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Welcome back Mr. Rudkin: differentiating *Papilio zelicaon* and *Papilio polyxenes* in Southern California (Lepidoptera: Papilionidae)

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Abstract

We studied wing pattern characters to distinguish closely related sympatric species *Papilio zelicaon* Lucas, 1852 and *Papilio polyxenes* Fabricius, 1775 in Southern California, and developed a morphometric method based on the ventral black postmedian band. Application of this method to the holotype of *Papilio* [*Zolicaon* variety] *Coloro* W. G. Wright, 1905, the name currently applied to the *P. polyxenes* populations, revealed that it is a *P. zelicaon* specimen. The name for western US *polyxenes* subspecies thus becomes *Papilio polyxenes rudkini* (F. & R. Chermock, 1981), **reinstated status**, and we place *coloro* as a junior subjective synonym of *P. zelicaon*. Furthermore, we sequenced mitochondrial DNA COI barcodes of *rudkini* and *coloro* holotypes and compared them with those of *polyxenes* and *zelicaon* specimens, confirming *rudkini* as *polyxenes* and *coloro* as *zelicaon*.

Key words: Taxonomy, field marks, swallowtail butterflies, desert, sister species

Introduction

Charles Nathan Rudkin, born 1892 at Meriden, Connecticut was a passionate scholar of history of the West, especially the Southwestern region. While he worked for the Southern California Edison Company, he has translated Spanish and French literature on history of California and published his translations while he was a member of the Los Angeles division ("Los Angeles Corral") of the Westerners, a group dedicated to the study of history and art of the old West (Dawson 1968).

Rudkin's interests were not confined to literature, he was passionate to study and collect butterflies. Comstock (1935) described Rudkin as one of the enthusiasts of the Lorquin Entomological Club, a gathering of lepidopterists in Natural History Museum of Los Angeles County at that time. Over 10,000 specimens of butterflies he collected during 1930-1945 were acquired by the University of California, Irvine after his death (Orsak 1974). It was 1934 when Rudkin brought up attention of the Club to a different-looking swallowtail he collected in Ivanpah Mountains of the Mojave Desert. Following year, it was published by John Comstock as a new form "rudkini" of *Papilio bairdii* which then was later elevated to full-species, *Papilio rudkini,* by the Chermock brothers (Chermock & Chermock 1937).

However, Ferris and Emmel (1982) later revised the status of *rudkini* to the subspecies of *Papilio polyxenes*, and at the same time, sunk *rudkini* as a junior synonym of *coloro*. In this article, Ferris and Emmel described that the "type of *coloro* represents what has been called *rudkini* since 1935". The name "*coloro*" was first named by Wright in his book, *The Butterflies of the West Coast of the United States* (1905), for the deep yellow *Papilio zelicaon* form from Colorado Desert.

Today, *Papilio polyxenes coloro* (Wright, 1905) is known as the western U.S. subspecies of *Papilio polyxenes* Fabricius, 1775, inhabiting Mojave and Colorado Deserts. Most of its distribution does not overlap with its close relative *Papilio zelicaon* Lucas, 1852, but at western edge of the desert, two species can be found in the same location (Monroe & Monroe 2004; Scott 1986). Unlike the darker eastern subspecies *P. polyxenes asterius*, high percentage of *coloro* is *zelicaon*-like yellow form (Scott 1992) which can be confused with *P. zelicaon* (Fig. 1).

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FIGURE 1. Live adults of *P. zelicaon* (left) and *P. polyxenes rudkini* (right).

Ways to distinguish *polyxenes* and *zelicaon* are discussed in several publications. Scott (1986) summarized them as 5 major characters.

1. Abdomen color patterns (Fig. 2 (a))
   polyxenes—one or two yellow bands on sides, often with yellow dots on top.
   zelicaon—one or two yellow bands with no yellow dots.
2. Marginal yellow spots on ventral forewing (Fig. 2 (b))
   polyxenes—round and rarely as continuous band. This feature is also used by Brock & Kaufman (2003). Pyle (1981) described it as "spots tends to be scalloped outward toward margin".
   zelicaon—mostly square and forming a continuous band, but some may be round and not continuous. Marginal spots "flattened distally" (Brock & Kaufman 2003) or "straight-edged" (Pyle 1981).
3. Orange ventral hindwing spots (Fig. 2 (c))
   polyxenes—orange in most of the yellow ventral hindwing postmedian and submarginal spots and the ventral forewing postmedian band.
   zelicaon—orange spots only at the apex and in the center of the postmedian band.
4. Yellow dorsal hindwing streak in cell CuA_2 (Fig. 2 (d))
   polyxenes—longer than the one in CuA_1.
   zelicaon—usually shorter than the one in CuA_1.
5. Black dorsal hindwing postmedian band (Fig. 2 (e))
   polyxenes—wider. Also in Garth (1986) "wider outer black wing bands".
   zelicaon—narrower.

Looking at the photographs of the *coloro* holotype, we noticed that it closely resembled *zelicaon* and not *polyxenes*. To investigate this similarity further, we conducted a study to develop a morphometric method for identification of these two species.

Materials and methods

Photographs of specimens for both dorsal and ventral were taken from KS and SDNHM collection. Specimen images in the Butterflies of America website (Warren *et al*. 2012) were also used. Each specimen photos were then viewed using Photoshop CS5, and with the Measure tool, ventral hindwing black postmedian band widths were measured in cells Sc, Rs, M_1, M_2, M_3 and CuA_1 (Fig. 3). The width is defined as the distance between distal ends of the black band at the midpoint between two wing veins. The measurement data was then normalized by the width of

Cell M_1. Ventral hindwing postmedian band was used for morphometrics instead of dorsal postmedian band because the dorsal band was found to be more variable than ventral band. Dorsal band also can fill up the entire wing cell in some of the specimens, which may give inaccurate data comparison. The other 4 characters listed above were also examined. DNA was isolated from a single leg and COI barcodes were determined as described previously (Shiraiwa *et al*. 2014). Barcode sequences and accompanying specimen data were submitted to GenBank and received accession numbers MW136698–MW136708.

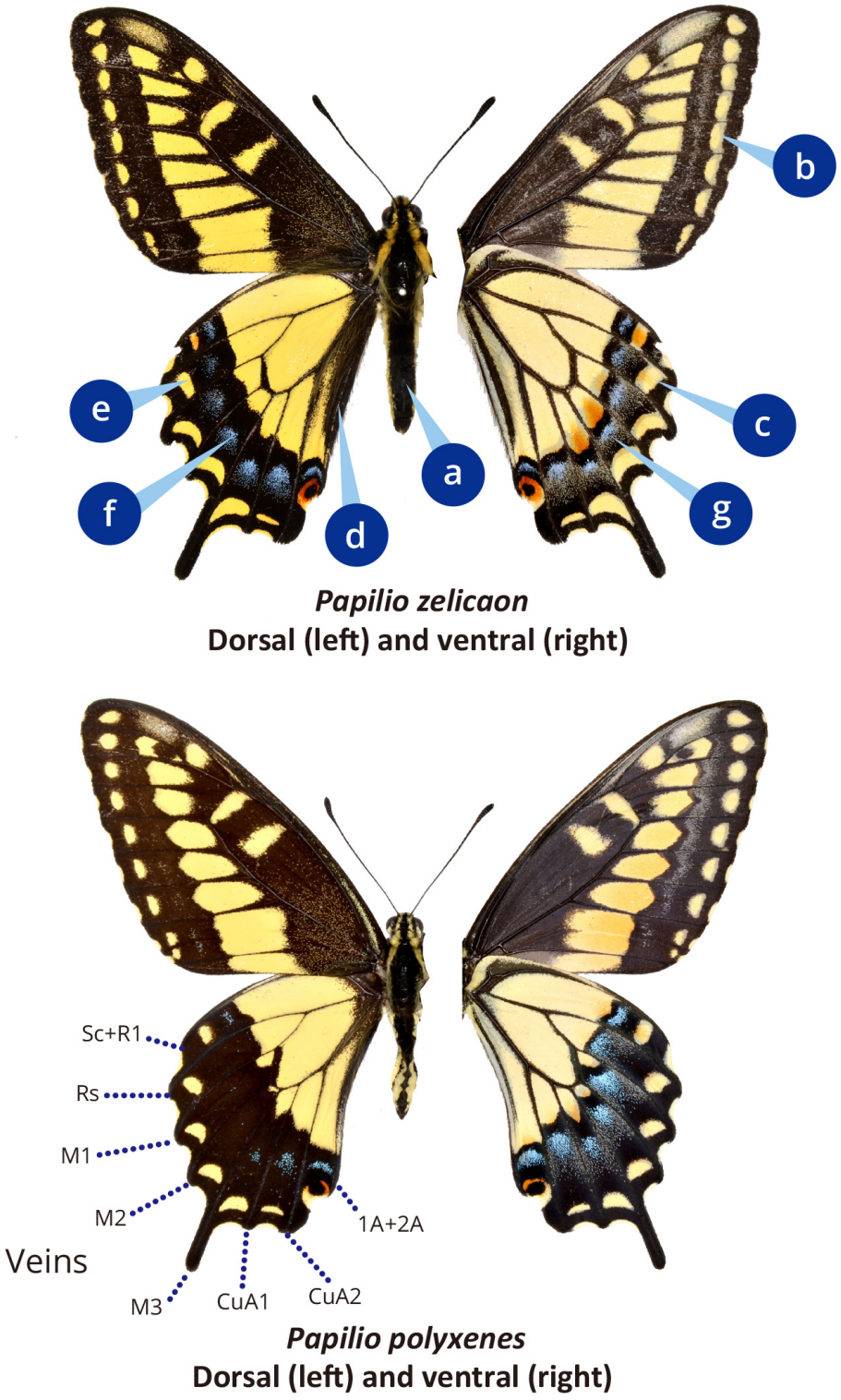

FIGURE 2. Fieldmarks of *P. zelicaon* and *P. polyxenes*. a: Abdomen color pattern. b: Marginal yellow spot shapes. c: Orange suffusion on ventral hindwing. d: Yellow dorsal hindwing streak in cell CuA_2. e: Black dorsal hindwing postmedian band width. f: Blue scales on ventral hindwing. g: Blue scales on dorsal hindwing.

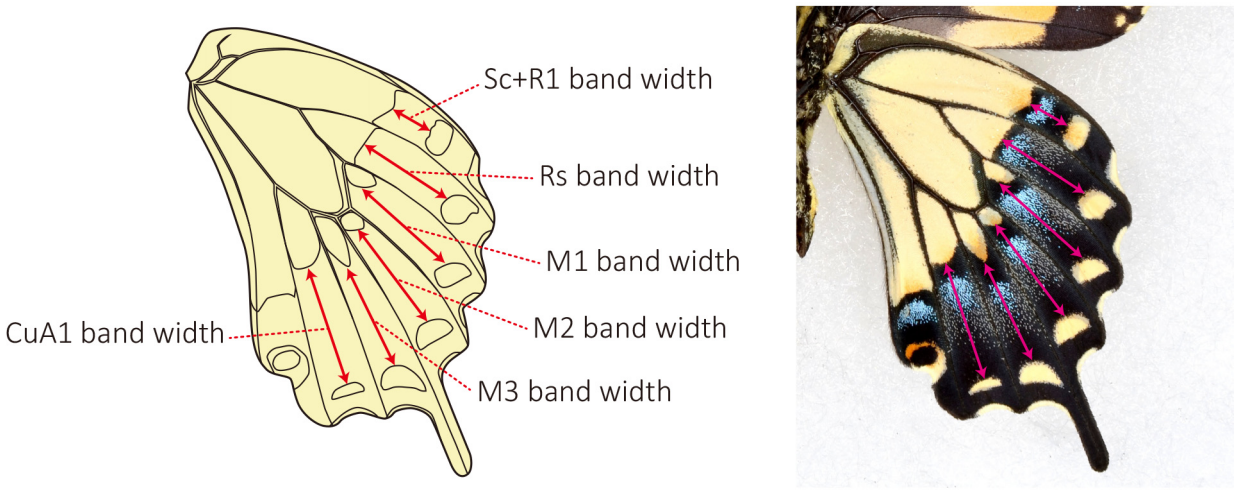

FIGURE 3. Measurements used for the width of ventral wing band.

Results

Based on our review, not all characters were found to be reliable.

1. Abdomen color patterns: The yellow dots above yellow stripes were not present in all *polyxenes* specimens, and moreover, such dots also found in some of *zelicaon* specimens.
2. Marginal yellow spots on ventral forewing: This character was found to be more reliable in distinguishing *zelicaon* and *polyxenes*. While some publications use dorsal side to evaluate this character, we found that ventral side is more reliable and differences are more prominent. Square and continuous marginal spots is a unique pattern found only in *zelicaon*, and it can be used to distinguish the two species. We found no *polyxenes* specimens with such character, however, some *zelicaon* specimens have *polyxenes*-like round spots, and for such specimens, this method cannot be used to identify them.
3. Orange under hindwing spots: The amount of orange scales on ventral hindwing is variable, and not all specimens of *polyxenes* have such coloring. Some *zelicaon* specimens have extensive orange scales, and therefore, this method cannot be used to differentiate between the two species.
4. Yellow upper hindwing streak in cell CuA_2: Exceptions were found in both species and it cannot be used for identification.
5. Black upper hindwing postmedian band: We found this character to be the most useful of. As discussed in the Materials and Methods, we choose to measure band widths on the ventral side instead of the dorsal. When the band width of each cell was compared, significant difference in band width pattern between cells M_1 and M_2 were confirmed. On average, *zelicaon* specimens showed M_1 band about 25% wider than M_2 band, where for *polyxenes*, M_1 and M_2 band were about the same width.

We noted two additional characters to differentiate between the two species. Blue spots on postmedian black band on dorsal hindwing are more developed in *zelicaon* than in *polyxenes* (Fig. 2(f)). Many *polyxenes* specimens lack the blue spots on cells Sc, Rs and M_1. Very few *zelicaon* specimens have reduced blue spots like *polyxenes*. Also, the blue-color scales on the ventral hindwing are more scattered in *polyxenes*, compared to *zelicaon* which the blue-color scales are dense at the basal side of the band (Fig. 2(g)). In *polyxenes*, but not in *zelicaon*, the distal half of the blue scales are often replaced by yellow scales.

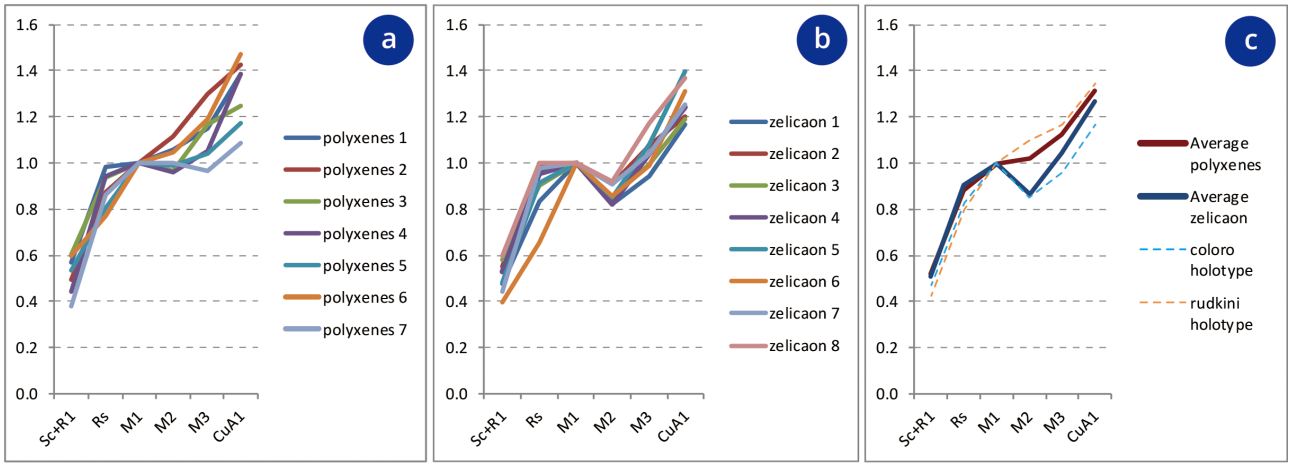

FIGURE 4. Morphometrics to distinguish between *P. zelicaon* and *P. polyxenes*:

(a) *P. polyxenes "coloro"* ventral hindwing band widths from each cell, indexing width of M_1 as 1. The width of M_2 is about the same or wider than the width of M_1.

(b) *P. zelicaon* ventral hindwing band widths from each cell, indexing width of M_1 as 1. The width of M_2 is narrower than the width of M_1.

(c) Average widths of ventral hindwing band from each cell for *P. polyxenes coloro* (bold red line) and *P. zelicaon*, (bold blue line) indexing width of M_1 as 1, compared with the *coloro* holotype specimen (thin green line) and the *P. rudkini* holotype specimen (thin orange line). This graph indicates that *coloro* is a *P. zelicaon* and *rudkini* is a *P. polyxenes*.

Discussion

"Papilio Coloro, n. v." was described by William G. Wright in his book, *The Butterflies of the West Coast of the United States*, from "Colorado Desert of Southeastern California" (Wright 1905), later defined as "Whitewater Hill, west end of the Coachella Valley, Colorado Desert, Riverside County, California" (Ferris & Emmel 1982). Wright named *coloro* as a "variety" of *zelicaon*, at that time referred to by the Boisduval name *zoliacaon* (see Wright 1905: 47, 48), and differentiated *coloro* from *zelicaon* by its color with *coloro* "having deep yellow ground color" than *zelicaon*. The holotype "specimen was taken ... in June 1883" (Wright 1905: 87) and thought to be lost in San Francisco earthquake, but it was re-discovered in the collection of the California Academy of Sciences by Ferris and Emmel (1982). Ferris and Emmel concluded that the type specimen is what then was called *rudkini*, and therefore sunk *rudkini* as a junior subjective synonym of *coloro*. What lead the authors to decide that *coloro* and *rudkini* are conspecific is not explained in the paper, but it is likely that the authors have determined so because *coloro* holotype was collected in the Colorado Desert where *zelicaon* was believed to be absent, and the deep yellow color and less developed blue spots suggested it was different from *P. zelicaon*. However, when the holotype of *coloro* was analyzed using the methods described here, the results suggested that *coloro* is *zelicaon*:

1. No yellow dots are found on abdomen (=*zelicaon*)
2. Ventral marginal yellow spots are square and continuous (=*zelicaon*)
3. No orange mark is found in underwings (=*zelicaon*)
4. Black hindwing postmedian band narrow (=*zelicaon*)
5. Blue spots on dorsal hindwing are less developed (=*polyxenes*)
6. Blue scales on ventral hindwing are not scattered (=*zelicaon*).

Out of six wing pattern traits, the only *polyxenes* trait in the *coloro* holotype is less extensive blue spotting on dorsal hindwing. To validate these patterns comparisons and morphometrics, we sequenced COI barcodes of the *coloro* and *rudkini* holotypes and of several *zelicaon* and *polyxenes* specimens. In addition, we used barcodes of *zelicaon*, *polyxenes*, *P. machaon* and *P. indra* from GenBank at NCBI (https://www.ncbi.nlm.nih.gov/), the last two

species were used as outgroups. Barcode results unambiguously revealed that the *coloro* holotype was *zelicaon*, but *rudkini* holotype was *polyxenes* (Fig. 5). In the maximum likelihood tree, *polyxenes* and *zelicaon* were partitioned into two distinct clades and the barcodes are nearly identical within each clade (Fig. 5 left). Barcode sequences visualized as variable positions colored by variations (Fig. 5 right) reveal the reason behind the clades in the tree and show that the barcode of the *coloro* holotype matches that of *zelicaon*, while the barcode of the *rudkini* holotype in the same as *polyxenes*. Agreement between morphological and DNA-based evidence enhances the strength of our argument.

Based on these results, we conclude that holotype specimen of *coloro* is indeed a *zelicaon*, and therefore propose to reinstate *rudkini* as the subspecies of *Papilio polyxenes* (*Papilio polyxenes rudkini* (F. & R. Chermock, 1981) **reinstated status**) and treat *coloro* as a junior subjective synonym of *Papilio zelicaon*. We believe that reinstating *rudkini* is a simpler alternative, because the conditions of the ICZN article 23.9.1. about the reversal of precedence and prevailing usage and not met, and we need to refer the matter to the Commission for a ruling, had we chosen to keep *coloro* as the name for the western U.S. population of *polyxenes* (article 23.9.3.). That would also involve *coloro* neotype designation. We think that the nearly 40 years since incorrect associations of *rudkini* with *coloro* and *coloro* with *polyxenes* (Ferris & Emmel 1982) do not warrant the change of the original concept of *coloro* ("*Zolicaon* [sic!] variety") proposed nearly 80 years prior to that (Wright 1905).

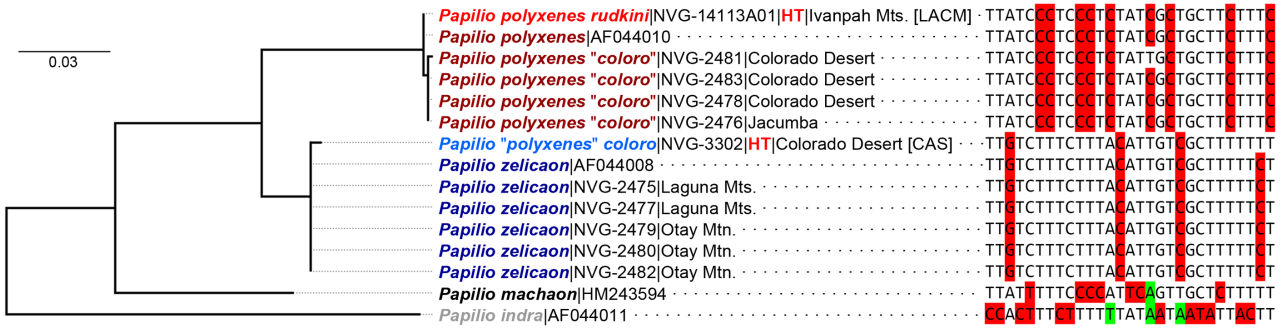

FIGURE 5. COI barcodes of *P. polyxenes* and *P. zelicaon*. A PhyML tree (http://www.phylogeny.fr/) is shown on the left and a segment of COI alignment to illustrate the differences between species is shown on the right. Only positions that exhibit variation are shown. The tree is rooted with *P. indra*. The holotypes are marked by "HT". The locality is given for specimens sequenced in this study, and barcodes of specimens without localities were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/), accession number is given after the name. Barcodes of specimens sequenced in this study (NVG-...) were assigned accession numbers MW136698–MW136708.

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