Zoological Journal of the Linnean Society, 2008, **152**, 297–314. With 12 figures

Phylogeny of the pollinating yucca moths, with revision of Mexican species (*Tegeticula* and *Parategeticula*; Lepidoptera, Prodoxidae)

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Received 2 October 2006; accepted for publication 30 May 2007

The yucca moths (*Tegeticula* and *Parategeticula*; Lepidoptera, Prodoxidae) are well known for their obligate relationship as exclusive pollinators of yuccas. Revisionary work in recent years has revealed far higher species diversity than historically recognized, increasing the number of described species from four to 20. Based on field surveys in Mexico and examination of collections, we describe five additional species: ***T. californica* Pellmyr sp. nov.**, ***T. tehuacana* Pellmyr & Balcázar-Lara sp. nov.**, ***T. tambasi* Pellmyr & Balcázar-Lara sp. nov.**, ***T. baja* Pellmyr & Balcázar-Lara sp. nov.** and ***P. ecdysiastica* Pellmyr & Balcázar-Lara sp. nov**. *Tegeticula treculeanella* Pellmyr is identified as a junior synonym of *T. mexicana* Bastida. A diagnostic key to the adults of all species of the *T. yuccasella* complex is provided. A phylogeny based on a 2104-bp segment of mitochondrial DNA (mtDNA) in the cytochrome oxidase I and II region supported monophyly of the two pollinator genera, and strongly supported monophyly of the 17 recognized species of the *T. yuccasella* complex. Most relationships are well supported, but some relationships within a recent and rapidly diversified group of 11 taxa are less robust, and in one case conflicts with a whole-genome data set (amplified fragment length polymorphism, AFLP). The current mtDNA-based analyses, together with previously published AFLP data, provide a robust phylogenetic foundation for future studies of life-history evolution and host interactions in one of the classical models of coevolution and obligate mutualism. © 2008 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2008, **152**, 297–314.

ADDITIONAL KEYWORDS: mitochondrial DNA – molecular phylogenetics – mutualism – pollination.

INTRODUCTION

The obligate mutualism between yucca moths (*Tegeticula*, *Parategeticula*; Lepidoptera, Prodoxidae) and yuccas (*Yucca*, *Hesperoyucca*; Agavaceae) is one of the most well-known models of coevolution (Riley, 1892; Baker, 1986; Powell, 1992; Pellmyr, 2003). In this association, the female moth actively gathers pollen from yucca stamens. She then oviposits into yucca ovaries and subsequently uses some of her pollen load to actively pollinate the flower. This is critical, as her larval progeny exclusively feed on developing yucca seeds and there are no other documented pollinators.

Early studies reported four species of pollinators (Riley, 1892; Davis, 1967; Frack, 1982; Powell, 1984), including three *Tegeticula* species and a single *Parategeticula* species, but considerable intraspecific variation was observed and interpreted as an indication that cryptic, more host-specific species may be found within *T. yuccasella* (Riley) (Davis, 1967; Miles, 1983; Powell, 1984, 1992). Indeed, extensive collection

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and examination indicated that *T. yuccasella* was a complex of at least 13 species (Pellmyr, 1999), and three additional species of *Parategeticula* were reported from Mexico (Pellmyr & Balcázar-Lara, 2000).

The yuccas are distributed from southernmost Canada southward at least to southernmost Belize (Clary, 1997; Pellmyr, 2003). The vast majority of all yucca moth collections have been made north of Mexico, even though a large proportion of yuccas are largely or entirely confined to Mexico (Matuda & Piña Luján, 1980; Clary, 1997). The 1999 revision of the *T. yuccasella* complex was almost completely confined to the northern species, simply because of the scarcity of other material. To remedy this situation, two of us (M.B.L., O.P.) performed extensive surveys of the Mexican yuccas for all prodoxid moths during the years 1996–2001. Three *Parategeticula* species have already been described (Pellmyr & Balcázar-Lara, 2000), and here we describe five additional species from both genera. With all recognized species described, we then use molecular data for all taxa to reconstruct the phylogeny of the radiation of the two genera of pollinator yucca moths, thus creating a foundation for future analysis of such issues as the role of coevolution in driving co-diversification of the yucca moth–yucca mutualism.

MATERIAL AND METHODS

The majority of the material used for the revisionary work was gathered during extensive fieldwork in Mexico during 1996–2001 by M.B.L. and O.P. In addition, we included specimens from collections known to have holdings, namely: University of California, Berkeley (UCB); Universidad Nacional Autónoma de México, Mexico City, Mexico (UNAM); and the National Museum of Natural History (Smithsonian Institution), Washington, DC (USNM). Specimens from our fieldwork will be divided between UNAM and USNM, with primary types to UNAM. Paratypes, when available, will be distributed to other major collections, including UCB, Natural History Museum of Los Angeles County (LACM) and Natural History Museum (BMNH).

MORPHOLOGY

Gross morphological data were collected from specimens representing the five species described or redescribed here, using an Olympus® SZX-9 dissection microscope when needed. From these specimens, genital dissections were made of three individuals of each sex when available. The entire abdomen was removed and boiled for 7 min in 10% aqueous potassium hydroxide. Female genitalia were stained for 3 min with Chlorazol black. After dissection, measurements were made using the Olympus microscope at 10–80× magnification fitted with a micrometre scale. Each specimen was subsequently mounted in polyvinyl lactophenol on a glass slide.

For figure preparation, wing images were scanned with a Nikon Super Coolscan 4000 from slide photographs produced with an Olympus OM-4T camera with a Tokina AT-X Macro lens and extender, and genitalic mounts of *Tegeticula* were captured using a Spot® 1.1.0 digital camera mounted on a Leica® DMR microscope. For *Parategeticula*, a Canon camera with an Olympus Zuiko 38-mm f/2.8 bellows macro lens was used to take a stack of images at 0.0127-mm focus steps, which were then compiled to generate images with extended focus.

DNA DATA ACQUISITION

A list of all samples is provided in supplementary Table S1. Prior to DNA extraction, the head, wings and genitalia were removed from adults and kept as voucher specimens. For the new *Parategeticula* species, the posterior half of a larva extracted from the same fruit as the holotype was used as the DNA source. For the sample of *P. elephantipella* from *Y. lacandonica*, DNA was recovered from a deceased 1st or young 2nd instar larva extracted from a 3-cm-long host fruit. Total genomic DNA from the remaining thorax and abdomen was extracted using Isoquick DNA Isolation Kits (Orca Research Inc., Bothell, WA, USA). Except for the sample mentioned above, for larvae, the entire individual was used. We used PCR to amplify the 3′ end of mtDNA cytochrome oxidase I, the intervening tRNA leucine, and the 5′ end of cytochrome oxidase II, which yielded a 2104-bp region. This region was amplified with four pairs of PCR primers that produced overlapping regions of sequence. The primer pairs were 1461F–2302R, 2231F–3020R, 2638F–3306R and 3252F–3371R, where the numbers refer to the nucleotide positions in the *Drosophila yakuba* mtDNA genome (Clary & Wolstenholme, 1985). Primer sequences are available upon request from the authors. PCR was conducted in 30-μL reaction volumes containing 50 mM KCl, 10 mM Tris (pH = 9.0), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 mM of each primer, one unit of Promega *Taq* polymerase and 10 ng of genomic DNA. The thermal cycler profile was one cycle at 95 °C for 2 min, 35 cycles at 95 °C for 1 min, 52 °C for 1 min and 72 °C for 90 s, and a final extension at 72 °C for 10 min. PCR products were cleaned using the Qiagen PCR purification columns (Qiagen Inc., Valencia, CA, USA). Dye terminator reactions were carried out following the Dye terminator protocol (Applied Biosystems, Foster City, CA, USA) with the exception that

one-quarter reactions were conducted with the addition of a Tris buffer (1 M Tris-acid, 1 M magnesium chloride, pH 9.0). Dye terminator reactions were cycled at 96 °C for 2 min, and 25 cycles at 96 °C for 30 s, 50 °C for 30 s and 60 °C for 4 min. Sequencing products were cleaned using Centri-sep Sephadex columns (Princeton Separations, Adelphia, NJ, USA), and both forward and reverse strands were sequenced on an ABI 377 automated DNA sequencer (Applied Biosystems). Forward and reverse sequences for each individual were combined into contigs using Sequencher 3.1 (Gene Codes Corp., Ann Arbor, MI, USA). The consensus sequence for each individual was then aligned by eye in PAUP* version 4.0b10 (Swofford, 2002).

Phylogenetic analysis

We conducted two separate DNA-based phylogenetic analyses. We first performed an analysis including ample intraspecific sampling to test the assumption that the somewhat morphologically cryptic yucca moth species indeed represent genetically distinct clusters. The second analysis was aimed at establishing phylogenetic relationships among the recognized taxa. In the first analysis, 114 samples were used to assess the monophyly of morphologically circumscribed *Tegeticula* and *Parategeticula* species. Sampling was done proportionally so as to reflect geographical range, with more widespread species having up to ten samples included. Individuals bearing the same mtDNA haplotype were represented by a single exemplar to speed the analysis; this reduced the sample size in the first analysis by 15 samples. In the second analysis, we used 20 species of *Tegeticula* and five species of *Parategeticula*, including those described in this paper. Three samples were included for *T. maculata* to incorporate strong intraspecific variation (Powell & Mackie, 1966; Segraves & Pellmyr, 2001; Althoff, Svensson & Pellmyr, 2007). *Prodoxus y-inversus* was used as the outgroup in both analyses based on prior studies of this group that consistently showed this genus to be sister to the pollinator moths (Pellmyr & Leebens-Mack, 1999). These analyses included mtDNA sequence data published previously (see supplementary Table S1). Samples and sequence references are listed in Table S1.

The DNA sequence data were analysed using maximum-likelihood (ML) analyses following the procedures and recommendations in Sullivan (2005). Studies have shown that there are trade-offs between the fit and performance of parameter-rich models and that the best strategy is to utilize the simplest model that fits the data (e.g. Buckley, Simon & Chambers, 2001; Minin *et al*., 2003). For our ML analyses, the model of sequence evolution was determined using the program DT-ModSel (Minin *et al*., 2003); the selected model was TIM+I+G for both data sets. This model was used in a heuristic search with random addition of taxa, ten replicate searches and TBR branch swapping in PAUP 4.0b10 (Swofford, 2002). Once the model was confirmed to be appropriate for the sequence data, another heuristic search was run using ML and the non-parametric bootstrap procedure (Felsenstein, 1985) to assess support for the nodes in the resulting topologies. To speed the analysis of the larger data set, the 100 non-parametric bootstrap replicates were conducted using a parallelized search strategy on a 108-node 2.8-GHz dual-processor Beowulf cluster.

RESULTS

Key to the *Tegeticula yuccasella* complex and descriptions of new pollinator species

The *T. yuccasella* complex constitutes 17 of 20 recognized species within the genus *Tegeticula*. Members of the complex can be distinguished by their white, or in a few taxa pale brown, forewing coloration. The remaining three species in the genus have forewings that are black, dark grey or white with black dots (Davis, 1967). A unique feature of *Tegeticula* and its sister genus *Parategeticula* is the presence of a prehensile tentacle-like structure on the basal segment of the maxillary palpus in the female (Riley, 1892; Davis, 1967; Pellmyr, 1999; Pellmyr & Krenn, 2002). It is used for pollen collection and in pollination of yucca flowers where the female has oviposited. The tentacles have been secondarily lost, but vestiges are often present, in two species within the *T. yuccasella* complex that oviposit into fruits. Species of the complex are generally distinguishable by genitalic characters. In the male, combinations of aedeagus length and diameter, together with the number and shape of spines that constitute a pectinifer at the posterioventral corner of the valva are sufficient for species identification. As the number of spines may differ on the two valvae of an individual, the summed numbers of spines of the two pectinifers provide more diagnostic power, and are used in this study. Females possess elaborate genitalia modified for cutting–sawing insertion into host plant tissue. Diagnostic traits include the length of the apophyses posteriores used for penetrating the host tissue to reach the oviposition site, the height and length of a dorsal serrated ridge on the ovipositor (the fused, posterior tip of the apophyses posteriores), and the dimensions of two stellate signa inside the corpus bursae, where spermatophores are deposited. Together with the state of the maxillary tentacles, these traits are sufficient to distinguish all taxa.

Males

1. Summed number of spines in pectinifers 37–50; forewing creamy white ..... *mojavella*
– Summed number of spines in pectinifers 11–33; forewing white or rarely brown 2
2. Aedeagus minimum diameter = 0.025 mm 3
– Aedeagus minimum diameter = 0.030 mm 5
3. Antennal integument brown ..... *baccatella*
– Antennal integument yellow, sometimes with darker apical segment 4
4. Aedeagus 1.7–2.0 mm long ..... *maderae*
– Aedeagus 2.7–3.0 mm long ..... *carnerosanella*
5. Hindwing white or very light grey 6
– Hindwing with at least apical region darker, often solid dark brown-grey 8
6. Aedeagus > 2.2 mm long ..... *rostratella*
– Aedeagus = 2.0 mm long 7
7. Aedeagus minimum diameter < 0.04 mm ..... *tehuacana*
– Aedeagus minimum diameter > 0.05 mm ..... *baja*
8. Aedeagus minimum diameter = 0.06 mm 9
– Aedeagus minimum diameter = 0.05 mm 13
9. Aedeagus > 2.2 mm long ..... *corruptrix*
– Aedeagus < 1.8 mm long 10
10. Summed number of spines in pectinifers 25–27; hindwing white with darker apical region ..... *elatella*
– Summed number of spines in pectinifers 13–20; hindwing dark brown to grey 11
11. Ventral base of valva to pectinifer 1.15–1.30 mm, ventral base of valva to apex 1.50–1.70 mm; forewing white, hindwing dark brown ..... *superficiella*
– Ventral base of valva to pectinifer 0.95–1.15 mm, ventral base of valva to apex 1.35–1.50 mm; forewing sometimes with brown scales, hindwing light with grey areas 12
12. Aedeagus diameter 0.07–0.08 mm; forewing often with brown scales ..... *intermedia*
– Aedeagus diameter 0.09–0.10 mm; forewing without brown scales ..... *cassandra*
13. Ventral edge of valva anterior of pectinifer with distinctive crescent shape 14
– Ventral edge of valva anterior of pectinifer at most slightly concave 15
14. Crescent ≤ 0.05 mm wide, < 0.1 mm deep; summed number of spines of both pectinifers ≥ 21 ..... *altiplanella*
– Crescent > 0.05 mm wide, >0.1 mm deep; summed number of spines of both pectinifers rarely > 18 .... *yuccasella*
15. Vinculum–saccus length = 1.35 mm ..... *tambasi*
– Vinculum–saccus length = 1.50 mm 16
16. Aedeagus minimum diameter 0.04 mm, forewing length 19.5–23.4 mm ..... *mexicana*
– Aedeagus minimum diameter 0.05 mm, forewing length 23.5–25.5 mm ..... *californica*

Females

1. Tentacles on maxillary palpi rudimentary or wholly absent 2
– Tentacles on maxillary palpi fully developed 3
2. Apophyses posteriores < 6 mm long, signa > 1.1 mm in diameter ..... *intermedia*
– Apophyses posteriores > 8 mm long, signa < 1.0 mm in diameter ..... *corruptrix*
3. Hindwing white or very light grey, general habitus an all-white moth 4
– Hindwing grey or brown in part or their entirety 7
4. Antennal integument brown or dark brown ..... *rostratella*
– Antennal integument yellow 5
5. Signa ≥ 0.8 mm in diameter ..... *baja*
– Signa ≤ 0.6 mm in diameter 6
6. Serrated ridge of ovipositor 0.04–0.08 mm in height; apophyses posteriores 5.1–5.7 mm in length ..... *tehuacana*
– Serrated ridge of ovipositor 0.01 mm in height; apophyses posteriores 7.6–8.3 mm in length ..... *carnerosanella*
7. Serrated ridge of ovipositor ≥ 0.06 mm high, signa >1.2 mm in diameter 8
– Serrated ridge of ovipositor ≤ 0.04 mm high, signa = 1.2 mm in diameter (usually much less) 10
8. Very prominent serrated ridge of ovipositor 0.06–0.07 mm in height, signa 1.2–1.3 mm in diameter, hindwing light with darker apical region, never uniformly dark brown ..... *elatella*
– Serrated ridge of ovipositor 0.07–0.09 mm in height, signa 1.3–1.7 mm in diameter; hindwing uniformly brown or dark brown or grey with darker apical region 9

9. Signa diameter 1.30–1.50 mm; hindwing uniformly brown or dark brown ..... *superficiella*
– Signa diameter 1.45–1.70 mm, hindwing light with darker grey apical region ..... *cassandra*
10. Signa 0.9–1.2 mm in diameter 11
– Signa ≤ 0.8 mm in diameter 13
11. Ovipositor ridge ≥ 0.09 mm in height ..... *yuccasella*
– Ovipositor ridge ≤ 0.03 mm in height 12
12. Apophyses posteriores ≥ 5.85 mm in length ..... *tambasi*
– Apophyses posteriores ≥ 6.3 mm in length ..... *californica*
13. Signa diameter ≤ 0.45 mm, apophyses posteriores > 7.5 mm in length; hindwing dark greyish brown ..... *baccatella*
– Signa diameter ≥ 0.55 mm, apophyses posteriores ≤ 7.3 mm (usually far less); hindwing dark brown or otherwise 14
14. Antennal integument yellow, with apical segment often darker ..... *maderae*
– Antennal integument brown or dark brown 15
15. Serrated ovipositor ridge height 0.030–0.035 mm, half that of ovipositor diameter ..... *altiplanella*
– Serrated ovipositor ridge so low that teeth appear to arise from ovipositor surface 16
16. Apophyses posteriores < 5.9 mm in length; forewing white ..... *mexicana*
– Apophyses posteriores > 6.6 mm in length; forewing creamy white ..... *mojavella*

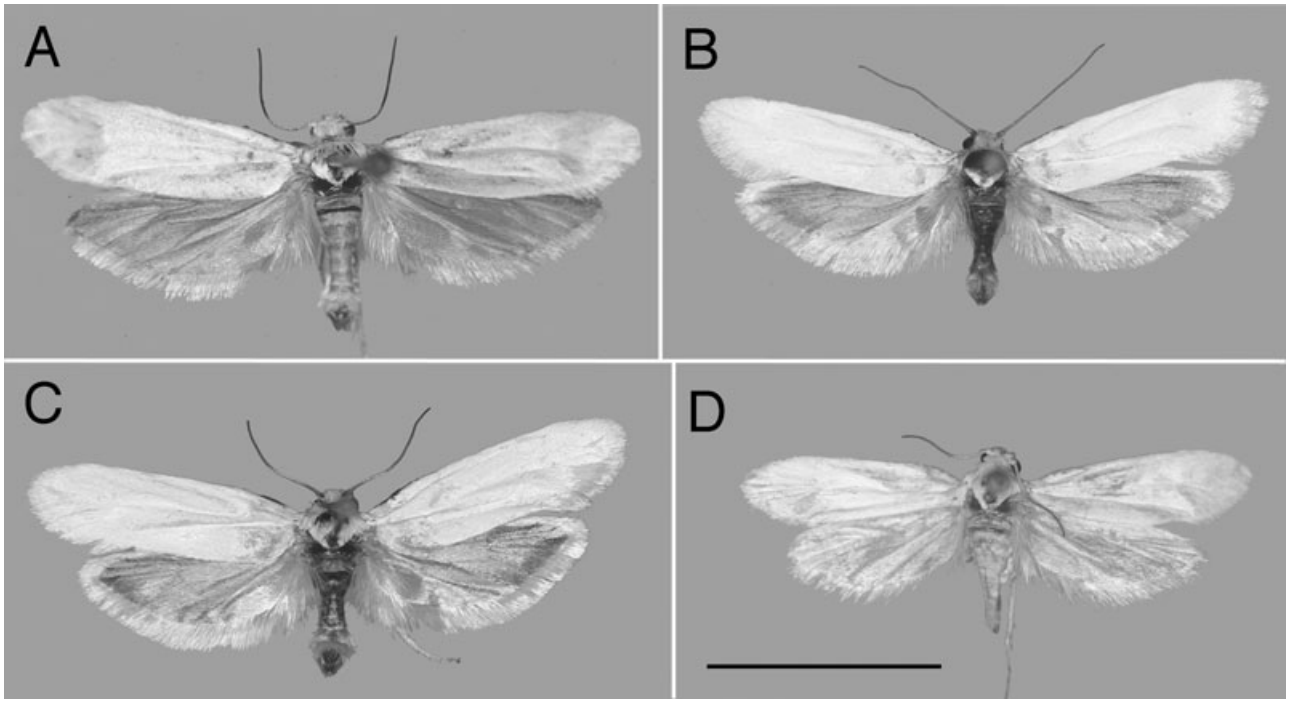

Figure 1. Representative adults of (A) ***Tegeticula californica* sp. nov.** (CA: E Encenitas), (B) ***T. tehuacana* sp. nov.** (Pue. N Azumbilla), (C) ***T. tambasi* sp. nov.** (Mich. San José Coapa), (D) ***T. baja* sp. nov.** (BCS. W La Paz). Location given in parentheses. Scale bar = 10 mm.

DESCRIPTIONS OF NEW SPECIES AND TAXONOMIC CHANGE

TEGETICULA CALIFORNICA PELLMYR, SP. NOV. (FIG. 1A)

Wingspan: m 23.4–25.5 mm, f 27.5–30 mm. Integument dark brown.

Head: With white scales. Maxillary palp with fully developed brown tentacle in female, in male absent or a minor protuberance. Labial palp with brown scales dorsally on all segments except terminal one, intermixed with white or tan scales ventrally on two segments and all-white terminal one, ventrally white on all segments; female with 20 or more sensory setae ventrally on second segment; 1–3 setae present in most males. Proboscis amber, lighter than maxillary palp. Antenna ~0.40–0.45× length of forewing, with 45–50 segments; white scales cover basal 20–25 segments, remainder dark brown.

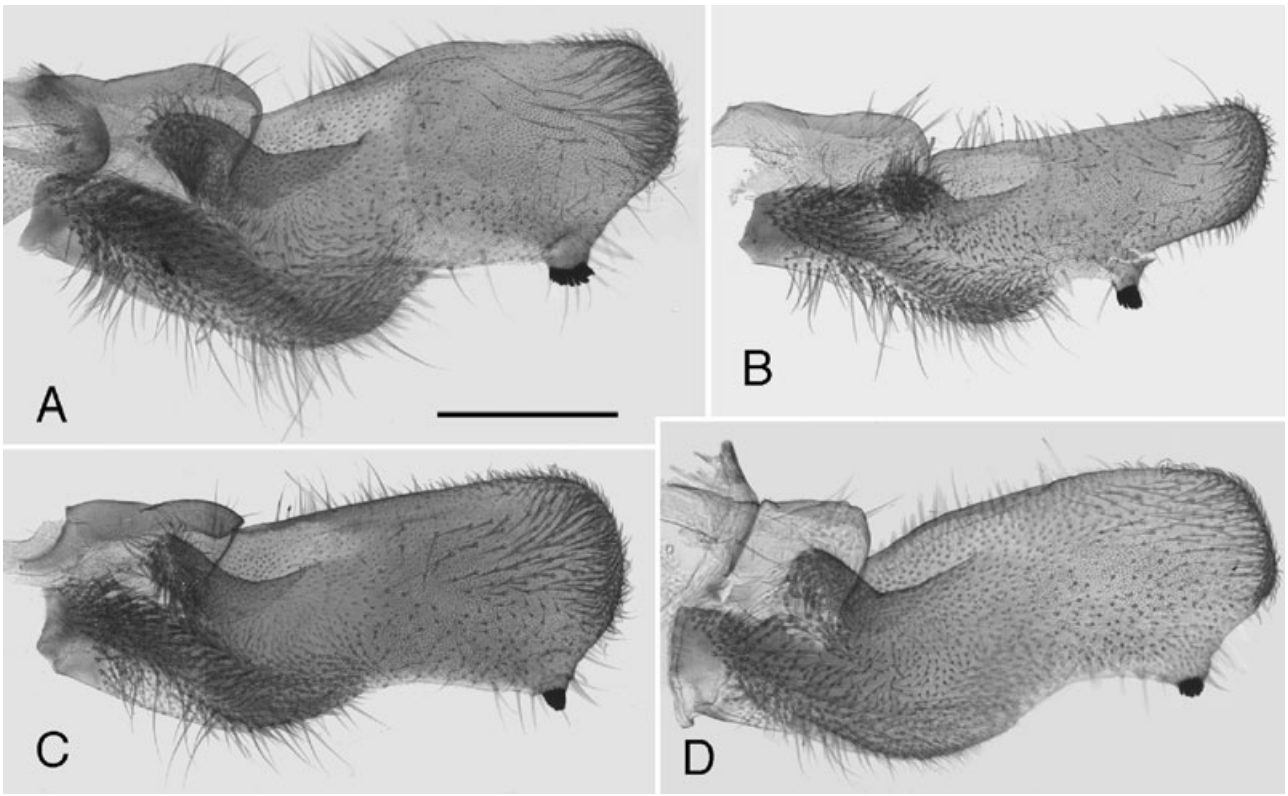

Figure 2. Right valva of (A) ***Tegeticula californica* sp. nov.** (CA: E Encenitas), (B) ***T. tehuacana* sp. nov.** (Pue. Acatepec), (C) ***T. tambasi* sp. nov.** (Mich. San José Coapa), (D) ***T. baja* sp. nov.** (BCS. W La Paz). Scale bar = 0.5 mm.

Thorax: With white scales. Legs medium to dark brown. Forewing length in male 10.5–11.5 mm, female 13–14.2 mm; width in male 2.4–3.5 mm, female 3.8–4.0 mm; dorsal surface white; all individuals with narrow band of dark brown scales on costa from base to 20–30% of entire length. Underside dark brown except for yellowish white portion overlapping hindwing. Forewing fringe white. Hindwing brownish grey, darkest by fore edge and apex, gradually lighter toward back corner. Underside scaled in brownish grey, with darker area along fore edge where overlapping with forewing, and near apex. Hindwing fringe light tan in female, in male tan in basal third, creating a relatively distinct line.

Abdomen: With dorsal scaling tan, with lighter scales along posterior edge of each segment; in both sexes last two segments with white erect scales forming brush. Underside white to light tan. In male, valva with white or light tan scales, often with darker scales on posterioventral edge near pectinifer.

Male genitalia: Vinculum–saccus 1.58–1.76 mm long, cucullus 1.42–1.54 mm from base to apex, with parallel upper and lower edges, bending dorsad and then ventrad, with outer edge slightly rounded (Fig. 2A), asymmetrical pectinifer consisting of 6–11 fused spines (Fig. 3A). Aedeagus 1.78–1.94 mm long, 0.05 mm in diameter (Fig. 4A).

Female genitalia (Fig. 5A)*:* Apophyses posteriores 6.33–7.03 mm long; ovipositor with 0.83–0.92-mm-long, 0.025-mm-high serrated dorsal ridge starting 0.03–0.07 mm behind tip (Fig. 6A); corpus bursae 2.40–2.80 mm long, 1.40–1.60 mm wide, with two 1.08–1.14-mm stellate signa.

GenBank accession number: DQ075470.

Etymology: The species epithet refers to the California cismontane floristic region (Raven & Axelrod, 1978), which contains the known sites of the species.

Material examined: 8m, 9f.

Holotype, f. USA: California. San Diego Co. 4.8 km [3 mi] E Encenitas. 15 m elev. 27–28.iv.1967. 'From flowers of *Yucca schidigera*'. (*Davis*) (USNM). *Paratypes*: Same data as holotype, 4m, 4f.

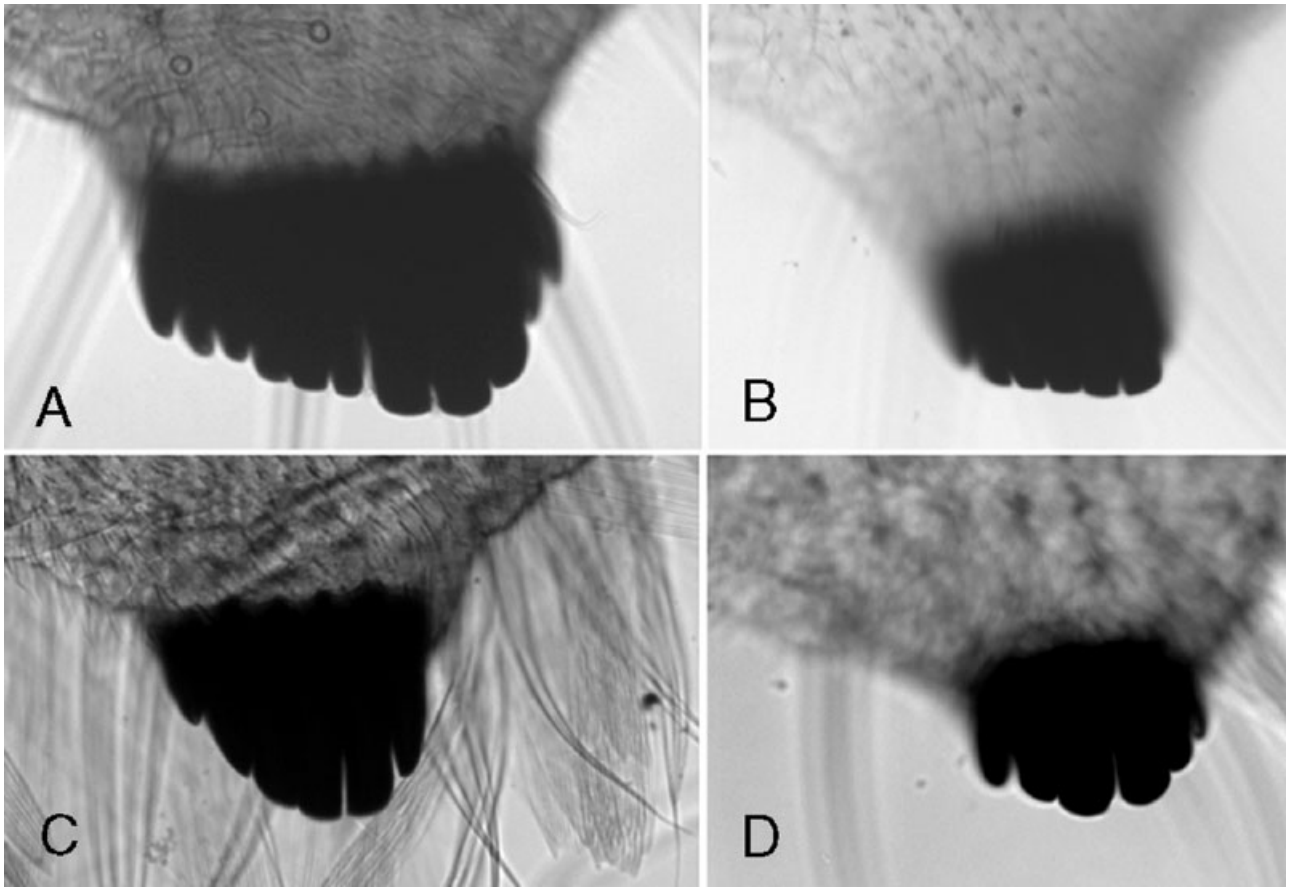

Figure 3. Pectinifer of (A) ***Tegeticula californica* sp. nov.** (CA: E Encenitas), (B) ***T. tehuacana* sp. nov.** (Pue. Acatepec), (C) ***T. tambasi* sp. nov.** (Mich. San José Coapa), (D) ***T. baja* sp. nov.** (BCS. W La Paz). For scale, see pectinifers in context in Figure 2.

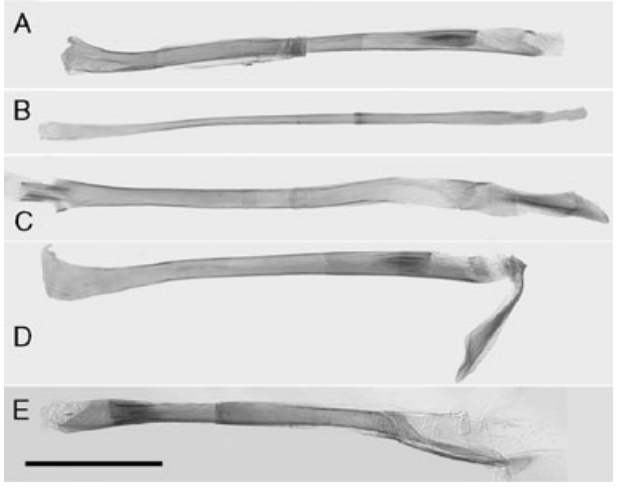

Figure 4. Aedeagus of (A) ***Tegeticula californica* sp. nov.** (CA: E Encenitas), (B) ***T. tehuacana* sp. nov.** (Pue. Acatepec), (C, D) ***T. tambasi* sp. nov.** (SLP. Pozo de Santa Clara; Mich. San José Coapa), latter with cornutus in everted vesica, (E) ***T. baja* sp. nov.** (BCS. W La Paz). Scale bar = 0.5 mm.

Other specimens: USA: San Diego Co. Torrey Pines State Park. 25.iv.2001, 1f (Udovic), *ibid*., 8.iv.2003, 3m, 4f (*Leebens-Mack*). All specimens cryopreserved in Pellmyr lab.

Known hosts and oviposition site: Yucca schidigera. The female oviposits into the ovary of the host, causing characteristic constrictions in the mature fruit.

Distribution (Fig. 9)*:* Thus far known only from a 40-km-long coastal stretch in southernmost California.

Flight period: April.

Comments: Although the host of *T. californica* is widespread across the Mojave desert, this rather large *Tegeticula* species appears to be confined to the coastal, cismontane region of southern California. This unusual region with coastal fog extends southward into Baja California, as does the host, and the

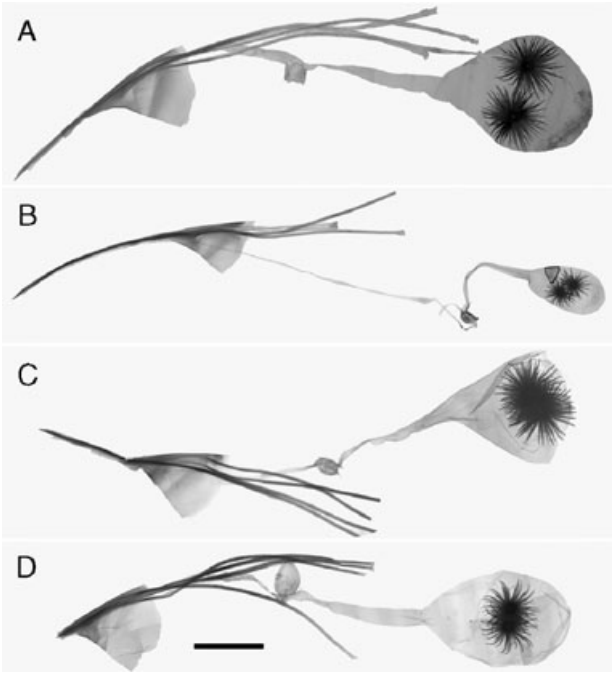

Figure 5. Female genitalia, including ovipositor to corpus bursae. (A) ***Tegeticula californica* sp. nov.** (CA: E Encenitas), (B) ***T. tehuacana* sp. nov.** (Pue. Zacatepec), (C) ***T. tambasi* sp. nov.** (anterior portion of c.b. ruptured) (Mich. San José Coapa), (D) ***T. baja* sp. nov.** (ovipositor concealed within conical VIIth–VIIIth abdominal segment) (BCS. W La Paz). Scale bar = 1 mm.

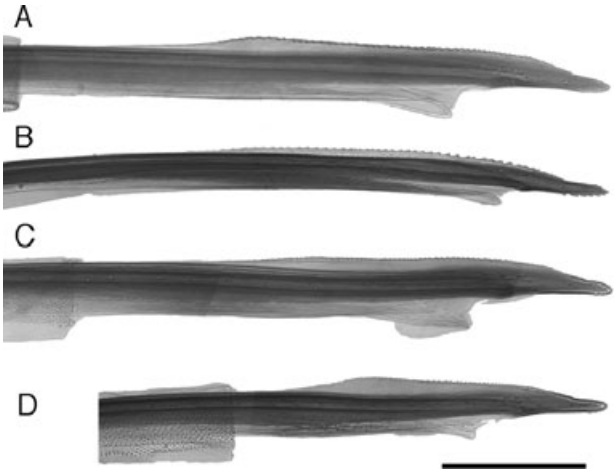

Figure 6. Ovipositor tip of (A) ***Tegeticula californica* sp. nov.** (CA: E Encenitas), (B) ***T. tehuacana* sp. nov.** (Pue. Zacatepec), (C) ***T. tambasi* sp. nov.** (Mich. San José Coapa), (D) ***T. baja* sp. nov.** (BCS. San Jacinto). Scale bar = 0.5 mm.

species should be sought there. A sample of this species is referred to under the label 'T. "california"' in Althoff *et al*. (2006).

TEGETICULA TEHUACANA PELLMYR & BALCÁZAR-LARA **SP. NOV.** (FIG. 1B)

Wingspan: m 20–24 mm, f 22.5–26 mm. Integument amber yellow.

Head: With white scales. Maxillary palp with fully developed brown tentacle in female, in male at most with minor protuberance at point of eruption in female, ventrodistad on first segment. Labial palp with brown scales dorsally on all segments, intermixed with white or tan scales on two distal segments, ventrally white on all segments; female with 20 or more sensory setae ventrally on second segment; 1–3 setae present in most males. Proboscis amber, concolorous with maxillary palp in male, slightly darker in female. Antenna ~0.5× length of forewing, with 45–50 segments; white scales cover basal 15–20 segments, remainder amber yellow.

Thorax: With white scales. Legs light brown. Forewing length in male 9–10.5 mm, female 10.3–11.2 mm; width in male 2.4–3.0 mm, female 2.9–3.3 mm; dorsal surface white; all individuals with narrow band of dark brown scales on costa from base to 20–30% of entire length. Underside dark brown except for yellowish white portion overlapping hindwing. Outer third of costa and forewing fringe white. Hindwing dark brownish grey, darkest by fore edge and apex, gradually lighter toward back corner. Underside scaled in brownish grey, with darker area along fore edge where overlapping with forewing. Hindwing fringe white.

Abdomen: With dorsal scaling tan to dark tan, with lighter scales along posterior edge of each segment; in both sexes last two segments with white (m) or tan (f) erect scales forming brush. Underside white to light tan. In male, valva with white or light tan scales, often with darker scales on posterioventral edge near pectinifer.

Male genitalia: Vinculum–saccus 1.5–1.53 mm long, valvae 1.28–1.30 mm from base to apex, with cucullus of nearly even width and nearly straight outer edge (Fig. 2B), nearly symmetrical, slightly convex pectinifer consisting of 6–7 fused spines on slight protrusion from cucullus (Fig. 3B). Aedeagus 1.99 mm long, 0.03 mm in diameter (Fig. 4B).

Female genitalia (Fig. 5B): Apophyses posteriores 5.1–5.7 mm long; ovipositor with 0.63–0.67-mm-long, 0.04–0.08-mm-high serrated dorsal ridge starting 0.075 mm behind tip (Fig. 6B), ductus bursae 2.0–2.5 mm long, corpus bursae 1.28–1.66 mm long, 0.71–0.77 mm wide, with two stellate 0.56-mm signa.

GenBank accession number: DQ924318.

Etymology: The species epithet refers to the Tehuacan desert region, which contains the known sites of the species.

Material examined: 10m, 27f, 30 larvae.

Holotype, m. MEX: Puebla. 6 km N Azumbilla along Rte 150. 2250 m 29.iv.1999. 18°40.981′N, 97°21.052′ W [*Pellmyr & Balcazar-Lara*] (UNAM). *Paratypes*: Same data as holotype, 3m, 5f.

Other specimens: MEX: Puebla. 2 km W Techachalco, 20.iv.2000, 1m, 1f; 7.5 km SW Tehuacan, 29.iv.1999, 2m, 22.vii.1996, 7 larvae; 6 km WSW Santiago Acantepec, 29.iv.1999, 2m, 1f; N San Salvador el Seco, 19.vii.1996, 7 larvae, SW Perote, 20.vii.1996, 14 larvae; Oaxaca. 22–25 km N Tepelmeme, 30.iv.1999, 1f; 7.5 km S Santiago Chazumba, 1f. All [*Pellmyr & Balcazar-Lara*]; specimens dry- or cryo-preserved in the Pellmyr lab.

Known hosts and oviposition site: Yucca periculosa Baker and the doubtfully distinct *Y. mixtecana* García-Mendoza (O. Pellmyr *et al.*, unpubl. data). Eggs are laid inside the ovary, generally creating a characteristic constriction at the point of oviposition in the maturing fruit.

Distribution (Fig. 10)*:* NW Oaxaca, western- and south-westernmost Veracruz, and central-northern portions of Puebla centred around the Tehuacan Valley. Elevational range 1660–2325 m.

Flight period: Late April.

Comments: This is the only known *Tegeticula* species throughout the Tehuacan portion of the Chihuahuan desert.

TEGETICULA TAMBASI PELLMYR & BALCÁZAR-LARA SP. NOV. (FIG. 1C)

Wingspan: m 20–24 mm, f 26–27.5 mm. Integument amber brown.

Head: With white scales. Maxillary palp with fully developed brown tentacle in female, in male at most with a minor protuberance ventrodistad on first maxillary palp segment. Labial palp with brown scales dorsally on all segments, intermixed with white or tan scales on two distal segments, ventrally white on all segments; female with 20 or more sensory setae ventrally on second segment; 1–3 setae present in most males. Proboscis amber, distinctly lighter than maxillary palp. Antenna ~0.40–0.45× length of forewing, with 45–50 segments; white scales cover basal 20–25 segments in full or in part; remainder bare, amber brown turning dark brown on terminal 3–5 segments.

Thorax: With white scales. Legs amber brown, with brown scales anteriodorsally on foretibia, tan hind tibial scales, and white elsewhere. Forewing length in male 9–11.5 mm, female 12–12.5 mm; width in male 2.8–3.2 mm, female 3.4–3.7 mm; dorsal surface white; all individuals with narrow band of dark brown scales on costa from base to 20–40% of entire length. Underside brown or dark brown except for off-white portion overlapping hindwing and distal portion of costa. Forewing fringe white. Hindwing brownish grey or light brownish grey, darkest by fore edge and apex, gradually lighter toward back corner. Underside sparsely scaled in brownish grey, with darker area along fore edge where overlapping with forewing. Hindwing fringe white, occasionally with basal third light tan.

Abdomen: With dorsal scaling tan, with lighter scales along posterior edge of each segment; in both sexes last two segments with white erect scales forming brush. Underside white to light tan. In male, valva with white and light tan scales, often with darker scales on posterioventral edge near pectinifer.

Male genitalia: Vinculum–saccus 1.15–1.35 mm long, valvae 1.40–1.43 mm from base to apex, with nearly parallel upper and lower edges (Fig. 2C), and a slightly concave margin anterior of mostly fused 5–6-spined, dome-shaped pectinifer (Fig. 3C). Aedeagus 1.50–1.86 mm long, 0.05 mm in diameter (Fig. 4C).

Female genitalia (Fig. 5C)*:* Apophyses posteriores 5.75–5.85 mm long; ovipositor with 0.64–0.66-mm-long, 0.023–0.030-mm-high serrated dorsal ridge starting 0.038–0.075 mm behind tip (Fig. 6C); corpus bursae 2.42–2.47 mm long, 1.76–1.91 mm wide, with two 1.16–1.18-mm signa.

GenBank accession number: DQ924343.

Etymology: The species epithet is derived from ‘tambasi’, the Purépecha (Tarascan) name for the sole

known host, *Yucca filifera*, in the region of the type locality.

Material examined: 27m, 9f.

Holotype, m. MEX: Michoácan. San Jose Coapa, rt 120 W Tiripetío near Morelia. 2080 m. 19°33.295′N, 101°23.443′W, 29.v.1999. (*Pellmyr & Balcázar-Lara*) (UNAM). *Paratypes*: Same data as holotype, 2m, 2f.

Other specimens: MEX. Querétaro. km 10 Pinal de Amoles, carr. de Bucareli, 1m, 28.v.1998 (*Balcázar-Lara & Ibarra*); San Luis Potosí. 4 km S Santa Maria, W Pozo de Santa Clara, 10.viii.1998, 14m, 1f; 11 km N Moctezuma, 11.viii.1998, 4m; 1 km E Gogorrón, 11.viii.1998, 1m (all *Pellmyr & Balcázar-Lara*); El Trinquete, 31.vii.1961, 1f (*Bastida*); Same data as holotype, 4m, 5f cryopreserved in the Pellmyr lab.

Known hosts and oviposition site: Yucca filifera Chabaud. Eggs are laid in the floral ovary, with exact location of the egg being unknown. Illustration of characteristic fruit damage from oviposition by the species is given in Villavicencio & Pérez-Escandón (1995; fig. 1). The egg is probably placed inside the locule or in the interior locule wall.

Distribution (Fig. 10): From northern central San Luis Potosí south-eastward to Querétaro, and south-westward beyond Morelia, Michoácan. Elevation 1500–2080 m.

Flight period: Late May to mid August.

Comments: Adults from the eastern populations are generally somewhat lighter and sometimes smaller than ones from the Michoácan region. This species coexists at least in part of the range with *T. mexicana* Bastida (Villavicencio & Pérez-Escandón, 1995). The two species often cause different fruit shape that can aid in rapid monitoring of moth presence. Fruits may be distinctly curved near the middle and have deep external scars ('curvos', *sensu* Villavicencio & Pérez-Escandón, 1995) when *T. tambasi* has oviposited into a subset of the carpels. Meanwhile, *T. mexicana* most commonly oviposits near the top of the ovary (Crabb & Pellmyr, 2004), causing a moderate, symmetrical constriction in the upper portion of the fruit.

TEGETICULA BAJA PELLMYR & BALCÁZAR-LARA **SP. NOV.** (FIG. 1D)

Wingspan: m 18.3–20 mm, f 20.5–23 mm. Integument amber yellow.

Head: With white scales. Maxillary palp with fully developed brown tentacle in female, and at most a prominent rudiment in the male. Labial palp with limited brown scales dorsally on all segments, intermixed with white or tan scales on two distal segments, ventrally white on all segments; female with 20 or more sensory setae ventrally on second segment; 1–3 setae present in most males. Proboscis amber, lighter than maxillary palp. Antenna ~0.4× length of forewing, with ~45 segments; white scales cover basal half, remainder bare and integument gradually darker toward tip.

Thorax: With white scales. Legs amber yellow. Forewing length in male 8–8.5 mm, female 9.5–12 mm; width in male 2.5–2.7 mm, female 2.8–3.2 mm; dorsal surface white, with very scattered cinnamon brown scales especially across upper half of wing; all individuals with narrow band of brown scales on costa from base to 20–30% of entire length. Underside light brown except for tan portion overlapping hindwing. Forewing fringe white. Hindwing light brownish grey, darkest by fore edge and apex, gradually lighter toward back corner. Underside scaled in off white, with darker brownish grey area along fore edge where overlapping with forewing. Hindwing fringe white.

Abdomen: With dorsal scaling white to light tan, always with white scales along posterior edge of each segment; in both sexes last two segments with white erect scales forming brush. Underside white. In male, valva with white scales.

Male genitalia: Vinculum–saccus 1.25–1.28 mm long, valvae 1.53–1.58 mm from base to apex, cucullus with most parallel upper and lower edges except for a moderate concavity anterior of six-spined pectinifer (Figs 2D, 3D). Aedeagus 1.66–1.86 mm long, 0.051–0.064 mm in diameter (Fig. 4D).

Female genitalia (Fig. 5D)*:* Apophyses posteriores 5.3–5.45 mm long; ovipositor with 0.45–0.51-mm-long, 0.03-mm-high serrated dorsal ridge starting 0.075–0.09 mm behind tip (Fig. 6D); corpus bursae 1.66–2.55 mm long, 0.89–1.40 mm wide, with two stellate 0.84–0.98-mm signa.

GenBank accession number: DQ924333.

Etymology: The species epithet refers to the Baja California peninsula of north-western Mexico, which circumscribes the range of the species.

Material examined: 5m, 10f, 2 larvae.

Figure 7. ***Parategeticula ecdysiastica*** **sp. nov.**, female (BCS. SW San Bartolo). Wing span = 25 mm.

Holotype, f. MEX*:* BCS. Sierra la Laguna. San Jacinto, 9.viii.2000, 150m. 23°14.562′N, 110°03.790′W, in flower of *Yucca capensis* [*Pellmyr & Balcazar-Lara*] (UNAM).

Other specimens: MEX: BCS. 42 km W La Paz, 10–13.viii.1966, 5m, 9f [*Doyen & Powell*] (UCB); BCS. Sierra La Laguna, above Rancho La Burrera, 20.xi.1999. 2 larvae [*Van Devender et al.*]. Latter specimens cryopreserved in Pellmyr lab.

Known hosts and oviposition site: Yucca valida Trelease and some lower-elevation populations of *Y. capensis* Lenz. The egg is laid inside the ovary, most likely between the wall and the ovules.

Distribution (Fig. 9)*:* The Baja California peninsula from the southernmost Cape region northward at least to Punta Prieta in southern Baja California state.

Flight period: August.

Comments: When on *Y. capensis*, the species may coexist with a *Parategeticula* species described below. The two species are readily distinguished based on gross morphology.

PARATEGETICULA ECDYSIASTICA PELLMYR & BALCÁZAR-LARA **SP. NOV.** (FIG. 7)

Described from the female only, as male is unknown.

Wingspan: f 25 mm. Integument pale amber brown.

Head: With off-white scales. All mouthparts amber. Maxillary palp four-segmented, with fully developed tentacle in female. Labial palp three-segmented, with scattered linear dark brown scales on two terminal segments. Antenna ~0.4× length of forewing, with ~40 segments; scattered, semi-translucent linear scales on basal half, remainder bare.

Thorax: With white scales. Legs sandy brown, with scattered white, linear scales and brown patches dorsally and near lower joint of third tibia; foretibial epiphysis absent. Forewing length in female 11.7 mm, width 3.9 mm; both dorsal and ventral side tannish white, with very light scattering of linear white scales (visible only under magnification); fringe reduced to a few scales at vertex. Hindwing clear-translucent, with very scattered white linear scales above and below; basal half with light fringe of white scales. Underside as upperside. Frenulum absent; humeral lobe present.

Abdomen: With light white scaling in all regions.

Female genitalia (Fig. 8)*:* Seventh tergite slightly elongated, with clusters of sensilla lining posterior edges; 20 sensilla on ventral edge, lateral edges with rows of 12–16 sensilla each, dorsally a much protruding blunt edge with ~20 sensilla arranged in a fan shape. Dark membrane of ninth segment forming a rim surrounding protruding ovipositor tip. Posterior apophyses 2.78 mm long, very heavily melanized and sclerotized, with nodular surface in outermost dorsal portion and then bent downward 90°, ending in a sharp trifid scraper with largest medial cusp; anterior apophyses 2.4 mm long, relatively slender; ductus bursae 0.5 mm long, without hard elements; moderately pyriform corpus bursae 1.21 mm long, 0.60–0.68 mm in diameter, wider in anterior portion; two vestigial signa in anterior portion clear round plates with ~75–100 tiny nodules on interior surface.

GenBank accession number: DQ924360.

Etymology: The species epithet is derived from Greek *ecdysiast*, one who sheds layers, referring to the near complete loss of all wing scales during adult emergence.

Material examined: 1f.

Holotype, female. Mexico: Baja California Sur. SW San Bartolo. Rd to Rancho Sierra de Antonio, 16.8 km from Hwy 1. 820 m. Open oak scrub, in fruit of *Yucca capensis*. 23°41.016′N, 109°56.449′W. 10.viii.2000, em. 1.xi.2000 (*Pellmyr & Balcázar-Lara*) (UNAM).

Known hosts and oviposition site: Yucca capensis Lenz. Oviposition site is not known, but strong similarities in the highly derived ovipositor between this species and that of the closely related *P. pollenifera* would suggest that the female ruptures the plant surface and deposits eggs near the yucca ovary.

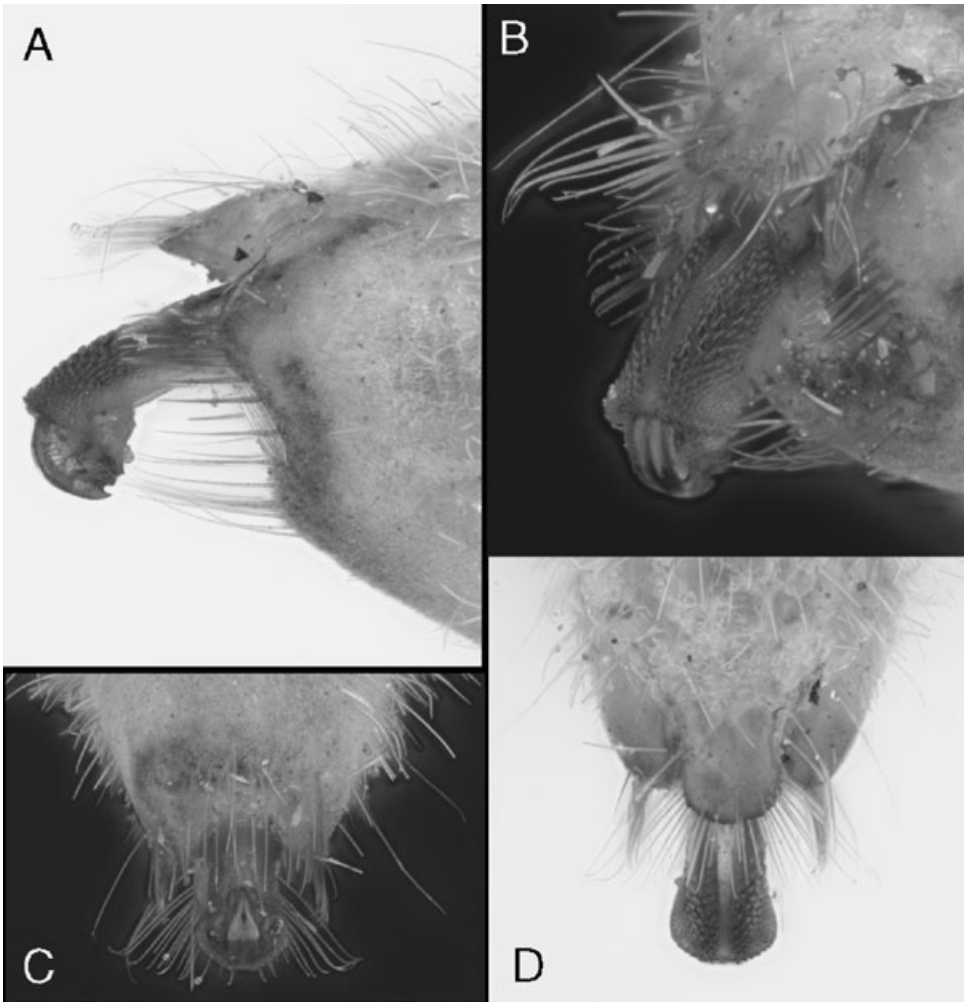

Figure 8. ***Parategeticula ecdysiastica*** **sp. nov.**, female external genitalia. Ovipositor in (A) lateral view, (B) dorsolateral view, (C) ventral view, (D) dorsal view.

Immature biology: Young larvae feed first inside gall-like tissue modified from three seeds ($N = 4$), much like the larvae of *P. pollenifera*. In three cases, they were located at the base of seed rows. One presumed entry hole was found near the base of a fruit, reaching a gall. Two full-size larvae had prepared exit paths to the fruit surface, and in one case consumed five developed seeds adjacent to the site of the consumed, modified tissue.

Distribution: Thus far only known from the type locality in the Sierra la Laguna Mountains of the Cape region of Baja California, Mexico. Elevation 820 m.

Flight period: Three presumed second-instar larvae, including the one reared to become the adult holotype specimen, were collected in early August. In the closely related *P. pollenifera*, the time from oviposition to larval exit takes 35–50 days (Powell, 1984). If similar in this species, it would suggest flight in July.

Comments: Most of the dark forewing scales are left behind in pupal exuviae after adult emergence. This moth most resembles the slightly larger *P. pollenifera*, which has an ovipositor tip bent downward, and similar texture of the eighth abdominal segment (Davis, 1967; Powell, 1984); the ovipositor of *P. ecdysiastica* is more strongly armored, and has a prominent tip bending partway forward. Whereas the three closely related species *pollenifera*, *elephantipella*, and *ecdysiastica* emerge with dark scales, *pollenifera* has far lower dark/light ratio than the others, and *ecdysiastica* loses virtually all forewing scales upon emergence.

Figure 9. Known locations for ***Tegeticula californica* sp. nov.** (squares) and ***T. baja* sp. nov.** (circles). Abbreviations: CA = California, BC = Baja California, BCS = Baja California Sur.

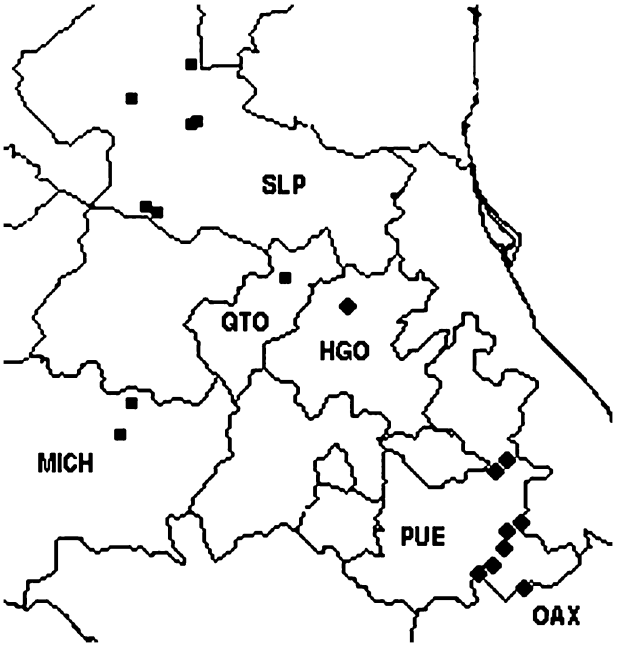

Figure 10. Known locations for ***Tegeticula tehuacana* sp. nov.** (squares) and ***T. tambasi* sp. nov.** (diamonds). Abbreviations: HGO = Hidalgo, MICH = Michoácan, OAX = Oaxaca, PUE = Puebla, QUE = Querétaro, SLP = San Luis Potosí.

Taxonomic change

Tegeticula mexicana Bastida, 1962.

T. treculeanella Pellmyr, 1999

Bastida (1962) described *Tegeticula mexicana* from a total of 23 specimens, deposited in the collections of the Universidad Nacional Autonoma de Mexico (UNAM) and in Instituto de Investigacion de Zonas Deserticas (IIZD; San Luis Potosí, SLP, Mexico). We were only able to recover and examine the material from UNAM, which included the holotype and six other specimens. The material was found to consist of two species. Dissection of the female holotype showed the genitalia to be identical to those of *T. treculeanella* Pellmyr, and DNA analyses of specimens from the geographical region of the *T. mexicana* type and from the type locality of *T. treculeanella* show them to be nested. Thus, *T. treculeanella* is a junior synonym.

PHYLOGENETIC RELATIONSHIPS AND DISCUSSION

The mtDNA sequencing resulted in a 2104-bp region spanning cytochrome oxidase I, the intervening tRNA leucine and the 5′ end of cytochrome oxidase II. The alignment required a single nucleotide insertion for *T. altiplanella* in the tRNA leucine and a single nucleotide deletion for a monophyletic 12-member group within the *yuccasella* complex (*T. californica*, *T. tambasi*, *T. rostratella*, *T. altiplanella*, *T. baccatella*, *T. superficiella*, *T. elatella*, *T. corruptrix*, *T. yuccasella*, *T. intermedia*, *T. cassandra* and *T. baja*) (Fig. 11). While informative, these indels were not used in the phylogenetic analyses. Using DT-ModSel (Minin *et al.*, 2003), we identified TIM+I+G as the simplest model fitting the data. The base frequencies were AT-biased (A = 0.35, C = 0.11, G = 0.11, T = 0.43), the proportion of invariable sites was 0.56, rate heterogeneity estimated as a gamma distribution was 0.86, and the rate matrix was AC and GT = 1.00, AG = 10.52, AT and CG = 2.02, CT = 18.49. The ML analysis resulted in a tree with a score of –lnL = 10207.696 (Fig. 11). The results showed that all but one taxon with two or more samples constituted monophyletic groups, mostly with strong support (Fig. 11). The exception is *T. intermedia*, which included two phenotypic *T. cassandra* (00.1325 and 00.1349) and two *T. elatella* (94.94 and 94.95). Studies using amplified fragment length polymorphism (AFLP) markers to complement the mitochondrial data show that the two *T. cassandra* samples reflect introgression between *T. intermedia* and *T. cassandra* in a recent contact zone in northern Florida (Segraves & Pellmyr, 2004), and the two *T. elatella* samples within *T. intermedia* reflect introgression between the two species in the Big Bend region of Texas (Segraves, Althoff & Pellmyr, 2005). For this

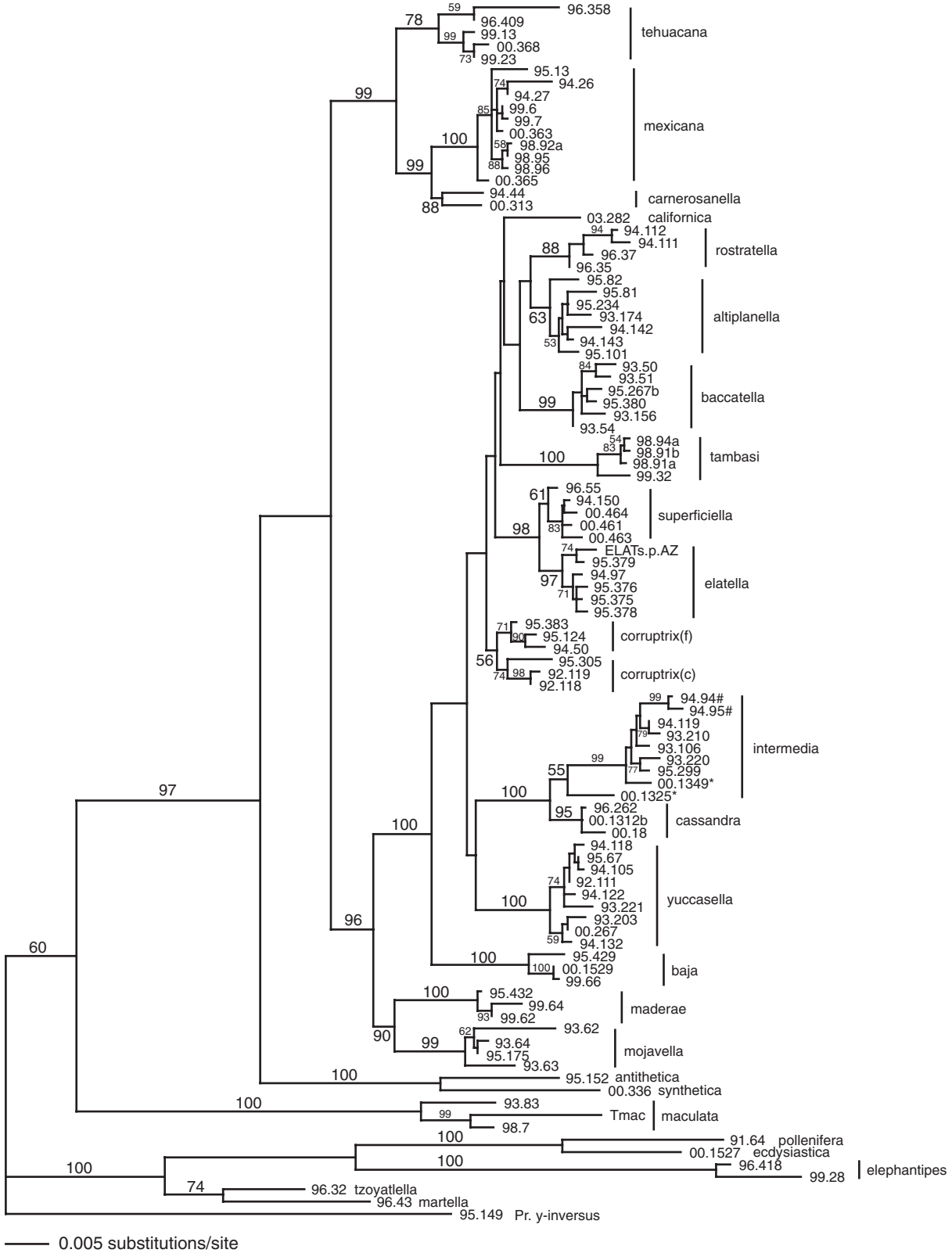
96.358
96.409
99.13
00.368
99.23
tehuacana
95.13
94.26
94.27
99.6
99.7
00.363
98.92a
98.95
98.96
00.365
mexicana
94.44
00.313
carnerosanella
03.282 californica
94.112
94.111
96.37
96.35
rostratella
95.82
95.81
95.234
93.174
94.142
94.143
95.101
altiplanella
93.50
93.51
95.267b
95.380
93.156
93.54
baccatella
98.94a
98.91b
98.91a
99.32
tambasi
96.55
94.150
00.464
00.461
00.463
superficiella
ELATs.p.AZ
95.379
94.97
95.376
95.375
95.378
elatella
95.383
95.124
94.50
corruptrix(f)
95.305
92.119
92.118
corruptrix(c)
94.94#
94.95#
94.119
93.210
93.106
93.220
95.299
00.1349*
00.1325*
intermedia
96.262
00.1312b
00.18
cassandra
94.118
95.67
94.105
92.111
94.122
93.221
93.203
00.267
94.132
yuccasella
95.429
00.1529
99.66
baja
95.432
99.64
99.62
maderae
93.62
93.64
95.175
93.63
mojavella
95.152 antithetica
00.336 synthetica
93.83
Tmac
98.7
maculata
91.64 pollenifera
00.1527 ecdysiastica
96.418
99.28
elephantipes
96.32 tzoyatlella
96.43 martella
95.149 Pr. y-inversus
0.005 substitutions/site

Figure 11. Results from maximum-likelihood-based bootstrap analysis of the phylogenetic relationships among 113 exemplars of all recognized species of *Tegeticula* and *Parategeticula*, based on a 2104-bp region of mtDNA. *Prodoxus y-inversus* was used as outgroup. Vertical bars on the right indicate morphological circumscription of named taxa; all taxa are monophyletic, except for *T. intermedia*, which includes two samples of *T. cassandra* (indicated by asterisks) and two samples of *T. elatella* (indicated by #), all of which are known to be the result of introgression. Numbers give non-parametric bootstrap values. For details of analysis see text.

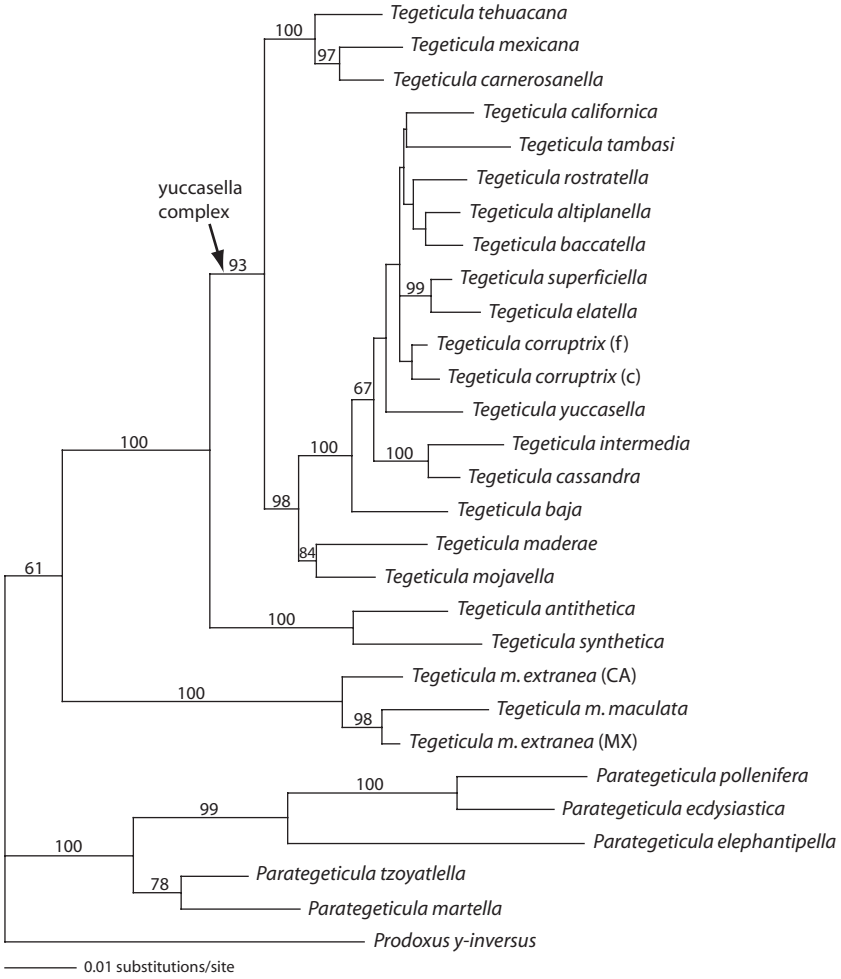

Figure 12. Maximum-likelihood-based reconstruction of the phylogenetic relationships among all recognized species of *Tegeticula* and *Parategeticula*, based on a 2104-bp region of mtDNA. *Prodoxus y-inversus* was used as outgroup. Taxa above arrow are members of the *yuccasella* complex. For details of analysis see text.

reason, when exemplars were drawn for the species-level analysis, specimens were chosen from sites well away from the known sites of introgression.

In the species-level analysis, raw sequence divergence within the ingroup ranged from 0.52 to 8.37%. The simplest model fitting the data in the second analysis was TIM+I+G. Base frequencies were AT-biased (A = 0.33, C = 0.12, G = 0.12, T = 0.42), the proportion of invariable sites was 0.60, rate heterogeneity estimated as a gamma distribution was 1.29, and the rate matrix was AC and GT = 1.00, AG = 10.57, AT and CG = 3.74, CT = 25.09. The ML-based analysis resulted in a tree with a score of –lnL = 7959.65 (Fig. 12). This tree provides important insight into diversification within the genera. The two traditionally recognized genera, *Parategeticula* and *Tegeticula*, are monophyletic. It is worth noting the modest support for *T. maculata*, the basal species of its genus, with the remainder of *Tegeticula*. This species utilizes the monobasic *Hesperoyucca whipplei*, a member of the sister group of *Yucca* (Bogler, Neff & Simpson, 1995), and appears to have diverged early

on from the remainder of *Tegeticula*, with which it shares a cutting ovipositor used for ovipositing inside host ovaries. In contrast, members of *Parategeticula* as a unique condition within the Prodoxidae oviposit externally, and the larvae bore into the ovary to feed on seeds and modified seed tissue. The three-species clade (*pollenifera*, *ecdysiastica* and *elephantipella*) use a highly derived, multicusped ovipositor tip (Fig. 9; Davis, 1967; Powell, 1984; Pellmyr & Balcázar-Lara, 2000) to rupture the plant epidermis and deposit eggs, whereas the two-species clade (*martella* + *tzoyatlella*) lacks the ripping structures (Pellmyr & Balcázar-Lara, 2000). The soft posterior edges of the ovipositor of the latter group suggest absence of cutting or puncturing ability, and larval entry holes in *P. tzoyatlella* suggest external oviposition without plant surface rupture. The two major clades in the group are confined to Sierra Madrean woodlands and the northern Chihuahuan desert, respectively.

Within *T. maculata*, there are three fairly distinctive phenotypes, including the type form with a white, black-spotted forewing, the form *extranea* to its south with an all-black forewing, and a smaller-sized, unnamed lead grey morph from Cataviña, Mexico, southward (Powell & Mackie, 1966; Segraves & Pellmyr, 2001; Althoff *et al*., 2007). Following this clade, there is very strong support for a clade containing *T. antithetica* and *T. synthetica*, the two dark grey pollinators of the highly distinctive *Yucca brevifolia*. These two species are mostly allopatric, but there is evidence for at least one contact zone (C.I. Smith *et al.*, unpubl. data).

The remaining 17 species are members of the *yuccasella* complex (Fig. 12), which share more or less completely white forewing. Basal relationships are well resolved, with a group of three species from different portions of the Chihuahuan desert at its base, then a two-species group from the Mojave desert (*mojavella*) and from the Sierra Madre Occidental pine–oak woodlands (*maderae*), and finally a species (*baja*) from the Baja California peninsula. In contrast to the strong resolution up to this point, there is evidence of rapid diversification and resulting poor resolution in the remaining clade above the other taxa. It contains primarily northern species, but also a single Mexican endemic species (*tambasi*). Furthermore, there has been life-history diversification in this group, with evolution of three distinctive oviposition modes that affect both the plant–moth interaction and interactions among moths. As a basal, most frequent condition among the moths, females oviposit directly into the floral locule, which selects against high egg numbers per flower by triggering a floral abscission mechanism (Pellmyr & Huth, 1994). Four species (*elatella*, *cassandra*, *superficiella* and *intermedia*) have evolved the habit of ovipositing superficially on the floral ovary, thus avoiding selecting abscission, but instead being susceptible to abiotic mortality factors, such as desiccation (Segraves, 2003). Two species have shifted to a less costly habit, namely cheating, by not pollinating but rather ovipositing directly into fruit; one of them is a superficially ovipositing species, whereas the latter cuts into fruits after the floral abscission has ceased to operate in the plant. The present analysis shows two strongly supported groups of superficially ovipositing species, but there is insufficient support of the basal relationships within this clade to reject a single origin of the habit.

A previous analysis based on AFLP data (Althoff *et al*., 2006) recovered a different set of relationships within the *yuccasella* complex, consisting of *cassandra*, *intermedia* and *elatella* but, again, there was insufficient data to reject a single origin of the habit. The recency and rapidity of diversification in this group is only one confounding factor, as there is evidence of geographically confined introgression between *intermedia* and the two other pollinators. Hybridization occurs in a secondary contact zone between *T. cassandra* and *T. intermedia* in northern Florida (Segraves & Pellmyr, 2004), and is also evident as ongoing unidirectional introgression of *T. intermedia* into the population of *T. elatella* in the Big Bend region of western Texas (Segraves *et al*., 2005). For the two cheater species, neither analysis shows particularly strong phylogenetic support for two origins of cheating. However, the very strong support for an origin of *intermedia* from a pollinating common ancestor with *cassandra* (Segraves & Pellmyr, 2004; Althoff *et al*., 2006), combined with two rather different modes of oviposition in the cheaters (superficial in young fruit, into locule of full-size fruit; Pellmyr, Leebens-Mack & Huth, 1996; Pellmyr, 1999), supports the dual-origin hypothesis. A second difference between the mtDNA and the AFLP phylogenies is the placement of *T. yuccasella*. In the present analysis, it is weakly supported as sister to a complex of eight species that includes both superficially and locule-ovipositing taxa, as well as one of the species of cheater yucca moths. In contrast, the AFLP analyses placed *T. yuccasella* within a morphologically and biologically homogeneous clade consisting of *T. rostratella*, *T. altiplanella* and *T. baccatella*, all of which oviposit into the locule of their hosts (Althoff *et al*., 2006). Bootstrap support was moderately strong (83–86%) in the AFLP analysis, and the reduced homoplasy in oviposition habit evolution also lends credence to this placement of *T. yuccasella*.

CONCLUSION

The obligate mutualism between yucca moths and yuccas was the first discovered instance of obligate

pollination mutualism involving a pollinating seed predator (Riley, 1872a, b), and it has been followed by more recent discovery of a few other similar associations (e.g. Holland & Fleming, 1999; Weiblen, 2002; Kawakita *et al*., 2004; Machado *et al*., 2005). They are excellent prospects for the study of coevolution, but prospects have been hampered by extraordinary species richness (figs and fig wasps; Machado *et al*., 2005) or species dearth (*Lophocereus* cactus and the senita moth; Holland & Fleming, 1999). The present concluding revision of known yucca moths documents an intermediate level of diversity for this group, making it highly useful for studies of coevolution. In addition, robust reconstruction of most relationships within pollinating and cheating yucca moths here, and the bogus yucca moths (*Prodoxus*) elsewhere (Pellmyr *et al*., 2006), together with imminent availability of a well-resolved phylogeny for the host yuccas (Pellmyr *et al*., 2007; Smith *et al*., 2007) now sets the stage for phylogenetically based analyses of the evolution of life histories and obligate mutualistic interactions of broad general interest for our understanding of obligate interspecific mutualism.

ACKNOWLEDGEMENTS

The results presented here result from over a decade of sampling, and has greatly benefited from the aid of many individuals. We especially thank Don Davis and Jerry Powell who have generously shared their experience with prodoxids over the years. Jack Sullivan has kindly taken time for discussions about molecular phylogenetics. Samples for the analyses were kindly provided by Jim Leebens-Mack, Ron Leuschner and Paul Wilson. The Florida Park Service, Ocala National Forest, The Nature Conservancy at Apalachicola Bluffs, Archbold Biological Station, Big Bend National Park, Black Gap Wildlife Management Area, Jornada Long-Term Ecological Research site, Torrey Pines State Reserve and White Sands National Monument provided permits and access to field sites. National Science Foundation grants DEB 024778, 024783 and 0516841 to O.P. funded much of the molecular work, and a National Geographic Society exploration grant to O.P. and M.B.L. funded the fieldwork in Mexico. K.A.S. was in part supported by a National Science Foundation Dissertation Improvement grant and a Joan DeWind Mosenthal Grant from the Xerces Society.

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SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Appendix S1. Samples used in phylogenetic analyses to test for monophyly and to establish species-level relationships.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1096-3642.2007.00361.x
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