ft). The type and one specimen collected in the Huachuca Mountains, Arizona by J. Powell were reared from cynipid galls on *Quercus*. Adults have been captured from April to November.

Remarks. The "TYPE" label affixed to the holotype by Walsingham suggests that he originally intended to name this species "Tortrix druidana."

Durangularia giganteana, **sp.n.**

Figs. 5–6, 9, 11

Diagnosis. *Durangularia giganteana* is separated from *D. druana* by its larger size (FWL 13.3–15.2 mm in *giganteana*, 6.2–11.5 mm in *druana*) and forewing color (reddish brown in *giganteana*, tan to light brown in *druana*). Male genitalia of the two species can be distinguished by the shape of the valva (rectangular in *giganteana*, more attenuate distally in *druana*). The female of *D. giganteana* is unknown.

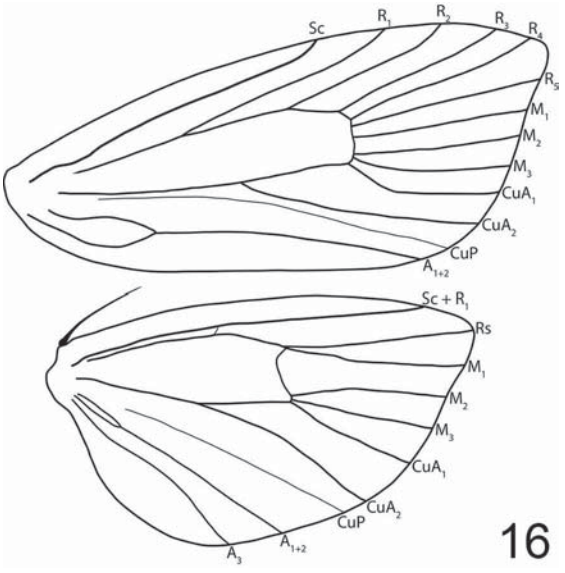


FIG. 16. Wing venation of *D. druana*.

Description. Male (Figs. 5–6, 9, 11). *Head* (Fig. 9): Vertex scales mixed brown and brownish tan; labial palpus ca. 2.2 times horizontal diameter of compound eye, segment II slightly curved, enlarged medially, rough scaled with tan to reddish brown, some scales banded with dark brownish gray, segment III smooth scaled with reddish brown; antenna brown with tan and reddish-brown scales, fasciculate-ciliated, sensory setae 0.75 times flagellomere diameter in male. *Thorax*: Dorsum scales brown and brownish tan, some banded with red-brown; legs tan, densely scaled, pro- and mesothoracic legs mottled with reddish brown. Forewing (Figs. 5–6) length 13.3–15.2 mm (mean 14.3 mm; n = 2); forewing ground color reddish brown; wing markings (costal strigulae, fasciae) gray to pale dark brown; costal strigulae weakly expressed as gray scales along costa; subbasal and median fasciae weakly expressed as rows of gray scales running from costa to dorsum; postmedian and preterminal fasciae expressed as weak mottling in the apical half of the wing; fringe scales gray to pale dark brown basally, tan apically. Hindwing pale yellowish brown, mottled with brown along the apex and outer margin; fringe scales pale yellowish brown, becoming darker and banded with brown near apex. *Abdomen*: Genitalia (Fig. 11) with uncus spatulate, ca. 2 times as long as wide, tapering to tegumen, apically bifid with deep U-shaped invagination; dorsolateral shoulders of tegumen slightly produced; socius fingerlike, projecting ventrolaterally, with long setae from base until before apex; gnathos V-shaped, arms joined distally forming numerous ridges; transtilla a weakly sclerotized sinuate band; valva rectangular, parallel-sided; cucullus sclerotized along costal margin, apex 90°, densely setose in distal 0.5, anal angle slightly rounded; sacculus well sclerotized, consisting of a basal setose lobe, a medial subtriangular process, and a large free distal lobe; juxta a pentagonal plate; phallus ca. as long as valve, curved in distal 0.5; vesica with two large basal non-deciduous cornuti, ventral surface microtrichiate.

Female. Unknown.

Holotype (Fig. 5) ♂, "Est. Cuerici, Sendero al Mirador, 4.6 Km al E. de Villa Mills, San Jose, Costa Rica, 2640 m, 17-22 Mar 1996, A. Picado, de Luz, L_S_389700_499600 #7026; Costa Rica INBIO CR1002, 431190; genitalia ♂, MCC-4; Genitalia slide, by MCC ♂, USNM 137,144" (INBio).

Paratype. COSTA RICA: Cartago Province: 1 km NE Cerro Asuncion, Cerro de la Muerte, 3100 m, 8 Apr 1984, D. H. Janzen & W. Hallwachs (1 ♂ INBio, slide USNM 137145).

Distribution and Biology. This species has been recorded from the provinces of Cartago and San José in Costa Rica at elevations of 3,100 and 2,640 m (10,170 and 8,660 ft), respectively. Adults were captured in March and April. Larval hosts are unknown.

Submitted for publication 4 September 2015; revised and accepted 21 January 2016.

ACKNOWLEDGEMENTS

We thank the following for providing access to specimens in their care: Peter Oboyski (EME), Eugenia Phillips-Rodriguez (INBio), Paul Opler (CSU), and Vitor Becker (VBC). Richard Brown, Mississippi Entomological Museum, provided access to Obraztsov's unpublished manuscripts. Jerry Powell (EME) provided useful discussion on *D. druana* and valuable review comments. Jason Dombroskie, Cornell University Insect Collection, also provided valuable review comments.

LITERATURE CITED

ANZALDO, S., J. DOMBROSKIE AND J. W. BROWN. 2014. Morphological variation, taxonomic distribution, and phylogenetic significance of cornuti in Tortricinae (Lepidoptera: Tortricidae). Proc. Entomol. Soc. Wash. 116: 1–31.

BROWN, J. W. 1990. Taxonomic distribution and phylogenetic significance of the male foreleg hairpencil in the Tortricinae (Lepidoptera: Tortricidae). Entomol. News 101: 109–116.

BROWN, J. W. 2005. World catalogue of insects: Tortricidae (Lepidoptera), Vol. 5. Apollo Books, Stenstrup, Denmark. 741 pp.

BROWN, J. W. AND J. A. POWELL. 1991. Systematics of the *Chrysoxena* group of genera (Lepidoptera: Tortricidae: Euliini). Univ. Calif. Pub. Entomol. 111, 87 pp.

GILLIGAN, T. M., D. J. WRIGHT AND L. D. GIBSON. 2008. Olethreutine moths of the Midwestern United States. An Identification Guide. Ohio Bio. Survey Bull. New Series. Vol. XVI, No. 2. 334 pp.

HORAK, M. 1984. Assessment of taxonomically significant structures in Tortricinae (Lep., Tortricidae). Mitt. Schweiz. Entomol. Ges. 57: 3–64.

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE (ICZN). 1999. International Code of Zoological Nomenclature, 4th edition. The International Trust for Zoological Nomenclature, London, United Kingdom, 306 pp.

POWELL, J. A., J. RAZOWSKI AND J. W. BROWN. 1995. Tortricoidea: Tortricidae, pp. 138–157. *In* J. B. Heppner (ed.). Atlas of Neotropical Lepidoptera. Checklist: Part 2. Hyblaeoidea-Pyraloidea-Tortricoidea. Assoc. Trop. Lepid., Sci. Publ., Gainesville.

REGIER, J., J. BROWN, C. MITTER, J. BAIXERAS, S. CHO, M. CUMMINGS, AND A. ZWICK. 2012. A molecular phylogeny for the leaf-roller moths (Lepidoptera: Tortricidae) and its implications for classification and life history evolution. PLoS ONE, 7, e35574.

WALSINGHAM, LORD T. DE GREY (1914) [1909-1915] Insecta. Lepidoptera-Heterocera, Vol. IV., pp. 1–482. *In* F. D. Godman and O. Salvin (eds.). Tineina, Pterophorina, Orneodina, and Pyralidina and Hepialina (part). Biologia Centrali-Americana. R. H. Porter, London.

Journal of the Lepidopterists' Society
70(2), 2016, 145–152

NEW INSIGHTS INTO THE SYSTEMATICS OF THE GENUS *POLYURA* BILLBERG, 1820 (NYMPHALIDAE, CHARAXINAE) WITH AN EMPHASIS ON THE *P. ATHAMAS* GROUP

EMMANUEL F.A. TOUSSAINT*
Department of Ecology & Evolutionary Biology & Division of Entomology, Biodiversity Institute, University of Kansas, Lawrence, KS 66045, USA
2SNSB-Bavarian State Collection of Zoology, Münchhausenstraße 21, 81247 Munich, Germany
*Corresponding author Email: Toussaint@ku.edu.

JÉRÔME MORINIÈRE, ATHENA LAM
2SNSB-Bavarian State Collection of Zoology, Münchhausenstraße 21, 81247 Munich, Germany

AND

MICHAEL BALKE
2SNSB-Bavarian State Collection of Zoology, Münchhausenstraße 21, 81247 Munich, Germany
3GeoBioCenter, Ludwig-Maximilians University, Munich, Germany

ABSTRACT. The genus *Polyura* comprises 32 species across the Oriental Region and the Indo-Australian archipelago. Its taxonomy and systematics have recently been studied using a comprehensive molecular phylogeny. Yet, certain elements of its fauna were not available for in depth study. Here, we provide a denser taxon sampling and reconstruct a new phylogenetic hypothesis for the *P. athamas* group, a morphologically cryptic complex. The known geographic range of the genus is extended to Eastern Pakistan where two species fly in sympatry. Endemics from Tawi Tawi archipelago off the northern tip of Borneo have more affinities with the fauna of Sunda Islands rather than with the Philippines. Our results also suggest three taxonomic changes; the recognition of a new species and the transfer of two subspecies to a different species.

Additional key words: Cryptic diversity; Pakistan; *Polyura attalus*; Sulu arc; Tawi Tawi archipelago.

The Nawab butterflies belong to the genus *Polyura* Billberg, 1820, a clade restricted to the Oriental Region and the Indo-Australian archipelago (IAA) (Smiles 1982, Toussaint et al. 2015). These charismatic butterflies are closely related to the genus *Charaxes* Ochsenheimer, 1816 which primarily occurs in the Afrotropics although about 30 species are found in the IAA. However, the relationships within the tribe Charaxini remain equivocal and additional work is needed to unveil the placement of *Polyura* with respect to *Charaxes* (Aduse-Poku et al. 2009, Müller et al. 2010, Toussaint et al. 2015). Recently, the first molecular phylogenetic hypothesis of *Polyura* was built to investigate species boundaries within the genus (Toussaint et al. 2015). Using a multimarker dataset and a comprehensive taxonomic coverage, Toussaint et al. (2015) recognized 32 valid species using an array of molecular species delimitation methods. Among the three species groups found in the genus, two new species were recognized, four subspecies were raised to species and one species was synonymized. Despite this stride toward a better understanding of the taxonomy and systematics of the genus, a few cases remain where the taxonomic status of genetic groupings is contentious to some degree (Toussaint et al. 2015). This is especially true in two species groups; the *P. athamas* (Drury, 1770) group and the *P. pyrrhus* (L., 1758) group. In the former, a dramatic revision was undertaken with the description of a new species *Polyura paulettae* (Toussaint 2015) and the elevation (re-elevation) of three subspecies to species rank, namely *P. alphius* (Staudinger 1886), *P. bharata* (Felder & Felder, 1867) and *P. luzonica* (Rothschild 1899). However, there is still a need to complete this taxonomic and systematic revision in the light of a denser geographic and taxonomic sampling.

The *P. athamas* group is notoriously difficult to work with because most species share a rather similar appearance and lack morphological synapomorphies (Smiles 1982, Toussaint et al. 2015). Two main monophyletic clades are found in the *P. athamas* group; (i) one including two very distinctive species with a blue wing pattern, *P. schreiber* (Godart 1824) and *P. luzonica*, and (ii) another including multiple species of elusive morphological limits, hereafter referred to as the "green species complex" (GSC) as most species present a rather similar appearance with green discal bands on the upperside of the wings (Smiles 1982, Toussaint et al. 2015). There is a clear genetic demarcation between

species of the GSC, and with morphology offering little for species delimitation, their correct identification is greatly facilitated by genetic assignment (Toussaint et al. 2015). Although most of the difficult cases in the *P. athamas* group were addressed in Toussaint et al. (2015), some challenging cases still need to be tackled. It is the case with *Polyura schreiber* whose Philippine populations were recognized as a separate species, *P. luzonica*. *Polyura schreiber* has a very widespread distribution, ranging from India to Borneo, and the demarcation of Philippine populations was not extremely surprising as this archipelago has a very different geological origin compared to the Indomalayan peninsula (Figure 1). Interestingly, some subspecies occur between the Philippines and Sunda, namely *P. schreiber lindae* Schroeder & Treadaway, 2008 from Tawi Tawi archipelago and *P. schreiber praedicta* (Schroder & Treadaway 1980) from Palawan. The latter was included in Toussaint et al. (2015) where it was recovered as sister to all other *P. schreiber* populations, a relationship nicely illustrating the geological affinity of Palawan with the Sunda arc (Hall 2012, 2013). Discovering whether the populations of *P. schreiber lindae* belong to *P. luzonica* or *P. schreiber* is important for understanding the biogeography of this species complex. In the GSC, there is some haziness regarding the geographic distribution of extant described species. This is especially the case for *P. paulettae* Toussaint, 2015, a genetically distinct lineage that belongs to a morphologically homogeneous group also comprising *P. agraria* (Swinhoe 1887), *P. alphius*, *P. athamas* and *P. bharata*. *Polyura paulettae* was described from specimens collected in Myanmar and Thailand, but it is not clear if its present distribution is actually broader than these two countries. Toussaint et al. (2015) revised the geographic distributions of most species belonging to the GSC, however the ranges of a few species are still nebulously understood and there is a need to refine these in order to fully understand the evolution of these lineages.

Here, we sampled and sequenced the holotype of the rare *P. schreiber lindae* from Tawi Tawi, as well as five specimens of the GSC from new localities, in order to investigate their placement with respect to the phylogenetic framework of Toussaint et al. (2015). We aim to (*i*) reconstruct the phylogenetic relationships among species of the *P. athamas* group; (*ii*) assign the newly sequenced specimens to extant species; (*iii*) provide revised maps of distribution for the GSC as well as revise the taxonomy of the group where needed.

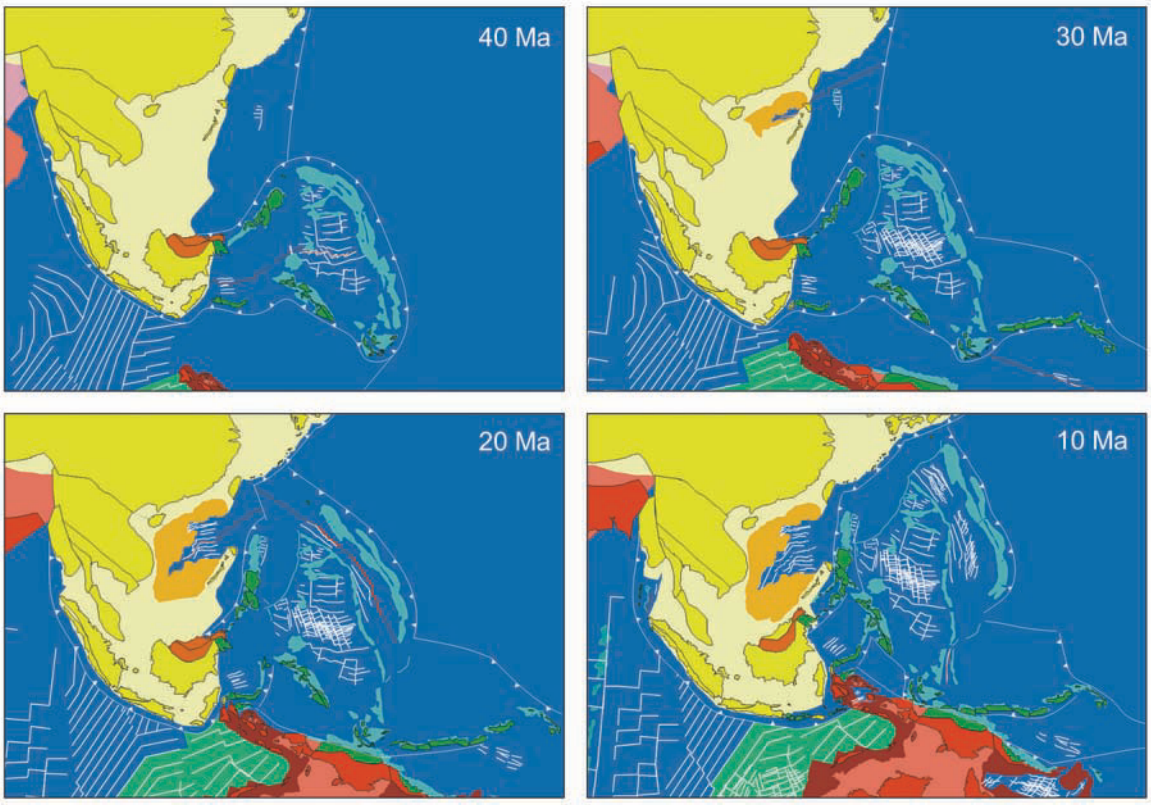


FIG. 1. Geological history of the Sunda region in the Cenozoic

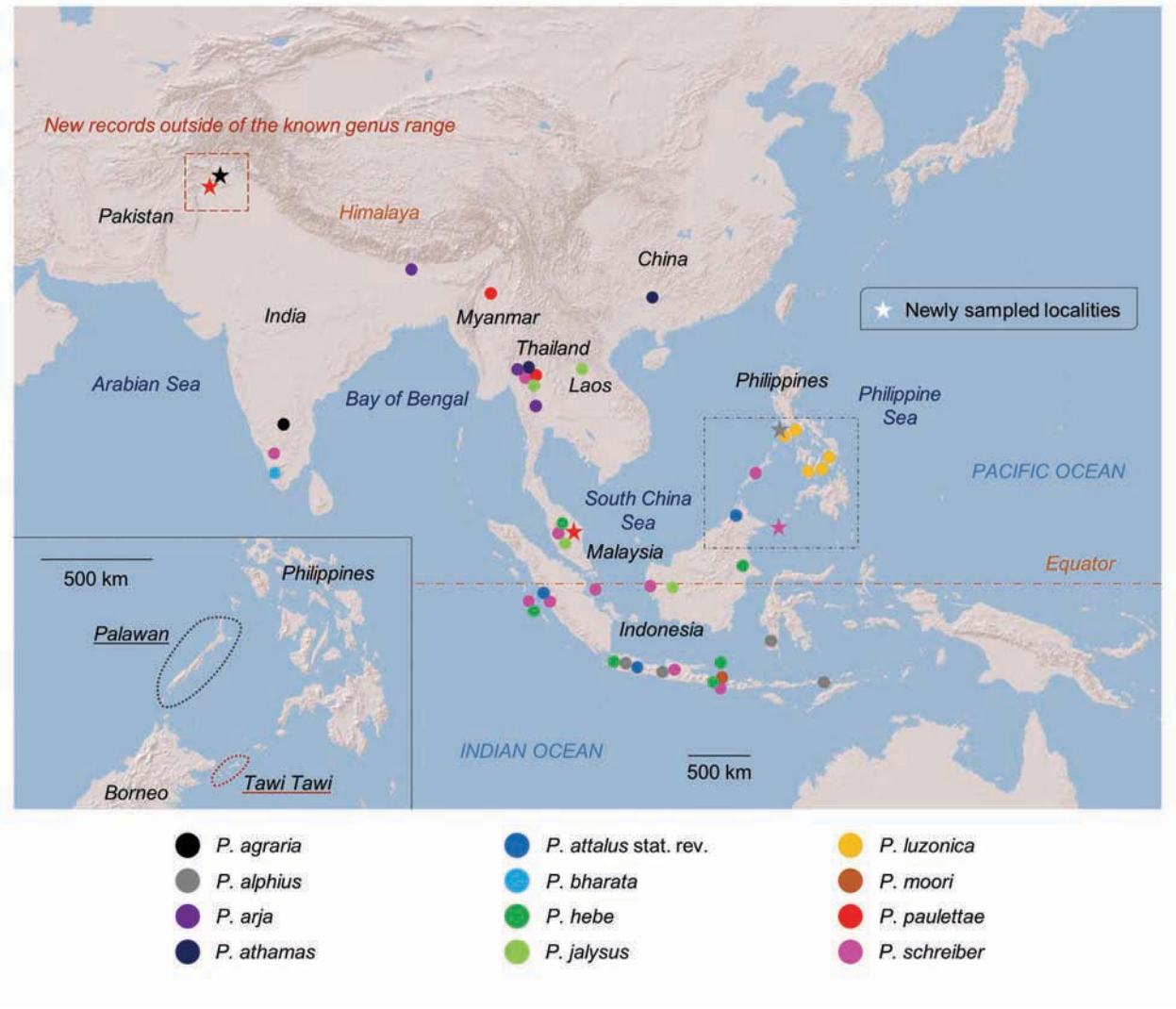


FIG. 2. Geographic sampling of the *P. athamas* group used in this study

METHODS

Taxon Sampling and Molecular Biology. We retrieved sequence data from Toussaint et al. (2015) and added several specimens of species belonging to the *P. athamas* group (Figure 2, Table 1). Total genomic DNA was extracted from leg tissues of dried collection specimens using the DNeasy kit (Qiagen, Hilden, Germany). Using PCR protocols described in Wahlberg and Wheat (2008) and Müller et al. (2010), we amplified and sequenced the following gene fragments: cytochrome oxidase subunit 1 (CO1, 471 bp), NADH dehydrogenase subunit 5 (ND5, 417 bp), ribosomal protein S5 (Rps5, 573 bp) and Wingless (396 bp). The DNA sequences were edited in Geneious R8 (Biomatters, http://www.geneious.com/), aligned using MUSCLE (Edgar 2004) and reading frames were checked under Mesquite 3.02 (http://mesquiteproject.org). The different datasets used to infer phylogenetic relationships were generated under Mesquite. *Charaxes viola* (Butler 1866) was used as outgroup in all analyses. All sequences were deposited in GenBank (accession Nos. KU170651–KU170664).

Molecular phylogenetics. We used Bayesian Inference (BI) to reconstruct the phylogenetic relationships of the *P. athamas* group. Partitions and corresponding optimal models of substitution were searched under PartitionFinder 1.1.1 (Lanfear et al. 2012) using the greedy algorithm, and the mrbayes set of models. All genes were divided by codon positions for a total of 12 partitions to analyze. The Akaike

TABLE 1. List of specimens newly sequenced for this study

Genus	Species	Subspecies	Country	Locality	Code	CO1	ND5	Rps5	Wingless
Polyura	*agraria*	-	Pakistan	Margalla hills, Islamabad	ET0252	-	X	-	X
Polyura	*alphius*	*acuta*	Philippines	Mindoro	ET0242	X	X	X	-
Polyura	*paulettae*	-	Malaysia	Cameron Highlands, Pahang	ET0070	X	X	-	-
Polyura	*paulettae*	-	Pakistan	Margalla hills, Islamabad	ET0253	X	X	X	X
Polyura	*paulettae*	-	Pakistan	Margalla hills, Islamabad	ET0254	X	X	X	X
Polyura	*schreiber*	*lindae*	Philippines	Tawi Tawi	ET0251	-	X	-	-

Information Criterion corrected (AICc) was used to compare the fit of the different models. The BI analyses were performed using MrBayes 3.2.3 (Ronquist et al. 2012). Two simultaneous and independent runs consisting of eight Metropolis-coupled Markov chain Monte Carlo (MCMC, one cold and seven incrementally heated) running 20 million generations were used, with a tree sampling every 1000 generations to calculate posterior probabilities (PP). In order to investigate the convergence of the runs we investigated the split frequencies and Effective Sample Size (ESS) of all the parameters, and plotted the log-likelihood of the samples against the number of generations in Tracer 1.5 (http://BEAST.bio.ed.ac.uk/Tracer). A value of ESS>200 was acknowledged as a good indicator of convergence. All posterior trees predating the time needed to reach a log-likelihood plateau were discarded as burn-in, and the remaining samples were used to generate a 50% majority rule consensus tree.

RESULTS

Phylogenetic Relationships. Under the AICc, PartitionFinder suggested a partitioning scheme comprised of seven partitions (Table 2). All MrBayes runs based on this partitioning scheme converged after only a few million generations and all parameters had an ESS>>200. The 50% majority rule consensus tree is presented in Figure 3 along with posterior probability nodal support.

Overall the phylogenetic tree is well resolved and nodal support is robust (Figure 3). We find the three species groups monophyletic with strong support. Within the *P. athamas* group, all extant described species are found monophyletic with strong support except *Polyura athamas* whose populations from mainland Asia (ssp *athamas*) are found sister to *P. bharata* + *P. arja* (Felder & Felder, 1867) + *P. hebe* (Butler, 1866) + the populations of *P. athamas* from Sunda (ssp *attalus* and *uraeus*). The latter are found sister to *P. arja* + *P. hebe* in a more derived part of the tree.

The endemic subspecies *P. schreiber lindae* from the Tawi Tawi archipelago is found nested in a derived clade of *P. schreiber* (Figure 3) and therefore has no affinity with the Philippine endemic *P. luzonica*. The three specimens of *Polyura* from Pakistan are found in two different parts of the tree. Two are recovered as belonging to *P. paulettae* along with the new specimen from Malaysia, whereas the remaining specimen from Pakistan is found within *P. agraria*. Finally, the Philippine specimen of *P. athamas acuta* from Mindoro is found as sister to *P. alphius*.

Taxonomic changes. Our results have three taxonomic consequences in the GSC as *P. athamas* was found polyphyletic. The Philippine endemic subspecies *P. athamas acuta* is found as sister to *P. alphius*, a species found in Sumatra, Java, the Lesser Sunda Islands and Sulawesi. Since we did not sample the other Philippine endemic subspecies, *P. athamas palawanica*, recognizing *P. athamas acuta* as a distinct valid species would be premature. Otherwise, the populations of *P. athamas* from Sunda Islands are found sister to *P. arja* and *P. hebe* as previously recovered in Toussaint et al. (2015). In this paper these populations were not elevated to species status because molecular species delimitation methods yielded incongruent results and because the nodal support of the entire *P. athamas*

TABLE 2. Best partitioning scheme for the molecular dataset as recovered under PartitionFinder

	Partition	Substitution model
P1	CO1 pos.1 + ND5 pos.1	GTR+Γ
P2	CO1 pos.2 + ND5 pos.2	HKY+I
P3	CO1 pos.3	GTR+Γ
P4	ND5 pos.3	GTR+I
P5	RPS5 pos.1 + RPS5 pos.2 + WGL pos.1 + WGL pos.2	JC+I
P6	RPS5 pos.3	HKY+Γ
P7	WGL pos.3	K80+Γ

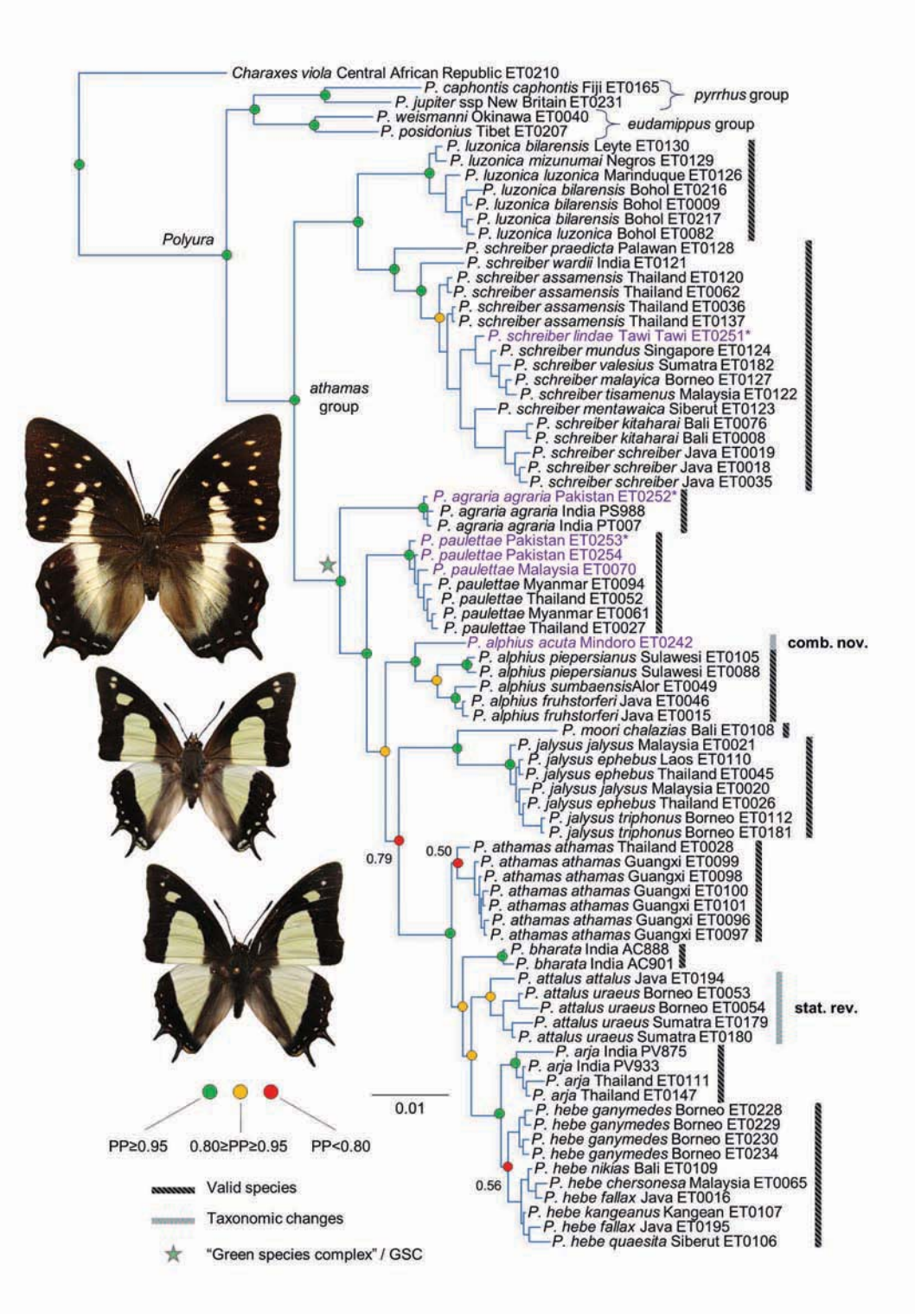


FIG. 3. Bayesian molecular phylogeny of the *Polyura athamas* group. The phylogenetic tree presented is a 50% majority rule consensus of post burnin posterior trees from the MrBayes analysis based on the best partitioning scheme selected under PartitionFinder (Table 2). Nodal support is highlighted for the major nodes of the phylogeny following the color code shown at the bottom left of the figure. Habitus of three specimens sampled in this study are presented as indicated with asterisks after the name of the specimens in the phylogeny. From top to bottom: *Polyura schreiber lindae* from Tawi Tawi archipelago, *Polyura agraria* from Pakistan and *Polyura paulettae* from Pakistan.

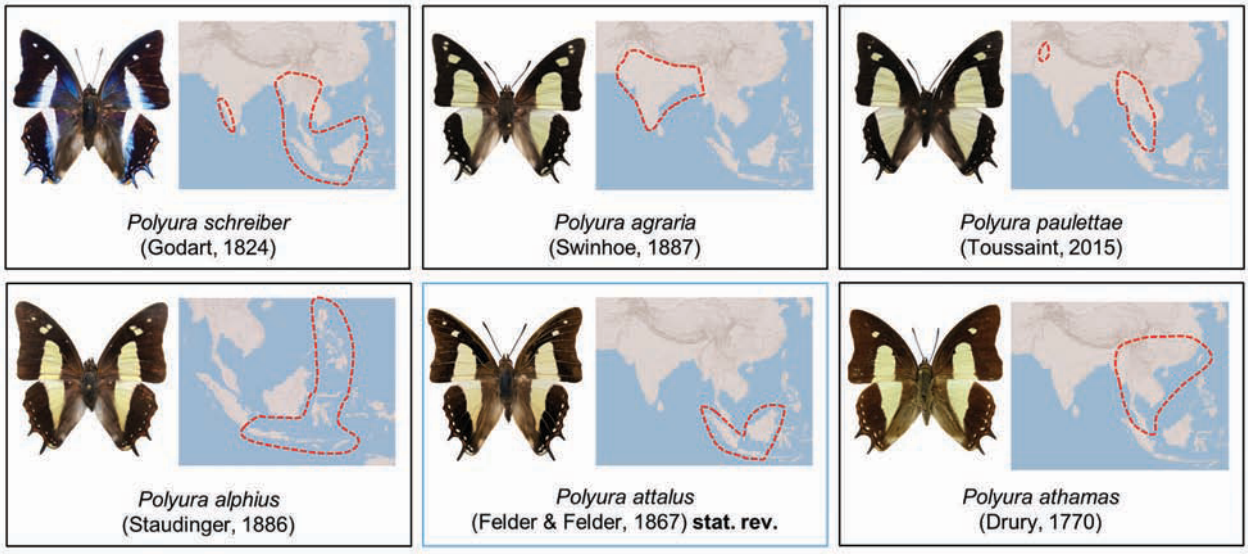


FIG. 4. Updated distribution maps of the species belonging to the *P. athamas* group

group was fairly low. Here, we recover a better resolution and better nodal supports compared to Toussaint et al. (2015). Moreover both clades from continental Asia and from Sunda Islands have been shown to be clearly differentiated genetically even when considering the nuclear genome only (Toussaint et al. 2015). Based on our results and need of a taxonomic act to solve the paraphyly of *P. athamas* we suggest the following changes:

POLYURA BILLBERG, 1820

Polyura attalus (Felder & Felder), 1867, **new status**

Charaxes attalus Felder & Felder, 1867: 438.
Eulepsis attalus (Felder & Felder): Moore 1896: 263.
Eulepsis athamas attalus (Felder & Felder): Rothschild & Jordan 1899: 257.
Eriboea athamas attalus (Felder & Felder): Fruhstorfer, 1914: 719; Roepke 1932: 95.
Charaxes athamas attalus (Felder & Felder): Roepke 1938: 348.
Polyura athamas attalus (Felder & Felder): Stichel 1939: 560; Smiles 1982: 172.

Polyura attalus uraeus (Rothschild), 1899, **new combination**

Eulepsis athamas uraeus Rothschild, 1899: 254.
Eriboea athamas uraeus (Rothschild): Fruhstorfer, 1914: 719.
Polyura athamas uraeus (Rothschild): Stichel 1939: 558; Smiles 1982: 169.

Polyura alphius acuta (Rothschild, 1899), **new combination**

Eulepis athamas acutus Rothschild, 1899: 256.
Eriboea athamas acutus (Rothschild): Fruhstorfer, 1914: 720.
Polyura athamas acutus (Rothschild): Stichel 1939: 563.
Polyura athamas acuta (Rothschild): Smiles 1982: 171.

DISCUSSION

Our phylogenetic reconstruction provides a clear resolution for the *P. athamas* group with interesting results regarding both species geographic ranges and taxonomy. First, we find that the endemic population from Tawi Tawi archipelago belongs to *P. schreiber* and is closely related with other populations from Borneo, Malaysia and Singapore. This pattern is similar to the one found in *P. schreiber praedicta* endemic to Palawan. The latter is found sister to all other populations of *P. schreiber* and therefore is more closely related to the fauna of Sunda than to the Philippine one. The affinity between this island endemic and the fauna of Sunda is congruent with the geological history of the region (Figure 1). Indeed, Palawan is mainly composed of continental margin material and is closely related to the Sunda shelf (Hall 2012, 2013). As a result, Palawan was likely connected with the rest of the Sunda shelf by land bridges during periods of decreased sea level (Hall 2012, 2013). Because *P. schreiber praedicta* is found as sister to all other *P. schreiber* populations, this subspecies might constitute a trace of a colonization

FIG. 5. Habitus of *Polyura attalus* (Felder & Felder, 1867) new status from Java. Dorsal and ventral pictures of a dry specimen from West Java stored at the Museum of Natural History of Toulouse (France). Picture credit: Didier Descouens.

event from the Philippines to continental Asia and the Sunda shelf. In the case of *P. schreiber lindae*, the geological history of the Tawi Tawi archipelago differs greatly from that of Palawan (Figure 1). The Tawi Tawi archipelago belongs to the Sulu arc, a young volcanic chain, which was a subduction-related arc from the Middle to Late Miocene (Hall 2012, 2013). The recent volcanic activity in this region has a somewhat unusual character: it is not obviously related to subduction and there is no subducted slab or seismicity beneath the islands today (Hall pers. comm.). The Tawi Tawi archipelago therefore has no clear geological affinity with either the Sunda arc or the Philippines. With respect to the directionality of biogeographic events, it is interesting to find that part of the fauna dwelling in this archipelago is more closely related to the fauna of Sunda than to the Philippines one. The Tawi Tawi archipelago was likely colonized from the Sunda shelf during periods of low sea level. Such sea level fluctuations have been recorded during the Quaternary climate change (QCC) that began 2.6 million years ago (Ma) (Voris 2000). The rather low genetic divergence between Tawi Tawi endemics and other *P. schreiber* populations, as well as the close relationships of this taxon with Sunda populations, might indicate that the origin of this subspecies could stem from the QCC and be associated with isolation caused by sea level fluctuations. Yet, the hypotheses regarding the origin and evolution of Palawan and Tawi Tawi endemics remain to be tested using a dated phylogeny and proper models of historical biogeography.

Second, we successfully assigned the Pakistani specimens to *P. agraria* and *P. paulettae*, two lineages that were not known to occur in sympatry. The presence of *P. agraria* in Eastern Pakistan is not surprising as this species flies throughout India. However, *P. paulettae* was only known from Thailand and Myanmar, from which this species was recently described (Toussaint et al. 2015). This finding, along with the assignment of a new specimen from Malaysia to *P. paulettae*, suggests that this species might be much more widespread than previously thought. This is relatively surprising considering that this is a recently described species and clearly highlights the complexity of this morphologically homogenous group. These results extend the range of *Polyura* westward to Pakistan (Figure 4). Additional fieldwork in India is needed to fully comprehend the distribution of *Polyura paulettae* as it presently has a disjunct distribution between Eastern Pakistan and Myanmar. Knowing if this gap is an artefact due to a sampling bias, or if it is real, would be crucial to understand the evolution of this species in Indomalaya. From our results we can hypothesize that the present-day disjunction is likely to be due to a sampling bias and *P. paulettae* should be present in India because we find a very shallow genetic difference between the populations across its geographic range. This hypothesis however remains to be tested in a proper phylogeographic framework with a denser geographic sampling.

In this study we revised the geographic distribution, taxonomy and systematics of *Polyura* species found in the *P. athamas* group. The genus range is extended to Pakistan and six species have an updated distribution. Most notably, the recently described *P. paulettae* has a putative disjunct distribution that requires additional sampling effort in India. *Polyura alphius* has also a wider geographic range encompassing the Philippines where the subspecies *P. alphius acuta* new combination is found. Finally, the "umbrella" species *P. athamas* has

a reduced distribution in mainland Asia, only because its populations from the Sunda Islands are reinstated as a valid species *P. attalus* new status. With these new insights, the *P. athamas* group now has a clearer taxonomy that will allow the study of biogeography and diversification dynamics of the entire genus.

ACKNOWLEDGEMENTS

We would like to warmly thank Bernard Turlin and Colin Treadaway for respectively providing legs of Pakistani specimens and of the unique female holotype of *P. schreiber lindae* from Tawi Tawi islands. We also want to particularly thank Robert Hall for fruitful discussion regarding the geological history of the Sulu arc. We would like to acknowledge Keith Summerville for his editorial work, Niklas Wahlberg for comments that improved an earlier version of this manuscript, and Stephen Baca for kindly revising the language. This work was supported by the German Science Foundation (DFG) grant BA2152/20-1. The authors declare no conflict of interest.

LITERATURE CITED

ADUSE-POKU, K., E. VINGERHOEDT & N. WAHLBERG. 2009. Out-of-Africa again: A phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. Mol. Phylogenet. Evol. 53:463–478.

EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

FELDER, C., R. FELDER. 1867. Reise der österreichischen Fregatte Novara um die Erde. Lepidoptera. Rhopalocera. 2: 379–535. Wien.

FRUHSTORFER, H. 1914. Familie: Nymphalidae. *In* Seitz, A. Die Gross-Schmetterlinge der Erde. Die Grossschmetterlinge des Indo-australischen Faunengebietes. 9: 453–766. Stuttgart.

HALL, R. 2012. Late Jurassic–Cenozoic reconstructions of the Indonesian region and the Indian Ocean. Tectonophys.570:1–41.

HALL, R. 2013. The palaeogeography of Sundaland and Wallacea since the Late Jurassic. J. Limnol. 72:1–17.

LANFEAR, R., B. CALCOTT, S. Y. HO & S. GUINDON. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29:1695–1701.

MOORE, F. 1896. Lepidoptera Indica. 2: 1–274. London.

MÜLLER, C. J., N. WAHLBERG & L. B. BEHEREGARAY. 2010. 'After Africa': the evolutionary history and systematics of the genus *Charaxes* Ochsenheimer (Lepidoptera: Nymphalidae) in the Indo–Pacific region. Biol. J. Linn. Soc. 100:457–481.

ROEPKE, W. 1932. De Vlinders van Java. [4]+5–142 pp. Batavia.

ROEPKE, W. 1938. Rhopalocera Javanica. Geillustreerd overzicht der Dagvlinders van Java. 3: [231–235]+236–262. Wageningen.

RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D. L. AYRES, A. DARLING, S. HÖHNA, B. LARGET, L. LIU, M. A. SUCHARD & J. P. HUELSENBECK. 2012. MrBayes 3.2:Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Syst. Biol. 61:539–542.

ROTHSCHILD, W. & K. JORDAN. 1899. A monograph of *Charaxes* and the allied prionopterous genera. Novit. Zool. 6: 220–286.

SMILES, R. L. 1982. The taxonomy and phylogeny of the genus *Polyura* Billberg (Lepidoptera: Nymphalidae). Bull. Br. Mus. (Nat. Hist.) Entomol. 44:115–237.

STICHEL, H. 1939. In: Bryk, F. Lepidopterorum Catalogus. 30(93):543–794.

TOUSSAINT, E. F. A., J. MORINIÈRE, C. J. MÜLLER, K. KUNTE, B. TURLIN, A. HAUSMANN & M. BALKE. 2015. Comparative molecular species delimitation in the charismatic Nawab butterflies (Nymphalidae, Charaxinae, *Polyura*). Mol. Phylogenet. Evol. 91:194–209.

VORIS, H. K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Biogeogr. 27:1153–1167.

WAHLBERG, N. & C. W. WHEAT. 2008. Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of Lepidoptera. Syst. Biol. 57:231–242.

Submitted for publication 4 August 2015; revised and accepted 20 November 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 153–157

LARVAL POLYCHROMATISM IN THE NEOTROPICAL HAIRSTREAK *STRYMON BUBASTUS* (STOLL) (LYCAENIDAE, THECLINAE, EUMAEINI) ASSOCIATED WITH TWO NEWLY DOCUMENTED HOST PLANTS IN THE ATACAMA DESERT

HÉCTOR A. VARGAS*, MARCELO VARGAS-ORTIZ, DANTE BOBADILLA

Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Casilla 6-D, Arica, Chile
*Corresponding author: havargas@uta.cl; lepvargas@gmail.com

MARCELO DUARTE

Museu de Zoologia, Universidade de São Paulo, Avenida Nazaré 481, Ipiranga, 04263-000 São Paulo, SP, Brazil

AND

WILSON HUANCA-MAMANI

Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile

ABSTRACT. Host-associated larval polychromatism is described for the first time for the Neotropical hairstreak *Strymon bubastus* (Stoll, 1780) (Lepidoptera, Lycaenidae, Theclinae, Eumaeini) based on larvae collected in the Atacama Desert of northern Chile on inflorescences of *Phyla nodiflora* (L.) Greene (Verbenaceae) and *Waltheria ovata* Cav. (Malvaceae) and reared to adult. This is the first record of a host plant in the family Verbenaceae for *S. bubastus*. Although other Malvaceae already have been recorded as its hosts, this is the first record of it feeding on *W. ovata*. Identical sequences (n=19) of the DNA barcode fragment (657 base pairs) of the cytochrome c oxidase subunit I (COI) gene were obtained from larvae collected on the two plants, providing additional support for conspecificity. However, deep divergence (>2%) was found among these sequences and others from geographically distant localities of the Neotropics. Deep divergence could be associated with phenotypic differentiation of *S. bubastus* over its wide geographic range.

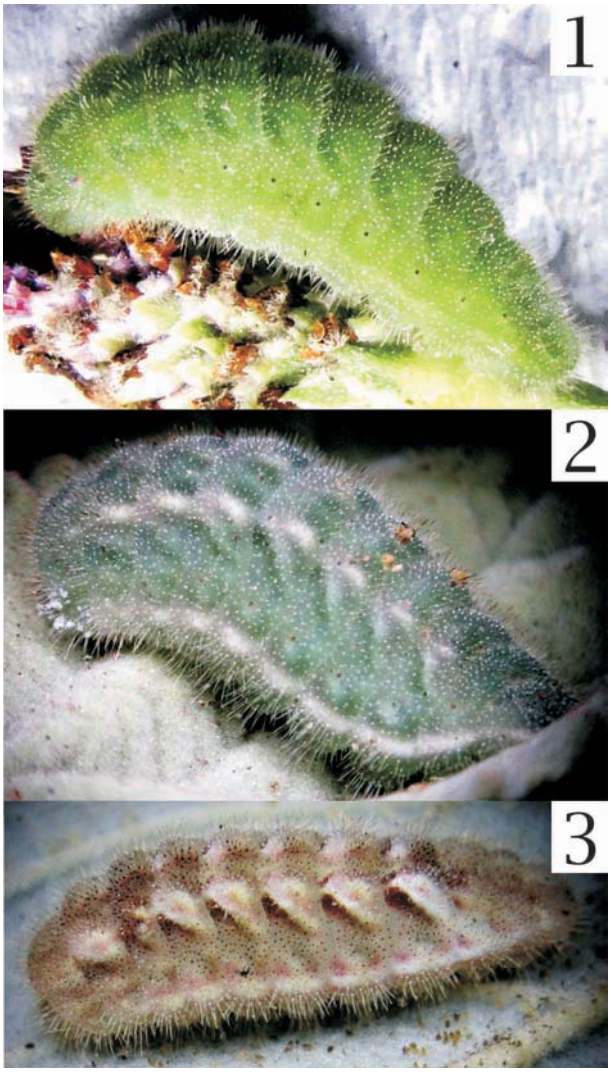
Additional key words: DNA barcoding, florivory, polyphagy, *Phyla nodiflora, Waltheria ovata*

The hairstreak *Strymon bubastus* (Stoll, 1780) (Lepidoptera, Lycaenidae, Theclinae, Eumaeini) is widely distributed in the Neotropical Region, ranging through a greater part of mainland South America and the Caribbean Islands (Robbins & Nicolay 2002, Nicolay & Robbins 2005). Several geographical adult phenotypes are recognized within this range, one of which, previously known as *S. sapota* (Hewitson, 1877), is endemic to the Atacama Desert (Nicolay & Robbins 2005). This phenotype is the only one recorded in Chile, where it is restricted to a few coastal valleys of the Atacama Desert, representing the southern limit for the range of *S. bubastus* along the Pacific coast (Peña & Ugarte 1996).

Despite the extensive geographic range of *S. bubastus*, its biology has been little studied (Silva et al. 2014). The available data are restricted mostly to some host records, including plants of the families Boraginaceae, Convolvulaceae, Fabaceae, Malvaceae and Portulacaceae (Beccaloni et al. 2008, Silva et al. 2011, 2014), suggesting a polyphagous habit for this species.

Polyphagy is common among larvae of Eumaeini, especially in flower-feeding species (Robbins & Aiello 1982, Brown 1993, Silva et al. 2011). For example, Monteiro (1991) recorded 30 host plants belonging to 10 families for larvae of *Rekoa marius* (Lucas, 1857) in a single locality in Brazil, while 44 host plants belonging to 19 families were recorded for *Parrhasius polibetes* (Stoll, 1781) by Kaminski et al. (2012). Some species of *Strymon* Hübner, 1818 also can be highly polyphagous (Robbins & Nicolay 2002, Silva et al. 2011). In addition, larvae of Eumaeini may display cryptic, host-associated color patterns (Ballmer & Pratt 1989, Monteiro 1991, Kaminski et al. 2012, Bächtold et al. 2013, Silva et al. 2014) due to the hypothesized accumulation of carotenoid and flavonoid pigments (Monteiro 1991). As a consequence, a wide range of color patterns is usually displayed by the larvae of some polyphagous species (Monteiro 1991, Kaminski et al. 2012).

Geographic variation in important biological features such as host plant use patterns, has been described for some butterflies (Rodrigues & Moreira 2002, Meister et al. 2015, Vilbas et al. 2015), including species inhabiting the arid landscapes of the Atacama Desert and neighboring areas of the Andes (Vargas 2013, 2014). Such geographic variation may be expected in widely distributed species, as is the case of *S. bubastus*, whose populations are separated from one another in different habitats along its range, where different plants



FIGS. 1–3. Polychromatic final instar of *Strymon bubastus*. **1**) unique color pattern recorded on *Phyla nodiflora*; **2**) dominant color pattern on *Waltheria ovata*; **3**) less frequent color pattern on *W. ovata*.

are available. The host plant records currently known for *S. bubastus* are based on collecting and rearing performed at different localities of its range (Beccaloni et al. 2008, Silva et al. 2011, 2014). However, the hosts of the Chilean populations have been unknown, impeding an understanding of the biology of this hairstreak at the local level.

The objective of this study is to provide the first data on the biology of the larvae of *S. bubastus* in northern Chile, including the first mention of host-associated polychromatism on two newly recorded host plants. In addition, the first sequences of DNA barcodes (sensu Hebert et al. 2003) are provided for the Atacama populations of *S. bubastus*.

MATERIALS & METHODS

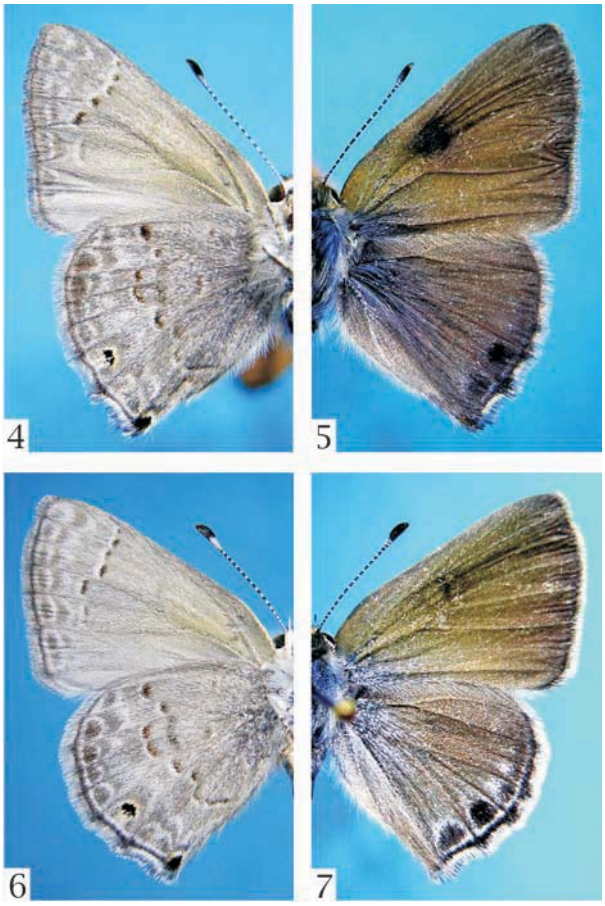
Final and penultimate instars of *S. bubastus* displaying host-associated color patterns were collected on inflorescences of *Phyla nodiflora* (Verbenaceae) and *Waltheria ovata* (Malvaceae) in the Azapa valley, Atacama Desert of northern Chile, between June 2008 and April 2015. The larvae were reared in the laboratory in individual plastic vials with inflorescences of their respective plants, which were changed daily until the larva stopped feeding and prepared for pupation. The adults obtained from the pupae were pinned and spread for species identification. Genitalia of two males and two females obtained from each plant were dissected following standard procedures (Winter 2000). Vouchers are deposited in the Colección Entomológica de la Universidad de Tarapacá (IDEA), Arica, Chile.

In order to assess the possible existence of genetic divergence between larvae displaying the two main color patterns (see below), the DNA barcode fragment (657 base pairs) of the mitochondrial cytochrome oxidase subunit I (COI) gene was sequenced for specimens collected on the two host plants in April 2015. Genomic DNA was extracted from pupae following the procedures described in Huanca-Mamani et al. (2015). PCR amplification and sequencing of the COI DNA barcode were performed by a commercial facility (Macrogen, South Korea) using the primers LEP-F1 (5'-ATTCAACCAATCATAAAGATAT-3') and LEP-R1(5'-TAAACTTCTGGATGTCCAAAAA-3') developed by Hebert et al. (2004). The sequences were aligned following the MUSCLE method (Edgar 2004) as implemented in the software MEGA6 (Tamura et al. 2013) to survey variable sites. A molecular identification was performed in BOLD v.3 Identification System (Ratnasingham & Hebert 2007).

RESULTS

Forty-two final and penultimate instars were collected on inflorescences of the two plants: 20 on *P. nodiflora*and 22 on *W. ovata*. All the larvae were reared successfully to the pupal stage, as parasitoids were not detected in the rearing. Only 23 adults were obtained from the pupae; the remaining 19 pupae were preserved in ethanol 95% to be used in DNA extraction.

Larval color pattern. All the larvae collected on *P. nodiflora*(n=20) were uniformly light green with short white secondary setae (Fig. 1), and six males and five females were reared from them. Twenty larvae collected on *W. ovata* were mostly pale green with one dorsal and one lateral cream white stripe, and with



FIGS. 4–7. Adults of *Strymon bubastus* reared from larvae collected on inflorescences of *Waltheria ovata*. **4**) Male, ventral view; **5**) Male, dorsal view; **6**) Female, ventral view; **7**) female, dorsal view.

short white secondary setae (Fig. 2), and they yielded five males and five females. An additional, less common (n=2) color pattern also was detected on *W. ovata* (Fig. 3), and was mostly pale yellow with the same dorsal and lateral stripes of the previous pattern, but with dark red blotches anteroventral to the dorsal stripe on A1–7, pink blotches dorsally to the lateral stripes on A1–8, and with the base of many secondary setae of the laterodorsal area black. One male and one female were reared from these larvae.

Wing pattern and genitalia. Rearing revealed no correlation between the color patterns of the larvae and the wing patterns or the morphology of the male and female genitalia. All the adults (12 males; 11 females) obtained from the larvae reared in the laboratory displayed the typical wing pattern of the Atacama Desert populations of *S. bubastus* regardless of the host plant on which the larvae were collected in the field, thus adults reared from only one host (*W. ovata*) are presented in Figs. 4–7. As well, no differences were found in the morphology of the male and female genitalia of the adults reared from the two sources.

DNA barcodes. Nineteen DNA barcode sequences (657 bp) were obtained, nine from the pupae reared as ex-larvae on *P. nodiflora* (GenBank accession: KT358184-KT358192), and 10 from pupae reared as ex-larvae on *W. ovata* (GenBank accession: KT358193-KT358202). Divergence among all sequences was 0%, as no variable sites were found. The nearest barcode sequence found in BOLD to the only Chilean haplotype was one "early release" of *S. bubastus* with 97.69% similarity, while the tree based identification tool of BOLD clustered the Chilean haplotype exclusively with *S. bubastus* sequences from Argentina, French Guiana and Puerto Rico.

DISCUSSION

The congruence in the wing pattern and the genitalia of the adults reared from the larvae collected on *P. nodiflora*and *W. ovata* suggests that larvae of the same hairstreak species (*S. bubastus*) feed on inflorescences of at least two plants in the Azapa Valley, and display host-associated color patterns. The only biological observation previously published on the populations of *S. bubastus* inhabiting the extremely arid environments of the Atacama Desert of northern Chile indicated that the adults visit flowers of *Alternanthera halimifolia* (Lam.) Standl. ex Pittier (Amaranthaceae) (Peña & Ugarte 1996). However, a survey of more than 60 plants of *A. halimifolia* plants yielded no larvae of *S. bubastus* in the Azapa Valley between the years 2010 and 2011, suggesting that this plant is used only as a nectar source by the adults, and not as a host by the larvae of this hairstreak.

Host plants. This is the first mention of the family Verbenaceae as a host plant for *S. bubastus*; and, even though Malvaceae already was reported as a host for this hairstreak (Beccaloni et al. 2008), this is the first record of its association with *W. ovata*. In addition, although *S. bubastus* is a polyphagous species (Beccaloni et al. 2008, Silva et al. 2011), *P. nodiflora* and *W. ovata* are the first host plants recorded for its Chilean populations, providing important information for future ecological studies or conservation projects at a local scale. The coastal valleys of the Atacama Desert of northern Chile are under strong anthropological pressures mostly associated with intensive agricultural activities that have strikingly modified the original habitats and restricted the natural vegetation to small, isolated patches (Luebert & Pliscoff 2006). The Chilean range of *W. ovata* is restricted to a few of these

coastal valleys (Muñoz-Pizarro 1966), where its presence is extremely rare, while at the same sites *P. nodiflora* is clearly more frequent. Additional field studies will be required to assess the relative importance of the two species in successfully supporting the local populations of *S. bubastus* under the environmental conditions of the Atacama Desert.

Larval polychromatism. This is the first mention of host-associated polychromatism for larvae of *S. bubastus*. The three color patterns reported here were cryptic on their respective plants. The only color pattern recorded on *P. nodiflora* provides the larva with excellent camouflage because its light green color perfectly matches that of the basal portion of the inflorescences and the leaves of the host. Thus, as penultimate and final instars eat externally with only the head introduced into the inflorescence, the thorax and abdomen are visually confused with the basal portion of the inflorescence and with the adjacent leaves. The commonest color pattern recorded on *W. ovata* is easily confused with flower buds and leaves of the host, enabling the larva to remain camouflaged when it is eating inflorescences at the flower bud stage; the less common color pattern provides the larva with camouflage when it is eating opened flowers. Monteiro (1991) reported similar variation in the larval color pattern of *Rekoa palegon* that was correlated with the age of the inflorescence on *Mikania stipulacea*. In addition, the three color patterns here detected are strikingly different from the one reported by Silva et al. (2014) for a mature larva of *S. bubastus*, which was cryptic on the inflorescences of its host, *Galactia* sp. (Fabaceae) in central Brazil. These findings suggest a great ability of the larvae of *S. bubastus* to display a variety of host-associated color patterns, in accordance with observations reported for other hairstreak species with polychromatic larvae (Monteiro 1991, Kaminski et al. 2012). As suggested by Brower (1958), patterns of host-associated polychromatism similar to that described here for *S. bubastus* break the phytophagous population into several "visual species" providing a useful strategy to avoid bird predation, because the predator bird must be able to learn each of these separate "prey images" before it can search for them.

DNA barcodes. The DNA barcode sequences reported here are the first available for the populations of *S. bubastus* of the Atacama Desert. The absence of variation among the sequences of larvae collected on the two plants indicates that all the sequences belong to the same species, in accordance with the identification based on wing pattern and genitalia morphology. The divergence (2.31%) between the Chilean haplotype and the nearest sequence deposited in BOLD is high compared to intraspecific divergences reported for other Eumaeini species (Faynel et al. 2012, Frye & Robbins 2015). Although deep COI divergences (>2%) may be indicative of unrecognized cryptic species (e.g. Landry & Hebert 2013, Huemer & Mutanen 2015), many cases of deep divergence involving clearly conspecific samples have been reported for Lepidoptera (Wiemers & Fiedler 2007, Hausmann & Huemer 2011, Hausmann et al. 2011, Huemer et al. 2014). Cases of deep divergence in Lycaenidae are mostly associated with samples from distantly located populations of widely distributed species (Wiemers & Fiedler 2007), a scenario similar to that reported here for *S. bubastus*, as the BOLD sequences are from locations extremely distant from northern Chile (Argentina, French Guiana and Puerto Rico). Additional DNA barcode sequences from populations of intermediate locations would be required to verify whether genetic variation is correlated with the geographic phenotypic variation already mentioned along the range of *S. bubastus* (Nicolay & Robbins 2005).

Further remarks. Larvae and pupae of myrmecophilous species of Lycaenidae may be associated with ants at a variable level ranging from facultative to obligate (Fiedler 1991, 1995). The life stages that interact with ants are characterized by the presence of some morphological specializations, such as the dorsal nectary organ (DNO) on the seventh abdominal segment, the pore cupola organs (PCO), tentacle organs or dendritic setae (Ballmer & Pratt 1991, Duarte et al. 2001, Duarte et al. 2005, Silva et al. 2014). The presence of DNO and PCOs was verified for the first time on the larvae of *S. bubastus* in this study regardless of the color pattern. However, attendant ants were not found in the field, suggesting that *S. bubastus* could be a facultative myrmecophilous species, at least at the local level. Ballmer & Pratt (1991) indicated that intra-specific geographic differences in myrmecophily might occur. Such geographic variation is expected in a widely distributed species such as *S. bubastus*, along whose range the ant composition and abundance can vary greatly.

Host plant ranges of the Lycaenidae of northern Chile still are only partially known. Based on the published records, Polyommatinae appear to be restricted to Fabaceae (Benyamini 1995, Vargas & Parra 2009, Vargas 2014), while Theclinae have been reared from Asteraceae (Vargas & Duarte 2014), Fabaceae (Vargas & Parra 2009), Malvaceae, and Verbenaceae (this study). Further studies would be required to reach a better understanding of the biology and evolution of the Lycaenidae of the arid

environments of the Atacama Desert and neighboring areas of the Andes.

ACKNOWLEDGMENTS

The authors would like to thank Annette Aiello, and Robert K. Robbins for kind and valuable comments that improved the manuscript; and to Lafayette Eaton for checking the English. Financial support was obtained from project DINV9710-15, Dirección General de Investigación, Universidad de Tarapacá. MD was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grants 2002/13898-0, 2010/14682-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grants 563332/2010-7, 305905/2012-0).

LITERATURE CITED

BÄCHTOLD, A., E. ALVES-SILVA, & K. DEL-CLARO. 2013. Lycaenidae larvae feeding on *Peixotoa parviflora* (Malpighiaceae) in a semideciduous forest in southeastern Brazil. J. Lepid. Soc. 67:65–67.

BALLMER, G.R. & G. F. PRATT. 1989. A survey of the last instar larvae of the Lycaenidae (Lepidoptera) of California. J. Res. Lepid. 27:1–81.

BALLMER, G.R. & G.F. PRATT. 1991. Quantification of ant attendance (myrmecophily) of lycaenid larvae. J. Res. Lep. 30:95–112.

BECCALONI, G.W., A.L. VILORIA, S.K. HALL & G.S. ROBINSON. 2008. Catalogue of the hostplants of the Neotropical butterflies. Monografias Tercer Milenio. Vol. 8. S.E.A., RIBES-CYTED, The Natural History Museum, Instituto Venezolano de Investigaciones Científicas, Zaragoza, Spain.

BENYAMINI, D. 1995. Synopsis of biological studies of the Chilean Polyommatini (Lepidoptera, Lycaenidae). Rep. Mus. Nat. Hist. Univ. Wisconsin (Stevens Point) 52:1–51.

BROWER, L.P. 1958. Bird predation and foodplant specificity in closely related procryptic insects. Amer. Naturalist 92:183–187.

BROWN, K.S., Jr. 1993. Neotropical Lycaenidae—an overview, pp. 45–61. *In* T. R. New (ed.), Conservation biology of Lycaenidae (Butterflies). IUCN, Gland, Switzerland.

DUARTE, M., G.L. DE ALMEIDA, M.M. CASAGRANDE & O.H.H. MIELKE. 2001. Notes on the last instar larva and pupa of *Hemiargus hanno* (Stoll) (Lepidoptera, Lycaenidae, Polyommatinae). Rev. Bras.Zool. 18:1097–1105.

DUARTE, M., R. K. ROBBINS & O. H. H. MIELKE. 2005. Immature stages of *Calycopis caulonia* (Hewitson, 1877) (Lepidoptera, Lycaenidae, Theclinae, Eumaeini), with notes on rearing detritivorous hairstreaks on artificial diet. Zootaxa 1063:1–31.

EDGAR, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

FAYNEL, C., R.C. BUSBY & R.K. ROBBINS. 2012. Review of the species level taxonomy of the neotropical butterfly genus *Oenomaus* (Lycaenidae, Theclinae, Eumaeini). ZooKeys 222:11–45.

FIEDLER, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonn. Zool. Monogr. 31:1–210.

FIEDLER, K. 1995. Lycaenid butterflies and plants: is myrmecophily associated with particular hostplant preferences? Ethol. Ecol. Evol. 7:107–132.

FRYE, J.A. & R. K. ROBBINS. 2015. Is the globally rare frosted elfin butterfly (Lycaenidae) two genetically distinct host plant races in Maryland? DNA evidence from cast larval skins provides an answer. J. Insect Conserv. 19:607–615.

HAUSMANN, A. & P. HUEMER. 2011. Taxonomic decision as a compromise: *Acasis appensata* (Eversmann, 1832) in Central Italy—a case of conflicting evidence between DNA barcode and morphology (Lepidoptera: Geometridae). Zootaxa 3070:60–68.

HAUSMANN, A., G. HASZPRUNAR & P.D.N. HEBERT. 2011. DNA barcoding the geometrid fauna of Bavaria (Lepidoptera): successes, surprises and questions. PloS ONE 6:e17134.

HEBERT, P. D. N., A. CYWINSKA, S. L. BALL & J. R. DEWAARD. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B 270:313–321.

HEBERT, P.D.N., E. H. PENTON, J. M. BURNS, D.H. JANZEN & W. HALLWACHS. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA 101:14812–14817.

HUANCA-MAMANI, W., D. RIVERA-CABELLO & J. MAITA-MAITA. 2015. A simple, fast and inexpensive CTAB-PVP-silica based method for genomic DNA isolation from single small insect larvae and pupae. Genet. Mol. Res.14:7990–8000.

HUEMER, P., M. MUTANEN, K. M. SEFC & P. D. N. HEBERT. 2014. Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic distances have small genetic impacts. PLoS ONE 9:e115774.

HUEMER, P. & M. MUTANEN. 2015. Alpha taxonomy of the genus *Kessleria* Nowicki,1864, revisited in light of DNA-barcoding (Lepidoptera, Yponomeutidae). ZooKeys 503:89–133.

KAMINSKI, L.A., D. RODRIGUES, & A. V. L. FREITAS. 2012. Immature stages of *Parrhasius polibetes* (Lepidoptera: Lycaenidae): host plants, tending ants, natural enemies and morphology. J. Nat. Hist. 46:645–667.

LANDRY, J.-F. & P.D.N. HEBERT. 2013. *Plutella australiana* (Lepidoptera, Plutellidae), an overlooked diamondback moth revealed by DNA barcodes. ZooKeys 327:43–63.

LUEBERT, F. & P. PLISCOFF. 2006. Sinopsis bioclimática y vegetacional de Chile. Editorial Universitaria, Santiago, Chile. 316 p.

MEISTER, H., L. LINDMAN &T. TAMMARU. 2015. Testing for local monophagy in the regionally oligophagous *Euphydryas aurinia* (Lepidoptera: Nymphalidae). J. Insect Conserv. 19:691–702.

MONTEIRO, R.F. 1991. Cryptic larval polychromatism in *Rekoa marius* Lucas and *R. palegon* Cramer (Lycaenidae: Theclinae). J. Res. Lepid. 29:77–84.

MUÑOZ-PIZARRO, C. 1966. Sinopsis de la flora Chilena: claves para la identificación de familias y géneros. Santiago, Ediciones de la Universidad de Chile, 500 p.

NICOLAY, S.S. & R. K. ROBBINS. 2005. Five new dry-area South American *Strymon* species (Lycaenidae: Theclinae) and their biogeographic significance. J. Res. Lepid. 38:35–49.

PEÑA G., L.E. & A.J. UGARTE P. 1996. Las mariposas de Chile. The butterflies of Chile. Santiago, Editorial Universitaria, 359 p.

RATNASINGHAM, S. & P.D.N. HEBERT. 2007. BOLD: the barcode of life data system (www.barcodinglife.org). Mol. Ecol. Notes 7:355–367.

ROBBINS, R. K. & A. AIELLO. 1982. Foodplant and oviposition records for Panamanian Lycaenidae and Riodinidae. J. Lepid. Soc. 36:65–75.

ROBBINS, R.K. & S.S. NICOLAY. 2002. An overview of *Strymon* Hübner (Lycaenidae: Theclinae: Eumaeini). J. Lepid. Soc. 55:85–100.

RODRIGUES, D. & G.R.P. MOREIRA. 2002. Geographical variation in larval host-plant use by *Heliconius erato* (Lepidoptera: Nymphalidae)and consequences for adult life history. Braz. J. Biol. 62:321–332.

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.

SILVA, N.A.P., M. DUARTE,E.B. ARAÚJO & H.C. MORAIS. 2014. Larval biology of anthophagous Eumaeini (Lepidoptera: Lycaenidae, Theclinae) in the Cerrado of central Brazil. J. Insect Sci. 14:184.

TAMURA. K., G. STECHER, D. PETERSON, A. FILIPSKI & S. KUMAR. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30:2725–2729.

VARGAS, H.A. 2013. Use of a native and an exotic Malvaceae by the little known skipper *Pyrgus bocchoris trisignatus* (Mabille) (Hesperiidae) in northern Chile. J. Lepid. Soc. 67: 225–226.

VARGAS, H.A. 2014. Lycaenid caterpillars (Lepidoptera, Lycaenidae) eating flowers of *Dalea pennellii* var. *chilensis* (Fabaceae) in the northern Chilean Andes. Rev. Bras. Entomol. 58:309–312.

VARGAS, H.A. & L.E. PARRA. 2009. Prospección de lepidópteros

antófagos asociados a *Acacia macracantha* Willd. (Fabaceae) en el norte de Chile. Rev. Bras. Entomol. 53:291–293.

VARGAS, H.A. & M. DUARTE. 2014. First host plant record for *Thaeides muela* (Lepidoptera, Lycaenidae: Theclinae: Eumaeini). Fla. Entomol. 97:288–290

VILBAS, M., T. TEDER, A. TIITSAAR, A. KAASIK &T. ESPERK. 2015. Habitat use of the endangered parasitic butterfly *Phengaris arion* close to its northern distribution limit. Insect Conserv. Diver. 8:252–260.

WIEMERS, M. & K. FIEDLER. 2007. Does the DNA barcoding gap exist?—a case study in blue butterflies (Lepidoptera: Lycaenidae). Front. Zool. 4:8.

WINTER, W. D. 2000. Basic techniques for observing and studying moths and butterflies. Memoirs of the Lepidopterists' Society 5:1–444.

Submitted for publication 3 August 2015; revised and accepted 7 September 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 159–163

A NEW FOODPLANT FOR *HISTORIS ODIUS DIOUS* LAMAS, 1995 (NYMPHALIDAE: NYMPHALINAE) WITH SOME NOTES ON THE LIFE HISTORY IN SURINAME

Additional key words: Surinam, Cecropiaceae, *Cecropia*, *obtusa*, host plant

Historis odius (Fabricius, 1795) (Nymphalidae: Nymphalinae) ranges from the southern USA and the Caribbean to Argentina. There are three subspecies (Lamas 2004): ssp. *odius* (Fabricius, 1775) and *caloucaera* Brévignon, 2003 are distributed in part of the Caribbean, ssp. *dious* Lamas, 1995 occurs in the remainder of its range. *Cecropia insignis*, *C. obtusifolia* and *C. peltata* (Cecropiaceae) have been reported as larval foodplants for *H. odius dious* (Beccaloni et al. 2008, Janzen & Hallwachs 2009). Its early stages were described from El Salvador (Muyshondt & Muyshondt 1979) and figured from Costa Rica (Janzen & Hallwachs 2009). In Suriname, the species has been recorded throughout the country in cultivated and secondary vegetation as well as primary forests. We describe a new foodplant and provide additional descriptive detail on some aspects of the life history in Suriname.

On 27 March 2015 in Paramaribo, Suriname (5°49'405"N, 55°10'404"W), the second author detected a fourth instar larva of *Historis odius dious* on the underside of a leaf of a *Cecropia* species. By providing the leaves of this tree as its only food, the larva was reared to an adult according to standard methods. A botanical collection was made of the foodplant. Stages were photographed with a Nikon D300s camera, an AF Micro Nikkor 105 mm 1:2.8 D lens and a SB-800 flash. Photographs were made in NEF-format and without adjustments converted to TIF-files in the same color space.

The foodplant (Fig. 1) was identified as *Cecropia obtusa* Trécul (Cecropiaceae). Description: Tree, to 15 m, usually inhabited by ants, with stilt roots (Fig. 1A). Leaves peltate (Fig. 1B), petioles whitish due to dense cobweb-like hairs, at its base a trichilium with white Müllerian bodies (Fig. 1D); leaf blade with 6–9 lobes, ca. 60 cm diam.; upperside green, rough to the touch due to presence of short, stiff hairs; underside whitish because of dense cobweb-like hairs, lateral veins loop-connected just inside leaf margin (Fig. 1B). Inflorescences spikes on common peduncle. Female inflorescense usually 4 spikes, each 3–10 cm long and ca. 0.4 cm thick (Fig. 1H–J), male inflorescences have 12–15 thinner spikes (Fig. 1D–G).

Fourth instar (Fig. 2a–c). Head capsule shiny black with on each side a more or less rectangular red patch between the stemmata and frons and below this at the left side a smaller red spot; above two stout black horns, each with two lateral and three superior, orange-red tipped spines; between the horns close to the epicranial notch two small black spines; at both sides a row of 5–6 light yellow spines with black base. Ground color thorax and abdomen dorsolaterally black to very dark brown, below spiracles and ventrally dark red-brown. Prolegs dark red-brown with black patch above planta. Prothoracic shield black, bordered on both sides by yellow transverse bands, extending to subdorsal area. Anal plate black with two spines. Thoracic and abdominal segments bordered by narrow, straight transverse bands with two or three wider, irregularly formed transverse bands in between, often laterally merging around spiracle, giving the impression of a black oval surrounded by yellow. Middorsally, a longitudinal yellow stripe, interrupted on T1, T3 and A3–A6. Middorsally on A1–A9, at junction of longitudinal and anterior irregular transverse band, a orange-based (A1, A2) or red-based (A3–A9) scolus with three (A7), four (A1–A6) or five (A9) terminal, unbranched yellow spines. Subdorsally on T2, T3 and A3, a dark yellow scolus with five (T2, T3) or three (A2) terminal, unbranched spines. Laterally on A1–A10, above and slightly anterior to the spiracle when present, a dark yellow scolus with 3–4 terminal, unbranched spines. Ventral and anterior to these, on A2–A8, a dark yellow scolus with a single, unbranched spine. Ventrally on T1–A8, caudad and ventral to spiracle when present, a dark yellow scolus with a single, bifid spine, except T3 where there are three spines. Finally, T1 and T2 have a single-spined scolus ventrally at the caudad end of the segment and T1 has a ventral single-spined scolus at the anterior end of the segment. Found on 27 March 2015, length 30 mm. It grew to 33 mm and molted on 30 March.

Fifth instar (Fig. 2d–f). Head capsule as in fourth instar, but with four conspicuous red patches, at each side a squared one at the base of the horns and a larger one between the stemmata and frons; spines on horns translucent to pink; tips of horns next to epicranial

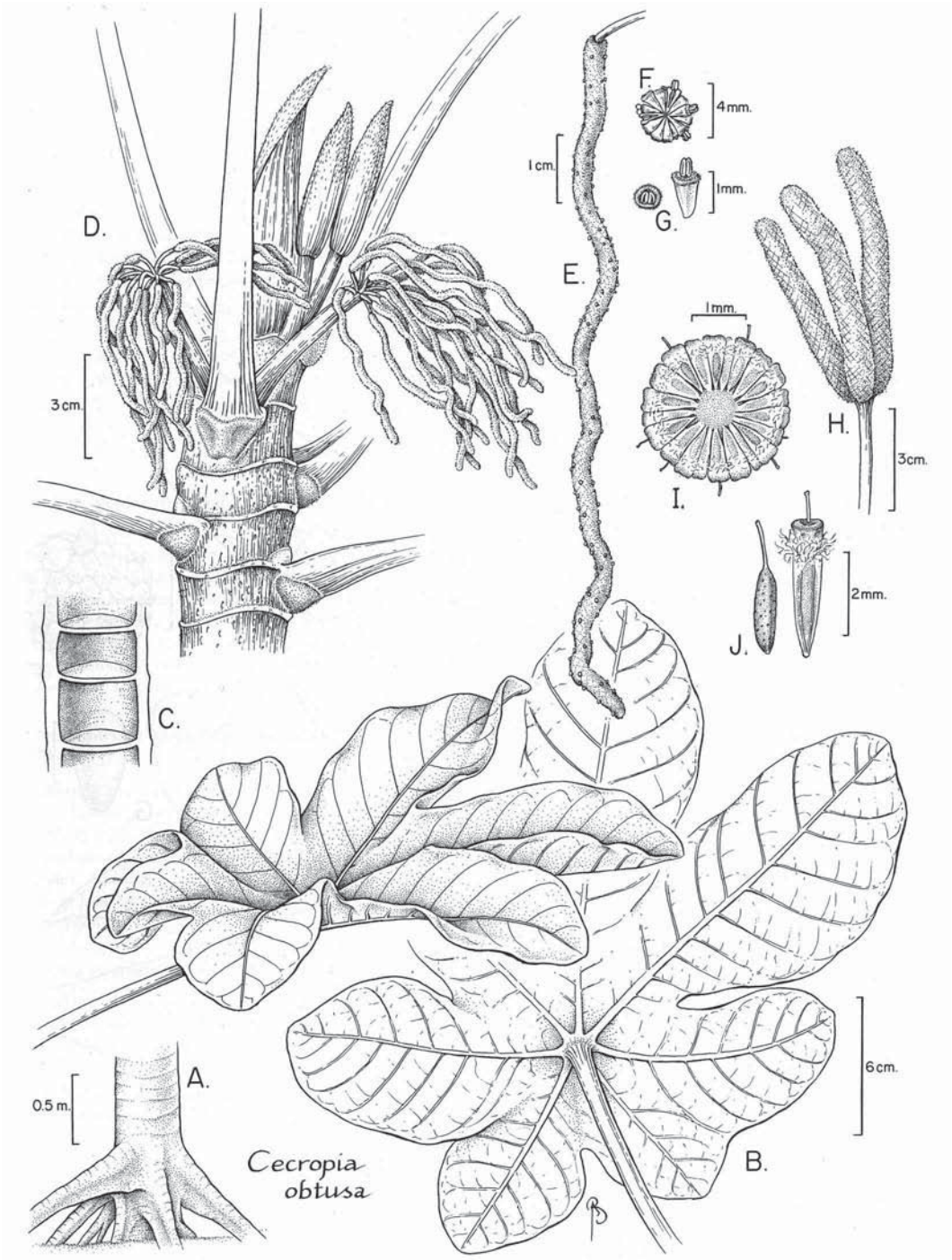


FIG. 1. *Cecropia obtusa* Trécul. **A**: roots and base of trunk; **B**: adaxial (left) and abaxial (right) sides of leaf; **C**: section of stem with hollow internodes; **D**: top of stem with male inflorescences and petioles with trichilia; note the spathes covering unopenend inflorescences; **E**: single spike of male inflorescence; **F**: transverse section of male inflorescence; **G**: detail of male flower; **H**: female inflorescence; **I**: transverse section of female inflorescence; **J**: detail of female flower. Drawing by Bobby Angell; reproduced with permission from Bobby Angell from Mori et al. (2002).

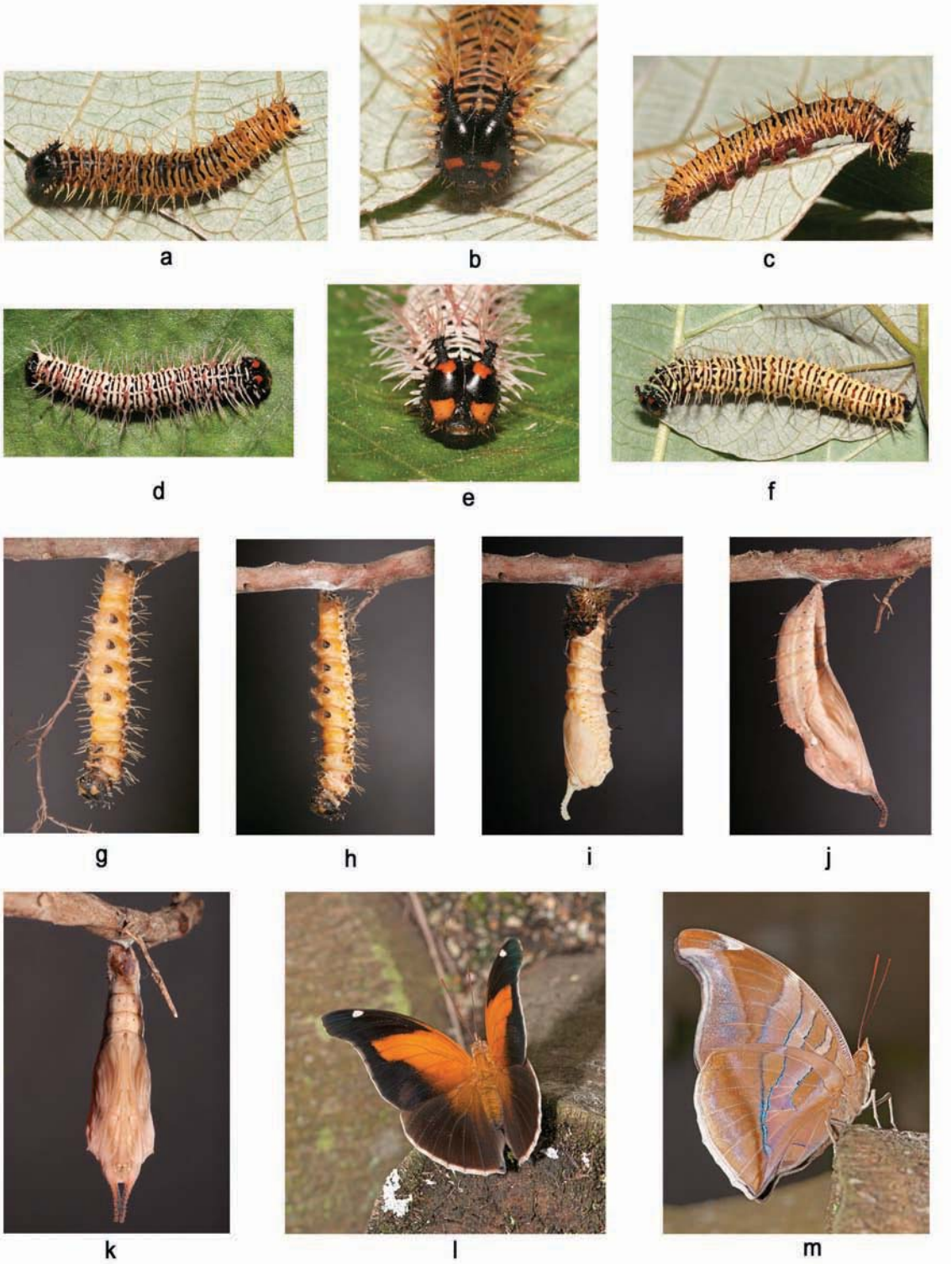


FIG. 2. Aspects of the life history of *Historis odius dious* on *Cecropia obtusa* Trécul, Paramaribo, Suriname. **a**: 4th instar, dorsal view (27-03-2015); **b**: 4th instar, anterior view (27-03-2015); **c**: 4th instar, lateral view (27-03-2015); **d**: 5th instar, dorsal view (01-04-2015); **e**: 5th instar, anterior view (01-04-2015); **f**: late 5th instar, dorsolateral view (04-04-2015); **g-h**: prepupa, ventrolateral view (07-04-2015); **i**: fresh pupa, lateral view with exuviae at caudal end (07-04-2015); **j**: pupa, lateral view (09-04-2015); **k**: pupa, ventral view (12-04-2015); **l**: adult male, dorsal view (18-04-2015); **m**: adult male, ventral view (18-04-2015). Photographs by second author.

notch translucent; spines on sides of head capsule translucent with black base. Thorax and abdomen as in fourth instar, including position of scoli; longitudinal lines and transverse bands white instead of yellow, middorsal longitudinal stripe more prominent, unbroken from T2 to A8; scoli and spines pink, translucent or white. On 1 April, the larva was 51 mm. On 4 April, it measured 62 mm and changed color (without molt): the ground color of the abdomen, and to a lesser extent the thorax, turned to dark brown, in some segments red-brown, and the longitudinal and transverse bands turned deep yellow, except for the bands on T2. On 6 April, it stopped eating and remained immobile horizontally on a twig. It measured 52 mm. No wandering phase was observed. On 7 April, it turned into the prepupa.

Prepupa (Fig. 2g–h). Head as in fifth instar. Ground color of thorax and abdomen dorsally light yellow, ventrally yellow-orange, between prolegs orange-red, some irregular brown patches lateroventrally on T1–A2. Armature as in fifth instar. The prepupa hung in vertical position for about 10 hrs.

Pupation (Fig. 2i). The pupal molt started on 7 April at 20.01 hrs and was finished at 20.05 hrs. The fresh skin was white-light yellow with darker yellow patches. It took the pupa about 5 minutes and much wriggling to get rid of its exuviae at its anal end. The pupa then started to curve, its lower two thirds turning ventrally, hinging at the A3–A4 junction.

Pupa (Fig. 2i–k). Lateral view: Dimensions: 50.5 ×13.2 mm (A2). From A10 gradual thickening to A8, more abruptly to A7, then more gradually to A2 (the pupa's widest segment), slightly constricting at A1 and especially at T3, humping dorsally at T2, then gradually tapering to head. Ground color pinkish-light brown. Head with two round, black with red, dorsally curved horns. Middorsally, from A2 to A7 near the junction of the anterior segment a brown-based 4-furcate spine. Subdorsal black wart on A2, T3 and T1. Laterally, two longitudinal lateral whitish stripes from A6 to A1 and a white rounded patch on T3. A black wart below the white line near the anterior border of A1–A7, A9 and A10, posteriorly on A8. Ventral and caudad to spiracle of A3–A7 a small circular brown-black spot. Ventral to these, a large dark brown patch from the ventral wing pad at T3 darkening and tapering to A6.

Ventral view: Dimensions: 50.5 × 12.3 mm (T2). From cremaster downwards abruptly thickening, gradually broadening to T2 with slight constriction at middle part of wing cases. A7–A10 fused ventrally forming an oval structure to which cremaster is attached dorsally. Midventrally, single short thorn on A3–A5 near junction with caudad segment. Subventrally on A4 and A5, laterally and caudally from thorn, a paired dark brown spot. T2 with lateral protuberances. Distal halves of head horns touching each other.

Ecdysis and adult (Fig. 2l–m). A male eclosed in the early morning on 18 April 2015.

C. obtusa is a new larval foodplant record for *H. o. dious*. It ranges from Panama to Peru and Bolivia. As a pioneer species it is common in secondary vegetation and in open areas in rain forest (van Roosmalen 1985, Heuret et al. 2002, Mori et al. 2002). In view of its wide distribution, it is likely to be found a larval foodplant in several other countries.

There were several morphological differences (color of head capsule and spines, ground color of thorax and abdomen, pattern of bands and stripes, middorsal longitudinal stripe, color changes of the late 5th instar and prepupa, pupal warts, thorns and spots) between earlier observations (Muyshondt & Muyshondt 1979) and our own. There were also behavioral differences (larva at underside of leaves, no wandering phase). Data from Costa Rica indicate some morphological characteristics are variable (Janzen & Hallwachs 2009). Further research is needed to assess the functional significance of larval and pupal characteristics, especially as to defensive capabilities.

ACKNOWLEDGMENTS

The authors wish to thank Frans Barten for preparing the figures and two anonymous reviewers for their constructive comments. We are grateful to Claudine Sakimin, Nature Conservation Division of the Suriname Forest Service, Paramaribo, for granting a research permit that includes the study of lepidopteran life histories. This research has been made possible by a grant from the Uyttenboogaart-Eliasen Foundation.

LITERATURE CITED

BECCALONI, G.W., Á.L. VILORIA, S.K. HALL & G.S. ROBINSON. 2008. Catalogue of the hostplants of the Neotropical butterflies. Catálogo de las plantes huésped de las mariposas neotropicales. The Natural History Museum. Monografías Tercer Milenio, volume 8, S.E.A., Zaragoza.

HEURET P., D. BARTHÉLÉMY, Y. GUÉDON, X. COULMIER & J. TANCRE. 2002. Synchronization of growth, branching and flowering processes in the South American tropical tree *Cecropia obtusa* (Cecropiaceae). Am. J. Bot. 89: 1180–1187.

JANZEN, D. H. & W. HALLWACHS. 2009. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservacion Guanacaste (ACG), northwestern Costa Rica (nn-SRNP-nnnnn voucher codes). http://janzen.sas.upenn.edu. Accessed April 2015.

LAMAS, G. (ed.) 2004. Atlas of Neotropical Lepidoptera. Checklist: part 4A. Hesperioidea – Papilionoidea. *In* Heppner, J.B. (ed.) Atlas of neotropical Lepidoptera. Association for Tropical Lepidoptera, Scientific Publishers, Gainesville.

MORI, S.A., G. CREMERS, C. GRACIE, J.J. DE GRANVILLE, S.V. HEALD, M. HOFF & J.D. MITCHELL. 2002. Guide to the vascular plants of central French Guiana. Part 2. Dicotyledons. Memoirs of the New York Botanical garden, vol 76, part 2, 2002.

MUYSHONDT, A. JR. & A. MUYSHONDT. 1979. Notes on the life cycle and natural history of butterflies of El Salvador. III. *Historis odius* and *Coea acheronta* (Nymphalidae-Coloburinae). J. Lepid. Soc. 33: 112-123.

VAN ROOSMALEN, M.G.M. 1985. Fruits of the Guianan flora. Institute of Systematic Botany, University of Utrecht, The Netherlands.

HAJO B.P.E. GERNAAT*, *Department of Taxonomy and Systematics, Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands; email: hajo.gernaat@naturalis.nl (*corresponding author).*

JOKE VAN DEN HEUVEL, *Marcusstraat 6, Paramaribo, Suriname; email: jokevandenheuvel58@gmail.com.*

TINDE VAN ANDEL, *National Herbarium, Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden; email: Tinde.vanAndel@naturalis.nl.*

Submitted for publication 18 July 2015; revised and accepted 19 December 2015.

Journal of the Lepidopterists' Society
70(2), 2016, 163–166

HOST PLANT AND LATE LARVAL STAGES OF *HYPERCOMPE CUNIGUNDA* (EREBIDAE: ARCTIINAE) IN SURINAME

Additional key words: Arctiini, Surinam, Cucurbitaceae, *Melothria*, neotropical

The genus *Hypercompe* (Erebidae: Arctiinae: Arctiini) currently encompasses 89 species, distributed from the USA through the Caribbean to Argentina and Bolivia (Vincent & Laguerre 2014). The early stages are only known for a handful of species (Dyer et al. 2004, Wagner 2005, Janzen & Hallwachs 2009). Here, we describe a hostplant and late larval stages of *H. cunigunda* (Stoll, 1781) from Suriname.

On 3 March 2014 at Colakreek, Suriname (05º 27' 50" N, 055° 13' 40" W, 15m asl; about 46 km S of Paramaribo), on white sand savanna with secondary vegetation next to a ditch, a black-and-red setose larva was observed on a herbaceous vine (Fig. 1). The plant was tentatively identified as a species within the Cucurbitaceae and a herbarium sample (voucher Gernaat044, deposited in Naturalis Biodiversity Center, Leiden, the Netherlands) was taken. The larva was collected and fed leaves of the host. When this material was finished, it was fed carefully cleaned and rinsed skin of commercially grown cucumber *Cucumis sativus* L. Measurements of length were taken including the setae. Photographs were made with a Nikon D-800 and an AF Micro Nikkor 105 mm 1:2.8 D lens on a tripod in NEF-format. They were converted to TIF-files in the same color space after minor adjustments of exposure, contrast and sharpening. Measurements of the last instar and photographs of the pupa were lost due to a computer crash in Suriname. Eclosion took place during a short field trip so that the imago could only be collected 1–2 days later.

The host plant (Fig. 1). The host plant was identified as *Melothria pendula* L. (Cucurbitaceae). Description after Mori et al. (2002) and Van Andel & Ruysschaert (2011): Delicate, annual vine, to 4 m long climbing over low vegetation. Tendrils simple, lateral to petioles. Leaves alternate, with long petiole, simple, thin, usually shallowly 3-lobed, 5–10 × 4–9 cm; base deeply cordate, apex acute, both sides pubescent, venation palmate. Inflorescences axillary, both sexes often found at the same node. Male inflorescences with 5–15 small, yellow flowers. Female inflorescences with 1–2 small, yellow flowers with elongated ovary and long pedicle. Berry cylindrical, juicy, about 1.4 × 1 cm, mottled green, then dark purple to almost black at maturity. Seeds 2–4, white. Distribution: E and SE USA and Caribbean to Argentina, has become an invasive species in SE Asia. In Suriname widespread in disturbed vegetation. Popular names: busikomkomro, sneki-komkomro (Suriname), creeping cucumber (USA).

Antepenultimate instar (Fig. 1, Fig. 2a, b). *Head*: vertices and frons black, no spines or scoli; light gray line over coronal sulcus and upper one-fourth of adfrontal sulci; clypeus light gray, upper border convex; labrum dirty yellow with in upper half a semicircular

FIG. 1: Antepenultimate instar of *Hypercompe cunigunda* (Stoll, 1781) on *Melothria pendula* L. (Cucurbitaceae), Colakreek, Suriname, 3 March 2014. Note pistillate yellow flower below.

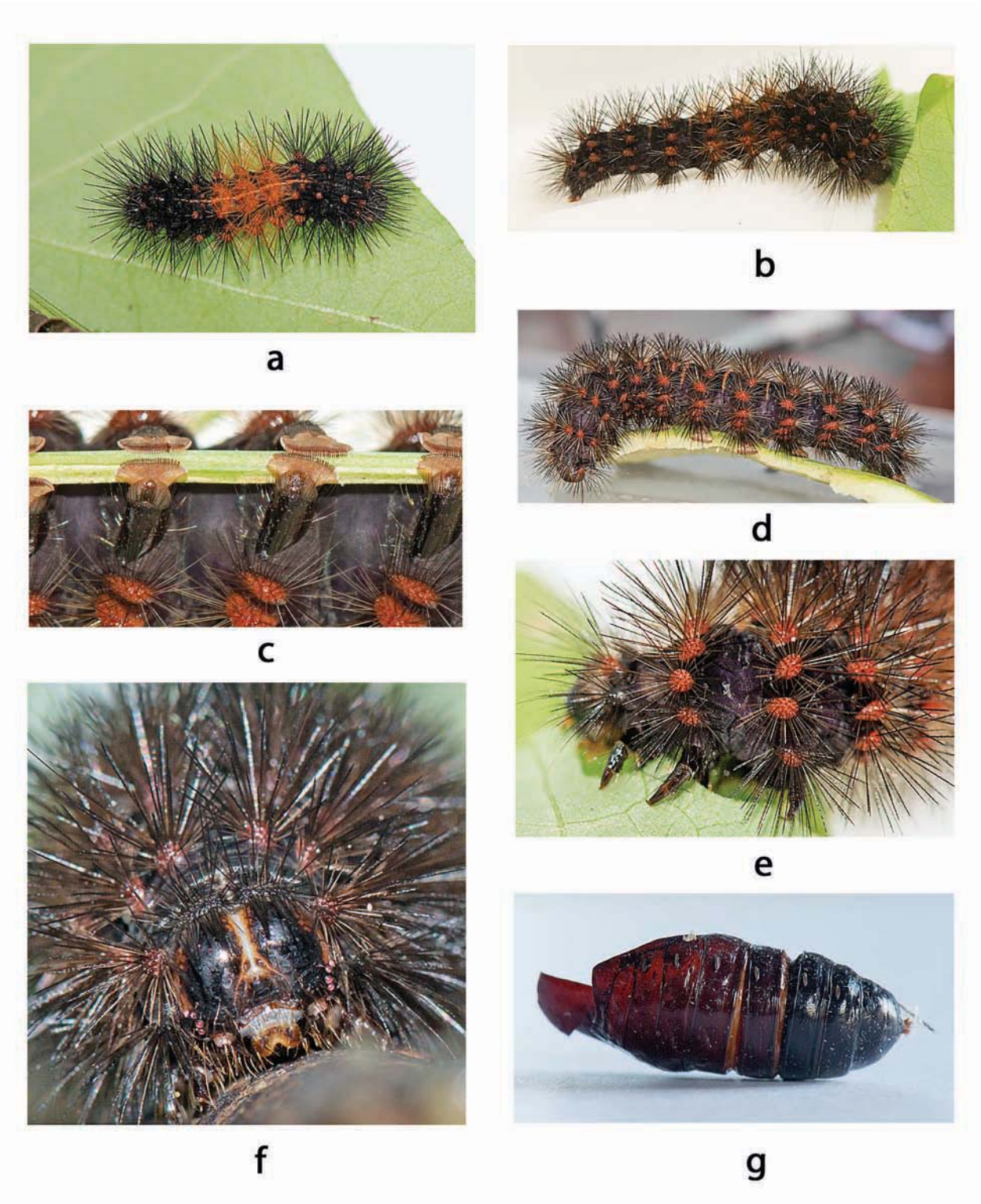


Fig. 2: Late larval stages of *Hypercompe cunigunda* in Suriname. **a**: antepenultimate instar, 3 March 2014; **b**: antepenultimate instar, 8 March 2014; **c**: penultimate instar, 12 March 2014, ventrolateral view of abdomen; note nonbarbed setae on subventral verrucae; **d**: ultimate instar, 19 March 2014; **e**: penultimate instar, 11 March 2014, left lateral view of thorax; **f**: ultimate instar, 26 March 2014, frontal view; **g**: pupal shell, 27–28 April 2014.

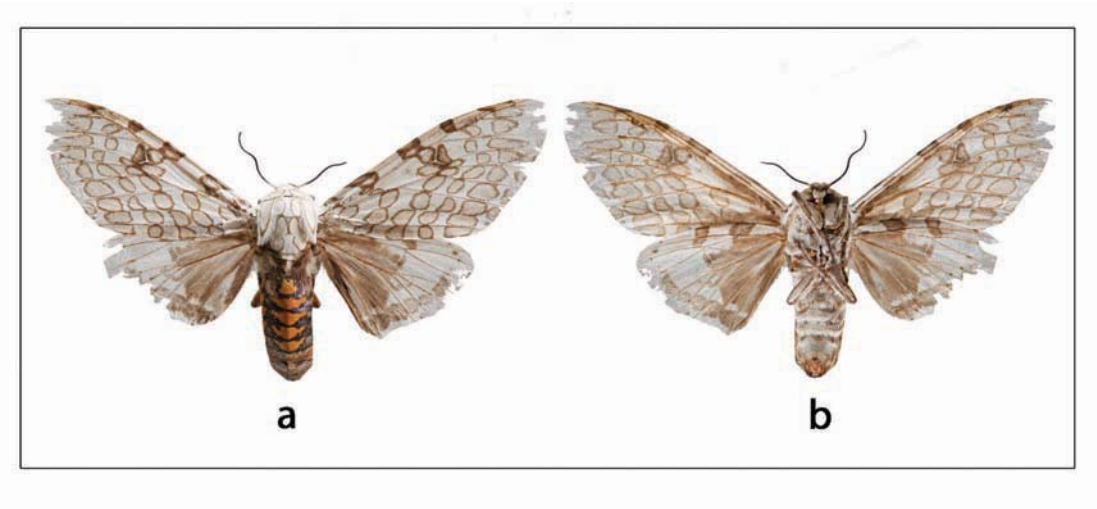


Fig. 3: Eclosed female of *Hypercompe cunigunda* (Stoll, 1781) from Suriname. **a**: dorsal view; **b**: ventral view.

row of black pinacula, notch to 37% of its length (Fig. 2f); first segment of antennae translucent-light gray, second segment black, elongated, with long seta (Fig. 2f). Thorax and abdomen black, middle third of body red with an abdominal middorsal black stripe (Fig. 1) or, depending on amount and angle of light, an orange longitudinal interrupted stripe from A2 to A8 (Fig. 2a). After two days, the red color disappeared without molt (Fig. 2b). On thorax and abdomen black or red verrucae, each with numerous black or red, sharp, unbranched setae, bearing paired barbs. On thorax, middorsally a light gray, irregular longitudinal stripe, anteriorly continuous with gray line over coronal sulcus, caudally ending at T3. Intersegmental membranes gray. Prothoracic plate gray, anterior margin concave. Lateral to prothoracic plate a small subdorsal gray verruca with short, unbarbed gray setae. Furthermore on T1, a red verruca laterally and a black verruca above and slightly anterior to the coxa. T2 and T3 each with five verrucae, one subventrally (above and slightly anterior to the coxa), two laterally, one subdorsally and one dorsally; verrucae on T2 all red, on T3 the subdorsal and lateral ones black. Abdomen with orange intersegmental membranes and prolegs on A3–A6 and A10 (Fig. 2b). Prolegs have an elongated black base with numerous soft setae on pinacula, the planta is gray without setae; crochets heteroideous, arranged in mesoseries (Fig. 2c). A1 has on either side ventrally two small black verrucae with soft non-barbed setae; subventrally two red ones, laterally one red and one black one, subdorsally a black one and dorsally one red and one black verruca, all with stiff barbed setae. A2 as A1, but with all verrucae red. A3–A6 each have seven red verrucae on either side; two are located dorsally, two subdorsally, two laterally and one subventrally, the latter just above the proleg; the subventral verruca has stiff, non-barbed setae; the setae directed downward from the lower lateral verruca are non-barbed, the other verrucae have barbed setae (Fig. 2b–d). A7 has ventrally two pinacula and one dark red to black small verruca with soft, non-barbed setae, other verrucae as A3–A6. A8 as A7 but with one ventral pinaculum. A9 has subventrally one pinaculum and an elongated black verruca with soft, non-barbed setae; laterally, subdorsally and dorsally one red verruca with barbed, stiff setae. Anal plate black.

Larva found on 3 March 2014, length 38 mm (Fig. 1, 2a). On 8 March 42 mm (Fig. 2b). The larva was inactive and did not eat on 9 and 10 March. It molted in the night of 10–11 March.

Penultimate instar (Fig. 2c, e). Similar to previous instar, but all verrucae on A1 and A2 red. On 11 March, the larva consumed its exuviae, including all barbed setae. It measured 36 mm and ate voraciously the next days, producing large amounts of frass. It molted during the night of 16–17 March.

Ultimate instar (Fig. 2d, f). Similar to previous instar, but head capsule (Fig. 2f) with gray line over coronal sulcus wide, irregularly bordered by dark yellow, extending unto upper part of frons and over the upper half of the adfrontal sulci; in the obtuse angle between the coronal and adfrontal sulci on both sides an oval black area, each with one black seta, bordered laterally by a narrow slightly irregular yellow line, which

runs between the coronal sulcus gray line and the lateral end of the clypeus; basal antennal segment gray.

On 17 March, the larva did not eat the exuviae and moved about actively for a few hours. On 2 April it stopped eating, became inactive and on 4 April it had pupated.

Larval behavior. When found, the solitary larva was fully exposed on the host plant. Generally, it stayed on the food provided, alternating periods of feeding with periods of inactivity. There were no bouts of restlessness other than a few hours at the start of the last instar. When molested, it curled itself head to tail, exposing the orange intersegmental membranes and barbed setae on all sides.

Pupa (Fig. 2g). Similar to the pupa of *H. scribonia*: very dark brown to black, oval, rounded, smooth, shiny, no setae on pupa; abdomen has a somewhat corrugated aspect due to constrictions between segments, the ones between A4 and A5 and between A5 and A6 orange-brown colored; exuviae attached to pupa at caudal end; no cocoon; length about 40 mm.

Imago (Fig. 3a, b). A female *H. cunigunda* eclosed on 27 or 28 April 2014. Forewing length about 27 mm, wingspan about 56 mm.

Duration of stages. Antepenultimate instar at least 9 days, penultimate instar 6 days, ultimate instar 18 days, pupa 23–24 days.

Melothria pendula is a new host plant record for *H. cunigunda*, the only previously known being the palm *Syagrus romanzoffiana* (Robinson et al 2010). *Hypercompe* spp. generally are polyphagous, in some species extremely so: in Costa Rica, *H. albescens* is reported to feed on 81 spp. from 31 families and *H. icasia* on 91 spp. from 40 families (Janzen & Hallwachs 2009). *H. cunigunda* is known from Ecuador, Venezuela, Suriname, French Guiana, Brazil and Bolivia (De Toulgoët & Navatte 2000). Therefore, other host plants are likely to be found. Another aspect of arctiine larval biology is individual polyphagy: a particular larva feeds on several individual plants and often on different species, even on the same day (Singer & Bernays 2009). For *H. cunigunda*, we had no evidence for this, although the short activity boost of the newly-molted last instar could be a sign that it normally would leave its host plant to forage at ground level, a behavior associated with polyphagy. Additional studies are required to understand more about the early instars and possible larval variation.

ACKNOWLEDGEMENTS

We would like to express our special thanks to Liz Steele, to Frans Barten for preparing the figures and to two anonymous reviewers for their constructive comments. We are grateful to Claudine Sakimin, Nature Conservation Division of the Suriname Forest Service, Paramaribo, for granting a research permit that includes the study of lepidopteran life histories. This research has been made possible by a grant from the Uyttenboogaart-Eliasen Foundation.

LITERATURE CITED

DE TOULGOËT, H & J. NAVATTE. 2000. Catalogue systématique et illustré des Lépidoptères Arctiidae, Arctiinae et Pericopinae de Guyane Française se trouvant dans la collection du Muséum National d'Histoire Naturelle. Available from: http://www7.inra.fr/papillon/arct_guy/arct_guy.htm (accessed 10 Aug 2015).

DYER, L.A., J.S. MILLER, S.B. RAB GREEN, G.L. GENTRY, H.F. GREENEY & T.W. WALLA. 2004. Caterpillars and parasitoids of the Eastern Andes in Ecuador. Available from: http://caterpillars.unr.edu/lsacat/ecuador/index.htm (accessed 9 Sept 2015).

JANZEN, D.H. & W. HALLWACHS. 2009. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservacion Guanacaste (ACG), northwestern Costa Rica (nn-SRNP-nnnnn voucher codes). Available from: http://janzen.sas.upenn.edu (accessed 8 Sept 2015).

MORI, S.A., G. CREMERS, C. GRACIE, J.J. DE GRANVILLE, S.V. HEALD, M. HOFF & J.D. MITCHELL. 2002. Guide to the vascular plants of central French Guiana. Part 2. Dicotyledons. Memoirs of the New York Botanical garden, New York, vol 76, part 2. 776 pp.

ROBINSON, G.S., P.R. ACKERY, I.J. KITCHING, G.W. BECCALONI & L.M. HERNÁNDEZ. 2010. HOSTS - A Database of the World's Lepidopteran Hostplants. Natural History Museum, London. Available from: http://www.nhm.ac.uk/hosts (accessed: 8 Sept 2015).

SINGER, M.S. & E.A. BERNAYS. 2009. Specialized generalists: behavioral and evolutionary ecology of polyphagous woolly bear caterpillars, pp. 103–114. *In* Conner, W.E. (ed.), Tiger Moths and Woolly Bears. Behavior, ecology, and evolution of the Arctiidae. Oxford University Press, New York.

STOLL, C. 1781. pp. 104–105 and Plate CCCXLIV Fig D and E. *In* Cramer P. 1782. De Uitlandsche Kapellen voorkomende in de drie Waereld-deelen Asia, Africa en America [Papillons Exotiques des Trois Parties du Monde l'Asie, l'Afrique et l'Amerique]. S.J. Baalde, Amsterdam/ B. Wild, Utrecht.

VAN ANDEL, T.R. & S. RUYSSCHAERT. 2011. Medicinale en rituele planten van Suriname. KIT Publishers, Amsterdam. 528 pp.

VINCENT, B. & M. LAGUERRE. 2014. Catalogue of the Neotropical Arctiini Leach, [1815] (except Ctenuchina Kirby, 1837 and Euchromiina Butler, 1876) (Insecta, Lepidoptera, Erebidae, Arctiinae) Zoosys. 36: 37–533.

WAGNER, D. 2005. Caterpillars of Eastern North America: A guide to identification and natural history. Princeton University Press, Princeton NJ, 512 pp.

HAJO B.P.E. GERNAAT* *(*corresponding author), Department of Taxonomy and Systematics, Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands; email: hajo.gernaat@naturalis.nl*; AUKE J. HIELKEMA, *Buenos Airesstraat 69 (Beni's Park 2), Paramaribo, Suriname; email: auke_hielkema @yahoo.co.uk;* ELKE NIJMAN, *Gantvoort 12, 7161 KL Neede, The Netherlands;* TINDE VAN ANDEL, *National Herbarium, Naturalis Biodiversity Center; email: Tinde.vanAndel@naturalis.nl.*

Submitted for publication 1 November 2015; revised and accepted 10 January 2016.

Journal of the Lepidopterists' Society
70(2), 2016, 167–169

FIRST NOTES ON THE LIFE HISTORY OF *EUPITHECIA TARAPACA* RINDGE (GEOMETRIDAE) ON THE WESTERN SLOPES OF THE ANDES OF NORTHERN CHILE

Additional key words: *Balbisia microphylla*, Florivory, Larentiinae, Vivianiaceae, Pupal diapause

The life histories of the Lepidoptera from the Atacama Desert and the Andes of northern Chile are in general poorly studied. However, as already shown for some butterflies inhabiting these arid environments, data dealing with biology and host ranges are essential to understand their abundance and distribution patterns, and also provide useful tools to assess adequately their conservation status (Despland 2014).

Eupithecia tarapaca Rindge (Lepidoptera, Geometridae, Larentiinae) is a little known geometrid moth originally described based only on the holotype male collected in Timar, a narrow ravine situated on the western slopes of the Andes of northern Chile (Rindge 1987, 1989). All aspects of its biology are presently unknown, mostly due to the scarce sampling for geometrid moths in these arid environments. However, some adults of this species were recently reared from larvae collected on a native plant close to the type locality. Accordingly, the objective of this contribution is to provide the first notes on the life history of *E. tarapaca*, including the first host plant record and the first record of facultative pupal dormancy for this desert moth.

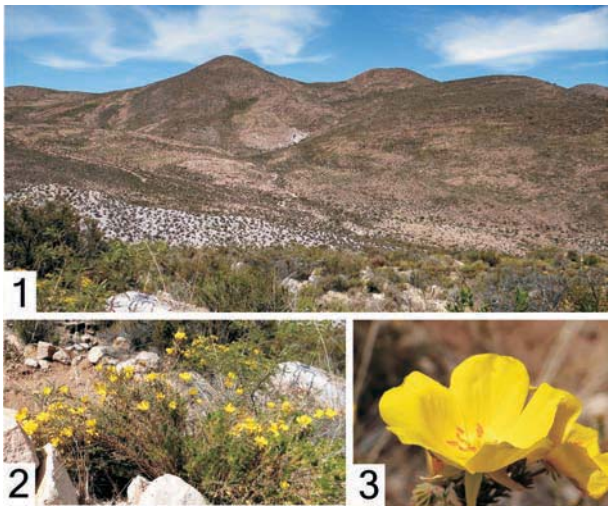
Sampling was performed in the area around Socoroma village (18°16' S, 69°35' W), Parinacota Province, at about 3,300 m elevation on the western slopes of the Andes of northern Chile (Fig. 1). The site is characterized by a tropical xeric bioclimate with a highly seasonal vegetation cover that generally reaches higher levels in March–April after the summer rains (Luebert & Pliscoff 2006). The larvae were collected between March 2011 and July 2015 on flower buds of *Balbisia microphylla* (Phil.) Reiche (Vivianiaceae) (Fig. 2–3). They were placed in plastic vials and kept in the laboratory. Additional flowers of the same plant were provided daily until the larvae finished eating and started to prepare for pupation. Pupae were periodically observed until adult emergence. Adults were pinned, spread, and dissected following standard procedures in order to provide a taxonomic identification. Voucher specimens are deposited in the Colección Entomológica de la Universidad de Tarapacá (IDEA), Arica, Chile.

Seventeen adults of *E. tarapaca* were reared, twelve males and five females. The identification was based on comparisons with the original description (Rindge 1987) and photographs of the genitalia of the holotype deposited in the American Museum of Natural History (AMNH).

Balbisia microphylla is the first host plant recorded for *E. tarapaca*. Furthermore, this is the first mention of the association of *Eupithecia* with Vivianiaceae (Robinson et al. 2010). At the local level, previous host plant records for *Eupithecia* of northern Chile mostly included Fabaceae, with only one species, *E. atacama* Vojnits, associated with Chenopodiaceae, and another, *E. yubitzae* Vargas & Parra, with larvae being able to feed on Anacardiaceae as well as Fabaceae (Vargas et al. 2015).

Detailed knowledge of host ranges of geometrid larvae is useful to understand the ecology of these organisms in these arid environments, either in their role as herbivores or as prey (Méndez-Abarca et al. 2014, Vargas et al. 2014). Although this study did not aim to determine the host specificity of *E. tarapaca*, it must be noted that other plant species, mostly of the Asteraceae and Fabaceae, have been surveyed for florivorous caterpillars for more than seven years in the study site, but no additional hosts have been detected for *E. tarapaca*, suggesting a close association with *B. microphylla*. Monophagy and oligophagy have been reported for other Neotropical representatives of the Larentiinae (Strutzenberger et al. 2010, Seifert et al. 2015), including *Eupithecia* (Parra & Ibarra-Vidal 2002, Bodner et al. 2010). However, polyphagy has been reported for several florivorous Nearctic and Palearctic *Eupithecia* (Bolte 1990, Mironov 2003, 2014). Further field and laboratory studies are required to assess better the host specificity of *E. tarapaca*. Since other species of *Balbisia* have distribution ranges close to *B. microphylla* both in northern Chile and southern Peru (Weigend 2005, 2011), it should be interesting to survey them for *E. tarapaca* larvae.

All larvae of *E. tarapaca* included in this study were collected in flower buds of the host. They mostly fed on the reproductive structures of the floral buds during rearing, eventually also eating the petals. Although leaves were also offered to the larvae, these organs were never consumed, suggesting a florivorous habit. Florivory appears to be the predominant feeding habit of the *Eupithecia* from the Atacama Desert, although



FIGS 1-3. Habitat and host plant of *Eupithecia tarapaca*. **1**) Study site near Socoroma village (18°16' S, 69°35' W), Parinacota Province, at about 3,300 m elevation on the western slopes of the Andes of northern Chile; **2**) the host plant *Balbisia microphylla*; **3**) open flower of *B. microphylla*.

larvae of *E. yubitzae* are able to eat leaves of Anacardiacaeae and flowers of Fabaceae (Vargas et al. 2015). Florivory is also the most important feeding habit in most species of *Eupithecia* globally (Mironov 2014).

The duration of the pupal stage of *E. tarapaca* was strikingly variable, ranging from about three weeks to more than two years. The most common duration of the pupal stage was 20–30 days (n = 13), but some pupae (n = 4) lasted 13–27 months in this stage, suggesting that *E. tarapaca* has facultative pupal dormancy with the possibility of an extremely prolonged duration of the pupal stage.

Many of the Nearctic and Palearctic species of *Eupithecia* overwinter as pupae with many cases of pupal diapause recorded (Bolte 1990, Mironov 2003, 2014). The northern populations of the Palearctic *Eupithecia abietaria* Goeze have biennial life cycles with a prolonged pupal diapause (Mironov 2014). Powell (1987) recorded 21 months of diapause for one pupa of the Nearctic *Eupithecia dichroma* McDunnough. Among the Neotropical *Eupithecia*, obligatory diapause was described for *E. tamarugalis* Vargas & Parra, whose larvae feed on flowers of *Prosopis tamarugo* (Fabaceae) in the Atacama Desert (Vargas & Parra 2005) about 300 km southwest of the study site. This strategy enables *E. tamarugalis* to synchronize its life cycle perfectly with the flowering of its host plant; adults and larvae are active only during the season with higher availability of flower buds and flowers for egg laying (female adults) and feeding (larvae).

Facultative diapause can have profound effects on the ecology and evolution of species (Liu et al. 2010, Mironov 2014). It has been recognized as a useful strategy to overcome adverse conditions in phytophagous Lepidoptera (Pessoa-Queiroz et al. 2008). Powell (1987) highlighted that extended diapause mostly occurs in species of Lepidoptera inhabiting areas with seasonal drought and with erratic food abundance. Studies dealing with the flowering phenology of *B. microphylla* were not found in the literature. However, in accordance with the patterns described for three other species of *Balbisia* in southern Peru (Weigend 2005), preliminary observations performed in the study site suggest that the main flowering of *B. microphylla* occurs following the summer rains, approximately in March–April, while additional flowers occur during the remainder of the year. The abundance of flowers produced outside the main flowering period appears to be extremely variable temporally and also highly clustered spatially. Thus based on the life history notes described here for *E. tarapaca*, it appears that this moth fits the bet-hedging strategy (Venable 2007, Rajon et al. 2014), characterized in this case by a variable duration of the pupal stage which enables *E. tarapaca* to use a highly variable resource. Further field and laboratory studies are required to understand the adaptive significance of the facultative pupal dormancy of *E. tarapaca* on the arid western slopes of the Andes of northern Chile.

ACKNOWLEDGMENTS

The author thanks Courtney Richenbacher and David Grimaldi for providing photographs of the holotype of *E. tarapaca* deposited in AMNH; Emma Despland for providing literature; Maximilian Weigend for confirming the identification of *B. microphylla*; Konrad Fiedler and an anonymous reviewer for constructive comments on a preliminary version and Lafayette Eaton for checking the English. This study was financed by project grant DGI-UTA 9710-13.

LITERATURE CITED

BODNER., F., G. BREHM, J. HOMEIER, P. STRUTZENBERGER & K. FIEDLER. 2010. Caterpillars and host plant records for 59 species of Geometridae (Lepidoptera) from a montane rainforest in southern Ecuador. J. Insect Sci. 10:67.

BOLTE, K. B. 1990. Guide to the Geometridae of Canada (Lepidoptera. VI. Subfamily Larentiinae. 1. Revision of the genus *Eupithecia*. Mem. Ent. Soc. Can. 151:1–253.

DESPLAND, E. 2014. Butterflies of the high-altitude Atacama Desert: habitat use and conservation. Front. Genet. 5:334.

LIU, Z., P. GONG, D. LI. & W. WEI. 2010. Pupal diapause of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) mediated by larval host plants: pupal weight is important. J. Insect Physiol. 56:1863–1870.

LUEBERT, F. & P. PLISCOFF. 2006. Sinopsis bioclimática y vegetacional de Chile. Editorial Universitaria, Santiago. 316 p.

MÉNDEZ-ABARCA, F., C. F. MÉNDEZ & E. A. MUNDACA. 2014. Host plant specificity of the moth species *Glena mielkei* (Lepidoptera, Geometridae) in northern Chile. Rev. Chil. Hist. Nat. 87:22.

MIRONOV, V. 2003. The Geometrid Moths of Europe. Volume 4: Larentiinae II (Perizomini and Eupitheciini). Apollo Books.

MIRONOV, V. G. 2014. Geometrid moths of the genus *Eupithecia* Curtis, 1825 (Lepidoptera, Geometridae): prerequisites and characteristic features of high species diversity. Entomol. Rev. 94:105–127.

PARRA, L. E. & H. IBARRA-VIDAL. 2002. A new species of *Eupithecia* (Lepidoptera: Geometridae) from the Juan Fernández Islands. Ann. Entomol. Soc. Am. 95:9–15.

PESSOA-QUEIROZ, R., H. C. MORAIS & I. R. DINIZ. 2008. Abundance and temporal distribution of *Gonioterma exquisita* Duckworth (Lepidoptera, Elachistidae, Stenomatinae) on *Byrsonima pachyphylla* Griseb. (Malpighiaceae) in the Brazilian Cerrado. Rev. Bras. Entomol. 52:62-67.

POWELL, J. A. 1987. Records of prolonged diapause in Lepidoptera. J. Res. Lepid. 25:83-109

RAJON, E., E. DESOUHANT, M. CHEVALIER, F. DÉBIAS, & F. MENU. 2014. The evolution of bet hedging in response to local ecological conditions. Am. Nat. 184:E1–E15.

RINDGE, F. H. 1987. The *Eupithecia* of Chile (Lepidoptera, Geometridae). Bull. Am. Mus. Nat. Hist. 186:269–363

RINDGE, F. H. 1989. An emended specific name in *Eupithecia* (Geometridae). J. Lepid. Soc. 43:71

ROBINSON, G. R., P. R. ACKERY, I. J. KITCHING, G. W. BECCALONI & L. M. HERNÁNDEZ. 2010. HOSTS—a database of the world's lepidopteran hostplants. Available at: http://www.nhm.ac.uk/research-curation/research/projects/hostplants/index.html (accessed 19 October 2015)

SEIFERT, C. L., F. BODNER, G. BREHM & K. FIEDLER. 2015. Host plant associations and parasitism of south Ecuadorian *Eois* species (Lepidoptera: Geometridae) feeding on *Peperomia* (Piperaceae). J. Insect Sci. 15:119.

STRUTZENBERGER, P., G. BREHM, F. BODNER & K. FIEDLER. 2010. Molecular phylogeny of *Eois* (Lepidoptera, Geometridae): evolution of wing patterns and host plant use in a species-rich group of Neotropical moths. Zool. Scr. 39:603–620.

VARGAS, H. A. & L. E. PARRA. 2005. Descripción de una nueva especie de *Eupithecia* Curtis (Lepidoptera: Geometridae) de la Pampa del Tamarugal, Chile. Neotrop. Entomol. 34:215–219.

VARGAS, H. A., M. VARGAS-ORTIZ, W. HUANCA-MAMANI & A. HAUSMANN. 2014. Prey identification in nests of the potter wasp *Hypodynerus andeus* (Packard) (Hymenoptera, Vespidae, Eumeninae) using DNA barcodes. Rev. Bras. Entomol. 58:157–160.

VARGAS, H. A., M. VARGAS-ORTIZ & W. HUANCA-MAMANI. 2015. First record of folivory on a newly documented host plant for the little known geometrid moth *Eupithecia yubitzae* Vargas & Parra (Lepidoptera, Geometridae) in northern Chile. Rev. Bras. Entomol. 59:121–123.

VENABLE, D. L. 2007. Bet hedging in a guild of desert annuals. Ecology, 88: 1086–1090.

WEIGEND, M. 2005. Notes on the floral morphology in Vivianiaceae (Geraniales). Pl. Syst. Evol. 253:125–131.

WEIGEND, M. 2011. The genus *Balbisia* (Vivianiaceae, Geraniales) in Peru, Bolivia and northern Chile. Phytotaxa 22:47–56

HÉCTOR A. VARGAS, *Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Casilla 6-D, Arica, Chile. havargas@uta.cl; lepvargas@gmail.com*

Submitted for publication 29 October 2015; revised and accepted 23 December 2015.

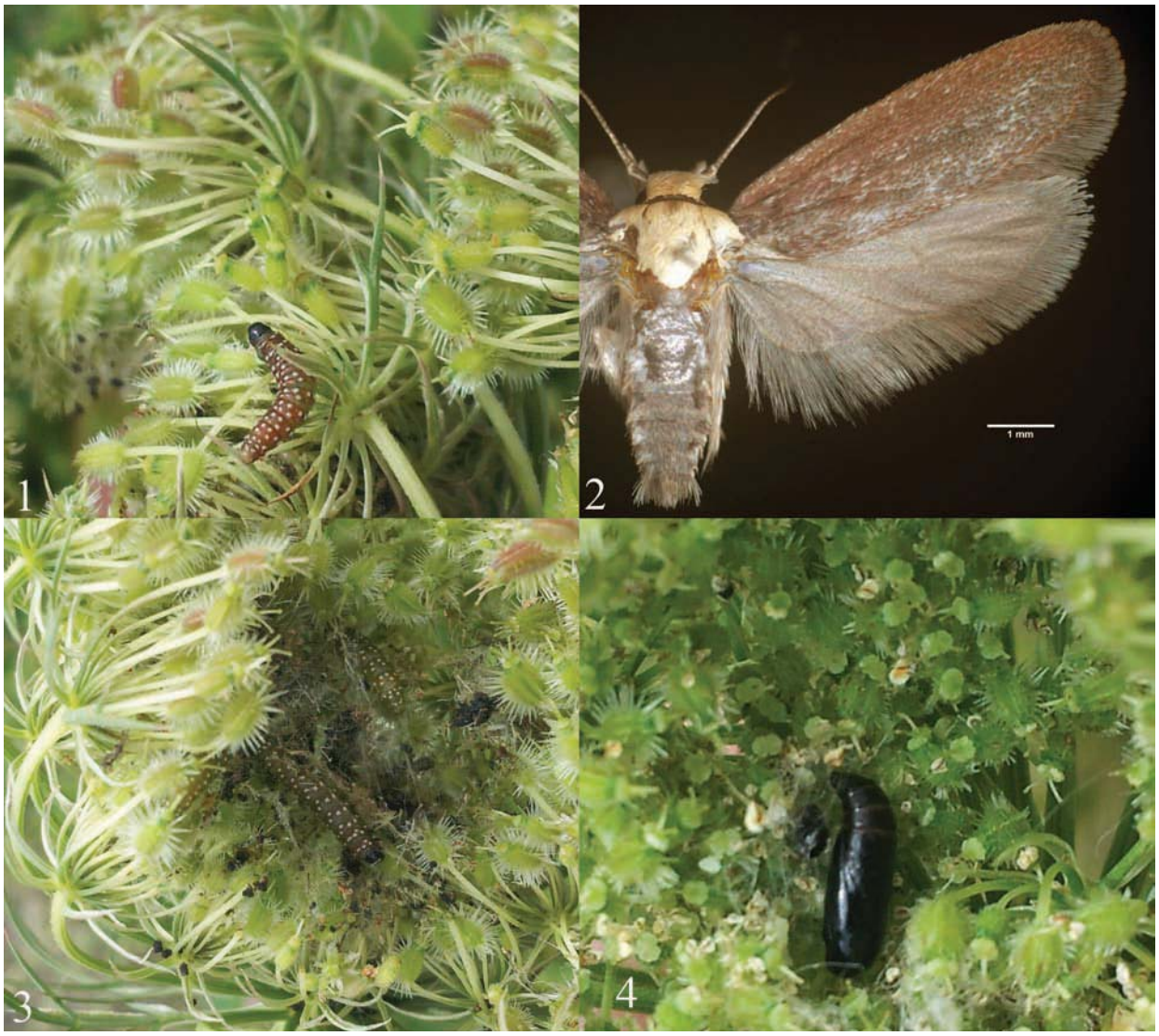
Journal of the Lepidopterists' Society
70(2), 2016, 169–173

DEPRESSARIA DEPRESSANA (FABRICIUS) (DEPRESSARIIDAE), NEW TO THE MIDWESTERN USA

Additional key words: Introduced species, Apiaceae, Ichneumonidae, Chalcididae

Characterization. *Depressaria depressana* (Fabricius) (Depressariidae), known as Blunt's flat-body or purple carrot-seed moth, is native to the Palearctic region, occurring from Europe to China and the Russian Far East (Landry et al. 2013). The larva (Fig. 1) is brownish red with irregularly-shaped dull green regions laterally and ventrally, as well as dorsally along the intersegmental margins; the head, prothoracic shield, thoracic legs, and spiracular peritremes are black, and the setal pinacula are white. The adult (Fig. 2) is uniformly purplish brown dorsally, with the head and thorax, including the tegulae, white. In Europe, the larva of *D. depressana* is recorded to feed on the inflorescences of many apiaceous hostplants, including *Daucus carota* L., *Pastinaca sativa* L., *Peucedanum oreoselinum* (L.) Moench, and several species in the genera *Carum* L., *Pimpinella* L., and *Seseli* L. (Palm 1989; Landry et al. 2013). The moth is univoltine, spending most of the year in the adult stage.

Occurrence in North America. Landry et al. (2013) appropriately concluded that *D. depressana* is a recent introduction into North America, on the basis of the fact that it was not included in monographs on Nearctic "Oecophoridae" (including Depressariidae) by Clarke (1941) and Hodges (1974) (of *Depressaria* species, Hodges stated that only "*cinereocostella, pastinacella, eleanorae*, and *alienella* occur in eastern North America"). Landry et al. (2013) recorded *D. depressana* from the Canadian provinces of Ontario and Quebec, with the earliest date being 2008. In addition, J.-F. Landry (in litt.) reports: "I have reared adults from maturing larvae collected in the flower umbels of *Daucus carota* near Ottawa on 20 Sep 2014, with adults emerging from 25 Sep to 20 Oct 2014. Eleven voucher specimens in the CNC." The moth also has been documented from the northeastern region of the USA. Photographs of the larva and adult of *D. depressana* from New York, Massachusetts, and Connecticut have been posted onto the BugGuide (2003–2015) web site, with the earliest date being 2010.



FIGS. 1–4. **1.** Larva of *Depressaria depressana* in umbel of *Daucus carota*, Champaign County Illinois, 11 July 2015. **2.** Adult *Depressaria depressana*, reared from larva collected in umbel of *Daucus carota*, Champaign County Illinois, 10 July 2015, emerged 21 July 2015. **3.** Multiple larvae of *Depressaria depressana* in umbel of *Daucus carota*, Champaign County Illinois, 11 July 2015. **4.** Pupa of *Depressaria depressana* in umbel of *Daucus carota*, Champaign County Illinois, 11 July 2015.

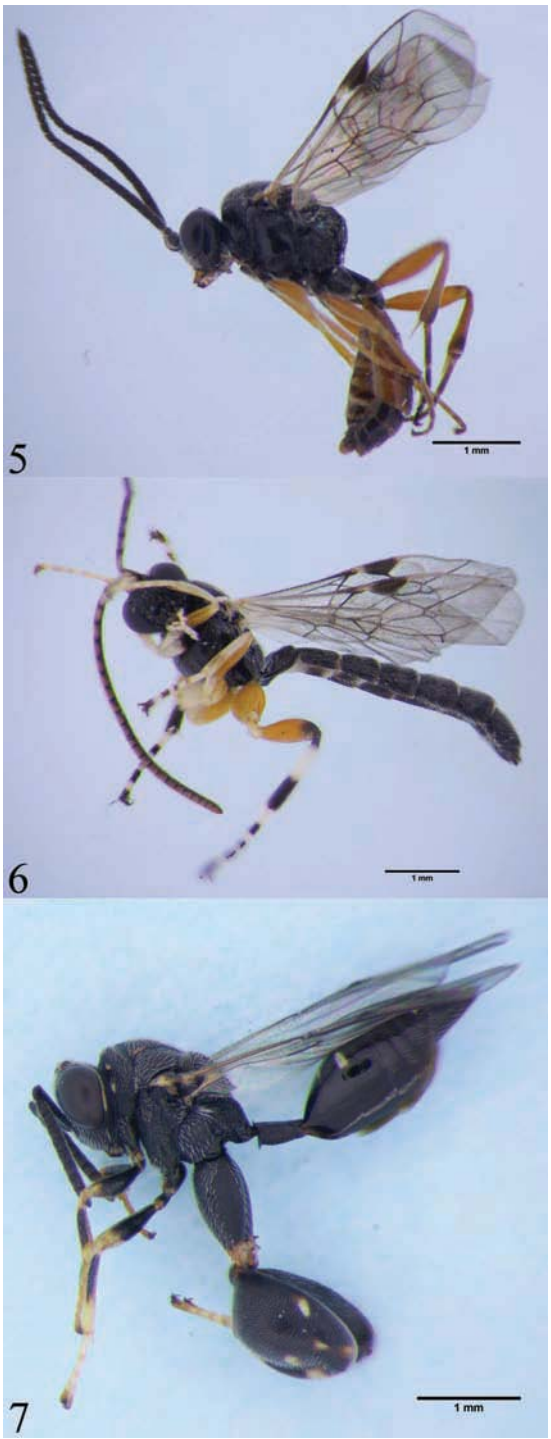
Very recently, it has become evident that *D. depressana* has expanded its range into the midwestern US. On 26 September 2014, James Vargo collected an adult *D. depressana* at UV light in Newton County in northern Indiana, and, on 2 August 2015, an adult *D. depressana* was photographed in Summit County in northern Ohio (photograph posted onto BugGuide). In July 2015, larvae and pupae of *D. depressana* were collected from inflorescences of *Pastinaca sativa* and *Daucus carota* in central Illinois, which at present represents the greatest southern and western range extension of the moth from its presumed point of introduction in southeastern Canada. A sample of two larvae on *P. sativa* was collected on 2 July 2015 near Urbana, along the entrance gate of Phillips Tract, a preserve owned by the University of Illinois at Urbana-Champaign. On 10 July 2015, an additional 21 individuals (11 larvae, 10 pupae) were collected on *P. sativa* and *D. carota* at Phillips Tract and also at a nearby overpass crossing Interstate Highway 74. In addition, on 13 July 2015, two *D. depressana* pupae were collected in a flower of *D. carota*, at a separate site approximately 5.5 km north of Urbana. Larvae and pupae were collected in situ on their hostplants and were reared to eclosion; adults were photographed and dissected, confirming their identity as *D. depressana*.

Similar species. *Depressaria alienella* Busck, the adult of which is externally similar to that of *D.*

depressana, is native to northeastern and far-western North America. The two species differ in a number of respects. Characters for differentiating adults of *D. alienella* and *D. depressana* on both external appearance and genital morphology were given by Landry et al. (2013). Furthermore, the host plants of *D. alienella* belong to the genera *Artemisia* L. and *Achillea* L. (Asteraceae) (Walsingham 1881; Clarke 1941). Also, Busck (1902) described the larva of *Depressaria emeritella* Stainton (a Palearctic species that in the adult stage closely resembles *D. alienella*, but now is believed not to occur in North America), as being "green with dorsal and subdorsal lines darker; head yellowish-green; first thoracic segment black dotted." It is not clear, however, whether this was a second-hand description based on European accounts of the larva of *D. emeritella* (it closely matches, e.g., Stainton's (1861) description and figure of that species) or if Busck actually examined the larva of the native North American moth that he later described as *D. alienella* (Busck 1904).

Depressaria pastinacella (Duponchel), another Eurasian invasive species, can also be found on umbels of *Pastinaca sativa* in central Illinois in July, but it is unlikely to be mistaken for *D. depressana*. Morphologically, it can be distinguished by its pale body color and dark pinacula. It is also considerably larger in size: preserved final-instar larvae of *D. pastinacella* from Illinois average 19.5 mm in length (n = 5, range = 18.0–21.0 mm), versus 10.8 mm in *D. depressana* (n = 2, range = 10.0–11.5 mm). The two species differ phenologically and ecologically as well. In central Illinois, *D. pastinacella* larvae feed on flowers and immature fruits of wild parsnip primarily in June and larval development is largely complete by early July (Nitao and Berenbaum 1988). By contrast, *D. depressana* larvae are most abundant in July, when *P. sativa* inflorescences contain primarily immature seeds and *D. carota* inflorescences have not yet fully opened. Another difference is that multiple *D. pastinacella* larvae are not commonly found in a single umbel; the larvae are territorial and actively exclude potential usurpers (Green et al. 1998). By contrast, multiple individuals of *D. depressana* can be found occupying the same umbel (Palm, 1989; Fig. 3). Moreover, *D. depressana* pupates within the umbel (Fig. 4), whereas *D. pastinacella* burrows into the hollow stem of its host plant and spins a silken cocoon in which to pupate (Zangerl et al. 2002).

Parasitoid associations. No parasitoid records have been reported previously for *D. depressana* in North America. Two of the 11 *D. depressana* caterpillars collected during this study produced a single specimen



FIGS. 5–7. **5.** Adult Phygadeuontinae sp. (Ichneumonidae) reared from *Depressaria depressana* larva collected on *Daucus carota*, Champaign County Illinois, 10 July 2015, parasitoid emerged 22 July 2015. **6.** Adult *Itoplectis conquisitor* (Ichneumonidae) reared from *Depressaria depressana* larva collected on *Daucus carota*, Champaign County Illinois, 10 July 2015, parasitoid emerged 22 July 2015. **7.** Adult *Conura* sp. (Chalcididae) reared from *Depressaria depressana* pupa collected on *Pastinaca sativa*, Champaign County Illinois, 10 July 2015, parasitoid emerged 10 August 2015.

of ichneumonid wasp. One of these ichneumonids is a phygadeuontine (Fig. 5). Phygadeuontinae is the largest subfamily of Ichneumonidae; species in this subfamily are typically idiobiont ectoparasitoids of holometabolous larvae, although life histories in this subfamily are diverse and include endoparasitoids and ectoparasitoids, koinobionts and idiobionts, hyperparasitoids, and some egg-sac parasitoids of chelicerates (Goulet & Huber 1993). The other larval parasitoid that we reared is a male *Itoplectis conquisitor* (Say) (Ichneumonidae: Pimplinae) (Fig. 6). *I. conquisitor* females typically oviposit in the mature larvae or prepupae of Lepidoptera from several families and are sometimes hyperparasitoids of other parasitoid wasps (Townes 1940). We do not know of any previous records of *I. conquisitor* attacking Depressariidae larvae; however, this species is highly polyphagous and widespread in North America (Townes 1940). *I. conquisitor* has been uncommonly reared from other Gelechioidea families, including Gelechiidae (Miller 1955; Pogue 1985; Loeffler 1994 (as a hyperparasitoid)) and Coleophoridae (Miller 1976). Other *Itoplectis* species, *I. quadricingulatus* (Provancher) and *I. melanocephalus* (Gravenhorst), attack several *Depressaria* species, including *D. pastinacella* and *D. daucella* (Denis & Schiffermüller) (Townes 1940; Gürbüz et al. 2009). In addition to the two ichneumonids that were reared from *D. depressana* larvae, a chalcidid of the genus *Conura* Spinola (Fig. 7) was reared from one of the 10 *D. depressana* pupae collected in this study. *Conura* species are typically parasites of the pupae of Lepidoptera, although some species attack Coleoptera or Hymenoptera, and some species are secondary parasites of braconid or ichneumonid-infested hosts (Bouček & Halstead 1997).

Ecological implications. In view of its status as more of an Apiaceae generalist than *D. pastinacella*, the possibility arises that in North America *D. depressana* may acquire native Apiaceae species as host plants. In fact, even the more specialized *D. pastinacella* has incorporated at least one North American apiaceous native species, *Heracleum sphondylium* subsp. *montanum* (Schleich. ex Gaudin) Briq. (reported in literature as *H. maximum* W. Bartram and *H. lanatum* Michx.), into its diet (Berenbaum and Zangerl 1991). A cursory examination of a population of *Zizia aurea* Koch, flowering in the general proximity of infested *D. carota* and *P. sativa* populations (within a 3-km radius), failed to reveal any evidence of colonization of this native hostplant by *D. depressana*. Given the likelihood that this species is expanding its range throughout the eastern and central states, however, other native Apiaceae may be at risk of becoming incorporated into the hostplant range of this invasive species.

ACKNOWLEDGMENTS

We thank Chris Grinter, for providing preserved larvae of *D. pastinacella* from the collection of the Illinois Natural History Survey, James Vargo, for the Indiana record of *D. depressana*, L. Field, D. Silsbee, J. Greenberg, and L. Armstrong, for posting photos of *D. depressana* from the USA onto BugGuide, and James Whitfield of the University of Illinois at Urbana-Champaign, for parasitoid identifications. This work was supported by National Science Foundation grant number NSFDEB1457731 to M.R.B.

LITERATURE CITED

BERENBAUM, M. R. & A. ZANGERL. 1991. Acquisition of a native host plant into the diet of an introduced oligophagous herbivore. Oikos 62:153-159.

BOUČEK, Z. & J. A. HALSTEAD. 1997. Chalcididae, pp. 151-164. *In* Gibson, G. A. P., J. Huber, & J. B. Woolley (eds.), Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). Research Press, National Research Council of Canada, Ottawa, Ontario.

BUGGUIDE.NET. 2003-2015. Iowa State University Department of Entomology. Accessed 9 August 2015.

BUSCK, A. 1902. A review of the American moths of the genus *Depressaria* Haworth, with descriptions of new species. Proc. U. S. Nat. Mus. 24:731-749.

———. 1904. Tineid moths from British Columbia, with descriptions of new species. Proc. U. S. Nat. Mus. 27:745-778.

CLARKE, J. F. G. 1941. Revision of the North American moths of the family Oecophoridae, with descriptions of new genera and species. Proc. U. S. Nat. Mus. 90:33-286.

GOULET, H. & J. T. HUBER. 1993. Hymenoptera of the World: An Identification Guide to Families. Agriculture Canada Research Branch, Ottawa, Ontario. 688 pp.

GREEN, E. S., A. R. ZANGERL, & M. R. BERENBAUM. 1998. Reduced aggressive behavior: A benefit of silk-spinning in the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Oecophoridae). J. Insect Behav. 11:761-772.

GÜRBÜZ, M. F., J. KOLAROV, & A. BUNCUKÇU. 2009. A Survey of *Itoplectis melanocephala* (Grav.)—A new ichneumonid endoparasite on wax moth *Galleria mellonella* L. J. Entomol. Res. Soc. 11:43-49.

HODGES, R.W. 1974. The Moths of America North of Mexico, Fascicle 6.2. Gelechioidea: Oecophoridae. E. W. Classey and R. B. D. Publications Inc., London. 142 + x pp. + 8 plates.

LANDRY, J.-F., V. NAZARI, J. R. DEWAARD, M. MUTANEN, C. LOPEZ-VAAMONDE, P. HUEMER, & P. D. N. HEBERT. 2013. Shared but overlooked: 30 species of Holarctic Microlepidoptera revealed by DNA barcodes and morphology. Zootaxa 3749:1-93.

LOEFFLER, C. C. 1994. Natural history of leaf-folding caterpillars, *Dichomeris* spp.(Gelechiidae), on goldenrods and asters. J. New York Entomol. Soc. 102:405-428.

MILLER, G. E. 1976. Native parasites of *Coleophora laricella* (Lepidoptera: Coleophoridae) in British Columbia. Ph.D. Dissertation. Simon Fraser University, Burnaby, British Columbia, Canada.

MILLER, W. E. 1955. Biology of *Anacampsis innocuella* (Zeller), a leafroller on aspen. J. Econ. Entomol. 48:622-623.

NITAO, J. K. & M. R. BERENBAUM. 1988. Laboratory rearing of the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Oecophoridae). Ann. Entomol. Soc. Am. 81:485-487.

PALM, E. 1989. [Oecophoridae (Lepidoptera) of northern Europe – with special reference to Denmark]. Danmarks Dyreliv Vol. 4. Apollo Books, Stenstrup. 247 pp. [in Danish]

POGUE, M. G. 1985. Parasite complex of *Archips argyrospilus, Choristoneura rosaceana* (Lepidoptera: Tortricidae) and *Anacampsis*

innocuella (Lepidoptera: Gelechiidae) in Wyoming shelterbelts. Entomol. News 96:83-86.

STAINTON, H. T. 1861. The Natural History of the Tineina. Volume VI. *Depressaria*. Part I. Van Voorst, London. 283 + vii pp. + 8 plates.

TOWNES, H. K. 1940. A revision of the Pimplini of eastern North America (Hymenoptera, Ichneumonidae). Ann. Entomol. Soc. Am. 33:283-323.

WALSINGHAM, LORD T. DE G. 1881. On some North-American Tineidae. P. Zool. Soc. London 1881:301-325 + 2 plates.

ZANGERL, A. R., T. HUANG, J. L. MCGOVERN, & M. R. BERENBAUM. 2002. Paradoxical host shift by *Depressaria pastinacella* in North America: is enemy-free space involved? Oikos 98:431-436.

TERRY HARRISON*, CHARLES A. E. DEAN, KYLE PARKS, AND MAY R. BERENBAUM, *Department of Entomology, University of Illinois at Urbana-Champaign, 320 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801 USA *Corresponding Author, E-mail: tharriso@illinois.edu*

Submitted for publication 15 October 2015; revised and accepted 20 November 2015.

A NEW GENERIC ASSIGNMENT FOR *TORTRIX BABOQUAVARIANA* KEARFOTT, 1907 (LEPIDOPTERA: TORTRICIDAE) WITH COMMENTS ON ITS TRIBAL ASSIGNMENT

Additional key words: Arizona, Cochylini, *Hyptiharpa*, Mexico, new synonymy, Sinaloa

Tortrix baboquavariana Kearfott, 1904 has a history of uncertain generic and tribal assignment. Described from three specimens collected in the Baboquivari (also spelled "Baboquavari") Mountains of southern Arizona, it was assigned by Kearfott (1904) to the "catch-all" tortricid genus *Tortrix* Linnaeus (Tortricidae: Tortricinae). Authors of early checklists of North American Lepidoptera (i.e., Barnes & McDunnough 1917, McDunnough 1939) continued to treat *baboquavariana* as a species of *Tortrix*. Although hundreds of species were described historically in *Tortrix*, the genus currently is narrowly circumscribed to include only two Palaearctic species (Brown 2005). Hence, *baboquavariana* belongs elsewhere.

In order to provide a more appropriate generic assignment for *baboquavariana*, Powell (1983) transferred the species (the spelling emended to "*baboquivariana*") to "*Incertae Sedis*" in Cochylidae (currently considered a tribe of Tortricidae). Poole and Gentili (1996) transferred all species treated by Powell (1983) as *Hysterosia* and Incertae Sedis to *Phtheochroa* because the latter subsequently was determined to be the senior synonym of *Hysterosia*. Brown (2005) listed *baboquivariana* (following Powell's emendation) under "Cochylini unplaced," and this treatment recently was followed by Metzler and Brown (2014). Therefore, the species continues to lack a contemporary generic assignment.

Meanwhile, in 1992 Razowski described *Hyptiharpa hypostas* (new genus and new species) from Sinaloa, Mexico. He assigned the genus to the tribe Euliini, with a few caveats, and Powell et al. (1995) followed that assignment in the Atlas of Neotropical Lepidoptera.

While trying to identify appropriate generic assignments for a few unplaced Cochylina, I recently stumbled upon the illustration of the male genitalia of *Hyptiharpa hypostas* in Razowski (1992) and realized that the genitalia are a perfect match with those on an old slide of *baboquavariana* in the National Museum of Natural History, Washington, DC. It took me only 23 years to put together these two simple pieces of the puzzle: the illustration of the male genitalia of *Hyptiharpa hypostas* in Razowski (1992) and the slide-mounted genitalia of the male of *Tortrix baboquavariana* in the USNM. This discovery results in a contemporary generic assignment for the species, i.e., *Hyptiharpa baboquavariana*, new combination; the synonymy of *hypostas* Razowski with the senior synonym *baboquavariana* Kearfott; and the possible assignment of the species to the subtribe Euliina (Cochylini), although the latter is still somewhat unresolved.

The lectotype of *Tortrix baboquavariana*, designated by Klots (1942), is deposited in the American Museum of Natural History (AMNH) and two co-types (paralectoypes) are in the National Museum of Natural History (USNM), Washington, DC. The holotype and a paratype of *Hyptiharpa hypostas* are deposited in the Essig Museum of Entomology (EME), University of California, Berkeley

Hyptiharpa baboquavariana (Kearfott, 1904), **new combination**

Figs. 1–3

Tortrix baboquavariana Kearfott, 1904: 82 (description); Barnes and McDunnough 1917: 177 (checklist); McDunnough 1938: 57 (checklist); Klots 1942: 412 (lectotype designation).

"Incertae Sedis" *baboquivariana*: Powell 1983: 42 (checklist). Unjustified emendation.

Hyptiharpa hypostas Razowski, 1992: 106 (description); Powell et al. 1995: 83 (checklist). **New Synonoymy.**

"Cochylini Unplaced" *baboquivariana*: Brown 2005: 208 (checklist).

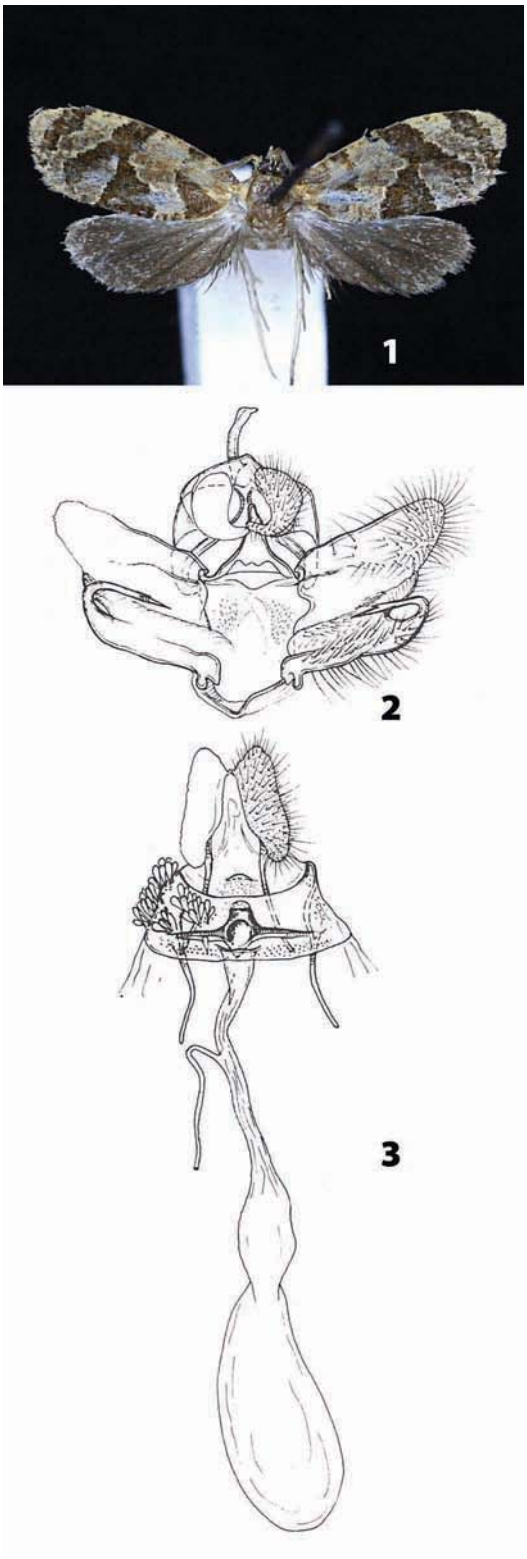
"Cochylina Unplaced" *baboquivariana*: Metzler and Brown 2014: 278 (checklist)

.

Diagnosis. *Hyptiharpa baboquavariana* is a small moth (forewing length mean 4.1 mm; n = 6) similar to many small Cochylini, but it is easily distinguished by the forewing pattern alone (Fig. 1; also see Moth Photographers Group 2015: #3855). The forewing ground color is pale orange ocherous and slightly shiny. The brown forewing markings are well defined and consist of the following: a subbasal fascia represented by an oblique, elongate-triangular spot from near the base of the hind margin, ending either in the vicinity of the discal cell (forming an incomplete fascia) or extending to the costa (forming a more-or-less complete fascia); a rounded-triangular blotch from the middle of the hind margin; an oblique median fascia extending from the costa, originating at about 0.6 the distance from the base to the apex, to the tornus; and a narrow preterminal fascia. The forewing pattern is slightly reminiscent of that of *Aethes interruptofasciata* (Robinson, 1869) (Cochylina) and *Atepa* Razowski, 1992 (Euliina), but the genitalia of *H. baboquavariana* have little in common with those taxa. Females are easily distinguished from all other species by the presence of a dense patch of long, somewhat blunt-tipped, specialized scales from the dorsum of the last abdominal segment which extend well beyond the papillae anales. Although the scales seem pale cream-colored in dorsal view, they are more orange in posterior view.

In the male genitalia (Fig. 2) the uncus is about 0.75 the length of the valva, slightly keel-shaped, curved, slightly broadened near the middle, and distinctly tapered distally; the socii are broad basally, attenuate and incurved medially and narrowed distally; the gnathos is absent; the valvae are broad and short, with a linear, crenulate, sclerotized ridge separating the sacculus from the costal portion of the valve; the sacculus is wide and bears a long, slender, distally curved, basal process with a long free distal portion, weakly bifurcate or toothed distally; the transtilla has a large median process, broad basally (nearly as broad as the transtilla) and tapering distally; and the phallus is small, tapered distally with a sharp apex; cornuti are absent.

In the female genitalia (Fig. 3) the papillae anales are small; the apophyses posteriores are slightly longer than the apophyses anteriores; the sterigma is mostly membranous with a long transverse sclerite near the anterior edge surrounding a rounded median pit (= ostium). The corpus bursae is long and membranous



FIGS. 1–3. *Hyptiharpa baboquavariana*. **1.** Holotype male of *H. hypostas*, which is a junior synonym of *H. baboquavariana*. **2.** Male genitalia from Razowski (1992). **3.** Female genitalia from Razowski (1992).

and undifferentiated from the slender membranous corpus bursae, the latter of which lacks a signum.

Distribution. The known range of *H. baboquavariana* includes Sinaloa, Mexico and the lower elevations of the mountains of southeastern Arizona (Baboquivari, Santa Rita, and Chiricahua mountains), ranging from about 250 to 1700 m in elevation. Capture records extend from mid-July to early August.

Specimens examined (14♂, 3♀). MEXICO: Sinaloa: 27 mi E Villa Union, 800', 27 Jul 1964 (holotype ♂ of *hypostas*), J. Powell (EME). USA: Arizona: Cochise Co.: 4 mi W Portal, Chiricahua Mountains, 5300', 3–6 Aug 1964 (3♂), D. R. Davis (USNM). Palmerlee, no date (1♂), no collector (USNM). Pima Co.: Baboquavari Mts, 15–30 July 1903 (3♂, including lectotype of *baboquavariana*), O. C. Poling (USNM, AMNH). Brown Canyon, Baboquavari Mts., 7 Aug 2005 (2♂), J. Brown (USNM). Santa Cruz Co.: Pena Blanca Canyon, 7 Aug 1959 (1♀), R. Hodges (USNM), 27–28 Jul 1964 (2♂, 2♀), D. R. Davis (USNM). Madera Canyon, 27 Jul 1959 (1♂), 5 Aug 1959 (1♂), R. W. Hodges (USNM).

Tribal Assignment. Razowski (1992) assigned *Hyptiharpa* to Euliini with some reservation, but because Euliini subsequently was relegated to a subtribe of Cochylini by Regier et al. (2012), that tribal assignment (Cochylini: subtribe Euliina) is compatible with its historical placement in Cochylini and our current understanding of the phylogeny of the group. However, it is less clear to which of the two subtribes (i.e., Euliina or Cochylina) the genus should be assigned. Morphological features that support the assignment of *H. baboquavariana* to Cochylina (formerly Cochylini, sensu stricto) include the absence of a gnathos and the presence of a prominent median process of the transtilla. However, the presence of a well-developed uncus, three acanthi in the female frenulum, and the absence of sclerotization in the corpus bursae argue for its assignment to Euliina. Although a well-developed uncus and three acanthi in the female frenulum are present in some presumably basal Cochylina (e.g., Phtheochroa Stephens), *Hyptiharpa* lacks other features of basal Cochylina; i.e., most are larger tortricids, males of many species have a forewing costal fold, and the phallus usually bears one or more large cornuti. *Hyptiharpa* are small moths, males lack a forewing costal fold, and the phallus lacks cornuti. A male foreleg hairpencil is characteristic of many, but not all, Euliina (Brown 1990), and this structure is absent in *Hyptiharpa*. Female genitalia are extremely simple and hence, provide little assistance in placing the genus to a subtribe. In conclusion, although *Hyptiharpa* can be assigned convincingly to Cochylini, its subtribal assignment remains somewhat enigmatic.

ACKNOWLEDGMENTS

I thank Pete Oboyski, EME, for the loan of the holotype of *Hyptiharpa hypostas*, and Józef Razowski, Polish Academy of Sciences, for allowing me to use his illustrations of the male and female genitalia of *H. hypostas*. The image of the adult was provided by Mark Metz, Systematic Entomology Laboratory, USDA, Washington, D.C. Eric Metzler and Richard Brown provided helpful comments on the brief manuscript.

LITERATURE CITED

BARNES, W., AND J. MCDUNNOUGH. 1917. Check list of the Lepidoptera of boreal America, 392 pp.

BROWN, J. W. 1990. Taxonomic distribution and phylogenetic significance of the male foreleg hairpencil in the Tortricinae (Lepidoptera: Tortricidae). Entomol. News 101: 109–116.

BROWN, J. W. 2005. World catalogue of insects. Volume 5. Tortricidae (Lepidoptera). Apollo Books, Stenstrup, Denmark, 742 pp.

KEARFOTT, W. D. 1904. North America Tortricidae. Trans. Am. Entomol. Soc. 30: 287–299.

KLOTS, A. B. 1942. Type material of North American microlepidoptera other than Aegeriidae in the American Museum of Natural History. Bull. Am. Mus. Nat. Hist. 79: 391–424.

MCDUNNOUGH, J. 1939. Check List of the Lepidoptera of Canada and the United States of America. II. Microlepidoptera. Mem. South. Calif. Acad. Sci. 2: 1–171.

METZLER, E. H. AND J. W. BROWN. 2014. An updated check list of the Cochylina (Tortricidae, Tortricinae, Euliini) of North America north of Mexico including Greenland, with comments on classification and identification. J. Lepid. Soc. 68: 274-282.

MOTH PHOTOGRAPHERS GROUP. 2015. North American Moth Photographers Group at the Mississippi Entomological Museum. Digital Guide to Moth Identification. http://mothphotographersgroup.msstate.edu/

POOLE, R.W., AND P. GENTILI. 1996. Nomina Insecta Nearctica. A check list of the insects of North America. Volume 3. Diptera, Lepidoptera, Siphonaptera. Entomological Information Services, Rockville, Maryland, 793 pp.

POWELL, J. A. 1983. Tortricoidea, pp. 31–42. *In* R. W. Hodges, ed., Check list of the Lepidoptera of America north of Mexico. E. W. Classey, Ltd., and Wedge Entomological Research Foundation, London.

POWELL, J. A., AND J. W. BROWN. 2012. The moths of North America, fascicle 8.1. Tortricoidea, Tortricidae (part), Sparganothini and Atteriini. Wedge Entomological Research Foundation, 230 pp.

POWELL, J. A., J. RAZOWSKI, AND J. W. BROWN. 1995. Tortricidae: Tortricinae, Chlidanotinae, pp. 138–151. *In* J. B, Heppner, ed., Atlas of Neotropical Lepidoptera. Checklist: Part 2. Hyblaeoidea-Pyraloidea–Tortricoidea. Scientific Publishers, Gainesville, Florida, 243 pp.

RAZOWSKI, J. 1992. Descriptions of some Neotropical Euliini and Archipini (Lepidoptera, Tortricidae). Misc. Zool. 14: 105–114.

REGIER, J., J. BROWN, C. MITTER, J. BAIXERAS, S. CHO, M. CUMMINGS, AND A. ZWICK. 2012. A molecular phylogeny for the leaf-roller moths (Lepidoptera: Tortricidae) and its implications for classification and life history evolution. PLoS ONE 7: art. no. e35574.

JOHN W. BROWN, *Entomology Department, National Museum of Natural History, P.O. Box 37012 Washington, DC 20013-7012, USA (e-mail: tortricidae.jwb@gmail.com)*

Submitted for publication 17 December 2015; revised and accepted 3 February 2016.

NOTICE TO CONTRIBUTORS

The Lepidopterists' Society has since its inception valued the contributions of both professional and amateur Lepidopterists. We maintain a policy of accepting for publication in the Journal articles from both groups, while maintaining high standards of scholarship. The Journal of The Lepidopterists' Society is a quarterly publication welcoming articles addressing any aspect of the study of Lepidoptera, including systematics, natural history, behavior, ecology, distribution and biogeography, and evolution. Categories are Articles, Profiles, General Notes, and Advances in Methodology. Instructions for electronic submission appear below.

Short articles, especially those of a less quantitative nature, may be more appropriate for publication in the ***News of the Lepidopterists' Society***. Content for the ***News*** typically includes natural history and life cycle observations, techniques for collecting, rearing and observation, photography, state records, book reviews and notices of new publications, and current events. Such material should be sent to the editor: James Adams, School of Sciences and Math, 650 College Dr., Dalton State College, Dalton, GA 30720-3778; email: **jadams@daltonstate.edu .**

Send ***Journal*** submissions electronically to: **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.

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). 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 a minimum of 1000 pixels wide; two-column illustrations should be at least 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 *News* editor.

PRINTED BY THE ALLEN PRESS, INC., LAWRENCE, KANSAS 66044 U.S.A.

CONTENTS