



## Molecular and morphological evidence reveals three species within the California sister butterfly, *Adelpha bredowii* (Lepidoptera: Nymphalidae: Limenitidinae)

KATHLEEN L. PRUDIC<sup>1,4</sup>, ANDREW D. WARREN<sup>2,3</sup> & JORGE LLORENTE-BOUSQUETS<sup>3</sup>

<sup>1</sup>Ecology and Evolutionary Biology, PO Box 210088, University of Arizona, Tucson, AZ 85721

<sup>2</sup>McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, SW 34<sup>th</sup> Street and Hull Road, P. O. Box 112710, Gainesville, FL 32611-2710

<sup>3</sup>Museo de Zoología, Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, Apdo. Postal 70-399, México D.F. 04510 México

<sup>4</sup>Current address: Ecology and Evolutionary Biology, Yale University. E-mail: kathleen.prudic@yale.edu

### Abstract

In recent decades the butterfly *Adelpha bredowii* (Lepidoptera: Nymphalidae), known in the USA as the California Sister, has been considered to be a polytypic array of taxa, composed of two, three or four subspecies. The most recent review of the genus (Willmott 2003a) recognized three: *A. b. bredowii*, *A. b. eulalia* (= *guatemalensis*) and *A. b. californica*. We used mitochondrial (*COII*) and nuclear (*Tpi*) DNA sequence data, coupled with a reevaluation of traditional morphological characters, to determine the phylogenetic relationships between members of the *A. bredowii* complex, and to elucidate their taxonomic status. Phylogenetic analysis of molecular data corroborated the monophyly of each of the three subspecies (sensu Willmott), with the following topology: (*bredowii* + (*eulalia* + *californica*)). Average levels of *COII* variation among these taxa were much greater than the average variation within each taxon (3.4% vs. 0.2%, respectively). There were no shared alleles among these taxa, even from localities where two lineages (*bredowii* and *eulalia*) were collected in exact sympatry and synchrony. The degree of genetic divergence, reciprocal monophyly, and absence of shared alleles between taxa, coupled with unique morphological and distributional attributes of each, strongly suggest that *A. bredowii*, *A. eulalia* and *A. californica* are all species-level taxa, as they are treated herein (rev. stat.). *Adelpha bredowii* is endemic to western, central and southern Mexico: the syntype female (herein designated Lectotype) most likely originated near Oaxaca City, Oaxaca. *Adelpha eulalia* occurs from Guatemala, through much of Mexico, to Arizona, New Mexico and Texas, United States (as permanent residents; further north as vagrants), and occurs in exact sympatry with *A. bredowii* at least in Oaxaca, Mexico, but potentially in much of western and southwestern Mexico. The syntype female of *eulalia* (herein designated Lectotype) most likely originated from south-central Mexico. *Adelpha californica* is allopatric with respect to the other two species, occurring in northern Baja California, much of California, far western Nevada, Oregon and Washington. Our results highlight the continuous need for systematic scrutiny of familiar taxa, and raise the prospect that the genus *Adelpha* may be comprised of many more hidden species.

**Key words:** phylogeny, DNA barcoding, Mexico, molecular systematics, taxonomy

### Introduction

Describing and classifying biodiversity has always been a major objective of the biological sciences. Recently, there has been an effort to promote the use of molecular sequence data as a central methodology in identifying species-level taxa, especially the use of a small segment of the mitochondrial gene Cytochrome Oxidase-I, or *COI* (e.g., Tautz *et al.* 2003; Hebert *et al.* 2003; Smith *et al.* 2006). This effort has encountered justifiable criticism from systematists who have questioned the analytical details and universal applicability of

this approach (e.g., Mallet & Willmott 2003; Lipscomb *et al.* 2003; Moritz & Cicero 2004; DeSalle *et al.* 2005; Brower 2006). However, both proponents and opponents of this approach seem to agree that DNA character analysis (currently known as barcoding when only a small segment of *COI* is studied) often can provide insights into species-level relationships, and that the methodology is most powerful when combined with ecological and morphological information.

The butterfly genus *Adelpha* (Hübner, 1819) displays a modest amount of morphological variation. According to the latest revision (Willmott 2003a), this genus includes about 85 species of medium to large-sized butterflies, generally characterized by a dark brown dorsal wing color broken by white, orange, or red spots and/or bands. *Adelpha* occur in a wide variety of forested habitats, from sea level to over 3000 meters, ranging from western North America to austral South America. Recent morphological study has provided a hypothesis of the evolutionary relationships among *Adelpha* species (Willmott 2003b), but little is known about the possibility of distinctive host-races or cryptic species within currently recognized species-level taxa.

In recent decades, *Adelpha bredowii* Geyer, 1837, has been considered a polytypic species, composed of two, three or four subspecies (Carpenter & Hobby 1944; Luis *et al.* 2003; Willmott 2003a; Lamas 2004; Llorente *et al.* 2006). Larvae of all taxa in this complex are thought to feed on oaks (*Quercus* spp.), and adults of all taxa display discernible morphological characters (Willmott 2003a). *Adelpha bredowii bredowii* (Figs. 1-6) is a Mexican endemic (described from “Mexico”), whose overall range has never been elucidated in detail, due to confusion with *A. bredowii eulalia* (Doubleday, [1848]) and due to sparse, imprecise museum specimen data. As indicated by Field (1940) and Carpenter & Hobby (1944), its range appears to include parts of the Sierra Madre del Sur and Eje Neovolcánico in southwestern and central Mexico.

*Adelpha bredowii eulalia* (Figs. 7–12), described from an erroneous locality of “Venezuela” (see Carpenter & Hobby 1944, p 316; Willmott 2003a, the syntype [or possible lectotype] specimen is from “Méxique”), occurs in southwestern North America, including far southeastern California (New York, Providence and Granite Mountains, San Bernardino County; J. Emmel & T. Emmel 1973, p. 46), much of Arizona and New Mexico, western, central and southern parts of Texas, and less frequently in western Oklahoma, western Kansas, southern Colorado, Utah, and southeastern Nevada (Stanford & Opler 1993). Its range in Mexico has remained unclear, due to confusion with *A. b. bredowii*, but it occurs widely in semi-arid habitats throughout the northern and central parts of the country, south at least to Guatemala (Carpenter & Hobby 1944; de la Maza 1987; Willmott 2003a). *Adelpha bredowii eulalia* is a taxonomic enigma. It was originally described as a species-level taxon (Doubleday 1848), but was subsequently treated as a synonym (e.g., Westwood 1850; Hoffmann 1940, p. 688), or subspecies (e.g., Carpenter & Hobby 1944) of *bredowii*. Confusion over the status of this taxon has persisted, with recent authors similarly considering *eulalia* to be either a subspecies (e.g., Ferris & Brown 1981; Bailowitz & Brock 1991; Willmott 2003a) or synonym (Hoffmann 1940; Luis *et al.* 2003, Llorente *et al.* 2006) of *A. bredowii*.

Carpenter & Hobby (1944) proposed the name *Limenitis bredowii guatemalensis* for populations occurring in Guatemala and Chiapas, with a type locality of San Gerónimo, Guatemala. This subspecies was described from a series of thirteen specimens, and was diagnosed by having narrower orange dorsal forewing subapical markings, broader white postdiscal bands and a better-developed white postdiscal spot in forewing cell M1-M2. De la Maza & de la Maza (1993) considered *guatemalensis* to be the subspecies of *A. bredowii* occurring in Chiapas, Mexico, but Willmott (2003a) downgraded this taxon to a synonym of *A. b. eulalia* (also see Lamas 2004), based on his analysis of morphological variation observed throughout the range of *eulalia*.

*Adelpha bredowii californica* (Butler, 1865) (Figs. 13–16), described from “California,” is endemic to far western North America, including northern Baja California, Mexico (Hoffmann 1940; Brown *et al.* 1992), most of California (including Santa Cruz Island; Emmel & Emmel 1973), western Nevada, western Oregon, and less often in southwestern Washington (Stanford & Opler 1993), United States, where its presence may be due to vagrant individuals from western Oregon (Pyle 2002). This taxon is geographically allopatric with respect to *A. b. eulalia*, although the two taxa approach each other in southern California (Emmel & Emmel

1973). Before the revision by Carpenter & Hobby (1944), *A. b. californica* was generally treated as a species-level taxon (e.g., Godman & Salvin 1879–1901), as originally described by Butler (1865), but it was considered to be a subspecies of *A. bredowii* by Skinner (1911) and Holland (1931), as well as by most authors since 1944 (e.g., dos Passos 1964; Miller & Brown 1981; Ferris 1989; Bailowitz & Brock 1991; Llorente *et al.* 2006).

In addition to the confusion at the species and subspecies levels, this group of taxa has variously been placed in the genera *Adelpha* (e.g., Geyer 1837; Kirby 1871; Godman & Salvin 1879–1901), *Limenitis* [Fabricius], 1987 (e.g., Doubleday 1848; Edwards 1868–1897; Seitz 1915; Carpenter & Hobby 1944, de la Maza *et al.* 1989), or *Heterochroa* Boisduval 1836 (e.g., Westwood 1850; Butler 1865). However, the placement of *bredowii* group taxa in the genus *Adelpha* was recently corroborated by Willmott (2003a; b).

The present study was initiated by A. Warren, on August 22nd, 2003, on the road leading north of Teotitlán del Valle (towards Benito Juárez), Oaxaca, Mexico. This road passes through seasonally dry semi-deciduous forest at lower elevations, roughly 1–8 kilometers north of Teotitlán del Valle, then enters into a pine-oak cloud forest at higher elevations, beginning roughly 9 kilometers north of Teotitlán del Valle. This day presented a unique opportunity, since *A. b. bredowii* and *A. b. eulalia* were found flying in exact sympatry at one point along the nearly four kilometers of road surveyed on foot. At the lowest elevations studied (*ca.* 2120 m), in semi-deciduous forest habitat, two somewhat damaged males of *A. b. eulalia* were sampled. A few hours later, further up the road at about 2500 m, various *Adelpha* were found flying in the canopy of roadside *Quercus* trees, and a few males were taken along the dirt road, as they sought damp ground. At one spot, where the downhill slope below the road was very steep, the canopy of three roadside *Quercus* trees was within net reach. In this small area, 4 males of *A. b. eulalia* were collected, along with 12 males and 3 females of *A. b. bredowii*, all within about forty-five minutes, under mostly cloudy conditions. Most of the *A. b. eulalia* were somewhat damaged, while essentially all of the *A. b. bredowii* had recently eclosed. Once these specimens were pinned and spread, their external morphology seemed to reinforce the idea generated in the field that two species-level taxa were represented. Two legs from each of these *Adelpha* specimens were removed and sent to the senior author for DNA extraction and sequencing, along with additional specimens of *A. b. bredowii* from Durango, Mexico, *A. b. eulalia* from another locality in Oaxaca, from Guanajuato State, Mexico, as well as from Arizona and New Mexico, United States. In addition, specimens of *A. b. californica* from California and Oregon, United States, were included to determine the taxonomic status of *A. b. californica*, with respect to the other two taxa.

To test the hypothesis that *A. bredowii* consists of three genetically distinct species-level taxa, we examined mitochondrial (*COII*) and nuclear (*Tpi*) DNA variation within all supposed subspecies of *A. bredowii* (*sensu* Willmott 2003a): *bredowii*, *eulalia*, and *californica*. We included specimens of each taxon collected in sympatry (for *bredowii* and *eulalia*) and allopatry in our analysis, in order to determine the distribution of genetic alleles within each taxon.

## Materials and methods

**Taxon Sampling.** We included a total of 60 *Adelpha* individuals in our analysis of *COII* variation: 17 *A. bredowii*, 25 *A. californica*, and 18 *A. eulalia*, from across the geographic range of each taxon (Table 1). We included all available (pre-2005) specimens of *A. bredowii* and *A. eulalia* from overlap or putative hybrid zones in Mexico, to investigate the possibility of shared alleles between taxa. Additionally, as outgroups we included three congeners: *Adelpha lycorias* (Godart, [1824]), *A. mesentina* (Cramer, [1777]), and *A. phylaca* (H. W. Bates, 1866). We sequenced only a subset (*n*=16) of these 60 individuals in our analysis of *Tpi* variation (Table 2). For our morphological analysis, in addition to specimens represented in our molecular studies, we examined a large number of Mexican specimens of *A. bredowii* and *A. eulalia*, in an effort to identify reli-

able morphological characters that may separate these two taxa, and to clarify the geographic distributions of each (Appendix I).

**TABLE 1.** List of specimens for *COII* DNA study

Genus species	Collection Date	Country	State	Location	N	Haplotype (N)	GenBank no.
<i>Adelpha bredowii</i>	Apr. 29, 1998	Mexico	Durango	Mpio. El Salto	2	Bre4 (2)	U851391
	Aug. 22, 2003	Mexico	Oaxaca	Teotitlán del Valle	15	Bre1 (4), Bre2 (1), Bre3 (1), Bre4 (9)	U851388- U851391
<i>Adelpha californica</i>	Jun. 19, 1998	USA	California	Mix Canyon, Coast Range	10	Cal1 (2), Cal2 (1), Cal4 (1), Cal5 (1), Cal6 (1), Cal7 (2), Cal10 (1), Cal13 (1)	DQ117896, U851375, U851377- U851380, U851383, U851386
	Jun. 20, 1998	USA	California	Pulga, Sierra Nevada Mtns.	2	Cal8 (1), Cal14 (1)	U851381, U851387
	Jun. 20, 1998	USA	California	Caribou, Sierra Nevada Mtns.	3	Cal1 (1), Cal7 (2)	DQ117896, U851380
	Jul. 11, 2003	USA	Oregon	Upper Powell Creek	10	Cal1 (4), Cal3 (2), Cal5 (1), Cal9 (1), Cal11 (1), Cal12 (1)	DQ117896, U851376, U851378, U851382, U851384, U851385
<i>Adelpha eulalia</i>	Feb. 11, 2001	Mexico	Guanajuato	Xichu Canyon	3	Eul5 (2), Eul6 (1)	U851396, U851397
	Aug. 16, 2003	Mexico	Oaxaca	5 mi N Oaxaca	3	Eul4 (3)	U851395
	Aug. 22, 2003	Mexico	Oaxaca	Teotitlán del Valle	6	Eul1 (1), Eul4 (4), Eul6 (1)	U851392, U851395, U851397
	Aug. 31, 2003	USA	Arizona	Wet Canyon, Pinaleno Mtns.	2	Eul2 (1), Eul3 (1)	U851393, U851394
	Sep. 07, 2003	USA	Arizona	Mt. Hopkins Wash, Santa Rita Mtns.	3	Eul2 (3)	U851393
	Aug. 04, 2000	USA	New Mexico	Mogollion	1	Eul3 (1)	U851394

**DNA Extraction and Sequencing.** We extracted total genomic DNA from leg or thoracic tissue using DNeasy Tissue Extraction (Qiagen Inc., CA, USA), per the manufacturer's protocol. We amplified the mitochondrial gene cytochrome oxidase subunit II (*COII*) using the primers (5' → 3') Pierre (AGA GCC TCT CCT TTA ATA GAA CA) and Eva (GAG ACC ATT ACT TGC TTT CAG TCA TCT) (Zakharov *et al.* 2004), and the nuclear gene triosephosphate isomerase (*Tpi*) using the primers Tpi1 (GGT CAC TCT GAA AGG AGA ACC ATC TT) and Tpi2 (CAC AAC ATT TGC CCA GTT GTT GCC AA) (Beltrán *et al.* 2002). PCR reactions (50 µl) were performed on a Mastercycler (Eppendorf, NY, USA), in 1X Eppendorf Hotmaster Taq Buffer containing 0.2 µM of each primer, 0.1 mM dNTPs, 2.5 mM MgCl<sub>2</sub>, and 1 unit of Eppendorf Hotmaster™ Taq DNA Polymerase. The thermal cycle profile to amplify *COII* was an initial 1.5 minute denaturation

at 94°C; 32 cycles of 40 seconds at 94°C, 40 seconds at 45°C, and 45 seconds at 72°C; and a 7 minute final extension at 72°C. The thermal cycling profile to amplify *Tpi* was an initial 2 minute denaturation at 94°C; 17 cycles of 20 seconds at 94°C, 20 seconds at 45°C, and 50 seconds at 65°C, with the annealing temperature increased by 0.5°C per cycle; 20 cycles of 20 seconds at 94°C, 20 seconds at 53.6°C, 50 seconds at 65°C; and a 5 minute final extension at 65°C. PCR products were purified with a Millipore Size Exclusion filtration (Millipore, MA, USA) prior to direct sequencing. Both strands were sequenced using an Applied Biosystems BigDye™ Terminator v3.1 cycle sequencing kit (Applied Biosystems, CA, USA) and run on an Applied Biosystems 3730XL DNA Analyzer (Applied Biosystems, CA, USA).

**Phylogenetic Analyses.** We generated consensus sequences from the two strands and aligned these sequences by eye. All alignments were unambiguous. Total lengths varied from 658–661 bases for *COII* and 325–341 bases for *Tpi*. The sequence matrix was trimmed of duplicate sequences and gaps were treated as missing data for all subsequent phylogenetic analyses (there were no shared alleles among the taxa). The partition homogeneity test (Farris *et al.* 1995) was implemented in PAUP\*4.0b10 (Swofford 2001) to test the homogeneity of the gene partitions before combining the two genes for phylogenetic analysis. We performed 1000 partition test replicates using tree bisection reconnection (TBR) branch swapping and saving 100 trees per replicate. Maximum Parsimony analyses were performed in PAUP\*4.0b10. We initially performed a heuristic tree search with 1000 random-addition replicates, saving all of the most parsimonious trees per replicate. To assess node support, we performed 1000 non-parametric bootstrap pseudoreplicates, each with 10 step-wise-addition, TBR heuristic search replicates, saving the first 1000 most parsimonious trees per heuristic search replicate. We used a hierarchical Likelihood Ratio Test (Huelsenbeck & Rannala 1997) to estimate the model of evolution for the Bayesian analysis. The best fit model, HKY+I with empirical base frequencies, was used for Bayesian inference. Using Mr.Bayes 3.01 (Huelsenbeck & Ronquist 2001), we ran four chains (three hot and one cold) for 4,000,000 generations, sampled trees every 100 generations, and discarded the trees sampled in the first 2,000,000 generations (burnin). To estimate the posterior probability of clades, we used the post-burnin sampled trees to generate a 50% majority rule consensus tree using the ‘sumt’ command in MrBayes. Our group taxa, *Adelpha lycorias*, *A. mesentina*, and *A. phylaca*, were used to root the trees.

**Morphological Analysis.** Pinned and papered specimens of all *Adelpha bredowii* taxa were examined in several institutional and private collections, as detailed in Appendix 1, for Mexican material. Several hundred specimens of *eulalia* and *californica* were examined from the United States, from institutional and private collections indicated in Appendix 1, but are not individually listed herein since the distributions of these taxa in the United States are well-known (see introduction); many of these records are listed by Willmott (2003a). All wing characters were studied, with special emphasis on those used by Carpenter & Hobby (1944) to diagnose the *A. bredowii* taxa. Male and female genitalia of each species were studied, following standard KOH saturation and micro-dissection protocols (*e.g.*, Burns 1997). The genitalia of eight males and one female of *A. bredowii*, twelve males and four females of *A. eulalia*, and eight males and two females of *A. californica* were studied, as indicated in Appendix 1 for Mexican material. Genitalia studied from specimens collected in the United States (thus excluded from Appendix 1) include three males and one female of *eulalia* (Arizona, Utah and New Mexico), and eight males and two females of *californica* (from California; all from the MGCL collection). Genitalic illustrations were prepared using a camera lucida attached to the dissecting microscope.

## Results

**COII.** The final aligned sequence yielded 658 characters: 572 were constant, 14 were noninformative, and 72 were phylogenetically informative. The maximum sequence divergence was 4.2% between *A. bredowii* and *A. californica*, 2.4% between *A. bredowii* and *A. eulalia*, and 3.7% between *A. eulalia* and *A. californica*.

Twenty-four different *COII* haplotypes were found in our three study taxa. *Adelpha californica* had the most haplotypes (14), while *A. bredowii* had 4 haplotypes and *A. eulalia* had 6 haplotypes (Table 1, with Gen-

Bank accession numbers). None of the three study taxa shared haplotypes, even when collected from the same locality, and no clear geographic patterns were correlated with haplotype variation within any taxon.

***Tpi***. The intron in *Tpi* ranged from 50–121 bp depending on the species. Thus direct sequencing and aligning those sequences was a straightforward process. The final aligned sequence yielded 324 characters: 277 were constant, 5 were noninformative, and 42 were phylogenetically informative. The maximum sequence divergence was 1.7% between *A. bredowii* and *A. eulalia*, and 1.4% between both *A. bredowii* and *A. californica*, and *A. californica* and *A. eulalia*. Seven different *Tpi* alleles were found in our three study taxa. *Adelpha eulalia* had the most alleles (3), while *A. bredowii* and *A. californica* each had 2 alleles (Table 2, with GenBank accession numbers). None of the taxa shared alleles, and no clear geographic patterns were correlated with the allelic variation within any taxon.

**TABLE 2.** List of specimens for *Tpi* DNA study

Genus species	Collection Date	Country	State	Location	N	Haplotype (N)	GenBank no.
<i>Adelpha bredowii</i>	Apr. 29, 1998	Mexico	Durango	Mpio. El Salto	2	Bre2 (2)	U851403
	Aug. 22, 2003	Mexico	Oaxaca	Teotitlán del Valle	3	Bre1 (1), Bre2 (2)	U851402, U851403
<i>Adelpha californica</i>	Jun. 19, 1998	USA	California	Mix Canyon, Coast Range	1	Cal (1)	U851401
	Jun. 20, 1998	USA	California	Pulga, Sierra Nevada Mtns.	1	Cal (1)	U851401
	Jun. 20, 1998	USA	California	Caribou, Sierra Nevada Mtns.	1	Cal (1)	U851401
	Jul. 11, 2003	USA	Oregon	Upper Powell Creek	2	Cal (2)	U851401
<i>Adelpha eulalia</i>	Feb. 11, 2001	Mexico	Guanajuato	Xichu Canyon	1	Eul4 (1)	U851407
	Aug. 16, 2003	Mexico	Oaxaca	5 mi N Oaxaca	1	Eul2 (1)	U851405
	Aug. 22, 2003	Mexico	Oaxaca	Teotitlán del Valle	2	Eul1 (1), Eul3 (1)	U851404, U851406
	Aug. 31, 2003	USA	Arizona	Wet Canyon, Pinaleno Mtns	1	Eul4 (1)	U851407
	Aug. 04, 2000	USA	New Mexico	Mogollion	1	Eul3 (1)	U851406

**Phylogenetic Trees Based on Both Gene Regions Combined.** The partition homogeneity test was not significant ( $P = 0.942$ ), thus the two gene regions were combined into a single matrix (982 bases) for all subsequent phylogenetic analyses. All samples included sequence data from *COII*, but not all included *Tpi*. Gaps were treated as missing data during the analyses. A maximum parsimony analysis resulted in 397 trees ( $L = 180$  steps,  $CI = 0.87$ , phylogram shown in Fig. 17). Our three study taxa were distributed across three clades, each with strong bootstrap support (Fig. 17), with the following topology: (*bredowii* + (*eulalia* + *californica*)). These clades corresponded exactly with the three taxa as identified before our analysis, even when individuals of different taxa were sampled in sympatry. Within each taxon there does not appear to be any clear geographic patterns of allelic variation; however, a more thorough sampling is required to better understand the phylogeography within each clade. The Bayesian analysis completely supported the MP results, also with high support values (Fig. 17).

**Adult Morphology. Wing characters.** Carpenter & Hobby (1944) provided an exhaustive review of the superficial differences between *A. bredowii*, *A. eulalia* and *A. californica* (even though these taxa were considered conspecific). While they studied a large number of wing characters, differences between the taxa were mostly related to the development of white wing markings, the development of orange wing markings, and in the coloration and development of ventral bluish areas. These authors provided a table summarizing these differences, which has been modified and expanded herein (Table 3), to include additional wing and genitalic characters.

The three taxa show consistent differences in the color, shape, and size of the orange subapical patch on the forewing. On *A. bredowii* (Figs. 1–6), this patch is a richer orange color, especially above, and is more linear in shape, widest along the costa and tapering towards the tornus. The subapical patch on *A. californica* (Figs. 13–16) is the roundest of all three taxa, and that of *A. eulalia* (Figs. 7–12) is roughly intermediate in shape between that of *A. bredowii* and *A. californica*, but similar to *A. californica* in coloration. On all three taxa, the forewing orange subapical patch tends to be better developed on the ventral wing surface, and often occupies additional wing cells (towards the tornus) on the ventral surface; this expanded development tends to be most pronounced on *A. bredowii* and least pronounced on *A. californica*.

The white postdiscal bands on all wings possess characters unique to each of the three taxa. The alignment of spots in the forewing postdiscal band of *A. bredowii* is nearly linear, whereas these spots are poorly aligned and better separated in *A. californica*; the spot band alignment of *A. eulalia* is roughly intermediate between that of *A. bredowii* and *A. californica*. The color of the forewing postdiscal band is creamy-white on fresh specimens of *A. californica* (fading to clear white on some older specimens), while it is clear white in the other two taxa. The width of the postdiscal bands on all wings shows geographic variation in *A. eulalia* and *A. bredowii* (as detailed below), with both taxa displaying narrower bands in the north and wider bands in the south; thus, some of the characters tabulated by Carpenter & Hobby (1944), subject to geographical variation, have been omitted from Table 3. The external outline of the hindwing postdiscal band is straight on *A. bredowii*, nearly straight on *A. eulalia*, and sinuous in *A. californica*. On both *A. bredowii* and *A. eulalia*, the inner and outer margins of the ventral hindwing postdiscal band are bordered by a bold dark band, followed by a well-developed bluish band, whereas all bordering bands are highly reduced and poorly-delineated on *A. californica*.

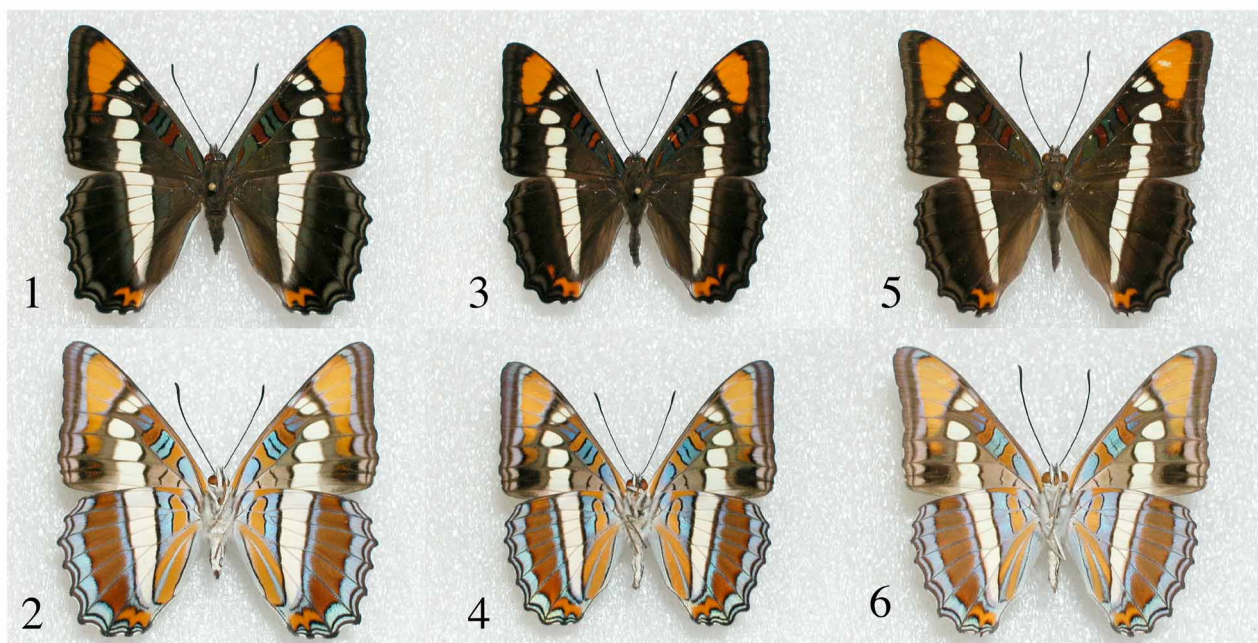
**TABLE 3.** Summary of morphological differences between *A. bredowii*, *A. eulalia* and *A. californica*. Abbreviations: d = dorsal, v = ventral, fw = forewing, hw = hindwing.

Character	<i>Adelpha bredowii</i>	<i>Adelpha eulalia</i>	<i>Adelpha californica</i>
fw orange subapical patch: shape	narrow, more linear	intermediate	rounded
dfw orange subapical patch: color	rich orange	pale orange	pale orange
fw white postdiscal band: spot alignment	nearly linear	intermediate	poorly aligned
dfw white postdiscal band: color	white	white	creamy-white
external outline of hw postdiscal band	straight	nearly straight	sinuous
dark and bluish vhw postdiscal bands	bold and straight	bold, nearly straight	poorly-developed, sinuous
bluish vhw coloration	pale blue	pale to dark blue	violet
vhw basal orange bars	one	one	two
vhw submarginal dark area	linear, dark ochraceous	near linear, warm brown	Irregular, broad, brownish
gap between ventral and distal teeth on valvae	prominent	prominent	inconspicuous-absent
shape of valvae	evenly upturned	abruptly upturned distally	evenly upturned
proximal end of tegumen in lateral view	rounded	pointed	fairly pointed
penis length	~ tegumen + uncus	> tegumen + uncus	~ tegumen + uncus



As summarized by Skinner (1911), Willmott (2003a) and Stout (2005), on *A. californica* there are two longitudinal orange bars in the basal part of the ventral hindwing (the basal-most of these may be broken into two portions), above Cu2A, basal of the bands bordering the postdiscal white stripe. On *A. bredowii* and *A. eulalia*, only one orange basal band exists in this region of the ventral hindwing. While the coloration of ventral hindwing bluish markings shows some geographic variation in *A. bredowii* and *A. eulalia* (see below), the markings on these taxa are decidedly bluish, whereas they are violet on *A. californica*. Finally, while it is subject to subtle geographic variation in *A. bredowii* and *A. eulalia*, the coloration and shape of the ventral hindwing submarginal dark areas differs on the three taxa. The dark submarginal area on *A. bredowii* is nearly linear, and ochraceous-brown in color, while on *A. californica* the dark area is irregularly shaped and rather broad throughout, with a duller brownish color; the dark region on *A. eulalia* is similar to that of *A. bredowii*, but somewhat broader, darker, and more irregularly-shaped.

There are several additional, less obvious differences in wing markings between these three taxa, some of which are not easily described. For example, as shown in Figs. 1–16, the taxa appear to have different average wing shapes, geographical variation in *A. bredowii* and *A. eulalia* considered (see below). At least, *A. bredowii* stands out as having a more “compact” wing shape, compared to the broader and more rounded wing shapes of *A. eulalia* and *A. californica*. While *A. bredowii* and *A. eulalia* show geographical variation in some wing characters (discussed below), we observed no geographical variation in any characters of *A. californica*, among specimens examined from throughout the range of the taxon (also see Hovanitz 1945), save the Channel Islands off the southern California coast. While the population on Santa Cruz Island might be slightly differentiated (J. F. Emmel, pers. comm. 2007), we have yet to examine specimens from there. This apparent lack of geographic variation in wing and genitalic characters contrasts the considerable diversity of *COII* haplotypes found in *A. californica* (Table 1).

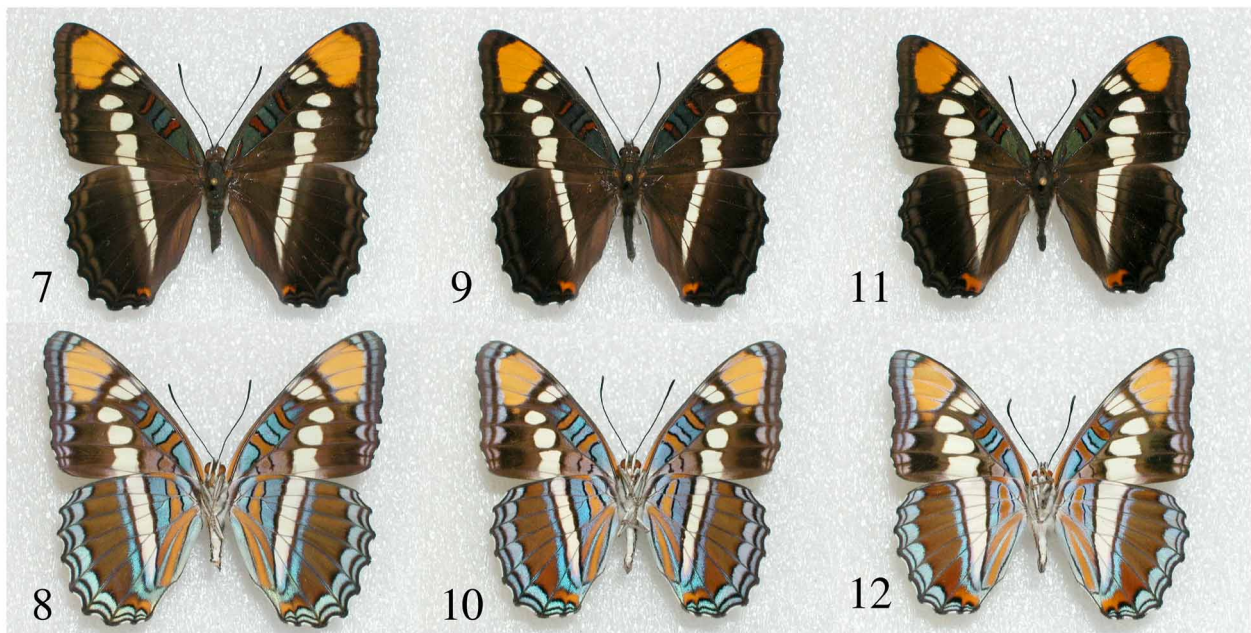


**FIGURES 1–6.** Adults of *Adelpha bredowii* from Mexico, collected by A. D. Warren. **Figs. 1** (dorsal), **2** (ventral), female, from Oaxaca: Mpio. Teotitlán del Valle / Díaz Ordaz: Rd. to Benito Juárez, ca. 8–10 mi N Teotitlán del Valle, ca. 8000', 22-VIII-2003, DNA voucher KLP238. **Figs. 3** (dorsal), **4** (ventral), male, from same locality and date as female in Figs. 1–2, DNA voucher KLP244. **Figs. 5** (dorsal), **6** (ventral), male, from Durango: Mpio. El Salto: Hwy. 40 at La Fragüita, 2350m., 29-IV-1998, DNA voucher KLP264.



A careful read of Carpenter & Hobby (1944) reveals that they identified 8 specimens among 218 examined as “intermediates” between *A. eulalia* (= *guatemalensis*) and *A. bredowii*. While we have not personally examined these specimens (housed in the Natural Museum of Natural History, London), we have seen images of two of them (provided by K. Willmott), which suggest they are simply variations of *A. eulalia*. Among the specimens examined in this study (Appendix I), no individuals appeared intermediate in wing or genitalic characters between *A. bredowii* and *A. eulalia*.

**Genitalic characters.** Each of the three *Adelpha* species possess distinctive male genitalia, although the male valvae on all three taxa are unusually variable in the number and positions of the ventral and distal “teeth” (Figs. 18–23). Carpenter & Hobby (1944) noted individual variation in tooth number in *A. bredowii* between three and four teeth per valve, while they found between one and two teeth per valve in *A. eulalia* (= *guatemalensis*). They also noted that valvae of a single individual quite often are not symmetrical in the number or placement of teeth. We were unable to define the number of teeth per valve in many cases, due to numerous smaller perforations along the distal edges of the valvae on some specimens (e.g., Figs. 18, 19, 21, 23), of various degrees of development. Variation in teeth number and placement was so great, both within and between individuals, that we failed to define any reliable traits related to these tooth that could be used to diagnose any of the three taxa, with one possible exception. On *A. bredowii* and *A. eulalia*, there tends to be a “gap” between the ventral set of teeth and the distal set of teeth on each valve (Figs. 18–21), where the edge of the valve is smooth. This gap is reduced or absent on *A. californica* (Figs. 22–23), and as a result, the distinction between ventral and distal teeth is ambiguous on many specimens.



**FIGURES 7–12.** Adults of *Adelpha eulalia* from Arizona and Mexico, collected by A. D. Warren. **Figs. 7** (dorsal), **8** (ventral), female, from USA: Arizona: Cochise County: Huachuca Mountains, Garden Canyon, 4-X-1997. **Figs. 9** (dorsal), **10** (ventral), male, from same locality and date as female in Figs. 7–8. **Figs. 11** (dorsal), **12** (ventral), male, from Mexico: Oaxaca: Mpio. Teotitlán del Valle / Díaz Ordaz: Rd. to Benito Juárez, ca. 8–10 mi N Teotitlán del Valle, ca. 8000', 22-VIII-2003, DNA voucher KLP253.

Despite the unusual variation seen in the teeth of the valvae of all three *Adelpha* taxa, the overall shape of the valvae, and of other genitalic structures, is apparently consistent within each species, and of diagnostic value. As shown in Figs. 18–19 and 22–23, valvae of *A. bredowii* and *A. californica* tend to be evenly upturned throughout their length, whereas on *A. eulalia* (Figs. 20–21), the distal ends of the valvae tend to be

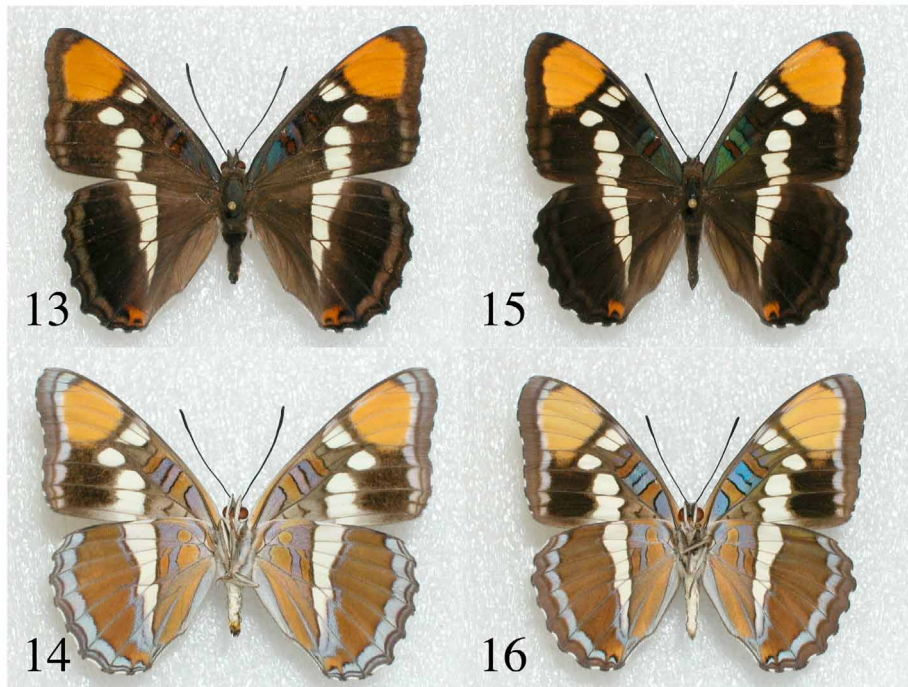
abruptly upturned. While this is a subtle difference, it is consistently expressed among the 12 individuals of *A. eulalia* examined. Overall, the genital capsule of *A. bredowii* is smaller than that of *A. eulalia* and *A. californica*, with a distinctly rounded proximal edge to the tegumen, in lateral view. Males of *A. eulalia* tend to have a proportionally longer penis than those of *A. bredowii* and *A. californica*, as shown in Figs. 18–23, which exceeds the length of the tegumen and uncus.

An insufficient number of females were dissected to know if observed differences in female genitalic characters are diagnostic of the three taxa, or subject to individual variation; therefore, female genitalic characters have not been summarized in Table 3. Unfortunately, too few females of *A. bredowii* were available for dissection to enable a significant comparison of characters across the three taxa. However, as shown in Figs. 24–26, each taxon appears to possess distinctive female genitalic features. As with male genitalia, the female genitalic structures of *A. bredowii* appear smaller than those of *A. eulalia* or *A. californica*, probably reflecting overall smaller adult size. The sterigma and lamella antevaginalis on *A. californica* are broad and well developed, while they are reduced in *A. bredowii* and roughly intermediate in *A. eulalia*. In addition, the ductus bursae and corpus bursae appear to be proportionally longer in *A. californica* than in *A. bredowii* or *A. eulalia*, where they are similar.

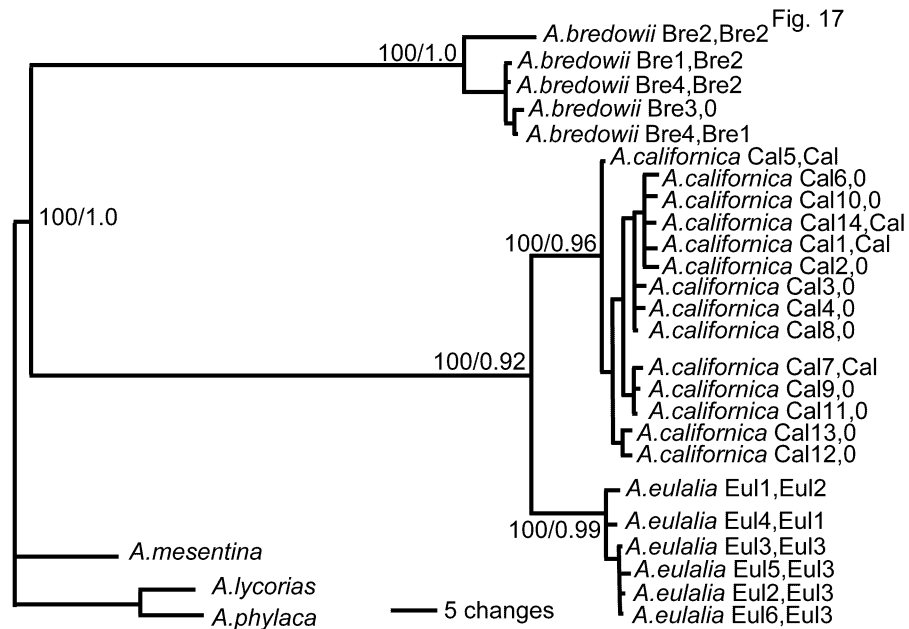
## Discussion

Our molecular results support the conclusion that *Adelpha bredowii* of previous authors, which included two, three or four subspecific taxa, is actually better treated as a complex of three species-level taxa, *A. bredowii* (Figs. 1–6, 18–19, 24, 27), *A. eulalia* (Figs. 7–12, 20–21, 25, 28) and *A. californica* (Figs. 13–16, 22–23, 26). The average levels of *COII* variation among these taxa were much greater than the average variation within each taxon (3.4% vs. 0.2%, respectively). Both mtDNA and nuclear DNA analyses recovered three strongly supported clades corresponding to the three taxa (Fig. 17), with *A. bredowii* as the most basal ingroup taxon. There were no shared alleles among taxa, even from localities where two taxa (*bredowii* and *eulalia*) were collected in exact sympatry and synchrony (Tables 1 and 2). Past research has provided conflicting perspectives on the efficacy of molecular markers for delineating species boundaries (e.g., Avise & Walker 1999; Lipscomb *et al.* 2003). However, the *A. bredowii* species complex represents a scenario where the molecular markers were expected to fail since the component taxa are all morphologically similar and sometimes sympatric, suggesting the possibility of gene flow. Thus, the reciprocal monophyly of the three taxa indicate there is either strict reproductive isolation or ongoing selection against genetic exchange between taxa.

The strongly supported basal position of *A. bredowii* in our phylogram (Fig. 17) is noteworthy. On one hand, this position corroborates the views of previous authors that have viewed *A. bredowii* as a more “typical” member of the genus *Adelpha*, with *A. eulalia* and *A. californica* as outliers (Poulton 1909; Carpenter & Hobby 1944). On the other hand, when the overall superficial similarities between *A. bredowii* and *A. eulalia* are considered (Figs. 1–6, 7–12), the position of *A. eulalia* and *A. californica* as sister species seems surprising, since *A. californica* (Figs. 13–16) is the most distinctive of the three taxa, in terms of superficial wing characters (Table 3).



**FIGURES 13–16.** Adults of *Adelpha californica* from Oregon, USA, collected by A. D. Warren. **Figs. 13** (dorsal), **14** (ventral), female, from Josephine County: Illinois River Road vic. Deer Creek, ca. 3 rd. mi W Selma, 1400', 25-VI-2000. **Figs. 15** (dorsal), **16** (ventral), male, from Josephine County: Upper Powell Creek, 5 rd. mi SW Provolt, 11-VII-2003, DNA voucher KLP271.



**FIGURE 17.** One of 397 most parsimonious trees obtained through parsimony analysis, based on 658 bp of *COII* and 325 bp of *Tpi* combined into a single matrix ( $L = 180$  steps,  $CI = 0.87$ ). Allele numbers (*COII*, *Tpi*) correspond to those listed in Tables 1 and 2, respectively. All samples included sequence data from *COII*, but not all included *Tpi*. Those without *Tpi* are listed as 0. Parsimony bootstrap/Bayesian posterior probabilities above 80/0.80 are indicated above or adjacent to branches. Trees rooted with *Adelpha lycorias*, *A. mesentina*, and *A. phylaca*.

Previous morphological studies on taxa in the *A. bredowii* complex (e.g., Carpenter & Hobby 1944; Willmott 2003a) have not indicated geographical variation in *A. bredowii* or *A. californica*, but have noted geographical variation within *A. eulalia*. However, because of confusion with *A. bredowii*, combined with limited Mexican material available for study, these studies have not clearly defined these pattern. Carpenter & Hobby (1944) described southern populations of *A. eulalia* as *Limenitis bredowii guatemalensis*, but examined very few specimens from central Mexico. The most obvious distinguishing features of *eulalia* specimens from Guatemala are the wider white postdiscal bands on all wings and the better developed white postdiscal spot in dorsal forewing cell M1–M2, compared to populations at the northern end of the species' range (e.g., Arizona, Figs. 7–10). Willmott (2003a) noted extensive variation in these characters throughout the range of *eulalia*, but did not indicate discernable patterns of geographical variation.

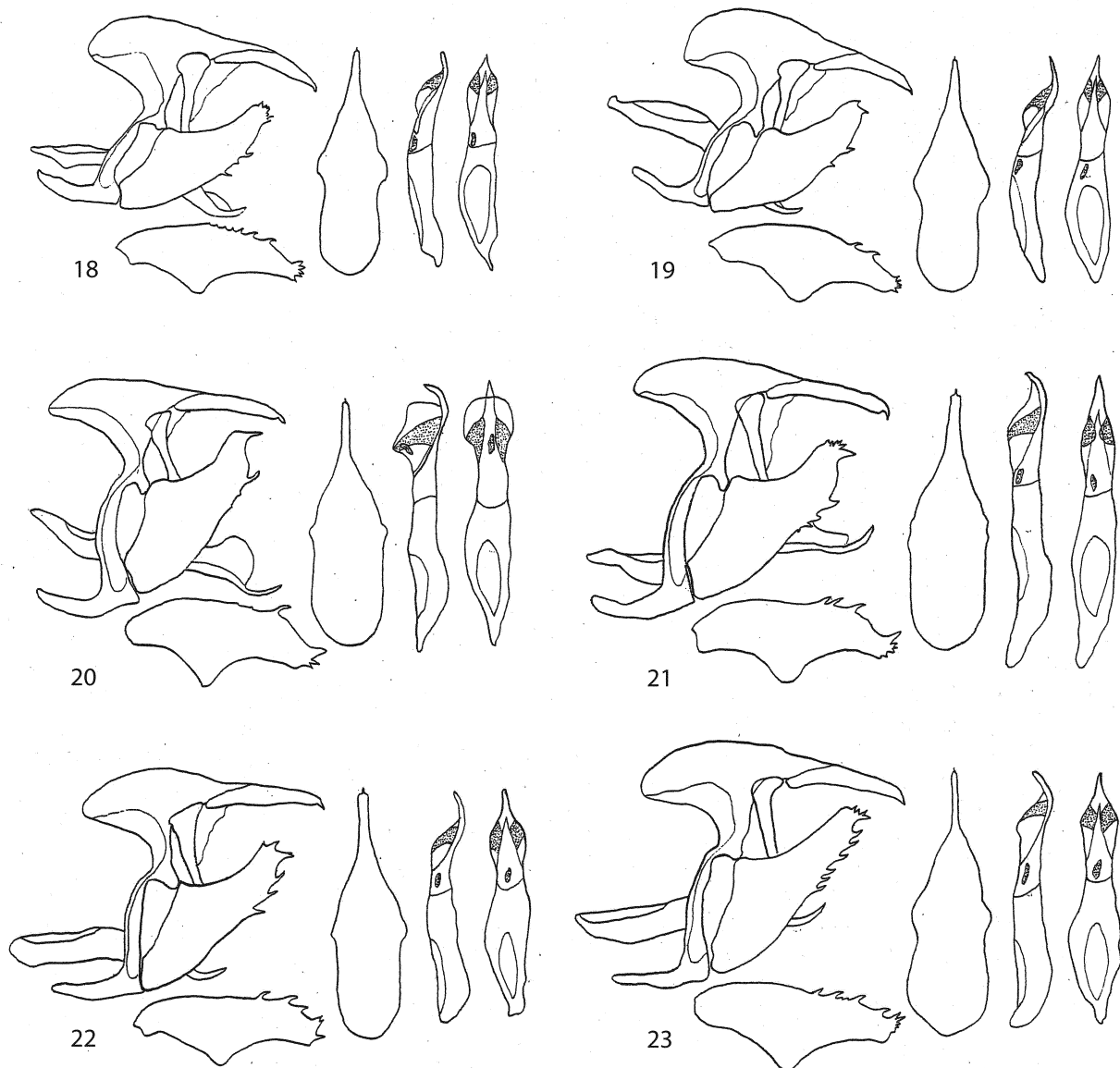
Specimens examined in this study (Appendix I) indicate that geographical variation in *A. eulalia* can be described as an approximate north-south cline in essentially all wing pattern elements. Adults from the United States, Sonora, Chihuahua, Durango and Nuevo León have narrower white postdiscal bands on all wings, a greater forewing length due to the longer forewing apex, better-developed black markings on the ventral hindwing, a deeper blue-violet ground color below, and tend to lack the dorsal forewing postdiscal spot in cell M1–M2, compared to populations to the south. Adults examined from Aguascalientes, Guanajuato, Querétaro, San Luis Potosí, Hidalgo, Puebla, Mexico, Morelos and Distrito Federal show a slight increase in the width of the white postdiscal bands and tend to have more rounded forewings, as well as a paler blue-violet coloration below. Individuals from Oaxaca, Chiapas and Guatemala generally have the widest white postdiscal bands and obviously rounded forewings, while the intensity of ventral coloration is variable. However, as correctly illustrated by Carpenter & Hobby (1944, p 341, pl. 1) and shown in Figs. 7–12, individuals from the extreme ends of the range of *A. eulalia* (e.g., USA vs. southern Mexico and Guatemala) show obvious differences in wing shape and markings.

As discussed above, the exact origin of the syntype of *A. eulalia* is unknown. Its label reads “Méxique,” which is uninformative considering the geographical variation known to exist in the species in Mexico. However, as figured in its original description, this specimen is a close match to various individuals examined in this study from central Mexico. We believe that the vicinity of Mexico City, Distrito Federal, is the most likely origin of this specimen, as indicated by its phenotype, the known distribution of *A. eulalia* in central Mexico (see below), and because of travel routes likely to have been accessible at the time the syntype was collected, sometime before 1848. In addition, the label on the syntype could be interpreted as referring to “Mexico City.” However, an origin as far south as Oaxaca City cannot be ruled out. Carpenter & Hobby's (1944, p 316) discussion of this type specimen might be interpreted to be a lectotype designation, although Willmott (2003a) called the specimen a syntype. To preserve stability of nomenclature, this specimen is here designated *Lectotype*, and an appropriate label will be added.

Considering that the lectotype of *eulalia* was apparently collected in central Mexico, where most adults are intermediate between extreme northern and southern phenotypes, the application of trinomials to northern and southern phenotypes seems unnecessary, and would be compromised by the gradual variation seen as one examines specimens to the north (e.g., Aguascalientes, Guanajuato, Querétaro, San Luis Potosí) and south (e.g., Guerrero, Oaxaca) of the Mexico City area. We therefore agree with Willmott (2003a) in his assessment of *guatemalensis* as a synonym of *A. eulalia*.

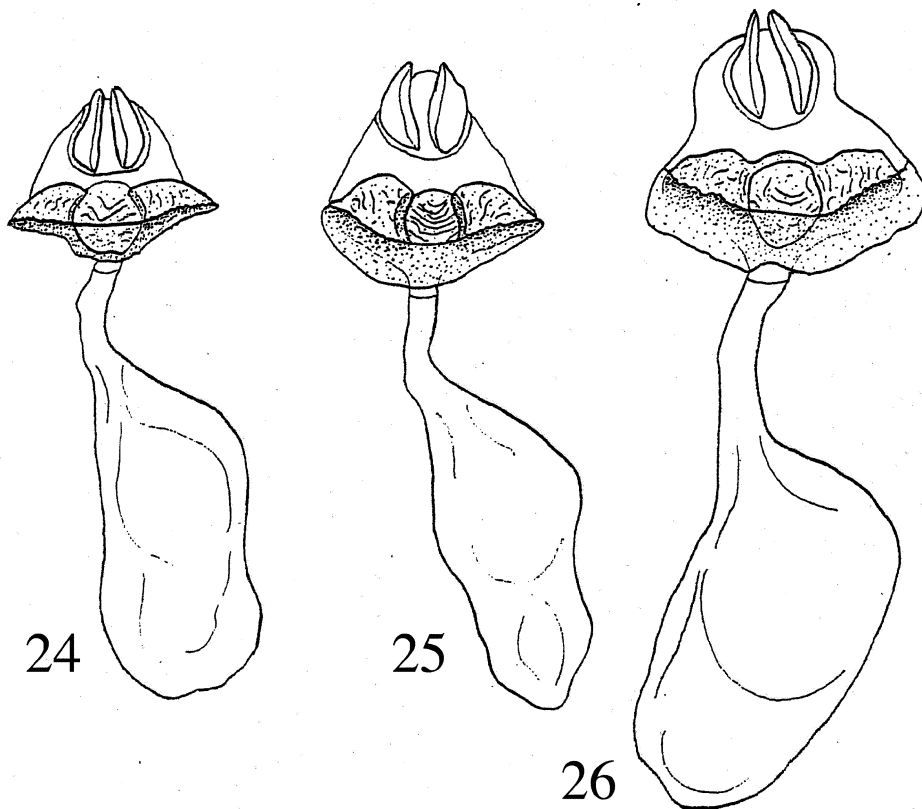
As noted above, previous studies have not indicated geographical variation within *A. bredowii*, but did not examine specimens from the apparent northern end of the species' range in Sinaloa and Durango. As shown in Figs. 1–6, patterns of geographical variation in adult wing morphology in *A. bredowii* are prominent, and are similar to those seen in *A. eulalia*, but are not exactly parallel. Like *A. eulalia*, adults of *A. bredowii* from the northern end of its range have narrower white postdiscal bands on all wings, and a longer forewing apex, compared to adults from the southern end of its range in Oaxaca. However, the patterns of variation seen in ventral blue-violet coloration and in the development of ventral hindwing black markings is the opposite of that seen

in *A. eulalia*, with southern populations of *A. bredowii* (e.g., Oaxaca) generally possessing the deeper blue-violet coloration and better-developed black markings.

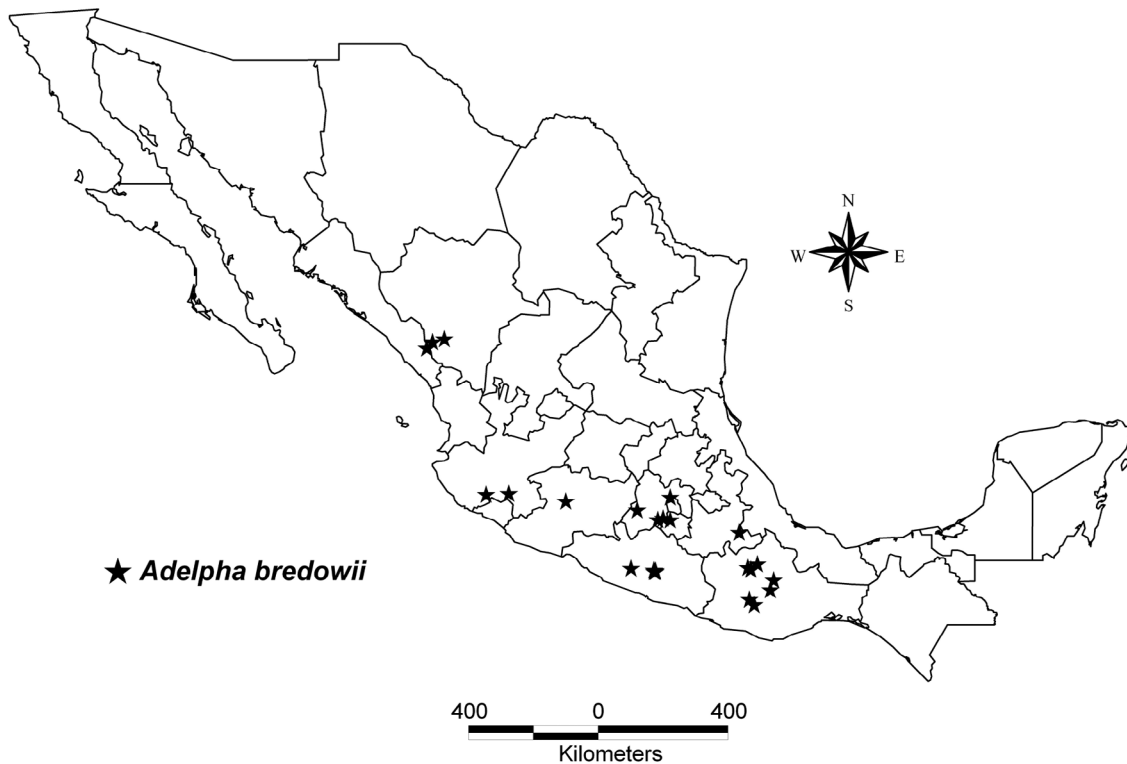


**FIGURES 18–23.** Male genitalia of *A. bredowii*, *A. eulalia*, and *A. californica*, showing genital capsule in left-lateral view (with left valve attached), inner surface of right valve, tegumen and uncus in dorsal view, and penis in left-lateral and dorsal views. **Figs. 18** (Mexico: Mexico State: km. 14, carretera Ocuilan – Cuernavaca, 15-III-1990), **19** (Mexico: Oaxaca, Xiacui, 22-III-1992) of *A. bredowii*. **Figs. 20** (Mexico: Puebla: Apulco, XI-1944; vesica slightly everted), **21** (Mexico: Chihuahua: Mesa de la Arena, 13-XI-1972) of *A. eulalia*. **Figs. 22** (USA: California: Siskiyou County: nr. McCloud, 24-VI-1967), **23** (USA: California: Maricopa County: Yosemite National Park, 14-VI-1961) of *A. californica*.





**FIGURES 24–26.** Female genitalia of *A. bredowii*, *A. eulalia*, and *A. californica*, in ventral view. **Fig. 24** (Mexico: Guerrero: Cueva del Borrego, 5-XI-1985) of *A. bredowii*. **Fig. 25** (Mexico: Chiapas: Montebello, IX-1947) of *A. eulalia*. **Fig. 26** (USA: California: Siskiyou County: nr. McCloud, 24-VI-1967) of *A. californica*.



**FIGURE 27.** Mexican distribution of *Adelpha bredowii*. Lines indicate state boundaries.

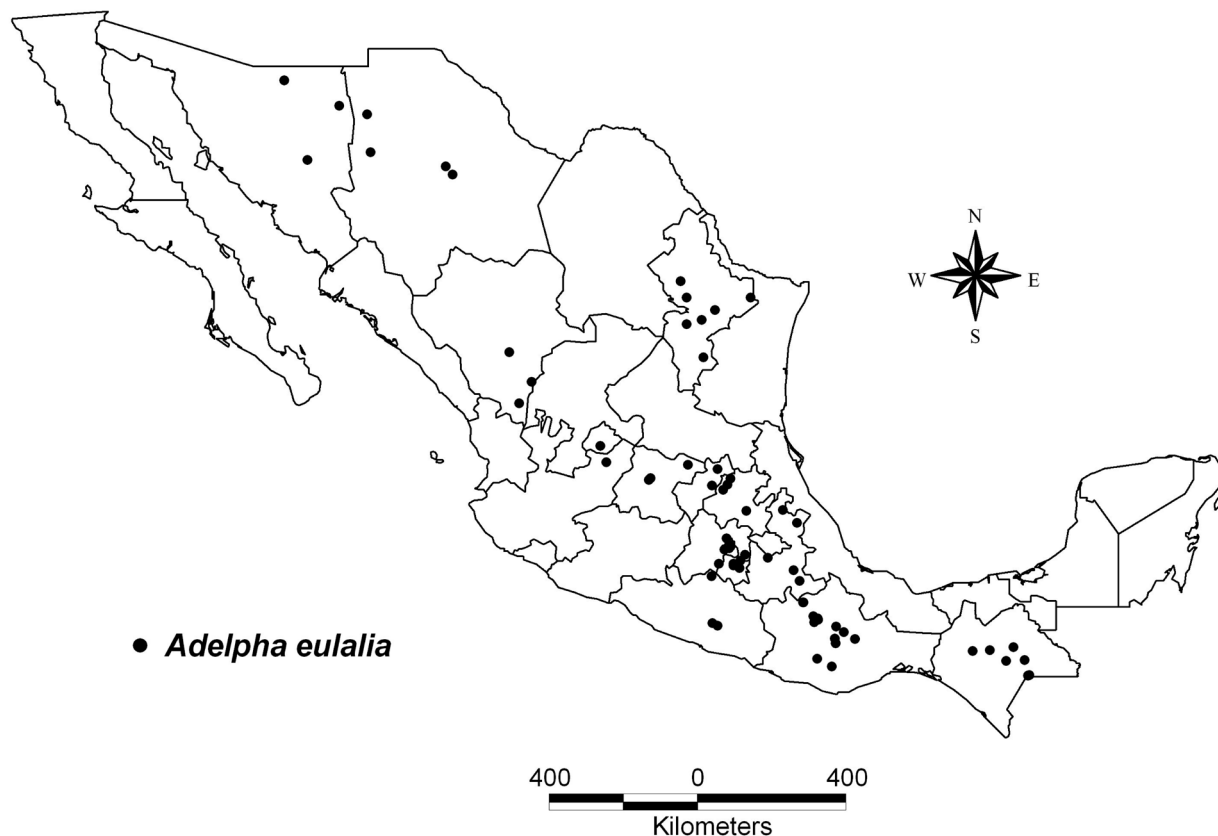
As noted in the introduction, *A. bredowii* was described from Mexico. The syntype female is an extremely close match to females of *A. bredowii* examined in this study from Oaxaca (e.g., Figs. 1–2) and Guerrero. Considering the phenotype of the syntype female of *A. bredowii* (color photograph provided by K. Willmott), patterns of geographic variation in the species, and travel routes likely to have been accessible when this specimen was collected before 1837, the most likely origin of this individual is near Oaxaca City, Oaxaca, where *A. bredowii* is currently known to be widespread and locally abundant (Appendix I). To preserve stability of nomenclature, this specimen is here designated *Lectotype*, and an appropriate label will be added.

Considering the results from our molecular and morphological analyses, we propose the following taxonomy: *Adelpha bredowii* Geyer, 1837 Mexican Sister, *Adelpha eulalia* **rev. stat.** (Doubleday, [1848]) Arizona Sister = *guatemalensis* (Carpenter & Hobby, 1944), and *Adelpha californica* **rev. stat.** (Butler, 1865) California Sister. *Adelpha bredowii* is endemic to western Mexico, occurring in the Sierra Madre Occidental, Sierra Madre del Sur, southern slopes of the Sierra de Juárez in Oaxaca and Puebla, and the Eje Neovolcánico east to southern México State and Morelos (Fig. 27). As indicated in Appendix I, we examined specimens of *A. bredowii* from the states of Durango, Sinaloa, Jalisco, México, Puebla, Guerrero and Oaxaca. All specimens studied were collected between 1800 and 2600 m elevation, in pine-oak or cloud forest habitats. Field (1940) reported *A. bredowii* (as “*Heterochroa bredowii*”) from Km. 90, road to Autlán, Jalisco (September), and Carpa, Michoacán (September). Carpenter & Hobby (1944) recorded specimens of *A. bredowii* (as “*Limenitis b. bredowii*”) from Uruapan, Michoacán; Cuernavaca, Morelos; Xocumanatlán (misspelled “Xucumanatlan”) and Omiltemi, Guerrero; and 12 miles southwest of Oaxaca, Oaxaca, 6100’, in addition to the records cited by Field (1940). De la Maza (1987) correctly identified *A. bredowii* (as “*A. b. bredowii*”), figured a male from Cruz de Ocote, Guerrero, and (p. 119) noted Valle de Bravo, México State; Cruz de Ocote and Filo de Caballo, Guerrero; Autlán, Jalisco; and Uruapan, Michoacán, as localities from which the taxon had been recorded, all from the month of March. Willmott (2003a) examined most of the specimens of *A. bredowii* (as “*A. b. bredowii*”) cited by Carpenter & Hobby (1944), and added Río Balsas, Guerrero (April), as an additional collection locality for the taxon. Willmott (2003a) also discussed the records of *A. bredowii* cited by Carpenter & Hobby (1944) from “Guatemala,” without specific locality data, and concurred that they are likely to be mislabeled Mexican specimens. *Adelpha bredowii* appears to fly in at least two annual broods, with most records from March to early May, and from August to early November, with a few records from July (Appendix I). The restricted distribution of *A. bredowii* suggests that it is likely to utilize only one or a few species of *Quercus* as larval foodplants, although to date, no larval foodplants for *A. bredowii* have been recorded.

Probably because of its restricted range, *A. bredowii* is uncommon in collections (as noted by Carpenter & Hobby 1944; Willmott 2003a), although adults are known to be locally and seasonally abundant at some sites in Guerrero and Oaxaca (see Appendix I). Only a few female specimens of *A. bredowii* are known (we examined only nine), yet ironically, the lectotype of *A. bredowii* is a female (Geyer 1837; Willmott 2003a). Carpenter & Hobby (1944) failed to recognize this, and erroneously claimed to have figured the only known female specimen of *A. bredowii*. In fact, the female specimen figured by Carpenter & Hobby (1944, p. 344), from “Mexico,” supposedly of *A. bredowii*, is a female of *A. eulalia*.

*Adelpha eulalia* is by far the most widespread of the three species in the *A. bredowii* complex. While its distribution north of the Mexican border is well known (see introduction and Willmott 2003a), confusion with *A. bredowii* in the past has obscured its distribution in Mexico (Fig. 28). As noted in Appendix I, we examined Mexican specimens of *A. eulalia* from the states of Sonora, Chihuahua, Durango, Nuevo León, San Luis Potosí / Querétaro, Guanajuato, Aguascalientes, Jalisco, Hidalgo, Puebla, Morelos, Distrito Federal, México, Guerrero, Oaxaca, and Chiapas. Carpenter and Hobby (1944, p. 322) recorded Mexican specimens of *A. eulalia* (as “*Limenitis b. eulalia*” and “*L. b. guatemalensis*”) from Chihuahua; Durango City, Durango; Pedregal de San Ángel, Distrito Federal; Amecameca (misspelled “Amecamecca”), México State; Puebla; Oaxaca; and Chiapas. De la Maza (1987, p. 247) correctly identified *A. eulalia* (as “*A. b. eulalia*”), figured a male from





**FIGURE 28.** Mexican distribution of *Adelpha eulalia*. Lines indicate state boundaries.

Pedregal, Distrito Federal, and noted its distribution in Mexico to include Madera, Chihuahua; Monterrey, Nuevo León; Michilía, Durango; La Encarnación, Hidalgo; Tepoztlán, Morelos; Ixtlán, Oaxaca; and Padierna and Tlalpan, Distrito Federal. Records were indicated from July to December. De la Maza & de la Maza (1993, p. 77) figured a live adult of *A. eulalia* (as "*Adelpha bredowi*") and a pinned male (p. 106) of *A. eulalia* (as *A. b. guatemalena* [sic!]) from Chiapas, and (p. 189) noted its distribution in Chiapas to include the "Macizo Central" and the "Depresión Central" of the state, in "cálido-templada semihúmeda," between 1000 and 2000 m. As indicated by Carpenter and Hobby (1944), the range of *A. eulalia* (as "*Limenitis b. guatemalensis*") also extends into Guatemala, and Willmott (2003a) suggested that it might also occur in western Honduras. Willmott (2003a) examined 386 males and 190 females of *A. eulalia*, mostly from the United States of America. Among the 59 males and 37 females he examined from Mexico, sixteen specimens were from Chiapas (February, August, September), twenty-four from Chihuahua (June, September), nineteen from Distrito Federal (April, August through October), two from Durango (June), seven from Guerrero (October, November), five from Hidalgo (April, August), four from México State (April, July), one from Morelos (August), two from Nuevo León (June), four from Oaxaca (September), four from Puebla (April, November), and four from Sonora (August, September), while three lacked specific locality data. One female examined from the "Bolaños-Guadalajara Rd." (July) is from either Jalisco or Zacatecas (specimens examined by Willmott from the Allyn Museum of Entomology [now the McGuire Center for Lepidoptera and Biodiversity] are also listed in Appendix I).

*Adelpha eulalia* occurs in a wide variety of habitats where *Quercus* species grow, from near sea level in southern Texas, United States (M. Overton pers. comm. 2005), to over 2670 m in central Mexico. As noted by Ferris & Brown (1981), preferred habitats include riparian canyons, and males are frequently found at moist

sandy areas along rivers and streams. This species appears to fly in at least two annual broods throughout much of its range. In central and southern Mexico, these appear to largely correspond with the timing of broods of *A. bredowii* (March through early May, August through early November), although records from San Luis Potosí, Oaxaca and Chiapas indicate that the species may fly in three or more annual broods at some localities (Appendix I; Beutelspacher 1983; de la Maza & de la Maza 1988). Records from Chihuahua, Sonora (see Appendix I; Willmott 2003a), New Mexico (Toliver *et al.* 1994), Arizona (Bailowitz & Brock 1991), and southern Nevada (Austin & Austin 1980) indicate a slightly later timing of the first brood at the northern end of the range of *A. eulalia*, roughly from April through June, with adults flying again from August to December. Emmel & Emmel (1973) reported a single annual brood of *A. eulalia* in far southeastern California, with adults from May to July.

The wide range of habitats inhabited by *A. eulalia* suggests that it is somewhat of a generalist in its food-plant preferences, undoubtedly utilizing a wide range of *Quercus* species. Harry (1994) reported *Quercus turbinella* and *Q. alba* as larval foodplants of *A. eulalia* in Utah, USA, and illustrated the life history in detail. *Quercus macrocarpa* and *Q. fusiformis* are known to be larval foodplants for *A. eulalia* in central Texas (Dale Clark, pers. comm. 2007). Sporadic freshly eclosed late-summer adults of *A. eulalia* in eastern Colorado (where the species is not a regular resident) suggest that it can also utilize *Quercus gambellii*, the only available *Quercus* in the region, at least for summer broods (A. Warren, pers. obs. 1990's).

Even though Carpenter & Hobby (1944) treated *A. bredowii* and *A. eulalia* as subspecies of a single species, they reported both taxa from Oaxaca. They seemed somewhat perplexed by the presence of both taxa in apparent sympatry, and (p. 323) noted "The race *b. eulalia* overlaps *b. bredowii* in South Mexico, and more specimens from Guerrero and Puebla are greatly desired." In his discussion of the geographic range of *A. bredowii*, Willmott (2003a) noted "Whether *A. b. bredowii* and *A. b. eulalia* are truly sympatric over such a large area, or locally allopatric, and whether the forms are connected by intergrades, requires field study and/or examination of Mexican collections with more material." We now know that *A. bredowii* and *A. eulalia* fly in exact sympatry and synchrony at four sites in the state of Oaxaca (Mpio. Cuicatlán: ca. 3 km E Tutepe-tongo, 5300'; Mpio. San Juan Ixtaltepec: camino a Almoloyas, 6700'; Mpio. Teotitlán del Valle / Díaz Ordaz: ca. 8–10 mi N Teotitlán del Valle, ca. 8000'; Mpio. Villa Sol de Vega / Santa María Jacatepec: 3–13 mi N Sol de Vega on the road to Las Grutas de San Sebastián, 5500–6500'; see Appendix I), and available records suggest the two species also fly in sympatry at least in México State and northern Morelos. *Adelpha bredowii* and *A. eulalia* potentially occur in sympatry over a large area in western Mexico, including parts of western Durango, Jalisco, Guerrero, Oaxaca, and possibly in northern Michoacán. It is hoped that future field workers will investigate this possibility.

*Adelpha californica* is endemic to western North America, occurring from northern Baja California, Mexico, northward to Washington State, and east only to far western Nevada (see introduction). While this species is resident throughout the western third of Oregon (Warren 2005), it is scarce in the Portland area (Newcomer 1964), and scattered records of *A. californica* from Washington State suggest it occurs there only as a rare vagrant (Pyle 2002). Despite the unpredictable nature of occasional vagrant individuals, the distribution of *A. californica* is well known, as summarized by Stanford & Opler (1993) and Willmott (2003a). This is the northernmost occurring species of *Adelpha*, and it is a prominent feature of Californian oak woodland habitats. Probably because of this, its immatures and ecology have been studied by various authors.

The pupa of *A. californica* was described by Edwards (1873). Dyar (1891) was the first to provide a relatively complete account of the early stages of *A. californica*, from Yosemite, California. Subsequently, Comstock (1927) illustrated a pupa of *A. californica*, and Comstock & Dammers (1932) described and illustrated some of the other immature stages. Dammers in Emmel & Emmel (1973, p. 46; also see Garth & Tilden 1986: pl. 1, i, j) figured a full-grown larva and pupa of *A. californica*, and a late-instar larva was illustrated by Miller & Hammond (2003). Larval foodplants for *A. californica* include various species of *Quercus*, including *Q. chrysolepis*, *Q. agrifolia*, *Q. kelloggii*, and *Q. garryana* (Comstock 1927; Emmel & Emmel 1973; Orsak

1977; Brown *et al.* 1992; Pyle 2002; Warren 2005), probably as well as additional *Quercus* species (Comstock 1927), and it has been reported to use *Chrysolepis chrysophylla* at higher elevations in the Cascade Mountains of Oregon (Warren 2005).

As with *Adelpha eulalia*, adults of *A. californica* can be abundant in areas where larval foodplants are common, from sea level, to over 6500' in Oregon (Warren 2005), and up to 9920' in the western Sierra Nevada of California (Davenport 2004). As summarized by Pyle (2002) and Warren (2005), males of *A. californica* frequently perch along riparian corridors and roads, usually in forested settings, recently eclosed males visit mud, and both sexes visit flowers, fermenting fruit, sap and scat. Depending on seasonal conditions and elevation, *A. californica* may produce between one and three broods annually, during the warmer months of the year (usually March through November.)

## Conclusion

The processes governing diversification within the *A. bredowii* complex are not currently understood. A common diversification mechanism in butterfly species is related to foodplant usage (*e.g.*, Pratt 1994; Pratt *et al.* 1994; Hebert *et al.* 2004), but we lack foodplant records for *A. bredowii*, and also lack foodplant records for *A. eulalia* from Mexico. A possible speciation mechanism for the *A. bredowii* complex may be geographical isolation followed by subsequent range expansions into areas of sympatry (*e.g.*, Hewitt 2004). The two sympatric taxa, *A. bredowii* and *A. eulalia*, may be experiencing stabilizing selection as putative Müllerian mimics. *Adelpha californica* is known to be an unpalatable, Batesian model for *Limenitis lorquini* Boisduval, 1852 (Prudic *et al.* 2002; also see Poulton 1909; Porter 1988). Assuming both *A. bredowii* and *A. eulalia* are also unpalatable, they may be co-models in areas of sympatry in Mexico. Regardless, future research on all three species is necessary to elucidate the precise diversification mechanisms and details of putative mimetic relationships.

This study has demonstrated cryptic diversity within the *Adelpha bredowii* species complex, and has exciting implications for rest of the genus *Adelpha*. This genus is one of the most diverse in the neotropics (Willmott 2003a; Lamas 2004), ranging from the northwestern USA to southern Uruguay. In the Andes Mountains of South America, 35 species of *Adelpha* have been recorded from a single location (Willmott 2003a). Even though dorsal wing patterns of many *Adelpha* species closely resemble one another, there is often marked variation in larval morphology and foodplant usage between species (Aiello 1984). Collectively, these patterns allude to the possibility of many cryptic lineages within *Adelpha*. It is hoped that future molecular studies will focus on additional groups of *Adelpha* taxa, in order to establish a framework through which important aspects of *Adelpha* biology, such as mimicry, adaptation, and speciation, may be investigated. Our results further emphasize that molecular techniques such as DNA sequence analysis, practiced together with traditional morphological methods, are powerful and necessary tools for taxonomic research and that these approaches will have a continuing, forcible impact on documenting and describing insect biodiversity.

## Acknowledgments

We especially thank Armando Luis-Martínez (Museo de Zoología "Alfonso L. Herrera," Universidad Nacional Autónoma de México, Mexico City) for collecting permits, transportation, and help in the field, as well as access to the MZFC collection. We thank John Kemner (Oaxaca City, in collaboration with MZFC) for field assistance in 2003 and 2005–2007, Thomas W. Ortenburger (Wheatridge, Colorado) and Jose Luis Salinas-Gutierrez (Chetumal, Quintana Roo) for field assistance in 2001, Ray E. Stanford (Medford, Oregon) for field assistance in 1998, and Sally J. Warren (Castle Rock, Colorado) for field assistance and funding in 2003. Spe-

cial thanks are extended to María Eugenia Díaz Batres (Museo de Historia Natural de la Ciudad de México), Patricia Escalante (Instituto de Biología, Universidad Nacional Autónoma de México) and Rafael Turrent (Mexico City) for allowing A. D. W. to examine specimens under their care. Thanks to George T. Austin, Thomas C. Emmel, Jacqueline Y. and Lee D. Miller, and Andrei Sourakov, McGuire Center for Lepidoptera and Biodiversity, for providing research facilities and collections access to A. D. W., and very special thanks to Marysol Trujano Ortega (MZFC) for providing distribution maps, and Isabel Vargas-Fernández (MZFC) for countless favors. We also thank Keith Willmott (McGuire Center) for providing a color photograph of the syn-type female of *A. bredowii* as well as other images and information, Richard S. Peigler for literature, and Jim P. Brock, John F. Emmel, Dale Clark and Mike D. Overton for information. George T. Austin, Andrew V. Z. Brower, Jeff Oliver, Jonathan P. Pelham and Keith Willmott provided many helpful comments on previous versions of the manuscript. This research was funded by NSF Doctoral Dissertation Improvement Grant DEB-0228701, a Center for Insect Science Graduate Research Award (University of Arizona), and a BIO5: Institute of Collaborative Bioresearch Fellowship (University of Arizona) to K.L.P. Additional funding was provided to A.D.W. through NSF Doctoral Dissertation Improvement Grant DEB-039005 and through DGAPA-UNAM during the conclusion of this study. DGAPA-IN 212006 provided very important funding to J.L.B.

## References

- Aiello, A. (1984) *Adelpha* (Lepidoptera: Nymphalidae): deception on the wing. *Psyche*, 91, 1–45.
- Austin, G.T. & Austin, A.T. (1980) Butterflies of Clark County, Nevada. *Journal of Research on the Lepidoptera*, 19(1), 1–63.
- Avise, J.C. & Walker D.E. (1999) Species realities and numbers in sexual vertebrates: Perspectives from an asexually transmitted genome. *Proceedings of the National Academy of Sciences USA*, 96, 992–996.
- Beutelspacher, C.R. (1983) Mariposas diurnas de “El Chorreadero”, Chiapas (Insecta: Lepidoptera). *Anales del Instituto de Biología, Universidad Nacional Autónoma de México (Zoología)*, 53(1), 341–366.
- Bailowitz, R.A. & Brock, J.P. (1991) *Butterflies of Southeastern Arizona*. Sonoran Arthropod Studies, Inc., Tucson. ix + 342 pp.
- Beltrán, M., Jiggins, C.D., Bull, V., Linares, M., Mallet, J., McMillan, W.O. & Bermingham, E. (2002) Phylogenetic discordance at the species boundary: comparative gene genealogies among rapidly radiating *Heliconius* butterflies. *Molecular Biology and Evolution*, 19, 2176–2190.
- Brower, A.V.Z. (2006) Problems with DNA barcoding for species delimitation: ‘ten species’ of *Astrartes fulgurator* reassessed (Lepidoptera: HesperIIDae). *Systematics and Biodiversity*, 4(2), 127–132.
- Brown, J.W., Real, H.G. & Faulkner, D.K. (1992) *Butterflies of Baja California. Faunal Survey, Natural History, Conservation Biology*. The Lepidoptera Research Foundation, Incorporated, 129 pp.
- Burns, J.M. (1997) Presidential address 1996: on the beauties, uses, variation, and handling of genitalia. *Journal of the Lepidopterists’ Society*, 51(1), 1–8.
- Butler, A.G. (1865) Description of the characters of six new species of Rhopalocerous Lepidoptera in the collection of the British Museum, with notes on the allied species. *Proceedings of the Zoological Society of London*, 1865(2), 484–486.
- Carpenter, G.D.H. & Hobby, B.M. (1944) On *Limenitis bredowii* Geyer (Lep., Nymphalidae) with description of a new subspecies and revival of another. A study in geographical distribution and speciation. *Transactions of the Royal Entomological Society of London*, 94, 311–346.
- Comstock, J.A. (1927) *Butterflies of California. A Popular Guide to a Knowledge of the Butterflies of California Embracing all of the 477 Species and Varieties at Present Recorded from the State*. Published by author, Los Angeles, California, USA, 63 pp.
- Comstock, J. A. & Dammers, C.M. (1932) The metamorphoses of *Heterochroa bredowii californica* Butl. (Lepid.). *Bulletin of the Southern California Academy of Sciences*, 30(3), 83–87.
- Davenport, K. (2004) The Yosemite butterflies. *The Taxonomic Report*, 5(1), 1–74.
- DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B*, 360(1462), 1905–1916.
- dos Passos, C.F. (1964) A synonymic list of the Nearctic Rhopalocera. *Memoirs of The Lepidopterists’ Society*, 1, 1–145.
- Doubleday, E. (1848) *The genera of diurnal Lepidoptera: comprising their generic characters, a notice of their habits and transformations and a catalogue of the species of each genus*. Longman, Green & Longmans, London, United

- Kingdom, 250 pp.
- Dyar, H.G. (1891) Descriptions of some butterfly larvae from Yosemite.- IV. *The Canadian Entomologist*, 23(12), 278–281.
- Edwards, W.H. (1868–1897) *The Butterflies of North America*. Boston, Houghton, Mifflin & Co., Boston, Massachusetts, USA.
- Edwards, W.H. (1870–1873) *Synopsis of North American butterflies*. The American Entomological Society, Philadelphia, Pennsylvania, USA.
- Emmel, T.C. & Emmel, J.F. (1973) *The Butterflies of Southern California*. Natural History Museum, Los Angeles, California, USA, 148 pp.
- Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. (1995) Testing significance of incongruence. *Cladistics*, 10, 315–319.
- Ferris, C.D. (1989) Supplement to: A Catalogue/Checklist of the Butterflies of America North of Mexico. *Memoirs of the Lepidopterists' Society* 3(8), 1–103.
- Ferris, C.D. & Brown, F.M. (1981) *Butterflies of the Rocky Mountain States*. University of Oklahoma Press. Norman, Oklahoma, USA, 442 pp.
- Field, W.D. (1940) A distributional note on *Heterochroa bredowii* (Gayer) (Lepidoptera, Nymphalidae). *Journal of the Kansas Entomological Society* 13, 123.
- Garth, J. S. & Tilden, J.W. (1986) *California Butterflies*. California Natural History Guide 51. University of California Press. Berkeley, California, USA, 246 pp.
- Geyer, C.M. (1837) *Zuträge zur Sammlung Exotischer Schmettlinge*. Berlin, Germany.
- Godman, F.D., & Salvin, O. (1879–1901) *Biologia Centrali Americana. Zoología, Insecta, Lepidoptera Rhopalocera. Vols. II and III*. Dulau & Co., Bernard Quaritch. London, UK, 487 pp.
- Harry, J.L. (1994) *Adelpha bredowi eulalia* in Utah: a photo life history (Lepidoptera: Nymphalidae). *Holarctic Lepidoptera*, 1, 62–63.
- Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B*, 270, S96–S99.
- Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly, *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, 101, 14812–14817.
- Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London B*, 359, 183–195.
- Hoffmann, C.C. (1940) Catálogo sistemático y zoogeográfico de los Lepidópteros Mexicanos. Primera parte, Papilionoidea. *Anales del Instituto de Biología. Universidad Nacional Autónoma de México*, 11(2), 639–739.
- Holland, W.J. (1931) *The Butterfly Book, New and Thoroughly Revised Edition. A Popular and Scientific Manual, Describing and Depicting All the Butterflies of the United States and Canada*. Doubleday, Doran & Co., Garden City, New York, USA, 424 pp.
- Hovanitz, W. (1945) Geographical regularity in the variation and supposed mimicry of a butterfly, *Limnitis bredowii*. *The American Naturalist*, 79(784), 472–474.
- Huelsenbeck, J.P. & Rannala, B. (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, 276, 227–232.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.
- Kirby, W.F. (1871) *A Synonymic Catalogue of Diurnal Lepidoptera*. Van Voorst, London, UK, 690 pp.
- Lamas, G. (2004) *Limnitiidae. Checklist: Part 4A. Atlas of Neotropical Lepidoptera*. Scientific Publishers, Gainesville, Florida, USA, 439 pp.
- Lipscomb, D., Platnick, N. & Wheeler, Q. (2003) The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends in Ecology and Evolution*, 18, 65–66.
- Llorente-Bousquets, J., Luis-Martínez, A. & Vargas-Fernández, I. (2006) Apéndice general de Papilionoidea: lista sistemática, distribución estatal y provincias biogeográficas, pp. 945–1009. In: Morrone, J. J. & J. Llorente-Bousquets (Eds.). *Componentes Bióticos Principales de la Entomofauna Mexicana*. Las Prensas de Ciencias, UNAM, Mexico City, Mexico, 1025 pp.
- Luis-Martínez, A., Llorente-Bousquets, J. & Vargas-Fernández, I. (2003) *Nymphalidae de México I (Danainae, Apaturinae, Biblidinae y Heliconiinae): distribución geográfica e ilustración* Facultad de Ciencias, Universidad Nacional Autónoma de México, and Comisión Nacional Para el Conocimiento y Uso de la Biodiversidad (CONABIO), Mexico City, Mexico, 249 pp.
- Mallet, J. & K.R. Willmott, K.R. (2003) Taxonomy: renaissance or tower of Babel? *Trends in Ecology and Evolution*, 18, 57–59.
- de la Maza, R.F. (1987) *Mariposas Mexicanas*. Fondo de Cultura Económica, S.A. de C.V., Mexico City, Mexico, 302 pp.
- de la Maza, R.G. & de la Maza, J. (1988) Notas sobre los Rhopalocera de la Sierra de Álvarez, San Luis Potosí, México (Lepidoptera). *Revista de la Sociedad Mexicana de Lepidopterología*, 11(2), 33–59.

- de la Maza, R.G. & de la Maza, J. (1993) *Mariposas de Chiapas*. Gobierno del Estado de Chiapas, Mexico, 223 pp.
- de la Maza, R.G., de la Maza, J. & White, A. (1989) La fauna de mariposas de México. Parte I. Papilionoidea (Lepidoptera: Rhopalocera). *Revista de la Sociedad Mexicana de Lepidopterología*, 12(2), 39–98.
- Miller, J.C. & Hammond, P.C. (2003) *Lepidoptera of the Pacific Northwest: caterpillars and adults*. United States Department of Agriculture, Forest Health Technology Enterprise Team, Washington, D.C. 324 pp.
- Miller, L.D. & Brown, F.M. (1981) A catalogue/checklist of the butterflies of America north of Mexico. *Memoirs of the Lepidopterists' Society*, 2, 1–280.
- Moritz, C. & Cicero, C. (2004) DNA barcoding: promise and pitfalls. *PLoS Biology*, 2(10), 1529–1531.
- Newcomer, E.J. (1964) Occurrence of certain Rhopalocera in Oregon and Washington. *Journal of the Lepidopterists' Society*, 18(1), 47–50.
- Orsak, L.J. (1977) *The butterflies of Orange County, California*. Museum of Systematic Biology Research Series no. 4, University of California, Irvine, USA, 349 pp.
- Porter, A.H. (1988) Courtship of a model (*Adelpha*; Nymphalidae) by its probable Batesian mimic (*Limenitis*; Nymphalidae). *Journal of Research on the Lepidoptera*, 26, 255–256.
- Poulton, E.B. (1909) Mimicry in the butterflies of North America. *Annals of the Entomological Society of America*, 11(4), 203–242.
- Pratt, G.F. (1994) Evolution of *Euphilotes* (Lepidoptera) by seasonal and host shifts. *Biological Journal of the Linnean Society*, 51, 387–416.
- Pratt, G.F., Wright, D.M. & Pavulaan H. (1994) The various taxa and hosts of the North American *Celastrina* (Lepidoptera: Lycaenidae). *Proceedings of the Entomological Society of Washington*, 96(3), 566–578.
- Prudic, K.L., Shapiro, A.M. & Clayton, N.S. (2002) Evaluating a putative mimetic relationship between two butterflies, *Adelpha bredowii* and *Limenitis lorquini*. *Ecological Entomology*, 27, 68–75.
- Pyle, R.M. (2002) *The Butterflies of Cascadia. A Field Guide to all the Species of Washington, Oregon, and Surrounding Territories*. Seattle Audubon Society, Seattle, Washington, USA. 420 pp.
- Seitz, A. (1907–1935) *Grossschmetterlinge der Erde*. Fritz Lehmann's Verlag, Stuttgart, Germany.
- Skinner, H. (1911) *Adelpha (Heterochroa) bredowii* and *californica* (Lepidoptera). *Entomological News*, 22(9), 414–415.
- Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W. & Hebert, P.D. (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences USA*, 103(10), 3657–3662.
- Stanford, R.E. & Opler, P.A. (1993) *Atlas of western USA butterflies, including adjacent parts of Canada and Mexico*. Published by authors, Denver, Colorado, USA, 275 pp.
- Stout, T.L. (2005) Fundamental differences between the California Sister and the Arizona Sister (*Adelpha bredowii californica* vs. *Adelpha bredowii eulalia*). *Bulletin of the Utah Lepidopterists' Society*, 12(1), 3.
- Swofford, D.L. (2001) PAUP\* Phylogenetic Analysis Using Parsimony and Other Methods. Version 4.0b10. Sinauer Associates, Sunderland, MA.
- Tautz, D., Arctander, P., Minelli, A.R.H. & Vogler, P. (2003) A plea for DNA taxonomy. *Trends in Ecology and Evolution*, 18, 70–74.
- Toliver, M.E., Holland, R. & Cary, S.J. (1994) *Distribution of Butterflies in New Mexico (Lepidoptera: Hesperioidea and Papilionoidea)*, Second Edition. Published by authors, Albuquerque, New Mexico, USA, 446 pp.
- Warren, A.D. (2005) *Lepidoptera of North America 6. Butterflies of Oregon. Their Taxonomy, Distribution, and Biology*. Contributions of the C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado, USA, 408 pp.
- Warren, A.D., Llorente-Bousquets, J.E., Luis-Martínez, J.A. & Vargas-Fernández, I. (2007) *Interactive listing of Mexican butterflies. Listado interactivo de la mariposas mexicanas*. <http://www.mariposasmexicanas.com>
- Westwood, J.O. (1850) *Genus LV. Heterochroa, the genera of diurnal Lepidoptera: comprising their generic characters, a notice of their habits and transformations and a catalogue of the species of each genus*. Longman, Green & Longmans, London, UK.
- Willmott, K.R. (2003a) *The genus Adelpha: its systematics, biology and biogeography (Lepidoptera: Nymphalidae: Limenitidini)*. Scientific Publishers, Gainesville, Florida, USA, 322 pp.
- Willmott, K.R. (2003b) Cladistic analysis of the Neotropical butterfly genus *Adelpha* (Lepidoptera: Nymphalidae), with comments on the subtribal classification of Limenitidini. *Systematic Entomology*, 28, 279–322.
- Zakharov, E.V., Smith, C.R., Lees, D.C., Cameron, A., Vane-Wright, R.I. & Sperling, F.A.H. (2004) Independent gene phylogenies and morphology demonstrate a Malagasy origin for a wide-ranging group of swallowtail butterflies. *Evolution*, 58, 2763–2782.

## Appendix I

Locality data for Mexican specimens of *A. bredowii* and *A. eulalia* examined in this study. Records marked with an asterisk (\*) indicate that the genitalia of that specimen were examined. ADW = Collection of Andrew D. Warren, Castle Rock, Colorado, United States; IBUNAM = Instituto de Biología, Universidad Nacional Autónoma de México Mexico City, Mexico; MGCL = McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, Florida, United States; MHNCM = Museo de Historia Natural de la Ciudad de México, Mexico City, Mexico; MZFC = Museo de Zoología, Departamento de Biología Evolutiva, Universidad Nacional Autónoma de México, Mexico City, Mexico; RT = Collection of Rafael Turrent, Mexico City, Mexico. m = male; f = female. Images of living individuals of *A. bredowii* and *A. eulalia* are provided by Warren *et al.* (2007) at: [http://www.mariposasmexicanas.com/adelpha\\_bredowii.htm](http://www.mariposasmexicanas.com/adelpha_bredowii.htm) and [http://www.mariposasmexicanas.com/adelpha\\_eulalia.htm](http://www.mariposasmexicanas.com/adelpha_eulalia.htm)

*Adelpha bredowii* (64 males, 9 females): **DURANGO**: Mpio. El Salto: Hwy. 40 at La Fragüita, 2350m, 29-IV-1998, A.D. Warren, 1m (ADW); Hwy. 40 at Mesa Redonda, 2370m, 29-IV-1998, 1m (ADW); Hwy. 40 at Palisades, 6500', 4-X-1970, R. Holland, 1m (ADW). **GUERRERO**: Mpio. Chilpancingo: Cueva del Borrego, Omiltemi, 1800m, 6-IV-1985, J. Llorente, A. Luis, 1m (MZFC); same locality and collectors, 18-VII-1985, 1m (MZFC); same locality and collectors, 5-IX-1985, 9m [1m\*] (MZFC); same locality, 18-X-1985, A. Luis, 1f (MZFC); same locality, 22-X-1985, J. Llorente, A. Luis, 1m (MZFC); same locality, 5-XI-1985, A. Luis, 1m\*, 2f [1f\*] (MZFC); Potrerillo, Omiltemi, 2300m, 5-V-1985, J. Llorente, A. Luis, 1m (MZFC); same locality and collectors, 1-VIII-1985, 2m [1m\*] (MZFC); same locality and collectors, 17-X-1985, 2m [1m\*] (MZFC); Omiltemi, 9-VII-1985, J. Llorente, A. Luis, 1m (MZFC); same locality, XI-1960, T. Escalante, 2m [1m\*] (MGCL). **JALISCO**: Mpio. Autlán: La Ordeñita, Las Joyas, 2000m, 22-III-1990, J. Llorente, A. Luis, 1m (MZFC); Mpio. Toliman: Floripandia, km 80, Carretera El Grullo – Cd. Guzmán, 25-III-1990, 2600m, J. Llorente, A. Luis, 1m\* (MZFC). **MÉXICO STATE**: Mpio. Atizapán de Zaragoza: cuenca vieja a Chinte, 27-IX-1997, A. González, 1m (RT); same locality and collector, 2-IV-1998, 1m (RT); Mpio. Tenancingo: Tenancingo, 20-VIII-1971, J. Llorente, 1m (MZFC); Mpio. Ocuilán: km 14, Carretera Ocuilán – Cuernavaca, 2000m, 15-III-1990, A. Luis, L. Rios, 1m\* (MZFC); same locality and collectors, 16-III-1990, 1m (MZFC); Mpio. Valle de Bravo: Valle del Bravo, IV-1984, J. Saldaña, 1f (IBUNAM). **OAXACA**: Mpio. San Juan Bautista Cuicatlán: ca. 3 km E Tutepetongo, 5300', N17°44' W96°52', 13-X-2006, J. Kemner, 4m (MZFC); same locality and collector, 18-X-2006, 5m (MZFC); same locality and collector, 6-XI-2006, 1m (MZFC); Mpio. San Juan Ixtaltepec: camino a Almoloyas, 6700', N17°35' W97°03', 13-III-2006, J. Kemner, 2m (MZFC); desviación Almoloyas, km. 2, arroyo, 6600', N17°38'992'' W97°08'387'', 10-VII-2005, J. Kemner, 1m (MZFC); Mpio. Santiago Xiacui: Xiacui, ca. 6000', 22-III-1992, J. Kemner (no. 422), 1m\* (MZFC); Mpio. Teotitlán del Valle / Díaz Ordáz: Rd. to Benito Juárez, ca. 8-10 mi N Teotitlán del Valle, ca. 8000', 22-VIII-2003, A.D. Warren, 14m, 3f (ADW); same locality and date, A. Luis, J. Kemner, 1m (MZFC); Mpio. Villa Sol de Vega / Santa Maria Jacatepec: 3-13 mi N Sol de Vega, camino a Las Grutas de San Sebastián, 5500-6500', 6-VII-1991, J. Kemner (no. 354), 1m (MZFC). **PUEBLA**: Mpio. Santiago Miahuatlán: Las Minas, Agua Escondida, 6800', N18°35' W97°22', 19-IX-2006, J. Kemner, 1m, 1f (MZFC). **SINALOA**: Mpio. Concordia: El Palmito, 1800m, 17-X-1987, A Luis & J. Llorente, 1f (MZFC); same locality, 16-IX-1987, G. Rodríguez, 1m (MZFC).

*Adelpha eulalia* (145 males, 68 females): **AGUASCALIENTES**: Picacho, III-1979, Reyes, 1m (IBUNAM); **CHIAPAS**: “Chiapas”, no date, J. Saldaña, 1f (IBUNAM); Montebello, IX-1947, T. Escalante, 3m, 3f [1f\*] (MGCL); Santa Rosa, 1300m, IX-1992, A. Ibarra, 1m (IBUNAM); Yagüech, Montebello, 1450m, 17-III-1980, J. Llorente, 1m, 1f (MZFC); Mpio. Comitán de Domínguez: Comitán, no date, J. Saldaña, 1m (IBUNAM); Santa Rosa Comitán, no date, T. Escalante, 1m\* (MGCL); same locality and collector, IV-1959, 1m, 1f\* (MGCL); same locality and collector, V-1961, 1m\* (MGCL); same locality and collector, IX-1963, 1f (MGCL); same locality and collector, VII-1965, 1m (MGCL); same locality and collector, VIII-1972, 3m, 2f (MZFC); Mpio. La Trinitaria: vic. Lagos de Montebello, 1500-1800m, 8-II-1969, L. D. & J. Y. Miller, 1f (MGCL); Mpio. Ocosingo: Campet, 7-10-VIII-1969, R. Wind, 1m (MGCL); Mpio. San Cristóbal de las Casas: San Cristóbal, IX-1983, J. Saldaña, 1m (IBUNAM); Mpio. Tuxtla Gutiérrez: El Chorreadero, XII-1975, C. Vázquez, 1m (IBUNAM); same locality, 7-VIII-1977, J. García P., 1f (IBUNAM); same locality and collector, 6-XI-1977, 1f (IBUNAM); same locality, 17-18-I-1980, C. R. Beutelspacher, 1m (IBUNAM). **CHIHUAHUA**: Ciénegas, 15-VI-1949, T. Escalante, 1f (MGCL); Mpio. Colonia Juárez: La Mesa de la Arema, SW of Colonia Juárez, 13-XI-1972, L. P. Gray, 1m\* (MGCL); Mpio. Madera: Madera, 4-III-1965, T. Escalante, 2m, 1f (IBUNAM), same locality, VI-1966, Díaz Francés, 1m, 4f (IBUNAM); same locality and date, T. Escalante, 6m, 7f (MGCL), 1f (MHNCM); same locality and collector, VIII-1966, 2f (MGCL); same locality and collector, VI-1967, 1f (MGCL). **DISTRITO FEDERAL**: “D. F.”, 1969, J. Llorente, 1f (MZFC); Cd. México, 18-X-1969, J. Llorente, 1f (MZFC); same locality and collector, 27-VIII-1970, 1m, 1f (MZFC); same locality and collector, 2-X-1970, 1m (MZFC); San Andrés Totolapan, 3-IV-1978, A. Ibarra, 1m (IBUNAM); Pedregal, VII-



1938, T. Escalante, 1f (MGCL); same locality and collector, VIII-1940, 2m (MGCL); same locality and collector, VIII-1959, 2m (MGCL); same locality and collector, IX-1961, 3m [1m\*], 3f [1f\*] (MGCL); same locality, date, and collector, 1f (IBUNAM); same locality and collector, 30-IX-1961, 1m (IBUNAM); same locality and collector, X-1961, 4m [1m\*], 2f (MGCL); same locality and collector, X-1961, 1m (MGCL); same locality and collector, IX-1962, 1m, 1f (IBUNAM); Delegación Coyoacán: Pedregal de San Ángel, 14-IV-1956, 1m (IBUNAM); same locality, 22-VIII-1959, 1m, 1f (MHNCM); same locality, 3-IX-1959, R. de la Maza, 1m (IBUNAM); same locality, XI-1959, 1f (IBUNAM); same locality, 29-VIII-1975, A. Turrent, 1m (RT); Delegación Magdalena Contreras: Dínamo 1, 2670m, 18-X-1982, A. Luis, 1f (MZFC); same locality and collector, 11-V-1983, 1m (MZFC); same locality and collector, 27-X-1983, 1f (MZFC); Delegación Tlalpan: Pedregal de Padierna, 19-VIII-1973, R. de la Maza, 1m (IBUNAM); Pedregal Tlalpan, 18-X-1969, J. Llorente, 1f (MZFC); same locality and collector, 18-VIII-1970, 1f (MZFC); Tlalpan, 26-VIII-1956, 1m (MHNCM); Villa Olímpica, 29-VIII-1971, J. Llorente, 1m (MZFC); same locality and collector, 25-IX-1971, 1f (MZFC). **DURANGO:** Mpio. La Michilia: Reserva La Michilia, 24-VIII-1983, M. E. Díaz Batres, 1f (MHNCM); same locality and collector, 10-IX-1983, 1m (MHNCM); same locality and collector, 14-IX-1983, 3f (MHNCM); same locality and collector, 13-X-1983, 3m, 1f (MHNCM); same locality and collector, 15-X-1983, 2m (MZFC), 1m, 1f (MHNCM); same locality and collector, 6-VI-1984, 1f (MHNCM); same locality and collector, 8-VI-1984, 2m, 3f (MHNCM). **GUANAJUATO:** Mpio. Dolores Hidalgo: hilltop ca. 5 km NE Santa Rosa on Hwy. 110, ca. 2600m, 28-III-2001, A.D. Warren, 1m (ADW); Mpio. Guanajuato: 3 hilltops ca. 6 km SW Santa Rosa on Hwy. 110, ca. 2400m, 28-III-2001, A.D. Warren, 1m (ADW); Mpio. Santa Catarina / Xichu: canyon below waterfall, ca. 16 km NW Misión Santa Rosa on Camino San Luis de la Paz – Xichu, 2300 – 2400m, 27-III-2001, A.D. Warren, T. W. Ortenburger, J. L. Salinas, 4m (ADW). **GUERRERO:** Balsas, IV, Müller, 1f (MHNCM); Torre de Microondas El Tejocote, 18-VIII-1984, A. Ibarra, 1m (IBUNAM); Mpio. Chilpancingo de los Bravo: Acahuizolta, VIII-1950, T. Escalante, 1m (MGCL); same locality and collector, X-1955, 2m [1m\*] (MGCL); same locality and collector, X-1958, 1m (MGCL); same locality, 20-IV-1978, A. Ibarra, 1m (IBUNAM); Mpio. Tetipac: Arroyo las Damas, Tetipac, 16-XII-1986, A. Luis, 1m (MZFC). **HIDALGO:** Mpio. Jacala de Ledezma: Puerto de Zorro, 18-IV-1972, H. L. King, 1f (MGCL); Mpio. Mineral del Chico: El Chico, 15-IX-1973, J. Llorente, 1m (MZFC); Mpio. Zimapán: La Encarnación, 14-III-1971, A. Turrent, 1f (RT); same locality, VII-1975, C. R. Beutelspacher, 3m (IBUNAM); same locality, 10-IV-1977, R. de la Maza, 1m (MHNCM); Mpio. Zimapán: 5 mi NE Zimapán, 2100m, 3-VIII-1973, 1f (MGCL). **JALISCO:** Mpio. Encarnación de Díaz: 10 km S Encarnación de Díaz, 2000m, 15-IX-1987, A. Luis, J. Llorente, 1m (MZFC). **MÉXICO STATE:** Mpio. Atizapán de Zaragoza: Atizapán, 11-VII-1964, Díaz Francés, 1f (IBUNAM); Mpio. Malinalco: Chalma, VII-1959, T. Escalante, 3m [1m\*] (MGCL). **MORELOS:** Cañada Acueducto Tilzapotla, 1150m, 27-I-1994, R. de la Maza, 1m (MHNCM); same locality and collector, 28-I-1994, 1m (MHNCM); Mpio. Cuautla: Cuautla, 12-I-1972, T. Carrajal M., 1f (IBUNAM); Mpio. Tepoztlán: Tepoztlán, I-1973, J. Saldaña, 1m (IBUNAM); same locality, 21-X-1984, L. Delgado, 1m (MZFC); Mpio. Yautepec: Cañón de Lobos, 25-IX-1965, A. Turrent, 1m (RT); Yautepec, VIII-1967, T. Escalante, 1m (MGCL); same locality and collector, VIII-1968, 2m (MZFC); same locality and collector, VIII-1973, 1m (MZFC). **NUEVO LEÓN:** Monterrey, 12-VII-1970, R. de la Maza, 1m (MHNCM); Mpio. China: Hacienda Vista Hermosa, Villa Santiago, 1500', 19-VI-1940, Hoogstraal & Knight, 2f (MGCL); same locality and collectors, 21-VI-1940, 1m\* (MGCL); Mpio. Dr. Coss: El Salto, 24-XI-1985, F. Arias, 1m (IBUNAM); Mpio. Galeana: Cerro El Potosí, 13-III-1989, I. Vargas, A. Luis, 1f (MZFC); Mpio. Linares: Raíces, 25-VII-1986, J. Kemner, 1m (MZFC); Mpio. Montemorelos / Rayones: Montemorelos – Rayones, 23-X-1986, J. Kemner, 4m (MZFC); Mpio. San Pedro Garza García: Chipinque, 8-X-1976, J. Saldana, 1m (IBUNAM); Mpio. Santiago: Cañón de la Virgen de Estanzuela San Tiago, 15-IX-1978, A. Ibarra, 1m (IBUNAM); Mpio. Te Pasas: Cola de Caballo, 22-VI-1977, C. R. Beutelspacher, 1m (IBUNAM); same locality, 30-III-1979, H. Pérez R., 2m (IBUNAM). **OAXACA:** Mpio. Cuicatlán: ca. 3 km E Tutepetongo, 5300', N17°44' W96°52', 18-X-2006, J. Kemner, 1m (MZFC); Mpio. Huauclilla: 2-3 km E Huauclilla, 6150', N17°26' W97°02', 4-VIII-2006, J. Kemner, 1m (MZFC); Mpio. Miahuatlán de Porfirio Díaz / Santo Tomás Tamazulapan: 8 mi S Miahuatlán, 7000', 4-V-1990, J. Kemner (no. 198), 1m (MZFC); Mpio. Nacaltepec: 1 km S Santiago Nacaltepec, 7000', N17°30' W96°56', 7-XII-2005, J. Kemner, 1f (MZFC); 1-3 km E Santiago Nacaltepec, 6500-7000', N17°30' W96°55', 22-IX-2006, J. Kemner, 1m (MZFC); 8 km N Santiago Nacaltepec, 6600', N17°32' W96°57', 11-III-2006, J. Kemner, 3m (MZFC); same locality and collector, 29-III-2006, 1m (MZFC); Mpio. San Andrés Huayapan / Tlalixtac de Cabrera: Hwy. 175, ca. 5 mi N Oaxaca, ca. 6000', 9/11-VII-1987, J. Kemner, 1m (MZFC); same locality and collector, 17-VII-1988, 3m (MZFC); same locality and collector, 28-VIII-1988, 1m (MZFC); same locality and collector, 6-VII-1989, 1m (MZFC); same locality and collector, 1-VIII-1990, 1m (MZFC); same locality and collector, 9-VII-1991, 1m (MZFC); same locality and collector, 11-VI-1992, 1m (MZFC); same locality, 16-VIII-2003, A.D. Warren, 3m (ADW); Mpio. San Juan Ixtaltepec: camino a Almoloyas, 6700', N17°35' W97°03', 13-III-2006, J. Kemner, 2m (MZFC); Mpio. Teotitlán del Valle: Rd. to Benito Juárez, ca. 5 mi N Teotitlán del Valle, ca. 7000', 22-VIII-2003, A.D. Warren, 2m (ADW); Mpio. Teotitlán del Valle / Díaz Ordaz: Rd. to Benito Juárez, ca. 8-10 mi N Teotitlán del Valle, ca. 8000', 22-VIII-2003, A.D. Warren, 4m (ADW); Mpio. Tepelmeme: El Manantial del Rosario, 7000', N17°56' W97°19', 4-X-2006, J. Kemner, 1m, 1f (MZFC); El Rosario – Puente Colosal, 6000-6600', N17°56' W97°18', 27-VIII-2006, 2m (MZFC); Mpio. Villa Sol

de Vega / Santa María Jacatepec: camino a Las Grutas de San Sebastián, 5500-6500', 10-VII-1991, J. Kemner, H. Romack (no. 358), 2m (MZFC). **PUEBLA**: Mpio. Jopala: Patla, 2000', 1992, local collectors (via J. Kemner), 1m (MZFC); Mpio. Tlacotepec de Juárez: camino Tlacotepec de Juárez – San Martín Esperilla, 7500-7700', N18°44' W97°33', 30-IX-2005, J. Kemner, 1f (MZFC); Mpio. Zacapoaxtla: Apulco, XI-1944, T. Escalante, 1m\* (MGCL). **QUERÉTARO**: Mpio. Cadereyta de Montes: Sierra del Doctor, vic. Tepozán, bosque de Piñón, 23-VIII-1995, A. Turrent, 1m (RT); same locality and collector, IV-1997, 1m (RT); **QUERÉTARO / SAN LUIS POTOSÍ**: Jalpan – Xilitla, 25-VI-1978, (as pupa on *Quercus*) J. Llorente, 1m (MZFC). **SONORA**: Canyon nr. El Novillo, V-1994, S. Prchal, 1m (MZFC).