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Cover Illustration: Adult male and early sixth instar larva of *Argynnis mormonia artonis* from Steens Mountain in SE Oregon, one of three subspecies of Mormon Fritillary occurring in the Pacific Northwest. High elevation populations of *A. mormonia* in the PNW are characterized by melanic late instars. Photos by David James. See article on page 199.

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DNA BARCODES AND INSIGHTS INTO THE RELATIONSHIPS AND SYSTEMATICS OF BUCKEYE BUTTERFLIES (NYMPHALIDAE: NYMPHALINAE: *JUNONIA*) FROM THE AMERICAS

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ABSTRACT. Nucleotide sequence data from a segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, known as the barcode segment, were used to examine phylogenetic relationships and systematics of buckeye butterflies (Nymphalidae: Nymphalinae: *Junonia*) from the New World, with emphasis on taxa from western North America. Three nominal species have been recognized for North America, *J. evarete* (Cramer), *J. genoveva* (Cramer), and *J. coenia* Hübner, with additional species recently proposed for the West Indies and northern South America. The distinctive Andean buckeye, *J. vestina* C. Felder & R. Felder, along with *J. evarete* and *J. genoveva*, are also components of the South American fauna. With the exception of *J. vestina*, butterflies comprising the New World *Junonia* have had a confused taxonomic history, and species assignments are often problematic. Our results show that the barcode segment resolves the two major clades of New World *Junonia*, referred to here as clades A and B, with similar high support seen in an earlier phylogenetic study using both mitochondrial and nuclear genes. Within clade A, *J. vestina* resolved in a basal position to *J. evarete* from South America and the Caribbean. The data further suggest that species assignments in some populations of New World *Junonia* clustering in clade B (*J. coenia* + *J. genoveva*) need to be reevaluated. DNA barcodes, although failing to resolve all recognized species and subspecies level taxa of New World *Junonia*, probably owing to relatively recent divergences, can provide valuable tools for identifying the two major lineages, and when used in conjunction with morphological, ecological, behavioral and life history information can provide insights into the taxonomy and evolution of this difficult group.

Additional key words: cytochrome c oxidase subunit I, dispersal, genetic distance, population structure, speciation.

Butterflies commonly known as buckeyes (Nymphalidae: Nymphalinae: *Junonia*) are widely distributed in the Americas, being found from southern Canada to South America. In an early treatment of the genus, Forbes (1928) recognized two species of *Junonia* in the New World, *J. vestina* C. Felder & R. Felder, a high altitude form found throughout the Andes of South America (Fig. 1), and *J. lavinia* (Cramer) [= *J. evarete* (Cramer)] in which he grouped all others forms that were morphologically similar and distinct from *J. vestina*. In the present paper we refer to buckeyes included in *J. lavinia* as the *J. evarete* complex [*Junonia lavinia* is now recognized as a permanently invalid synonym of *J. evarete* (Comstock 1942)]. The genus *Precis* also has been used for the New World buckeyes, but butterflies belonging to this genus are now known to be restricted to Africa (Wahlberg et al. 2005). Recently, Pelham (2008) recognized three nominal species of *Junonia* belonging to the *J. evarete* complex as defined here: *J. evarete* (Cramer), *J. genoveva* (Cramer) and *J. coenia* Hübner, as well as three subspecies: *J. evarete nigrosuffusa* W. Barnes & McDunnough, *J. evarete zonalis* C. Felder & R. Felder and *J. coenia grisea* Austin & J. Emmel. In addition, ongoing taxonomic studies on *Junonia* from the West Indies and northern South America suggest that additional species level taxa

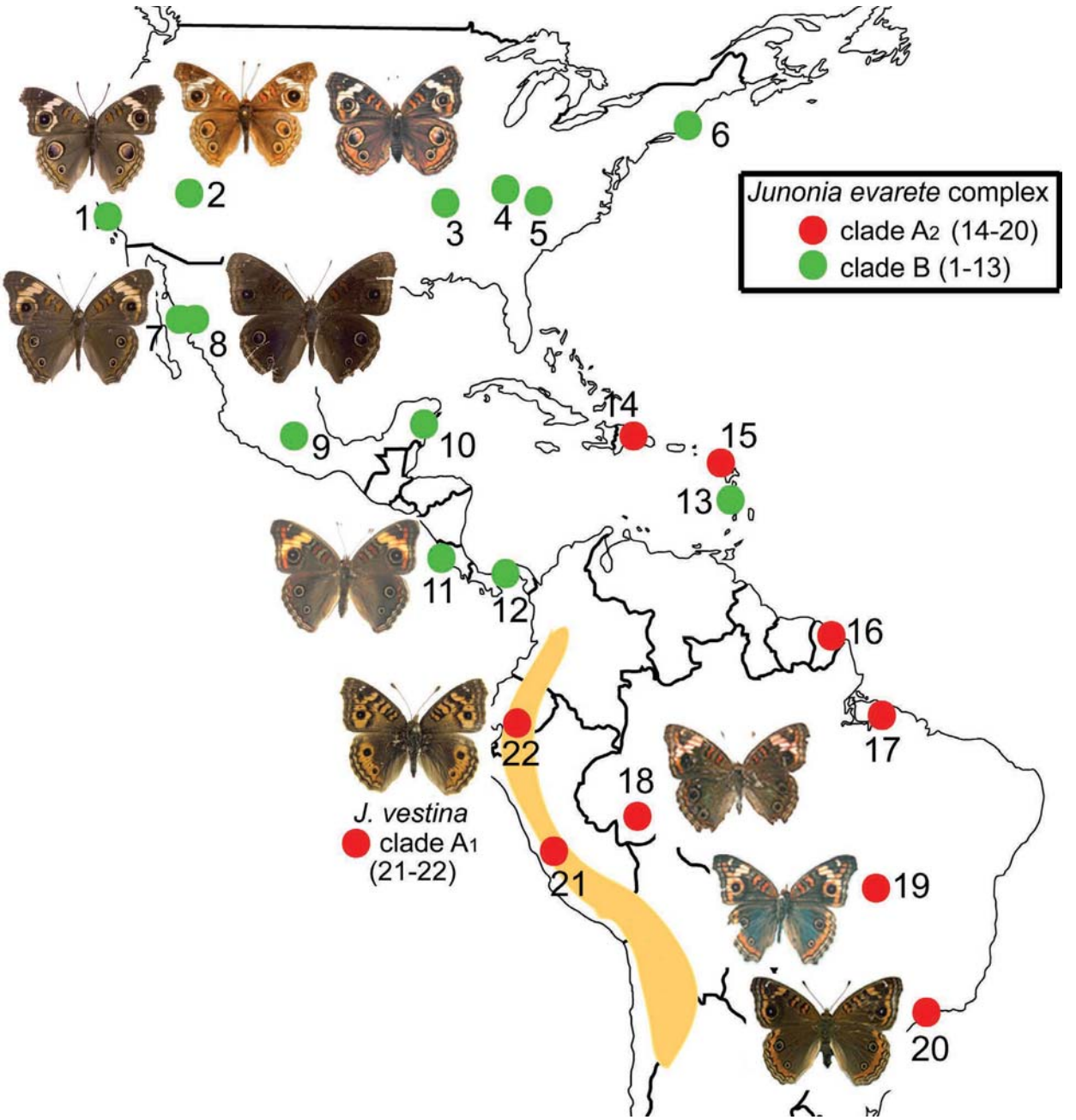

FIG. 1. Map of North and South America showing collection localities and phenotypic variability of *Junonia* spp. at selected localities where dorsal images were available. Red and green dots correspond to the two main clades (A and B, respectively) of New World *Junonia* (see Fig. 2). The shaded area represents the approximate geographic distribution of the Andean buckeye, *J. vestina*. Voucher codes for each species are given below (see Table 1 for details). Scientific names in parentheses are suggested changes in assignment based on data presented here (see Discussion regarding the assignment of *J. nigrosuffusa*) or unpublished data (C. Brévignon, pers. com.). **1,** *J. coenia grisea* (CIAD 10–B03); **2,** *J. coenia coenia* (NW38–18); **3,** *J. coenia coenia* (female) (NW85–13); **4,** *J. coenia coenia* (no image) (Bio175–17); **5,** *J. coenia coenia* (no image) (DNA–ATBI–0802 and –0816); **6,** *J. coenia coenia* (no image) (TDWG–0126); **7,** *J. evarete* (= *J. genoveva*; CIAD 10–B19; Estero del Soldado); **8,** *J. evarete nigrosuffusa* (= *J. nigrosuffusa*; CIAD 10–B24); **9,** *J. evarete* (no image) (= *J. genoveva*; JM6–10); **10,** *J. evarete* (no image) (= *J. genoveva*; MAL–02877); **11,** *J. evarete* (= *J. genoveva*; 05–SRNP–58293); **12,** *J. evarete* (no image) (= *J. genoveva*; YB–BCI12765); **13,** *J. genoveva* (no image) (= *J. neildi* Brévignon [C. Brévignon, pers com.]; NW136–16); **14,** *Junonia* sp. (no image) (= *J. evarete*; NW153–12); **15,** *J. evarete* (no image) (NW136–17); **16,** *Junonia* spp. (no images) (UK4–14, –15, –16); **17,** *J. evarete* (no image) (NW151–3); **18,** *J. evarete* (NW126–20); **19,** *J. evarete* (NW84–15); **20,** *J. genoveva*? (= *J. evarete*; NW155–2); **21,** *J. vestina* (no image) (NN07); **22,** *J. vestina* (Las Culebrillas, Cañar, Ecuador; DNA not extracted). Photograph credits: **2, 3, 18–20,** Nymphalidae Systematics Group (2009); **1, 7, 8,** Wain Evans; **11,** Janzen and Hallwachs (2009); **22,** Jean-Claude Petit.

are also present (Brévignon 2008, 2009).

Although the buckeyes are a well-known and much studied group of nymphalid butterflies, especially with respect to genetic factors involved in evolution and development of eyespots and color patterns (Nijhout 1980; Reed et al. 2007; Monteiro 2008; Kodandaramaiah 2009; Monteiro & Prudic 2010), and the chemical ecology and evolution of hostplant preferences (Bowers 1984; Bowers & Puttick 1989; Bowers & Stamp 1997), the systematics of the *J. evarete* complex has been plagued with uncertainty, with species assignments often questionable and unreliable. The confusion can be traced, at least in part, to the pronounced phenotypic variability in wing maculation and coloration within the genus *Junonia* (Tilden 1971; Hafernik 1982). The apparent loss of type specimens, vague or unknown type localities, and non-standardized use of common names have also added to the taxonomic confusion. Molecular phylogenetic evidence suggests that the ancestor of the *J. evarete* complex probably colonized the New World from Africa or Asia relatively recently, ~2–4 million years ago (Ma) (Kodandaramaiah & Wahlberg 2007), implying that subsequent speciation in this group also is relatively recent. Thus, the possibility for incomplete lineage sorting among diversifying taxa may be high. The many observations of hybridization among phenotypic variants of *Junonia* (Rutkowski 1971; Hafernik 1982) are consistent with this possibility.

In the only comprehensive (worldwide) molecular phylogenetic study conducted to date on *Junonia*, based on 3090 base pairs (bp) from both mitochondrial (cytochrome c oxidase subunit I; COI) and nuclear genes (*wingless* and elongation factor-1α), the three nominal species of the *J. evarete* complex partitioned into two well-supported clades, one comprised of *J. evarete* (Brazil and Guadeloupe) and the other consisting of *J. coenia coenia* (Utah and Tennessee, USA) + *J. genoveva* (Martinique) (Kodandaramaiah & Wahlberg 2007). Because total sample size from the two New World clades was low ($N = 8$), and did not include any populations from western North America (with the exception of a single individual from Utah), the relationships of these previously studied taxa to western populations of *Junonia* remain unclear. We also wished to assess whether molecular data from western populations would provide any additional insights into the results of the hybridization studies of Hafernik (1982) who found high genetic similarity among western taxa.

Given the increase in available COI sequence data for *Junonia* from the DNA barcode initiative (Ratnasingham & Hebert 2007), and the fact that most (633 bp) of the 658 bp barcode region was sequenced by Kodandaramaiah and Wahlberg (2007), we were particularly interested in determining if the barcode segment alone could provide informative characters for inferring phylogenetic relationships and addressing taxonomic uncertainties in *Junonia* from the Americas. DNA barcodes, although sometimes of limited usefulness (Elias et al. 2007; Yassin et al. 2010), have been shown to be highly reliable at species-level identifications within the Lepidoptera in the eastern USA and northwestern Costa Rica, with a success rate of >97% for ~2000 morphologically-defined taxa (Hebert et al. 2003, 2010; Janzen et al. 2005; Hajibabaei et al. 2006). In the present study, we analyzed both new and previously published COI sequences from a total of 85 individuals of New World *Junonia*.

Materials and Methods

Sampling. The new taxa of *Junonia* treated here include (1) *J. coenia grisea* from far western USA (California and southern Oregon) and the Baja California Peninsula, Mexico (type locality: South Pasadena, Los Angeles County, California) (Austin & Emmel 1998); specimens for the present study were collected at a residential development site in Santa Barbara, California, USA, (2) a population from northwestern Mexico that feeds on black mangrove *Avicennia germinans* (L.) L. (Acanthaceae) (Pfeiler 2011). This population is listed as *J. evarete* by Brown et al. (1992) (an assignment initially followed here) and referred to as an intermediate between *J. evarete zonalis* and *J. coenia* by Hafernik (1982); our samples were collected at a mangrove estuary (Estero del Soldado) near San Carlos, Sonora, Mexico, (3) the taxon currently recognized as *J. evarete nigrosuffusa* (Luna-Reyes et al. 2008; Pelham 2008), a large, dark subspecies inhabiting southwestern USA and Mexico, generally inland from the immediate coast (type locality: southeastern Arizona); our specimens were collected in the coastal foothills of the Sierra El Aguaje at San Carlos, Sonora, Mexico. GenBank sequences were available for a population of *Junonia* from the Area de Conservación Guanacaste (ACG), Guanacaste Province in northwestern Costa Rica assigned to *J. evarete*, whose foodplants include *Dyschoriste valeriana* Leonard (Acanthaceae) and *Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae) (DeVries 1987; D.H. Janzen & M. Hajibabaei unpublished). Additional GenBank sequences were obtained for specimens collected in southern Mexico, Panama, Brazil, Peru, French Guiana, central and eastern USA, and the Caribbean. Details on taxa analyzed, collection data and GenBank accession numbers for the complete data set are given in Table 1.

TABLE 1. List of species of *Junonia* analyzed for COI, with collection data and GenBank accession numbers.

Species	Voucher code	Locality	Geographic Coordinates	Altitude (m)	Collection date	GenBank access. no.	Ref [a]
J. coenia coenia (USA)	NW38–18	Washington Co. UT			29 May 1998	AY248777	1
(*N* = 6)	NW85–13	Shelby Co., TN			1992 (reared)	AY788643	1
	DNA–ATBI–0802	Swain Co., NC	35.520 N, 83.304 W	607	20 July 2004	GU089962	2
	DNA–ATBI–0816	"	35.550 N, 83.308 W		19 July 2004	GU089963	2
	Bio175–17	Hart Co., KY				EU271674	
	TDWG-0126	Barnstable Co., MA	41.642 N, 70.558 W	36	29 Sept. 2010	HQ964540	6
J. coenia grisea (USA)	CIAD 10–B03	Santa Barbara, CA	34.459 N, 119.759 W	130	19 June 2010	JQ430685	3
(*N* = 7)	CIAD 10–B04	"	"	"	"	JQ430686	3
	CIAD 10–B05	"	"	"	"	JQ430687	3
	CIAD 10–B06	"	"	"	"	JQ430688	3
	CIAD 10–B07	"	"	"	"	JQ430689	3
	CIAD 10–B08	"	"	"	"	JQ430690	3
	CIAD 10–B09	"	"	"	"	JQ430691	3
J. evarete (Brazil)	NW36–2	Minaçu, Goiás			1 Dec. 1998	EU053298	1
(*N* = 5)	NW84–15	Minaçu, Goiás			"	EU053299	1
	NW126–20	Acre				EU053295	1
	NW129–30	Acre				EU053296	1
	NW151–3	Vigia, Pará	0.858 S, 48.142 W		19 July 2006	JQ430732	5
J. evarete (Guadeloupe)	NW136–17	Guadeloupe			16 Dec. 2003	EU053297	1
J. evarete (Mexico)	CIAD 10–B01	San Carlos, Sonora	27.959 N, 110.981 W	<2	8 Jan. 2010	JQ430692	3
(*N* = 22)	CIAD 10–B10	"	"	"	21 July 2010	JQ430693	3
	CIAD 10–B11	"	"	"	16 Aug. 2010	JQ430694	3
	CIAD 10–B12	"	"	"	"	JQ430695	3
	CIAD 10–B13	"	"	"	20 Aug. 2010	JQ430696	3
	CIAD 10–B14	"	"	"	"	JQ430697	3
	CIAD 10–B15	"	"	"	"	JQ430698	3
	CIAD 10–B16	"	"	"	25 Sept. 2010	JQ430699	3
	CIAD 10–B17	"	"	"	"	JQ430700	3
	CIAD 10–B18	"	"	"	"	JQ430701	3
	CIAD 10–B19	"	"	"	"	JQ430702	3

Table 1. (continued)

	CIAD 10–B20	"	"	"	"	JQ430703	3
	CIAD 10–B21	"	"	"	"	JQ430704	3
	CIAD 10–B22	"	"	"	"	JQ430705	3
	CIAD 10–B23	"	"	"	"	JQ430706	3
	CIAD 10–B27	"	"	"	11 Oct. 2010	JQ430707	3
	CIAD 10–B28	"	"	"	"	JQ430708	3
	CIAD 10–B29	"	27.983 N, 111.043 W	42	1 Nov. 2010	JQ430709	3
	CIAD 10–B37	"	27.959 N, 110.981 W	<2	9 Nov. 2010	JQ430710	3
	JM6–10	El Limón, Morelos		1200	29 June 2007	JQ430733	5
	NW162–7	Cerro Frio, Morelos		1600	13 July 2007	JQ430731	5
	MAL–02877	Tulum Pueblo, QR	20.146 N, 87.575 W		7 Feb. 1993	JN201293	7
J. e. nigrosuffusa (Mexico)	CIAD 10–B24	San Carlos, Sonora	27.997 N, 111.047 W	70	1 Oct. 2010	JQ430711	3
(N = 10)	CIAD 10–B25	"	"	"	8 Oct. 2010	JQ430712	3
	CIAD 10–B26	"	"	"	11 Oct. 2010	JQ430713	3
	CIAD 10–B30	"	27.983 N, 111.043 W	42	1 Nov. 2010	JQ430714	3
	CIAD 10–B31	"	"	"	"	JQ430715	3
	CIAD 10–B32	"	"	"	"	JQ430716	3
	CIAD 10–B33	"	"	"	"	JQ430717	3
	CIAD 10–B34	"	27.997 N, 111.047 W	70	2 Nov. 2010	JQ430718	3
	CIAD 10–B35	"	27.968 N, 111.045 W	30	5 Nov. 2010	JQ430719	3
	CIAD 10–B36	"	"	"	"	JQ430720	3
J. evarete (Costa Rica)	03–SRNP–28069	ACG, Guanacaste	11.018 N, 85.450 W	410	9 Sept. 2003	GU334034	4
(N = 22)	03–SRNP–28076	"	"	"	5 Sept. 2003	GU334037	4
	04–SRNP–15110	"	10.838 N, 85.619 W	295	31 Oct. 2004	GU157294	4
	05–SRNP–31184	"	11.019 N, 85.410 W	440	23 April 2005	GU157298	4
	05–SRNP–31186	"	"	"	13 April 2005	GU157295	4
	05–SRNP–31187	"	"	"	26 April 2005	GU157296	4
	05–SRNP–31188	"	"	"	27 April 2005	GU157297	4

TABLE 1. (continued)

J. evarete (Costa Rica) (continued)	05–SRNP–31215	"	"	"	7 April 2005	GU157299	4
	05–SRNP–57995	"	10.767 N, 85.433 W	325	24 July 2005	GU157288	4
	05–SRNP–57996	"	"	"	"	GU157285	4
	05–SRNP–57998	"	"	"	22 July 2005	GU157287	4
	05–SRNP–57999	"	"	"	23 July 2005	GU157286	4
	05–SRNP–58001	"	"	"	"	GU157290	4
	05–SRNP–58009	"	"	"	"	GU157291	4
	05–SRNP–58011	"	"	"	22 July 2005	GU157280	4
	05–SRNP–58208	"	10.768 N, 85.426 W	480	28 July 2005	GU157292	4
	05–SRNP–58220	"	"	"	6 Aug. 2005	GU157284	4
	05–SRNP–58221	"	"	"	29 July 2005	GU157300	4
	05–SRNP–58241	"	"	"	"	GU157283	4
	05–SRNP–58257	"	"	"	28 July 2005	GU157282	4
	05–SRNP–58293	"	"	"	"	GU157289	4
	05–SRNP–32756	"	11.019 N, 85.410 W	440	16 Aug. 2005	GU157281	4
J. evarete (Panama)	YB-BCI12765	Barro Colorado Is.	9.155 N, 79.848 W	150	24 May 2009	HM416470	6
J. evarete (French Guiana)	UK4–15	Montsinéry				JQ430730	5
Junonia sp. (Fr. Guiana)	UK4–16	Macouria				JQ430722	5
J. genoveva (Fr. Guiana)	UK4–14	Kaw-Roura				JQ430728	5
J. genoveva (Martinique)	NW136–16	Les Salines, Sainte-Anne			15 Feb. 2004	EU053300	1
J. genoveva (Guadeloupe)	UK4–18	Port-Louis				JQ430727	5
(*N* = 2)	UK4–19	Port-Louis				JQ430726	5
J. genoveva? (Brazil)	NW155–2	Ubatuba, São Paulo			21 May 2005	JQ430729	5
Junonia sp. (Dominican	NW153–11	Pedernales			8 Nov. 2006	JQ430725	5
(Republic) (*N* = 3)	NW153–12	Pedernales			"	JQ430724	5
	NW153–14	Hatillo, Azua			9 Nov. 2006	JQ430723	5
J. vestina (Peru)	NN07	near Laguna Huacracocha		4000	26 Oct. 2009	JQ430721	5

[a]References: 1, Kodandaramaiah and Wahlberg (2007); 2, Hebert et al. (2010); 3, this study; 4, Janzen and Hallwachs (2009); 5, Nymphalidae Systematics Group (2009); 6, Ratnasingham and Hebert (2007); Barcode of Life Data System (http://www.barcodinglife.org); 7, Prado et al. (2011).

Molecular protocol and data analysis. Total genomic DNA was extracted from two legs of each butterfly using the DNeasy™ (QIAGEN Inc., Valencia, CA) protocol. The polymerase chain reaction (PCR) was used to amplify the barcode segment of the COI gene with primers LCO1490f and HCO2198r using standard PCR conditions (Folmer et al. 1994). Sequencing reactions were performed on an Applied Biosystems (Foster City, CA) ABI 3730XL DNA sequencer at the DNA Sequencing Facility, University of Arizona, Tucson using the amplifying primers. Sequences were proofread and aligned in ClustalX 1.81 (Thompson et al. 1997) followed by manual editing.

Calculations of Kimura (1980) 2–parameter (K2P) genetic distances (*d*) among sequences were carried out in MEGA version 4.0 (Tamura et al. 2007). Calculations of genetic diversity indices and Tajima's (1989) *D* were performed in DnaSP version 5.00.04 (Librado & Rozas 2009). Relative rate tests (Tajima 1993) of sequence evolution were carried out in MEGA using *J. orithya* as the outgroup. Analysis of molecular variance (AMOVA, Excoffier et al. 1992) performed in ARLEQUIN version 3.5.1.3 (Excoffier & Lischer 2010) was used to test for structure among selected populations of *Junonia.* The calculation of significance ($\alpha = 0.05$) of the fixation index Φ_{ST} was based on 10,000 permutations of the data matrix. Estimates of the number of migrants per generation (N_m) among populations were also calculated in ARLEQUIN.

Phylogenetic analyses. For phylogenetic analyses all COI sequences were trimmed to 633 bp to correspond to the barcode region reported in Kodandaramaiah and Wahlberg (2007). Relationships among haplotypes were assessed with the neighbor-joining (NJ) algorithm of Saitou and Nei (1987) carried out in MEGA using a matrix of K2P distances. We used two African species of *Junonia* as outgroups, *J. orithya* (GenBank EU053315) and *J. westermanni* (GenBank EU053319). Both African species show a close relationship with the New World *Junonia* (Kodandaramaiah & Wahlberg 2007). *Junonia orithya*, in particular, shares similarities in both wing pattern and morphology of male genitalia with New World *Junonia* (Corbet 1948; Tilden 1971). Statistical support for nodes was obtained by bootstrap analyses using 1000 pseudoreplicates (Felsenstein 1985). Confirmation of clades identified from NJ analysis was obtained by constructing phylogenetic trees with (a) Bayesian inference implemented in MrBayes version 3.1 (Huelsenbeck & Ronquist 2001), sampling 4000 trees and using both HKY and GTR nucleotide substitution models, and (b) maximum parsimony (MP) carried out in MEGA using the CNI heuristic search option and 100 random additions of sequences. Clade support for Bayesian trees was estimated utilizing a Markov chain Monte Carlo (MCMC) algorithm and expressed as posterior probabilities; relative support for MP tree topology was obtained by bootstrapping using 500 pseudoreplicates.

RESULTS

Sequence data and genetic diversity. A complete barcode segment (658 bp) was available for 68 of the 85 individuals of *Junonia* shown in Table 1. No stop codons or indels were found in any of the sequences. There were 53 variable sites. Nucleotide composition was nearly identical in the 68 sequences (mean values: 38.7% T, 14.6% C, 31.4% A and 15.2% G). There was a strong bias against G at the third codon position (mean G content 1.4%; range 0.5–2.7%). Inspection of the 658 bp segment in the 68 samples revealed that of the 53 variable sites, none were present in the first 25 bases that were deleted for phylogenetic analyses.

Genetic diversity indices for *Junonia* are shown in Table 2. Two different patterns were observed. Haplotype diversity (h) and nucleotide diversity (π) were relatively high ($h \geq 0.900$; $\pi > 0.003$) in *J. evarete* from Costa Rica and South America (including the Caribbean) and in *J. coenia coenia* from the USA, but were lower ($h < 0.700$; $\pi < 0.002$) in *J. evarete* and *J. evarete nigrosuffusa* from Sonora, Mexico and in *J. coenia grisea* from southern California, USA. The differences in h and π seen in *J. evarete* from Sonora, Mexico and Costa Rica are notable given that sample sizes from the two localities were similar. Tajima's *D* was not significant in any of the taxa. None of the relative rates tests (Tajima 1993) were significant, indicating that a molecular clock could not be rejected for *Junonia.* The AMOVA revealed significant structure among populations of *J. evarete* from Costa Rica ($N = 22$) and Estero del Soldado, Mexico ($N = 19$) ($\Phi_{ST} = 0.398$; $P < 0.0001$). The estimated number of individuals migrating between the two regions per generation (N_m) was 0.756. The AMOVA also showed significant structure between the subspecies *J. coenia coenia* ($N = 6$) from eastern USA and *J. coenia grisea* ($N = 7$) from California ($\Phi_{ST} = 0.787$; $P < 0.001$; $N_m = 0.135$).

Phylogenetic relationships. Preliminary phylogenetic analyses of the three New World taxa (*J. evarete*, *J. genoveva* and *J. coenia coenia*) from Kodandaramaiah and Wahlberg (2007), using only the 633 bp COI barcode segment and *J. orithya* and *J. westermanni* as outgroups, resolved the *J. evarete* and (*J. coenia* + *J. genoveva*) clades (referred to below as clades A and B, respectively) in NJ, MP and Bayesian trees (not shown) with similar (MP) or identical (Bayesian) clade support

values reported by those workers from the combined mitochondrial and nuclear data set of 3090 bp.

The NJ tree of New World *Junonia* based on barcodes, and representing both new and previously published data, is shown in Fig. 2. The NJ tree again resolved clades A and B with high statistical support. In addition to the single *J. genoveva* and two *J. coenia coenia* from Kodandaramaiah and Wahlberg (2007), all sequences of *Junonia* from the USA, Mexico, Costa Rica and Panama clustered in clade B, including those from taxa currently assigned to *J. evarete* and *J. evarete nigrosuffusa*. A short COI sequence (290 bp) assigned to *J. evarete* from Quintana Roo, Mexico (Prado et al. 2011) also clustered in clade B (not shown). Within clade B, a weakly-supported subclade consisting of *J. coenia grisea* from southern California was found. All other populations within clade B were unresolved. The same topology, with similar support values, was obtained on a representative subset of sequences from all taxa using MP and Bayesian analyses (not shown).

All populations of *Junonia* from South America and the Caribbean, with the exception of a single *J. genoveva* (= *J. neildi* Brévignon) from Martinique (NW136–16), clustered in Clade A, including individuals identified as *J. evarete* and *J. genoveva*. The resolution of *J. vestina* in a basal position in clade A (referred to here as clade A_1) was highly supported. The remaining clade A individuals were all closely related (see below) and are grouped into clade A_2.

Pairwise comparisons of K2P genetic distances (d) among New World *Junonia* are shown in Table 3. Mean genetic distances were low ($d \leq 1.1\%$) for all comparisons between taxa within clade B. Genetic distance between the subspecies *J. coenia coenia* and *J. coenia grisea* was $d = 1.0\%$. Mean values in all pairwise comparisons between clades A and B, including comparisons with individuals assigned to *J. evarete* which appear in both clades, ranged from d = 4.0–4.5%. These values are higher than the genetic distances found between the two species from Africa used as outgroups, *J. orithya* and *J. westermanni* (d = 3.4%; not shown in Table 3), and also are higher than the value found between *J. orithya* and clade A_2 (d = 3.9%); the values are slightly lower than d = 5.0 % found between *J. orithya* and clade B. Within clade A, the genetic distance between the distinctive *J. vestina* (clade A_1) and *J. evarete* (clade A_2) was d = 2.1 %. The mean value between *J. evarete* from Costa Rica and Sonora, Mexico, localities separated by ~3250 km, was d = 0.5%. Within population d values for *J. evarete* were 0.0–0.5% (mean d = 0.1%) for Sonora and 0.0–1.4% (mean d = 0.5%) for Costa Rica. One individual of *J. evarete* from Sonora shared the same haplotype with an individual from Costa Rica (see Fig. 3).

DISCUSSION

We have shown that phylogenetic analysis of a 633 bp segment of the mitochondrial COI gene, comprising most of the barcode segment, resolves the two main clades of New World *Junonia* reported previously using a larger data set of both mitochondrial and nuclear genes (Kodandaramaiah & Wahlberg 2007). Barcodes thus provide an informative and relatively inexpensive tool for phylogenetic studies of this group. Assigning individuals of the *J. evarete* complex to their respective clade using morphological characters alone is unreliable and has probably contributed much to the taxonomic

Table 2. Summary of genetic diversity indices and results of neutrality tests (Tajima's D) in theCOI gene segment in *Junonia*.

Species	N	L	k	K	h (± SD)	π (± SD)	Tajima's D
J. evarete (clade A_2)*	12	633	20	10	0.970 ± 0.044	0.00824 ± 0.00105	–0.97
J. evarete (Costa Rica)	22	658	18	14	0.926 ± 0.039	0.00455 ± 0.00075	–1.45
J. evarete (Sonora, Mexico)	19	658	3	5	0.696 ± 0.077	0.00133 ± 0.00023	0.06
J. e. nigrosuffusa (Mexico)	9	658	3	3	0.639 ± 0.126	0.00186 ± 0.00042	0.41
J. coenia coenia (USA)	5	633	4	4	0.900 ± 0.161	0.00253 ± 0.00076	–1.09
J. coenia grisea (USA)	7	658	2	3	0.667 ± 0.160	0.00116 ± 0.00035	–0.27

N, number of sequences; L = sequence length (number of bases); k = number of variable sites; K, number of haplotypes; h, haplotype diversity; π, nucleotide diversity. Values for Tajima's D were not significant for any species at the 0.05 level. *All species from South America and the Caribbean clustering in clade A_2, including those originally assigned to *J. evarete*, *J. genoveva* or *Junonia* sp. (see Fig. 2), were combined under *J. evarete* in Tables 2 and 3. Five shorter sequences (≤600 bp) from *J. evarete* (clade A_2) (NW36–2, UK4–15, UK4–18), *J. e. nigrosuffusa* (CIAD 10–B25) and *J. coenia coenia* (NW38–18) were omitted from Tables 2 and 3.

TABLE 3. Mean K2P genetic distances (*d*) among taxa and geographic populations of New World *Junonia* based on the COI gene (633 bp). Values for *d* within taxa are shown along the diagonal. Shaded area shows taxa included in clade A, *J. vestina* (clade A_1) and *J. evarete* (clade A_2) from South America and the Caribbean (see footnote to Table 2). The remaining taxa all cluster in clade B from North America and the Caribbean.

		1	2	3	4	5	6	7	8
1	*J. coenia coenia* (USA) (*N* = 5)	0.003							
2	*J. coenia grisea* (USA) (*N* = 7)	0.010	0.001						
3	*J. e. nigrosuffusa* (MX) (*N* = 9)	0.003	0.010	0.002					
4	*J. genoveva* (Martinique) (*N = 1*)	0.004	0.009	0.003	-----				
5	*J. evarete* (Sonora, MX) (*N* = 19)	0.004	0.008	0.003	0.003	0.001			
6	*J. evarete* (Costa Rica) (*N = 22*)	0.007	0.011	0.006	0.006	0.005	0.005		
7	*J. evarete* (S. Amer.) (*N* = 12)	0.043	0.043	0.042	0.045	0.044	0.042	0.008	
8	*J. vestina* (Peru) (*N* = 1)	0.040	0.044	0.040	0.043	0.041	0.042	0.021	-----

confusion. Because of evidence for relatively recent divergences in the New World *Junonia*, however, barcodes alone may be of limited usefulness for inferring intra-clade relationships and species identifications, especially within clade B. All new barcode sequences from populations from western North America, comprising several recognized taxa, clustered in clade B and most showed low genetic divergences ($d < 1\%$). The western *J. coenia grisea*, however, resolved as a weakly-supported subclade within clade B, supporting its designation as a subspecies of *J. coenia* (Austin & Emmel 1998). The AMOVA showed significant population structure among *J. coenia grisea* and *J. coenia coenia*, also consistent with subspecies status. Additionally, our analyses revealed that none of the North American *Junonia* from Mexico and Central America currently recognized as *J. evarete*, including *J. evarete nigrosuffusa* from Mexico, clustered with *J. evarete* from South America and the Caribbean (clade A_2). These results suggest that either the taxon currently recognized as *J. evarete* is paraphyletic, or taxonomic assignments of the western populations need to be reconsidered (see below).

Assuming a molecular clock rate of ~2% pairwise sequence divergence per million years for insect COI (Brower 1994; Craft et al. 2010; Pfeiler et al. 2010) we estimate that clades A and B began to diverge ~2.2 Ma. Based on fossil evidence, *Junonia* is thought to have colonized the New World about 2–4 Ma (Kodandaramaiah & Wahlberg 2007). Mean genetic distances between clade A and the outgroup taxa from Africa were 3.9 and 4.3% for *J. orithya* and *J. westermanni*, respectively, suggesting the ancestor of the clade A lineage began to diverge from the African taxa ~2 Ma. Thus, molecular clock considerations and fossil evidence provide estimated dates which are in relatively close agreement, implying that clades A and B began to diverge shortly after colonization of the New World. Because we found no evidence that nucleotide substitution rate in the COI gene in *Junonia* is different from that typically seen in many insects, the low genetic divergences within clade B likely indicate a relatively recent (late Pleistocene or Holocene) radiation and speciation within this group. The low genetic divergences also could result from incomplete lineage sorting and extensive hybridization among diversifying taxa, possibly suggesting just a single, polytypic species. There is evidence, however, apart from the pronounced intra-clade phenotypic variability (Fig. 3), to support recognizing distinct species level taxa within clade B that barcodes are unable to detect.

The low genetic divergences and presumed recent speciation among recognized taxa of *Junonia* comprising clade B are consistent with the conclusions of laboratory hybridization studies showing a high degree of genetic similarity among North and Central American *Junonia* (Hafernik 1982). The taxa used in the hybridization experiments and phenetic analyses of Hafernik (1982) included *J. coenia* (populations from both Texas and California representing what are now recognized as subspecies *J. coenia coenia* and *J. coenia grisea*, respectively), *J. evarete nigrosuffusa* (southern Texas and southeastern Arizona; treated as a full species by Hafernik) and *J. evarete zonalis* (southern Guatemala and northwestern Costa Rica). Caribbean populations, including *J. genoveva*, were excluded from the study [*J. genoveva* is currently listed for southern Texas (Opler et al. 2011; Warren et al. 2011)]. Several lines of circumstantial evidence, however, suggest that the reference populations of *Junonia* from Central America used by Hafernik (1982) may have been from

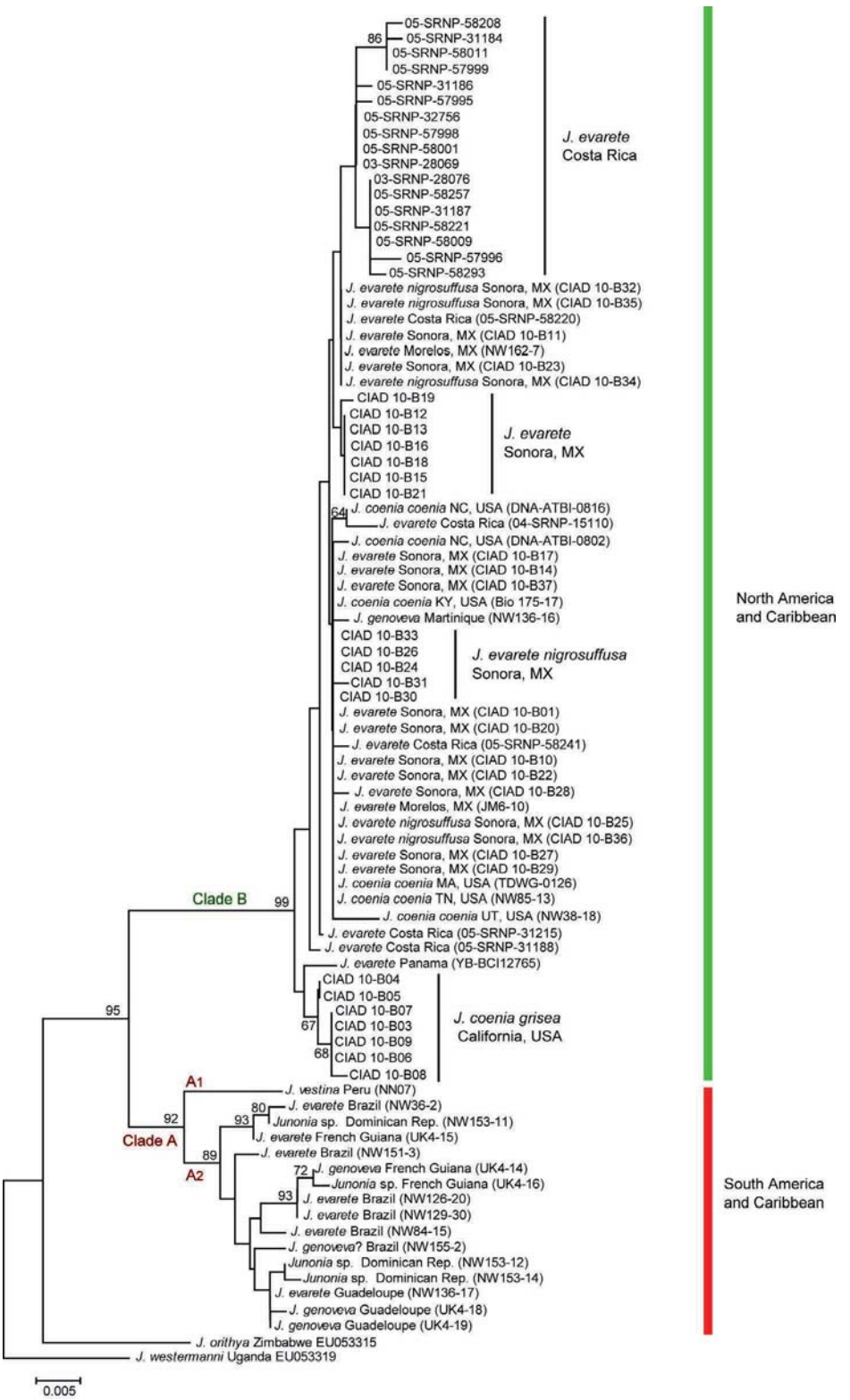

FIG. 2. Neighbor-joining (NJ) tree showing relationships among New World *Junonia* based on COI barcode sequences. Voucher codes are listed for each of the ingroup species (see Table 1 for details). GenBank accession numbers are shown for the outgroups, *J. orithya* and *J. westermanni* from Africa. Red and green bars represent the two main clades (A and B, respectively) of New World *Junonia*. Clade A_1 is comprised of *J. vestina*; clades A_2 and B contain the members of the *J. evarete* complex. Bootstrap support values are shown on branches; values <60% were omitted. Scale bar indicates sequence divergence.

the clade B lineage, most probably from the taxon *J. genoveva*. Specimens from these reference populations were taken at Escuintla, Guatemala and Cañas, Costa Rica, both from the Pacific slope and ~700 and ~75 km, respectively, from the Area de Conservación Guanacaste (ACG). Although multiple species of *Junonia* occur in certain regions, no COI genotypes similar to those found in South American and Caribbean populations of *J. evarete* have thus far been detected in the 45 barcode sequences obtained for *Junonia* from the ACG (D.H. Janzen & M. Hajibabaei, unpublished). *Junonia evarete* genotypes also were not present in the two samples from Morelos, Mexico, or in the samples from Quintana Roo, Mexico (Prado et al. 2011) and Panama (Fig. 1). Because of the genetic similarities and lack of reproductive isolation, Hafernik (1982) concluded that *J. evarete nigrosuffusa* and *J. evarete zonalis* represented a cline from Central America to southern Texas and should be considered conspecific. We have shown, however, that *J. evarete nigrosuffusa* from Mexico and *J. evarete* from South America and the Caribbean show a mean genetic divergence (d = 4.2%; Table 3) well within the range of values seen for species level taxa in Lepidoptera based on barcodes (Hajibabaei et al. 2006; Hebert et al. 2010). Finding high genetic identity in hybridization studies between individuals of clades A_2 and B would not be expected in two distinct taxa with relatively high genetic divergences. For example, in Jamaica where *J. evarete* and *J. genoveva* both occur, no evidence was found for natural hybridization among the two taxa (Turner & Parnell 1985). However, the conclusions of Hafernik (1982) are consistent with our findings if the Central America taxon used in that study was from the *J. genoveva* lineage and not a subspecies of *J. evarete*. Our argument assumes that *J. evarete* was correctly identified in the earlier molecular study of Kodandaramaiah and Wahlberg (2007). Photographs of *J. evarete* studied by those authors (see Fig. 1) match closely the phenotype of the recently assigned neotype of *J. e. evarete* from Suriname, South America (Neild 2008), suggesting that the identification was correct.

Based on the above arguments we propose that the population of *Junonia* from Mexico that utilizes black mangrove (*Avicennia germinans*) as a larval host, as well as the specimens shown in Table 1 from Morelos

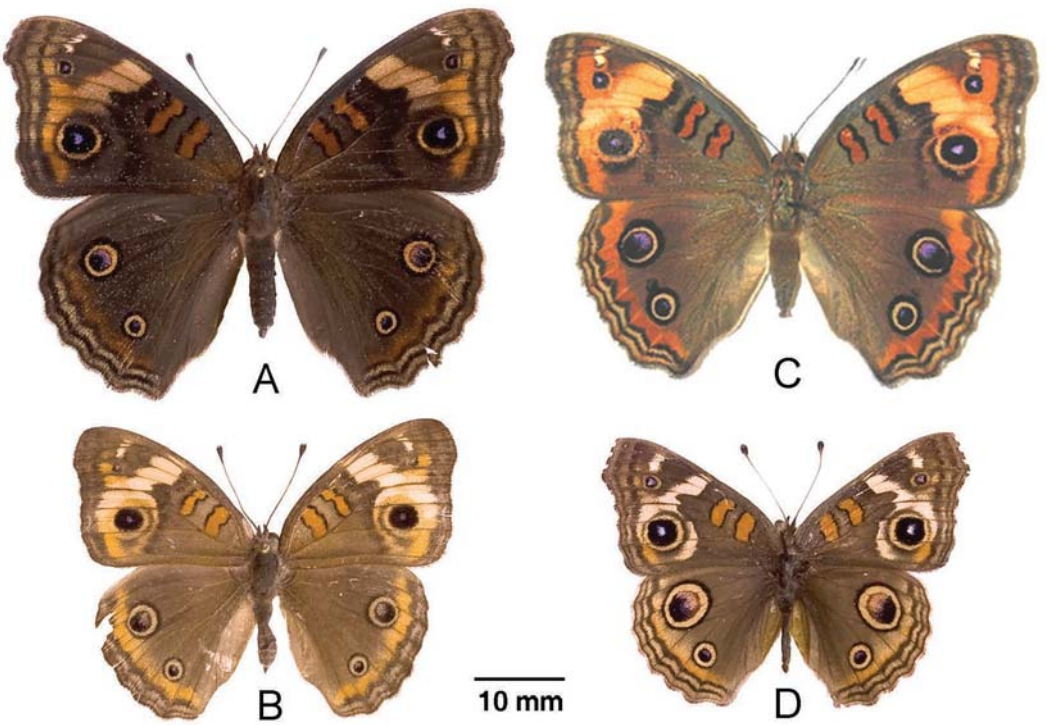

FIG. 3. Comparison of adult females of *Junonia* from western North America showing phenotypic variability. **(A)** *J. evarete nigrosuffusa* (= *J. nigrosuffusa*; see Discussion) (San Carlos, Sonora, Mexico; CIAD 10–B32); **(B)** *J. evarete* (= *J. genoveva*) (Estero del Soldado, near San Carlos, Sonora, Mexico; CIAD 10–B11); **(C)** *J. evarete* (= *J. genoveva*) (Area de Conservación Guanacaste, Guanacaste Province, Costa Rica; 05–SRNP–58220); (D) *J. coenia grisea* (Santa Barbara, California, USA; CIAD 10–B04). Haplotypes for COI were identical for specimens A, B and C; specimen D differs by 5 nucleotide substitutions. Specimens A, B and D are wild-caught; specimen C was reared. Specimen B from Estero del Soldado is a worn individual; ground color of recently eclosed specimens is deep brown (Pfeiler 2011). Scientific names in parentheses are suggested changes in assignment based on data presented here. Photograph credits: **(A), (B)** and **(D),** Wain Evans; **(C),** Janzen and Hallwachs (2009).

(JM6–10 and NW162–7) and Quintana Roo, Mexico (MAL–02877), Panama (YB-BCI12765), and the population from Costa Rica that utilizes *Dyschoriste valeriana* and *Stachytarpheta jamaicensis*, be removed from *J. evarete* and provisionally reassigned to *J. genoveva*. These new assignments agree with an earlier observation that a possible subspecies of *J. genoveva* occurs in coastal regions of western Mexico (Vargas et al. 1996). Ongoing research on *Junonia* from the Caribbean, however, suggests that the mangrove buckeye probably consists of more than one species, including the recently-named *J. litoralis* Brévignon and *J. neildi* Brévignon (Brévignon 2009). In addition, the clade A_2 individual from São Paulo Brazil (Fig. 1, locality 20), was reared on *Avicennia* sp. indicating that representatives of both clades A and B have adapted to feeding on black mangrove. A more thorough examination of relationships among taxa of *Junonia* in the Americas that utilize black mangrove and other host plants may ultimately require revision of our provisional assignment.

Although significant structure was found between the populations of *Junonia* from Estero del Soldado, Mexico and Costa Rica, the low mean genetic distance between the two populations (d = 0.5%) agrees well with intraspecific divergences in Lepidoptera based on barcodes (Hajibabaei et al. 2006; Hebert et al. 2010). Phenotypic differences of adults, however, together with the different host plants utilized by larvae, suggest that these two populations may warrant recognition as distinct subspecies. Also, the higher haplotype and nucleotide diversities of the Costa Rica population compared with the Sonora population (Table 2) suggest that dispersal and colonization proceeded from a southern source population northward along the Pacific slope of North America (Pfeiler et al. 2012). Haplotype and nucleotide diversities were also relatively low in *J. evarete nigrosuffusa* and *J. coenia grisea* (Table 2), but low sample sizes did not allow for unambiguous interpretations of demographic patterns.

The barcode data also suggest that *J. evarete nigrosuffusa* be removed as a subspecies of the *J. evarete* lineage, as it clearly nests within clade B rather than clade A_2 (Fig. 2). Two possible alternative assignments, previously proposed by others, are consistent with the genetic data. These include recognizing *nigrosuffusa* as a subspecies of *J. genoveva* (Vargas et al. 1996; Warren et al. 1998; Glassberg 2001), or as a subspecies of *J. coenia* [as originally described by Barnes and McDunnough (1916)]. A third possibility, also previously proposed but supported only by morphological and ecological data, is to recognize the taxon as a full species (Tilden 1971; Emmel & Emmel 1973; Miller & Brown 1981; Bailowitz & Brock 1991; Brown et al. 1992; Brown 2004). In northwestern Mexico, *J. genoveva* and *J. nigrosuffusa* are generally ecologically isolated and morphologically distinct (Fig. 3), with larvae of the two species utilizing different host plants (Tilden 1971; Hafernik 1982; Bailowitz & Brock 1991; Brown et al. 1992; Vargas et al. 1996; Warren et al. 1998; Pfeiler 2011). Our field observations in the San Carlos region of Sonora have revealed no evidence for hybridization, although adults of both lineages are occasionally encountered feeding together (Pfeiler 2011). There are reports, however, of intermediates between the coastal *J. genoveva* and *J. nigrosuffusa* in other regions of western Mexico (Vargas et al. 1996), as well as intermediates between *J. coenia* and *J. nigrosuffusa* from southeastern Arizona (K. Hansen pers. com.). We suggest that, at least for northwestern Mexico, *J. nigrosuffusa* and *J. genoveva* meet the two basic criteria consistent with ecological speciation, i.e. evidence for ecologically-based divergent selection and assortative mating (Chamberlain et al. 2009). Strong adult dispersal capability (Adler & Dudley 1994), together with the ability of larvae to adapt to a variety of host plants from different families, are traits that would favor survival and potentially lead to ecological speciation during the radiation of the New World *Junonia*.

In summary, we have shown that COI barcodes can distinguish *J. vestina* from members of the *J. evarete* complex, and can resolve the two subspecies of *J. coenia*, but overall are of limited usefulness in species identifications within the complex itself. Nonetheless, barcodes are a valuable tool in taxonomic studies of this group for their ability to easily identify the two major clades of the *J. evarete* complex found in the New World, which is difficult, if not impossible, by morphological analysis alone. The ability to unambiguously identify clades A and B will contribute to our understanding of the degree of phenotypic variability and larval host plant preferences within each lineage. More extensive sampling will be required to determine the complete distribution of the two clades in the New World [e.g., records of *J. evarete zonalis* in southern Florida (Warren et al. 2011) suggest the presence of clade A in the USA, and clade B probably occurs South America], but given the widely separated geographic localities in the Americas sampled to date (Fig. 1), it seems unlikely that barcodes will demonstrate additional deep divergences within the *J. evarete* complex. Other molecular markers, however, such as amplified fragment length polymorphisms (AFLPs), show promise of being able to reveal recent divergences that barcodes fail to detect (Dasmahapatra et al. 2010).

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IMMATURE STAGES OF *ARGYNNIS MORMONIA ARTONIS* (LEPIDOPTERA: NYMPHALIDAE) COMPARED TO *ARGYNNIS MORMONIA ERINNA* AND *ARGYNNIS MORMONIA WASHINGTONIA* IN THE PACIFIC NORTHWEST, WITH EVIDENCE FOR HIGH ELEVATION-MEDIATED MELANISM

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ABSTRACT. *Argynnis mormonia artonis* from Steens Mountain (2207 m) in southeast Oregon was reared and the immature stages illustrated and compared to those of two other *mormonia* subspecies resident in the Pacific Northwest, *A. m. erinna* and *A. m. washingtonia.* Gravid females oviposited readily in captivity on desiccating Common Dog Violet (*Viola riviniana*) and Western Bistort (*Polygonum bistortoides*) leaves and twigs. Larvae were reared on *V. riviniana* and after overwintering developed from first instar to pupa in 51–59 days at 24–27 ºC. Eggs, pupae and early instars (first-early fourth) of *A. m. artonis* are similar to those of *A. m. erinna* and *A. m. washingtonia*, but the fifth and sixth instars differ by being concolorously black instead of gray, brown or black with white markings. Late instars of *A. m. erinna* from a high elevation site (Mt Howard, northeast Oregon, 2445 m) were similarly dark colored. These and other observations indicate that larval populations of *A. mormonia* and perhaps other *Argynnis* spp. are polymorphic with a greater incidence of melanic late instars occurring in high-elevation populations.

Additional key words: development, coloration, Mormon fritillary, larvae, host plants

Argynnis mormonia (Boisduval) (Mormon Fritillary) ranges from Alaska south to Arizona and east to Manitoba, South Dakota, Utah and Colorado occupying mid-high elevation habitats. Pelham (2008) lists nine subspecies, three of which (*washingtonia*, *erinna*, *artonis*) occur in the Pacific Northwest. *Argynnis mormonia washingtonia* (Barnes & McDunnough) and *Argynnis mormonia erinna* (W. H. Edwards) are relatively widespread in the Pacific Northwest but *A. m. artonis* (W. H. Edwards) occurs only in southeast Oregon. Warren (2005) recorded *A. m. artonis* from Harney County, primarily in the Trout Creek Mountains and on Steens Mountain. Elsewhere, *A. m. artonis* is found in northern Nevada, Colorado and Montana (Scott 1986, Pelham 2008). James and Nunnallee (2011) described and illustrated the immature stages of *A. m. washingtonia* and *A. m. erinna* from the Pacific Northwest but did not feature *A. m. artonis*. I also present additional images and information on *A. m. erinna* and *A. m. washingtonia* from additional rearing studies.

MATERIALS AND METHODS

Argynnis mormonia artonis was reared from gravid females obtained from a population near the summit of Steens Mountain (2207 m), Harney County in southeast Oregon. Nine females were obtained on August 9, 2009 and 16 females on August 18, 2010 and confined a few days later in plastic boxes (32 × 20 × 9 cm) with desiccating leaves of *Viola riviniana* Rchb. (Common Dog Violet). In 2009, 5 of the 9 females were confined with *Polygonum bistortoides* Pursh (Western Bistort). *Argynnis mormonia erinna* was reared from gravid females obtained from near the summit of Mt Howard (2445 m), (Wallowa County) in the Wallowa Mountains of northeast Oregon. Two females were obtained on August 20 2010 and confined with desiccating *V. riviniana* leaves in a plastic box (32 × 20 × 9 cm) on August 22. *Argynnis mormonia washingtonia* was reared from gravid females obtained from near Bear Creek Mountain (1871 m), Yakima County, in the Cascade Mountains of central Washington. Three females were obtained on August 26, 2010 and confined with desiccating *V. riviniana* leaves in a plastic box (32 × 20 × 9 cm) on August 27. Newly hatched larvae of all species were held in the plastic boxes at 20–26 ºC, natural daylength for ~ 1 month before overwintering at 4–5 ºC and 80–90 % relative humidity in darkness. Larvae were removed from overwintering after 107 (*artonis* 2009), 130 (*artonis* 2010) or 138 (*erinna*, *washingtonia*) days and held at 24–26 ºC in 2009 and 27 ºC in 2010. The 2009 *A. m. artonis* larvae were held under continuous illumination while the 2010 larvae and those of *A. m. washingtonia* and *A. m. erinna* were held under naturally increasing photophases from April 22. All early instars were reared on plastic arenas (15 × 9 cm) placed on saturated cotton wool in a plastic box. Each species cohort consisted of 40–50 first instars resulting in 5–10 adults. Leaves of *V. riviniana* with cut stems in the saturated cotton wool were provided for food and shelter. Later instars were reared in plastic cylinders (12 × 13 cm) with a gauze lid. Cut stems and leaves of *V. riviniana* in a small jar were provided for food and shelter with 6–8 larvae/cylinder. Observations were made on larval feeding, molting and pupating.

Estimates of the length of larvae at the beginning and end of each instar are given. Individuals showed little variation (± 1 mm) in these lengths. High resolution images were taken of eggs, all instars and pupae using a Canon digital SLR camera (EOS 1DS Mark II) mounted on a tripod. A Canon MP-E 65mm 1X–5X macro lens was used together with a Macro Twin Lite MT – 24 EX flash lighting system.

RESULTS

Immature stages. The egg of *A. m. artonis* (Fig. 1) is very similar to those of *A. m. erinna* and *A. m. washingtonia* (James and Nunnallee 2011). It is broad-based, measuring 0.8–0.9 mm at its base, conical and creamy white when first laid becoming orange-brown with indistinct red spots prior to hatching. There are 22–26 vertical ribs merging to ~ 12 at the top.

Larval instars of *A. m. artonis*, *A. m. erinna* and *A. m. washingtonia* reared in this study are shown in Fig. 2. The first instar of *A. m. artonis* measures 1.25–1.5 mm after hatching and is very similar to the other subspecies, medium-dark brown becoming lighter with maturity with a shiny black head and 8 prominent black bullae across each segment. Each bulla carries a long dark seta sometimes with a droplet at the tip (see James and Nunnallee 2011). The prothoracic shield is sclerotized and black as is the anal plate. At maturity the first instar measures 3 mm. The second instar of *A. m. artonis* is black with a wide pale stripe dorsally and a shiny black head. Six prominent black spines traverse each segment each bearing ~ 10 setae. Laterally, the spines have pale bases which become orange during the instar. Prior to molting the second instar is flecked with white laterally. The second instar develops from 3 to 6 mm in length and is very similar to those of *A. m. erinna* and *A. m. washingtonia* (Fig. 2). The third instar of *A. m. artonis* is also very similar to the other two subspecies and grows from 6 to 9 mm. It is predominantly black with a pale dorsal stripe bisected by a dark line. The bases of all spines are orange and lateral white flecking is prominent. The black spines each bear 14–16 setae. Initially, the fourth instar of *A. m. artonis* is similar to the third instar but most individuals darken during the instar with the orange bases of spines contracting and white flecking diminishing. This differs from fourth instar *A. m. erinna* and *A. m. washingtonia* which are grayer with increased white markings (Fig. 2 and James and Nunnallee 2011). Some fourth instar *A. m. artonis* have distinct brown markings on the head dorsolaterally, similar to the other subspecies, but other individuals retain a completely black head. Fourth instar *A. m. artonis* develop from 9 to 15 mm in length. The fifth instar of *A. m. artonis* (growing from 15 to 20 mm) is dark but laterally there are whitish-orange vermiform markings and the orange bases of spines are more prominent. The dorsal white band bisected by a dark line is prominent against the dark ground color. The fifth instar of *A. m. washingtonia* is distinctly lighter than *A. m. artonis* due to increased areas of white flecking (Fig. 2 and James and Nunnallee 2011). Fifth instars of *A. m. erinna* reared in this study were almost as dark as *A. m. artonis*, unlike *A. m. erinna* reared and described by James and Nunnallee (2011) which had 'extensive white

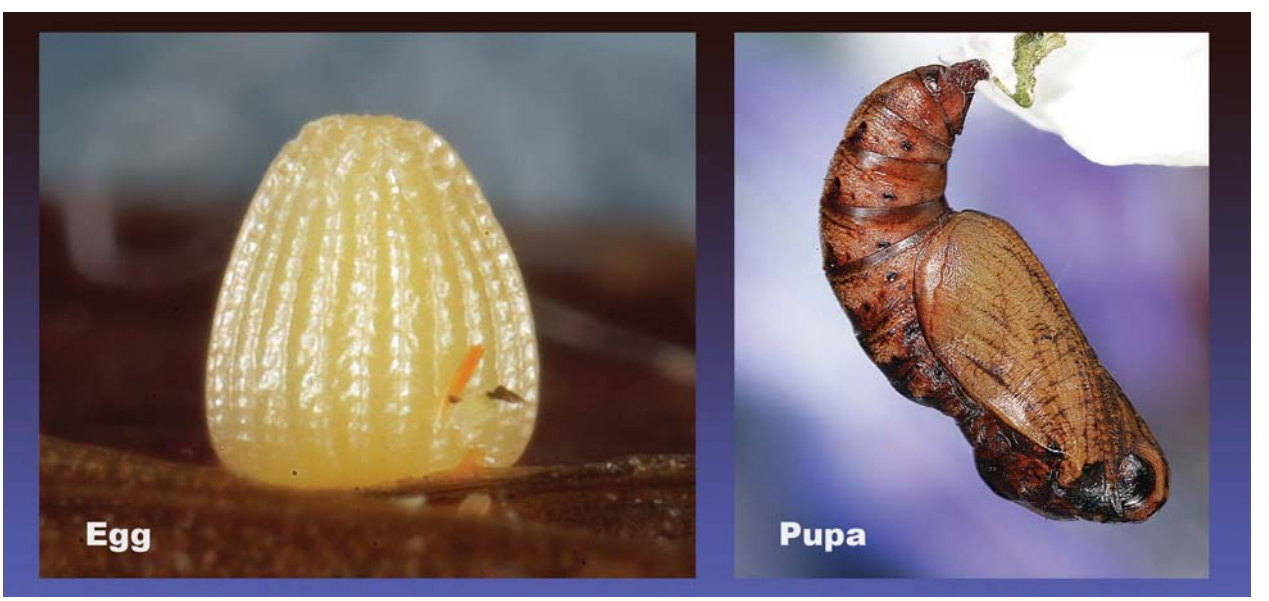

FIG. 1. Egg and pupa of *Argynnis mormonia artonis* from Steens Mountain, Oregon.

FIG. 2. First to sixth instars of *Argynnis mormonia artonis*, *A. m. erinna* and *A. m. washingtonia* from Steens Mountain (OR), Mt Howard (OR) and Bear Creek Mountain (WA), respectively.

vermiform' markings, similar to fifth instar *A. m. washingtonia*. Most individuals of *A. m. artonis* and *A. m. erinna* in early sixth instar were shiny jet black with whitish to orange spines, contrasting with the brownish-gray final instar of *A. m. washingtonia* (Figs. 2 and 3). Sixth instar *A. m. erinna* in this study were substantially darker than in the reared individuals of this subspecies described in James and Nunnallee (2011). In the current study the dorsal pale stripe prominent in sixth instar *A. m. washingtonia*, was much reduced or absent in sixth instar *A. m. erinna* and *A. m. artonis*. Some individuals of *A. m. artonis* and *A. m. erinna* had fine brown speckling overlying the black ground color creating a more mottled black appearance but these larvae are still substantially darker than sixth instars of *A. m. washingtonia*. The heads of sixth instars of all three subspecies are black with orange-brown markings dorsolaterally that vary in extent (Fig. 4). A summary of phenotypic differences in late instars of *A. m. artonis*, *A. m. erinna* and *A. m. washingtonia* is provided in Table 1. Sixth instars of *A. m. artonis* grow from 20 to 32 mm in length, comparable to the other two subspecies.

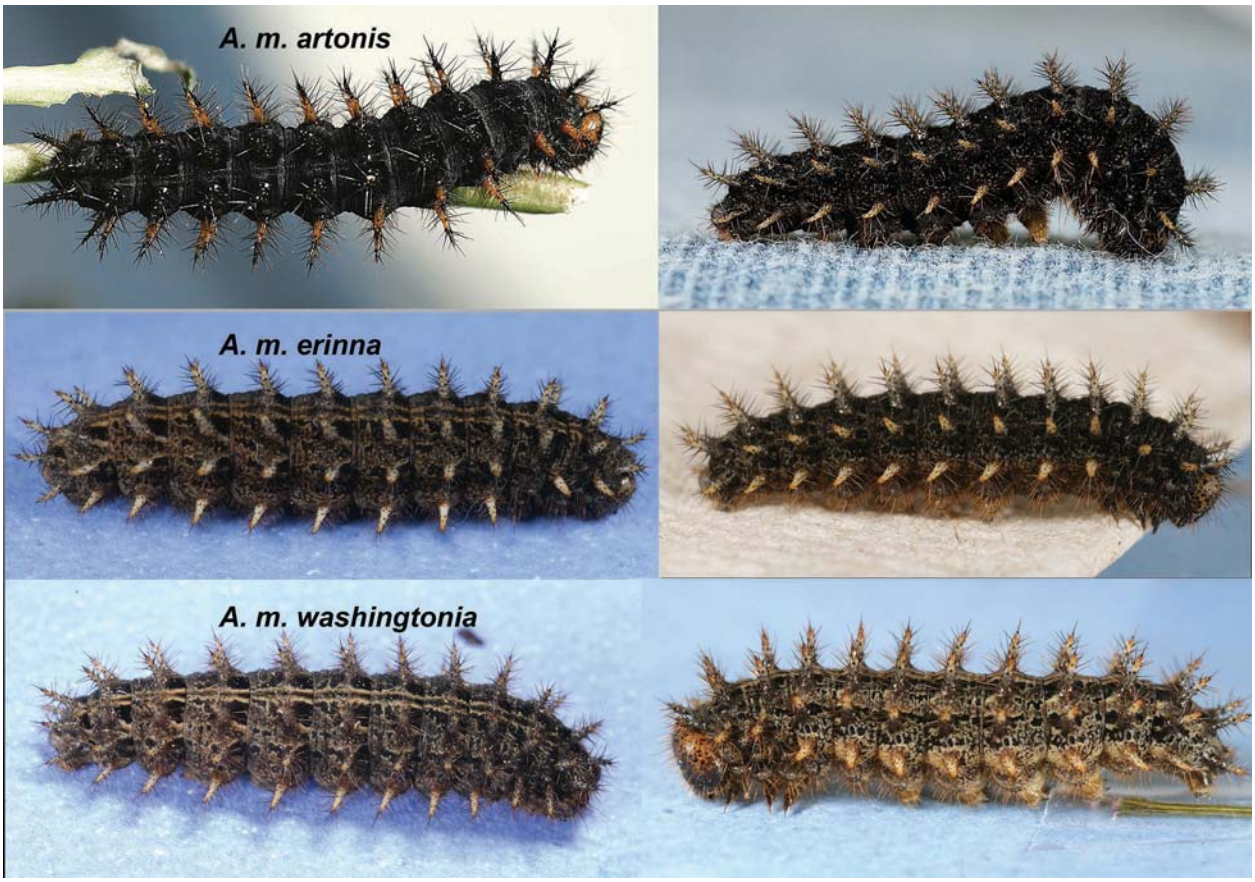

FIG. 3. Dorsal (left) and lateral (right) views of sixth instar *Argynnis mormonia artonis* (Steens Mountain, OR), *A. m. erinna* (Mount Howard, OR) and *A. m. washingtonia* (Bear Creek Mountain, WA).

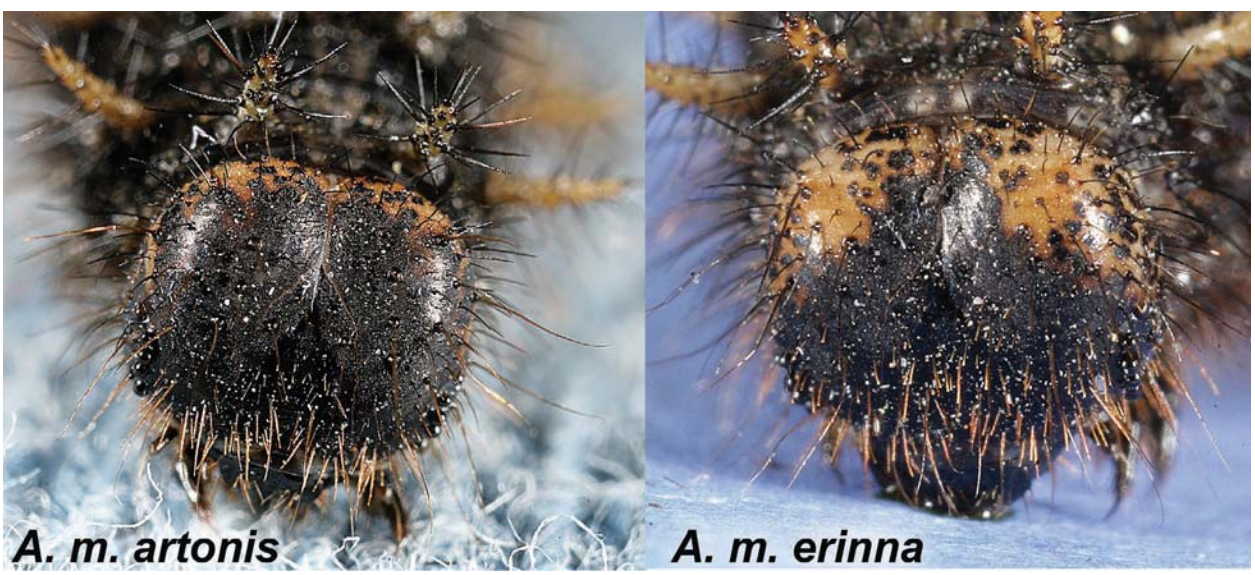

FIG. 4. Head capsules of sixth instar *Argynnis mormonia artonis* (Steens Mountain, OR) and *A. m. erinna* (Mount Howard, OR).

The pupa of *A. m. artonis* is light-medium brown with variable dark markings and measures 17–18 mm in length (Fig. 1), very similar to the pupae of *A. m. erinna* and *A. m. washingtonia* (James and Nunnallee 2011).

Biology of immature stages. Accounts of the biology of *A. m. erinna* and *A. m. washingtonia* immature stages are presented in James and Nunnallee (2011). The biology of immature stages of *A. m. artonis* is likely to be similar. The host plant used by *A. m. artonis* at Steens Mountain is uncertain, although *Viola purpurea* Kellogg (Goosefoot Violet) appears to be the only violet occurring at the upper elevations where *A. m. artonis* is found. However, this violet or any other violet species do not appear to be present in many of the locations where *A. m. artonis* is abundant. Western Bistort (*P. bistortoides*) is abundant in all of the locations where *A. m. artonis* flies and gravid females readily oviposited on this plant in captivity, suggesting it should be investigated as a potential host plant.

First instars of *A. m. artonis* overwintered well with a low mortality rate (<5 %). Feeding commenced after 3–7 days at 24–27 ºC. All instars readily accepted *V. riviniana* as a host plant but first instars rejected *Viola odorata* L. (Sweet Violet) and *Vaccinium* sp. (Blueberry). First instars fed well on Pansy (*Viola tricolor* L.) but this was discontinued as a host after this instar. Development from commencement of feeding to pupation took 59–65 days at 24–26 ºC (2009) and 51–55 days at 27 ºC (2010) (53–55 and 55–58 days respectively, for *A. m. washingtonia* and *A. m. erinna* at 27 ºC). Six to eight days were spent in each of the first four instars with 20–30 days spent in the final two instars. Pupation occurred near the bottom of containers usually under a leaf in a shelter constructed with sparse strands of silk. Pupae were held at 18–21 ºC and adult individuals eclosed after approximately 4 weeks.

DISCUSSION

The early immature stages (egg–early fourth instar) of *A. m. artonis* from Steens Mountain in southeast Oregon are similar to those of *A. m. erinna* and *A. m. washingtonia*, but late instars (fifth, sixth) are substantially darker than those of *A. m. washingtonia* and previously described populations of *A. m. erinna* (James and Nunnallee 2011). Late instars of *A. m. erinna* reared in this study from Mt Howard in northeast Oregon were as dark as those of *A. m. artonis*. The final instars of *A. m. artonis* from Steens Mountain and *A. m. erinna* from Mt Howard are almost concolorously black and very similar to the late instars of *Argynnis hydaspe* (Boisduval) and *Argynnis hesperis* (W. H. Edwards) (James and Nunnallee 2011). The *A. m. artonis* and *A. m. erinna* larvae reared in this study originated from populations that occur above 2200m, significantly higher than the *A. m. washingtonia* population (1877 m) reared in this study. The *A. m. washingtonia* larvae described by James and Nunnallee also originated from approximately 1800 m and their *A. erinna* larvae came from a population at 990 m. The only other published images available of final instar *A. m. mormonia* (Guppy and Shepard 2001; *opis*, Miller and Hammond 2007, *erinna*, no elevation data) show lighter-colored larvae similar to those in James and Nunnallee (2011). It seems likely that the darker color of high elevation final instars of *A. m. artonis* and *A. m. erinna* may be due to increased melanism developed as an adaptation to enhance body temperatures and development under cool conditions. Increased melanism at high elevations is a predominant adaptation in insects generally and Lepidoptera in particular (Hodkinson 2005). Caterpillars of a number of butterfly and moth species have been shown to be more melanic under cooler conditions and better able to attain optimal body temperatures for development than their

TABLE 1. Summary of phenotypic differences in late instars of *A. m. artonis*, *A. m. erinna* and *A. m. washingtonia* in populations from high (> 2000 m) or low (< 2000 m) elevations in Washington and Oregon.

	A. m. artonis (high elev.)	*A. m. erinna* (high elev.)	*A. m. erinna* (low elev.)	*A. m. washingtonia* (low elev.)
Fourth instar	Darkens late in instar	Gray with pale markings	Gray with pale markings	Gray with pale markings
Fifth instar	Dark	Dark	Gray with pale markings	Gray with pale markings
Sixth instar	Jet black. Dorsal stripe absent or much reduced	Jet black. Dorsal stripe absent or much reduced	Brownish-gray with prominent white dorsal stripe	Brownish-gray with prominent white dorsal stripe

less melanic counterparts (James 1986, Goulson 1994, Hazel 2002, Solensky and Larkin 2003, Davis et al. 2005, Nice and Fordyce 2006). Larval populations of all *A. mormonia* subspecies likely express color polymorphism with darker phenotypes rare at low-mid elevations becoming increasingly prevalent at higher elevations as the need for solar-mediated enhancement of development increases.

The final two instars of six northwestern *Argynnis* spp., including *A. mormonia*, generally occupy 30–50% of the larval developmental period (James 2008), thus the greatest need to accelerate development should occur during these instars. I suggest that increased melanism in late instars may not be confined to *A. mormonia* but may also occur in some other western North America *Argynnis* spp. that occupy a range of elevations. For example, *Argynnis hesperis* (Gunder) and *Argynnis atlantis* (W. H. Edwards) occur in mid-high elevation habitats in the Pacific Northwest and have larvae that appear to vary considerably in the amount of black coloration in late instars (Scott et al. 1998). The sixth instar of *A. atlantis* is shown with substantial areas of white markings in James and Nunnallee (2011) but sixth instars from Mt. Howard are entirely black (James in prep.). Color polymorphisms in *Argynnis* larvae clearly have the potential to confuse species identifications. Much of the described larval color variation in *A. hesperis, A. atlantis* (Scott et al. 1998) and *A. mormonia* (Dunford 2009) may simply be a consequence of different temperatures affecting different populations during development.

The commercially available violet used in this study, *V. riviniana*, is invariably mislabeled as *Viola labradorica* Schranck (Labrador Violet). Thus, recent publications reporting acceptability of *V. labradorica* to *Argynnis* larvae (James 2008, James and Nunnallee 2011 actually refer to *V. riviniana. Viola riviniana* is a suitable host for *A. m. artonis* as it is for *A. m. erinna, A. m. washingtonia, Argynnis egleis* (Gunder), *Argynnis hesperis dodgei* (Gunder) and *A. atlantis*. Its acceptance in other Pacific Northwest *Argynnis* spp. is limited to late instars (James 1986, James and Nunnallee 2011). The possibility of *A. m. artonis* using *P. bistortoides* as a natural host at Steens Mountain needs investigating. *Polygonum bistorta* L. is recorded as a larval host for the European Fritillary *Mesoacidalia aglaja* L. and a number of *Boloria* and *Clossiana* spp. also use various *Polygonum* spp. as larval hosts (Robinson et al. 2010). The larval development period of *A. m. artonis* of 51–59 days at 24–27 ºC was similar to that of *A. m. washingtonia* and *A. m. erinna* at 27 ºC (James and Nunnallee 2011). It was also comparable to the developmental rates of *Argynnis coronis simaetha* dos Passos and Grey, *Argynnis zerene picta* (McDunnough), *A. e. macdunnoughii* and *Argynnis cybele leto* (Behr) (James 2008).

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ADAPTIVE SIGNIFICANCE OF PREVIOUSLY MATED MONARCH BUTTERFLY FEMALES (*DANAUS PLEXIPPUS* (LINNEAUS)) OVERWINTERING AT A CALIFORNIA WINTER SITE

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ABSTRACT. In the fall, migrating monarch butterflies are in reproductive diapause when they arrive at winter sites in California. Approximately 1/3 of the overwintering females contain sperm of summer males within their spermatheca. When these females were subjected to conditions that terminated diapause, they were able to produce offspring with near equal fecundity throughout the overwintering period. The mated females also benefited by the added male nutrients to produce eggs when their body energy reserves were low. Spring matings of virgin females are necessary to obtain spermatozoa to fertilize their eggs and possibly to receive male nutrients to supplement their energy reserves. Several possible survival advantages of early mating are: (1) previously mated females are better able to withstand the rapid ovarian development towards the end of the overwintering season than virgin females; (2) females need not mate again to insure the fecundity of her eggs; and (3) mating with previous summer males broadens the genetic plasticity of the species.

Additional key words: Diapause, fecundity, multiple mating, spermatheca, sperm

In North America, the monarch butterflies, *Danaus plexippus* (Linneaus), have evolved an adaptive strategy for utilization of larval host plants, the milkweeds (*Asclepias* spp), that grow abundantly over much of the United States and as far north as lower regions of Canada during spring and summer months. In the fall, before the milkweeds die back to their rhizomes for the winter, monarch butterflies east of the Rocky Mountains begin their long distance migration to winter sites located in the high mountains of Mexico while monarchs west of this divide, migrate to selected forested areas along the California coastline (Leong et al. 2004). During the winter, monarchs are found in groves that offer protection against environmental extremes (Leong 1990) and are physiologically different from the summer generation in that they are long lived (5–6 months versus 4–6 weeks) and in reproductive diapause (Herman 1985; Herman et. al 1989; Herman & Tatar 2001).

In California, approximately 30% of the females captured from winter aggregations were mated (contained spermatophores) and this percentage remained statistically unchanged through most of the overwintering months until just prior to the spring migration (Leong et. al. 1995, 2008). Since overwintering butterflies are in reproductive diapause upon emergence (Herman & Peng 1976; Herman & Tatar 2001), the mated overwintering females had to have mated with the previous summer's non-diapausing males prior to the migration to winter sites. Mating between two physiologically different generations can occur because of the temporal overlap of diapausing females with non-diapausing males and the mating behavior of this species. Unlike many danaine species, the monarch male employs comparatively simple mating behavior where male pheromone plays a minor role and in which "force mating" is common (Boppré 1993). Males locate females visually and capture them in flight (Urquhart 1960; Pliske 1975; Hill et al. 1976; Frey et al. 1998) or while sunning on foliage (Leong 1995). Mating between diapausing females and non-diapausing males is not unique to the monarch butterfly. The autumn morph females of the common grass yellow butterfly, *Eurema hecabe* (Linnaeus) often mated with summer non-diapausing males (Kato 1986). A similar mated condition may exist among monarch females migrating to the Mexican overwintering sites because "old" spermatophores were recovered within the bursa copulatrix of females in spring (Van Hook 1999).

The purpose of this study was to investigate the adaptive significance of previously mated overwintering females by comparing the fecundity, fertility and longevity of these mated females with virgin females under laboratory conditions.

METHODS AND MATERIALS

Winter site. The monarch butterflies were collected during the 2009–2010 overwintering season from the Pismo North Beach winter grove, located in the southeastern section of Pismo State Park, Pismo Beach, San Luis Obispo County, California (35°07'46" latitude; 120°37'53" longitude). The site consists mainly of blue gum trees, *Eucalyptus globulus* Labillardiere, few Monterey cypress, *Cupressus marocarpa* Gordon, and willow, *Salix* sp. on the northern edge and Monterey pines, *Pinus radiata* D. Don, on the southeastern corner. The grove (180 × 120 m) supports little to no understory beneath the eucalyptus canopy and slopes downward northwest toward a small creek.

Collection and laboratory rearing. Twenty female monarch butterflies were randomly collected from their

winter aggregation during the early morning hours (0700–0730 h PST) every 21 d starting on 6 November 2009 until 1 February 2010. The butterflies were placed in a large paper bag and stored in a cooler for transportation to the laboratory. The laboratory is located in the Biological Sciences Department, California Polytechnic State University, San Luis Obispo, California.

In the laboratory, the butterflies were initially placed in a cool room (10°C) to lower their body temperature so that they could be more easily handled. Each butterfly was tagged by writing a sequence number on the right hind wing with a marking pen and placed in a 18.8 liter ice cream container, one female per container, with the top open and covered with a plastic house screen (36 cm^2). A door "flap," 10 cm square, was cut on the bottom side of the cage. The door "flap" was secured to the side of the cage with tape.

Diapause was terminated by keeping the butterflies under laboratory conditions (temperature 20.6°C ± 3.2 SE; RH = 56.5 ± 6.2 SE) with continuous light. Lights were standard ceiling fluorescent lights approximately 2 meters above the containers. A honey-water mixture in 200 ml plastic tissue culture dishes with 1 mm perforations on top was provided and changed every 3 d. These conditions have been used previously to successfully rear several generations of monarch butterflies from eggs to adults. The butterflies were misted daily to provide water for them to imbibe. A bouquet of milkweed, *Calotropis gigantean* (L.), leaves was placed in each cage and changed every 2 d. Number of eggs laid and the emerging neonates were recorded daily for each female for a 21-d period.

Upon death or when killed at the termination of the 21 d period, the female's abdomen was removed, immersed in insect Yeager's ringer solution, and examined under a dissecting microscope (60–100X).

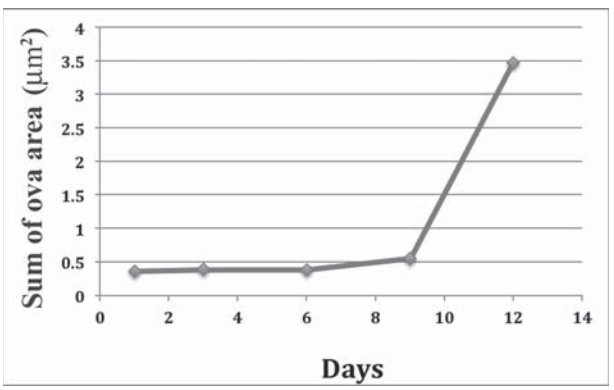

FIG. 1. The sum of the average area of the three largest ova measured with a compound microscope (100X) over a 12-d period after terminating diapause.

Each female was dissected and observed for the presence of spermatophores and sperm within the spermatheca. A female was considered to be virgin if it lacked spermatophores within the bursa copulatrix, and if sperm was not found within the spermatheca. Females were considered to be mated if spermatophores or sperm were detected.

To determine the rate of ovarian development under laboratory and 24 h light conditions an additional 15 females were collected on 27 November 2009. After holding intervals of 1, 3, 6, 9, and 12 d, three females were randomly selected per interval, killed and their left ovary dissected, placed on a glass slide and examined with a compound microscope (100X). The length and width of the three largest ova within one of the four ovarian strands were measured to the nearest 0.01-micrometer and the data presented as the sum of the product of these 2 measurements.

Statistical analyses. Data were analyzed using the statistical program of Biostat 1 (Pimentel & Smith 1990) for analysis of variance (ANOVA). Data that did not satisfy the assumption of ANOVA were subjected to nonparametric tests (chi-square and randomization test).

RESULTS AND DISCUSSION

The sample size for each collection date varied, due to errors in the number of females collected and to butterflies escaping from cages. The resulting sample sizes for 6 and 27 November and 18 December were 18, and for 8 January and 1 February were 19.

The proportion of non-mated to mated overwintering females collected from winter aggregations from November to January was 3:1 and remained statistically unchanged through most of the winter season (Table 1. $X^2 = 4.02$; df = 3; p=0.259). This relationship agreed with earlier investigations of California winter populations where mating among overwintering butterflies is infrequent (Hill et al 1976; Tuskes & Brower 1978; Leong et al. 1995, 2008). Mating among overwintering monarch butterflies in California is generally limited to the last few weeks prior to their spring migration. The inclusion of the 1 February (last) sample with the November to January data resulted in a deviation from the 3:1 proportional relationship between the non-mated to mated females (Table 1. $X^2 = 17.10$; df = 4; p> 0.01). All of the females in the February sample were mated which agreed with the previously reported truncated mating activity period prior to their spring dispersal (Frey et al.1998; Leong et al. 1995).

Under laboratory conditions where diapause was terminated, the longevity of virgin females collected

TABLE 1. Proportion of non-mated to mated individuals collected from winter aggregations from November 2009 to February 2010.

Collection date	Total/mated females	% mated
6 Nov	18/5a	28
27 Nov	18/10a	56
18 Dec	18/6a	33
8 Jan	19/11a	61
1 Feb	19/19b	100

Proportions within a column with different letters deviated significantly from a 3:1 ratio ($X^2 > 0.01$).

TABLE 2. Average longevity (days) of mated and virgin females fed honey water mixture, sprayed daily with water mist, and held under laboratory conditions (20.6 °C ± 3.2 SE, RH 56.5 ± 6.2 and 24 h light).

Collected	Mated	days ± SE	Virgin	days ± SE
6 Nov	5	14.8a ± 3.56	13	13.3a ± 0.8
27 Nov	10	14.9a ± 1.6	8	12.9a ± 2.5
18 Dec	6	12.3a ± 3.5	12	13.0a ± 2.2
8 Jan	11	17.5a ± 1.8	8	5.2b ± 1.5
1 Feb	19	11.1a ± 1.7		

Means within a column followed by a different letter were significantly different ($P > 0.01$).

TABLE 3. Number of virgin females collected from winter aggregations laying eggs under laboratory conditions.

Collected	N	No. laying eggs	Eggs laid (mean ± SE)
6 Nov	13	4	5.5 ± 1.3
27 Nov	8	6	17.5 ± 6.6
18 Dec	12	2	1.5 ± 0.5
8 Jan	8	0	0

TABLE 4. Number of eggs laid, hatches and % fecundity of mated overwintering females under laboratory conditions.

Collection date	No. females laying eggs	No. Eggs laid (mean ± SE)	No. Hatches (mean ± SE)	% Fecundity
6 Nov	4	139.0 ± 49.34	122.7 ± 55.26	88.3
27 Nov	5	71.2 ± 14.31	69.6 ± 12.21	90.4
18 Dec	3	102.3 ± 17.90	69.0 ± 13.58	67.6
8 Jan	10	117.1 ± 29.10	75.8 ± 14.58	64.8
1 Feb	10	142.2 ± 49.56	114.2 ± 39.12	80.3

from November to December was similar, ≈13 d, but was significantly shorter, 5.2 d ± 1.4 SE (F = 4.59; df 3, 37; p=0.008; Table 2) for those collected in January. In contrast, longevity among mated females showed no such decline (Table 2). Reduced longevity in January among the virgin females may be attributed to the demands of ovarian development when body energy reserve is low (Wells et al. 1990). The egg development of overwintering females under laboratory conditions showed little change for the first 6 days but began to increase geometrically starting by the 9th day, and by the 12th day the ovaries were filled with fully developed eggs with chorion (Fig. 1). Death occurred at an average of 5.2 days that corresponded well to the transitional period of rapid ovarian development. The marked decline in the survival in January among the virgin females suggests that under laboratory conditions favoring ovarian development, the amount of fat (energy) reserve remaining in these females (Wells et al. 1990) was not enough to accommodate both the full ovum development and survival.

Under field conditions, virgin females may be able to survive with low fat reserve in January because temperatures that induce ovarian development are not constant. The females can conserve their body energy and delay ovarian development by returning to the winter grove where temperatures are cool, thereby lowering their metabolic rate. In the spring or just prior to their migration, the available evidence suggests that they must mate not only to fertilize their eggs but also to gain added nutritional energy from the males (Boggs & Gilbert 1979; Boggs 1981; Marshall 1982; Shapiro 1982; Oberhauser 1989). Previous mated overwintering females, on the other hand, have the nutritional advantage to survive the winter and perhaps to compete better for mates than virgin females in the spring.

Virgin females collected from their winter aggregations in November and December were able to lay infertile eggs (1 to 45 eggs/female; Table 3) under laboratory conditions. None of the virgin females collected in January produced eggs. Seven of 8 females in the January sample died about 5 days after being collected from the winter grove and placed under laboratory conditions that would break diapause. Their deaths seem to correspond well to the interval prior to rapid maturation of eggs within the ovaries (Fig. 1), suggesting that their premature deaths were attributed to physiological demands of ovarian development when their body energy reserves were low. The sole survivor did not lay eggs, possibly due to the lack of sufficient body energy reserves.

Since one-third of the overwintering females were mated when they arrived at the California winter site and winter matings were infrequent, the fertility (reproductive ability, i.e. eggs laid) and fecundity (number of offspring) of these females collected from November to January were the product of earlier mating with summer males. The average number of eggs laid (F = 0.89; df = 3, 18; p = 0.53), hatches (F=1.33; df = 3,18; p=0.29) and % fecundity (Rant test F = 1.01; p = 0.40) during this period were not significantly different (Table 4), indicating that overwintering females were able to store viable spermatozoa for more than four months and produced fertilized eggs with equal fecundity. This long-term storage of viable sperm in monarch butterflies has not been previously reported. The physiological aspect of storage and the maintenance of the viable sperm within the female's spermatheca should be investigated to determine if seminal fluids from the male (Shapiro 1982; Tram & Wolfner 1999; Gillot 2003; Baer & Schmid-Hempel 2005; Poiani 2006; den Boer et al 2008), spermathecal gland secretions (Filosi & Perotti 1975; Pitnick et al. 1999; den Boer et al. 2008), or a combination of the seminal fluid and spermathecal fluids (Baer et al. 2009) are involved in keeping the sperm viable.

The February sample of females deviated significantly from the 3:1 proportion of non-mated to mated females (Table 1. X^2 = 17.09; df = 4; p=0.002) because all were mated. The average number of eggs laid (F = 0.44; df = 4, 27; p=0.78), hatches (F = 0.72; df = 4, 27; p=0.59), and fecundity (Randomization test; F = 1.48; p = 0.26) did not, however, differ significantly from earlier samples of mated females. Although differences were not detected, the reduced fecundity of approximately 65% of the December and January samples (Table 4) suggests that the viability of the stored spermatozoa in fall-mated females was diminishing.

Mating occurred earlier this season due to mild January temperatures. The February sample may reflect an early mating behavior of California monarch butterflies. Prior to the monarch's spring migration, the monarchs at California winter sites undergo a short intense mating activity period where males capture females in flight or while they sun on foliage. Multiple mated females are common. Leong et al. (1995) reported that 80% of the females collected during the latter phase of this mating period had 2+ spermtophores within their bursa copulatrix. Although all of the females of the February sample were mated (Table 1), only 1 of 19 was multiple mated. Assuming mating is random in a winter population consisting of 3 virgin to 1 previously mated females, the expected number of multiple mated females in the February should have been 6 out of 19. The low frequency of multiple mated females in the February sample suggests that virgin females were

mated before the previously (fall) mated females. Another possibility is that the previously mated females had already left the winter grove.

We believe that the 1 February sample was taken during the initial phase of the mating activity period. Another sample on 8 February was unsuccessful because few butterflies remained and were clustered at heights too high to be captured by our 25 ft extension net.

This study revealed several possible survival advantages to mating in late summer. Firstly, the nutrients associated with the male's spermatophore improve the female's winter survival and possibly contribute towards maintenance of viable spermatozoa. Mated females are better able to withstand the physiological stresses of rapid egg maturation toward the end of the overwintering season than the virgin females, when their body lipid levels are at the lowest (Wells et al. 1990). A similar circumstance may exist for the females overwintering in Mexico since Van Hook (1999) reported finding many "old" spermatophores in overwintering females. This added survival advantage may also play a significant role for females overwintering in Mexico because they undergo a longer period of reproductive diapause (Herman et al. 1989).

Secondly, previously mated females need not mate again to secure fecundity and possibly begin spring migration earlier to be the first to exploit re-emerging milkweeds. Kato (1986) reported that the mated overwintering pierid butterfly, *Eurema hecabe*, began laying eggs in the spring without remating. By comparison, virgin females require spring mating to fertilize their eggs.

Thirdly, earlier mating connects the genes of late summer and the overwintering generations and thereby broadens genetic plasticity of the species, although numbers of offspring resulting from spermatozoa of the late summer males has not been determined. How many of the offspring will be sired by spermatozoa of the late summer males is yet to be determined.

By mating with females before they arrive at the overwintering site, males from the summer generation avoid competition with other males for females in the spring and the subsequent competition among spermatophores of different males within the bursa copulatrix for alignment with the ductus seminalis, the narrow pathway the spermatozoa must travel to the spermatheca (Drummond 1984; Solensky & Oberhauser 2009a, b). Once the spermatozoa gain entry into the spermatheca, however, mixing (Solensky & Oberhauser 2009a, b) and competition among the spermatozoa of different mating may occur before successfully uniting with the ova.

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FUNCTIONALITY OF A REDUCED PROBOSCIS: FLUID UPTAKE BY *PHIGALIA STRIGATARIA* (MINOT) (GEOMETRIDAE: ENNOMINAE)

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ABSTRACT. The structure and functionality of the reduced proboscis of males of *P. strigataria* was studied. Scanning electron microscopy revealed a proboscis structurally similar to functional proboscises of other lepidopteran species, including chemo- and mechanosensilla and a tip-region with larger spaces between the dorsal legulae. Drops of red food coloring applied to the dorsal legulae entered the proboscis. Subsequent dissections exposed a complete and functional gut containing food coloring. We suggest that Lepidoptera with reduced proboscises might rely on capillarity as an initial step for fluid to enter the proboscis for subsequent uptake. Field observations are needed to determine if *P. strigataria*, and other Lepidoptera with reduced proboscises, feed in their natural habitats.

Additional key words: feeding habits, fluid uptake, capillarity

Most extant Lepidoptera possess a coilable proboscis that is used for fluid uptake (Scoble 1992; Krenn 2010). The proboscis is composed of two elongated maxillary galeae connected by dorsal and ventral legulae to form a food canal for fluid transport (Eastham & Eassa 1955; Krenn et al. 2001). The lepidopteran proboscis has been assumed to function like a drinking straw (Eberhard & Krenn 2005), only using the sucking pump in the head for fluid uptake (Daniel et al. 1989; Kingsolver & Daniel 1995). According to the drinking-straw model, a proboscis must be a sealed tube to properly function (Borrell & Krenn 2006; Krenn 2010); proboscises that are small and might lack a sealed tube-like arrangement are described as reduced, rudimentary, or vestigial (Rindge 1975; Krenn & Kristensen 2000), implying a lack of functionality without experimental evidence. The lepidopteran proboscis, however, does not have to be completely sealed to be functional (Monaenkova et al. 2012), which suggests that the drinking-straw model is incorrect or incomplete.

Moths in the genus *Phigalia* are medium-sized and have a reduced proboscis; most additional members of the Bistonini have a proboscis described as vestigial or absent (Rindge 1975). Adults of *Phigalia strigataria* (Minot) (Lepidoptera: Geometridae) (Fig. 1) are active from January to March in the southeastern USA (excluding peninsular Florida), and their range continues north into Canada (Rindge 1975). Although adult Lepidoptera use a variety of dietary sources (Adler 1982; Scoble 1992), foods such as floral and extrafloral nectar, fruit, and tree sap, are scarce or unavailable during the winter when adults of *P. strigataria* are active.

The reduced proboscis of *P. strigataria* suggests a lack of functionality; however, studies of *Gluphisia septentrionis* and *Clostera albosigma* (Notodontidae) provide an example of a reduced, but functional proboscis (Adler 1982; Smedley & Eisner 1995). Considering that a reduced proboscis can retain functionality (e.g., *G. septentrionis*), and that functionality is not limited to the drinking-straw model, we hypothesized that the proboscis of *P. strigataria* is functional, even though food sources are scarce when the adults are active.

MATERIALS AND METHODS

Twelve males of *Phigalia strigataria* were captured at lights from 1900 to 2300 h in Central, SC, USA, in February 2011, and placed in glassine envelopes. Two males were deposited in the Clemson University Arthropod Collection. The remaining males were used to study the proboscis with scanning electron microscopy (SEM) or to perform feeding experiments followed by dissections.

Scanning electron microscopy and proboscis measurements. The heads of four males were prepared for SEM using a series of ethanol washes (24

hrs in 80, 95, and 100% each) followed by chemical drying with hexamethyldisilazane. Heads were placed on stubs with carbon-graphite tape and gold sputter-coated for approximately 90 seconds. A Hitachi TM3000 scanning electron microscope was set to full vacuum and 15 kv for imaging. The galea length (= proboscis length), galea width at its widest point, and tip-region length were measured using SEM images and ImageJ software (http://rsbweb.nih.gov/ij/download.html). The tip-region was characterized by larger spaces between the dorsal legulae (Krenn et al. 2001, 2010) and where only a single layer of dorsal legulae existed. The tip-region was measured from the proximal end of the drinking-slit region (sensu Krenn 2010).

Feeding trials and dissections. Four live males of *P. strigataria* were secured with insect pins on dissecting trays, leaving the ventral side exposed. With the aid of a dissecting microscope, a microsyringe (Hamilton Co. Inc., Whittier, CA.) was used to place drops of diluted red-food coloring (Southern Homer Assorted Food Coloring Set, Mauldin, SC) (ca. 5:1 water:food coloring) on the dorsal side of the proboscis. Drops of food coloring (less than 1 µl each) were placed on the proboscis, and larger drops (greater than 1 µl each) were placed near the base of the proboscis, covering the labrum.

After the feeding trials, the dissecting tray was filled with physiological saline, and the abdomen and thorax were dissected to expose the alimentary canal. The crop was removed from one specimen, placed on a slide with 50% acetic acid, and viewed under a light microscope. Two unfed males were stored for three months in a refrigerator and later rehydrated in water and dissected to compare the alimentary canal to males in feeding trials. A Scion Corporation Color Digital Camera (Model CFW 1310C, Scion Corporation, Frederick, MD) was used to acquire images of specimens under the dissecting and light microscope, and ImageJ software was used for measurements.

RESULTS

Proboscis morphology and feeding trials. The average length of the proboscis was less than 0.50 mm (n = 4, Table 1), and appeared fleshy, pliable, and was light tan (Fig. 2a). The proboscis could bend in all directions. SEM imagery revealed dorsal and ventral legulae (Fig. 2b); however, the galeae separated from each other when nudged with an insect pin. Dorsal legulae were flat and lancet-shaped, with a pointed extension at each tip (Fig. 2c). The architecture of the ventral legulae suggested interlinking capability (Fig. 2b), but this could not be verified because the galeae were separated in all SEM images.

The proboscis had at least two distinct structural regions: a tip-region characterized by a single layer of dorsal legulae with larger spaces between them than those more proximal, and a region proximal to the tip-region characterized by a second row of smaller dorsal legulae (Fig. 2d,e); the proximal region comprised most of the proboscis length (76.4%, n = 4, Table 1). The dorsal legulae in the tip-region were shorter (mean = 11.9 µm, n = 4, 2 individuals) than those proximal to this region (30.2 µm). The proximal region of the galeae was covered by microtrichia that declined in number near the transition to the tip-region.

An irregular row of sensilla trichodea lined the dorsal side of the galeae and occurred in two rows in the mid-region of the proboscis, but became a single row in the tip-region; the tip region had an average of 8.3±0.88 sensilla trichodea (12.6±0.92 µm in length, n = 3 individuals, Table 1) (Fig. 2e). Four sensilla basiconica, each with an elongated stylus, occurred on the lateral galeal wall in the tip-region. A sensillum styloconicum with 4 longitudinal ridges and an extended stylus was at the tip of the proboscis (Fig. 2d). The food-canal wall was annulated and had a row of sensilla basiconica (Fig. 2f).

When a drop of red food coloring was applied to the proboscis, it entered the proboscis, leaving no food

TABLE 1. Measurements (µm) of proboscises of males of *P. strigataria* collected in Central, SC, in February 2011.

Male ID #	Length of proboscis	Max. width of galea	Length of tip region	% of tip-region	# of sensilla trichodea in tip-region	Mean length of sensilla trichodea in tip-region
1	481.8	75.3	111.3	23.1	8	13.6
2	496.2	63.6	119.8	24.1	-	-
3	488.4	54.8	110.9	22.7	10	13.4
4	495.5	63.0	121.7	24.6	7	10.7
Mean±SE	490.5±3.38	64.2±4.21	115.9±2.81	23.6±0.43	8.3±0.88	12.6±0.92

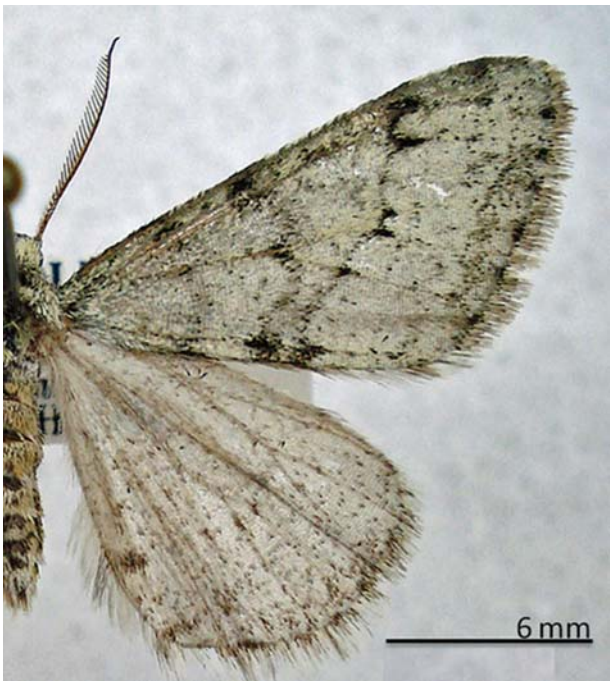

FIG. 1. Photograph of male of *P. strigataria* captured in February 2011 in Central, SC (Canon Rebel XTI digital camera).

coloring on the external galeal walls; however, the dorsal legulae subsequently had a pink-tinted stain. Fluid entered all tested regions of the proboscis, and antiparallel movements of the galeae occurred irregularly during the feeding trials. The food canal was stained red after a drop of food coloring was administered. Larger drops placed at the base of the proboscis pulsated and decreased in size until they disappeared.

Gut morphology and functionality. After the feeding trials, red portions of the gut were visible through the cuticle and epidermal layers at the juncture of the thorax and abdomen, and between the abdominal segments. Subsequent dissections revealed a gut with sections of red fluid (Fig. 3). The foregut consisted of a thin tube and the crop, which attached as a separate compartment and was filled with red liquid. Light microscopy revealed spines on the interior crop wall (Fig. 3).

The midgut was wider (323 µm, n = 2) and shorter (986.5 µm, n = 2) than the foregut (52 µm, 3471 µm, respectively) and appeared to be filled with red fluid. Two sets of three Malpighian tubules extended from the juncture of the midgut and hindgut, and each set branched from a single stalk. One Malpighian tubule

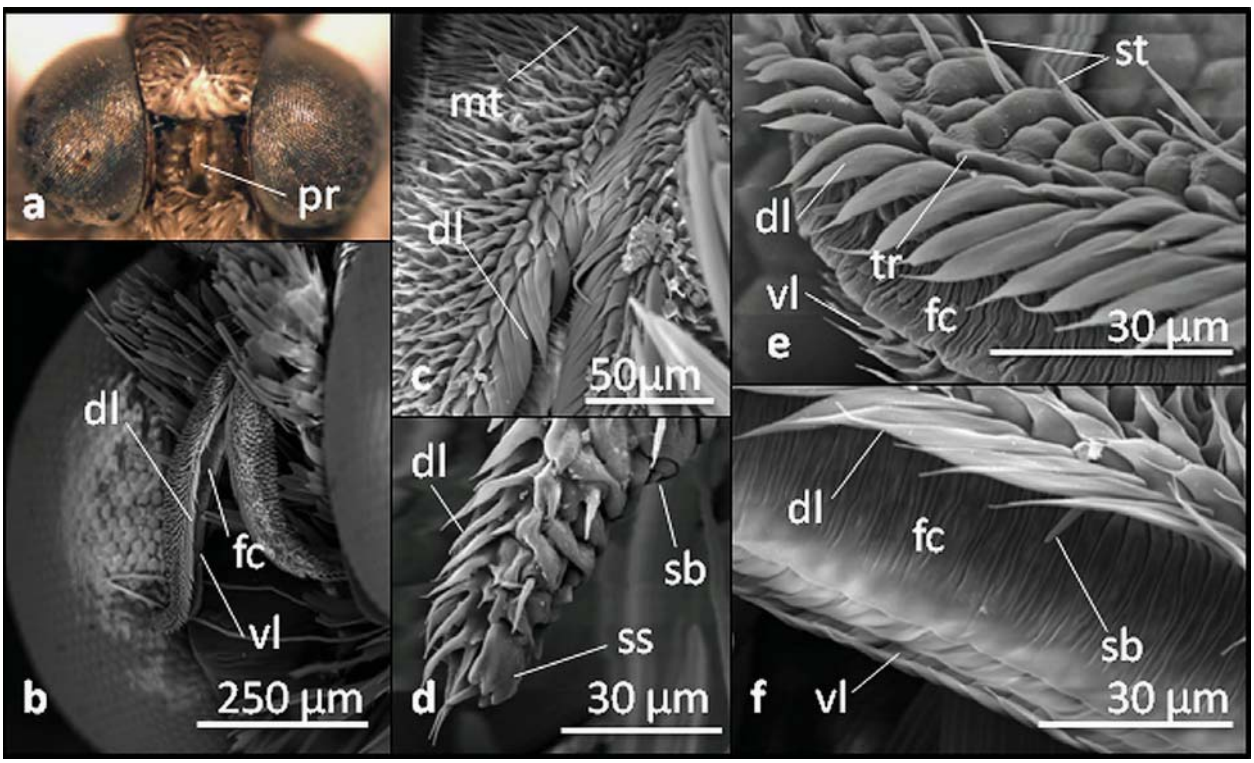

FIG. 2. Reduced proboscises of males of *P. strigataria* (Geometridae). **a.** head of a male of *P. strigataria* displaying the reduced proboscis (pr). b – f SEM photomicrographs of the proboscis of males of *P. strigataria*. **b.** proboscis in its entirety, showing dorsal legulae (dl), ventral legulae (vl), and food canal (fc). **c.** overlapping dorsal legulae and microtrichia (mt) near the base of the proboscis. **d.** tip-region of the proboscis; sensilla basiconica (sb) are at the tip-region and a sensillum styloconicum (ss) is located at tip. **e.** the transition (tr) marking the proximal beginning of the tip-region where two rows of dorsal legulae become a single row, also shown with sensilla trichodea (st). **f.** annulations and sensillum basiconicum in the food canal.

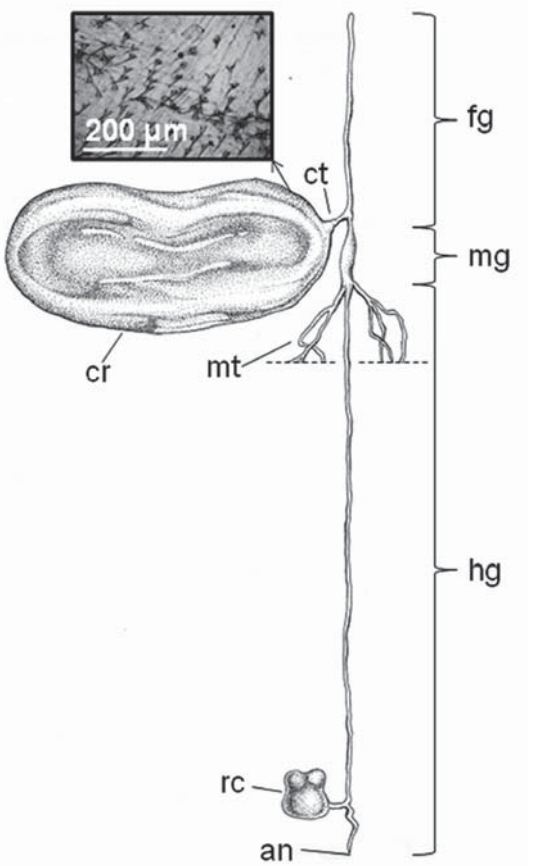

FIG. 3. Gut of a male of *P. strigataria*. The foregut (fg), midgut (md), and hindgut (hg), represent approximately 27%, 9%, and 64% of the entire gut, respectively. The crop (cr) was connected to the foregut by a crop tube (ct). In this illustration, the crop was partially filled with food coloring. The insert from the crop shows a photograph of the cuticular spines on the internal wall of the crop. The Malpighian tubules (mt) (actual length not shown) are at the pyloric juncture of the midgut and hindgut. A rectal caecum arises as an offshoot of the hindgut anterior to the anus (an). of Los Angeles County, science series No. 38.

separated from each base approximately 200 µm from the gut, and the main stalk branched into 2 Malpighian tubules approximately 100 µm distal to the first branch. The longest Malpighian tubule was approximately 3807 µm long (n = 2).

The hindgut was about 8000 µm in length and 50 µm wide (n = 1). Sections of red liquid were interspersed throughout the hindgut, and peristaltic activity moved them toward the rectum. The most noticeable feature of the hindgut was the rectal caecum (Kristensen 2003) (Fig. 3), represented as a red pouch. One male defecated red fluid during dissection.

DISCUSSION

Microtrichia, sensilla trichodea (mechanosensory, Krenn 1998), and sensilla basiconica (chemosensory, Walters et al. 1998) are plesiomorphic in the glossatan Lepidoptera, and are found in the most primitive clades including the Micropterigidae (Krenn & Kristensen 2000; Krenn 2010); *Phigalia strigataria*, in addition to other Lepidoptera with a proboscis considered vestigial or reduced, also retains these structures. Males of *P. strigataria* also possess derived structures, such as a sensillum styloconicum (chemo-mechanosensory, Altner & Altner 1986; Petr & Stewart 2004), implying that the proboscis can use a combination of sensory equipment for feeding. The occurrence of only a single sensilla styloconicum at the tip of the proboscis might be a trait found in other lepidopteran species with a reduced proboscis, but this requires further study. The presence of a tip-region, considered previously the only place where fluid uptake occurs (Krenn, 2010), and chemo- and mechano-sensilla suggest fluid uptake capability. Males of *P. strigataria*, unlike other Lepidoptera, had smaller dorsal legulae in the tip-region than basally. The galeae, however, were malleable and separated easily, suggesting that if *P. strigataria* feeds in the wild, it might rely on a mechanism of fluid uptake other than the drinking-straw model (Kingsolver & Daniel 1995), which requires a tightly sealed tube (Eberhard & Krenn 2005; Borrell & Krenn 2006).

The feeding trials also indicate a proboscis capable of fluid uptake. Food coloring placed on the surface of the proboscis stained the food canal red. Unlike most reports of fluid uptake being restricted to the drinking-slit region of the proboscis (Krenn et al. 2001; Krenn 2010), fluid also entered the proboscis of *P. strigataria* proximal to the tip-region. The noticeable pulsing of the larger drops placed near the base of the proboscis suggests activity of the sucking pump.

The anti-parallel movements of the galea indicate that these movements are not unique to Lepidoptera with proboscises for specialized feeding, as described for blood-feeding and fruit-piercing moths (Bänziger 1970; Krenn 2010), or as a method for initial proboscis alignment following ecdysis (Krenn 1997). In addition to the movements and fluid uptake by the proboscis, the functional gut provides supporting proof that *P. strigataria* can feed. A functional crop with cuticular spines suggests that these moths are not subjected to selection for a reduced crop in a non-feeding species (Kristensen, 2003).

The morphology, feeding trials, and dissections provide evidence that males of *P. strigataria* are capable of fluid uptake. We suggest that short or reduced proboscises employ capillarity as an important step for fluid uptake into the proboscis, before the sucking pump is used to transport the fluid to the gut. This dual-functioning system has been demonstrated with *Danaus*

plexippus L. (Monaenkova et al. 2012), a butterfly with a relatively long, coilable, and tube-like proboscis. Reduced proboscises that lack a straw-like appearance and are not completely sealed, demonstrated here with *P. strigataria*, probably require capillarity when feeding. The dual-functioning system of capillarity and the sucking pump warrants a reevaluation of proboscises previously labeled as vestigial or reduced.

The lack of available nectar sources during the winter, coupled with the short proboscis, suggests that *P. strigataria* might use sources of nutrition other than floral nectar. Winter temperatures rise above freezing in South Carolina during the flight period of *P. strigataria*, which might provide sources of water for uptake. The fluctuating temperatures might provide adequate humidity for dew droplets to precipitate on the proboscis; however, this method of fluid uptake has not been investigated. Droplets of water available on the surfaces of substrates in the habitat also might be acquired by *P. strigataria*. The uptake of water could be important for longevity of this species, as demonstrated with *Parapediasia teterrella* (Pyralidae) (Marshall 1988). We suggest the need for field observations of *P. strigataria* and other moths with proboscises labeled as reduced or vestigial to determine feeding habits that could provide new insights into the biology of these species.

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PALMISTICHUS ELAEISIS (HYMENOPTERA: EULOPHIDAE) PARASITIZING PUPAE OF *CITIOICA ANTHONILIS* (LEPIDOPTERA: SATURNIIDAE) COLLECTED ON *PIPTADENIA GONOACANTHA* (FABACEAE)

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ABSTRACT. The moth *Citioica anthonilis* (Herrich-Schaeffer, [1854]) (Lepidoptera: Saturniidae: Ceratocampinae) occurs in areas of preserved forests, where it is a significant defoliator of *Piptadenia gonoacantha* (Martius) Macbride (Fabaceae) trees. In this study, caterpillars of fourth instar *C. anthonilis* were collected from the ground after falling from a *P. gonoacantha* tree in a herbarium and were reared in the laboratory. Pupae of *C. anthonilis*, the velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), and the flour beetle *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) were each parasitized by mated parasitoid females wasp *Palmistichus elaeisis* Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae). Data were collected relating to the levels of parasitism and emergence rates of *P. elaeisis* per host pupa, and the size of the host pupae. Our results show that the fecundity of *P. elaeisis* was highest in *C. anthonilis* hosts, probably because of the greater size of these pupae, which supported the development of an increased number of parasitoids. Therefore, *C. anthonilis* is a suitable host for rearing *P. elaeisis* in the laboratory, which could be a means of rearing parasitoids for the biological control of this defoliator of *P. gonoacantha* and other pests in Brazil.

Additional key words: biological control, forest insects, host, parasitism, pupal parasitoid

Citioica anthonilis (Herrich-Schaeffer, [1854]) (Lepidoptera: Saturniidae: Ceratocampinae) occurs in Central America, Guyana, Amazonia, the Andean region from Mexico to Bolivia and southern Brazil, in tropical forests with an annual rainfall of between 250 mm and 2500 mm, and is a bioindicator of habitat conservation (Regier et al. 2008, Stefanescu et al. 2009). *Citioica anthonilis* completes its larval development on *Robinia pseudoacacia* L. (Fabaceae) and *Salix caprae* L. (Salicaceae); its larvae are green in color with a black stripe on each side (Prestes et al. 2009). The length of the forewing *C. anthonilis* females collected in Iraí, Rio Grande do Sul State, Brazil (Atlantic Forest biome) was 36–43 mm, in contrast to that of the male, which was 26–31 mm long; the flight period of this insect is January in this region (Prestes et al. 2009).

The parasitoid wasp *Palmistichus elaeisis* Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae) is an important natural enemy of pests on palms (Arecaceae), eucalyptus (Myrtaceae), passion fruit (Passifloraceae) and *Terminalia catappa* L. (Combretaceae) (Gil-Santana & Tavares 2006, Pereira et al. 2011, Tavares et al. 2012a),

attacking the pupae of lepidopteran defoliators (Delvare & LaSalle 1993, Pereira et al. 2010a, Tavares et al. 2012b). Importantly, it can also be reared in the laboratory on alternative lepidopteran and coleopteran host pupae (Zanuncio et al. 2008, Pereira et al. 2009, 2010b), including those of species of the genus *Hylesia* Hübner, [1820] and *Dirphia moderata* Bouvier, 1919 (Lepidoptera: Saturniidae) (Pereira et al. 2008, Soares et al. 2009).

The objective of this study was to assess the suitability of alternatives hosts for rearing *P. elaeisis* in the laboratory. We used pupae of its natural host, *C. anthonilis*, and also of the velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), and the flour beetle *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae). We recorded the rates of parasitism, and emergence of *P. elaeisis* from pupae of these species, as well as the length and width of each host pupa.

Materials and Methods

On May 4, 2011, 25 fourth-instar caterpillars of *C. anthonilis* were collected from the ground after falling from a *Piptadenia gonoacantha* (Martius) Macbride (Fabaceae) tree in the herbarium of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil (20º45'S, 42º51'W, 651 m above sea level; Atlantic Forest biome) (Tavares et al. 2011a). The occurrence of *P. gonoacantha* is widespread throughout the Atlantic Forest biome of Brazil (Marques et al. 2009, Braga et al. 2011). The caterpillars were brought to the Laboratory of Biological Control of Insects (LCBI) from UFV and kept at 25 ± 1ºC, under a 12-h photoperiod and 70 ± 10% relative humidity (RH) in 1L plastic cups with a thin layer of sand in the bottom and *P. gonoacantha* branches until they pupated, which occurred on May 10 and 11, 2011.

In total, 20 newly formed *C. anthonilis* pupae were each placed in a test tube (14 cm length × 2.2 cm diameter) closed with a cotton swab and kept for 10 days with 40 mated *P. elaeisis* females of a first generation emerged from pupa of *Thagona tibialis* Walker, 1855 (Lepidoptera: Lymantriidae). This number of females was used based on results of a preliminary test using 10, 20, 30, 40 or 50 *P. elaeisis* females per *C. anthonilis* pupa. Pupae of *T. tibialis* had been collected from a *T. catappa* tree in the campus of UFV (Tavares et al. 2012a, 2012b). A drop of honey was placed inside the test tubes as food for the parasitoid females. Recordings were made of the rates of parasitism and emergence, as well as the number of parasitoids that emerged per *P. elaeisis* pupa. In addition, the width and length of each *C. anthonilis* pupa were recorded.

To study the parasitism by *P. elaeisis* of *A. gemmatalis* and of *T. molitor*, 20 newly formed pupae of each host species were each placed in a test tube (14 cm length × 2.2 cm diameter) for 48 hours with six mated *P. elaeisis* females, based on the methodology proposed for *A. gemmatalis* (Pereira et al. 2010c) and *T. molitor* (Zanuncio et al. 2008). The pupae were obtained from the mass rearing of these insects from the LCBI of the UFV (Pereira et al. 2010b, Zanuncio et al. 2011). The same data recordings were made as for *C. anthonilis* detailed above.

The parasitism of *C. anthonilis*, *A. gemmatalis* and *T. molitor* pupae by *P. elaeisis* was evaluated according to the following criteria: parasitized pupae turned caramel in color, whereas unviable pupae were black in color, lost weight, became hollow and then died and non-parasitized pupae had emergence of adult (Zanuncio et al. 2008, Pereira et al. 2010c). The design was entirely randomized with three treatments represented by *C. anthonilis*, *A. gemmatalis* and *T. molitor*. Each treatment had 20 replications. Data of the mean ± standard error of mean of parasitism and emergence rates were obtained. Data relating to the number of parasitoids of *P. elaeisis* that emerged per host pupa, and the width and length of the pupae were submitted to Analysis of Variance (ANOVA) and the means (± standard error of mean) compared between species by Tukey test at 5% probability, using the software Statistical Analysis Software (SAS/STAT 1989) (Supplier: UFV).

Adult females of *P. elaeisis* were photographed in the UFV. These pictures were sent to the Department of Biology of Lund University in Sölvegatan, Lund, Sweden for identification by Dr. Christer Hansson. Five pupae of *C. anthonilis* were kept in plastic cups until adult emergence. Four adult females were sent to the Department of Zoology of Federal University of Paraná in Curitiba, Paraná State, Brazil for identification by Dr. Olaf Hermann Hendrik Mielke. Second (Fig. 1), fourth (Fig. 2) and fifth instar (Fig. 3) caterpillars, and adult (Fig. 4) *C. anthonilis* were photographed by Leroy Simon and these pictures are available at http://www.silkmoths.bizland.com/phlsimon.htm.

Results

Pupae of *C. anthonilis* were longer (3.82 ± 0.28 cm) and wider (0.8 ± 0.04 cm) than those of *A. gemmatalis* (1.68 ± 0.19 cm and 0.67 ± 0.02 cm, respectively), which, in turn, were longer and wider than those of *T. molitor* (1.47 ± 0.12 cm and 0.59 ± 0.01 cm, respectively) ($F_{2,57}$; $P<0.05$ in both cases).

In total, *P. elaeisis* parasitized 100% of *C. anthonilis* pupae, 90% of *T. molitor* pupae and 70.0% of *A.*

FIG. 1. Caterpillar of second instar of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

FIG. 3. Caterpillar of fifth instar of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

gemmatalis pupae. The same values applied to the emergence of *P. elaeisis* from the pupae of each species (i.e. parasitoids emerged from each of the pupae that had been parasitized).

More *P. elaeisis* emerged per *C. anthonilis* pupa (286 ± 29 insects) than from *A. gemmatalis* pupae (108 ± 17 insects), totals that were both higher than from *T. molitor* pupae (69 ± 7 insects) ($F_{2,57}$; $P<0.05$).

DISCUSSION

This is the first report of the parasitism of *C. anthonilis* pupae by *P. elaeisis* in the laboratory. The parasitism of *C. anthonilis* by progeny of *P. elaeisis* collected from *T. tibialis* pupa sampled from *T. catappa* trees in Viçosa confirms the ability of a wild strain of *P. elaeisis* to parasitize *C. anthonilis* pupae in the laboratory. However, wild strains of parasitoids might require several generations in the laboratory to develop an adequate parasitic ability on alternative hosts, including *P. elaeisis* collected in the field in Viçosa on pupae of *A. gemmatalis*, *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) and *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) (Pereira et al. 2010b, 2011).

Parasitism of *C. anthonilis* pupae by *P. elaeisis* suggests that this host is suitable for rearing this parasitoid in the laboratory. The occurrence of *C. anthonilis* in preserved areas of native forests in several

FIG. 2. Caterpillar of fourth instar of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

FIG. 4. Adult of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

States and in the Federal District of Brazil and the adaptation of *P. elaeisis* and other hymenopteran parasitoids in urban areas to *T. catappa* and to plantations of crop species from the Arecaceae, Myrtaceae and Passifloraceae suggests the need to maintain areas of original vegetation near agricultural and forest crops to increase natural biological control (Woodcock & Vanbergen 2008, Pickett et al. 2009), particularly by eulophid parasitoids on pupae of arctiid, geometrid, lymantriid, noctuid and saturniid defoliators (Murakami & Hirao 2010). This was shown by fewer outbreaks of lepidopterous pests on *Eucalyptus grandis* Hillex Maiden (Myrtaceae) plantations near areas of native forest due to the refuges for natural enemies (Zanuncio et al. 2001). Natural enemies might be more frequent in mixed environments, as plants of *Crotalaria juncea* L. (Fabaceae) grown near to *Zea mays* L. (Poaceae) act as a refuge for these organisms and so aid the natural biological control of herbivores of this important crop (Tavares et al. 2011b, 2012c, 2012d).

As in our investigation, more individuals of *P. elaeisis* have been recorded to emerge per *C. anthonilis* pupa than from *T. molitor* pupae in other studies [only 71 individuals (Zanuncio et al. 2008)] or from pupae of *Thyrinteina leucoceraea* Rindge, 1961 (Lepidoptera: Geometridae) [only 194 individuals (Pereira et al. 2008)]. This can be explained by the larger size in terms of length and width of *C. anthonilis* pupae compared with those of *T. molitor*, *T. leucoceraea* and *A. gemmatalis*. Pupae of *B. mori*, which are of a similar or larger size to those of *C. anthonilis*, produced 550 individuals of *P. elaeisis*, but rearing this host is difficult in the laboratory (Pereira et al. 2009).

Our study verified that a wild strain of *P. elaeisis* was more successful at parasitizing pupae of *C. anthonilis* than those of *A. gemmatalis* and *T. molitor* in the laboratory. This is the first report of parasitism by *P. elaeisis* of *C. anthonilis* pupae in the laboratory and provides background information for the laboratory rearing of *P. elaeisis* as a means of biological control of *C. anthonilis* and other pests.

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MODIFIED POLLARD TRANSECTS DO NOT PREDICT ESTIMATED DAILY POPULATION SIZE FOR THE SECRETIVE BUTTERFLY, *NEONYMPHA MITCHELLII MITCHELLII* FRENCH.

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ABSTRACT. Over two years, we concurrently assessed two populations of Mitchell's satyr butterfly using mark-release-recapture (MRR) and modified Pollard transects (MPT) in order to calibrate the low intensity MPT method to high intensity quantitative MRR population estimates. We found no correlation between daily MRR population estimates and MPT counts. We attribute this to the sedentary behavior of Mitchell's satyr. We strongly suggest that researchers and managers understand the nature of this relationship before interpreting MPT data and other low intensity monitoring methods if these data are used for population management and recovery programs.

Additional key words: Mark-release-recapture, population monitoring, endangered species

Because of simplicity, population assessments using Pollard transects are in widespread use as monitoring tools for butterfly communities (Caldas and Robbins 2003; Thomas 1983) and imperiled species (e.g., Seidl 1999; Mattoni et al. 2001). Relative to the other more quantitative assessment tool in widespread use for estimating butterfly populations, mark-release-recapture (MRR), Pollard Transects are generally billed as a reliable and easy method for estimating population trends over time. As originally defined, the method involves weekly walks along a transect route making counts of butterflies seen within defined limits. Transects are divided into sections related to habitat or management units. Walks are made only when weather conditions satisfy specified minimum requirements (Pollard 1977). These observation counts are a measure of abundance because they are positively correlated with the abundances of individual species as estimated by mark-recapture studies (Pollard 1979).

Although originally intended as an assessment technique for butterfly communities, it has been widely adopted by those monitoring endangered species. Again, because of the ease of implementation and the relative lack of disturbance to imperiled species from handling (Murphy 1988), Pollard transects have been widely adapted by the butterfly conservation community (e.g., Thomas 1983; Seidl 1999; Mattoni et al. 2001; Gross et al. 2007).

For our work with the federally endangered Mitchell's satyr (*Neonympha mitchellii mitchellii* French), we investigated the use of a modified Pollard transect (MPT) relative to time intensive MRR to determine the feasibility of using a less intrusive population monitoring technique to assess daily population trends and status. By generating simultaneous assessments, we assumed that we could calibrate daily MPT data to reflect quantitative estimates based on MRR. Gross et al. (2007) and Haddad et al. (2008) discuss in detail the issues associated with monitoring populations of the closely related subspecies, St. Francis satyr (*Neonympha mitchellii franscisci* Parshall & Kral), in North Carolina. These authors specifically recommend that MPT estimates be calibrated using MRR. Ten years before their recommendation, we did exactly that to determine the relationships for daily population estimates.

METHODS

We conducted field research at two sites in Berrien County, Michigan, U.S.A., during 1997 and 1998 (Szymanski et al. 2004). Blue Creek Fen and Sarett Nature Center are located within the St. Joseph River drainage approximately 3 km apart. Both sites are complex habitat mosaics best characterized as fen habitats over peat, clay and sandy soils in oak-forested river valleys. Blue Creek Fen is a 10.4 ha linear wetland with distinct vegetation communities. Two occupied habitat patches (1.4 ha and 0.9 ha) were assessed as part of this study, separated by 230m of dense shrub carr habitat. Sarett Nature Center includes a 6.8 ha peatland with suitable habitat limited to two distinct areas (1.4 ha and 0.2 ha) separated by 290m of dense shrub dominated carr and swamp forest.

During the summers of 1997 and 1998 we conducted simultaneous evaluations of population trends at two sites. We used MRR to provide a quantitative estimate of Mitchell's satyr population size, demographics and spatial ecology. Each sampling day two people conducted the MRR for approximately 3 hours at each site walking through all suitable habitat patches. The

details of our methods, analyses and discussion of the results are documented in Szymanski et al. (2004).

Concurrently, we used modified Pollard techniques to provide a less time intensive assessment of population trends for Mitchell's satyr. At each fen, we established set transect routes that passed through the occupied habitats patches. The routes were designed to assess all occupied habitat patches and ecotones in each fen. Occupied habitat is limited at both sites (Szymanski et al. 2004) and transects were approximately 175m long at both sites. The same transect routes were used in 1997 and 1998. Transects were performed daily near mid-day for 30 minutes each under favorable flight conditions. We performed our MPT routes either before daily MRR efforts, or at least two hours post MRR at the sites to minimize the potential impact of MRR efforts on butterfly behavior. We counted only butterflies in a forward direction to minimize the possibility of double counting.

For this paper, we used simple correlation analysis by site and by year for all days with both MRR and MPT population estimates to determine if the there was a relationship between the results generated by these two methods.

RESULTS AND DISCUSSION

There are no discernible correlations between population trends for Mitchell's satyr using MRR and MPT (Figure 1, Table 1). The near randomness of the relationship between these two assessment methods is startling, given that both are widely accepted and used for measuring populations (e.g., Brussard et al. 1974; Southwood 1978; Schultz 1998). We believe that our MRR estimates are a reflection of the actual population levels present in the fens during our efforts and these estimates are comfortably robust (Szymanski et al. 2004). On the other hand, butterflies counted on the daily routes on our set transect routes were consistently low. If our MRR estimates reflect reality (and given our relatively high recapture rates we believe they do) for Mitchell's satyr, daily counts generated by MPTs provide no insight into actual population size.

We believe that the ecology and behavior of Mitchell's satyr explains the disparate results produced by MRR and MPT. Mitchell's satyr is perhaps the most sedentary butterfly known in the central United States (Shuey 1997; Gross et al. 2004; Hamm et al., in press). Both sexes spend a considerable amount of time at rest in sedges near low shrubs. When they do fly, their flight is generally low and hidden by tall sedges. During this two year study period, we found that the total distance moved by individuals averaged approximately 35m – indicative of a very sedentary species (Szymanski et al. 2004). Nectar feeding is rare, and neither sex visits flowers on a regular basis (Hamm et al., in press). The few adults typically encountered are generally flushed

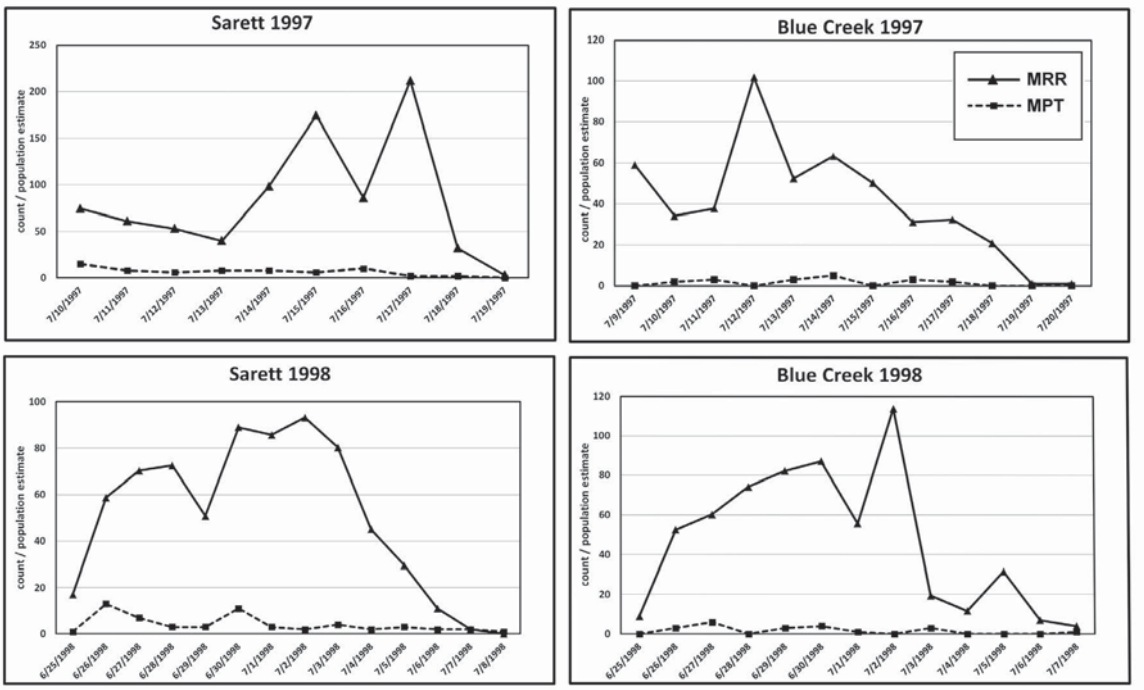

Fig. 1. The daily relationship between MRR population estimates and MPT counts.

out from shaded areas and quickly settle into sedges or shrubs (Shuey 1997). In short, Mitchell's satyr behavior makes it difficult to observe them.

To compensate for this, for our MRR assessment two people canvassed the entire occupied habitat (and adjacent non-habitat edges) for three hours to find, capture, and mark or record as many butterflies as possible. This intense effort was specifically designed to compensate for the sedentary behavior of the butterfly, and to produce the most accurate population estimates possible by flushing as many adults from the vegetation as possible. MPT efforts were considerably less time intensive and were specifically intended to be the counterpoint to time intensive MRR efforts. One person performed the MPT in 30 minutes on a predetermined route and no attempt was made to flush butterflies from habitats away from the transect route. We expected to encounter fewer butterflies during MPT than we

TABLE 1. Population assessments (MRR) and transect counts of Mitchell's satyr at two sites in 1997 and 1998. Correlation analysis indicates that there are no significant trends for any of the data sets. [P is the probability that the two variables are uncorrelated and ranges from 1.0 (no relationship between variables) to values approaching 0.00 (strong non-random relationship between variables). R^2 is the square of the sample correlation coefficient between the outcomes and their predicted values and ranges between 1.0 (perfect correlation between two variable) and 0.0 (no correlation between variables)]

Sarett Nature Center			**Blue Creek Fen**		
Date	Estimated Daily Popuation	Transect Count	Date	Estimated Daily Popuation	Transect Count
			7/9/1997	59.1	0
7/10/1997	74.2	15	7/10/1997	34.2	2
7/11/1997	61.0	8	7/11/1997	37.8	3
7/12/1997	53.1	6	7/12/1997	101.9	0
7/13/1997	39.9	8	7/13/1997	52.5	3
7/14/1997	98.1	8	7/14/1997	63.4	5
7/15/1997	174.4	6	7/15/1997	50.3	0
7/16/1997	85.8	10	7/16/1997	31.3	3
7/17/1997	211.8	2	7/17/1997	32.5	2
7/18/1997	32.0	2	7/18/1997	21.0	0
7/19/1997	3.0	0	7/19/1997	1.0	0
			7/20/1997	1.0	0
P = 0.99			**P = 0.62**		
R^2 = 0.0%			**R^2 = 2.6%**		
6/25/1998	17.1	1	6/25/1998	9.0	0
6/26/1998	58.8	13	6/26/1998	52.5	3
6/27/1998	70.3	7	6/27/1998	60.3	6
6/28/1998	72.5	3	6/28/1998	74.2	0
6/29/1998	50.8	3	6/29/1998	82.2	3
6/30/1998	89.0	11	6/30/1998	87.0	4
7/1/1998	85.7	3	7/1/1998	55.7	1
7/2/1998	93.1	2	7/2/1998	113.8	0
7/3/1998	80.3	4	7/3/1998	19.4	3
7/4/1998	45.0	2	7/4/1998	11.7	0
7/5/1998	29.5	3	7/5/1998	31.5	0
7/6/1998	11.0	2	7/6/1998	7.0	0
7/7/1998	2.0	2	7/7/1998	4.0	1
7/8/1998	0.0	1			
P = 0.11			**P = 0.33**		
R^2 = 20.3%			**R^2 = 8.5%**		

estimated were present. But, in keeping with the literature, we assumed that MPT numbers would be related to MRR estimates, and that our same-day assessments would allow us to calibrate MPT into a tool that provides insight into real Mitchell's satyr populations at the sites.

The two assessment tools produced data that cannot be reconciled for this species at these two sites. Although this has not been previously reported, most researchers have realized that quick habitat assessments do not provide an adequate picture for Mitchell's satyr. In Michigan, more time intensive "meander surveys" are typically used to assess the presence and relative abundance of the species during annual monitoring (Hyde et al. 2001). These meander surveys are specifically intended to flush sedentary butterflies from dense vegetation. Unfortunately, these estimates have not been calibrated to quantitative population estimates either, but because they are standardized relative to the length of observation time, and the observers walk through as much of the occupied habitat as possible to flush butterflies from their perches, intuitively these meander surveys seem much more likely to encounter a higher percentage of resident butterflies.

We urge caution when using Pollard Transects to blindly assess populations and trends of single species. As generally accepted, these assessments generate an index of abundance which is produced for each brood of the species. Pollard (1977) notes that "this index is correlated with abundance, although the precise nature of the relationship will vary from species to species". We caution that the relationship may be weak or non-existent for species such as Mitchell's satyr that have secretive behaviors. We suspect that too often, the exact nature of the relationship between counts generated by MPT and estimated population levels are inadequately explored by people using the assessment tool. Although we did our absolute best to establish transect routes using prevailing best practices as reported in the literature to guide us, our transect counts had little relationship with reality. While Murphy (1988) speculates that the relative densities of butterflies within their habitats can "nearly always be ascertained through simple observation and use of low impact sampling techniques such as that of Pollard (1977)", we believe that this is a gross oversimplification. Managers and researchers must clearly understand the nature of that relationship before interpreting MPT data and other low impact assessment methods (such as meander surveys or distance sampling efforts [Buckland et al. 2001]) for imperiled butterfly species, especially if these data are used for population management and recovery programs.

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GEOGRAPHICAL DISTRIBUTION OF *APEPLOPODA MECRIDA* (DRUCE, 1889) (EREBIDAE: ARCTIINAE: ARCTIINI: EUCHROMIINA) WITH NOTES ABOUT ITS NATURAL HISTORY

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ABSTRACT. An analysis of curatorial information of *Apeplopoda mecrida* (Druce, 1889) (Erebidae: Arctiinae: Arctiini: Euchromiina) from specimens collected between 1889 and 2010 and deposited at several collections is presented. The species is distributed along the heights of 1100 to 2300 m from Arizona (USA) to Punteras (Costa Rica). This makes the species particularly vulnerable to climatic changes. Even though specimens were not found while collecting during the month of March, the species flies throughout the entire year.

Additional key words: Biogeography, wasp moths, México, Guatemala, USA, Costa Rica, biological conservation

Wasp moths belong to the subtribes Ctenuchina and Euchromiina (Erebidae: Arctiinae: Arctiini) (Lafontaine & Fibiger 2006). They fly mainly at night, although there are some species that might fly during the day (Hernández-Baz & Bailey 2006). They are basically Neotropical, reaching their highest diversity in the Amazon forest and the oriental slopes of the South American Andes. Some taxa, however, are distributed in the Neartic and the genus *Euchromia* Hübner is distributed in certain regions of Africa and Asia (Hernández-Baz & Grados 2004; Hernández-Baz & Bailey 2006)

The richness of these moths in the Americas is substantial. Out of 2482 described species, 2446 are Neotropical (Heppner 1991), whereas 36 are Nearctic (Lafontaine & Schmidt 2010). The last comprehensive taxonomic revision of the Euchromiina in the Americas was published almost 100 years ago (Draudt 1915). Revisions for the genera *Macrocneme* Hübner (Dietz 1994), *Horama* Hübner, *Poliopastea* Hampson (Dietz & Duckworth 1976), *Sphecosoma* Butler (Simmons & Weller 2006), *Mallodeta* Butler and *Erruca* Walker (Pinheiro & Duarte 2010) have been done more recently. Mexico is considered a megadiverse country with several hotspots for biodiversity (Myers et al. 2000). However, little is known about the total richness of Lepidoptera in Mexico to date, with the exception of the butterflies in the superfamilies Papilionoidea and Hesperioidea, and certain families of larger-bodied moths such as Sphingidae and Saturniidae.

The Mexican wasp moths (Ctenuchina and Euchromiina) contain 240 known species (Hernández-Baz 1992). One hundred and twenty eigth of them are in 39 genera of Ctenuchina, whereas the remaining 112 species are within the 31 genera of Euchromiina (Hernández-Baz 2008, 2009, 2010, 2011a, b). Not much has been published about the life cycles, ecology, trophic relations, parasites, and geographic distribution of the Euchromiina. As far as we know, the genus *Apeplopoda* (Watson 1980) contains two species found in Mexico: *A. mecrida* (Druce 1889) and *A. ochracea* (Felder 1894) (Hernández-Baz 1992), however nothing has been previously reported about the geographic distribution of the genus. Thus the main aim of this study is to present ecological and geographic information about *Apeplopoda mecrida* (Druce, 1889) in the Americas.

MATERIAL AND METHODS

Our data is derived from four primary sources: a) specimens collected by the first author (FHB) and deposited in the code collection SEMARNAT/CITES/CP-0026-VER/05, Xalapa, Veracruz, Mexico; b) information obtained at two institutional collections:

FIG. 1. Male *Apeplopoda mecrida* (Druce, 1889) specimen deposited at Semarnat / Cites/CP-0026-Ver/05 Collection, Mexico. Picture: F. Hernández-Baz.

ECOSUR's Entomological Collection, San Cristobal de las Casas' Unit (ECO-SC-E); Natural History Museum of Mexico City (MHNCM) and the private colection Lepidoptera Collection (SEMARNAT / CITES/CP-0026-VER/05) in Xalapa, Veracruz. (CPFHB), all from Mexico; c) information published in: Druce (1889), Hampson (1898), Dyar, (1907), Draudt (1915), Hernández-Baz (1992, 2009, 2011a,b) and CONABIO's research projects P-080 (Maza 1998); and d) the database "polilla" with information on Euchromiina collected and reported for the period of time covering the years 1854–2010. This database contains the most complete information on wasp moths from Mexico.

All records (data from bibliograpy and collections) were georeferenced using the Mexican National Institute of Statistics, Geography and Computer science catalogue of names and the 1:250000 topographic map of Mexico 1:250 (INEGI 2012). For USA and Guatemala data, we used information obtained in http//www.googleearth.com. The information taken from the "Polilla" database was converted into sexagesimal data for inclusion in a geographical information system for the Arc view 2.0 program (Esri, 1998).

RESULTS AND DISCUSSION

Localities of examined material. MEXICO: Chiapas: Ángel Albino Corzo, Reserva "El Triunfo", road to Mapastepec, 2180m, 19-xi-2001, A. Molina & Lind (ECO-SC-E); Ángel Albino Corzo, Reserva "El Triunfo", cerro "El Triunfo", 2050m, 18-xi-2001, A. Molina & Lind (ECO-SC-E); Ángel Albino Corzo, Reserva "El Triunfo", cerro "El Triunfo", 2050m, 18-xi-2001, J. León-C, A. Molina (ECO-SC-E). Distrito Federal: Mexico City, Chapultepec, 2302m (Hampson, 1898); Mexico City, Chapultepec, 2302m (Draudt, 1915); Coyoacán City, Ajusco, 2276m (Hernández-Baz 1992); Mexico City, 2302m, 9-x-1929; R. Mueller (MHNCM); Mexico City; Ciudad Universitaria, 2299m, 23-i-1979, R. Turrent (Maza 1998); Mexico City, Pedregal, 2307M, 6-x-1939, R. Turrent (Maza 1998); Mexico City, Pedregal, 2307m, 18-iv-1980, R. Turrent (Maza 1998); Mexico City, Pedregal, 2307M, 2-viii-1979, R. Turrent (Maza 1998); Mexico City, San Ángel, 2300m, 9-vi-1929, R. Mueller (MHNCM); Mexico City, Chapultepec, 2302m (Druce 1889). Durango state: Victoria de Durango, 1891m (Druce 1889); Durango City, 1891m (Hampson 1898); Durango City, 1891m, (Draudt 1915); Durango City, 1891m (Hernández-Baz 1992). Mexico state: Valle de Bravo, 1820m, 25-vii1992, R. Turrent (Maza, 1998); Valle de Bravo, 1820m 9-xii-1988, R. Turrent (Maza 1998); Valle de Bravo, 1853m, 24-vii-1985, R. Turrent (Donahue 1993); Valle de Bravo, 1820m, 21-8-1982, R. Turrent (Maza 1998). Hidalgo state: Tepeji del Río de Ocampo, 2150m, 1-ix-1981, R. Turrent (Maza 1998); Jacala de Ledezma, 1900m, 10-i-1966, L. D. Miller (Maza 1998); Zimapán, 5 miles N, 1780m, 12-i-1966, L.D. Miller (Maza 1998). Michoacán state: Contepec, Contepec, 2936m, 9-ix-1939, R. Mueller (MHNCM); Contepec, 2480m, 9-ix-1929, R. Mueller (MHNCM). Puebla state: Puebla City, 2 km de Cañada Morelos, 2271m, 20-vii-1976, E. Giesbert (Donahue 1993). Veracruz state: Orizaba (Dyar 1907). GUATEMALA: Quezaltenango, Cantel, 2200m, 23-vi-1987, E.C. Welling M. (LACM), Sacatepequez, Antigua Guatemala, Finca el Pilar, 1900m, 20.-ii-2005, ♀, 21-ii-2005, 1 ♀, F. Hernández-Baz, UV light, Light Trap (CPFHB). COSTA RICA: Punteras, Monteverde, 1400m, 22-23-v-1974, E. Giesbert (LACM). USA: Arizona: Cochise County, Douglas, 1220m, 7-x-1945, W.W. Jones, 1♀, (LACM); Cochise County, Douglas, 1220m, 4-v-1986, 1 ♂ UV, Light (LACM).

Distribution. *Apeplopoda mecrida* (Euchromiina) (Figure 1) is a species barely mentioned in the specialized literature. It was described by Druce (1889) based on specimens collected in Mexico City, its type locality. Hampson (1898) also mentions it from Durango State. Dyar (1907) extended its distribution to the Orizaba region, in Veracruz State of Veracruz. Draudt (1915) and Hernández-Baz (1992) corroborate Hampson's localities (1898), but included detailed information about the flying season. Hernández-Baz (2009, 2011b) also increases information on its distribution by including the mountainous regions of the State of México, and other locations in Chiapas State. Hernández-Baz & Bailey (2008) collected specimens in the highest regions of Sacatepequez Department, Guatemala. Donahue (1993) reports it from Cochise, Arizona, USA, but also from Quezaltenango, Guatemala, and Punteras Province, Costa Rica (Figure 2).

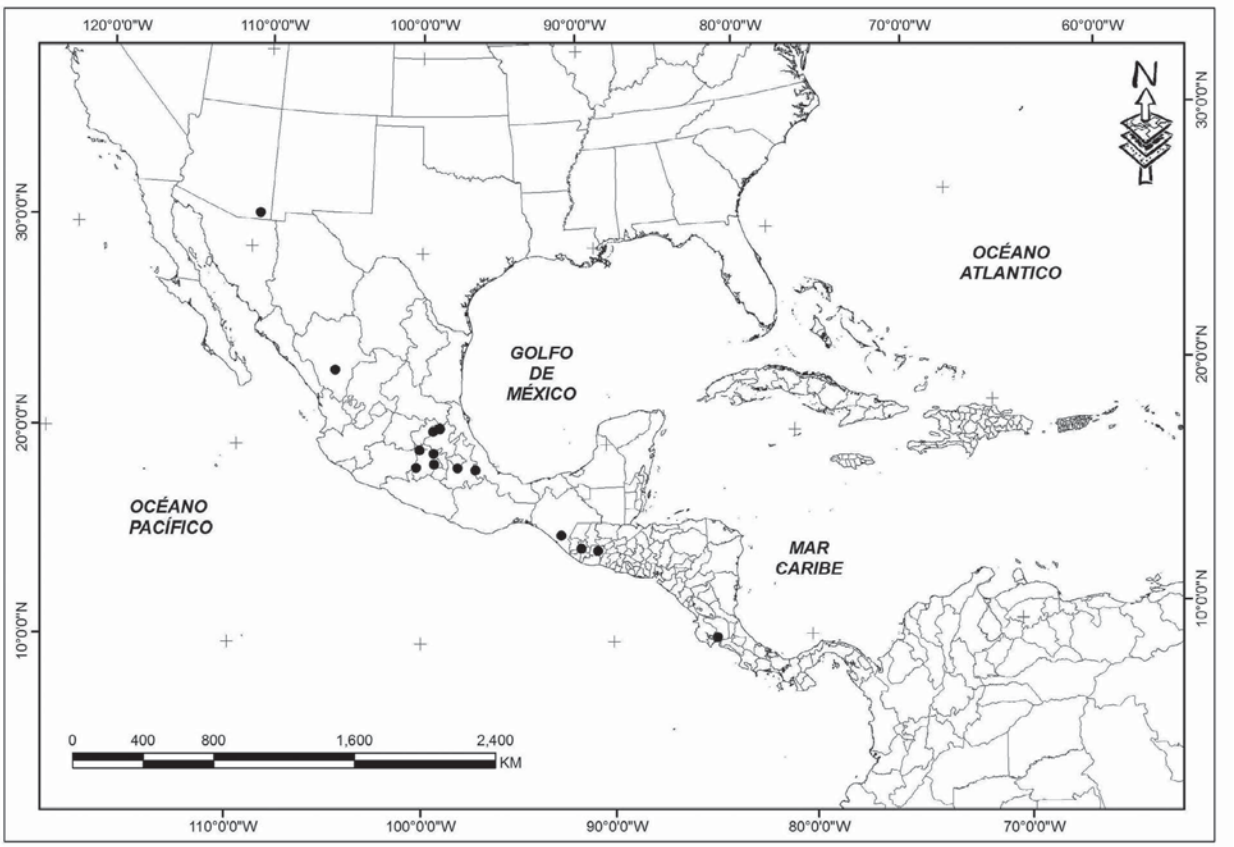
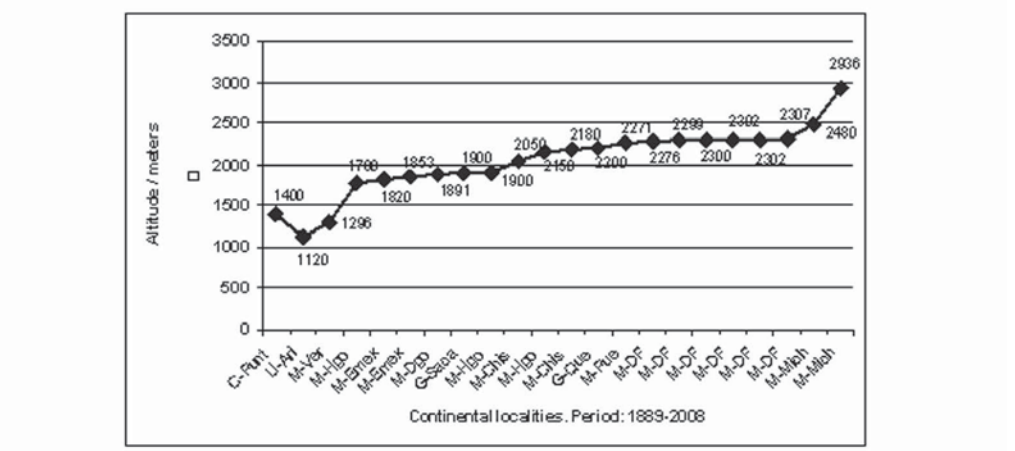


FIG. 2. Distribution of *Apeplopoda mecrida*, in the Americas, from data base "Polilla", of the Lepidoptera Collection: Semarnat / Cites/CP-0026-Ver/05, Mexico.

Thirty-two records from 17 locations were found for the Americas. From Mexico, we found information for eight states: Chiapas, with three records and one location; Distrito Federal: 10 records, four locations; Durango: four records, one location; Mexico State: four records, one location; Hidalgo: three records, three locations; Michoacán: two records, one location; Puebla: one record, one location; Veracruz: one record, one location; Guatemala: two records, two locations. From Costa Rica and the US we found one record and one location in each country.

It appears that the ecological niche of *A. mecrida* resides within the mountainous range of the central region of Mexico. This large expanse generated a total of 27 specimens (or 84.4% of total number of specimens) from 12 locations (70.6%). Some records extend the distribution from Mexico up to Arizona, in the Cochise County area at 1120 m (Donahue 1993). We can also appreciate the presence of the species on the corridor that is occupied by the highlands above 1100m of the western Sierra Madre going south down to Guatemala. Hernández-Baz & Bailey (2006) reported that this species flies along the Sierra Madre through Chiapas, which is an altitudinal corridor connecting the Mexican highlands with Central America (Halffter 1964, 1987). Thus, the species enters Guatemala and moves along the Sierra of the Chucumanes. The Sierra Madre, along the Pacific forms the Meseta Central with two branches: the Sierra Chuacús and the Sierra Merendón, where we can find two collecting localities of the species: Quetzaltenango (2200m) (Donahue 1993) and Sacatepequez (1900m) (Hernández-Baz et al. 2008). On the other side, the Sierra of the Chucumanes becomes the Sierra Chamá, crossing Guatemala through the East and becoming a corridor for wasp moths crossing Central America and entering South America.

We also consulted other important Latin-american insect collections in case there was the possibility of finding specimens collected south of their known distribution. The first of those collections was Museo de Zoología Agrícola, campus Maracay, Universidad Central de Venezuela, in Venezuela; the second were the insect collections of Universidad Nacional de Colombia; one at Instituto de Ciencias Naturales, Facultad de Agronomía, in Bogotá, and the other at Facultad de Agronomía, Medellín. The entomological



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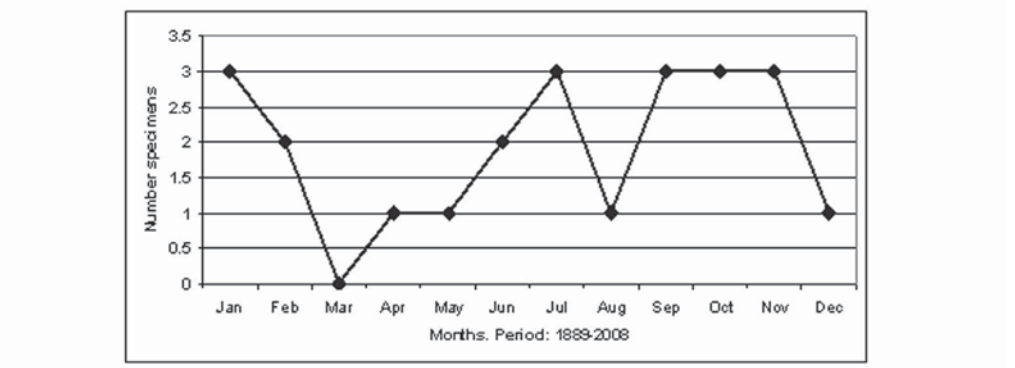

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FIGS. 3–4. (**3**) Altitudinal distribution of *Apeplopoda mecrida* in the Americas. C-Punt (Costa Rica, Punteras); U-Ari (USA, Arizona); M-Ver (México, Veracruz); M-Hgo (Mexico, Hidalgo); M-Emex (Mexico, Mexico State); M-Dgo (México, Durango); G-Sac (Guatemala, Sacatepequez); M-Chis (Mexico, Chiapas); G-Que (Guatemala, Quezaltenango); M-Pue (Mexico, Puebla); M-DF (Mexico, Distrito Federal); M-Mich (Mexico, Michoacán). Period of time: 1889-2008. Source: Data Base "Polilla." (**4**) Flight period of *Apeplopoda mecrida* in the Americas. Period of time: 1889–2008. Source: Data Base "Polilla."

collection of Instituto de Biología, of Universidad de Antioquia, campus Medellín, was also reviewed. We did not find records of *A. mecrida* in any of them. In this study, the distribution ranges of *A. mecrida* were found to be between 1120 and 2936m (Fig. 3), with the highest range from 2200 to 2936m found only in the Mexican mountains.

Biology and Behavior. Not much is known about the biology of *A. mecrida*. Its flight time seems to be highly variable. It was collected at 07:30 hrs in Arizona (USA), but the information provided for specimens collected in Hidalgo, Mexico was obtained from butterfly collectors. Thus, we assumed the moths fly during the day. However, Hernández et al. (2008) reports two specimens in Sacatepequez, Guatemala that were collected using ultraviolet ligth from 22:00 to 00:00 hrs. There are reports of several specimens which were seen at 03:00 hrs (CPFHB). Specimens had been collected with ultraviolet light from 20:30 to 23:30 hrs in the Ocote Reserve in Chiapas (ECO-SC-E). The collected information apparently indicates that the species could fly from 22:00 to 07:00 hrs.

As mentioned above *A. mecrida* flies all year round. The population density of the species is very low during the year with the highest records during the months of January, July and from September to November. These findings seem to indicate that the species is multivoltine (Fig. 4). *Apeplopoda mecrida* is distributed in USA, Mexico and Guatemala in areas where the type of vegetation includes oaks, pines and other conifers; however, in Costa Rica, the species is found within higher elevational cloud forest.

Final remarks. The mountainous ranges of Mexico play a relevant function as a biological corridor for many North and South American species (Halffter 1964, 1987). This passageway seems to be especially important for *A. mecrida* which flies above 1100 m. This indicates that a higher elevation is one of the main components that will favor or limit the distribution of this wasp moth.

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FUNGI FEEDING BY THE AGREEABLE TIGER MOTH (*SPILOSOMA CONGRUA* WALKER) (EREBIDAE: ARCTIINAE)

Additional key words: *Spilosoma congrua*, Agreeable Tiger Moth, mycophagy, fungivory.

Mycophagy in caterpillars from numerous moth families is well known, but for many species, including arctiines, basic information on fungal hosts and feeding behavior is lacking (Rawlins 1984; Wagner et al. 2008; D. Wagner pers. comm.). Although fungi provide nutrients (Jonsell & Norlander 2004; Rawlins 1984), availability of fruiting bodies can be unpredictable, and fungi are often only part of a polyphagous diet (Jonsel & Nordlander 2004).

Many species of caterpillars utilize chemical defenses against predators (Bowers 2009; Hristov & Conner 2005), and parasitoids (Bowers 2009), or use compounds produced *de novo* and/or secondary metabolites sequestered from host plants for reproductive functions (Hristov & Conner 2005; Rawlins 1984; Wagner 2005). Many sequestered compounds have been identified in arctiines (Hristov & Conner 2005; Wagner 2009), including *Spilosoma congrua* caterpillars which are highly effective at sequestering iridoid glycosides (Conner 2009; Hristov & Conner 2005). Other arctiines are known to sequester pyrrolizidine alkaloids (Woolley 2001), azoxyglycosides, lichen phenolics, biogenic amines, iridoid glycosides, pyrrolizidine glycosides and cardiac glycosides (Nishida 2002; Weller et al. 1999; Bowers 2009) A variety of bioactive secondary metabolites are widely produced by fungi (Zjawiony 2004; Mahmood 2010; Kukor & Martin 1987) and although there are few reports that insects sequester fungal toxins (Wicklow 1988), sequestration of fungal secondary compounds from lichens was shown in 24 lichen feeding lithosiines (Hesbacher et al. 1995). The purpose of this note is to document mycophagy in a primarily phytophagous arctiine, *Spilosoma congrua* Walker. Anecdotal and unpublished accounts, coupled with a single-confirmed published report indicate that this caterpillar occasionally feeds on fungi and maybe a useful candidate for exploring fungivory in Lepidoptera.

On 30 July 2010 at 22:15, we observed an Agreeable Tiger Moth caterpillar (*Spilosoma congrua* Walker) feeding on a small bracket fungus on a fallen log in a mature deciduous forest in Community Park, East Brunswick Township, Middlesex County, New Jersey (40.40887° N, 74.44360° W). The caterpillar was observed for approximately five minutes before being collected along with its host fungus. The identification of the caterpillar as *Spilosoma congrua* was confirmed by David Wagner (David Wagner pers. comm.).

The caterpillar we observed was feeding on the bracket polypore fungus *Trichaptum biforme* (Fr. in Kl.) Ryvarden (Polyporaceae). The fungus was also covered by two algal species, possibly a *Chlorococcum* (Meneghini) and another unidentified filamentous species (Dorothy Smullen pers. comm.). Our observations suggest that the caterpillar was feeding on the mushroom as well as the algae. The algal consumption may have been incidental to feeding on the mushroom but algivory has been reported in other Arctiidae (Moskowitz & Westphal 2002; Rawlins 1984; Robinson, et al. 2001; Wagner 2005). A short supplemental video of the feeding is available at http://www.youtube.com/watch?v=uaYkUqQmGG4.

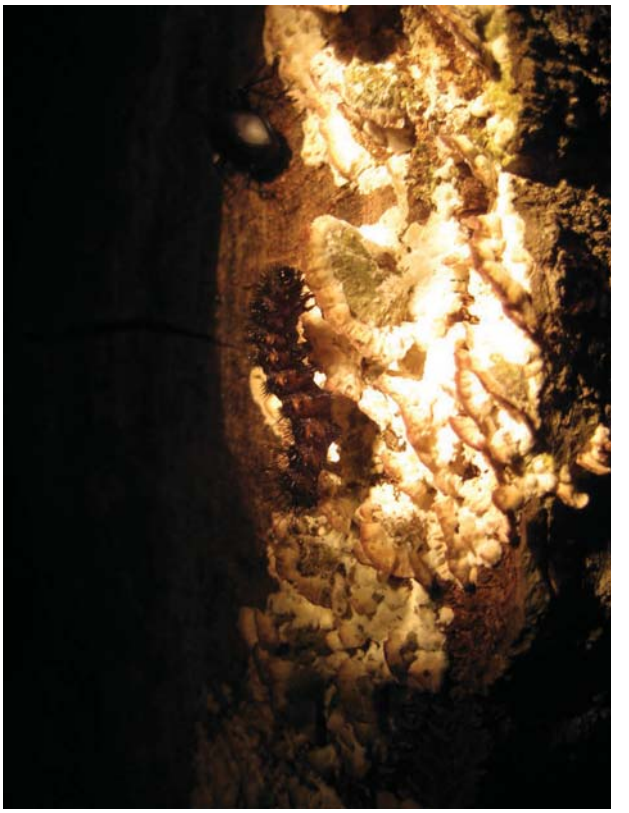

FIG. 1. Agreeable Tiger Moth caterpillar (*Spilosoma congrua* Walker) feeding on the bracket polypore fungus *Trichaptum biforme* (Fr. in Kl.) Ryvarden (Polyporaceae) in Community Park, East Brunswick Township, Middlesex County, New Jersey

While the caterpillar was in captivity before succumbing to an unknown cause, it was fed fresh common dandelion (*Taraxacum officinale* F.H. Wigg.) leaves and white clover (*Trifolium repens* L.) leaves. It readily accepted the dandelion but not the clover.

Spilosoma congrua is typically described as phytophagous (Covell 1984; Robinson, et al. 2001; Wagner 2005). There are also a few reports of the caterpillars feeding on mushrooms. We are aware of three published reports that note the caterpillar feeding on the mushroom *Agaricus campestris* L. (Tietz 1972; Handfield 1999; Robinson 2001). It is likely that these three accounts all originated from the same fungal host report noted in Thomas (1939) as originally reported by Beutenmüller (1890) and are also the source of many other general references to this species feeding on mushrooms. Unpublished reports, supported by photographs, also note feeding by *Spilosoma congrua* caterpillars on other mushrooms including *Fomitopsis spraguei* (Berk. & M.A. Curtis) (Fomitopsidaceae) (Woods and Woods 2008) and possibly on *Boletes* sp. (E. M. Fries) (Ramos 2006) (Boletaceae). Sargent (pers. comm.) also observed an unconfirmed *S. congrua* feeding on *Boletus bicolor* Peck. This caterpillar was also offered a *Russula* sp. (C.H. Persoon) (Russulaceae) but feeding on this host was sparse and inconclusive. A few other references note feeding by *S. congrua* on mushrooms but are more general without identification of the species; "feeding in mushrooms" (Beutenmüller 1890), "larva also bores in mushroom stems" (Zhang 1994) and "Attacks mushrooms but is rare" (Thomas 1939).

The paucity of reports of *Spilosoma congrua* feeding on fungi over the past century suggest that fungivory in this species must be only part of a broader generalist diet. It is possible that *Spilosoma congrua* utilizes fungi, when available, as a source of defensive chemicals and/or for reproductive purposes. Despite the limited number of accounts of fungivory in *Spilosoma congrua*, feeding trials with various fungi may be informative and help to understand the ability of the caterpillars to utilize these abundant food sources.

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FORCIPOMYIA (*MICROHELEA*) *ERIOPHORA* (WILLISTON) (DIPTERA: CERATOPOGONIDAE) AN ECTOPARASITE OF LARVAL *ANAEA TROGLODYTA FLORIDALIS* (NYMPHALIDAE)

Additional key words: Florida Keys, Florida leafwing, threatened species.

The Florida leafwing, *Anaea troglodyta floridalis* F. Johnson and Comstock (Nymphalidae), occurs locally within the pine rocklands of southern Florida and the lower Florida Keys (Minno & Emmel 1993; Smith et. al 1994). Hennessey and Habeck (1991) and Worth et al. (1996) described many aspects of *A. t. floridalis* natural history. Salvato and Hennessey (2003) and Salvato and Salvato (2010) also discussed *A. t. floridalis* ecology and provided a review of known parasites and predators for the species.

On 11 December 2010 MHS and HLS observed a female biting midge (Diptera: Ceratopogonidae) attached to the cuticle of a late instar *A. t. floridalis* larva in the Long Pine Key region of the Everglades National Park (Miami-Dade County, Florida). The midge was collected at approximately 0930 h, shortly after the initial observation. The larva, which was first encountered on 27 November 2010, remained in the field for additional monitoring.

The midge was preserved in 100% ethanol and sent to WLG who cleared it in phenol-alcohol, dissected and mounted it onto a microscope slide in Canada balsam and identified it as *Forcipomyia* (*Microhelea*) *eriophora* (Williston). *Forcipomyia* (*M.*) *eriophora* is an ectoparasite known to occur in Maryland and Florida, the West Indies and Mexico, Central America south to Bahia, Brazil (Wirth 1972; Wilkening et al. 1985; Hribar & Grogan 2005; Borkent & Spinelli 2000, 2007; Borkent & Grogan 2009; Grogan et al. 2010). This observation represents the first known report of *F.* (*M.*) *eriophora* parasitism on *A. t. floridalis*. Larvae of *Manduca sexta jamaicensis* Butler (Sphingidae) (Wirth 1972), *Corula geometroides* Walker (Geometridae) (Wirth 1956), and more recently *Papilio demoleus* L. (Papilionidae) (WLG, pers. obs.) have also been previously identified as hosts of *F.* (*M.*) *eriophora*. The slide-mounted *F.* (*M.*) *eriophora* will be deposited in the South Florida Collection Management Center at Everglades National Park.

On 17 December 2010 the host *A. t. floridalis* larva was encountered moribund possibly as a result of unseasonably cold temperatures within the Everglades over the preceding days. Therefore it could not be determined if *F.* (*M.*) *eriophora* parasitism influenced *A. t. floridalis* larval development. Salvato et al. (2008) reported that an early instar *A. t. floridalis* died following parasitism from *F.* (*M.*) *fuliginosa* (Meigen), perhaps the result of the midge vectoring microbes during feeding (Wirth 1972). Conversely, MHS and HLS (unpublished data) have reared late instar *A. t. floridalis* larvae (n = 2) to the adult stage following instances of *F.* (*M.*) *fuliginosa* parasitism. Similar instances of sub-lethal *Forcipomyia* ectoparasitism on lepidopteran larvae have been noted by Sevastopulo (1973) and Young (1983).

The distribution of *F.* (*M.*) *eriophora* in Florida and other southeastern states, as well as the role of this ectoparasitic biting midge on the natural history of *A. t. floridalis* and other Lepidoptera requires further examination.

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MOMPHA EPILOBIELLA (MOMPHIDAE), A EUROPEAN MOTH IN THE PACIFIC NORTHWEST, WITH NOTES ON ASSOCIATED PARASITOIDS

Additional key words: introduced species, biological control agent, noxious weeds.

Momphidae is a primarily Holarctic gelechioid family, comprising 60 described species in six genera (Koster & Sinev 2003; Pohl et al. 2010). About 40 named species are known from the United States and Canada, although there are potentially many species yet to be described (Powell & Opler 2009). The North American fauna is represented predominantly by the genus *Mompha*, unique among lepidopteran genera for specializing on the family Onagraceae.

Adult *Mompha* are small moths, with forewing lengths varying from ~2.5 to 8 mm. Larvae are typically leaf miners or stem miners, while a few species induce galls in plant stems and tips (Koster & Sinev 2003). *Oenothera* and *Epilobium* are the most common host genera in western North America (Powell & Opler 2009). Host damage varies, from little or no obvious damage to significant depredation that can reduce host plant fecundity (Doak 1992; DeWalt 2006). The host specificity displayed by some *Mompha* species makes them potentially valuable biological control agents for weeds (Bradley et al. 1973; Winder 2002; Culliney et al. 2003).

Mompha epilobiella ([Denis & Schiffermüller] 1775) is common throughout Europe, parts of Asia Minor, and low-lying regions of Central Asia (Koster & Sinev 2003). The species is typically associated with marshes and wetlands in Europe, as is its primary host plant, *Epilobium hirsutum* L. Other infrequent hosts include *E. montanum* L., *E. palustre* L., *Chamaenerion angustifolium* (L.), and *Oenothera* sp. (Koster & Sinev 2003). Ivinskis (1982) records larvae of *M. epilobiella* from *Lythrum salicaria* L. (purple loosestrife) and *Eupatorium cannibinum* L. in the 1970s but provides no evidence of successful rearing. Early instars mine leaves and bore into flower buds, whereas later-stage larvae feed in clumps of webbed apical leaves (Koster & Sinev 2003). Mature larvae pupate between leaves, and the species overwinters in the adult stage (Koster & Sinev 2003).

Epilobium hirsutum is a Eurasian introduction to North America and a widespread weed of natural and man-made wetlands in the northeastern United States and adjacent Canada, as well as Oregon, Washington, and British Columbia in the Pacific Northwest. The plant is commonly associated with ballast sites and was sometimes introduced inadvertently via that pathway; in some of the earliest records from the eastern seaboard it was recorded sporadically in "waste sites" (Trelease 1891). Its spread was likely aided by its status as an ornamental plant (Stuckey 1970). At one time the species was sold in nurseries, and some Washington gardeners considered it an attractive replacement for purple loosestrife (L. Baldwin pers. comm.). The plant has been known from Washington State since the 1930s, with the earliest detections made in Klickitat County (WTU 2011).

To date, *E. hirsutum* has been found in eleven counties in Washington. Franklin, Island, Klickitat, and Whatcom have infestations of up to 40 hectares, while the remaining counties all report infestations under four hectares (WSDOE 2010). The plant is categorized as a class B noxious weed in Washington State, and its sale and transport are prohibited (WAC 16-752-505). In Whatcom County in particular, *E. hirsutum* has become established in several wetlands, often co-occurring with purple loosestrife, another class B noxious weed. When established, *E. hirsutum* can form large, nearly monotypic stands, often replacing more diverse assemblages of native species.

Mompha epilobiella was collected relatively recently in New York and Quebec (Sinev 1996). We report here the first records of *M. epilobiella* from the western

Fig. 1. *Epilobium hirsutum* infestation (pink-tinged vegetation) near Crockett Lake, Whatcom County, WA (photo by S. Horton).

TABLE 1. *Mompha epilobiella* specimens, parasitoids, and damage localities, 2005–2007.

Locality	Collection Date	Method	Species					
			Mompha epilobiella		*Elasmus setosiscutellatus*		*Temelucha* sp.	
			F	M	F	M	F	M
Island Co. WA N48.169942 W122.637361	23 May 2005	hand collected / reared	0	1	0	0	0	0
Whatcom Co. WA N48.773547 W122.437183	22 Aug. 2007 emerged 1-10 Sept. 2007	hand collected / reared	38	26	25	3	3	0
Klickitat Co. WA N45.70404 W121.43746	August, 2007	damage only	-	-	-			
Franklin Co. WA N46.4459 W119.2331	26 Sept. 2010	*Lobesia botrana* pheromone trap	0	1	0	0	0	0
Franklin Co. WA N46.327881 W119.120119	August, 2010	damage only	-	-	-			

United States, from four counties in Washington State, all associated with its host plant, *E. hirsutum*. The first specimens of *M. epilobiella* from Washington were collected in Island County in 2005. On 23 May 2005, JA collected samples of non-flowering *E. hirsutum* along the side of a highway adjacent to Crockett Lake in Island County, WA. The *E. hirsutum* infestation at this locality is approximately 20 hectares, and spreads along both sides of road throughout a wetland area (Fig. 1). Plant samples were collected to document the weed's occurrence in the county, and the presence of the insects was neither noticed nor expected at the time. JA later discovered larvae in apical stems while pressing the Crockett Lake samples, and removed and placed both a larva and plant material at room temperature for rearing. The larva pupated on 30 May 2005, and an adult moth emerged around 6 June 2005.

In June 2006, JA discovered pupae and late instar larvae in an *E. hirsutum* patch in Bellingham, WA (Whatcom County). The patch was less than 0.5 hectares in extent and was located in a small drainage ditch along a walking/bike trail in a semi-urban area. The site has likely been subject to herbicide sprays in the past for control of *E. hirsutum* and other weeds. Plants were between 1.5 and 2 meters tall and were in full flower and seed set. We returned to this location in August 2007 and collected plant material with *M. epilobiella* larvae and pupae for lab rearing and identification. The majority of the plant damage was concentrated in auxiliary shoots; although the damage was noticeable, there were no obvious impacts on individual plant vigor. In August 2007, JA noted damage to *E. hirsutum* consistent with *M. epilobiella* feeding in an infestation east of Bingen, Klickitat County, WA. There are several *E. hirsutum* infestations, generally less than a half hectare each, along a state highway in this area. We collected samples from plants between 1-1.8 m tall. Most damage was restricted to auxiliary shoots and was readily visible. One *M. epilobiella* specimen was recovered as a non-target catch in a *Lobesia botrana* moth pheromone trap from Franklin County, WA, in 2010, and damage to *E. hirsutum* consistent with *M. epilobiella* feeding was observed in Pasco, WA in August 2010. Locality data for adult *M. epilobiella* specimens and damage are provided in Table 1.

In addition to *M. epilobiella*, we reared two species of parasitoid wasps, all from the Whatcom County site. Three female *Temelucha* sp. (Hymenoptera: Ichneumonidae: Cremastinae) were reared from three *M. epilobiella* pupae. *Temelucha* species typically parasitize leaf-mining and stem-boring Hymenoptera and other insect orders (Townes 1971). Twenty-eight specimens (3 male, 25 female) of *Elasmus setosiscutellatus* Crawford (Hymenoptera: Eulophidae) were reared from the *M. epilobiella* pupae (Table 1). *Elasmus* species are primary parasitoids of several species of Lepidoptera, and less commonly, ichneumonids and braconids parasitizing Lepidoptera (Gibson et al. 1997). *Elasmus setosiscutellatus* is a

widely distributed species in the US (Burks 1979), known from habitats as diverse as marshes in Florida (McCoy & Rey 1987) to pine plantations in Kansas (McKnight 1973). *Elasmus* specimens were identified using Burks (1965). All moth and parasitoids specimens are retained in the Washington State Department of Agriculture Collection, in Olympia, WA.

Based on reared specimens, visible signs of damage, and the non-target catch in eastern Washington, *M. epilobiella* appears to be widely distributed among Washington *E. hirsutum* infestations. It is unclear how long the moth has been in Washington State. We have not located any *M. epilobiella* specimens in museum collections at Washington State University or other regional collections. *Mompha epilobiella* was only recently recorded from the eastern United States (Sinev 1996), although this group of moths is often poorly collected and prone to being ignored in collections. The close proximity of our *M. epilobiella* detections to British Columbia and Oregon suggests that it could be even more widespread than our data show. The concealed habit and small size of this moth suggest that it could initially have been transported with its host plant, with subsequent spread in the region through natural dispersal. Generally, *E. hirsutum* has not been as high a priority for weed control as many other species in Washington and has historically escaped vigorous treatment, which may have facilitated the establishment and spread of *M. epilobiella*.

The introduction of this moth could potentially impact any of the 14 native *Epilobium* species, eight of which are in the same section of the genus as *E. hirsutum* (Wagner & Hoch 2005). Most species are widespread and of no conservation concern. A potential exception is *E. pygmaeum*, an uncommon species associated with vernal pools and under review for potential conservation action in Washington State. We have not observed *M. epilobiella* feeding on native *Epilobium* species in limited survey efforts to date. The host record for *Oenothera* sp. (Koster & Sinev 2003) is

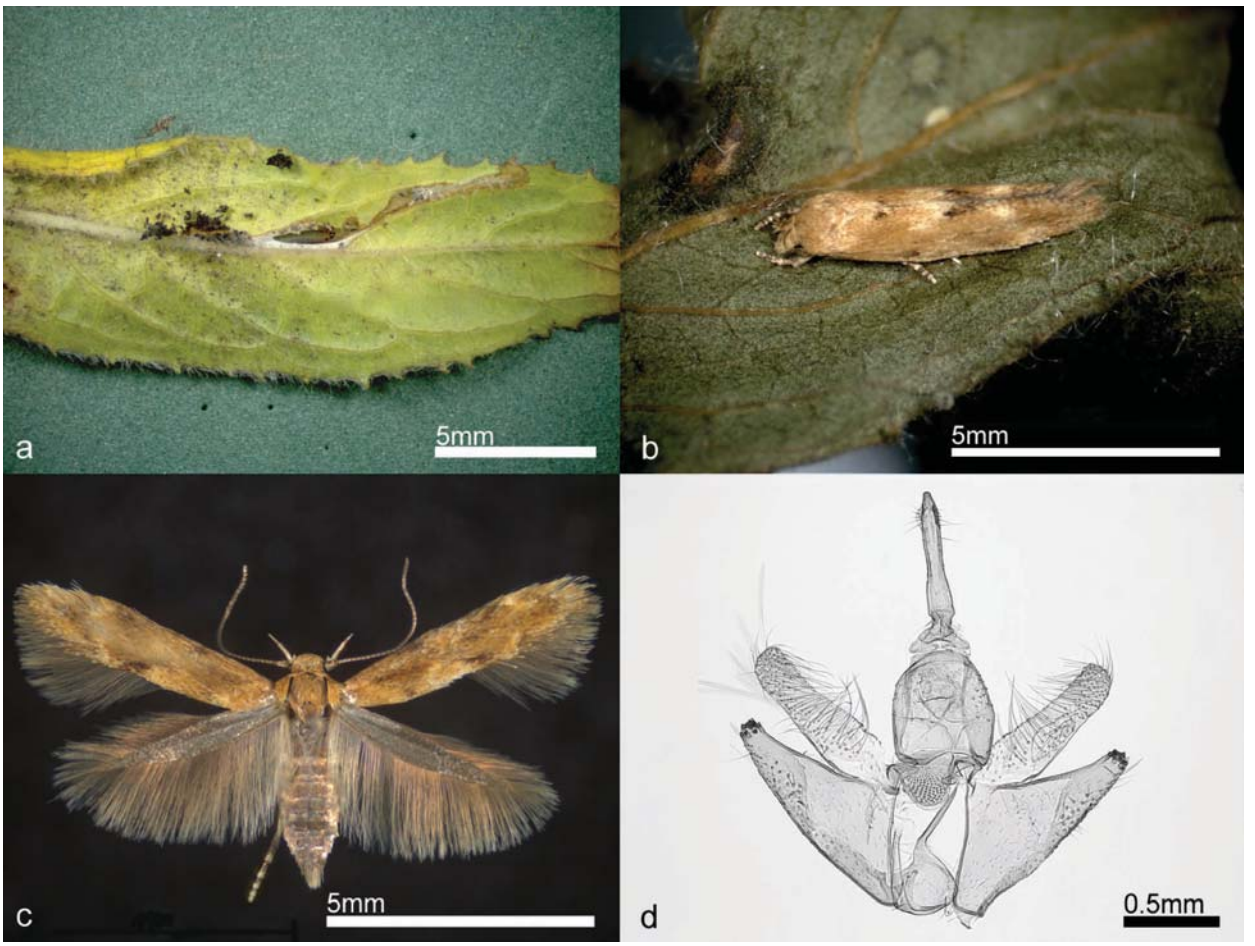

FIG. 2. *Mompha epilobiella.* **a,** larva in mine; **b,** adult in repose; **c,** adult, spread; **d,** male genitalia (aedeagus removed).

potentially alarming; Washington State has two State Threatened and one State Sensitive *Oenothera* species that occur near some of the *M. epilobiella* detections. While *Oenothera* is not a preferred host, land managers and researchers should be aware of potential impacts to these species.

Some regional field biologists have recently noted populations of *E. hirsutum* that appear to be in poor health and have reduced density, despite lack of active weed control (L. Baldwin pers. comm.). This suggests that *M. epilobiella* could be affecting plant vigor, either alone or synergistically with plant competition or other disease agents. For example, JA observed *E. hirsutum* populations in Klickitat County that were also attacked by a rust fungus (*Pucciniastrum* sp.) that appeared to be the primary factor in reducing plant health. The impact of *M. epilobiella* on *E. hirsutum* may be worth investigating, particularly given the scientific and institutional challenges of screening and importing novel biological control agents (Delfosse 2005; Messing & Wright 2006; Scoles et al. 2008).

Adventive insects such as *M. epilobiella* could be a component of biocontrol programs and noxious weed control in the future. Although host-specificity testing would still be needed prior to interstate transport, the "real-world" open-field testing opportunities afforded to researchers by already naturalized populations might provide novel and valuable information about a control agent's environmental safety. The results of open-field experiments and broad surveys for nontarget attack could provide more nuanced assessment of potential biocontrol agents than could be achieved with controlled pre-release trials alone. There is some precedent for this—after accidental introduction and establishment in eastern North America, the seed-feeding bruchid *Bruchidius villosus* (F.) underwent host-specificity testing and was ultimately approved and released as a biocontrol agent for *Cytisus scoparius* (L.) in the Pacific Northwest (Coombs et al. 2008).

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AN ALTERNATE METHOD FOR COLLECTING NOCTURNAL INSECTS IN TREE-LESS HABITATS

Additional key words: black light, prairie

Black light collecting sheet set ups are considered a standard technique for collecting nocturnal insects and are used by researchers and amateur collectors alike. Many guides suggest using a 15 watt ultraviolet light with a sheet for insects to rest upon (Covell 2005, Powell & Opler 2009). In forested habitats, sheets can be easily be hung by tying a rope between two trees, draping the sheet over the rope, and securing it with clothespins. However, this technique for hanging a sheet is not feasible when collecting in habitats without tall, sturdy vegetation upon which to tie a sheet using the rope and clothespin method. Commercial pop-up sheets are available for collecting in flat areas, but they are expensive and can deplete limited funds unnecessarily. I have devised a new method for constructing a low-cost frame on which to hang a twin sheet; the frame can be constructed with materials from any local hardware store for less than $30.

The frame consists of a total of 9.1 m of 1.9 cm (¾ inch) polyvinyl chloride (PVC) pipe (measurement also given in English units in parentheses as this is how PVC pipe is labeled for commercial sale in the United States). The PVC pipe needs to be cut into twelve pieces and trimmed to the measurements given in Table 1. I have determined that a chop saw is the easiest method for cutting the PVC pipe, but a hand saw or PVC pipe cutter will work as well. Additional materials that are necessary are: one straight PVC pipe connector, two 90-degree PVC pipe connectors, six 'T' shape PVC pipe connectors, and a fitted sheet for a twin bed. Tent stakes may also be purchased to anchor the frame to the ground if collection takes place in a windy environment.

To assemble the frame, connect the A and B PVC pipe pieces to form the façade of the frame (Fig. 1). The C and D PVC pipe pieces form the rear support for the façade and attach to the top of the frame at a ~25° angle to the plane of the façade (Fig. 2). After assembling the frame, a fitted twin sheet can be slipped over the frame and the tent stakes can be used to anchor pieces B4, D1, and D2 to the ground. The black light battery can also be used to weigh the frame down by resting it on piece B4. When ready to begin collecting, simply drape the black light over the top of the frame (Fig. 3).

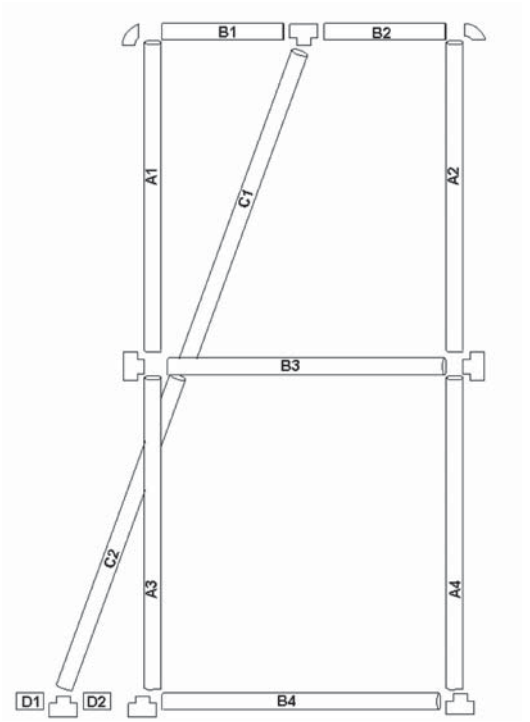

FIG. 1. Frame Assembly from front view.

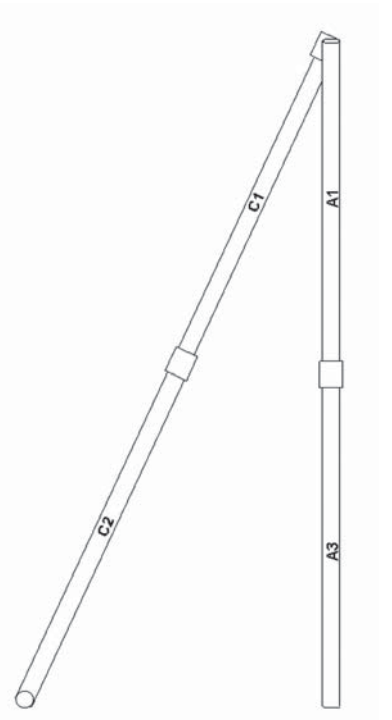

FIG. 2. Frame Assembly from side view.

TABLE 1. Lengths of PVC pipe necessary to make a frame that will be sized to fit a fitted twin sheet.

Poles	Length (cm)
A1, A2, A3, A4	89
B1, B2	49
B3, B4	104
C1, C2	111
D1, D2	13

FIG. 3. The author using one of the frame assemblies to collect Lepidoptera in Colorado.

There are several advantages to this frame over the commercial versions that are available. First, it is made entirely of pieces that can be found in any local hardware store. Second, the entire cost of the frame is less than $30 and thus is an affordable option for collectors of more limited means (e.g. graduate students and amateur collectors). Finally, the frame, as I have designed it, is light and weighs about four pounds. Thus, when disassembled, the entire frame can be carried in a large mesh laundry sack and is easy to transport over rough terrain and long distances.

I would like to thank Jenny McCarty and Andrew Haertzen for their help in constructing the prototype versions of this frame. I would also like to thank Shannon Murphy and two anonymous reviewers for comments on previous versions of the manuscript.

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BASIC PATTERN OF LEPIDOPTERA DIVERSITY IN SOUTHWESTERN AFRICA by Wolfram Mey. 316 pages, ca. 270 line drawings, 25 black and white plates of genitalia photographs, 15 color plates of adult moths. Esperiana, Buchreihe zur Entomologie, Memoir 6, series editor Hermann H. Hacker, ISBN 3-938249-02-4. Hardbound. Copies of Esperiana can be ordered from the homepage at www.esperiana.net. 125 Euro (~ $164). Publication date: December 2011.

The Ethiopian Region has seldom received the attention it deserves in most areas of Lepidoptera, particularly in the more primitive, usually smaller species generally referred to as Microlepidoptera. This lack of attention, of course, is largely due to the paucity of researchers focused on this enormous region. The pioneering works by A. J. T. Janse over many years on the South African Lepidoptera and the more recent Catalogue of the Lepidoptera of southern Africa (Vari, et al 2002) have provided valuable introductions to this fauna. The latest effort to increase our knowledge of the Ethiopian Region was initiated by Wolfram Mey of the Museum für Naturkunde, Humboldt Universität, Berlin, Germany, and several collaborators with a multiyear entomological survey of one of the most poorly known areas of southwestern Africa, a largely arid and semiarid region between Angola and Capetown. The high endemism of Lepidoptera in this area reflects the unique vegetation present in the Desert, Nama Karoo, Succulent Karoo, Fynbos, and dry Savanna biomes. Prior to this survey, Mey and his colleagues had focused on the Lepidoptera of the Brandberg Massif in Namibia (Mey 2004, 2007, Davis 2008).

For several years a major biological survey of southwestern Africa, involving cooperating institutions from Germany, Namibia, and South Africa, has been the focus of the German BIOTA [an acronym for Biological Transect Analysis] of South Africa Project. The primary purpose of the project was to investigate the relationship between different land use systems and biodiversity. With the publication of a three volume monograph, "Biodiversity in southern Africa", the initial phase of the project was completed. However, the taxonomic results from this major survey were excluded from the series. Consequently, efforts are now being conducted to complete this portion of the study, with the present volume representing a first step toward this goal.

Memoir 6 on the basic patterns of Lepidoptera diversity is divided into eight sections, including a brief introduction and a final section of references. Section 2 on Materials and Methods summarizes the 109 collecting sites (Table B), accompanied by a map showing these locations extending along the northern border of Namibia south to Capetown. Section 3 on the Study Area provides climatic and topographic details of the area as well for the entire African continent. Section 4 treats the Lepidoptera diversity from the light trap samples representing 36 localities included in Table B of Section 2. Numerous tables are included which list the species by family for each locality as well as a list of the most common species encountered. Enumeration of the tables in this section is according to the locality number listed in Table B from Section 2. Each of the 36 localities is typified by 1-2 unlabeled color photographs. Although butterflies were not one of the target groups of this survey over the last few years, Section 5 includes a summation of the faunistic data for about 100 species of butterflies gathered from irregular collecting efforts over southwestern Africa. Tables are provided which lists the species and their collection data. Section 6 discusses the basic pattern of Lepidoptera diversity in southwestern Africa. Tables are also included here to help summarize species diversity according to annual rainfall and/or the five biomes surveyed. Attention is also given to relative host specificity according to plant biomes. Finally, Section 7 discusses the "New and little known species of Lepidoptera of southwestern Africa". The great majority of those species described within the 28 families treated represent new taxa (118) of mostly Microlepidoptera. One of the most interesting discoveries reported was that of the first African record for the small, homoneurous family Acanthopteroctetidae, a primitive family previously known only from North America, Peru, and the Crimea. Approximately 270 line drawings, 25 half tone plates of genitalia illustrations, and 15 colored plates of adults supplement the species descriptions in this section.

From his thorough review of current and previous work in southwest Africa, the author of this volume has established an excellent foundation for future reports on the Lepidoptera fauna of this interesting region. We look forward to seeing the continuation of this series and the new discoveries resulting from these surveys.

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MARIPOSAS DIURNAS DE LA COMUNITAT VALENCIANA by Sergio Montagud Alario and José Antonio García Alamá. Publisher: Generalitat Valenciana, Spain, 471 pp; numerous color illustrations and distribution maps throughout, plus 40 composite color plates of adults, and 9 of larvae. Publication 2010; Soft cover ISBN 9788448252557; Price 20 Euros (approx. $25 US). Available from the author: Sergio Montagud Alario, C/ Mar 43, 1º–4ª, 46003, Valencia · SPAIN. E-mail: sergio.montagud@uv.es

The Region of Valencia, Spain embraces 23,259km^2 of the Iberian Peninsula where elevations rise from sea level on the coast, to over 1,800 m on some inland mountains. The area of Valencia contains a diverse patchwork of Mediterranean habitats and microclimates that support a surprising diversity of butterflies. One needs to consider that the butterfly fauna of Valenciana embraces over 70% of all butterfly species known from the entire Iberian Peninsula. By employing scholarship, enthusiastic field and museum experience, and digital technology the authors of *Mariposas Diurnas de la Comunitat Valenciana* provide us with a richly detailed and beautifully illustrated treatment of the 159 butterfly species. Nearly all pages in the book have color photographs that illuminate butterfly identification, natural history, ecology, habitat distribution, and concerns about habitat conservation.

The book is composed of five major sections. Section 1 introduces the reader to the motivation behind the book, advice on how to use the book, its geographical context, and a section on butterfly morphology useful for identifying them, and how the Natural History Museum of Valencia and numerous collaborators supported the work. Section 2 represents the largest section and devoted to what I think of as the species accounts. Each richly illustrated account includes detailed sections on identification, distribution, habitats, biology, larval food plants, concerns for conservation, and additional field observations on behaviors. The photographs of adults, early stages and habitats in nature are all uniformly good, and despite their small size; the range maps are easily readable. Section 3 consists of two parts. First there are traditional color plates of adults of museum specimens for identification. These are very good, comprehensive and often depict multiple specimens showing the range of variation in wing coloration for each species. In the second part we are treated to color photographic plates of the caterpillars for nearly every one of the 159 species. These are uniformly excellent. Section four is comprised of an epilogue detailing the conservation status and likely fate of particular species and habitats, a bibliography, and an alphabetical index.

Lets be honest, there are many treatments of European butterflies. Some are books for the field or library, and nowadays there are others that exist as web sites. Given tangible book offerings over the past century and current offerings using digital technology, it is fair to say the European butterfly fauna is rather well known. But well known doesn't necessarily translate as pedestrian. In talented hands the well known can be transformed into something new and inspiring. The current offering, *Mariposas Diurnas de la Comunitat Valenciana*, represents a book that, in my opinion, lifts the field guide to a new height. It is an identification guide, field biology and conservation tool all in one that can be used beneficially even by those who do not read Spanish. The enthusiastic and talented authors have created an outstanding book that is useful, creative and thought provoking. Everyone interested in the biology of Lepidoptera should own this book.

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