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DNA barcodes reveal different speciation scenarios in the four North American *Anthocharis* Boisduval, Rambur, [Duménil] & Graslin, [1833] (Lepidoptera: Pieridae: Pierinae: Anthocharidini) species groups

PAUL A. OPLER[1,6], TODD L. STOUT[2,7], WERNER BACK[3,8], JING ZHANG[4,5,9], QIAN CONG[5,10], JINHUI SHEN [4,11] & NICK V. GRISHIN[4,12]

[1]*Department of Agricultural Biology, C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, CO 80523-1177*
[2]*McGuire Center for Lepidoptera and Diversity, Florida Museum of Natural History, Gainesville, FL 32611-2710*
[3]*Technische Universität München, Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt, Weihenstephaner Steig 20, 85354 Freising, Germany*
[4]*Departments of Biophysics and Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, USA 75390-9050*
[5]*Eugene McDermott Center for Human Growth & Development and Department of Biophysics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, USA 75390-9050*
[6] *paul.opler@colostate.edu;* *https://orcid.org/0000-0002-3672-5746*
[7] *todd@raisingbutterflies.org;* *https://orcid.org/0000-0002-7703-209X*
[8] *drwernerback@icloud.com;* *https://orcid.org/0000-0003-1393-4286*
[9] *Jing.Zhang@UTSouthwestern.edu;* *https://orcid.org/0000-0003-4190-3065*
[10] *Qian.Cong@UTSouthwestern.edu;* *https://orcid.org/0000-0002-8909-0414*
[11] *Jinhui.Shen@UTSouthwestern.edu;* *https://orcid.org/0000-0001-8395-7841*
[12] *grishin@chop.swmed.edu;* *https://orcid.org/0000-0003-4108-1153*

Abstract

The mitochondrial DNA COI barcode segment sequenced from American *Anthocharis* specimens across their distribution ranges partitions them into four well-separated species groups and reveals different levels of differentiation within these groups. The *lanceolata* group experienced the deepest divergence. About 2.7% barcode difference separates the two species: *A. lanceolata* Lucas, 1852 including *A. lanceolata australis* (F. Grinnell, 1908), from *A. desertolimbus* J. Emmel, T. Emmel & Mattoon, 1998. The *sara* group consists of three species distinctly defined by more than 2% sequence divergence: *A. sara* Lucas, 1852, *A. julia* W. H. Edwards, 1872, and *A. thoosa* (Scudder, 1878). Our treatment is fully consistent with morphological evidence largely based on the characters of fifth instar larvae and pupal cone curvature (Stout, 2005, 2018). In barcodes, it is not possible to see evidence of introgression or hybridization between the three species, and identification by morphology of immature stages always agrees with DNA barcode identification. Interestingly, *A. thoosa* exhibited the largest intraspecific divergence in DNA barcodes, and several of its metapopulations are identifiable by haplotypes. The *cethura* group is characterized by the smallest divergence and is best considered as a single species variable in expression of yellow coloration: *A cethura* C. Felder & R. Felder, 1865. Notably, the most sexually dimorphic subspecies *A. cethura morrisoni* W. H. Edwards, 1881 is the most distinct by the barcodes. Finally, the *midea* group barcodes do not always separate *A. midea* (Hübner, [1809]) and *A. limonea* (A. Butler, 1871) and we observe gradual accumulation of differences from north (northeastern USA) to south (Hidalgo, Mexico). This barcode gradient suggests a recent origin of the two *midea* group species and provides another example of vicariant sister species well defined by morphology, ecology and geography, but not necessarily by DNA barcodes.

Key words: cryptic species, biodiversity, DNA barcodes, mtDNA, COI, species groups

Introduction

Anthocharis Boisduval, Rambur, [Duménil] & Graslin, [1833] is a genus of showy species that attract attention due to their unique appearance and mostly early spring flight. The genus is Holarctic with most recently recognized species in Eurasia (Back *et al*. 2006; Back 2008). The genus seems particularly rich in areas with a Mediterranean

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or arid climate. Regions where the genus is especially rich include China (5), the Mediterranean region and Middle East (6), and western North America (6). *Anthocharis* is a genus within the Anthocharini which has been defined by male genitalia, and pupal morphology (Klots 1930; Gorbunov 2001). The tribe is defined by the presence of a flap-like harpe on the inner face of each valve. Other included genera are *Euchloe* Hübner, *Elphinstonia* Klots, 1930, *Iberochloe* Back, Knebelsberg & Miller, 2008, and *Zegris* Boisduval, 1836. *Microzegris* is best considered as a subjective synonym of *Zegris*, according to findings reported by Back (2020). *Anthocharis* has been defined as having male genitalia with valvae more elongate, a larger rounded harpe, and a narrower aedeagus (Gorbunov 2001). It is not known which of the other four genera is most closely related to *Anthocharis*, and an answer to this question will have to wait for a morphological and genetic study of the tribe.

Overview of North American *Anthocharis*. The presently recognized North American *Anthocharis* species—*cethura* C. Felder & R. Felder, 1865, *sara* Lucas 1852, *julia* W.H. Edwards, *thoosa* (Scudder), *midea* (Hübner, 1809), *limonea* Butler, 1871, and *lanceolata* Lucas, 1852, with the exception of *julia*, *limonea*, and *thoosa* were included with what we now know as *Euchloe* species in a review and key by Beutenmüller (1898). He included illustrations of adults, synonymies, and descriptions of all species and subspecies (as varieties) then recognized. In the intervening years many more subspecies were described, some as species-level taxa as was the practice of the day (Pelham 2008). Since Beutenmüller's study, no comprehensive study of the genus was undertaken and periodic catalogues and books (e.g. Barnes & McDunnough 1917; Comstock 1927; Hoffmann 1940; dos Passos 1964; Miller & Brown 1981; and others) usually with scant explanation for their species-level decisions were the only guides for an understanding of the species. Most often, *A. pima* W.H. Edwards, 1888 was the only recognized species-level taxon in addition to those included by Beutenmüller. Rudkin (1936) was the first to recognize that *pima* might be more properly considered as a subspecies of *cethura*. A treatment of *A. midea* with description of a new subspecies *annickae* was published by dos Passos & Klots (1969).

A suggestion that the *sara* group might be more than a single species began with Geiger & Shapiro's (1986) electrophoretic study and the observation that *sara* and *stella* W.H. Edwards, 1879, occurred parapatrically in the western foothills of California's Sierra Nevada without apparent intermediate individuals. Their study of enzymes by electrophoresis supported this species pair, and Colorado *A. julia* W.H. Edwards, 1872, though based on a small sample size, also appeared distinct in their study. Opler (1999), following the suggestion of Geiger & Shapiro, considered the *sara* group to be comprised of four species, including the more austral *thoosa* (Scudder, 1878) that had not been considered so previously. Distribution maps were presented for these 'species.' for the first time, and these were based in large part of maps compiled over many years by Stanford & Opler (1993). Most recently, Stout (2010, 2012, 2018) has undertaken an extensive and intensive investigation of all named taxa in the *sara*-group, including studies of the early stages.

Each of the three species has adults which tend to differ morphologically, although a statistical character analysis has never been performed. Within each species features also vary geographically as a number of subspecies of each has been described. These species are also sexually dimorphic.

Anthocharis thoosa adults tend to have the most extensive black dorsally with the widest black discal bar and the most extensive black apically. Ventrally, *A. thoosa* adults have relatively more black scales in the hindwing marbling giving a blackish green appearance. At the other extreme *Anthocharis julia* adults have the least extensive black with narrow black discal bars and fewest black scales amidst the ventral hindwing marbling giving it a yellowish green appearance. *Anthocharis sara* is intermediate between the two extremes having darkish first brood individuals, though with less extensive black than *A. thoosa*, having the ventral hindwing marbling blackish green. Not all *A. sara* populations have a facultative second generation but the phenotype of such late individuals is to have larger overall size and more restricted black dorsally with fewer black scales on the ventral hindwing giving it a yellowish green appearance.

Stout (2018) points out that in several geographic areas, e.g. Klamath County, Oregon (A. Warren, personal communication) and southwestern Colorado and adjacent northwestern New Mexico, where two *A. sara* complex species come in contact (parapatric) or overlap (sympatric), most adults are assignable to species, and some may not be readily assignable to species. And it is possible that this has resulted from occasional cross-species mating and introgression. In most other near contact zones such introgression has not been detected.

Fifth instar larvae of the sara group demonstrate three consistent color forms which represent the species level taxa of *Anthocharis sara*, *Anthocharis thoosa*, and *Anthocharis julia* – dark green, medium green, and light green, respectively. Larval coloration of each described subspecies conforms to one of these color forms. It should be noted that because last instar larvae of all species of Anthocharini change color as they advance through fifth instar, it is important that any larval coloration comparisons be measured using the same length of time after a fifth instar has recently molted from the fourth instar. For Stout's larval comparison studies, he compares larvae that have been fifth instars for 48–54 hours as larvae darken soon after this period before seeking pupation sites.

Stout (2018) found that fifth instar larvae of all four described subspecies of *Anthocharis sara,* including nominotypical *sara*, *gunderi*, *pseudothoosa*, and *sempervirens,* are dark green with small black chalazae and pinacula; exceptional were populations of *A. sara* in northwest California and southwest Oregon, whose fifth instar larvae were lighter green. In contrast, fifth instars of all four described subspecies of *Anthocharis thoosa,* including nominotypical *thoosa*, *inghami*, *colorado*, and *coriande,* have a different, lighter shade of green with larger green pinnaculi surrounding the setae or tubercles. On most larvae examined the width of the white lateral stripe as it extends along the head capsule of *A. thoosa* is narrower than that of *A. sara*.

Fifth instar larvae of all *Anthocharis julia,* including nominotypical *julia*, *browningi*, *prestonorum*, *sulfuris*, *stella*, *flora*, and *alaskensis,* have a broader lateral white stripe than that of *A. thoosa* and the ground color is lighter green as compared to either *A. thoosa* or *A. sara* (Stout 2018). The transitional color change from the white lateral stripe to the base green color of a fifth instar larva of *A. julia* is subtler compared to that of *A. thoosa* or *A. sara*. (See figures 17–19).

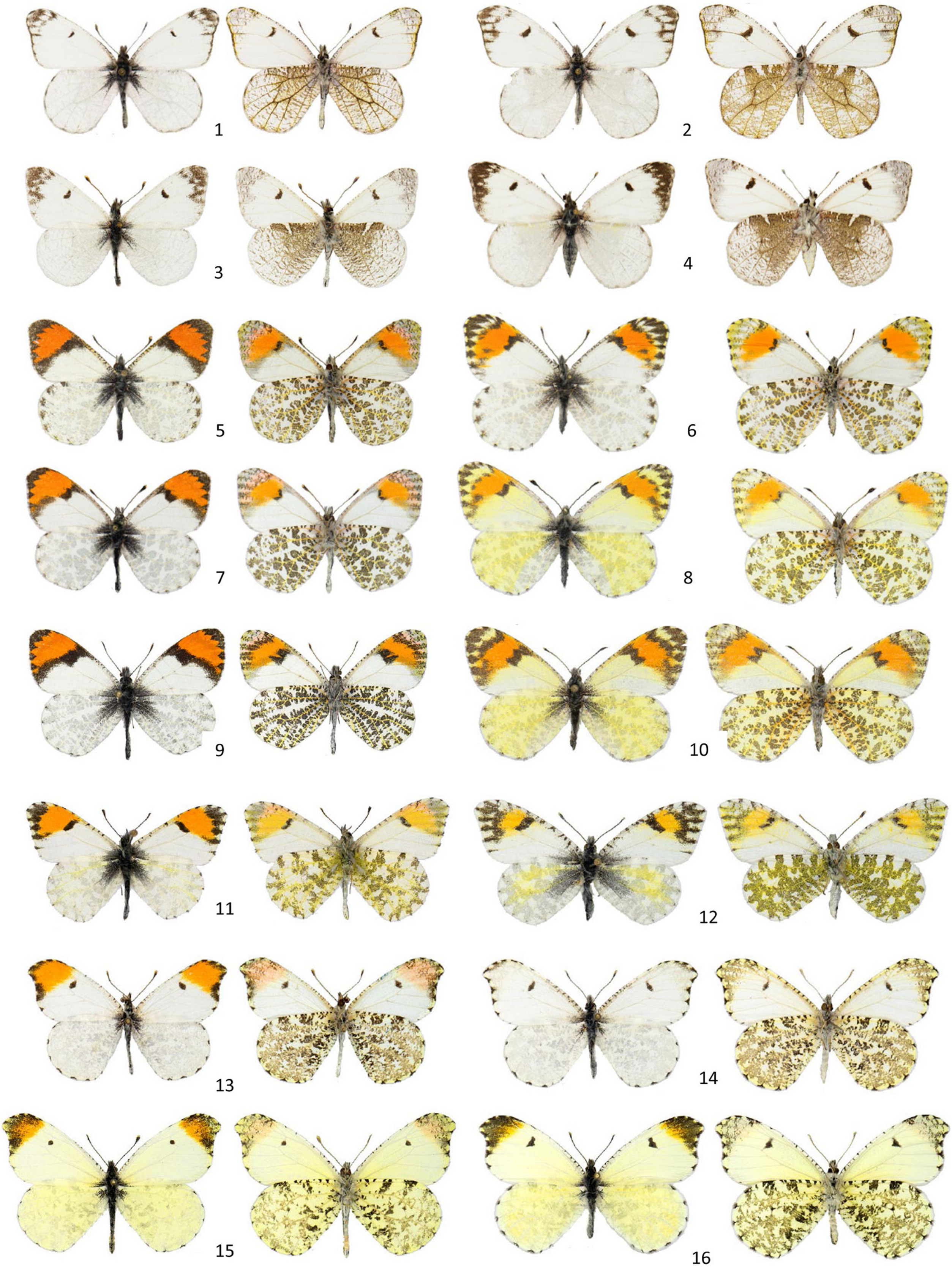
A pupal character is that the anterior 'cone' is distinctly curved dorsad in pupae of *Anthocharis julia*, and not bent dorsad or only slightly so in *Anthocharis thoosa*. However, this character is variable in *Anthocharis sara* populations varying from either curved dorsad, only slightly so or not at all (Stout 2018). Pupal color is dimorphic in various *Anthocharis sara* complex populations being either tan or green (Stout 2018). Stout, who reared many hundreds of broods, found that the tan morph was dominant in *Anthocharis sara* and *A. thoosa*, but that the green morph was dominant in *Anthocharis julia* populations.

Pupal diapause is a feature of most A*nthocharis sara* complex populations (Stout 2018). Stout found that the average number of years that pupae remained in diapause under laboratory condition was least in *Anthocharis julia* populations (1.07 years, n=385), intermediate for *Anthocharis sara*, which often has a second generation (1.41 years, n=123), and most for *Anthocharis thoosa* which lives in the most arid habitats (2.83 years, n=207). Some pupae of *A. thoosa* remained in diapause as long as six overwintering cycles. Only *Anthocharis sara* is bivoltine and seems to be facultatively so; moreover, *Anthocharis sara* is seasonally diphenic (see above).

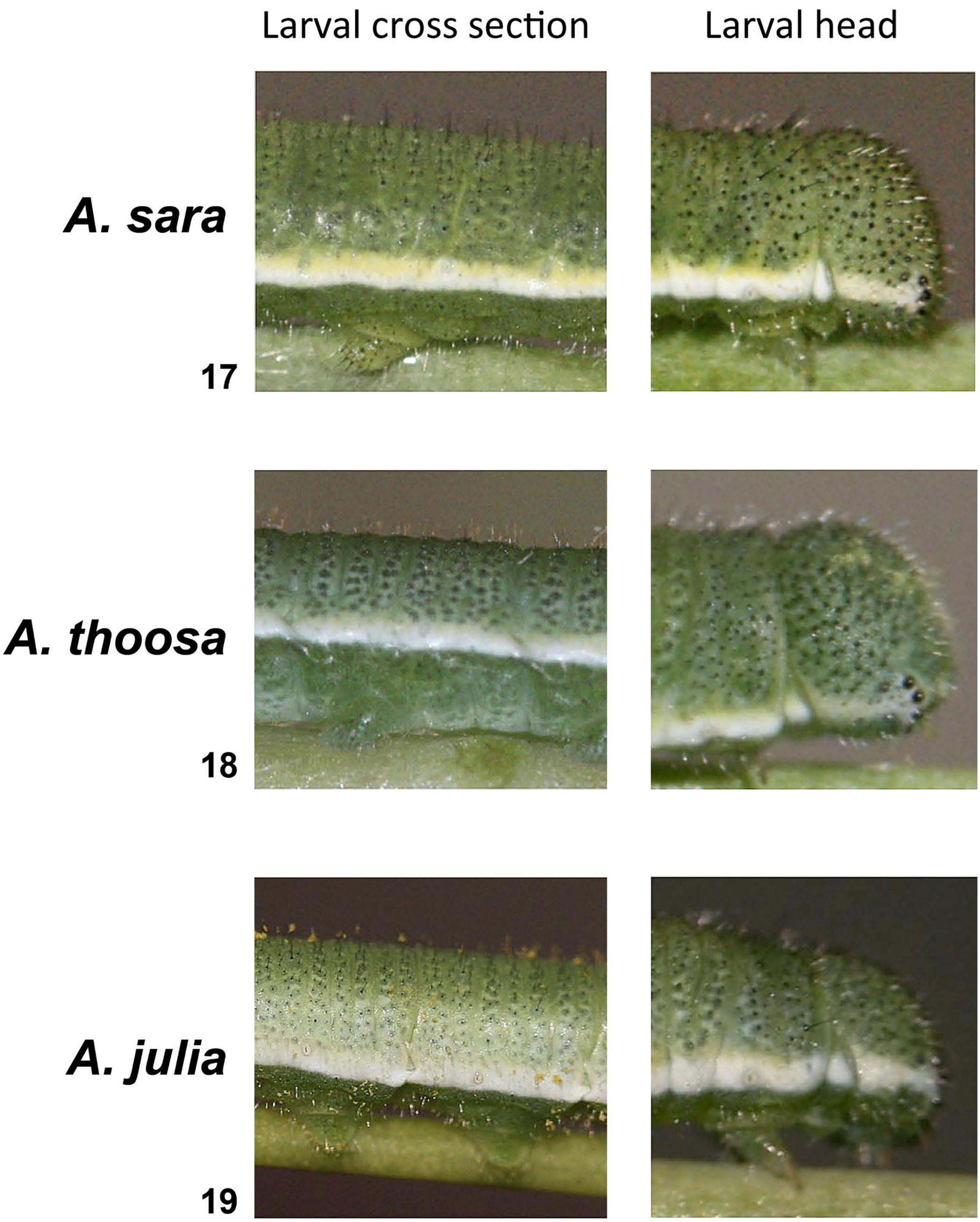
Previously, Emmel & Emmel (1973) treated *A. lanceolata australis* and *A. cethura*, including life history details, as they occur in southern California. Finally, Back (2010) was the first to recognize that *A. lanceolata* and *A. desertolimbus*, which he referred to as '*A. australis'*, were actually separate species based on evidence from DNA barcode analysis and adult morphology.

DNA methods in systematics often provide an additional dimension. DNA barcodes of COI (Cox I gene) are easy to obtain even from small tissue samples of older specimens and have been determined for many species of animals to facilitate comparisons (Hebert *et al*. 2004a, 2004b). They usually correlate with the time of divergence between taxa and, thus, are frequently indicative of speciation (e.g., Sperling 2003). Divergences equal to or greater than 2% are frequently indicative of distinct species (Hebert *et al*. 2004a), but see also van Nieukerken *et al*. (2012). For instance, as we have applied to *Anthocharis*, indicate that *A*. *lanceolata*, including its subspecies *A*. *australis* (F. Grinnell, 1908), and *A*. *desertolimbus* J. Emmel, T. Emmel, & Mattoon, 1998, are likely to be distinct species (Back 2010). However, for recently diverged species, because of insufficient time since speciation, no difference in the barcode gene may accumulate and barcodes might be identical (d'Ercole *et al*. 2020), and due to introgression and hybridization, a species may be paraphyletic in barcodes with different individuals of the same population carrying more than 2% different barcode sequences (Zakharov *et al.* 2009). Thus, caution is always in order when interpreting DNA barcode data and it is essential to correlate this data with other character states such as morphology and ecology. Finally, DNA barcodes are relatively short sequences—just 654 base pairs compared to more than 15,000 bp of complete mitochondrial sequences and 200,000,000 to 300,000,000 base pairs of complete genome sequences (Gregory & Hebert 2003; Honeybee Genome Sequencing Consortium 2006), so it may be tenuous to support phylogenetic conclusions with such short sequences, so all the results need careful interpretation. However, combined with all available evidence, DNA barcodes are extremely valuable in deriving sensible conclusions about species and their evolution (Hebert *et al.* 2004b).

In this work, we performed DNA barcode analysis of North American *Anthocharis* taxa. DNA barcode comparison strongly supported the four morphologically obvious species groups and revealed very different scenarios of speciation within these groups.



FIGURES 1–16. Adults of North American *Anthocharis* species. Male D, V, Female D, V—1–2 *A. lanceolata*, 3–4 *A. desertolimbus*, 5–6 *A. sara*, 7–8 *A. julia*, 9–10 *A. thoosa*, 11–12 *A. cethura*, 13–14 *A. midea*, 15–16 *A. limonea*.



FIGURES 17–19. Fifth instar larval phenotypes of *Anthocharis sara* group species—17. *A. sara*, 18. *A. thoosa*, and 19. *A. julia*.

Materials and methods

Adult specimens used in this study were from the following collections: California Academy of Sciences (CASC); C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, CO (CSUC); National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM); American Museum of Natural History, New York, NY (AMNH); Los Angeles County Museum of Natural History, Los Angeles, CA (LACM); Oregon State University, Corvallis, OR (OSUC); and Werner Back, Germany (WB). Standard entomological techniques were used for dissection (Robbins 1991), i.e., the distal part of adult abdomen was broken off, soaked for 40 minutes (or until ready) in 10% KOH at 60°C (or overnight at room temperature), dissected, and subsequently stored in a small glycerol-filled vial on the pin under the specimen. Genitalia and wing venation terminology follows Klots (1970) and Heppner (2008). Length measurements are in metric units and were made from photographs of specimens taken with a scale and magnified on a computer screen. Photographs of specimens and dry genitalia were taken with Nikon D200 and Nikon D800 cameras through a 105 mm f/2.8G AF-S VR Micro- Nikkor lens; dissected genitalia were photographed in glycerol with the Nikon D200 camera without the lens and through microscopes at 2x, and 5x magnifications. Images were assembled and edited in Photoshop CS5.1. Genitalia photographs were taken in several focus slices and stacked in Photoshop to increase depth of field.

Up to four legs (cut with scissors into tiny pieces in lysis buffer), or an abdomen (dropped into lysis buffer as a whole, and after overnight incubation at 56°C transferred into 10% KOH for genitalia dissection) (Knölke *et al*. 2005) of older specimens were used to extract genomic DNA with QIAGEN DNeasy blood and tissue kit complemented with EconoSpin columns from Epoch, or Macherey-Nagel (MN) NucleoSpin® tissue kit following the manufacturers' protocol. Genomic DNA was eluted in a total volume of 120–150 µl QIAGEN AE buffer (concentration of DNA as measured by Promega QuantiFluor® dsDNA System was from 0.01 to 10 ng/µl for legs and from 0.005 to 60 ng/µl for abdomens, depending on specimen age and storage conditions) and was stored at -20°C.

PCR was performed using Invitrogen AmpliTaq Gold 360 master mix in a 20 µl total volume containing less than 10 ng of template DNA and 0.5 µM of each primer. For recently collected specimens (10 years or less), the following primers were used to obtain the complete barcode: LepF: 5'-TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG-3' and LepR: 5'-CAGGAAACAGCTATGACCTAAACTTCTGGATGTCCAAAAAATCA-3'. For older specimens and those for which PCR reactions with the above-mentioned primers did not yield product, the following pairs of primers were used: sCOIF (forward, 5'-ATTCAACCAATCATAAAGATATTGG-3')—smCOIR (reverse, 5'-CCTGTTCCAGCTCCATTTTC-3') and bat-smCOIF2 (forward, 5'-CCTCGTATAAATAATATAAGATTTTG-3')—sCOIR (reverse, 5'-TAAACTTCTGGATGTCCAA AAAATCA-3'), to amplify the barcode in two overlapping segments (307, 398 bp).

The PCR reaction product was cleaned up by enzymatic digestion for the whole barcode amplifications of DNA from freshly collected or alcohol-preserved specimens and ID tag amplification of old specimens with 4 µl Shrimp Alkaline Phosphatase (20 U/µl) and 1 ul Exonuclease I (1 U/µl) from New England Biolabs. For older specimens that are barcoded in multiple segments, due to the frequent presence of primer dimers and other short non-specific PCR products, Agencourt Ampure XP beads or Invitrogen E-Gel® EX Agarose Gels (followed by Zymo gel DNA recovery kit) were used to select the DNA products of expected length. Sequences were obtained using the M13 primers (for amplification from LepF and LepR primers): 5'-TGTAAAACGACGGCCAGT-3' or 5'-CAGGAAACAGCTATGACC-3' or with primers used in PCR. Sanger sequencing was performed with Applied Biosystems Big Dye Terminator 3.1 kit on ABI capillary instrument in the DNA Sequencing Core Facility of the McDermott Center at the University of Texas—Southwestern Medical Center, or on an ABI 377XL DNA Sequencer at kmbioservices.com, respectively. The resulting sequence traces were proofread in FinchTV <http://www.geospiza.com/Products/finchtv.shtml>. We obtained complete or partial DNA barcode sequences from 166 *Anthocharis* specimens. COI barcode sequences obtained in the work have been deposited in GenBank with accessions OP231473–OP231626. Haplotype tree for these specimens is provided in Figure 20.

Other DNA sequences were downloaded from GenBank http://genbank.gov/ or BOLD (http://www.boldsystems.org/), aligned by hand since they matched throughout their length without insertions or deletions, and analyzed using the Phylogeny.fr server at http://www.phylogeny.fr/ with default parameters (Dereeper *et al.* 2008). Photographs of many specimens are available from the BOLD database (Ratnasingham & Hebert 2007).

Results and discussion

Four species groups in North American *Anthocharis*. The four species groups are clearly defined in distance analysis of DNA trees (Figure 20).

The *lanceolata* group: deep barcode divergence with limited morphological divergence.

Traditionally considered the single species *A. lanceolata* with subspecies *australis* and *desertolimbus* (e.g. Comstock 1927; Emmel *et al.* 1998; Opler 1999; Warren *et al.* 2021), our results show a deep divergence between the CO I of *lanceolata* including the southern subspecies *A. lanceolata australis* and *A. desertolimbus*. These results corroborate Back's (2010) species-level distinction by our detailed COI haplotype data. *Anthocharis lanceolata* ranges from 42°north latitude in southern Oregon (Dornfeld 1980; Hinchliff 1994; Warren 2005) and thence south in the Cascade Province, Klamath Mountains, Trinity Alps, North Coast Ranges, Warner Mountains, and Sierra Nevada of California. Except for an unsubstantiated specimen from near Mt. Diablo, Contra Costa County (Steiner 1990), the species is absent from the entire south coast range south of San Francisco Bay, California (Steiner 1990). The species is found on the western slope of the Sierra Nevada in the Transition and Canadian life zones, where it overlaps both *A. sara* and *A. julia* (see below). The species is also found sparingly along the lower eastern slope of the Sierra Nevada from the Carson Range of Nevada and other locations in Mono and Inyo counties, California (K. Davenport and J. Emmel, pers. comm.). Its southern terminus seems to be along the Kern River drainage of Tulare and Kern Counties (Davenport 2004b). Further south, the species continues as the subspecies *australis* in the Transverse Ranges in the San Gabriel and San Bernardino Mountains of Los Angeles and San Bernardino County (Figure 21).

The butterfly described as *A. lanceolata desertolimbus* which extends along the desert edge of the Laguna Mountains south into the arid eastern slope of the Sierra Juarez. (Emmel & Emmel 1973), is a distinct species according to our DNA CO I result but genomic sequencing (Grishin lab, unpublished) reveals less genetic differentiation than usually observed for distinct species. A population in the Sierra San Pedro Martir of Baja California Norte, Mexico (30°north latitude) is included with *desertolimbus* in our results.

Back (2010) has suggested that the *A. lanceolata* group be considered as two species—*lanceolata* and its subspecies *australis* (F. Grinnell) as opposed to the subspecies *desertolimbus* J. Emmel, T. Emmel and Mattoon found in the desert edge of the Colorado Desert in San Diego County, California, which Back considered as a separate species. His decision was based on some phenotypic differences between these populations together with some differences in mitochondrial genes.

Back's description of these characters included large size of typical *lanceolata* Lucas, together with an extended forewing apex, lightening of forewing apical markings, a half-moon shaped black discal spot, and contrasting darkened veins on the ventral hindwing. These stand in contrast to smaller size of the more southern *desertolimbus* together with its lack of extended forewing apex, darker apical forewing maculation, reduced discal black spot, and barely darkened ventral hindwing veins. In addition, Back found that the pupae of *A. desertolimbus* lack the scattered tiny black spots.

Additionally, there appear to be some larval differences between *A. lanceolata*, including subspecies *australis*, and *A. desertolimbus*. In later instars of *A. lanceolata*, including subspecies *australis*, there are 6 shallow annulets per abdominal segment, whereas in late instar larvae of *A. l. desertolimbus* there are 7-8 deeper annulets per abdominal segment. Moreover, there seem to be minor color pattern differences in the advanced instar larvae. Larvae of all populations have a subspiracular lateral band of yellow subtended by white. In *A. lanceolata*, included *australis*, both components of this band are relatively even in width throughout, whereas, in contrast, on late instar larvae of *A. l. desertolimbus*, the yellow portion of the band is uneven in width throughout.

An additional factor arguing for the distinctness of *Anthocharis desertolimbus* is its occurrence in low elevation desert-like habitats along the western Colorado Desert edge and Laguna Mountains of San Diego County, California and the eastern desert edge of the Sierra San Pedro Martir of Baja California Norte, Mexico.

Our thought on how to represent the taxonomic status of these named and un-named entities is that it may be an individual choice. Our perception of larval differences and CO I would lean toward treating *A. lanceolata* and *A. desertolimbus* as separate species-level entities, whereas genomic results and the seeming clinal relationship of adult characters would lead one to treat all of these populations as conspecific. Occasional rare field-collected individuals appear to be intermediate between *A. sara* and *A. lanceolata* (Comstock 1929; Shields & Mori 1979; Warren 2005). We have not personally examined or sequenced these specimens and their barcodes are unknown.

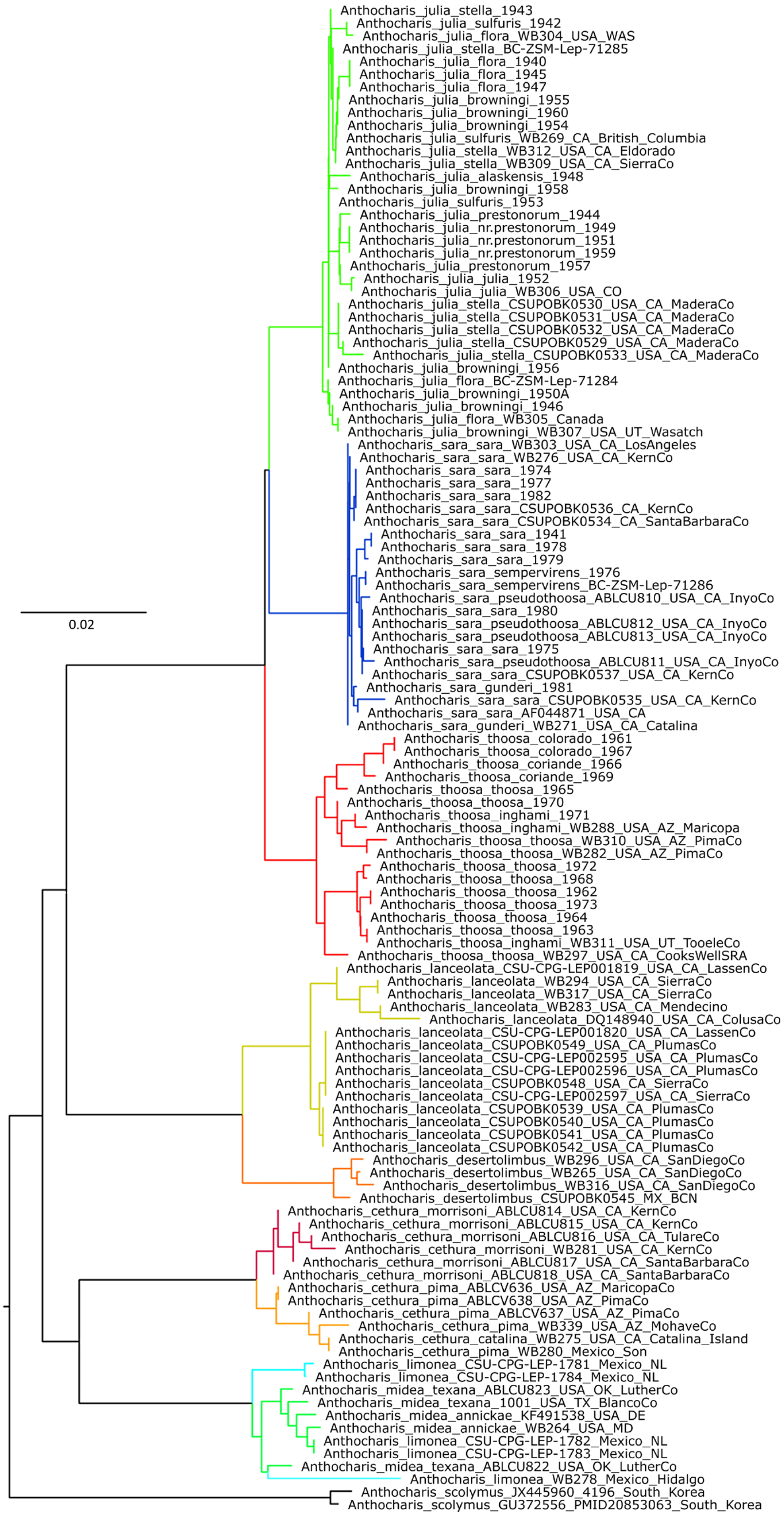


FIGURE 20. Haplotype tree for North American *Anthocharis*. *Sara* group (Green—*A*. *julia*, Blue—*A*. *sara*, Red—*A*. *thoosa*), *lanceolata* group (Brown—nominotypical *lanceolata*, Orange—*A*. *desertolimbus*), *cethura* group (Red—*A*. *cethura morrisoni*, Orange—*A*. *cethura pima* and *A*. *cethura catalina*), *midea* group (Green—*A*. *limonea* and *A*. *midea*), Black—*A*. *scolymus* [outgroup].

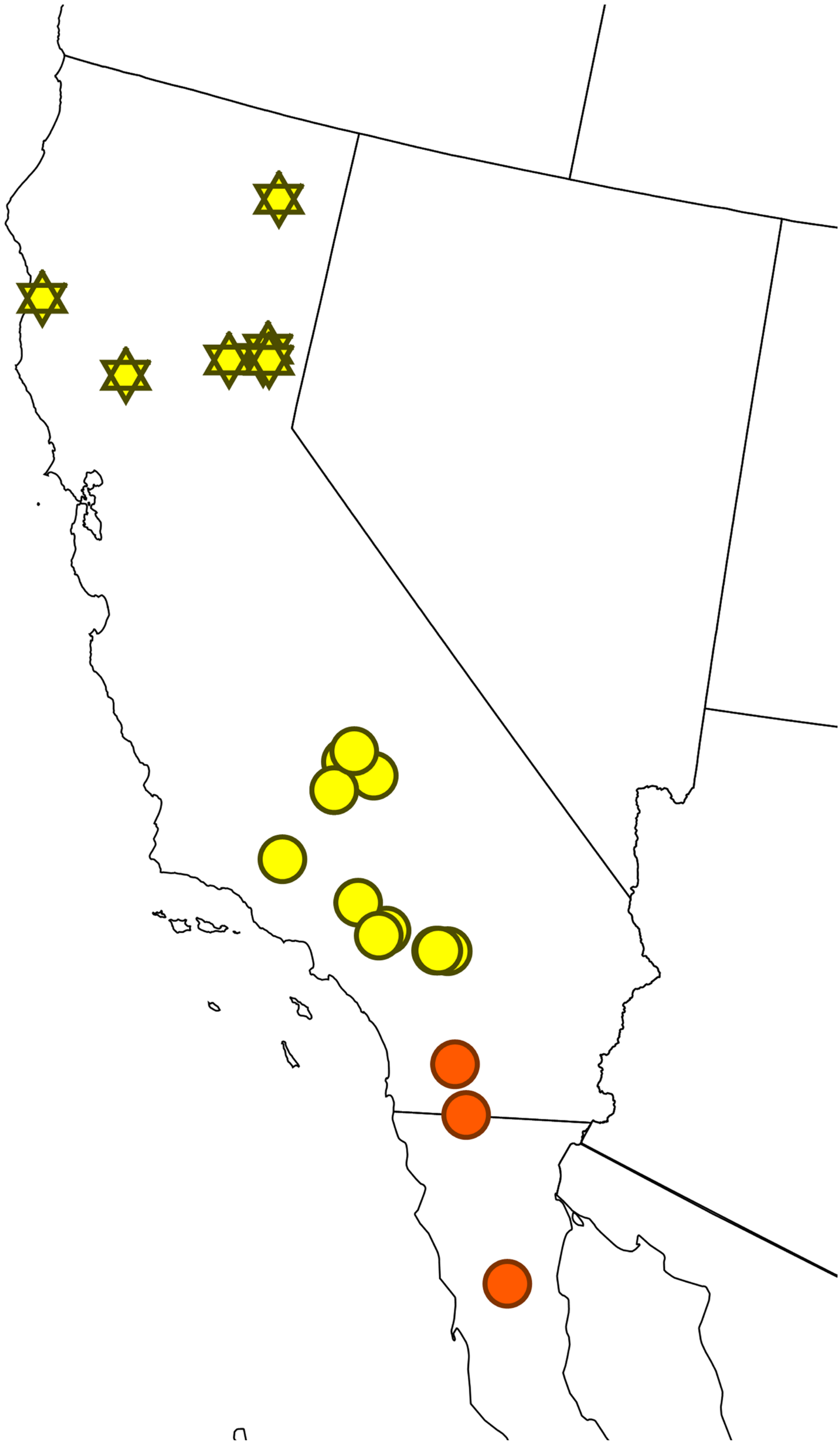


FIGURE 21. Distribution of *Anthocharis lanceolata* group, six-pointed stars—nominotypical *lanceolata*, yellow-filled circle—southern Sierra Nevada *lanceolata* and subspecies *australis*, orange-filled circles—*desertolimbus* and San Pedro Martir population.

The *sara* group: three wing-pattern cryptic species best separated by larvae and are cleanly identifiable by DNA barcodes.

In agreement with Stout (2018), DNA barcodes suggest that the *sara* group is best treated as three species—*A. julia*, *A. sara*, and *A. thoosa.*

Adult Phenotypes

As mentioned, the historical treatment of the sara group relied heavily on adult morphological characters to infer species level distinctions (Opler 1999; Scott & Fisher 2008). This is problematic because overlap in adult morphological characters of all three species make it challenging to identify wing traits exclusive to any single species. For example, all three species show variation which includes white to yellow dorsal wing colors, thin to thick dorsal and ventral discal cell bars, dorsal forewing black apical borders that either connect or disconnect with the dorsal discal cell bar, weak to strong dorsal hindwing black marginal spots, greenish to grayish ventral hindwing mottling, etc. These similar phenotypes are distributed randomly throughout the *sara* group taxa and are not regionally correlated except for those which are phenotypically similar and fly in near sympatry, for example *A. julia* nr. *prestonorum* and *A. thoosa colorado* in SW Colorado. Examples of these similar individual variants are shown in Figure 20.

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                                                                    1122233333345555666 | 3
                                                                    44589360370122368225582 24 | 8
                                                                    39821302514925740790602 86 | 0
 Anthocharis_lanceolata_lanceolata|CSU-CPG-LEP001819|USA_CA_LassenCo ATGCCTACCTCTTAGTGTTCAATAT | V
 Anthocharis_lanceolata_lanceolata|CSU-CPG-LEP001820|USA_CA_LassenCo ATGCCTACCTCTTAGTGTCCAATAT | V
 Anthocharis_lanceolata_lanceolata|CSUPOBK0548|USA_CA_SierraCo       ATGCCTACCTCTTAGTGTCCAATAT | V
 Anthocharis_lanceolata_lanceolata|CSU-CPG-LEP002597|USA_CA_SierraCo ATGCCTACCTCTTAGTGTCCAATAT | V
 Anthocharis_lanceolata_lanceolata|WB294|USA_CA_SierraCo             ---------TCTTAGTGTCCAA--- | V
 Anthocharis_lanceolata_lanceolata|WB317|USA_CA_SierraCo             ---------TCTTAGTGTCCAA--- | V
 Anthocharis_lanceolata_lanceolata|CSUPOBK0549|USA_CA_PlumasCo       ATGCCTACCTCTTAGTGTCCAATAT | V
 Anthocharis_lanceolata_lanceolata|CSU-CPG-LEP002595|USA_CA_PlumasCo ATGCCTACCTCTTAGTGTCCAATAT | V
 Anthocharis_lanceolata_lanceolata|CSU-CPG-LEP002596|USA_CA_PlumasCo ATGCCTACCTCTTAGTGTCCAATAT | V
 Anthocharis_lanceolata_lanceolata|WB283|USA_CA_MendeocinoCo         ---------TCTTAGTGTTCAA--- | V
 Anthocharis_lanceolata_lanceolata|DQ148940|USA_CA_ColusaCo          ----------CTTTGTGTTCAA--- | V
 Anthocharis_lanceolata_australis|CSUPOBK0539|USA_CA_TulareCo        ATGCCTACCTCTTAGTGTCCAATAC | V
 Anthocharis_lanceolata_australis|2946|CA-TulareCo                   ATGCCTACCTCTTAGTGTCCAATAC | V
 Anthocharis_lanceolata_australis|CSUPOBK0540|USA_CA_KernCo          ATGCCTACCTCTTAGTGTCCAATAC | V
 Anthocharis_lanceolata_australis|CSUPOBK0541|USA_CA_KernCo          ATGCCTACCTCTTAGTGTCCAATAC | V
 Anthocharis_lanceolata_australis|CSUPOBK0542|USA_CA_KernCo          ATGCCTACCTCTTAGTGTCCAATAC | V
 Anthocharis_lanceolata_australis|2344|CA-Ventura                    ATGCCTACCT--------------- | -
 Anthocharis_lanceolata_australis|2347|CA-Kern                       ATGCCTACCT--------------- | -
 Anthocharis_lanceolata_australis|2348|CA-SanBrnrd                   ATGCCTACCT--------------- | -
 Anthocharis_lanceolata_australis|2343|CA-SanBrnrd                   ATGCCTACCTCTCAGTGTCCAATAC | V
 Anthocharis_lanceolata_australis|2042|CA-SnBernardinoCo             ATGCCTACCT--------------- | -
 Anthocharis_lanceolata_australis|2726|M|CA-LosAngelesCo             ATGCCTACCTC-------------- | -
 Anthocharis_lanceolata_australis|2945|CA-LAngelesCo-MtBoldy         ATGCCTGCCTCTTAGTGTCCAATAC | V
 Anthocharis_desertolimbus|WB265|USA_CA_SanDiegoCo_SciCors           ---------CTCTATCGCATAC--- | V
 Anthocharis_desertolimbus|WB296|USA_CA_SanDiegoCo_Jacumba           ---------CTCTATCGCATGC--- | V
 Anthocharis_desertolimbus|WB316|USA_CA_SanDiegoCo_Jacumba           ---------TTCTATCGCATAC--- | V
 Anthocharis_desertolimbus|CSUPOBK0545|Mexico_BCN                    GCATTCATTTTCTATCACATACCTT | M
 Anthocharis_desertolimbus|2040|Mexico-BCN-SierraSanPedroMartir      GCATTCATTTTCTATCGCATACCTT | V
                                                                    3333333333333333133333333
```

FIGURE 22. Haplotype array for *A. lanceolata* group.

Regional adult comparisons. Distinguishing species-specific adult wing characters is more reliable on a regional level where two species fly in or near sympatry. For example, Davenport (pers. comm. 2007), discussed the differences between *A. julia stella* and *A. sara sara* in central California, where *A. sara* males were dorsally white and *A. julia stella* males were off white with yellowish over scaling just above the dorsal hindwing marginal spots. Geiger & Shapiro (1986), also provided observations of both taxa at Donner Pass, Lang Crossing, and Castle Peak in the Sierra Nevada Range of California. Davenport (pers. comm. 2007) also reviewed the distribution of *A. sara sara, A. sara pseudothoosa,* and *A. julia stella* (as *A. stella stella*) from Yosemite National Park and neighboring regions of central California and stated the possibility of intergradation between species and the need for further research. Warren (2005) discussed the relationship among *A. julia sulfuris, A. julia flora* (as *A. sara* nr. *stella* and

A. sara flora, respectively) and *A. sara sara* in Oregon. He observed notable variation in the ventral hindwing mottling of a long series of adults collected from Klamath River Canyon, Klamath County, where he suspected

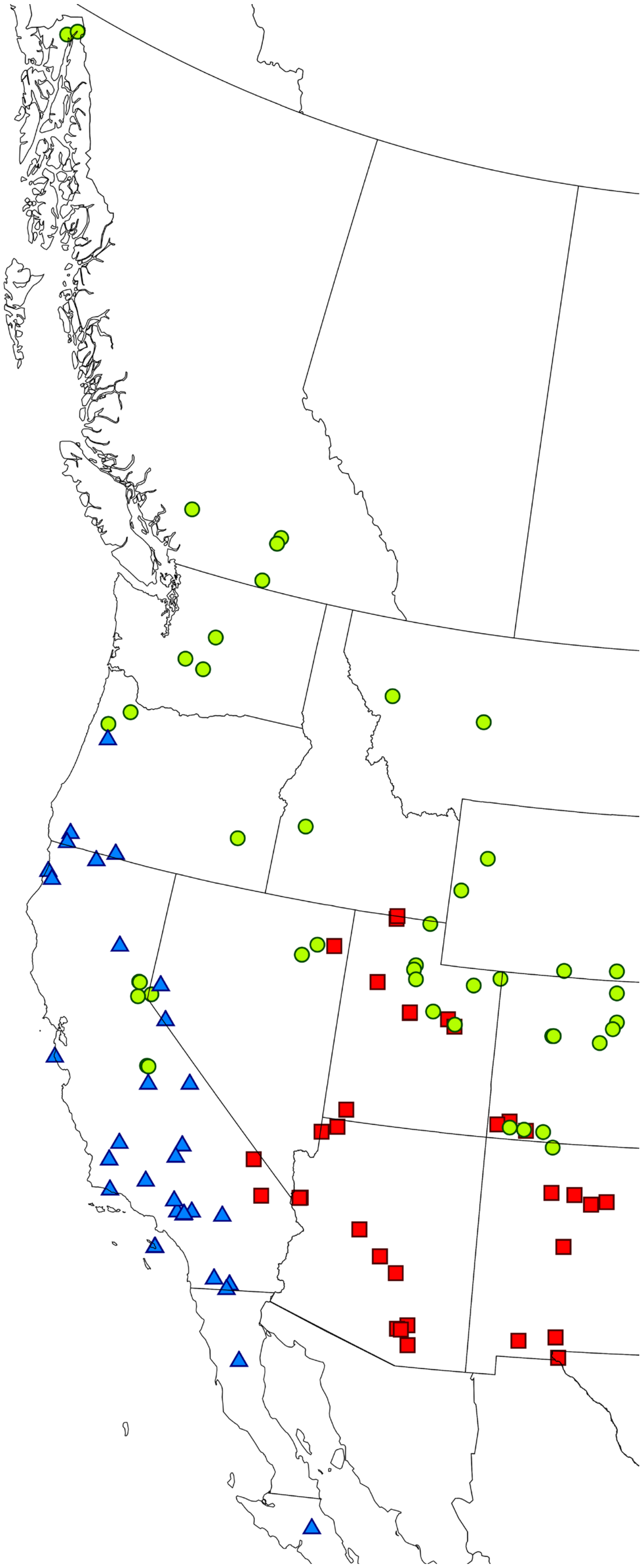

FIGURE 23. Distribution of *Anthocharis sara* group. Small red-filled circles—type localities of various names. Green-filled circles—*Anthocharis julia*, Blue-filled triangles—*Anthocharis sara*, Red-filled squares—*Anthocharis thoosa*.

A. sara sara flies with *A. julia sulfuris*. This observation of two species flying in sympatry is supported through larval examination and is discussed in the interspecific contact zones section. Austin (1998) discussed adult differences between *A. sara pseudothoosa* and *A. thoosa thoosa (*as *A. sara thoosa)* where *A. sara pseudothoosa* has a paler orange forewing apical patch, narrower discal cell bars that extend more narrowly to the outer margin that generally disconnect from the black apical border, and a lighter shade of ventral hindwing mottling as compared to *A. thoosa thoosa*. Fisher (2012) provided an extensive overview of the adult differences in Colorado among *A. julia julia, A. julia prestonorum, A. thoosa coriande* and *A. thoosa colorado.*

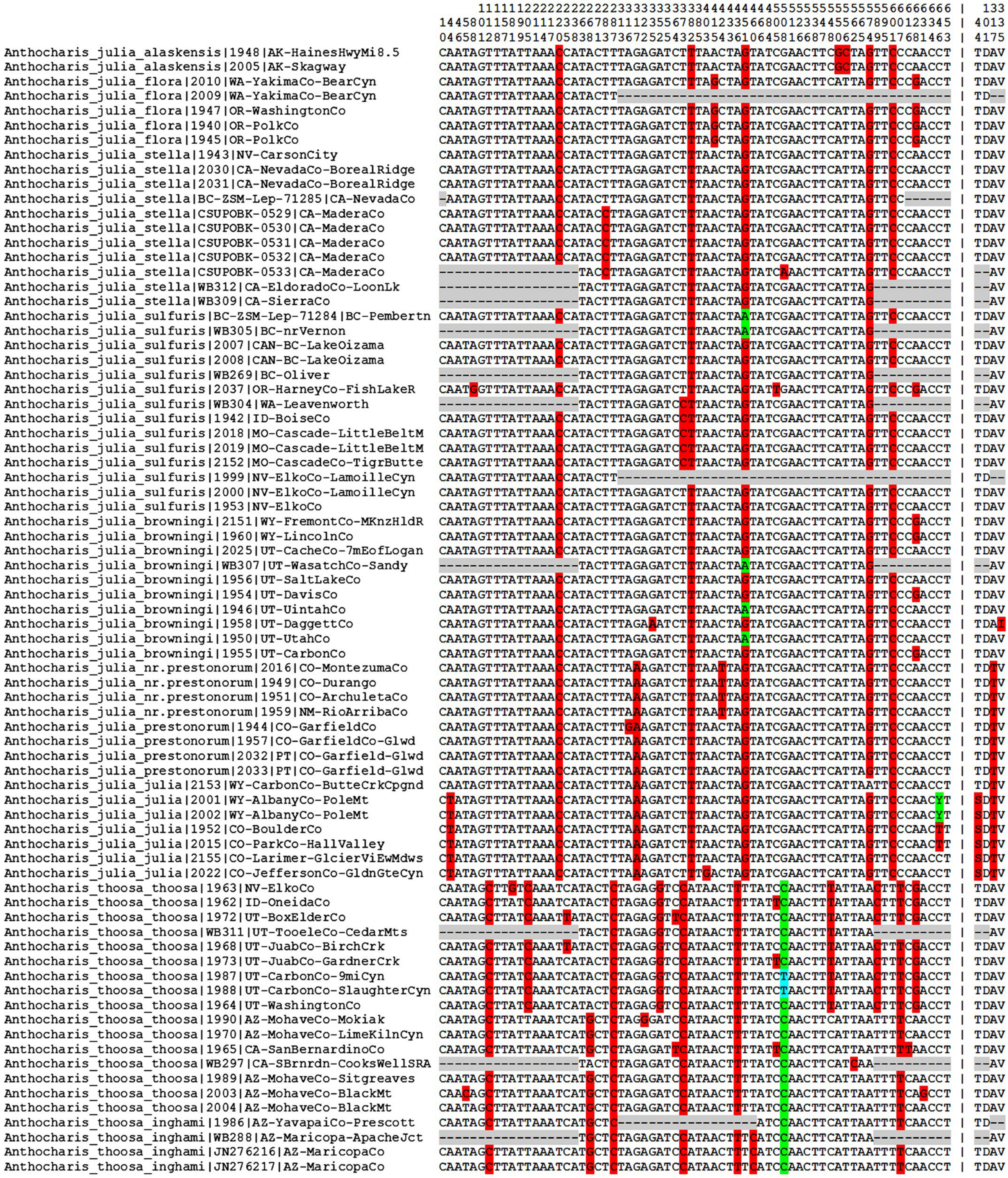

FIGURE 24. part 1. Haplotype array for "*sara* group".

FIGURE 24. part 2. Haplotype array for "*sara* group".

Anthocharis julia stella is parapatric with *A. sara* along the western slope of the Sierra Nevada (Geiger & Shapiro 1986; Garth & Tilden 1963; Davenport 2004a; Scott & Fisher 2008) and *A. julia browningi* is parapatric with *thoosa* in canyons along the west of the Wasatch Front in Juab County, Utah (Stout 2010, 2018). We have found further examples of elevational parapatry in the eastern Great Basin ranges in Nevada*, e.g*. Ruby Mountains of Elko County and the Snake Range in White Pine County, where *A. thoosa* flies at lower elevations in the Pinyon-Juniper zone and *A. julia* occurs at higher elevations in mixed conifer forest. In all of these situations, the two species are also mostly or completely allochronic with *A. julia* always flying about a month later than *A. sara* or

A. thoosa. Moreover, montane populations of *A. julia*, at least in California and Nevada are polytopic with respect to populations of *A. sara* or *A. thoosa*—perched at high elevations while being surrounded by lower elevation populations of the other two species.

Anthocharis julia is a species of more boreal habitats from southwestern Alaska (ssp. *alaskensis*) south through British Columbia and montane western Alberta to the montane cordillera (Canadian and Hudsonian life zones) of western North America as far south as California's Sierra Nevada (ssp. *stella*), northern Nevada, northern Utah (ssp. *browningi*) and Colorado (ssps. *julia* and *prestonorum*).

Populations of *A. sara* extend along the Pacific coast from southwestern Oregon (Jackson and Josephine counties), primarily but not entirely in cismontane habitats (typically Upper Sonoran and Transition life zones), south through cismontane California, including Catalina and Santa Cruz islands (ssp. *gunderi*), and south at least to central Baja California, Mexico, including Isla de Cedros. Transmontane populations are found in the hills near Carson City and Virginia City, Nevada (ssp. *pseudothoosa*), south through the Owens River drainage, and as subspecies *sara* along the western edge of the Mojave Desert, and the east slope of the Laguna Mountains in San Diego County. Note that in California populations of *A. julia* occur in the higher elevations of the Cascade and Sierra Nevada, separating the populations of *A. sara sara* and *A. sara pseudothoosa.*

Intriguingly, there may be a small second non-overwintering flight of *A. sara* in parts of the range where introduced European *Brassica* species and native *Thysanocarpus* species (fringepods) enable a longer period of larval host availability. These second brood individuals have yellow-green marbling and are significantly larger than first brood individuals from the same localities. The increased yellow scaling must be due to increased production of the pteridine compounds responsible for the white, yellow, and orange colors of *Anthocharis* (Opler, unpublished).

Anthocharis thoosa is found primarily in Pinyon-Juniper woodland habitat of the Great Basin ranges of extreme southern Idaho, eastern Nevada and Utah ranging south through the more arid ranges of southeastern California, Arizona (ssps. *thoosa* and *inghami*), New Mexico and Chihuahua (ssp. *coriande*). Our results show that this species has three distinctive groupings that could be referred to as semispecies as their CO I divergence probably does not justify species-level distinction. These groupings do not agree with extant subspecies designations, and coincide with Arizona populations of *thoosa* and *inghami*, New Mexican populations of ssp. *coriande*, and Utah populations that have been referred to nominotypical *A. thoosa*.

The *cethura* group—a single species with variable expression of yellow color on wings.

The wide-ranging cethura-group occurs in the southwestern arid desert region, primarily though not entirely Sonoran. The species ranges from southeastern California, central Nevada, southern Arizona, southern New Mexico, and extreme west Texas (El Paso County) south to the tip of Baja California, northern Sonora, and northwestern Chihuahua (Figures 25, 26).

The species does not display much variation in the CO I gene region, if anything, subspecies *morrisoni* (lower Kern River drainage, southern San Joaquin Valley, and western Mojave Desert) shows some slight divergence of its CO I. Despite this seeming lack of diversity, there are several remarkable morphological trends that do vary geographically and one may postulate that the extreme of these character states developed in different Pleistocene refugia. In any event, at the eastern extreme of its distribution (Chihuahua, Texas, New Mexico, Arizona, and Sonora) adults are uniformly pale yellow, and sexually monomorphic. At the western extreme (southern California and Baja California including subspecies *morrisoni*, *catalina*, *cethura*, and *bajacalifornica*) the vast majority of adults have a white ground color and the sexes are strikingly dimorphic as to their apical forewing patterns. An enigmatic population of all-yellow is indicated by one pale yellow specimen from the tip of Baja California Sur. Additionally, females of subspecies *morrisoni* are diphenic with some individuals lacking orange and others possessing orange scaling. Proceeding southward there is a cline with an increasing proportion of females with orange apical forewing scaling (subspecies *catalina*, *cethura*, and *bajacalifornica*). Between these eastern and western extremes populations of *mojavensis* and *hadromarmorata* show a mixing of these conditions. There is less sexual monomorphy and a varying mixture of yellow and white individuals.

The *midea* group: speciation without barcode differentiation

Populations of *A. midea* extend southwestwardly from Connecticut and the Great Lakes states through the eastern and Midwestern United States to northern Florida, the Gulf coast states and Texas to northeastern Mexico (Tamaulipas), while *Anthocharis limonea* is found in southern Nuevo Leon south along the Sierra Madre Orientale to the states of Puebla and Mexico (Llorente *et al.* 1997) (Figures 27–28). The existence of a small population of *A. limonea* in the Sierra Madre Occidental is shown by the collection of two specimens collected by the late Richard W. Holland in western Durango; these specimens are deposited in the McGuire Center for Lepidoptera, Gainesville, FL.

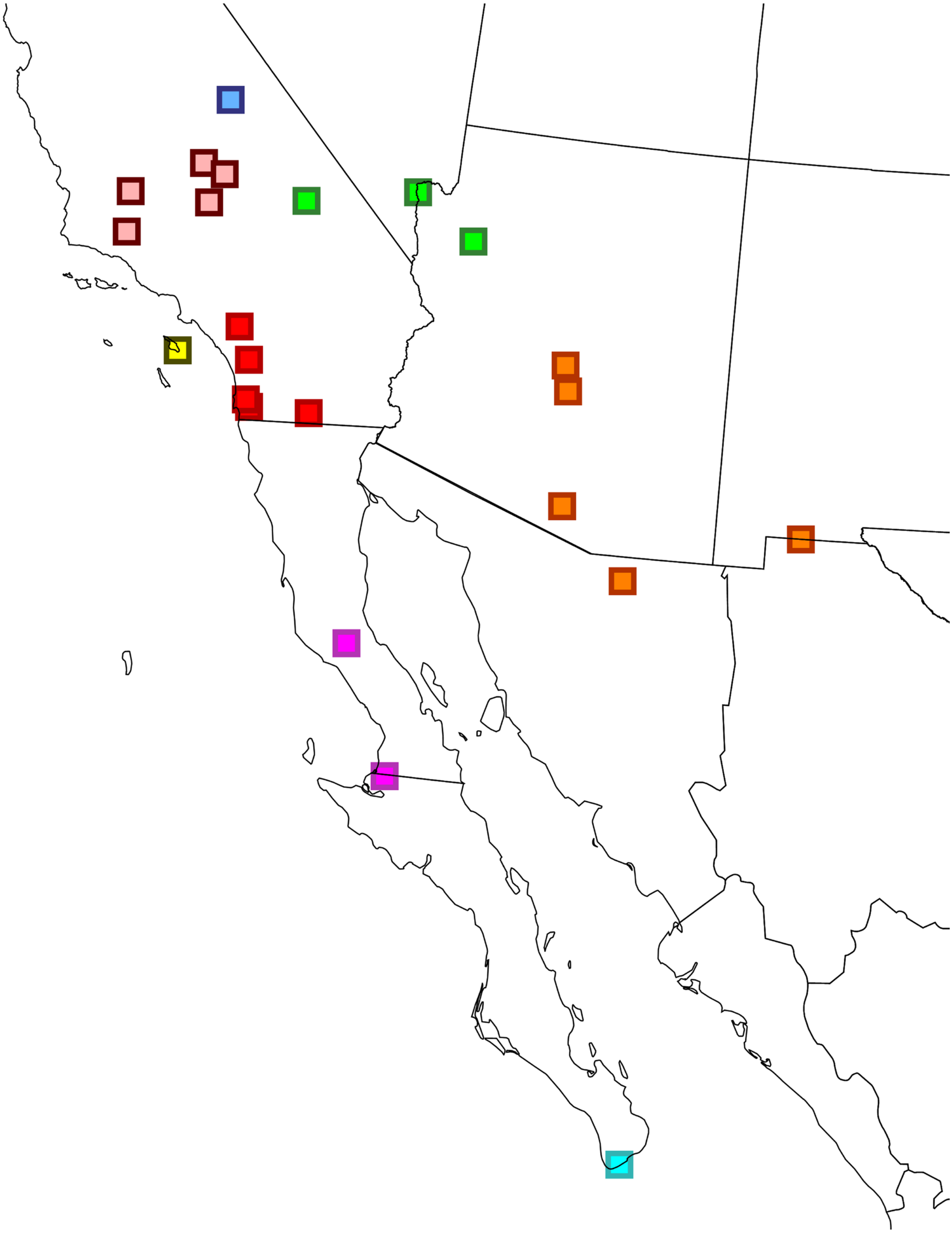

FIGURE 25. Distribution of "*cethura* group". Blue-filled square—subspecies *morrisoni*, red-filled squares—nominotypical subspecies *cethura*, pink-filled squares—subspecies *hadromarmorata*, green-filled squares—subspecies *mohavensis*, orange-filled squares—subspecies *pima*, yellow-filled square—subspecies *catalina*, violet-filled squares—subspecies *bajacalifornica*, pale-blue filled square—un-named population similar to *pima* from Baja California Cape region.

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                                                                   1122244455556 | 44
                                                                  6593676770258 1 | 77
                                                                  4738616078909 6 | 06
Anthocharis_cethura_morrisoni|ABLCU816|USA_CA_TulareCo            TTTCTCAGGCTTTC | VS
Anthocharis_cethura_morrisoni|ABLCU815|USA_CA_KernCo              TTTCTCAGGCTTTC | VS
Anthocharis_cethura_morrisoni|ABLCU814|USA_CA_KernCo              TTTCTCAGACTTTC | VN
Anthocharis_cethura_morrisoni|WB281|USA_CA_KernCo                 ----TCAGGCTT-- | VS
Anthocharis_cethura_morrisoni|ABLCU817|USA_CA_SantaBarbaraCo      TTTCTCAGGCTTTC | VS
Anthocharis_cethura_morrisoni|ABLCU818|USA_CA_SantaBarbaraCo      TTTCTCAGACTTTC | VN
Anthocharis_cethura_bajacalifornica|2133|MX-BCN                   CCTCTTAGATTTCT | VN
Anthocharis_cethura_bajacalifornica|2134|MX-BCN                   CTTCTCAGATTTTT | VN
Anthocharis_cethura_bajacalifornica|2342|MX-BCN                   CTTCTCAGATTTTT | VN
Anthocharis_cethura_ssp.|2340|MX-BCS                              CTTCTCAGATTTTT | VN
Anthocharis_cethura_cethura|2143|CA-RiversideCo-GavilanHills      CTTCTCAGATTTTT | VN
Anthocharis_cethura_cethura|2142|CA-SanDiegoCo-Pala               CTCCTCAGATTTTT | VN
Anthocharis_cethura_cethura|DMTRN-0189|CA-ImperialCo-2mNOcotillo  CTTCCCGGATCTTC | VN
Anthocharis_cethura_cethura|DMTRN-0190|CA-ImperialCo-2mNOcotillo  -TTCCCGGATCTTC | VN
Anthocharis_cethura_cethura|2144|CA-SnDiegoCo-DictionaryHill      CTTCTCGAATCTTC | IN
Anthocharis_cethura_cethura|2150|CA-SanDiegoCo-MissionGorge       CTTCTC-------- | --
Anthocharis_cethura_cethura|DMTRN-0193|CA-ImperialCo-2mNOcotillo  CTTCTCGAATCTTC | IN
Anthocharis_cethura_cethura|BIOUG00719-C02|CA-ImperialCo          CTTCTCGAATCTTC | IN
Anthocharis_cethura_cethura|BIOUG00719-C03|CA-ImperialCo          CTTCTCGAATCTTC | IN
Anthocharis_cethura_catalina|WB275|USA_CA_CatalinaIsland          ----TCGAATCT-- | IN
Anthocharis_cethura_hadromarmorata|2345|CA-InyoCo                 CTTCTCGAATCTTC | IN
Anthocharis_cethura_mojavensis|2140|CA-SnBrnrdno-GranitePass      CTTCTCGAATCTTC | IN
Anthocharis_cethura_mojavensis|2147|AZ-MohaveCo-SitgrvsPass       CTTCTCGAATCTTT | IN
Anthocharis_cethura_pima|BIOUG00716-E01|AZ-MaricopaCo             CTTCTCGAATCTTC | IN
Anthocharis_cethura_pima|BIOUG00716-E06|AZ-MaricopaCo             CTTTTCGAATCTTC | IN
Anthocharis_cethura_pima|BIOUG00716-E08|AZ-MaricopaCo             CTTCTCAAATCTTC | IN
Anthocharis_cethura_pima|WB339|USA_AZ_MohaveCo                    ----TCGGATCC-- | VN
Anthocharis_cethura_pima|ABLCV636|USA_AZ_MaricopaCo               CTTCTCAAATCTTC | IN
Anthocharis_cethura_pima|ABLCV637|USA_AZ_PimaCo                   CTTCTCGAATCTTT | IN
Anthocharis_cethura_pima|ABLCV638|USA_AZ_PimaCo                   CTTCTCAAATCTTC | IN
Anthocharis_cethura_pima|WB280|Mexico_Son                         ----TCGAATCT-- | IN
Anthocharis_cethura_pima|2141|MX-Chihuahua-PalomasRanch           CTTCTCRAATCTTY | IN
                                                                  33331331233333
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FIGURE 26. Haplotype array for "*cethura* group".

The voltinism of *Anthocharis limonea* is uncertain with its flights ranging from mid-June until November depending on the locality and year. The species flies most consistently in September (Llorente et al. 1997; Back, pers. comm.; Opler, unpublished), but emergence of adults in November resultant from eggs found in September suggest that is bivoltine (Back, pers. comm.). Flights appear to be timed to occur after heavy wet season rains, in particular those associated with hurricanes coming from the Caribbean. We presume there is a pupal diapause broken by the rains which result in a single flight per season, though it is possible that the species could have more than one generation per year at some localities, but this is unknown and remains to be documented.

The two species most likely arose in the past from a common ancestor and a distributional disjunction must have allowed the two species to evolve under somewhat to very different selective pressures. At present, the two taxa's closest reported occurrences are northern Tamaulipas for *A. midea texana* and southern Nuevo Leon for *A. limonea*, a linear distance of 175 kilometers. They differ in the following character states: flight period, geography, habitat, mate location, adult ground color, and adult dimorphism. They are no doubt each other's closest relatives and may be relatively recently separated, Pliocene—Miocene. Other examples of such vicariant populations or species-pairs are *Chlosyne harrisii* and *C. kendallorum*, *Papilio glaucus* and *P. alexiares*. [*Papilio palamedes* and *P. palamedes leontis* is an example of disjunct subspecies.]

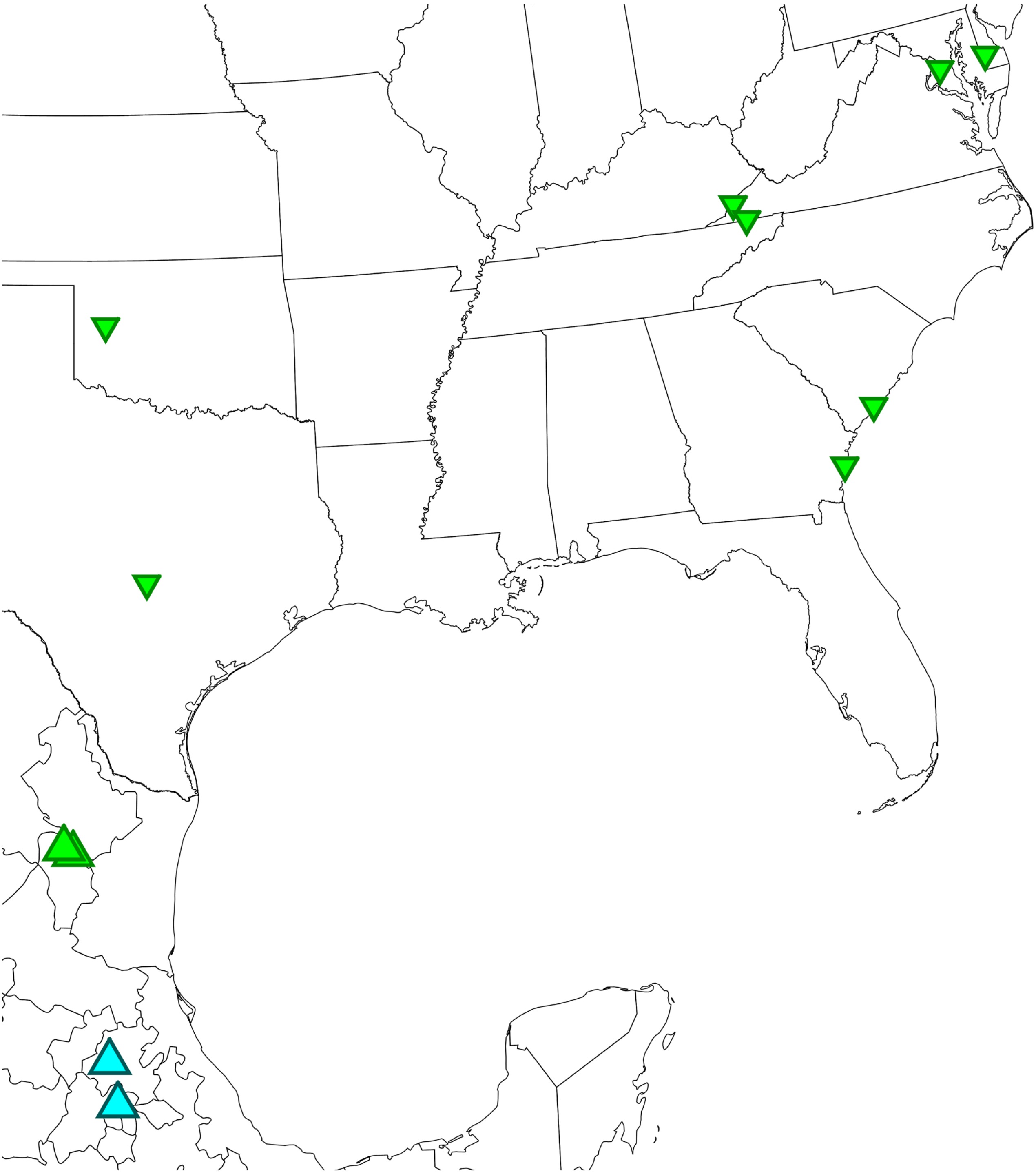

FIGURE 27. Distribution of sampled individuals in the *midea* group. *Anthocharis midea*—upside down green triangles, *Anthocharis limonea*—pale blue triangles, erect green triangle—*Anthocharis limonea* phenotype with *Anthocharis midea* haplotype.

```
                                                       11112222222223333444444445555566666 |  22
                                                     22344012601123788067811246668466822334 | 201
                                                     23439087307808106740228123691225328146 | 308
Anthocharis_midea_midea|2122|M|SC-HuntingIsldSP      GAAATATTTGAGCTTATCTATTTCTTAGACTATGAATC | IVA
Anthocharis_midea_midea|2123|M|SC-HuntingIsldSP      GAAATATTTGAGCTTATCTATTTCTTAGACTATGAATC | IVA
Anthocharis_midea_midea|2124|F|SC-HuntingIsldSP      GAAATATTTGAGCTTATCTATTTCTTAAACTATGAATC | IVA
Anthocharis_midea_annickae|2767|GA-SapeloIs          GAAATATTTGAGCTTATCTATTTCTTAGACTATGAATC | IVA
Anthocharis_midea_annickae|2125|M|KY-BigBlackMnt     GAAATATTTGAGCTCACTTCTTTCCTAAACTATAAATC | IVA
Anthocharis_midea_annickae|2126|F|VA-ScottCo         GAAATATTTGAGCTTATTTATTTTCTAGACTATGAATC | IVA
Anthocharis_midea_annickae|2127|M|VA-ScottCo         GAAATATTTAAGCTTATTTATTTTTTAGACTATGAATC | IIA
Anthocharis_midea_annickae|2128|F|VA-ScottCo         GAAATATTTGAGTTTATTTATTTCCTAGATTATGAATC | IVA
Anthocharis_midea_annickae|KF491538|USA_DE           GAAATTTTTGAGCTCATTTATTCCCTAGACTATGAATC | IVA
Anthocharis_midea_annickae|WB264|USA_MD              --------------TATTTATTTCCTAGACTAT----- | ---
Anthocharis_midea_texana|ABLCU822|USA_OK_LutherCo    GAAATATTTGAGCTCATTCAGTTCCTAGACTATAAATC | IVA
Anthocharis_midea_texana|ABLCU823|USA_OK_LutherCo    GAAATATTTGAACTTATCTATTTCTTAGACTATGAATC | IVT
Anthocharis_midea_texana|1001|USA_TX_BlancoCo_PedernalesSP GAAATATTTGAGCCTATCTATCTCCTAGACTATGAATC | IVA
Anthocharis_midea_texana|5591|USA_TX_BlancoCo_PedernalesSP GAAATATTTGAGCCTATCTATCTCCTAGACTATGAATC | IVA
Anthocharis_midea_texana|5592|USA_TX_WiseCo_LBJgrassland GAAATATTTGAGCTCATTTATTTCCTAGACTATGAATC | IVA
Anthocharis_limonea|4579|Mexico_NuevoLeon             GAAATATTTGAGCTTATTTATTTCCTGGACTATGAATC | IVA
Anthocharis_limonea|4585|Mexico_NuevoLeon             GAAATATTTGAGCTTATTTATTTCCTGGACTATGAATC | IVA
Anthocharis_limonea|CSU-CPG-LEP-1782|Mexico_NuevoLeon GAAATATTTGAGCTTATTTATTTCCTGGACTATGAATC | IVA
Anthocharis_limonea|CSU-CPG-LEP-1783|Mexico_NuevoLeon GAAATATTTGAGCTTATTTATTTCCTGGACTATGAATC | IVA
Anthocharis_limonea|4581|Mexico_NuevoLeon             GAAATATTTGAGCTCATTTATTTCCTGGACTATGAATC | IVA
Anthocharis_limonea|4582|Mexico_NuevoLeon             GAAATACTTGAGCTTATTTATTTCTTGGACTATGAACC | IVA
Anthocharis_limonea|4583|Mexico_NuevoLeon             GAAATACTTGAGCTTATTTATTTCTTAGACTATGAACC | IVA
Anthocharis_limonea|4584|Mexico_NuevoLeon             GAAATACTTGAGCTTATTTATTTCTTAGACTATGAACT | IVA
Anthocharis_limonea|2139|Mexico_NuevoLeon             AAAACATTAAAGCTTATTTATTTTCCAGACTATGAGTC | IIA
Anthocharis_limonea|4578|Mexico_NuevoLeon             AAAACATTAAAGCTCATTTATTTTCCAGACTATGAGTC | IIA
Anthocharis_limonea|4580|Mexico_NuevoLeon             AAAACATTAAAGCTTATTTATTTTCCAGACTATGAGTC | IIA
Anthocharis_limonea|CSU-CPG-LEP-1781|Mexico_NuevoLeon AAAACATTAAAGCTTATTTATTTTCCAGACTATGAGTC | IIA
Anthocharis_limonea|CSU-CPG-LEP-1784|Mexico_NuevoLeon AAAACATTAAAGCTCATTTATTTTCCAGACTATGAGTC | IIA
Anthocharis_limonea|WB278|Mexico_Hidalgo              GGGGTATATGGGCTCGTTCATTTCCTAAGTCGAGGATC | VVA
Anthocharis_limonea|2947|MX-Hidalgo-Ixmuquilpan       GGGGTATATGGGCTCGTTTATTTCCTAAGTCGAGGATC | VVA
Anthocharis_limonea|2948|MX-DF-Ecatepec-Tulpetlac     GGGGTATATGGGCTCGTTTATTTCCTAAGTCGAGGATC | VVA
Anthocharis_limonea|2949|MX-DF-Ecatepec-Tulpetlac     GGGGTATATGGGCTCGTTTATTTCCTAAGTCGAGGATC | VVA
                                                     31333333131333333333333333313333333
```

FIGURE 28. Haplotype array for *Anthocharis midea* group.

Taxonomic implications and conclusions

This taxonomic arrangement directly follows from the DNA barcode tree on Fig. 20, except that *A. limonea* is given species status despite the lack of barcode differences and in agreement with morphological, ecological and geographic differences, as discussed above. Since this work did not fully evaluate the subspecies, but merely determined DNA barcode haplotypes of topotypical or near topotypical populations of each name, subspecies and synonyms are listed together below each species name, ‡ denotes unavailable names.

Anthocharis Boisduval, Rambur, Duménil & Graslin, [1833]

lanceolata group

- ***A. lanceolata*** Lucas, 1852
  - ‡*lanceolata* Boisduval, 1852; *edwardsii* Behr, 1869; *australis* (F. Grinnell, 1908)
- ***A. desertolimbus*** J. Emmel, T. Emmel & Mattoon, 1998

sara group

- ***A. sara*** Lucas, 1852
  - ‡*sara* Boisduval, 1852; *reakirtii* W. H. Edwards, 1869; *mollis* W. G. Wright, 1905; *dammersi* J. A. Comstock, 1929; ‡*wrighti* J. A. Comstock, 1924; ‡*sternitzkyi* Gunder, 1925; ‡*corcorani* Gunder, 1931; ‡*broweri* Gunder, 1932; ‡*flavicoloris* Gunder, [1934]; ‡*pallida* Scott, 1986; *gunderi* Ingham, 1933; *sempervirens* J. Emmel, T. Emmel & Mattoon, 2008; *pseudothoosa* Austin, 1998
- ***A. julia*** W. H. Edwards, 1872
  - *sulfuris* Pelham, 2008; *columbia* J. Scott & Kondla, 2008; ‡*sulfuris* Gunder, 1931; *browningi* Skinner, 1906; *stella* W. H. Edwards, 1879; *alaskensis* Gunder, 1932; *flora* W. G. Wright, 1892; *prestonorum* Stout, 2012

A. thoosa (Scudder, 1878)

inghami Gunder, 1932; ‡*duncani* Gunder, 1932; *colorado* J. Scott & M. Fisher, 2008; *coriande* M. Fisher & Scott, 2008

cethura group

A. cethura C. Felder & R. Felder, 1865

cooperii Behr, 1869; *angelina* Boisduval, 1869; *deserti* W. G. Wright, 1905; *caliente* W. G. Wright, 1905; *catalina* Meadows, 1937; *bajacalifornica* J.F. Emmel, T.C. Emmel & S.O. Mattoon, 1998; *morrisoni* W. H. Edwards, 1881; *hadromarmorata* J. Emmel, T. Emmel & Mattoon, 1998; *mojavensis* J. Emmel, T. Emmel & Mattoon, 1998; *pima* W. H. Edwards, 1888

midea group

A. midea (Hübner, [1809])

annickae dos Passos & Klots, 1969; ‡*genutia* (Fabricius, 1793); *lherminieri* (Godart, 1819); *flavida* Skinner, 1917; *texana* Gatrelle, 1998

A. limonea (A. Butler, 1871)

ellena Dyar, 1920

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