UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

Sistemática y evolución del género *Taeniopoda* (Orthoptera: Romaleidae: Romaleinae)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS BIOLÓGICAS

PRESENTA:

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Lic. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, celebrada el día 20 de agosto de 2018, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del alumno **DE JESÚS BONILLA VLADIMIR SALVADOR** con número de cuenta **99095813** con la tesis titulada: **"Sistemática y evolución del género *Taeniopoda* (Orthoptera: Romaleidae: Romaleinae)"**, realizada bajo la dirección del **DR. ALEJANDRO ZALDÍVAR RIVERÓN:**

Presidente:	DR. ADOLFO GERARDO NAVARRO SIGÜENZA
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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 24 de octubre de 2018.

POSGRADO EN CIENCIAS BIOLOGICAS
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DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA

c.c.p. Expediente del (la) interesado (a).

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MÉXICO, CD. MX. **NOVIEMBRE 2018**

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-Juan Gabriel, el divo de Juárez

ÍNDICE

Resumen

Taeniopoda Stål es un género de saltamontes que se distribuye desde el sur de EEUU hasta Panamá. A pesar de su amplia distribución e importancia económica, la taxonomía y sistemática del género han sido pobremente estudiadas. La última revisión taxonómica de *Taeniopoda* fue publicada hace casi un siglo y algunos trabajos sugirieron que el género monotípico *Romalea* Serville es su grupo hermano, aunque esto no ha sido puesto a prueba. En este trabajo se utilizó evidencia morfológica, marcadores moleculares puntuales y genómicos para investigar la sistemática de *Taeniopoda* y su biogeografía histórica. En el capítulo 2 se evaluaron los límites entre las especies en *Taeniopoda* utilizando morfología y dos marcadores mitocondriales (COI, cyt *b*), se reconstruyeron sus relaciones filogenéticas adicionando dos marcadores nucleares (28S, H3) y se estimaron sus tiempos de origen y divergencia. Se detectaron pseudogenes mitocondriales (*numts*) y probable introgresión mitocondrial de *T. tamaulipensis* en especímenes de *T. eques* del centro de México. Se delimitaron entre seis y 14 especies dependiendo del método y marcador empleado, de las cuales cuatro fueron consideradas "estables" por estar soportadas por al menos uno de los análisis moleculares y morfología. *Taeniopoda* se recuperó como parafilética con respecto a *Romalea* y se recuperaron tres clados principales, dos con especies con una cresta pronotal considerablemente elevada y otro en cuyos miembros la está poco elevada. Se estimó que el origen y posterior diversificación de especies en *Taeniopoda* ocurrió en el Mioceno-Plioceno. La diversidad actual de especies en el género pudo haberse originado durante el Pleistoceno, probablemente influenciada por las oscilaciones climáticas ocurridas durante ese período y la elevación de las cordilleras en Centroamérica. En el tercer capítulo se evaluaron los límites entre especies y las relaciones filogenéticas en *Taeniopoda* utilizando datos genómicos de 3RAD, así como la existencia de flujo genético y sobrelapamiento de nicho ecológico entre *T. eques* y *T. tamaulipensis.* Los datos de 3RAD se ensamblaron *de novo* con diferentes parámetros para explorar cómo diferentes configuraciones afectan las reconstrucciones obtenidas. El umbral de similitud y número mínimo de muestras que deben tener información de un locus para ser retenido en la matriz final tienen impacto en la cantidad de *loci* y datos faltantes obtenidos en las matrices. Sin embargo, las relaciones obtenidas utilizando diferentes matrices son congruentes entre sí, incluso con gran cantidad de datos faltantes. Diez especies fueron consistentemente delimitadas, con *T. picticornis* y *T. stali* consideradas conespecíficas, mientras que las poblaciones de *T. auricornis* del centro

de México y Guatemala se consideraron como diferentes especies. A pesar de mantener su estado específico, *T. eques* y *T. tamaulipensis* no se recuperaron consistentemente como especies diferentes. Los resultados sugieren que estas dos especies representan una “especie anillo”, ya que sus poblaciones examinadas parecen variar gradualmente en "forma de bucle" a lo largo de su distribución geográfica. Los análisis filogenómicos confirman la parafilia de *Taeniopoda* con respecto a *Romalea* y la presencia de tres clados principales dentro del grupo. Este estudio demuestra la utilidad de 3RAD para detectar el flujo de genes y resolver límites entre especies y relaciones filogenéticas entre taxones estrechamente relacionados. En el cuarto capítulo se realiza una revisión taxonómica del grupo de estudio con base en las inferencias taxonómicas realizadas en los dos capítulos anteriores. *Taeniopoda* se considera como sinónimo de *Romalea* y se reconocen 12 especies dentro del género.

Abstract

Taeniopoda Stål is a genus of grasshoppers that is distributed from the south of the USA to Panama. Despite its wide distribution and economic importance, the taxonomy and systematics of this genus have been poorly studied. The last taxonomic revision of *Taeniopoda* was published almost a century ago, and some works have also suggested that the monotypic genus *Romalea* Serville is its sister group, although this has not been tested. In this work, morphological evidence, punctual molecular markers and genomic data were used to investigate the systematics of *Taeniopoda* as well as its historical biogeography. In the second chapter, the limits between species of *Taeniopoda* were evaluated using morphology and two mitochondrial markers (COI, cyt b), their phylogenetic relationships were reconstructed adding two nuclear markers (28S, H3), and their times of origin and divergence were also estimated. Mitochondrial pseudogenes (*numts*) and the probable introgression of mitochondrial DNA in specimens of *T. tamaulipensis* and *T. eques* from central Mexico were detected. Between six and 14 species were delimited based on the method and examined marker, of which four were considered "stable" since they were supported by at least one of the molecular approaches performed and morphology. *Taeniopoda* was recovered as paraphyletic with respect to *Romalea*, and three main clades were recovered, two with species with an elevated pronotal crest and another one whose members have a low elevated crest. The origin and posterior diversification in *Taeniopoda* occurred from the Miocene to the Pliocene. The current species diversity in the genus occurred during the Pleistocene, probably influenced by the climatic oscillations that occurred during that period and the elevation of the mountain ranges in Central America. In the third chapter, the limits among species and phylogenetic relationships in *Taeniopoda* were evaluated using 3RAD genomic data, as well as the existence of genetic flow and ecological niche overlapping between *T. eques* and *T. tamaulipensis*. The 3RAD data were assembled de novo with different parameters to explore how different configurations affect the phylogenetic reconstructions obtained. The similarity threshold and the minimum number of samples that must have information from a locus to be retained in the final matrix have an impact on the quantity of loci and missing data obtained in the matrices. However, the relationships obtained using different matrices are congruent with each other, even with a large amount of missing data. Ten species were consistently delimited; with *T. picticornis* and *T. stali* being considered as conspecific, whereas the populations of *T. auricornis* from central Mexico and Guatemala were divided into two species. Despite maintaining its specific status, *T. eques* and *T.*

tamaulipensis were not consistently recovered as different species. The results suggest that these two species represent a "ring species", since their populations seem to gradually vary following a "loop form" along their geographical distribution. The phylogenomic analyses performed confirm the paraphyly of *Taeniopoda* with respect to *Romalea,* and the presence of three main clades within the group. This study demonstrates the utility of 3RAD for detecting gene flow and resolving species boundaries and phylogenetic relationships among closely related taxa. In the fourth chapter, a taxonomic revision of the group of study was performed based on the taxonomic inferences made in the two previous chapters. *Taeniopoda* is considered as a synonym of *Romalea* and 12 species are recognized within this genus.

Capítulo I: Introducción general

Sistemática y la delimitación de especies

La sistemática es la base del conocimiento de la biodiversidad. Reconstruir las relaciones filogenéticas entre los organismos y la clasificación de éstos es la base de casi cualquier estudio biológico (Contreras-Ramos *et al*., 2007). Los estudios en sistemática biológica nos permiten en primera instancia caracterizar la biodiversidad; es decir, investigar cuántas especies existen (Sites & Marshall, 2003), y en el contexto del estudio del origen de la diversidad es posible estudiar la historia evolutiva de los taxones y comprender su distribución espacial (Santos & Amorim, 2007).

La especie es la unidad fundamental de la biodiversidad y generalmente es el punto de partida para el estudio de la ecología, evolución, conservación y clasificación de los organismos (Hohenegger, 2014; Hull, 1977; Mace, 2004; Mayr, 1996; Miller III, 2001). La delimitación de especies es la identificación de los límites a nivel de especie de la diversidad biológica (Carstens *et al*., 2013), y actualmente es un campo fructífero que propicia la discusión sobre sus bases teóricas y metodológicas (Camargo & Sites, 2013; Rannala, 2015; Sites & Marshall, 2003). La delimitación de especies comprende dos aspectos complementarios: el concepto de especie y el criterio operacional para la delimitación de especies (de Queiroz, 2007). El concepto de especie es la definición teórica de la entidad que se considerará una especie, mientras que el criterio operacional son los atributos que permiten establecer y probar los límites de especie en la biodiversidad observada (Sites & Marshall, 2003). En la literatura se pueden encontrar de veinte a treinta conceptos de especie, y para cada uno se proponen diferentes criterios operacionales (Cracraft, 1983; Mayden, 1997; Zachos, 2016). Esta variedad de conceptos de especie y criterios operacionales puede implicar dificultades de forma que al abordarse el problema con base en diferentes conceptos se pueden generar delimitaciones diferentes con los mismos datos (e. g. Peterson & Navarro-Sigüenza 1999; Dillon & Fjeldså 2005).

Para conciliar los diferentes conceptos de especie y los criterios de delimitación, se propuso el Concepto de Especie de Linaje General, en el que las especies son consideradas líneas directas de ancestría-descendencia, es decir linajes históricos de metapoblaciones o segmentos de metapoblaciones de evolución independiente (de Queiroz, 1998, 2005, 2007). En el marco de esta propuesta, los otros conceptos de especie hacen énfasis en propiedades

particulares del mismo atributo. Por ejemplo, en los conceptos que consideran a las especies como poblaciones interfértiles (como el Concepto Biológico) y aquellos que las consideran como linajes (como el Concepto Genealógico de Especie) no se consideran diferentes atributos de una especie, sino el mismo atributo limitado o extendido en el tiempo. El Concepto de Linaje General no invalida, sino que incluye los otros conceptos utilizados en la descripción y delimitación de especies así que permite conciliar distintas interpretaciones conceptuales de lo que es una especie y permite integrar en los estudios distintas líneas de evidencia y métodos de análisis. Sin embargo, en la literatura científica actual continúa el intenso debate sobre los conceptos de especie y los diversos métodos para delimitarlas (Barberousse & Samadi, 2010; Frankham *et al*., 2012; Hausdorf, 2011; Leavitt *et al*., 2015). Por su importancia, la delimitación de especies es una de las actividades más activas en el estudio de la biodiversidad y no existen criterios o métodos universales para estudiar la biodiversidad. Para tratar de establecer puentes de comunicación entre diferentes disciplinas se propuso el enfoque de la taxonomía integrativa para delimitar las especies desde perspectivas múltiples y complementarias (Dayrat, 2005).

La morfología es la línea de evidencia tradicional utilizada en la delimitación de especies, y sigue siendo la base fundamental en muchos estudios (Elewa, 2010; Hillis, 1987). No obstante, los avances en biología molecular y en particular en la secuenciación de ADN han permitido el uso de secuencias genéticas en los estudios de delimitación de especies. Uno de estos avances fue el descubrimiento de la reacción en cadena de la polimerasa (PCR), que permitió complementar decenas de caracteres morfológicos con cientos o miles de caracteres moleculares (Avise, 2004). Los marcadores moleculares genéticos nos permiten obtener información sobre la historia evolutiva de los organismos que no siempre se ve reflejada por la morfología, por lo que su uso en la delimitación de especies es complementario a otras líneas de evidencia. Se han propuesto métodos de delimitación de especies basados en la divergencia de las secuencias genéticas. Estos métodos se basan en el supuesto de que la divergencia genética entre organismos de una misma especie será menor que entre los organismos de diferentes especies (Hebert *et al*., 2003). También se han propuesto métodos de delimitación de especies basados en procesos biológicos y que tengan soporte estadístico; estos métodos están basados en la Teoría de la Coalescencia y buscan identificar linajes evolutivos independientes, y considera a estos linajes como especies (Fujita *et al*., 2012).

En los últimos años, se han desarrollado técnicas de secuenciación que permiten aumentar el número de datos a secuencias de decenas o cientos de miles de pares de bases (Metzker, 2010). Con los avances en técnicas de secuenciación que permiten obtener información a escala genómica los métodos de delimitación de especies enfrentan dos retos: el primero es hacer frente al esfuerzo computacional implica una cantidad masiva de datos, por otro lado, se deben implementar estrategias que permitan el análisis de estos datos en un marco estadístico (Wiens, 2008). Para enfrentar estos retos los datos genómicos pueden analizados con algunos ajustes a los algoritmos de los métodos existentes para la reconstrucción filogenética y delimitación de especies (Whelan, 2011).

El origen de la biodiversidad en la zona de transición Neotropical y Neártica.

La zona de transición Neártica-Neotropical es un sistema complejo en el que interactúan las biotas de ambas regiones y que tiene un pasado geológico y climático complejo. La región Neotropical comprende la mayor parte de Sudamérica, Centroamérica, las Antillas y parte de México (Morrone, 2014), y se caracteriza por ser una región biogeográfica que concentra una gran riqueza de especies (e.g. Antonelli & Sanmartín, 2011; Mound & Marullo, 1996; Patterson & Costa, 2012). En el territorio mexicano la región Neotropical adquiere otro nivel de complejidad por su interacción con la región Neártica en una zona de transición (Espinosa *et al*., 2008; Halffter & Morrone, 2017; Morrone 2014).

La región Neotropical ha sido influenciada por distintos eventos paleogeológicos y paleoclimáticos. Eventos geológicos como la tectónica de placas han provocado el movimiento de masas continentales, la aparición y desaparición de puentes y barreras geográficas que han favorecido la migración y aislamiento de grupos de organismos (Gutiérrez-García & Vázquez Domínguez, 2013). También se ha propuesto que periodos de transgresión y regresión marina (Haq *et al*., 1987; Brett, 1998), la formación del puente GAARlandia (Iturralde-Vinent & MacPhee 1999), y el cierre del Istmo de Panamá (Schmittner *et al*., 2004, Lessios, 2008) como eventos que han influido en el origen de la biodiversidad.

Los eventos paleoclimáticos también han sido importantes en la conformación de la biodiversidad de la región Neotropical y la zona de transición. Los eventos glaciales e interglaciales del Pleistoceno tuvieron como consecuencia cambios climáticos que influyeron en la distribución de la biota al promover migraciones y extinciones, y conexiones y desconexiones entre poblaciones de organismos (Hewitt, 2000). Estos eventos propiciaron

cambios entre los organismos a nivel de estructura poblacional y especiación (Simpson, 1971, Moritz *et al*., 2000). Además de estos eventos pelogeológicos y paleoclímaticos los mecanismos de adaptación al suelo, vagilidad y conservación del nicho son importantes en la configuración de la biodiversidad (Antonelli & Sanmartín, 2011).

El origen de la biodiversidad en esta zona es complejo y no se puede circunscribir a un solo modelo, periodo de tiempo o evento geológico en particular (Rull 2008; Rull, 2014). El origen de la biodiversidad en la región Neotropical y la zona de transición es complejo. Buena parte de los estudios sobre el tema ponen énfasis en plantas, aves y mamíferos mientras que otros grupos, como los insectos, están menos estudiados (Rull, 2008).

Los ortópteros

Los insectos pertenecen al phylum Arthoproda y se caracterizan por tener un par de antenas, tres pares de patas y en organismos adultos tener el cuerpo dividido en cabeza, tórax y abdomen (Resh & Cardé, 2009). Los insectos son el grupo más diverso del planeta con alrededor de un millón de especies reconocidas, pero se calcula que podrían existir entre 10 y 80 millones de especies (Foottit & Alder, 2009; Stork, 1988). La clasificación actual de los insectos consta de 29 órdenes, siendo Coleoptera, Diptera, Hymenoptera y Lepidoptera los órdenes en los que se concentra el 81% de las especies reconocidas por la ciencia (Grimaldi & Engel, 2005).

Los ortópteros son un orden de insectos hemimetábolos, comúnmente llamados chapulines, saltamontes, langostas, esperanzas y grillos, con amplia distribución en las zonas tropicales (Fontana *et al*., 2008). Presentan aparato bucal masticador bien desarrollado, su cuerpo es generalmente alargado, cilíndrico, robusto y de tamaño, en promedio, mayor que otros órdenes de insectos; una característica peculiar de los ortópteros son las patas posteriores largas, robustas y dotadas de una fuerte musculatura, lo que les permite actividad saltatoria (Gillott, 2005). Se trata de un orden antiguo, los primeros registros fósiles datan del Carbonífero superior (Chopard, 1920). Actualmente se han registrado más de 28000 especies (Eades *et al*., 2016). Las especies se agrupan en dos subórdenes: Caelifera que presenta antenas usualmente más cortas que el cuerpo, los órganos timpánicos ausentes o en el costado del abdomen, estridulación por frotación de las tegminas y patas traseras, y ovipositor reducido; y el suborden Ensifera que está caracterizado por antenas largas compuestas de numerosos segmentos antenales usualmente más largas que el cuerpo, órganos timpánicos en las tibias anteriores, estridulación por frotamiento de

tegminas, y ovipositor largo (Capinera, 2008).

Los ortópteros son de gran importancia económica ya que algunas de sus especies son plagas de cultivos y otras tienen uso alimenticio por lo que han sido objeto de estudios desde siglos pasados (Ingrisch & Willemse, 2004). Sin embargo, hoy en día el conocimiento del orden Orthoptera está disperso y proviene en general de trabajos poco recientes y son pocos los géneros que han sido revisados recientemente (Fontana *et al*., 2008). En el suborden Caelifera, la familia Romaleidae es un grupo de organismos de tamaño mediano a grande, usualmente de colores llamativos, y que se distribuye en las zonas tropicales de América (Rehn & Grant, 1959a). Las especies de Romaleidae se caracterizan por tener cabeza hipognota con fastigio no dividido, una espina inmóvil en el ápice de la superficie externa de las tibias posteriores y un sistema estridulatorio altamente especializado (Amedegnato, 1977; Dirsh & Dirsh, 1961). Se ha planteado que los romaleidos y otras familias como Ommexechidae y Tristiridae tienen su origen en la región Neotropical (Carbonell, 1977).

Actualmente, la familia Romaleidae contiene dos subfamilias, Bactrophorinae y Romaleinae, y está conformada por 64 géneros y más de 400 especies (Cigliano & Langle, 1998). Se ha planteado que el centro de origen y diversificación de la subfamilia Romaleinae en Sudamérica es la zona comprendida entre el sur de Brasil, Paraguay, Uruguay y el norte de Argentina. Es probable que el grupo surgiera en la región Amazónica y que posteriormente se extendiera progresivamente hacia Centroamérica y Norteamérica, así como hacia el sur de Sudamérica (Carbonell, 1977; Rehn & Grant, 1959a).

El género Taeniopoda

Taeniopoda Stål es un género de romaleinos que se distribuye desde el sur de Estados Unidos de Norteamérica hasta Panamá (Figura 1). Se caracteriza por tener un fastigio fuertemente declivente y la parte occipital de la cabeza abultada, carina facial lateral fuertemente marcada, pronoto cibroso-puntulado con carina media tectada y bien definida, tegmina y alas grandes, y proceso prosternal espiniforme ligeramente elevado (Rehn & Grant, 1959a, 1959b). En la última revisión del género se consideraron como válidos doce taxones (Hebard, 1924): *Taeniopoda eques* (Burmeister, 1838), *T. picticornis* (Walker, 1870), *T. stali* Bruner, 1907, *T. tamaulipensis* Rehn, 1904, *T. auricornis* (Walker, 1870), *T. gutturosa*

Bolívar, 1901, *T. varipennis* Rehn, 1905, *T. centurio* (Drury, 1770), *T. reticulata* (Fabricius, 1781), *T. bricristata* Bruner, 1907, *T. obscura* Bruner, 1907 y *T. citricornis* Bruner, 1907. En dicha revisión se reconocen tres grupos, uno formado por *T. eques*, *T. picticornis* y *T. stali*, otro grupo lo constituye solamente *T. oscura* y un tercer grupo formado por el resto de las especies. Los problemas en la sistemática del género incluyen la validez de *T. citricornis*, la cual es incierta; la sugerencia de que el grupo formado por *T. eques*, *T. picticornis y T. stali* podrían representar cromatomorfos y variación geográfica de una misma especie y no especies diferentes; y la sugerencia de que *T. reticulata* puede ser una variación de *T. centurio* y *T. varipennis* una variación de *T. gutturosa* (Hebard, 1924).

Con base en la morfología y genitalia el género *Taeniopoda* se ha considerado cercanamente relacionado con el género monotípico *Romalea* Serville, que se distribuye en el sureste de Estados Unidos (Rehn & Grant, 1959b). Estos últimos autores plantean la hipótesis de que el género *Taeniopoda* pudo haberse originado en México y que durante los periodos cálidos antes y después del Pleistoceno extendió su distribución hacia el norte llegando hasta la parte sur de Estados Unidos de América. Dada la similitud de la especie *Taeniopoda eques*, cuya distribución alcanza el sur de Estados Unidos de América, y la especie *Romalea microptera* se ha sugerido que esta última surgió por aislamiento de la primera, o que ambas tienen un ancestro común (Rehn & Grant, 1959a).

El objetivo general de esta tesis es investigar la sistemática y evolución del género *Taeniopoda* (Orthoptera: Romaleidae) con base en evidencia morfológica, marcadores genéticos puntuales y de secuenciación masiva. Los objetivos particulares son: a) investigar los límites entre las especies del género *Taeniopoda* empleando marcadores mitocondriales y morfología, b) reconstruir las relaciones filogenéticas entre sus especies e investigar su afinidad filogenética con *Romalea* empleando marcadores mitocondriales y de secuenciación masiva e c) investigar su origen y diversificación.

Figura 1. Mapa de distribución del género *Taeniopoda* (Tomado de Eades *et al*., 2016).

Capítulo II: Sequence-based species delineation and molecular phylogenetics of the transitional Nearctic–Neotropical grasshopper genus *Taeniopoda* (Orthoptera, Romaleidae)

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Research Article

Sequence-based species delineation and molecular phylogenetics of the transitional Nearctic–Neotropical grasshopper genus *Taeniopoda* (Orthoptera, Romaleidae)

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Taeniopoda is a genus of grasshoppers currently represented by 12 species distributed from southern USA to Panama, with most of them occurring along the transitional Nearctic–Neotropical region in central and southern Mexico. Despite being a small group of conspicuous, colourful species, the systematics of *Taeniopoda* has been largely neglected, including its phylogenetic affinity with the morphologically similar, monotypic genus *Romalea*. Here, we assessed the species limits in 11 of the species of *Teniopoda* based on two mitochondrial (mt) markers (COI, cyt *b*). Phylogenetic relationships were reconstructed adding two nuclear gene markers (28S, H3). A relaxed molecular clock analysis was performed based on the mt markers. We detected nuclear mt paralogues (*numts*) and the probable introgression of *T. tamaulipensis* mtDNA in specimens of *T. eques* from central Mexico. Between six and 14 species of *Taeniopoda* were delimited by the sequence-based approaches performed (COI divergence with thresholds of 1 and 2%; General Mixed Yule-Coalescent (GMYC) model). The GMYC and 1% threshold analyses with COI were more congruent with the currently recognized morphology-based taxonomy with 10 and 11 putative species, respectively. Four of these species were regarded as 'stable', since they were supported by at least one of the molecular analyses and by diagnostic morphological features. The species-based phylogeny recovered *Taeniopoda* as paraphyletic with respect to the monotypic genus *Romalea*. Three morphologically and geographically congruent major clades were recovered, two with species having a considerably elevated pronotal crest and one with its members having it less elevated. The origin and subsequent diversification of *Taeniopoda* were estimated to occur from the mid and late Miocene to Pliocene, respectively. The current species diversity in *Taeniopoda* was estimated to occur during the Pleistocene, which was probably influenced by the climatic oscillations that occurred during this period and the uplift of mountain ranges in Central America.

Key words:: DNA barcoding, Insecta, Neotropics, Mexico, phylogeny, species delimitation

Introduction

Species delimitation is defined as the act of identifying species-level biological diversity (Carstens, Pelletier, Reid, & Satler, 2013), and currently is one of the main areas of study in systematics (Hohenegger, 2014; Ruane, Bryson, Pyron, & Burbrink, 2014). Traditionally, the most important source of information to delimit species has been through the examination of morphological features (Abebe, Mekete, & Thomas, 2011; Mutanen & Pretorius, 2007). However, now it is widely accepted that morphology does not always reflects species boundaries, because species could sometimes be indistinguishable morphologically (Bickford et al., 2007; Leavitt, Starrett, Westphal, & Hedin, 2015; Massimino Cocuzza, & Cavalieri, 2014), or their intra- and interspecific variation might be difficult to discern (Gittenberger & Gittenberger, 2011; Katz, Giordano, & Soto-Adames, 2015). For this reason, the simultaneous use of various lines of evidence is now being largely employed to cross-validate species delineation (Berta & Churchill, 2012; Lecocq et al., 2015; Phuong, Lim, Wait, Rowe, & Moritz, 2014).

Mitochondrial (mt) DNA markers currently play a crucial role to complement and corroborate species delimitation based on morphology (Chan et al., 2014; Price et al., 2015; Schmidt, Schmid-Egger, Morinière, Haszprunar, &

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Hebert, 2015; Toussaint et al., 2015). The mt genome has features that make it particularly useful in systematics, since it usually does not recombine and has a higher substitution rate than most nuclear DNA markers (Avise, 2000; Ballard & Whitlock 2004). Mt markers also represent an important source of information used to investigate evolutionary patterns and processes that have sculpted the planet's biodiversity (Joly et al., 2014; Kress, García-Robledo, Uriarte, & Erickson, 2015), including evolutionary processes in complex scenarios between biogeographic areas (e.g., Rodríguez-Gómez & Ornelas, 2015; Tänzler, Toussaint, Suhardjono, Balke, & Riedel, 2014; Teske, Papadopoulos, Barker, Mcquaid, & Beheregaray, 2014). Some factors, however, can affect the performance of mt markers, such as heteroplasmy (White, Wolff, Pierson, & Gemmell, 2008), presence of incomplete lineage sorting and introgression (Choleva, Musilova, Kohoutova-Sediva, Paces, & Janko, 2014; Funk & Omaland, 2003), nuclear mt pseudogenes (*numts*) (Bensasson, Zhang, & Hewitt, 2000; Song, Buhay, Whiting, & Crandall, 2008) or *Wolbachia* infection (Smith et al., 2012).

The Nearctic and Neotropical regions are two adjacent biogeographic areas that are in contact along the central part of the Mexican territory, where there is a transitional confluence of northern and southern biotas (Halffter, 1976; Lomolino, Riddle, & Brown, 2005; Morrone, 2015a, 2015b). This transitional zone comprises the highlands of Mexico and Guatemala (Morrone, 2015b), and is characterized by having a complex geological history and different environments and climates (Ferrusquía, 1998). Various studies have investigated the origin and evolution of the biota that occur in the transitional Nearctic–Neotropical zone, though most of them have focused on vascular plants and vertebrates (e.g., Kobelkowsky-Vidrio, Ríos-Muñoz, & Navarro-Sigüenza, 2014; Munguía-Lino, Vargas-Amado, Vázquez-García, & Rodríguez, 2015; Sanginés-Franco et al., 2015). These studies have revealed the existence of multiple speciation patterns and a vast number of endemisms in the region (Flores-Villela & Gerez, 1994; Miguez-Gutiérrez, Castillo, Márquez, & Goyenechea, 2013; Morrone, 2010).

The genus *Taeniopoda* Stål, 1873 (Orthoptera: Romaleidae) is a group of conspicuous, colourful grasshoppers (Figs 1, 2) distributed from the southern United States to Panama (Fig. 3), with most of its species occurring along the Nearctic–Neotropical transitional zone (Hebard, 1924). Members of *Taeniopoda* are of economic importance because they represent pests in some of the regions where they occur (King & Saunders, 1984; Mariño-Pérez, Fontana, & Buzzetti, 2011). The few studies that have been carried out for members of this genus have mainly focused on their behaviour and physiology (e.g., Bernays, Bright, Howard, Raubenheimer, & Champagne, 1992; Stauffer, Hatle, & Whitman, 2011; Whitman, 1988, 2010), whereas its taxonomy has been largely neglected. The only taxonomic revision for *Taeniopoda* was performed almost a century ago (Hebard, 1924), where a total of 12 species were recognized and divided into three informal groups but without any clear justification.

Romalea microptera (Palisot de Beauvois), of the monotypic genus *Romalea* Serville, is a species restricted to the south-east United States that has been proposed to be closely related to *Taeniopoda* based on external morphology and genitalia (Hebard, 1925; Rehn & Grant, 1959a). Given the overall morphological similarity between *R. microptera* and *T. eques* (Burmeister), which occurs in the southern United States, it was suggested that the former species emerged from the latter by isolation, or both have a common ancestor (Rehn & Grant, 1959b). The genetic diversity of *Romalea* has been studied (Mutun & Borst, 2004), though its relationships with the members of *Taeniopoda* remain to be investigated.

Here we generated sequences of two mt markers to investigate the species boundaries in *Taeniopoda* using two DNA sequence-based approaches. We first searched for potential cases of *numts*, introgression and/or incomplete lineage sorting in the mt sequences in order to exclude them from the analyses. We then evaluated the congruence between the species delimited by the molecular information and the morphology-based taxonomy. For this, we examined the morphological diagnostic features that are employed to distinguish the currently recognized species of *Taeniopoda*, as well as two additional external morphological and six male genitalia characters. We also carried out a species-based phylogenetic analysis for *Taeniopoda* adding two nuclear markers, assessed its phylogenetic affinity with *R. microptera* and estimated the times of origin and subsequent diversification within the group to infer the vicariant events that could have originated its current species diversification.

Materials and methods

Taxon sampling

A total of 211 specimens assigned to 11 of the 12 currently recognized species of *Taeniopoda* were processed for DNA sequencing. Species assignation of specimens was performed following Bruner's (1906) and Hebard's (1924) keys to species. Specimens were collected in localities situated along most of the known geographic distribution of the genus (Fig. 3). The only species that could not be sampled, *T. bicristata* Bruner, is only known from its type material and has ambiguous locality ('Mat, Matamoros, Mexico'). We also generated sequences of five specimens of *R. microptera*, as well as sequences of four species belonging to the romaleid genera *Brachystola* Scudder, *Chromacris* Walker, *Tropidacris* Scudder and

Fig. 1. Photographs of *Taeniopoda* species: (1.1) *T. citricornis* Bruner, (1.2) *T. gutturosa* Bolívar, (1.3) *T. varipennis* Rehn, (1.4) *T. reticulata* (Fabricius), (1.5) *T. auricornis* (Walker), and (1.6) *T. centurio* (Drury).

Cibotopteryx Rehn, using the latter taxon to root all the generated trees.

All sequenced specimens are deposited at the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México (IB UNAM). The mounted material that was examined for the morphological part of this work is deposited at IB UNAM, Instituto Tecnológico de Ciudad Victoria, México (ITCV), Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), the Natural History Museum, London, UK (NHM), Museo de Historia Natural de la Universidad de San Carlos, Guatemala (MUSHNAT), Universidad del Valle de Guatemala (UVG), and Instituto Nacional de Biodiversidad, Costa Rica (InBio). A list with the examined specimens, their species assignment, locality details and DNA voucher and GenBank accession numbers for the four gene markers obtained is provided in Table S1 (see online supplemental material, which is available from the article's Taylor & Francis Online page at https://doi.org/10.1080/14772000.2017.1313792). The mtDNA data generated from this work can also be retrieved from the project file 'Species boundaries in *Taeniopoda*', which is found in the projects section of the Barcode of Life Data Systems (www.boldsystems.org).

Laboratory protocols

Genomic DNA extraction was obtained from a hind leg of each specimen. The genomic DNA was extracted using both the DNeasy Blood & Tissue (QIAGEN®: Austin, EUA) and the EZ-10 Spin Column Genomic

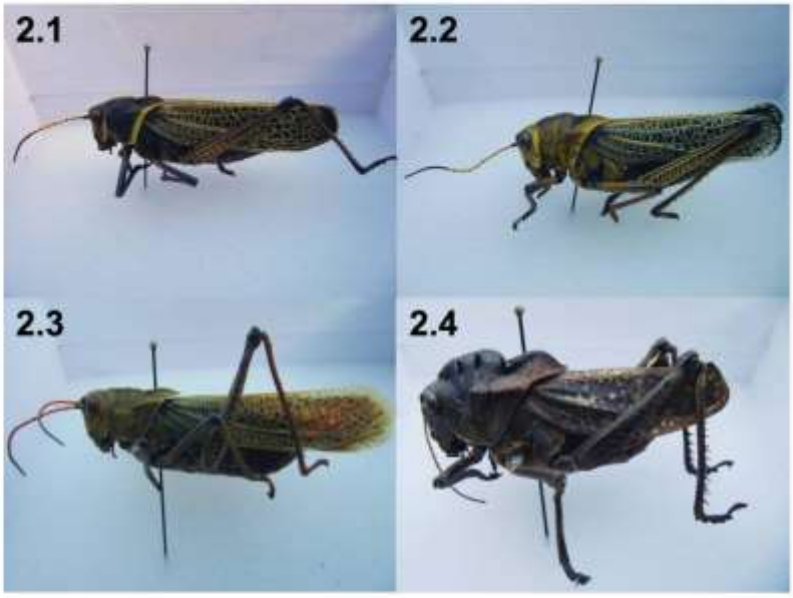

Fig. 2. Photographs of *Taeniopoda* species: (2.1) *T. eques* (Burmeister), (2.2) *T. tamaulipensis* Rehn, (2.3) *T. stali* Bruner, and (2.4) *T. obscura* Bruner.

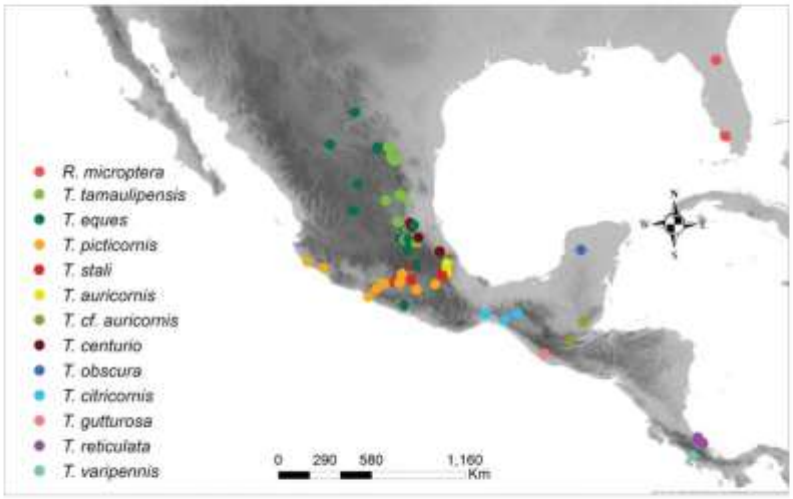

Fig. 3. Map of the sampled localities for the specimens of *Taeniopoda* and *Romalea* examined in this study.

DNA Minipreps (BIO BASIC®; Toronto, Canada) kits following the manufacturers' protocols. Two mt and two nuclear markers were amplified. The mt markers examined included a 626 bp fragment of the Cytochrome Oxidase I and 423 bp of the Cytochrome *b* DNA genes. These two are the most widely employed mt markers for species delimitation analyses (Ceccarelli, Sharkey, & Zaldívar-Riverón, 2012; Grill, Gkiokia, & Alvarez, 2006; Vanhaecke et al., 2012). We also amplified two nuclear DNA markers, a 652 bp fragment belonging to

the 28S ribosomal (r) and 314 bp of the Histone 3 protein DNA genes.

The primers used to amplify the gene fragments were the following: COI.-LCO (5'-GTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994); cyt *b*.-CB1_5 (5'-TATGTACTACCATGAGGACAAATATC-3)' and CB2_5 (5'-ATTACACCTCCTAATTTATTAGGAAT-3') (Jermiin & Crozier, 1994); 28S.-28SFwd (5'-GCGAACAAGTAACCGTGAGGG- 3') (Belshaw & Quicke, 1997) and 28SRev_Int (5'- GGAGTGCGGAGGCCGCCGCCCMC-3') (this study); and H3.-H3F (5′-ATGGCTCGTACCAAGCAGACVGC-3′) and H3R (5′-ATATCCTTRGGCATRATRGTGAC-3′) (Colgan et al., 1998).

PCRs were carried in 15 μl of total volume containing 2.5 μl of 10× PCR buffer, 1.5 μl of $MgCl_2$ 50mM, 0.5 μ of dNTPS 10mM, 1 μl of each primer 10μM, 0.1 μl of Taq Polymerase (Taq Platinum; Invitrogen®; Carlsbad, EUA), 2 μl of DNA template and ddH_2O to bring volume to 15 μl. The temperature conditions for COI amplification were: 3 minutes of initial denaturation at 95°C; 35 cycles with 1 minute denaturation at 94°C, 1 minute annealing at 50°C and 1 minute of extension at 72°C; and 10 minutes final extension at 72°C. Cyt *b* was amplified using a touchdown with the following temperatures: initial denaturation at 95°C; 10 cycles with 1 minute denaturation at 94°C, 1 min annealing at 61°C with reduction of 0.5°C each cycle and 1 minute of extension at 72°C; followed by 20 cycles with 1 minute denaturation at 94°C, 1 minute annealing at 48°C and 1 minute of extension at 72°C; and a final extension of 10 minutes at 72°C. The PCR conditions for 28S and H3 were the same used for COI, but using 58°C and 55°C for the annealing step, respectively.

Unpurified PCR products were sent for sequencing to the High-Throughput Genomics Center of the University of Washington (www.htseq.org) and to the genomics unit at IB UNAM. All sequences were edited with Sequencher 4.14 (Genecodes®, USA) and aligned with the program ClustalW (Larkin et al., 2007) implemented in Bioedit 7.1.3.0 (Hall, 1999).

Detection of numts, mt introgression and incomplete lineage sorting

Species delineation analyses based on mtDNA sequence data could be biased by the presence of mt introgression or incomplete lineage sorting (Leaché, 2009). Moreover, species boundaries based on mtDNA sequence data are particularly difficult to assess in orthopterans due to the frequent presence of *numts* within this order (Bensasson et al., 2000; Song, Moulton, & Whiting, 2014). We therefore carried out two different approaches to detect sequences that could represent the above three phenomena.

We first searched for *neonumts* (Song et al., 2014), i.e., recent duplications that did not have time to accumulate enough mutations, in the mt datasets based on the presence of stop codons, indels, polymorphism (double peaks) in chromatograms, codon position substitution bias and variation in rates of evolution (Calvignac, Konecny, Malard, & Douady, 2011; Song et al., 2014). We also carried out separate Bayesian analyses for the two mt markers to detect incorrect phylogenetic placement of taxa that could potentially represent *paleonumts*, i.e., ancient paralogues with long branch lengths nested in separate clades distantly related to the orthologue (Song et al., 2014), or possible cases of introgression or incomplete lineage sorting (Leaché, 2009). Incorrect phylogenetic placement was established based on our morphological examination. All sequences that potentially represented cases of incomplete lineage sorting, mt introgression or *numts* were excised from the species delimitation analyses.

Bayesian phylogenetic analyses were carried out separately for the two mt and the two nuclear markers with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Two databases were analysed for each mt marker, one including and the other one excluding the detected *numts* and potential cases of introgression or incomplete lineage sorting. The nuclear markers only included a subset of the specimens sequenced for the mt genes (Table S1, see supplemental material online). Three partitions were each considered for COI and cyt *b*. Substitution models for each partition were calculated with jModeltest (Darriba, Taboada, Doallo, & Posada, 2012) using the Bayesian criterion. Each analysis consisted of two simultaneous runs of 100 million generations each, sampling trees every 1000 generations and saving branch lengths. We discarded the 25% of the sampled trees as burn-in based on convergence of the two simultaneous runs, according to the values of the average standard deviation of split frequencies (a value < 0.01 was considered as convergence of the two simultaneous runs). The remaining trees were employed to build a phylogram with posterior probabilities of clades, considering them as significantly supported if they had a posterior probability value ≥ 0.95. The separate and concatenated matrices analysed in this study can be retrieved from Table S2 (see supplemental material online).

DNA sequence-based species delimitation

Two DNA sequence-based methods for species delineation were performed for specimens of 11 of the described species of *Taeniopoda* and for *R. microptera*. We first

used the 2% genetic divergence criterion for the Barcoding locus, which comprises a fragment of the COI gene (Hebert, Cywinska, Ball, & DeWaard, 2003). This has been shown to be a reliable approach for the exploration of putative species in various groups of animals (Kerr, Lijtmaer, Barreira, Hebert, & Tubaro, 2009). We also employed the threshold of 1% genetic divergence for the above marker, which represents a more conservative approach to delimit species (Ratnasingham & Hebert, 2007). Corrected genetic distances for COI were calculated using the Kimura 2-parameter (K2P) model with MEGA 7.0 (Kumar, Secher & Koichiro, 2016).

The Generalized Mixed Coalescent Yule (GMYC) model also was implemented separately for the COI and cyt *b* markers and for the concatenated mt dataset. An ultrametric tree, required for the GMYC model to distinguish a branching pattern of intraspecific coalescence and speciation events (Pons et al., 2006), was generated for each matrix with BEAST version 2.2.1 (Bouckaert et al., 2014). Duplicated haplotypes were excised with Collapse version 1.2 (Posada, 2004). Analyses were run for 50 million generations, saving trees every 1,000 generations, using a lognormal distribution relaxed clock and the Yule prior. The resulting maximum credibility ultrametric trees were employed to delimit species with the GMYC method implemented in the SPLITS package in the R programming environment (http://r-forge.r-project.org/projects/splits), using the single threshold optimization. We did not perform a multi-threshold GMYC analysis because it has been shown to have a poor performance both with simulations and empirical data (Fujisawa & Barraclough, 2013; Kekkonen, Mutanen, Kaila, Nieminen, & Hebert, 2015; Monaghan et al., 2009).

Morphological examination

We evaluated the congruence between the species delineation approaches based on mtDNA sequence data and the species currently recognized by the morphology-based taxonomy. For this, we recorded the morphological diagnostic features reported by Hebard (1924) to distinguish species in *Taeniopoda* in all our examined specimens. These include nine colour, shape and sculpture adult features. Moreover, we recorded two additional external morphological characters that were potentially informative to delimit species in the group (male and female adult body size, pronotal crest sculpture).

We also recorded six internal male genitalia characters for a subsample of the two to four specimens for each of the putative species delimited by any of the DNA sequence-based species delineation approaches performed. Internal male genitalia was dissected for each specimen immersing the distal part of the abdomen in 10% KOH for 10 minutes, and subsequently removing it with a hook, immersing it again in 10% KOH for 20 minutes and then removing the muscle tissue. These features were observed with a ZEISS® Stemi DV4 stereomicroscope and photographed in a Leica Z16 APO. The morphological features examined in this work are listed in Table S3 (see supplemental material online). Digital pictures of male genitalia of representative species of *Taeniopoda* are shown in Fig. S1 (see supplemental material online).

Phylogenetic relationships and molecular clock estimates

The phylogenetic relationships among specimens belonging to the putative species that were delimited by at least one of the species delineation approaches based on molecular evidence were reconstructed carrying out a concatenated Bayesian analysis using the mt and nuclear markers with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). The analysis employed the above parameters, as well as the same partitions and evolutionary models for the mt markers. Three partitions were considered for the nuclear H3 gene according to their codon positions, whereas the nuclear 28S rDNA marker was regarded as a single partition. The terminal taxa included in the Bayesian analysis only included a subset of the specimens sequenced for the mt genes.

A relaxed molecular clock analysis was also performed for a concatenated mt dataset with BEAST version 2.2.1 (Bouckaert et al., 2014). We did not include the two nuclear markers since their scarce variation does not have an impact in the derived topology but considerably affects the molecular divergence time estimates. The analysis was run for 100 million generations, sampling trees every 10,000 generations, using a Death-Birth tree prior, an uncorrelated relaxed lognormal rate, considering one partition for each gene marker (GTR+G for both mt genes) and burn-in was established after 10 million generations. Absolute node ages (percentage of change per million years) were obtained using the insect mutation rates reported by Pons and Vogler (2005) and Papadopoulou, Anastasiou, and Vogler (2010) for COI (3.36%) and cyt *b* (4.22%) [COI ucld.mean (subst/site/my): lognormal distribution in real space, with initial value = 0.0168; Log (Mean) = 0.0168; Log (Stdev) = 0.2; cyt *b* ucld.mean (subst/site/my): lognormal distribution in real space, with initial value = 0.0211; Log (Mean) = 0. 0211; Log (Stdev) = 0.17]. Effective sample size (ESS) $\geq$ 200 for each parameter was confirmed with Tracer version 1.6 (Drummond & Bouckaert, 2015). A maximum clade credibility tree with the associated Bayesian 95% High Probability Density Interval was then built with TreeAnnotator version 1.8.1 contained in the BEAST Package.

Results

Detection of *numts* and gene genealogies

A total of 212, 194, 42, and 66 sequences were generated for COI (626 bp), cyt *b* (423pb), 28S (652 bp), and H3 (314 bp), respectively. The evolutionary models selected for each partition and the main characteristics of the four gene markers are provided in Table S4 (see supplemental material online).

The Bayesian analyses performed with all the generated sequences for the two mt markers showed that 15 and 13 COI and cyt *b* sequences had an apparent incorrect phylogenetic placement, respectively (Fig. S2, see supplemental material online). Of these, five COI and three cyt *b* sequences had highly polymorphisms in their chromatograms. Four cyt *b* sequences were considered as *paleonumts* because they were placed at the base of a clade with all members of *Taeniopoda*. The two mt phylograms had a clade with specimens of *T. eques* from Central Mexico intermingled with those of *T. tamaulipensis* Rehn, two clades containing specimens of *T. stali* Bruner and *T. picticornis* (Walker), and a clade with *T. varipennis* Rehn, *T. reticulata* (Fabricius), *T. citricornis* Bruner, and *T. gutturosa* Bolívar. We consider that the sequences of *T. eques* from Central Mexico represent a case of mt introgression with *T. tamaulipensis* based on the consistent morphological differences that exist between these two species. The above sequences of *T. eques* therefore were excised from the species delineation analyses.

The lack of consistent morphological differences between *T. stali* and *T. picticornis*, and *T. citricornis* and *T. gutturosa* (see below) on the other hand led us to maintain all their sequences. The generated sequences of *T. varipennis* and *T. reticulata* shared single COI and cyt *b* haplotypes or had considerably low variation between them; however, they are morphologically distinct and we thus maintained all their sequences for the subsequent analyses.

The Bayesian phylograms obtained from separate COI and cyt *b* analyses excluding all *numts* and presumable cases of mt introgression are congruent in their significantly supported relationships (Fig. 4). The monophyly of *Taeniopoda* was not recovered in the COI phylogram, since *R. microptera* appeared deeply nested within it. Only two species of *Taeniopoda* were significantly recovered as exclusive with the two mt markers: *T. tamaulipensis* and *T. centurio* (Drury). *Taeniopoda obscura* Bruner and *T. eques* were also recovered as exclusive in the COI topology (PP = 0.98 and 0.99).

The above two mt genealogies recovered the specimens assigned to *T. picticornis* and *T. stali* intermingled in a significantly supported clade, each having at least two of the following three groups: group (A) composed by specimens of *T. picticornis* from the Pacific Mountain Ranges subprovince (Sierra Madre del Sur Province); group (B) containing species of both species from the Pacific Coastal Plains, Balsas Depression and Septentrional Balsas highlands (Sierra Madre del Sur province) and the Meridional Extension subprovince [Trans-Mexican Volcanic Belt (TMBV) province]; and group (C) comprising specimens assigned to both species from the Septentrional Balsas highlands, Oaxaca and Puebla Highlands and Balsas Depression subprovinces in the Sierra Madre del Sur province, and the Eastern Portion subprovince in the TMBV province.

The specimens assigned to *T. auricornis* (Walker) appeared divided into two separate, geographically congruent clades, one with specimens from Veracruz and the other one from Guatemala. The specimens of *T. reticulata*, *T. varipennis*, *T. citricornis*, and *T. gutturosa* formed a single, largely unresolved clade (COI, cyt *b*: PP = 1.0 and 1.0).

The phylograms derived from the two nuclear markers, though largely unresolved, recovered a paraphyletic *Taenipoda* with respect to *R. microptera* (Fig. S3, see supplemental material online). The 28S phylogram recovered two main clades, one with *T. citricornis*, *T. gutturosa*, and *T. reticulata* (PP = 0.62), and the other with the remaining species of the genus and *R. microptera* (PP = 0.79). The H3 phylogram recovered the specimens of *Romalea* forming a clade with *T. guttorosa* and one specimen of *T. eques* (PP = 0.74).

DNA sequence-based species delimitation

The threshold of 2% for species delimitation using the COI corrected distances delimited six species of *Taeniopoda* (Table S5, see supplemental material online; Fig. 5). COI corrected distances among specimens assigned to *T. gutturosa*, *T. reticulata*, *T. varipennis*, and *T. citricornis*, and between specimens of *T. obscura* and *T. auricornis*, were lower than the above threshold. Use of the 1% threshold of genetic divergence on the other hand delimited 11 species, of which only three were concordant with the 2% threshold. Under the 1% threshold, the specimens assigned to *T. picticornis* and *T. stali* appear divided into the same three groups mentioned above.

A total of 10 and 14 species were delimited by the separate COI and cyt *b* GMYC analyses, respectively (Fig. 5; Fig. S4, see supplemental material online). The concatenated mt GMYC analysis showed a considerable species oversplitting (Fig. S4, see supplemental material online). We therefore only considered the results obtained by the separate mt markers.

Only two of the species delimited by the GMYC analyses with COI and cyt *b* corresponded to currently recognized species, *T. tamaulipensis* and *T. centurio*. The

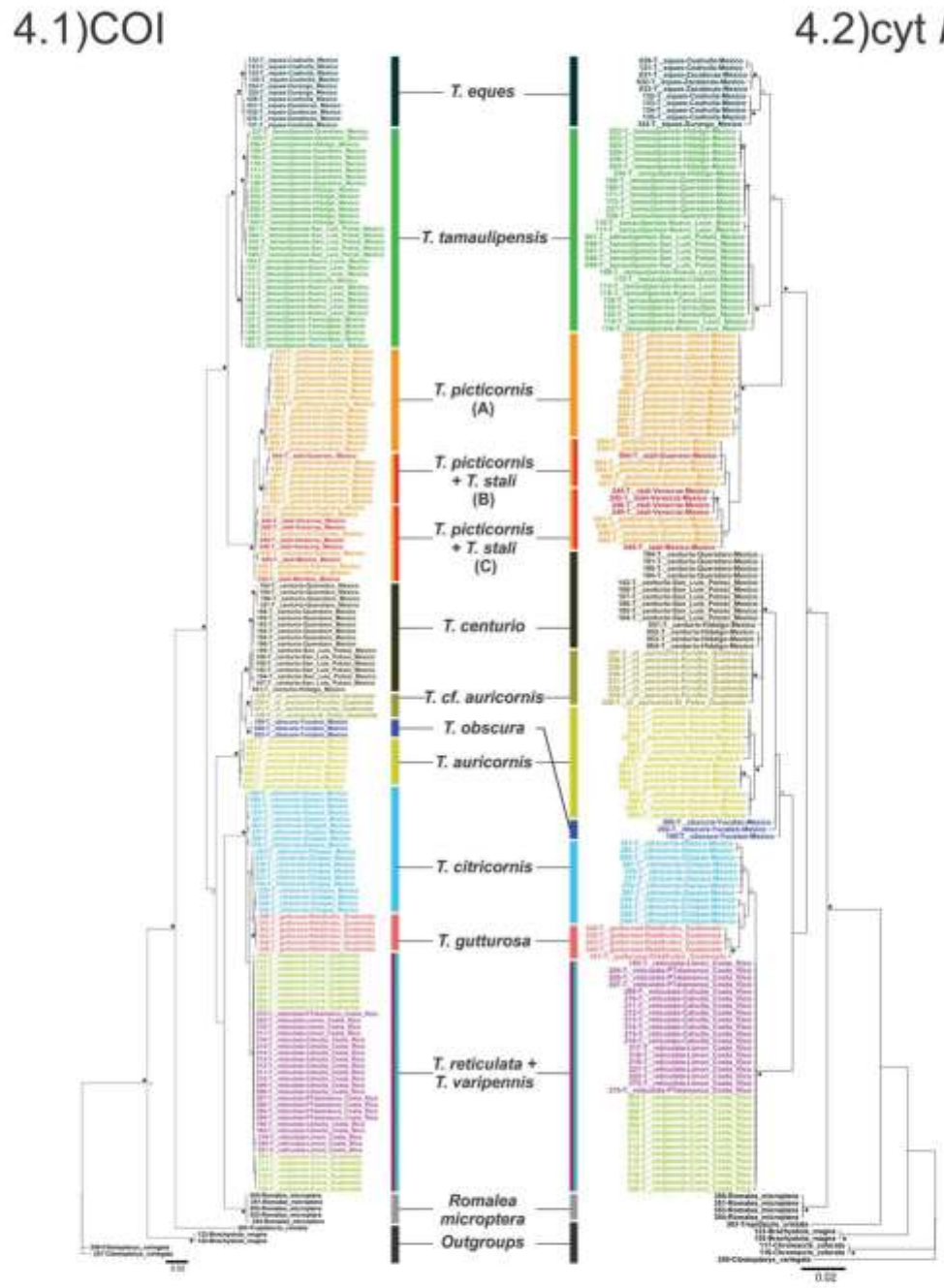

Fig. 4. Phylograms reconstructed with the Bayesian analysis based on the (4.1) COI and (4.2) cyt *b* mitochondrial markers excluding probable cases of introgression/incomplete lineage sorting events and *numts*. Black and white circles near branches indicate Bayesian posterior probabilities ≥ 0.95 and $\geq 0.90 \leq 0.94$, respectively.

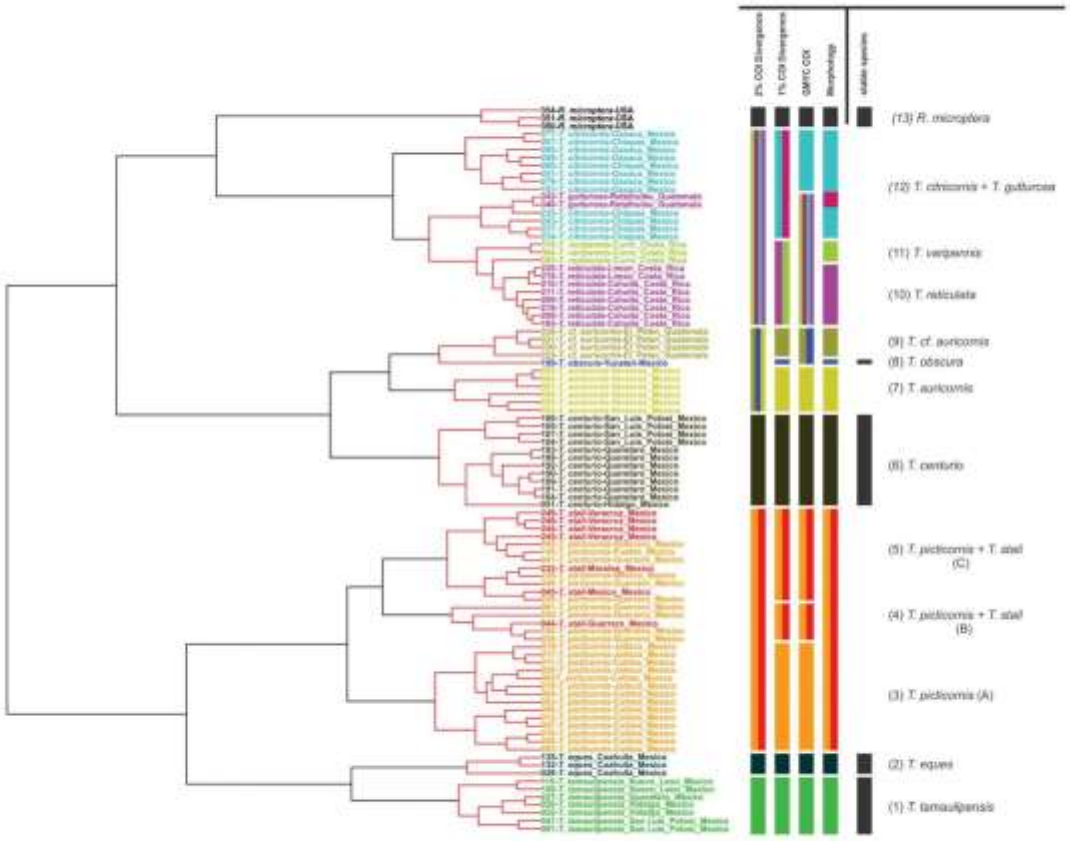

Fig. 5. Ultrametric tree constructed with the COI dataset used for the species delimitation with the GMYC method. Bars beside the tree summarize the results obtained for the species delineation analyses. Black bars indicate the stable species (*sensu* Padial et al., 2009); i.e., species delimited by the molecular analyses and the species-based taxonomy.

specimens assigned *T. picticorns* and *T. stali* were split into the same three species delimited by the COI 1% divergence threshold, whereas those of *T. reticulata* and *T. varipennis* appeared fused as a single GMYC species.

The GMYC analysis with COI was more congruent with the morphology-based taxonomy. This analysis clustered the specimens of *T. eques* as a single evolutionary unit. *Taeniopoda auricornis* was divided into two GMYC species, one with members from Veracruz and the other one with specimens from Guatemala. Four currently recognized species were on the other hand intermingled in two GMYC species with COI, one comprising specimens of *T. citricornis* from Chiapas and Oaxaca, and the other one with specimens of *T. citricornis* from Chiapas, specimens of *T. gutturosa* from Guatemala, and the included specimens of *T. reticulata* and *T. varipennis*.

Morphological confirmation of species boundaries

The morphological features and the states recorded for each taxon are shown in Table S3 (see supplemental material online). The male genitalia features had considerable intraspecific variation and thus were not informative to delimit species in the group. The colour of pronotum and pronotal crest sculpture help to distinguish the three species delimited by the two genetic distance thresholds and the GMYC model, *T. eques*, *T. tamaulipensis*, and *T. centurio*. *Taeniopoda obscura*, on the other hand, can be distinguished from the remaining species of the genus by two exclusive external morphological features, pronotum black and lateral carina of pronotum distinctly prominent, though it was only recovered as a distinct species by the COI 1% threshold.

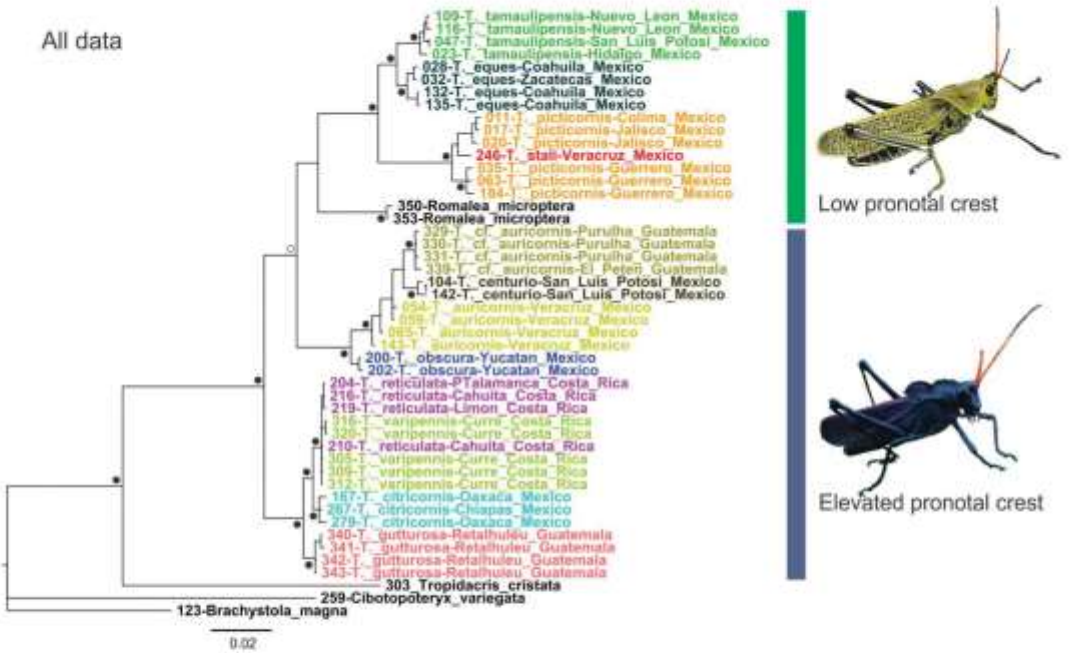

Fig. 6. Bayesian phylogram derived from the concatenated COI + cyt *b* + 28S + H3 datasets. Black circles near branches indicate Bayesian posterior probabilities ≥ 0.95.

Taeniopoda reticulata and *T. varipennis* are morphologically distinguishable from each other; however despite the DNA sequence-based species delineation analyses fused them as a single evolutionary lineage. The two geographically isolated populations assigned to *T. auricornis* that appeared as separate species by the GMYC and COI 1% threshold analyses could only be morphologically distinguished from each other by the female and male body size.

There were no consistent morphological features that helped to distinguish the specimens assigned to *T. picticornis* from those of *T. stali*. The only character that was proposed to distinguish these two taxa, colour of the antenna, was found to be highly variable even among individuals from same localities. All but one (see Discussion section) of the specimens assigned to *T. gutturosa* and *T. citricornis* could be distinguished from each other by two features, colour of antenna and pronotum.

Phylogenetic relationships and divergence-time estimates

The phylogram derived from the Bayesian concatenated analysis employing four genetic markers and including representatives of the putative species recovered by the different species delineation approaches based on mt sequence data is shown in Fig. 6. *Taeniopoda* was significantly supported as paraphyletic with respect to *R. microptera* (PP = 1.0). A clade with *T. varipennis*, *T. reticulata*, *T. citricornis*, and *T. gutturosa* (PP = 1.0) was recovered as sister to two clades (PP = 0.99), one with the two lineages assigned to *T. auricornis*, *T. obscura*, and *T. centurio* (PP = 1.0), and the other one with *T. eques*, *T. tamaulipensis*, *T. picticornis*, *T. stali*, and with *R. microptera* at the base (PP = 0.88). The former two clades are represented by taxa distributed from central Mexico to Central America, and are characterized by having a considerably elevated pronotal crest (at least 0.3 times total height of pronotum). The remaining clade, on the other hand, has taxa that occur from the southern United States to central Mexico and have a lower pronotal crest elevation (≤ 0.25 times total height of pronotum).

The chronogram derived from the relaxed molecular clock analysis (Fig. S5, see supplemental material online) indicates that *Taeniopoda* could have originated during the mid Miocene to Pliocene, 12.66–2.71 MYa (mean = 6.81 MYa). In this analysis the genus was divided into two main clades, one with the species with elevated pronotal crest including *R. microptera* at the base, and the

other one with species lacking this morphological feature. These two clades were estimated to have diverged during the late Miocene to Pliocene, 7.48–2.40 MYa, and started to diversify from the late Miocene to early Pleistocene, 6.12–1.35 MYa. The species diversification that resulted in the currently recognized species of *Taeniopoda* was on the other hand estimated to occur during the early to mid Pleistocene, 2.35–0.29 MYa.

Discussion

Detection of *numts*, introgression and incomplete lineage sorting

Species delineation methods based on mtDNA sequence data are widely employed in systematics to draw taxonomic inferences along with morphological evidence (e.g., Álvarez-Presas & Riutort, 2014; Cruz-Barraza, Vega & Carballo, 2014; Jiruskova & Bocak, 2015). However, *numts* represent a potential source of error in this kind of studies, and its presence has been documented in several animal groups at intra- and interspecific levels (Ahmed & Jaffar Ali, 2015; Haran, Koutroumpa, Magnoux, Roques, & Roux, 2015; Jordal & Kambestad, 2014; Song et al., 2008).

Here we found that some cyt *b* sequences of specimens assigned to *T. centurio*, *T. picticornis*, *T.citricornis*, and *T. tamaulipensis* could represent *paleonumts*, since they were recovered at the base of a clade containing the remaining sequences of *Taeniopoda* (Song et al., 2014). We also report the existence of a number of putative *neonumts* in our COI and cyt *b* datasets, which were detected based on the presence of polymorphisms in chromatograms. Our study therefore notes the importance of carrying out approaches to detect *numts* in phylogenetic studies in order to avoid the inclusion of paralogues that lead to the reconstruction of wrong evolutionary relationships as well as species overestimation, especially in groups like Orthoptera, which have been reported to contain a large number of mt pseudogenes.

Our gene genealogies including all the generated sequences recovered four cases of non-reciprocal monophyly, one in the clade with sequences of *T. eques* and *T. tamaulipensis*, another one represented by two clades with members of *T. picticornis* and *T. stali*, and the remaining two in the clade containing *T. citricornis*, *T. reticulata* and *T. varipennis*. Non-reciprocal monophyly can be explained by recent divergence leading to incomplete differentiation and incomplete lineage sorting (Funk & Omland, 2003; Mao, Zhang, Nakamura, Guan, & Qiu, 2014; Welch, Yoshida, & Fleischer, 2011), introgression, which implies the movement of genes from one species into the genome of another one (Peters, Zhuravlev, Fefelov, Logie, & Omland, 2007; Rheindt & Edwards, 2011; Wang et al., 2014), or taxonomic error, where species are incorrectly identified (Funk & Omland, 2003).

In the case of the non-reciprocal monophyly between *T. eques* and *T. tamaulipensis*, we propose that this could be due to mt introgression, since the two species are morphologically distinguishable from each other (completely black pronotum and slightly crushed antennal segments in *T. eques*; pronotum laterally green and never completely black and antennal segments not crushed in *T. tamaulipensis*). For *T. picticornis* and *T. stali*, their non-reciprocal monophyly is probably due to their taxonomic uncertainty, since the only feature that distinguishes them, the colour of antenna, is highly variable. Further studies including additional markers and populations will also help to understand the low mt variation between *T. reticulata* and *T. varipennis*, which are morphologically well differentiated and appear to be allopatric.

Performance of species delineation approaches

Species delimitation based on genetic distances has been mainly criticized because the proposed thresholds are not linked to any biological phenomenon and are not universal (Cognato, 2006; Fregin, Haase, Olsson, & Alström, 2012). However, it has been shown that it represents an efficient approach as a first assessment of species richness, especially in megadiverse, poorly studied taxa (Zaldívar-Riverón et al., 2010; Ceccarelli et al., 2012; Gutiérrez-Arellano, Gutiérrez-Arellano, & Zaldívar-Riverón, 2015). Moreover, it is a practical tool to reassess and confirm delimitations proposed by traditional taxonomy (Schmidt et al., 2015). We found discordant results between the species limits recovered with the COI 1 and 2% thresholds and those obtained by the GMYC method, though the COI 1% threshold was more congruent with the latter approach and with our morphospecies discrimination.

The methods for species delimitation based on coalescence involve a particular biological process and have the advantage of having statistical support (Fujita, Leaché, Burbrink, McGuire, & Moritz, 2012). Currently, the GMYC model is one of the most widely used approaches based on coalescence due to its stability regarding the proportion of unique haplotypes, ultrametric tree reconstruction conditions and taxonomic sampling (Ceccarelli et al., 2012; Talavera, Dincă, & Vila, 2013). This approach, however, tends to overestimate the number of species in the presence of high population structure or considerably high values of effective population size (Esselstyn, Evans, Sedlock, Khan, & Heaney, 2012; Tänzler, Sagata, Surbakti, Balke, & Riedel, 2012). Here we found that the concatenated mt dataset overestimated the number of species with the GMYC method, probably due to the increase of genetic structure in the data.

Taxonomic inferences

Establishing species limits in *Taeniopoda* has been hampered by the lack of consistent diagnostic morphological features. Most of the diagnostic morphological features employed within the genus are differences in colour pattern, which were proposed in the only taxonomic revision of the genus carried out almost a century ago (Hebard, 1924). Some of these colour pattern features are known to be highly variable, which highlights the taxonomic uncertainty of some of the species involved. Here we employed mtDNA sequence data and two DNA sequence-based species delineation approaches to assess the number of species diversification events that occurred within this Nearctic–Neotropical group of grasshoppers, and compared our results with the main diagnostic morphological features that are currently employed for its recognized species. This molecular study has helped to clarify the actual number of species within this morphologically conserved group of romaleids, and will serve as a robust basis to carry out further studies using additional molecular markers and morphological information from different character systems.

We summarized the species of *Taeniopoda* that are delimited by the molecular evidence (Fig. 5). Between six and 14 species of *Taeniopoda* were discriminated depending on the molecular approach performed. The GMYC and the 1% threshold analyses with COI were more congruent with the currently recognized morphology-based taxonomy with 10 and 11 putative species, respectively. We regard four of these delimited species as 'stable' (*sensu* Padial et al., 2009), since they were supported by at least one of the molecular species delineation approaches and by consistent diagnostic morphological features. Below we list the four stable species together with their known geographic distribution based on examined museum material (De Jesús-Bonilla et al., unpubl. data): (1) *T. tamaulipensis*, distributed along the Sierra Madre Oriental and the Mexican Plateau in the states of Coahuila, Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, and Queretaro; (2) *T. eques*, occurring from Arizona, Nuevo Mexico and Texas in southern USA to central Mexico; (3) *T. centurio*, recorded for southern Sierra Madre Oriental in the states of San Luis Potosí, Queretaro, Hidalgo and Puebla, Mexico, to Nicaragua in Central America; and (4) *T. obscura*, with most of its records restricted to the Yucatán Peninsula and Guatemala, but with an unconfirmed record in San Luis Potosí in central Mexico.

The population groups assigned to *T. auricornis* that were recovered by some of the molecular analyses as two separate evolutionary lineages could only be morphologically distinguished from each other by the male and female adult body size. As in other orthopteran taxa, adult body size in *Taeniopoda* has been reported to be considerably variable intraspecifically (Hebard, 1924; Rehn and Grant, 1959b). We therefore maintained these taxa as a single species. *Taeniopoda auricornis* had so far been reported for the Mexican states of Hidalgo, Veracruz, and the southern portion of Tamaulipas (Hebard, 1924). The specimens from Guatemala reported here therefore represent the first confirmed record of the species for this country.

The DNA sequence-based approaches for species delineation did not recover the exclusivity of *T. citricornis* with respect to *T. gutturosa*. Only two diagnostic features, antennal and pronotal colour, distinguish these two species. In *T. gutturosa* these structures have been reported to be scarlet red to orange, whereas in *T. citricornis* they are olivaceous green (Bruner, 1906; Hebard, 1924). In our morphological examination we assigned the specimens from Santiago Ixtaltepec, Oaxaca, to the latter species based on their colour features. However, one specimen (DNA voucher no. R167), had a scarlet red to orange pronotum and antenna. The actual status of these two taxa therefore needs to be further investigated.

Taeniopoda reticulata and *T. varipennis* are morphologically distinguishable from each other, and apparently have a disjunct geographic distribution separated by mountain ranges of recent formation (Abratis & Wörner, 2001; Bergoeing, 2006). However, all the species delineation analyses performed fused them as a single species. *Taeniopoda reticulata* occurs from the eastern side of the Central Mountainous Axis of Costa Rica to the Atlantic Coast of Costa Rica and Panama, whereas *T. varipennis* is mainly distributed along the Pacific coastal regions in Costa Rica and Nicaragua. Additional studies are needed to investigate whether the lack of mtDNA sequence variation in these two species is due to their recent divergence or due to mt introgression.

The specific status of the three separate groups containing the specimens assigned to *T. picticornis* and *T. stali* requires to be assessed in more detail. These groups were consistently delimited as separate species in some of our molecular analyses, and they are geographically congruent with respect to each other, since they are exclusively composed of specimens from separate localities along the Trans-Mexican Volcanic Belt (TMVB) and the Sierra Madre del Sur provinces. However, we did not find any consistent diagnostic morphological feature that helps to distinguish them from each other. In his revision of the genus, Hebard (1924) questioned the validity of *T. picticornis*, since he found that the only feature that distinguished it from *T. stali*, yellow to orange antenna in the former one and scarlet in the latter, is highly variable. In this study we observed that the scarlet colour of the antenna in the specimens assigned to *T. stali* appears to decolour after they are mounted or preserved in ethanol, changing to yellow or orange.

Phylogenetic relationships and biogeographic inferences

Our concatenated phylogenetic analysis based on four gene markers significantly supports the paraphyly of *Taeniopoda* with respect to *R. microptera*, though the relationships of this species within the group remain to be clarified. Previous studies based on genitalia, external morphology and physiological features had suggested a close relationship between the members of these two genera (Hebard, 1925; Rehn & Grant, 1959a, 1959b; Roberts, 1941; Stauffer et al., 2011; Stauffer & Whitman, 2007). Thus, the synonymy of *Taeniopoda* with *Romalea* needs to be formally established.

The relationships recovered in this study within *Taeniopoda* do not correspond with the three species-groups mentioned by Hebard (1924), who proposed them without any morphological justification. One of these groups contained *T. eques*, *T. picticornis*, and *T. stali*, another one only comprised *T. obscura*, and the third had the remaining species. Our best estimate of phylogeny instead recovered three morphologically and geographically congruent major clades, two with species having an elevated pronotal crest and with a Mesoamerican distribution, and another one with species having a lower elevated pronotal crest and distributed from the southern United States to central Mexico.

The chronogram reconstructed shows that *Taeniopoda* probably originated during the Miocene, with its subsequent diversification occurring from the late Miocene to Pliocene. During these geological periods there was an intense geological activity in Mesoamerica, which shaped its current physiography (Castillo, 1991; Ferrusquia, 1998). Based on these time estimates, we suggest that the recent stages of formation of the TMVB (7.5–3 My) could have influenced the origin of the major clades within *Taeniopoda*, since the species having or lacking a considerably elevated pronotal crest are mostly distributed to the south and north of this mountain range, respectively. The TMBV began its formation 20 million years ago, and it has been in change until recent times (Ferrari, Orozco-Esquivel, Manea, & Manea, 2012). This province has been associated with the formation and/or diversification of various groups of plants (Gándara & Sosa, 2014), reptiles (Bryson, García-Vázquez, & Riddle, 2012a, 2012b), fishes (Kallman & Kazianis, 2006; Mateos, 2005; Ornelas-García, Domínguez-Domínguez, & Doadrio, 2008), and even other orthopterans (Pedraza-Lara, Barrientos-Lozano, Rocha-Sánchez, & Zaldívar-Riverón, 2015).

The current species of *Taeniopoda* appear to have diversified during the Pleistocene. This period was characterized by having a global climate change composed by a series of glacial and interglacial periods (Ehlers & Gibbard, 2008), which promoted the expansion-contraction and/or the isolation-reconnection of the biota (Hewitt, 2000). These climatic oscillations also have been suggested as the main speciation event that led to the current species diversity in other groups of insects of recent divergence in North and Central America (Callahan & McPeek, 2016; Knowles & Alvarado-Serrano, 2010; Pedraza-Lara et al., 2015).

The probable recent speciation event that led to the origin of *T. reticulata* and *T. varipennis* on the other hand could have been promoted by recent, rapid mountain uplifts of the Talamanca, Tilarán, Guanacaste, and Central mountain ranges that occurred in the Costa Rican and Panamanian territory. These mountain uplifts have been estimated to have started from mid to recent Pleistocene, and thus agree with the low genetic differentiation that has been observed between the above two species (Bergoeing, 2006; Castillo, 1991).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplemental data

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References

Abebe, E., Mekete, T., & Thomas, W. K. (2011). A critique of current methods in nematode taxonomy. *African Journal of Biotechnology, 10*, 312–323. doi:10.5897/AJB10.1473

Abratis, M., & Wörner, G. (2001). Ridge collision, slab-window formation, and the flux of Pacific Asthenosphere into the Caribbean Realm. *Geology, 29*, 127–130. doi:10.1130/0091-7613(2001)029<0127:RCSWFA>2.0.CO;2

Ahmed, N. S., & Jaffar Ali H. A. (2015). Numts: An impediment to DNA barcoding of Polyclinids,Tunicata. *Mitochondrial DNA, 27*, 1–4. doi:10.3109/19401736.2015.1018238

Álvarez-Presas, M., & Riutort, M. (2014). Planarian (Platyhelminthes, Tricladida) diversity and molecular markers: A new view of an old group. *Diversity, 6*, 323–338. doi:10.3390/d6020323

Avise, J. C. (2000). *Phylogeography, the history and formation of species*. Cambridge: Harvard University Press.

Ballard, J. W. O., & Whitlock, M. C. (2004). The incomplete natural history of mithocondria. *Molecular Ecology, 13*, 729–744. doi:10.1046/j.1365-294X.2003.02063.x

Belshaw, R., & Quicke, D. L. J. (1997). A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution, 7*, 281–293. doi:10.1006/mpev.1996.0400

Bensasson, D., Zhang, D., & Hewitt, G. M. (2000). Frequent assimilation of mitochondrial DNA by grasshopper nuclear genomes. *Molecular Biology and Evolution, 17*, 406–415. doi:10.1093/oxfordjournals.molbev.a026320

Bergoeing, J. P. (2006). El Cuaternario en Costa Rica. Proposición Cronológica [The Quaternary in Costa Rica. Chronological Proposition]. *Revista Reflexiones, 85*, 207–226. Retrieved from: http://revistas.ucr.ac.cr/index.php/reflexiones/article/view/11445 (accessed 23 March 2017).

Bernays, E. A., Bright, K., Howard, J. J., Raubenheimer, D., & Champagne, D. (1992). Variety is the spice of life: Frequent switching between foods in the polyphagous grasshopper *Taeniopoda eques* Burmeister (Orthoptera: Acrididae). *Animal Behaviour, 44*, 721–731. doi:10.1016/S0003-3472(05)80298-2

Berta, A., & Churchill, M. (2012). Pinniped taxonomy: Review of currently recognized species and subspecies, and evidence used for their description. *Mammal Review, 42*, 207–234. doi:10.1111/j.1365-2907.2011.00193.x

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., … Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution, 22*, 148–155. doi:10.1016/j.tree.2006.11.004

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T. G., Wu, C. H., Xie, D., … Drummond, A. J. (2014). BEAST2: A software platform for Bayesian evolutionary analysis. *Public Library of Science Computational Biology, 10*, e1003537. doi:10.1371/journal.pcbi.1003537

Bruner, L. (1906). *Biologia Centrali-Americana. Insecta: Orthoptera* (Vol *II*). London: Taylor and Francis.

Bryson, R. W., García-Vázquez, U. O., & Riddle, B. R. (2012a). Diversification in the Mexican horned lizard *Phrynosoma orbiculare* across a dynamic landscape. *Molecular Phylogenetics and Evolution, 62*, 87–96. doi:10.1016/j.ympev.2011.09.007

Bryson, R. W., García-Vázquez, U. O., & Riddle, B. R. (2012b). Relative roles of neogene vicariance and quaternary climate change on the historical diversification of bunchgrass lizards (*Sceloporus scalaris* group) in Mexico. *Molecular Phylogenetics and Evolution, 62*, 447–457. doi:10.1016/j.ympev.2011.10.014

Callahan, M. S., & McPeek, M. A. (2016). Multi-locus phylogeny and divergence time estimates of *Enallagma* damselflies (Odonata: Coenagrionidae). *Molecular Phylogenetics and Evolution, 94*, 182–195. doi:10.1016/j.ympev.2015.08.013

Calvignac, S., Konecny, L., Malard, F., & Douady, C. J. (2011). Preventing the pollution of mitochondrial datasets with nuclear mitochondrial paralogs (numts). *Mitochondrion, 11*, 246–254. doi:10.1016/j.mito.2010.10.004

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology, 22*, 4369–4383. doi:10.1111/mec.12413

Castillo, R. (1991). Geología de Costa Rica [Geology of Costa Rica]. In D. Janzen (Ed.), *Historia Natural de Costa Rica* [*Natural History of Costa Rica*] (pp. 47–61). Costa Rica: Editorial de la Universidad de Costa Rica.

Ceccarelli, F. S., Sharkey, M. J., & Zaldívar-Riverón, A. (2012). Species identification in the taxonomically neglected, highly diverse, Neotropical parasitoid wasp genus *Notiospathius* (Braconidae: Doryctinae) based on an integrative molecular and morphological approach. *Molecular Phylogenetics and Evolution, 62*, 485–495. doi:10.1016/j.ympev.2011.10.018

Chan, A., Chiang, L. P., Hapuarachchi, H. C., Tan, C.-H., Pang, S.-C., Lee, R., … Lam-Phua, S. -G. (2014). DNA barcoding: Complementing morphological identification of mosquito species in Singapore. *Parasites & Vectors, 7*, 569. doi:10.1186/s13071-014-0569-4

Choleva, L., Musilova, Z., Kohoutova-Sediva, A., Paces, J., Rab, P., & Janko, K. (2014). Distinguishing between incomplete lineage sorting and genomic introgressions: Complete fixation of allospecific mitochondrial DNA in a sexually reproducing fish (Cobitis; Teleostei), despite clonal reproduction of hybrids. *Public Library of Science ONE, 9*, e80641. doi:10.1371/journal.pone.0080641

Cognato, A. I. (2006). Standard percent DNA sequence difference for insects does not predict species boundaries. *Journal of Economic Entomology, 99*, 1037–1045. doi:10.1603/0022-0493-99.4.1037

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., … Gray, M. R. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology, 46*, 419–437. doi:10.1071/ZO98048

Cruz-Barraza, J. A., Vega, C., & Carballo, J. L. (2014). Taxonomy of family *Plakinidae* (Porifera: Homoscleromorpha) from eastern pacific coral reefs, through morphology and cox1 and cob mtDNA data. *Zoological Journal of the Linnean Society, 171*, 254–276. doi:10.1111/zoj.12137

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods, 9*, 772. doi:1038/nmeth.2109

Drummond, A. J., & Bouckaert, R. R. (2015). *Bayesian evolutionary analysis with BEAST*. United Kingdom: Cambridge University Press.

Ehlers, J., & Gibbard, P. (2008). Extent and chronology of Quaternary glaciation. *Episodes, 31*, 211–218. Retrieved from: http://www.episodes.org/index.php/epi/article/view/64281 (accessed 23 March 2017).

Esselstyn, J. A., Evans, B. J., Sedlock, J. L., Khan, F. A. A., & Heaney, L. R. (2012). Single-locus species delimitation: A test of the mixed Yule-coalescent model, with an empirical application to Philippine round-leaf bats. *Proceedings of the Royal Society B: Biological Sciences, 279*, 3678–3686. doi:10.1098/rspb.2012.0705

Ferrari, L., Orozco-Esquivel, T., Manea, V., & Manea, M. (2012). The dynamic history of the Trans-Mexican Volcanic

Belt and the Mexico Subduction Zone. *Tectonophysics*, *522-523*, 122–149. doi:10.1016/j.tecto.2011.09.018

Ferrusquía, I. (1998). Geología de México: Una sinopsis [Geology of Mexico: A synopsis]. In T. P. Ramamoorthy, R. Bye, A. Lot, & J. Fa (Eds.), *Diversidad Biológica de México* [*Biological Diversity of Mexico*] (pp. 3–108). México: Instituto de Biología, UNAM.

Flores-Villela, O., & Gerez, P. (1994). *Biodiversidad y conservación en México: Vertebrados, vegetación y uso del suelo* [*Biodiversity and Conservation in Mexico: Vertebrates, vegetation and land use*]. México: CONABIO-UNAM.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial Cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.

Fregin, S., Haase, M., Olsson, U., & Alström, P. (2012). Pitfalls in comparisons of genetic distances: A case study of the avian family Acrocephalidae. *Molecular Phylogenetics and Evolution*, *62*, 319–328. doi:10.1016/j.ympev.2011.10.003

Fujisawa, T., & Barraclough, T. G. (2013). Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology*, *62*, 707–724. doi:10.1093/sysbio/syt033

Fujita, M. K., Leaché, A. D., Burbrink, F. T., McGuire, J. A., & Moritz, C. (2012). Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology and Evolution*, *27*, 480–488. doi:10.1016/j.tree.2012.04.012

Funk, D. J., & Omland, K. E. (2003). Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 397–423. doi:10.1146/annurev.ecolsys.34.011802.132421

Gándara, E., & Sosa, V. (2014). Spatio-temporal evolution of *Leucophyllum pringlei* and allies (Scrophulariaceae): A group endemic to North American xeric regions. *Molecular Phylogenetics and Evolution*, *76*, 93–101. doi:10.1016/j.ympev.2014.02.027

Gittenberger, A., & Gittenberger, E. (2011). Cryptic, adaptive radiation of endoparasitic snails: Sibling species of *Leptoconchus* (Gastropoda: Coralliophilidae) in corals. *Organisms Diversity and Evolution*, *11*, 21–41. doi:10.1007/s13127-011-0039-1

Grill, A., Gkiokia, E., & Alvarez, N. (2006). Evolutionary history and patterns of differentiation among European *Maniola* butterflies (Lepidoptera: Satyrinae). *European Journal of Entomology*, *103*, 613–618. doi:10.14411/eje.2006.082

Gutiérrez-Arellano, D., Gutiérrez-Arellano, C. R., & Zaldívar-Riverón, A. (2015). DNA Barcoding of the parasitoid wasp subfamily Doryctinae (Hymenoptera: Braconidae) from Chamela, Mexico. *Biodiversity Data Journal*, *3*, e5109. doi:10.3897/BDJ.3.e5109

Halffter, G. (1976). Distribución de los insectos en la zona de transición mexicana: Relaciones con la entomofauna de Norteamérica [Distribution of Insects in the Mexican Transition Zone: Relations with the North American entomofauna]. *Folia Entomológica Mexicana*, *35*, 1–64. Retrieved from: http://www.folia.socmexent.org/revista/folia/Num%2035/1-64.pdf (accessed 23 March 2017).

Hall, T.A. (1999). BioEdit: A user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98. doi:10.1021/bk-1999-0734.ch008

Haran, J., Koutroumpa, F., Magnoux, E., Roques, A., & Roux, G. (2015). Ghost mtDNA haplotypes generated by fortuitous NUMTs can deeply disturb infra-specific genetic diversity and phylogeographic pattern. *Journal of Zoological Systematics and Evolutionary Research*, *53*, 109–115. doi:10.1111/jzs.12095

Hebard, M. (1924). A Revision of the Genus *Taeniopoda* (Orthoptera, Acrididae, Cyrtacanthacrinae). *Transactions of the American Entomological Society*, *50*, 253–274. Retrieved from: http://www.jstor.org/stable/25077112 (accessed 23 March 2017).

Hebard, M. (1925). The Group Taeniopodae as Found in the United States (Orthoptera). *Transactions of the American Entomological Society*, *51*, 1–12. Retrieved from: http://www.jstor.org/stable/25077119 (accessed 23 March 2017).

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 313–321. doi:10.1098/rspb.2002.2218

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, *405*, 907–913. doi:10.1038/35016000

Hohenegger, J. (2014). Species as the basic units in evolution and biodiversity: Recognition of species in the recent and geological past as exemplified by larger foraminifera. *Gondwana Research*, *25*, 707–728. doi:10.1016/j.gr.2013.09.009

Jermiin, L. S., & Crozier, R. H. (1994). The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: Sequence divergence in Hymenoptera may be associated with nucleotide content. *Journal of Molecular Evolution*, *38*, 282–294. doi:10.1007/BF00176090

Jiruskova, A., & Bocak, L. (2015). Species Delimitation in *Cautires* (Coleoptera: Lycidae) from Peninsular Malaysia using DNA data and morphology. *Annales Zoologici*, *65*, 239–248. doi:10.3161/00034541ANZ2015.65.2.007

Joly, S., Davies, T. J., Archambault, A., Bruneau, A., Derry, A., Kembel, S. W., … Wheeler, T. A. (2014). Ecology in the age of DNA barcoding: The resource, the promise and the challenges ahead. *Molecular Ecology Resources*, *14*, 221–232. doi:10.1111/1755-0998.12173

Jordal, B. H., & Kambestad, M. (2014). DNA barcoding of bark and ambrosia beetles reveals excessive NUMTs and consistent east-west divergence across Palearctic forests. *Molecular Ecology Resources*, *14*, 7–17. doi:10.1111/1755-0998.12150

Kallman, K. D., & Kazianis, S. (2006). The genus *Xiphophorus* in Mexico and Central America. *Zebrafish*, *3*, 271–285. doi:10.1089/zeb.2006.3.271

Katz, A. D., Giordano, R., & Soto-Adames, F. N. (2015). Operational criteria for cryptic species delimitation when evidence is limited, as exemplified by North American *Entomobrya* (Collembola: Entomobryidae). *Zoological Journal of the Linnean Society*, *173*, 818–840. doi:10.1111/zoj.12220

Kekkonen, M., Mutanen, M., Kaila, L., Nieminen, M., & Hebert, P. D. N. (2015). Delineating species with DNA barcodes: A case of taxon dependent method performance in moths. *Public Library of Science ONE*, *10*, e0122481. doi:10.1371/journal.pone.0122481

Kerr, K. C. R., Lijtmaer, D. A., Barreira, A. S., Hebert, P. D. N., & Tubaro, P. L. (2009). Probing evolutionary patterns in Neotropical birds through DNA barcodes. *Public Library of Science ONE*, *4*, e4379. doi:10.1371/journal.pone.0004379

King, A. B. S., & Saunders, J. L. (1984). *Las plagas invertebradas de cultivos alimenticios anuales en América Central* [*Invertebrate Pests of Annual Food Crops in Central America*]. London: Overseas Development Administration.

Knowles, L., & Alvarado-Serrano, D. F. (2010). Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: Insights from coupled ecological, demographic and genetic models in montane grasshoppers. *Molecular Ecology*, *19*, 3727–3745. doi:10.1111/j.1365-294X.2010.04702.x

Kobelkowsky-Vidrio, T., Ríos-Muñoz, C. A., & Navarro-Sigüenza, A. G. (2014). Biodiversity and biogeography of the avifauna of the Sierra Madre Occidental, Mexico. *Biodiversity and Conservation*, *23*, 2087–2105. doi:10.1007/s10531-014-0706-6

Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015). DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology and Evolution*, *30*, 25–35. doi:10.1016/j.tree.2014.10.008

Kumar, S., Secher, G., & Koichiro, T. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*, 1870–1874. doi:10.1093/molbev/msw054

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., … Higgins, D. G. (2007). ClustalW and ClustalX, version 2. *Bioinformatics*, *23*, 2947–294. doi:10.1093/bioinformatics/btm404

Leaché, A. D. (2009). Species tree discordance traces to phylogeographic clade boundaries in North American fence lizards (*Sceloporus*). *Systematic Biology*, *58*, 547–559. doi:10.1093/sysbio/syp057

Leavitt, D. H., Starrett, J., Westphal, M. F., & Hedin, M. (2015). Multilocus sequence data reveal dozens of putative cryptic species in a radiation of endemic Californian mygalomorph spiders (Araneae, Mygalomorphae, Nemesiidae). *Molecular Phylogenetics and Evolution*, *91*, 56–67. doi:10.1016/j.ympev.2015.05.016

Lecocq, T., Dellicour, S., Michez, D., Dehon, M., Dewulf, A., De Meulemeester, T., … Rasmont, P. (2015). Methods for species delimitation in bumblebees (Hymenoptera, Apidae, Bombus): Towards an integrative approach. *Zoologica Scripta*, *44*, 281–297. doi:10.1111/zsc.12107

Lomolino, M. V., Riddle, B. R., & Brown, J. H. (2005). *Biogeography*. (Third ed). Sunderland, USA: Sinauer Associates.

Mao, Y., Zhang, Y., Nakamura, K., Guan, B., & Qiu, Y. (2014). Developing DNA barcodes for species identification in *Podophylloideae* (Berberidaceae). *Journal of Systematics and Evolution*, *52*, 487–499. doi:10.1111/jse.12076

Mariño-Pérez, R., Fontana, P., & Buzzetti, F. M. (2011). Identificación de plagas de chapulín en el norte–centro de México [Identification of grasshopper pests in north-central Mexico]. In C. García-Gutierrez C & J. Lozano-Gutierrez (Eds.), *Control biológico de plagas de chapulín en el norte~centro de México* [*Biological control of grasshopper pests in the north~central Mexico*] (pp. 35–55). México: Universidad Autónoma de Zacatecas.

Massimino Cocuzza, G. E., & Cavalieri, V. (2014). Identification of aphids of *Aphis frangulae*-group living on *Lamiaceae* species through DNA barcode. *Molecular Ecology Resources*, *14*, 447–457. doi:10.1111/1755-0998.12199

Mateos, M. (2005). Comparative phylogeography of livebearing fishes in the genera *Poeciliopsis* and *Poecilia* (Poeciliidae: Cyprinodontiformes) in Central Mexico. *Journal of Biogeography*, *32*, 775–780. doi:10.1111/j.1365-2699.2005.01236.x

Miguez-Gutiérrez, A., Castillo, J., Márquez, J., & Goyenechea, I. (2013). Biogeografía de la Zona de Transición Mexicana con base en un análisis de árboles reconciliados [Biogeography of the Mexican Transition Zone based on a reconciled trees analysis]. *Revista Mexicana De Biodiversidad*, *84*, 215–224. doi:10.7550/rmb.32119

Monaghan, M. T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D. J. G., … Vogler, A. P. (2009). Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology*, *58*, 298–311. doi:10.1093/sysbio/syp027

Morrone, J. J. (2010). Fundamental biogeographic patterns across the Mexican Transition Zone: An evolutionary approach. *Ecography*, *33*, 355–361. doi:10.1111/j.1600-0587.2010.06266.x

Morrone, J. J. (2015a). Biogeographical regionalisation of the world: A reappraisal. *Australian Systematic Botany*, *28*, 81–90. doi:10.1071/SB14042

Morrone, J. J. (2015b). Halffter's Mexican transition zone (1962-2014), cenocrons and evolutionary biogeography. *Journal of Zoological Systematics and Evolutionary Research*, *53*, 249–257. doi:10.1111/jzs.12098

Munguía-Lino, G., Vargas-Amado, G., Vázquez-García, L. M., & Rodríguez, A. (2015). Riqueza y distribución geográfica de la tribu *Tigridieae* (Iridaceae) en Norteamérica [Richness and geographic distribution of the tribe Tigridieae (Iridaceae) in North America]. *Revista Mexicana de Biodiversidad*, *86*, 80–98. doi:10.7550/rmb.44083

Mutanen, M., & Pretorius, E. (2007). Subjective visual evaluation vs. traditional and geometric morphometrics in species delimitation: A comparison of moth genitalia. *Systematic Entomology*, *32*, 371–386. doi:10.1111/j.1365-3113.2006.00372.x

Mutun, S., & Borst, D. W. (2004). Intraspecific mitochondrial DNA Variation and Historical Biogeography of the Eastern Lubber Grasshopper, *Romalea microptera*. *Annals of the Entomological Society of America*, *97*, 681–696. doi:10.1603/0013-8746(2004)097[0681:IMDVAH]2.0.CO;2

Ornelas-García, C. P., Domínguez-Domínguez, O., & Doadrio, I. (2008). Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in mesoamerica reveals multiple morphological homoplasies. *BioMedCentral Evolutionary Biology*, *8*, 340. doi:10.1186/1471-2148-8-340

Padial, J. M., Castroviejo-Fisher, S., Köhler, J., Vilà, C., Chaparro, J. C., & De La Riva, I. (2009). Deciphering the products of evolution at the species level: The need for an integrative taxonomy. *Zoologica Scripta*, *38*, 431–447. doi:10.1111/j.1463-6409.2008.00381.x

Papadopoulou, A., Anastasiou, I., & Vogler, A. P. (2010). Revisiting the Insect Mitochondrial Molecular Clock: The Mid-Aegean trench calibration. *Molecular Biology and Evolution*, *27*, 1659–1672. doi:10.1093/molbev/msq051

Pedraza-Lara, C., Barrientos-Lozano, L., Rocha-Sánchez, A. Y., & Zaldívar-Riverón, A. (2015). Montane and coastal species diversification in the economically important Mexican grasshopper genus *Sphenarium* (Orthoptera: Pyrgomorphidae). *Molecular Phylogenetics and Evolution*, *84*, 220–231. doi:10.1016/j.ympev.2015.01.001

Peters, J. L., Zhuravlev, Y., Fefelov, I., Logie, A., & Omland, K. E. (2007). Nuclear loci and coalescent methods support ancient hybridization as cause of mitochondrial paraphyly between gadwall and falcated duck (*Anas* spp.). *Evolution*, *61*, 1992–2006. doi:10.1111/j.1558-5646.2007.00149.x

Phuong, M. A., Lim, M. C. W., Wait, D. R., Rowe, K. C., & Moritz, C. (2014). Delimiting species in the genus *Otospermophilus* (Rodentia: Sciuridae), using genetics, ecology, and morphology. *Biological Journal of the Linnean Society*, *113*, 1136–1151. doi:10.1111/bij.12391

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., … Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology, 55*, 595–609. doi:10.1080/10635150600852011

Pons, J., & Vogler, A. P. (2005). Complex pattern of coalescence and fast evolution of a mitochondrial rRNA pseudogene in a recent radiation of tiger beetles. *Molecular Biology and Evolution, 22*, 991–1000. doi:10.1093/molbev/msi085

Posada, D. (2004). *Collapse* ver. 1.2. *A tool for collapsing sequences to haplotypes*. Vigo: Universidad de Vigo.

Price, B. W., Henry, C. S., Hall, A. C., Mochizuki, A., Duelli, P., & Brooks, S. J. (2015). Singing from the grave: DNA from a 180 year old type specimen confirms the identity of *Chrysoperla carnea* (Stephens). *Public Library of Science ONE, 10*, e0121127. doi:10.1371/journal.pone.0121127

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system: Barcoding. *Molecular Ecology Notes, 7*, 355–364. doi:10.1111/j.1471-8286.2007.01678.x

Rehn, J. A. G., & Grant, Jr. H. J. (1959a). An analysis of the tribes of the Romaleinae with special reference to their internal genitalia (Orthoptera: Acrididae). *Transactions of the American Entomological Society, 85*, 233–271. Retrieved from: http://www.jstor.org/stable/25077781 (accessed 23 March 2017).

Rehn, J. A. G., & Grant, Jr. H. J. (1959b). A review of the *Romaleinae* (Orthoptera: Acrididae) found in America north of Mexico. *Proceedings of the Academy of Natural Sciences, Philadelphia, 111*, 109–271. Retrieved from: http://www.jstor.org/stable/4064509 (accessed 23 March 2017).

Rheindt, F. E., & Edwards, S. V. (2011). Genetic introgression: An integral but neglected component of speciation in birds. *Auk, 128*, 620–632. doi:10.1525/auk.2011.128.4.620

Roberts, H. R. (1941). A Comparative Study of the Subfamilies of the *Acrididae* (Orthoptera) Primarily on the Basis of Their Phallic Structures. *Proceedings of the Academy of Natural Sciences of Philadelphia, 93*, 201–246. Retrieved from: http://www.jstor.org/stable/4064333 (accessed 23 March 2017).

Rodríguez-Gómez, F., & Ornelas, J. F. (2015). At the passing gate: Past introgression in the process of species formation between *Amazilia violiceps* and *A. viridifrons* hummingbirds along the Mexican Transition Zone. *Journal of Biogeography, 42*, 1305–1318. doi:10.1111/jbi.12506

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*, 1572–1574. doi:10.1093/bioinformatics/btg180

Ruane, S., Bryson, R. W., Pyron, R. A., & Burbrink, F. T. (2014). Coalescent species delimitation in milksnakes (genus *Lampropeltis*) and impacts on phylogenetic comparative analyses. *Systematic Biology, 63*, 231–250. doi:10.1093/sysbio/syt099

Sanginés-Franco, C., Luna-Vega, I., Contreras-Medina, R., Espinosa, D., Tejero-Díez, J. D., & Rivas, G. (2015). Diversity, endemism and conservation of ferns (Polypodiales) in the Mexican Mountain Component. *Journal of Mountain Science, 12*, 891–904. doi:10.1007/s11629-014-3070-9

Schmidt, S., Schmid-Egger, C., Morinière, J., Haszprunar, G., & Hebert, P. D. N. (2015). DNA barcoding largely supports 250 years of classical taxonomy: Identifications for central European bees (Hymenoptera, Apoidea partim). *Molecular Ecology Resources, 15*, 985–1000. doi:10.1111/1755-0998.12363

Smith, M. A., Bertrand, C., Crosby, K., Eveleigh, E. S., Fernandez-Triana, J., Fisher, B. L., … Zhou, X. (2012). *Wolbachia* and DNA barcoding insects: Patterns, potential, and problems. *Public Library of Science ONE, 7*, e36514. doi:10.1371/journal.pone.0036514

Song, H., Buhay, J. E., Whiting, M. F., & Crandall, K. A. (2008). Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 13486–13491. doi:10.1073/pnas.0803076105

Song, H., Moulton, M. J., & Whiting, M. F. (2014). Rampant nuclear insertion of mtDNA across diverse lineages with in Orthoptera (Insecta). *Public Library of Science ONE, 9*, e110508. doi:10.1371/journal.pone.0110508

Stauffer, T. W., Hatle, J. D., & Whitman, D. W. (2011). Divergent egg physiologies in two closely related grasshopper species: *Taeniopoda eques* versus *Romalea microptera* (Orthoptera: Romaleidae). *Environmental Entomology, 40*, 157–166. doi:10.1603/EN10200

Stauffer, T. W., & Whitman, D. (2007). Divergent Oviposition Behaviors in a Desert Vs a Marsh Grasshopper. *Journal of Orthoptera Research, 16*, 103–114. doi:10.1665/1082-6467(2007)16[103:DOBIAD]2.0.CO;2

Talavera, G., Dincă, V., & Vila, R. (2013). Factors affecting species delimitations with the GMYC model: Insights from a butterfly survey. *Methods in Ecology and Evolution, 4*, 1101–1110. doi:10.1111/2041-210X.12107

Tänzler, R., Sagata, K., Surbakti, S., Balke, M., & Riedel, A. (2012). DNA Barcoding for community ecology - how to tackle a hyperdiverse, mostly undescribed melanesian fauna. *Public Library of Science ONE, 7*, e28832. doi:10.1371/journal.pone.0028832

Tänzler, R., Toussaint, E. F., Suhardjono, Y. R., Balke, M., & Riedel, A. (2014). Multiple transgressions of Wallace's line explain diversity of flightless *Trigonopterus* weevils on Bali. *Proceedings of the Royal Society. Biological Sciences, 281*, 20132528. doi:10.1098/rspb.2013.2528

Teske, P. R., Papadopoulos, I., Barker, N. P., Mcquaid, C. D., & Beheregaray, L.B. (2014). Mitonuclear discordance in genetic structure across the Atlantic/Indian ocean biogeographical transition zone. *Journal of Biogeography, 41*, 392–401. doi:10.1111/jbi.12201

Toussaint, E. F. A., Morinière, J., Müller, C. J., Kunte, K., Turlin, B., Hausmann, A., … Balke, M. (2015). Comparative molecular species delimitation in the charismatic Nawab butterflies (Nymphalidae, Charaxinae, Polyura). *Molecular Phylogenetics and Evolution, 91*, 194–209. doi:10.1016/j.ympev.2015.05.015

Vanhaecke, D., de Leaniz, C. G., Gajardo, G., Young, K., Sanzana, J., Orellana, G., … Consuegra, S. (2012). DNA Barcoding and Microsatellites Help Species Delimitation and Hybrid Identification in Endangered Galaxiid fishes. *Public Library of Science ONE, 7*, e32939. doi:10.1371/journal.pone.0032939

Wang, W., Dai, C., Alström, P., Zhang, C., Qu, Y., Li, S. –H., … Lei, F. (2014). Past hybridization between two East Asian long-tailed tits (*Aegithalos bonvalotti* and *A. fuliginosus*). *Frontiers in Zoology, 11*, 40. doi:10.1186/1742-9994-11-40

Welch, A. J., Yoshida, A. A., & Fleischer, R. C. (2011). Mitochondrial and nuclear DNA sequences reveal recent divergence in morphologically indistinguishable petrels. *Molecular Ecology, 20*, 1364–1377. doi:10.1111/j.1365-294X.2011.05008.x

White, D. J., Wolff, J. N., Pierson, M., & Gemmell, N. J. (2008). Revealing the hidden complexities of mtDNA inheritance. *Molecular Ecology, 17*, 4925–4942. doi:10.1111/j.1365-294X.2008.03982.x

Whitman, D. W. (1988). Function and evolution of thermoregulation in the desert grasshopper *Taeniopoda eques*. *Journal of Animal Ecology, 57*, 369–383. doi:10.2307/4911

Whitman, D. W., & Richardson, M. L. (2010). Necrophagy in grasshoppers: *Taeniopoda eques* feeds on mammal carrion. *Journal of Orthoptera Research, 19*, 377–380. doi:10.1665/034.019.0228

Zaldívar-Riverón, A., Martínez, J. J., Ceccarelli, F. S., De Jesús-Bonilla, V. S., Rodríguez-Pérez, A. C., Reséndiz-Flores, A., & Smith, M. A. (2010). DNA barcoding a highly diverse group of parasitoid wasps (Braconidae: Doryctinae) from a Mexican nature reserve. *Mitochondrial DNA, 21*(Suppl. 1), 18–23. doi:10.3109/19401736.2010.523701

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Capítulo III: 3RAD-based systematics of the transitional Nearctic-Neotropical lubber grasshopper genus *Taeniopoda* (Orthoptera: Romaleidae) [Enviado a Molecular Phylogenetics and Evolution, en revisión]

3RAD-based systematics of the transitional Nearctic-Neotropical lubber grasshopper genus *Taeniopoda* (Orthoptera: Romaleidae)

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Abstract

The genus *Taeniopoda* Stål (Romaleidae) is a group of Nearctic-Neotropical grasshoppers whose systematics has been largely neglected. A recent phylogenetic study based on morphology and mitochondrial and nuclear markers failed to resolve the species limits in this genus, and showed a lack of reciprocal exclusivity between two of its species, *T. eques* (Burmeister) and *T. tamaulipensis* Rehn. Here we assessed the species limits and phylogenetic relationships in *Taeniopoda* based on 3RAD data, and the presence of gene flow and niche overlap between the latter species using clustering and ecological niche modelling (ENM) analyses. We performed de novo assembly of different 3RAD data sets to explore how distinct parameters settings could impact the recovered relationships. Similar to previous studies, we found that the relationships derived from the matrices were mostly congruent despite their wide range of missing data. Moreover, the similarity threshold and minimum number of samples that must share a locus for it to be retained have an impact in the amount of loci and missing data obtained in the matrices. Ten species were consistently delimited, with *T. picticornis* and *T. stali* regarded as conspecific and the populations of *T. auricornis* from Central Mexico and Guatemala divided into two species. We also maintained the specific status of *T. eques* and *T. tamaulipensis*, though our results suggest that they could represent a ring species since their examined populations appear to change gradually following a "loop form" along their geographical distribution. The phylogenomic analyses confirmed the paraphyly of *Taeniopoda* with respect to *Romalea*, and the presence of three major clades within the group. This study demonstrates the utility of 3RAD to detect gene flow and resolve species limits and phylogenetic relationships among closely related taxa.

1. Introduction

Improving our knowledge of the biodiversity that inhabits our planet and understanding the processes that are involved in its origin and evolution are among the major goals in systematics and evolutionary biology. These tasks are particularly relevant for invertebrate taxa, most of which continue being largely neglected. A clear example of this is the insect order Orthoptera, whose studies addressing the systematics and evolution of most of its groups are considerably scarce compared to other insect orders.

The genus *Taeniopoda* Stål (Romaleidae) is a small group of conspicuous, colourful grasshoppers (known as "lubber grasshoppers") that are distributed in the Nearctic and Neotropical regions, from southern United States to northern Panama, though most of its species occur along the transitional Nearctic-Neotropical zone from Central Mexico to Guatemala (Eades et al., 2016; De Jesús-Bonilla et al., 2017). Members of this genus are of economic importance because in high densities they are considered as agricultural pests, although they have also been used as food resource and in traditional medicine (King and Saunders, 1984; Mariño-Pérez et al., 2011; Paul et al., 2016; Ramos-Elorduy et al., 2008).

Despite its wide distribution and economic importance, the systematics and evolutionary history of *Taeniopoda* have been largely neglected. The only revision carried out for the genus recognised 12 species, though it was suggested that some of them could actually represent chromatomorphs instead of valid species (Hebard, 1924). Moreover, in the latter study the author suggested a close relationship between the species of *Taeniopoda* and the monotypic genus *Romalea* Serville, though this hypothesis was not tested in a phylogenetic context.

We recently employed mitochondrial (mt) and nuclear DNA sequence data and morphological features to investigate the species limits and phylogenetic relationships in

Taeniopoda (De Jesús-Bonilla et al., 2017). The species delineation based on two mt gene markers (COI and cyt *b*) was not conclusive, discriminating between six and 14 species depending on the method and parameters employed. Moreover, the mito-nuclear phylogeny recovered a paraphyletic *Taeniopoda* with respect to the member of the monotypic genus *Romalea* Serville, *R. microptera* (Palisot de Beauvois), as well as three morphologically and geographically congruent major clades. However, various of the relationships involved were not significantly supported.

The mt genealogy reconstructed in the above study also showed a lack of reciprocal exclusivity (term employed instead monophyly at species level *sensu* De Queiroz, 2007) between *T. tamaulipensis* Rehn and *T. eques* (Burmeister), suggesting the presence of mitochondrial introgression or incomplete lineage sorting between them. These two species have a wide geographic distribution along southern USA to central Mexico, have been recorded to occur in sympatry in central Mexico and are morphologically distinguished from each other only by their colour of pronotum (Hebard, 1924; Eades et al., 2016; De Jesús-Bonilla et al., 2017). The taxonomic status and existence of gene flow among their populations therefore needs to be assessed in more detail using nuclear markers.

Restriction site-associated DNA sequencing (RADseq) methods allow a reduced random representation of the full genome with a large amount of homologous loci (Andrews et al., 2016; Baird et al., 2008; Rowe et al., 2011). One of the main advantages of these techniques is that it is not necessary to have a reference genome to align and assembly reads, so it can be used in non-model organisms (Andrews et al., 2016; Peterson et al., 2012; Toonen et al., 2013). The RADseq techniques were initially developed for population genetics studies (Baird et al., 2008; Davey and Blaxter, 2010), though they have also been successfully employed to reconstruct phylogenetic relationships among both closely (e.g.

Wagner et al., 2013; Wessinger et al., 2016) and distantly (Leaché et al., 2015a; Nieto-Montes de Oca et al., 2017; Rubin et al., 2012) related species.

In this study, we generated RADseq data using 3RAD, a three-enzyme protocol that reduces the adapter-dimer formation (Glenn et al., in press) to assess the species limits and phylogenetic relationships within *Taeniopoda*. We also assessed the genealogical relationships and the existence of genetic admixture among populations of *T. eques* and *T. tamaulipensis* using both phylogenetic and clustering analyses.

Different pipelines (*e.g.* Stacks: Catchen et al., 2011; RADTools: Baxter et al., 2011; Pyrad: Eaton, 2014), have been developed for raw reads bioinformatic processing of RADseq data. The main parameters affecting RADseq assembly are the threshold of similarity for clustering reads into a locus, the number of raw reads needed to form a stack and the minimum number of samples in which a locus must be retained it in the final matrix (Paris et al., 2017; Takahashi and Sota 2016). It is known that combination of these parameters directly affects the number of loci retrieved, coverage and the amount of missing data in the final matrices (Chattopadhyay et al., 2014; Hou et al., 2016; Paris et al., 2017). We therefore performed a de novo assembly of the gathered 3RAD data implementing a protocol that optimizes the similarity threshold, and explored how the minimum number of samples that must have data at a given locus for it to be retained in the final data set could impact the retrieved data in terms of shared loci and missing data.

2. Methods

2.1 Taxon sampling

We generated 3RAD data for a subsample of 64 of the 223 specimens that were sequenced for few mitochondrial and nuclear markers in a previous phylogenetic study of *Taeniopoda*

(De Jesús-Bonilla et al., 2017) (Supplementary Material 1). These specimens belong to the 12 described species that were included in the above study (11 species of *Taeniopoda* and *R. microptera*), containing representatives of their different sampled localities and including five specimens of *T. eques* that appeared intermingled in a clade together with specimens of *T. tamaulipensis*. We also generated data for the following four additional romaleid genera as outgroups: *Brachystola magna* Bruner (two specimens), *Chromacris colorata* Serville (two specimens), *Cibotopteryx variegata* Rehn (two specimens) and *Tropidacris cristata* Linnaeus (one specimen).

2.2 Laboratory protocols

The genomic DNA of each sample was extracted from muscle of hind leg using the DNeasy Blood and Tissue (QIAGEN: Austin, EUA) or EZ-10 Spin Column Genomic DNA Minipreps (BIO BASIC; Toronto, Canada) kits following the manufacturer's protocols. RADseq libraries were prepared following the Adapterama III protocol (Glenn et al., in press), which uses a three enzymes digestion. The genomic DNA was digested with XbaI and EcoRI-HF (New England Biolabs, Massachusetts, USA), whereas NheI (New England Biolabs, Massachusetts, EUA) was added to suppress phosphorylated ends in XbaI recognition sites and to avoid the dimer adaptors formation.

For each sample, 1000 ng of genomic DNA were digested for 1 hour at 37°C in a solution with 1.5 µl of 10x Cutsmart® buffer, 0.25 µl (NEB®) of XbaI at 20 U/µl, 0.25 µl of EcoRI-HF 20 at 20 U/µl, 0.25 µl of NheI at 20 U/µl, 1 µl of i5Tru adapter at 5 µM, 1 µl of i7Tru adapter at 5 µM and 0.75 µl of dH2O. After digestion, we added in the same solution 2.5 µl of H2O, 1.5 µl ATP at 10µ M, 0.5 µl of 10x Ligase Buffer (NEB®) and 0.25 µl of T4 DNA Ligase at 400 U/µl, and incubated at 22°C for 20 min and 37°C for 10

min for two cycles and a final cycle of 80°C for 20 min. After digestion/ligation, samples were pooled and cleaned with 1.8x Agencourt AMPure XP Beads (Agencourt Bioscience Corporation, Beverly, Massachusetts). An enrichment PCR of each pool was carried with 10 µl of 5x Kapa Long Range Buffer (Kapa Biosystems, Massachusetts, EUA), 0.25 µl of KAPA LongRange DNA Polymerase at 5 U/µl, 1.5 µl of dNTPs mix (10 mM each dNTP), 3.5 µl of $MgCL_2$ at 25 mM, 2.5 µl of iTru5 primer at 5 µM, 2.5 µl of iTru7 primer at 5 µM and 5µl of pooled DNA.

The temperature conditions for PCR enrichment were 94°C for 2 min of initial denaturation, followed by 10 cycles of 94°C for 20 sec, 57°C for 15 sec and 72° for 30 sec, and a final cycle of 72°C for 5 min. The enriched pools were cleaned and quantified with a Qubit Fluorometer (Life Technologies, Inc., Grand Island). Cleaned and quantified pools were sent to the Georgia Genomics and Bioinformatics Core for size selection using Pippin Prep (Sage Science, Beverly, MA) to capture 550 bp +/- 10% fragments using a 1.5% agarose cassette, and paired end 150bp sequencing in an Illumina HiSeq 2500 platform. The Indexing strategy is provided in the Supplementary Material 2.

2.3 3RAD bioinformatic processing

Raw sequence quality was evaluated with FASTQC 0.11.5 (Andrews, 2010). Raw sequences were demultiplexed with iPyrad 0.7.18 (Eaton, 2014), allowing one mismatch in barcodes. We only used the R1 reads files and excised samples with < 100,000 reads from subsequent analyses. A crucial step for recovering orthologous loci from RADseq data is the similarity threshold for clustering reads into a locus. A high similarity threshold tends to split alleles into separate loci (false homozygosity), whereas a lower one could merge paralogous loci into a single locus (false heterozygosity) (Ilut et al., 2014; McKinney et al.,

2017). We therefore implemented the strategy proposed by Ilut et al. (2014) to avoid the use of arbitrary clustering threshold, which identifies the similarity threshold that minimizes false homozygosity and heterozygosity. We carried out clustering threshold series allowing similarity thresholds from 0.99 to 0.85 for each sample with the custom scripts developed by Ilut et al. (2014). Then, we identified the similarity threshold that maximizes and minimizes the number of two allele loci and single allele loci, respectively. All Illumina raw data were archived in Genbank Sequence Read Archive in Bioproject XXXXXX.

2.4 De novo assembly

Raw Illumina reads were processed with the software pipeline iPyrad 0.7.18 to generate de novo assemblies. For quality control and filtering of reads, bases with phred Q score lower that 33 were considered as unknown and replaced with Ns, and reads with more than five unknown bases were discarded from subsequent analyses. Reads were subsequently clustered considering at least 6x depth coverage for statistical base calling and a maximum of 100,000 reads depth within samples. Consensus sequences were generated considering a maximum of two alleles per locus in individual consensus sequences, and allowing five and eight uncalled bases and heterozygotes sites, respectively. We regarded a maximum of 20 SNPs and 8 indels per locus to cluster consensus sequences between samples.

We explored how the minimum number of samples that must have data at a given locus for it to be retained in the final data set (min_samples_locus parameter) affects the matrix output. For this, a total of 13 matrices were generated with 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 63 min_samples_locus values (named m5, m10, m15, m20, m25, m30, m35, m40, m45, m50, m55, m60 and m63, respectively). For each matrix, the

proportion of shared loci among samples was calculated with the R package RADami 1.1-2 with locus.dist function (Hipp et al., 2014). Missing data were calculated with the program VCFtools 0.1.14 (Danecek et al., 2011). SNPs plots were generated with the glPlot function of the adegenet v2.0.1 R package (Jombart, 2008; Jombart and Ahmed, 2011) to visually observe the degree of dispersion of the matrices.

2.5 Phylogenetic analyses and genetic distances

We carried out phylogenetic analyses for the 13 generated matrices with the Maximum Likelihood (ML) and Bayesian probabilistic methods using the programs RAxML version 8.0 (Stamatakis, 2014) and Exabayes version 1.4.1 (Aberer et al., 2014), respectively. The matrices included all concatenated loci with SNPs and invariant sites to improve branch length and topological accuracy in phylogenetic reconstructions (Leaché et al., 2015b).

For the ML analyses, we carried out a simultaneous search to obtain the best--scoring ML tree. Rapid bootstrap analyses were also conducted with the GTRGAMMA model, using 1,000 bootstrap replicates starting from random seeds. Clades were considered strongly supported if they had a bootstrap value ≥ 70.

The Bayesian analyses had two independent runs and were carried out with four chains. Each chain was run with the GTR model for 1,000,000 generations or until they reached convergence (Average Standard Deviation of Split Frequencies $\leq 1\%$). Trees were sampled every 500 generations, and the 25% of the sampled trees were discarded as burn-in. Effective Sample Size (>200) and convergence were inspected with the program Tracer version 1.6 (Rambaut and Drummond, 2009). We built for each analysis a majority rule consensus tree with posterior probabilities (PP) of clades with the Exabayes consensus option, considering clades as significantly supported when they had a value ≥ 0.95. The ML

and Bayesian phylograms were edited with the program FigTree version 1.4.3 (Rambaut, 2009).

We could not obtain shared loci between any of the outgroup and the ingroup taxa in the final assembly. We therefore rooted the trees based on the best estimate of phylogeny recovered in our previous mito-nuclear phylogenetic study (De Jesús-Bonilla et al., 2017). This topology places the *T. reticulata* (Fabricius) + *T. varipennis* Rehn + *T. citricornis* Bruner + *T. gutturosa* Bolivar clade as sister to the remaining taxa, including *R. microptera*.

We estimated whole genome pairwise distances among the examined samples calculating the intra and interspecific Nei's Genetic Distances (Nei, 1972) for the m20 matrix (see below) with the StAMPP version 1.5.1 R package (Pembleton et al., 2013) using the stamppNeisD function.

2.6 Genetic admixture between T. eques and T. tamaulipensis

We used two approaches to investigate the presence of genetic admixture among the examined samples, with an emphasis between the specimens assigned to *T. eques* and *T. tamaulipensis*. First, we identified clusters of genetically related individuals of all included species performing a Principal Components Analysis (PCA) and a Discriminant Analysis of Principal Components (DAPC) following the method proposed by Jombart et al. (2010). The SNPs matrices were analyzed in the adegenet v2.0.0 R package (Jombart, 2008; Jombart and Ahmed, 2011) using the glPca and dapc functions. PCA results were used to conduct DAPC. We implemented the optimization by cross-validation using a training set of 90% of the data to determine the number of Principal Components (PCs) retained in each dataset. PCA and DAPC were performed including all samples, with three subsets

containing samples of the three main clades identified in our phylogenetic analyses, and with a subset of samples of *T. eques* and *T. tamaulipensis*.

We further investigated the existence of admixture between *T. eques* and *T. tamaulipensis* using a bayesian clustering analysis. We generated a subset with the 17 specimens assigned to *T. eques* and *T. tamaulipensis* (min sample locus 10; 14,110 unlinked snps). The resulting matrix of unlinked snps was analyzed with Structure version 2.3.4 (Pritchard et al., 2000) under the admixture model, running 500,000 iterations and discarding 100,000 as burnin. We evaluated K values from 2 to 5, and for each K we ran 10 independent analyses. The parallel analyses were summarized with CLUMP v1.1.2 using 'greedy' algorithm (Jakobsson and Rosenberg, 2007). We used the ΔK method to determine the optimal K value (Evanno et al., 2005).

2.7 Niche overlap between T. tamaulipensis and *T. eques*

We modeled the ecological niches of *T. tamaulipensis* and *T. eques* to determine the degree of overlap in their distribution. Ecological niche models (ENMs) for both species were generated with the program Maxent version 3.3.3k (Phillips and Dudik, 2008), which implements a maximum entropy algorithm (Phillips et al., 2006). We first generated occurrence points files of *T. eques* and *T. tamaulipensis* from previously published data (De Jesús-Bonilla et al., 2017) and iNaturalist/GBIF research-grade observations (Gbif.Org, 2017) (Supplementary Material 3). Occurrence points were projected into an ecoregions map to create a mask for extracting data from 19 layers of WorldClim2 and altitude (Hijmans et al., 2005; Fick and Hijmans 2017) only for ecoregions containing *T. eques* or *T. tamaulipensis*. Extracted layers were analysed with the program SDMtoolbox via ArcMap (Brown, 2014) to retain layers without high collinearity (< 0.8). ENMs were

generated with 10 layers (Bio02, Bio03, Bio05, Bio06, Bio09, Bio12, Bio15, Bio19 and alt), with 30% of subsample for training and test model, 50,000 iterations and 15 replicates.

The ENMs of *T. eques* and *T. tamaulipensis* were compared with the program ENMTools version 1.3 (Warren et al., 2010) to test whether the habitat suitability scores generated by ENM models from these two species exhibit statistically significant ecological differences. This program measures overlap both with the Schoener's D and I indexes (Warren et al., 2008, 2010), and their values range from 0 to 1, where 0 indicates no overlap and 1 total overlap. We conducted an identity test to statically assess the overlap values. The Identity test contrasts the null hypothesis in which two species have identical niches by generating null distribution of expected values of D and I under the assumption of the interchangeability of ENMs of the compared species. Comparing the null distribution and the overlap values of I and D allows to determine whether the ENMs of two species are more different than expected by chance (Warren et al., 2008, 2010). The Identity test was carried out with the program ENMTools 1.3 running 500 pseudoreplicates.

2.8 Species delimitation

We carried out species delimitation analyses with the program BPP version 3.3 (Yang, 2015) and the m20 matrix. We used as species guide tree the topology derived from our bayesian analysis performed with the m20 matrix, but considering *T. picticornis* (Walker) and *T. stalli* Bruner and *T. eques* and *T. tamaulipensis* each as sister terminal taxa in order to test whether they represent separate evolutionary lineages. Due to the computational demand of the BPP analyses, we carried out separate analyses for three different data sets that were generated from a random subsample of 50 loci of the m20 matrix. All analyses were run for 100,000 generations with the reverse-jump Markov-chain Monte Carlo

(rjMCMC), using a gamma prior □ G (2, 1000) and τ G (2, 2000), a sampling interval of 10 and discarding trees obtained during the first 10,000 generations as burn-in. We also carried out the analyses excising the putative introgressed specimens of *T. eques* and *T. tamaulipensis*.

2.9 Species tree analysis

We reconstructed a species tree with the SVDquartets approach (Chifman and Kubatko, 2014, 2015) that is implemented in the program PAUP* version 4.0a159 (Swofford, 2002). The SVDquartets method reconstructs phylogenetic relationships under coalescence from the quartets of unlinked nucleotide sites (Chifman and Kubatko, 2014). We excised from the analysis three samples with evidence of admixture or extreme low shared loci. The updated matrix had 53 samples and contained all loci present in at least 15 samples (19,279 loci). All possible quartets derived from this updated matrix were evaluated, selecting trees using the QFM quartet assembly (Reaz et al., 2014), setting a taxon partition based on the BPP results (considering the split between *T. eques* and *T. tamaulipensis*) and carrying out 1,000 bootstrap replicates. The species tree was rooted as above.

3. Results

3.1 3RAD data

The 71 examined samples yielded 153,155,892 reads. The FASTQC revision did not show poor quality reads. An average of 870,029 reads per sample were obtained after demultiplex, with a minimum of 83,552 and a maximum of 2,441,262 reads per sample (Supplementary Material 4). The optimization of clustering threshold across samples converged to 0.94 of similarity. The percentage of loci with two copies were asymptotic

with a 0.94 similarity threshold and lower values. One copy loci on the other hand decreased at lower threshold values (Supplementary material 5). The outgroup taxa were excised from the analyses because they only shared few loci (10-28 loci) with the ingroup.

Table 1 summarizes the number of loci and SNPs obtained in each matrix under different min_sample_locus values. At low values, more loci and SNPs are retained, but when this value increases the number of loci and SNPs retained decreases. Also, the parameter min_sample_locus affects the dispersion of the data in the matrices, since the amount of missing data in the matrices increases with lower values, whereas the proportion of shared loci among the samples decreases (Table 1; Supplementary Material 6).

3.2 Phylogenetic relationships and genetic distances

Phylogenetic reconstructions with the m5 and m10 matrices could not be completed due to limitations on computational power (> 2,300 cpu hours), and we could not obtain a tree from the m63 matrix for the Bayesian analysis. The ML and Bayesian phylograms derived from the remaining matrices had similar topologies and generally all their clades were significantly supported (Supplementary Material 7). The exceptions were the bayesian analyses with the m15, m55 and m60 matrices and the ML analysis with m15 matrix, where *T. gutturosa* and *T. citricornis* were not reciprocally exclusive, and the ML topologies with the m60 and m63 matrices, which had distinct, poorly supported or unresolved relationships. Below we only refer to the topology derived from the Bayesian analysis performed with the m20 matrix.

The Bayesian phylogram derived from the matrix built with each locus shared by at least 20 individuals (12,703 loci) recovered three main clades (Figure 1). One of them has the species of *Taeniopoda* with low pronotal crest (*T. stali*, *T. picticornis*, *T. eques* and *T.*

tamaulipensis; PP = 1.0), with the specimens assigned to *T. stali* and *T. picticornis* intermingled in a subclade (PP = 1.0). Similarly, the specimens assigned to *T. eques* and *T. tamaulipensis* were not recovered as reciprocally exclusive (PP=1.0). The other two clades include the *Taeniopoda* species with elevated pronotal crest. One of them is formed by four exclusive, well-supported species (PP= 1.0): *T. reticulata, T. varipennis, T. citricornis* and *T. gutturosa*. The third clade (PP = 1.0) recovered *R. microptera* at the base, followed by *T. obscura* Bruner, a clade with the specimens of *T. auricornis* from Guatemala (thereafter referred as *T.* sp. *auricornis* "Guatemala") and *T. centurio* as sister to the remaining samples of *T. auricornis*, in that order.

The Nei genetic distances obtained with the m20 matrix ranged from 0.011 to 0.3155 across all samples (Table 2). The mean intraspecific distance has a maximum of 0.0617 in *T. citricornis,* and minimum of 0.0133 in *T. varipennis*. Interspecific distances (mean=0.2153) ranged from 0.3044 between *T. tamaulipensis* and *R. microptera* to 0.0444 between *T. varipennis* and *T. reticulata*. The mean genetic distance between species that were recovered within the three main clades was of 0.1071, ranging from 0.0779 in the clade with species with low pronotal to 0.142 in the clade that contains *Romalea*.

3.4 Genetic admixture between T. eques and T. tamaulipensis

In the PCA that includes all the samples contained in the m20 matrix, 32% of the total variation among samples was explained by the first and second principal components (PC1 and PC2, respectively). Samples were grouped into clusters that are concordant with the three main clades recovered in above the phylogenetic analyses. The PC1 separates the clade of species with low pronotal crest from the two with species having elevated pronotal

crest and *Romalea*, whereas PC2 divides the two clades with the species with elevated pronotal crest into two groups (Figure 2A).

In the PCA of the clade formed by the species with low pronotal crest, the PC1 and PC2 explain 37% of the total variation. *Taeniopoda picticornis* and *T. stali* are separated from *T. eques* and *T. tamaulipensis* by PC1 (Figure 2B). The subset of *T. eques* and *T. tamaulipensis* were divided into three clusters, two corresponding to samples of *T. eques*, and a third one to samples assigned to both species from the Sierra Madre Oriental (SMO), southern Central Mexican Plateau (CMP) and northern part of the Trans-Mexican Volcanic Belt (TMVB) morphotectonic provinces. In the subset formed by *T. citricornis*, *T. gutturosa*, *T. reticulata* and *T. varipennis*, PC1 and PC2 account for 35% of the total variation, and PC1 separates the first two species from the latter two (Figure 2C). In the third subset, 46% of the total variation is explained by PC1 and P2, whereas PC1 separates *Romalea* from *T. centurio*, *T. auricornis*, *T.* sp. *auricornis* "Guatemala" and *T. obscura*. *Taeniopoda centurio* and *T. auricornis* were also separated from *T. obscura* and *T.* sp. *auricornis* "Guatemala" by PC2 (Figure 2D).

The membership probability evaluated with DAPC grouped the specimens of *R. microptera*, *T. citricornis*, *T. gutturosa*, *T. obscura*, *T. reticulata* and *T. varipennis* each in independent clusters. In contrast, six specimens assigned to *T. eques* showed mixed probability of membership with *T. tamaulipensis*. The examined specimens of *T. picticornis* and *T. stali* and those assigned to *T. auricornis*, *T.* sp. *auricornis* "Guatemala" and *T. centurio* neither clustered exclusively (Supplementary Material 8).

The Structure analysis and the Evanno test K determined an optimal K=3 for the specimens assigned to *T. eques* and *T. tamaulipensis* (Figure 3A). These three clusters are congruent with the geographical distribution of their examined specimens, but not with

their species assignation in one of the clusters (Figure 3B). Two clusters have specimens morphologically assigned to *T. eques* from northern CMP and southern CMP and Sierra Madre del Sur (SMS) provinces, respectively, whereas the third one has specimens assigned to *T. tamaulipensis* from the SMO and southern CMP provinces. The Structure analysis shows gene flow among specimens of the cluster of *T. eques* from southern CMP and SMS with specimens of the remaining two clusters from occurring in various localities along central Mexico in the CMP, TMVB and SMO provinces.

The D and I indices were of 0.39 and 0.70, respectively, for the overlap between the ENMs of *T. eques* and *T. tamaulipensis* (Figures 4A, B). These values were significantly lower than their null distributions (D: 0.66-0.87, and I: 0.89-0.98; Figure 4C), therefore refuting the null hypothesis of identity that proposed that the ENMs of *T. eques* and *T. tamaulipensis* are identical in their use of niche space.

3.5 Species delimitation

The three BPP species delimitation analyses performed with matrices generated from a random subsample of 50 loci of the m20 matrix significantly support the specific status of *T. varipennis*, *T. reticulata*, *T. citricornis*, *T. gutturosa*, *T. centurio*, *T. obscura*, *T. auricornis*, *T.* sp. *auricornis* "Guatemala" and *R. microptera* (PP= 0.96-1.0) (Figure 5). In contrast, the posterior probability values for the split between *T. eques* and *T. tamaulipensis* varied from 0.69 to 1.0, whereas the allospecificity between *T. picticornis* and *T. stali* was consistently rejected (PP = 0.26-0.04). *Taeniopoda eques* and *T. tamaulipensis* were significantly supported as separate species (PP = 0.97-1.0) in the analyses excising the samples of *T. eques* with admixture.

3.6 Species tree

The species tree based on coalescence reconstructed with SVDquartets is generally congruent with the topology obtained both with the concatenated matrices and with the best estimate of phylogeny reconstructed in De Jesús-Bonilla et al.'s (2017) study. Three main clades were recovered, one with species of *Taeniopoda* with low pronotal crest and the remaining two with species of *Taeniopoda* with elevated pronotal crest, with one of them including *R. microptera* (Figure 5). The relationships between the taxa within three main clades are fully congruent with the ones obtained by the BI and ML analyses with the concatenated matrices. The 12 species delimited in the BPP analyses were fully bootstrap supported in the species tree, including *T. eques* and *T. tamaulipensis* as separate species.

4. Discussion

4.1 3RAD data assembly

The large amount of data generated with high-throughput sequencing techniques implies the challenge of establishing standardised methodologies for their analysis (da Fonseca et al., 2016; Pervaiz et al., 2017). In the case of RADseq data, there is still no consensus for its treatment, though two main strategies have been adopted. One uses fixed parameters for the assembly a single matrix (e.g. Dimond et al., 2017; Fischer et al., 2015; Hipp et al., 2018), whereas the other one employs a complete exploration of the space of parameters (e.g. Hou et al., 2016; Leaché et al., 2015a; Takahashi et al., 2014; Takahashi and Sota, 2016).

The similarity threshold and the minimum number of samples that must have data at a given locus for it to be retained in the final data set are two key parameters to assemble the RADseq data (Takahashi and Moreno, 2015). We avoided the use of arbitrary similarity threshold to clustering reads by implementing a strategy that identifies the optimal

threshold using a ploidy-based clustering series (Ilut et al., 2014). This strategy reduces the probability to assemble artifacts originated by the split of loci due a strict threshold, or by the merge of non-orthologous loci in the same locus due a lower threshold (Harvey et al., 2015). Samples with low number of reads were more sensitive to clustering threshold. At lower clustering values, the proportion of one copy loci is inversely proportional to the 3+ copy loci due the merge of different loci into the same locus (Supplementary Material 5). Thus, the estimation of appropriate clustering threshold avoids biases and optimizes the computational work by not having to perform an assembly with each improper threshold.

It has been observed that matrices with large amounts of missing data generally have well-resolved and supported phylogenies, even with up to 90% of missing data (Eaton et al., 2016; Tripp et al., 2017). However, other studies with empirical data indicate that missing data can imply biases in the resulting topology (Hou et al., 2016). In our analyses, the matrices with different amounts of missing data resulted in a topology that consistently recovered the same three well-supported clades and most of their internal relationships (Figure 1; Supplementary Material 7). However, the specimens of *T. gutturosa* and *T. citricornis* appeared mixed in the most extreme matrices (m15, m55, m60). These biases reflect that the RADseq data may show gene/species tree incongruence (Rubin et al., 2012). It is therefore advisable to explore the results through different amounts of missing data and to evaluate any inconsistency with other type of evidence such as geographic distribution or morphology.

To reduce bias by samples with low reads, samples having low number of reads are usually removed. We used <100,000 as the limit to discard samples from the analysis. However, we found that in the PCA three samples assigned to *T. auricornis* (Tau060; 137,714 reads), *T. centurio* (Tce089; 276,492 reads) and *T.* sp. *auricornis* "Guatemala"

(TauG339; 344,242 reads) appeared near the intersection of the principal components independently of their species assignation (Figure 2A). These samples also had a mixed membership with *T. tamaulipensis* and *T. eques* in the DAPC (Supplementary Material 8). The PCA makes a correction of missing data, replacing them by the average of all data. Thus, the results of these samples could be an artefact due to the amount of missing data caused by the low count of shared loci with the rest of the samples. This bias is particularly evident in the larger matrices. With extremely few reads, the probability to cluster reads in loci shared with other specimens is reduced. For instance, in the matrix m20 the average of shared loci among samples was of 0.211, whereas the average of shared loci in samples Tau060, Tce089 and TauG339 were 0.008, 0.06 and 0.07, respectively. We excised this samples with extreme low shared loci from the matrix to construct the species tree with SVDquartets. The difference on shared loci among samples must therefore be taken into account to develop sample removal strategies based on the shared loci rather than on the number of reads *per se*.

4.2 Gene flow between T. eques and T. tamaulipensis

In the previous molecular phylogenetic study of *Taeniopoda* mainly based on mt markers the lack of exclusivity between *T. tamaulipensis* and *T. eques* was suggested to occur due either by the presence of mt introgression or incomplete lineage sorting (De Jesús-Bonilla et al., 2017). Here we used 3RAD data, which provide a vast amount of nuclear loci suitable for phylogenetic reconstruction among closely related species even in presence of hybridization or mt introgression (Good et al., 2015; Hou et al., 2015).

Our phylogenomic analyses did not recovered the exclusivity of *T. eques* and *T. tamaulipensis*. However, the clustering and Structure analyses showed that the members of

the *T. eques* + *T. tamulipensis* clade form three geographically congruent genetic clusters, two exclusively composed of specimens morphologically assigned to *T. eques* and the remaining one of specimens assigned to *T. tamaulipensis*. Moreover, we found evidence of extensive gene flow along central Mexico between the genetic cluster of *T. eques* from southern CMP and SMS and the remaining two (Figure 3B).

Our niche model reconstruction on the other hand showed that *T. eques* is widely distributed from central Mexico to the southern United States (Figure 4A), whereas *T. tamaulipensis* is restricted to the SMO and the adjacent areas of the CMP morphotectonic provinces (Figure 4B). However, the ENM analysis detected a statistically significant overlap among populations of these two taxa (0.39, D index; 0.70, I index; Figure 4C), indicating the existence of areas in which they could co-occur. This co-occurrence comprises physiographic zones of the southern part of the SMO and northern TMVB provinces in the states of Querétaro and Hidalgo in central Mexico. Specimens assigned to both species have been collected in sympatry in this zone, and the ENMs reveals ecological conditions for their coexistence.

The above genetic and niche modelling evidence thus do not conclusively support the allospecificity between *T. eques* and *T. tamaulipensis*. Our results instead suggest that these two taxa could represent a "ring species" (*sensu* Irwin and Wake, 2016) with a high genetic structure and phenotypic variation in its colour of pronotum. This scenario is mainly suggested by the allele frequencies of the examined populations, which appear to change gradually following a "loop form" along their geographical distribution in the CMP, TMBV and TMVB provinces (Fig. 4B). Interestingly, the geographic distribution of the taxa involved fits with one of the 100 candidate cohesive barriers that were proposed by Monahan et al. (2012) based on a global topographical analysis. Further studies including a

complete sampling along the entire geographic distribution of these taxa are therefore necessary to know the processes that were implicated in their evolution.

4.3 Species limits and phylogeny of Taeniopoda

Our analyses based on 3RAD data recovered a fully supported estimate of phylogeny for the genus and consistently delimited species of *Taeniopoda* except for the case of *T. eques* and *T. tamaulipensis*, which were not significantly supported as allospecific in some of the BPP analyses. All our species delineation analyses significantly supported 10 species within the genus with the inclusion of *R. microptera*. We also maintain the specific status of *T. eques* and *T. tamaulipensis*, pending further population genetics and phylogenetic studies. The 12 delimited species and the taxonomic changes mentioned here will be formally proposed in a forthcoming taxonomic revision of the group. Only one currently recognized species could not be included in this study, *T. bicristata* Bruner. This species was described based on a single female from an uncertain locality (Bruner, 1907; Hebard, 1924), though, according to its external morphology, it probably represents a synonym of *T. obscura*.

Our best estimate of phylogeny and the low genetic distances observed among *R. microptera* and some species of *Taeniopoda* confirms the paraphyly of the latter genus with respect to *R. microptera*, which was previously proposed based on mitochondrial and nuclear markers (De Jesús-Bonilla et al., 2017). The topology derived from the 3RAD data, however, significantly supported *R. microptera* at the base of one of the two clades having species with a high pronotal crest, and not at the base of the clade with species with low pronotal crest, as in the above study.

The three main clades previously reported in the De Jesús-Bonilla et al.'s (2017) phylogenetic study of *Taeniopoda* were fully resolved by the 3RAD data. Two of these clades are geographically and morphologically congruent. The species with low pronotal crest, grouped in a single clade, are distributed from southern United States to central Mexico, whereas a second clade has species with high pronotal crest that are distributed from southern Mexico to Panama. The third clade has the remaining species with high pronotal crest and *R. microptera*, and includes the most morphologically distinctive species of the genus.

The above three major clades probably originated by recent stages of the TMVB formation occurred during late Miocene to Pleistocene, whereas the current species in the group could have diverged as a result of the Pleistocene climatic oscillations occurred (De Jesús-Bonilla et al., 2017). Four species of *Taeniopoda* and *R. microptera* are distributed in the Nearctic region, whereas the remaining eight species occur along the Transitional Nearctic-Neotropical zone and the adjacent part of Neotropics in Costa Rica and Panama (De Jesús-Bonilla et al., 2017; Hebard, 1924; Gbif.Org, 2017). This pattern is congruent with a complex scenario in this region, which was influenced by Neogene active tectonics and the Pleistocene paleoclimatic oscillations (Gutiérrez-García and Vázquez-Domínguez, 2013; Rull, 2011).

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Appendix A. Supplementary material associated to this work is available in the online version at XXXXXXXXXXX.

References

Aberer, A. J., Kobert, K., Stamatakis, A., 2014. ExaBayes: massively parallel bayesian tree inference for the whole-genome era. Mol. Biol. Evol. 31, 2553–2556. https://doi.org/10.1093/molbev/msu236

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., Hohenlohe, P. A., 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nat. Rev. Genet. 17, 81-92. https://doi.org/10.1038/nrg.2015.28

Andrews, S., 2010. FastQC: A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3, e3376. https://doi.org/10.1371/journal.pone.0003376

Baxter, S.W., Davey, J.W., Johnston, J.S., Shelton, A.M., Heckel, D.G., Jiggins, C.D., Blaxter, M.L., 2011. Linkage mapping and comparative genomics using next-generation rad sequencing of a non-model organism. PLoS One 6, e19315. https://doi.org/10.1371/journal.pone.0019315

Brown, J.L., 2014. SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. Methods Ecol. Evol. 5, 694-700. https://doi.org/10.1111/2041-210X.12200

Bruner, L., 1907. *Biologia Centrali-Americana. Insecta: Orthoptera (Vol II).* London: Taylor and Francis.

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J. H., 2011. Stacks : Building and Genotyping Loci De Novo From Short-Read Sequences. G3-Genes | Genomes | Genetics. 1, 171-182. https://doi.org/10.1534/g3.111.000240

Chattopadhyay, B., Garg. K. M., Ramakrishnan, U., 2014. Effect of diversity and missing data on genetic assignment with RAD-Seq markers. BMC Res. Notes. 7, 841. https://doi.org/10.1186/1756-0500-7-841

Chifman, J., Kubatko, L., 2015. Identifiability of the unrooted species tree topology under the Coalescent Model with time-reversible substitution processes, site-specific rate variation, and invariable sites. J. Theor. Biol. 374, 35-47. https://doi.org/10.1016/J.JTBI.2015.03.006

Chifman, J., Kubatko, L., 2014. Quartet Inference from SNP Data Under the Coalescent

Model. Bioinformatics. 30, 3317-3324. https://doi.org/10.1093/bioinformatics/btu530

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. Bioinformatics 27, 2156-2158. https://doi.org/10.1093/bioinformatics/btr330

Davey, J. L., Blaxter, M. W., 2010. RADseq: Next-generation population genetics. Brief. Funct. Genomics. 9, 416-423. https://doi.org/10.1093/bfgp/elq031

da Fonseca, R. R., Albrechtsen, A., Themudo, G. E., Ramos-Madrigal, J., Sibbesen, J. A., Maretty, L., Zepeda-Mendoza, M. L., Campos, P. F., Heller, R., Pereira, R. J., 2016. Next-generation biology: Sequencing and data analysis approaches for non-model organisms. Mar. Genomics. 30, 3-13. https://doi.org/10.1016/j.margen.2016.04.012

De Jesús-Bonilla, V. S., Barrientos-Lozano, L., Zaldívar-Riverón, A., 2017. Sequence-based species delineation and molecular phylogenetics of the transitional Nearctic–Neotropical grasshopper genus *Taeniopoda* (Orthoptera, Romaleidae). Syst. Biodivers. 15, 600-617. https://doi.org/10.1080/14772000.2017.1313792

De Queiroz, K., 2007. Species Concepts and Species Delimitation. Syst. Biol. 56, 879-886. https://doi.org/10.1080/10635150701701083

Dimond, J. L., Gamblewood, S. K., Roberts, S. B., 2017. Genetic and epigenetic insight into morphospecies in a reef coral. Mol. Ecol. 26, 5031-5042. https://doi.org/10.1111/mec.14252

Eades, D. C., Otte, D., Cigliano, M. M., Braun, H., 2016. Orthoptera Species File. Version 5.0/5.0. 2016. 536. http://orthoptera.speciesfile.org/HomePage/Orthoptera/HomePage.aspx

Eaton, D. A. R., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics. 30,1844-1849. https://doi.org/10.1093/bioinformatics/btu121

Eaton, D. A. R., Spriggs, E. L., Park, B, Donoghue, M. J., 2016. Misconceptions on Missing Data in RAD-seq Phylogenetics with a Deep-scale Example from Flowering Plants. Syst. Biol. 66, 399-412. https://doi.org/10.1093/sysbio/syw092

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14, 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fick, S. E., Hijmans, R. J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302-4315. https://doi.org/10.1002/joc.5086

Fischer, G., Azorsa, F., Hita Garcia, F., Mikheyev, A. S., Economo, E. P., 2015. Two new phragmotic ant species from Africa: Morphology and next-generation sequencing solve a caste association problem in the genus *Carebara* Westwood. Zookeys. 525, 77-105. https://doi.org/10.3897/zookeys.525.6057

Gbif.Org, 2017. iNaturalist Research-grade Observations. https://doi.org/10.15468/AB3S5X

Glenn, T.C., Bayona-Vasquez, N.J., Kieran, T.J., Pierson, T.W., Hoffberg, S.L., Scott, P.A., Bentley, K.E., Finger, J.W., Watson, P.R., Louha, S., Troendle, N., Diaz-Jaimes, P., Mauricio, R., Faircloth, B.C., 2017. Adapterama III: Quadruple-indexed, triple-enzyme RADseq libraries for about $1USD per Sample (3RAD). In press

Good, J. M., Vanderpool, D., Keeble, S., Bi, K., 2015. Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. Evolution. 69, 1961-1972. https://doi.org/10.1111/evo.12712

Gutiérrez-García, T. A., Vázquez-Domínguez, E., 2013. Consensus between genes and stones in the biogeographic and evolutionary history of Central America. Quat. Res. 79,

311-324. https://doi.org/10.1016/j.yqres.2012.12.007

Harvey, M.G., Judy, C.D., Seeholzer, G.F., Maley, J.M., Graves, G.R., Brumfield, R.T., 2015. Similarity thresholds used in DNA sequence assembly from short reads can reduce the comparability of population histories across species. PeerJ. 3, e895. https://doi.org/10.7717/peerj.895

Hebard, M., 1924. A Revision of the Genus *Taeniopoda* (Orthoptera, Acrididae, Cyrtacanthacrinae). Trans. Am. Entomol. Soc. 50, 253-274.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965-1978. https://doi.org/10.1002/joc.1276

Hipp, A.L., Eaton, D.A.R., Cavender-Bares, J., Fitzek, E., Nipper, R., Manos, P.S., 2014. A Framework Phylogeny of the American Oak Clade Based on Sequenced RAD Data. PLoS One 9, e93975. https://doi.org/10.1371/journal.pone.0093975

Hipp, A.L., Manos, P.S., González-Rodriguez, A., Hahn, M., Kaproth, M., McVay, J.D., Avalos, S.V., Cavender-Bares, J., 2018. Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. New Phytol. 217, 439-452. https://doi.org/10.1111/nph.14773

Hou, Y., Nowak, M.D., Mirré, V., Bjorå, C.S., Brochmann, C., Popp, M., 2016. RAD-seq data point to a northern origin of the arctic-alpine genus *Cassiope* (Ericaceae). Mol. Phylogenet. Evol. 95, 152-160. https://doi.org/10.1016/j.ympev.2015.11.009

Hou, Y., Nowak, M.D., Mirré, V., Bjorå, C.S., Brochmann, C., Popp, M., 2015. Thousands of RAD-seq loci fully resolve the phylogeny of the highly disjunct arctic-alpine genus *Diapensia* (Diapensiaceae). PLoS One. 10, 1-14. https://doi.org/10.1371/journal.pone.0140175

Ilut, D. C., Nydam, M. L., Hare, M. P., 2014. Defining loci in restriction-based reduced representation genomic data from nonmodel species: Sources of bias and diagnostics for optimal clustering. Biomed Res. Int. 2014, 675158. https://doi.org/10.1155/2014/675158

Irwin, D. E., Wake, D.B., 2016. Ring Species, in: Kliman, R. M. (ed.), Encyclopedia of Evolutionary Biology. vol. 3. Academic Press, Oxford, pp. 467-475.

Jakobsson, M., Rosenberg, N. A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 23, 1801-1806. https://doi.org/10.1093/bioinformatics/btm233

Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 24, 1403-1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Ahmed, I., 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics. 27, 3070-3071. https://doi.org/10.1093/bioinformatics/btr521

Jombart T, Devillard S, Balloux F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11, 94. https://doi.org/10.1186/1471-2156-11-94

King, A. B. S., Saunders, J. L., 1984. *The Invertebrate Pests of Annual Food Crops in Central America: A Guide to Their Recognition and Control*. London: Overseas Development Administration.

Leaché, A. D., Chavez, A. S., Jones, L. N., Grummer, J. A., Gottscho, A.D., Linkem, C. W., 2015a. Phylogenomics of phrynosomatid lizards: Conflicting signals from sequence capture versus restriction site associated DNA sequencing. Genome Biol. Evol. 7, 706-719. https://doi.org/10.1093/gbe/evv026

Leaché, A. D., Banbury, B. L., Felsenstein. J., Nieto-Montes De Oca, A., Stamatakis, A., 2015b. Short tree, long tree, right tree, wrong tree: New acquisition bias corrections for

inferring SNP phylogenies. Syst. Biol. 64, 1032-1047. https://doi.org/10.1093/sysbio/syv053

Mariño-Pérez, R., Fontana, P., Buzzetti, F. M., 2011. Identificación de plagas de chapulin en el norte–centro de México. in: García-Gutiérrez C, Lozano-Gutiérrez J, (Eds.), Control biológico de plagas de chapulín en el norte–centro de México. Universidad Autónoma de Zacatecas, México, pp. 35-55.

McKinney, G. J., Waples, R. K., Seeb, L. W., Seeb, J. E., 2017. Paralogs are revealed by proportion of heterozygotes and deviations in read ratios in genotyping-by-sequencing data from natural populations. Mol. Ecol. Resour. 17, 656-669. https://doi.org/10.1111/1755-0998.12613

Monahan, W.B., Pereira, R.J., Wake, D.B., 2012. Ring distributions leading to species formation: A global topographic analysis of geographic barriers associated with ring species. BMC Biol. 10, 20. https://doi.org/10.1186/1741-7007-10-20

Nei, M., 1972. Genetic Distance between Populations. Am. Nat. 106, 283-292. https://doi.org/10.2307/2459777

Nieto-Montes de Oca, A., Barley, A.J., Meza-Lázaro, R.N., García-Vázquez, U.O., Zamora-Abrego, J.G., Thomson, R.C., Leaché, A.D., 2017. Phylogenomics and species delimitation in the knob-scaled lizards of the genus *Xenosaurus* (Squamata: Xenosauridae) using ddRADseq data reveal a substantial underestimation of diversity. Mol. Phylogenet. Evol. 106, 241-253. https://doi.org/10.1016/j.ympev.2016.09.001

Paris, J. R., Stevens, J. R., Catchen, J. M., 2017. Lost in parameter space: a road map for stacks. Methods Ecol. Evol. 8, 1360-1373. https://doi.org/10.1111/2041-210X.12775

Paul, A., Frederich, M., Uyttenbroeck, R., Hatt, S., Malik, P., Lebecque, S., Hamaidia, M., Miazek, K., Goffin, D., Willems, L., Deleu, M., Fauconnier, M., Richel, A., Pauw, E.D.,

Blecker, C., Monty, A., Francis, F., Haubruge, É., Danthine, S., 2016. Grasshoppers as a food source? A review. Biotechnol. Agron. Société Environ. 20, 337-352.

Pembleton, L. W., Cogan, N. O. I., Forster, J. W., 2013. StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Mol. Ecol. Resour. 13, 946-952. https://doi.org/10.1111/1755-0998.12129

Pervaiz, T., Lotfi, A., Salman Haider, M., Haifang, J., Fang, J., Fang1, J., 2017. High Throughput Sequencing Advances and Future Challenges. J. Plant Biochem. Physiol. 05, 2. https://doi.org/10.4172/2329-9029.1000188

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., Hoekstra, H. E., 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS One. 7,e37135. https://doi.org/10.1371/journal.pone.0037135

Phillips, S. J., Anderson, R. P., Schapire, R. E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190, 231-259. https://doi.org/10.1016/J.ECOLMODEL.2005.03.026

Phillips, S. J., Dudik, M., 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography. 31, 161-175. https://doi.org/10.1111/j.0906-7590.2008.5203.x

Pritchard, J. K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics. 155, 945-959. http://www.ncbi.nlm.nih.gov/pubmed/10835412

Rambaut, A., 2009. FigTree v1.4.3: Tree figure drawing tool. http//tree.bio.ed.ac.uk/software/figtree.

Rambaut, A., Drummond, A. J., 2009. Tracer V1.6. http//beast.bio.ed.ac.uk/Tracer

Ramos-Elorduy, J., Landero-Torres, I., Murguia-González, J., Pino M, J. M., 2008. Biodiversidad antropoentomofágica de la región de Zongolica, Veracruz, México. Rev. Biol. Trop. 56, 303-316.

Reaz, R., Bayzid, M. S., Rahman, M.S., 2014. Accurate Phylogenetic Tree Reconstruction from Quartets: A Heuristic Approach. PLoS One. 9,e104008. https://doi.org/10.1371/journal.pone.0104008

Rowe, H. C., Renaut, S., Guggisberg, A., 2011. RAD in the realm of next-generation sequencing technologies. Mol. Ecol. 20, 3499-3502. https://doi.org/10.1111/j.1365-294X.2011.05197.x

Rubin, B. E. R. , Ree, R. H., Moreau, C. S., 2012. Inferring Phylogenies from RAD Sequence Data. PLoS One. 7, e33394. https://doi.org/10.1371/journal.pone.0033394

Rull, V. 2011. Neotropical biodiversity: Timing and potential drivers. Trends Ecol. Evol. 26, 508-513. https://doi.org/10.1016/j.tree.2011.05.011

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30, 1312-1313. https://doi.org/10.1093/bioinformatics/btu033

Swofford, D. L., 2002. PAUP: Phylogenetic Analysis Using Parsimony. version 4.0a159. Sinauer Associates, Sunderland, Massachusetts. Smithson. Inst. http://paup.csit.fsu.edu/

Takahashi, T., Moreno, E., 2015. A RAD-based phylogenetics for *Orestias* fishes from Lake Titicaca. Mol. Phylogenet. Evol. 93, 307-317. https://doi.org/10.1016/J.YMPEV.2015.08.012

Takahashi, T., Nagata, N., Sota, T., 2014. Application of RAD-based phylogenetics to complex relationships among variously related taxa in a species flock. Mol. Phylogenet. Evol. 80, 77-81. https://doi.org/10.1016/j.ympev.2014.07.016

Takahashi, T., Sota, T., 2016. A robust phylogeny among major lineages of the East African cichlids. Mol. Phylogenet. Evol. 100, 234-242. https://doi.org/10.1016/j.ympev.2016.04.012

Toonen, R.J., Puritz, J.B., Forsman, Z.H., Whitney, J.L., Fernandez-Silva, I., Andrews, K.R., Bird, C.E., 2013. ezRAD: a simplified method for genomic genotyping in non-model organisms. PeerJ. 1, e203. https://doi.org/10.7717/peerj.203

Tripp, E. A., Tsai, Y.-H. E., Zhuang, Y., Dexter, K.G., 2017. RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. Ecol. Evol. 7, 7920-7936. https://doi.org/10.1002/ece3.3274

Wagner, C.E., Keller, I., Wittwer, S., Selz, O.M., Mwaiko, S., Greuter, L., Sivasundar, A., Seehausen, O., 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Mol. Ecol. 22, 787-798. https://doi.org/10.1111/mec.12023

Warren, D. L., Glor, R. E., Turelli, M., 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography. 33, 607-611. https://doi.org/10.1111/j.1600-0587.2009.06142.x

Warren, D. L., Glor, R. E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution. 62, 2868-2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x

Wessinger, C. A., Freeman, C. C., Mort, M. E., Rausher, M. D., Hileman, L. C., 2016. Multiplexed shotgun genotyping resolves species relationships within the North American genus *Penstemon*. Am. J. Bot. 103, 912-922. https://doi.org/10.3732/ajb.1500519

Yang, Z., 2015. The BPP program for species tree estimation and species delimitation. Curr. Zool. 61, 854-865. https://doi.org/10.1093/czoolo/61.5.854

Table 1. Data obtained in the matrices with 94% of clustering threshold and different min_sample_locus values.

Matrix	Loci	Average proportion shared loci	Total no. of SNPs	informative SNPs	Ratio info/total SNPs	Missing data (%)
m5	113,887	0.04	840,083	420,419	0.50	78.85
m10	49,784	0.08	492,492	274,907	0.56	71.40
m15	23,886	0.15	279,478	163,794	0.59	63.61
m20	12,703	0.21	160,722	97,218	0.60	56.02
m25	6,885	0.29	91,584	56,616	0.62	48.40
m30	3,759	0.37	51,693	32,712	0.63	40.66
m35	2,225	0.46	30,732	19,561	0.64	33.92
m40	1,315	0.54	18,164	11,604	0.64	27.88
m45	709	0.64	9,995	6,440	0.64	22.09
m50	339	0.73	4,724	3,047	0.65	16.70
m55	130	0.84	1,761	1,122	0.64	11.19
m60	26	0.94	292	198	0.68	6.44
m63	6	1.00	24	15	0.63	14.29

Table 2. Intra and inter-specific distances (Nei) among species of *Romalea* and *Taeniopoda* (mean, minimum and maximum) obtained from the m20 matrix. Distances were calculated between specimens (pop=FALSE) and between groups (pop=TRUE).

		1	2	3	4	5	6	7	8	9	10	11	12
1	*Romalea microptera*	0.0306 (0.0355-0.0242)											
2	*Taeniopoda auricornis*	0.2158 (0.221-0.2098)	0.043 (0.0625-0.0271)										
3	*Taeniopoda* sp. *auricornis* Guatemala	0.2209 (0.2282-0.211)	0.0773 (0.0805-0.0733)	0.0184 (0.0257-0.0121)									
4	*Taeniopoda centurio*	0.2208 (0.2285-0.2121)	0.0711 (0.079-0.0637)	0.0886 (0.0961-0.0828)	0.0339 (0.0454-0.0178)								
5	*Taeniopoda citricornis*	0.2494 (0.2548-0.2417)	0.2279 (0.2517-0.2201)	0.2315 (0.2409-0.213)	0.2539 (0.2437-0.2263)	0.0617 (0.0713-0.045)							
6	*Taeniopoda equus*	0.3064 (0.3144-0.2807)	0.2776 (0.298-0.2506)	0.2837 (0.3015-0.247)	0.2854 (0.3025-0.2668)	0.244 (0.2569-0.2242)	0.0528 (0.0694-0.0293)						
7	*Taeniopoda tamaulipensis*	0.3044 (0.3155-0.2945)	0.2785 (0.2878-0.2632)	0.2863 (0.3033-0.2514)	0.2864 (0.3-0.2636)	0.2487 (0.2561-0.2402)	0.0526 (0.0805-0.0265)	0.0292 (0.0365-0.0197)					
8	*Taeniopoda guttierosa*	0.2538 (0.26-0.2472)	0.232 (0.2432-0.2269)	0.2376 (0.2452-0.2231)	0.238 (0.2475-0.2306)	0.0716 (0.0778-0.0652)	0.2517 (0.262-0.2364)	0.2561 (0.2617-0.2486)	0.0434 (0.0578-0.0189)				
9	*Taeniopoda obscura*	0.2553 (0.2212-0.2093)	0.1016 (0.1105-0.0792)	0.0984 (0.1015-0.0898)	0.1107 (0.1149-0.1066)	0.2266 (0.2333-0.2136)	0.2782 (0.2922-0.2589)	0.2824 (0.2911-0.2559)	0.2343 (0.2392-0.2274)	0.0249 (0.026-0.0235)			
10	*Taeniopoda picticornis* + *T. stali*	0.28 (0.3037-0.2743)	0.2719 (0.2844-0.2573)	0.2755 (0.2939-0.2478)	0.2792 (0.2959-0.2625)	0.2301 (0.2439-0.2137)	0.1231 (0.1343-0.1061)	0.1284 (0.145-0.1051)	0.2370 (0.2532-0.2252)	0.2719 (0.2851-0.2506)	0.0596 (0.0768-0.044)		
11	*Taeniopoda reticulata*	0.2573 (0.2655-0.245)	0.2384 (0.2674-0.2278)	0.2414 (0.2562-0.2245)	0.2426 (0.2501-0.2329)	0.0922 (0.0981-0.0862)	0.2585 (0.266-0.2496)	0.2637 (0.271-0.2582)	0.0841 (0.0877-0.0812)	0.238 (0.2448-0.2301)	0.2443 (0.2552-0.2292)	0.0162 (0.0187-0.0118)	
12	*Taeniopoda variipennis*	0.253 (0.2661-0.2407)	0.238 (0.2829-0.2251)	0.2428 (0.2581-0.2302)	0.242 (0.2602-0.2333)	0.0917 (0.0986-0.0852)	0.2494 (0.2626-0.2204)	0.2553 (0.2655-0.2408)	0.0838 (0.0879-0.0805)	0.2352 (0.2484-0.2284)	0.2358 (0.2511-0.2098)	0.0444 (0.0459-0.0428)	0.0133 (0.015-0.011)

FIGURE LEGENDS

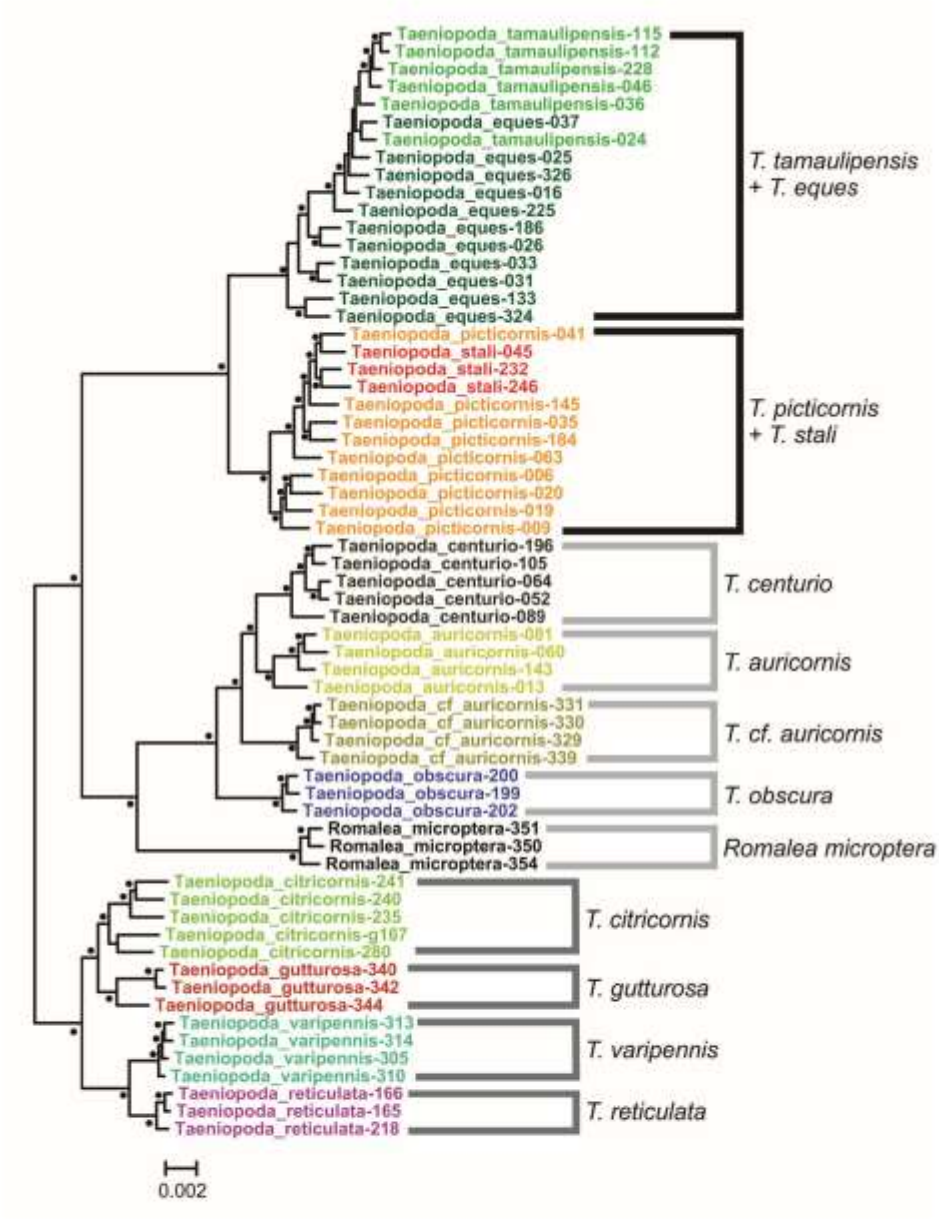
Figure 1. Phylogram of *Taeniopoda* and *Romalea* derived from the Bayesian analysis with the program Exabayes version 1.4.1 using the concatenated matrix m20 (see details in the methods section). The tree topology was created with consensus post-processing tool. Black circles near branches indicate posterior probabilities ≥ 0.95.

Figure 2. PCA plot of the m20 matrix with all included samples (A). (B-D) PCA of subsets of the three recover main clades in *Taeniopoda* and *Romalea*; (B) subset including samples assigned to *T. eques*, *T. tamaulipensis*, *T. picticornis* and *T. stali* (PCA of *T. eques* and *T. tamaulipensis* is included in the box); (C) subset including samples assigned to *T. varipennis*, *T. reticulata*, *T. gutturosa* and *T. citricornis*; (D) subset of samples assigned to *T. auricornis*, *T.* sp. *auricornis* "Guatemala", *T. centurio* and *R. microptera*.

Figure 3. A) Bayesian clustering analysis of specimen assigned to *T. eques* and *T. tamaulipensis* carried out with the program Structure (K= 3). Sample ID of examined specimens and morphotectonic provinces are showed (CMP= Central México Plateau; SMS= Sierra Madre del Sur; TMVB= Trans Mexican Volcanic Belt; SMO= Sierra Madre Oriental). Coloured bars indicate specific assignation (dark green= *T. eques*, light green= *T. tamaulipensis*). B) Distribution map of genetic clustering. Outer and inner circles indicate specific assignation and membership probability, respectively.

Figure 4. A) ENM of *T. eques*; B) ENM of *T. tamaulipensis*; C) histograms of null distribution for D and I indexes for identity test. Arrows indicate the observed overlap values for D and I indexes.

Figure 5. Species tree reconstructed with the SVDquartets coalescence method, including the posterior probabilities derived from the BPP (with putative introgressed excised) species delimitation analyses. Left (red) and right (black) numbers represent posterior probabilities derived from the BPP species delimitation analyses and bootstrap support values obtained by the SVDquartets approach, respectively.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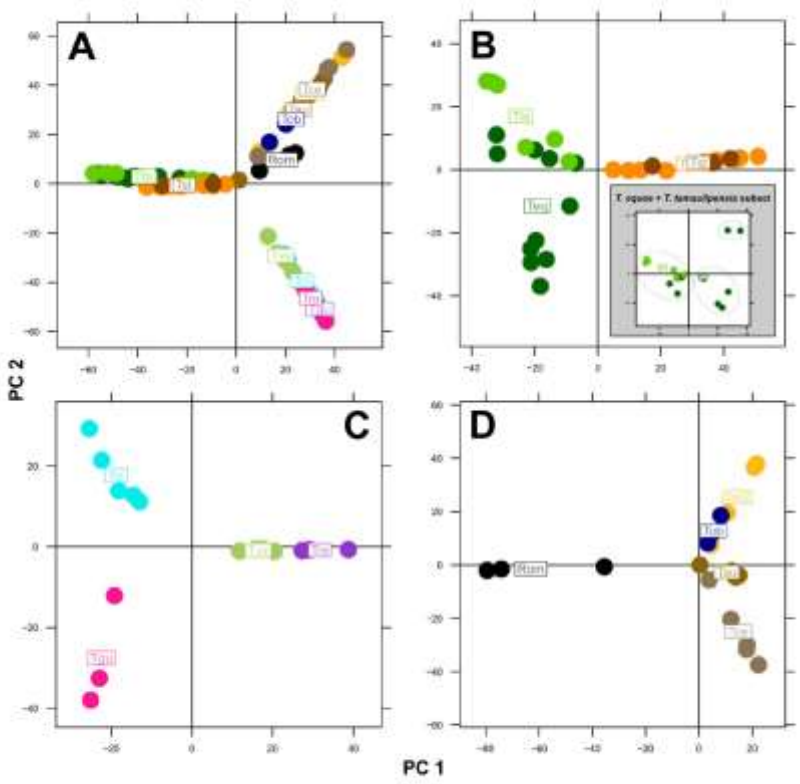
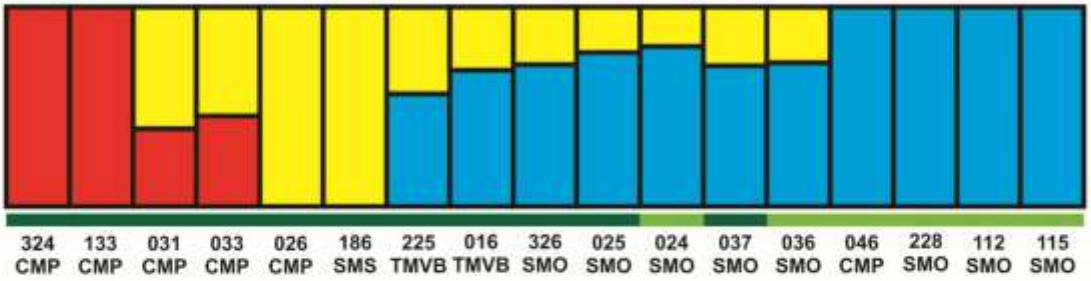
Taeniopoda_tamaulipensis-112
Taeniopoda_tamaulipensis-228
Taeniopoda_tamaulipensis-046
Taeniopoda_tamaulipensis-036
Taeniopoda_eques-037
Taeniopoda_tamaulipensis-024
Taeniopoda_eques-025
Taeniopoda_eques-326
Taeniopoda_eques-016
Taeniopoda_eques-225
Taeniopoda_eques-186
Taeniopoda_eques-026
Taeniopoda_eques-033
Taeniopoda_eques-031
Taeniopoda_eques-133
Taeniopoda_eques-324
T. tamaulipensis + T. eques
Taeniopoda_picticornis-041
Taeniopoda_stali-045
Taeniopoda_stali-232
Taeniopoda_stali-246
Taeniopoda_picticornis-145
Taeniopoda_picticornis-035
Taeniopoda_picticornis-184
Taeniopoda_picticornis-063
Taeniopoda_picticornis-006
Taeniopoda_picticornis-020
Taeniopoda_picticornis-019
Taeniopoda_picticornis-009
T. picticornis + T. stali
Taeniopoda_centurio-196
Taeniopoda_centurio-105
Taeniopoda_centurio-064
Taeniopoda_centurio-052
Taeniopoda_centurio-089
T. centurio
Taeniopoda_auricornis-081
Taeniopoda_auricornis-060
Taeniopoda_auricornis-143
Taeniopoda_auricornis-013
T. auricornis
Taeniopoda_cf_auricornis-331
Taeniopoda_cf_auricornis-330
Taeniopoda_cf_auricornis-329
Taeniopoda_cf_auricornis-339
T. cf. auricornis
Taeniopoda_obscura-200
Taeniopoda_obscura-199
Taeniopoda_obscura-202
T. obscura
Romalea_microptera-351
Romalea_microptera-350
Romalea_microptera-354
Romalea microptera
Taeniopoda_citricornis-241
Taeniopoda_citricornis-240
Taeniopoda_citricornis-235
Taeniopoda_citricornis-g107
Taeniopoda_citricornis-280
T. citricornis
Taeniopoda_gutturosa-340
Taeniopoda_gutturosa-342
Taeniopoda_gutturosa-344
T. gutturosa
Taeniopoda_varipennis-313
Taeniopoda_varipennis-314
Taeniopoda_varipennis-305
Taeniopoda_varipennis-310
T. varipennis
Taeniopoda_reticulata-166
Taeniopoda_reticulata-165
Taeniopoda_reticulata-218
T. reticulata
0.002


A
B
C
D
PC 2
PC 1

A)


324 CMP
133 CMP
031 CMP
033 CMP
026 CMP
186 SMS
225 TMVB
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326 SMO
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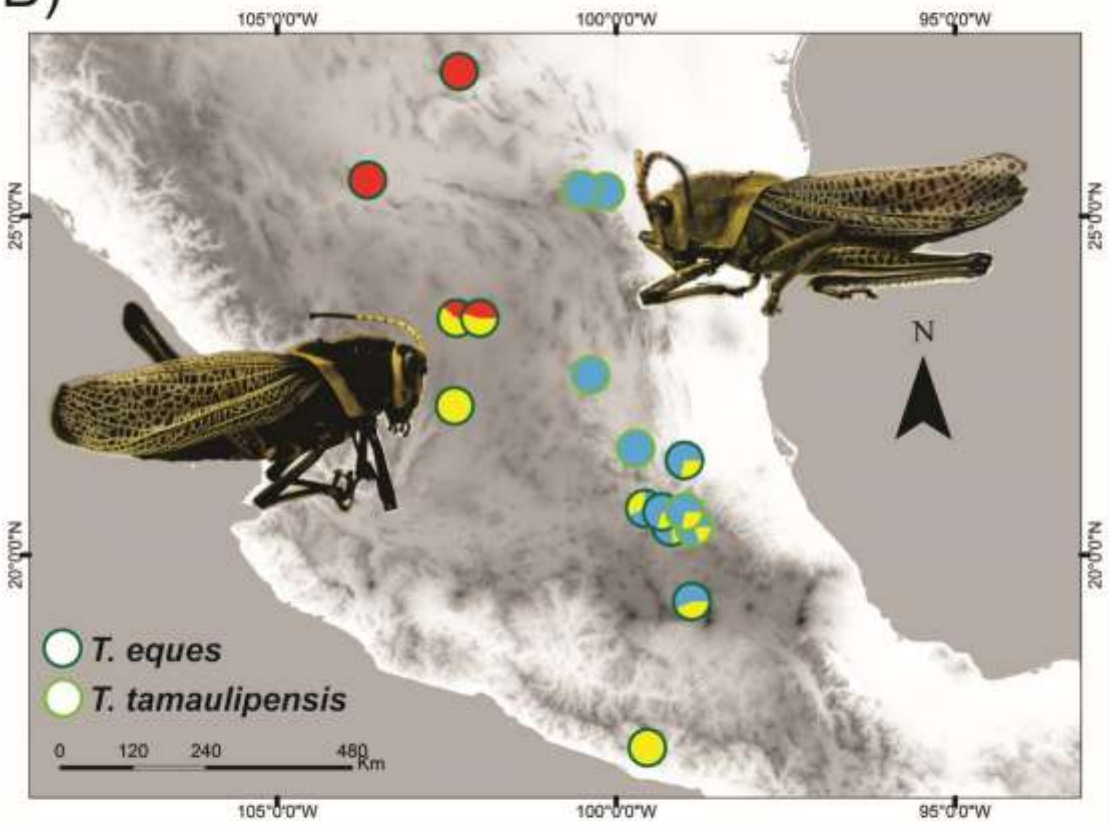
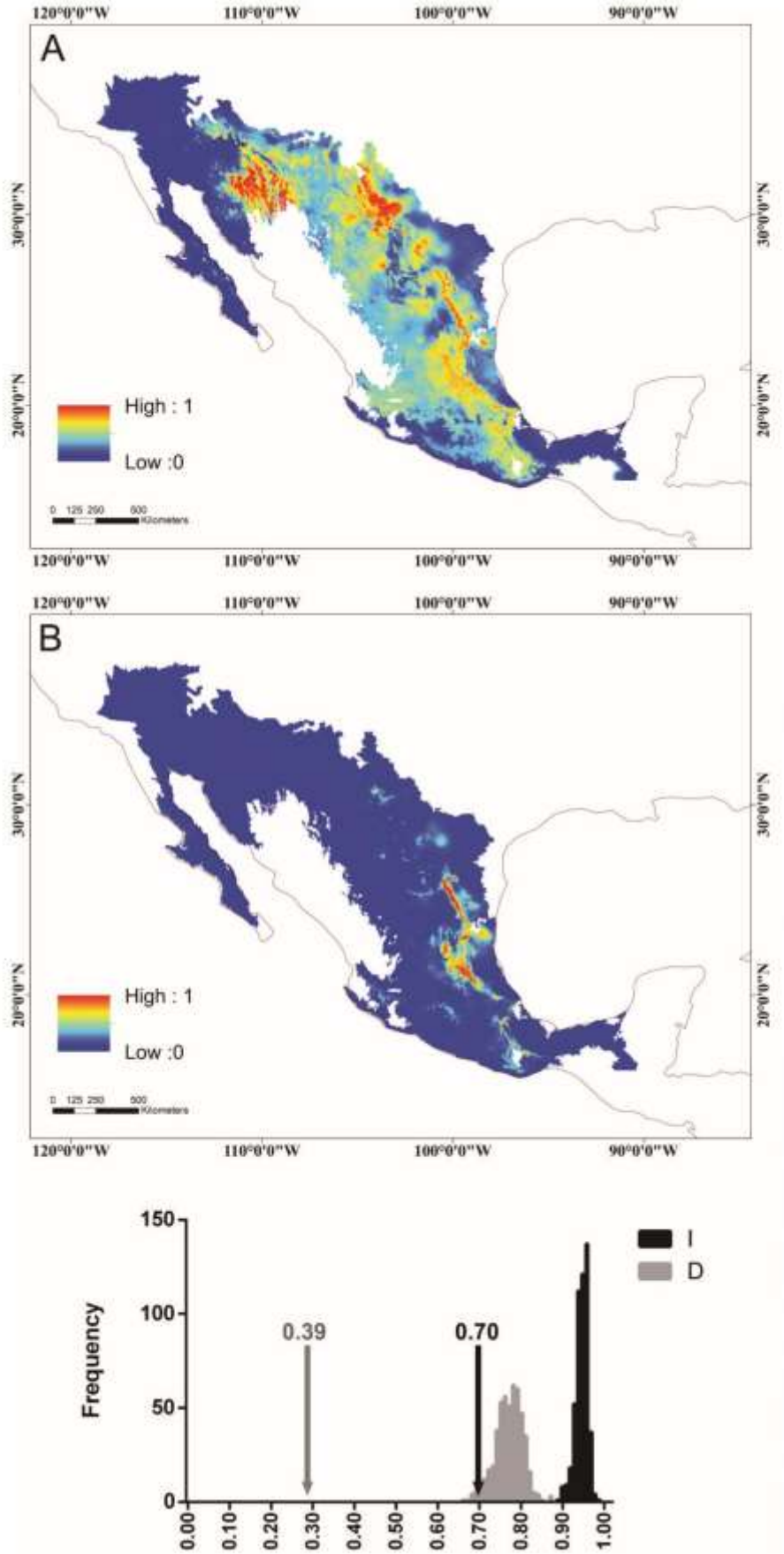
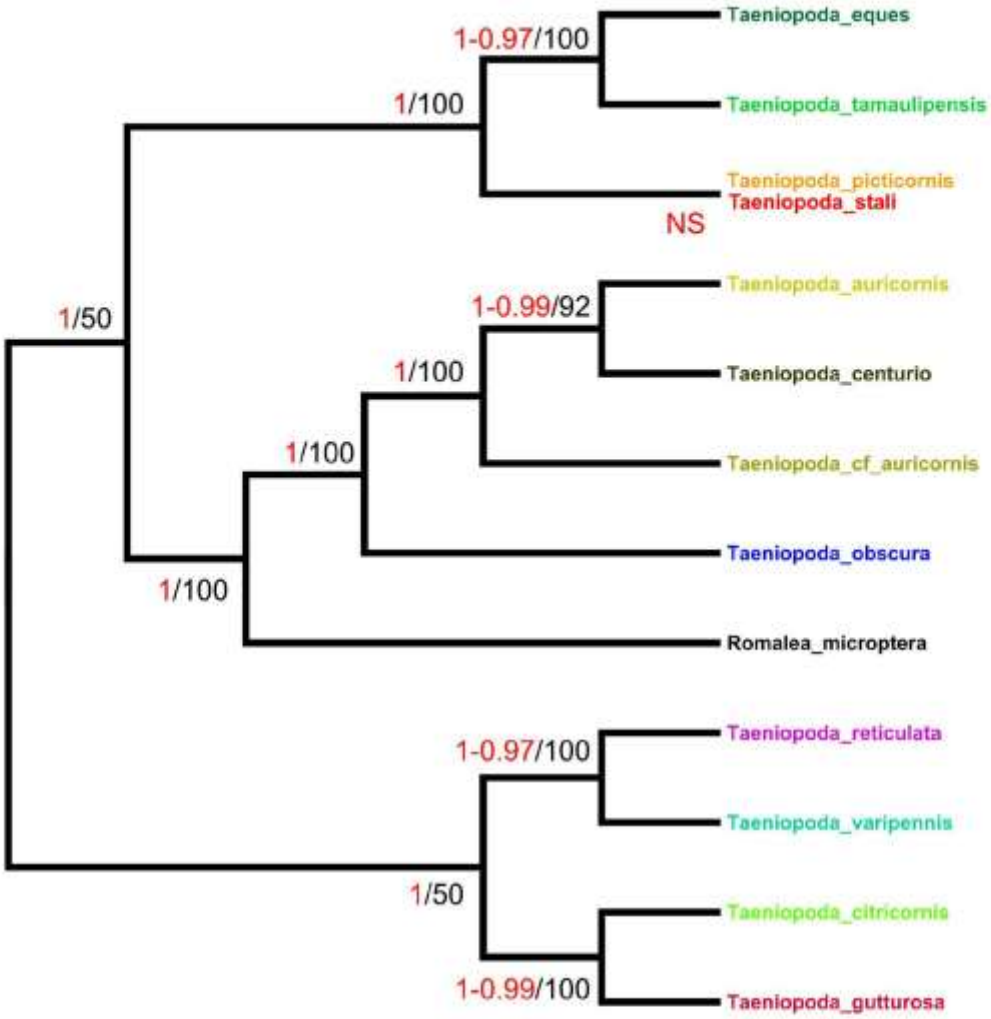
036 SMO
046 CMP
228 SMO
112 SMO
115 SMO

B)


105°0'0"W
100°0'0"W
95°0'0"W
25°0'0"N
20°0'0"N
N
T. eques
T. tamaulipensis
0 120 240 480
Km


A
B
120°0'0"W
110°0'0"W
100°0'0"W
90°0'0"W
30°0'0"N
20°0'0"N
High : 1
Low :0
0 125 250 500
Kilometers
150
100
50
0
Frequency
0.39
0.70
I
D
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00


Taeniopoda_eques
1-0.97/100
Taeniopoda_tamaulipensis
1/100
Taeniopoda_picticornis
Taeniopoda_stali
NS
Taeniopoda_auricornis
1-0.99/92
1/50
Taeniopoda_centurio
1/100
Taeniopoda_cf_auricornis
1/100
Taeniopoda_obscura
1/100
Romalea_microptera
Taeniopoda_reticulata
1-0.97/100
Taeniopoda_varipennis
1/50
Taeniopoda_citricornis
1-0.99/100
Taeniopoda_gutturosa

SUPPLEMENTARY MATERIAL

Supplementary Material 1. List of specimens included in this study, their localities and sample ID numbers.

Supplementary Material 2. Project design of RAD sequencing. List of I5 and I7 adapters ligated to each sample, and itru7 and itru5 primers used to build pools.

Supplementary Material 3. Occurrence data of *T. tamaulipensis* and *T. eques* used for Ecological Niche Modelling specific assignation. Latitude and longitude are in decimals.

Supplementary Material 4. Reads obtained for each sample. Samples in red were excised from the study (see Results and Discussion sections).

Supplementary Material 5. Results of optimal clustering threshold series for each concatenated matrix. The proportions of 1, 2 and 3+ copy (putative alleles) clusters under 85% to 99% similarity threshold are reported for each matrix.

Supplementary Material 6. SNPs matrices plot constructed with the glPlot function of package adegenet version 2.0.1. Shared loci among samples plots were obtained with the RADami 1.1-2 package with the locus.dist function.

Supplementary Material 7. ML and Bayesian Phylograms obtained for all concatenated matrices reconstructed with the programs RAxML (simultaneous 1000 rapid bootstrap analysis and search for the best ML tree) and Exabayes (1,000,000 or until convergence, ASDSF $\leq$ 1%).

Supplementary Material 8. Barplot of DAPC based on PCA of m20 matrix with membership probabilities of all samples.

Supplementary Material 1. Specimens of *Taeniopoda*, *Romalea* and outgroups species included in this study

Sample ID	Species	Locality
Tpi006	*Taeniopoda picticornis*	México: Colima: Coquimatlán: Arroyo El Tanque del General
Tpi009	*Taeniopoda picticornis*	México: Colima: Coquimatlán: Arroyo El Tanque del General
Tau013	*Taeniopoda auricornis*	México: Veracruz: Xico: Cascada de Texolo
Teq016	*Taeniopoda eques*	México: Querétaro: Cadereyta: Bellavista del Río
Tpi019	*Taeniopoda picticornis*	México: Jalisco: La Huerta, Estación de Biología de Chamela
Tpi020	*Taeniopoda picticornis*	México: Jalisco: La Huerta, Estación de Biología de Chamela
Tta024	*Taeniopoda tamaulipensis*	México: Hidalgo: El tablón
Teq025	*Taeniopoda eques*	México: Hidalgo: El tablón
Teq026	*Taeniopoda eques*	México: Aguascalientes: San José de García-Paredes
Teq031	*Taeniopoda eques*	México: Zacatecas: Sierra Vieja
Teq033	*Taeniopoda eques*	México: Zacatecas: Sierra Vieja
Tpi035	*Taeniopoda picticornis*	México: Guerrero: Carretera Teleolapan-Iguala
Tta036	*Taeniopoda tamaulipensis*	México: Hidalgo: Xhita
Teq037	*Taeniopoda eques*	México: Hidalgo: Xhita
Tpi041	*Taeniopoda picticornis*	México: Guerrero: Olinalá: Xixila
Tst044	*Taeniopoda stali*	México: Guerrero: Teleolapan
Tst045	*Taeniopoda stali*	México: Estado de México México: Ixtapaluca
Tta046	*Taeniopoda tamaulipensis*	México: San Luis Potosí: Guadalcázar: Abrego, Campamento Monternach
Tce052	*Taeniopoda centurio*	México: Hidalgo: Molango
Tau060	*Taeniopoda auricornis*	México: Veracruz: Barranca de San Miguel
Tpi063	*Taeniopoda picticornis*	México: Guerrero: Coyuca de Catalán: Arroyo parado
Tce064	*Taeniopoda centurio*	México: Hidalgo: Molango
Tau081	*Taeniopoda auricornis*	México: Veracruz: Ixhuatlan del café
TcQ089	*Taeniopoda centurio*	México: Puebla: Cuetzalan
Tce105	*Taeniopoda centurio*	México: San Luis Potosí: Xilitla: Cueva de potrerillos
Tta112	*Taeniopoda tamaulipensis*	México: Coahuila: 30 km antes de Santiago Aserradero
Tta115	*Taeniopoda tamaulipensis*	México: Nuevo León: Santiago: Cola de Caballo
Cco117	*Chromacris colorata*	México: Nuevo León: Santiago: Cola de Caballo
Cco118	*Chromacris colorata*	México: Nuevo León: Santiago: Cola de Caballo
Bma123	*Brachystola magna*	México: Nuevo León: Galeana: Buenavista
Bma125	*Brachystola magna*	México: Nuevo León: Galeana: Buenavista
Teq133	*Taeniopoda eques*	México: Coahuila: Cuatro Ciénegas: Rancho Casita
Tau143	*Taeniopoda auricornis*	México: Veracruz: Ixhuatlan del café
Tpi145	*Taeniopoda picticornis*	México: Puebla: Totoltepec de Guerrero
Tre165	*Taeniopoda reticulata*	Costa Rica: Limón: Cahuita
Tre166	*Taeniopoda reticulata*	Costa Rica: Limón: Cahuita
Tci167	*Taeniopoda citricornis*	México: Oaxaca: Asunción Ixtaltepec: Santiago Ixtaltepec
Tpi184	*Taeniopoda picticornis*	México: Guerrero: Teleoloapan
Teq186	*Taeniopoda eques*	México: Guerrero: Tierra Colorada

Tce196	*Taeniopoda centurio*	México: Querétaro: Jalpan de Serra: Valle verde, el Pilón
Tob199	*Taeniopoda obscura*	México: Yucatán: Kaxil kiuic
Tob200	*Taeniopoda obscura*	México: Yucatán: Kaxil kiuic
Tob202	*Taeniopoda obscura*	México: Yucatán: Kaxil kiuic
Tre218	*Taeniopoda reticulata*	Costa Rica: Limón: Limón
Teq225	*Taeniopoda eques*	Mexico: Estado de México: Ixtapaluca
Tta228	*Taeniopoda tamaulipensis*	México: Querétaro: Sierra Gorda: Arroyo Seco
Tst232	*Taeniopoda stali*	México: Morelos: Tepalcingo: El Limón
Tci235	*Taeniopoda citricornis*	México: Chiapas: Tuxtla Gutiérrez: Copoya
Tci240	*Taeniopoda citricornis*	México: Chiapas: Tuxtla Gutiérrez: Copoya
Tci241	*Taeniopoda citricornis*	México: Chiapas: Tuxtla Gutiérrez: Copoya
Tst246	*Taeniopoda stali*	México: Veracruz: Acultzingo: Tecamalucan
Cva257	*Cibotopteryx variegata*	Guatemala: Guatemala: Villa Canales
Cva259	*Cibotopteryx variegata*	Guatemala: Sacatepequez: Alotenango
Tci280	*Taeniopoda citricornis*	México: Oaxaca: Asunción Ixtaltepec: Santiago Ixtaltepec
TRc303	*Tropidacris cristata*	México: Querétaro: Pinal de Amoles
Tva305	*Taeniopoda varipennis*	Costa Rica: Puntarenas: Curré
Tva310	*Taeniopoda varipennis*	Costa Rica: Puntarenas: Curré
Tva313	*Taeniopoda varipennis*	Costa Rica: Puntarenas: Curré
Tva314	*Taeniopoda varipennis*	Costa Rica: Puntarenas: Curré
Teq324	*Taeniopoda eques*	México: Durango: Carretera Torreón
Teq326	*Taeniopoda eques*	México: San Luis Potosí: Xilitla
TaG329	*Taeniopoda cf. auricornis*	Guatemala: Baja Verapaz: Purulhá: Orejuela
TaG330	*Taeniopoda cf. auricornis*	Guatemala: Baja Verapaz: Purulhá: Orejuela
TaG331	*Taeniopoda cf. auricornis*	Guatemala: Baja Verapaz: Purulhá: Orejuela
TaG339	*Taeniopoda cf. auricornis*	Guatemala: El Petén: Poptún: Finca las Jarrillas
Tgu340	*Taeniopoda gutturosa*	Guatemala: Retalhuleu: Finca Los Brillantes
Tgu342	*Taeniopoda gutturosa*	Guatemala: Retalhuleu: Finca Los Brillantes
Tgu344	*Taeniopoda gutturosa*	Guatemala: Retalhuleu: Finca Los Brillantes
Rom350	*Romalea microptera*	USA: Florida: Collier Co: Big Cypress Bend
Rom351	*Romalea microptera*	USA: Florida: Collier Co: Port of the Islands Marina
Rom354	*Romalea microptera*	USA: Florida: Putnam Co: Katharine Ordway Preserve

Supplementary Material 2. Project design of RAD sequencing. List of I5 and I7 adapters ligated to each sample, and itru7 and itru5 primers used to build pools.

microplate position	Sample ID	Adapter I5	sequence	Adapter I7	sequence	itru7 primer	itru5 primer	Pool
A1	Tau060	A	CCGAATG	1	CTAACGT	12-1	5A	1
B1	Tta046	B	TTAGGCAG	2	TCGGTACT	12-1	5A	1
C1	Tst044	C	AACTCGTCG	3	GATCGTTGT	12-1	5A	1
D1	Teq033	D	GGTCTACGTG	4	AGCTACACTT	12-1	5A	1
E1	Teq031	E	GATACCG	5	ACGCATT	12-1	5A	1
F1	Tpi020	F	AGCGTTGG	6	GTATGCAT	12-1	5A	1
G1	Tpi009	G	CTGCAACTG	7	CACATGTCT	12-1	5A	1
H1	Tpi006	H	TCATGGTCAG	8	TGTGCACGAT	12-1	5A	1
A2	Teq225	A	CCGAATG	2	TCGGTACT	12-2	5B	2
B2	Tta228	B	TTAGGCAG	3	GATCGTTGT	12-2	5B	2
C2	Tce196	C	AACTCGTCG	4	AGCTACACTT	12-2	5B	2
D2	Tob200	D	GGTCTACGTG	5	ACGCATT	12-2	5B	2
E2	Tst232	E	GATACCG	6	GTATGCAT	12-2	5B	2
F2	Tst246	F	AGCGTTGG	7	CACATGTCT	12-2	5B	2
G2	Tpi145	G	CTGCAACTG	8	TGTGCACGAT	12-2	5B	2
H2	TauG339	H	TCATGGTCAG	9	GCATCAT	12-2	5B	2
A3	Tgu340	A	CCGAATG	3	GATCGTTGT	12-3	5C	3
B3	Tgu342	B	TTAGGCAG	4	AGCTACACTT	12-3	5C	3
C3	Tgu344	C	AACTCGTCG	5	ACGCATT	12-3	5C	3
D3	Rom350	D	GGTCTACGTG	6	GTATGCAT	12-3	5C	3
E3	Tre166	E	GATACCG	7	CACATGTCT	12-3	5C	3
F3	Tre165	F	AGCGTTGG	8	TGTGCACGAT	12-3	5C	3
G3	Teq016	G	CTGCAACTG	9	GCATCAT	12-3	5C	3
H3	TauG331	H	TCATGGTCAG	10	ATGCTGTT	12-3	5C	3
A4	TauG330	A	CCGAATG	4	AGCTACACTT	12-4	5D	4
B4	TauG329	B	TTAGGCAG	5	ACGCATT	12-4	5D	4
C4	Teq324	C	AACTCGTCG	6	GTATGCAT	12-4	5D	4
D4	Tva313	D	GGTCTACGTG	7	CACATGTCT	12-4	5D	4
E4	Tva310	E	GATACCG	8	TGTGCACGAT	12-4	5D	4
F4	Tro303	F	AGCGTTGG	9	GCATCAT	12-4	5D	4
G4	Tva305	G	CTGCAACTG	10	ATGCTGTT	12-4	5D	4
H4	Tci280	H	TCATGGTCAG	11	CATGACCTT	12-4	5D	4
A5	Tci235	A	CCGAATG	5	ACGCATT	12-5	5E	5
B5	Tob199	B	TTAGGCAG	6	GTATGCAT	12-5	5E	5
C5	Tpi184	C	AACTCGTCG	7	CACATGTCT	12-5	5E	5
D5	Tau143	D	GGTCTACGTG	8	TGTGCACGAT	12-5	5E	5
E5	Teq133	E	GATACCG	9	GCATCAT	12-5	5E	5
F5	Tta115	F	AGCGTTGG	10	ATGCTGTT	12-5	5E	5
G5	Tce105	G	CTGCAACTG	11	CATGACCTT	12-5	5E	5
H5	Tce064	H	TCATGGTCAG	12	TGCAGTGAGT	12-5	5E	5
A6	Rom351	A	CCGAATG	6	GTATGCAT	12-6	5F	6
B6	Rom354	B	TTAGGCAG	7	CACATGTCT	12-6	5F	6
C6	Tci240	C	AACTCGTCG	8	TGTGCACGAT	12-6	5F	6
D6	Tva314	D	GGTCTACGTG	9	GCATCAT	12-6	5F	6
E6	Tre218	E	GATACCG	10	ATGCTGTT	12-6	5F	6

F6	Tta112	F	AGCGTTGG	11	CATGACCTT	12-6	5F	6
G6	Tcig167	G	CTGCAACTG	12	TGCAGTGAGT	12-6	5F	6
H6	Teq326	H	TCATGGTCAG	1	CTAACGT	12-6	5F	6
A7	Teq186	A	CCGAATG	7	CACATGTCT	12-7	5G	7
B7	Tce052	B	TTAGGCAG	8	TGTGCACGAT	12-7	5G	7
C7	Tau013	C	AACTCGTCG	9	GCATCAT	12-7	5G	7
D7	Tci241	D	GGTCTACGTG	10	ATGCTGTT	12-7	5G	7
E7	Cib257	E	GATACCG	11	CATGACCTT	12-7	5G	7
F7	Cib259	F	AGCGTTGG	12	TGCAGTGAGT	12-7	5G	7
G7	Tob202	G	CTGCAACTG	1	CTAACGT	12-7	5G	7
H7	Tau081	H	TCATGGTCAG	2	TCGGTACT	12-7	5G	7
A8	Tpi041	A	CCGAATG	8	TGTGCACGAT	12-8	5H	8
B8	Tpi019	B	TTAGGCAG	9	GCATCAT	12-8	5H	8
C8	Tpi035	C	AACTCGTCG	10	ATGCTGTT	12-8	5H	8
D8	Bra125	D	GGTCTACGTG	11	CATGACCTT	12-8	5H	8
E8	Chr118	E	GATACCG	12	TGCAGTGAGT	12-8	5H	8
F8	Teq037	F	AGCGTTGG	1	CTAACGT	12-8	5H	8
G8	Tpi063	G	CTGCAACTG	2	TCGGTACT	12-8	5H	8
H8	Teq026	H	TCATGGTCAG	3	GATCGTTGT	12-8	5H	8
A9	Tce089	A	CCGAATG	9	GCATCAT	12-10	6A	9
B9	Bra123	B	TTAGGCAG	10	ATGCTGTT	12-10	6A	9
C9	Chr117	C	AACTCGTCG	11	CATGACCTT	12-10	6A	9
D9	Teq025	D	GGTCTACGTG	12	TGCAGTGAGT	12-10	6A	9
E9	Tst045	E	GATACCG	1	CTAACGT	12-10	6A	9
F9	Tta024	F	AGCGTTGG	2	TCGGTACT	12-10	6A	9
G9	Tta036	H	TCATGGTCAG	3	GATCGTTGT	12-10	6A	9

Supplementary Material 3. Occurrence data of *T. tamaulipensis* and *T. eques* used for Ecological Niche Modelling specific assignation. Latitude and longitude are in decimals.

species	latitude	longitude
T. tamaulipensis	26.047715	-100.371437
T. tamaulipensis	25.914642	-100.479185
T. tamaulipensis	25.745777	-100.424351
T. tamaulipensis	25.648623	-100.252001
T. tamaulipensis	25.637657	-100.200291
T. tamaulipensis	25.630907	-100.207863
T. tamaulipensis	25.630886	-100.210848
T. tamaulipensis	25.629435	-100.209829
T. tamaulipensis	25.620195	-100.354826
T. tamaulipensis	25.617677	-100.359451
T. tamaulipensis	25.617263	-100.352763
T. tamaulipensis	25.594263	-100.457612
T. tamaulipensis	25.571266	-100.28717
T. tamaulipensis	25.559747	-100.261192
T. tamaulipensis	25.559058	-100.252792
T. tamaulipensis	25.557929	-100.254021
T. tamaulipensis	25.549778	-100.270485
T. tamaulipensis	25.546414	-100.270334
T. tamaulipensis	25.532383	-100.2767
T. tamaulipensis	25.40369	-100.107474
T. tamaulipensis	25.3891	-100.253
T. tamaulipensis	25.368	-100.1606
T. tamaulipensis	25.365685	-100.16407
T. tamaulipensis	25.364	-100.1594
T. tamaulipensis	24.8839	-100.0944
T. tamaulipensis	24.7455	-99.8152
T. tamaulipensis	24.7019	-99.9079
T. tamaulipensis	24.655941	-99.684877
T. tamaulipensis	23.592228	-99.234352
T. tamaulipensis	22.97	-99.6117
T. tamaulipensis	22.9478	-99.6045
T. tamaulipensis	22.8376	-99.3413
T. tamaulipensis	22.299261	-98.952484
T. tamaulipensis	22.276389	-98.931885
T. tamaulipensis	22.186133	-98.943558
T. tamaulipensis	21.91512	-100.21089
T. tamaulipensis	21.5708	-99.712

T. tamaulipensis	21.451151	-100.209732
T. tamaulipensis	21.285895	-100.060387
T. tamaulipensis	20.739258	-98.830776
T. tamaulipensis	20.723043	-98.785114
T. tamaulipensis	20.632354	-98.754391
T. tamaulipensis	20.63235	-98.75439
T. tamaulipensis	20.42798	-98.67868
T. tamaulipensis	20.341302	-98.713199
T. tamaulipensis	20.250182	-98.561048
T. eques	33.0758	-108.32157
T. eques	32.83371	-110.0472
T. eques	32.76303	-108.38905
T. eques	32.35156	-110.24261
T. eques	32.34708	-110.67884
T. eques	32.34052	-110.67455
T. eques	32.3404	-110.68565
T. eques	32.34023	-110.68457
T. eques	32.34004	-110.78386
T. eques	32.3122	-106.7778
T. eques	32.30412	-109.78738
T. eques	32.1949	-110.9162
T. eques	32.07108	-110.0667
T. eques	31.9679	-110.2945
T. eques	31.95056	-109.13801
T. eques	31.92561	-106.04196
T. eques	31.92534	-106.04231
T. eques	31.92039	-106.04116
T. eques	31.91627	-106.05169
T. eques	31.9137	-109.14145
T. eques	31.91	-106
T. eques	31.90797	-109.0316
T. eques	31.87044	-109.03506
T. eques	31.87018	-109.03527
T. eques	31.81793	-106.23973
T. eques	31.75999	-110.84338
T. eques	31.72509	-110.88009
T. eques	31.5949	-110.3185
T. eques	31.556	-110.46053
T. eques	31.44785	-110.30697
T. eques	31.429	-111.1906
T. eques	31.4255	-111.1942
T. eques	31.39237	-111.13089
T. eques	30.90219	-108.43657

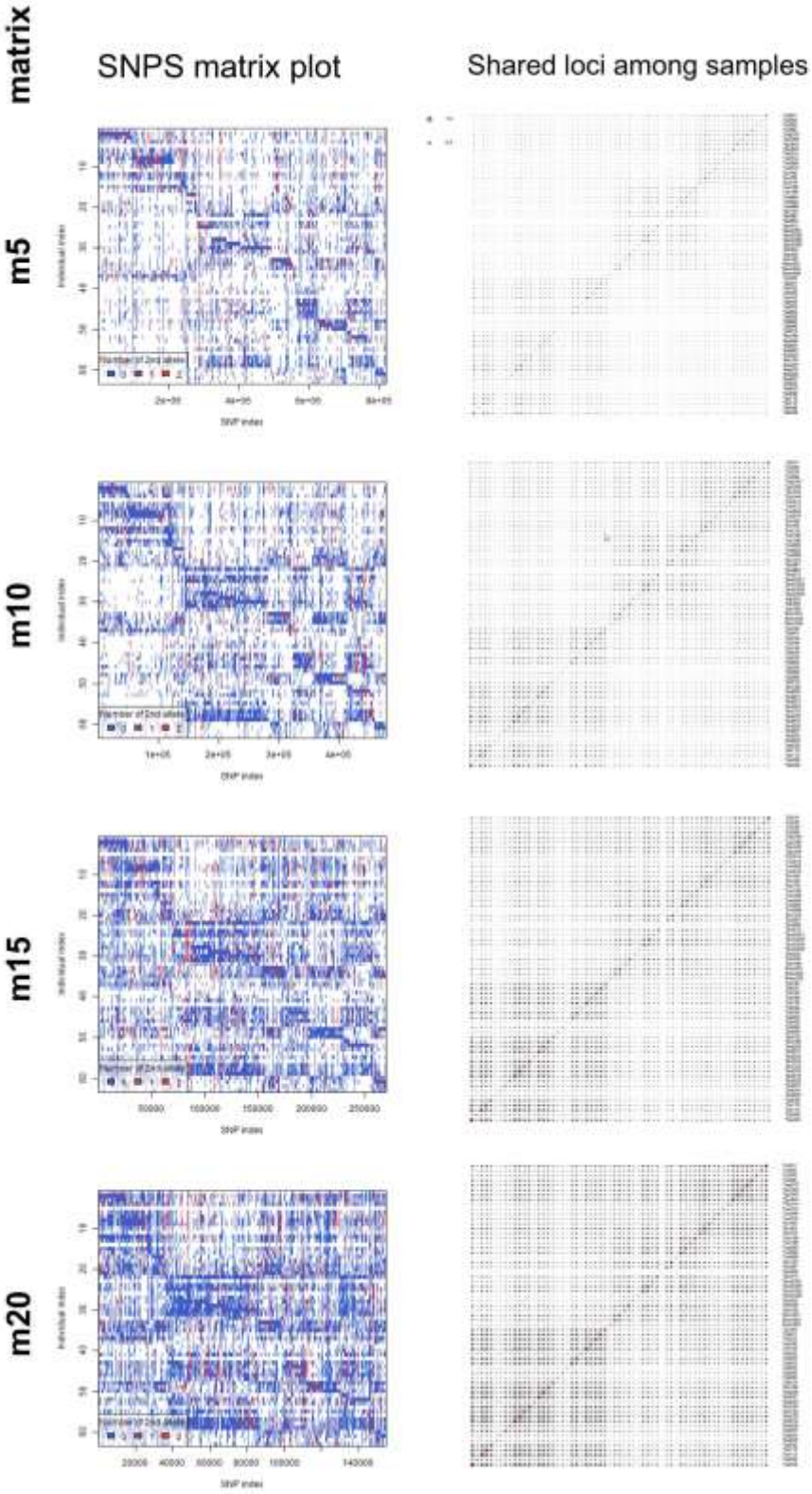
T. eques	30.60064	-103.89931
T. eques	30.59024	-103.92795
T. eques	30.54103	-103.8365
T. eques	30.44464	-104.72069
T. eques	30.28372	-103.58806
T. eques	30.22	-103
T. eques	30.17518	-102.79552
T. eques	30.05764	-103.46485
T. eques	30.01	-104
T. eques	29.90489	-104.46381
T. eques	29.87	-104
T. eques	29.83869	-104.36686
T. eques	29.82	-104
T. eques	29.81167	-104.3055
T. eques	29.8058	-104.20292
T. eques	29.78571	-104.20819
T. eques	29.42838	-102.91184
T. eques	29.37632	-103.15288
T. eques	29.33	-103
T. eques	29.31986	-103.25801
T. eques	29.29594	-103.27778
T. eques	29.27013	-103.30034
T. eques	28.61583	-106.13583
T. eques	28.43	-99.7
T. eques	27.1344	-102.316
T. eques	26.0582	-100.37762
T. eques	25.64544	-100.26234
T. eques	25.62	-100
T. eques	25.54504	-100.27064
T. eques	25.53995	-100.2738
T. eques	25.5302	-103.6666
T. eques	25.52901	-100.20373
T. eques	25.3275	-101.026
T. eques	25.22236	-100.15364
T. eques	22.2502	-98.28391
T. eques	22.08386	-102.46028
T. eques	21.3957	-98.9921
T. eques	21.17011	-103.01881
T. eques	20.83435	-102.74504
T. eques	20.83387	-102.74579
T. eques	20.7452	-103.88363
T. eques	20.74208	-99.94016
T. eques	20.72883	-99.40067

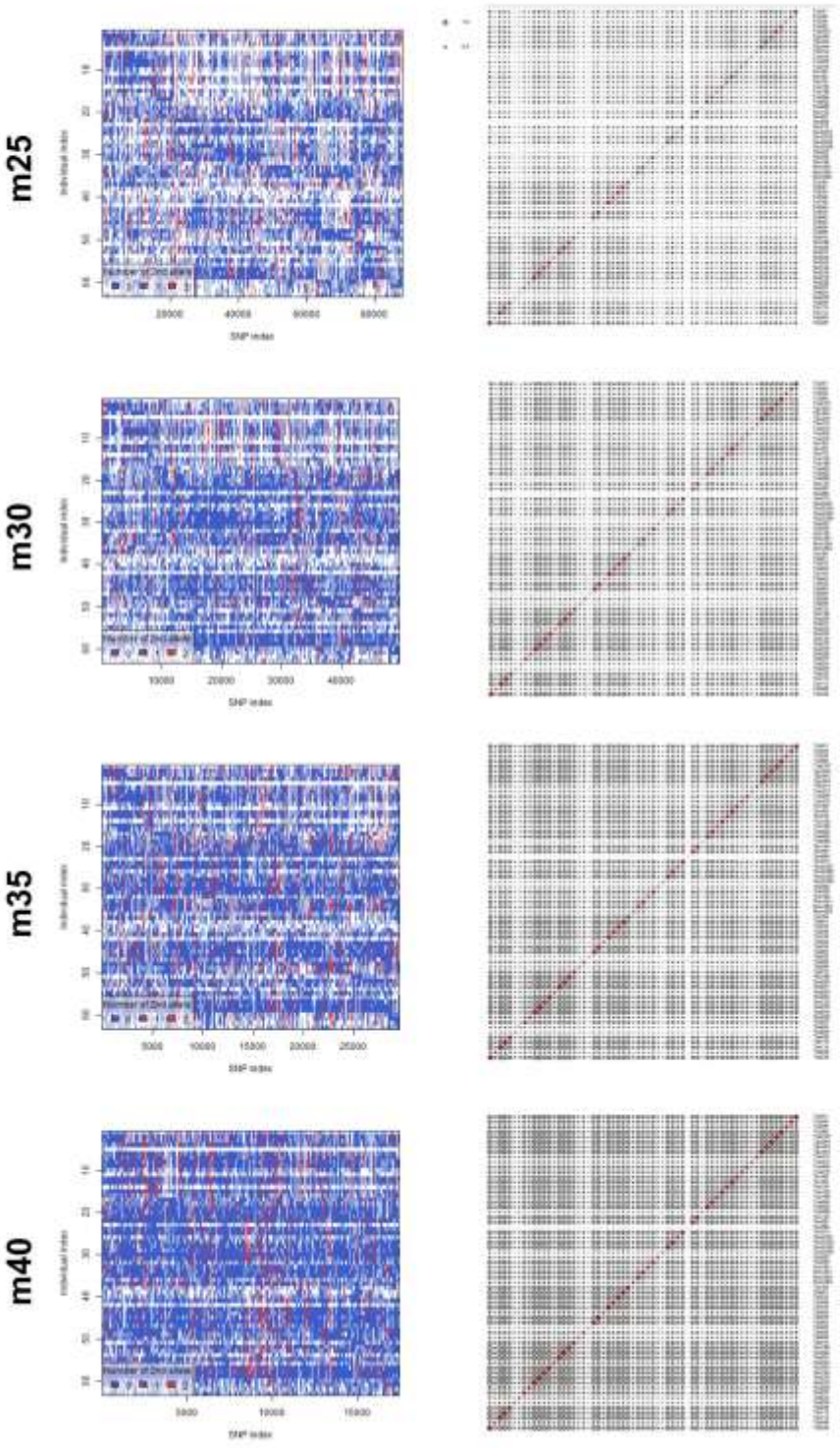
T. eques	20.7	-100
T. eques	20.68575	-99.80391
T. eques	20.68342	-99.53028
T. eques	20.6822	-99.80268
T. eques	20.60148	-100.25015
T. eques	20.59801	-100.04527
T. eques	20.50289	-99.68335
T. eques	20.25385	-98.55036
T. eques	20.0786	-99.3527
T. eques	19.86372	-102.76882
T. eques	19.69658	-101.1405
T. eques	19.43584	-96.94986
T. eques	19.42368	-96.96567
T. eques	19.3145	-98.8845
T. eques	18.9916	-99.08964
T. eques	18.74783	-99.67917
T. eques	17.1569	-99.5408

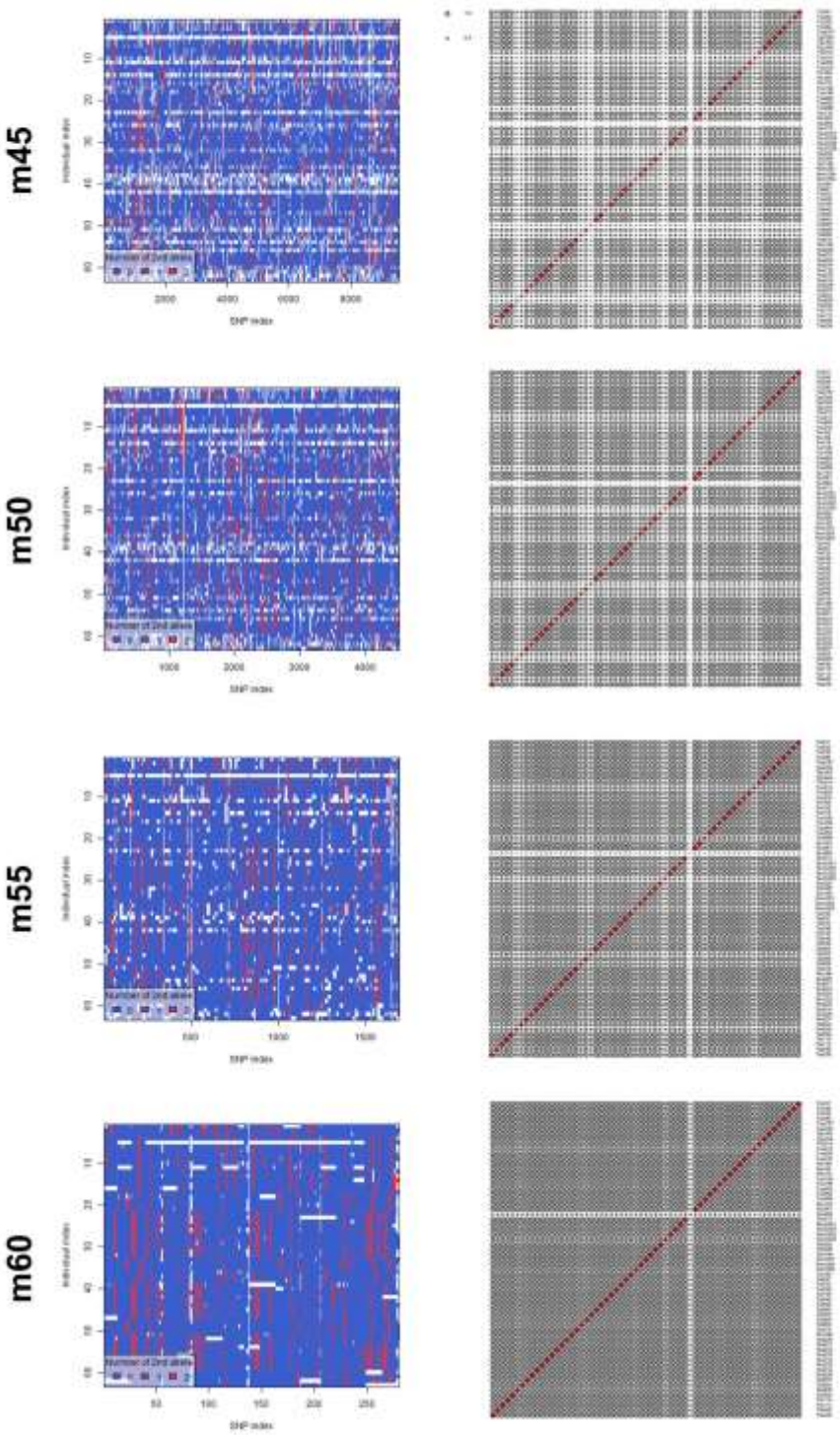
Supplementary Material 4. Reads obtained by each sample. Red samples were excised from the study (see Results and Discussion sections).

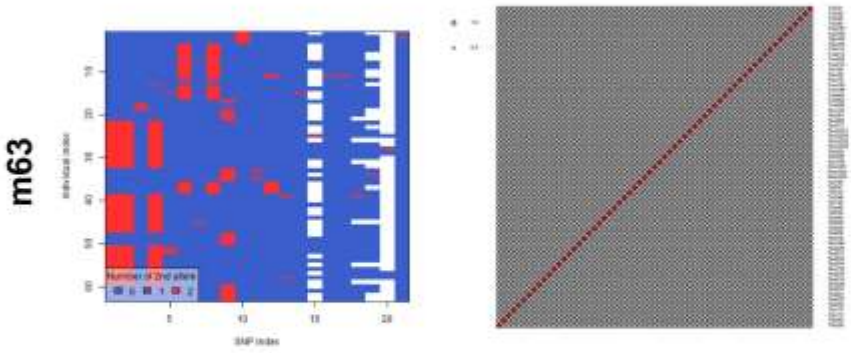
sample	reads	sample	reads	sample	reads	sample	reads
Bra123	565048	Tce105	1048711	Tgu344	1378640	Tst246	727962
Bra125	898356	Tce196	601155	Tob199	571166	Tta024	283096
Chr117	668362	Tci235	553787	Tob200	1292482	Tta036	757379
Chr118	1241862	Tci240	608793	Tob202	467677	Tta046	389236
Cib257	779360	Tci241	1210503	Tpi006	378140	Tta112	1128372
Cib259	676068	Tci280	1056184	Tpi009	461170	Tta115	1046670
Rom350	454856	Tcig167	1335589	Tpi019	585056	Tta228	2046727
Rom351	2441262	Teq016	1365151	Tpi020	228106	Tva305	668873
Rom354	1275174	Teq025	264258	Tpi035	1072646	Tva310	602599
Tau013	835329	Teq026	1031635	Tpi041	953741	Tva313	468852
Tau060	137714	Teq031	1371005	Tpi063	811399	Tva314	496277
Tau081	724952	Teq033	392502	Tpi145	1913156		
Tau143	1154231	Teq037	515176	Tpi184	716629		
TauG329	1967016	Teq133	877450	Tre165	1305956		
TauG330	1367574	Teq186	807735	Tre166	710394		
TauG331	481942	Teq225	1560631	Tre218	769847		
TauG339	344242	Teq324	1352804	Tro303	643878		
Tce052	1732775	Teq326	467080	Tst044	83552		
Tce064	1082085	Tgu340	901564	Tst045	369823		
Tce089	276492	Tgu342	1160365	Tst232	855795		

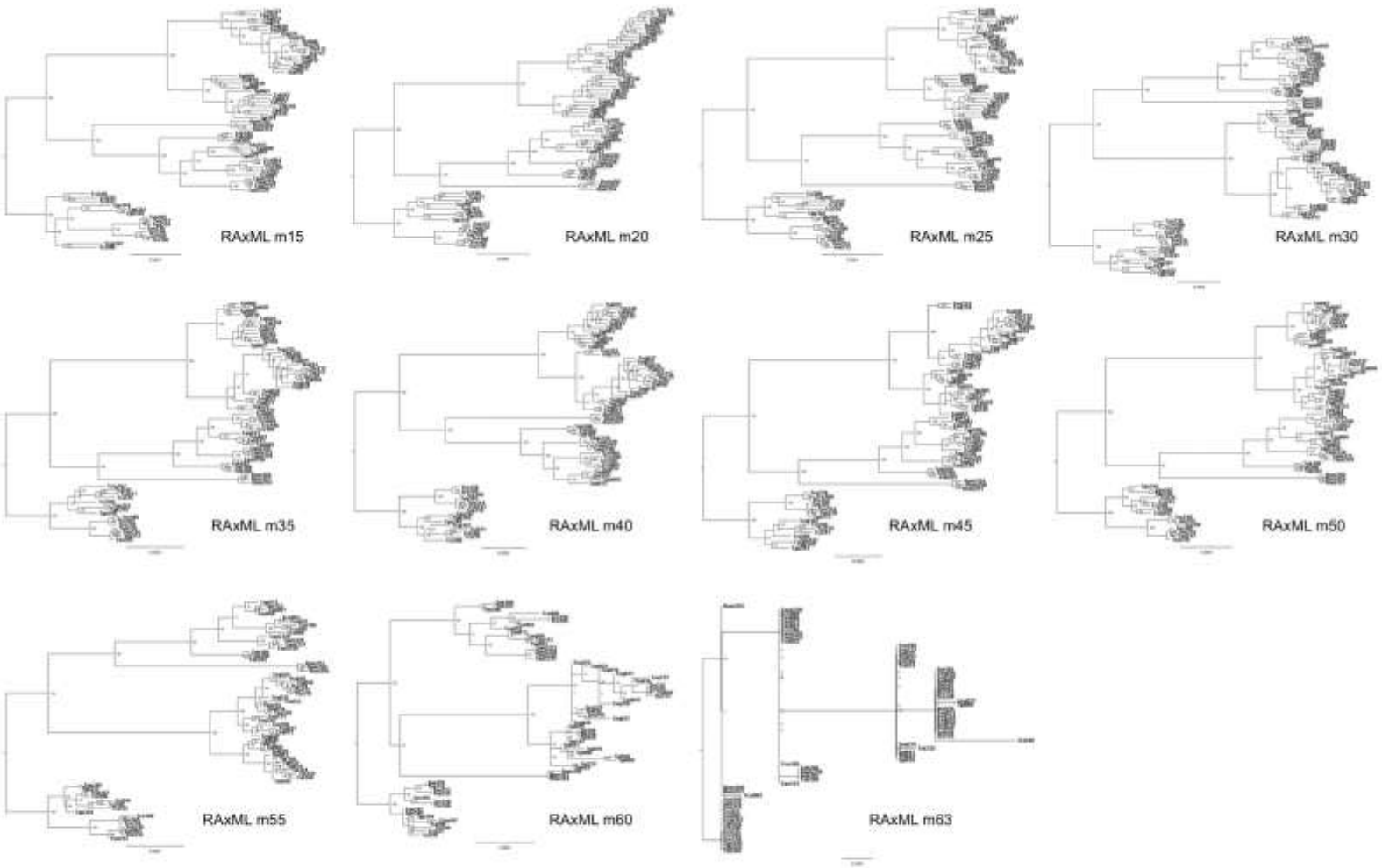
Supplementary Material 6. SNPs matrices plot constructed with the glPlot function of package adegenet v2.0.1. Shared loci among samples plots were obtained with the RADami 1.1-2 package with the locus.dist function.




m25
m30
m35
m40

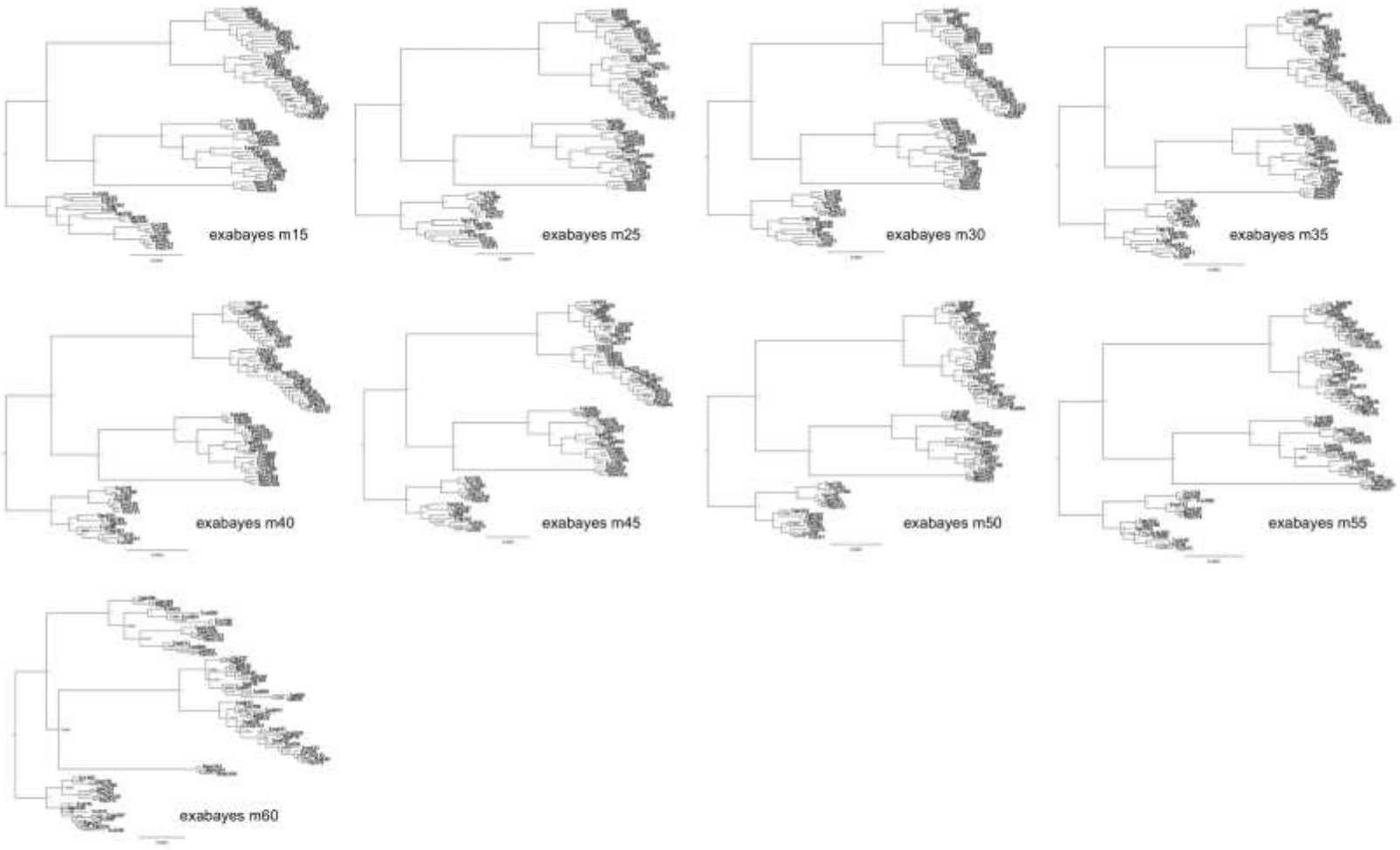

m45
m50
m55
m60


m63



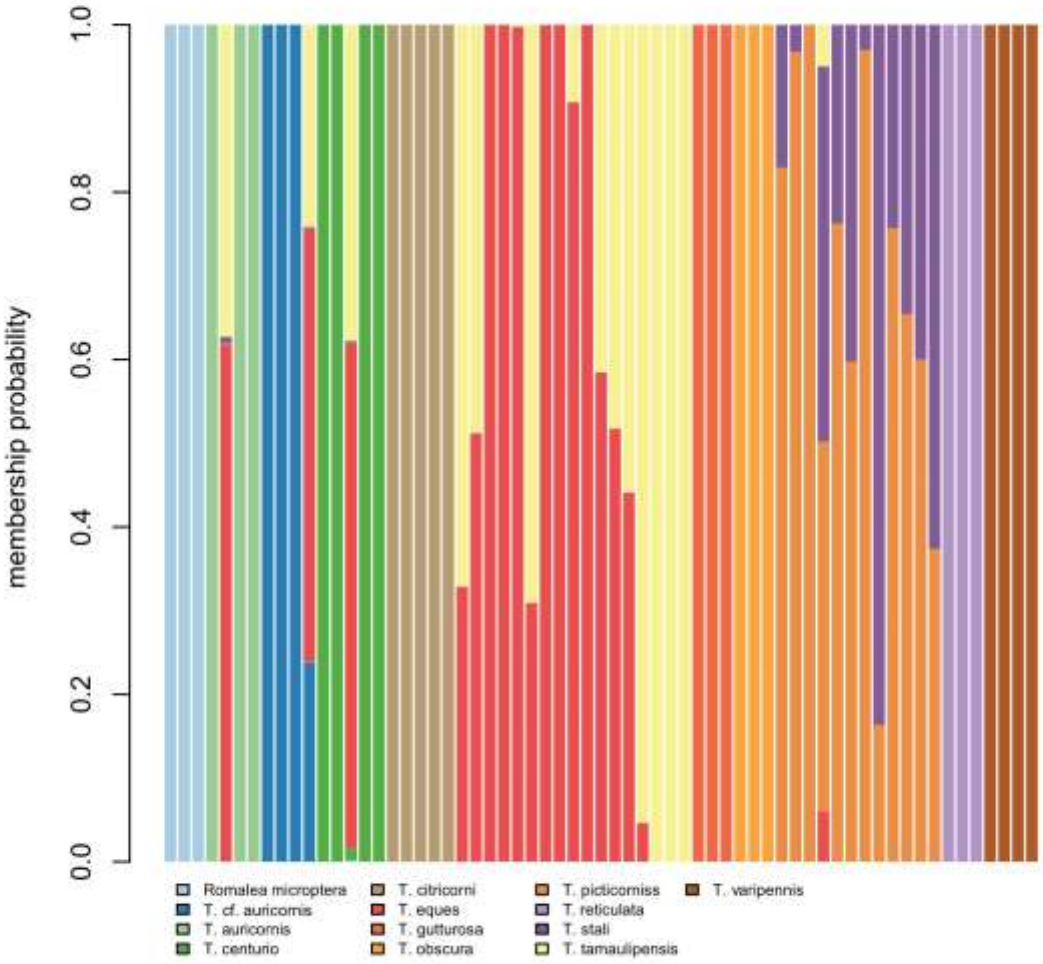
Phylograms reconstructed by Maximum Likelihood in RAxML

Supplementary Material 7. ML and Bayesian Phylograms obtained for all concatenated matrices reconstructed with the programs RAxML (simultaneous 1000 rapid bootstrap analysis and search for the best ML tree) and Exabayes (1,000,000 or until convergence, ASDSF ≤ 1%).



Phylograms reconstructed by Bayesian Inference in exabayes

Supplementary Material 8. Barplot of DAPC based on PCA of m20 matrix, with membership probabilities of all samples.



Capítulo IV: Taxonomic revision of the Transitional Nearctic-Neotropical lubber grasshopper genus *Romalea* (Romaleidae)
[En preparación para ser enviado a Zookeys]

Taxonomic revision of the Transitional Nearctic-Neotropical lubber grasshopper genus *Romalea* (Romaleidae)

Vladimir Salvador De Jesús-Bonilla[1], Ludivina Barrientos-Lozano[2], Alejandro Zaldívar-Riverón[1]

Abstract

The taxonomy of the transitional Nearctic-Neotropical lubber grasshopper genus *Romalea* (Romaleidae) is revised, considering *Taeniopoda* as its junior synonym (**syn. n.)**. A total of 12 species of *Romalea* are recognised, whose distribution extend from the south of the USA to Panama. The following species are redescribed: *R. auricornis* Walker **comb. n.**, *R. citricornis* (Bruner) **comb. n.**, *R. centurio* (Drury) **comb. n.**, *R. eques* Burmeister **comb. n.**, *R. gutturosa* (Bolívar) **comb. n.**, *R. microptera* (Palisot de Beauvois), *R. obscura* (Bruner) **comb. n.** (=*Taenipoda bicristata*), *R. picticornis* Walker **comb. n.** (=*Taeniopoda stali* **syn. n.**), *R. reticulata* (Fabricius) **comb. n.,** *R. tamaulipensis* (Rehn) **comb. n.** and *R. varipennis* (Rehn) **comb. n.** A new species is described, *R.* sp. 1.

Introduction

The grasshopper family Romaleidae is mainly distributed in the Neotropical region, with its species being medium to large sized and rich alar coloration (Rehn and Grant Jr. 1959). Members of Romaleidae are characterized by having a hypognatous head with undivided fastigium, an immobile spine at the apex of the outer surface of the posterior tibia and a highly specialized stridulatory system (Dirsh 1961, Amedegnato 1977).

The family Romaleidae takes its name from the monotypic genus *Romalea* Serville, which only is represented by *R. microptera* Palisot de Beauvois. The geographic distribution of this species is restricted to south-eastern United States (Hebard 1925, Rehn and Grant 1959). *Romalea* was always considered a monotypic genus; however, recent molecular phylogenetic studies of the morphologically similar genus *Taeniopoda* Stål based on punctual and genomic markers have revealed that the latter genus is paraphyletic with respect to *Romalea* (De Jesús-Bonilla et al. 2017; submitted).

Taeniopoda is distributed from the south of the United States to Panama, though the majority of its species occur in México (Hebard 1924, Eades et al. 2016). *Taeniopoda* contains twelve recognized species; nevertheless, De Jesús-Bonilla et al. (2017; submitted) concluded that *T. picticornis* (Walker) and *T. stali* Bruner actually are conspecific. Moreover, these studies supported the existence of a new species from Guatemala morphologically similar to *T. auricornis* (Walker). Here we therefore formally followed the taxonomic inferences obtained in the aforementioned molecular phylogenetic studies carrying out a taxonomic revision of the genus *Romalea*, considering *Taeniopoda* as its junior synonym **syn. n.**

Material and methods

A total of 346 adult specimens of *Romalea* were examined. The majority of specimens were collected by the author along the known geographical distribution of the genus. The collected material is deposited at the Colección Nacional de Insectos (CNIN), Universidad Nacional Autónoma de México (IB UNAM). We also examined specimens deposited from the following entomological collections: Instituto Tecnológico de Ciudad Victoria, México (ITCV), Universidad de San Carlos de Guatemala, Guatemala (USAC), Instituto Nacional de Biodiversidad, Costa Rica (INBio), Museo Nacional de Ciencias Naturales, España (MNCN), and British Museum of Natural History, United Kingdom (BMNH), the Entomology Collection of the Academy of Natural Sciences of Drexel University (ANSP).

External morphological features were observed with a ZEISS Stemi DV4 stereomicroscope, and measurements were made with a digital caliper (Figure 1a-b). Internal male genitalia were dissected by immersing the distal part of the abdomen in 10% KOH for 10 minutes and then removing it with a hook. After removal, the genitalia were kept immersed in 10% KOH for 20 minutes, muscle tissue was removed and morphological characteristics of the internal genitalia were observed with a ZEISS Stemi DV4 stereomicroscope (Figure 1c-d). Digital photographs of the entire specimens were taken with a Fujifilm FinePix S1600 digital camera. Photos of diagnostic external morphological features and internal genitalia were taken with Leica Z6 APO stereoscope. The terminology used was the proposed by Snodgrass (1935) and Jones (1981) for external morphology; and the genitalia terminology of Dirsh (1956).

Results

Genus *Romalea* Serville. (= *Taeniopoda* Stål)

Romalea Serville 1831: 280; Hebard 1925: 2; Rehn and Grant 1959: 252; Rehn and Grant 1961: 231; Amédégnato 1974: 198; Eades 2000: 204; Eades et al. 2016: online

Rhomalea (misspelling) Burmeister 1838: 619; Saussure 1859: 392; Pictet and Saussure 1887: 348; Kirby 1890: 588; Kirby 1910: 369

Taeniopoda Stål 1873: 50; Brunner von Wattenwyl 1893: 134; Bolívar 1901: 264; Rehn 1904: 530; Bruner 1907: 231; Kirby 1910: 371; Hebard 1924: 253; Hebard 1925: 7; Rehn and Grant 1959: 252; Rehn and Grant 1961: 240; Ortega and Márquez 1988: 327; Eades 2000: 204; Rowell 2013: 106; Eades et al. 2016: online; De Jesús-Bonilla et al. 2017: 601

Teniopoda (misspelling) Buzzetti and Barrientos-Lozano 2011: 210

Description: Size varies from medium to large, (Head-tegmina mm) females 70.00-31.83 mm, males 57.90-32.07 mm. **Head**: Antennae filiform with 19-22 segments; first 7-17 antennal segments pale, the rest of antennal segments black. The antennal segments 1 and 2, and/or 5 and 6 tend to be fused (Figure 2a-b). Gena rounded, vertex globose, fastigium declivent. Lateral ridges of fastigium obtuse-angulate. Frontal costa marked, narrow and sulcate from apical frons to median ocellus, evanescent inferiorly. Frons with marked facial carina. Eye oval shaped and prominent. Fastigium and frons punctate; vertex sparsely punctate, gena and circumocular area sparsely punctate to smooth. **Pronotum**: Tectiform with a median carina forming a pronotal crest, and lateral carinas with distinct lateral angles. Lateral lobes slightly longer than deep, the inferior margin slightly emarginated anteriorly. Anterior margin obtuse-angulate; posterior margin rectangulate to acute-angulate. Pronotum divided in prozona and metazona by a major transverse sulcus. Prozona with three transverse sulci; the anterior sulcus does not cut the median carina and not touch the bottom

margin of the pronotum, the mid sulcus cut the median carina and it extends to the middle of pronotum, the posterior sulcus of metazona cut the median carina and it extends more than two thirds of height of pronotum. The sulci are associated to furrows or scars. Pronotal disk and lateral lobes cribose-punctate to rugose-punctate. Prosternal process developed, spiniform. **Tegmina**: Macropterous to moderately reduced. Reticulated veins and venilets, fenestrated areas black, or partially to completely bleeded by veins colour. Costal margin arcuate, anal margin straight to arcuate. Apex semi-circular. **Wings**: Equally as long as the tegmina or reduced. Apex slightly falcate. Costal and apical margin black, posterior margin narrowly black. Disk with central red area. **Hind leg**: Femur almost equal to the abdomen in length, rather slender. Medial area of femur with two series of black spots. Tibia with two series of 8-10 posterior spines. **Male Genitalia**: Endophallic plates large. Dorsal and ventral aedeagal valves and aedagal process relatively short. Aedeagal valves transverse sulcated.

Distribution: From Southern and Eastern of United States to Panama.

Key to species of *Romalea*

1 With a median carina 0.25 or less times the maximum height of pronotum (Figure 2a), forming low elevated pronotal crest …….…….…… 2

- With a median carina elevated at least 0.3 times total height of pronotum (Figure 2b), forming an elevated pronotal crest, without black ring at apex of antennal segments (Figure 2c) …… 5

2 Tegmina reduced 3/4 length of pronotum, with central red to pink area ……. *R. microptera*

- Tegmina not reduced, with black ring at apex (Figure 2d) ……..…….. 3

3 Head and pronotum background colour, gree…….…….. 4

Head and pronotum background colour, black ……..…….…… *R. eques*

4 Sides of median carina and pronotal sulci black, with slightly excavated black furrow in the caudal segment of prozona ……..……..…… *R. tamaulipensis*

- Sides of median carina, pronotal sulci, and slightly excavated furrow in the caudal segment of prozona of same colour of pronotum ……...… *R. picticornis*

5 Pronotum heavy robust, lateral heavy carina marked. Pronotal disk deplanated, table shaped ……..……..… *R. obscura*

- Pronotum robust, lateral carina marked. Pronotal disk not deplanated ……..…….. 6

6 Posterior margin of pronotum slightly acute-angulate to rectangulate ……..……. 7

- Posterior margin of pronotum very acute-angulate, produced caudal……..……... 9

7 Pronotum and head ground colour, green. Tegmina veins and venilets green to brown yellowish ……..…...… 8

- Pronotum and head ground colour, dark brown to black. Tegmina veins and venilets brown to light brown ……..……..…… *R. centurio*

8 Robust. Large size. With broadly apical black margin in tegmina .... *R. guatemalensis*

Slender. Medium size. Without apical or narrowly margin in tegmina …….. *R. auricornis*

9 Medial and lateral carinas black ……..……..…… 10

- Medial and lateral carinas lighter than pronotum ……..……..... *R. varipennis*

10 Pronotum and head ground colour, purple to black ……..…… *R. reticulata*

- Pronotum and head ground colour, green or red ……..……. 11

11 Pronotum and head ground colour, red. Pale portion of antenna red ……. *R. gutturosa*

- Pronotum and head ground colour, green. Pale portion of antenna yellow to lemon-yellow ……..…… *R. citricornis*

***Romalea microptera* (Palisot de Beauvois, 1817) (Figure 3a-c)**

Acridium micropterum Palisot de Beauvois 1817: 146; Scudder 1901: 9

Romalea microptera (misspelling) Serville 1831: 280; Rehn and Hebard 1912: 256; Hebard 1916: 19; Roberts 1941: 217; Rehn and Grant 1959: 249; Rehn and Grant 1961: 233; Dirsh 1961: 394; Kevan 1980: 139; Jones 1981; Helms et al. 2003: 135-140; Capinera et al. 2004: 149; Mefferd et al. 2005: 31-32; Stauffer and Whitman 2007: 103-114; Capinera 2008: 1268-1270; Schowalter 2018: 1-17

Rhomalea microptera Charpentier 1845: 49; Kirby 1910: 369

Dictyophorus micropterus Pictet and Saussure 1887: 347; Bruner 1907: 230

Rhomalea gigantea Burmeister 1838 (synonym)

Romalea gloveri Kirby, 1910 (synonym)

Gryllus (Locusta) guttata (Stoll, 1813) (synonym)

Romalea marci M. A. Serville, 1838 (synonym)

Dictyophorus reticulatus (Thunberg, 1815) (synonym)

Description: Size (Head-tegmina mm): Females 70.00-50.00 mm, males 55.00-43.00 mm. **Head**: Scape and pedicel pale yellow to orange; first 7-9 antennal segments orange to yellow with black dorsal coloration, the rest of antennal segments black. Fastigium slightly declivent

(Figure 3b). Vertex and fastigium orange or green yellowish, orange or black; coronal suture yellow to orange. Frons green olivaceous to orange or black; facial carina and epistomal margin yellow to orange, lighter than frons; vertex and gena usually with black spots. **Pronotum**: With a median carina 0.25 or less times the maximum height of pronotum. Median carina transversely porcate in prozona, and slightly rugose in metazona. Sides of carina and transverse sulci broadly black. Pronotal disk and lateral lobes green olivaceous, orange yellowish, orange or black. Median carina and posterior margins of pronotum yellow to orange, lighter than pronotum. Caudal segment of prozona with 1 to 5 dorsal slightly excavated black dots. Posterior margin rectangulate. **Tegmina**: Tegmina reduced to 3/4 of the abdomen, with red to pink central coloration. Veins and venilets green yellowish, yellow, orange or black. **Abdomen**: Colour of tergites variable in combinations green, grey, yellow or black, with yellow to orange dorsomedial line. Sternites colour variable as the tergites, with anterior black margin, and yellow to orange caudal margin. **Hind leg**: Medial area of femur yellow to orange, in melanistic specimens all black. Carinula and keel yellow to orange, lower carinula and keel paler than upper carinula and keel. Upper marginal area variable, all black, same colour of carinula and keel or discontinuously black; lower marginal area with black dots series. Semilunar process black, cover plate yellow to orange, insertion of extensor tibia muscle paler than rest of femur.

Diagnosis: This species has great variation in color with general yellow or orange ground colour forms, melanistic variations are almost entirely black; but can be distinguished from the other species of *Romalea* by the following combination of characters: (1) low pronotal crest, (2) tegmina and wing reduced to 3/4 of abdomen length, (3) tegmina with a red to pink central area, (4) caudal segment of prozona with 1 to 5 dorsal slightly excavated black dots (Figure 3b-c), and (5) vertex slightly declivent.

Material examined: 1 female, USA: Florida: Collier Co: Big Cypress Bend | 25.9424, -81.4697 | 5-VIII-2009 | leg. Jensen, Mugleston; 1 female, USA: Florida: Collier Co: Port of the Islands Marina | 25.9571, -81.5132 | 7-VIII-2009 | Jensen, Mugleston.

Distribution: The species are distributed in the southeast of the United States. Distributed from Texas to Florida, and northwards to Tennessee.

Remarks: This is the best-known species of the genus, with numerous works on morphology, physiology and ecology. This species has great variation in colour, it is usual that the melanistic specimens are confused with *R. eques* but can be separated by the characteristics of the key.

***Romalea tamaulipensis* (Rehn, 1904), comb. n. (Figure 4a-c)**

Taeniopoda tamaulipensis Rehn 1904: 531; Bruner 1907: 234; Hebard 1924: 261; Buzzetti and Barrientos-Lozano 2011: 210; De Jesús-Bonilla et al. 2017: 600-617

Description: Size (Head-tegmina mm): Females 57.32-43.31mmm, males 48.79-41.82 mm. **Head**: Scape yellow, green yellowish or black; pedicel black; first 9-11 antennal segments yellow with a black ring at the apex, the rest of antennal segments black. Fastigium strong declivent. Vertex and fastigium green yellowish to dark green; coronal suture yellow. Frons green olivaceous to dark green; facial carina and epistomal margin yellow to green olivaceous, lighter than frons. Gena with a distinct vertical yellow band. **Pronotum**: With a median carina 0.25 or less times the maximum height of pronotum. Median carina of pronotum rugose or slightly rugose. Sides of media carina black, transverse sulci narrowly black. Pronotal disk green olivaceous to almost dark green; lateral lobes green olivaceous to dark green, lighter than pronotal disk. Median carina and posterior margins of pronotum

yellow. Caudal segment of prozona with a slightly excavated black furrow. Posterior margin rectangulate. **Tegmina**: Veins and venilets green yellowish, fenestrated areas black. Apical margin usually narrowly black (Figure 4c). **Abdomen**: Tergites green to black, with yellow dorsomedial line. Sternites green with yellow to olivaceous green with yellow caudal margin. **Hind leg**: Medial area of femur yellow to green yellowish. Carinula and keel yellow to green yellowish. Marginal areas black. Semilunar process, cover plate and insertion of extensor tibia muscle black. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus lobiform shaped with interior or right orientation. Epiphallus bridge sculpture strong developed or absent. Margin of lateral plate of epiphallus sub-angular or rounded.

Diagnosis: This species can be recognized by the following combination of characters: (1) low pronotal crest, (2) general ground colour green olivaceous (Figure 4a), (3) antennal segments with a black ring at the apex, (4) sides of medial carina black coloured extends to lateral lobes of the pronotum (Figure 4b), and (5) segment caudal of the prozona with black groove slightly excavated.

Type material examined: 1 male, *Taeniopoda tamaulipensis* **|** vii.i.1903 Altamira Tamaulipas | leg. M.E. Hoag | Type 5113 (holotype).

Other material examined: 3 females 2 males, México: San Luis Potosí: Guadalcazar: Ábrego, Campamento Monternach | Coords: 22.65644, -100.38007 | 1567msnm | 22-ix-2012; 1 male, México: Coahuila: 30 km antes de Santiago Aserradero | Coords: 25°23.347'N, 100°15.180'W | 1373msnm | 10-xi-2013 | leg. L. Barrientos-Lozano, V.S. De Jesús-Bonilla, A.Y. Rocha-Sanchez, L. Cortéz; 1 female, México: Hidalgo: El tablón | 11-x-2009 | leg. M. García-París, N. Percino; 1 male, México: Hidalgo: Metznoxtla | Coords: 20°37'47.8N, 98°51'28.0"W | 1612msnm | 12-ix-2012 | leg. M. García-París, N. Percino; 1 female, México:

Hidalgo: Pedregal | Coords: 20°39'05.7"N, 98°48'22.8"W | 1290msnm | 12-ix-2012 | leg. M. García-París, N. Percino; 3 females, México: Hidalgo: RMO Xhita | Coords: 20.634467, -99.326451 | 2048msnm | 10-x-2009 | leg. M. García-París, N. Percino; 1 female, México: Hidalgo: Venados | Coords: 20°28'31.4"N, -98°39'24.9"W | 1597msnm | 11-ix-2012 | leg. M. García-París, N. Percino; 1 female 1 male, México: Nuevo León: Iturbide: Santa Rosa Km 4 | Coords: 24°42.113'N, 99°54.472'W | 1513msnm | 8-xi-2013 | leg. L. Barrientos-Lozano, V.S. De Jesús-Bonilla, A.Y. Rocha-Sanchez, L. Cortéz; 1 female, México: Nuevo León: Linares-San Roberto: Carretera 58 Km 30 | Coords: 24° 44.732'N, 99°48.910'W | 852msnm | 8-xi-2013 | leg. L. Barrientos-Lozano, V.S. De Jesús-Bonilla, A.Y. Rocha-Sanchez, L. Cortéz; 2 females 2 males, México: Nuevo León: Santiago: Arriba de la Cola de Caballo, 6km antes de Santiago | Coords: 25°21.841'N, 100°9.566'W | 455msnm | 10-xi-2013 | leg. L. Barrientos-Lozano, V.S. De Jesús-Bonilla, A.Y. Rocha-Sanchez, L. Cortéz; 1 female, México: Nuevo León: Santiago: Horse tail fall | Coords: 25°22.081'N, 100°09.637'W | 701msnm | 10-vii-2013 | leg. L. Barrientos-Lozano; 1 male, México: Nuevo Leoón: Galeana: Puente de Dios | 15-viii-2015 | leg. M. Trujano-Ortega; 4 females 2 males, México: Querétaro: Camino a Santa María Cocos | 18-xi-2013 | leg. C. Perdaza Lara, E. Recuero; 1 female, México: Querétaro: Sierra Gorda, 3km N de Arroyo Seco | Coords: 21.570842, -99.711956 | 1010msnm | 14-ix-2014 | leg. U.O. García-Vázquez, M. Trujano-Ortega; 1 female, México: Querétaro: Sierra Gorda, Carretera hacía Sotano de Barro | 24-vii-2014 | leg. A. Ramírez-Ponce; 1 female 1 male, México: Tamaulipas: Ocampo: Libramiento poniente Km 1.5 | Coords: 22°50'15.25"N, 99°20'28.81"W | 351msnm | 12-x-2013 | leg. L. Barrientos-Lozano; 2 females 1 male, Tamaulipas: Rd. 66, Tula-Ocampo a 11 km de Tula | Coords: 22°56.867'N, 99°36.267'W | 1481msnm | 12-x-2013 | leg. L. Barrientos-Lozano.

Distribution: Mainly in the Sierra Madre Occidental, with some occurrences in the Tran-

Mexican Volcanic Belt and the south of Mexican Plateau.

Remarks: This information complements Rehn's description (1904), based only on single adult female, with information of a mayor series of specimens. The colours reported in the original description correspond to decolouration observed after fixation, we report recently collected specimens that preserve the original colours.

***Romalea eques* Burmeister, 1838, comb. n. (Figure 5a-c)**

Rhomalea eques Burmeister 1838: 620; Saussure 1859: 392;

Taeniopoda eques Kirby 1910: 372; Hebard 1924: 256; Hebard 1925: 7; Hebard 1932: 270; Rehn and Grant 1959: 253; Rehn and Grant 1961: 243; Whitman and Loher 1984: 1-12; Richman et al. 1993: 95; Eades 2000: 204; Capinera et al. 2004: 150; Rivera 2006: 135; Stauffer and Whitman 2007: 103-114; Fontana et al. 2008: 225-226; Whitman and Richardson 2010: 377–380; Barrientos-Lozano et al. 2013: 323-324; De Jesús-Bonilla et al. 2017: 600-617.

Taeniopoda burmeisteri (Bolívar, 1901) (synonym)

Description: Size (Head-tegmina mm): Females 50.26-41.78 mm, males 50.97-38.24 mm **Head**: Scape black to green; pedicel black; first 9-11 antennal segments yellow with black ring at the apex (in some specimens segments 1-3 black), the rest of antennal segments black. Fastigium strong declivent. Vertex and fastigium black; coronal suture yellow. Frons black or occasionally yellow, facial carina and epistomal margin yellow. Gena with a distinct vertical yellow band. **Pronotum**: With a median carina 0.25 or less times the maximum height of pronotum. Median carina of pronotum rugose. Pronotal disk black; lateral lobes black. Median carina and posterior margins of pronotum yellow. Posterior margin rectangulate.

Tegmina: Veins and venilets green yellowish, fenestrated areas black. Apical margin usually broadly black (Figure 5c). **Hind leg**: Medial area of femur yellow to green yellowish. Carinula and keel yellow to green yellowish. Marginal areas black. Semilunar process, cover plate and insertion of extensor tibia muscle black. **Abdomen**: Tergites black, with yellow dorsomedial line. Sternites black with yellow caudal margin. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus lobiform shaped with interior, right or exterior orientation. Epiphallus bridge sculpture strong, slightly or absent. Margin of lateral plate of epiphallus sub-angular or rounded.

Diagnosis: This species can be recognized by the following combination of characters: (1) low pronotal crest, (2) general ground colour black (Figures 5a-b), (3) antennal segments with a black ring at the apex, and (4) lateral lobes and disk of the pronotum entirely black.

Material examinated: 1 female 1 male, México: Aguascalientes: San José de García-Paredes | Coords: 20°08'34.70"N, -102°22'53.94"W | 2157msnm | 23-ix-2010 | leg. M. García-París, N. Percino; 4 males, México: Coahuila: Cuatro Ciénegas: Rancho Casita, Ojo de agua | Coords: 27.134419, -102.315979 | 935msnm | leg. U.O. García-Vázquez, M. Trujano-Ortega; 1 male, México: Coahuila: La angostura Saltillo | Coords: 25°21'38.48"N, 101° 1'21.44"W | 1566msnm | 22-ix-2010 | leg. M. García-París, N. Percino; 1 male, México: Coahuila: Saltillo 3 km SE de La Angostura | Coords: 25.32748°N, 101.026°W | 1931msnm | 21-x-2013 | leg. U.O. García-Vázquez, M. Trujano-Ortega; 2 females 1 male, México: Durango: Carretera Torreón | Coords: 25.53015, -103.666616 | 1355msnm | 11-viii-2015 | leg. M. Trujano-Ortega; 1 female, México: Durango: Carretera Torreón | Coords: 25.53015, -103.666616 | 1355msnm | 11-viii-2015 | leg. M. Trujano-Ortega; 1 female 2 males, México: Guanajuato: Carretera Juventino Rosas, rumbo a Celaya al NE de Potrerillos | 2292msnm | 19-vi-2013 | E. O. Martínez-Luque; 2 females, México: Guanajuato: El Moro de Barajas |

Coords: 20.317739, -101.695879 | 1825msnm | 17-vii-2014 | leg. E.O. Martínez-Luque, M. Canchola, J.J. Castro-Sanchez; 1 female, México: Guerrero: Tierra Colorada | Coords: 17.156894, -99.54083 | 620msnm | vii-2012 | leg. E.O. Martínez-Luque; 1 female, México: Hidalgo: RMO Xhita | Coords: 20.634467, -99.326451 | 2048msnm | 10-x-2009 | leg. M. García-París, N. Percino; 1 female 1 male, México: Hidalgo: Tula de Allende, carretera Tula-Sn. Fco. Bojay | Coords: 20°04'43.08"N, 99°21'9.8"W | 2196msnm | 24-ix-2014 | A. Ramírez-Ponce; 1 female, México: Hidalgo: El tablon | 11-x-2009 | leg. M. García-París, N. Percino; 1 female 2 males, México: Michoacán: Emiliano Zapata, Cerro grande cara sur | Coords: 20.007086, -102.596889 | 1669msnm | 7-viii-2014; 1 female, México: Querétaro: Bellavista del Río (Cadereyta) | Coords: 20.68504, -99.577019 | 1945msnm | 11-x-2009 | leg. M. García-París, N. Percino; 1 female; México: Querétaro: Cadereyta | Coords: 20.702676, -99.783551 | 2072msnm | 11-x-2009 | leg. M. García-París, N. Percino; 1 male, México: San Luis Potosí: Xilitla: Los pozos de Edward James | Coords: 21.3957, -98.9921 | 17-vii-2015 | leg. O. Pérez-Flores; 3 females, México: Zacatecas: Sierra Vieja | Coords: 23°29'40.78"N, 102°7'42.69"W | 2085msnm | 22-ix-2010 | leg. M. García-París, N. Percino.

Distribution: Widely distributed from the semi-arid highlands of the southern of the USA to the Mexican Plateau, Sierra Madre Oriental, Trans-Mexican Volcanic Belt and Pacific Coast of México.

Remarks: This species is the closest to *T. tamaulipensis* and it is distinguished from that by the black general coloration of pronotum and head. De Jesús-Bonilla et al. (in preparation) suggest the existence of two lineages in *T. eques* of south and northern Mexican Plateau.

***Romalea picticornis* Walker 1870 [= *Taeniopoda stali* (Bruner, 1907)], comb. n. (Figure 6a-c)**

Rhomalea picticornis (misspelling*)* Walker 1870: 538; Thomas 1873: 240

Taeniopoda pecticornis (misspelling*)* Scudder and Cockerell 1902: 39; Caudell 1903: 795

Taeniopoda picticornis Bruner 1907: 234; Kirby 1910: 371; Hebard 1924: 256; De Jesús-Bonilla et al. 2017: 600-617.

Taeniopoda stali Bruner 1907: 234 *(Taeniopoda ståli,* Incorrect original spelling*)*; Hebard 1924: 261; Hebard 1932: 270; Fontana et al. 2008: 277; De Jesús-Bonilla et al. 2017: 600-617.

Taenipoda steali (misspelling) Kirby 1910.

Description: Size (Head-tegmina mm): Females 59.44-42.35 mm, males 51.21-43.51 mm. **Head**: Scape green yellowish or black, pedicel black; first 11-12 antennal segments yellow, or orange to scarlet with black ring at the apex, the rest of antennal segments black. Fastigium strong declivent. Vertex and fastigium green to yellowish-olivaceous; coronal suture yellow. Frons green to yellowish-olivaceous, facial carina and epistomal margin yellow, lighter than frons. **Pronotum**: With a median carina 0.25 or less times the maximum height of pronotum. Medial and lateral carina rugose. Pronotal disk and lateral lobes green to yellow-olivaceous. Sides of carina and transverse sulci of the same colour of pronotum. Posterior margin rectangulate. **Tegmina**: Veins and venilets green, fenestrated areas black. **Abdomen**: Tergites olivaceous-yellowish, with yellow dorsomedial line. Sternites olivaceous-yellowish. **Hind leg**: Medial area of femur yellow to olivaceous-yellowish. Carinula and keel yellow to olivaceous-yellowish. Upper marginal area variable black, lower marginal area all black or with black dots series. Semilunar process black, cover plate black or olivaceous-yellowish, insertion of extensor tibia muscle grey to black. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus angular or lobiform shaped

with interior orientation. Epiphallus bridge sculpture slightly or absent. Margin of lateral plate of epiphallus sub-angular or rounded.

Diagnosis: This species resembles *T. eques* and *T. tamaulipensis* but can be distinguished from latter species by the (1) low pronotal crest, (2) green to olivaceous-yellowish ground colour, (3) antennal segments with a black ring at the apex, and (4) transverse sulci o the same colour of pronotum.

Type material examined: 1 male, *Rhomalea puncticornis* Walker | Mexico (stali) (syntype); 1 male 1 female*, Rhomalea puncticornis* | Mexico 58.135 Oajaca (syntype).

Other material examined: 4 females 3 males, México: Colima: Coquimatlan: Arroyo El Tanque del General | Coords: 19.14167, -104.02424 | 504msnm | 25.26-viii-2013 | leg. J.C. Arenas-Monroy; 1 male, México: Estado de México México: Ixtapaluca | Coords: 19°18'52.24"N, 98°53'4.30"W | 2485msnm | xi-2012; 1 female, México: Estado de México: Ixtapan de la sal | 1850msnm | 5,6-x-2013 | leg. E. O. Martínez-Luque; 1 male, México: Guerrero: Teloloapan | Coords: 18°22'1"N, -99°52'8" | 1575msnm | 03-xi-2012; 1 female 1 male, México: Guerrero: Carretera Teloloapan-Iguala, 44 km E? de Xalostoc | Coords: 18°26'01.17"N, 99°45'15.99"W | 16 82msnm; 2 females 1 male, México: Guerrero: Olinalá: Xixila | Coords: 18.001944, -98.836527 | 154msnm | 11-ix-2013; 2 males, México: Guerrero: Teloloapan, entrada al pueblo | Coords: 18°21'5.6"N, 99°50'37.2"W | 1562msnm | 23-xi-2013 | leg. A. Zaldívar-Riverón, J. J. Martínez, M. García-París; 3 females 2 males, México: Jalisco: La Huerta, Estación de Biología de Chamela | Coords: 19° 29.620' N, 105° 02.749' W | 87msnm | 6-ix-2009 | leg. M. García-París, N. Percino; 1 female, México: Morelos: Tepalcingo: El Limón, Rio de El limón | Coords: 18.53277, -98.93905 | 1255msnm | 25-vii-2014 | leg. E. O. Martínez-Luque; 1 male, México: Puebla: Totoltepec de Guerrero | Coords: 18°16'59.51"N, 97°48'25.80"W | 1508msnm | 30-xi-2013 | leg. H. Alvarez-García; 1

female, México: Veracruz: Acultzingo: Acultzingo | Coords: 18.7005, -97.31350 | 1840msnm | 24-vii-2014 | leg. A.G. Clause; 1 female 4 males, México: Veracruz: Acultzingo: Tecamalucan | Coords: 18°45'36.0"N, 97°12'36.0"W | 1384msnm | 12-ix-2014 | leg. A. Zadívar-Riverón, D. Dubovikoff, V.S. De Jesús-Bonilla et al.

Distribution: Pacific Mountain Ranges and Pacific Coastal plains from México, Balsas depression and highlands, Oaxaca and Puebla highlands and Trans-Mexican Volcanic Belt.

Remarks: This species was separated from its synonym *T. stali* by the coloration of the pale portion of the antennae, but the colour of the antennae in this species is variable, even in fixed specimens the antennae can be fading from scarlet to yellow (Figure 6b-c).

***Romalea obscura* (Bruner, 1907) [= *Taeniopoda bicristata* (Bruner, 1907)], comb. n. (Figure 7a-c)**

Taeniopoda obscura Bruner 1907: 235; Kirby 1910: 372; Hebard 1924, 273; Maes 1998: 103; Barrientos-Lozano et al. 2013: 325; De Jesús-Bonilla et al. 2017: 600-617.

Taeniopoda bicristata Bruner 1907: 236; Kirby 1910: 372; Hebard 1924, 271.

Description: Size (Head-tegmina mm): Females 50.89-48.76 mm, males 46.14 mm. **Head**: Scape and pedicel grey to dark yellow; first1 15-17 antennal segments yellow, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons brown to black mate; coronal suture, facial carina and epistomal margin cinnamon, lighter than frons. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Lateral carinas heavy rugose. Pronotal disk robust and deplanated, table shaped in metazona (Figure 7b-c). Pronotal disk and lateral lobes dark brown to black mate. Pronotal crest, lateral carinas, and posterior margins of pronotum light

brown. Median carina rugose. Sides of pronotal crest, and lower margin of lateral carina black polished. Posterior margin rectangulate. **Tegmina**: Finely reticulate. Veins and venilets light brown; fenestrated areas black and light brown, mostly light brown in anal area**. Abdomen**: Tergites brown and black, with light brown dorsomedial line. Sternites brown to black. **Hind leg:** Medial area of femur brown or entirely black. Carinula and keel light brown to black, lower carinula and keel paler than upper carinula and keel. Upper marginal area variable, same colour of upper carinula and keel; lower marginal area same colour of lower carinula and keel with black dots series. Semilunar process black, cover plate and insertion of extensor tibia muscle same colour of upper marginal area. **Male genitalia**: Posterior margin of the endophallus plate rounded. Ancora of epiphallus lobiform shaped with interior orientation. Epiphallus bridge sculpture strong developed. Margin of lateral plate of epiphallus sub-angular.

Diagnosis. This is the most robust species within the genus *Romalea*, it can be distinguished by the following combination of characters: (1) elevated pronotal crest, (2) dark brown to black ground colour, (3) presence of elevated pronotal crest, (4) the unusually robust pronotum, and (5) pronotal disc deplanated, table shaped.

Type material examined: 1 male, *T. obscura* | Temax, Yucatán | Bruner (syntype, possible type); 1 male, *T. obscura* | Merida Yucatan | leg. Gaumer (paratype); 1 male, *T. obscura* | Temax, Yucatán Mex (leg. Gaumer) (paratype); 1 female 1 male, *T. obscura* | Temax N. Yucatan | leg. Gaumer (paratype); 1 female, *Taeniopoda bicristata* | Syntype | Type H299 | Mat. | Matamoros, Puebla | Type Bruner (holotype).

Other material examined: 2 females, México: San Luis Potosí: Taninul | Coords: 21.955525, -98.8888353 | 77msnm | 09-iv-2001 | leg. L. Barrientos-Lozano; 3 females 1 male, México: Yucatán: Kaxil kiuic | Coords: 20.0929, -89.5638 | 85msnm | 30-vii-2014 | leg. C.N. Ibarra-

Cerdeña; 1 female, Guatemala: Petén: Flores: Dos Lagunas | 17-vi-1989 | Sergio Perez; 1 female, Guatemala: Petén: Tikal | 2-x-1999 | Byron González; 2 males, Merida Yucatan | leg. Gaumer; 1 male, Chichen Itza Yucatan | Col&pres by E. Thomposon | F.M.N. Coll; 1 female, Merida Yucatan Gaumer; 1 female, Northern Yucatan.

Distribution: Yucatán Peninsula, and a single report from San Luis Potosí, México.

Remaks: *T. bicristata* was described from a single female from an uncertain locality (the label reads "Mat", probably Izúcar de Matamoros). However, we consider that the characters that separate *T. bicristata* from *R. obscura*, pronotal crest lower in the metazona in *R. obscura* and higher in *T. bicristata,* deplanate portions on pronotal disk and sculpture of lateral carina, actually represent an individual proportion of the only known specimen assigned to *T. bicristata*, and hence are not sufficient to be considered as separated species.

***Romalea centurio* Drury 1770, comb. n. (Figure 8)**

Romalea centurio Drury, 1770, 78;

Rhomalea centurio Saussure 1859: 392;

Taeniopoda centurio Pictet and Saussure 1887: 348; Bolívar 1901; 268; Rehn 1903: 12; Bruner 1907: 236; Kirby 1910: 372; Hebard 1924: 265; Barrientos-Lozano et al. 2013: 322-323; De Jesús-Bonilla et al. 2017: 606, 608, 610

Taeniopoda reticularis Bolívar 1901 (synonym)

Taeniopoda superba (Stål, 1855) (synonym)

Description: Size (Head-tegmina mm): Females 52.00-37.70 mm, males 44.56-33.17 mm

Head: Scape and pedicel grey to dark yellow; first1 14-15 antennal segments pale yellow, the

rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons brown to dark brown; coronal suture, facial carina and epistomal margin same colour of frons or slightly lighter. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Pronotal disk and lateral lobes dark brown to black. Median carina surcate-rugose at the prozona, rugose at metazona. Lateral carinas rugose. Pronotal crest and lateral carinas dark brown to black, darker than pronotum. Sides of pronotal crest, and lateral carinas dark brown to black. Posterior margin slightly acute-angulate. **Tegmina**: Veins and venilets brown to light brown. Apical margin narrowly dark brown to black. **Abdomen**: Tergites brown and black, with light brown dorsomedial line. Sternites dark brown to black. **Hind leg:** Medial area of femur brown to light brown. Carinula and keel same colour of medial area. Upper marginal area same colour of carinula and keel; lower marginal area same colour of lower carinula and keel or with black dots series. Semilunar process black, cover plate and insertion of extensor tibia muscle same colour of upper marginal area or black. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus lobiform shaped with interior, right or exterior orientation. Epiphallus bridge sculpture strong or slightly developed. Margin of lateral plate of epiphallus sub-angular or rounded.

Diagnosis: This species is similar to *T. auricornis* but can be recognized by (1) elevated pronotal crest, (2) the head and pronotum dark brown to black ground colour, and (3) the tegmina coloration black and yellowish brown, and (4) tegmina with apical black margin.

Material examined: 1 female 1 male, México: Hidalgo: S. de Molango | Coords: 20°44'10.9"N, 98°43'02.3"W | 1426msnm | 11-ix-2012 | leg. M. García-París, N. Percino; 1 female 2 males, México: Puebla: Cuetzalan | Coords: 20.016829, -97.527118 | 983msnm | 11-x-2013; 5 females 2 males, México: Querétaro: Jalpan de Serra: Valle verde, el Pilón |

Coords: 21.503, -99.1705 1152msnm | 16-viii-2014 | leg. D. Dubovikoff, A. Zaldívar-Riverón; 2 females 3 males, México: San Luis Potosí: Mpio: Xilitla: Afuera de cueva de potrerillos | Coords: 21.30721, -99.06622 1176msnm | 25-x-2013 | leg. O. Franke, C. Santibañes, J. Crúz, A. Guzmán; 1 female, México: San Luis Potosí: Xilitla: Los pozos de Edward James | Coords: 21° 23' 45.6"N, 98°59'47.8"W | 621msnm | 26-x-2013 | leg. L. Barrientos-Lozano.

Distribution. Southern Sierra Madre Oriental in the states of San Luis Potosí, Querétaro, Hidalgo and Puebla, Mexico, to Nicaragua in Central America

Remarks: Some populations assigned to this species possess a ground colour violet, almost like *T. reticulata*; however, it can be distinguished from the latter species by having the veins and venilets brown to light brown.

***Romalea auricornis* Walker 1870, comb. n. (Figure 9a-c)**

Rhomalea auricornis (misspelling) Walker 1870: 538

Taeniopoda auricornis Rehn 1904: 532; Bruner 1907: 237; Kirby 1910: 372; Hebard 1924: 261; Hebard 1932: 270; COPR 1982: 114; Descamps 1975: 51; Fontana et al. 2008: 224-225; Barrientos-Lozano et al. 2013: 321-322; De Jesús-Bonilla et al. 2017: 606, 608, 609.

Taeniopoda pulchella Bolívar 1901: 269; Bruner 1907: 237; Kirby 1910: Kirby 1910: 72; Hebard 1924: 263; Descamps 1975: 51.

Description: Size (Head-tegmina mm): Females 47.57-31.83 mm, males 38.96-32.07 mm

Head: Scape and pedicel grey to pale yellow; first1 13-17 antennal segments yellow, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons dark green to yellowish green; coronal suture, facial carina and epistomal margin green to

yellowish green. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Pronotal disk and lateral lobes green to dark green. Median carina rugose or slightly rugose. Lateral carinas slightly prominent and rugose. Pronotal crest and lateral carinas same colour of pronotum or slightly dark. Sides of pronotal crest, and lateral carinas same colour of pronotum or slightly dark. Pronotal disk and lateral lobes green to dark green. Posterior margin slightly acute-angulate. **Tegmina**: Veins and venilets green to yellowish green. **Abdomen**: Tergites grey and black, with green yellowish dorsomedial line. Sternites grey or same colour of the tergites. **Hind leg:** Medial area of femur green to yellowish green. Carinula and keel same colour of medial area. Upper marginal area same colour of carinula and keel or finely black; lower marginal area same colour of lower carinula with black dots series. Semilunar process black, cover plate and insertion of extensor tibia muscle black or paler green. Posterior margin slightly acute-angulate. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus angular or lobiform shaped with right or interior orientation. Epiphallus bridge sculpture strong developed. Margin of lateral plate of epiphallus sub-angular.

Diagnosis: This species is similar to *T. centurio* but can be recognized by having (1) elevated pronotal crest, (2) green to dark green ground colour, and (3) veins and venilets green to yellowish green.

Type material examined: 1 male*, Taeniopoda pulchella* | MNCN_ent 147350 Tipos 7479 (possible holotype).

Other material examined: 2 females 2 males, Veracruz: (Fortín) Hotel | Coords: 18.907188°N, 97.011801°W | 1057msnm | leg. M. García-París, N. Percino; 2 females, 1 male, Veracruz: Barranca de San Miguel | Coords: 18.884722, -97.001944 | 10-xi-2011 | leg. M. García-París, N. Percino; 3 females 3 males, México: Veracruz: Ixhuatlan del café |

Coords: 19°4'56''N, 97°1'47''W | 1507msnm | 6-x-2013 | leg. A. Zaldívar-Riverón; 1 female, México: Veracruz: Santiago Tuxtla | Coords: 18.463483, -95.28942 | 312msnm | 3-x-2014; 1 female 1 male, México: Veracruz: Teocelo | Coords: 19°23'04.3''N, 96°58'51.6''W | 1176msnm | 26-x-2014 | leg. E. Recuero, A. Soto; 1 female 1 male, México: Veracruz: Xico: Cascada de Texolo | Coords: 19.4063, -96.9919 | 1182msnm | 13-ix-2013; 3 males, Santa Lucrecia, Mex (White) Veracruz | x.1922.

Distribution: Mexican states of Hidalgo, Veracruz, and the southern portion of Tamaulipas.

Remarks: *T. centurio* and *T. auricornis* are morphologically similar. The most common variation of *T. auricornis* resembling to specimens *T. centurio*; however, *T. centurio* can be distinguished from *T. auricornis* by the dark brown to black background colour in *T. centurio* and green to dark green in *T. auricornis*.

Romalea **sp. 1. sp. nov. De Jesus Bonilla (Figure 10a-c)**

Description: Size (Head-tegmina mm): Females 55.75-53.33 mm, males 52.80-49.67 mm **Head**: Scape and pedicel black; first1 15-16 antennal segments yellow, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons yellowish green to dark green; coronal suture, facial carina and epistomal margin yellow or yellowish orange. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Median carina rugose or slightly rugose. Lateral carinas slightly prominent and rugose. Pronotal crest and lateral carinas yellowish orange. Sides of pronotal crest, and lateral carinas yellowish orange. Pronotal disk and lateral lobes yellowish green to green. Posterior margin acute-angulate. **Tegmina**: Veins and venilets brown yellowish. Apical margin broadly black. **Abdomen**: Tergites grey and black, with yellowish dorsomedial line.

Sternites grey or same colour of the tergites. **Hind leg:** Medial area of femur green to pale yellow. Carinula and keel same colour of medial area. Upper marginal area same colour of carinula and keel or finely black; lower marginal area same colour of lower carinula with black dots series. Semilunar process, cover plate and insertion of extensor tibia muscle black. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus angular or lobiform shaped with right or interior orientation. Epiphallus bridge sculpture strong developed. Margin of lateral plate of epiphallus sub-angular.

Diagnosis: This species morphologically resembles *T. auricornis*, but it can be distinguished from this species by having: (1) elevated pronotal crest, (2) pronotum green with yellowish lateral and median carinas (lateral and median carinas same colour of pronotum in *R. auricornis*), (3) apical margin broadly black (narrowly black in *R. auricornis*), and (4) metazona strong produced caudal (sligthly produced in *T. auricornis*).

Types: **Holotype** 1 female, Guatemala: Baja Verapaz: Purulha: Orejuela | Coords: 15.24963, -90.1559 | 1359msnm | 26-ix-2015 | leg. V.S. De Jesús-Bonilla, O. Pérez-Flores, Carmen L. Yurritia. **Paratypes** 2 females 4 males, Guatemala: Baja Verapaz: Purulha: Orejuela | Coords: 15.24963, -90.1559 | 1359msnm | 26-ix-2015 | leg. V.S. De Jesús-Bonilla, O. Pérez-Flores, Carmen L. Yurritia; 1 male, Guatemala: El Petén: Poptún: Finca la Jarrilla | 16.305125, -89.409619 | 4-vii-2015 | leg. Carmen L. Yurritia.

Etymology: From Guatemala, country where the species has been collected.

Remarks: This species is closely related phylogenetically to *R. microptera*, R. *auricornis*, *R. centurio* and *R. obscura*. In phylogenetic analysis they are part of the same major clade, but is delimited as different species. This species is similar to *T. auricornis*, but it can be separated from that by the characteristics described above, it is also larger.

***Romalea citricornis* (Bruner 1907), comb. n. (Figure 11)**

Taeniopoda citricornis Bruner 1907: 234, Kirby 1910: 371; Hebard 1924: 270; Hebard 1932: 270; De Jesús-Bonilla et al. 2017: 606, 608, 611.

Description: Size (Head-tegmina mm): Females 47.90-39.07 mm, males 47.04-36.68 mm **Head**: Scape and pedicel black; first1 13-15 antennal segments yellow to lemon-yellow, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons green or lemon green; coronal suture, facial carina and epistomal margin same colour of frons or slightly lighter. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Pronotal disk and lateral lobes olivaceous. Median carina rugose or slightly rugose. Lateral carinas rugose. Pronotal crest and lateral carinas black. Sides of pronotal crest, and lateral carinas black. Posterior margin acute-angulate. **Tegmina**: Veins and venilets brown or green. **Abdomen**: Tergites brown and green, with brown yellowish dorsomedial line. Sternites brown to light green. **Hind leg:** Medial area of femur brownish to light green. Carinula and keel same colour of medial area. Upper marginal area same colour of carinula and keel or black; lower marginal area black, or same colour of lower carinula and keel with black dots series. Semilunar process black, cover plate and insertion of extensor tibia muscle black or same colour of upper marginal area. **Male genitalia**: Posterior margin of the endophallus plate or rounded. Ancora of epiphallus angular or lobiform shaped with right or exterior orientation. Epiphallus bridge sculpture strong or slightly developed. Margin of lateral plate of epiphallus rounded.

Material examined: 8 females 7 males, México: Oaxaca: Municipio Asunción Ixtaltepec: Santiago Ixtaltepec | Coords: 16°41'18"N, 94°54'17"W | 213msnm | x-2014 | leg. A. Villaluz; 1 male; México: Oaxaca: Municipio Asunción Ixtaltepec: Santiago Ixtaltepec | Coords:

16°41'18''N, 94°54'17''W | 213msnm | 22-xii-2012 | A Zaldívar Riverón, V Salinas Ramos; 3 females 4 males, México: Chiapas: Tuxtla Gutierrez: Copoya | Coords: 16.70608, -93.1173 | 1273msnm | 13-ix-2014 | leg. A. Zadívar-Riverón, D. Dubovikoff, V. S. De Jesús-Bonilla et al.; 1 female 1 male, México: Chiapas: Tierra y Libertad | Coords: 16.368928, -93.868474 | 687msnm | ix-2014; 1 male, Guatemala: Jutiapa: Asuncion Mita | 8-x-1993 | leg. X. Leiva; 1 male, Guatemala: Guatemala: Guatemala | 12-x-1985 | leg. M. Zepeda; 2 females, Guatemala: Santa Rosa: Estanzuela de Ixhuatán | 23-ix-2000 | leg. Carlos Avila Ramos; 1 male, Guatemala: Santa Rosa: Barberena: El Naranjito | 30-x-1990 | leg. C. MacVean; 1 female, Guatemala: Quetzaltenango: Finca Lozano | 1-vii-1991 | leg. E. Lozano; 1 male, Guatemala: Jalapa: Mataquescuintla: Finca Buena Vista | |4-xii-1996 | D. Rodríguez; 1 male, Guatemala: Jalapa: Mataquescuintla | 15-ix-1991 | leg. Claudia Maza; 1 male, Guatemala: Guatemala: Puerta parada | 19-vii-1984 | leg. J. Schuster; 1 male, Guatemala: Escuintla: Palín | 16-x-1999 | leg. R. Juarez; 1 male, Guatemala: Coatepeque: Finca Santa Gertrudis | 21-v-1983 | leg. Hazard.

Diagnosis: This species can be distinguished from the remaining species of the genus by having the following combination of characters: (1) elevated pronotal crest, (2) green ground colour, (3) pronotal crest and lateral carina black, (4) yellow or lemon-yellow pale portion of antenna, and (5) posterior margin acute-angulate.

Distribution: Southern Mexico to Guatemala.

Remarks: The validity of this species was previously uncertain. The original figure of Bruner (1907) was poor or was based on a specimen with a low pronotal crest. Hebard (1932) records that the pronotal crest in this species is high, resembling *T. varipennis*. The examination of more populations from southern Mexico and Central America show that it has a high pronotal ridge.

***Romalea gutturosa* (Bolívar 1901), comb. n. (Figure 12a-b)**

Taeniopoda gutturosa Bolívar 1901: 268; Kirby 1910: 372; Hebard 1924: 269; COPR 1982: 115; De Jesús-Bonilla et al. 2017: 606, 608, 611.

Taeniopoda aurantia Bruner 1907 (synonym)

Description: Size (Head-tegmina mm): Females 53.75-42.77 mm, males 46.19-42.86 mm **Head**: Scape and pedicel black; first1 11-15 antennal segments scarlet red, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons scarlet red or dark orange; coronal suture, facial carina and epistomal margin same colour of frons or slightly lighter. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Pronotal disk and lateral lobes scarlet red to dark orange. Median carina rugose or slightly rugose. Lateral carinas rugose. Pronotal crest and lateral carinas black. Sides of pronotal crest, and lateral carinas black. Posterior margin acute-angulate. **Tegmina**: Veins and venilets brown or yellowish brown. **Abdomen**: Tergites black and grey, with brown yellowish dorsomedial line. Sternites brown to red. **Hind leg**: Medial area of femur red, orange or brownish. Carinula and keel same colour of medial area. Upper marginal area same colour of carinula and keel or black; lower marginal area black, or same colour of lower carinula and keel. Semilunar process black, cover plate, insertion of extensor tibia black. **Male genitalia**: Posterior margin of the endophallus plate rounded. Ancora of epiphallus angular shaped with interior orientation. Epiphallus bridge sculpture strong developed. Margin of lateral plate of epiphallus rounded.

Diagnosis: This species can be recognized by the following combination of characters: (1) elevated pronotal crest, (2) scarlet red to dark orange ground colour, (3) pronotal crest and

lateral carina black, (4) scarlet red to orange red pale portion of antenna, and (5) posterior margin acute-angulate.

Type material examined: 1 female, *Taenipoda gutturosa* Allolectotype *C S* Carbonell 1966 | Escuintla Guatemala | MNCN Cat. Tipos No 7244 | MNCN_Ent 147347 (syntype); 1 male, *Taenipoda gutturosa* Allolectotype *C S* Carbonell 1966 | Escuintla Guatemala | MNCN Cat. Tipos No 7244 | MNCN_Ent 147346 (syntype).

Material examined: 2 females, Guatemala: Santa Rosa: Estanzuela de Ixhuatán | 24-ix-2000 | leg. Carlos Avila Ramos; 1 male, Guatemala: Santa Rosa: El Naranjito | 8-xi-1989 | leg. R. Perez; 1 male, Guatemala: Santa Rosa: El cerrinal | i-1993| leg. A. C. Bailey, J. Monzon; 1 male, Guatemala: Santa Rosa: Barberena: El Naranjito | 2-viii-1989 | leg. R. Perez; 3 males, Guatemala: Santa Rosa: Barberena: El Cerinal | i-1993 | leg. A.C. Bailey, J. Monzon; 1 female 1 male, Guatemala: Retalhuleu: Finca Los Brillantes | 14.55754158, -91.618463 | 14-v-2016 | leg. V.S. De Jesús-Bonilla, Eliam Percha; 1 female, Guatemala: Quetzaltenango: Hda Batzá | 6-vii-1985 | leg. M. R. Saenz; 1 male, Guatemala: Peten: Sayaxché | 7-vii-1975 | leg. José M; 1 male, Guatemala: Guatemala: San Miguel Petapa | 8-x-1988 | leg. C. Taracena; 1 male, Guatemala: Guatemala: Amatitán |xii-78 | leg. M. C. Vean; 1 male, Guatemala: Suchitepequez: Cuyotenango: La encantadora | 26-viii-1978 | leg. Martin Minundo; 2 females 2 males, El Salvador: Depto. La libertad | Coords: 13°40'59.68"N, 89°17'0.63"W | 341msnm | 14-viii-2009 | leg. L. Barrientos-Lozano.

Distribution: Central America, in Guatemala and El Salvador.

Remarks: It is a species close to *T. citricornis*, it can be distinguished by the first with the above key. Its distribution is more restricted than *T. citricornis*.

***Romalea varipennis* (Rehn 1905), comb. n. (Figure 13a-c)**

Taeniopoda varipennis Rehn 1905: 410; Bruner 1907: 237; Hebard 1924: 271; Hebard 1932: 271; COPR 1982: 115; Maes 1998: 115; Rowell 2013: 108; De Jesús-Bonilla et al. 2017.

Taeniopoda flavida (Bruner 1907) (synonym)

Description: Size (Head-tegmina mm): Females 59.54-43.67 mm, males 55.34-46.91 mm **Head**: Scape and pedicel black; first1 12-15 antennal segments yellow to orange, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons green yellowish to orange; coronal suture, facial carina and epistomal margin yellowish to orange. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Pronotal disk and lateral lobes orange yellowish to greenish yellow. Median carina slightly rugose and polished. Lateral carinas rugose. Pronotal crest, lateral carinas and posterior margin orange to orange yellowish, lighter than rest of pronotum. Posterior margin acute-angulate. **Tegmina**: Veins and venilets green yellowish. **Abdomen**: Tergites brown and green, with brown yellowish dorsomedial line. Sternites green to orange. **Hind leg:** Medial area of femur green yellowish to orange yellowish. Carinula and keel same colour of medial area. Upper marginal area same colour of carinula and keel; lower marginal area same colour of lower carinula and keel with black dots series. Semilunar process black, cover plate and insertion of extensor tibia muscle black or same colour of upper marginal area. **Male genitalia**: Posterior margin of the endophallus plate sub-angular or rounded. Ancora of epiphallus angular or lobiform shaped with right or exterior orientation. Epiphallus bridge sculpture strong or slightly developed. Margin of lateral plate of epiphallus sub-angular.

Diagnosis: This species can be distinguished by having the following combination of characters: (1) elevated pronotal crest, (2) Ground colour orange yellowish to greenish yellow, (3) pronotal crest and lateral carinas lighter than rest of pronotum, and (4) posterior margin

acute-angulate.

Type material examined: 1 male, *Taeniopoda varipennis* Rehn Hebard Collection | *Taeniopoda veripennis* Rehn H300 Type | Central America | Figured (syntype); 1 male, *Taeniopoda vaipennis* | Central America (paratype); 1 female, *Taeniopoda varipennis* | Gulf of Nicoya Costa Rica (homotype).

Other material examined: 1 female, Costa Rica: Guanacaste: Bagaces, Est. Palo Verde | Coords: 10.3491, -85.3523 | 10msnm | viii-1991 | leg. D. Acevedo; 1 female 1 male, Costa Rica: Guanacaste: Bagaces, Est. Palo Verde | Coords: 10.3491, -85.3523 | 1-vii-1991 | leg. G. Dauphin; 2 males, Costa Rica: Guanacaste: Bagaces, Est. Palo Verde | Coords: 10.3491, -85.3523 | 12.24-viii-1992 | leg. U. Chavarria; 1 female, Costa Rica: Guanacaste: La Cruz, Santa Elena Finca Jenny | Coords: 10.8655, -85.5735 | 9-vii-1993 | leg. E. Araya; 1 female 2 males, Costa Rica: Guanacaste: Liberia, PN Sta Rosa | Coords: 10.8364, -85.6155 | 6-vii-1978 | leg. D. Janzen; 1 male, Costa Rica: Guanacaste: Liberia, Sector Las Pailas | Coords: 10.7768, -85.3519 | 24-viii-1992 | leg. C. Cano; 1 male, Costa Rica: Guanacaste: Nicoya: Barra honda, Los mesones | Coords: 10.1701, -85.3508 | 1-vii-1995 | leg. M. Reyes; 5 females 10 males, Costa Rica: Puntarenas: A 3km S de Curré | Coords: 8.9754, -83.3036 93msnm | 25-vi-2015 | leg. A. Zaldívar-Riveron, J.J. Martínez, V. Salinas-Ramos, V.S. De Jesús-Bonilla; 1 male, Costa Rica: San José: San Antonio de Escazú | leg. WGE; 1 female, Costa Rica: Puntarenas: Garabito, Estación Quebrada Bonita | 9.76745, -84.6081 | 1-vi-1992 | leg. J. C. Saborio; 5 female 2 female, Camoapa Nicaragua; 1 female 3 males Ciruelas Costa Rica | vii-15-1915 | leg. A. Alfaro; 1 male, Ciruelas Costa Rica | vi-19123| leg. A. Alfaro; 1 male, Ciruelas Costa Rica | leg. A. Alfaro; 5 females 6 males, Gulf of Nicoya Costa Rica; 1 female, Nicaragua; 1 female, Orotinac Costa Rica | x-12-1915 | In Cementery #58 | leg. A. Alfaro; 1 female, Parismina C.R. | vii,26,’28 5m | leg. M. Valerio; 1 male, Pozo Azul Costa

Rica (underwood); 1 female 1 male, San José Costa Rica (Underwood); 2 females, San José Costa Rica 19 | leg. A. Alfaro; 1 male, San José Costa Rica | 1161m | leg. P. Biolley; 1 female, San José, Costa Rica; 3 males, Ujurras de Terraba Costa Rica | ix-10-07 | leg. M. A. Carriker Jr.

Distribution: Nicaragua, Costa Rica and Panama.

Remarks: Is the sister species of *T. reticulata*, in Costa Rica its distributions are separated by the mountain ranges that cross the country centrally.

***Romalea reticulata* (Fabricius 1781) (Figure 14)**

Gryllus reticulatus Fabricius 1781: 362; Donovan 1800

Acheta reticulata Fabricius 1787, 231

Taeniopoda reticulata Kirby 1910: 372, Hebard 1924: 103; Rowell 2013: 106; De Jesús-Bonilla et al. 2017: 600-617.

Taeniopoda maxima (Bruner 1907)

Description: Size (Head-tegmina mm): Females 61.66-51.15 mm, males 57.90-51.18 mm **Head**: Scape and pedicel black; first1 10-14 antennal segments yellow to orange, the rest of antennal segments black. Fastigium strong declivent. Vertex, fastigium and frons purple to black; coronal suture, facial carina and epistomal margin crimson to purple. **Pronotum**: With a median carina elevated at least 0.3 times total height of pronotum, forming an elevated pronotal crest. Pronotal disk and lateral lobes purple to black. Median carina slightly rugose and polished. Lateral carinas heavy rugose. Pronotal crest and lateral carinas dark purple to black. Posterior margin acute-angulate. **Tegmina**: Veins and venilets red brown to purple.

Abdomen: Tergites and sternites purple to black. **Hind leg:** Medial area of femur purple. Carinula and keel same colour of medial area, black at apex. Upper marginal area same colour of carinula and keel or black; lower marginal area same colour of lower carinula and keel with black dots series. Semilunar process black and cover plate black; insertion of extensor tibia muscle black or grey. **Male genitalia**: Posterior margin of the endophallus plate rounded. Ancora of epiphallus angular or lobiform shaped with exterior orientation. Epiphallus bridge sculpture strong or slightly developed. Margin of lateral plate of epiphallus rounded.

Diagnosis: This species can be recognized by the following combination of characters: (1) elevated pornotal crest, (2) ground colour purple to black, (3) pronotal crest and lateral carinas darker than rest of pronotum, and (4) posterior margin acute-angulate.

Type material examined: 1 male, *Taeniopoda maxima* | Type H297 | Limon, Costa Rica | leg. M.A. Carriker, Jr. (syntype).

Material examined: 1 male, Costa Rica: Puntarenas: Cabo Blanco: Est. San Miguel | 173174, 411412 | INBio 1651349 | leg. M. Ramírez; 1 female, Costa Rica: Limón: Talamanca, Sixaola | 9.632582, -82.659053 | 7-viii-1992 | INBio 803105 | leg. K. Taylor; 2 females 3 males, Costa Rica: Limón: Puerto Viejo de Talamanca | Coords: 9.652882, -82.739620 | 1 msnm | 9-ix-2014 | leg. V.S. De Jesús-Bonilla; 1 female, Costa Rica: Limón: Pococi, Colorado | 10.64405, -83.742 | 16-vii-1993 | INBio 1685036 | F. Araya; 4 females 4 males, Costa Rica: Limón: Limón, La cieneguita | Coords: 9.982908, -83.031418 | 1msnm | 9-ix-2014 | leg. V.S. De Jesús-Bonilla; 1 male, Costa Rica: Limón: Est. Hitoy-Carere | 184200, 643300 | xi-1990 | INBio 269359 | leg. G. Carballo; 5 female 6 male, Costa Rica: Limón: Cahuita | Coords: 9.734267, -82.827943 | 1 msnm | 9-ix-2014 | V.S. De Jesús-Bonilla; 1 male, Costa Rica: Limón; 1 male, Costa Rica: Heredia: Sarapiquí, La Virgen | 10.40126, -84.0493 | 1-x-1990 | INBio 526096 | leg. R. Aguilar; 1 male, Costa Rica: Heredia: Sarapiquí, La Virgen |

10.40126, -84.0502 | 1-vii-1990 | INBio 1377644 | leg. A. Fernandez; 1 female, Costa Rica: Heredia: Braulio Carrillo: Est. Magsasay | 264600, 531100 | vii-1990 | INBio 299017 | leg. D. Acevedo; 1 female 2 male, Costa Rica: Alajuela: Los Chiles, Caño Negro | 10.89381, -84.7889 | 12-viii-1993 | INBio 1976539, 1976540, 1976542 | leg. K. Martinez; 1 male, Costa Rica: Alajuela: Los Chiles, Caño Negro | 10.89381, -84.7889 | 29-vi-1992 | INBio 439523 | K. Quesada; 1 female, Costa Rica: Alajuela: Los Chiles, Caño Negro | 10.401256, -84.049314 | 1-vi-1990 | INBio 257197 | leg. G. Carballo; 1 X, Costa Rica: Alajuela: Los Chiles, Caño Negro | 10.89381, -84.7889 | 29-vi-1992 | INBio 439524 | leg. K. Quesada; 1 female, Costa Rica: Alajuela: Los Chiles, Caño Negro | 10.89381, -84.7889 | 18-viii-1992 | INBio 696655 | leg. K. Martinez; 1 male, Costa Rica: Alajuela: Los Chiles, Caño Negro | 10.955344, -84.7496 | 12-ix-1993 | INBio 1947371 | leg. K. Quesada; 1 male, Carrillo Costa Rica | vii-ix-03; 1 female, Darien Panama 1914 | leg. J. Zetek; 1 female, Guapiles Costa Rica | vi-5-1909 | leg. P. P. Calvert; 1 female, La Emilia Costa Rica | xi-19-1909 | leg. P. P. Calvert; 1 male, San Carlos Costa Rica | leg. Schils & Burgdorf; 1 male, Santa Clara (Atl) | leg. C.R. P. Biolley; 1 female 4 males, Siquirres CR vii.2-3.1903 | leg. MA CarrikerJr; 1 male, Chontales Nicaragua Janson.

Distribution. Nicaragua, Costa Rica in the Caribbean slope, and Panama.

Remarks. This species has the southernmost geographic distribution for the genus.

Acknowledgments

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for their help at the Colección Nacional de Insectos (CNIN), IB UNAM. Specimens were collected in Costa Rica under the permit given by the Sistema Nacional de Áreas de Conservación (SINAC-SE_GASP-PI-R-109-2014; scientific passport 03460) to VSJB. This study was supported by grants given by the Consejo Nacional de Ciencia y Tecnología (CONACyT: convocatoria SEP-Ciencia Básica 2014, No. 220454; Red Temática del Código de Barras de la Vida) to AZR. This study is part of VSDB's PhD thesis, who received a PhD scholarship given by CONACyT. VSDB also thanks the Posgrado en Ciencias Biológicas, UNAM, for its support during his studies.

References

Amedegnato C (1977) Etude des Acridoidea Centre et Sud Americains (Catantopinae, Sensu Lato) Anatomie des Genitalia, Classification, Repartition, Phylogenie. Universite Pierre et Marie Curie, Paris. 385 pp.

Amédégnato C (1974) Les genres d'acridiens neotropicaux, leur classification par familles, sous-families et tribus. Acrida 3: 193–204.

Barrientos-Lozano L, Rocha-Sánchez AY, Buzzetti FM, Méndez-Gómez BR, Horta-Vega J V. (2013) Saltamontes y Esperanzas del Noreste de México. MAPorrúa (Ed), Ciudad de México. 388 pp.

Bolívar I (1901) El género *Taeniopoda* Stål. Boletín de la Real Sociedad Española de Historia Natural 1: 264–270.

Bruner L (1907) Biologia Centrali-Americana. Insecta: Orthoptera (Vol II). Taylor & Francis, London. 412 pp.

Brunner von Wattenwyl C (1893) Revision du systeme des Orthopteres et description des especes rapportees par M. Leon Fea de Birmanie. Annali del Museo civico di Storia naturale di Genova 2: 1–230.

Burmeister HC (1838) Handbuch der Entomologie 2 Handbuch der Entomologie. G. Reimer, Berlin, 397-756 pp. Available from: https://www.biodiversitylibrary.org/item/82123 (April 3, 2018).

Buzzetti FM, Barrientos-Lozano L (2011) Bioacoustics of some Mexican Orthoptera (Insecta: Orthoptera: Ensifera, caelifera). Bioacoustics 20: 193–213. doi: 10.1080/09524622.2011.9753643

Capinera JK, Scott RD, Walker TJ (2004) Field guide to grasshoppers, katydids, and crickets of the United States. Comstock Publishing Associates, New York. 249 pp.

Capinera JL (2008) Encyclopedia of entomology. Springer, Netherlands. 4346 pp.

Caudell AN (1903) Notes on Orthoptera from Colorado, New Mexico, Arizona, and Texas, with descriptions of new species. Proceedings of the United States National Museum 26: 775–809. doi: 10.5479/si.00963801.26-1333.775

Charpentier T de (1845) Orthoptera descripta et depicta. Lipsiae , 243 p. Available from: http://hdl.handle.net/2027/uiuc.2859184 (April 3, 2018).

COPR (1982) The Locust and Grasshopper Agricultural Manual. Centre for Overseas Pest Research, London. 690 pp.

De Jesús-Bonilla VS, Barrientos-Lozano L, Zaldívar-Riverón A (2017) Sequence-based species delineation and molecular phylogenetics of the transitional Nearctic–Neotropical grasshopper genus *Taeniopoda* (Orthoptera, Romaleidae). Systematics and Biodiversity

15: 600–617. doi: 10.1080/14772000.2017.1313792

Descamps M (1975) Étude du peuplement acridien de l’état de Veracruz (Mexique). Folia Entomológica Mexicana 31–32: 3–98.

Dirsh VM (1956) The phallic complex in Acridoidea (Orthoptera) in relation to taxonomy. Transactions of the Royal Entomological Society of London 108: 223-270.

Dirsh VM (1961) A preliminary revision of the families and subfamilies of Acridoidea (Orthoptera, Insecta). Bulletin of the British Museum (Natural History). 10: 351–419. doi: 10.5962/bhl.part.16264

Donovan E (1800) An epitome of the natural history of the insects of India, and the islands in the Indian seas. printed fo. London.

Drury D (1770) Illustrations of Natural History, wherein are exhibited upwards of 240 figures of Exotic Insects 1. by the Author, London.

Eades D (2000) Evolutionary Relationships of Phallic Structures of Acridomorpha (Orthoptera). Journal of Orthoptera Research 9: 181–210. Available from: http://www.jstor.org/stable/3503648.

Eades DC, Otte D, Cigliano MM, Braun H (2016) Orthoptera Species File. Version 5.0/5.0. 2016: 536. Available from: http://orthoptera.speciesfile.org/HomePage/Orthoptera/HomePage.aspx (February 3, 2018).

Fabricius JC (1781) Species insectorum, exhibentes eorum differentias specificas, synonyma auctorum, loca natalia, metamorphosin, adjectis observationibus, descriptionibus. I. Carol Ernest Bohnii, Hamburg & Kiel.

Fabricius JC (1787) Mantissa insectorvm sistens eorvm species nvper detectas adiectis characteribvs genericis, differentiis specificis, emendationibvs, observationibvs. I. Christ. Gottl. Proft., Copenhague.

Fontana P, Buzzetti FM, Mariño-Pérez R, World Biodiversity Association (2008) Chapulines, langostas, grillos y esperanzas de México: guía fotográfica = Grasshoppers, locusts, crickets and katydids of Mexico: photographic guide. World Biodiversity Association, Verona, Italy, 272 pp. Available from: https://searchworks.stanford.edu/view/8802743 (February 3, 2018).

Hebard M (1916) Spring Orthoptera found on the islands in the vicinity of Charlotte Harbor, Florida. Entomological News 27: 14–21.

Hebard M (1924) A Revision of the Genus *Taeniopoda* (Orthoptera, Acrididae, Cyrtacanthacrinae). Transactions of the American Entomological Society 50: 253–274. Available from: https://www.jstor.org/stable/pdf/25077112.pdf (November 21, 2017).

Hebard M (1925) The Group Taeniopodae as Found in the United States (Orthoptera). Transactions of the American Entomological Society 51: 1–12. Available from: http://www.jstor.org/stable/25077119 (November 21, 2017).

Hebard M (1932) New Species and Records of Mexican Orthoptera. Transactions of the American Entomological Society (1890-) 58: 201–371. doi: 10.2307/25077283

Helms JB, Booth CM, Rivera J, Siegler JA, Wuellner S, Whitman DW (2003) Lubber grasshoppers, *Romalea microptera* (Beauvois), orient to plant odors in a wind tunnel. Journal of Orthoptera Research 12: 135–140. doi: 10.1665/1082-6467(2003)012[0135:LGRMBO]2.0.CO;2

Jones JC (1981) The Anatomy of the Grasshopper (*Romalea microptera*). Ch. Thomas

Publisher, Springfield. 281 pp.

Kevan DKM (1980) *Romalea guttata* (Houttuyn), name change for well-known eastern lubber grasshopper (Orthoptera: Romaleidae). Entomological News 91: 139–140. doi: 10.5962/bhl.part.3239

Kirby WF (1890) On the employment of names proposed for genera of Orthoptera, previous to 1840. Proceedings of the Royal Dublin Society 6: 556–597.

Kirby WF (1910) A synonymic catalogue of Orthoptera Vol III. Taylor & Francis, London, 674 pp.

Maes JM (1998) Insectos de Nicaragua I. Setab BOSAWAS, MARENA, León, Nicaragua. 1899 pp.

Mefferd CL, Hatch W, Burries RL, Whitman DW (2005) Plasticity in the length of the ovulation-oviposition interval in the lubber grasshopper *Romalea microptera*. Journal of Orthoptera Research 14: 31–32. doi: 10.1665/1082-6467(2005)14[31:PITLOT]2.0.CO;2

Ortega G, Márquez C (1988) Ortópteros de la Estación de Biología “Chamela” Jalisco (Insecta: Orthoptera). Anales del Instituto de Biología de la Universidad Nacional Autónoma de México. Serie Zoología. 58: 327–340.

Palisot de Beauvois AMFJ (1817) Palisot de Beauvois. 1817. Insectes recueillis en Afrique et en Amerique. Faint et compagnie, Paris.

Pictet A, Saussure H (1887) Catalogue d’Acridiens. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 7: 331–376.

Rehn JAG (1903) A Contribution to the Knowledge of the Orthoptera of Mexico and Central America. Transactions of the American Entomological Society (1890-) 29: 1–34. doi:

10.2307/25076743

Rehn JAG (1904) Notes on Orthoptera from Arizona, New Mexico and Colorado. Proceedings of the Academy of Natural Sciences of Philadelphia 56: 562–575. doi: 10.2307/4062994

Rehn JAG (1905) A Contribution to the Knowledge of the Acrididæ (Orthoptera) of Costa Rica. Proceedings of the Academy of Natural Sciences of Philadelphia 57: 400–454. doi: 10.2307/4063032

Rehn JAG, Grant HJ (1961) A Monograph of the Orthoptera of North America (north of Mexico) V1. Monographs Of The Academy Of Natural Sciences Of Philadelphia 12: 1–257.

Rehn JAG, Grant Jr. HJ (1959) A Review of the Romaleinae (Orthoptera; Acrididae) Found in America North of Mexico. Proceedings of the Academy of Natural Sciences of Philadelphia 111: 271. Available from: https://www.jstor.org/stable/pdf/4064509.pdf (November 21, 2017).

Rehn JAG, Hebard M (1912) On the Orthoptera found on the Florida Keys and in extreme southern Florida, II. Proceedings of the Academy of Natural Sciences of Philadelphia 66: 373–412. doi: 10.2307/4063465

Richman DB, Lightfoot DC, Sutherland CA, Fergurson DJ (1993) A manual of the grasshoppers of New Mexico (Orthoptera: Acrididae and Romaleidae). New Mexico State University, New Mexico. 112 pp.

Rivera García E (2006) An annotated checklist of some orthopteroid insects of Mapimi Biosphere Reserve (Chihuahuan desert), Mexico. Acta zoologica Mexicana n. s. 22: 131–149. Available from: http://www.scielo.org.mx/pdf/azm/v22n3/v22n3a11.pdf (April 4, 2018).

Roberts HR (1941) A Comparative Study of the Subfamilies of the Acrididae (Orthoptera) Primarily on the Basis of Their Phallic Structures. Proceedings of the Academy of Natural Sciences of Philadelphia 93: 201–246.

Rowell CHF (2013) The Grasshoppers (Caelifera) of Costa Rica and Panama. The Orthopterists' Society, San Martín de los Andes. 609 pp.

Saussure H (1859) Orthoptera Nova Americana (Diagnoses praeliminares). Revue et Magasin de Zoologie Pure et Appliquée 2: 201–212, 315–317, 390–394.

Schowalter TD (2018) Biology and Management of the Eastern Lubber Grasshopper (Orthoptera: Acrididae). Journal of Integrated Pest Management 9. doi: 10.1093/jipm/pmy004

Scudder SH (1901) Alphabetical index to North American Orthoptera described in the eighteenth and nineteenth centuries. Boston Society of Natural History, Boston. 456 pp.

Scudder SH, Cockerell TDA (1902) A first list of the Orthoptera of New Mexico. Proceedings of the Davenport Academy of Natural Sciences 9: 1–60.

Serville JGA (1831) Revue méthodique des Insectes de l'ordre des Orthoptères. Annales des sciences naturelles: comprenant La physiologie animale et végétale, l'anatomie comparée des deux règnes, la zoologie, la botanique, la minéralogie et la géologie 22: 28–65, 134–167, 262–292.

Serville MA (1838) Histoire naturelle des insectes. Orthoptères. Fonderie de fain, Paris.

Snodgrass (1935) Principles of insect morphology. Mc Grw Hill, New York. 667 pp.

Stål C (1855) entomologiska notiser. Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlinger 12: 343–353.

Stål C (1873) Recencio Orthopterorum. Revue critique des Orthoptères décrits par Linné, de Geer et Thunberg. Norstedt & Söner, Stockholm.

Stauffer TW, Whitman DW (2007) Divergent oviposition behaviors in a desert vs a marsh grasshopper. Journal of Orthoptera Research 16: 103–114. doi: 10.1665/1082-6467(2007)16[103:DOBIAD]2.0.CO;2

Stoll C (1813) Représentation exactement colorée d'après nature des Spectres ou Phasmes, des Mantes, des Sauterelles, des Grillons, des Criquets et des Blattes qui se trouvent dans les quatre parties du monde. J.C. Sepp et Fils, Amsterdan.

Thomas C (1873) Synopsis of the Acrididae of North America Part I. In: Hayden F V. (Ed), V. Zoology and Botany. Government Printing Office, Washington.

Thunberg CP (1815) Hemipterorum Maxillosorum genera illustrata plaurimisque novis speciebus ditata ae descripta. Mémoires de l'Académie impériale des sciences de St.-Pétersbourg 5: 211–301.

Walker F (1870) Catalogue of the specimens of Dermaptera Saltatoria and supplement of the Blattariæ in the collection of the British museum III. Trustees of the Brirish Museum, London.

Whitman DW, Loher W (1984) Morphology of male sex organs and insemination in the grasshopper *Taeniopoda eques* (Burmeister). Journal of Morphology 179: 1–12. doi: 10.1002/jmor.1051790102

Whitman DW, Richardson ML (2010) Necrophagy in Grasshoppers: *Taeniopoda eques* Feeds on Mammal Carrion. Journal of Orthoptera Research 19: 377–380. doi: 10.1665/034.019.0228

Figure legends

Figure 1. Morphological features of the *Romalea* genus: a) head, b) body, c) endophallus, and d) ephiphallus.

Figure 2. Pronotum and antennae variation in the *Romalea* genus: Scheme representing a) pronotum with low pronotal crest, b) pronotum with high elevated pronotal crest; antenna with c) antennal segments without black ring at apex, d) antennal segments with black ring at apex.

Figure 3. Adult female of *R. microptera*: a) lateral view of body, b) pronotum and head lateral view, c) pronotum and head lateral view.

Figure 4. Adult female of *R. tamaulipensis,* a) lateral view of body (holotype), b) pronotum in dorsal view, and c) apical margin of tegmina.

Figure 5. Adult female of *R. eques,* a) lateral view of body, b) pronotum in dorsal view, and c) apical margin of tegmina.

Figure 6. Adult male of *R. picticornis*: a) dorsal view of body (syntype), b) antenna with yellow colouration, and c) antenna with scarlet colouration.

Figure 7. Male of *R. obscura*: a) lateral view of body (syntype), b) dorsal view of pronotum, and c) lateral view of pronotum.

Figure 8. Lateral view of adult female of *R. centurio.*

Figure 9. Female of *R. auricornis*: a) lateral view of body, b) dorsal view of pronotum, and c) apical margin of tegmina.

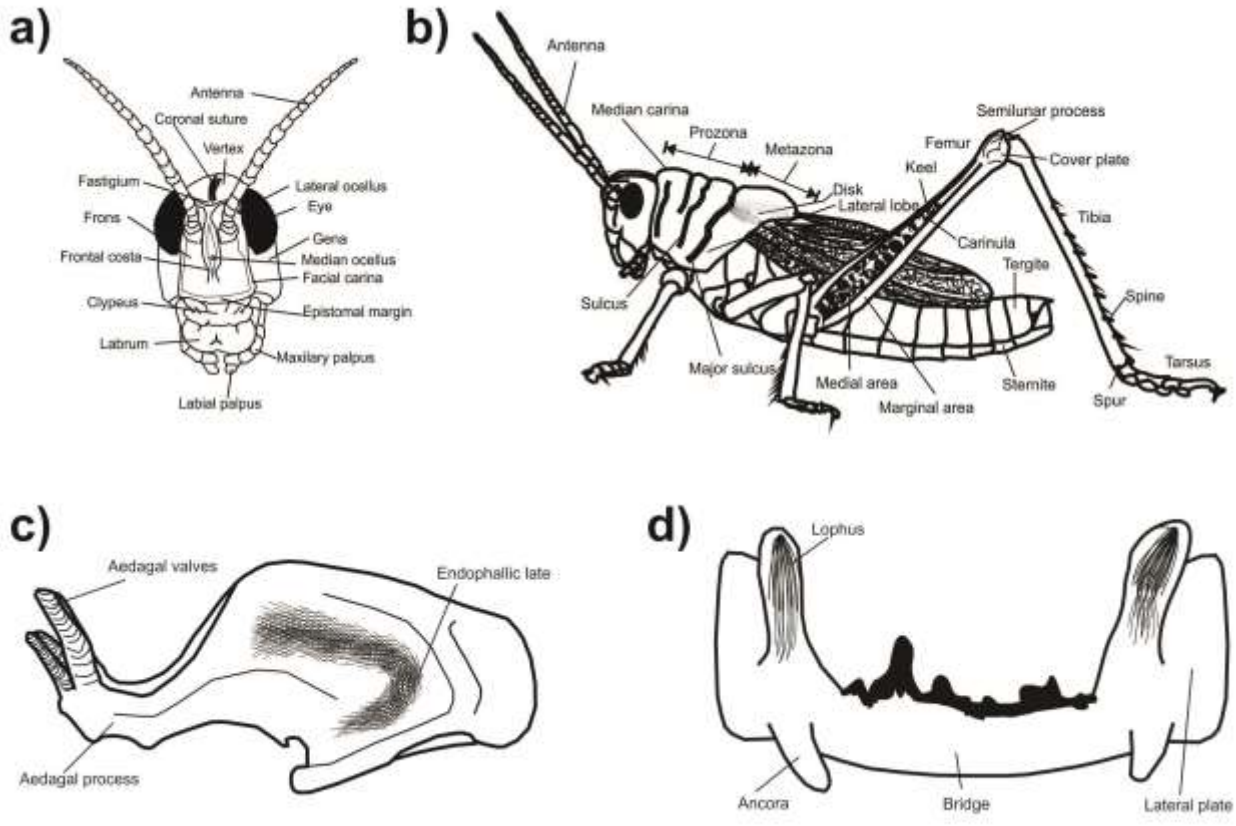
Figure 10. Male adult of *R.* sp.1: a) lateral view of body, b) dorsal view of pronotum, and c) apical margin of tegmina.

Figure 11. Lateral view of adult female of *R. citricornis.*

Figure 12. Lateral view of a) female of *R. gutturosa (*syntype), and b) lateral view of alive female.

Figure 13. Female of *R. varipennis:* a) lateral view of body (syntype), b) dorsal view of pronotum, and c) lateral view of pronotum.

Figure 14. Lateral view of adult female of *R. reticulata.*



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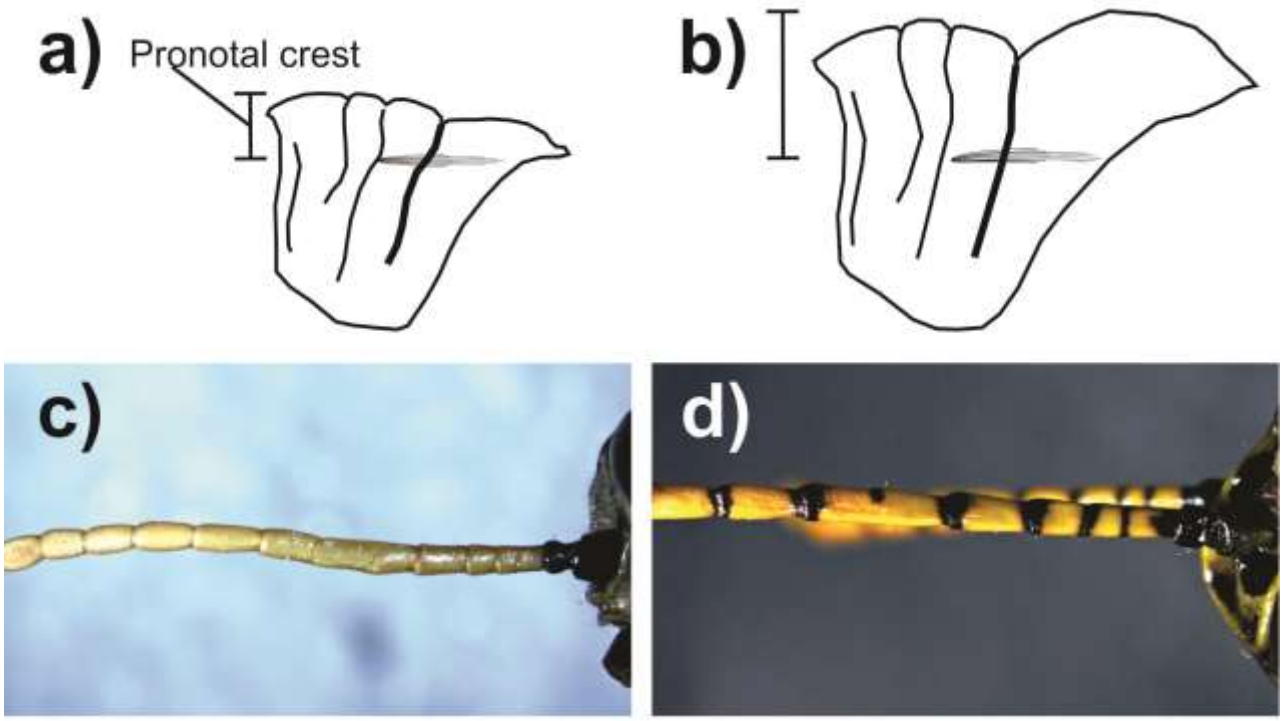

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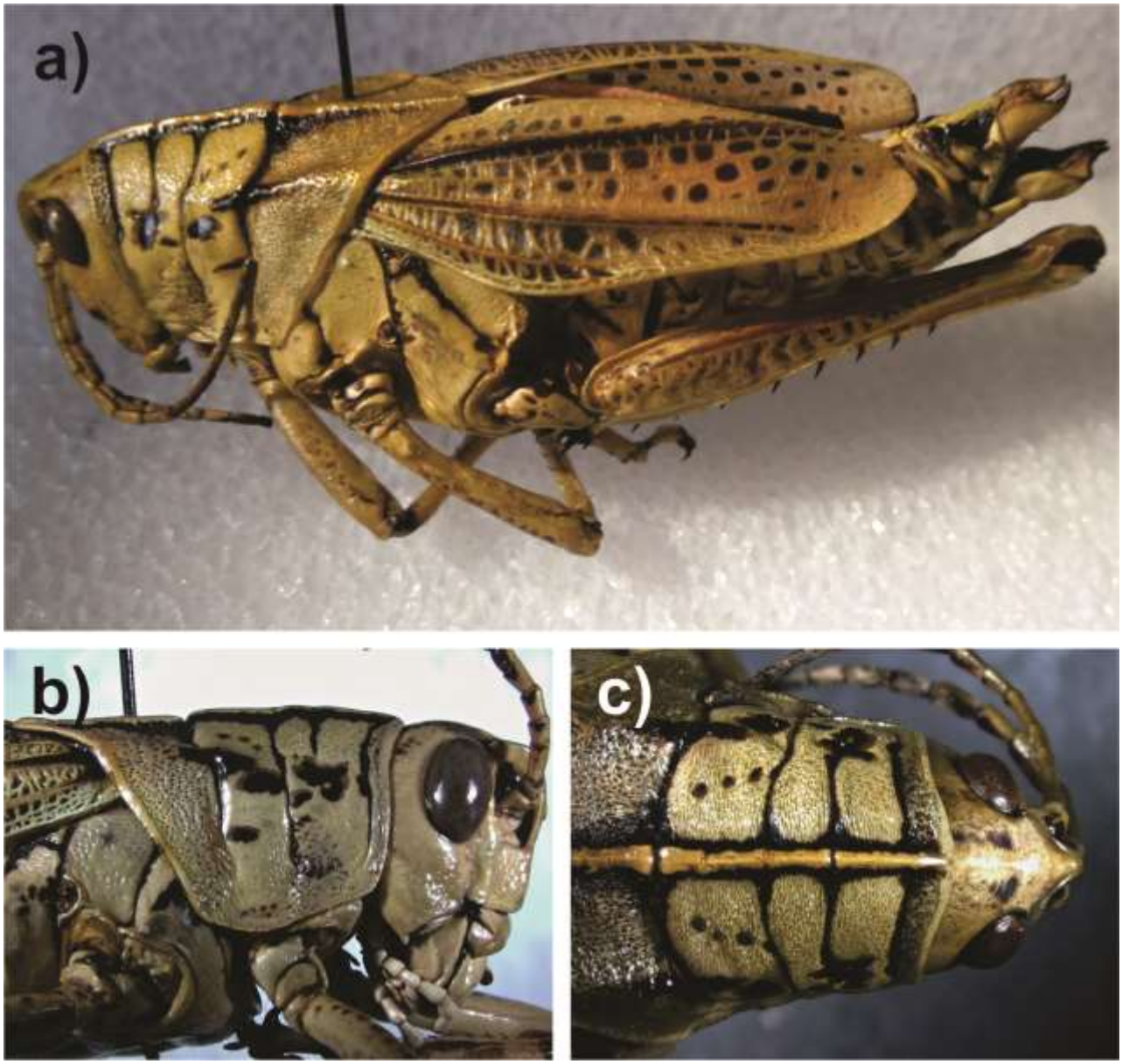

Figure 3. Adult female of *R. microptera*: a) lateral view of body, b) pronotum and head lateral view, c) pronotum and head lateral view.

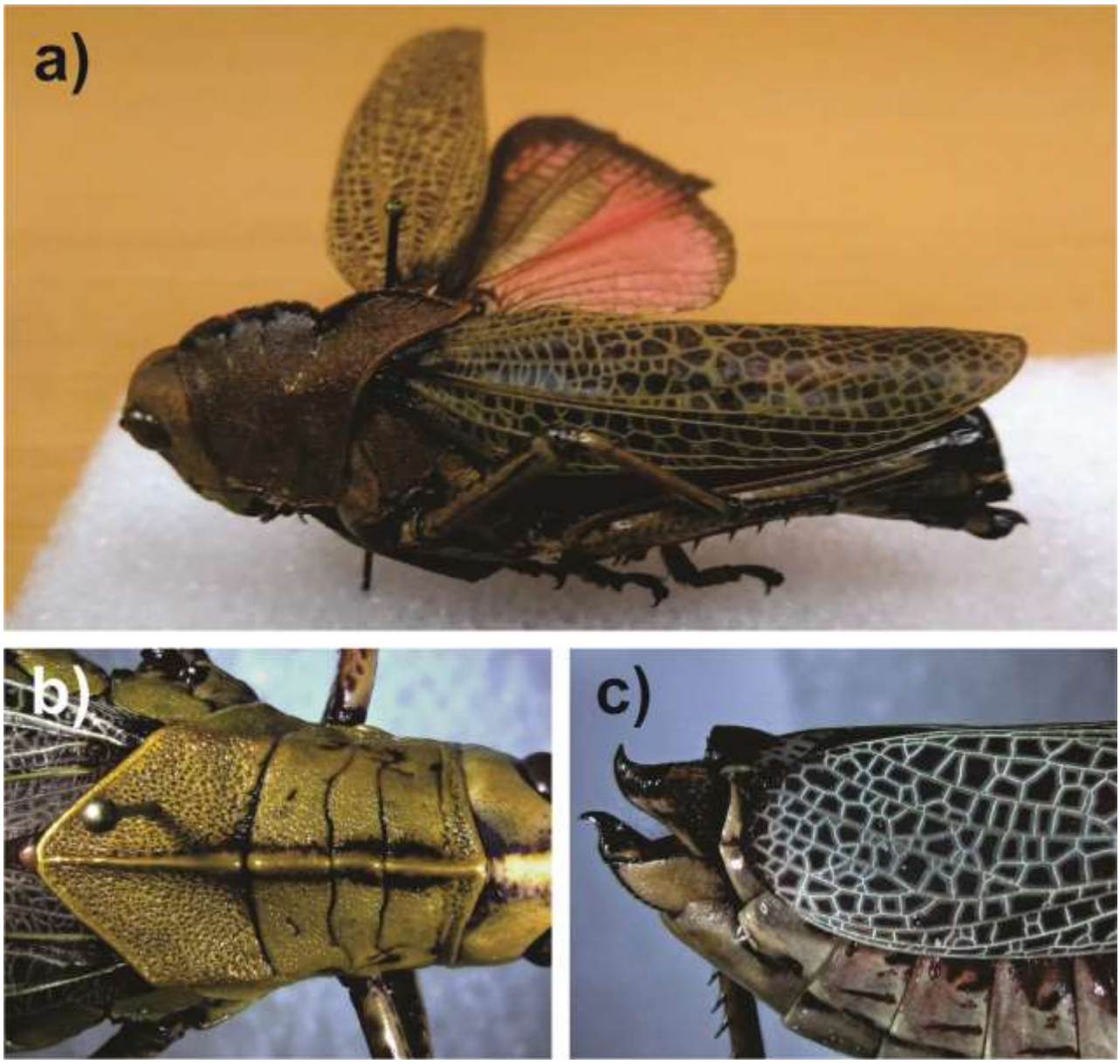

Figure 4. Adult female of *R. tamaulipensis,* a) lateral view of body (holotype), b) pronotum in dorsal view, and c) apical margin of tegmina.

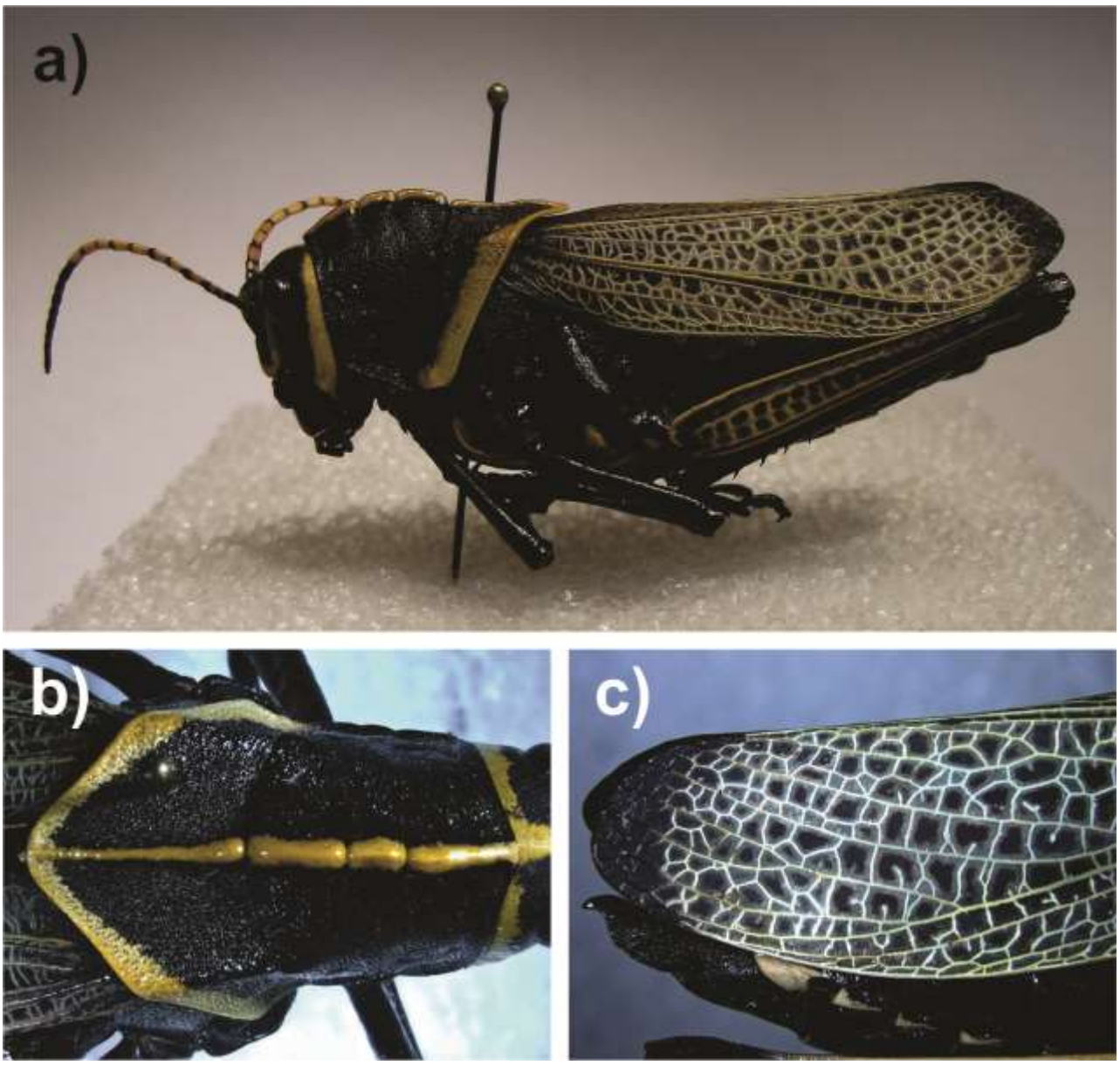

Figure 5. Adult female of *R. eques,* a) lateral view of body, b) pronotum in dorsal view, and c) apical margin of tegmina.

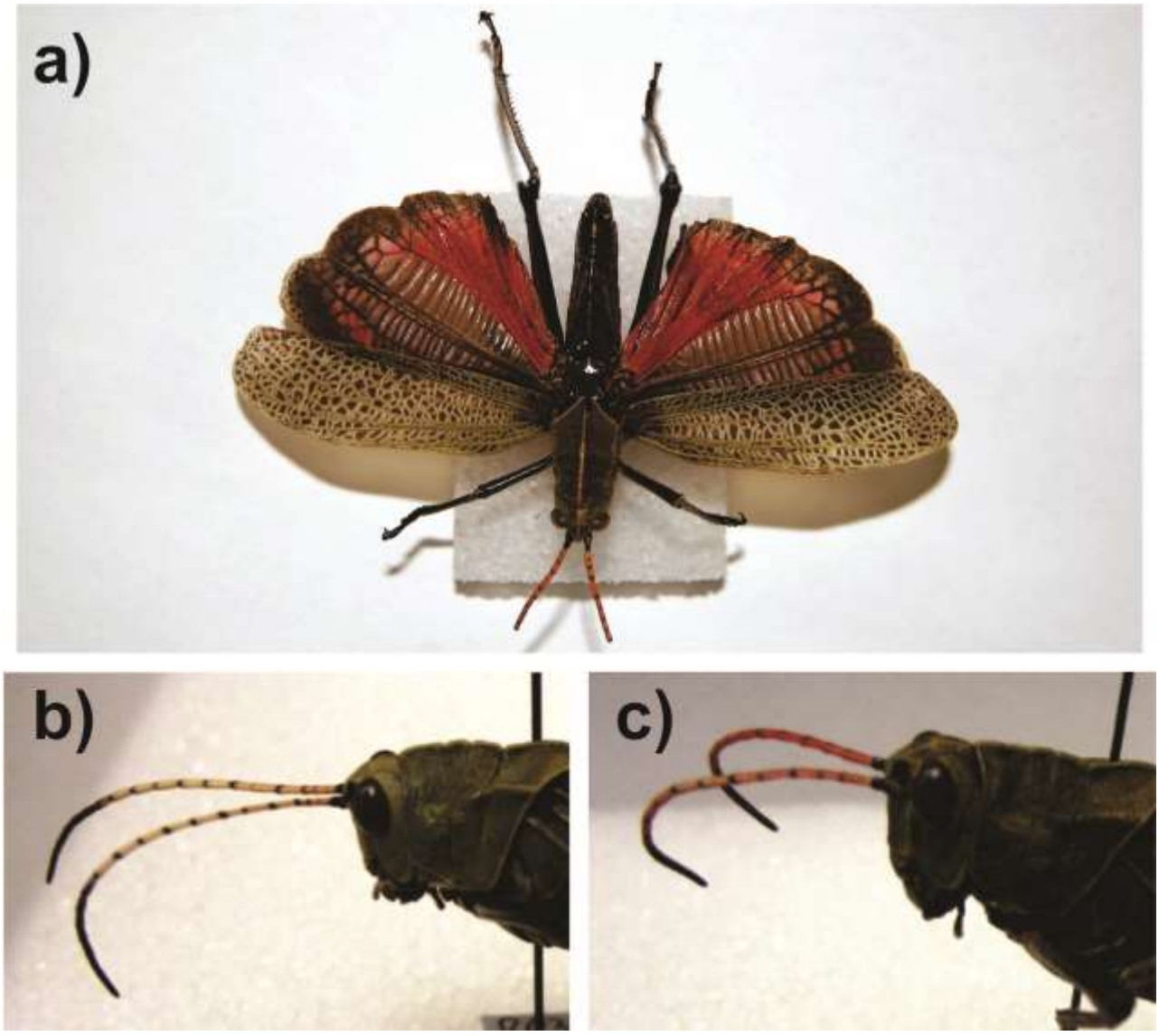

Figure 6. Adult male of *R. picticornis*: a) dorsal view of body (syntype), b) antenna with yellow colouration, and c) antenna with scarlet colouration.

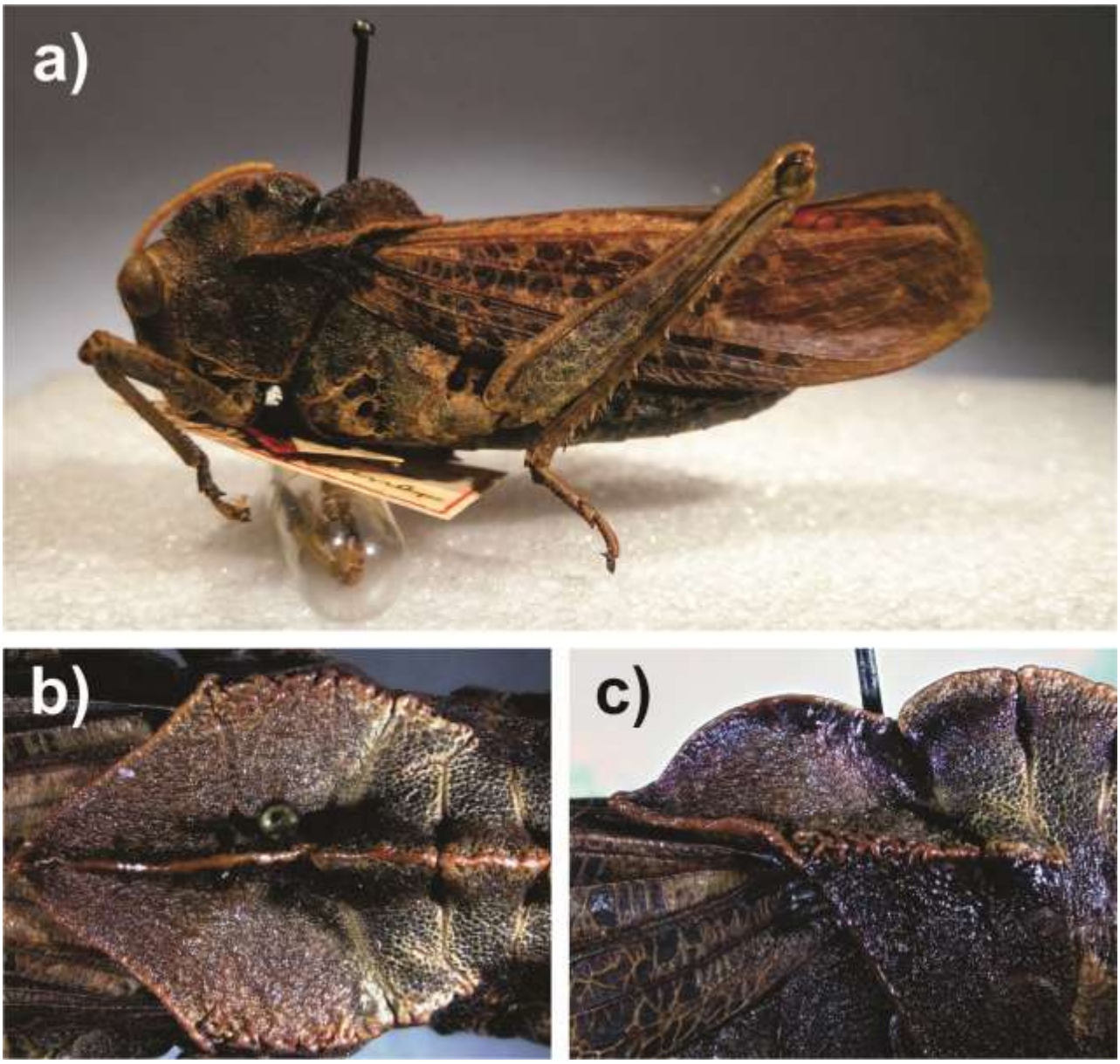

Figure 7. Male of *R. obscura*: a) lateral view of body (syntype), b) dorsal view of pronotum, and c) lateral view of pronotum.

Figure 8. Lateral view of adult female of *R. centurio.*

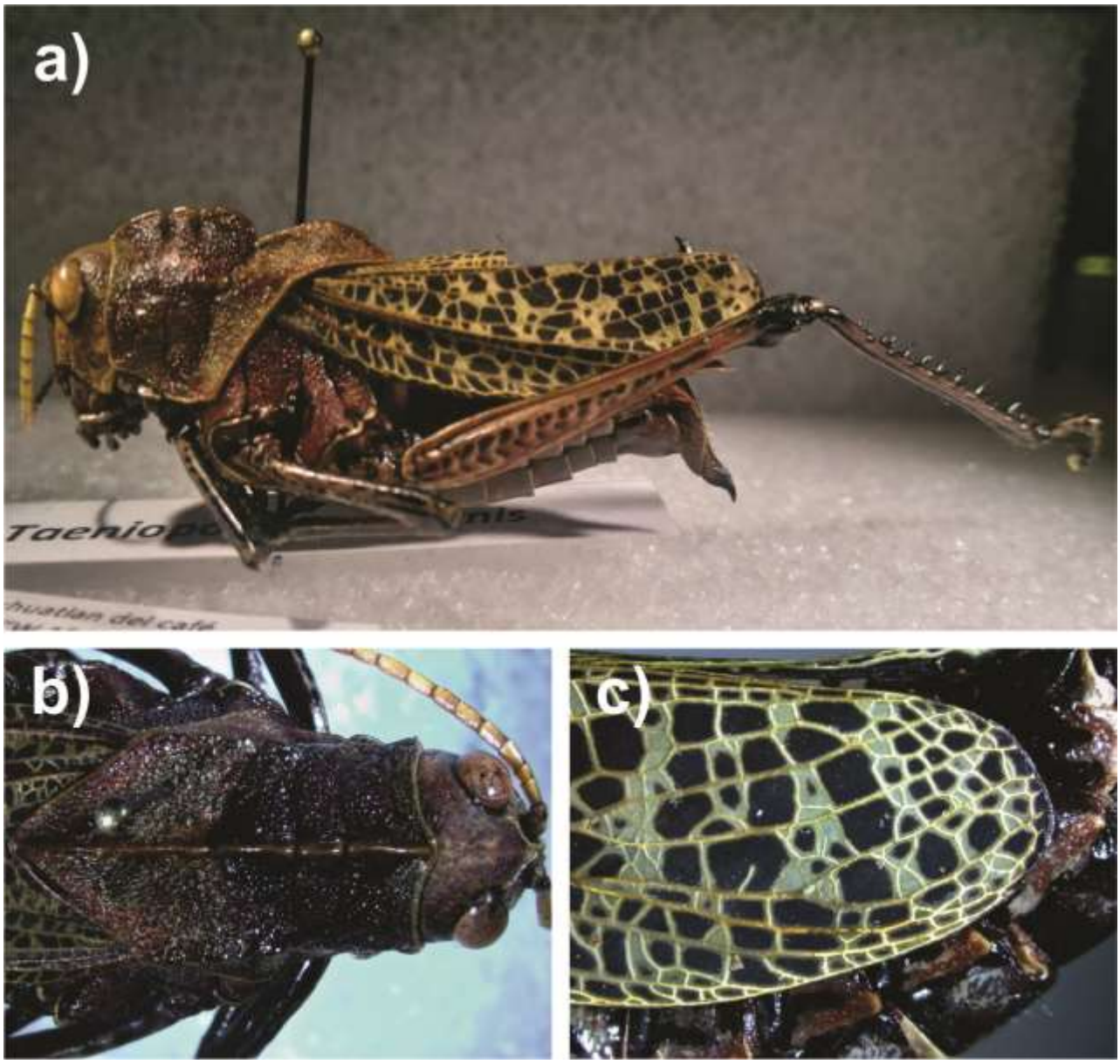

Figure 9. Female of *R. auricornis*: a) lateral view of body, b) dorsal view of pronotum, and c) apical margin of tegmina.

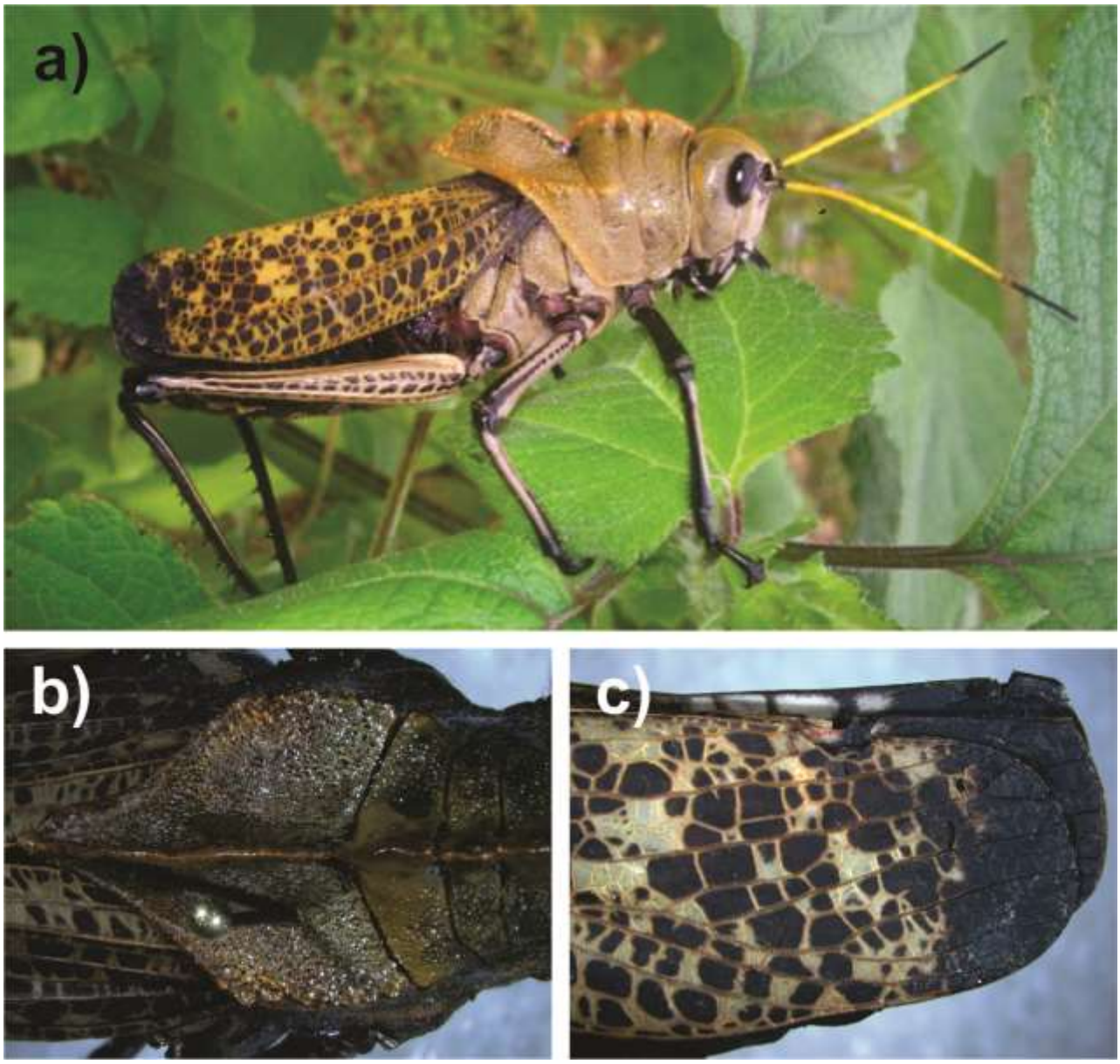

Figure 10. Male adult of *R.* sp.1: a) lateral view of body, b) dorsal view of pronotum, and c) apical margin of tegmina.

Figure 11. Lateral view of adult female of *R. citricornis.*

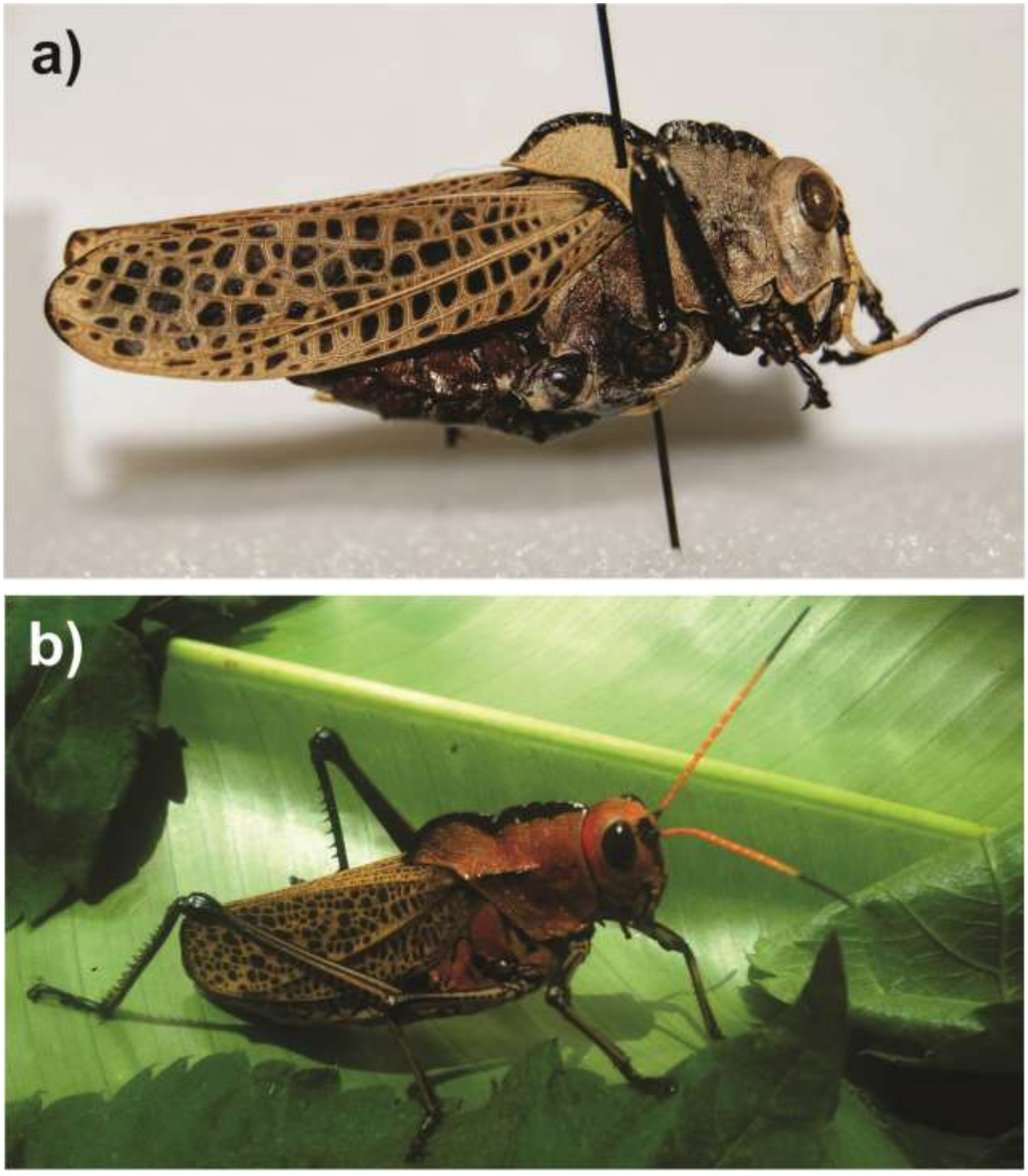

Figure 12. Lateral view of a) female of *R. gutturosa (*syntype), and b) lateral view of alive female.

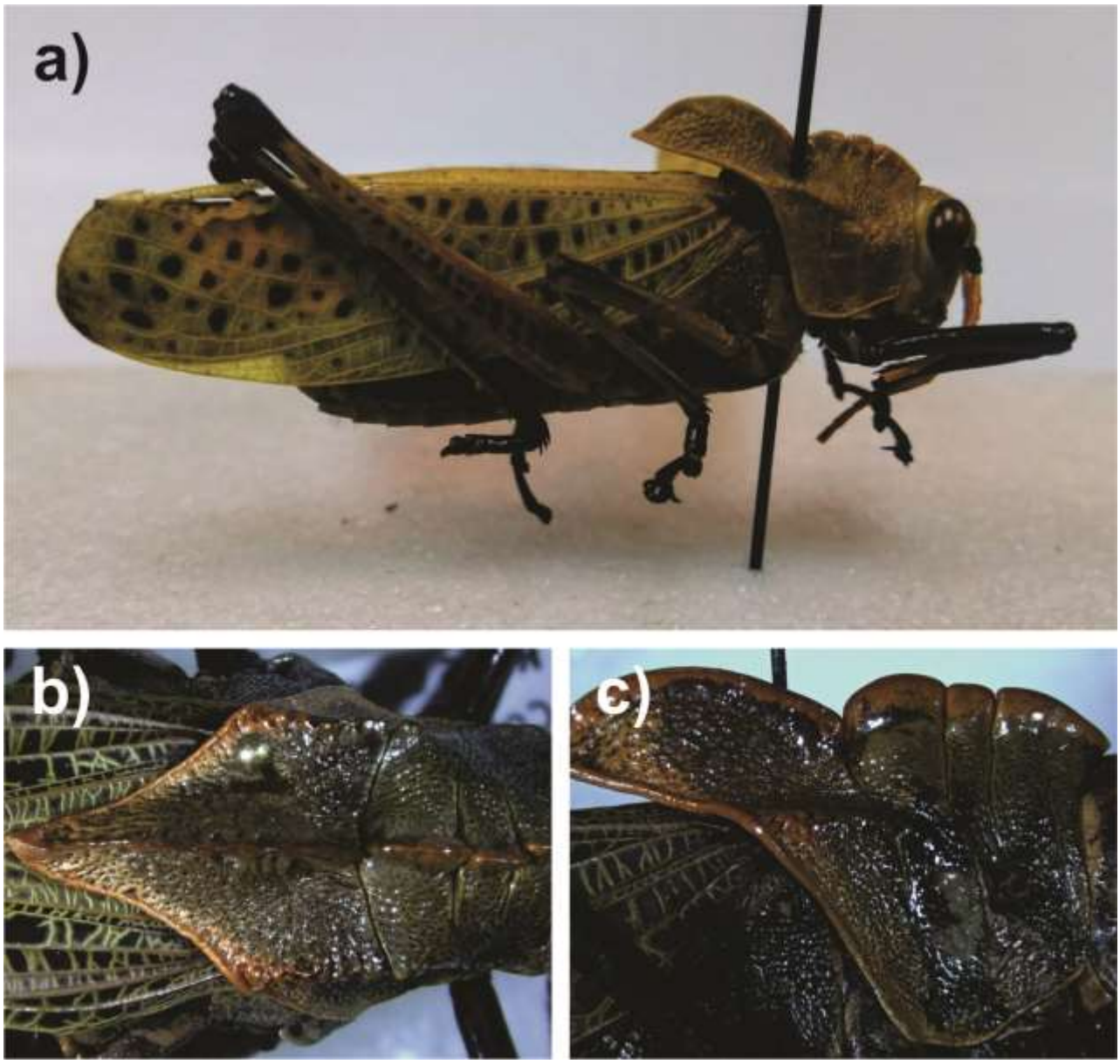

Figure 13. Female of *R. varipennis:* a) lateral view of body (syntype), b) dorsal view of pronotum, and c) lateral view of pronotum.

Figure 14. Lateral view of adult female of *R. reticulata.*

Discusión y conclusiones generales

Delimitación de especies en el género Taeniopoda

La delimitación de especies en el género *Taeniopoda* Stål se realizó utilizando evidencia morfológica, de marcadores puntuales y datos genómicos. Después de reevaluar las características morfológicas para la identificación de especies del género *Taeniopoda* (Hebard, 1924) se encontró que las características de color de cabeza, pronoto y tegminas, combinadas con características morfológicas del pronoto fueron de utilidad para identificar a la mayoría de las especies.

Los marcadores genéticos puntuales, en particular el marcador COI, han sido utilizados exitosamente para realizar delimitaciones de especies utilizando distintos enfoques (Pons *et al*., 2006; Ratnasingham & Hebert, 2007). En nuestro sistema de estudio estas delimitaciones tuvieron cierta congruencia con las asignaciones basadas en morfología. La congruencia entre las delimitaciones obtenidas no fue total, lo que puede deberse a que los marcadores mitocondriales pueden ser afectados por introgresión mitocondrial, sorteo incompleto de linajes, estructura genética o la presencia de pseudogenes (Dupuis, 2012; Song *et al*., 2014). Sin embargo, son una herramienta bastante útil para establecer hipótesis de especies basadas en marcadores puntuales que pueden ser sometidas a prueba con evidencia adicional.

El uso de marcadores genómicos contribuyó a una mayor resolución y soporte de las delimitaciones en el género *Taeniopoda*. Con los datos de 3RAD se delimitaron consistentemente las especies *R. microptera* (Palisot de Beauvois), *T. auricornis* (Walker), *T. centurio* (Drury), *T. obscura* Bruner, *T. varipennis* Rehn, *T. reticulata* (Fabricius), *T. citricornis* Bruner y *T. gutturosa* Bolívar. Se confirmó la coespecificidad de *T. picticornis* (Walker) y *T. stali* Bruner, por lo que se propone su sinonimización; la característica propuesta originalmente para separarlas, el color de la porción clara de la antena, es variación intraespecífica. La variación de color en ortópteros puede estar relacionada con factores genéticos, ecológicos, fisiológicos o etológicos (Dearn 1990, Song *et al*., 2017; Valverde & Schielzeth, 2015). En el caso de *T. stali* y *T. picticornis* la variación del color de la antena se encontraba incluso en individios de la misma población, por lo que es probable que la variación este relacionada con factores que afectan diferencialmente a cada individuo, por ejemplo, la cantidad de alimento consumido.

Las especies *T. eques* (Burmeister) y *T. tamaulipensis* Rehn plantean un escenario

más complejo, su aloespecificidad no se fue apoyada consistentemente y los análisis de agrupamiento bayesiano y de componentes principales sugieren la existencia de tres grupos genéticos diferenciados que mantienen flujo genético en el centro de México. Esto es compatible con un escenario en el que los especímenes asignados a *T. tamaulipensis* y *T. eques* podrían representar una especie clinal o especie anillo. Tomando un enfoque conservador, se decidió mantener el estatus específico de *T. tamaulipensis* y *T. eques* hasta que estudios a nivel poblacional y filogeográfico investiguen las causas de la estructura observada. Por otro lado, como ya se había reportado para *T. reticulata* y *T. varipennis* (Rowell, 2013), se encontró que dada la variación intra e interespecífica de la genitalia de los machos no es viable para la identificación de especies en el género *Taeniopoda*. Ante la vaguedad de la localidad tipo no se logró obtener material fresco para la obtención de secuencias de *T. bicristata* Bruner; sin embargo, la comparación de material tipo permitió establecer que *T. bicristata* es un sinónimo de *T. obscura*.

Historia evolutiva del género Taeniopoda y Romalea

En la última revisión del género *Taeniopoda* se sugiere que las especies forman tres grupos y se delinean algunas relaciones entre los taxones (Hebard, 1924); sin embargo, hasta antes del presente trabajo no se había hecho una prueba formal de dichas hipótesis. También se había sugerido una relación cercana con *Romalea* (Rehn & Grant, 1956a), sin probarla en un contexto filogenético. Los marcadores puntuales y los datos genómicos permitieron poner a prueba la relación entre *Taeniopoda* y *Romalea* Serville, se encontró que tienen una relación parafilética ya que *Romalea* está anidada dentro del clado con las especies de *Taeniopoda*.

Los marcadores puntuales (dos mitocondriales y dos nucleares) y datos genómicos permitieron determinar la existencia de tres clados mayores formados por las especies de *Romalea* y *Taeniopoda*. Estos clados tienen congruencia geográfica: un clado está formado por *T. eques*, *T. picticornis*, *T. stali* y *T. tamaulipensis*. Estas son especies con cresta pronotal baja, un anillo negro en la parte apical de los segmentos antenales y se distribuyen del centro de México al sur de Estados Unidos. Otro clado está formado por *T. gutturosa, T. citricornis, T. varipennis y T. reticulata*, que se distribuyen del sur de México hasta Panamá y tienen cresta pronotal alta sin un anillo negro en la parte aplical de los segmentos antenales. El tercer clado incluye a *R. microptera* que tiene la cresta pronotal baja, y las especies con cresta pronotal alta *T. obscura*, *T. auricornis*, *T. centurio* y una nueva especie de Guatemala; estas especies se distribuyen del sur de México a América Central, estas especies carecen

de un anillo en la parte apical de los segmentos antenales. Este último clado incluye a especies con gran disimilitud morfológica; por ejemplo *R. microptera* es una especie con cresta pronotal baja mientras que *T. obscura* presenta una cresta pronotal alta y un pronoto inusualmente robusto. Las relaciones internas en los tres clados principales se recuperan consistentemente con los marcadores puntuales y los datos genómicos, estos últimos resultaron en reconstrucciones con mejor soporte estadístico.

El análisis de tiempos de divergencia sugiere que el género *Taeniopoda* probablemente se originó durante el Mioceno; la posterior diversificación y origen de los tres clados mayores dentro de *Taeniopoda* coincide con las etapas recientes de la formación de la Faja Volcánica Trans-Mexicana (hace 7.5-3 millones de años) (Ferrari *et al*., 2012. La diversidad específica actual de *Taeniopoda* es reciente, teniendo su origen durante el Pleistoceno probablemente bajo la influencia de los eventos glaciales e interglaciales de este periodo, así como el reciente levantamiento de las cordilleras en Costa Rica y Panamá durante el mismo periodo (Bergoeing, 2006; Castillo, 1991).

Análisis de datos genómicos

Confirmamos la utilidad de los datos genómicos provenientes de 3RAD para probar los límites entre especies y resolver relaciones filogenéticas. También se comprobó que en el ensamble *de novo* el número mínimo de muestras en las que debe haber datos para retener un *locus* afectan la cantidad de *loci* y de datos faltantes en la matriz final. Encontramos que las secuencias con pocos *reads* y pocos *loci* compartidos con el resto de las muestras pueden sesgar los análisis y generar cambios en la topología o topologías con bajo sporte, por lo que es necesario desarrollar estrategias para retirarlas de los análisis finales.

Perspectivas

La delimitación de especies es parte primordial del estudio de la biodiversidad y se pueden utilizar distintos tipos de evidencia (Padial, 2010). El uso conjunto de datos morfológicos y genéticos o genómicos ha permitido la precisa delimitación de especies en distintos grupos, en particular se ha mostrado que el uso de datos genómicos mejora la resolución de las delimitaciones (e. g. Chen *et al*., 2017; Herrera & Shank, 2016; Yu *et al*., 2017). En este trabajo se ha utilizado evidencia morfológica, de marcadores moleculares puntuales e información a escala genómica. El uso de diferentes tipos de evidencia permitió delimitar

especies y reconstruir la historia evolutiva de las mismas. Actualmente el uso de marcadores moleculares puntuales sigue siendo pertinente como primera aproximación al estudio de la biodiversidad; asimismo, la gran cantidad de datos genómicos nos permiten obtener delimitaciones y reconstrucciones filogenéticas con mayor resolución. La morfología sigue siendo parte importante en la delimitación de especies, y en este trabajo se usó en conjunto con la evidencia genética y genómica. Con la información de marcadores genéticos puntuales se encontraron algunas inconsitencias atribuibles al sorteo incompleto de linajes o flujo genético; sin embargo, con el uso de datos genómicos la delimitación de especies y reconstrucciones filogenéticas fueron congruentes.

Los resultados de este trabajo son relevantes dada la importancia social y económica de las especies involucradas. La correcta identificación específica es fundamental para la proyección de planes de manejo en aquellas especies que pueden ser una plaga para los cultivos. Asimismo, en aquellos organismos que tienen utilidad alimentaria e incluso médica, la asignación específica permitirá un estudio adecuado de sus propiedades nutricionales y terapéuticas. La nueva especie descrita para Guatemala tiene las implicacioens citadas y adicionalmente representa un nuevo registro a tener en cuenta en inventarios zoológicos.

Conclusiones generales

- Las reconstrucciones filogenéticas con marcadores puntuales y datos genómicos muestran que el género *Taeniopoda* es parafilético respecto a *Romalea.* Se recuperó a las especies formando tres clados principales, un conformado por especies con cresta pronotal baja distribuidas del sur de Estados Unidos al centro de México, otro por especies con cresta pronotal alta que se distribuyen del sur de México hasta Panamá, y un tercero que incluye a *R. microptera* y especies con cresta poronotal alta que se distribuyen del sur de México a América Central.
- Se delimitaron doce especies originalmente asignadas a *Taeniopoda* y *Romalea* integrando la evidencia morfológica, así como con marcadores puntuales mitocondriales y nucleares y con datos genómicos nucleares obtenidos con la técnice de 3RAD.
- *Taeniopoda stali* es un sinónimo de *T. picticornis.*

- Las poblaciones asignadas originalmente a *T. auricornis* de Guatemala representan una nueva especie.
- *Taeniopoda eques* y *T. tamaulipensis* podrían representar una especie anillo, ya que sus poblaciones examinadas parecen variar gradualmente en "forma de bucle" a lo largo de su distribución geográfica.
- El origen y diversificación de especies en *Taeniopoda* ocurrió durante Mioceno - Plioceno. La diversidad de especies actual en el género se produjo durante el Pleistoceno, probablemente influenciada por eventos paleoclimáticos y la elevación de las cordilleras en Centroamérica.
- Al amplificar marcadores mitocondriales se detectó la presencia de pseudogenes.
- Los datos de 3RAD sirvieron para resolver totalmente las relaciones filogenéticas, incluso con gran cantidad de datos faltantes.
- Los parámetros de ensamble *de novo* de los datos de 3RAD tienen impacto en la cantidad de *loci* y datos faltantes obtenidos en las matrices.
- Se realizó una revisión taxonómica del grupo, la cual considera a *Taeniopoda* como un sinónimo de *Romalea* y se incluyen las siguientes especies: *Romalea auricornis* Walker **comb. n.**, *R. citricornis* (Bruner) **comb. n.**, *R. centurio* (Drury) **comb. n.**, *R. eques* Burmeister **comb. n.**, *R. gutturosa* (Bolívar) **comb. n.**, *R. microptera* (Palisot de Beauvois), *R. obscura* (Bruner) **comb. n.** (=*Taenipoda bicristata*), *R. picticornis* Walker **comb. n.** (=*Taeniopoda stali* **syn. n.**), *R. reticulata* (Fabricius) **comb. n.,** *R. tamaulipensis* (Rehn) **comb. n.** and *R. varipennis* (Rehn) **comb. n.** Se describe una nueva especie, *R.* sp. 1

Referencias generales

Amedegnato, C. (1977). *Etude des Acridoidea Centre et Sud Americains (Catantopinae, Sensu Lato) Anatomie des Genitalia, Classification, Repartition, Phylogenie.* Universite Pierre et Marie Curie. 385 pp.

Antonelli, A., & Sanmartín, I. (2011). Why are there so many plant species in the Neotropics? *Taxon*, 60, 403-414. https://doi.org/10.1111/jbi.12228

Avise, J. C. (2004). *Molecular markers, natural history and evolution*. Sinauer Associates Inc., Massachusetts, USA. 511 pp.

Barberousse, A., & Samadi, S. (2010). Species from Darwin onward. *Integrative Zoology*, 5, 187-197. https://doi.org/10.1111/j.1749-4877.2010.00204.x

Bergoeing, J. P. (2006). El Cuaternario en Costa Rica. Proposición Cronológica. Revista *Reflexiones*, 85, 207-226.

Brett, C. E. (1998). Sequence stratigraphy, paleoecology, and evolution: Biotic clues and responses to sea-level fluctuations. *Palaios*, 13, 241-262. https://doi.org/10.1043/0883-1351(1998)013<0241:SSPAEB>2.0.CO;2

Camargo, A., & Sites, J. (2013). Species Delimitation: A Decade After the Renaissance. En: Pavlinov, I (Ed), *The Species Problem - Ongoing Issues*, pp. 225-247, In Tech. https://doi.org/10.5772/52664

Capinera, J. L. (2008). *Encyclopedia of entomology*. Springer; Heidelberg, Germany. 4346 pp.

Carbonell, C. S. (1977). Origin, evolution and distribution of the neotropical acridomorph fauna (Orthoptera): a preliminary hypotesis. *Revista de La Sociedad Entomológica Argentina*, *36*, 153-175.

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, *22*, 4369-4383. https://doi.org/10.1111/mec.12413

Castillo, R. (1991). Geología de Costa Rica. En: Janzen, D. (Ed*) Historia Natural de Costa Rica* pp. 47-61. Costa Rica: Editorial de la Universidad de Costa Rica.

Chen, Y-T., Tseng, H-Y., Jeng, Y-C., Huang, W-S. y Lin, C-P. (2017). Integrated species delimitation and conservation implications of an endangered weevil *Pachyrhynchus sonani* (Coleoptera: Curculionidae) in Green and Orchid Islands of Taiwan. *Systematic Entomology,* 42, 796-813.

Chopard, L. (1920). Recherches sur la conformation et la developpement des derniers segments abdominaux chez les Orthepteres. *Insecta Rennes*, 10, 1-112. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201300453851

Cigliano, M. M., & Langle, C. E. (1998). Orthoptera. En: Morrone J. J. & Coscarón, S. (Eds), *Biodiversidad de Artrópodos Argentinos*, pp. 67–83. Ediciones SUR; La Plata, Argentina.

Contreras-Ramos, A. & Goyenechea, I. (2007). *La sistemática, base del conocimiento de la biodiversidad.* En: Contreras-Ramos, A., Cueva Cardona, C., Goyenechea, I., & Iturbe, U. (Eds), *La sistemática, base del conocimiento de la biodiversidad*, pp. 11-21.

UAEH; Hidalgo, México.

Cracraft, J. (1983). Species Concepts and Speciation Analysis. En: Johnston R. F. (Ed), *Current Ornithology*, pp. 159-187. Springer US; Boston, MA https://doi.org/10.1007/978-1-4615-6781-3_6

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, *85*, 407-415. https://doi.org/10.1111/j.1095-8312.2005.00503.x

Dearn, J. M. (1990). Color pattern polymorphism. En: Chapman, R. F. & Joern, A. (Eds), Biology of grasshoppers, pp. 517-550. John Wiley & Sons, Inc.; Hoboken, EUA.

de Queiroz, K. (1998). The General Lineage Concept of Species, Species Criteria, and the Process of Speciation. En: Howard, D. J. & Berlocher, S. H. (Eds.), *Endless Forms: Species and Speciation*, pp. 57-75. Oxford University Press; Oxford, UK.

de Queiroz, K. (2005). Different species problems and their resolution. *BioEssays*, *27*, 1263-1269. https://doi.org/10.1002/bies.20325

de Queiroz, K. (2007). Species Concepts and Species Delimitation. *Systematic Biology*, *56*, 879-886. https://doi.org/10.1080/10635150701701083

Delsuc, F., Brinkmann, H., & Philippe, H. (2005). Phylogenomics and the reconstruction of the tree of life. *Nature Reviews Genetics*, *6*, 361–375. https://doi.org/10.1038/nrg1603

Dillon, S., & Fjeldså, J. (2005). The implications of different species concepts for describing biodiversity patterns and assessing conservation needs for African birds. *Ecography*, *28*, 682–692. https://doi.org/10.1111/j.2005.0906-7590.04344.x

Dirsh, V. M., & Dirsh, V. M. (1961). A preliminary revision of the families and subfamilies of Acridoidea (Orthoptera, Insecta). *Bulletin of the British Museum (Natural History)*, 10, 351-419. https://doi.org/10.5962/bhl.part.16264

Dupuis, J. R., Roe, A. D., & Sperling, F. A. H. (2012). Multi-locus species delimitation in closely related animals and fungi: One marker is not enough. *Molecular Ecology*, 21, 4422-4436. https://doi.org/10.1111/j.1365-294X.2012.05642.x

Eades, D. C., Otte, D., Cigliano, M. M., & Braun, H. (2016). Orthoptera Species File. Version 5.0/5.0. Retrieved February 3, 2018, from http://orthoptera.speciesfile.org/HomePage/Orthoptera/HomePage.aspx

Elewa, A. M. T. (2010). *Morphometrics for Nonmorphometricians*. Springer; Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-95853-6

Espinosa, D., Ocegueda, S., Aguilar, C., Flores, O., & Llorente-Bousquets, J. (2008). El conocimiento biogeográfico de las especies y su regionalización natural. En: Sarukhán, J. (Ed), *Capital natural de México, vol. I: Conocimiento actual de la biodiversidad*, pp. 33-65. Conabio; México.

Ferrari, L., Orozco-Esquivel, T., Manea, V. & Manea, M. (2012). The dynamic history of the Trans-Mexican Volcanic Belt and the Mexico Subduction Zone. *Tectonophysics*, 522-523, 122-149.

Fontana, P., Buzzetti, F. M., & Mariño-Pérez, R. (2008). *Chapulines, langostas, grillos y esperanzas de México: guía fotográfica.* World Biodiversity Association; Verona, Italy.

286 pp.

Fujita, M. K., Leaché, A. D., Burbrink, F. T., McGuire, J. A. & Moritz, C. (2012). Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology and Evolution*, 27, 480-488. https://doi.org/10.1016/j.tree.2012.04.012

Frankham, R., Ballou, J. D., Dudash, M. R., Eldridge, M. D. B., Fenster, C. B., Lacy, R. C. Mendelson III, J. R., Porton, I. J., Ralls, K., & Ryder, O. A. (2012). Implications of different species concepts for conserving biodiversity. *Biological Conservation*, *153*, 25-31. https://doi.org/10.1016/J.BIOCON.2012.04.034

Foottit R. G., & Adler, P. H. (2009). Insect Biodiversity: Science and Society. Blackwell Publishing Ltd.; West Sussex, UK. 632 pp.

Gillott, C. (2005). *Entomology*. Springer; Netherlands. 816 pp.

Grimaldi, D. A., & Engel, M. S. (2005). *Evolution of the insects*. Cambridge University Press; Cambridge and New York. 755 pp.

Gutiérrez-García, T. A., & Vázquez-Domínguez, E. (2013). Consensus between genes and stones in the biogeographic and evolutionary history of Central America. *Quaternary Research*, 79, 311-324. https://doi.org/10.1016/j.yqres.2012.12.007

Halffter, G., & Morrone, J. J. (2017). An analytical review of Halffter's Mexican transition zone, and its relevance for evolutionary biogeography, ecology and biogeographical regionalization. *Zootaxa*, 4226, 001-046. http://dx.doi.org/10.11646/zootaxa.4226.1

Haq, B. U., Hardenbol, J., & Vail, P. R. (1987). Chronology of fluctuating sea levels since the Triassic. *Science*, 235, 1156-1167. http://science.sciencemag.org/content/235/4793/1156

Hausdorf, B. (2011). Progress toward a general species concept. *Evolution*, *65*, 923-931. https://doi.org/10.1111/j.1558-5646.2011.01231.x

Hebard, M. (1924). A Revision of the Genus *Taeniopoda* (Orthoptera, Acrididae, Cyrtacanthacrinae). *Transactions of the American Entomological Society*, *50*, 253-274. Retrieved from https://www.jstor.org/stable/pdf/25077112.pdf

Hebert, P. D. N., Ratnasingham, S. & DeWaard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences*, 270, S96-S99. https://doi.org/10.1098/rsbl.2003.0025

Herrera, S. & Shank, T. M. (2016). RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. Molecular Phylogenetics and Evolution, 100, 70-79. https://doi.org/10.1016/j.ympev.2016.03.010

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907-913. https://doi.org/10.1038/35016000

Hillis, D. M. (1987). Molecular Versus Morphological Approaches to Systematics. *Annual Review of Ecology and Systematics*, *18*, 23-42. https://doi.org/10.1146/annurev.es.18.110187.000323

Hohenegger, J. (2014). Species as the basic units in evolution and biodiversity: Recognition of species in the Recent and geological past as exemplified by larger foraminifera. *Gondwana Research*, *25*, 707-728. https://doi.org/10.1016/j.gr.2013.09.009

Hull, D. L. (1977). The Ontological Status of Species as Evolutionary Units. En: Butts R.E., Hintikka J. (Eds), *Foundational Problems in the Special Sciences*, pp. 91–102. Springer; Dordrecht. https://doi.org/10.1007/978-94-010-1141-9_6

Ingrisch, S., & Willemse, F. (2004). *Bibliographia systematica Orthopterorum saltatoriorum = Systematic bibliography of saltatorial Orthoptera from Linnaean times to the end of the 20th century (about 1750 to 2000)*. Pensoft; Sofia.

Iturralde-Vinent, M. A. & MacPhee, R. D. E. (1999). Paleogeography of the caribbean region: Implications for cenozoic biogeography. *Bulletin of the American Museum of Natural History,* 238, 1-95. http://hdl.handle.net/2246/1642

Leavitt, S. D., Moreau, C. S., & Thorsten Lumbsch, H. (2015). The Dynamic Discipline of Species Delimitation: Progress Toward Effectively Recognizing Species Boundaries in Natural Populations. En: Upreti, D. K., Divakar, P. K., Shukla, V., & Bajpai, R. (Eds), *Recent Advances in Lichenology*, pp. 11–44. Springer India; New Delhi. https://doi.org/10.1007/978-81-322-2235-4_2

Lessios, H. A. (2008). The great american schism: Divergence of marine organisms after the rise of the central american isthmus. *Annual Review of Ecology, Evolution, and Systematics,* 39, 63-91. https://doi.org/10.1146/annurev.ecolsys.38.091206.095815

Mace, G. M. (2004). The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359, 711-719.https://doi.org/10.1098/rstb.2003.1454

Mayden, R. L. (1997). A hierarchy of species concepts: the denouement in the saga of the species problem. En: Claridge, M. F., Dawah, H. A., & Wilson, M. R. (Eds.), *Species: The units of diversity*, pp. 318-423, Chapman & Hall.; London.

Mayr, E. (1996). What Is a Species, and What Is Not? *Source: Philosophy of Science*, *63*, 262-277. Retrieved from http://www.jstor.org/stable/188473

Metzker, M. L. (2010). Sequencing technologies the next generation. *Nature Reviews Genetics*, 11, 21-46. https://doi.org/10.1038/nrg2626

Miller III, W. (2001). The structure of species, outcomes of speciation and the species problem: ideas for paleobiology. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *176*, 1-10. Retrieved from http://www.sciencedirect.com/science/article/pii/S0031018201003467

Moritz, C., Patton, J. L., Schneider, C. J. & Smith, T. B. (2000). Diversification of rainforest faunas: An integrated molecular approach. *Annual Review of Ecology and Systematics*, 31, 533-563. https://doi.org/10.1146/annurev.ecolsys.31.1.533

Morrone, J.J. (2014). Biogeographical regionalisation of the Neotropical region. *Zootaxa*, 3782, 1-110. http://dx.doi.org/10.11646/zootaxa.3782.1.1

Mound L.A. y R. Marullo. (1996). The Thrips of Central and South America: An Introduction. *Memory of Entomology, International*. Associated Publishers; Gainesville,

Florida. 487 pp.

Padial, J. M., Miralles, A., De la Riva, I. & Vences, M. (2010). The integrative future of taxonomy. Frontiers in Zoology, 7. https://doi.org/10.1186/1742-9994-7-16

Patterson, B. D., & Costa, L. P. (2012). Introduction to the history and geography of Neotropical mammals. En: Patterson, B. D., & Costa, L. P. (Eds), *Bones, Clones and Biomes. The History and Geography of Recent Neotropical Mammals*, pp. 1-5. The University of Chicago Press; Chicago.

Peterson, A. T., & Navarro-Sigüenza, A. G. (1999). Alternate Species Concepts as Bases for Determining Priority Conservation Areas. *Conservation Biology*, *13*, 427-431. https://doi.org/10.1046/j.1523-1739.1999.013002427.x

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D., & Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, 55: 595-609.

Rannala, B. (2015). The art and science of species delimitation. *Current Zoology*, *61*, 846-853. Retrieved from https://doi.org/10.1093/czoolo/61.5.846

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system: Barcoding. *Molecular Ecology Notes*, 7, 355-364. https://dx.doi.org/10.1111%2Fj.1471-8286.2007.01678.x

Rehn, J. A. G., & Grant Jr., H. J. (1959a). A Review of the Romaleinae (Orthoptera; Acrididae) Found in America North of Mexico. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *111*, 271. Retrieved from https://www.jstor.org/stable/pdf/4064509.pdf

Rehn, J. A. G., & Grant Jr., H. J. (1959b). An Analysis of the Tribes of the Romaleinae with Special Reference to Their Internal Genitalia (Orthoptera; Acrididae). *Transactions of the American Entomological Society*, *85*, 233–271. Retrieved from http://www.jstor.org/stable/25077781

Resh, V. H., & Cardé, R. T. (2009). *Encyclopedia of insects*. Elsevier/Academic Press; San Diego. 1169 pp.

Rowell, C. H. F. (2013). *The Grasshoppers (Caelifera) of Costa Rica and Panama.* The Orthopterist's Society; San Martín de los Andes. 609 pp.

Rull, V. (2008). Speciation timing and Neotropical biodiversity: The tertiary-quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology,* 17, 2722-2729. https://doi.org/10.1111/j.1365-294X.2008.03789.x

Rull, V. (2014). Biodiversity, mountains and climate change. *Collectanea Botanica*. 33, e006. http://dx.doi.org/10.3989/collectbot.2013.v33.006

Santos, C. M. D., & Amorim, D. S. (2007). Why biogeographical hypotheses need a well supported phylogenetic framework: a conceptual evaluation. *Papéis Avulsos de Zoologia (São Paulo)*, *47*, 63–73. https://doi.org/10.1590/S0031-10492007000400001

Schmittner, A., Sarnthein, M., Klnkel, H., Bartoli, G., Bickert, T., Crucifix, M., Crudeli, D., Groeneveld, J., Kosters, E., Mikolajewicz, U., Millo, C., Reijmer, J., Schafer, P., Schmidt, D., Schneider, B., Schulz, M., Steph, S., Tiedemann, R., Weinelt, M. & Zuvela, M. (2004).

Global impact of the panamanian seaway closure. *Eos*, 85, 526-527. https://doi.org/10.1029/2004EO490010

Simpson, B. (1971). Pleistocene changes in the fauna and flora of South America. *Science*, 173, 771-780. https://doi.org/10.1126/science.173.3999.771

Sites, J. W., & Marshall, J. C. (2003). Delimiting species: A Renaissance issue in systematic biology. *Trends in Ecology & Evolution*, *18*, 462-470. https://doi.org/10.1016/S0169-5347(03)00184-8

Song, H., Foquet, B., Mariño-Pérez, R. & Woller, D. A. (2017). Phylogeny of locusts and grasshoppers reveals complex evolution of density-dependent phenotypic plasticity. Scientific Reports, 7, 6606.

Song, H., Moulton, M. J., & Whiting, M. F. (2014). Rampant nuclear insertion of mtDNA across diverse lineages with in Orthoptera (Insecta). *PLoS ONE*, 9, e110508. https://doi.org/10.1371/journal.pone.0110508

Stork, N. E. (1988). Insect diversity: facts, fiction and speculation. *Biological Journal Ofthe Linnean Society*, *35*, 321-337.

Valverde, J. P. & Schielzeth, J. P. (2015). What triggers colour change? Effects of background colour and temperature on the development of an alpine grasshopper. BMC Evolutionary Biology, 15, 168.

Whelan, N. V. (2011). Species tree inference in the age of genomics. *Trends in Evolutionary Biology*, 3, 23-28. https://doi.org/10.4081/eb.2011.e5

Wiens, J. J. (2008). Systematics and herpetology in the age of genomics. Bioscience, 58, 297-307. https://doi.org/10.1641/B580405

Yu, G., Rao, D., Matsui, M. & Yang, J. (2017). Coalescent-based delimitation outperforms distance-based methods for delineating less divergent species: the case of Kurixalus odontotarsus species group. *Scientific Reports*, 7, 16124

Zachos, F. E. (2016). *Species Concepts in Biology*. Springer International Publishing; Switzerland. 220 pp.