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Sistemática filogenética y diversificación de los chapulines neotropicales del género *Sphenarium* Charpentier, 1842 (Orthoptera; Pyrgomorphidae)

TESIS

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Índice

Resumen

Los chapulines del género *Sphenarium* Charpentier, 1842 destacan por su importancia biológica, económica y cultural. Además, representan un modelo ideal para explorar los efectos de eventos históricos y procesos de adaptación local sobre la diversificación de los insectos neotropicales, lo cual ha sido escasamente estudiado. Sin embargo, su diversidad, relaciones evolutivas y patrones de diversificación han permanecido poco comprendidos hasta este momento. Los objetivos de este trabajo fueron determinar la diversidad específica de *Sphenarium*, inferir sus relaciones evolutivas, y explorar el impacto relativo de los eventos históricos y de las fuerzas evolutivas en su diversificación. Usando conjuntamente evidencia genética, filogenética y morfológica se determinó el número de especies en el género, se revisó su taxonomía y se reconocieron 17 especies de las cuales ocho son nuevas para el género. Las relaciones filogenéticas, la estructura filogeográfica y los tiempos de divergencia inferidos sugieren una diversificación reciente del género probablemente asociada a las fluctuaciones climáticas del Cuaternario y las últimas etapas de formación del Eje Volcánico Transmexicano. Simultáneamente, se identificaron diferentes patrones de divergencia genética y morfológica que sugieren una compleja interacción entre las fuerzas evolutivas durante la evolución del género. Además, se encontró una relación significativa entre variación climática y tamaño corporal de las especies de *Sphenarium*, lo cual sugiere que la selección natural actuando sobre atributos de historias de vida también pudo haber jugando un papel importante en la diversificación del género mediante procesos de adaptación local a la compleja heterogeneidad ambiental del país.

Abstract

The grasshoppers of the genus *Sphenarium* Charpentier, 1842 stand out because of its biological, economical and cultural importance. Moreover, they represent an ideal model to explore the effects of historic events and local adaptation processes on the diversification of Neotropical insects, which has been so far barely studied. However, there has been little understanding on the diversity, the evolutionary relationships and patterns of diversification of this genus. For these reasons, this study aimed to determine the species diversity, to infer the evolutionary relationships and to explore the relative impact of historic events and evolutionary forces on the diversification of *Sphenarium*. The number *Sphenarium* species was determined using jointly genetic, phylogenetic and morphological evidence. After revising the taxonomy of the genus 17 species were recognized, eight of which are new taxa. The phylogenetic relationships, the phylogeographic structure and the divergence times inferred suggest a recent diversification of this group, which is probably associated to the Quaternary climatic shifts and to the latest formation stages of the Mexican Volcanic Belt. Simultaneously, different patterns of genetic and morphologic divergence were identified suggesting a complex interplay between evolutionary forces during the evolution of the genus. In addition, a significant relationship between climatic variation and body size of *Sphenarium* species was found suggesting that natural selection on life history traits may also have played an important roll on the diversification of the genus by local adaptation processes to the complex environmental heterogeneity of Mexico.

Introducción general

Uno de los principales objetivos de la biología evolutiva es explicar la diversidad biológica, la cual resulta del balance entre los procesos de formación de especies (especiación) y de extinción. Así, el estudio de la especiación tiene un papel central en la investigación evolutiva (Bultin *et al.*, 2012), el cual involucra principalmente el estudio de los mecanismos responsables de generar y mantener el aislamiento reproductivo a lo largo de la formación de las especies (Coney & Orr, 2004). Sin embargo, es necesario disponer de un conocimiento sólido sobre la diversidad y relaciones evolutivas de los miembros de un grupo determinado que permita establecer un marco biológico e histórico para el estudio posterior de los mecanismos de especiación.

El Neotrópico forma parte de una de las regiones con mayor diversidad biológica, la cual está representada principalmente por la fauna de insectos. En México se encuentran aproximadamente el 5.2% de las especies de insectos descritas en el mundo (Llorente-Bousquets & Ocegueda, 2008). Además, la entomofauna mexicana destaca por ser una compleja mezcla de componentes bióticos con diferentes orígenes biogeográficos (e.g. neártico y neotropical), así como de grupos cuya diversificación ha ocurrido principalmente dentro del territorio mexicano (Halffter, 1987; Morrone, 2010).

La diversidad biológica de México ha sido atribuida a la heterogeneidad ambiental y la compleja historia geológica y climática del país. Durante las últimas dos décadas diversos estudios moleculares filogenéticos y filogeográficos en vertebrados y plantas (para una síntesis ver Bryson *et al*. 2011; Bryson *et al*. 2012; Ornelas *et al.*, 2013; Masttreta-Yanes *et al*. 2015) han encontrado patrones de divergencia interespecíficos congruentes espacial y temporalmente con la ocurrencia de los eventos geológicos neogénicos (e.g. formación del Eje Volcánico Transmexicano y hundimiento del Istmo de Tehuantepec) y climáticos cuaternarios (ciclos glaciares e inter-glaciares) en México. Sin embargo, hasta el momento este tipo de estudios en insectos son escasos e involucran principalmente especies neártcias desérticas (e.g. Hurtado *et al.*, 2004; Morse & Farrell, 2005; Smith *et al.*, 2011; Pfeiler & Markow, 2011) y con gran capacidad de dispersión (e.g. Anducho-Reyes *et al.*, 2008; Duennes *et al.*, 2012), o especies con rangos geográficos limitados en el país (Bargues *et al.*, 2008; Pringle *et al.*, 2012). Debido a esto, no se ha distinguido concluyentemente el efecto relativo de los eventos históricos sobre la diversificación de la entomofauna mexicana. Además, la compleja heterogeneidad ambiental de México brinda un mosaico de oportunidades ecológicas para sus especies que pueden en resultar de procesos de adaptación local que generen diferenciación interpoblacional y especiación (Darwin, 1859; Coney & Orr, 2004). No obstante, hasta el momento el efecto relativo de la selección natural en la diversificación de insectos Mexicanos no ha sido explorado.

Los chapulines del género *Sphenarium* Charpentier, 1842 pueden representar un modelo ideal para explorar los efectos de los eventos históricos y procesos de adaptación local sobre la diversificación de la entomofauna en México. El género *Sphenarium* se distribuyen desde la porción central de México hasta el noroeste de Guatemala a lo largo de un gradiente altitudinal y ambiental amplio. Las especies de este género se encuentran desde el nivel del mar hasta aproximadamente 2800 m s.n.m. Estos chapulines son polífagos y se asocian principalmente a los estratos herbáceos y arbustivos de múltiples ambientes, tales como bosques templados, xerófilos, tropicales perennifolios y caducifolios. Los principales sistemas montañosos de México delimitan en gran medida la distribución parapátrica de las especies del género, lo cual sugiere que estos elementos geográficos han jugado un papel importante en la evolución del grupo a través del aislamiento de sus linajes. Además, el patrón biogeográfico de la subtribu Sphenariina, que incluye a los géneros *Sphenarium*, *Prosphena* Bolivar, 1884 (restringido a América Central) y *Jaragua* Perez-Gelabert, Dominici & Hierro, 1995 (restringido a las Antillas orientales), sugiere que los ancestros de esta subtribu pudieron estar presentes en Mesoamérica desde hace aproximadamente 50 Ma (Kevan, 1990; Perez *et al.*, 1995), por lo que es probable que los eventos históricos vicariantes del Neógeno y Cuaternario hayan provocado diversificación de linajes dentro de *Sphenarium*.

Al ser ectotermos, los rangos de distribución de estos chapulines pueden ser muy sensibles a los cambios de temperatura (Howden, 1969; Hewitt, 1996). Además, su fenología se asocia a los cambios de estacionalidad de los ambientes en los que se distribuyen. Generalmente, el fin de sus ciclos anuales de vida coincide con el inicio de la estación invernal (*obs. pers.*). Estos chapulines son ápteros y tienen aparentemente capacidades de dispersión limitada, lo cual reduce la posibilidad de que los patrones genéticos generados por procesos demográficos y eventos vicariantes históricos sean afectados por eventos de dispersión secundaria (Avise, 2000). Además, este género incluye a *S. purpurascens* una de las especies de insectos mexicanos que más ha sido estudiada en términos de su biología general y ecología evolutiva (para una síntesis ver Cano-Santana & Castellano-Vargas, 2009). En esta especie se han identificando atributos morfológicos y conductuales bajo intensa selección natural y sexual (Cueva del Castillo & Núñez-Farfán, 1999; Cueva del Castillo *et al*., 1999; Cueva del Castillo, 2003). Esto nos lleva a cuestionarnos el impacto que han tenido estas fuerzas evolutivas y su importancia relativa en la diferenciación y formación de las especies de *Sphenarium*. Además, las regiones con topografía heterogénea, como en la que se distribuye *Sphenarium*, brindan la oportunidad de estudiar procesos de adaptación local a condiciones climáticas diferentes (Blanckenhorn, 1997; Berner *et al*., 2004; Karl et al. 2008). En estas regiones, las condiciones climáticas pueden variar considerablemente en distancias cortas. Esto hace que las adaptaciones locales sean posibles únicamente si la selección es lo suficientemente fuerte para contrarrestar el flujo genético esperado entre poblaciones adyacentes (Berner *et al*., 2004; Hodkinson, 2005).

A pesar del potencial del género *Sphenarium* para ser empleado como un grupo modelo para estudios de biología evolutiva, previamente al desarrollo de esta investigación, existía una escasa compresión de la diversidad del grupo, que resultaba en una taxonomía incompleta. Además se desconocían las relaciones evolutivas entre las especies y los patrones generales de diversificación del grupo. Por lo tanto, los objetivos de este trabajo fueron: i) determinar la diversidad específica del género, ii) inferir las relaciones las evolutivas entre las especies, iii) explorar los efectos de los eventos geológicos y climáticos en su diversificación y iv) explorar el impacto relativo de las fuerzas evolutivas (e.g. selección natural, selección sexual y deriva génica) en la diversificación del grupo.

En el primer capítulo de este trabajo se abordan los primeros tres objetivos mediante un análisis conjunto de información genética, filogenética y morfológica. Los resultados de este análisis permitieron determinar el número de especies en el género. A partir de estos resultados se realizó una revisión taxonómica de *Sphenarium* a fin de redefinir los conceptos de especies previamente definidos y describir a las especies nuevas encontradas. El análisis filogenético realizado permitió inferir las relación evolutivas y obtener estimaciones aproximadas de los tiempos de divergencia interespecífica en el género. Estos resultados permitieron examinar la coincidencia espacial y temporal de los eventos de diversificación del grupo con los eventos históricos del territorio Mexicano. En el segundo capítulo se aborda el último objetivo mediante un estudio comparativo que exploró el impacto relativo de las presiones selectivas asociadas a la heterogeneidad ambiental y la evolución del tamaño corporal y dimorfismo sexual en las especies de *Sphenarium* considerando sus relaciones filogenéticas.

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Capítulo 1: Integrative taxonomy reveals cryptic diversity in the Neotropical grasshoppers: taxonomy, phylogenetics, and evolution of the genus *Sphenarium* Charpentier, 1842 (Orthoptera; Pyrgomorphidae)

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Integrative taxonomy reveals cryptic diversity in the Neotropical grasshoppers: taxonomy, phylogenetics, and evolution of the genus Sphenarium Charpentier, 1842 (Orthoptera; Pyrgomorphidae)

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Abstract

The genus *Sphenarium* Charpentier, 1842 comprises the most diverse group of the New World Pyrgomorphidae. These grasshoppers show an extensive variation in external morphology, and are culturally and economically important for Mexican people since pre-Hispanic times. Nevertheless, the taxonomy of *Sphenarium* has been chaotic and remained incompletely resolved until now. Following an integrative taxonomic framework, we infer the number of species in this genus by species delimitation based on morphological, phylogenetic, and geographic information. Based on our results, we revise the genus and redefine 9 species and describe 8 new species (*S. adelinae* **sp.n.**, *S. crypticum* **sp.n.**, *S. infernalis* **sp.n.**, *S. miztecum* **sp.n.**, *S. occidentalis* **sp.n.**, *S. tarascum* **sp.n.**, *S. totonacum* **sp.n.** and *S. zapotecum* **sp.n.**). Moreover, we update the knowledge of the evolutionary relationships and biogeographic patterns of *Sphenarium* species. Our results also demonstrate the importance of historic geological and climatic events on the lineage diversification of this genus. Different levels of morphological and genetic differentiation among species suggest a complex interplay between evolutionary forces during the evolution of these Neotropical grasshoppers.

Keywords: Integrative taxonomy, Neotropical region, Pyrgomorphidae, *Sphenarium*, male genitalia morphology, molecular phylogenetics, species phylogeny.

Introduction

The genus *Sphenarium* Charpentier, 1842 comprises a group of fusiform and flightless grasshoppers. This genus belongs to the mainly tropical family Pyrgomorphidae (Orthoptera), which is primarily distributed in the Old World. Only 15 out of 146 genera of this family occur in the New Wold (Kevan 1964, 1977), and *Sphenarium* represents the most diverse pyrgomorph genus in the New World (Kevan 1977). The genus *Sphenarium* is part of the Mesoamerican endemic subtribe Sphenariina, which also includes the genera *Prosphena* Bolivar, 1884 and *Jaragua* Perez-Gelabert, Dominici & Hierro, 1995.

Sphenarium grasshoppers are distributed from central Mexico to northwest Guatemala. Across this region the Neogene formation of the Mexican Volcanic Belt (19 - 3 Ma) and the Quaternary climatic shifts (2.6 - 0.01 Ma) have been proposed as main drivers of biotic diversification (e.g. Bryson *et al.* 2011, 2012a; Mastretta-Yanes *et al.* 2015; Ornelas *et al.* 2013). The first event increased topographical and environmental complexity across central Mexico causing isolation and divergence in multiple species (e.g. Bryson *et al.* 2012a; Mulcahy *et al.* 2006; Ruiz-Sanchez & Specht 2013), whereas the latest event promoted recurrent shifts in ranges of ancestral biotas across highlands and lowlands in Mexico causing lineage diversification in several taxa (e.g. Bryson *et al.* 2011; Castoe *et al.* 2009; Duennes *et al.* 2012; Ornelas & González 2014; Pringle *et al.* 2012). Considering the distribution of *Sphenarium* grasshoppers, as well as their relatively low dispersal capabilities and tropical origin, this genus provide the opportunity to explore the relative importance of past geological and climatic events on the diversification of Neotropics.

The species of *Sphenarium* show an extensive variation on body size, colouration and life history traits (Kevan 1977; Sanabria-Urbán *et al.* 2015) suggesting high levels of adaptation to environmental heterogeneity (e.g. Cueva del Castillo & Cano-Santana 2001; Sanabria-Urbán *et al.* 2015). Moreover, morphological and behavioural traits appear to be under strong sexual selection (Cueva del Castillo *et al.* 1999; Cueva del Castillo & Nunez-Farfan 1999; Cueva del Castillo & Núñez-Farfán 2002). For instance, in *S. purpurascens,* larger males have advantage in accessing females (Cueva del Castillo *et al.* 1999) and show prolonged female guarding behaviour that may suggest strong sperm competition (Cueva del Castillo 2003). Males can spend from 17 to 22 days mounted on females, which can represent half of the average life expectancy of typical adults (Cueva del Castillo 2003; Cueva del Castillo & Nunez-Farfan 1999). This prolonged mate guarding behaviour also can be found in other *Sphenarium* species, such as *S. rugosum* and *S. histrio* (Cueva del Castillo, pers. obs.), and other pyrgomorphs (Descamps & Wintrebert 1966; Uvarov 1977), including *Prosphena scudderi* (Cueva del Castillo, pers. obs.) and *Zonocerus elegans* (Wickler & Seibt 1985). Although prolonged mate guarding is found in other members of the family, this opens the question if this behaviour is shared for all Pyrgomorphidae.

Sphenarium grasshoppers also have strong cultural and economical significance for Mexican people. For instance, these grasshoppers are traditional elements of the human diet since pre-Columbian times, in central and southern Mexico (Ramos-Elorduy & Moreno 1989). On the other hand, S*phenarium* is the only genus of the New World Pyrgomorphidae known to be a serious crop pest that can outbreak, principally in central Mexico (Cerritos & Cano-Santana 2008; Kevan 1977; SENASICA 2017). In fact, *Sphenarium* grasshoppers have long been regarded as agricultural pests of corn (*Zea mays*) and beans (*Phaseolus vulgaris*), both of which are fundamental elements of the Mexican diet (Kevan 1977).

Despite the biological, economical, and cultural importance of *Sphenarium*, its taxonomy has remained incompletely resolved. Since the erection of the genus by Charpentier in 1842 until 1932, taxonomists focused entirely on highly variable characters in recognising species, such as colouration, body morphology and size, which led to the ambiguous description of 15 species within the genus (see Table 1). During that period, only Bolivar (1884, 1904) and Bruner (1906) thoroughly revised the taxonomy of the group redefining the genus and species concepts and recognising morphological relationships between species. However, the definition and geographic ranges of *Sphenarium* species were largely unclear. After these studies, few taxonomic changes in the group were conducted and the validity of some taxa remained uncertain (Table 1).

The study of male genitalia in the genus led to the discovery of new species and allowed the resolution of some previous taxonomic problems. Márquez (1962) conducted the first study on interspecific variation of female and male genitalia in *Sphenarium*, adding a new taxon and synonymising some of the previously described species (Table 1). However, his taxonomic rearrangements were poorly supported because he did not examine all *Sphenarium* species and his descriptions were ambiguous, based only on few specimens and genital characters. Later, Boyle (1974) studied the taxonomy of *Sphenarium* based on a larger number of male genitalia characters, specimens and localities, and revised the type material of all described species. This author identified two new taxa and re-defined the genus and species concepts synonymising most of the previously described taxa (Table 1). Moreover, he identified general biogeographic patterns within the genus. Unfortunately, this study was never properly published and only available as a graduate thesis. Kevan (1977) summarized Boyle's results publishing only distribution maps of his recognised taxa, synonyms and preliminary diagnosis of his two new taxa (Table 1). After this last study, the *Sphenarium* taxonomy remained steady complicating the identification its species for almost four decades.

Recently, Pedraza-Lara *et al*. (2015) and Sanabria-Urbán *et al*. (2015) proposed changes to this last classification of *Sphenarium* based on morphological and genetic information (Table 1). Moreover, in each study, at least eight different taxa had been identified that could represent new species in the genus, but these still await proper taxonomic descriptions. Therefore, so far there is no consensus on the number and definition of species, previously described or new, to be recognised within the genus. Moreover, these last studies on *Sphenarium* have pointed out that the levels of genetic and morphological differentiation do not always reflect each other, resulting in incongruences in the number of taxa inferred by genetic or morphological data in the group. These kinds of incongruences have been observed in other groups of closely related and recently diverged lineages (e.g. Allegrucci *et al.* 2014; López *et al.* 2007; Pocco *et al.* 2015). High levels of morphological divergence associated to low levels of genetic differentiation between taxa can be explained by incomplete linage sorting due to genetic drift and/or gene flow (e.g. Allegrucci *et al.* 2013; Knowles & Carstens 2007). Moreover, if speciation has been mainly driven by natural selection (e.g. causing morphological divergence), low levels of genetic differentiation in neutral genetic markers could be expected (Knowles & Carstens 2007). Conversely, high levels of genetic differentiation associated to low morphological differentiation can be explained if divergence mainly occurred in isolation under neutral process such as genetic drift (Padial *et al.* 2010). For these reasons, studies attempting to determine species boundaries in groups of closely related taxa with recent diversification, such as *Sphenarium*, need the consensus from different sources of evidence.

Integrative taxonomy has provided a framework under which several sources of evidence are simultaneously used in inferring numbers and limits of species (Dayrat 2005; Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). Any inference of species boundaries strongly depends on the species concept adopted; which determines the delimitation criterion to be used, as well as the choice, analysis, and interpretation of data (Schlick-Steiner *et al.* 2010). Therefore, under integrative taxonomy, a unified species concept is adopted (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). This concept equates species with separately evolving lineages of populations or meta-populations (De Queiroz 2007). Under this concept the most reliable species boundaries are those supported by multiple independent sources of evidence. Nonetheless, any of the organismal attributes that provide evidence of lineage separation can also support the existence of species (De Queiroz 2007).In this study we use an integrative taxonomic framework to determine boundaries and numbers of species in *Sphenarium* based on a detailed analysis of morphological and genetic characters, in combination with geographic information. Based on this information we revise the genus, redefine species concepts and describe the new discovered species. In addition, we aim to update the knowledge of the evolutionary relationships among *Sphenarium* members and contribute the understanding of the process implicated on the diversification of this genus. In this context, the complex geological and climatic history of the Mexican territory can explain the diversification of the genus. Therefore, we expecte that phylogenetic relationships and divergence times between *Sphenarium* species reflect the spatial and temporal occurrence of these historical events. Specifically, we expect that basal divergences occurred in lineages distributed along the Mexican Volcanic Belt during the formation of this mountain range. Moreover, due to climatic fluctuations we expect closer relationships and more recent divergences on lineages distributed in low and highlands of central and southern Mexico.

Materials and methods

Material examined

We examined the type material of all described species of *Sphenarium*. In most cases, we were able to examine only the external morphology of the type, except in cases where the genital structures were previously dissected or paratypes were available to dissect (see description of species for details). In addition, we examined external and genital morphology of specimens collected from the type localities and/or across the recognised distribution ranges of the described species. These material were loaned from the National Autonomous University of Mexico (Biology Institute: IBUNAM & FESI: CAFESI) in Mexico City, Mexico; the Academy of Natural Sciences of Drexel University (ANSP) in Philadelphia, Pennsylvania, USA; the University of Michigan Museum of Zoology (UMMZ) in Ann Arbor, Michigan, USA; the Florida State Collection of Arthropods (FSCA) in Gainesville, Florida, USA. Moreover, we also examined specimens collected between 2008 and 2016 across the geographic distribution of the genus in Mexico by the senior author. All collected specimens were vouchered and individually stored at –80 ºC in the Laboratory of Genetic and Molecular Ecology, IIES (UNAM, Campus Morelia). All collected specimens are available upon request to corresponding author. All type specimens designated here were deposited in the IBUNAM; whereas paratypes and the remaining examined material were deposited also at IBUNAM, as well as in the Texas A&M University Insect Collection (TAMUIC) in College Station, Texas, USA.

Analysis of morphology

We based our morphological analysis, descriptions, and diagnosis of species principally on male genital structures because they provide diagnostic characters that can be easily verifiable (Boyle 1974; Kevan 1977). Moreover, females of this genus are very difficult to differentiate (Boyle 1974). Thus, so far, only analysing male genitalia we can achieve an unmistakable identification of *Sphenarium* taxa. Following the method described by Boyle (1974), we extracted the entire male genitalia complex from adult specimens. The excised structures were cleared of all non-sclerotized tissue by placing them into vials with 10% KOH solution in warm water bath at 50º C, for approximately 10 minutes. After clearing, we separated epiphallus from ectophallus by gently removing the ectophallic membrane. We analysed the morphology of these structures before separating endophallus from the ectophallus, which sometimes damaged structures. All observations were conducted using a DISCOVERY V.8 ZEISS stereoscope. Depending on the availability of material we analysed male genital structures of up to 5 specimens per locality. The structures from each specimen were preserved in glycerine in a single vial after the analysis.

We also examined external traits such as colouration patterns and morphology of antennae, head, tegmina and abdomen apex that are sometimes useful in identifying *Sphenarium* species. We used a digital calliper (Mitutoyo Corp., Tokyo, Japan) to measure the following traits of the type material: total body size (BS) considering the length from the tip of the fastigium of vertex to the apex of the folded hind femur; fastigium length (FL) from apex to anterior dorsal margin of vertex; pronotum length (PL) measured along the dorsal midline from anterior to posterior margins; hind femur length (HF) measured from dorsoproximal lobe to the posterior margin of the knee. We followed the terminology used in Kevan *et al.* (1969) in our descriptions of genital and external morphology.

We took high-resolution photographs of male genitalia structures and specimens of each identified taxa using a Visionary Digital BK Plus Imaging System in combination with a Canon EOS 7D camera with 65mm (for genital structures) or 100mm lens (for specimens). We followed procedures described in Woller *et al.* (2014) to export and adjust light levels, background colouration and sharpness when necessary for the final images.

Molecular data

We newly generated nucleotide sequence data for 64 *Sphenarium* specimens to combine with our previously published dataset (Sanabria-Urbán *et al.* 2015), which consisted of 81 terminals (including both ingroups and outgroups) and five loci (cytochrome *c* oxidase subunit 1 [*CO1*] and subunit 2 [*CO2*], small ribosomal RNA [*12S*], histone 3 [*H3*] and internal transcribed spacer between *5.8S* rRNA and *28S* rRNA [*ITS2*]). We provide information about identification, voucher numbers, sampling locality and GenBank accession numbers of all included specimens in Table 2. We did not include the nucleotide sequences of *Sphenarium* published by Pedraza-Lara *et al*. (2015) because we were unable to verify the taxonomic identity of their analysed specimens.

Phylogenetic analysis

We aligned the sequences of each locus using MUSCLE (Edgar 2004). The final alignments included the following number of aligned positions: 1065 for *CO1*, 486 for *CO2*, 336 for *12S*, 318 for *H3* and 311 for *ITS2*. The combined dataset included the genetic information of 145 terminals, 2527 nucleotide aligned positions. Using JMODELTEST 2 V 0.1.1 (Darriba *et al.* 2012) we estimated the best substitution model for each locus according with the Bayesian Information Criterion (BIC): HKY+I+G for *CO1*, GTR+I+G for *CO2*, GTR+G for *12S*, HKY+G for *H3* and *ITS2*. We conducted a concatenated Bayesian analysis (CA) in MRBAYES V. 3.2.6 (Ronquist *et al.* 2012) with this dataset, individually applying the specific substitution model estimated for each locus. This analysis consisted of four independent runs, each of them with 10,000,000 generations and four chains, sampling each 1,000 generations. We used default priors for other parameters in the analysis. We assessed parameter convergence and proper mixing of independent runs using TRACER V.1.6 (Rambaut & Drummond 2013). All parameters in the combined analysis resulted in ESS values higher than 200. We discarded 25% of the samples obtained prior to stability (burn-in) to obtain the final consensus tree.

Gene phylogenies and species phylogeny can disagree because of incomplete lineage sorting, gene introgression and duplication/extinction; which is especially problematic for closely related species or species with high population sizes (Heled & Drummond, 2010; Knowles & Kubatko, 2010). Moreover, concatenating sequences of multiple genes may lead to poor estimation of the species phylogeny (Knowles & Kubatko 2010; Kubatko & Degnan 2007). For this reason, we also estimated the species phylogeny using the multilocus coalescent-based Bayesian approach implemented in *BEAST V. 2.4.2 (Bouckaert *et al.* 2014). This species phylogeny approach jointly estimates posterior distribution of the species tree from posterior distribution of individual genes, incorporating uncertainty associated with gene trees due to incomplete lineage sorting, nucleotide substitution model parameters and coalescent process (Heled & Drummond 2010). Moreover this approach allowed us to infer divergence times between major clades in the genus. For this species phylogeny analysis (SA) we used only the genetic information of *Sphenarium* specimens and its sister genera *Prosphesna* and re-estimated the substitution models for each locus for this dataset as previously described. We applied the following substitution models: TPM2uF+I+G for *CO1*, TIM2+I+G for *CO2*, TPMuF+G for *12S*, F81+G for *H3* and HKY+G for *ITS2*. A preliminary analysis on this dataset using uncorrelated lognormal clock and similar conditions as specified below indicated that a strict clock behaviour could not be rejected for both nuclear loci (Drummond & Rambaut 2015) [coefficients of variation: *H3* = 0.119 (95% High Posterior Density, HDP: 7.449E^{-8}- 0.417); *ITS2* = 0.419 (95% HDP: 4.293E^{-8}- 1.06)]. For this reason we only applied an uncorrelated lognormal clock prior for all mitochondrial loci and individual strict clock priors for *H3* and *ITS2*. The species assignments in this analysis followed our results in the inference of species boundaries section (see results section). We applied Yule model and Piecewise linear and constant root as tree and population size priors, respectively. The analysis comprised two independent runs, each one with 200 million generations and sampling every 20,000 generations. We verified parameter convergence and proper mixing of the independent runs using TRACER V 1.6 (Rambaut & Drummond 2013). All parameters in the combined analysis resulted in ESS values higher than 200. Single consensus tree was estimated setting a burn-in at 25%.

Fossil evidence or reliable events of geological vicariance to calibrate a molecular clock in *Sphenarium* are not available. Hence, in order to approximate the absolute interspecific divergence ages in the genus we used the available insect substitution rates in literature. In the analysis we specified two ranges of clock rates: the first one, for all mitochondrial loci together, varying from 0.0115 to 0.0134 substitutions/site/million years (Ma), which involved average substitution rates estimated for insect mitochondrion (Brower 1994; Papadopoulou *et al.* 2010); and the second one, for *ITS2* sequences, varying form 0.002 to 0.005 substitutions/site/Ma, which was estimated for this locus in Hemiptera (Bargues *et al.* 2000).

Inferences of species boundaries

We followed a unified species concept in delimiting species, under which any organismal property that provides evidence of lineage separation is relevant in determining boundaries and numbers of species of *Sphenarium* (De Queiroz 2007). For instance, reciprocal monophyly based on genetic data is widely assumed as evidence of species separation, considering the time and conditions it needs to be accomplished (Barrowclough & Zink 2009; Knowles & Carstens 2007). Therefore, we recognised as different species those groups of specimens that formed well-supported (posterior probabilities values > 95%) and geographically-structured monophyletic groups in the concatenated Bayesian phylogenetic analysis. On the other hand, if speciation involves selection-driven divergence, then taxonomic decisions based on neutral genetic information will tend to underestimate diversity (Knowles & Carstens 2007). In this context, male genital structures are assumed to evolve under strong sexual selection and sexual selected characters are known to diverge rapidly (Eberhard 1985; Hosken & Stockley 2004; Simmons 2014). Moreover, male genital morphology is widely used in determining species boundaries in grasshoppers (Song 2009). For these reasons we also recognised as different species of *Sphenarium* those groups of specimens that differed from each other by showing unique and constant combination of male genital characters.

Results

Morphological analysis

We examined a total of 3,869 *Sphenarium* specimens collected from 503 Mexican and Guatemalan localities. We identified 17 groups of specimens that could be separated by constant and unique combination of male genital characters (Table 3). Some of these groups were slightly differed from each other in their male genital morphology but showed unique combinations of external characters, both in females and males, that supported their recognition as separated groups. Nine of these identified groups corresponded to previously recognised species (including *S. borrei*, *S. histrio*, *S. macrophallicum*, *S. mexicanum*, *S. minimum*, *S. planum*, *S. purpurascens*, *S. rugosum*, and *S. variabile*); whereas the remaining eight groups were considered as independent operational taxonomic units (OTU1 to OTU8). In addition, we identified several morphotypes within *S. histrio, S. mexicanum, S. purpurascens, S. rugosum,* and *S. variabile*. These morphotypes represented groups of specimens that slightly differed in their male genital morphology and genetically (see below) from other morphotypes within the same taxa and in most cases were geographically restricted to particular geographic regions (see the description of species section).

Molecular data

We obtained nucleotide sequence data for 129 *Sphenarium* specimens collected from 94 Mexican localities and 16 outgroup terminals (Table 2). Each of the identified morphological taxa of *Sphenarium* was represented from two to 21 individuals in our dataset. In total we obtained the following number of sequences per loci: 134 for *CO1*, 107 for *CO2*, 62 for *12S*, 73 for *H3* and 62 for *ITS2* (Table 2). Within *Sphenarium CO2* sequences showed the highest percentage of parsimony informative sites (31.28%), followed by *CO1* (29.67%), *12S* (15.07%), *ITS2* (6.71%) and *H3* (5.35%). We identified shared haplotypes in the three independent loci used (all mitochondrial loci together, *ITS2* and *H3*). However, the number and combinations of taxa sharing haplotypes was inversely related to levels of polymorphism of these loci. Specifically, three shared mitochondrial haplotypes occurred only between individuals from geographically distant localities of the following taxa pairs: *S. purpurascens*/OTU8 (h01); *S. purpurascens*/*S. variabile* (h02); and *S. variabile*/OTU8 (h03) (see Table 2 & Fig. 1). In less polymorphic nuclear loci more shared haplotypes were observed but only between closely related taxa (for *ITS2*) or taxa within the same major clade (for *H3*) (see phylogenetic relationships section for details). In *ITS2* sequences shared haplotypes occurred between *S. mexicanum*/*S. histrio*/OTU1 (h01); *S. minimum*/OTU8 (h02); and *S. rugosum*/OTU5 (h03) (Table 2; Fig. 1). In *H3* sequences shared haplotypes occurred between *S. mexicanum*/*S. histrio* (h01); *S. mexicanum*/OTU1 (h02); *S. macrophallicum*/*S. purpurascens*/OTU7 (h03); *S. minimum*/*S. planum*/*S. purpurascens*/*S. variabile*/OUT3/OUT8 (h04); and *S. purpurascens* /*S. rugosum*/*S. variabile* (h05) (Table 2; Fig. 1).

Using *CO1* sequences we estimated *P-distance* values commonly used in interspecific comparisons in insects (Hebert *et al.* 2003a) between and within the identified taxa of *Sphenarium* (Table 3). In nine taxa (*S. borrei, S. histrio, S. mexicanum, S. planum,* OTU1, OTU2, OTU3, OUT4, OTU8) we observed *P-distance* values ≥ 3%. The most differentiated taxon was *S. borrei* followed by OTU4 and OTU7 with genetic distances ranging from 7.43 to 10.79 %; whereas the lowest values, ranging from 0.88 to 0.97%, were observed between *S. purpurascens*, *S. variabile*, and OTU8 (Table 3). *P-distance* values ranging from 1.29 to 2.99 % were obtained in comparisons between *S. macrophallicum, S. minimum, S. purpurascens, S. variabile,* OTU5, OTU7, and OTU9 (Table 3). Mean divergence within taxa resulted in *P-distance* values lower than 2.5% in all cases. The highest levels of within taxa mean divergence were observed within *S. borrei, S. histrio, S. mexicanum,* and OTU1 (Table 3); whereas the lowest were observed in *S. minimum, S. purpurascens, S. variabile,* OTU2, OTU5, OTU6, and OTU8. In addition, genetic distances between the identified morphotypes within *S. histrio, S. mexicanum, S. purpurascens, S. rugosum,* and *S. variabile* were in all cases lower than 3% (Table 4).

Phylogenetic analysis

In our CA phylogeny of the total evidence obtained (Fig. 2), we recovered strong support (posterior probability, *PP* ≥ 0.95) for the monophyly of *Sphenarium* and the following taxa in the genus: *S. borrei, S. minimum, S. planum,* OTU2, OTU3, OTU4, OTU5, OTU6, and OTU7; whereas only a weak support was obtained for OTU1 (*PP* = 0.76). The remaining taxa (*S. histrio, S. macrophallicum, S. mexicanum, S. purpurascens, S. rugosum, S. variabile,* and OTU8) were recovered as paraphyletic. In both phylogenetic analyses (CA and SA, Figs. 2, 3, respectively), we recovered a close phylogenetic relationship between specimens of *S. histrio* and *S. mexicanum* (CA, *PP* = 0.56; SA, *PP* = 0.99); *S. rugosum* and OTU5 (CA, *PP* = 0.84; SA, *PP* = 0.57); *S. purpurascens, S. variabile,* and *OTU8* (CA, *PP* = 0.96; SA, *PP* = 0.99); as well as sister relationships between *S. macrophallicum* and OTU6 (CA, *PP* = 0.97; SA, *PP* = 0.97), and OTU2 and OTU3 (CA, *PP* = 1; SA, *PP* = 0.98). Moreover, in both analyses we recovered three major clades of lineages that were geographically structured (Figs. 1, 2): Clade 1 comprised solely *S. borrei* lineages (*PP* = 1 in both CA & SA) distributed in the inner highlands of western-central Mexico; Clade 2 comprised *S. histrio*, *S. mexicanum*, OTU1, and OTU4 lineages (CA, *PP* = 1; SP, *PP* = 0.99) that are distributed from the central costal lowlands to the highlands of southern Mexico; Clade 3 comprised all other remaining lineages (CA, *PP* =0.86; SA, *PP* = 0.72), which are distributed principally in the inner highlands and basins of central Mexico. Within the Clade 2, OTU4 occupied the basal position and OTU1 was more closely related to the *S. histrio* and *S. mexicanum* lineages, in all cases with *PP* values higher than 0.95. Within the Clade 3, *S. minimum*, *S. macrophallicum*, *S. planum*, *S. purpurascens*, *S. rugosum*, *S. variabile*, OTU5, OTU6, and OTU8 formed a monophyletic group (CA, *PP* = 1; SA, *PP* = 0.81). However, the inferred phylogenetic relationships between these last taxa were different in both analyses and poorly supported (Figs. 2, 3). Other differences between both phylogenetic analyses were that in CA the relationships between the three major clades were unresolved; whereas in SA, *S. borrei* occupied the basal position in the genus but with a weak support (*PP* = 0.55). Moreover, in the CA analysis, OTU2 and OTU3 were more closely related to the monophyletic group comprising *S. minimum*, *S. macrophallicum*, *S. planum*, *S. purpurascens*, *S. rugosum*, *S. variabile*, OTU5, OTU6, and OTU8 (*PP* =1); whereas in SA this specious group was more closely related to OTU7 but with a weak support (*PP* = 0.55). In addition, we observed a remarkable geographic structure of the monophyletic taxa within the three major clades of the genus, which in most cases were delimited by the principal mountain ranges in Mexico (Fig. 1).

Divergence time estimation

According to our approximate estimates of mean divergence ages (Fig. 3) the separation between *Sphenarium* and *Prosphena* occurred around 14.08 Ma and initial divergences within *Sphenarium* occurred around 7.22 to 2.91 Ma. Moreover, most lineage diversification within the clades of *Sphenarium* occurred principally around 2.51 to 0.19 Ma.

Inferences of species boundaries

Nine taxa were consistently recognised by both morphological and phylogenetic criteria: *S. borrei*, *S. minimum*, *S. planum*, OTU2, OTU3, OTU4, OTU5, OTU6, and OTU7. Moreover, most of them (*S. borrei*, *S. planum*, OTU2, OTU3, OTU4, and OTU7) showed genetic differentiation values congruent with the threshold values (≥ 3%) usually considered in interspecific comparisons in insects (Allegrucci *et al.* 2013; Hebert *et al.* 2003a; b) . Therefore, we considered that these nine taxa to be well-separated lineages within the genus. As for the remaining taxa (*S. histrio*, *S. macrophallicum*, *S. mexicanum*, *S. purpurascens*, *S. variabile*, OTU1, and OTU8), we considered that there exist enough morphological (see description of species) and/or genetic differences (Table 2) among them to be considered as different lineages even if they were paraphyletic or their monophyly were weakly supported. Therefore, we recognise a total of 17 taxa as valid species within the genus. Hereafter, we refer to the eight identified OTUs as new species within the genus and name them as follows: *S. occidentalis* **sp.n.** (OTU1), *S. adelinae* **sp.n.** (OTU2), *S. miztecum* **sp.n.** (OTU3), *S. totonacum* **sp.n.** (OTU4), *S. tarascum* **sp.n.** (OTU5), *S. crypticum* **sp.n.** (OTU6), *S. infernalis* **sp.n.** (OTU7) and *S. zapotecum* **sp.n.** (OTU8). Because new species were discovered, we update the description of the genus and provide a key for its species in Appendix. Below, we redefine and describe all recognised species of *Sphenarium*, comparing our results with the previous taxonomic works.

Taxonomic description

Subfamily Pyrgomorphinae Brunner von Wattenwyl, 1874

Tribe Sphenariini Bolívar, 1884

Subtribe Sphenariina Bolívar, 1884

Genus *Sphenarium* Charpentier, 1842

Type species: *Sphenarium purpurascens* Charpentier, by original monotypy.

External morphology: Body: strongly fusiform, not compressed, broader at the region of metathorax; larger and wider in females than in males, body size notably variable ranging approximately from 18.05 to 42.59 mm in females and from 16.04 to 41.67mm in males; integument smooth or granulose. Antennae: filiform (Fig. 4A) to weakly ensiform (Fig. 4B); notably shorter or longer than head and pronotum together, generally shorter in females than in males; antennal segments longer than wide, ranging from 10 to 14 in both sexes; last antennal segments as long as the three previous segments together. Head: in dorsal view subtriangular-compressed (wider than long, Fig. 4C), subtriangular-elongated (nearly as wide as long, Fig. 4D) or conical (notably longer than wide, Fig. 4E); generally wider in females than in males; vertex prolonged anteriorly, horizontal to slightly ascending; frontal costa narrow, widely sulcate, prominent between the base of the antennae, obsolescent towards the clypeus; fastigium with a median carinula, acute, tapered or rounded at the apex, shorter or nearly as long as the interocular space; eyes spherical to ovate. Thorax: pronotum dorsally straight to slightly humped in lateral view, longer than wide, narrower anteriorly, notably widened posteriorly, usually with conspicuous median carina and little or not trace of lateral carina; pronotal disc with lateral anterior margins strongly convex, weakly excavated in the middle and lateral posterior margins somewhat sinuous, slightly emarginated or not dorsally, covering mesonotum almost entirely in females and partially in males. Wings: tegmina minute, spatula-like with divergent borders narrow toward the base (Fig. 4G) or strap-like with nearly parallel borders (Fig. 4H); no trace of hind wings. Legs: anterior pair slightly shorter than middle pair, anterior and middle femora rounded in section, notably more robust in males than in females; hind femora stout, flattened, generally extending beyond the apex of the abdomen in both sexes; tibia of all legs as long as their respective femur, with two ventral rows spines; anterior and middle tibia with 5 to 7 small spines per row, hind tibia with 9 to 13 large or small spines per row, pretarsus large, arolium disc-like. Abdomen: tapered towards the apex whit an obvious median carina notably pointer dorsally in the first abdominal segment of females; epiproct triangular as long as broad; cercus conical, pointed at their apices, somewhat shorter than epiproct; sub-genital plate of males tapered (Fig. 4J) or rounded moderately (Fig. 4K) or notably (Fig. 4L) developed posteriorly towards the apex in lateral view; dorsal ovipositor valves rounded (Fig. 4M), or lanceolate moderately (Fig. 4N) or notably elongated (Fig. 4O) towards the apex.

Male genitalia: Epiphallus: in dorsal view (Fig. 5A) transverse with short lateral plates broader at the base; anterior process divergent; lophi dorsolaterally or anteriorly directed with apices laterally expanded to interiorly curved; appendices short and heavy, narrowed anteriorly, wide in the middle and rounded or compressed posteriorly; bridge as long or slightly longer than lateral plates. Ectophallus: in dorsal view (Fig. 5B) with basal emargination of cingulum slightly to very developed and wide basal thickening; suprazygomal plate broad to undeveloped; zygoma with posterior margin subcovex and/or irregularly produced; apodemal plates of cingulum broad, laterally rounded with blunt apices inwardly directed, slightly to widely spaced; central membrane with two arms diverging anteriorly to varying distances on either side of the zygoma; lateral borders of ramus of cingulum notably concave, convergent (slightly rounded or straight) or parallel; ventral processes variable in length often prolonged beyond the base of ectophallus with rounded or pointed apices; ventral transverse thickening present or not. In lateral view (Fig. 5C) apodemal plates rounded to pointed. In posterior view (Fig. 5D) inflections of supraramus reduced to notably developed; valves of cingulum in apical view small to large, variable in form, stout or slender, sometimes highly sclerotized towards inner-central portion of the sheath forming a conspicuous sclerotized hollow, close or widely open. Endophallus: in lateral view (Fig. 5E) pseudoarch short or long, tightly or loosely joining the endophallus to the valves of cingulum; aedeagal valves ranging from very short to very long; slender or stout; horizontal to strongly curved upwards; ventrally smooth of somewhat serrated, in the apex widely or slightly rounded, sharply pointed or with an apical spine; aedeagal valves and sclerites together shorter to nearly 3 fold the length of dorsal inflections of endophallic apodemes; ventral inflections of endophallic apodemes slightly to quite developed, quadrate, rectangular or triangular; dorsal inflections of endophallic apodemes pointed upwards, varying from rectangular to somewhat triangular in dorsal view (Fig. 5G).

Colouration: Notably variable even between individuals from the same population; ground colours varying from different kinds of green (e.g. olive, lime green, yellow), yellow (e.g. dark yellow buff, warm buff, cream) and brown (e.g. dark salmon, Mikado, cinnamon). Body uniformly coloured, principally in females, or with different colouration traits including body parts and lines, shades and blotches distinctly coloured, contrasting again the ground colourations of the body. Colouration traits: antenna darker dorsally and apically than ventrally and posteriorly; fastigium distinctly coloured (Fig. 6, T1); head with lateral postocular bands, wide or narrow, lightly coloured (Fig. 6, T2); eyes with six longitudinal darker lines (Fig. 6, T3), usually with dark speckling on the upper third; dorsomedial line narrow or wide, running from pronotum to abdomen, lightly coloured (Fig. 6, T4); dorsal shades covering partially or entirely the dorsal portion of the body from head to abdomen, vaguely or distinctly delimited in pronotum; darkly coloured (Fig. 6, T5); lateral shades with the same colouration as dorsal shades, running from head to abdomen, mostly evident on head and abdomen (Fig 6, T6); lateral bands of blotches (Fig 6, T7) distinctly coloured, running from pronotum to abdomen, interrupted at the 1st abdominal segment by lateral light blotches (Fig. 6, T8); ventral margins of pronotum with conspicuous light bands (Fig 6, T9) continuous with the lateral postocular band of head; lateral carinas of pronotum (Fig. 6, T10) highlighted whit lighter colouration; mesonotum partially or entirely dark dorsally (Fig. 6, T11); mesonotum and metanotum lightly coloured laterally below tegmina (Fig. 6, T12); tegmina uniformly coloured or darker basally than apically, often with the same colouration tan dorsal shades or background colouration; fore and middle legs darker or equally coloured than hind legs; hind femora with upper (Fig. 6, T13) and lower (Fig. 6, T14) medial area darkly and lightly coloured, respectively; lower marginal area (Fig. 6, T15) distinctly coloured; knees of hind femora uniformly dark or darker laterally than dorsally (Fig. 6, T16); hind tibia darkly coloured or distinctly coloured (Fig. 6, T17).

Biology: Most biological studies in the genus have focused principally on *S. purpurascens* (e.g. Cano-Santana & Castellanos-Vargas, 1999; and the references therein)(Cano-Santana & Castellanos-Vargas 2009); whereas other species have only been partially considered in ecological studies (Descamps 1975; Márquez 1965a; b). Nevertheless, direct observations in the wild and the information associated with museum specimens indicate that all *Sphenarium* species are biologically similar. These grasshoppers are univoltine. The nymphs mainly emerge and develop during the raining season (mid-May to mid-September); adults appear and reproduce mainly during the fall (from mid-September to mid-December), and oviposition and death occur during the winter (approximately from mid-December to mid-February). *Sphenarium* grasshoppers occur in a wide variety of plant communities including xerophytic, temperate, tropical deciduous and rain forests where they are mainly found in open areas and border vegetation. They feed on a wide variety of herbaceous plants including seasonal (e.g. *Dahlia coccinea, Verbesina virgate, Datura stramonium* & *Tithonia* sp.) and perennial species (e.g. *Montanoa tomentosa, Eupatorium petiolare* & *Budleia cordata*), as well as trees (e.g. *Burcera* sp. & *Gliricidia* *sepium*) and crop species (e.g. *Zea mays, Medicago sativa* & *Phaseolus vulgaris*) (Cano-Santana & Castellanos-Vargas 2009; Descamps 1975; Márquez 1962, 1965b; Oyama *et al.* 1994; SSU, pers. obs.).

Geographic distribution: *Sphenarium* grasshoppers are distributed from central Mexico to northwest Guatemala along a wide altitudinal gradient ranging approximately from 0 to 2700 m a.s.l. They are mainly distributed parapatrically with apparently narrow sympatric zones at their latitudinal and altitudinal distribution limits.

Diagnosis: The sister genera *Sphenarium* and *Prosphena* clearly differentiate genetically (Figs. 2, 3) and morphologically. For instance, in *Prosphena,* the head and fastigium are conical considerably more elongated than in *Sphenarium* (Fig. 4F), the tegmina are tongue-like, always wider at the base than in the apex, and the pronotal disc is strongly sinuous and emarginated covering entirely the mesonotum in both sexes (Fig. 4I). Moreover, *Prosphena* grasshoppers are geographically restricted to Central America, from northwest Guatemala to northwest Costa Rica (Kevan *et al.* 1964). So far, no sympatric populations between *Sphenarium* and *Prosphena* have been found.

Description of species

Sphenarium purpurascens **Charpentier, 1842**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:36986)

Description: External morphology (Fig. 7A, B, C, D, E, F): total body length ranging from 18.01 to 30.24 mm in females and 16.04 to 28.14 mm in males. In most cases: antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-compressed, wider than long with spherical eyes in females or subtriangular-elongated moderately longer than wide with oval eyes in males; fastigium notably reduced, less than a half the length of interocular space in females or moderately elongated, nearly half the length of interocular space in males; tegmina spatula-like; subgenital plate of males rounded, moderately developed posteriorly; dorsal ovipositor valves lanceolate, moderately elongated towards the apex. Male genitalia: bridge of epiphallus as long as the length of lateral plates in most cases (Figs. 5A; 8A-I, D-I, G-I). Ectophallus in dorsal view (Fig 8A-II, D-II, G-II) with lateral borders of ramus strongly concave; basal emargination of cingulum moderately developed; interspace between apodemal plates of cingulum moderately open. Ectophallus in posterior view (Fig. 8B, E, H) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus moderately developed with distal margins dorsally directed in most cases; valves of cingulum small, triangular, slightly developed (morphotypes 1 & 2; Figs. 8C, F, respectively) or notably developed posteriorly (morphotype 3; Fig. 8I). In lateral view of endophallus (Fig. 8A-III, D-III, G-III) pseudoarch elongated, loosely joined to the valves of cingulum; aedeagal valves long with smooth ventral borders and an apical spine slightly longer (morphotypes 1 & 3; Fig. 8A-III, G-III, respectively) or shorter (morphotype 2; Fig. 8D-III) than the base of aedeagal sclerites (see Fig. 5E); aedeagal valves and sclerites together about twice (morphotypes 1 & 3; Fig. 8A-III, G-III, respectively) or 1½ fold (morphotype 2; Fig. 8D-III) the length of dorsal inflections of endophallic apodemes (see Fig. 5F).

Colouration: Ground colours vary from green, beige, brown or grey (Fig. 7A, B, C, D, E, F). Body uniformly coloured with ground colours (Fig. 7F) or with the following colour traits: antennae black, yellow or orange; fastigium reddish or brownish; lateral postocular bands whitish or yellowish; dorsomedial line frequently present, narrow, whitish or reddish; dorsal shades frequently present, black, purple, brown or grey, covering partially (Fig. 7E) or entirely (Fig. 7A, C) the dorsal portion of the body; lateral shades often present; lateral bands of blotches not evident; ventral bands of pronotum generally present, wide and whitish; mesonotum partially or entirely black; light lateral blotches of 1st abdominal segment generally present and whitish; hind femora frequently with upper medial area black to brown and lower medial area whitish or yellowish; frequently knees of hind femora black laterally, brownish to reddish dorsally; hind tibia black, yellow or orange.

Diagnosis: *S. purpurascens* mainly differs from its congeners in the following combination of male genitalia characters: lateral borders of ramus of cingulum strongly concave, inflections of supraramus moderately developed, aedeagus valves moderately long and its apical spine of aedeagus always present slightly longer or notably shorter than the base of aedeagal sclerites..

Distribution: This species is distributed in elevations ranging approximately from 800 to 2700 m a.s.l. from the southern Altiplano to the Sierra Madre del Sur in the Mexican states of Guanajuato, Hidalgo, Mexico, Mexico City, Michoacan, Oaxaca, Puebla, Queretaro, Tlaxcala, and Veracruz (Fig. 9A). The distribution of *S. purpurascens* is interrupted at the Tehuacan valley and somewhat delimited by the higher mountains of the Mexican Volcanic Belt, Sierra Madre Oriental and Sierra Madre del Sur. Across this geographic range the morphotype 1 of this species has the widest distribution, whereas the morphotype 2 is restricted to the central valleys of Oaxaca and the morphotype 3 is found in separate populations in the inner slopes of the Sierra Madre del Sur, Oaxaca (Fig. 9A & C). Previously, lower and southern ranges were recognized for *S. purpurascens* (Kevan, 1977). However, these assumptions were based on two misidentified male nymphs from 1.0 mi W Puerto Angel, Oaxaca (resembling *S. histrio* nymphs); and three adult females from Las Margaritas, Chiapas, apparently corresponding to *S. purpurascens* but probably mislabelled. Moreover, during our fieldwork we did not find *S. purpurascens* beyond the above-specified ranges.

Material examined: Lectotype *m* (Appendix Fig. 18A) and paralectotype *f* (Appendix Fig. 18B) from Mexico (V. Charpentier); designation: Kevan (1960, unpublished results); location: Berlin Zoological Museum (BZM), Berlin, Germany. We were able to examine only the external morphology of this material. *Additional material*: 459 *m*, 361 *f*, from 100 localities. Locality information and depositories of these examined specimens is provided in Appendix Table 5.

Taxonomic discussion: Charpentier (1842) described this species apparently based on two females and males syntypes from an unspecified Mexican locality. After its original description the taxonomic status of *S. purpurascens* remained unchanged until Boyle (1974) and Kevan (1977) recognised it as a subspecies, *S. purpurascens purpurascens.* Recently, Pedraza-Lara *et al.* (2015) and Sanabria-Urbán *et al.* (2015) proposed the elevation of this species at the species level considering principally its morphological distinctiveness. Based on the morphological, genetic and geographical cohesiveness of this species we also consider *S. purpurascens* as a valid species within the genus.

Sphenarium zapotecum **Sanabria-Urbán, Song & Cueva del Castillo sp.n.**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495075)

Description: External morphology (Fig. 7G, H): total body length ranging from 22.13 to 26.73 mm in females and from 19.29 to 24.26 in males; antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-elongated slightly longer than wide in females (Fig. 7H) or conical notably loner than wide in males (Fig. 7G), with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina spatula-like in both sexes; subgenital plate of males rounded, moderately developed posteriorly; dorsal ovipositor valves lanceolate, moderately elongated towards the apex. Male genitalia: bridge of epiphallus as long or slightly longer than the length of lateral plates in most cases (Fig. 8J-I). Ectophallus in dorsal view (Fig. 8J-II) with lateral borders convergent slightly rounded; basal emargination of cingulum moderately developed; interspace between apodemal plates of cingulum moderately open. Ectophallus in posterior view (Fig. 8K) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus reduced or not developed laterally (Fig. 8K, arrow); valves of cingulum with unique tongue-like form, notably developed posteriorly (Fig. 8L). Endophallus in lateral view (Fig. 8J-III) with an elongated pseudoarch loosely joined to the valves of cingulum; aedeagal valves long with smooth ventral borders and an apical spine slightly longer than the width of the base of aedeagal sclerites; aedeagal valves and sclerites together about twice the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours varying from green to brown. Body uniformly coloured with ground colours (Fig. 7H) or with the following colour traits (Fig. 7G): antennae generally light brown; fastigium brown to black; lateral postocular bands whitish; dorsomedial line narrow and pinkish in eastern populations or wide and yellowish in western populations; dorsal shades black, grey or brown, frequently covering partially the dorsal portion of the body; lateral black shades generally absent or restricted to head; lateral bands of blotches not evident; ventral bands of pronotum generally absent, instead ventral borders of pronotum lightly-coloured; mesonotum partially or entirely black; lateral blotches of 1st abdominal segment if present white; hind femora uniformly coloured with knees laterally black, dorsally brownish; hind tibia orange.

Diagnosis: Externally this species closely resembles *S. purpurascens* and *S. variabile*. However, *S. zapotecum* **sp.n.** generally differs from these two species by its more elongated head in both sexes. At the male genitalia level *S. zapotecum* **sp.n.** more closely resembles *S. purpurascens* and *S. tarascum* **sp.n.**, which also show an apical spine in the aedeagus and lack the sclerotized hollow in the sheath of ectophallus. Nevertheless, *S. zapotecum* **sp.n.**, differs from these latter species by its reduced or undeveloped inflections of supraramus, as well as the unique form of its valves of cingulum notably projected posteriorly.

Distribution: This species is apparently restricted to the outer southern slope of the Sierra Madre del Sur in Oaxaca, Mexico, occurring in elevations ranging from 1016 to 1457 m a.s.l. (Fig. 9C).

Material examined: Holotype *m* (Figs. 7G; 8J, K & L) from Mexico: Oaxaca, Pluma Hidalgo (1), 15.93987876ºN, -96.42996051ºW, 1153 m a.s.l., XII-11-2013 (Sanabria-Urbán S., Fontana P. & Mariño-Pérez R. #L27); measurements: BS = 23.74 mm, FL = 1.29 mm, PL = 4.68 mm, HF = 11.77 mm. Paratypes from Mexico: Oaxaca: 4 *m*, 3 *f*, same data as holotype; 2 *m*, 2 *f*, Carr. 175 Km. 172, XII-12-2013, 16.01864845ºN, -96.5303105ºW, 1457 m a.s.l. (Sanabria-Urbán S., Fontana P. & Mariño-Pérez R. #L31); 1 M, Oaxaca road ca. 85km N Pto. Angel, IX-1-1981 (Otte, Azuma & Newlin # 43); 2 *m*, 1 *f*, 24-25 mi. N Pto. Escondido rd. to Oaxaca, IX-2-1981 (Otte, Azuma & Newlin # 45). The holotype was deposited at IBUNAM and the paratypes were deposited at the ANSP, IBUNAM and TAMUIC. *Additional material*: 5 *m*, 4 *f*, from three additional localities (Appendix Table 5).

Taxonomic discussion: We observed that *S. zapotecum* **sp.n.** is mainly related morphologically and genetically to *S. purpurascens*. Nevertheless, its unique combination of morphologic traits (both in external and male genitalia structures), as well as its geographic isolation supports its recognition as an independent species within the genus. *Sphenarium zapotecum* **sp.n.** was not included in previous studies on the genus except for Pedraza-Lara *et al.* (2015), who identified specimens from a locality within the ranges of this new species as *Sphenarium* sp. Oax6. Using the *CO1* sequences of Pedraza-Lara *et al.* (2015) and the present study, the only locus represented in both studies, we conducted a combined Bayesian phylogenetic analysis, in which all *Sphenarium* sp. Oax6 samples clustered (*PP* > 0.95) with *S. zapotecum* **sp.n.** samples (Appendix Fig. 22). Considering their geographical and phylogenetic proximity it is probable that *Sphenarium* sp. Oax6 and *S. zapotecum* **sp.n.** represent the same species.

Etymology: Named in honour of the Zapotecos, an ancient Native American people still living in the area where this species is distributed.

Sphenarium tarascum **Sanabria-Urbán, Song & Cueva del Castillo sp.n.**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495075)

Description: External morphology (Fig. 7I, J): total body length ranging from 21.41 to 30.07 mm in females and from 19.44 to 25.6 mm in males; antennae filiform, slightly shorter in females or notably longer than head and pronotum together in males; head subtriangular-elongated slightly longer than wide in females or conical notably longer than wide in males, with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina are spatula-like in both sexes; subgenital plate of males rounded moderately developed posteriorly; dorsal ovipositor valves lanceolate, notably elongated towards the apex. Male genitalia: bridge of epiphallus as long or slightly longer than the length of lateral plates in most cases (Fig. 10A-I). Ectophallus in dorsal view (Fig. 10A-II) with lateral borders of ramus convergent slightly rounded; basal emargination of cingulum moderately; interspace between apodemal plates of cingulum moderately open. Ectophallus in posterior view (Fig. 10B) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus moderately developed with distal margins dorsally directed; valves of cingulum triangular to slightly quadrangular, moderately developed posteriorly (Fig. 10C). Endophallus in lateral view (Fig. 10A-III) with elongated pseudoarch, loosely joined to the valves of cingulum; aedeagal valves long with smooth ventral borders and an apical spine slender, slightly bent, and notably longer than the width of the base of aedeagal sclerites; aedeagal valves and sclerites together about 1½ fold the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours varying from green to brown; body uniformly coloured (Fig. 7J) or with the following colour traits (Fig. 7I): antennae brown to magenta; fastigium often reddish or brownish; lateral postocular bands yellowish; dorsomedial line frequently absent, if present very narrow, whitish, almost entirely restricted to the abdomen; dorsal shades brown, grey or magenta covering almost entirely the dorsal portion of thorax and abdomen, generally less evident in pronotum; lateral shades generally absent or restricted to head; lateral bands of blotches not evident; ventral bans of pronotum generally absent, instead ventral borders of pronotum lightly-coloured; mesonotum partially or entirely black; lateral blotches of 1st abdominal segment if present, whitish or yellowish; in most cases hind femora uniformly coloured with knees laterally brown, dorsally reddish; hind tibia sometimes brown or yellowish.

Diagnosis: Externally this species resembles other species with adjacent distribution such as *S. purpurascens*, *S. rugosum*, and *S. infernalis* **sp.n.**, as well as other more geographically distant species such as *S. minimum* and *S. zapotecum* **sp.n.** Generally, *S. tarascum* **sp.n.** differs from the first three species by its longer antenna, and elongated head (in both sexes) and dorsal ovipositor valves. The male genitalia of *S. tarascum* **sp.n.** more closely resemble those of *S. purpurascens* and *S. zapotecum* **sp.n.**, which also have an apical spine in the aedeagus. However, *S. tarascum* **sp.n.**, differs from these species by the following combination of male genital characters: lateral borders of ramus convergent slightly rounded, inflections of supraramus moderately developed and apical spine of aedeagus slender, slightly bent, and longer than in any other species in the genus (Fig. 10A-III, arrow).

Distribution: This species is restricted north of the central portion of the Balsas River Basin in Michoacan, Mexico (Fig. 9A), in elevations ranging from 1394 to 1759 m a.s.l.

Material examined: Holotype *m* (Fig. 7I) from Mexico: Michoacan, Las Cañas sobre Carr. 120, 19.08443296ºN, -101.768692ºW, 1394 m a.s.l., X-8-2015 (Sanabria-Urbán S. # M011-11ARO); measurements: BS = 22.19 mm, FL = 1.04 mm, PL = 4.31 mm, HF = 10.60 mm. Paratypes from Mexico: Michoacan: 9 *m*, 10 *f* same data as holotype; 3 *m*, 1 *f*, Rancho el Mirador, 19.20727001ºN, -101.735125ºW, 1759 m a.s.l., X-8-2015 (Sanabria-Urbán S. #M012). The holotype was deposited at IBUNAM and the paratypes were deposited at the IBUNAM and TAMUIC. *Additional material*: 5 *m*, 2 *f*, from the same locality as holotype but different date; and 3 *m*, 1 *f*, from two additional localities (see Appendix Table 5).

Taxonomic discussion: This species is closely related morphologically to *S. purpurascens* and genetically to *S. rugosum*. Nevertheless, its unique combination of morphologic traits (both in external and male genitalia structures), monophyly and geographic isolation supports its recognition as a separate species within the genus. Previously, Sanabria-Urbán *et al*. (2015) identified specimens of *S. tarascum* **sp.n.** as a putatively new species, *Sphenarium* sp.n. 5. For other studies in the group this species was unknown.

Etymology: Named in honour of the Tarascos, an ancient Native American people still living in the area where this species was found.

***Sphenarium planum* Bruner, 1906**
(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:36998)

Description: External morphology (Fig. 7K, L): total body length ranging from 21.30 to 29.04 mm in females and 18.29 to 26.24 mm in males; antennae filiform, notably shorter in females or slightly longer than head and pronotum together in males; head subtriangular-compressed, wider than long with spherical eyes in females or subtriangular-elongated moderately longer than wide with oval eyes in males; fastigium notably reduced, less than a half the length of interocular space in females or moderately elongated, nearly half the length of interocular space in males; tegmina spatula-like in both sexes; subgenital plate of males rounded, moderately developed posteriorly; dorsal ovipositor valves lanceolate, moderately elongated towards the apex. Male genitalia*:* bridge of epiphallus as long as the length of lateral plates in most cases (Fig. 10D-I). Ectophallus in dorsal view (Fig. 10D-II) with lateral borders of ramus convergent slightly rounded; basal emargination of cingulum notably reduced; interspace between apodemal plates of cingulum widely open. Ectophallus in posterior view (Fig. 10E) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus reduced; ramus of cingulum distinctly developed ventrally; valves of cingulum with unique form notably developed posteriorly (Fig. 10F). In lateral view of endophallus (9D-III) pseudoarch elongated loosely joined to the valves of cingulum; aedeagal valves slender, long, with smooth ventral margins and moderately rounded in the apex without apical spine; aedeagal valves and sclerites together about 2 ½ fold the length of endophallic apodemes.

Colouration: Ground colours vary from green, beige, brown or grey, yellow or brown. Body uniformly coloured (Fig. 7L) or with the following colour traits: antennae often yellow; fastigium frequently brownish to reddish; lateral postocular bands yellow; dorsomedial line frequently absent, if present very narrow, restricted almost entirely to the abdomen and yellowish; dorsal shades black, grey or brown, frequently less evident in head and pronotum; lateral shades frequently absent, if present very narrow and restricted to the head; lateral bands of blotches sometimes evident and yellowish; ventral bands of pronotum if present yellowish, wide or narrow; mesonotum partially or entirely black; lateral blotches of 1st abdominal segment whitish; hind femora frequently with upper medial area dark green and lower medial area yellow; frequently knees of hind femora laterally black, dorsally brownish; frequently hind tibia yellow or brown.

Diagnosis: Externally this species closely resembles *S. purpurascens* and *S. rugosum*. Generally, females of *S. planum* have smaller antennae than any other its congeners. Moreover, when present, the yellow colourations of the lateral bands of blotches and the medial area of the hind femora of *S. planum* distinguish it from *S. purpurascens.* On the other hand, the male genitalia of *S. planum* more closely resemble that of *S. rugosum,* and *S. crypticum* **sp.n.**, which also lack the apical spine of aedeagus. However, *S. planum* differs from all these species by the following combination of male genital traits: lateral borders of ramus convergent slightly rounded, basal emargination of cingulum notably reduced with a wide open interspace between the apodemal plates of cingulum, inflections of supraramus reduced, ramus of cingulum is distinctly developed ventrally, aedeagal valves moderately rounded in the apex without apical spine, and aedeagal valves and sclerites relatively long and slender.

Distribution: This species is apparently restricted to the Tehuacan valley with sparse records in the surrounding mountain ranges of the valley in Puebla, Oaxaca and Veracruz, Mexico (Fig. 9A) in elevations ranging from approximately 1200 to 2095 m a.s.l.

Material examined: Lectotype *m* (Appendix Fig. 18C) and paralectotype *f* (Appendix Fig. 18D) from Mexico: Puebla, Tehuacan, XI (L. Bruner); designation: Rehn and Hebard (1912); location: ANSP. We could examine only the external morphology of this type material. *Additional material*: 10 *m*, 10 *f*, from the type locality; 45 *m*, 16 *f*, from 12 adjacent localities (Appendix Table 5).

Taxonomic discussion: Bruner (1906) originally described this species from two syntypes (male and female) erroneously specifying Tehuantepec as its type locality rather than Tehuacan as it is labelled in the type material. Bruner also recognised a close relationship between *S. planum* and *S. purpurascens* indicating that the former species differed from the latter in its more rotund form and in the absence of lateral carina of pronotum. However, these traits are ambiguous and variable within the genus. Posteriorly, Boyle (1974) identified differences in endophallic morphology between these two species, but he interpreted this differentiation as geographic variation of *S. p. purpurascens* and/or probable hybridization with *S. p. minimum*, recognising *S. planum* only as an intermediate form between these two later taxa. Previously, we proposed the re-establishment of *S. planum* as valid species mainly based on genetic evidence (Sanabria-Urbán *et al.* 2015). In this study we found that *S. planum* shows a unique combination of male genitalia characters, a well-supported monophyly (Fig. 2), relatively high levels of interspecific genetic differentiation (Table 3) and apparent geographic isolation. All these lines of evidence support the recognition of *S. planum* as a valid species.

Sphenarium macrophallicum **Kevan and Boyle, 1977**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:37035)

Description: External morphology (Fig. 7M, N): total body length total body length ranging from 23.47 to 38.88 mm in females and 23.12 to 36.15 mm in males; antennae filiform; notably shorter in females or slightly longer than head and pronotum together in males; head subtriangular-elongated nearly as wide as long in females or moderately longer in males wide, with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in both sexes; tegmina spatula-like in both sexes; subgenital plate of males rounded notably developed posteriorly; dorsal ovipositor valves rounded or lanceolate moderately elongated towards the apex. Male genitalia: bridge of epiphallus shorter or as long as the length of lateral plates (Fig. 10G-I). Ectophallus in dorsal view (Fig. 10G-II) large with lateral borders of ramus parallel; basal emargination of cingulum notably reduced; interspace between apodemal plates notably open. Ectophallus in posterior view (Fig. 10H) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus reduced; ramus of cingulum distinctly developed ventrally; valves of cingulum small with unique form, centred at the middle of the sheath. In lateral view of ectophallus valves of cingulum slightly developed posteriorly (Fig. 10I). Endophallus in lateral view (Fig. 10G-III) with elongated pseudoarch, loosely joined to the valves of cingulum; aedeagal valves very slender and long, with smooth ventral margins and moderately rounded in the apex without apical spine; aedeagal valves and sclerites together about 2 ½ to 3 fold the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from green to brown. Body uniformly coloured or with the following colour traits: antennae generally black or dark brown; fastigium frequently reddish; lateral postocular bands frequently present, wide and yellow; dorsomedial line frequently absent, if present very narrow and yellowish; dorsal shades generally absent; lateral shades frequently absent, if present narrow and black, principally evident in head, metanotum, 1st abdominal segment and apex of abdomen; lateral bands of blotches not evident; ventral bands of pronotum often absent, if present very narrow and yellowish; mesonotum partially or entirely black; lateral whitish blotches of 1st abdominal segment frequently absent; hind femora mostly uniformly coloured, sometimes with lower medial area yellowish and knees laterally black, dorsally reddish; hind tibia generally black.

Diagnosis: Externally this species closely resembles *S. crypticum* **sp.n.** and *S. infernalis* **sp.n.**, which are distributed contiguously. Generally, males of *S. macrophallicum* differ from males of other species by its rounded subgenital plate more notably developed posteriorly. Moreover, the male genitalia of *S. macrophallicum* conspicuously differ from any other congener by the following combination of characters: ectophallus notably large with lateral borders of ramus of ectophallus parallel, inflections of supraramus reduced, ramus of cingulum distinctly developed ventrally, valves of cingulum small with distinct form and centred at the middle of the sheath, aedeagal valves very long and slender with smooth ventral margins and moderately rounded in the apex, and aedeagal valves and sclerites together longer than in any other species ranging from 2 ¾ to 3 fold the length of dorsal inflections of endophallic apodemes.

Distribution: This species is distributed northwest of the middle portion of the Balsas River Basin in elevations ranging from 207 to 1607 m a.s.l. in Michoacan and Guerrero, Mexico (Fig. 9A).

Material examined: Holotype *m* (Appendix Fig. 18E) from Mexico: Guerrero, 11 rd. mi. NE. of Arcelia, XII-8-1958, 3000ft± (T. J. Cohn #360). Allotype *f* (Appendix Fig. 18F) from Mexico: Guerrero, Cd. Altamirano, X-20-1957. Paratypes from Mexico: Guerrero: 1 *m* same locality as holotype (Figs. 7M; 10G, H, I), 1 *m*, 1 *f*, Temisco, XI-10-1928 (T. W: Bouchelle). Designation: Kevan and Boyle (1974) and location: UMMZ for all these specimens. We were able to examine both external and genital morphology of this type material. *Additional material*: 45 *m*, 43 *f*, from six localities. Locality information and depositories of these additional specimens examined is provided in Appendix Table 5.

Taxonomic discussion: This species was partially described from eight specimens collected from three different localities in Guerrero, Mexico (Kevan 1977). After the original description, the validity of this species has remained unchanged, even in the most recent studies in the genus (Pedraza-Lara *et al.* 2015; Sanabria-Urbán *et al.* 2015). Our phylogenetic analysis recovered *S. macrophallicum* as a paraphyletic species and we observed relatively low levels of genetic differentiation between *S. macrophallicum* and other adjacent (*S. crypticum* **sp.n.**, *S. tarascum* **sp.n.**, *S. rugosum*, *S. purpurascens*) and distant species (*S. variabile* and *S. zapotecum* **sp.n.**). Nevertheless, the decidedly different and unique combination of male genital traits, and restricted geographic distribution of this species supports its recognition as an independent species within the genus.

Sphenarium minimum **Bruner, 1906**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:36988)

Sphenarium affine Bruner, 1906

Description: External morphology (Figs. 7Q, R): total body length ranging from 23.67 to 28.57 mm in females and 18.05 to 24.89 mm in males; antennae filiform, slightly shorter in females or notably longer than head and pronotum together in males; head subtriangular-elongated slightly longer than wide in females or conical notably loner than wide in males, with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in both sexes; tegmina spatula-like in both sexes; subgenital plate of males rounded moderately developed posteriorly; dorsal ovipositor valves lanceolate, notably elongated towards the apex. Male genitalia: bridge of epiphallus as long as the length of lateral plates in most cases (Fig. 10J-I). Ectophallus in dorsal view (Fig. 10J-II) with lateral borders of ramus strongly concave; basal emargination of cingulum moderately developed; interspace between apodemal plates of cingulum moderately open. Ectophallus in posterior view (Fig. 10K) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus moderately developed with distal margins laterally directed in most cases; valves of cingulum notably small, triangular and slightly developed posteriorly (Fig. 10K, L). In lateral view of endophallus (Fig. 10J-III) pseudoarch elongated loosely joined the valves of cingulum; aedeagal valves medium sized with smooth ventral margins and moderately rounded in the apex, without apical spine (Fig. 10K, L); aedeagal valves and sclerites together about 1 ¼ fold the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from green, yellow or brown. Body uniformly coloured or with the following colour traits: antennae and fastigium frequently brown; lateral postocular bands frequently present, narrow and yellow; dorsomedial line generally absent, if present very narrow almost restricted to the abdomen, whitish, yellowish or pinkish; dorsal shades frequently absent, if present very narrow in head and thorax, wider in the dorsal portion of abdomen, light to dark brown; lateral shades often absent, if present very narrow, black or dark brown, almost restricted to head and abdomen; lateral bands of blotches not evident; ventral bands of pronotum often absent, if present very narrow and yellowish; mesonotum partially or entirely black; lateral whitish blotches of 1st abdominal segment sometimes evident; in most cases hind femora uniformly coloured, sometimes with lower medial area whitish or yellowish and brown knees; hind tibia frequently yellow or pale orange.

Diagnosis: *S. minimum* closely resembles *S. planum*, *S. purpurascens*, *S. tarascum* **sp.n.**, and *S. zapotecum* **sp.n.** in both external and genital morphology. Externally *S. minimum* only differs from the former two species by its more elongated head (in both sexes) and its lanceolate and more notably elongated dorsal ovipositor valves. Nevertheless, more conspicuous differences exist between male genital structures of all these species. *S. minimum* differs from these species by the following combination of male genital traits: ectophallus with lateral borders of ramus notably concave, valves of cingulum triangular and notably small, medium-sized aedeagal valves and moderately rounded in the apex without apical spine, and aedeagal valves and sclerites about 1 ¼ fold the length of dorsal inflections of endophallic.

Distribution: This species is distributed in elevations ranging from approximately 1000 to 1596 m a.s.l. in the outer slope of southern portion of the Sierra Madre in Veracruz, Mexico (Fig. 9A).

Material examined: *S. minimum*: lectotype *m* (Appendix Fig. 18G) from Mexico: Veracruz, Jalapa, XII (O.W. Barrett); *S. affine*: lectotype *m* (Appendix Fig. 18H) from Mexico: Veracruz, Orizaba, XI-1887 (L. Bruner). Designation: Rehn and Hebard (1912) and location: ANSP for both type specimens. We examined only the external morphology of this type material. *Additional material*: 7 *m*, 5 *f*, from Jalapa (L76); 2 *m* from Orizaba (L292), 35 *m*, 26 *f*, from 12 adjacent localities in Veracruz (Appendix Table 5).

Taxonomic discussion: Bruner (1906) originally described this species apparently based on a single male from Jalapa, Veracruz. Bolivar (1909) and Hebard (1932) recognized *S. minimum* as a valid species. Particularly, the later author proposed a closer relationship between *S. minimum* and *S. histrio* and *S. mexicanum* mainly based on their head morphology. Boyle (1974) also identified differences in head morphology and colouration patterns between *S. minimum* and *S. purpurascens*. However, this author only recognized the former taxon as a subspecies of the later, *S. purpurascens minimum*, principally based on the similarity between their epiphallic and ectophallic structures. Recently, Pedraza-Lara *et al.* (2015) and Sanabria-Urbán *et al.* (2015) proposed again the re-establishment of S. minimum as valid species based principally on genetic evidence. In this study we found that despite the notable similarity in the male genitalia between *S. minimum* and *S. purpurascens* the former taxon shows a unique combination of external and male genitalia characters, a well-supported monophyly (Fig. 2), and a well-separated geographic distribution from *S. purpurascens*. All these lines of evidence support the recognition of *S. minimum* as a valid species.

Sphenarium affine was only briefly described by Bruner (1906) apparently based on one female and male from Orizaba, Veracruz. Originally, Bruner (1906) recognized a close relationship between *S. affine* and *S. minimum*, only differentiating the former from the later in highly variable traits of head morphology. Later *S. affine* was synonymised within *S. marginatum* (Hebard 1932), despite the notable external differentiation between both taxa. Posteriorly, Boyle (1974) synonymized *S. affine* within *S. p. minimum*. In this study we examined several specimens collected in type localities of *S. minimum* and *S. affine*; which were practically similar among them in their male genital morphology. Therefore, we also recognise *S. affine* as a synonym of *S. minimum.*

***Sphenarium infernalis* Sanabria-Urbán, Song & Cueva del Castillo sp.n.**
(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495096)

Description: External morphology (Fig. 7Q, R): body size ranging from 25.74 to 38.48mm in females and from 25.94 to 38.13mm in males; antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-elongated slightly longer than wide in females or conical notably longer than wide in males, with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina spatula-like in both sexes; subgenital plate of males rounded moderately developed posteriorly; dorsal ovipositor valves rounded towards the apex. Male genitalia: bridge of epiphallus moderately longer or as long as the length of lateral plates (Fig. 11A-I). Ectophallus in dorsal view (Fig. 11A-II) broad at the base, with lateral borders of ramus convergent; dorsal borders of ramus with lateral projections notably developed interiorly, closing the central membrane of ectophallus somewhat far from the sheath (Fig. 11A-II, arrow); basal emargination of cingulum moderately developed; interspace between apodemal plates of cingulum moderately open to notably close. Ectophallus in posterior view (Fig. 11B) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus moderately developed with distal margins laterally or dorsally directed; valves of cingulum with distinct form (Fig. 11B), small and slightly developed posteriorly (Fig. 11C). Endophallus in lateral view (Fig. 11A-III) with elongated pseudoarch, loosely joined to the valves of cingulum; aedeagal valves somewhat broad, medium-sized, with smooth ventral margins and moderately rounded in the apex (Fig. 11B) without apical spine (Fig. 11C); aedeagal valves and sclerites together about the same length than dorsal inflections of endophallic apodemes (Fig. 11A-III).

Colouration: Ground colours vary from green or brown. Body uniformly coloured (Fig. 7R) or with the following colour traits (Fig. 7Q): antennae mostly black; fastigium frequently reddish; lateral postocular bands frequently present, wide, whitish to yellow; dorsomedial line mostly absent, if present very narrow and yellowish; dorsal shades often absent, if present reduced and restricted to the apex of abdomen, brown to black; lateral shades often present, narrow, black or brown; lateral bands of blotches not evident; ventral bands of pronotum very narrow and yellow; mesonotum is partially or entirely black; whitish lateral blotches of 1st abdominal segment frequently present; tegmina green, black to magenta; generally hind femora uniformly coloured with knees laterally black and dorsally reddish; hind tibia usually black.

Diagnosis: Externally this species more closely resembles *S. macrophallicum*, and *S. crypticum* **sp.n.** In most cases, females of *S. infernalis* **sp.n.** differs from females of these other species by their rounded dorsal ovipositor valves. Nonetheless, the male genitalia of *S. infernalis* **sp.n.** clearly differ from other *Sphenarium* species by the following combination of traits: lateral borders of ramus convergent, dorsal borders of ramus with lateral projections developed towards the central membrane that are not evident in other species in the genus, inflections of supraramus moderately developed, and aedeagal valves somewhat broad, medium-sized, moderately rounded in the apex.

Distribution: This species is apparently restricted to the western portion of the Balsas River Basin in elevations ranging from 335 to 1450 m a.s.l. Colima, Jalisco and Michoacan, Mexico (Fig. 9A).

Material examined: Holotype *m* (Fig. 7Q) from Mexico: Jalisco, 5mi. NE. Tecalitlan, 19.540895ºN, -103.3147204ºW, 4250ft, XI-25-1958 (T. J. Cohn #313); measurements: BS = 28.01 mm, FL = 1.03 mm, PL = 5.99 mm, HF = 13.30 mm. Paratypes from Mexico: Jalisco: 1 *m*, 1 *f*, same data as holotype; 3 *m*, 2 *f*, 1mi S Pihuamo, 869m a.s.l., XI-26-1958 (T.J. Conh #314); 2 *m*, 2 *f*, 6mi NE Tecalitlan, 4250ft, XI-30-1958 (T.J. Cohn #331); 1 *m*, 1 *f*, Tecalitlan, 19.52045714ºN, -103.3007948ºW, 1213 m a.s.l., X-2-2013 (Sanabria-Urban #P78); 2 *m*, 4 *f*, Pihuamo, 19.24430234ºN, -103.4002227ºW, 800 m a.s.l., X-2-2013 (Sanabria-Urban S. & Rivera-Ortiz F. #P79 [L58 MS1]). Colima: 1 *m*, 1 *f*, 9 mi E Colima, 1620ft, XI-22-1958 (T.J. Cohn #328); 1 *m*, 6 mi E Colima, 1250ft, XI-29-1958 (T.J. Cohn #327); 1 *m*, 1 *f*, Entrada a Colima, 19.18950356ºN, -103.6827591ºW, 468 m a.s.l., X-3-2013 (Sanabria-Urban S. & Rivera-Ortiz F. #M58). Michoacan: 1 *m*, 1 *f*, 15 mi W Apatzingan (2 mi E. Santa Ana), 1600ft, XII-2-1958 (T.J. Cohn #340); 1 *m*, 23rd mi WSW Ario de Rosales, 4200ft, XII-4-1958 (T.J. Cohn #348); 2 *m*, 1 *f*, 16.7mi W Apatzingan, 1100ft, IX-26-1959 (Cantral & Cohn #179); 1 *m*, 11mi S Uruapan on 37, IX-8-1981 (Otte, Azuma, Newlin #57); 1 *m*, 26rd mi NE Arteaga on Hwy. 37, 3 rd. mi. SW Rancho Nuevo, 2060ft, XI-3-1974 (T.J. & J. W. Cohn #121); 10 *m*, 8 *f*, Las Majadas Carr 120, 19.134653ºN, -102.463237ºW, 301 m a.s.l., IX-23-2012 (Sanabria-Urban S. #P43 [L57 MS1]); 1 *m*, 1 *f*, Periban, 19.55953625ºN, -102.4384873ºW, 1450 m a.s.l., X-1-2013 (Sanabria-Urban S. & Rivera-Ortiz F. #P75). The holotype was deposited at UMMZ and the paratypes were deposited at the IBUNAM, TAMUIC and UMMZ.

Taxonomic discussion: This species is closely related morphologically to *S. rugosum*. In deed material of this new species was identified as *S. rugosum* in previous studies (Boyle 1974; Kevan 1977). Nevertheless, *S. infernalis* **sp.n.** shows a unique combination of morphologic traits, in both external and male genitalia structures, a well-supported monophyly (Fig. 2b), relatively high levels of interspecific genetic differentiation (Table 3). All these lines of evidence support the recognition of *S. infernalis* **sp.n**. as a valid species. Sanabria-Urbán *et al*. (2015) identified specimens of *S. infernalis* **sp.n**. as the putatively new taxon, *Sphenarium* sp.n. 8. For other studies in the genus this new species was unknown.

Etymology **–** The specific name “infernalis” is derived from Latin and means “belonging to the lower regions”. It refers to the distribution of this species in the lowest portion of the Balsas River Basin, which is also one of the warmest regions in Mexico.

Sphenarium rugosum Bruner, 1906

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:37030)

Sphenarium barretti Bruner, 1906.

Description: External morphology (Fig. 7S, T; 12A, B, C, D): total body length ranging from 25.46 to 36.14 mm in females and from 19.8 to 35.07 mm in males; antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-elongated nearly as wide as long in females or moderately longer than wide in males with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in both sexes; tegmina spatula-like in both sexes; subgenital plate of males rounded moderately developed posteriorly; dorsal ovipositor valves lanceolate moderately elongated towards the apex. Male genitalia: bridge of epiphallus moderately longer than the length of lateral plates (Fig. 11D-I, G-I, J-I). Ectophallus in dorsal view (Fig. 11D-II, G-II, J-II) with lateral borders of ramus convergent; basal emargination of cingulum moderately or not developed (morphotypes 1 & 3; Fig. 11D-II, J-II, respectively) or notably developed (morphotype 2; Fig. 11G-II); interspace between apodemal plates of cingulum moderately open (morphotypes 1 & 3; Fig. 11D-II, J-II, respectively) or notably close (morphotype 2; Fig. 11G-II). Ectophallus in posterior view (Fig. 11E, H, K) without a conspicuous sclerotized hollow in the sheath;

inflections of supraramus moderately developed with distal margins laterally or dorsally directed; valves of cingulum triangular to slightly quadrangular, relatively small (morphotypes 1 & 2; Fig. 11E, H, respectively) or large (morphotype 3; Fig. 11K), slightly developed posteriorly (Fig. 11F, I, L). Endophallus in lateral view (Fig. 11D-III, G-III, J-III) with elongated pseudoarch, loosely joined to the valves of cingulum; aedeagal valves moderately (morphotype 1; Fig. 11D-III) or notably (morphotype 2 & 3; Fig. 11G-III, J-III, respectively) broad, medium-sized (morphotype 1 & 3) or short (morphotype 2), with ventral margins smooth (morphotypes 1 & 2) or somewhat serrated (morphotype 3; Fig. 11J-IV, arrow), broadly rounded apically (Fig. 11E, I, K, arows); aedeagal valves and sclerites together varying from ¾ (morphotype 2; Fig. 11G-III) to 1 ½ (morphotypes 1 & 3; Fig. 11D-III, J-III) fold the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from olive green to brown. Body uniformly coloured (Fig. 7T, 12B) or with the following colour traits: antennae frequently black or dark brown; fastigium generally reddish; lateral postocular bands frequently present, wide and yellow; dorsomedial line frequently present, narrow and yellowish; dorsal shades often present, covering partially (Fig. 7S) or entirely (Fig. 12C) the dorsal part of the body, black to brown; lateral shades often present, black or dark brown, mostly evident in the abdomen (Fig. 7S); lateral bands of blotches often present with distinct yellow colouration; ventral bands of pronotum often present and yellow; sometimes lateral carinas of pronotum highlighted with yellow (Fig. 7S); mesonotum partially or entirely black; lateral blotches of 1st abdominal segment frequently present, whitish or yellowish; hind femora uniformly coloured or with upper medial area black and lower medial area yellowish: knees of hind femora laterally black, dorsally reddish; hind tibia frequently black.

Diagnosis: Externally this species closely resembles *S. macrophallicum*, *S. planum*, *S. tarascum* **sp.n.**, *S. crypticum* **sp.n.**, and *S. infernalis* **sp.n.** Sometimes *S. rugosum* differs from all these species by its more conspicuous yellow colourations in the dorsomedial line, lateral carinas and lateral bands of blotches, in combination with darker dorsal colourations in the body (Fig. 12C). Nevertheless, *S. rugosum* clearly differs from all these species by the following combination of male genitalia characters: lateral borders of ramus convergent, inflections of supraramus moderately to notably developed, and aedeagal valves moderately or notably broad, medium sized or small, broadly rounded apically.

Distribution: This species is distributed in elevations ranging from 457 to 2344 m a.s.l. and is apparently confined to the eastern Balsas River Basin and Sierra Madre del Sur in Guerrero, Mexico, Michoacan, Morelos, Oaxaca, and Puebla, Mexico (Fig. 9A). The morphotype 1 of this species is widely distributed in the western portion of the Balsas River Basin; whereas the morphotype 2 is apparently restricted to inner slope of middle portion of the Sierra Madre del Sur (Fig. 9A, B). The morphotype 3 is only known from three localities east of the middle portion of the Balsas River Basin (Fig. 9A).

Material examined: *S. rugosum*: lectotype *m* (Appendix Fig. 19A) and paralectotype *f* (Appendix Fig. 19B) from Mexico: Morelos, Cuernavaca, I-4-1899 (C. C. Deam); designation: Rehn and Hebard (1912); location: ANSP. *S. barretti*: lectotype *m* (Appendix Fig. 19C), from Mexico: Guerrero, Rio Cocula, XII (O. W. Barrett); designation: Rehn and Hebard (1912); location: ANSP. We examined only external morphology of this type material. *Additional material*: 355 *m*, 201 *f*, from 80 localities (see Appendix Table 5).

Taxonomic discussion: Bruner (1906) originally described this species based on one male and female syntypes from Cuernavaca, Morelos. He considered the dull granulose and pubescent surface of the type specimen of *S. rugosum* as the main evidence for separating this species from its congeners, though this trait is variable across the species and the genus. Bolivar (1909) and Hebard (1932) recognized *S. rugosum* as a valid species. Márquez (1962) synonymized this species within *S. purpurascens* based on variable and ambiguous morphologic traits of the epiphallus and endophallus of both species. Later, Boyle (1974) and Kevan (1977) re-established *S. rugosum* as valid species based on more detailed analysis of its male genital morphology. Recent genetic studies (Pedraza-lara *et al.* 2015; Sanabria-Urbán *et al.* 2015) have also supported the validity of *S. rugosum*. In this study we analysed several specimens of *S. rugosum*, some of them collected at or near the type locality of this species (L414, L435, L440 & L449; Appendix Table 5). Our phylogenetic analysis recovered *S. rugosum* as a paraphyletic species and we observed relatively low levels of genetic differentiation between *S. rugosum* and other morphologically different species including adjacent (*S. crypticum* **sp.n.**, *S. macrophallicum*, *S. purpurascens* and *S. tarascum* **sp.n.**) and distant taxa (*S. minimum S. variabile* and *S. zapotecum* **sp.n.**) (see Table 3). Nevertheless, the unique combination of morphologic traits, and restricted geographic distribution of this species supports its recognition as an independent species within the genus. Moreover, we observed considerable morphological similarity (external and in male genitalia), low genetic differentiation (*CO1 P-distances* < 3%; Table 3) and close phylogenetic relationships (Appendix Fig. 22) between *S. rugosum* and the putative new taxa *Sphenarium* sp. Gro1+Gro8 and *Sphenarium* sp.n. 6 recognized in Pedraza-Lara *et al.* (2015) and Sanabria-Urbán *et al.* (2015) respectively. Therefore, we consider that these taxa represent part of the same species, *S. rugosum* (morphotype 2 and 3, respectively).

Sphenarium barretti was originally described by Bruner (1906) based on an unstated number of specimens, from Rio Cocula, Guerrero. Bruner (1906) originally considered a closer relationship between *S. barretti* and *S. purpurascens*. Posteriorly, *S. barretti* was initially synonymised with *S. purpurascens* (Márquez 1962) and later with *S. rugosum* (Boyle 1974; Kevan 1977). In this revision we examined several specimens collected near the type locality of this species (L428, L427 & L423; Appendix Table 5); which were similar in their male genitalia to other *S. rugosum* specimens (morphotype 1). Therefore, we agree in considering *S. barretti* as a synonym of *S. rugosum.*

***Sphenarium crypticum* Sanabria-Urbán, Song & Cueva del Castillo sp.n.**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495097)

Description: External morphology (Fig. 12E, F): total body length ranging from 32.22 to 35.92 mm in females and from 19.04 to 34.35 mm in males; antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-elongated nearly as wide as long in females or moderately longer than wide in males wide with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina spatula-like in both sexes; subgenital plate of males rounded moderately developed posteriorly; dorsal ovipositor valves rounded or lanceolate moderately elongated towards the apex. Male genitalia: bridge of epiphallus as long or slightly longer than the length of lateral plates (Fig. 13A-I). Ectophallus in dorsal view (Fig. 13A-II) with lateral borders of ramus convergent; basal emargination of cingulum moderately developed; interspace between apodemal plates moderately open. Ectophallus in posterior view (Fig. 13B) without a conspicuous sclerotized hollow in the sheath; inflections of supraramus moderately developed with distal margins laterally or dorsally directed; valves of cingulum triangular to slightly quadrangular, slightly developed posteriorly (Fig. 13C). Endophallus in lateral view (Fig. 13A-III) with elongated pseudoarch, loosely joined to the valves of cingulum; aedeagal valves slender, long, with smooth ventral margins and moderately rounded in the apex without apical spine; aedeagal valves and sclerites together ranging from 1¾ to 2 fold the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from green to brown. Body uniformly coloured or with the following colour traits: antennae frequently black or dark brown; fastigium frequently reddish; lateral postocular bands frequently present, wide and yellow; dorsomedial line frequently absent, if present very narrow and yellowish; dorsal black shades frequently absent, if present restricted principally to apex of the abdomen; lateral shades often present, narrow, black or dark brown; lateral bands of blotches not evident; ventral bands of pronotum often absent, if present very narrow and yellowish; mesonotum partially or entirely black; lateral whitish blotches of 1st abdominal segment frequently absent; generally hind femora uniformly coloured with knees laterally black, dorsally reddish; hind tibia often black.

Diagnosis: Externally this species closely resembles *S. macrophallicum*, *S. rugosum,* and *S. infernalis* **sp.n.** In most cases, males of *S. crypticum* **sp.n.** differ from males of *S. macrophallicum* by their rounded moderately developed posteriorly subgenital plate. *S. crypticum* **sp.n.** differs from *S. rugosum* by lacking the dorsomedial line and the yellow lateral bands of blotches; whereas the former species is almost indistinguishable from *S. infernalis* **sp.n.** externally. Nevertheless, *S. crypticum* **sp.n.** conspicuously differs from other *Sphenarium* species by the following combination of male genitalia traits: lateral borders of ramus of ectophallus convergent, inflections of supraramus moderately developed, and aedeagal valves and sclerites long, moderately rounded in the apex without apical spine.

Distribution: This species is apparently restricted to the western portion of the inner slope of the Sierra Madre del Sur in Guerrero, Mexico (Fig. 9A), in elevations ranging approximately from 395 to 1662 m a.s.l.

Material examined: Holotype *m* (Fig. 12E, 12A, B, C) from Mexico: Guerrero, El Pinzan Morado Carr. 134, 18.114954ºN, -100.945988ºW, 730 m a.s.l., X-13-2012 (Sanabria-Urbán S.#P66 L47-MS1); measurements: BS = 34.04 mm, FL = 1.32 mm, PL = 7.25 mm, HF = 15.66 mm). Paratypes from Mexico: Guerrero: 2 *m*, 5 *f*, same locality as holotype; 1 *m*, 1 *f*, Vallecitos Carr. 134, 18.039971ºN, -101.046337ºW, 916 m a.s.l., X-13-2012 (Sanabria-Urban S. #P67); 2 *m*, Coyuca de Catalan Microondas El Nopal, 18.30111ºN, -100.798333ºW, 395 m, XI-17-2006 (G. Ortega, L. Cervantes & C. Mayora). The holotype was deposited at IBUNAM and the paratypes were deposited at IBUNAM and TAMUIC. *Additional material*: 8 *m*, 5 *f*, same locality as holotype; 6 *m*, 1 *f*, same localities as paratypes (Appendix Table 5).

Taxonomic discussion: *S. crypticum* **sp.n.** is mainly related genetically to *S. macrophallicum*. Nevertheless, these two species clearly differ each other in their male genital structures. Moreover, *S. crypticum* **sp.n.** shows a unique combination of morphologic traits, in both external and male genitalia structures, a well-supported monophyly (Fig. 2b) and separated geographic distribution. All these lines of evidence support the recognition of *S. crypticum* **sp.n.** as a valid species.

Peviously, we identifed material of *S. crypticum* **sp.n.** as *Sphenarium* sp.n. *7* (Sanabria-Urbán *et al.* 2015); whereas the specimens collected within the ranges of *S. crypticum* **sp.n.**, were considered as an unambiguous new species, *Sphenarium* sp. Gro3, in Pedraza-Lara *et al*. (2015). During this study we examined specimens collected approximately 7 km apart from the *Sphenarium* sp. Gro3 population (L88; Appendix Table 5) that were undoubtedly *S. crypticum* **sp.n.** Moreover, *CO1* sequences of *Sphenarium* sp. Gro3 clustered with our *S. macrophallicum* and *S. crypticum* **sp.n.** samples ($PP \geq 0.95$). Although *Sphenarium* sp. Gro3 and *S. crypticum* **sp.n.** did not form a monophyletic group, we consider that these taxa should represent the same species, considering their phylogenetic and geographic proximity. For other taxonomic studies in the genus *S. crypticum* **sp.n.** was unknown.

Etymology: The specific name "crypticum" is derived from Latin and means "concealed". It refers to the condition in which this species remained undiscovered and also the close similarity with other adjacent species

***Sphenarium borrei* Bolivar, 1884**
(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:37036)

Sphenarium bruneri Bolivar, 1909

Description: External morphology (Fig. 12G, H): total body length ranging from 26.82 to 31.76 mm in females and from 21.08 to 30.62 mm in males; antennae filiform, notably shorter in females or slightly longer in males than head and pronotum together; head conical notably longer than wide and with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina spatula-like in both sexes; subgenital plate of males rounded moderately developed posteriorly; dorsal ovipositor valves lanceolate, notably elongated towards the apex. Male genitalia: bridge of epiphallus as long as the length of lateral plates (Fig. 13D-I). Ectophallus in dorsal view (Fig. 13D-II) broad at the base, with lateral borders of ramus convergent; basal emargination of cingulum notably developed towards the base reducing completely the interspace between the apodemal plates. Ectophallus in posterior view (Fig. 13E) with a conspicuous sclerotized hollow in the sheath notably closed; inflections of supraramus notably developed anterolaterally; valves of cingulum triangular, stout and notably developed posteriorly (Fig. 13F). Endophallus in lateral view (Fig. 13D-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, tapered in the apex without apical spine; aedeagal valves and sclerites about ¾ the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from green, yellow or brown. Body uniformly coloured or with the following colour traits (Fig. 12G, H): antennae frequently black or brown; fastigium often reddish to pinkish; lateral postocular bands frequently present, narrow, yellowish, whitish or pinkish; dorsomedial line absent; dorsal shades sometimes absent, if present almost entirely restricted to the head, metanotum and abdomen apex, brown or black; lateral shades often present, black or dark brown, principally restricted to head and pronotum; lateral bands of blotches absent; ventral bands of pronotum often present, narrow and pinkish; frequently posterior margin of pronotum pinkish; mesonotum is partially or entirely black; tegmina green, black or magenta; lateral blotches of 1st abdominal segment absent; fore and middle femora pinkish; sometimes hind femora with upper and lower medial areas black and whitish, respectively; knees of hind femora laterally black, dorsally reddish; hind tibia frequently black.

Diagnosis: Externally this species sometimes resembles *S. purpurascens* and *S. infernalis* **sp.n.** However, *S. borrei* differs from these species principally by its notably elongated head (conical in both sexes) and dorsal ovipositor valves, as well as its pinkish colourations in the ventral and posterior margins of pronotum when present. At the level of male genitalia, *S. borrei* more closely resembles other species with a conspicuous sclerotized hollow in the sheath (all species described below). Nevertheless, *S. borrei* differs from other species in the genus by the following combination of male genitalia characters: lateral borders of ramus convergent, basal emargination of cingulum notably developed, sclerotized hollow of the sheath conspicuous and closed, inflections of supraramus notably developed anterolaterally, valves of cingulum triangular and stout, posteriorly developed, and aedeagal valves very small.

Distribution: This species is distributed in elevations ranging from 304 to 2225 m a.s.l. and is principally restricted to the western portion of the Mexican Volcanic Belt in Colima, Guanajuato, Jalisco, Michoacan, Nayarit and Zacatecas, Mexico (Fig. 9A).

Material examined: *S. borrei*: lectotype *m* (Appendix Fig. 19D) and paralectotype *f* (Appendix Fig. 19E), from Mexico: Guanajuato (E. Duges); designation: Kevan (1960, unpublished results); Location: Royal Institute of Natural Sciences of Belgium (RISCNB), Brusseles, Belgium. *S. bruneri*: lectotype *m* (Appendix Fig. 19F) and paralectotype *f*(Appendix Fig. 19G), from Mexico (Coneradt); designation: Kevan (1960, unpublished results); location: Spanish Institute of Entomology (SIE), Madrid, Spain. We could only examine the external morphology of all these type specimens. *Additional material*: 214 *m*, 178 *f* from 65 localities (Appendix Table 5).

Taxonomic discussion: Bolivar (1884) originally described this species from one male and female syntypes from Guanajuato, Mexico. He recognized this species based on the morphology of head, antenna and ovipositor valves, as well as coloration patterns. For posterior taxonomic studies this species remain valid until

Márquez (1962) synonymised it within *S. purpurascens* despite he did not examined specimens of this species. Later, Boyle (1974) re-established the status of this species and this status remain stable for posterior molecular analysis (Pedraza-lara *et al.* 2015; Sanabria-Urbán *et al.* 2015). According to our results, *S. borrei* is the most differentiated species in the genus in both morphological and genetic characters. Moreover, its well-supported monophyly and geographic restrictiveness support his recognition as a valid species.

Sphenarium bruneri was only briefly described based on unstated number of specimens (Bolivar 1909), probably one male and two females from an unspecified Mexican locality. Originally, Bolivar (1909) stated a close relationship between this species and *S. rugosum*. However, he mainly considered highly variable external traits in differentiating *S. brunerri* from its congeners. The validity of *S. bruneri* is questionable and its taxonomic position has remained uncertain. This species has been considered a synonym of *S. histrio* (Boyle 1974) or *S. purpurascens* (Kevan 1977). Nevertheless, considering close resemblance in the morphology of head, tegmina and ovipositor valves between *S. borrei* and *S. bruneri*, it is probable that *S. bruneri* represents a junior synonym of *S. borrei.*

***Sphenarium variabile* Kevan & Boyle, 1977**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:37029)

Description: External morphology (Fig. 12I, J, K, L): total body length ranging from 21.38 to 25.57 mm in females and from 16.29 to 28.22 mm in males; antennae filiform, slightly shorter in females or notably longer than head and pronotum together in males; head subtriangular-compressed, wider than long with spherical eyes in females or subtriangular-elongated moderately longer than wide with oval eyes in males; fastigium notably reduced, less than half the length of interocular space in females or moderately elongated, nearly half the length of interocular space in males; tegmina spatula-like in both sexes; subgenital plate of males rounded, moderately developed posteriorly; dorsal ovipositor valves lanceolate, moderately elongated towards the apex. Male genitalia: bridge of epiphallus moderately longer or as long as the length of lateral plates (Fig. 13G-I, J-I). Ectophallus in dorsal view (Figs. 13G-II, J-II) with lateral borders of ramus convergent, slightly sinuous; basal emargination of cingulum notably developed reducing completely the interspace between the apodemal plates. Ectophallus in posterior view (Fig. 13H, K) with a conspicuous sclerotized hollow in the sheath moderately closed or open; inflections of supraramus moderately developed laterally (morphotype 1; Fig. 13H, arrow) or anteriorly (morphotype 2; Fig. 13K, arrow); valves of cingulum small whit distinct finger-like form, slender (morphotype 1; Fig. 13H) or somewhat broad (morphotype 2; Fig. 13K); notably developed posteriorly (morphotype 1; Fig. 13I) or not (morphotype 2; Fig. 13L) in lateral view of ectophallus. Endophallus in lateral view (Fig. 13G-III, J-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, tapered in the apex without apical spine; aedeagal valves and sclerites about ¾ or the same length as the dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from green to brown. Body uniformly coloured (Fig. 12J, L) or with the following colour traits (Fig. 12I, K): antennae black, brown or pale orange; fastigium often brownish; lateral postocular bands frequently present, narrow, yellowish or whitish; dorsomedial line often present, narrow and whitish; dorsal shades frequently present, black or brown, covering partially the dorsal part of the body, frequently well delimited in pronotum by the lateral carinas (Fig. 12I); lateral shades frequently present, black or dark brown, restricted to head and pronotum; lateral bands of blotches not evident; ventral bands of pronotum often present, wide and whitish; mesonotum partially or entirely black; lateral blotches of 1st abdominal segment whitish frequently present; hind femora with lower medial area sometimes whitish with knees laterally black, dorsally brownish; hind tibia black, green or orange.

Diagnosis: Externally this species is very similar to *S. purpurascens*. Sometimes, principally in sympatry, these species differ from each other in their colouration patterns. For instance, in *S. variabile* males (e.g. Fig. 13I), the dark dorsal shades are more conspicuous and notably delimited by the lateral carinas of pronotum than in *S. purpurascens* males (Fig. 7A, C). Moreover, in *S. variabile* the dorsomedial line of body frequently lack the pinkish colourations that are commonly found in *S. purpurascens*. Nonetheless, *S. variabile* clearly differs from other species of *Sphenarium* by its unique lateral borders of ectophallus convergent, slightly sinuous and valves of cingulum that are finger-like.

Distribution: This species is restricted to inner valleys and highlands of the Sierra Madre del Sur in Oaxaca, Mexico, in elevations ranging from 838 to 2279 m a.s.l. (Fig. 9A, C). According to our results, the two morphotypes of this species are distributed separately northeast (morphotype 1) and southwest (morphotype 2) of the southern portion of the Sierra Madre del Sur.

Material examined: Holotype *m* (Appendix Fig. 19H) from Mexico: Oaxaca, 18 mi. NW. La Reforma (Km695 onHwy. 190; 32mi WNW. Tequisistlan), 2750ft, IX-14-1959 (I. J. Cantrall & T. J. Cohn #116). Allotype *f* (Appendix Fig. 20A) same data as holotype. Paratypes from Mexico: Oaxaca: 1 *f*, same locality as holotype; 1 *m*, 45 mi. NW. Tequisistlan (1 mi. S. El Camaron), 2000ft, XII-21- 1958 (T.J. Cohn #384) (Fig. 12I; 13G, H, F); 1 *m*, 13mi. SE. Tlacolula, XII-21-1958 (T.J. Cohn #383); 1 *m*, 1.0 mi. NW. Tamazulapan at Km 388, IX-9-1958 (T.J. Hubell, I.J. Cantrall & T.J. Cohn #87); 1 *m*, 2 *f*, 2 mi. SE. Tlacolula on Hwy. 190, 5700ft, IX-11-1961 (T. J. Hubell, I.J. Cantrall & T.J. Cohn #91); 1 *m*, 3 *f*, 7mi. SE. El Camaron (37mi. NW. Tequesistlan on Hwy. 190), 3500ft, IX-14-1959 (I.J. Cantrall & T.J. Cohn #117); 1 *m*, Mitla, 5000ft, VIII-11-1938 (H.R.Roberts). Designation: Kevan and Boyle (1974); location: UMMZ for all these type specimens. We examined both external and genital morphology of the type material. *Additional material*: 89 *m*, 38 *f*, from 15 new localities (Appendix Table 5).

Taxonomic discussion: This species was briefly described based on at least 102 specimens designating the holotype, allotype and 68 paratypes from seven different localities from Oaxaca, Mexico (Kevan 1977). Since its original description the validity of *S. variabile* has remained unchanged for later studies (Pedraza-lara *et al.* 2015; Sanabria-Urbán *et al.* 2015). According to our results, *S. variabile* is genetically close and paraphyletic with respect to *S. purpurascens* and *S. zapotecum* **sp.n.** lineages. However, the differentiation between their male genital structures is greater than that observed between other congeners with greater genetic differentiation. Therefore, we also agree in considering *S. variabile* a valid species.

Sphenarium mexicanum **Saussure, 1859**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:37003)

Opomala crassipes Walker, 1870
Sphenarium ictericum Gerstaecker, 1873
Sphenarium bolivari Bruner, 1906
Sphenarium marginatum Bruner, 1906
Sphenarium magnum Márquez, 1962

Description: External morphology (Fig. 12M, N, O, P): total body length ranging from 33.23 to 46.66 mm in females and from 30.99 to 41.58 mm in males. In most cases: antennae filiform to weakly ensiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-elongated slightly longer than wide in females or conical notably loner than wide in males, with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina strap-like in both sexes; subgenital plate of males rounded, moderately developed posteriorly; dorsal ovipositor valves rounded or lanceolate moderately elongated towards the apex. Male genitalia: bridge of epiphallus slightly longer than the length of lateral plates (Fig. 14A-I, D-I). Ectophallus in dorsal view (Fig. 14A-II,D-II) large with lateral borders of ramus convergent; basal emargination of cingulum notably or slightly developed; interspace between the apodemal plates close. Ectophallus in posterior view (Fig. 14B, E) with a conspicuous sclerotized hollow in the sheath notably open; inflections of supraramus notably reduced; valves of cingulum claw-like rather stout (Fig. 14A-III, D-III). Ectophallus in lateral view (Fig. 14C, F) with valves of cingulum notably (morphotype 1, Fig. 14C) or moderately (morphotype 2, Fig. 14F) developed posteriorly, always evident. Endophallus in lateral view (Fig. 14A-III, D-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, tapered in the apex without apical spine; aedeagal valves and sclerites as long as (morphotype 1, Fig. 14A-III) or about ¾ (morphotype 2, Fig. 14D-III) the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from olive green to brown. Body uniformly coloured (Fig. 12N) or with the following colour traits (Fig. 12M, O, P): antennae black, gray or dark blue; fastigium often blue or black; lateral postocular bands frequently present, narrow and yellowish; dorsomedial line frequently present, wide and yellowish; dorsal shades if present black to dark blue, generally absent in most of the range of the species except in populations in the Isthmus of Tehuantepec (Fig. 12O, P); lateral shades often present, black or dark blue; lateral bands of blotches frequently present and reddish; ventral bands of pronotum often present, wide, whitish, rarely bluish; frequently pronotum with white lateral carinas and small stripes and dots in the posterior dorsal margin; mesonotum partially or entirely red; lateral blotches of 1st abdominal segment frequently present and whitish; generally hind femora uniformly coloured with knees laterally black, dorsally bluish; hind tibia light brown to reddish.

Diagnosis: This species is similar to *S. histrio* and *S. totonacum* **sp.n.** in both male genitalia and external morphology. Nevertheless, *S. mexicanum* clearly differs from other *Sphenarium* species by the following combinations of male genital characters: ectophallus relatively large, notably conspicuous sclerotized hollow in the sheath, inflections of supraramus notably reduced, and valves of cingulum claw-like, rather stout, notably or moderately developed posteriorly.

Distribution: This species is distributed in elevations ranging approximately from 6 to 1280 m a.s.l. in the southern portion of the Golf of Mexico Cost, outer slope of the Sierra de Oaxaca and the Isthmus of Tehuantepec in Oaxaca, Veracruz and Tabasco, Mexico (Fig. 9B). The morphotype 1 of this species is widely spread across most parts of the range of the species; whereas morphotype 2 was only observed in a single population near Tehuantepec (L14) (Fig. 9B).

Material examined: *S. mexicanum*: lectotype *f* (Appendix Fig. 20B) and paralectotype *m* (Appendix Fig. 20C) from Mexico: Litoral du Mexique (M. H. Saussure); designation: Kevan (1960, unpublished results); located: Natural History Museum Geneva (NHMG), Geneva, Sweden. *O. crassipes*: holotype *m* (Appendix Fig. 20D) from Mexico: Veracruz; designation by monotypy; location: British Museum of Natural History (BMNH), London, England. *S. ictericum*: lectotype *m* (Appendix Fig. 20E)and paralectotype *f* (Appendix Fig. 20F) from Mexico; designation: Kevan (1962, unpublished results); location: BZM. *S. bolivari*: lectotype *m* (Appendix Fig. 20G)and paralectotype *m* (Appendix Fig. 20H) from Mexico: Oaxaca, Salina Cruz, XII-1898 (C.C. Deem); designation: (Rehn & Hebard 1912); location: ANSP. *S. marginatum*: lectotype *m* (Appendix Fig. 21A) from Mexico: Veracruz, Orizaba, XI and paralectotype *f* (Appendix Fig. 21B) from Mexico: Veracruz, Medellin (T. Heyde); designation: (Rehn & Hebard 1912); location: ANSP. *S. magnum*: holotype *m*, allotype *f* and *paratypes* (2 *m*, 2 *f*), from Mexico: Oaxaca, Santo Domingo, XII-16-1953 (L. Vázquez); designation: Márquez (1962); location: IBUNAM. For *S. bolivari* we were able to examine the external and male genital morphology of the paratype; whereas for the remainder taxa we could examine only their external morphology. *Additional material*: 212 *m*, 169 *f*, form 50 Mexican localities (Appendix Table 5).

Taxonomic discussion: Saussure (1859) briefly described *S. mexicanum* based on an unstated number of male and female syntypes from "Mexico Calida", probably Veracruz, Mexico (Saussure 1859). The validity of *S. mexicanum* remained unchanged until Boyle (1974) ans Kevan (1977) considered this taxon as the subspecies *S. mexicanum mexicanum* (Boyle 1974; Kevan 1977). Recently, Pedraza-lara *et al.* (2015) and Sanabria-Urbán *et al.* (2015) have proposed the elevation of this taxon at the species level. According to our results, this species is closely related and/or paraphyletic with respect to *S. histrio* and *S. occidentalis* **sp.n.** lineages. However, *S. mexicanum* clearly differs from these species genetically (*CO1 P-distance* > 3%) and in male genital structures. Therefore, we also agree in considering *S. mexicanum* a valid species.

Opomala crasipes was initially described from a single male (Walker 1870) from Veracruz, Mexico, that was later transferred to the genus *Sphenarium* (Kirby 1910). Later, Uvarov (1925) independently confirmed Kirby's taxonomic rearrangement and synonymised *S. crasipes* within *S. mexicanum.* Considering the type locality and external similarity, we agree in considering *S. crassipes* as a synonym of *S. mexicanum.*

Sphenarium ictericum was originally described apparently from a single pair, one male and female, form an unspecified Mexican locality (Gerstaecker 1873). After its description the taxonomic position of *S. ictericum* has been variable. In some studies this taxon has been considered a synonym of *S. histrio* (Hebard 1932; Márquez 1962); whereas in others it was considered a synonym of *S. mexicanum* (Bolivar 1904; Boyle 1974; Bruner 1906; Kevan 1977). It is undoubtedly difficult to determine the taxonomic position of *S. ictericum* without examination of the male genitalia. In fact, we consider that there is a remarkable similarity between this species and *S. mexicanum*, *S. histrio,* and *S. occidentalis* **sp.n.** Nevertheless, in the absence of further evidence we accept synonymising *S. ictericum* with *S. mexicanum* as in the last taxonomic revision of the genus (Boyle 1974).

Sphenarium bolivari was originally described from two male syntypes from Salina Cruz, Oaxaca (Bruner 1906). After its description the validity and taxonomic position of *S. bolivari* has been variable. In some studies this taxon was considered a synonym of *S. histrio* (Hebard 1932; Márquez 1962); whereas in others it was considered a synonym of *S. mexicanum* and sometimes as an intermediate from between *S. mexicanum mexicanum* and *S. mexicanum histrio* (Boyle 1974; Kevan 1977). In this study, we observed that the male genitalia of the *S. bolivari* paralectotype were similar to those of other *S. mexicanum* specimens. Thus, we agree in considering the former species as a synonym of the latter.

Sphenarium marginatum was initially described based on four specimens (Bruner 1906) from three localities in Veracruz: one male and female from Cordova, one male from Orizaba and one female from Medellin. After its description *S. marginatum* was synonymised within *S. mexicanum* (Boyle 1974; Kevan 1977). During this study we examined several specimens collected near to the localities of the original description of *S. marginatum* (L252, L260, L261, L273, L274 & L279; Appendix Table 5); which were similar in their male genitalia to other specimens assigned to *S. mexicanum.* Therefore, we agree in recognising *S. maginatum* as a synonym of *S. mexicanum*.

Sphenarium magnum was originally described based on six specimens from a single locality (Santo Domingo, Oaxaca), with designation of the type material (Márquez 1962). The validity and taxonomic position of *S. magnum* has been variable after its description. This species was considered an intermediate from between *S. m. mexicanum* and *S. m. histrio,* being synonymised with *S. m. mexicanum* (Boyle 1974) or *S. m. histrio* (Kevan 1977). Moreover, recently this taxon was proposed as a valid species (Sanabria-Urbán *et al.* 2015). During this study, we conducted a more detailed examination of several specimens collected in the type locality of *S. magnum* and surrounding areas (L14, L246, L250 & L231; Appendix Table 5). These specimens were similar or slightly differed genetically and in their male genitalia from other *S. mexicanum* individuals. Therefore, we consider *S. magnum* as a synonym of *S. mexicanum*.

Sphenarium histrio **Gerstaecker, 1873**
(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:37005)

Sphenarium carinatum Bolivar, 1904

Description: External morphology (Figs. 12Q, R, S, T; 15A, B, C, D, E, F, G, H, I, J): total body length ranging from 22.01 to 41.8 mm in females and from 18.57 to 38.87 mm in males. In most cases: antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head subtriangular-elongated slightly longer than wide in females or conical notably loner than wide in males, with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina strap-like in both sexes; subgenital plate of males somewhat tapered in the apex; dorsal ovipositor valves rounded or lanceolate slightly elongated towards the apex. Male genitalia: bridge of ectophallus slightly longer than the length of lateral plates (Figs. 14G-I, J-I; 16A-I, D-I, G-I). Ectophallus in dorsal view small with lateral borders of ramus convergent, straight (morphotypes 1, 3, 4; Figs. 14G-II, 16A-II, D-II, respectively) or slightly rounded (morphotypes 2, 5; Figs. 14J-II; 16G-II, respectively); basal emargination of cingulum notably or slightly developed; interspace between the apodemal plates notably closed. Ectophallus in posterior view with a conspicuous sclerotized hollow in the sheath closed (morphotype 4; Fig. 16E) or moderately open (other morphotypes; Figs. 14H, K; 16B, H); inflections of supraramus moderately developed, with distal borders ventrally (morphotypes 1, 2, 5; Figs. 14H, K; 16H, respectively) or laterally directed (morphotypes 3, 4; Fig. 14B, E, respectively); valves of cingulum principally triangular but with distinct variations, each morphotype with its own form; Figs. 14H, K; 16B, E, H), small (morphotypes 1-4) or moderately large (morphotype 5). Ectophallus in lateral view with valves of cingulum moderately developed dorsoposteriorly (morphotype 5; Fig. 16I) or not developed posteriorly (morphotypes 1-4; Figs. 14I, L; 16C, F, respectively). Endophallus in lateral view (Figs. 14G-III, J-III; 16A-III, D-III, G-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, tapered in the apex without apical spine; aedegala valves and sclerites as long as (morphotype 5, Fig. 16G-III) or about ¾ (the remainder morphotypes; Figs. 14G-III, J-III; 16A-III, D-III) the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from olive green, yellow or brown. Body uniformly coloured (Fig. 12R, T; 15B) or with the following colour traits (Figs. 12Q, S; 15A, B, C, D, E, F): antennae black, gray or dark blue; fastigium blue, brown or black; lateral postocular bands frequently present, narrow and yellowish; dorsal medial line frequently present, wide, yellowish or whitish; dorsal shades frequently present black, brown to dark blue, in southern populations covering entirely the dorsal portion of the body (Fig. 15C, D); lateral shades often present, black or dark blue; lateral bands of blotches frequently present, mostly reddish, in some populations in northern ranges of the species yellowish (Fig. 12S); ventral bands of pronotum often present, wide, whitish or bluish; pronotum with white lateral carinas and small strips and dots in the posterior margin; mesonotum dorsally red; frequently mesonotum and metanotum laterally white or yellow; lateral blotches of 1st abdominal segment frequently present and whitish; tegmina green, red, magenta or blue; generally hind femora uniformly coloured with knees laterally black, dorsally bluish or brownish; hind tibia green, brown, reddish or blue.

Diagnosis: Externally *S. histrio* is very similar to *S. mexicanum*, *S. totonacum* **sp.n.**, and *S. occidentalis* **sp.n.** Nevertheless, the phallic structures of *S. histrio* differ from other species as follows: ectophallus small, conspicuous sclerotized hollow of the sheath moderately open or close, inflections of supraramus moderately developed with distal portions ventrally directed in most cases (except in morphotypes 3 & 4), and valves of cingulum with distinct form not developed or slightly developed dorsoposteriorly.

Distribution: This species is distributed in elevations ranging approximately from 15 to 2225 m a.s.l. in Chiapas, Guerrero, Oaxaca, Tabasco, Veracuz, in southern Mexico; and in the states of Huehuetenango and

Santa Rosa in Guatemala (Fig. 9B). We observed that the five morphotypes identified within the species are apparently restricted to different geographic areas. The morphotype 1 is mainly distributed across the southern Pacific Cost and the outer slope of the Sierra Madre del Sur in Oaxaca. The morphotype 2 is distributed in the Pacific Cost south of the Isthmus of Tehuantepec, as well as in the mountain ranges of Chiapas and north-western Guatemala. The remaining morphotypes are more geographically restricted: the morphotype 3 is distributed principally in the central Valleys of Oaxaca in the Sierra Madre del Sur (Fig. 9B, C), the morphotype 4 was observed in the south-eastern portion of the Sierra Madre del Sur also in Oaxaca (Fig. 9B, C), and morphotype 5 was observed only few localities in the Pacific Cost of Chiapas, Mexico (Fig. 9B). Overall, here we recognised smaller distribution ranges for *S. histrio* than in Boyle (1974) and Kevan (1977). Within the examined material we identified two *S. histrio* males from Sonora (morphotype 2) and Sinaloa (morphotype 3). However, we considered these specimens as mislabelled records considering that multiple collectors, including us, have not recorded *S. histrio* beyond the above-specified limits. Moreover, according to the revised material, S. *histrio* is apparently absent from the lowlands of the Isthmus of Tehuantepec where *S. mexicanum* is distributed. Additional fieldwork will clarify if these two species are sympatric in this geographic region.

Material examined: S. histrio: holotype *m* (Appendix Fig. 21C) from Mexico; designation by monotypy; location: BZM. *S. carinatum*: holotype *m* (Appendix Fig. 21D) from Guatemala: Santa Rosa, Testuaco; designation by monotypy; location: SIE. We could examine only the external morphology of this type material. *Additional material*: 398 *m*, 326 *f*, form 91 Mexican and Guatemalan localities (Appendix Table 5).

Taxonomic discussion: Gerstaecker (1873) originally described *S. histrio* based on a single male from an unknown Mexican locality considering mainly coloration traits as diagnostic characters of this species. Bolivar (1884) re-described this species based on a male and a female, but also from an unspecified Mexican locality. The validity of this species remained constant in posterior taxonomic studies. Hebard (1932) recognized a closer relationship between *S. histrio* and *S.mexicanum* based on their morphological resemblance. Later, Márquez (1962) and Boyle (1974) identified differences in endophallic morphology between these two species. However, Boyle (1974) and Kevan (1977) only recognized *S. histrio* as a subspecies of *S. mexicanum* (*S. mexicanum histrio*). Recently, Pedraza-Lara *et al.* (2015) and Sanabria-Urbán *et al.* (2015) have proposed the recognition of *S. histrio* as an independent and valid species within the genus based on morphologic and genetic evidence. In our phylogenetic analysis *S. histrio* was recovered as a paraphyletic species despite its notable morphologic cohesiveness. Moreover, we observed notable genetic (with *CO1 P-distance* > 3%) and morphological differentiation in the male genitalia between *S. histrio* and *S. mexicanum*. Therefore, here we also agreed in considering *S. histrio* as a valid species. Moreover, during this revision we examined several specimens collected near to the reported localities of two putative new taxa recently identified, *Sphenarium* sp. Oax9 and *Sphenarium* sp. Oax2 (Pedraza-lara *et al.* 2015) (L11, L167, L172, L173; Appendix Table 5). The male genitalia of these specimens were similar to those of other *S. histrio* individuals. Moreover, *CO1* sequences of these taxa intermingled with our samples of *S. histrio*. Therefore, we consider that these putative new taxa probably represent part of *S. histrio*.

Sphenarium carinatum was originally described based on a single male from an uncertain Guatemalan locality, Testuaco, probably Tecuaco (Bolivar 1904). In later taxonomic studies this species was synonymised with *S. m. histrio* (Boyle 1974; Kevan 1977). In this study we examined multiple specimens from north-western Guatemala; which were similar to other *S. histrio* individuals in southern Mexico. Therefore, in the lack of additional evidence we agree in recognising *S. carinatum* as a synonym of *S. histrio*.

Sphenarium occidentalis **Sanabria-Urbán, Song & Cueva del Castillo sp.n.**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495098)

Description: External morphology (Fig. 15K, L, M, N): total body length ranging from 32.58 to 43.67 mm in females and from 30.84 to 43.03 mm in males; antennae filiform, slightly shorter in females or longer than head and pronotum together in males; head conical notably longer than wide with oval eyes in both sexes; fastigium moderately elongated, nearly half the length of interocular space in females or notably elongated, nearly as long as the interocular space in males; tegmina strap-like in both sexes; subgenital plate of males somewhat tapered in the apex; dorsal ovipositor valves rounded or lanceolate slightly elongated towards the apex. Male genitalia: bridge of epiphallus slightly longer than the length of lateral plates (Fig. 16J-I). Ectophallus in dorsal view (Fig. 16J-II) small with lateral borders of ramus convergent, straight or slightly rounded; basal emargination of cingulum mostly slightly developed; interspace between the apodemal plates notably closed. Ectophallus in posterior view (Fig. 16K) with a conspicuous sclerotized hollow in the sheath moderately open; valves of cingulum arrow-like, relatively small; inflections of supraramus moderately developed whit distal borders laterally directed. Ectophallus in lateral view (Fig. 16L) with valves of cingulum not developed posteriorly. Endophallus in lateral view (Fig. 16J-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, tapered in the apex without apical spine; aedeagal valves and sclerites ranging from one half to ¾ the length of dorsal inflections of endophallic apodemes.

Colouration: round colours vary from yellow to green. Body uniformly coloured or with the following colour traits: antennae black, gray or dark blue; fastigium blue or brown; lateral postocular bands frequently present, wide and yellowish; dorsomedial line frequently present, wide and yellowish; dorsal shades dark blue to dark green covering partially the dorsal portion of the body, frequently present in northern populations (Fig. 15K, L) or absent in southern populations (Fig. 15M, N); lateral shades often present, black or dark blue; lateral bands of blotches frequently present, yellow to pale orange; ventral bands of pronotum often present, wide, whitish to bluish; pronotum sometimes with white lateral carinas and small strips and dots in the posterior margin; mesonotum yellow or pale orange; frequently mesonotum and metanotum laterally white; lateral blotches of 1st abdominal segment frequently present and whitish; hind femora uniformly coloured with knees laterally black laterally, dorsally bluish or brownish.

Diagnosis: This species is similar to *S. histrio* both externally and in male genital structures. Nevertheless, *S. occidentalis* **sp.n.** differs from *S. histrio* by the following combination of characters: if present lateral bands of blotches yellow to pale orange but never red, sclerotized hollow in the sheath moderately open, inflections of supraramus moderately developed whit distal borders laterally directed, and valves of cingulum although similar in form (specially to *S. histrio* morphotype 1) somewhat larger than in *S. histrio*.

Distribution: This species is distributed in elevations ranging approximately from 30 to 750m a.s.l. and is apparently restricted to the western portion of the Balsas River Basin and Pacific Cost in Michoacan and Guerrero, Mexico (Fig. 9A).

Material examined: Holotype *m* (Fig. 15K) from Mexico: Michoacan, Cuchurumuco, 18.67008ºN, -101.670625ºW, 241 m a.s.l., IX-22-2012 (Sanabria-Urbán S. #P39); measurements: BS = 41.36 mm, FL = 1.66 mm, PL = 9.98 mm, HF = 19.25 mm. Paratypes from Mexico: Michoacan: 7 *m*, 7 *f*, same data as holotype; 1 *m*, 20 mi E Nueva Italia (on La Huacana rd.), IX-25-1959 (I. J. Cantrall & T. J. Cohn #174); 1 *m*, 23 mi E Nueva Italia (on La Huacana rd.), IX-25-1959 (I. J. Cantrall & T. J. Cohn #175); 1 *m*, 31mi S Nueva Italia (on Arteaga rd), 550 ft, IX-26-1959 (I. J. Cantrall & T. J. Cohn #182); 1 *m*, 11 mi SW La Huacana, 1600 ft, XII-4-1959 (T. J. Cohn #346); 1 *m*, 26 rd. mi NE Arteaga on Hwy. 37 (rd. mi SW Rancho Nuevo), 2060 ft, XI-3-1974 (T.J. & J. W. Cohn #121); 1 *m*, 3rd mi SW Arteaga church (0.1mi E Hwy. 37), 2460 ft, XI-3-1974 (T.J. & J. W. Cohn #122); 1 *m*, 7.2 mi NE Playa Azul (on Hwy. 37) 2.8 mi. NE La Mira Jct., 700 ft, XI-6-1974 (T.J. & J. W. Cohn #123); 1 *m*, 1 *f*, 15mi W Caleta de Campos (W playa Azul), X-9-1981 (Otte #62); 1 *m*, 1 *f*, 12mi W Caleta de Campos (W playa Azul), X-9-1981 (Otte #63);1 *m*, 1 *f*, 26mi n La Mira, VIII-9-1981 (Otte #59); 1*m*, 1 *f*, 16-

20km NE Rt 200 Ixtapa-Altamirano Rd., VIII-9-1981 (Otte #60); 6 M, 6 F, Las Peñitas, 17.990621ºN, -102.026924ºW, 36 m a.s.l., IX-23-2012 (Sanabria-Urbán S. # P40 [L7 MS1]); 1 M, 1 *f*, Zicuirán Carr. 120 Km 144, 18.881308ºN, -101.967336ºW, 239 m a.s.l., IX-22-2012 (Sanabria-Urbán S. # P38 [L8 MS1]); 1 *m*, 1 *f*, Carr. 37D Km 236, 18.3868ºN, -101.89475ºW, 197 m a.s.l., IX- 23-2012 (Sanabria-Urbán S. # M055). Guerrero: 1 *m*, 5mi N Acapulco, IX-15-1940 (C. Bolivar & H. R. Roberts); 2 *m*, 1 *f*, 13mi SW Tierra Colourada, 1000 ft, XII-11-1958 (T. J. Cohn # 368); 6 *m*, 4 *f*, Rio Papagayo Carr. 200, 16.773689ºN ,-99.608538ºW, 56 m a.s.l., X-5-2011 (Sanabria-Urbán S. # 16ACA [L9 MS1]). The holotype was deposited at IBUNAM and paratypes were deposited at IBUNAM and TAMUIC. *Additional material*: 3 *m*, 3 *f*, same locality as holotype; 64 *m*, 59 *m*, from other 25 localities (Appendix Table 5).

Taxonomic discussion: This species is closely related morphologically to *S. histrio*. In deed material of this new species was identified as *S. m. histrio* in previous studies (Boyle 1974; Kevan 1977). Nevertheless, *S. occidentalis* **sp.n.** shows a unique combination of morphologic traits, in both external and male genitalia structures, relatively high levels of interspecific genetic differentiation (Table 3) and is geographically separated from its congeners. All these lines of evidence support the recognition of *S. occidentalis* **sp.n**. as a valid species.

Previusly, we identified specimens of this new species as *Sphenarium* sp.n. 1 (Sanabria-Urbán *et al*. 2015), whereas Pedraza-Lara et al. (2015) recognised two taxa of uncertain identity, *Sphenarium* sp. Gro6 and *Sphenarium* sp. Gro9, collected within the distribution ranges of *S. occidentalis* **sp.n.** During this revision we examined multiple specimens collected near to the localities of *Sphenarium* sp. Gro6 and Gro9 (e.g. L09, L309, L307, L315; Appendix Table 5) that were undoubtedly *S. occidentalis* **sp.n.** Moreover, *CO1* sequences of *Sphenarium sp*. Gro6 of *S. occidentalis* **sp.n.** formed a well supported (*PP* ≥ 0.95) monophyletic group (Appendix Fig. 22). Therefore, we consider that *Sphenarium* sp. Gro6 and *Sphenarium* sp. Gro9 probably represent additional populations of *S. occidentalis* **sp.n.**

Etymology: This species is named after its distribution in the occidental region of Mexico.

Sphenarium totonacum **Sanabria-Urbán, Song & Cueva del Castillo sp.n.**

(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495099)

Description: External morphology (Fig. 15O, P): total body length ranging from 32.77 to 40.47 mm in females and from 30.04 to 38.75 mm in males; antennae weakly ensiform, slightly shorter in females or longer than head and pronotum together in males; head conical notably longer than wide with oval eyes in both sexes; fastigium notably elongated, nearly as long as the interocular space in both sexes; tegmina strap-like in both sexes; subgenital plate of males somewhat tapered in the apex; dorsal ovipositor valves lanceolate, elongated towards the apex. Male genitalia: bridge of epiphallus slightly longer than the length of lateral plates (Fig. 17A-I). Ectophallus in dorsal view (Fig. 17A-II) small with lateral borders of ramus convergent, somewhat straight; basal emargination of cingulum notably developed; interspace between the apodemal plates notably closed. Ectophallus in posterior (Fig. 17K) view with a conspicuous sclerotized hollow in the sheath closed; valves of cingulum drop-like (Fig. 17B, arrow); inflections of supraramus notably developed whit distal borders ventrolateral directed. Ectophallus in lateral view (Fig. 17C) with valves of cingulum not developed posteriorly. Endophallus in lateral view (Fig. 17A-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves small, sharply pointed apically, without apical spine; aedeagal valves and sclerites about half the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours vary from green or brown. Body uniformly coloured or with the following colour traits (Fig. 15O, P): antennae black, gray or dark brown; lateral postocular bands frequently present, narrow and yellowish; dorsomedial line frequently absent, if present very narrow, yellowish or whitish; dorsal shades absent; lateral shades often present and black; lateral bands of blotches frequently present and reddish;

ventral bands of pronotum often present, wide and whitish; pronotum with white small stripes and dots in the posterior margin; dorsal portion of pronotum, metanotum and 1st abdominal segment with darker green colouration; mesonotum red; lateral blotches of 1st abdominal segment if present whitish; generally hind femora uniformly coloured with knees laterally black, dorsally brownish; hind tibia reddish.

Distribution: This species is apparently restricted to the outer slope of the Sierra Madre Oriental in elevations ranging from 440 to 1145 m a.s.l. in Veracruz and probably Puebla, Mexico (Fig. 9A).

Diagnosis: Externally this species closely resembles *S. mexicanum*, whereas it is more similar to *S. histrio* in terms of the male genitalia. Nevertheless, *S. totonacum* **sp.n**. differs from other *Sphenarium* species by the following male genitalia characters: sheath of ectophallus with a conspicuous sclerotized hollow closed, inflexion of supraramus notably developed and ventrolateral directed, and valves of cingulum with distinct drop-like form.

Material examined: Holotype *m* (Fig. 15O) from Mexico: Veracruz, Plan de Hayas, 19.74138ºN, -96.64531ºW, 1145 m a.s.l., XI-3-2012 (Sanabria-Urbán S. & Jímenez-Arcos V. H. # P72 [L19 MS1]); measurements: BS = 34.63 mm, FL = 1.38 mm, PL = 7.27 mm, HF = 15,22 mm. Paratypes from Mexico: Veracruz: 5 M, 5 F same data as holotype; 3 *m*, 3 *f*, Km 21 Carr. 131, ca. 7km SO de Tlapacoyan, 19.90252398ºN, -97.22993698ºW, 751 m a.s.l., IX-19-2015 (Sanabria-Urbán S. # M010-L52); 1 *f*, Tlapacoyan Eytepequez, 11-9-1995 (Delgadillo J.). The holotype was deposited at IBUNAM and the paratypes were deposited at the IBUNAM and TAMUIC. *Additional material*: 13 *m*, 16 *f*, from seven localities (Appendix Table 5).

Taxonomic discussion: This species is also closely related morphologically to *S. histrio*. Indeed, specimens of this new species were identified as an isolated population of *S. m. histrio* in the cost of the Golf of Mexico (Boyle 1974; Kevan 1977). Sanabria-Urbán *et al*. (2015) identified specimens of this new species as *Sphenarium* sp.n. 4. For other studies in the genus this species was unknown. In this study we found that *S. totonacum* **sp.n.** shows a unique combination and notably different male genitalia structures. Moreover, this new species has a well-supported monophyly (Fig. 2), shows relatively high levels of interspecific genetic differentiation (Table 3), and it is considerably separated geographically from *S. histrio*. All these lines of evidence support the recognition of *S. totonacum* **sp.n**. as a valid species.

Etymology: Named in honour of the Totonacos, an ancient Native American people still living in the area where this species was found.

Sphenarium adelinae **Sanabria-Urbán, Song & Cueva del Castillo sp.n.**

(http://lsid.speciesfile.org/*urn:lsid:Orthoptera.speciesfile.org:TaxonName:495100*)

Description: External morphology (Fig. 15Q, R): total body length ranging from 27.91 to 35.25 mm in females and from 26.47 to 33.1 mm in males; antennae weakly ensiform, slightly shorter in females or longer than head and pronotum together in males; head conical notably longer than wide with oval eyes in both sexes; fastigium notably elongated, nearly as long as the interocular space in both sexes; tegmina strap-like in both sexes; subgenital plate of males somewhat tapered in the apex; dorsal ovipositor valves lanceolate slightly elongated towards the apex. Male genitalia: bridge of epiphallus slightly longer than the length of lateral plates in most cases (Fig. 17D-I). Ectophallus in dorsal view (Fig. 17D-II) small with lateral borders of ramus convergent, straight or slightly rounded; basal emargination of cingulum notably developed closing the interspace between the apodemal plates. Ectophallus in posterior (Fig. 17E) view with a conspicuous sclerotized hollow in the sheath notably closed; valves of cingulum small with distinct form; inflections of supraramus notably developed laterally and dorsally forming a dorsal fold; dorsal borders of inflections of supraramus very close but not fused (Fig. 17E, arrow). Ectophallus in lateral view with valves of cingulum barely evident slightly developed posteriorly (Fig. 17F). Endophallus in lateral view (Fig. 17D-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, sharply pointed apically, without apical spine; aedeagal valves and sclerites about half the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours green or light brown. Body uniformly coloured or with the following colour traits: antennae dark to light brown; fastigium brownish; lateral postocular bands frequently present, wide and yellowish, whitish or cyan; dorsomedial line frequently present, wide and yellowish; dorsal shades brown to dark magenta, frequently covering entirely the dorsal portion of the abdomen; lateral shades often present, dark to light brown; lateral bands of blotches absent; ventral bands of pronotum often present, wide, yellowish or cyan; pronotum sometimes with small dots in the dorsal posterior margin; mesonotum partially or entirely brownish; lateral blotches of 1st abdominal segment frequently present and yellowish; hind femora with medial area uniformly coloured and lower marginal area with distinct blue colouration, specially in the apex of femur; knees of hind femora without dark colouration; hind tibia intense yellow.

Diagnosis: Externally this species closely resembles *S. histrio* and *S. miztecum* **sp.n.** In most cases, *S. adelinae* **sp.n.** differs from *S. histrio* by its weakly ensiform antennae and the blue colourations in the lower marginal area of the hind femora; whereas it differs from *S. miztecum* principally by lacking the cyan lateral band of blotches. Nevertheless, more conspicuous differences exist among the male genital structures of these species. *S. adelinae* **sp.n.** differs from these species by the following combinations of male genital traits: sclerotized hollow in the sheath notably closed, valves of cingulum small with distinct form, and inflections of supraramus notably developed laterally and dorsally with dorsal borders almost fused above the valves of cingulum.

Distribution: This species is only known from a small region in the outer slope of the Sierra Madre del Sur in Guerrero, Mexico (Fig. 9B). The altitudinal distribution of this species ranges approximately from 420 to 1210 m a.s.l.

Material examined: Holotype *m* (Fig. 18K) from Mexico: Guerrero, 14 mi. S. Chilpancingo, IX-4-1981 (Otte, Azuma & Newlin #52); measurements: BS = 32.94 mm, FL = 1.38 mm, PL = 6.28 mm, HF = 15.51 mm. Paratypes from Mexico: Guerrero: 12 *m*, 9 *f*, Acahuizotla ca. de Ocotito, Carr. 95, 17.355494ºN, -99.480226ºW, 1018 m a.s.l., X-4-2011 (Sanabria-Urbán S. # 13OC [L16 MS1]); 5 *m*, 4 *f*, Tierra Colourada Carr. 95, 17.204383ºN, -99.508199ºW, 534 m a.s.l., X-4-2011 (Sanabria-Urbán S. & Días de la Vega A. # 15TC); 5 *m*, 5 *f*, Palo Blanco Carr. 95, 17.409805ºN, -99.466767ºW, 1210 m a.s.l., X-4-2011 (Sanabria-Urbán S. # 14ZC [L17 MS1]); 1*m*, Tierra Colourada, 1800 ft, IX-16-1940 (C. Bolivar & H. R. Roberts); 2 *m*, 16 rd. mi S Chilpancingo (Km 298 on Hwy. 95), 3800 ft (I. J. Cantrall & T. J. Cohn # 147); 1*m*, 2 mi S Tierra Colourada (Km 335 on Hwy. 95), 1400 ft, IX-19-1959 (I. J. Cantrall & T. J. Cohn # 148). The holotype was deposited at ANSP and the paratypes were deposited at IBUNAM and TAMUIC. *Additional material*: 22 *m*, 24 *f*, form the same first three paratypes localities (Appendix Table 5).

Taxonomic discussion: This species is also closely related morphologically to *S. histrio*. Specimens of this new species were identified as *S. m. histrio* in previous studies (Boyle 1974; Kevan 1977). In this study we found that *S. adelinae* **sp.n.** shows a unique combination of morphologic traits, both external and on male genitalia, differencing this new species from *S. histrio*. Moreover, this new species has a well-supported monophyly (Fig. 2), shows relatively high levels of interspecific genetic differentiation (Table 3). We consider that all these lines of provide evidence for the recognition of *S. adelinae* **sp.n**. as a valid species.

Previously, we identified specimens of this new species as *Sphenarium* sp.n. 2 (Sanabria-Urbán *et al.*, 2015). Pedraza-Lara *et al.* (2015) recognised a putative new species, *Sphenarium* sp. Gro7, externally similar and geographically close to *S. adelinae* **sp.n**. Indeed, we examined several specimens collected less than 9 km apart from the *Sphenarium* sp. Gro7 locality (L86, L100, L103; Appendix Table 5), which were invariably *S.*

adelinae **sp.n.** Nevertheless, the *CO1* sequences of *Sphenarium* sp. Gro7 are different but closely and strongly related (*PP* ≥ 0.95) to the *S. adelinae* **sp.n.**/*S. miztecum* **sp.n** group (Appendix Fig. 22). Considering their geographical and phylogenetic proximity, both *Sphenarium* sp. Gro7 and *S. adelinae* **sp.n.** probably represent the same species. The paraphyletic relationships between these taxa suggest a greater genetic diversity within *S. adelinae* **sp.n.** than the one we recognised during our genetic analysis. Another possibility is that the genetic differentiation observed between *CO1* sequences of *Sphenarium* sp. Gro7 and *S. adelinae* **sp.n.** is due to the different methodology used to obtain the sequences. We obtain mitochondrial sequences following a long-PCR amplification protocol in order to avoid coamplification nuclear mitochondrial pseudogenes; whereas Pedraza-Lara *et al*. (2015) followed standard *CO1* barcoding protocols, which usually results in coamplification of multiple paralogous numts haplotypes of different divergences, leading to the overestimation of genetic diversity (Song *et al*., 2008).

Etymology: This species is dedicated to the memory of Adelina Cedillo-Franco, who always supported and encouraged the senior author in his biological studies. The specific name is a female noun in the genitive case.

Sphenarium miztecum **Sanabria-Urbán, Song & Cueva del Castillo sp.n.**
(http://lsid.speciesfile.org/urn:lsid:Orthoptera.speciesfile.org:TaxonName:495094)

Description: External morphology (Fig. 15S, T): total body length ranging from 31.09 to 32.07 mm in females and from 24.87 to 33.35 mm in males; antennae ensiform, slightly shorter in females or longer than head and pronotum together in males; head conical notably longer than wide with oval eyes in both sexes; fastigium notably elongated, nearly as long as the interocular space in both sexes; tegmina strap-like in both sexes; subgenital plate of males somewhat tapered in the apex; dorsal ovipositor valves lanceolate slightly elongated towards the apex. Male genitalia: bridge of epiphallus as long or slightly longer than the length of lateral plates in most cases (Fig. 17G-I). Ectophallus in dorsal view (Fig. 17G-II) small with lateral borders of ramus convergent, straight; basal emargination of cingulum mostly notably developed closing the interspace between the apodemal plates. Ectophallus in posterior (Fig. 17H) view with a conspicuous sclerotized hollow in the sheath notably closed; valves of cingulum small with form, not developed posteriorly (Fig. 17I); inflections of supraramus notably developed laterally and dorsally whit dorsal borders evidently fused above the valves of cingulum (Fig. 17H, arrow). Endophallus in lateral view (Fig. 17D-III) with a short pseudoarch tightly joined to the valves of cingulum; aedeagal valves very small, sharply pointed apically, without apical spine; aedeagal valves and sclerites about half the length of dorsal inflections of endophallic apodemes.

Colouration: Ground colours green or brown. Body uniformly coloured (Fig. 15T) or with the following colour traits (Fig. 15S): antennae dark brown; fastigium brownish; lateral postocular bands frequently present, wide and yellowish, whitish or bluish; dorsomedial line frequently present, wide and yellowish; dorsal shades brown to dark magenta, covering entirely the dorsal portion of pronotum and abdomen in most specimens; lateral shades often present, dark to light brown; lateral bands of blotches if present, cyan or distinct emerald green; ventral bands of pronotum often present, wide, whitish to bluish; pronotum sometimes with small dots in the dorsal posterior margin; mesonotum brownish; lateral blotches of 1st abdominal segment frequently present, cyan to whitish; tegmina red or green; hind femora with medial area uniformly coloured and lower marginal area with distinct blue colouration, specially in the apex of femur; knees of hind femora darkly coloured laterally, lighter dorsally, black to bluish; hind tibia intense yellow or green.

Diagnosis: *S. miztecum* **sp.n.** is very similar to its sister species *S. adelinae* **sp.n.** both in external and male genital morphology. Externally *S. miztecum* **sp.n.** differs from other *Sphenarium* species by its cyan or emerald green colourations in the lateral bands of blotches, when present. The main difference in their male genital structures is that in *S. miztecum* **sp.n.** the dorsal borders of the inflections of supraramus are fused above the valves of cingulum.

Distribution: This species is only known from a single locality from in the Pacific Cost of northern Oaxaca (Fig. 9B). Additional fieldwork on this region is necessary to accurately delimit the distribution rages of this species.

Material examined: Holotype *m* (Fig. 15S) from Mexico: Oaxaca, ca. de Pinotepa Nacional Carr 200, 16.37195ºN, -98.177102ºW, 216 m a.s.l., X-5-2011 (Sanabria-Urbán S. # 18CPI [L18 MS1]). Paratypes from, 11 *m*, 6 *f*, same data as holotype. The holotype was deposited at IBUNAM and the paratypes were deposited at IBUNAM and TAMUIC.

Taxonomic discussion: This species is also closely related morphologically and genetically to *S. adelinae* **sp.n.** Nevertheless, this new species show a unique combination of morphologic traits (both in external and male genitalia structures) in combination with a well-supported monophyly and relatively high levels of genetic differentiation (Table 3). Therefore, we considered *S. miztecum* **sp.n.** as separate lineage from *S. adelinae* **sp.n.** Previously, we recognized specimens *S. miztecum* **sp.n.** as the putative new taxa *Sphenarium* sp.n. 3 (Sanabria-Urbán *et al*. 2015). For other studies in the genus, this new species was unknown.

Etymology: Named in honour of the Mixtecos, an ancient Native American people still living in the area where this species was found.

Discussion

Taxonomic implications

In this study, we have defined the number and identity of *Sphenarium* species jointly using morphological, phylogenetic, and genetic evidence. We have conducted more detailed analyses of male genitalia and genetic characters of *Sphenarium* grasshoppers than the previous studies, based on a larger number of specimens and populations. Our results indicate that there are at least 17 independent lineages, nine of which correspond to previously described species and eight representing new species concepts for the genus.

Undoubtedly, the combined use of morphological and genetic information has allowed the recognition of cryptic diversity in *Sphenarium* that has been unnoticed for decades. Indeed, most of the new species here described were previously examined but recognised as part of the taxa with the largest geographic distribution (Boyle 1974). On the other hand, we have recognised less number of putative new species than Pedraza-Lara *et al*. (2015) and Sanabria-Urbán *et al*. (2015). The former study identified only from Guerrero and Oaxaca eight putative new taxa (*Sphenarium sp.* Gro1+ Gro8, Gro6+Gro9, Gro2, Gro3, Gro4, Gro7, Oax2, Oax9); whereas Sanabria-Urbán *et al*. (2015) recognised two additional taxa (*Sphenarium* sp.n. 6 & *S. magnum*) from Michoacan and Oaxaca. However, according to our results, most of these putative taxa represent part of the species recognised in this revision. In those two studies, the taxonomic delimitations were based on the analysis of fewer specimens and populations than this study. Moreover, Pedraza-Lara *et al.* (Pedraza-Lara *et al.* 2015) based their species delimitations on few ambiguous and variable morphological characters; and used General Mixed Yule-Coalescent methods that tend to estimate a greater diversity than other approaches (Fujisawa & Barraclough 2013; Pocco *et al.* 2015; Talavera *et al.* 2013).

Two taxa identified by Pedraza-Lara *et al*. (2015) could represent additional new species in the genus, *Sphenarium* sp. Gro2 and Gro4. The first one was recognised as an unambiguous new species supported by morphological, genetic, and coalescent criteria. In our combined Bayesian analysis of *CO1* sequences, this taxon represents a well-supported monophyletic group closely related to *S. macrophallicum* and *S. crypticum* **sp.n.** (Appendix Fig. 22). Nonetheless, after analysing several specimens collected near to the locality *Sphenarium* sp.

Gro2 was collected, we find that all of them are morphologically and genetically identified as *S. rugosum*. The second taxon is closely related to *S. rugosum* by Pedraza-Lara *et al*. (2015), as well as in our combined Bayesian analysis of *CO1* sequences (Appendix Fig. 22). However, if this taxon were part of *S. rugosum*, it would represent the most distantly and isolated population of the species.

In addition, our study demonstrates the existence of notable intraspecific variation in both morphological and genetic characters in species with wide (*S. borrei*, *S. histrio*, *S. mexicanum*, *S. purpurascens*, *S. rugosum*) and narrow (*S. variabile*) distribution ranges. Interestingly, these species show slight intraspecific genetic differentiation (≤3%; Table 3 & 4) and geographically restricted male genital morphotypes are slightly differed from each other (except in *S. borrei*), which may suggest an ongoing speciation process. Currently we are conducting more detailed studies in order to determine if this intraspecific variation represents incipient speciation events.

We have discovered that morphology and structures of male ectophallus provide important diagnostic characters in the group, which were previously overlooked and would be useful for further taxonomic studies. Moreover, we have also identified that geographic information and some external characters (such as antennae, head, tegmina, male subgenital plate, ovipositor valves, and colour patterns) are useful in differentiating *Sphenarium* species. In general, we support previous indications of species validation for *S. mexicanum*, *S. histrio*, *S. minimum*, *S. purpurascens*, and *S. planum* (Pedraza-Lara *et al.* 2015; Sanabria-Urbán *et al.* 2015); and we also propose *S. bruneri* as a synonym of *S. borrei*, rather than of *S. histrio*. For other species described before 1960's, we accept the synonymies proposed by Kevan (1977) because we were unable to examine the male genitalia and/or detailed sampling localities were unknown. Therefore, we recognise that future examination of the male genitalia would undoubtedly validate the taxonomic classification of these taxa.

Phylogeny and biogeographic history

We have conducted a more robust phylogenetic analysis than in the previous studies (Pedraza-Lara *et al.* 2015; Sanabria-Urbán *et al.* 2015). Our phylogeny is mostly congruent with the previous inferences, which suggests that upon divergence from *Prosphena*, *Sphenarium* diversified into three major clades within which most lineage diversification occurred subsequently (Figs. 2, 3). These results also show that phylogenetically close species are geographically close as well (Fig. 1). Interestingly, our phylogeny also suggest that ancestral *Sphenarium* probably occupied outer lowlands of Mexico and that younger lineages have more recently colonized inner basins and highlands of central Mexico.

In our phylogeny, we have recovered four mostly well-supported monophyletic groups comprising paraphyletic and/or weakly supported monophyletic species, within which shared haplotypes occurred: *S. occidentalis* **sp.n.**/*S. histrio*/*S. mexicanum*; *S. macrophallicum*/*S. crypticum* **sp.n.**; *S. rugosum*/*S. tarascum* **sp.n.**; and *S. purpurparcens*/*S. variabile*/*S. zapotecum* **sp.n.** (Fig. 2; 3; Table 2). Incomplete lineage sorting and gene flow can explain the occurrence of shared haplotypes, weak monophyly and paraphyletic relationships between these morphologically different species. Incomplete lineage sorting and gene flow can result in similar genetic and phylogenetic patterns (McGuire *et al.* 2007; McKay & Zink 2010; Nater *et al.* 2015). Nevertheless, there are two main expectations that allow differentiating these processes (McGuire *et al.* 2007). First, incomplete lineage sorting requires that gene tree bifurcations precede species divergences. Therefore, if species divergence occurred so rapidly that there has not been enough time for genetic divergence and reciprocal monophyly, little or not genetic differentiation is expected between species sharing haplotypes or with paraphyletic relationships (Knowles & Carstens 2007; McGuire *et al.* 2007). Second, if descendent species have retained multiple alleles from their common ancestor, a random distribution of these alleles would be expected in the descendent populations. Therefore, shared or slightly differed alleles are not expected to be geographically concentrated near species boundaries (Hare & Avise 1998; McGuire *et al.* 2007).

In *Sphenarium*, incomplete lineage sorting rather than gene flow seems to be a more plausible explanation for the low genetic divergence between diagnosable and morphologically different species. In most of the above mentioned monophyletic groups, there are relatively low levels of intraspecific genetic differentiation (< 2.4% in *CO1 P-distance* values) (except for the *S. occidentalis* **sp.n.**/*S. histrio*/*S. mexicanum* group, where interspecific *CO1 P-distance* values range from 3.16 – 4.65%; see Table 2). In addition, all shared haplotypes (mitochondrial and nuclear) are randomly distributed in geographically distant populations of closely related taxa (i.e. within the same monophyletic group for mitochondrial and *ITS2* haplotypes or within the same major clade for *H3* haplotypes) (Fig. 1; Table 2). Exceptionally, *S. rugosum* and *S. tarascum* **sp.n.** share *ITS2* haplotypes between adjacent populations suggesting gene flow among them. Moreover, during fieldwork we have observed occasional interspecific pairs in copula in the contact zones of some species (e.g. between *S. variabile*, *S. purpurascens* and *S. histrio*; and *S. rugosum* and *S. adelinae* **sp.n.**), suggesting the possibility of hybridization even between morphologically and genetically different species. Mitochondrial introgression has been suggested in *S. variabile* because of putative hybridization with *S. hitrio* and/or *S. mexicanum* populations (Pedraza-Lara *et al.* 2015). In this case, the possibility of hybridization may be dismissed because the species could have been misidentified due to the lack of enough morphological and geographic information. Moreover, if hybridization occurs it might not necessarily result in mitochondrial introgression (McGuire *et al.* 2007). Nevertheless, recognising and distinguishing the relative importance of incomplete lineage sorting and gene flow in the evolution of *Sphenarium* would necessarily involve examining dense population samplings and more multi-locus information (Bryson *et al.* 2010; McGuire *et al.* 2007).

In our phylogeny, the basal relationships between clades and within clade 3 conflict or are weakly supported (Figs. 2, 3). These phylogenetic uncertainties probably reflect incomplete lineage sorting due to a recent origin (Talavera *et al.* 2013), or nearly simultaneous cladogenetic events (Allegrucci *et al.* 2013). The basal divergences in S*phenarium* occurred around 7.22 to 2.91Ma, whereas the most of the diversification events occurred between 2.51 and 0.19 Ma (Fig. 3). In congruence with our phylogenetic hypothesis these episodes correlate with the third major formation of the Mexican Volcanic Belt (MVB), around 7.5 to 3 Ma (Ferrari *et al.* 2012), and the climate-driven range shifts around 2.6 to 0.01 Ma, which have been proposed as drivers of simultaneous divergences in other co-distributed taxa (Bryson *et al.* 2011, 2012b; Duennes *et al.* 2012; Leaché & Mulcahy 2007; Mulcahy *et al.* 2006; Pringle *et al.* 2012). The several volcanic episodes during the formation of the MVB (Ferrari *et al.* 2012) probably sundered ancestral populations of *Sphenarium*, whereas Quaternary climatic changes probably caused several and recurrent isolation events in ancestral populations across high and lowlands. In addition, the strongly supported monophyletic groups of *Sphenarium* are geographically restricted to well-defined biogeographic provinces, supporting that vicariance events could have played a fundamental role during the diversification of the group. Therefore, current parapatric and narrow sympatric distribution of the species have probably resulted from secondary dispersal events.

Evolutionary forces and diversification of Sphenarium

We have identified three broad patterns of differentiation that reflect the relative importance of evolutionary forces in the diversification of *Sphenarium*. Firstly, we find that despite some species are genetically close (e. g. *S. histrio*/*S. mexicanum*; *S. macrophallicum*/*S. crypticum* **sp.n.**; *S. rugosum/S. tarascum* **sp.n.**; and *S. purpurascens*/*S. variabile*), they strongly differ from each other by male genital morphology. Because male genitalia are known to be under strong sexual selection (Eberhard 1985), and sexually selected characters tend to diverge very rapidly (Hosken & Stockley 2004), we can infer that sexual selection may have played a major role in the divergence among these *Sphenarium* species. The molecular data have revealed a second pattern; cryptic diversity among species that are morphologically similar (e. g. *S. miztecum* **sp.n.**/*S. adelinae* **sp.n.**; *S. histrio/S. occidentalis* **sp.n.**; and *S. infernalis* **sp.n.**/*S. rugosum*). In these cases, we postulate that evolutionary processes different from sexual selection on male genitalia could have played an important role in the divergence of lineages. The recurrent isolation and genetic drift in ancestral populations could have generated genetic rather than morphological divergence. Finally, we find that some species are both morphologically and genetically differed (e. g. *S. borrei* and *S. totonacum* **sp.n.**). In this pattern, we assume that the interplay between evolutionary forces is driving species differentiation in the group. Moreover, correlative evidence in the genus suggests that natural and sexual selection on body size in response to altitudinal climatic variation could have also promoted the diversification of the group (Sanabria-Urbán *et al.* 2015).

The results of this study shows that the genus *Sphenarium* is an interesting assemblage of lineages with different levels of morphological and genetic divergence suggesting a complex interplay between evolutionary forces during the evolution of the genus. Moreover, the observed patterns of intraspecific variation suggest several instances of on going speciation processes in the genus. This study opens to an opportunity to further explore the mechanisms involved in diversification of *Sphenarium* members. Due to their low vagility, these flightless grasshoppers can be an excellent model to further understand the relative impact of historical geological and climatic events, as well as local adaptation processes in the diversification of Neotropical insects.

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Figures

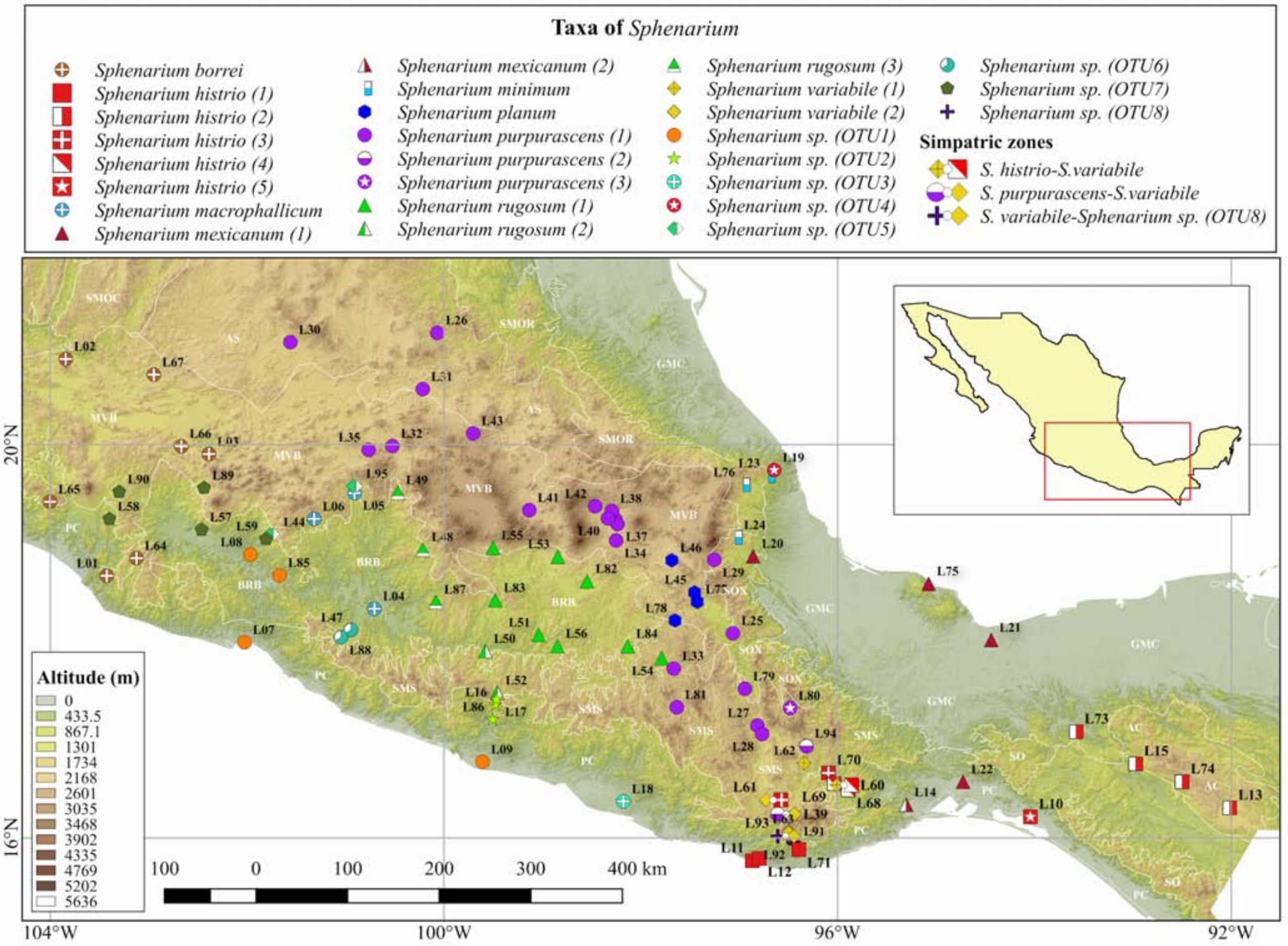

Figure 1. Sampled localities (bold numbers in map) for the genetic analysis and geographic distribution of the identified morphologic taxa in *Sphenarium* (different symbols) in Mexico. Numbers within parenthesis in front of taxon names indicate different morphotypes within the given species. White surrounded areas and upper case abbreviations denote the Mexican biogeographic provinces. AC, Altos de Chiapas; AL, Altiplano Sur; BRB, Balsas River Basin; GMC, Gulf of Mexico Coast; PC, Pacific Coast; SMOC, Sierra Madre Occidental; SMOR, Sierra Madre Oriental; SMS, Sierra Madre Sur; SO, Soconusco; and SOX, Sierra de Oaxaca.

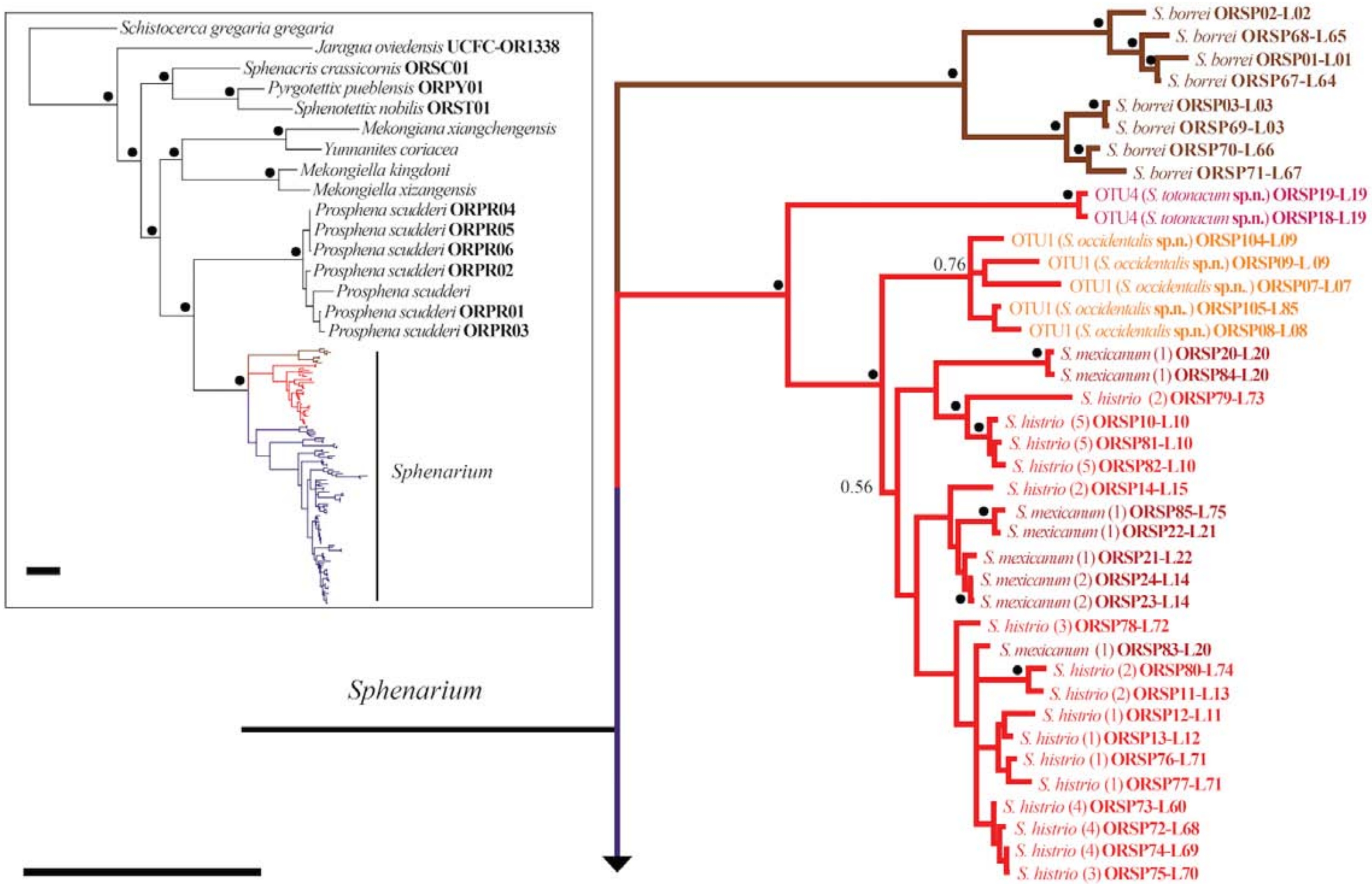

Figure 2. Bayesian phylogeny of *Sphenarium* based on a concatenated analysis of 2527 nucleotide positions from five loci and 145 terminals (129 ingroup and 16 outgroup terminals). Higher-level phylogenetic relationships are shown on left-top box and ingroup relationships are magnified. Voucher and locality identifier numbers of the included specimens are indicated in bold characters in terminals names, except for those cases in which genetic information was retrieved from the GenBank. Different branch colours highlight the three mayor clades within the genus (brown, Clade 1; red, Clade 2; and purple, Clade 3). Different colours of the *Sphenarium* terminals represent the 17 taxa identified during the morphologic analysis. Black circles behind the nodes indicate *PP* values ≥ 95%. For some important nodes we also showed the *PP* values in bold numbers. Black bottom bars in all cases are equal to 0.03 substitutions per site.

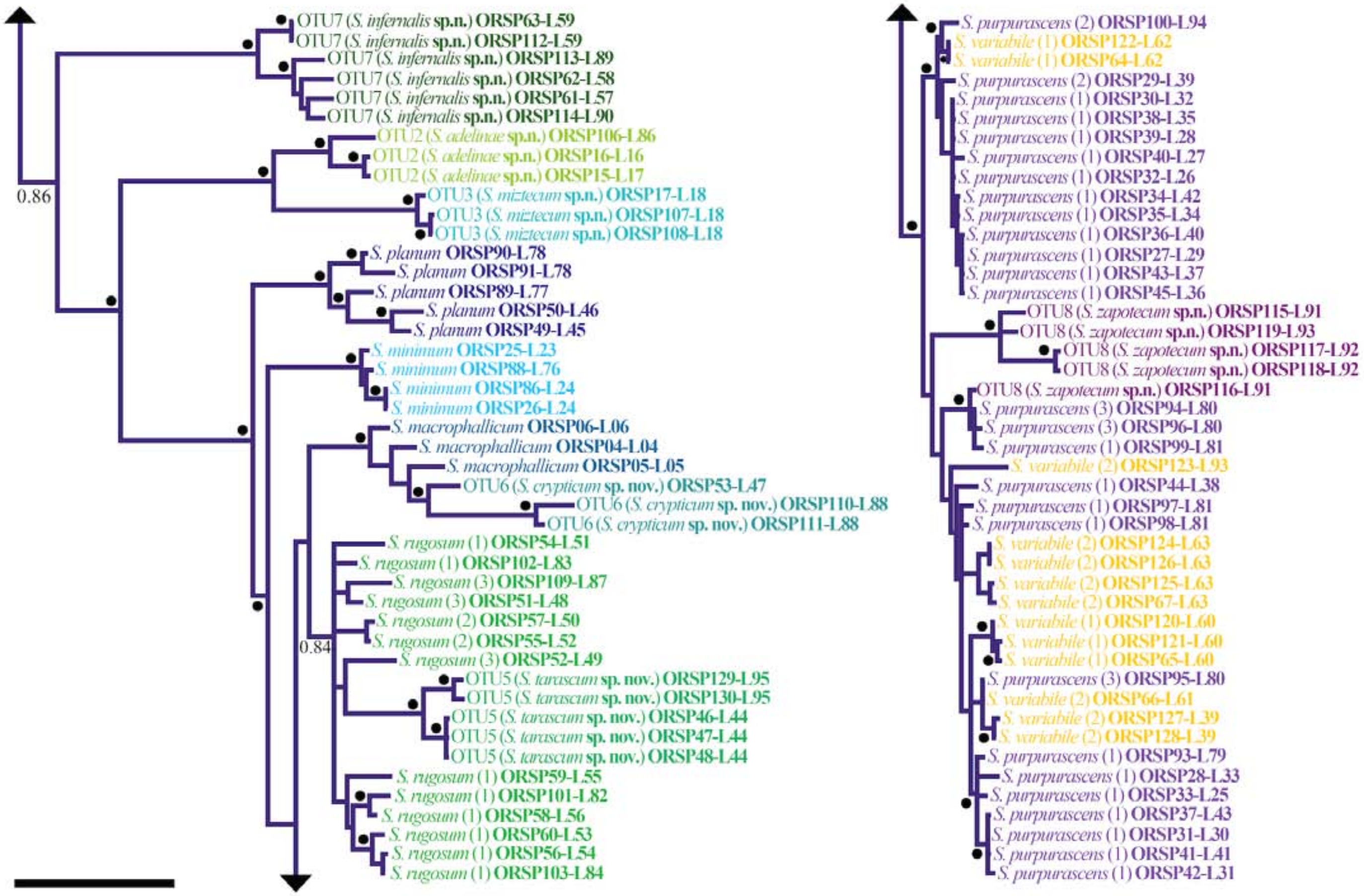

Figure 2. Continuance.

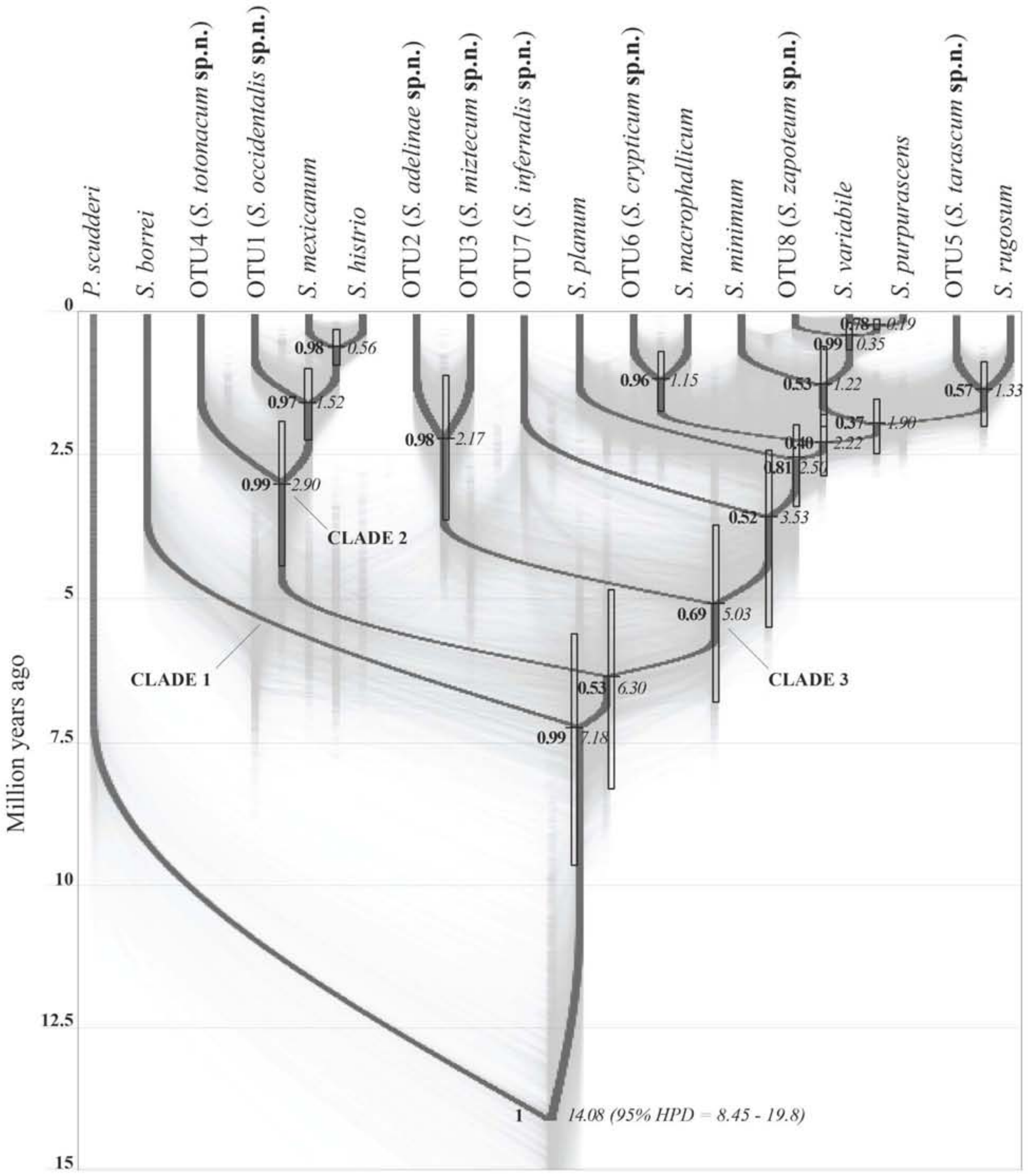

Figure 3. Species phylogeny and approximate divergence times between *Sphenarium* lineages. The consensus tree is shown in dark grey; whereas other possible trees are denoted in light grey. Numbers behind the nodes indicate their *PP* values (left numbers in bold) and mean divergence time (Ma) (right numbers in italics). The lengths of the bar in the nodes indicate the 95% HDP interval values for the divergence time estimations, except for the first basal node where this interval is indicated in numbers.

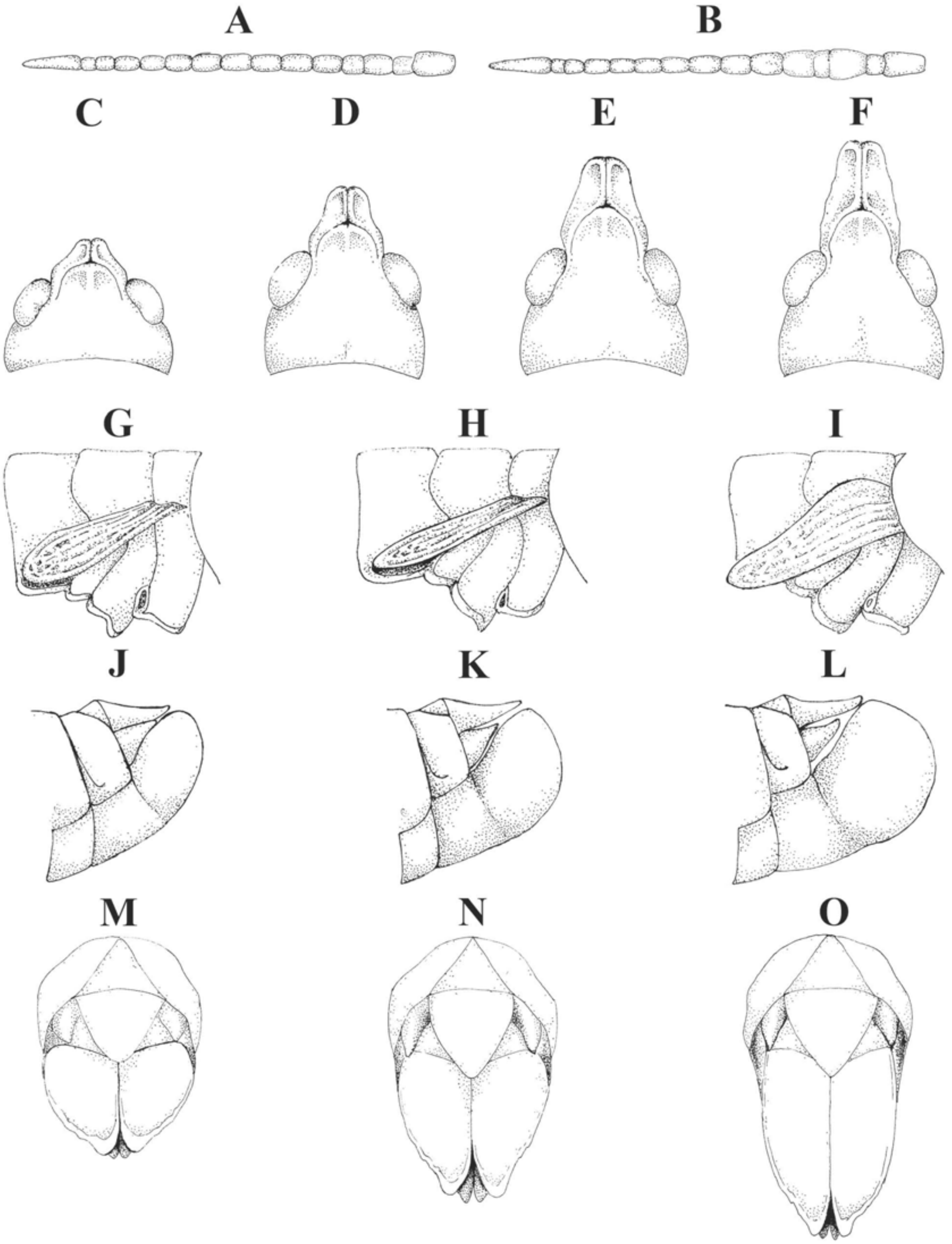

Figure 4. External morphologic characters of *Sphenarium* and *Prosphena*: antennae filiform (A) or weakly ensiform (B); head subtriangular-compresed (C), subtriangular-elongated (D) or conical (E, F); tegmina spatula-like (G), strap-like (H) or tongue-like (I); subgenital plate of males tapered (J) or rounded moderately (K) or notably (L) developed posteriorly; dorsal ovipositor valves rounded (M), moderately lanceolate (N) or notably elongated (O).

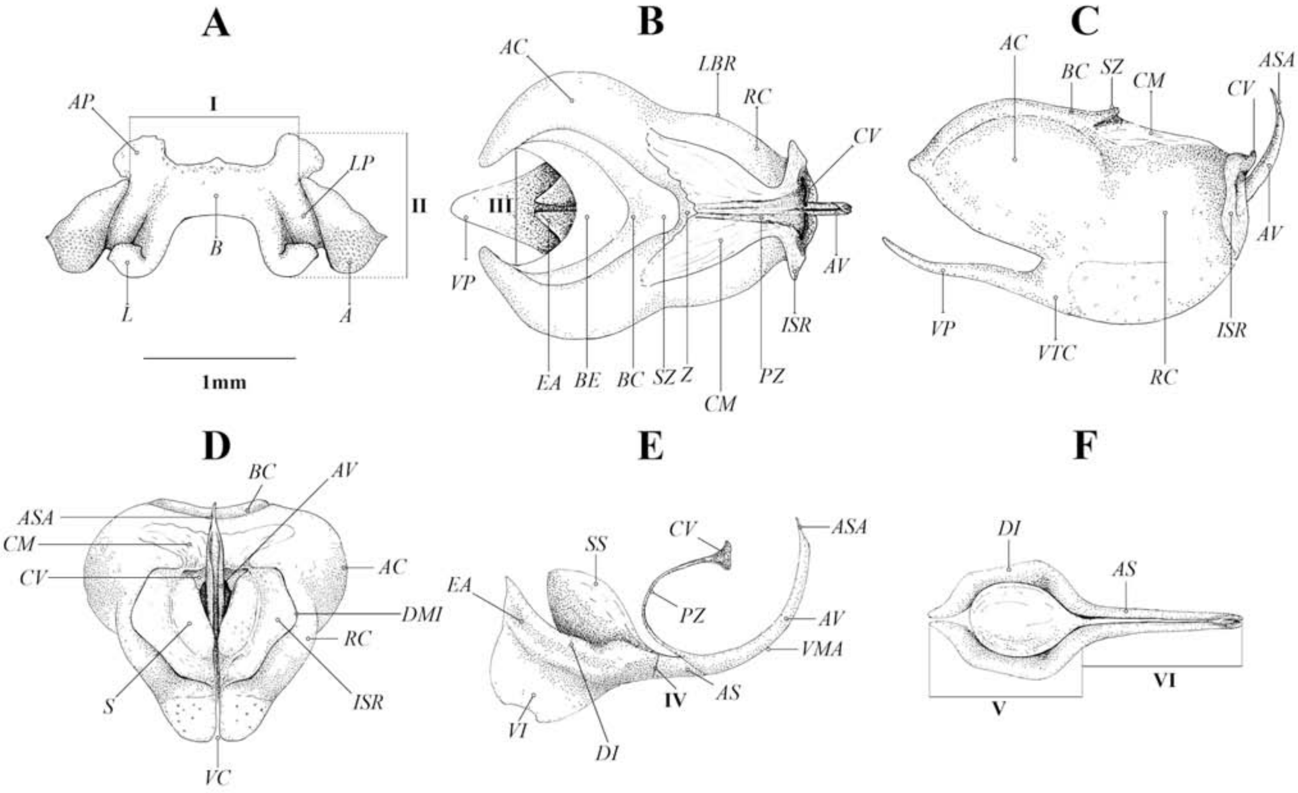

Figure 5. Male genitalia of *Sphenarium*: epiphallus in dorsal view (A); ectophallus in dorsal (B), lateral (C) and posterior (D) view; and endophallus in lateral (E) and dorsal (F) view. *A*, appendix of epiphallus; *AC*, apodemal plate of cingulum; *AP*, anterior projection of epiphallus; *AS*, aedeagal sclerites; *ASA*, apical spine of aedeagus; *AV*, aedeagal valve; *B*, bridge of epiphallus; *BAS*, base of aedeagal sclerites; *BC*, basal thickening of cingulum; *BE*, basal emargination of cingulum; *CM*, central membrane; *CV*, cingulum valve; *DI*, dorsal inflection of endophallic apodeme; *EA*, endophallic apodeme; *ISR*, inflection of supraramus; *L*, lophus of epiphallus; *LBR*, lateral borders of ramus of cingulum; *LP*, lateral plate of epiphallus; *PZ*, pseudoarch of ectophallus; *RC*, ramus of cingulum; *S*, sheath of ectophallus; *SS*, spermatophore sac; *SZ*, suprazygomal plate of cingulum; *VC*, ventral cleft of cingulum; *VI*, ventral inflection of endophallic apodeme; *VMA*, ventral margins of aedeagal valves; *VP*, ventral process of cingulum; *VTC*, ventral transverse thickening of cingulum; *Z*, zygoma of cingulum; **I**, length of bridge of epiphallus; **II**, length of lateral plate of epiphallus; **III**, interspace between apodemal plates of cingulum; **IV**, width of base of aedeagal sclerites; **V**, length of dorsal inflections of endophallus; **VI**, length of aedeagal sclerites and valves together (from the tip of aedeagus to the base of aedeagal sclerites).

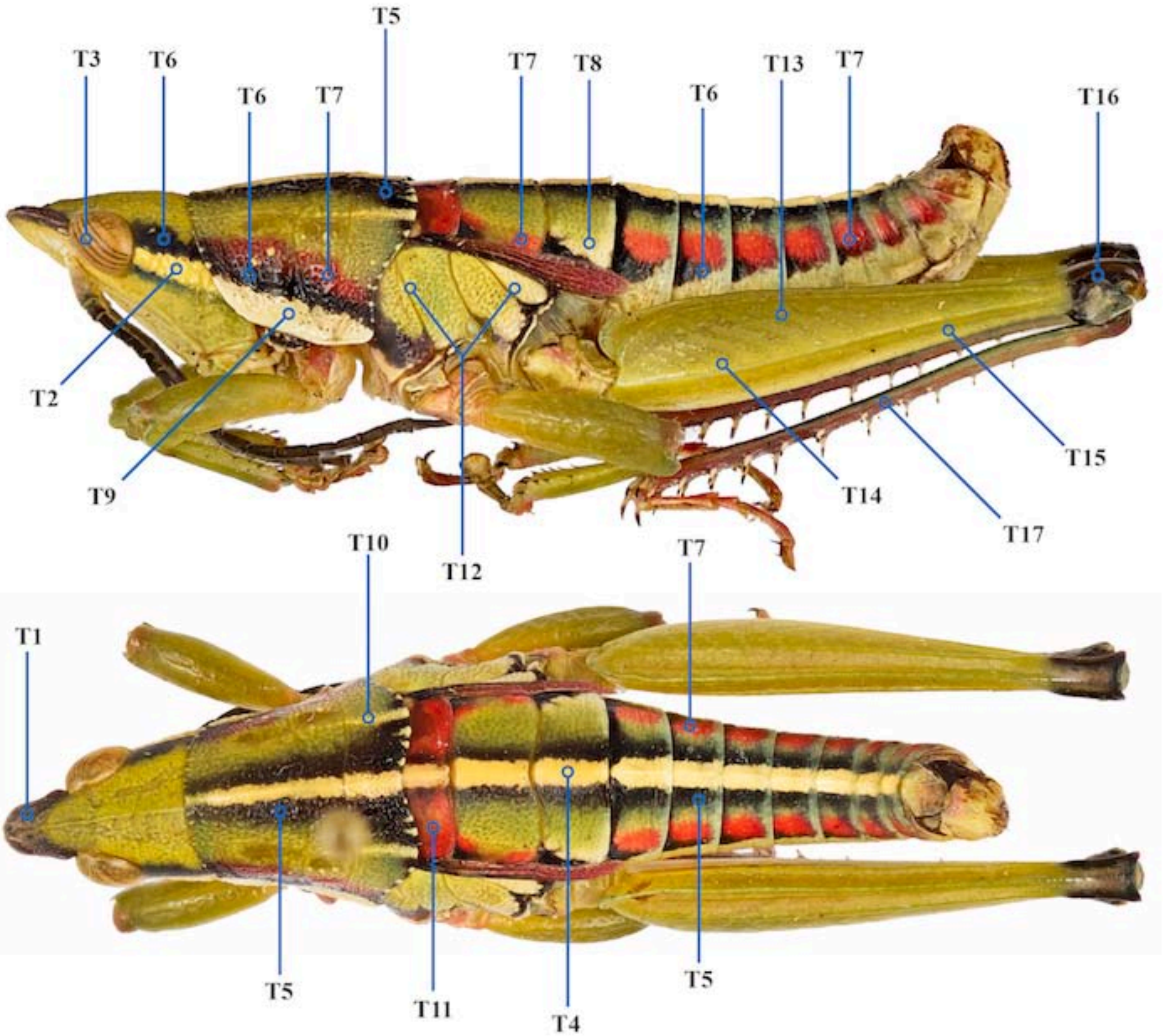

Figure 6. General colour traits of *Sphenarium* grasshoppers: T1, fastigium; T2, lateral postocular bands; T3, longitudinal lines of eyes; T4, dorsomedial line; T5, dorsal shades; T6, lateral shades: T7, lateral bands of blotches; T8, lateral light blotches of the 1st abdominal segment; T9 ventral bands of pronotum; T10, lateral carinas of pronotum; T11, mesonotum; T12, lateral segments of mesonotum and metanotum; T13, upper medial area of hind femora hind femora; T14, lower medial area of hind femora hind femora; T15, lower marginal area of hind femora hind femora; T16, knees of hind femora; and T17 hind tibia.

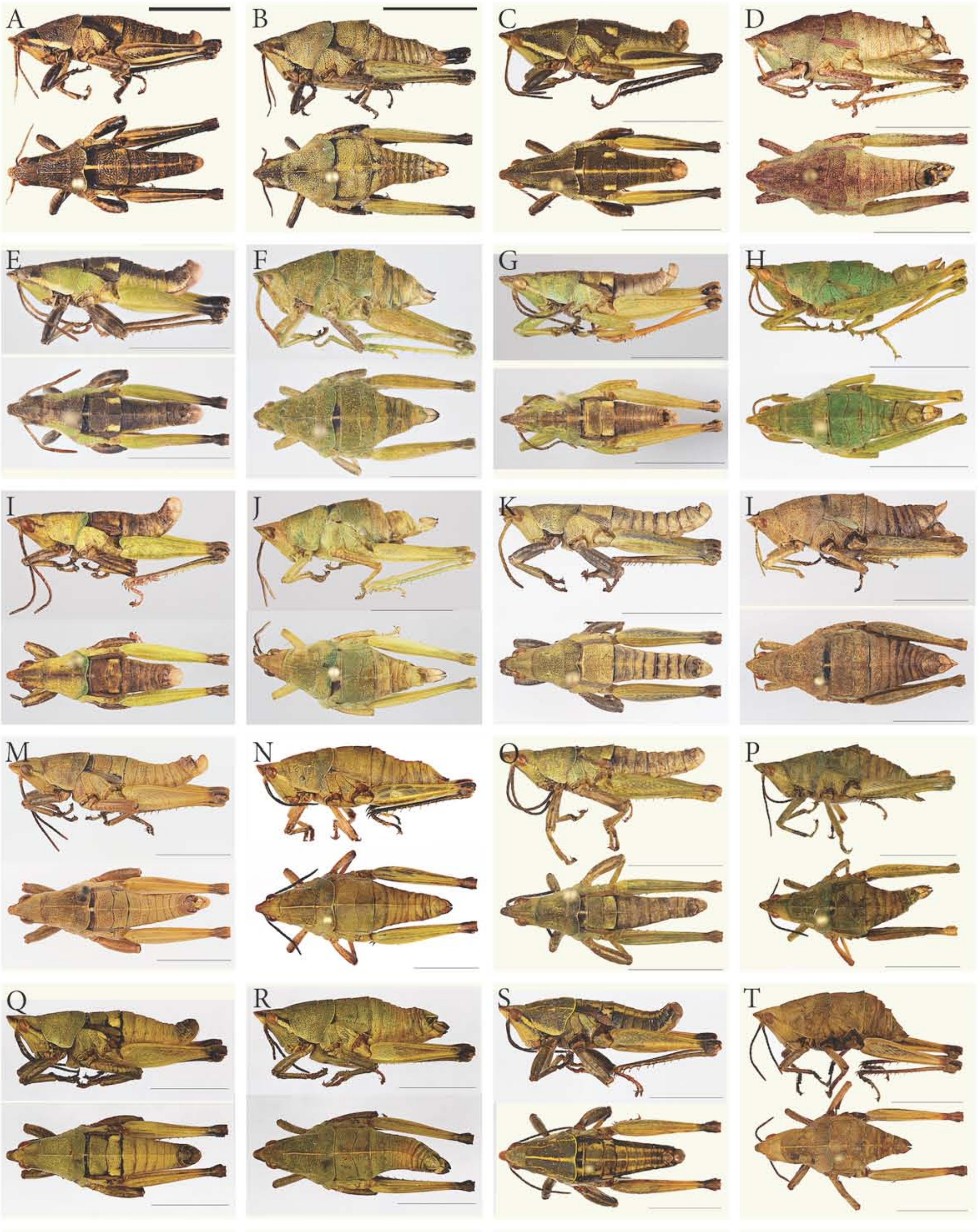

Figure 7. External morphology of *S. purpurascens*: morphotype 1 *m* (A) and *f* (B), morphotype 2 *m* (C) and *f* (D), and morphotype 3 *m* (E) and *f* (F); *S. zapotecum* **sp.n.** holotype *m* (G) and paratype *f* #23 (H); *S. tarascum* **sp.n.** holotype *m* (I) and paratype *f* #2 (J,); *S. planum m* (K) and *f* (L); *S. macrophallicum* paratype *m* #230 (M) and *f* (N); *S. minimum m* (O) and *f* (P); *S. infernalis* **sp.n.** holotype *m* (Q) and paratype *f* #2 (R); *S. rugosum* morphotype 1 *m* (S) and *f* (T) (Scale bars= 1cm).

Figure 8. Male genital structures of *S. purpurascens*: morphotype 1 (A-C), morphotype 2 (D-F), morphotype 3 (G-I); and *S. zapotecum* **sp.n.** holotype (J-l). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

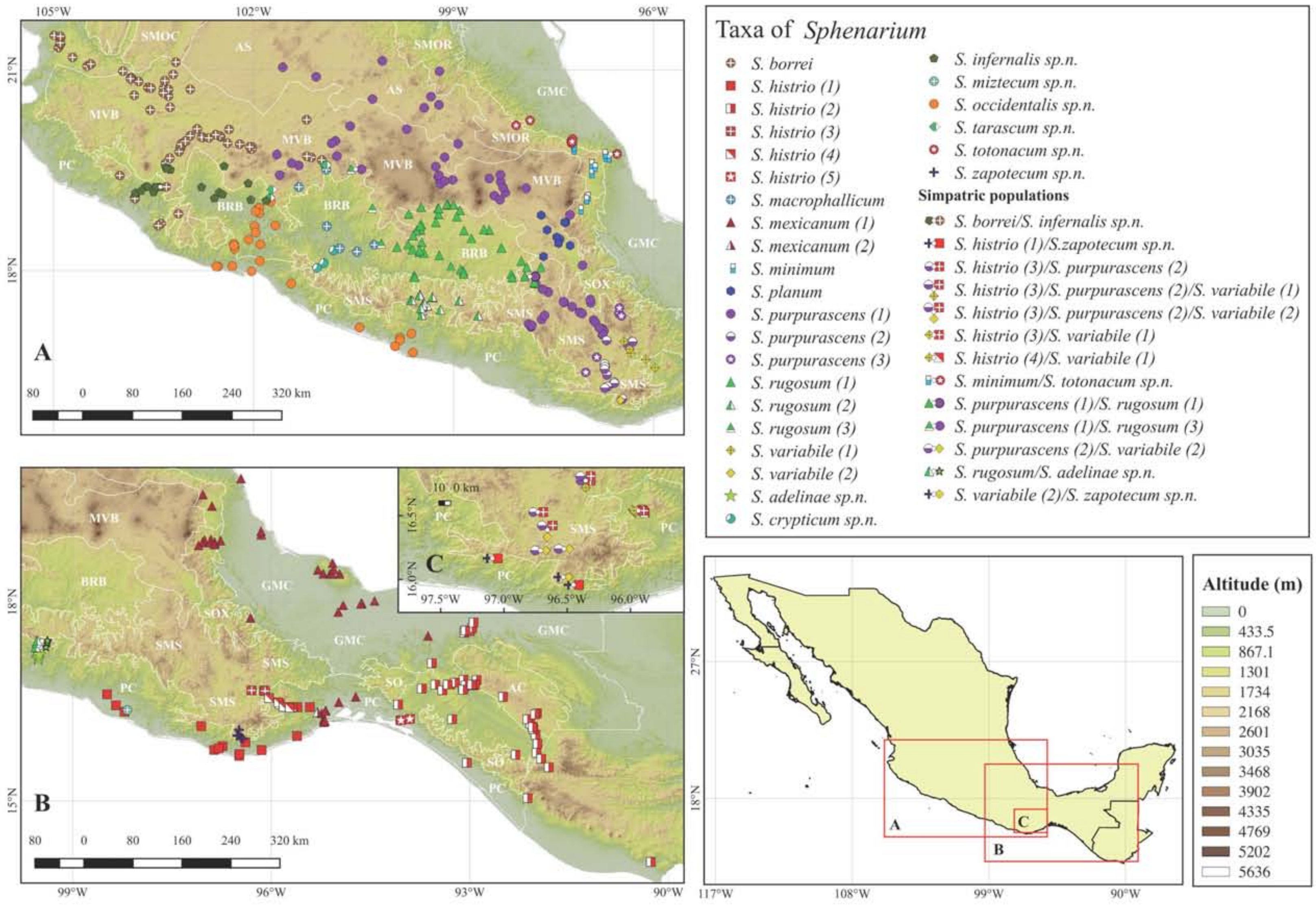

Figure 9. Geographic distribution of *Sphenarium* species. Numbers within parenthesis in front of taxa names indicate the number of identified morphotype within the species. White surrounded areas and upper case abbreviations denote the Mexican biogeographic provinces. AC, Altos de Chiapas; AL, Altiplano Sur; BRB, Balsas River Basin; GMC, Gulf of Mexico Coast; MVB, Mexican Volcanic Belt; PC, Pacific Coast; SMOC, Sierra Madre Occidental; SMOR, Sierra Madre Oriental; SMS, Sierra Madre Sur; SO, Soconusco; and SOX, Sierra de Oaxaca.

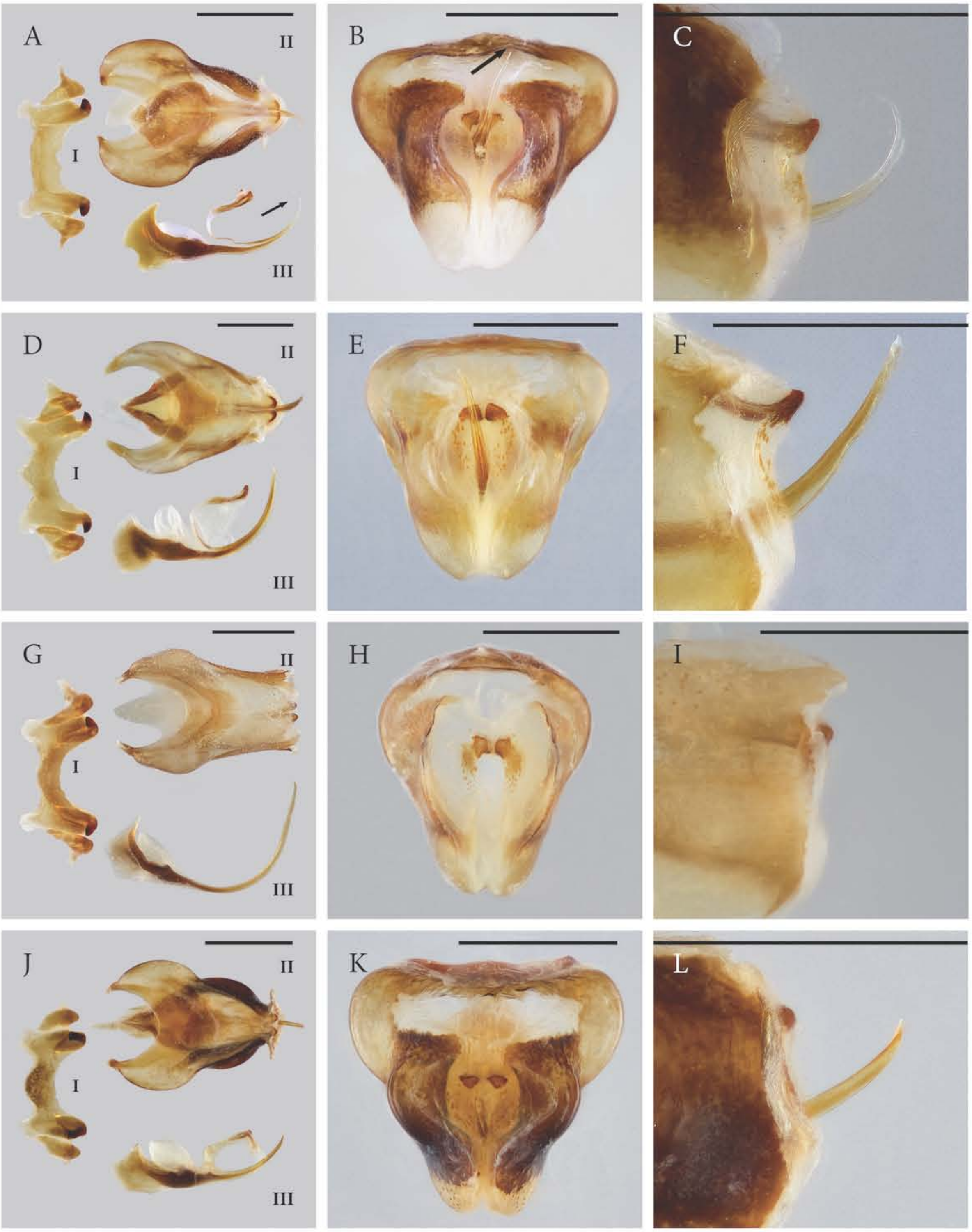

Figure 10. Male genital structures of *S. tarascum* **sp.n.** holotype (A-C); *S. planum* (D-F); *S. macrophallicum* paratype #234 (G-I); and *S. minimum* (J-L). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

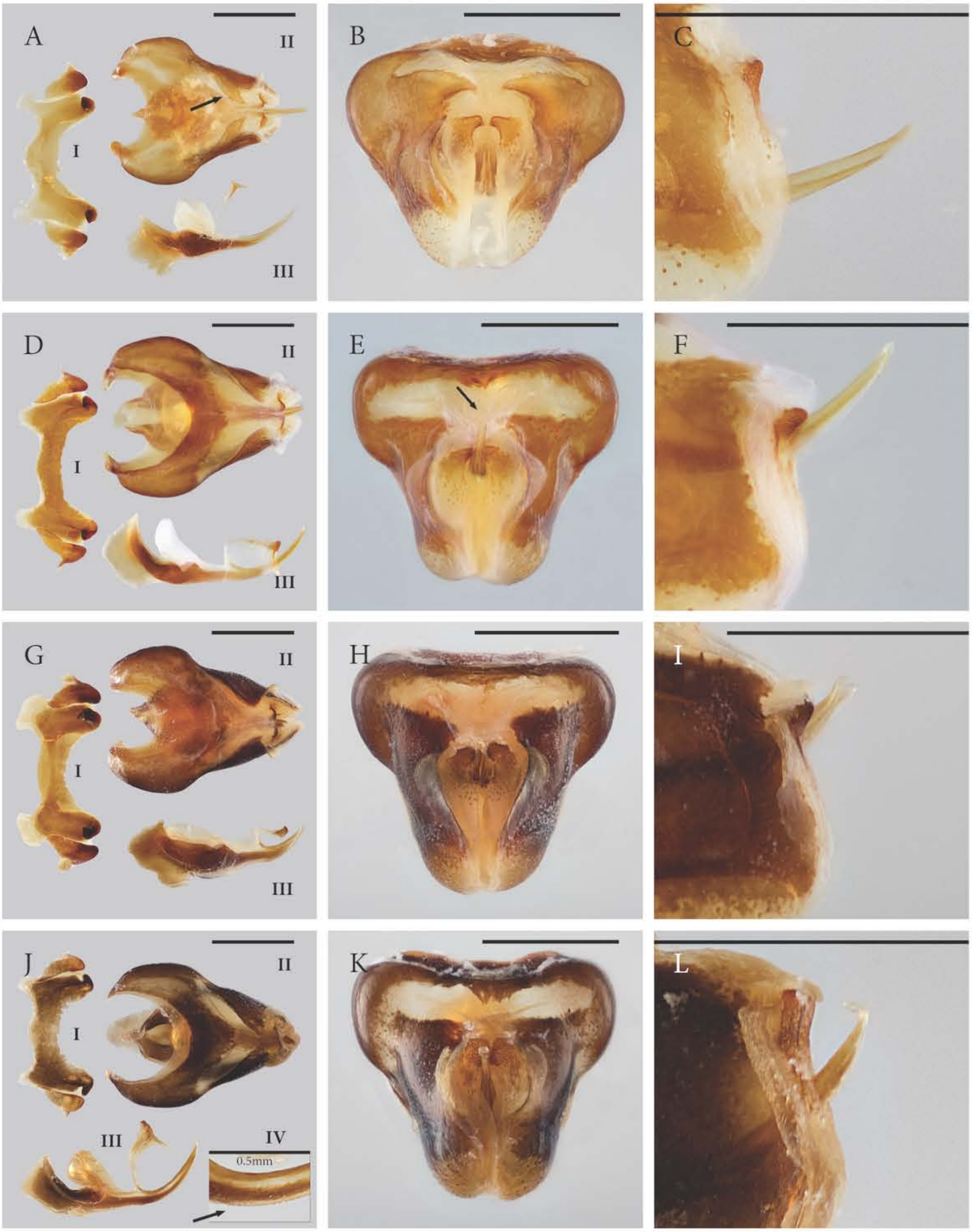

Figure 11. Male genital structures of *S. infernalis* **sp.n.** holotype (A-C); *S. rugosum*: morphotype 1 (D-F), morphotype 2 (G-I) and morphotype 3 (J-l). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

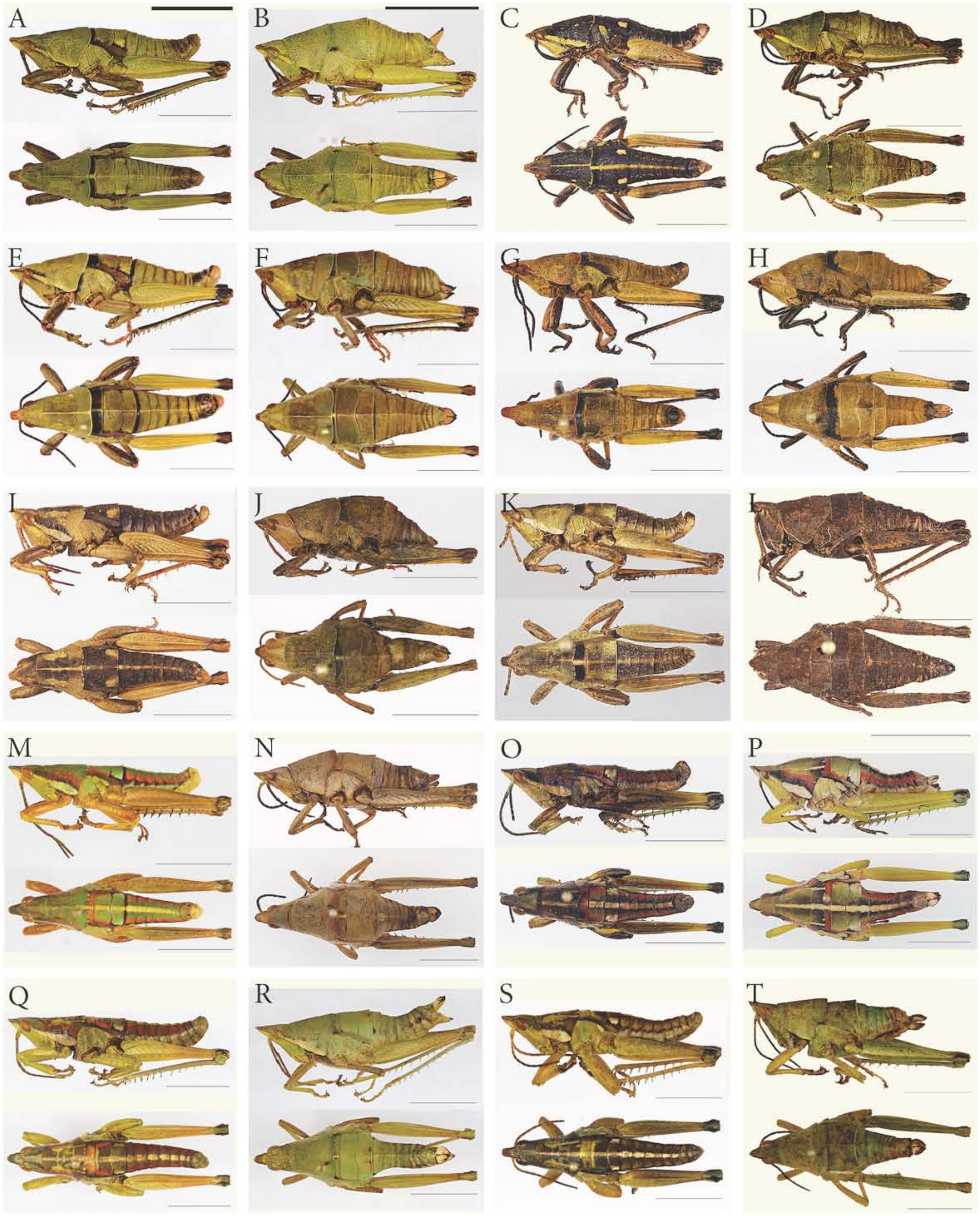

Figure 12. External morphology of *S. rugosum*: morphotype 2 *m* (A) and *f* (B), morphotype 3 *m* (C) and *f* (D); *S. crypticum* **sp.n.** holotype *m* (E) and paratype *f* #45 (F); *S. borrei m* (G) and *f* (H); *S. variabile*: morphotype 1 paratype *m* #134 (I) and *f* (J), and morphotype 2 *m* (K) and *f* (L); *S. mexicanum*: morphotype 1 *m* (M) and *f* (N), and morphotype 2 *m* (O) and *f* (P); *S. histrio*: morphotype 1 *m* (Q & S) and *f* (R & T) (Scale bars = 1cm).

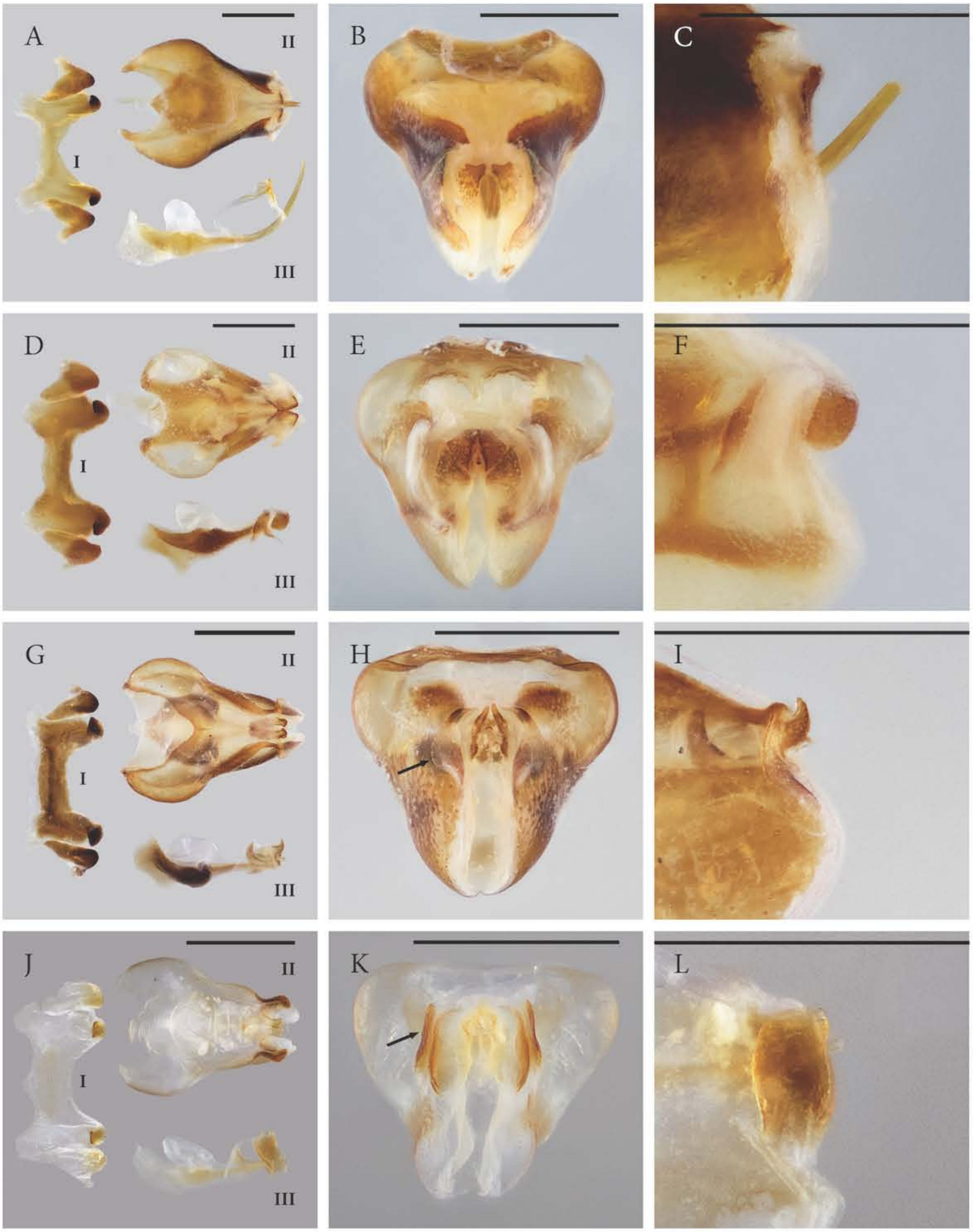

Figure 13. Male genital structures of *S. crypticum* **sp.n.** holotype (A-C); *S. borrei* (D-F); *S. variabile*: morphotype 1 paratype #234 (G-I) and morphotype 2 (J-l). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

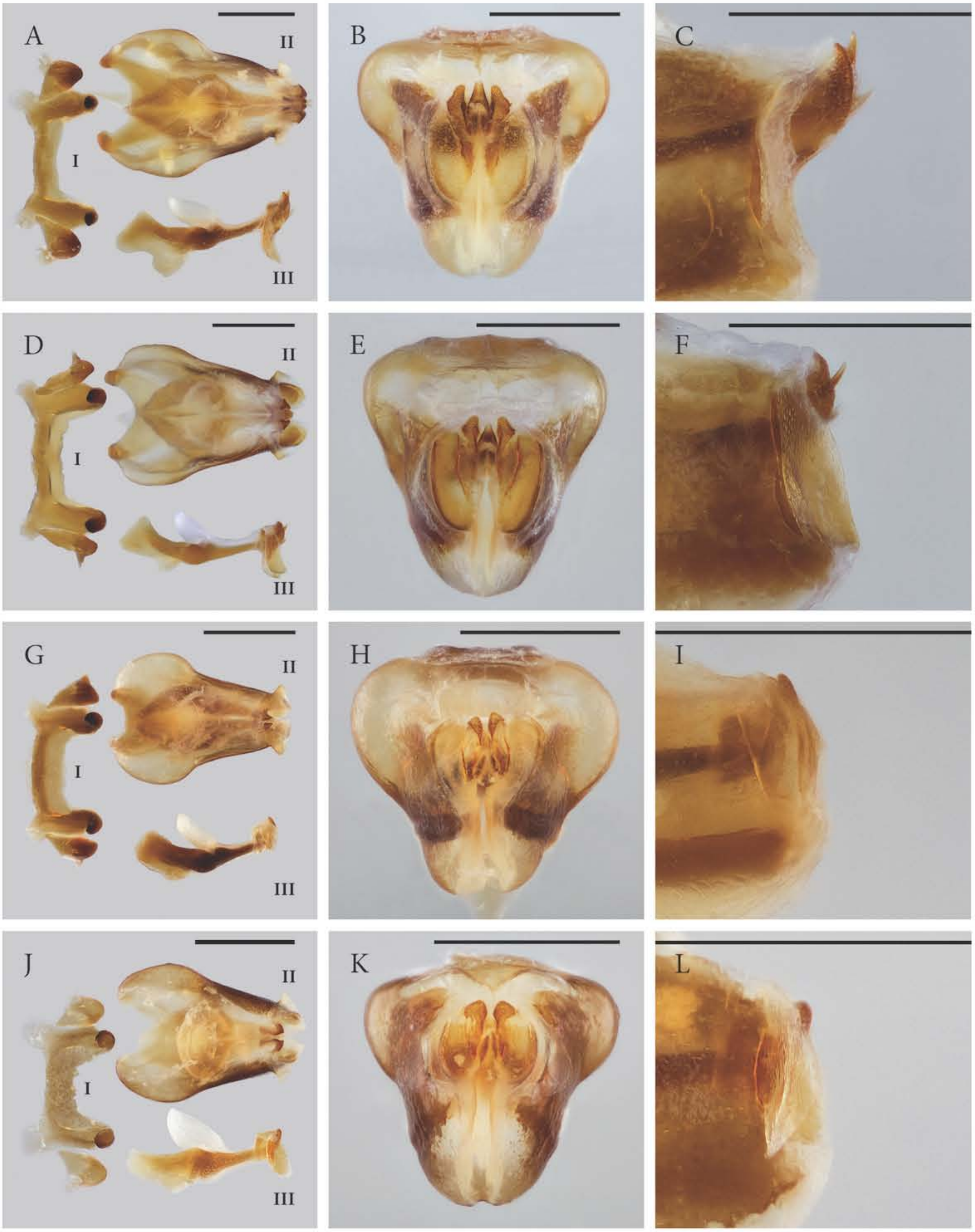

Figure 14. Male genital structures of *S. mexicanum*: morphotype 1 (A-C) and morphotype 2 (D-F); *S. histrio*: morphotype 1 (G-I) and morphotype 2 (J-l). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

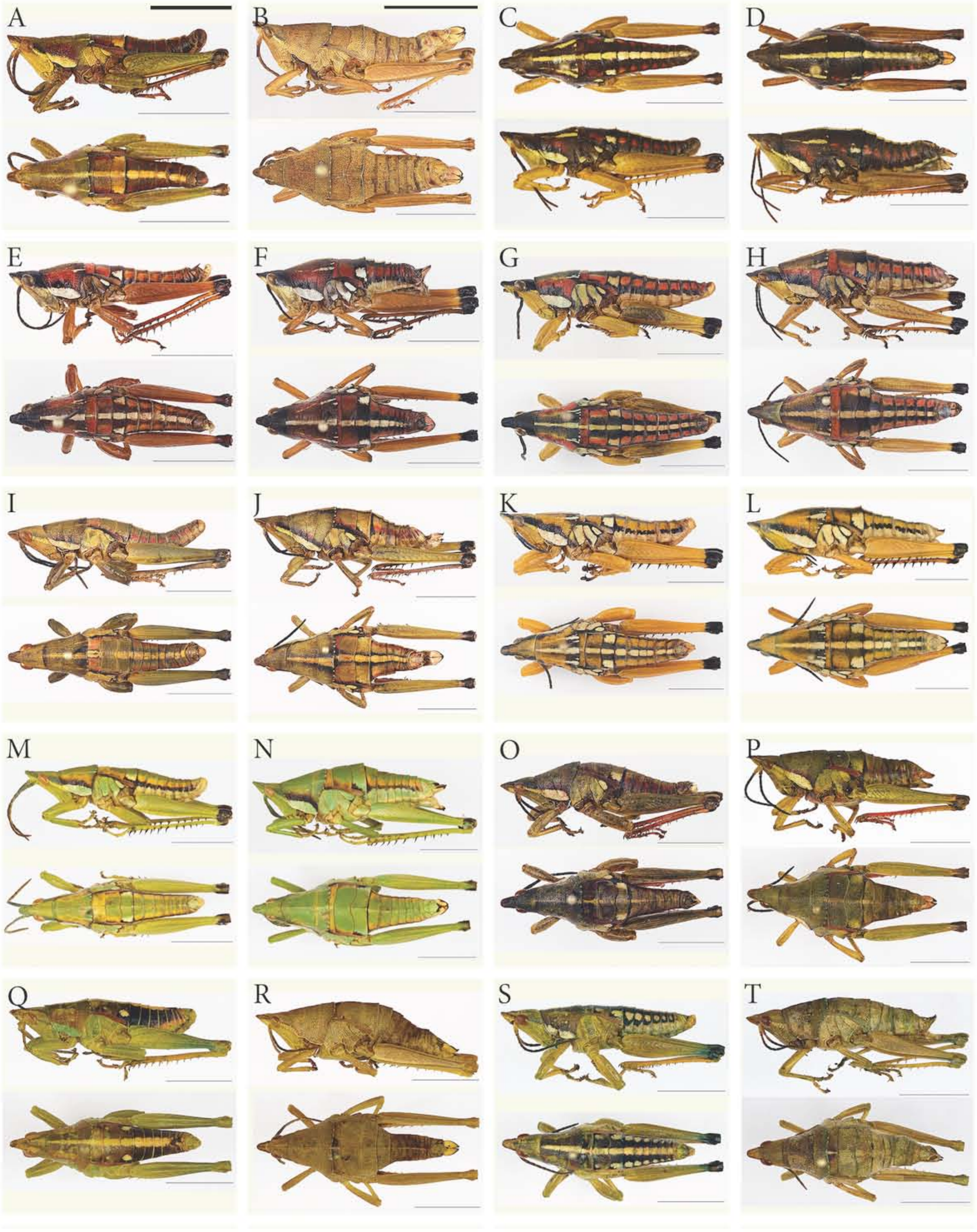

Figure 15. External morphology of *S. histrio*: morphotype 2 *m* (A & C) and *f* (B & D), morphotype 3 *m* (E) and *f* (F), morphotype 4 *m* (G) and *f* (H), and morphotype 5 *m* (I) and *f* (J); *S. occidentalis* **sp.n.** holotype *m* (K) and paratypes *m* #2 (M) f #3 (L) and *f* #4 (N); *S. totonacum* **sp.n.** holotype *m* (O) and paratype *f* #1 (P); *S. adelinae* **sp.n.** holotype *m* (Q) and paratype *f* #1 (R); *S. miztecum* **sp.n.** holotype *m* (S) and paratype *f* #1 (T) (Scale bars = 1cm).

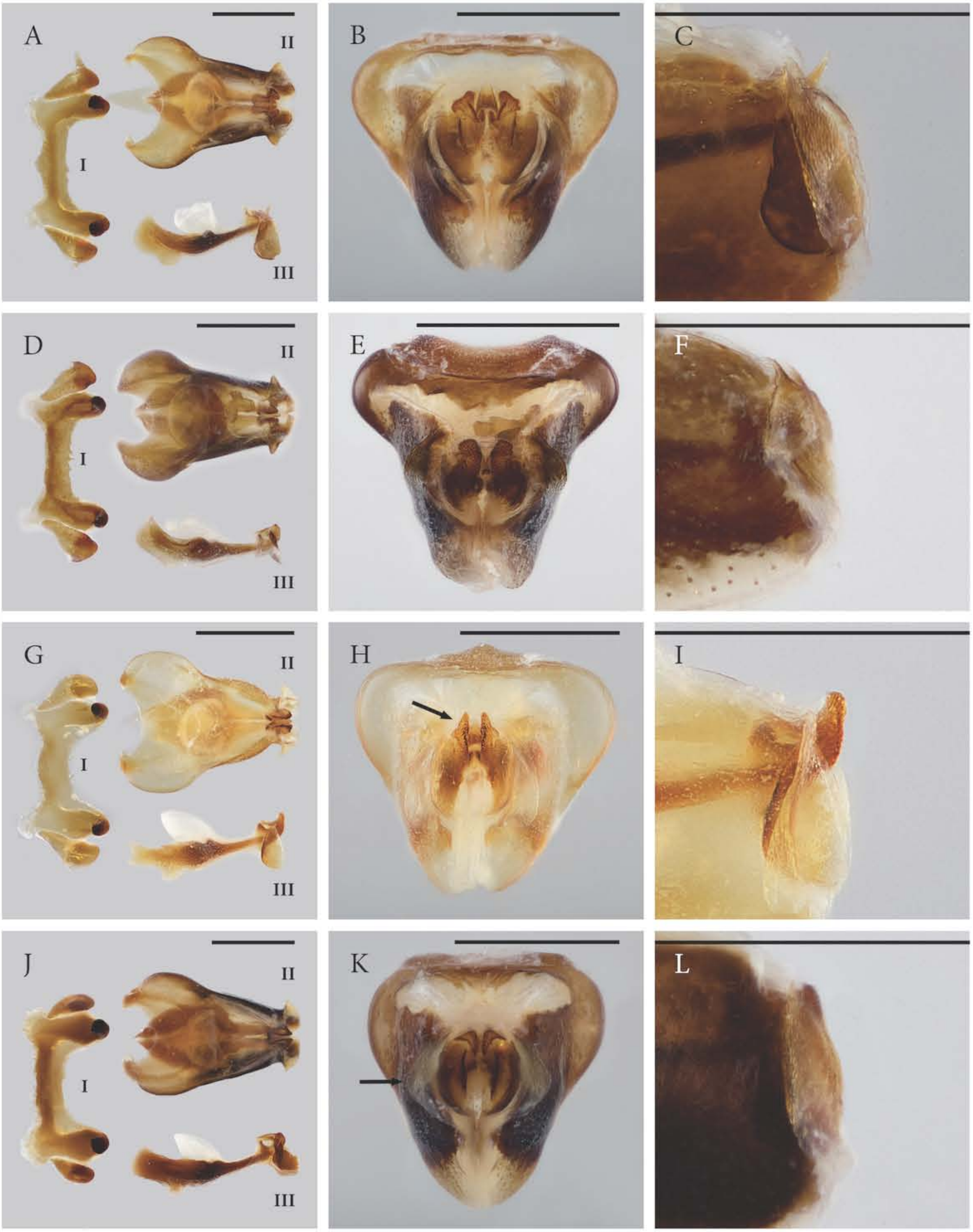

Figure 16. Male genital structures of *S. histrio*: morphotype 3 (A-C), morphotype 4 (D-F), and morphotype 5 (G-I); and *S. occidentalis* **sp.n.** holotype (J-l). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

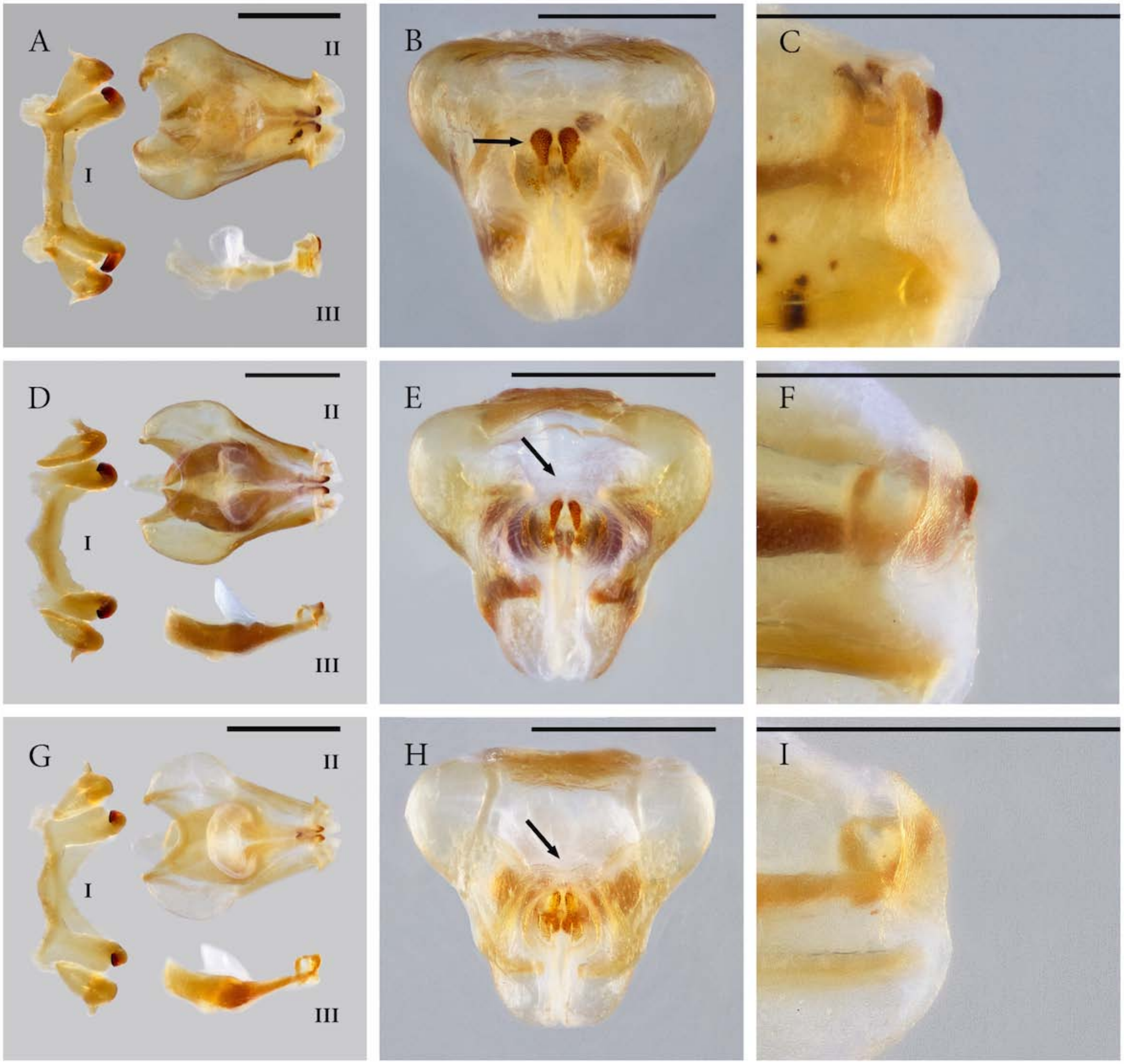

Figure 17. Male genital structures of *S. totonacum* **sp.n.** holotype (A-C), *S. adelinae* **sp.n.** holotype (D-F); and *S. miztecum* **sp.n.** holotype (G-I). For all taxa left squares show epiphallus (I) and ectophallus (II) in dorsal view, and endophallus in lateral view (III); meddle squares show ectophallus in posterior view; and right squares show a close up of ectophallus in lateral view (Scale bars = 1mm).

Tables

Table 1. List of taxonomic studies on *Sphenarium* indicating their additions (+), recognised taxa (·), junior synonyms (-), transferences (››) and tentative changes proposed (?).

1842 - 1906	1909 - 1962	1974 - 2015
Charpentier (1842)	**Bolivar (1909)**	**Boyle (1974) & Kevan (1977)**
+ *Sphenarium*	+ *Sphenarium bruneri*	+ *Sphenarium macrophallicum*
+ *Sphenarium purpurascens*	· *S. affine*	+ *Sphenarium variabile*
	· S. *barretti*	· *S. rugosum*:
Saussure (1859)	· *S. bolivari*	**-** *S. barretti*
+ *Sphenarium mexicanum*	· *S. marginatum*	· *S. borrei*
	· *S. minimum*	· *S. mexicanum histrio*:
Gerstaecker (1869)	· *S. rugosum*	**-** *S. histrio*
+ *Sphenarium pulchripes*	· *S. planum*	**-** *S. magnum*
	· *S. carinatum*	**-** *S. carinatum*
Gerstaecker (1873)	· *S. borrei*	· *S. mexicanum mexicanum*:
+ *Sphenarium histrio*	· *S. histrio*	**-** *S. mexicanum*
+ *Sphenarium ictericum*	· *S. mexicanum*	**-** *S. bolivari*
	- *S. ictericum*	**-** *S. marginatum*
Bolivar (1884)		**-** *S. crassipes*
›› *Parasphena pulchripes*:	**Kirby (1910)**	**-** *S. ictericum*
- *S. pulchripes*	›› *S. crassipes*:	· *S. purpurascens minimum*:
+ *Sphenarium borrei*	**-** *Opomala crassipes* Walker, 1870	**-** *S. minimum*
· *S. histrio*		**-** *S. affine*
· *S. mexicanum*:	· *S. mexicanum*:	· *S. purpurascens purpurascens*:
- *S. ictericum*?	**-** *S. crassipes*?	**-** *S. purpurascens*
· *S. purpurascens*		**-** *S. bruneri*
	Uvarov (1925)	**-** *S. planum*
Bolivar (1904)	· *S. mexicanum*:	
+ *Sphenarium carinatum*	**-** *S. crassipes*	**Pedraza-Lara *et al*. (2015)**
· *S. borrei*		· *S. variabile*
· *S. histrio*	**Hebard (1932)**	· *S. macrophallicum*
· *S. mexicanum*:	· *S. minimum*	· *S. minimum*
- *S. ictericum*	· *S. rugosum*	· *S. rugosum*
· *S. purpurascens*	· *S. histrio*:	· *S. borrei*
	- *S. bolivari*	· *S. histrio*
Bruner (1906)	**-** *S. ictericum*	· *S. mexicanum*
+ *Sphenarium affine*	· *S. mexicanum*:	· *S. purpurascens*
+ *Sphenarium barretti*	**-** *S. affine*	
+ *Sphenarium bolivari*	**-** *S. marginatum*?	**Sanabria-Urbán *et al*. (2015)**
+ *Sphenarium marginatum*	· *S. purpurascens*	· *S. variabile*
+ *Sphenarium minimum*		· *S. macrophallicum*
+ *Sphenarium rugosum*	**Márquez (1962)**	· *S. magnum*
+ *Sphenarium planum*	+ *Sphenarium magnum*	· *S. planum*
· *S. carinatum*	· *S. histrio*:	· *S. minimum*
· *S. borrei*	**-** *S. bolivari*	· *S. rugosum*
· *S. histrio*	**-** *S. ictericum*	· *S. borrei*
· *S. mexicanum*:	· *S. mexicanum*	· *S. histrio*
- *S. ictericum*	· *S. purpurascens*:	· *S. mexicanum*
· *S. purpurascens*	**-** *S. barretti*	· *S. purpurascens*
	- *S. rugosum*	
	- *S. borrei*	

Table 2. List of *Sphenarium* and outgroup specimens included in the genetic analysis. For each individual we provide GenBank accession numbers, haplotype and locality information. Shared haplotypes between taxa are denoted in bold.

Taxa	GenBank accession information	Haplotypes			Locality information		
		Mt	I2	H3	LN	Lat. (N)	Long. (W)
Sphenarium borrei	VN: ORSP01; C1: KU146933	5	--	--	L01	18.66667	-103.42653
Sphenarium borrei	VN: ORSP02; C1: KU146934; C2: KU147003; SR: KU147050; I2: KU147150; H3: KU147103	6	4	6	L02	20.86985	-103.84343
Sphenarium borrei	VN: ORSP131; C1: XX-XX; C2: XX-XX; SR: XX-XX; I2: XX-XX; H3: XX-XX	89	--	7	L64	18.84503	-103.12431
Sphenarium borrei	VN: ORSP68; C1: XX-XX; C2: XX-XX; SR: XX-XX; I2: XX-XX; H3: XX-XX	8	6	7	L65	19.41908	-104.00369
Sphenarium borrei	VN: ORSP03; C1: KU146935; C2: KU147004; SR: KU147051; H3: KU147104	4	--	6	L03	19.90387	-102.38818
Sphenarium borrei	VN: ORSP69; C1: XX-XX	4	--	--	L03	19.90387	-102.38818
Sphenarium borrei	VN: ORSP70; C1: XX-XX	9	--	--	L66	19.9819	-102.66916
Sphenarium borrei	VN: ORSP71; C1: XX-XX	10	--	--	L67	20.71085	-102.94811
Sphenarium histrio (1)	VN: ORSP12; C1: KU146943; C2: KU147011; SR: KU147060; H3: KU147112	13	--	8	L11	15.7691	-96.86683
Sphenarium histrio (1)	VN: ORSP13; C1: KU146944; C2: KU147012; SR: KU147061; I2: KU147158; H3: KU147113	14	**1**	**1**	L12	15.79067	-96.79722
Sphenarium histrio (1)	VN: ORSP76; C1: XX-XX; I2: XX-XX; H3: XX-XX	15	**1**	**1**	L71	15.88261	-96.39463
Sphenarium histrio (1)	VN: ORSP77; C1: XX-XX; I2: XX-XX; H3: XX-XX	16	**1**	9	L71	15.88261	-96.39463
Sphenarium histrio (3)	VN: ORSP75; C1: XX-XX; C2: XX-XX; H3: XX-XX	11	--	10	L70	16.66029	-96.09411
Sphenarium histrio (3)	VN: ORSP78; C1: XX-XX; C2: XX-XX	17	--	--	L61	16.38622	-96.65603
Sphenarium histrio (4)	VN: ORSP73; C1: XX-XX; C2: XX-XX	18	--	--	L60	16.53892	-95.93637
Sphenarium histrio (4)	VN: ORSP72; C2: XX-XX; I2: XX-XX	19	**1**	--	L68	16.48714	-95.89375
Sphenarium histrio (4)	VN: ORSP74; C1: XX-XX; C2: XX-XX	11	--	--	L69	16.55201	-96.03512
Sphenarium histrio (2)	VN: ORSP11; C1: KU146942; C2: KU147010; SR: KU147059; I2: KU147157; H3: KU147111	20	**1**	11	L13	16.30636	-92.01643
Sphenarium histrio (2)	VN: ORSP14; C1: KU146945; C2: KU147013; SR: KU147062; H3: KU147114	21	--	11	L15	16.75459	-92.9716
Sphenarium histrio (2)	VN: ORSP79; C1: XX-XX; C2: XX-XX	22	--	--	L73	17.07904	-93.57688
Sphenarium histrio (2)	VN: ORSP80; C2: XX-XX	23	--	--	L74	16.57094	-92.50021
Sphenarium histrio (5)	VN: ORSP10; C1: KU146941; C2: KU147009; SR: KU147058; I2: KU147156; H3: KU147110	12	**1**	12	L10	16.21464	-94.0425
Sphenarium histrio (5)	VN: ORSP81; C1: XX-XX; C2: XX-XX	12	--	--	L10	16.21464	-94.0425
Sphenarium histrio (5)	VN: ORSP82; C1: XX-XX; C2: XX-XX	12	--	--	L10	16.21464	-94.0425
Sphenarium macrophallicum	VN: ORSP04; C1: KU146936; C2: KU147005; SR: KU147052; I2: KU147151; H3: KU147105	24	7	**3**	L04	18.33056	-100.70654
Sphenarium macrophallicum	VN: ORSP05; C1: KU146937; C2: KU147006; SR: KU147053; I2: KU147152; H3: KU147106	25	7	13	L05	19.50757	-100.90914
Sphenarium macrophallicum	VN: ORSP06; C1: KU146938; C2: KU147007; SR: KU147054; I2: KU147153; H3: KU147107	26	7	14	L06	19.24499	-101.3195
Sphenarium mexicanum (1)	VN: ORSP20; C1: KU146951; C2: KU147019; SR: KU147068; I2: KU147164; H3: KU147120	28	**1**	**1**	L20	18.86428	-96.85898
Sphenarium mexicanum (1)	VN: ORSP83; I2: XX-XX; H3: XX-XX	--	**1**	**1**	L20	18.86428	-96.85898
Sphenarium mexicanum (1)	VN: ORSP84; C1: XX-XX; C2: XX-XX	28	--	--	L20	18.86428	-96.85898
Sphenarium mexicanum (1)	VN: ORSP22; C1: KU146953; C2: KU147021; SR: KU147070; H3: KU147122	29	--	**2**	L21	18.01328	-94.44017
Sphenarium mexicanum (1)	VN: ORSP21; C1: KU146952; C2: KU147020; SR: KU147069; I2: KU147165; H3: KU147121	30	**1**	**2**	L22	16.57204	-94.72675
Sphenarium mexicanum (1)	VN: ORSP85; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	31	8	15	L75	18.58467	-95.0762
Sphenarium mexicanum (2)	VN: ORSP23; C1: KU146954; SR: KU147071	27	--	--	L14	16.33906	-95.30373

Sphenarium mexicanum (2)	VN: ORSP24; C1: KU146955; C2: KU147022; SR: KU147072; H3: KU147123	27	--	**2**	L14	16.33906	-95.30373
Sphenarium minimum	VN: ORSP25; C1: KU146956; C2: KU147023; SR: KU147073; I2: KU147166; H3: KU147124	33	**2**	**4**	L23	19.68323	-96.70412
Sphenarium minimum	VN: ORSP26; C1: KU146957; C2: KU147024; SR: KU147074; I2: KU147167; H3: XX-XX	32	**2**	**4**	L24	19.05665	-97.03934
Sphenarium minimum	VN: ORSP86; C1: XX-XX; C2: XX-XX	32	--	--	L24	19.05665	-97.03934
Sphenarium minimum	VN: ORSP88; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	34	**2**	**4**	L76	19.59034	-96.96003
Sphenarium planum	VN: ORSP49; C1: KU146980; C2: KU147032; SR: KU147083; I2: KU147174; H3: KU147131	35	9	**4**	L45	18.49668	-97.45423
Sphenarium planum	VN: ORSP50; C1: KU146981; C2: KU147033; SR: KU147084; I2: KU147175; H3: KU147132	36	10	**4**	L46	18.82347	-97.68564
Sphenarium planum	VN: ORSP89; C1: XX-XX; C2: XX-XX	37	--	--	L77	18.40188	-97.42706
Sphenarium planum	VN: ORSP90; C1: XX-XX; C2: XX-XX	38	--	--	L78	18.21141	-97.65216
Sphenarium planum	VN: ORSP91; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	39	11	16	L78	18.21141	-97.65216
Sphenarium purpurascens (1)	VN: ORSP33; C1: KU146964; C2: KU147030; SR: KU147081; I2: KU147172; H3: KU147129	43	12	**3**	L25	18.08045	-97.06484
Sphenarium purpurascens (1)	VN: ORSP32; C1: KU146963; C2: KU147029; SR: KU147080; I2: KU147171; H3: KU147128	40	12	17	L26	21.13617	-100.06848
Sphenarium purpurascens (1)	VN: ORSP40; C1: KU146971	44	--	--	L27	17.14361	-96.81583
Sphenarium purpurascens (1)	VN: ORSP39; C1: KU146970	40	--	--	L28	17.05722	-96.76672
Sphenarium purpurascens (1)	VN: ORSP27; C1: KU146958; C2: KU147025; SR: KU147075	41	--	--	L29	18.82787	-97.25417
Sphenarium purpurascens (1)	VN: ORSP31; C1: KU146962; C2: KU147028; SR: KU147079; I2: KU147170; H3: KU147127	42	12	**4**	L30	21.04154	-101.56044
Sphenarium purpurascens (1)	VN: ORSP42; C1: KU146973	45	--	--	L31	20.56416	-100.21541
Sphenarium purpurascens (1)	VN: ORSP30; C1: KU146961; C2: KU147027; SR: KU147078; I2: KU147169; H3: KU147126	40	12	18	L32	19.98444	-100.52472
Sphenarium purpurascens (1)	VN: ORSP28; C1: KU146959; C2: KU147026; SR: KU147076; I2: KU147168; H3: KU147125	46	12	17	L33	17.72168	-97.66633
Sphenarium purpurascens (1)	VN: ORSP35; C1: KU146966	41	--	--	L34	19.025	-98.25167
Sphenarium purpurascens (1)	VN: ORSP38; C1: KU146969	40	--	--	L35	19.94694	-100.76689
Sphenarium purpurascens (1)	VN: ORSP45; C1: KU146976	41	--	--	L36	19.2375	-98.25806
Sphenarium purpurascens (1)	VN: ORSP43; C1: KU146974	41	--	--	L37	19.19202	-98.23796
Sphenarium purpurascens (1)	VN: ORSP44; C1: KU146975	47	--	--	L38	19.3247	-98.29475
Sphenarium purpurascens (1)	VN: ORSP36; C1: KU146967	41	--	--	L40	19.25	-98.33531
Sphenarium purpurascens (1)	VN: ORSP41; C1: KU146972	42	--	--	L41	19.33333	-99.13333
Sphenarium purpurascens (1)	VN: ORSP34; C1: KU146965	41	--	--	L42	19.37351	-98.4653
Sphenarium purpurascens (1)	VN: ORSP37; C1: KU146968	42	--	--	L43	20.11361	-99.70306
Sphenarium purpurascens (1)	VN: ORSP93; C1: XX-XX; C2: XX-XX	48	--	--	L79	17.51683	-96.94341
Sphenarium purpurascens (1)	VN: ORSP97; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	49	12	**5**	L81	17.32931	-97.63512
Sphenarium purpurascens (1)	VN: ORSP98; C1: XX-XX; C2: XX-XX	50	--	--	L81	17.32931	-97.63512
Sphenarium purpurascens (1)	VN: ORSP99; I2: XX-XX; H3: XX-XX	--	12	**5**	L81	17.32931	-97.63512
Sphenarium purpurascens (2)	VN: ORSP29; C1: KU146960; SR: KU147077	51	--	--	L39	16.24167	-96.52472
Sphenarium purpurascens (2)	VN: ORSP100; C1: XX-XX; C2: XX-XX	52	--	--	L94	16.93418	-96.3173
Sphenarium purpurascens (3)	VN: ORSP94; C1: XX-XX; C2: XX-XX	**1**	--	--	L80	17.32114	-96.48401
Sphenarium purpurascens (3)	VN: ORSP95; C1: XX-XX; C2: XX-XX	**2**	--	--	L80	17.32114	-96.48401
Sphenarium purpurascens (3)	VN: ORSP96; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	**1**	13	19	L80	17.32114	-96.48401
Sphenarium rugosum (1)	VN: ORSP54; SR: KU147088	54	--	--	L51	18.06543	-99.03882
Sphenarium rugosum (1)	VN: ORSP60; C1: KU146989	55	--	--	L53	18.85972	-98.84444
Sphenarium rugosum (1)	VN: ORSP56; C1: KU146985; C2: KU147038; SR: KU147090; I2: KU147180; H3: KU147137	56	15	21	L54	17.8273	-97.7889

Sphenarium rugosum (1)	VN: ORSP59; C1: KU146988	57	--	--	L55	18.95139	-99.50167
Sphenarium rugosum (1)	VN: ORSP58; C1: KU146987	58	--	--	L56	17.95266	-98.84848
Sphenarium rugosum (1)	VN: ORSP101; C1: XX-XX; C2: XX-XX	59	--	--	L82	18.61056	-98.54639
Sphenarium rugosum (1)	VN: ORSP102; C1: XX-XX; C2: XX-XX	60	--	--	L83	18.41797	-99.47938
Sphenarium rugosum (1)	VN: ORSP103; C1: XX-XX; C2: XX-XX	61	--	--	L84	17.95063	-98.13279
Sphenarium rugosum (2)	VN: ORSP57; C1: KU146986; C2: KU147039; SR: KU147091; I2: KU147181; H3: KU147138	53	14	**5**	L50	17.89879	-99.58002
Sphenarium rugosum (2)	VN: ORSP55; C1: KU146984; C2: KU147037; SR: KU147089; I2: KU147179; H3: KU147136	54	14	20	L52	17.47676	-99.45952
Sphenarium variabile (1)	VN: ORSP65; C1: KU146994; C2: KU147043; SR: KU147096; I2: KU147185; H3: KU147143	**3**	23	**4**	L60	16.53892	-95.93637
Sphenarium variabile (1)	VN: ORSP120; C1: XX-XX; C2: XX-XX	**3**	--	--	L60	16.53892	-95.93637
Sphenarium variabile (1)	VN: ORSP121; C1: XX-XX; C2: XX-XX	**3**	--	--	L60	16.53892	-95.93637
Sphenarium variabile (1)	VN: ORSP64; C1: KU146993; C2: KU147042; SR: KU147095; H3: KU147142	88	--	**4**	L62	16.76203	-96.34334
Sphenarium variabile (1)	VN: ORSP122; C1: XX-XX; C2: XX-XX	88	--	--	L62	16.76203	-96.34334
Sphenarium variabile (2)	VN: ORSP127; C1: XX-XX; C2: XX-XX	**2**	--	--	L39	16.24167	-96.52472
Sphenarium variabile (2)	VN: ORSP128; C1: XX-XX; C2: XX-XX	**2**	--	--	L39	16.24167	-96.52472
Sphenarium variabile (2)	VN: ORSP66; C1: KU146995; C2: KU147044; SR: KU147097; H3: KU147144	**2**	--	**4**	L61	16.38622	-96.65603
Sphenarium variabile (2)	VN: ORSP67; C1: KU146996; SR: KU147098; H3: KU147145	7	5	**5**	L63	16.05869	-96.49885
Sphenarium variabile (2)	VN: ORSP124; C1: XX-XX; C2: XX-XX	91	--	--	L63	16.05869	-96.49885
Sphenarium variabile (2)	VN: ORSP125; C1: XX-XX	89	--	--	L63	16.05869	-96.49885
Sphenarium variabile (2)	VN: ORSP126; C1: XX-XX; C2: XX-XX	91	--	--	L63	16.05869	-96.49885
Sphenarium variabile (2)	VN: ORSP123; C1: XX-XX; C2: XX-XX; SR: XX-XX; H3: XX-XX	90	--	**4**	L93	16.01865	-96.53031
Sphenarium rugosum (3)	VN: ORSP51; C2: KU147034; SR: KU147085; I2: KU147176; H3: KU147133	62	**3**	21	L48	18.93259	-100.21336
Sphenarium rugosum (3)	VN: ORSP52; C1: KU146982; C2: KU147035; SR: KU147086; I2: KU147177; H3: KU147134	63	**3**	21	L49	19.51256	-100.46963
Sphenarium rugosum (3)	VN: ORSP109; C1: XX-XX; C2: XX-XX	64	--	--	L87	18.40031	-100.083
Sphenarium sp. (OTU1)	VN: ORSP07; C1: KU146939; SR: KU147055; I2: KU147154; H3: KU147108	66	**1**	**2**	L07	17.99062	-102.02692
Sphenarium sp. (OTU1)	VN: ORSP08; SR: KU147056; I2: XX-XX	65	**1**	23	L08	18.88131	-101.96734
Sphenarium sp. (OTU1)	VN: ORSP09; C1: KU146940; C2: KU147008; SR: KU147057; I2: KU147155; H3: KU147109	67	**1**	22	L09	16.77369	-99.60854
Sphenarium sp. (OTU1)	VN: ORSP104; I2: XX-XX; H3: XX-XX	--	**1**	23	L09	16.77369	-99.60854
Sphenarium sp. (OTU1)	VN: ORSP105; C1: XX-XX; C2: XX-XX	68	--	--	L85	18.67008	-101.67063
Sphenarium sp. (OTU2)	VN: ORSP16; C1: KU146947; C2: KU147015; SR: KU147064; I2: KU147160; H3: KU147116	69	16	24	L16	17.35549	-99.48023
Sphenarium sp. (OTU2)	VN: ORSP15; C1: KU146946; C2: KU147014; SR: KU147063; I2: KU147159; H3: KU147115	70	16	24	L17	17.40981	-99.46677
Sphenarium sp. (OTU2)	VN: ORSP106; C1: XX-XX; C2: XX-XX	71	--	--	L86	17.20438	-99.5082
Sphenarium sp. (OTU3)	VN: ORSP17; C1: KU146948; C2: KU147016; SR: KU147065; I2: KU147161; H3: KU147117	72	17	**4**	L18	16.37195	-98.1771
Sphenarium sp. (OTU3)	VN: ORSP107; C2: XX-XX; I2: XX-XX; H3: XX-XX	73	17	25	L18	16.37195	-98.1771
Sphenarium sp. (OTU3)	VN: ORSP108; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	74	17	25	L18	16.37195	-98.1771
Sphenarium sp. (OTU4)	VN: ORSP18; C1: KU146949; C2: KU147017; SR: KU147066; I2: KU147162; H3: KU147118	75	18	26	L19	19.74138	-96.64531
Sphenarium sp. (OTU4)	VN: ORSP19; C1: KU146950; C2: KU147018; SR: KU147067; I2: KU147163; H3: KU147119	76	18	26	L19	19.74138	-96.64531
Sphenarium sp. (OTU5)	VN: ORSP46; C1: KU146977; C2: KU147031	77	--	--	L44	19.08633	-101.76425
Sphenarium sp. (OTU5)	VN: ORSP47; C1: KU146978	77	--	--	L44	19.08633	-101.76425
Sphenarium sp. (OTU5)	VN: ORSP48; C1: KU146979; SR: KU147082; I2: KU147173; H3: KU147130	77	**3**	27	L44	19.08633	-101.76425

Sphenarium sp. (OTU5)	VN: ORSP129; C1: XX-XX; I2: XX-XX; H3: XX-XX	78	**3**	28	L95	19.57722	-100.92381
Sphenarium sp. (OTU5)	VN: ORSP130; C1: XX-XX; H3: XX-XX	78	--	28	L95	19.57722	-100.92381
Sphenarium sp. (OTU6)	VN: ORSP53; C1: KU146983; C2: KU147036; SR: KU147087; I2: KU147178; H3: KU147135	79	19	29	L47	18.11495	-100.94599
Sphenarium sp. (OTU6)	VN: ORSP110; C1: XX-XX; C2: XX-XX	80	--	--	L88	18.03997	-101.04634
Sphenarium sp. (OTU6)	VN: ORSP111; C1: XX-XX; C2: XX-XX	81	--	--	L88	18.03997	-101.04634
Sphenarium sp. (OTU7)	VN: ORSP61; C1: KU146990; SR: KU147092; I2: KU147182; H3: KU147139	83	20	30	L57	19.13465	-102.46324
Sphenarium sp. (OTU7)	VN: ORSP62; C1: KU146991; C2: KU147040; SR: KU147093; I2: KU147183; H3: KU147140	84	21	30	L58	19.2443	-103.40022
Sphenarium sp. (OTU7)	VN: ORSP63; C1: KU146992; C2: KU147041; SR: KU147094; I2: KU147184; H3: KU147141	82	20	**3**	L59	19.04115	-101.80892
Sphenarium sp. (OTU7)	VN: ORSP112; C1: XX-XX; C2: XX-XX	82	--	--	L59	19.04115	-101.80892
Sphenarium sp. (OTU7)	VN: ORSP113; C1: XX-XX; C2: XX-XX	85	--	--	L89	19.55954	-102.43849
Sphenarium sp. (OTU7)	VN: ORSP114; C1: XX-XX; C2: XX-XX	86	--	--	L90	19.52046	-103.30079
Sphenarium sp. (OTU8)	VN: ORSP115; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	**3**	22	**4**	L91	15.93988	-96.42996
Sphenarium sp. (OTU8)	VN: ORSP116; C1: XX-XX; C2: XX-XX; I2: XX-XX; H3: XX-XX	**1**	**2**	31	L91	15.93988	-96.42996
Sphenarium sp. (OTU8)	VN: ORSP117; C1: XX-XX; C2: XX-XX	87	--	--	L92	15.95663	-96.45034
Sphenarium sp. (OTU8)	VN: ORSP118; C1: XX-XX; C2: XX-XX	87	--	--	L92	15.95663	-96.45034
Sphenarium sp. (OTU8)	VN: ORSP119; C1: XX-XX; C2: XX-XX	**1**	--	--	L93	16.01865	-96.53031
Prosphena scudderi	VN: ORPR01; C1: KU146927; C2: KU146998	--	--	--	--	--	--
Prosphena scudderi	VN: ORPR02; C1: KU146928; C2: KU146999	--	--	--	--	--	--
Prosphena scudderi	VN: ORPR03; C1: KU146929	--	--	--	--	--	--
Prosphena scudderi	VN: ORPR04; C1: KU146930; C2: KU147000;	--	--	--	--	--	--
Prosphena scudderi	VN: ORPR05; C1: KU146931; C2: KU147001; SR: KU147047; I2: KU147147; H3: KU147100	--	--	--	--	--	--
Prosphena scudderi	VN: ORPR06; C1: XX-XX; C2: XX-XX; SR: XX-XX; I2: XX-XX; H3: XX-XX	--	--	--	--	--	--
Prosphena scudderi	SR: Z93261.1	--	--	--	--	--	--
Jaragua oviedensis	VN: UCFC-OR1338; C1: KU146926; C2: KU146997; SR: KU147046; H3: KU147099	--	--	--	--	--	--
Mekongiana xianchengensis	C1: NC_014450.1; C2: NC_014450.1; SR: NC_014450.1;	--	--	--	--	--	--
Mekongiella kingdoni	C1: NC_023921.1; C2: NC_023921.1; SR: NC_023921.1;	--	--	--	--	--	--
Mekongiella xizangensis	VN: --; C1: NC_014451.1; C2: NC_014451.1; SR: NC_014451.1;	--	--	--	--	--	--
Yunnanites coriacea	VN: --; C1: JQ301463.1; C2: GQ421456.1;	--	--	--	--	--	--
Sphenacris crassicornis	VN: ORSC01; C1: KU146932; C2: KU147002; SR: KU147049; I2: KU147149; H3: KU147102	--	--	--	--	--	--
Sphenotettix nobilis	VN: ORST01; C2: KU147045; I2: KU147186; H3: KU147146	--	--	--	--	--	--
Pygotettix pueblensis	VN: ORPY01; SR: KU147048; I2: KU147148; H3: KU147101	--	--	--	--	--	--
Schistocerca gregaria gregaria	C1: NC_013240.1; C2: NC_013240.1; SR: NC_013240.1; I2: JQ796355.1; H3: JQ796332.1	--	--	--	--	--	--

(*n*), morphotype number; VN, voucher number; C1, cytochrome *c* oxidase subunit 1; C2, cytochrome *c* oxidase subunit 2; SR, small ribosomal RNA; H3, histone 3; I2, internal transcribed spacer between *5.8S* rRNA and *28S* rRNA; Mt, mitochondrial haplotypes considering all available mitochondrial sequences together; LN, locality number (same as in Figure 1 and Table 6); Lat., latitude; Long., longitude.

Table 3. Percentages of base differences per site (*P-distance*) from averaging over all *CO1* sequence pairs within (first column values) and between (values below the diagonal) identified taxa of *Sphenarium*. The standard error (values within parenthesis and above the diagonal in within and between taxa comparisons, respectively) was estimated using the bootstrap method (500 replicates) implemented in MEGA 6 (Tamura *et al.* 2013). *P-distance* values lower than 3% in between species comparisons are denoted in bold.

	Within taxa	**Between taxa**																
Taxa		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1 *S. borrei*	2.30 (0.003)	--	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2 *S. histrio*	2.20 (0.003)	9.08	--	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
3 *S. macrophallicum*	1.46 (0.003)	9.77	9.06	--	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00
4 *S. mexicanum*	2.20 (0.004)	9.06	3.16	9.30	--	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
5 *S. minimum*	0.56 (0.002)	9.24	8.71	3.52	8.75	--	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6 *S. planum*	1.06 (0.002)	10.13	9.22	4.68	9.28	4.18	--	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
7 *S. purpurascens*	0.83 (0.002)	9.67	8.47	**2.83**	8.60	3.26	4.49	--	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00
8 *S. rugosum*	1.55 (0.003)	9.39	8.56	**2.53**	8.72	**2.99**	4.45	**2.10**	--	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00
9 *S. variabile*	0.70 (0.002)	9.70	8.77	**2.79**	8.82	3.28	4.52	**0.97**	**2.08**	--	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00
10 *Sphenarium* sp. (OTU1)	2.08 (0.004)	9.93	4.39	10.07	4.65	9.54	10.04	9.50	9.24	9.70	--	0.01	0.01	0.01	0.01	0.01	0.01	0.01
11 *Sphenarium* sp. (OTU2)	0.95 (0.003)	10.38	8.16	8.62	8.65	7.47	8.90	8.71	8.59	9.10	9.73	--	0.01	0.01	0.01	0.01	0.01	0.01
12 *Sphenarium* sp. (OTU3)	*NA*	10.50	8.42	8.98	9.17	8.38	9.41	9.02	8.77	9.27	9.92	4.34	--	0.01	0.01	0.01	0.01	0.01
13 *Sphenarium* sp. (OTU4)	*NA*	10.79	7.75	9.84	7.68	9.28	10.13	9.46	9.47	9.68	8.28	10.48	10.70	--	0.01	0.01	0.01	0.01
14 *Sphenarium* sp. (OTU5)	0.52 (0.002)	9.91	8.98	**2.71**	8.63	3.20	4.53	**2.49**	**2.34**	**2.51**	9.65	8.53	9.05	9.87	--	0.01	0.01	0.00
15 *Sphenarium* sp. (OTU6)	0.17 (0.001)	10.04	9.22	**1.29**	9.26	3.93	4.90	3.00	**2.80**	3.03	9.99	8.59	9.02	9.41	3.04	--	0.01	0.01
16 *Sphenarium* sp. (OTU7)	1.25 (0.003)	10.35	9.06	7.90	9.21	7.43	8.39	7.75	7.77	7.92	10.02	9.17	9.11	10.55	7.50	8.03	--	0.01
17 *Sphenarium* sp. (OTU8)	0.64 (0.002)	9.72	8.51	**2.42**	8.65	**2.94**	4.27	**0.91**	**1.65**	**0.88**	9.46	8.63	9.10	9.41	**2.25**	**2.61**	7.72	--

NA, taxa in which all individuals shared the same *CO1* sequences.

Table 4. Percentages of base differences per site (*P-distance*) from averaging over all *CO1* sequence pairs between the morphotypes (numbers within parenthesis) identified within *S. histrio*, *S. mexicanum*, *S. purpurascens*, *S. rugosum* and *S. variabile*. In all comparisions the standard error values were lower than 0.01.

Taxon 1	**Taxon 2**	***P-distance***
S. histrio (1)	*S. histrio* (2)	2.99
S. histrio (1)	*S. histrio* (3)	1.48
S. histrio (1)	*S. histrio* (4)	1.29
S. histrio (1)	*S. histrio* (5)	2.99
S. histrio (2)	*S. histrio* (3)	2.64
S. histrio (2)	*S. histrio* (4)	2.56
S. histrio (2)	*S. histrio* (5)	2.51
S. histrio (3)	*S. histrio* (4)	0.58
S. histrio (3)	*S. histrio* (5)	2.79
S. histrio (4)	*S. histrio* (5)	2.86
S. mexicanum (1)	*S. mexicanum* (2)	1.93
S. purpurascens (1)	*S. purpurascens* (2)	0.95
S. purpurascens (1)	*S. purpurascens* (3)	0.96
S. purpurascens (3)	*S. purpurascens* (2)	1.01
S. rugosum (1)	*S. rugosum* (2)	1.76
S. rugosum (1)	*S. rugosum* (3)	2.03
S. rugosum (2)	*S. rugosum* (3)	2.26
S. variabile (1)	*S. variabile* (2)	0.89

Appendix

Key to *Sphenarium* species

1. Tegmina spatula-like, wider in the apex than in the base (Fig. 4F)..…………………………………….....**2**

– Tegmina strap-like, as wide in the apex as in the base (Fig. 4G)...…………..……………….……………**12**

2. Ectophallus in posterior view without sclerotized hollow in the inner-central portion of the sheath (Figs. 8, 10, 11, 13B). Endophallus in lateral view with short to very long aedeagal valves and elongated pseudoarch loosely joined to the valves of cingulum (Figs. 8, 10, 11; 13A-III)…………………………………..….... **3**

– Ectophallus in posterior with a conspicuous sclerotized hollow in the sheath (Figs. 13E, H, K; 14, 16, 17). Endophallus in lateral view always with short aedeagal valves and short pseudoarch tightly joined to the valves of cingulum (Figs. 13D, G, J; 14, 16, 17)…………..**11**

3. Aedeagal valves with an apical spine in lateral view of endophallus (Figs. 8; 10A)………..…..……………**4**

– Aedeagal valves without apical spine in lateral view of endophallus (Figs. 10D; 11, 13A).………..................**6**

4. Ectophallus in dorsal view with lateral borders of ramus strongly concave (Fig. 8A, D, G). Apical spine of aedeagus slightly longer or shorter than the base of aedeagal sclerites. Widely distributed from the southern Altiplano to the Sierra Madre del Sur in central and southern Mexico (Fig. 9A)……….........***S. purpurascens***

– Ectophallus in dorsal view with lateral borders of ramus convergent slightly rounded (Fig. 8J; 10A)……....**5**

5. Ectophallus in posterior view with inflections of supraramus reduced or not developed laterally; valves cingulum tongue-like (Fig. 8K). Apical spine of aedeagus as long or shorter than the base endophallic apodemes (Fig. 8J-III, L). Restricted to outer slope of the Sierra Madre del Sur in southern Mexico (Fig. 9C)……………………………………….....…………………………………..…..***S. zapotecum*** sp.n.

– Ectophallus in posterior view with inflections of supraramus moderately developed laterally; valves of cingulum triangular to slightly quadrangular (Fig. 10B-C). Apical spine of aedeagus slender, slightly bent, and notably longer than the base of aedeagal sclerites (Fig. 10A-III). Restricted to the northern-central portion of the Balsas River Basin in western Mexico (Fig. 9A, B)……………………….. ***S. tarascum*** sp.n.

6. Ectophallus in posterior view with inflections of supraramus reduced (Fig. 10E, H)……………………....**7**

– Ectophallus in posterior view with inflections of supraramus moderately developed laterally (Figs. 10K; 11; 13B)…………………………..……………………………………………………..………..................**8**

7. Ectophallus in dorsal view with lateral borders of ramus convergent slightly rounded; interspace between apodemal plates of cingulum widely open (Fig. 10D-II). In posterior view of ectophallus valves of cingulum with unique form notably developed posteriorly, placed dorsally (Fig. 10E). In lateral view of endophallus aedeagal valves and sclerites together about 2 ½ fold the length of endophallic apodemes (Fig. 10D-III). Restricted to the Tehuacan Valley in southern Mexico (Fig. 9A)……………………………….…. ***S. planum***

– Ectophallus in dorsal view with lateral borders of ramus nearly parallel; interspace between apodemal plates of cingulum widely open (Fig. 10G-II). In posterior view of ectophallus valves of cingulum with unique form, centered in the middle of the sheath (Fig. 10H, I). In lateral view of endophallus aedeagal valves and sclerites together longer than in any other *Sphenarium* species, ranging from 2¾ to 3 fold the length of endophallic apodemes (Fig. 10G-III). Restricted to the northern-middle portion of the Balsas River Basin, in central-western Mexico (Fig. 9B)…………………………………………………..…….***S. macrophallicum***

8. Ectophallus with lateral borders of ramus of cingulum strongly concave in dorsal view (Fig. 10J-II). Ectophallus in posterior view with valves of cingulum triangular notably small (Fig. 10K). In lateral view of endophallus aedeagal valves and sclerites together about 1 ¼ fold the length of endophallic apodemes (Fig. 10J-III). Restricted to the outer slope of southern portion of the Sierra Madre Oriental in central-eastern Mexico (Fig. 9A)...……….……………………………………………………….………... ***S. minimum***

– Ectophallus with lateral borders of ramus of cingulum convergent in dorsal view (Fig. 11)……………….. **9**

9. Dorsal borders of ramus with lateral projections notably developed interiorly, closing the central membrane of ectophallus somewhat far from the sheath in dorsal view ectophallus (Fig. 11A-II). In posterior view of ectophallus valves of cingulum with distinct form (Fig. 11B). In lateral view of endophallus aedeagal valves somewhat broad, medium-sized; aedeagal valves and sclerites together about as long as the length of endophallic apodemes (Fig. 11A-III). Restricted to the western portion of the Balsas River Basin in central-western Mexico (Fig. 9B)……………………………………………..……………… ***S. infernalis*** sp.n.

– Dorsal borders of ramus without lateral projections in dorsal view of ectophallus (Figs. 11D, G, J, 13A)...**10**

10. Aedeagal valves broadly rounded apically (Fig. 11E, I, K). In lateral view of endophallus aedeagal valves moderately or notably broad, short or medium sized; aedeagal valves and sclerites together ranging approximately from ¾ to 1½ the length of dorsal inflections of endophallic apodemes (Fig. 11 D-III, G-III, J-III). Restricted to the eastern Balsas River Basin and the Sierra Madre del Sur in central and southern Mexico (Fig. 9B) ……………………………………………………………….…………………... ***S. rugosum***

– Aedeagal valves moderately rounded in the apex. In lateral view of endophallus aedeagal valves slender, relatively long; aedeagal valves and sclerites together ranging approximately from 1¾ to 2 fold the length of dorsal inflections of endophallic apodemes (Fig. 13A-III). Restricted to the western portion of the inner slope of the Sierra Madre del Sur in central-western Mexico (Fig. 9A, B)………….......... ***S. crypticum*** sp.n.

11. In posterior view of ectophallus the sclerotized hollow in the sheath notably closed, inflections of supraramus notably developed anterolaterally, valves of cingulum triangular, stout and notably developed interiorly (Fig. 13E). Distributed in the northern-western portion of the Mexican Volcanic Belt in central-western Mexico (Fig. 9A)………………………………….. ***S. borrei***

– In posterior view of ectophallus the sclerotized hollow in the sheath closed or moderately opened, inflections of supraramus moderately developed laterally or anteriorly, valves of cingulum small with unique finger-like forms (Fig. 13H, K). Restricted to the Sierra Madre del Sur in southern Mexico (Fig. 9C)..……………………………………………………………………………………… ***S. variabile***

12. Ectophallus in dorsal view relatively large (Fig. 14A, D). In posterior view of ectophallus the sclerotized hollow of the sheath notably open; inflections of supraramus reduced; valves of cingulum claw-like rather stout (Fig. 14B, E), notably or moderately developed posteriorly, very conspicuous in lateral view of ectophallus (Fig. 14C, F). Restricted to southern Golf of Mexico Cost and the Isthmus of Tehuantepec in southern Mexico (Fig. 9A)……………………………………………………………………... ***S. mexicanum***

– Ectophallus relatively small. In posterior view of ectophallus the sclerotized hollow of the sheath closed or moderately open; inflections of supraramus moderately to notably developed laterally; valves of cingulum very slightly developed posteriorly or developed dorsoposteriorly (Figs. 14G, J; 16; 17A, D, G)………... **13**

13. In posterior view of ectophallus inflections of supraramus without forming a dorsal fold (Figs. 14H, K; 16B, E, H, K; 17B)…………………………….…………………………………………………..……… **14**

– In posterior view of ectophallus inflections of supraramus forming a dorsal fold (Fig. 17E, H)………….. **17**

14. In posterior view of ectophallus inflections of supraramus moderately developed laterally (Figs. 14H, K; 16B, E, H, K)…………………………………………….. **15**

– In posterior view of ectophallus inflections of supraramus notably developed laterally (Fig. 17B)…..…... **16**

15. Ectophallus mostly with distal borders of inflections of supraramus ventrally directed and a moderately open sclerotized hollow in the sheath (Figs. 14H, K; 16H). In some cases (morphotypes 3 & 4, see description of species) distal borders of inflections of supraramus laterally directed but with a closed sclerotized hollow in the sheath (Fig. 16B, E). Valves of cingulum triangular but with distinct variations. Distributed in the Pacific Cost and Sierra Madre del Sur and mountain ranges in southern Mexico and north-western Guatemala (Fig. 9A)………………………………………………………………………………………………***S. histrio***

– Ectophallus with distal borders inflections of supraramus laterally directed and a moderately open sclerotized hollow in the sheath, valves of cingulum arrow-like (Fig. 16K). Restricted to the Pacific Cost and western portion of the Balsas River Basin in central-western Mexico (Fig. 9A)…... ***S. occidentalis*** sp.n.

16. In posterior view of ectophallus sclerotized hollow of the sheath closed; valves of cingulum drop-like, stouter than in other species with similar form (Fig. 17B). Restricted to outer slope of the central portion of the Sierra Madre Oriental in central-eastern Mexico (Fig. 9A)……………………….…... ***S. totonacum*** sp.n.

17. In posterior view of ectophallus dorsal borders of inflections of supraramus very close but not fused (Fig. 17E). Restricted to the outer slope of the Sierra Madre del Sur in southern Mexico (Fig. 9A)…………………………………………………………….…………………..… ***S. adelinae*** sp.n.

– In posterior view of ectophallus dorsal borders of inflections of supraramus fused (Fig. 17H). Restricted to the Pacific Cost in southern Mexico (Fig. 9A)…………………………………………….. ***S. miztecum*** sp.n

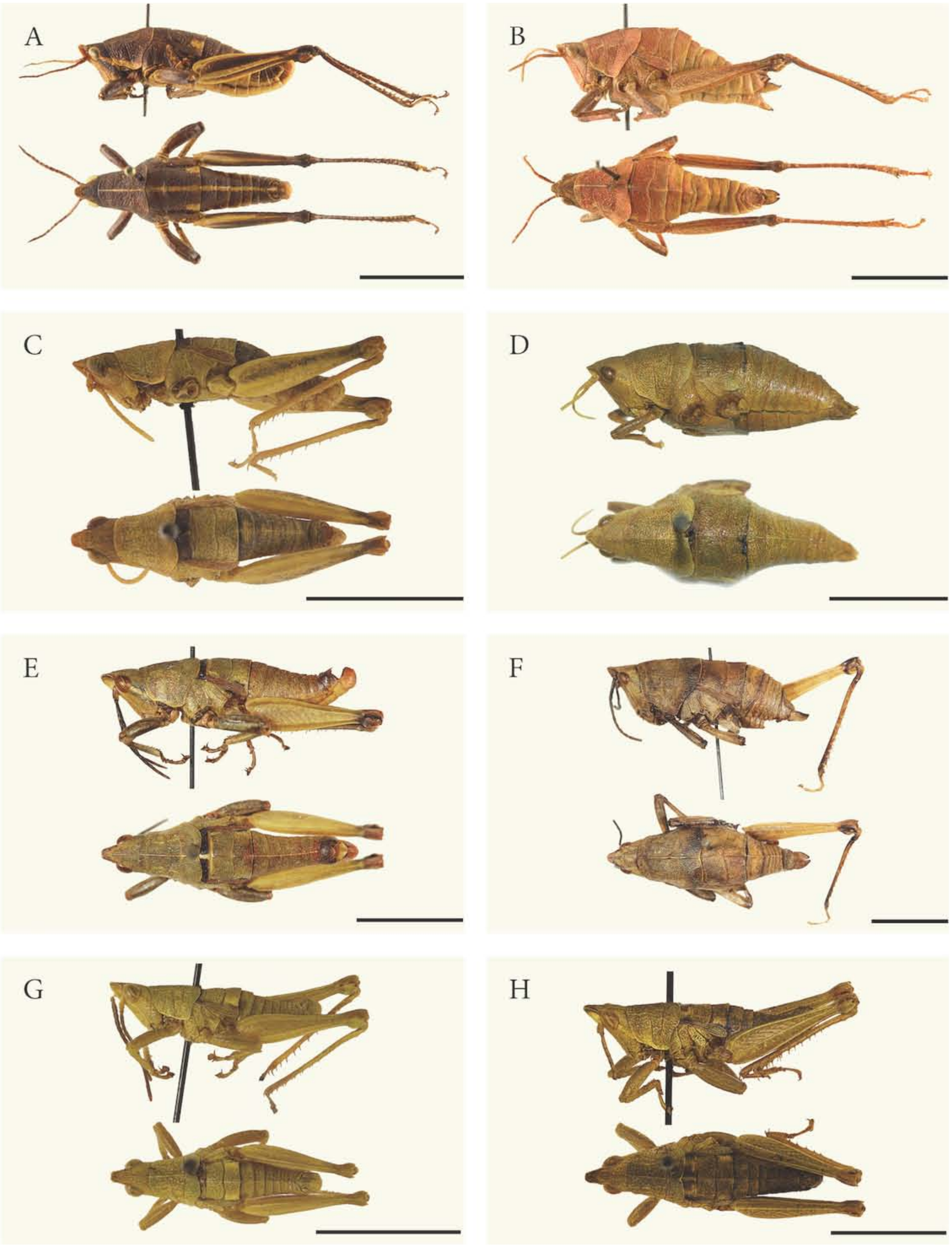

Figure 18. Type specimens of *S. pururascens*: lectotype *m* (A) and paralectotype *f* (B); *S. planum*: lectotype *m* (C) and paralectotype *f* (D); *S. macrophallicum*: holotype *m* (E) and allotype *f* (F); *S. minimum* lectotype *m* (G); *S. affine* lecototype *m* (H) (Scale bars = 1cm).

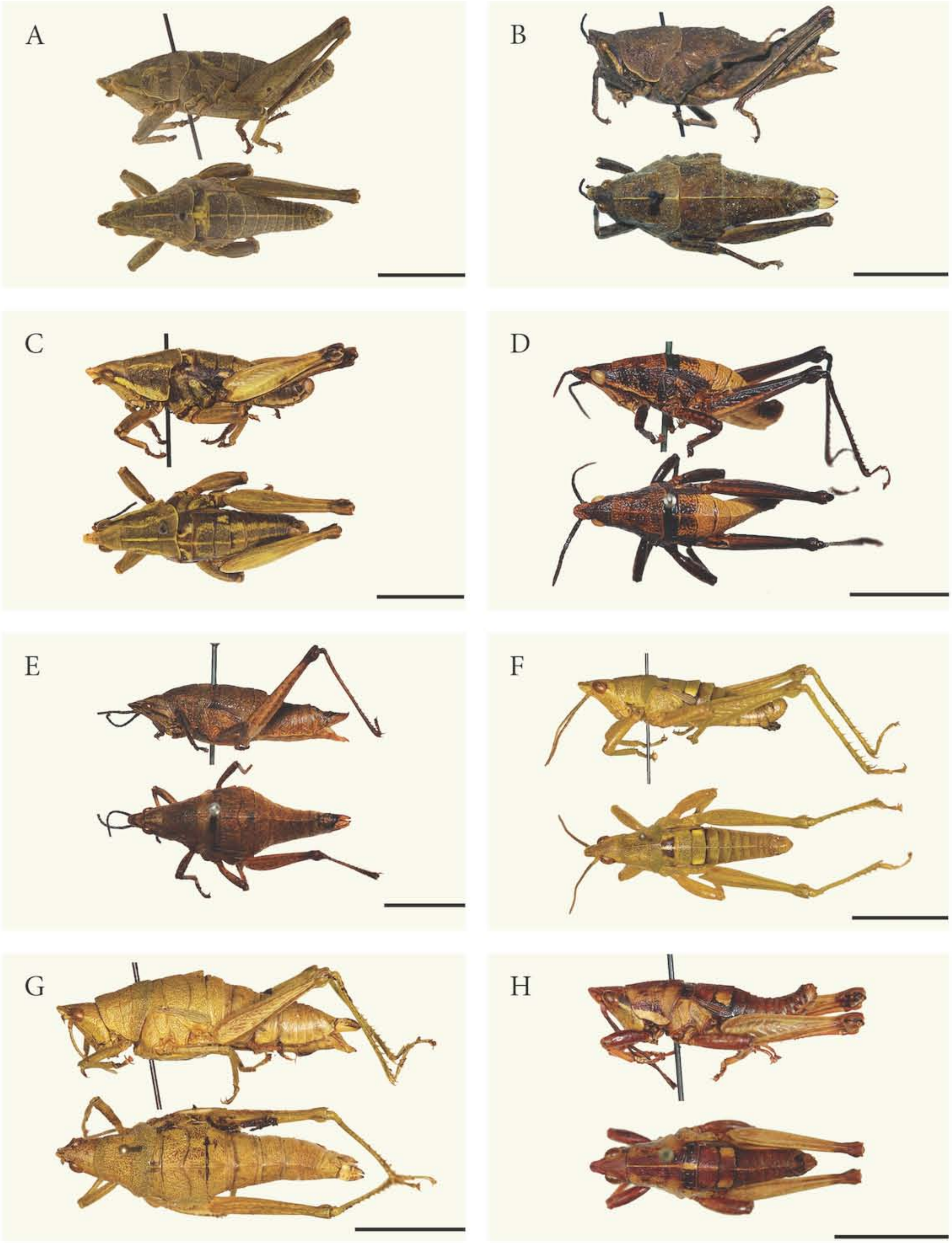

Figure 19. Type specimens of *S. rugosum*: lectotype *m* (A) and paralectotype *f* (B); *S. barrettii*: lectotype *m* (C); *S. borrei*: lectotype *m* (D) and paralectotype *f* (E); *S. bruneri* lectotype *m* (F) and paralectotype *f* (G); *S. variabile*: holotype *m* (H) (Scale bars = 1cm).

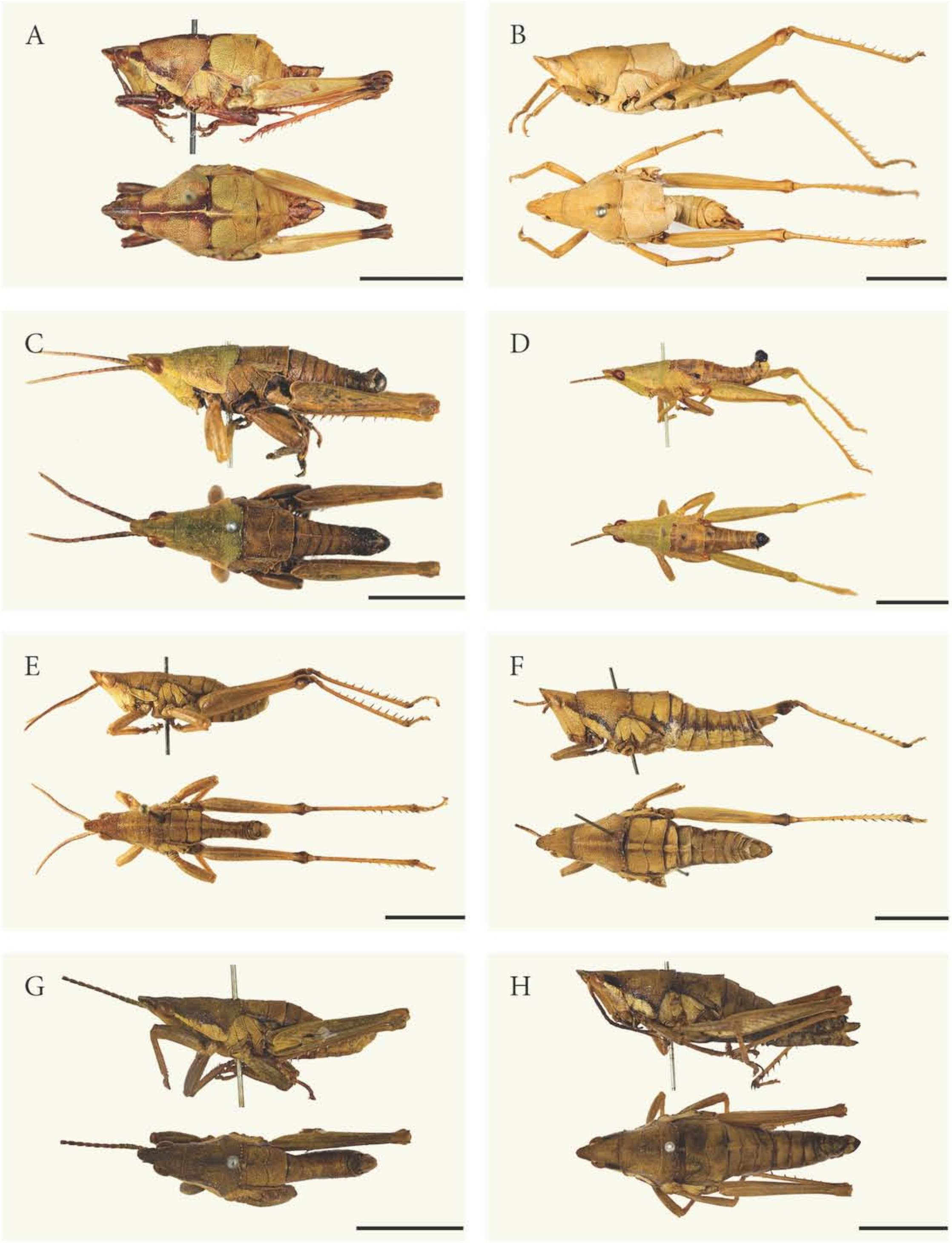

Figure 20. Type specimens of *S. variabile*: allotype *f* (A); *S. mexicanum* lectotype *f* (B) and paralectotype *m* (C); *O. crassipes* holotype *m* (D); *S. ictericum*: lectotype *m* (E) and paralectotype *f* (F); *S. marginatum* lectotype *m* (G) and paralectotype *f* (H) (Scale bars = 1cm).

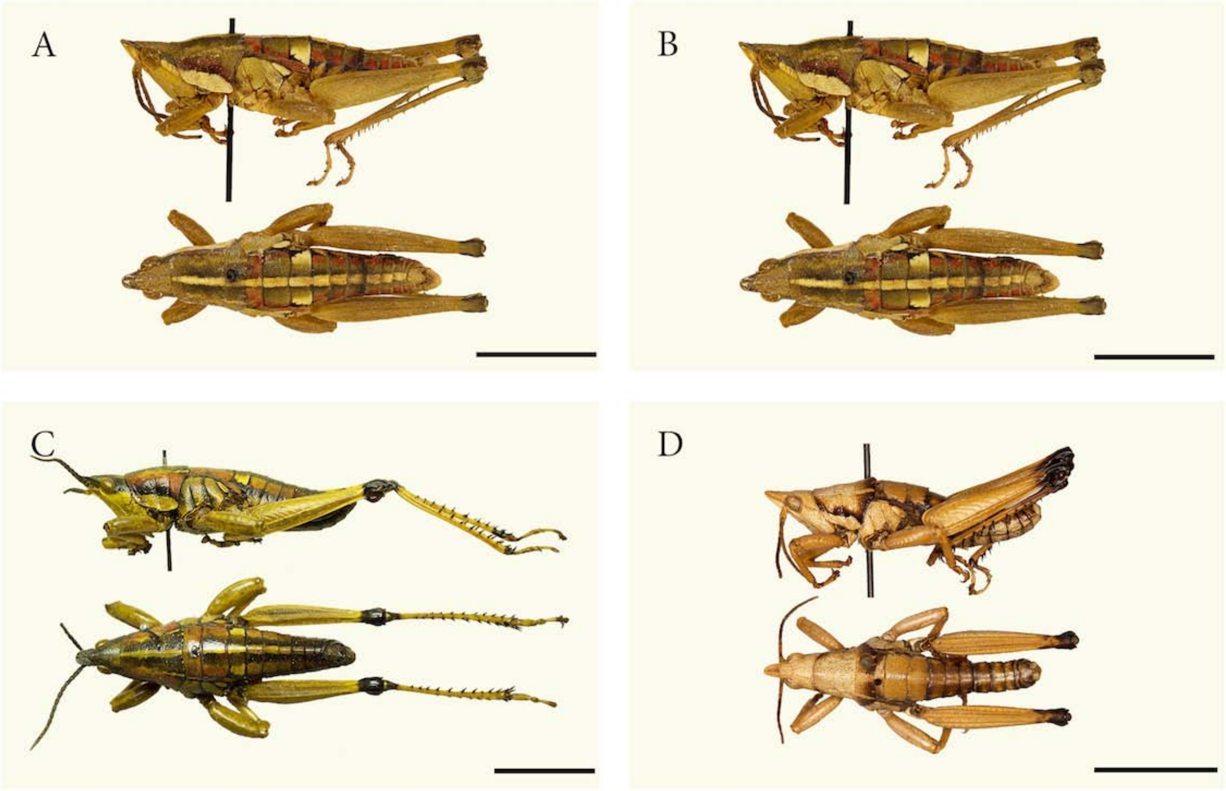

Figure 21. Type specimens of *S. bolivari*: lectotype *m* (A) and paralectotype *m* (B); *S. histrio* holotype *m* (C) and *S. carinatum* holotype *m* (D) (Scale bars = 1cm).

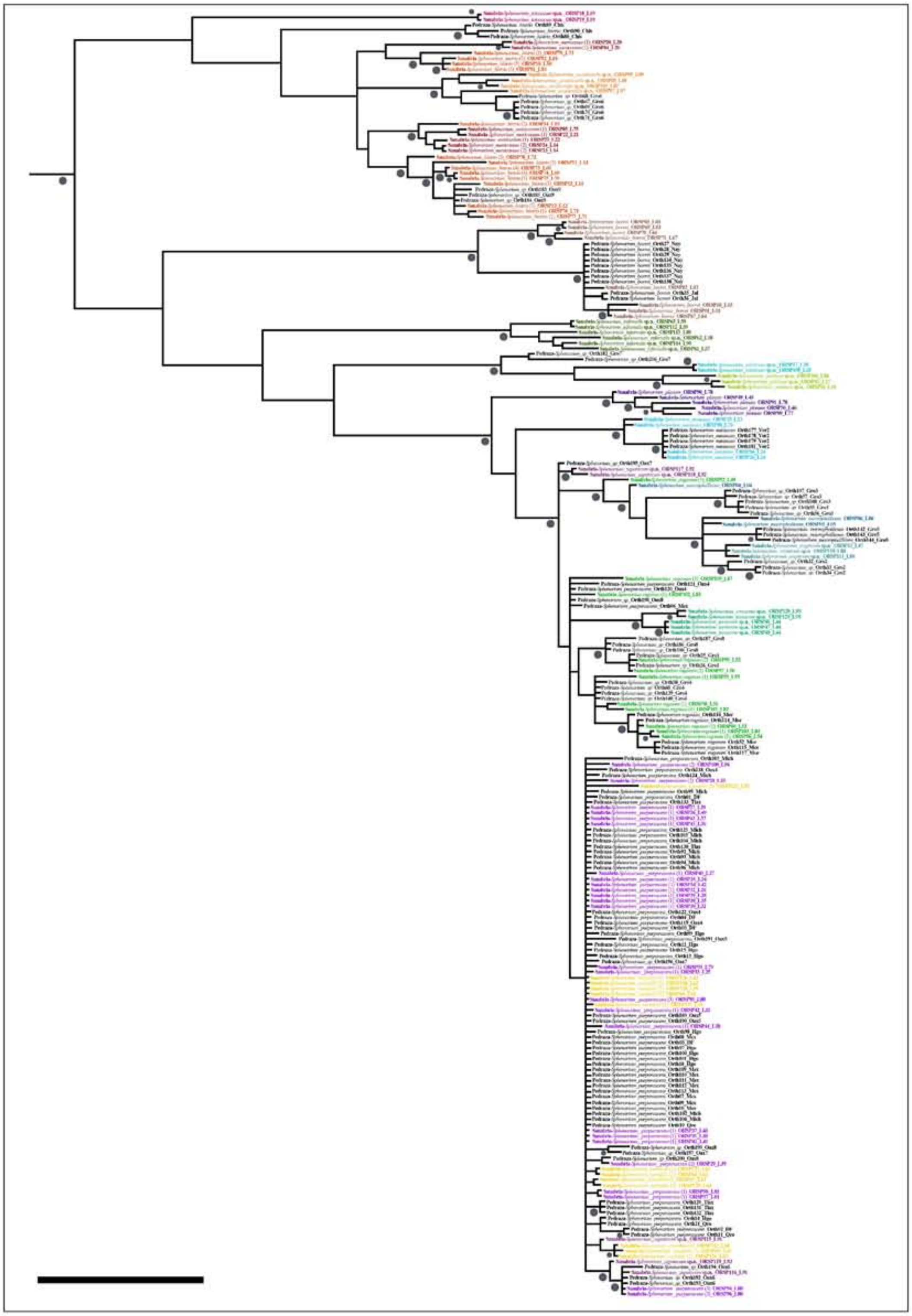

Figure 22. Bayesian phylogeny of *Sphenarium* species based on *CO1* sequences from Pedraza-Lara *et al*. (2015) and this study. The analysis was conducted using the substitution model estimated previously and under the same conditions above specified for the concatenated phylogenetic analysis in methods section. Terminal of different colours represent the 17 recognized species in this study. Black dots behind the nodes indicate *PP* values higher than 95%.

Table 5. List of localities revised of each *Sphenarium* species and morphotypes (numbers within parenthesis) identified. Localities included our genetic analysis are denoted in bold. For each locality we indicate its locality number (LN), description, number specimens (F, females & M, males) examined, depository of the examined material (D) and the type of record (T).

Species	LN	Locality description	F	M	D	T
S. borrei	L109	Mexico, Colima, 11.3mi SW Colima, on Hwy 110; XI-28-1958 (T.J. Conh)	9	7	UMMZ	A
S. borrei	L118	Mexico, Colima, 13mi S Colima ; XI-28-1958 (T.J. Cohn)	3	2	UMMZ	A
S. borrei	L131	Mexico, Colima, Volcano ; 1961 (T.A. Peters)		1	UMMZ	B
S. borrei	L142	Mexico, Colima, Volcano (Conrad)		1	ANSP	B
S. borrei	**L65**	**Mexico, Colima, Manantlan Carr 8 ; X-2-2013 (S. Sanabria & F. Rivera)**	2		IIES	A
S. borrei	L151	Mexico, Guanajuato , 10mi S Valle de Santiago #55; IX-7-1982 (Otte)	1	1	ANSP	A
S. borrei	L106	Mexico, Jalisco, 0.4mi S Huentintan del Bajo, 5mi N Guadalajara ; IX-30-1959 (Cantral & Cohn)	2	3	UMMZ	A
S. borrei	L107	Mexico, Jalisco, 1.4rd mi SE Amatitlan ; X-2-1959 (T.J. Conh)		2	UMMZ	A
S. borrei	L108	Mexico, Jalisco, 10mi SW Mazamitla, on Hwy 110; IX-28-1959 (Cantral & Cohn)	3	7	UMMZ	A
S. borrei	L110	Mexico, Jalisco, 11mi NE (NW) Tacalitlan ; XI-30-1958 (T.J. Conh)		1	UMMZ	A
S. borrei	L111	Mexico, Jalisco, 1mi S. Pihuamo ; XI-26-1958 (T.J. Conh)	3	7	UMMZ	A
S. borrei	L113	Mexico, Jalisco, 22mi SW Guadalajara, arch, km 643 on Hwy 15; XI-30-1958 (T.J. Conh)	6	1	UMMZ	A
S. borrei	L120	Mexico, Jalisco, 15mi W Zapotlanejo ; XI-23-1958 (T.J. Conh)		2	UMMZ	A
S. borrei	L121	Mexico, Jalisco, 1mi NW Mazamitla; II-6-1953 (I.J Cantral)	4	5	UMMZ	A
S. borrei	L127	Mexico, Jalisco, 9mi S Mazamitla ; XI-30-1958 (T.J. Conh)	2	5	UMMZ	A
S. borrei	L129	Mexico, Jalisco, Tamuzula de Gordiano ; II-10-1954 (R.R. Dreisbach)		1	UMMZ	A
S. borrei	L132	Mexico, Jalisco, 15.4mi S Mazamitla, on Hwy 110; XI-15-1974 (T.J. & J. W. Cohn)	1	2	UMMZ	A
S. borrei	L137	Mexico, Jalisco, 4.5mi S Mazamitla plaza, on Hwy 110; XI-16-1974 (T.J. & J. W. Cohn)	3	5	UMMZ	A
S. borrei	L139	Mexico, Jalisco, NE Guadalajara, barranca de los oblatos; XI-12-1974 (T.J. & J. W. Cohn)	5	4	UMMZ	A
S. borrei	L140	Mexico, Jalisco, 26mi W Guadalajara ; IX-11-1938 (Roberts)		1	ANSP	A
S. borrei	L143	Mexico, Jalisco, Pine forest 7mi S Mazamitla; XII--1948 (Leech)	1		ANSP	A
S. borrei	L145	Mexico, Jalisco, Between Guadalajara & Chapala ; IX-13-1938 (Roberts)	1		ANSP	A
S. borrei	L146	Mexico, Jalisco, 12mi NW Tequila ; XI-22-1948 (Leech)		1	ANSP	A
S. borrei	L147	Mexico, Jalisco, Tequila; XI-12-1938 (Roberts)		1	ANSP	A
S. borrei	L148	Mexico, Jalisco, 12-15mi W Jiquilpan ; XII-30-1948 (Leech)		1	ANSP	A
S. borrei	L150	Mexico, Jalisco, Guadalajara; VII--1908 (McClendon)		1	ANSP	A
S. borrei	L152	Mexico, Jalisco, 11mi S Zacatecas-Jalisco border on Rt 54 #70; IX-12-1982 (Otte)		1	ANSP	A
S. borrei	L154	Mexico, Jalisco, N side Rio Verde (N Guadalajara) on Rd to Zacatecas #69; IX-12-1981 (Otte, Azuma, Newlin)	1		ANSP	A
S. borrei	L157	Mexico, Jalisco, Entrada a Tequila; X-29-2010 (S. Sanabria)	2	2	IIES	A
S. borrei	L158	Mexico, Jalisco, Desviación a Tepic Carr 15 km1; X-28-2010 (S. Sanabria)	1		IIES	A
S. borrei	L161	Mexico, Jalisco, Entrada a Mazamitla Carr 110 ; X-1-2013 (S. Sanabria & F. Rivera)	7	3	IIES	A
S. borrei	**L02**	**Mexico, Jalisco, Volcan Tequila ; X-5-2013 (S. Sanabria & F. Rivera)**	16	14	IIES	A
S. borrei	L162	Mexico, Jalisco, La Venta de Aztillero Carr 15 ; X-5-2013 (S. Sanabria & F. Rivera)	11	11	IIES	A
S. borrei	**L67**	**Mexico, Jalisco, Rumbo a Acatic Carr 80 ; X-5-2013 (S. Sanabria & F. Rivera)**	21	14	IIES	A
S. borrei	L112	Mexico, Michoacan, 2.1mi E Carapan, on Hwy 15; XI-24-1959 (Cantral & Cohn)	9	9	UMMZ	A

S. borrei	L114	Mexico, Michoacan, 3mi S Carapan; XII-1-1958 (T.J. Cohn)	3	2	UMMZ	A
S. borrei	L119	Mexico, Michoacan, 15mi W Jacona ; XII-1-1958 (T.J. Conh)		2	UMMZ	A
S. borrei	L123	Mexico, Michoacan, 3.5mi W Jiquilpan, on Hwy 110; IX-28-1959 (Cantral & Cohn)	1		UMMZ	A
S. borrei	L124	Mexico, Michoacan, 3mi S Carapan, on Hwy 39; XI-24-1959 (Cantral & Cohn)	3	5	UMMZ	A
S. borrei	L125	Mexico, Michoacan, 4.2mi E Morelia, on Hwy 15; XI-24-1959 (Cantral & Cohn)	1	1	UMMZ	A
S. borrei	L126	Mexico, Michoacan, 4rd mi W Jiquilpan; XI-25-1959 (Cantral & Cohn)	3	1	UMMZ	A
S. borrei	L130	Mexico, Michoacan, Tangancicuaro; II-8-1954 (R.R. Dreisbach)	2	1	UMMZ	A
S. borrei	L133	Mexico, Michoacan, 15.9mi W. Jacona Church, on Hwy 15; XII-15-1972 (J. C. Lee)	5	7	UMMZ	A
S. borrei	L134	Mexico, Michoacan, 16.4rd mi E Morelia ; XI-8-1977 (T.J. & J. W. Cohn)	1	5	UMMZ	A
S. borrei	L135	Mexico, Michoacan, 22.2mi SE Brisenas Plaza, 4.3mi El Salitre; XI-9-1974 (T.J. & J. W. Cohn)		3	UMMZ	A
S. borrei	L136	Mexico, Michoacan, 3.2mi S Carapan on Hwy 37; XI-2-1974 (T.J. & J. W. Cohn)	2	2	UMMZ	A
S. borrei	L144	Mexico, Michoacan, Cojumatlan, Lake chapala ; IX-8-1938 (Roberts)		1	ANSP	A
S. borrei	L149	Mexico, Michoacan, Morelia ; IX-4-1938 (Roberts)		1	ANSP	A
S. borrei	**L64**	**Mexico, Michoacan, Maruata cerca de Coacolman Carr 120 ; X-31-2010 (S. Sanabria)**	1	1	IIES	A
S. borrei	L159	Mexico, Michoacan, Rumbo a Coacolman 2 Carr 120 ; X-31-2010 (S. Sanabria)	1	2	IIES	A
S. borrei	**L01**	**Mexico, Michoacan, Rumbo a Coacolman 1 Carr 120 ; X-31-2010 (S. Sanabria)**	2	4	IIES	A
S. borrei	L160	Mexico, Michoacan, Cerca de Carapan Carr 15; XII-5-2013 (S. Sanabria, P. Fontana & R. Mariño)	10	10	IIES	A
S. borrei	**L03**	**Mexico, Michoacan, El Nopalito rumbo a Jacona Carr 16; IX-30-2013 (S. Sanabria & F. Rivera)**	11	10	IIES	A
S. borrei	**L66**	**Mexico, Michoacan, Rumbo a Jiquilpan Carr 15 ; X-1-2013 (S. Sanabria & F. Rivera)**	11	17	IIES	A
S. borrei	L163	Mexico, Michoacan, 19.4mi W. Jacona; X-31-1977 (T.J. & J. W. Cohn)		1	UMMZ	A
S. borrei	L115	Mexico, Nayarit, 7mi W Ixtlan del Rio ; XI-22-1958 (T.J. Conh)	3	2	UMMZ	A
S. borrei	L116	Mexico, Nayarit, 9mi N Compostela ; XI-4-1958 (T.J. Conh)		1	UMMZ	A
S. borrei	L117	Mexico, Nayarit, Cerro de San Juan, 4.5rd mi SW Jalisco (Xalisco); XI-3-1958 (T.J. Conh)	2	9	UMMZ	A
S. borrei	L122	Mexico, Nayarit, 2.5mi N Jalisco (Xalisco), 18mi N compostela ; XI-4-1958 (T.J. Conh)		1	UMMZ	A
S. borrei	L128	Mexico, Nayarit, Cerro de San Juan, 3.5rd mi SW Jalisco (Xalisco); XI-3-1958 (T.J. Conh)		1	UMMZ	A
S. borrei	L138	Mexico, Nayarit, 7.3rd mi W Tepec ; X-4-1959 (T.J. Cohn)		2	UMMZ	A
S. borrei	L141	Mexico, Nayarit, Tepic; XI--1894 (Bruner)		1	ANSP	A
S. borrei	L155	Mexico, Nayarit, Camino a Comostela Carr 68D km11; X-30-2010 (S. Sanabria)	1	3	IIES	A
S. borrei	L156	Mexico, Nayarit, Desviacion a Jala sobre Carr 15; X-30-2010 (S. Sanabria)	1	1	IIES	A
S. borrei	L153	Mexico, Zacatecas, 4mi N Zacatecas-Jalisco border on Rt. 54 #71; IX-12-1982 (Otte)		1	ANSP	A
S. histrio (1)	L168	Mexico, Guerrero, 5mi E Cuajinicuilapa #50; IX-3-1981 (Otte, Azuma, Newlin)	1		ANSP	A
S. histrio (1)	L179	Mexico, Guerrero, Salida Ometepec; X-25-2011 (S. Sanabria & A. Díaz)		1	IIES	A
S. histrio (1)	L164	Mexico, Oaxaca, 28mi WNW Tehuantepec, on Hwy 190; IX-13-1959 (Cantral & Cohn)		1	UMMZ	A
S. histrio (1)	L165	Mexico, Oaxaca, Tehuantepec; XI-12-1948 (H.O. Wagner)		1	UMMZ	A
S. histrio (1)	L166	Mexico, Oaxaca, 1mi W Puerto Angel ; VII-21-1958 (H.O. Wagner)		2	UMMZ	A
S. histrio (1)	L167	Mexico, Oaxaca, 20mi N Pto. Escondido Rt. 131 #46; IX-2-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. histrio (1)	L169	Mexico, Oaxaca, 24-25mi N Pto Escondido Rd to Oaxaca #45; IX-2-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. histrio (1)	L170	Mexico, Oaxaca, Lagunillas N (W) Pinotepa Nacional #48; IX-3-1981 (Otte, Azuma, Newlin)	1		ANSP	A

S. histrio (1)	L171	Mexico, Oaxaca, 2mi E Jalapa del Marquez #35; X-31-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. histrio (1)	**L11**	**Mexico, Oaxaca, Cerca del Rosedal Carr 200 km 165; X-26-2011 (S. Sanabria & A. Díaz)**	7	7	IIES	A
S. histrio (1)	L172	Mexico, Oaxaca, Puerto Angel ; X-26-2011 (S. Sanabria & A. Díaz)	2	1	IIES	A
S. histrio (1)	L173	Mexico, Oaxaca, Rumbo a Pochutla Carr 175; X-26-2011 (S. Sanabria & A. Díaz)	8	7	IIES	A
S. histrio (1)	L174	Mexico, Oaxaca, La Crucecita Huatulco; X-26-2011 (S. Sanabria & A. Díaz)		1	IIES	A
S. histrio (1)	L175	Mexico, Oaxaca, San Pedro Huamelula; IX-30-2012 (S. Sanabria & F. Rivera)	3	3	IIES	A
S. histrio (1)	L176	Mexico, Oaxaca, Cozoaltepec 1; IX-30-2012 (S. Sanabria & F. Rivera)	9	13	IIES	A
S. histrio (1)	**L12**	**Mexico, Oaxaca, Cozoaltepec 2; IX-31-2012 (S. Sanabria & F. Rivera)**	10	11	IIES	A
S. histrio (1)	**L71**	**Mexico, Oaxaca, Rumbo a Pluma Hidalgo ; XII-11-2013 (S. Sanabria, P. Fontana & R. Mariño)**	6	5	IIES	A
S. histrio (1)	**L92**	**Mexico, Oaxaca, Pluma Hidalgo 2 ; XII-11-2013 (S. Sanabria, P. Fontana & R. Mariño)**	3	1	IIES	A
S. histrio (1)	L178	Mexico, Oaxaca, Tequisistlan; X-27-2011 (S. Sanabria & A. Díaz)	1	1	IIES	A
S. histrio (2)	L210	Guatemala (Hebard)		1	ANSP	B
S. histrio (2)	L204	Guatemala , Huehuetenango, 7.8rd mi SE Cd. Cuahutemoc; IX-14-1961 (T.J. Cohn & P. Hubbell)	2	8	UMMZ	A
S. histrio (2)	L205	Guatemala , Huehuetenango, 13.6mi NW Los Naranjoles on Panamericana Hwy, 27.3mi NW jct. N spur rd to Huehuetenango; IX-14-1961 (T.J. Cohn & P. Hubbell)		14	UMMZ	A
S. histrio (2)	L181	Mexico, Chiapas, Parque Cerro Hueco, 4.1mi S Tuxtla Gutierrez; X-17-20-1961 (T.J. Cohn & P. Hubbell)	3	2	UMMZ	A
S. histrio (2)	L182	Mexico, Chiapas, 4.2mi N Cd. Cuauhutemoc ; X-11-1961 (Hubbell, Cantral, Cohn)	1	2	UMMZ	A
S. histrio (2)	L183	Mexico, Chiapas, 9mi SE Comitan on Panamericana Hwy; X-19-1961 (Hubbell, Cantral, Cohn)	4	1	UMMZ	A
S. histrio (2)	L184	Mexico, Chiapas, Cintalapa; X-16-1948 (H.O. Wagner)	4	4	UMMZ	A
S. histrio (2)	L185	Mexico, Chiapas, Las Margaritas, 25km W Comitan ; XII-8-1948 (H.O. Wagner)		4	UMMZ	A
S. histrio (2)	L186	Mexico, Chiapas, 8mi W Teapa on Pichucalco Rd; IX-8-1959 (Cantral & Cohn)	10	7	UMMZ	A
S. histrio (2)	L187	Mexico, Chiapas, 1.7mi SW Berriozabal on Hwy 190; X-13-1961 (Hubbell, Cantral, Cohn)	3	4	UMMZ	A
S. histrio (2)	L188	Mexico, Chiapas, 19.2rd mi SE Comitan on Panamericana Hwy; X-19-1961 (Hubbell, Cantral, Cohn)	1	1	UMMZ	A
S. histrio (2)	L190	Mexico, Chiapas, 5.6 mi SE of Comitan ; X-11-1961 (Hubbell, Cantral, Cohn)		2	UMMZ	A
S. histrio (2)	L191	Mexico, Chiapas, 1.9mi N Ixtapa on Hwy 195; X-12-1961 (Hubbell, Cantral, Cohn)	3	15	UMMZ	A
S. histrio (2)	L192	Mexico, Chiapas, El Sumidero, 13.5rd mi N Tuxtla Gutierrez ; X-13-1961 (Hubbell, Cantral, Cohn)	2	4	UMMZ	A
S. histrio (2)	L193	Mexico, Chiapas, 13.9mi SE Comitan on Panamericana Hwy; X-19-1961 (Hubbell, Cantral, Cohn)		7	UMMZ	A
S. histrio (2)	L194	Mexico, Chiapas, El zapotal, 3.3mi S Tuxtla Gutierrez ; IX-12-1959 (Cantral & Cohn)		1	UMMZ	A
S. histrio (2)	L195	Mexico, Chiapas, 7mi W Teapa on Pichucalco Rd; IX-8-1959 (Cantral & Cohn)	3	4	UMMZ	A
S. histrio (2)	L196	Mexico, Chiapas, 12mi W Teapa on Pichucalco Rd; IX-8-1959 (Cantral & Cohn)	36	30	UMMZ	A
S. histrio (2)	L197	Mexico, Chiapas, 7mi W Chiapa de Corzo, on hwy 190; IX-11-1959 (Cantral & Cohn)		1	UMMZ	A
S. histrio (2)	L198	Mexico, Chiapas, 9mi W Tuxtla Gutierrez, plaza; IX-11-1959 (Cantral & Cohn)	2	6	UMMZ	A
S. histrio (2)	L199	Mexico, Chiapas, 3.6mi W Los Amates at Km 947, 34mi SW Cintalapa on Hwy 190; IX-12-1959 (Cantral & Cohn)	6	9	UMMZ	A
S. histrio (2)	L200	Mexico, Chiapas, Cerro Hueco, 4.5mi S Tuxtla Gutierrez ; X-16-20-1961 (T.J. Cohn & P. Hubbell)	3	4	UMMZ	A
S. histrio (2)	L201	Mexico, Chiapas, Villa Flores ; XII--1954 (O. Macias)	1	3	UMMZ	A
S. histrio (2)	L202	Mexico, Chiapas, Aguacero, N from Mex 190 to Aguacero Nat. Pk.; X-24-1976 (J.G. Chemminck)		2	UMMZ	A
S. histrio (2)	L203	Mexico, Chiapas, 17.2mi N Mexican border station on Mex 190; X-30-1976 (J.G. Chemminck)	2	2	UMMZ	A

S. histrio (2)	L211	Mexico, Chiapas, La Zacualpa		1	ANSP	A
S. histrio (2)	L212	Mexico, Chiapas, Comitan; III-3-1953 (Betchel)	1		ANSP	A
S. histrio (2)	L213	Mexico, Chiapas, La Trinitaria, 13mi S Chiapas; III-4-1953 (Betchel)	1		ANSP	A
S. histrio (2)	L214	Mexico, Chiapas, 8rd mi E Chapa de Corzo #28; X-30-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. histrio (2)	L215	Mexico, Chiapas, 28rd mi W San Cristobal #26; X-30-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. histrio (2)	L216	Mexico, Chiapas, 12rd mi E Chiapa de Corzo, near Tuxtla Gut. #27; X-30-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. histrio (2)	L217	Mexico, Chiapas, Chiapas-Oaxaca border on Rt. 200. # 33; X-30-1981 (Otte, Azuma, Newlin)		2	ANSP	A
S. histrio (2)	L219	Mexico, Chiapas, Ocozocoautla 16 mi E Tuxtla Gut. # 29; X-30-1981 (Otte, Azuma, Newlin)		2	ANSP	A
S. histrio (2)	L220	Mexico, Chiapas, Carca del Chorreadero Carr 190; IX-26-2012 (S. Sanabria & F. Rivera)	2	3	IIES	A
S. histrio (2)	**L15**	**Mexico, Chiapas, EL Chorreadero ; IX-26-2012 (S. Sanabria & F. Rivera)**	11	8	IIES	A
S. histrio (2)	**L74**	**Mexico, Chiapas, Teopisca Carr 190; IX-27-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. histrio (2)	**L13**	**Mexico, Chiapas, Las Margarita Carr 190; IX-27-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. histrio (2)	L180	Mexico, Oaxaca, 7mi W Tequisistlan; IX-13-1959 (Cantral & Cohn)	51	35	UMMZ	A
S. histrio (2)	L218	Mexico, Oaxaca, 7mi S Ocozocoautla, Rd to Villa Flores #30; X-30-1981 (Otte, Azuma, Newlin)	1		ANSP	A
S. histrio (2)	L189	Mexico, Sonora, 39mi SSW Magdalena on Mex15; VIII-31-1957 (T.J. Cohn & E.R. Tinkham)		1	UMMZ	C
S. histrio (2)	L206	Mexico, Tabasco, 3.5mi W Teapa on Pichucalco Rd; IX-8-1959 (Cantral & Cohn)	1	4	UMMZ	A
S. histrio (2)	L207	Mexico, Tabasco, 3mi ± NE Teapa; X-27-1961 (T.J. Cohn & P. Hubbell)		3	UMMZ	A
S. histrio (2)	L208	Mexico, Tabasco, 8.2mi W Teapa on Pichucalco Rd; X-28-1961 (T.J. Cohn & P. Hubbell)		1	UMMZ	A
S. histrio (2)	L209	Mexico, Tabasco, 9.5mi N Teapa ; VII-15-1958 (W.E. Duellman & J. Tulecke)	3		UMMZ	A
S. histrio (2)	**L73**	**Mexico, Veracruz , Amador Hernandez Carr 187; IX-26-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. histrio (3)	L221	Mexico, Oaxaca, 32mi NW Oaxaca, main Plaza, on Hwy 190; X-15-1959 (Cantral & Cohn)	1	1	UMMZ	C
S. histrio (3)	L222	Mexico, Oaxaca, 45mi NW Tequisistlan (1mi S El Camaron); XII-21-1958 (T.J. Cohn)		8	UMMZ	A
S. histrio (3)	L224	Mexico, Oaxaca, 12.3rd mi NW San Jose, 0.4 mi NW km 636, on Hwy 190; IX-12-1961 (Hubbell, Cantral, Cohn)		1	UMMZ	B
S. histrio (3)	L225	Mexico, Oaxaca, 46mi W Jalapa del Marquez at Portillo Nejapam pass #37; X-31-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. histrio (3)	**L60**	**Mexico, Oaxaca, Pasando San Juan Lajarcia Carr 190 km 150; X-27-2011 (S. Sanabria & A. Díaz)**	12	11	IIES	A
S. histrio (3)	**L70**	**Mexico, Oaxaca, San Jose de García Carr 190; X-27-2011 (S. Sanabria & A. Díaz)**	7	8	IIES	A
S. histrio (3)	**L61**	**Mexico, Oaxaca, Carr 175 Km87 ; XII-13-2013 (S. Sanabria, P. Fontana & R. Mariño)**	2	9	IIES	A
S. histrio (3)	L226	Mexico, Oaxaca, Portillo San Dionisio; XI-21-2015 (S. Sanabria)		1	IIES	A
S. histrio (3)	L227	Mexico, Oaxaca, San Baltazar Guelavilla; XI-21-2015 (S. Sanabria)		1	IIES	A
S. histrio (3)	L228	Mexico, Oaxaca, Km 67, Carr. 175; XI-23-2015 (S. Sanabria)		1	IIES	A
S. histrio (3)	L229	Mexico, Oaxaca, San Pedro Totolapan, Carr. 190; X-27-2011 (S. Sanabria & A. Díaz)	1		IIES	A
S. histrio (3)	L223	Mexico, Sinaloa, 19mi NW Guasave ; IX-2-1957 (T.J. Cohn & E.R. Tinkham)	1	1	UMMZ	C
S. histrio (4)	L230	Mexico, Oaxaca, 7mi SE El Camaron (37mi NW Tequisistlan onhwy 190); IX-14-1959 (Cantral & Cohn)	4	3	UMMZ	A
S. histrio (4)	L232	Mexico, Oaxaca, 12.3rd mi NW San Jose, 0.4 mi NW km 636, on Hwy 190; IX-12-1961 (Hubbell, Cantral, Cohn)	5	1	UMMZ	B
S. histrio (4)	L233	Mexico, Oaxaca, 34rd mi W Jalapa del Marquez on 190 # 36; X-31-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. histrio (4)	**L68**	**Mexico, Oaxaca, Las Minas Carr 190 km 160; X-27-2011 (S. Sanabria & A. Díaz)**	2	4	IIES	A
S. histrio (4)	**L69**	**Mexico, Oaxaca, El Camaron Yautepec Carr 190; X-27-2011 (S. Sanabria & A. Díaz)**	6	9	IIES	A

S. histrio (4)	L234	Mexico, Oaxaca, Km 175, Carr. 190, M117; XII-10-2013 (S. Sanabria, P. Fontana & R. Mariño)	2	2	IIES	A
S. histrio (4)	L235	Mexico, Oaxaca, Km 193, Carr. 190, M118; XII-10-2013 (S. Sanabria, P. Fontana & R. Mariño)	2	2	IIES	A
S. histrio (4)	L236	Mexico, Oaxaca, Las Majadas	3	3	IIES	A
S. histrio (4)	L251	Mexico, Oaxaca, 1.7mi SE El Camaron, 42mi NW Tequisistlan onhwy 190; IX-14-1959 (Cantral & Cohn)		1	UMMZ	C
S. histrio (5)	L237	Mexico, Chiapas, Arriaga; II-1-1947 (H.O. Wagner)	3	1	UMMZ	A
S. histrio (5)	L238	Mexico, Chiapas, Arriaga; X-30-1981 (Otte, Azuma, Newlin)	1		ANSP	A
S. histrio (5)	**L10**	**Mexico, Chiapas, Salida de Arriaga Carr 200; IX-30-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. macrophallicum	L239	Mexico, Guerrero, 11rd mi NE of Arcelia ; XII-8-1958 (T.J. Cohn)		1	UMMZ	A
S. macrophallicum	**L04**	**Mexico, Guerrero, Coyuca de Catalán Carr 134; X-13-2012 (S. Sanabria & A. Pingarroni)**	10	10	IIES	A
S. macrophallicum	L242	Mexico, Guerrero, Monumento a Cardenas Carr 51; X-13-2012 (S. Sanabria & A. Pingarroni)	1	4	IIES	A
S. macrophallicum	L240	Mexico, Michoacan, Salida de Tzitzio Carr 49 ; X-7-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)	2	1	IIES	A
S. macrophallicum	**L05**	Mexico, Michoacan, Rumbo al Debanadero Carr 49; X-7-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)	10	10	IIES	A
S. macrophallicum	**L06**	Mexico, Michoacan, Sta Barbara Carr 521 ; X-12-2012 (S. Sanabria & A. Pingarroni)	10	10	IIES	A
S. macrophallicum	L241	Mexico, Michoacan, Huetamo Carr 51; X-12-2012 (S. Sanabria & A. Pingarroni)	10	10	IIES	A
S. mexicanum (1)	L440	Mexico, Morelos, Cuernavaca (Deam)		1	ANSP	B
S. mexicanum (1)	L231	Mexico, Oaxaca, 4.5mi E Tehuantepec on Hwy 190; IX-13-1959 (Cantral & Cohn)	2	6	UMMZ	A
S. mexicanum (1)	L243	Mexico, Oaxaca, 1mi N of Salina Cruz ; IX-13-1959 (Cantral & Cohn)	5	7	UMMZ	A
S. mexicanum (1)	L244	Mexico, Oaxaca, 3mi N os Salina Cruz; IX-13-1959 (Cantral & Cohn)	9	16	UMMZ	A
S. mexicanum (1)	L245	Mexico, Oaxaca, 13mi W Miltepec; XII-22-1955 (F. Thompson)		1	UMMZ	C
S. mexicanum (1)	L246	Mexico, Oaxaca, Tehuantepec; XII-11-1948 (H.O. Wagner)	1	6	UMMZ	A
S. mexicanum (1)	L247	Mexico, Oaxaca, Salina Crus; VIII-27-1947 (H.O. Wagner)	1	1	UMMZ	A
S. mexicanum (1)	L248	Mexico, Oaxaca, Salina Cruz; XII--1898 (Deam)		1	ANSP	A
S. mexicanum (1)	L249	Mexico, Oaxaca, 4mi NNE Juchitan (Hwy 190); IX-10-1959 (Cantral & Cohn)	14	12	UMMZ	A
S. mexicanum (1)	L250	Mexico, Oaxaca, 4.5mi E Tehuantepec on Hwy 190; IX-13-1961 (Hubbell, Cantral, Cohn)	3	6	UMMZ	A
S. mexicanum (1)	L278	Mexico, Oaxaca, Almoloyas; XI--1922 (White)		1	ANSP	B
S. mexicanum (1)	L280	Mexico, Oaxaca, Betwenn Zanatepec & Juchitan # 34; X-31-1981 (Otte, Azuma, Newlin)		1	ANSP	B
S. mexicanum (1)	L283	Mexico, Oaxaca, Betwenn Zanatepec & Juchitan # 34; X-31-1981 (Otte, Azuma, Newlin)		1	ANSP	B
S. mexicanum (1)	**L22**	**Mexico, Oaxaca, Paque Heolico La ventosa Carr 200; IX-30-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. mexicanum (1)	L252	Mexico, Veracruz, 1mi ± S Medellin de Bravo on Hwy 150; IX-6-1959 (Cantral & Cohn)	15	14	UMMZ	A
S. mexicanum (1)	L253	Mexico, Veracruz, 1.4mi N Santiago Tuxtla ; IX-6-1959 (Cantral & Cohn)	2	10	UMMZ	A
S. mexicanum (1)	L254	Mexico, Veracruz, 3mi WSW Minatitlan on Hwy 180; IX-6-1959 (Cantral & Cohn)	14	10	UMMZ	A
S. mexicanum (1)	L255	Mexico, Veracruz, 4mi WSW Minatitlan on Hwy 180; IX-6-1959 (Cantral & Cohn)	5	4	UMMZ	A
S. mexicanum (1)	L256	Mexico, Veracruz, Acayucan ; X-23-1957 (B. & K. Dreisbach)	2	3	UMMZ	A
S. mexicanum (1)	L257	Mexico, Veracruz, 4.9mi S Veracruz Mocambo Beach ; VIII-8-1948 (P.P. Dowling)		9	UMMZ	B
S. mexicanum (1)	L258	Mexico, Veracruz, 2km S San Andres Tuxtla ; X-19-1957		2	UMMZ	A
S. mexicanum (1)	L259	Mexico, Veracruz, Catemaco, halfway up El Mono; I-20-1961 (K. Bailey)		1	UMMZ	A
S. mexicanum (1)	L260	Mexico, Veracruz, 1mi N Fortin ; VII-25-1951 (U. R. Mosiman)	1		UMMZ	A
S. mexicanum (1)	L261	Mexico, Veracruz, 3.3mi NE Orizaba, 2.3mi N of east gate; IX-3-1959 (Cantral & Cohn)	5	4	UMMZ	A
S. mexicanum (1)	L262	Mexico, Veracruz, 3.3mi NE Cordoba , on quarry rd; IX-4-1959 (Cantral &		2	UMMZ	A

		Cohn)				
S. mexicanum (1)	L263	Mexico, Veracruz, 3.3mi NE Cordoba , below quarry; IX-4-1959 (Cantral & Cohn)		4	UMMZ	A
S. mexicanum (1)	L264	Mexico, Veracruz, Atoyac, E eadge; IX-5-1959 (Cantral & Cohn)	1	3	UMMZ	B
S. mexicanum (1)	L265	Mexico, Veracruz, 3.4mi S Veracruz, Post office ; IX-5-1959 (Cantral & Cohn)		3	UMMZ	B
S. mexicanum (1)	L266	Mexico, Veracruz, 7mi S Veracruz, Post office ; IX-5-1959 (Cantral & Cohn)	6	2	UMMZ	B
S. mexicanum (1)	L267	Mexico, Veracruz, 6mi SW Veracruz, Hwy 140; IX-6-1959 (Cantral & Cohn)		4	UMMZ	B
S. mexicanum (1)	L268	Mexico, Veracruz, 4.5mi S Sayul (Sayula); X-21-1961 (T.J. Cohn & P. Hubbell)	7	10	UMMZ	A
S. mexicanum (1)	L269	Mexico, Veracruz, 1mi NE Atoyac; XI-7-1961 (T.J. Cohn & P. Hubbell)	1	1	UMMZ	A
S. mexicanum (1)	L270	Mexico, Veracruz, 5.4mi SE Las Vigas on Hwy 140; XI-11-1961 (T.J. Cohn & P. Hubbell)		1	UMMZ	A
S. mexicanum (1)	L271	Mexico, Veracruz, Fortin, Km 329 W Cordoba ; VII-27-1936 (Roberts)	1		ANSP	B
S. mexicanum (1)	L272	Mexico, Veracruz, 5mi E Cordoba; VII-31-1936 (Roberts)		1	ANSP	A
S. mexicanum (1)	L273	Mexico, Veracruz, Medellin (Heyde)		1	ANSP	A
S. mexicanum (1)	L274	Mexico, Veracruz, Cordoba; X-19-1924 (Hebard)		1	ANSP	A
S. mexicanum (1)	L275	Mexico, Veracruz, Santa Lucrecia ; X--1922 (White)		1	ANSP	B
S. mexicanum (1)	L276	Mexico, Veracruz, 10mi S Jalapa; XII-28-1940 (Bohart)	1		ANSP	A
S. mexicanum (1)	L277	Mexico, Veracruz, Medellin (Heyde)		1	ANSP	A
S. mexicanum (1)	L279	Mexico, Veracruz, Orizaba; XII--1987		1	ANSP	A
S. mexicanum (1)	L281	Mexico, Veracruz, 11mi N Palma Sola, N Veracruz #7; X-22-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. mexicanum (1)	L282	Mexico, Veracruz, Mountain forest near Sontecomapan 10 mi E Catemaco #9; X-23-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. mexicanum (1)	L284	Mexico, Veracruz, Lake Catemaco, Hotel Playa Azul ; VII-7-1963 (H.V. Weems. Jr.)	1	1	FSCA	A
S. mexicanum (1)	L285	Mexico, Veracruz, Catemaco, Dos Amates; VII-8-1973 (H.V. Weems. Jr.)	1		FSCA	A
S. mexicanum (1)	**L75**	**Mexico, Veracruz , Los Tuxltas; VII-30-2011 (R. Cueva)**	1	2	IIES	A
S. mexicanum (1)	L286	Mexico, Veracruz , Cerca de Presa Malpaso Carr 187D; IX-25-2012 (S. Sanabria & F. Rivera)		1	IIES	A
S. mexicanum (1)	**L20**	**Mexico, Veracruz , Salida de Cordoba Carr 150; IX-25-2012 (S. Sanabria & F. Rivera)**	5	5	IIES	A
S. mexicanum (1)	**L21**	**Mexico, Veracruz , Puente Coatzacoalcos 2 Minatitlán Carr 145D; IX-26-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. mexicanum (2)	**L14**	**Mexico, Oaxaca, Tehuantepec Carr 190 km 244; X-27-2011 (S. Sanabria & A. Díaz)**	34	26	IIES	A
S. minimum	L288	Mexico, Veracruz, El mirador, 13mi NE Huatusco; IX-3-1959 (Cantral & Cohn)	1		UMMZ	B
S. minimum	L289	Mexico, Veracruz, 7.5mi SW Huatusco on Hwy 132 at km 30; XI-8 to 10-1961 (T.J. Cohn & P. Hubbell)	2	3	UMMZ	A
S. minimum	L290	Mexico, Veracruz, 30km E Fortin Ver. Cerca de Coscomatepec; X-17-1957 (W Robert)		2	UMMZ	A
S. minimum	L291	Mexico, Veracruz, 0.3mi S Jalapa-Veracruz Hwy, on Teocelo Rd 6; VIII-6-1964 (T.J. Cohn)		4	UMMZ	B
S. minimum	L295	Mexico, Veracruz, Carr. 131, ca. 4.5Km al NE de Altotonga; IX-19-2015 (S. Sanabria)	1	1	IIES	A
S. minimum	L296	Mexico, Veracruz, Carr. 131, ca. 9.5Km al NE de Altotonga; IX-19-2015 (S. Sanabria)	1	1	IIES	A
S. minimum	L297	Mexico, Veracruz, Coatepec; VI--1922 (Conradt)		1	ANSP	A
S. minimum	L298	Mexico, Veracruz, Jalapa; VII-- (O. W. Barrett)	1		ANSP	A
S. minimum	L292	Mexico, Veracruz , Mariano Escobedo Orizaba; XI-1-2012 (S. Sanabria & V.H. Jimenéz)		1	IIES	A
S. minimum	L293	Mexico, Veracruz , Enríquez ; XI-1-2012 (S. Sanabria & V.H. Jimenéz)	3	3	IIES	A
S. minimum	**L24**	**Mexico, Veracruz , Entre Chocamán y Coscomatepec Carr 143; XI-2-2012 (S. Sanabria & V.H. Jimenéz)**	14	11	IIES	A
S. minimum	**L19**	**Mexico, Veracruz , Plan de Hayas ; XI-3-2012 (S. Sanabria & V.H. Jimenéz)**		3	IIES	A

S. minimum	**L23**	**Mexico, Veracruz , La Reforma ; XI-3-2012 (S. Sanabria & V.H. Jimenéz)**	3	7	IIES	A
S. minimum	**L76**	**Mexico, Veracruz , Banderilla Jalapa Carr 140; XI-3-2012 (S. Sanabria & V.H. Jimenéz)**	5	7	IIES	A
S. planum	**L45**	**Mexico, Oaxaca, Tehuacan Carr 135D; X-3-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. planum	**L78**	**Mexico, Oaxaca, Chazumba Carr 125 Km 60 ; XII-8-2013 (S. Sanabria, P. Fontana & R. Mariño)**	1	2	IIES	A
S. planum	L320	Mexico, Puebla, 3km W. Of Cacaloapan at Km 226 (11km SE Tlacotepec); IX-1-1959 (Cantral & Cohn)	7	25	UMMZ	A
S. planum	L321	Mexico, Puebla, 0.6 mi S of Ajalpan ; IX-1-1959 (Cantral & Cohn)		2	UMMZ	A
S. planum	L322	Mexico, Puebla, Tehuacan ; XI-- (Bruner)	1		ANSP	A
S. planum	L323	Mexico, Puebla, Km 226 NW Tehuacan; VII-28-1936 (Roberts)	1	3	ANSP	B
S. planum	**L46**	**Mexico, Puebla, Despues de Tecamachalco Carr ; XII-7-2013 (S. Sanabria, P. Fontana & R. Mariño)**	2	2	IIES	A
S. planum	**L77**	**Mexico, Puebla, Basurero Tehuacan ; XII-8-2013 (S. Sanabria, P. Fontana & R. Mariño)**	4	8	IIES	A
S. planum	L327	Mexico, Veracruz, Acultzingo ; XII-1-1936 (Roberts)		1	ANSP	A
S. planum	L328	Mexico, Veracruz, La Cumbre, adv, Acultzingo ; VII-24-1936 (Roberts)		1	ANSP	A
S. purpurascens (1)	L332	Mexico, Chiapas, Las Margaritas, 25km W Comitan ; XII-7-1948 (H.O. Wagner)	3		UMMZ	C
S. purpurascens (1)	**L32**	**Mexico, Guanajuato , Tarandacuaro (Acambaro); IX-8-2008 (S. Sanabria)**	8	6	IIES	A
S. purpurascens (1)	**L35**	**Mexico, Guanajuato , Rumbo a Guanajuato; IX-8-2008 (S. Sanabria)**	10	6	IIES	A
S. purpurascens (1)	L378	Mexico, Guanajuato , Desviacion Dolores ; X-6-2013 (S. Sanabria & F. Rivera)	6	3	IIES	A
S. purpurascens (1)	**L30**	**Mexico, Guanajuato , Leon ; X-6-2013 (S. Sanabria & F. Rivera)**	13	13	IIES	A
S. purpurascens (1)	**L26**	**Mexico, Guanajuato , Sta Catarina ; X-6-2013 (S. Sanabria & F. Rivera)**	7	3	IIES	A
S. purpurascens (1)	L385	Mexico, Guerrero, Colotlipa		1	CAFESI	C
S. purpurascens (1)	L338	Mexico, Hidalgo, 15mi W Ixmiquilpan; VIII-30-1959 (Cantral & Cohn)	14	7	UMMZ	A
S. purpurascens (1)	L353	Mexico, Hidalgo, 5mi N Tizayuca; XI-13-1946 (Ross & Skinner)		1	ANSP	A
S. purpurascens (1)	L354	Mexico, Hidalgo, Agua Fria Grnade ; IX--1950 (Wienanid)		1	ANSP	A
S. purpurascens (1)	L379	Mexico, Hidalgo, Carr 85 entre Tasquillo y Aguacatal; X-6-2013 (S. Sanabria & F. Rivera)	10	10	IIES	A
S. purpurascens (1)	L339	Mexico, Mexico, San Rafael ; IX-15-1950 (H.O. Wagner)		1	UMMZ	B
S. purpurascens (1)	L355	Mexico, Mexico, Atizapan ; VII-10-1898 (Barret)		1	ANSP	A
S. purpurascens (1)	L358	Mexico, Mexico, Ixmiquilpan ; IX--1950 (Wiegan)		1	ANSP	A
S. purpurascens (1)	**L43**	**Mexico, Mexico, Desviasión a Amealco ; IX-8-2008 (S. Sanabria)**	3	5	IIES	A
S. purpurascens (1)	L371	Mexico, Mexico, Tultepec ; XI-5-2011 (S. Sanabria)	6	10	IIES	A
S. purpurascens (1)	L333	Mexico, Mexico City, San Geronimo, Pedregal; IX-24-1959 (H.O. Wagner)	22	10	UMMZ	A
S. purpurascens (1)	L334	Mexico, Mexico City, Sacatepec, Pedregal ; IX-24-1959 (H.O. Wagner)	2	4	UMMZ	A
S. purpurascens (1)	L335	Mexico, Mexico City, Acolma ; VII-29-1946 (H.O. Wagner)		1	UMMZ	B
S. purpurascens (1)	L336	Mexico, Mexico City, Lomas Altas; X-12-1957 (W.W. Gibson)		9	UMMZ	A
S. purpurascens (1)	L337	Mexico, Mexico City, Tlalpan	2	5	UMMZ	A
S. purpurascens (1)	L361	Mexico, Mexico City, Lomas de Chapultepec; X-27-1933 (Dampf)		1	ANSP	A
S. purpurascens (1)	L365	Mexico, Mexico City, Tlalpan; IX-5-1936 (Roberts)		1	ANSP	A
S. purpurascens (1)	**L41**	**Mexico, Mexico City, Pedregal San Ángel; X-5-2008**		1	IIES	A
S. purpurascens (1)	L366	Mexico, Michoacan, Rumbo a Morelia Carr14 Km 19; XI-2-2010 (S. Sanabria)	10	8	IIES	A
S. purpurascens (1)	L367	Mexico, Michoacan, Pasando Sta Clara del Cobre Carr120; XI-1-2010 (S. Sanabria)	11	10	IIES	A
S. purpurascens (1)	L368	Mexico, Michoacan, Desviasión a Araro Carr 15 ; XI-1-2010 (S. Sanabria)	3	6	IIES	A
S. purpurascens (1)	L372	Mexico, Michoacan, Penuelas Carr. 15 Km 5 ; XII-6-2013 (S. Sanabria, P. Fontana & R. Mariño)	3	7	IIES	A

S. purpurascens (1)	**L49**	**Mexico, Michoacan, Rumbo a Zitacuaro ; XII-6-2013 (S. Sanabria, P. Fontana & R. Mariño)**		3	IIES	A
S. purpurascens (1)	L380	Mexico, Michoacan, Desviación Quiroga; XII-5-2013 (S. Sanabria, P. Fontana & R. Mariño)		1	IIES	A
S. purpurascens (1)	L381	Mexico, Michoacan, Carr 15 Km15, cerca de Zacapú; XII-5-2013 (S. Sanabria, P. Fontana & R. Mariño)		1	IIES	A
S. purpurascens (1)	L356	Mexico, Morelos, Cañon de Lobos; IX-25-1952 (Boush)		1	ANSP	C
S. purpurascens (1)	L363	Mexico, Morelos, Nepantla; XI-- (Barret)		1	ANSP	B
S. purpurascens (1)	L340	Mexico, Oaxaca, Nochixtlan; IX-17-1948 (H.O. Wagner)	3	8	UMMZ	A
S. purpurascens (1)	L341	Mexico, Oaxaca, Huajuapan; IX-13-1948 (H.O. Wagner)		2	UMMZ	A
S. purpurascens (1)	L342	Mexico, Oaxaca, 1.0mi NW Tamazulapan at Km 388 (Km38.8) ; IX-9-1961 (Hubbell, Cantral, Cohn)	1	4	UMMZ	A
S. purpurascens (1)	L343	Mexico, Oaxaca, 15.5mi NW Oaxaca; IX-9-1961 (Hubbell, Cantral, Cohn)		1	UMMZ	A
S. purpurascens (1)	L344	Mexico, Oaxaca, 9.1mi E Oaxaca on Papaloapan rd at Km 221; IX-10-1961 (Hubbell, Cantral, Cohn)	5	4	UMMZ	B
S. purpurascens (1)	L345	Mexico, Oaxaca, 7.2mi E Oaxaca on Papaloapan rd at Km 224; IX-10-1961 (Hubbell, Cantral, Cohn)	2	7	UMMZ	B
S. purpurascens (1)	L346	Mexico, Oaxaca, 6mi NW Oaxaca, main Plaza, on Hwy 190; IX-14-1959 (Cantral & Cohn)		3	UMMZ	A
S. purpurascens (1)	L347	Mexico, Oaxaca, Tlaxiaco ; XI-23-1961 (B. Longacre)	10	6	UMMZ	A
S. purpurascens (1)	L348	Mexico, Oaxaca, 10mi SE Nochixtlan ; XII-20-1958 (T.J. Conh)	4	4	UMMZ	A
S. purpurascens (1)	L349	Mexico, Oaxaca, 31mi SE Nochixtlan on Hwy 190; X-15-1959 (T.J. Conh)	1	4	UMMZ	A
S. purpurascens (1)	L350	Mexico, Oaxaca, Yanhuitlan ; I-27-1980 (A. S. Fuentes)		7	UMMZ	A
S. purpurascens (1)	L351	Mexico, Oaxaca, San Andres Chicohuaxtla ; XI-28-1961 (B. Longacre)		3	UMMZ	A
S. purpurascens (1)	L352	Mexico, Oaxaca, 12 mi SE Nochixtlan ; XII-13-1948 (Leech)		1	ANSP	A
S. purpurascens (1)	L357	Mexico, Oaxaca, Cerro San Felipe; VII-20-1988 (Roberts)		1	ANSP	A
S. purpurascens (1)	L359	Mexico, Oaxaca, Jalpan		1	ANSP	B
S. purpurascens (1)	L362	Mexico, Oaxaca, Monte Alban; XII-11-1948 (Leech)		1	ANSP	A
S. purpurascens (1)	L364	Mexico, Oaxaca, Oaxaca; 1903 (Hebard)		1	ANSP	B
S. purpurascens (1)	**L25**	**Mexico, Oaxaca, Rumbo a Teotitlan Carr 131 Km 71 ; XII-14-2013 (S. Sanabria, P. Fontana & R. Mariño)**	6	5	IIES	A
S. purpurascens (1)	**L28**	**Mexico, Oaxaca, Monte Alban; X-2-2008 (S. Sanabria)**	3	4	IIES	A
S. purpurascens (1)	L369	Mexico, Oaxaca, Yautitlan Carr 190; X-28-2011 (S. Sanabria & A. Díaz)	3	1	IIES	A
S. purpurascens (1)	L370	Mexico, Oaxaca, Tamazulapan Carr 190; X-28-2011 (S. Sanabria & A. Díaz)	10	10	IIES	A
S. purpurascens (1)	**L33**	**Mexico, Oaxaca, Entre Huajuapan y Tamazulapan Carr 190; X-28-2011 (S. Sanabria & A. Díaz)**	8	8	IIES	A
S. purpurascens (1)	L376	Mexico, Oaxaca, Frontera Oaxaca Puebla Carr 190; XII-9-2013 (S. Sanabria, P. Fontana & R. Mariño)		3	IIES	A
S. purpurascens (1)	**L37**	**Mexico, Oaxaca, El Capulín ; XII-14-2013 (S. Sanabria, P. Fontana & R. Mariño)**	7	6	IIES	A
S. purpurascens (1)	L377	Mexico, Oaxaca, Rumbo a Tonaltepec ; XII-14-2013 (S. Sanabria, P. Fontana & R. Mariño)	2	3	IIES	A
S. purpurascens (1)	L382	Mexico, Oaxaca, San Anrés Chicahuaxtla; X-18-2015 (S. Sanabria)	10	10	IIES	A
S. purpurascens (1)	L383	Mexico, Oaxaca, San Martin Itunyoso; X-18-2015 (S. Sanabria)	3	3	IIES	A
S. purpurascens (1)	**L81**	**Mexico, Oaxaca, Carr. 125, ca. 8Km NE de Tlaxiaco; X-19-2015 (S. Sanabria)**	2	5	IIES	A
S. purpurascens (1)	L384	Mexico, Oaxaca, Entrada a San Andrés Xochixtlan; X-19-2015 (S. Sanabria)	1	2	IIES	A
S. purpurascens (1)	**L27**	**Mexico, Oaxaca, Entrada Oaxaca; IV-10-2008**		1	IIES	A
S. purpurascens (1)	L360	Mexico, Puebla, Km 226 NW Tehuacan; VII-28-1936 (Roberts)		1	ANSP	B
S. purpurascens (1)	**L40**	**Mexico, Puebla, Cacaxtla; IX-13-2008 (S. Sanabria)**	6	8	IIES	A
S. purpurascens (1)	**L34**	**Mexico, Puebla, Cholula; IX-12-2008 (S. Sanabria)**	7	6	IIES	A
S. purpurascens (1)	**L31**	**Mexico, Queretaro, El paraiso, Peña de Bernal; IX-8-2008 (S. Sanabria)**	17	9	IIES	A
S. purpurascens (1)	**L37**	**Mexico, Tlaxcala, Papalotla; IX-13-2008 (S. Sanabria)**	11	24	IIES	A

S. purpurascens (1)	**L42**	**Mexico, Tlaxcala, Atotonilco; IX-13-2008 (S. Sanabria)**	7	13	IIES	A
S. purpurascens (1)	**L38**	**Mexico, Tlaxcala, Techachalco; IX-13-2008 (S. Sanabria)**	8	5	IIES	A
S. purpurascens (1)	**L36**	**Mexico, Tlaxcala, Zacualpan; IX-13-2008 (S. Sanabria)**	6	8	IIES	A
S. purpurascens (1)	L374	Mexico, Tlaxcala, Camino a Huiloapan ; XII-7-2013 (S. Sanabria, P. Fontana & R. Mariño)	3	7	IIES	A
S. purpurascens (1)	L375	Mexico, Tlaxcala, Ixtenco pop Victor ; XII-7-2013 (S. Sanabria, P. Fontana & R. Mariño)	1	4	IIES	A
S. purpurascens (1)	**L29**	**Mexico, Veracruz , Cumbres de Maltrata Carr 150D; IX-25-2012 (S. Sanabria & F. Rivera**)	10	10	IIES	A
S. purpurascens (2)	L386	Mexico, Oaxaca, Miahuatlan; X-13-1948 (H.O. Wagner)	17	24	UMMZ	A
S. purpurascens (2)	L387	Mexico, Oaxaca, Ejutla; X-17-1948 (H.O. Wagner)	2	3	UMMZ	A
S. purpurascens (2)	L386	Mexico, Oaxaca, Miahuatlan; X-5-1948 (H.O. Wagner)	6	12	UMMZ	A
S. purpurascens (2)	L388	Mexico, Oaxaca, Ejutla; X-16-1948 (H.O. Wagner)	7	13	UMMZ	A
S. purpurascens (2)	L389	Mexico, Oaxaca, 13mi S Ocotlan, rd to Pto Angel #39; IX-1-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. purpurascens (2)	L390	Mexico, Oaxaca, 7mi S Oaxaca rd to Pto. Angel #38 ; IX-1-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. purpurascens (2)	L391	Mexico, Oaxaca, Ejutla		1	ANSP	A
S. purpurascens (2)	L392	Mexico, Oaxaca, Mitla; VII-19-1988 (Roberts)		1	ANSP	A
S. purpurascens (2)	L393	Mexico, Oaxaca, Oaxaca-Pto Angel rd, 49rd mi S Ejutla # 41; IX-1-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. purpurascens (2)	L394	Mexico, Oaxaca, Oaxaca-Pto Angel rd, ca 10 mi S Ejutla # 40; IX-1-1981 (Otte, Azuma, Newlin)	1	2	ANSP	A
S. purpurascens (2)	L395	Mexico, Oaxaca, Tlacolula Carr 190; X-27-2011 (S. Sanabria & A. Díaz)	11	10	IIES	A
S. purpurascens (2)	**L39**	**Mexico, Oaxaca, San Adres Paxtlan Carr 175 km 116; X-2-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	10	10	IIES	A
S. purpurascens (2)	L396	Mexico, Oaxaca, Miahuatlan Carr 175; X-2-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)	10	10	IIES	A
S. purpurascens (2)	L397	Mexico, Oaxaca, Ejutla Carr 175; X-2-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)	10	10	IIES	A
S. purpurascens (2)	**L94**	**Mexico, Oaxaca, Rumbo a Hierve el Agua; X-3-2012 (S. Sanabria & F. Rivera)**	10	10	IIES	A
S. purpurascens (2)	**L61**	**Mexico, Oaxaca, Carr 175 Km 87 ; XII-13-2013 (S. Sanabria, P. Fontana & R. Mariño)**		8	IIES	A
S. purpurascens (2)	L399	Mexico, Oaxaca, Camino a San Pablo Coatlan 1; X-11-2015 (V. H. Jiménez)	1	1	IIES	A
S. purpurascens (2)	L400	Mexico, Oaxaca, Camino a San Pablo Coatlan 2; X-11-2015 (V. H. Jiménez)	2	1	IIES	A
S. purpurascens (2)	L401	Mexico, Oaxaca, Camino a San Pablo Coatlan 3; X-11-2015 (V. H. Jiménez)	3	2	IIES	A
S. purpurascens (2)	L226	Mexico, Oaxaca, Portillo San Dionisio; XI-21-2015 (S. Sanabria)		1	IIES	A
S. purpurascens (2)	L227	Mexico, Oaxaca, San Baltazar Guelavilla; XI-21-2015 (S. Sanabria)		1	IIES	A
S. purpurascens (2)	L228	Mexico, Oaxaca, Km 67, Carr. 175; XI-23-2015 (S. Sanabria)		1	IIES	A
S. purpurascens (3)	L405	Mexico, Oaxaca, 26mi NE Sola de Vega on Mex 131, rd E to Santiago Textitlan ; X-20-1976 (J.G. Chemminck)	1	4	UMMZ	A
S. purpurascens (3)	L406	Mexico, Oaxaca, 12mi SW Sola de Vega on Mex 131; X-19-1976 (J.G. Chemminck)		2	UMMZ	A
S. purpurascens (3)	L407	Mexico, Oaxaca, Santa Maria Jalteanguis; X-14-2015 (S. Sanabria)	10	10	IIES	A
S. purpurascens (3)	L408	Mexico, Oaxaca, San Juan Atepec; X-16-2015 (S. Sanabria)	9	10	IIES	A
S. purpurascens (3)	**L80**	**Mexico, Oaxaca, Ixtlán; X-17-2015 (S. Sanabria)**	5	9	IIES	A
S. rugosum (1)	L415	Mexico, Guerrero, 16rd mi ENE Taxco, 4.7rd mi NE Acuitlapan; IX-16-1959 (Cantral & Cohn)		2	UMMZ	A
S. rugosum (1)	L416	Mexico, Guerrero, 9rd mi NE Taxco, 1.7rd mi SW Acuitlapan; IX-17-1959 (Cantral & Cohn)		4	UMMZ	A
S. rugosum (1)	L417	Mexico, Guerrero, 1mi S Rio Balsas on Hwy 95, 33mi N Chilpancingo; IX-17-1959 (Cantral & Cohn)		1	UMMZ	A
S. rugosum (1)	L418	Mexico, Guerrero, 6mi S Iguala on Hwy 95; IX-20-1959 (Cantral & Cohn)	1		UMMZ	A
S. rugosum (1)	L419	Mexico, Guerrero, 9rd mi NE Taxco, 2rd mi SW Acuitlapan; XII-7-1958 (T.J. Cohn)	1	1	UMMZ	A
S. rugosum (1)	L422	Mexico, Guerrero, 8.5mi SE Iguala on Huitzuco rd; IX-20-1959 (Cantral &	3	3	UMMZ	A

		Cohn)				
S. rugosum (1)	L423	Mexico, Guerrero, 19mi W Iguala on Arcelia rd; IX-21-1959 (Cantral & Cohn)		2	UMMZ	A
S. rugosum (1)	L425	Mexico, Guerrero, 16rd mi NE Taxco, 5rd mi NE Acuitlapan; XII-7-1958 (T.J. Cohn)	1	1	UMMZ	A
S. rugosum (1)	L426	Mexico, Guerrero, 1mi S Taxco; XII-7-1958 (T.J. Cohn)	3	3	UMMZ	A
S. rugosum (1)	L427	Mexico, Guerrero, 12rd mi NW of Iguala; XII-7-1958 (T.J. Cohn)	1	1	UMMZ	A
S. rugosum (1)	L428	Mexico, Guerrero, 6mi W Iguala; XII-7-1958 (T.J. Cohn)	1	1	UMMZ	A
S. rugosum (1)	L429	Mexico, Guerrero, 6mi W Acatlan; XII-18-1958 (T.J. Cohn)		1	UMMZ	A
S. rugosum (1)	L436	Mexico, Guerrero, Km 234, btw. Iguala & R. Balsas; IX-10-1940 (Roberts)		1	ANSP	B
S. rugosum (1)	L438	Mexico, Guerrero, Guerrero ; X-12-1928 (Hebard)		1	ANSP	B
S. rugosum (1)	**L83**	**Mexico, Guerrero, Rumbo a Iguala Carr 95D km 44; X-24-2011 (S. Sanabria & A. Díaz)**	8	9	IIES	A
S. rugosum (1)	L444	Mexico, Guerrero, Pasando Iguala Carr 95; X-24-2011 (S. Sanabria & A. Díaz)	10	11	IIES	A
S. rugosum (1)	**L50**	**Mexico, Guerrero, Pasando Rio Mezcala Carr 95 km 175; X-24-2011 (S. Sanabria & A. Díaz)**	8	12	IIES	A
S. rugosum (1)	**L54**	**Mexico, Guerrero, Huajuapan de León Carr 190; X-28-2011 (S. Sanabria & A. Díaz)**	7	10	IIES	A
S. rugosum (1)	L445	Mexico, Guerrero, Desviasión a Atenango del Río Carr 1; X-15-2011 (S. Sanabria & V.H. Jimenéz)	4	2	IIES	A
S. rugosum (1)	**L51**	**Mexico, Guerrero, Copalillo desviación a Papalutla Carr 7; X-15-2011 (S. Sanabria & V.H. Jimenéz)**	9	22	IIES	A
S. rugosum (1)	L446	Mexico, Guerrero, Barranca Papalutla; X-16-2011 (S. Sanabria & V.H. Jimenéz)	11	15	IIES	A
S. rugosum (1)	L447	Mexico, Guerrero, Rumbo a Xixila ; X-15-2011 (S. Sanabria & V.H. Jimenéz)	8	8	IIES	A
S. rugosum (1)	**L56**	**Mexico, Guerrero, Xixila; X-16-2011 (S. Sanabria & V.H. Jimenéz**)	16	20	IIES	A
S. rugosum (1)	L455	Mexico, Guerrero, Tixtla Km 29 Chilpancingo-Chilapa		1	IBUNAM	C
S. rugosum (1)	L431	Mexico, Mexico, Ixtapa de la Sal; X-10-1958 (E.G. Matthews)	5	2	UMMZ	A
S. rugosum (1)	**L55**	**Mexico, Mexico, Malinalco; IX-12-2008 (S. Sanabria)**		17	IIES	A
S. rugosum (1)	L441	Mexico, Mexico City, Mexico City (Juda)		1	ANSP	C
S. rugosum (1)	L443	Mexico, Mexico City, Tlalpan; IX-5-1936 (Roberts)		1	ANSP	C
S. rugosum (1)	L411	Mexico, Morelos, 1mi E Tepoztlan; XII-26-1957 (K. Bailey)	1		UMMZ	A
S. rugosum (1)	L414	Mexico, Morelos, 9mi S Cuernavaca on Hwy 95; IX-16-1959 (Cantral & Cohn)		1	UMMZ	A
S. rugosum (1)	L424	Mexico, Morelos, 18rd mi NE Taxco, 7rd mi NE Acuitlapan; XII-7-1958 (T.J. Cohn)	1	1	UMMZ	A
S. rugosum (1)	L439	Mexico, Morelos, Tepoztlan; XII-26-1957 (K. Bailey)	3	4	UMMZ	A
S. rugosum (1)	L435	Mexico, Morelos, Cuernavaca ; IX-7-1936 (Roberts)		1	ANSP	A
S. rugosum (1)	L439	Mexico, Morelos, Tepoztlan; XII-26-1957 (Bailey)		1	ANSP	A
S. rugosum (1)	L440	Mexico, Morelos, Cuernavaca (Deam)	1	1	ANSP	A
S. rugosum (1)	**L53**	**Mexico, Morelos, Yecapixtla; IX-12-2008 (S. Sanabria)**	2	8	IIES	A
S. rugosum (1)	L448	Mexico, Morelos, Axochiapan ; X-17-2011 (S. Sanabria & V.H. Jimenéz)	10	7	IIES	A
S. rugosum (1)	L449	Mexico, Morelos, Alpuyeca Carr 95D; X-15-2011 (S. Sanabria & V.H. Jimenéz)	4	9	IIES	A
S. rugosum (1)	L452	Mexico, Morelos, Tlayacapan, Sn. Jose de los Laureles		1	IBUNAM	A
S. rugosum (1)	L453	Mexico, Morelos, Tepalcingo		1	IBUNAM	A
S. rugosum (1)	L454	Mexico, Morelos, Cuautla		1	IBUNAM	A
S. rugosum (1)	L410	Mexico, Oaxaca, Huajuapan; I-25-1949 (H.O. Wagner)	1	5	UMMZ	A
S. rugosum (1)	L450	Mexico, Oaxaca, Rumbo a Huajuapan ; XII-8-2013 (S. Sanabria, P. Fontana & R. Mariño)		7	IIES	A
S. rugosum (1)	L376	Mexico, Oaxaca, Frontera Oaxaca Puebla Carr 190; XII-9-2013 (S. Sanabria, P. Fontana & R. Mariño)	4	7	IIES	A
S. rugosum (1)	**L84**	**Mexico, Oaxaca, Freznillo Trujano ; XII-9-2013 (S. Sanabria, P. Fontana & R. Mariño)**		9	IIES	A

S. rugosum (1)	L456	Mexico, Oaxaca, Rumbo a Freznilo Trujano, Carr. 34; XII-9-2013 (S. Sanabria, P. Fontana & R. Mariño)		1	IIES	A
S. rugosum (1)	L412	Mexico, Puebla, 3mi SE Petlalcingo; IX-15-1959 (Cantral & Cohn)	2	10	UMMZ	A
S. rugosum (1)	L413	Mexico, Puebla, 1.2mi W Tehuitzingo on Hwy 190; IX-16-1959 (Cantral & Cohn)	1	1	UMMZ	A
S. rugosum (1)	L420	Mexico, Puebla, 15.3mi N jtc between Mex 140 & Mex 190; X-16-1976 (J.G. Chemminck)		14	UMMZ	B
S. rugosum (1)	L421	Mexico, Puebla, 6mi NW Petlalcingo on Hwy 190; IX-15-1959 (Cantral & Cohn)	3	10	UMMZ	A
S. rugosum (1)	L432	Mexico, Puebla, San Lucas ; X--1931 (Fraire)	1		UMMZ	B
S. rugosum (1)	L433	Mexico, Puebla, Matamoros; IX-10-1948 (H.O. Wagner)		1	UMMZ	A
S. rugosum (1)	L434	Mexico, Puebla, San Lucas ; X--1931 (Fraire)	1	1	ANSP	B
S. rugosum (1)	L442	Mexico, Puebla, Puebla		1	ANSP	C
S. rugosum (1)	**L82**	**Mexico, Puebla, Tilapa; X-17-2011 (S. Sanabria & V.H. Jimenéz)**	23	22	IIES	A
S. rugosum (2)	L457	Mexico, Guerrero, 3rd mi SW Almolonga, 8mi NE Tixtla; IX-18-1959 (Cantral & Cohn)		3	UMMZ	A
S. rugosum (2)	L458	Mexico, Guerrero, 10mi S Chilpancingo on Hwy 95, 1.5mi N Mazatlan turnoff; XI-20-1959 (Cantral & Cohn)		2	UMMZ	A
S. rugosum (2)	L459	Mexico, Guerrero, 2.4mi SW Almolonga, 9.4mi NE Tixtla ; IX-18-1959 (Cantral & Cohn)	3	3	UMMZ	A
S. rugosum (2)	L102	Mexico, Guerrero, 16rd mi S Chilpancingo, Km 298 on Hwy 95 (Cantral & Cohn)	2	5	UMMZ	A
S. rugosum (2)	L461	Mexico, Guerrero, 4.3mi E Petaquillas, 1.3mi E Tepechicotlan; IX-19-1959 (Cantral & Cohn)	1	1	UMMZ	A
S. rugosum (2)	L462	Mexico, Guerrero, 2.2rd mi S Petaquillas; XII-13-1958 (T.J. Cohn)	1		UMMZ	A
S. rugosum (2)	L463	Mexico, Guerrero, 4mi SE Chilpancingo; XII-12-1958 (T.J. Cohn)		2	UMMZ	A
S. rugosum (2)	L464	Mexico, Guerrero, 6mi S Petaquillas	2	1	UMMZ	A
S. rugosum (2)	L465	Mexico, Guerrero, 3mi S Acahuizlotla; XI-17-1946 (Dyke)		1	ANSP	A
S. rugosum (2)	L466	Mexico, Guerrero, Km 335, 16mi S Chilpancingo ; IX-14-1940 (Roberts)		1	ANSP	A
S. rugosum (2)	L467	Mexico, Guerrero, 4mi N Chilpancingo ; XI-17-1940 (Roberts)		1	ANSP	A
S. rugosum (2)	L468	Mexico, Guerrero, 3mi N Chilpancingo; XI-18-1946 (Dyke)	2	1	ANSP	A
S. rugosum (2)	L469	Mexico, Guerrero, 14mi S Chilpancingo #52; IX-4-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. rugosum (2)	**L52**	**Mexico, Guerrero, Petaquillas Carr 95 km 14; X-24-2011 (S. Sanabria & A. Díaz)**	7	13	IIES	A
S. rugosum (2)	**L17**	**Mexico, Guerrero, Palo Blanco Carr 95; X-24-2011 (S. Sanabria & A. Díaz)**	10	12	IIES	A
S. rugosum (2)	L471	Mexico, Guerrero, Atixtac Km 52 Tlapa-Chilapa		1	IBUNAM	A
S. rugosum (2)	L472	Mexico, Guerrero, Chilapa Km39 Tlapa-San luiz Acatlan		1	IBUNAM	A
S. rugosum (2)	L473	Mexico, Guerrero, Chilpancingo Der. El alquitran		1	IBUNAM	A
S. rugosum (2)	L474	Mexico, Guerrero, Las Grutas, Omiltemi; X-27-2015 (S. Sanabria)	1	2	IIES	A
S. rugosum (2)	L475	Mexico, Guerrero, Camino a Amojileca; X-27-2015 (S. Sanabria)	1	2	IIES	A
S. rugosum (2)	L476	Mexico, Oaxaca, Santa Fe, Quechaltenango		1	CAFESI	A
S. rugosum (3)	**L87**	**Mexico, Guerrero, Ceibitas Carr 51; X-14-2012 (S. Sanabria & A. Pingarroni)**	10	11	IIES	A
S. rugosum (3)	**L48**	**Mexico, Mexico, Tejupilco Carr 134; X-14-2012 (S. Sanabria & A. Pingarroni)**	4	7	IIES	A
S. rugosum (3)	**L49**	**Mexico, Michoacan, Rumbo a Zitacuaro ; XII-6-2013 (S. Sanabria, P. Fontana & R. Mariño)**	2	2	IIES	A
S. variabile (1)	L505	Mexico, Oaxaca, 2mi SE Tlacolula on Hwy #190; IX-11-1961 (Hubbell, Cantral, Cohn)	5	19	UMMZ	A
S. variabile (1)	L506	Mexico, Oaxaca, 45mi NW Tequisistlan (1mi S El Camaron); XII-21-1958 (T.J. Cohn)		1	UMMZ	A
S. variabile (1)	L507	Mexico, Oaxaca, 18mi Nw La Reforma (Km695 onHwy 190; 31mi WNW Tequisistlan); IX-14-1959 (Cantral & Cohn)		3	UMMZ	A
S. variabile (1)	L508	Mexico, Oaxaca, 13mi SE Tlacolula; XII-21-1958 (T.J. Cohn)		2	UMMZ	A
S. variabile (1)	L230	Mexico, Oaxaca, 7mi SE El Camaron (37mi NW Tequisistlan onhwy 190); IX-14-1959 (Cantral & Cohn)	5	20	UMMZ	A

S. variabile (1)	L225	Mexico, Oaxaca, 46mi W Jalapa del Marquez at Portillo Nejapam pass #37; X-31-1981 (Otte, Azuma, Newlin)	1	2	ANSP	A
S. variabile (1)	**L62**	**Mexico, Oaxaca, Carr 190 km 65; X-27-2011 (S. Sanabria & A. Díaz)**	13	16	IIES	A
S. variabile (1)	**L60**	**Mexico, Oaxaca, Pasando San Juan Lajarcia Carr 190 km 150; X-27-2011 (S. Sanabria & A. Díaz)**	9	8	IIES	A
S. variabile (1)	L226	Mexico, Oaxaca, Portillo San Dionisio; XI-21-2015 (S. Sanabria)		1	IIES	A
S. variabile (1)	L513	Mexico, Oaxaca, Entre km 55 y km54, Carr. 190; XI-21-2015 (S. Sanabria)	1	1	IIES	A
S. variabile (2)	**L93**	**Mexico, Oaxaca, Carr 175 Km 172 ; XII-12-2013 (S. Sanabria, P. Fontana & R. Mariño)**		1	IIES	A
S. variabile (2)	**L63**	**Mexico, Oaxaca, Carr 175 Km 158; XII-12-2013 (S. Sanabria, P. Fontana & R. Mariño)**	4	2	IIES	A
S. variabile (2)	**L61**	**Mexico, Oaxaca, Carr 175 Km 87 ; XII-13-2013 (S. Sanabria, P. Fontana & R. Mariño)**		10	IIES	A
S. variabile (2)	**L39**	**Mexico, Oaxaca, San Adres Paxtlan Carr 175 km 116; X-2-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**		2	IIES	A
S. variabile (2)	L400	Mexico, Oaxaca, Camino a San Pablo Coatlan 2; X-11-2015 (V. H. Jiménez)		1	IIES	A
S. adelinae **sp.n.**	L100	Mexico, Guerrero, 2mi S Tierra Colorada, Km 335 on Hwy 95; IX-19-1959 (Cantral & Cohn)	3		UMMZ	A
S. adelinae **sp.n.**	L102	Mexico, Guerrero, 16rd mi S Chilpancingo, Km 298 on Hwy 95 (Cantral & Cohn)	2	7	UMMZ	A
S. adelinae **sp.n.**	L103	Mexico, Guerrero, Tierra Colorada		1	UMMZ	A
S. adelinae **sp.n.**	L104	Mexico, Guerrero, 14mi S Chilpancingo #52; IX-4-1982 (Otte, Azuma, Newlin)		1	ANSP	A
S. adelinae **sp.n.**	**L17**	**Mexico, Guerrero, Palo Blanco Carr 95; X-24-2011 (S. Sanabria & A. Díaz)**	10	10	IBUNAM, TAMUIC, IIES	A
S. adelinae **sp.n.**	**L16**	**Mexico, Guerrero, Acahuizotla rumbo al Ocotito Carr 95; X-24-2011 (S. Sanabria & A. Díaz)**	20	25	IBUNAM, TAMUIC, IIES	A
S. adelinae **sp.n.**	**L86**	**Mexico, Guerrero, Rumbo a Tierra Colorada Carr 95; X-24-2011 (S. Sanabria & A. Díaz)**	9	7	IBUNAM, TAMUIC, IIES	A
S. crypticum **sp.n.**	**L47**	**Mexico, Guerrero, El Pinzan Morado Carr 134; X-13-2012 (S. Sanabria & A. Pingarroni)**	10	10	IBUNAM, TAMUIC, IIES	A
S. crypticum **sp.n.**	**L88**	**Mexico, Guerrero, Vallecitos Carr 134; X-13-2012 (S. Sanabria & A. Pingarroni)**	2	7	IBUNAM, IIES	A
S. crypticum **sp.n.**	L105	Mexico, Guerrero, Coyuca de Catalan		2	IBUNAM	A
S. infernalis **sp.n.**	L489	Mexico, Colima, 8mi SW Colima; XI-28-1958 (T.J. Cohn)		1	UMMZ	A
S. infernalis **sp.n.**	L492	Mexico, Colima, 6mi E Colima ; XI-29-1958 (T.J. Cohn)	1	1	UMMZ	A
S. infernalis **sp.n.**	L493	Mexico, Colima, 9mi E Colima; XI-22-1958 (T.J. Cohn)	7	7	UMMZ	A
S. infernalis **sp.n.**	L498	Mexico, Colima, Rio Naranjo ; X-2-2013 (S. Sanabria & F. Rivera)	2	1	IIES	A
S. infernalis **sp.n.**	L499	Mexico, Colima, Entrada Colima ; X-2-2013 (S. Sanabria & F. Rivera)	1	1	IBUNAM, IIES	A
S. infernalis **sp.n.**	L496	Mexico, Guerrero, 11mi S Uruapan on 37 #57; IX-8-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. infernalis **sp.n.**	L111	Mexico, Jalisco, 1mi S Pihuamo ; XI-26-1958 (T.J. Conh)	3	7	UMMZ	A
S. infernalis **sp.n.**	L488	Mexico, Jalisco, 5mi NE Tecalitlan; XI-25-1958 (T.J. Cohn)	1	1	UMMZ	A
S. infernalis **sp.n.**	L494	Mexico, Jalisco, 6mi NE Tecalitlan; XI-30-1958 (T.J. Cohn)	2	1	UMMZ	A
S. infernalis **sp.n.**	**L90**	**Mexico, Jalisco, Tecalitlan ; X-1-2013 (S. Sanabria & F. Rivera)**	3	4	IBUNAM, IIES	A
S. infernalis **sp.n.**	**L58**	**Mexico, Jalisco, Pihuamo ; X-2-2013 (S. Sanabria & F. Rivera)**	13	7	IBUNAM, IIES	A
S. infernalis **sp.n.**	L490	Mexico, Michoacan, 15mi W Apatzingan (2mi E Santa Ana); XII-2-1958 (T.J. Cohn)	3	2	UMMZ	A
S. infernalis **sp.n.**	L491	Mexico, Michoacan, 16.7mi W Apatzingan; IX-26-1959 (Cantral & Cohn)	8	11	UMMZ	A
S. infernalis **sp.n.**	L495	Mexico, Michoacan, 23rd mi WSW Ario de Rosales; XII-4-1958 (T.J. Cohn)		2	UMMZ	A
S. infernalis **sp.n.**	**L59**	**Mexico, Michoacan, El Cangrejo Carr 120 km 105; IX-22-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	7	5	IIES	A
S. infernalis **sp.n.**	**L57**	**Mexico, Michoacan, Las Majadas Carr 120; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	13	23	IBUNAM, TAMUIC,	A

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S. infernalis **sp.n.**	L497	Mexico, Michoacan, Tepalcatepec Carr 120; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)		2	IIES	A
S. infernalis **sp.n.**	**L89**	**Mexico, Michoacan, Periban ; IX-30-2013 (S. Sanabria & F. Rivera)**	17	21	IBUNAM, TAMUIC, IIES	A
S. infernalis **sp.n.**	L305	Mexico, Michoacan, 26rd mi NE Arteaga, on Hwy 37, 3rd mi SW Rancho Nuevo; XI-3-1974 (T.J. & J. W. Cohn)		1	UMMZ	A
S. miztecum **sp.n.**	**L18**	**Mexico, Oaxaca, Cerca de Pinotepa Nacional Carr 200; X-25-2011 (S. Sanabria & A. Díaz)**	6	12	IBUNAM, TAMUIC, IIES	A
S. occidentalis **sp.n.**	L299	Mexico, Guerrero, 13mi SW Tierra Colorada; XII-11-1958 (T.J. Cohn)		3	UMMZ	A
S. occidentalis **sp.n.**	L307	Mexico, Guerrero, 5mi N Acapulco; IX-15-1940 (Roberts)		1	ANSP	A
S. occidentalis **sp.n.**	L308	Mexico, Guerrero, Ridge, 2mi N Acapulco; IX-15-1940 (Roberts)		1	ANSP	A
S. occidentalis **sp.n.**	L309	Mexico, Guerrero, Acapulco		1	ANSP	A
S. occidentalis **sp.n.**	L310	Mexico, Guerrero, 15mi E Tecpan ; VIII-25-1950 (Peters)		1	ANSP	A
S. occidentalis **sp.n.**	**L09**	**Mexico, Guerrero, Salida Acapulco cerca de Rio Papagayo Carr 200; X-25-2011 (S. Sanabria & A. Díaz)**	7	12	IBUNAM, TAMUIC, IIES	A
S. occidentalis **sp.n.**	**L08**	**Mexico, Guerrero , Las Peñitas ; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	20	15	IBUNAM, TAMUIC, IIES	A
S. occidentalis **sp.n.**	L319	Mexico, Guerrero , Carr 37D 13Km antes de frontere con Michoacán; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)	10	12	IIES	A
S. occidentalis **sp.n.**	L300	Mexico, Michoacan, 23mi E Nueva Italia, on La rd. rd.; IX-25-1959 (Cantral & Cohn)	1	1	UMMZ	A
S. occidentalis **sp.n.**	L301	Mexico, Michoacan, 31mi S Nueva Italia, on Arteaga rd.; IX-26-1959 (Cantral & Cohn)	1	1	UMMZ	A
S. occidentalis **sp.n.**	L302	Mexico, Michoacan, 11mi SW La Huacana; XII-4-1958 (T.J. Cohn)	2	1	UMMZ	A
S. occidentalis **sp.n.**	L303	Mexico, Michoacan, 20mi E Nueva Italia, on La Huacana rd.; IX-25-1959 (Cantral & Cohn)	10	14	UMMZ	A
S. occidentalis **sp.n.**	L304	Mexico, Michoacan, 3rd mi SW Arteaga church, 0.1mi E Hwy 37; XI-3-1974 (T.J. & J. W. Cohn)		2	UMMZ	A
S. occidentalis **sp.n.**	L305	Mexico, Michoacan, 26rd mi NE Arteaga, on Hwy 37, 3rd mi SW Rancho Nuevo; XI-3-1974 (T.J. & J. W. Cohn)	8	6	UMMZ	A
S. occidentalis **sp.n.**	L306	Mexico, Michoacan, 7.2mi NE Playa Azul, on Hwy 37, 2.8mi NE la mira jct ; XI-6-1974 (T.J. & J. W. Cohn)		3	UMMZ	A
S. occidentalis **sp.n.**	L311	Mexico, Michoacan, 15mi W Caleta de Campos #62; IX-10-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. occidentalis **sp.n.**	L312	Mexico, Michoacan, 12mi W Caleta de Campos #63; IX-10-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. occidentalis **sp.n.**	L313	Mexico, Michoacan, 26mi n La Mira #59; IX-8-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. occidentalis **sp.n.**	L314	Mexico, Michoacan, Few mi N Arteaga #38; IX-8-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. occidentalis **sp.n.**	L315	Mexico, Michoacan, 16-20km NE Rt 200, Ixtapa-Altamirano Rd. #60; IX-9-1981 (Otte, Azuma, Newlin)	1	1	ANSP	A
S. occidentalis **sp.n.**	L316	Mexico, Michoacan, Preza el Infiernillo ; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)		1	IIES	A
S. occidentalis **sp.n.**	L317	Mexico, Michoacan, Carr 37D Km 236; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)		1	IIES	A
S. occidentalis **sp.n.**	**L07**	**Mexico, Michoacan, Zicuiran Carr 120 Km 144; IX-22-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	10	10	IBUNAM, TAMUIC, IIES	A
S. occidentalis **sp.n.**	**L85**	**Mexico, Michoacan, Cuchurumuco ; IX-22-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	10	10	IBUNAM, TAMUIC, IIES	A
S. occidentalis **sp.n.**	L318	Mexico, Michoacan, Caseta Las Cañas Carr 37D; IX-23-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)	5	2	IIES	A
S. tarascum **sp.n.**	L329	Mexico, Michoacan, 0.3mi S Tzitzio; IX-24-1959 (Cantral & Cohn)		1	UMMZ	A
S. tarascum **sp.n.**	**L44**	**Mexico, Michoacan, Las cañas rumbo a Ario de Rosales Carr 120 ; XI-1-2010 (S. Sanabria)**	2	5	IIES	A
S. tarascum **sp.n.**	**L95**	**Mexico, Michoacan, Tzitzio Carr 140; X-7-2012 (S. Sanabria, A. Blanco, P. Gómez & A. Pingarroni)**	1	2	IIES	A
S. tarascum **sp.n.**	L330	Mexico, Michoacan, Las cañas, sobre Carr. 120; X-8-2015 (S. Sanabria)	10	10	IBUNAM, TAMUIC,	A

					IIES	
S. tarascum **sp.n.**	L331	Mexico, Michoacan, Rancho El Mirador, sobre Carr 120; X-8-2015 (S. Sanabria)	1	3	IBUNAM, TAMUIC	A
S. totonacum **sp.n.**	L482	Mexico, Puebla, Huauchinango	1		CAFESI	A
S. totonacum **sp.n.**	L501	Mexico, Veracruz, Km 21, Carr. 131, ca. 7km SO de Tlapacoyan; IX-19-2015 (S. Sanabria)	3	3	IBUNAM	A
S. totonacum **sp.n.**	L502	Mexico, Veracruz, Tlapacoyan, Eytepequez; XI-9-1995 (Delgadillo J.)	4	1	IBUNAM	A
S. totonacum **sp.n.**	L503	Mexico, Veracruz, Tlapacoyan	2	1	CAFESI	A
S. totonacum **sp.n.**	L504	Mexico, Veracruz, Tlapacoyan, Rancho La Tomata; I-3-1984	1		CAFESI	A
S. totonacum **sp.n.**	**L19**	**Mexico, Veracruz , Plan de Hayas ; XI-3-2012 (S. Sanabria & V.H. Jimenéz)**	12	16	IBUNAM, TAMUIC, IIES	A
S. zapotecum **sp.n.**	L169	Mexico, Oaxaca, 24-25mi N Pto Escondido Rd to Oaxaca #45; IX-2-1981 (Otte, Azuma, Newlin)	1	2	ANSP	A
S. zapotecum **sp.n.**	L516	Mexico, Oaxaca, Oaxaca road ca. 85km N Pto Angel #43; IX-1-1981 (Otte, Azuma, Newlin)		1	ANSP	A
S. zapotecum **sp.n.**	L517	Mexico, Oaxaca, Copalita Carr 175 ; X-2-2012 (S. Sanabria & F. Rivera)	1	1	IIES	A
S. zapotecum **sp.n.**	**L91**	**Mexico, Oaxaca, Plum a Hidalgo 1 ; XII-11-2013 (S. Sanabria, P. Fontana & R. Mariño)**	3	3	IBUNAM, TAMUIC, IIES	A
S. zapotecum **sp.n.**	**L92**	**Mexico, Oaxaca, Pluma Hidalgo 2 ; XII-11-2013 (S. Sanabria, P. Fontana & R. Mariño)**	1	4	IIES	A
S. zapotecum **sp.n.**	L518	Mexico, Oaxaca, Carr 175 Km184 ; XII-12-2013 (S. Sanabria, P. Fontana & R. Mariño)	2	1	IIES	A
S. zapotecum **sp.n.**	**L93**	**Mexico, Oaxaca, Carr 175 Km 172 ; XII-12-2013 (S. Sanabria, P. Fontana & R. Mariño)**	2	2	IBUNAM, TAMUIC, IIES	A

A, used records to construct distribution maps; B, records not accurately located; C, suspicious records of probable mislabelled specimens.

Capítulo 2: Body size adaptations to altitudinal climatic variation in Neotropical grasshoppers of the genus *Sphenarium* (Orthoptera: Pyrgomorphidae)

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RESEARCH ARTICLE

Body Size Adaptations to Altitudinal Climatic Variation in Neotropical Grasshoppers of the Genus *Sphenarium* (Orthoptera: Pyrgomorphidae)

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Abstract

Altitudinal clines in body size can result from the effects of natural and sexual selection on growth rates and developing times in seasonal environments. Short growing and reproductive seasons constrain the body size that adults can attain and their reproductive success. Little is known about the effects of altitudinal climatic variation on the diversification of Neotropical insects. In central Mexico, in addition to altitude, highly heterogeneous topography generates diverse climates that can occur even at the same latitude. Altitudinal variation and heterogeneous topography open an opportunity to test the relative impact of climatic variation on body size adaptations. In this study, we investigated the relationship between altitudinal climatic variation and body size, and the divergence rates of sexual size dimorphism (SSD) in Neotropical grasshoppers of the genus *Sphenarium* using a phylogenetic comparative approach. In order to distinguish the relative impact of natural and sexual selection on the diversification of the group, we also tracked the altitudinal distribution of the species and trends of both body size and SSD on the phylogeny of *Sphenarium*. The correlative evidence suggests no relationship between altitude and body size. However, larger species were associated with places having a warmer winter season in which the temporal window for development and reproduction can be longer. Nonetheless, the largest species were also associated with highly seasonal environments. Moreover, large body size and high levels of SSD have evolved independently several times throughout the history of the group and male body size has experienced a greater evolutionary divergence than females. These lines of evidence suggest that

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natural selection, associated with seasonality and sexual selection, on maturation time and body size could have enhanced the diversification of this insect group.

Introduction

Body size relates to many aspects of an organism's biology, such as local adaptations to different climatic conditions, female fecundity and male mating success [1]. Local adaptations to different climatic conditions (e.g. temperature and season length) affect body size through the regulation of growth rates and development times [2,3]. Growth rates are positively affected by temperature, food quantity and quality [4]; whereas development time is largely constrained by seasonality, which determines the duration of optimal developmental conditions, such as temperature and food availability [5,6]. The regulation of growth rates and development times are under strong natural selection due to seasonality. Generally, seasonality increases with elevation, constraining the available time for development and reproduction [7,8]. This can favor the evolution of shorter development times, decreased time to reach maturity, and smaller adult body size at high elevations than in lowlands [7–10]. Nonetheless, the fitness benefits of decreasing the time to reach maturity may be counterbalanced by the costs of small size on the reproductive success of females and males [10–12].

Female fecundity and male mating success usually increase with body size [13–15]. However, the size that conveys maximal fitness (i.e. the optimal body size) often differs between the sexes, thus generating sexual size dimorphism (SSD). Male-biased SSD can result when male mating success increases with size due to male–male competition or female choice [16], or due to allocation to reproductive reserves, such as nuptial gifts or ejaculate size [17]. Conversely, female-biased SSD results when large females have higher fecundity [15], or small males have advantages in mate searching or courting due to a higher agility [18–20]. In most taxa examined for these relationships, strong correlations between the sexes have been found (typically > 0.9) [16,21], which are likely to arise because of high genetic correlations between males and females [22]. Despite these high correlations, the magnitude of SSD often varies considerably among closely related species, indicating some independence in the evolutionary trajectories of body size between the sexes [23].

In many taxa, the magnitude of SSD changes systematically with mean body size, either increasing or decreasing as body size increases [21,24,25]. The former pattern is common in taxa where males are larger than females, while the latter occurs in some species in which females are the larger sex. Both patterns are explained by greater evolutionary divergence in male size, compared with female size; such pattern is known as Rensch's rule [23,24]. For many taxa this allometric trend can be attributed to sexual selection acting on male body size [3,21,26]. The converse trend, where female size varies more than male size, is less common, but seems to be the result of strong fecundity selection acting on females [25,27,28].

Despite the evolutionary implications of natural selection and sexual selection on population differentiation and speciation, few studies have explored the relative impact of altitudinal climatic variation on the diversification of Neotropical insects [7,8,29–31]. Regions with topographical heterogeneity provide opportunities to study the adaptive value of body size in response to climatic variables [8,31,32]. In these regions, seasonality, temperature, and precipitation regimes can vary considerably with altitude, allowing the settlement of different climates in short distances [8,33]. This makes local adaptations possible only if selection is strong enough to neutralize the expected continual gene flow from adjacent populations [32]. Most comparative studies addressing the interspecific variation of body size and SSD in relation to altitudinal climatic gradients have not considered the phylogenetic relationships among species [34], which allows separating the effects of a common evolutionary history from the relative impact of natural and sexual selection [35].

In this study, we investigate the relationship between altitudinal climatic variation and body size, and the divergence rates of body size between females and males in Neotropical grasshoppers of the genus *Sphenarium* (Orthoptera: Pyrgomorphidae), taking into account their phylogenetic relationships. Because the season length limits the body size grasshoppers can achieve, we would expect large adult sizes at lower elevations associated with high temperature and precipitation regimens. In addition, if sexual selection acting on male body size has been stronger than fecundity selection acting on females, a greater evolutionary divergence in male size than in female size would be expected.

Methods

Study species

The genus *Sphenarium* Charpentier, 1842 is distributed from central Mexico to northwestern Guatemala and represents the most diverse group of the American Pyrgomorphidae [36,37]. Currently, eight taxa are recognized within this genus: *S. mexicanum mexicanum*, *S. mexicanum histrio*, *S. purpurascens purpurascens*, *S. purpurascens minimum*, *S. borrei*, *S. macrophallicum*, *S. rugosum* and *S. variabile* [38]. These species are flightless, polyphagous, and univoltine [37]. Their nymphs emerge principally in the beginning of the rainy season (around mid-May) and adults die in the winter (from mid-December to mid-February) [39–42].

Sphenarium grasshoppers represent a good model system to explore the relative impact of altitudinal climatic variation on body size adaptations. These grasshoppers have a wide altitudinal distribution, ranging from the sea level to approximately 2600 m, across the climatically heterogeneous Mexican topography, with extensive inter- and intraspecific body size variation [36,37,43]. Moreover, males and females of this genus are highly dimorphic (e. g. the thorax is wider in females and forelegs are wider in males). In addition, in *S. purpurascens*, maturation time and body size are under strong natural and sexual selection [44,45], and there is a positive relationship between body size and fecundity [46].

Ethical statement

In Mexico grasshoppers specimens were collected under the permit SGPA/DGVS/032887/13 issued by Secretaría de Medio Ambiente y Recursos Naturales; Dirección General de Vida Silvestre. Samples from Guatemala were provided by the Universidad del Valle de Guatemala Collection of Arthropods and exported through the permit granted to Enio Cano by Consejo Nacional de Áreas Protegidas. The Dominican Republic government through Ministerio del Medio Ambiente y Recursos Naturales provided the necessary permits for collecting and exportation in Jaragua National Park to Hojun Song. The field studies did not involved endangered or protected species.

Fieldwork

Between 2008 and 2013 we collected *Sphenarium* grasshoppers from 63 localities across their geographic distribution throughout central and southern Mexico. Collection sites varied in elevation from 15 to 2571 m above the sea level (m.a.s.l.) (See S1 Table). Geographic position and elevation of each locality was recorded during fieldwork with a GPS-map 60CSx (Garmin, Kansas City, USA). All collected specimens were stored at -80°C in individual vials and vouchered

(See S1 Table) in the Laboratory of Genetic and Molecular Ecology, IIES [Universidad Nacional Autónoma de México (UNAM), Morelia] and Laboratory of Ecology, UBIPRO (FES-Iztacala; UNAM). Specimens are available upon request to the corresponding author.

In this study we included the eight recognized taxa of *Sphenarium*, as well as the two intermediate forms (between *S. p. purpurascens* and *S. p. minimum*; and between *S. m. mexicanum* and *S. m. histrio*) identified by Boyle [36] and Kevan [37]. Our taxonomic identifications were based on the most recent taxonomic work for the genus [36,37] and by comparing our samples with identified museum specimens and types housed at UNAM's Collection of Insects (Mexico City), the University of Michigan Museum of Zoology (Ann Arbor, USA), and the Academy of Natural Sciences of Drexel University (Philadelphia, USA). S1 Table further provides information on the taxonomic identification of collected specimens.

Acquisition of genetic information

We extracted genomic DNA from single hind femur of one to three specimens from each sampled locality using Qiagen DNeasy kit (Qiagen, Valencia, USA). We amplified fragments of three mitochondrial loci [*Cytochrome c Oxidase subunit 1* (CO1) and *subunit 2* (CO2), and the *12 Subunit of ribosomal RNA* (12S)] and two nuclear loci [*Histone 3* (H3) and the *Internal Transcribed Spacer between 5.8S rRNA and 28S rRNA* (ITS2)]. For mitochondrial loci, we followed the recommendations of Song et al. [47] to avoid co-amplification of nuclear mitochondrial pseudogenes. Two long fragments of 3600 (F1) and 2100 (F2) base pairs (bp) of the mitochondrial genome were amplified and used as templates for nested-PCR amplifications of mitochondrial targets (F1 for CO1 and CO2; and F2 for 12S). We provide information about the primers used on Table 1. All PCR reactions were performed using Elongase Enzyme mix (Invitrogen Corporation, Carlsbad, USA) following the manufacturer's recommendations. For nested-PCRs we used as a template: 1:10 dilution for each Long-PCR product (F1 and F2). Long-PCR conditions included 2 min of initial denaturation at 92°C; 39 cycles of 92°C for 30 s, 50°C for 30 s, 60°C for 5 min; and a final extension at 60°C for 20 min. Other PCR reactions were performed using an initial denaturation at 92°C for 2 min, followed by 34 cycles of 92°C for 30 s, 30 s at the specific Tm for each primer combination (see Table 1), 2 min at 60°C; and a final extension at 60°C for 10 min. Single-band PCR products were purified using PrepEase Purification 96-well plate kit (USB Corporation, Santa Clara, USA) and samples containing more than a single band were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, USA).

We sequenced both strands of each purified products using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using Applied Biosystems 3730XL DNA Analyzer (Applied Biosystems, Foster City, USA). We analyzed forward and reverse sequences of each sample with SEQUENCHER v. 4.2 (Gene Codes Corporation, Ann Arbor, USA) and aligned them in MUSCLE [52], using default parameters. We translated and checked for stop-codons the coding loci sequences (CO1, CO2 and H3) using MEGA v. 6.0.6 [53]. All sequences were deposited on GenBank (see S1 Table for accession numbers).

In addition, we incorporated genetic information from the closest relatives of *Sphenarium* in America (including *Prosphena scudderi* and *Jaragua oviedensis*) and Asia (including *Mekongiana xiangchengensis*, *Mekongiella kingdoni*, *M. xizangensis* and *Yunnanites coriacea*) [37,54], as well as other American Pyrgomorphidae (*Pyrgotettix pueblensis*, *Sphenotettix nobilis*, *Sphenacris crassicornis*) and Acridoidea (*Schistocerca gregaria gregaria*). Genetic information of these outgroup taxa were primarily obtained for the present study or by retrieving the available information from the GenBank (See S1 Table for outgroup species information).

We constructed a dataset comprising the total genetic information obtained from 67 ingroup and 15 outgroup individuals. We subdivided this dataset in 11 partitions corresponding to the

Table 1. List of amplified loci indicating their approximate size (bp), annealing temperature (Tm) and the pairs of primers used for the PCR reactions.

Loci	Size (bp)	Tm (°C)	Primer [A]	Sequence (5'-3')
F1	3600	50	ORMET [48]	CATAAGCTAATGGGTTCATAC
			ORRLYS [48]	GAGACCAGTACTTGCTTTCAGTCATC
F2	2100	50	OR16SN [B]	AGAAACCGACCTGGCTCACGC CGG
			OR12SN [B]	CGTGCCAGCAGCCGCGGTTATACG
CO1	1180	58	SPHCO1F [C]	TAGATCATCAATGGTTAATACAGG
			SPHCO1R [C]	CTGATATGAGTGTTCTGCAGGAGG
CO2	550	58	C2J3138 [49]	GGAGCTTCACCATTAATAGAACA
			C2N3661 [49]	CCACAAATTTCTGAACATTGACCA
12S	360	58	SRJ14233 [49]	AAGAGCGACGGGCGATGTGT
			SRN14588 [49]	AAACTAGGATTAGATACCCTATTAT
H3	329	60	HexAF [50]	ATGGCTCGTACCAAGCAGACGGC
			HexAR [50]	ATATCCTTGGGCATGATGGTGAC
ITS2	320	60	CAS5p8sFc [51]	TGAACATCGACATTTYGAACGCACAT
			CAS28sB1d [51]	TTCTTTTCCTCCSCTTAYTRATATGCTTAA

[A] source of primers is indicated within brackets and superscript letters.

[B] designed by H. Song

[C] designed by S. Sanabria-Urbán

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1st, 2nd and 3rd codon positions of each coding loci (CO1, CO2 and H3) and the two non-coding loci (12S and ITS2). We estimated the best partitioning scheme for this dataset and models of nucleotide substitution for each partition using the greedy algorithm implemented in PARTITIONFINDER v. 1.1.1 [55]. The final dataset was subdivided in seven partitions (P1-P7) corresponding to the 1st codon position of CO1 (P1), the 2nd codon position of CO1 and CO2 (P2), the 3rd codon position of CO1 and CO2 (P3), the 1st codon position of CO2 and the 12S locus (P4), the 1st and 2nd codon position of H3 (P5), the 3rd codon position of H3 (P6), and the ITS2 locus alone (P7). We individually applied five substitution models to each partition: JC+I for P5, HKY+G for P6 and P7, HKY+I+G for P2, GTR+G for P1 and P3 and GTR+I+G for P4.

Phylogenetic reconstruction

We conducted a concatenated Bayesian inference (BI) analysis in MRBAYES v. 3.2.6 [56] with the total genetic evidence dataset obtained, applying the specific substitution model estimated for each partition. This analysis consisted of four independent runs, each of them with 10,000,000 generations and four chains, sampling each 1000 generations. We used default priors for other parameters in the analysis. We assessed parameter convergence and proper mixing of independent runs using TRACER v.1.6 [57]. We also discarded 25% of the samples obtained prior to stability as burn-in.

This initial phylogenetic analysis (Fig 1) indicated that current taxonomic classification [38] of *Sphenarium* species did not reflect their evolutionary relationships. Most currently recognized species were paraphyletic, and only *S. borrei* and *S. p. minimum* were recovered as monophyletic taxa. In addition, we identified three broad patterns of divergence in *Sphenarium*. The first pattern was that some taxa were well-defined by male genital morphology, despite the fact that they were genetically close. The second pattern was that our molecular data revealed cryptic diversity among genetic lineages that were morphologically similar. Finally, the third pattern was that some taxa were both morphologically and genetically differentiated.

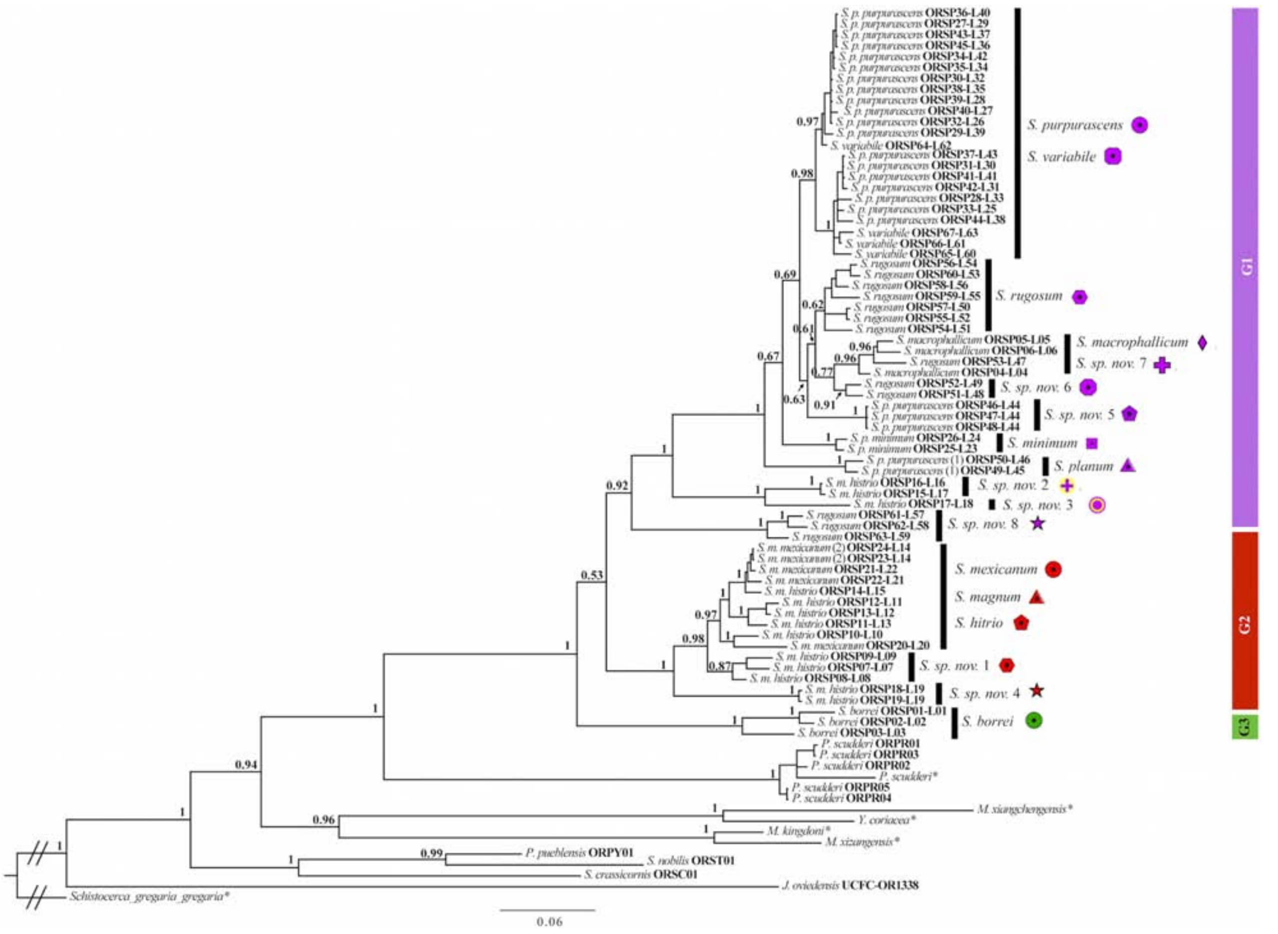

Fig 1. Phylogeny based on a concatenated Bayesian analysis of the total genetic evidence retrieved from 67 *Sphenarium* and 15 outgroup taxa. Tip labels indicate current taxonomic classification, voucher numbers and locality ID for all included terminals, except for those whose genetic information was retrieved from GenBank (*). Black vertical bars indicate the phylogenetic position of the identified species based on our integrative taxonomy approach (names and coloured symbols in front of the black bars).

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In order to define the taxonomic units for our comparative analysis, we adopted an integrative taxonomy framework in identifying lineages that would potentially represent valid species within *Sphenarium*. Specifically, we recognized as species those taxa that could be consistently identified on the basis of their male genital morphology, as noted previously by Boyle [36] and Kevan [37]. We followed this criterion because the male genitalia morphology is widely used in defining species concepts in grasshoppers [58]. Moreover, these morphological species shared no mitochondrial haplotype in our genetic dataset despite they were genetically close. We also recognized as different species those specimens that formed well-supported monophyletic groups (posterior probability values higher than 0.85) and/or divergent lineages that were geographically structured in the concatenated phylogenetic reconstruction (Fig 1). These identified genetic species also diverged from other species with *P-distance* values greater than 2% in pairwise comparisons of their *CO1* sequences (See S2 Table), which is concordant with the *CO1* interspecific levels of differentiation observed in other invertebrates [59]. Finally, we considered as species those taxa that were both morphologically and genetically differentiated. After we identified the lineages that could represent valid species within *Sphenarium* using the integrative taxonomy framework (see Results section for details), we used this taxonomic classification for further analyses.

Particularly, the black vertical bars indicating the phylogenetic position of *S. purpurascens*, *S. variabile*, *S. macrophallicum*, *S. sp. nov. 7*, *S. mexicanum*, *S. magnum* and *S. histrio* represent cases where species differentiation was primarily morphological and they did not separate in individual monophyletic groups (See methods and results sections for details). The numbers positioned closely to the nodes indicate posterior probability values. G1, Monophyletic Group 1; G2, Monophyletic Group 2; G3, Monophyletic Group 3. *S. p. purpurascens* (1) intermediate form between *S. p. purpurascens* and *S. p. minimum*. *S. m. mexicanum* (2) intermediate form between *S. m. mexicanum* and *S. m. histrio*.

We also estimated a species tree of the *Sphenarium* taxa identified using the multilocus coalescent-based Bayesian approach implemented in *BEAST v. 1.8.1 [60]. This species tree approach incorporates uncertainty associated with gene trees due to incomplete lineage shorting, nucleotide substitution model parameters and coalescent process [61]. For this analysis, we used a smaller dataset comprising all nucleotide sequences of *Sphenarium* (67 individuals) and *P. scudderi* (6 individuals), which were recovered as sister taxa in our previous concatenated phylogenetic reconstruction. We used the same partitioning scheme and nucleotide substitution models previously specified. We applied an uncorrelated relaxed clock with lognormal distribution and set a Birth Death Model as the tree prior, using a Piecewise linear and constant root for population size prior. We set the length of the Markov chain Monte Carlo at 100 million generations, sampling every 10,000 generations and two independent runs. We verified parameter convergence and proper mixing of the independent runs using TRACER 1 v 1.6 [57]. We constructed a consensus species tree setting a burn-in at 25%.

Estimation of climatic parameters

Adult body size is affected by seasonality, temperature, and food availability. Food availability for primary consumers in food webs relies on plant primary production, which strongly depends on precipitation regimens [62,63]. For this reason, we considered the mean regional temperature and precipitation parameters associated with each collecting site. We obtained Mean Temperatures of the Wettest (MTWT) and the Coldest Trimesters (MTCT); and Mean Annual Precipitation (MAP) and Temperature Seasonality (TS) values from high-resolution monthly climate surfaces for Mexico [64] (Table 2). TS represents the amount of temperature variation over a year based on the ratio of the standard deviation of the monthly mean temperatures to the mean monthly temperature (also known as the coefficient of variation, CV). Thus, the larger the TS values the greater the variability of the temperature [65]. Similar climatic parameters have been used in other studies on body size variation in altitudinal clines [e.g. 34]. On the other hand, we used MTWT and MTCT since they encompass approximately the temperature regimens associated with *Sphenarium* life cycles, from the beginning of the rainy season (MTWT) to the beginning of the winter (MTCT). For each identified species, we estimated mean values of elevation and climatic parameters considering the collecting point information of each individual within the taxa (Table 2). All values, except for TS, were log-transformed for subsequent analysis.

Morphological measurements

Using a digital calliper (Mitutoyo Corp., Tokyo, Japan), we measured Femur I Width, Femur III Length, and Thorax Length and Width of each collected adult male and female of

Table 2. Mean values of elevation and climatic parameters of the identified *Sphenarium* species.

Species	*N*	NL	Elevation (m.a.s.l.)	TS (CV)	MTWT (°C)	MTCT (°C)	MAP (mm)
S. borrei	50	3	1477.24	0.82	21.67	16.44	950.57
S. histrio	91	5	671.32	0.50	23.76	20.98	1011.40
S. macrophallicum	60	3	953.66	0.65	24.17	21.56	1047.60
S. magnum	58	1	68.13	0.50	28.40	25.55	878.23
S. mexicanum	48	3	134.75	0.73	26.35	22.30	1901.24
S. minimum	33	2	1458.96	0.68	18.92	14.73	1725.30
S. planum	24	2	1760.83	0.69	19.75	15.15	476.74
S. purpurascens	3967	19	2147.70	0.72	17.79	13.36	749.93
S. rugosum	790	8	1635.25	0.54	20.01	17.49	1017.38
S. variabile	63	4	1539.10	0.48	19.42	16.90	697.75
S. sp. nov. 1	59	3	111.25	0.49	27.99	25.34	956.02
S. sp. nov. 2	62	2	1073.74	0.46	23.41	21.22	1338.79
S. sp. nov. 3	17	1	216.00	0.40	26.32	24.80	1421.49
S. sp. nov. 4	27	1	1145.00	0.83	22.14	16.88	1356.36
S. sp. nov. 5	7	1	1336.00	0.53	21.44	18.93	1189.35
S. sp. nov. 6	23	2	1619.82	0.59	20.48	17.74	955.05
S. sp. nov. 7	20	1	730.00	0.53	24.84	23.37	1141.21
S. sp. nov. 8	42	3	621.30	0.60	25.88	22.87	987.43

n, number of individuals considered; NL, number of localities considered; TS, Temperature Seasonality; MTWT, Mean Temperature of The Wettest Trimester; MTCT, Mean Temperature of The Coldest Trimester; MAP, Mean Annual Precipitation; CV, Coefficient of Variation.

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Sphenarium from the 63 sampled localities. These traits are known to be under natural and sexual selection in *S. purpurascens* [44,45]. In this study we assumed that body size was positively correlated with maturation time in all species. This assumption is true for at least two species in the genus, *S. purpurascens* and *S. histrio* (Cueva del Castillo, *Obs. Pers*.). Considering the total number of individuals for each taxon, we averaged the values of the four morphological traits per species and sex (Table 3). These values were then log-transformed before they were used in the comparative analyses. In *Sphenarium* species Femur I is larger in males than females, whereas thorax width and length are larger in females than males. However, Femur III shows a mixed SSD pattern (see below). Due to this interspecific variation in morphological traits, we used the Lovich and Gibbons Sexual Dimorphism Index (SDI) [66] to estimate the magnitude and direction of SSD in *Sphenarium*. We obtained the SDI for each morphological trait and species (Table 3) dividing females' (the larger) on males' (the shorter) mean trait values, and then subtracting 1. Thus, positive values indicate female biased SSD and negative values indicate male biased SSD (Table 3).

Comparative analyses

To test whether climatic and elevation variables influenced male and female body size, we fitted Generalized Linear Mixed Models (GLMMs) using Markov Chain Monte Carlo algorithms, as implemented in the R [67] package 'MCMCglmm' [68]. The package makes use of the flexible and widely employed GLMMs whilst marginalizing the random effects in a robust manner (compared to other, currently available packages). For models including phylogenetic effects, such as ours, a vector containing a tree topology must be associated with the inverse relationship matrix A^{-1}. This matrix is, in turn, formed by assigning the tree topology to the pedigree argument of MCMCglmm [68]. The method has been used in a variety of studies, such as to

Table 3. Mean values of body size measurements and Sexual Dimorphism Index (SDI) of identified *Sphenarium* species. Numbers in bold and underlined indicate maximum and minimum mean values observed for each trait measured, respectively.

	n		**Females (mm)**				**Males (mm)**				**Species (mm)**				**Sexual dimorphism index (♀/♂)**			
Species	♀	♂	FIW	FIIIL	TL	TW	FIW	FIIIL	TL	TW	FIW	FIIIL	TL	TW	FIW	FIIIL	TL	TW
S. borrei	24	26	1.07	13.64	7.21	9.56	1.28	12.94	5.75	6.74	1.18	13.27	6.45	8.09	-0.16	0.05	0.25	0.42
S. histrio	46	45	1.05	14.19	7.1	9.1	1.31	12.97	5.74	6.39	1.17	13.59	6.43	7.76	-0.20	0.09	0.24	0.42
S. macrophallicum	30	30	1.19	15.27	8	9.96	1.53	14.67	7.2	7.96	1.36	14.97	7.6	8.96	-0.22	0.04	0.11	0.25
S. magnum	32	26	1.18	15.99	7.33	9.51	1.62	15.7	6.77	7.69	1.39	15.86	7.07	8.67	-0.27	0.02	0.08	0.24
S. mexicanum	24	24	**1.38**	**18.51**	**9**	**11.42**	**1.76**	**16.73**	**7.59**	8.42	**1.57**	**17.62**	**8.29**	9.92	-0.22	0.11	0.19	0.36
S. minimum	16	17	0.99	12.85	6.62	8.82	1.17	11.45	4.96	5.77	1.08	12.13	5.76	7.25	-0.15	0.12	0.33	0.53
S. planum	12	12	0.96	12.33	6.66	9.08	1.11	10.69	4.77	5.87	1.03	11.51	5.71	7.48	-0.14	**0.15**	**0.40**	**0.55**
S. purpurascens	1797	2170	0.85	12.07	6.02	7.94	1.3	12.62	5.54	6.64	1.09	12.37	5.76	7.23	**-0.35**	-0.04	0.09	0.20
S. rugosum	378	412	1.08	14.62	7.06	9.04	1.44	14.38	6.5	7.64	1.27	14.49	6.77	8.31	-0.25	0.02	0.09	0.18
S. variabile	29	34	0.91	11.82	5.9	8.8	1.17	10.95	4.74	5.99	1.05	11.35	5.27	7.28	-0.22	0.08	0.24	0.47
S. sp. nov. 1	27	32	1.29	17.15	8.6	10.56	1.62	15.67	7.18	8.13	1.47	16.35	7.83	9.24	-0.20	0.09	0.20	0.30
S. sp. nov. 2	29	33	1.14	14.59	7.78	9.98	1.45	14.05	6.47	7.68	1.31	14.3	7.08	8.76	-0.21	0.04	0.20	0.30
S. sp. nov. 3	6	11	1.02	13.8	6.93	8.89	1.27	12.76	5.8	6.51	1.18	13.13	6.2	7.35	-0.20	0.08	0.19	0.37
S. sp. nov. 4	10	17	1.25	15.22	8.06	10.98	1.57	14.45	6.85	8.42	1.46	14.74	7.29	9.36	-0.20	0.05	0.18	0.30
S. sp. nov. 5	5	2	0.89	12.59	6.04	8.13	1.13	11.74	4.89	5.96	0.96	12.34	5.71	7.51	-0.21	0.07	0.24	0.36
S. sp. nov. 6	10	13	0.99	13.41	6.82	9.19	1.31	12.67	5.63	6.84	1.17	12.99	6.15	7.86	-0.24	0.06	0.21	0.34
S. sp. nov. 7	10	10	1.28	15.94	8.38	10.99	1.65	15.33	7.59	**8.89**	1.46	15.63	7.98	**9.94**	-0.22	0.04	0.10	0.24
S. sp. nov. 8	23	19	1.02	13.67	6.99	8.77	1.35	13.92	6.67	7.61	1.17	13.78	6.85	8.25	-0.24	-0.02	0.05	0.15

n, number of individuals considered; FW1, Femur I width; F3L, Femur III Length; TL, Thorax Length; TW, Thorax Width; ♀/♂, measurements of females over males'.

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estimate the patterns of evolution in anuran vocal sexual signals [69] and to test Darwin's naturalization hypothesis in plants [70]. We used Femur I Width, Femur III Length, and Thorax Length and Width as dependent variables, and Sex, Elevation, TS, MTWT, MTCT and MAP as independent variables, and a vector containing the species tree topology (which resulted from the *BEAST analysis) as a random variable. Note that the latter allowed us to account for the phylogenetic non-independence of species [71]. The models fitted a univariate normal response. The full, saturated models included the first order interaction between sex and all other independent variables, and we removed non-significant interaction terms by backward elimination. Models were run for 5,500,000 iterations after a burn-in of 1000 iterations and a thinning interval of 500 iterations. The proportion of the total variance in a given model was accounted for by the random variable tree topology, which was calculated for each model. We further ensured that Effective Sampling Sizes (ESS) were adequate (> 10000). The significance of the predictors was determined when the 95% credible intervals of the effect size excluded zero [e.g. 68]. Finally, for each model, we also determined the extent of the phylogenetic signal by calculating Pagel'sλ [72].

To test if the divergence of male body size has been greater than female body size in *Sphenarium* species (Rensch's rule), we used the phylogenetic independent contrasts method [35], as implemented by the R package 'caper' [73], to control for the phylogenetic non-independence of species [71]. Since outliers can seriously affect the parameter estimates for any regression model we removed automatically outliers with studentized residuals $> \pm 3$ [74]. A key assumption of the contrasts method is that the standardized contrasts are independent from their estimated nodal values [35]. This assumption was verified by plotting the standardized contrasts against their estimated nodal values using the 'plot' function provided by 'caper'. We then tested the allometric relationship between *log*(male) (dependent variable) and *log*(female) (independent variable) body size (Femur I Width, Femur III Length, Thorax Width and Length) by fitting four major axis regressions (model II regression, MA [75]) using the phylogenetic independent contrasts [76]. Rensch's rule predicts the slope of male on female size to be significantly larger than 1. Since the mean value of contrasts is expected to be zero, the MA regression was forced through the origin [70]. We provided the slope of major axis regressions (β), as well as their 95% lower and upper confidence intervals, which were calculated using the R package 'smatr' [77,78].

Results

Genetic data

We successfully sequenced 1065 bp of CO1, 486 bp of CO2, 336 bp of 12S, 318 bp of H3 and 311 bp of ITS2. For some individuals, mostly outgroup taxa, we obtained shorter CO2, 12S and ITS2 sequences because indels were present. We could not obtain reliable sequences from all five loci for some individuals. In some cases, multiple copies (for ITS2) or pseudogenes (for H3 and CO2) were detected. The number of sequences per locus obtained was as follows: 76 for CO1, 54 for CO2, 58 for 12S, 49 for H3 and 41 for ITS2. The final dataset for the phylogenetic analysis comprised 82 terminals and 2,524 aligned nucleotides. In this dataset CO2 sequences showed the highest percentage of parsimony informative sites (39.8% of 1065 bp), followed by CO1 (32.7% of 486 bp), 12S (24% of 336 bp), H3 (10% of 318 bp) and ITS2 (8.2% of 311 bp).

Definition of taxonomic units

We identified a total of 18 morphologically and/or genetically distinct lineages within *Sphenarium* (Table 4). Eight lineages represented probably new species within the genus; other wise, they corresponded to previously recognized species, but in most cases they only comprised individuals from particular geographic provinces (Fig 2). Within the morphological pattern of differentiation, we identified eight species: *S. macrophallicum*, *S. variabile*, *S. mexicanum*, *S. histrio* (*S. m. histrio* localities in southern Mexico), *S. magnum* (previously synonymized within the intermediate form between *S. m. mexicanum* and *S. m. histrio*), *S. purpurascens* (*S. p. purpurascens* localities in central Mexico highlands), *S. rugosum* (*S. rugosum* localities in the eastern portion of Balsas River Basin), and *S. sp. nov. 7* (*S. rugosum* in the southern middle portion of the Balsas River Basin). Within the cryptic genetic pattern of differentiation, we identified eight species, most of them probably representing new taxa: *S. planum* (*S. p. purpurascens* localities from the Tehuacan Valley, previously synonymized within the intermediate form between *S. p. purpurascens* and *S. p. minimum*), *S. sp. nov. 1* (*S. m. histrio* localities in northern Pacific Costal Plains and western portion of the Balsas River Basin), *S. sp. nov. 2* (*S. m. histrio* localities in the Sierra Madre del Sur of Guerrero), *S. sp. nov. 3* (*S. m. histrio* found in the Pacific Costal Plain of Oaxaca), *S. sp. nov. 4* (*S. m. histrio* localities in the northeastern portion the Mexican Volcanic Belt), *S. sp. nov. 5* (*S. p. purpurascens* found in the western portion of the Balsas River Basin), *S. sp. nov. 6* (*S. rugosum* localities found in north-eastern portion of the middle Balsas River Basin) and *S. sp. nov. 8* (*S. rugosum* localities found in the northwestern portion of the Balsas River Basin). Finally, within the morphological and genetic pattern of differentiation we recognized *S. borrei* and *S. minimum* (*S. p. minimum* localities) as separate species.

Table 4. Identified *Sphenarium* species using the integrative taxonomy framework. The current taxonomic classification and localities included within each identified species are also shown.

Identified species	Pattern of differentiation	Current species [A]	Localities
S. borrei	M & G	*S. borrei*	L1—L3
S. macrophallicum	M	*S. macrophallicum*	L4—L6
S. histrio	M	*S. m. histrio*	L10—L13, L15
S. mexicanum	M	*S. m. mexicanum*	L20—L22
S. magnum	M	*S. m. mexicanum*[B]	L14
S. minimum	M & G	*S. p. minimum*	L23, L24
S. purpurascens	M	*S. p. purpurascens*	L25—L43
S. planum	G	*S. p. purpurascens*[C]	L45, L46
S. rugosum	M	*S. rugosum*	L50—L56
S. variabile	M	*S. variabile*	L60—L63
S. sp. nov. 1	G	*S. m. histrio*	L7—L9
S. sp. nov. 2	G	*S. m. histrio*	L17, L16
S. sp. nov. 3	G	*S. m. histrio*	L18
S. sp. nov. 4	G	*S. m. histrio*	L19
S. sp. nov. 5	G	*S. p. purpurascens*	L44
S. sp. nov. 6	G	*S. rugosum*	L48, L49
S. sp. nov. 7	M	*S. rugosum*	L47
S. sp. nov. 8	G	*S. rugosum*	L57—L59

M, morphological differentiation; G, genetic differentiation; M & G, morphological and genetic differentiation.

[A] taxonomic classification recognized by Eades [38] based on Boyle [36] and Kevan [37].

[B] specimens corresponding to the intermediate form between *S. m. mexicanum* and *S. m. histrio*, within which *S. magnum* was synonymized.

[C] specimens corresponding to the intermediate form between *S. p. purpurascens* and *S. p. minimum*, within which *S. planum* was synonymized.

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Phylogenetic relationships

The Bayesian analysis based on total evidence data completely resolved the higher-level phylogenetic relationships with most nodes well supported with posterior probability (*PP*) values higher than 0.94 (Fig 1). All included Pyrgomorphidae and species within the tribe Sphenariini (*P. scudderi*, *M. xiangchengensis*, *M. kingdoni*, *M. xizangensis* and *Y. coriacea*), with the exception of *J. oviedensis*, formed a monophyletic group, and a close phylogenetic relationship between most Sphenariini species and other American Pyrgomorphidae (*P. pueblensis*, *S. nobilis* and *S. crassicornis*) was identified. Despite the fact that our results indicated that the subtribe Sphenariina (comprising the genus *Sphenarium*, *Prosphena* and *Jaragua*) was paraphyletic, a sister relationship between the genus *Sphenarium* and *Prosphena*, as well as the monophyly of these genera were strongly supported (*PP* = 1).

Within the genus *Sphenarium*, we identified three major geographically structured monophyletic groups in the Concatenated Analysis (CA) and Species Tree Analysis (STA) (Fig 3). The Group 1 [*PP* = 0.92 (CA) and 0.87 (STA)] comprised species distributed in the inner basins and highlands of central Mexico (*S. machiphallicum*, *S. minimum*, *S. planum*, *S. purpurascens*, *S. rugosum*, *S. variabile*, *S. sp. nov. 2*, *S. sp. nov. 3* *S. sp. nov. 5*, *S. sp. nov. 6*, *S. sp. nov. 7* and *S. sp. nov. 8*). The Group 2 [*PP* = 1 (CA and STA)] included the species distributed in the costal lowlands of central and southern Mexico, and Sierra Madre de Chiapas (*S. histrio*, *S. magnum*, *S. mexicanum*, *S. sp. nov. 1* and *S. sp. nov. 4*). The Group 3 [*PP* = 1 (CA and STA)] comprised solely *S. borrei* lineages restricted to northwestern portions of the Mexican Volcanic Belt (see Fig 2 for species distribution and Fig 3 for phylogenetic relationships).

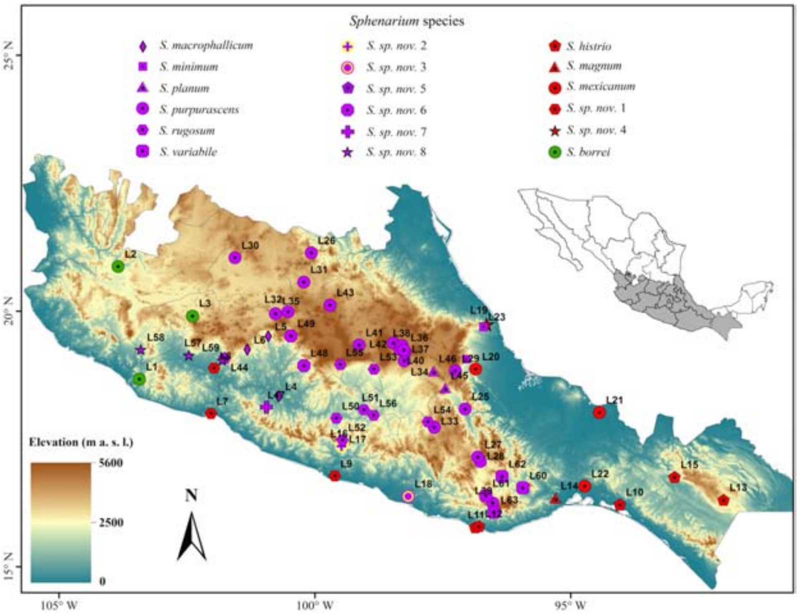

Fig 2. Sampling localities of *Sphenarium* (L1-L63) included in this study and geographic distribution of the 18 identified species within the genus. This map is based on the digital elevation model developed for Mexico, including only the Mexican states where the genus *Sphenarium* is distributed.

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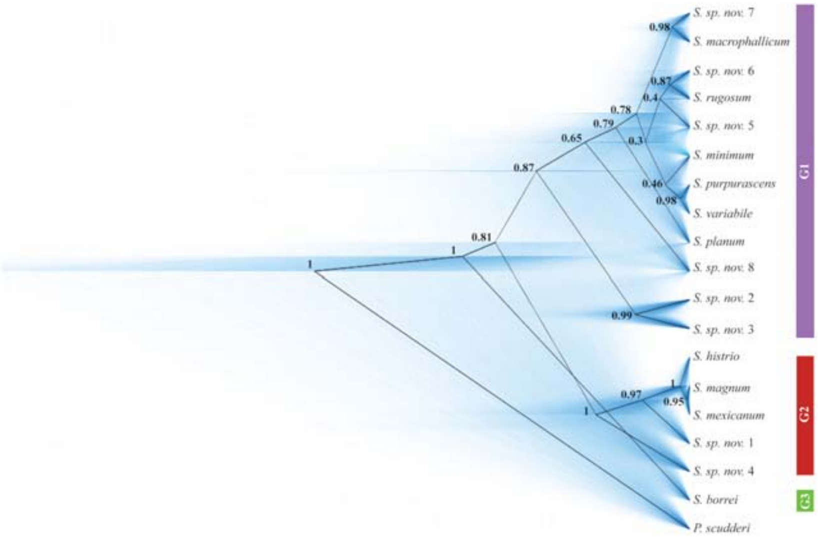

Fig 3. Consensus species tree of the 18 identified taxa of *Sphenarium*. The consensus species tree (in black) is embedded in all sampled trees (in blue) of the Markov Chain Monte Carlo chain of the species tree analysis including the total genetic evidence obtained from *Sphenarium* and *Prosphena* individuals. Higher tree densities represent high levels of certainty. Numbers before the nodes indicate posterior probabilities values. G1, Monophyletic Group 1; G2, Monophyletic Group 2; G3, Monophyletic Group 3.

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In both analyses we also recovered a close phylogenetic relationship between Group 1 and Group 2 [*PP* = 0.53 (CA) and 0.81 (STA)], and *S. borrei* lineages (Group 3) as the basal species in the genus. Moreover, within Group 1 the species *S. machiphallicum*, *S. minimum*, *S. purpurascens*, *S. rugosum*, *S. variabile*, *S. sp. nov. 5*, *S. sp. nov. 6* and *S. sp. nov. 7* formed a monophyletic group [*PP* = 0.67 (CA) and 0.78 (STA)], which was closely related to *S. planum* [*PP* = 1 (CA) and 0.79 (STA)]. In both analyses, we also observed a strong ($PP > 0.95$) sister relationship among three pairs of species comprising *S. purpurascens*–*S. variabile*, *S. machiphallicum*–*S. sp. nov. 7*, *S. sp. nov. 2* –*S. sp. nov. 3*, and *S. mexicanum*–*S. magnum*. In both analyses, phylogenetic relationships were similar and strongly supported (PP > 0.95) among species within Group 2. Within this group *S. sp. nov. 4* was placed in the basal position, *S. sp. nov. 1* was closely related to *S. histrio*, *S. mexicanum* and *S. magnum*.

The main difference between the two analyses performed was the placement of the species pair *S. sp. nov. 2* –*S. sp. nov. 3*. In the CA the species *S. sp. nov. 2* –*S. sp. nov. 3* was more closely related to the species group comprising *S. machiphallicum*, *S. minimum*, *S. planum*, *S. purpurascens*, *S. rugosum*, *S. variabile*, *S. sp. nov. 5*, *S. sp. nov. 6* and *S. sp. nov. 7* (*PP* = 1); whereas in the STA the same species pair was placed in the basal position within Group 1. In addition, different relationships with *PP* values lower than 0.67 were obtained between the species and species pairs within the group comprising *S. machiphallicum*, *S. minimum*, *S. planum*, *S. purpurascens*, *S. rugosum*, *S. variabile*, *S. sp. nov. 5*, *S. sp. nov. 6* and *S. sp. nov. 7* in both analyses.

Morphological analyses

A total of 5441 *Sphenarium* grasshoppers (2508 females and 2933 males) were measured from the 63 sampled localities. The number of individuals considered within each species and sex ranged from 2 to 2204 (Table 3). *S. purpurascens* and *S. rugosum* were the species with the largest samples size (> 300 individuals each sex), whereas *S. sp. nov. 5* had the lowest sample size (5 females and 2 males). Females and males showed considerable variation among the 18 taxa in all traits measured. In females, mean Femur I Width ranged from 0.85 to 1.38 mm, the Femur III Length ranged from 11.82 to 18.56 mm, the Thorax Length ranged from 5.9 to 9 mm, and the Thorax Width ranged from 7.94 to 11.42 mm. In males, mean Femur I Width ranged from 1.11 to 1.76 mm, the Femur III Length ranged from 10.69 to 16.73 mm, the Thorax Length ranged from 4.74 to 7.59 mm, and the Thorax Width ranged from 5.77 to 8.89 mm. For the measured traits, the largest species (*S. magnum*, *S. mexicanum*, *S. sp. nov. 1* and *S. sp. nov. 7*) were nearly 1.38 to 1.63-fold larger than the smallest ones (*S. minimum*, *S. planum*, *S. purpurascens*, *S. variabile* and *S. sp. nov. 5*; Table 3).

In all species the Femur I was wider in males than in females, whereas the Thorax Length and Width was larger in females than males. The Length of the Femur III was larger in females than in males, except for S. *purpurascens* and *S. sp. nov. 8*, which showed the inverse pattern (Table 3). The magnitude of sexual dimorphism varied notably among species and traits. For instance, *S. purpurascens*, showed the highest levels of sexual dimorphism in Femur I Width, whereas *S. planum* and *S. minimum* showed the highest values of sexual dimorphism in the other three traits. The magnitude of sexual dimorphism in the Femur I Width ranged from -0.14 to -0.35. For the Femur III Length varied from -0.04 to 0.15, in the Thorax Length ranged from 0.05 to 0.40, and in the Thorax Width ranged from 0.15 to 0.55 (Table 3).

Altitude, climatic variation and body size

After controlling for phylogenetic non-independence among *Sphenarium* species, the results of the MCMCglmm analysis indicated significant differences between females and males for the four morphological traits (Table 5). We found a positive and significant relationship between temperature during winter (MTWT) and Femur III Length and Thorax Width. In addition, temperature seasonality was positively related to the four traits. The elevation, Mean Temperature of the Wettest Trimester and Mean Annual Precipitation had no significant effect on body size (Table 5). The interactions between sex and all other independent variables were not significant (data not shown in simplified models), indicating a similar body size response to elevation and climatic variables between sexes. All models showed high λ values ($\lambda > 0.94$), indicating a strong phylogenetic effect on the relationships between the ecological and morphological variables. A character reconstruction of the altitudinal distribution of the species (Fig 4) indicated a mid elevation origin of the genus *Sphenarium*, considering that the inferred common ancestor and most species within the genus occupied intermediated elevations (around 800–1500 m), whereas low (<800 m) and highlands (>1500 m) distributions have been occupied independently several times in the genus.

Rensch's rule

The results of model II regressions of independent contrasts analysis indicated strong coevolution between females and males (Fig 5). Regressions of males' over females' traits resulted in slopes greater than 1.0 for Femur III and Thorax Lengths. However, only Thorax Length significantly differed from isometry ($\beta = 1.0$). In addition, in order to explore the evolutionary trends on body size and SSD we performed an ancestral character reconstruction using the mean species values and SDI from each trait. All traits measured resulted in similar pattern (Fig 6). Large body size and high levels of SSD have evolved independently several times throughout the evolutionary history of the group. Small body size and high levels of SSD occurred more frequently in species within Group 1 (Fig 6).

Discussion

Sphenarium grasshoppers show considerable divergence in size during the diversification of the genus. Despite the fact that phylogenetic relationships are heavily affecting body size and climatic niche of *Sphenarium* species, we find (according to our hypotheses) that large *Sphenarium* species are associated with high temperatures during the winter. However, they are also associated with highly seasonal environments. Body size is not significantly related to precipitation or temperatures during the rainy season. In addition, *Sphenarium* females and males respond similarly to the climatic differences, and the evolutionary divergence in the thorax length has been greater in males than in females.

Our results suggest that during benign winters, the window for development and reproduction may increase, allowing grasshoppers to achieve larger body sizes. Conversely, when mean temperatures are lower, body sizes become smaller. Similar body size clines associated with decreasing temperatures have been observed in other insects at higher latitudes [7–10]. Smaller body sizes at low temperatures are commonly explained by natural selection favouring faster development by decreasing development time (reducing the number of nymphal instars or diapause [7] or increasing growth rates [33]).

The climatic body size cline of *Sphenarium* grasshoppers probably reflects their life history adaptability. The nymphs of *Sphenarium* emerge mainly in the beginning of the rainy season and adults die in the winter. However, there is considerable variation in emergence times and life cycle lengths at inter- and intraspecific levels. In *S. purpurascens*, the taxa with the highest altitudinal distribution, hatching occurs in the middle of June in central Mexico (<2200 m.a.s.l.). The first organisms reach sexual maturity at the end of August, the peak of the reproductive season occurs by middle October, and they die off when temperatures drop drastically at the

Table 5. MCMCglmm models for the body size indicators and independent variables (sex, elevation and climatic parameters) of *Sphenarium* species. Significant interactions are denoted in bold numbers.

Morphologic trait	Source	Posterior Mean	Lower CI	Upper CI	ESS	*PP*
FW1, λ = 0.99 (0.98–1)						
	Intercept	1.48	-3.75	6.51	11308	0.56
	Sex	-0.25	-0.29	-0.20	10998	**0.00**
	Elevation	-0.16	-0.40	0.10	10998	0.21
	TS	1.22	0.23	2.16	10998	**0.02**
	MTWT	-5.50	-13.35	2.01	10998	0.14
	MTCT	4.87	-0.04	10.10	10998	0.06
	MAP	0.00	-0.31	0.33	11325	0.99
F3L, λ = 0.98 (0.88–1)						
	Intercept	3.23	-0.44	7.26	11507	0.08
	Sex	0.06	0.02	0.10	10998	**0.01**
	Elevation	-0.16	-0.34	0.04	10998	0.10
	TS	1.07	0.30	1.80	11474	**0.01**
	MTWT	-4.71	-10.25	1.45	11546	0.10
	MTCT	4.21	0.20	7.98	11421	**0.04**
	MAP	0.03	-0.21	0.30	10998	0.81
TXL, λ = 0.95 (0.70–1)						
	Intercept	0.57	-3.78	4.93	10998	0.79
	Sex	0.17	0.12	0.22	10998	**0.00**
	Elevation	-0.04	-0.27	0.18	10998	0.68
	TS	1.19	0.31	2.08	10998	**0.01**
	MTWT	-3.64	-10.37	2.91	10998	0.25
	MTCT	4.14	-0.33	8.60	10998	0.07
	MAP	0.03	-0.27	0.34	10998	0.84
TXW, λ = 0.94 (0.68–1)						
	Intercept	1.65	-2.25	6.10	10998	0.41
	Sex	0.28	0.23	0.34	10998	**0.00**
	Elevation	-0.05	-0.27	0.17	10998	0.61
	TS	1.19	0.30	2.04	10998	**0.01**
	MTWT	-4.56	-11.28	1.83	10998	0.14
	MTCT	4.49	0.23	9.10	10998	**0.03**
	MAP	-0.04	-0.33	0.27	11100	0.80

CI, 95% confidence interval; ESS, Effective Sampling Size; *PP*, Posterior probabilities values of Markov chain Monte Carlo analysis; FW1, Femur I Width; F3L, Femur 3 Length; TL, Thorax Length; TW, Thorax Width; TS, Temperature Seasonality; MTWT, Mean Temperature of The Wettest Trimester; MTCT, Mean Temperature of The Coldest Trimester; MAP, Mean Annual Precipitation; λ, Phylogenetic Signal of the model with lower and upper confidence interval values within parenthesis.

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beginning of December [46]. On the other hand, in *S. sp. nov. 1* at lowlands of the western portion of the Balsas River Basin (239 m.a.s.l) the peak of the reproductive season occurs in middle September, whereas in *S. sp. nov. 8* few adults and mostly last instar nymphs at higher altitudes (1074 m.a.s.l.) can be found at that time (Sanabria-Urban *pers. obs.*).

In general, smaller body sizes are favored in seasonal environment because both maturation time and body size are constrained by weather and food availability [8,80]. However, contrary to our initial expectation, our results indicate that larger grasshopper species are associated, on average, with a greater variability on temperature (i.e. high seasonality). The pattern found in

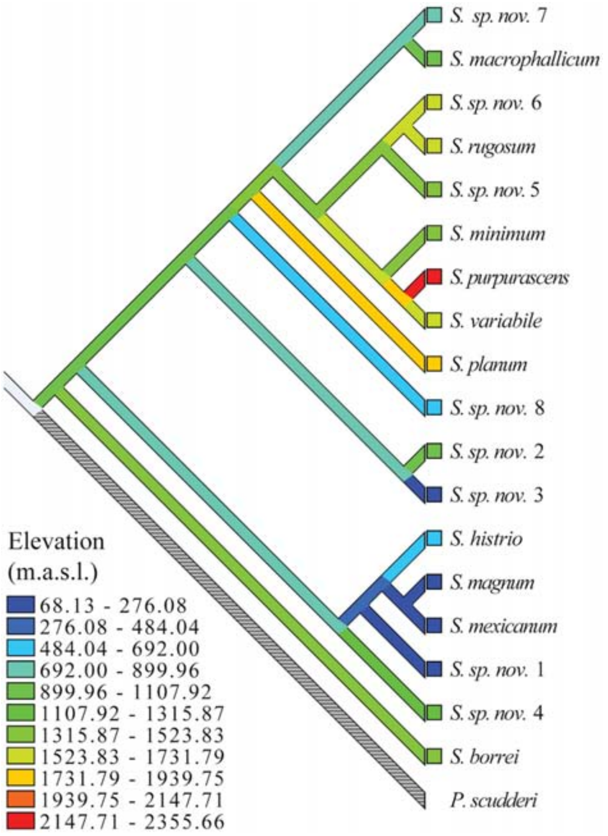

Fig 4. Parsimony ancestral reconstruction of the altitudinal distribution of the *Sphenarium* species performed in MESQUITE v. 3.0.2 [79]. For this analysis we used the species tree analysis topology and the mean elevation values estimated for each species.

doi:10.1371/journal.pone.0145248.g004

Sphenarium could differ from those found in other taxa mainly because species with a wider spatial and altitudinal distribution can be exposed to more heterogeneous environments (which accounted for greater variation in seasonality) than species with a narrow distribution. Moreover, species with a narrow distribution can be exposed to low or high temperature seasonality (see Table 2). Thus, as result of the high levels of environmental variation, high levels of phenotypic variation would be expected too. In order to test this possibility, we correlated the coefficients of variation of phenotypic traits of *Sphenarium* species on their estimated temperature seasonality. In general high levels of phenotypic variation were positively related with high temperature seasonality (S3 Table).

The extensive interspecific variation in morphological traits could suggest local adaptation, which could be the result of adaptive genetic variation, and/or phenotypic plasticity. Biotic and abiotic factors like temperature and food availability can affect growth and development times. For instance, insects grown under high temperatures and high quality and/or quantity of food resources can reach large body sizes [81,82] and females and males can respond in different ways to the environment [26]. However, in *Sphenarium* females and males respond similarly to the environmental differences.

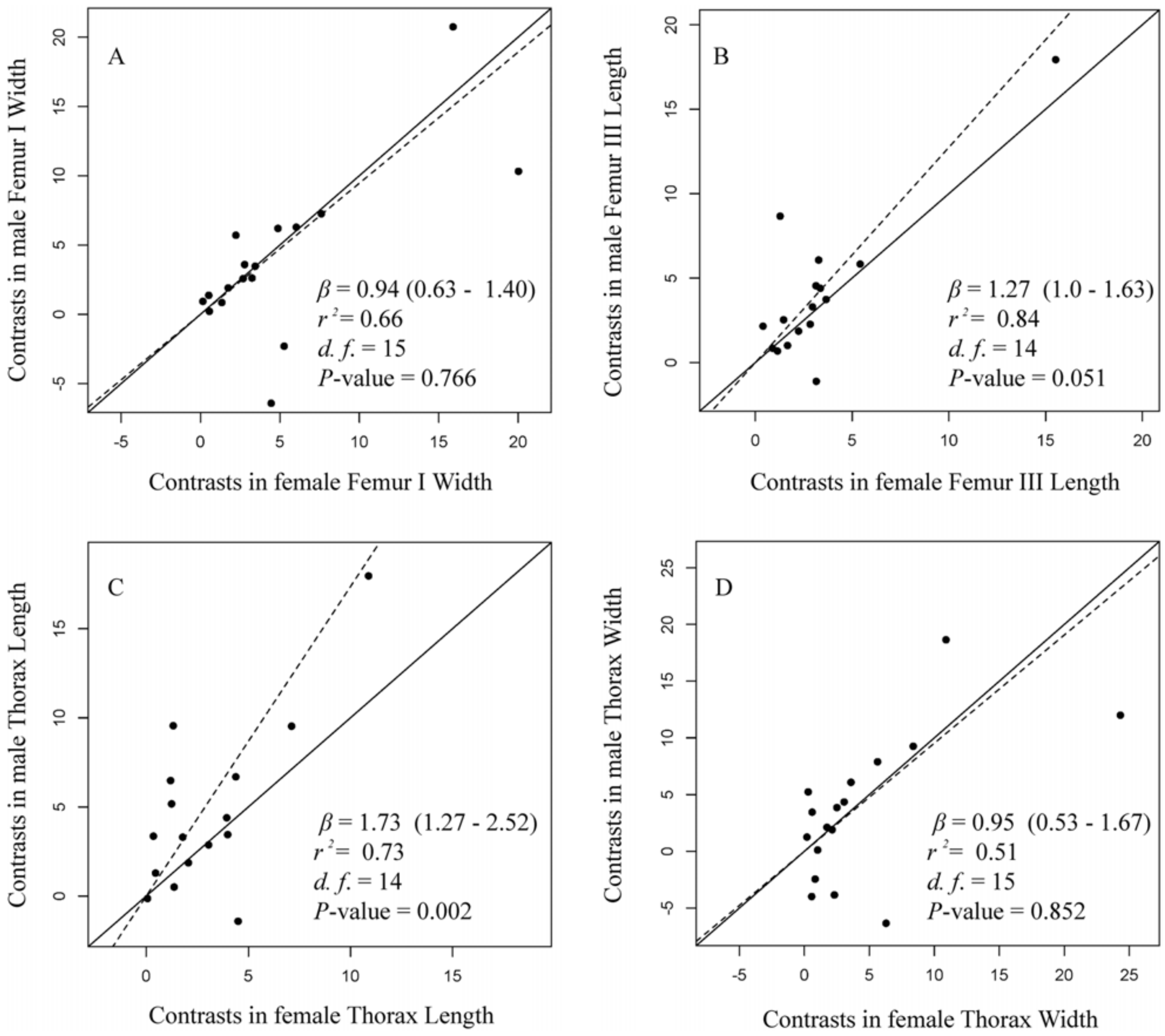

Fig 5. Model II major axis regressions of independent contrasts of body size indicators of males and females of *Sphenarium* species. The *P*-value from the comparison of each calculated slope *vs* slope = 1 (H_O: slope not different from 1). β, slope with lower and upper confidence intervals at 95% probability within parenthesis; r^2, explained variance of the model; *d.f.*, degrees of freedom.

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In Mexico, western and eastern mountain ranges generate large environmental heterogeneity. Temperate, dry and rain forest and even desserts occur in short distance apart at the same latitude. This variation in plant communities results in differential availability of both, food quantity and quality. Because *Sphenarium* are generalist herbivores, they can feed on a wide variety of plants across their altitudinal range. Their diet includes seasonal species that produce leaves and flowers during the growing season of the grasshoppers (e.g. *Dahlia coccinea*, *Verbesina virgate*, *Datura stramonium*, *Tithonia sp*.), perennial species (e.g. *Montanoa tomentosa*, *Eupatorium petiolare*, *Budleia cordata*) and even crop plant and trees, which provide a

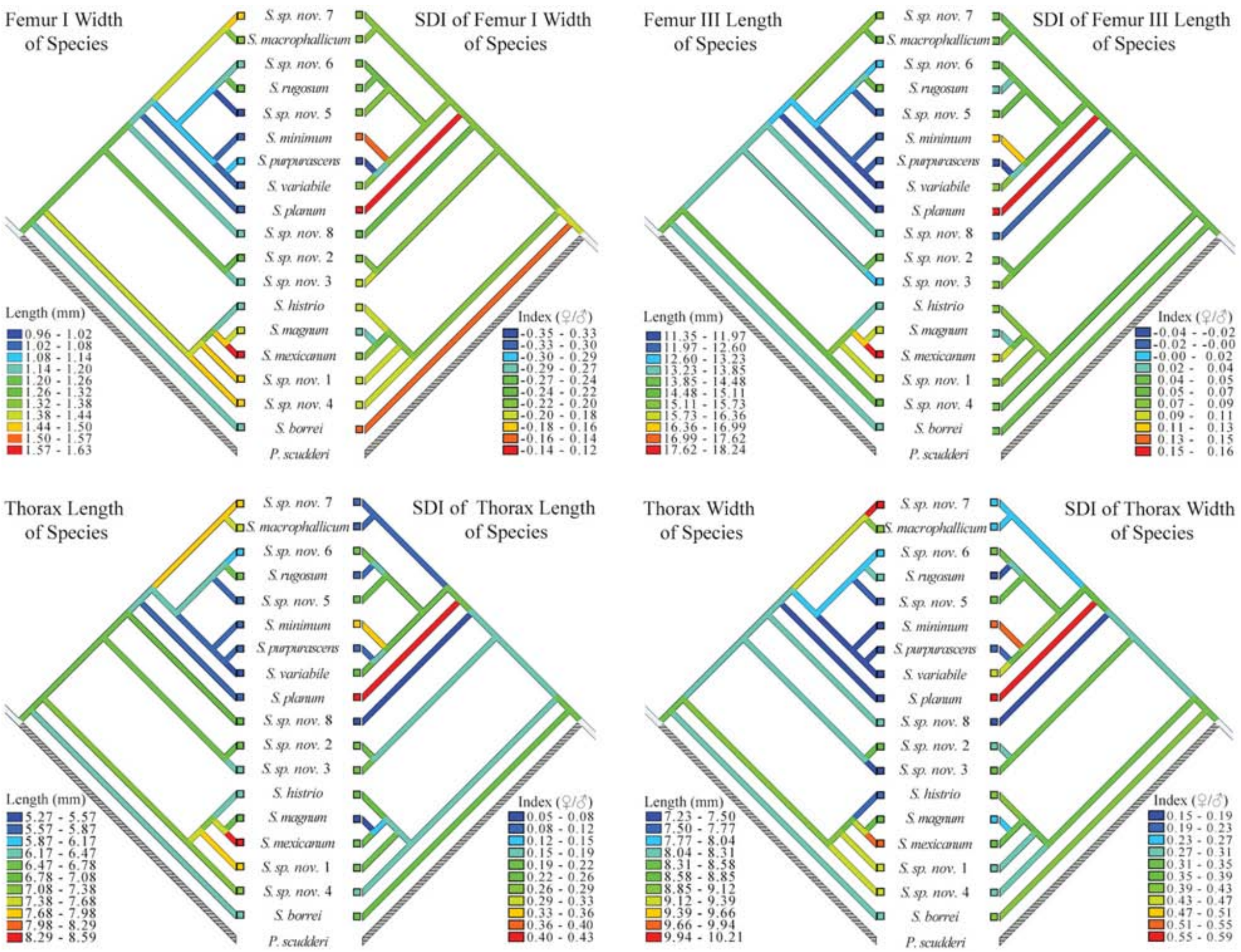

Fig 6. Parsimony ancestral reconstruction of the body size and the magnitude of SSD of the *Sphenarium* species performed in MESQUITE v. 3.0.2 [79]. For this analysis we used the species tree analysis topology and the mean values of SDI estimated for each species (considering females over males) for each trait.

doi:10.1371/journal.pone.0145248.g006

continuum of food supply during development and reproductive season of grasshoppers [83,84]. The potential continuum of food supply during the life cycle of the grasshoppers may reduce the indirect relationship between precipitation and size that has been found in other grasshoppers [34], and may explain why we did not find the significant relationship between body size and precipitation that has been found in other ectotherm species [62,85].

Decreasing the time to maturity at low temperatures may have negative effects on the fitness of individuals by reducing reproductive success via small body sizes [10,12,86]. The smallest species of *Sphenarium* (*S. purpurascens*, *S. minimum*, *S. planum*, *S. variabile and S. sp. nov. 5*) probably have lower fecundity than larger species, but they have been able to colonize highlands. However, at intraspecific level in *S. purpurascens* this trade-off between maturation time and body size does not seem to exist. Early maturation and large body size are associated with high mating success in both sexes [44,45].

The divergence in body size among *Sphenarium* species can be explained by natural selection operating on their life cycles, but also by sexual selection. The relationship between male and female thorax length was significantly hyperallometric, indicating a greater evolutionary divergence in male body size, even though the other traits showed isometric relationships. Hyperallometry is typically explained by strong sexual selection acting on male body size [21,23,25,87]. According to the Rensch's rule, because thorax length is larger in females than males, the greater divergence on males may explain why there is a low SSD in thorax length of larger species (Fig 6). In *S. purpurascens* body size, including thorax length increase mating success in both, females and males [44,45], even thought the impact of sexual selection on males can be stronger than in females. On the other hand, isometry in Femurs I and III and thorax width can be explained by genetic [88], phylogenetic [89], developmental [90], and/or physiological [91] constraints, differences in the magnitude and/or direction of selection between species or even that size is not under any evolutionary pressure.

SSD is the result of differential selection among sexes, and the balance between natural selection and sexual selection in a given species [16,92]. In any case, the different patterns associated with the evolution of body size and SSD in *Sphenarium* (see Fig 6) may suggest that in some species natural selection has been stronger than sexual selection. Natural selection on developing time associated with a short reproductive season can favour small body sizes. However, positive directional sexual selection on thorax length could explain a greater divergence in males than in females. Small body sizes and high SSD levels on thorax length (e. g. *S. planum* and *S. minimum*) can be explained by strong natural selection associated with a short reproductive season, and low levels of sexual selection on male body size. On the other hand, large body sizes and low SSD levels (e. g. *S. magnum* and *S. mexicanum*) can be expected under strong sexual selection on males and lower levels of natural selection associated with a long growing and reproductive season, which also favors large body sizes in both females and males. Moreover, small body sizes and low SSD (e. g. *S. purpurascens* and *S. variabile*) could result from strong sexual and natural selection on body size and maturation time in places with short reproductive seasons [44,45].

In *S. purpurascens* body size and maturation times are under strong sexual and natural selection in both males and females. Individuals maturing earlier and with larger body sizes usually attain high mating success [45,46]. However, pre- and post-copulatory sexual selection could be stronger on males than on females. Female and males can mate multiple times, and sperm competition is very likely [93,94]. After copulation, large males remain in a post-insemination association with their mates. A male can spend as many as 17 days mounted on a female, and guarding duration is related to both male and female body sizes [93].

The diversification of the genus *Sphenarium* could be associated with Quaternary climatic fluctuations, which probably cause the vicariance of ancestral populations throughout recurrent shifts in their altitudinal and spatial distribution. The low mobility of these univoltine and flightless grasshoppers, plus the combination of strong natural and sexual selection on adult body size and maturation times could enhance the genetic isolation and consequently the speciation of these Neotropical grasshoppers. Genetic differentiation, but also high levels of phenotypic plasticity could explain the diversification of the clade. We are currently conducting the taxonomic revision of the genus and evaluating the genetic variance and plasticity levels in body size and maturation time within and between species. Perhaps widely distributed and more variable species will show the highest levels of phenotypic plasticity.

Supporting Information

S1 Table. Sampling localities, specimens and GenBank accession numbers of the *Sphenarium* individuals and outgroup species included in this study. ID, locality identifier; VN,

voucher number. ^{A} species identification based on Boyle [36] and Kevan [37,95] and by comparing our samples with museum specimens. ^{B} Intermediate form between *S. m. mexicanum* and *S. m. histrio*. ^{C} Intermediate form between *S. p. purpurascens* and *S. p. minimum*.
(XLSX)

S2 Table. *P-distance* values between *CO1* sequences of the identified *Sphenarium* species. *P-distance* percentages were estimated using pairwise comparisons of evolutionary divergence implemented in MEGA v. 6.0.6 [53]. Standard errors were base on a 500 replicates bootstrap analysis. Bold numbers indicate *P-distance* values lower than 2%.
(XLSX)

S3 Table. Coefficients of variation associated to the mean values of species of the morphologic traits and temperature seasonality. ^{A} Correlation analysis between coefficients of variation of Temperature Seasonality and all morphologic traits in both females and males, of *Sphenarium* species (*d.f.* 16). Significant correlations are denoted in bold. CV, coefficient of variation; FW1, Femur I Width; F3L, Femur 3 Length; TL, Thorax Length; TW, Thorax Width; TS, Temperature Seasonality; *r*, correlation values; *P*-values, significance values.
(XLSX)

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Author Contributions

Conceived and designed the experiments: SSU RCdC. Performed the experiments: SSU HS KO AGR. Analyzed the data: SSU HS KO AGR MASM RCdC. Contributed reagents/materials/analysis tools: RCC HS KO. Wrote the paper: SSU HS KO AGR MASM RCdC.

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Discusión general y conclusiones

Diversidad del género *Sphenarium*

La combinación de caracteres morfológicos y la sistemática filogenética ha permitido identificar una diversidad mayor a la que fue reconocida en estudios taxonómicos previos en *Sphenarium*. En total se reconocen 17 especies morfológicamente y/o genéticamente diferenciadas dentro del género, nueve de las cuales corresponden a especies previamente descritas (*S. borrei*, *S. histrio*, *S. macrophallicum*, *S. mexicanum*, *S. minimum*, *S. planum*, *S. purpurascens*, *S. rugosum* y *S. variabile*) y ocho representan nuevas especies para el grupo (*S. adelinae* **sp.n.**, *S. crypticum* **sp.n.**, *S. infernalis* **sp.n.**, *S. miztecum* **sp.n.**, *S. occidentalis* **sp.n.**, *S. tarascum* **sp.n.**, *S. totonacum* **sp.n.** y *S. zapotecum* **sp.n**.). Este estudio también demuestra la existencia de una notable variación morfológica y/o genética dentro de las especies con amplia distribución geográfica (*S. histrio*, *S. mexicanum*, *S. purpurascens* y *S. rugosum*) y en una especie con una distribución reducida (*S. variabile*), lo cual sugiere altos niveles de adaptación local y eventos de especiación incipiente en el grupo. Además, se reconoce la posibilidad de existencia de una mayor diversidad específica, principalmente en aquellas regiones geográficas inexploradas y aisladas dentro de los rangos de distribución del género.

Con base en los resultados obtenidos, se actualizó y redefinió el concepto de *Sphenarium*, de sus especies previamente reconocidas y se describió a las nuevas especies identificadas. Así, se propone un nuevo esquema de clasificación taxonómica y se proporciona una mayor claridad para la identificación de las especies del género y de sus rangos de distribución geográfica. Además, se reconoce utilidad diagnostica en estructuras genitales de machos previamente ignoradas, así como de atributos morfológicos externos y de la distribución geográfica de las especies reconocidas. Este estudio taxonómico representa uno de los más amplios realizados en los ortópteros mexicanos y revela patrones de variación morfológica y genética que podrían ser comunes en otros grupos con distribución y características biológicas similares a *Sphenarium*.

Relaciones evolutivas en *Sphenarium*

Las reconstrucciones filogenéticas indican que la subtribu Sphenariina es parafilética con respecto a los otros pyrgomórfidos incluidos en los análisis. Esto sugiere la posibilidad de un origen independiente y más complejo al propuesto para los pyrgomórfidos del nuevo mundo (i.e. a parir de incursiones a América de pyrgomórfidos asiáticos por el estrecho de Bering; Kevan, 1977). Sin embargo, la estrecha relación entre los géneros *Sphenarium* y *Prosphena*, así como su monofilia fue fuertemente sustentada. Además, los resultados filogenéticos sugieren que después de la separación de estos dos géneros hermanos, *Sphenarium* diversificó en tres clados principales (Clado 1, 2 y 3), dentro de los cuales ocurrió subsecuentemente la mayor diversificación en el grupo.

Dentro de los clados 2 y 3 se reconocen cuatro grupos monofiléticos de especies morfológicamente diferenciadas cuya monofilia está escasamente sustentada o son parafiléticas (*S. occidentalis* **sp.n.**/*S. histrio*/*S. mexicanum*; *S. macrophallicum*/S. *crypticum* **sp.n.**; *S. rugosum*/S. *tarascum* **sp.n.**; y *S. purpurascens*/*S. variabile*/*S. zapotecum* **sp.n.**). Las especies dentro de estos grupos mostraron algunos haplotipos mitocondriales y nucleares compartidos. Estos resultados pueden explicarse por sorteo incompleto de linajes y/o flujo genético, los cuales pueden resultar en patrones genéticos y filogenéticos similares (McGuire *et al.*, 2007; McKay & Zink, 2010; Nater *et al*., 2015) . Sin embargo, se reconocen dos expectativas que permitirían diferenciar estos procesos (McGuire *et al.*, 2007; Toews & Brelsford, 2012). Primero, si la divergencia entre especies ocurrió rápidamente es probable que no haya transcurrido el tiempo suficiente para generar diferenciación genética y monofilia reciproca en ellas. Por lo tanto, se esperaría diferenciación genética escasa o nula entre especies con alelos compartidos y parafiléticas. Segundo, si las especies descendientes han retenido múltiples alelos de su ancestro en común se espera una distribución aleatoria de esos alelos en las poblaciones descendientes. Por lo tanto se esperaría que alelos compartidos o escasamente diferenciados no estuvieran geográficamente concentrados cerca de los límites de las especies (Hare & Avise, 1998; Funk & Omland 2003; McGuire *et al.*, 2007; Toews & Brelsford, 2012).

El sorteo incompleto de linajes parece ser una explicación más probable a las relaciones parafiléticas y ocurrencia de haplotipos compartidos entre especies morfológicamente diferentes de *Sphenarium*. En la mayoría de los cuatro grupos antes mencionados, sus especies están escasamente diferenciadas genéticamente. Además, todos los haplotipos compartidos identificados están aleatoriamente distribuidos en poblaciones geográficamente distantes de especies estrechamente relacionadas. No obstante, excepcionalmente dos especies compartieron haplotipos nucleares entre poblaciones geográficamente cercanas, sugiriendo la posibilidad de flujo génico entre ellas. Además, durante el trabajo de campo se observaron parejas heterospecíficas en copula en las zonas de contacto de algunas especies, sugiriendo la posibilidad de hibridación entre especies morfológica y genéticamente bien diferenciadas. Se reconoce que para poder reconocer y distinguir la importancia relativa del sorteo incompleto de linajes y flujo génico en la evolución de *Sphenarium* es necesario examinar una mayor cantidad muestras poblaciones y genéticas de múltiples loci (e.g. McGuire *et al.*, 2007; Bryson *et al.*, 2010; Toews & Brelsford, 2012; Nater *et al*. 2015).

Finalmente, en las filogenias inferidas, las relaciones entre los clados y dentro del clado 3 son conflictivas o están escasamente soportadas. Estas incertidumbres filogenéticas pueden explicarse también de un sorteo incompleto de linajes asociado a una divergencia reciente (e.g. Talavera *et al.*, 2013) o a eventos de divergencia simultanea en el género (e.g. Allegrucci *et al.*, 2013).

Eventos históricos asociados a la diversificación

Las posiciones basales en la filogenia son ocupadas por especies distribuidas alrededor de los extremos orientales y occidentales de la Faja Volcánica Transmexicana. En tanto que las especies distribuidas en zonas costeras, cuencas y tierras altas de centro y sur de México han derivado más recientemente. Las estimaciones aproximadas de tiempos de divergencia indican que las divergencias iniciales, entre clados y dentro de ellos, ocurrieron entre 7.22 a 2.91 Ma, mientras que la mayor diversificación de los clados ocurrió durante los últimos 2.5 Ma. Estos episodios de diversificación concuerdan con la tercera etapa de mayor formación de la Faja Volcánica Transmexicana, entre 7.5 y 3 Ma aproximadamente (Ferrari *et al*., 2012), y las fluctuaciones climáticas del Cuaternario, entre 2.6 y 0.01 Ma. Estos resultados sugieren que los episodios de vulcanismo durante la formación de la Faja Volcánica Transmexicana fragmentaron las poblaciones ancestrales de *Sphenarium* a lo largo del centro de México causando los episodios de divergencia inicial. Por otro lado, los cambios recurrentes de distribución altitudinal de las biotas y del nivel del mar promovidos por los ciclos glaciares e interglaciares del Cuaternario en combinación con la orografía del territorio mexicano probablemente causaron la divergencia de los linajes más recientes mediante la fragmentación y reducción poblacional de sus poblaciones ancestrales en tierras altas y costeras. Además, el hecho que los grupos monofiléticos fuertemente sustentados de *Sphenarium* estén restringidos a regiones geográficas delimitadas (e.g. cuencas, valles y sistemas montañosos) indica que los eventos vicariantes han jugado un papel importante en la evolución del grupo (Avise, 2000). Por lo tanto, es probable que la distribución parapátrica actual y la existencia de zonas de contacto estrechas entre sus especies sean resultado de procesos de dispersión secundaria.

La reconstrucciones filogenéticas inferidas muestran que especies geográficamente cercanas son también especies filogenéticamente cercanas, lo cual es congruente con las capacidades limitadas de dispersión de estos chapulines. Además, estos resultados sugieren que las poblaciones ancestrales de *Sphenarium* ocuparon inicialmente las vertientes externas del centro y sur de México y que estos chapulines han colonizaron más recientemente las cuencas y tierras altas del interior del país.

Impacto relativo de las fuerzas evolutivas

Se identificaron tres patrones generales de diferenciación que podrían reflejar el papel relativo de fuerzas evolutivas en la diversificación del grupo. En el primer patrón se encuentran especies escasamente diferenciadas genéticamente pero que difieren marcadamente en la morfología genital de los machos. La genitalia de machos está bajo presiones intensas de selección sexual en múltiples insectos (Eberhard, 1985; Simmons, 2014) y los caracteres bajo selección sexual tienden a divergir rápidamente (Hosken & Stockley, 2004; Eberhard, 2010; Simmons, 2014). Por lo tanto, se puede inferir que la selección sexual ha jugado un papel principal en la divergencia de las especies bajo este patrón. El segundo patrón involucra especies marcadamente diferenciadas a nivel genético pero que son morfológicamente similares a otras especies. En este caso, se infiere que otros procesos evolutivos han sido más importantes para su divergencia que la selección sexual sobre la genitalia masculina. El aislamiento y la deriva génica pudieron jugar un papel predomínate en la divergencia de estos linajes. El tercer patrón comprende especies que son marcadamente diferenciadas genética y morfológicamente. En estos casos, se puede asumir una interacción conjunta de las fuerzas evolutivas promoviendo la divergencia de estas especies.

Los patrones encontrados en el estudio comparativo entre la variación climática altitudinal y el tamaño corporal de las especies de *Sphenarium* sugieren una considerable divergencia en tamaño corporal durante la evolución del grupo asociada a las diferencias climáticas en su área de distribución. A pesar de la fuerte inercia filogenética del tamaño corporal de las especies de *Sphenarium* se observa que tamaños corporales grandes están asociados a temperaturas altas durante el invierno. Esto sugiere que en inviernos más cálidos la ventana de tiempo para el desarrollo y reproducción de estos chapulines es más amplia permitiéndoles alcanzar tallas corporales mayores. Por lo contrario, cuando las temperaturas promedio son más bajas la selección natural favorecería la evolución de un tamaño corporal pequeño. Comúnmente, tamaños corporales pequeños en temperaturas bajas se explican por selección natural favoreciendo un desarrollo rápido mediante la disminución del número de estadios ninfales, diapausa, y/o incrementando las tasas de crecimiento (Dingle *et al.*, 1990; Hodkinson, 2005).

La clina climática en el tamaño corporal de las especies de *Sphenarium* puede reflejar adaptación local en sus atributos de historias. En general, las ninfas de estos chapulines anuales eclosionan al inicio de la temporada de lluvias y los adultos mueren al durante el invierno. No obstante, en sus poblaciones también se ha observado variación en los tiempos de emergencia, longitud de los ciclos de vida y reproducción (*obs. pers.*) . Por lo tanto, es probable que esta variación morfológica sea resultado de variación genética adaptativa y/o plasticidad fenotípica en sus atributos de historias de vida.

La divergencia en el tamaño corporal de las especies de *Sphenarium* puede ser explicada por selección natural sobre atributos de historias de vida pero también por selección sexual. La relación hiperalométrica encontrada entre el largo del tórax de machos y hembras de *Sphenarium* indican una mayor divergencia evolutiva en el tamaño corporal de machos que puede explicarse por selección sexual intensa (Fairbain, 1997; Dale *et al.*, 2007; Fairbain *et al.*, 2007; Webb & Freckleton, 2007).

El dimorfismo sexual en tamaño es resultado de selección diferencial entre los sexos y del balance entre selección natural y sexual en una especie determinada (Blanckenhorn *et al.* 2007; Lehmann & Lehmann, 2008). Los diferentes patrones observados de divergencia entre tamaño corporal y en la magnitud de dimorfismo sexual en tamaño pueden explicarse por este balance entre estas fuerzas evolutivas. En los insectos generalmente el tamaño de las hembras está positivamente relacionado con su fecundidad. Las hembras generalmente son más grandes que los machos y se asume que esto es resultado correlativo de una intensa selección natural favoreciendo un incremento en fecundidad (Ridley, 1983). Sin embargo, en muchas especies de insectos la selección sexual favorece a los machos grandes, ya sea por competencia o elección de pareja (Thornhill & Alcock, 1983). En ambientes estacionales con temporadas reproductivas cortas la selección natural sobre el tiempo de desarrollo favorece la evolución de tamaños corporales pequeños. Asimismo, la selección sexual, direccional y positiva, en *Sphenarium* puede explicar una divergencia mayor en el tamaño de machos que en hembras. Por lo tanto, tamaños corporales pequeños y un dimorfismo sexual sesgado a hembras pueden explicarse por selección natural intensa asociada a temporadas reproductivas cortas y selección sexual débil en el tamaño de machos. Por otra parte, tamaños corporales grandes y un dimorfismo sexual reducido pueden estar asociados a ambientes con una baja estacionalidad en la cual machos y hembras tienen más tiempo para desarrollarse y además hay una intensa selección sexual sobre el tamaño de los machos. Finalmente, tamaños corporales pequeños y dimorfismo sexual reducido pude explicarse por intensa selección sexual y natural en tamaño corporal y tiempos de desarrollo asociados a temporadas reproductivas cortas.

Así, se reconoce que los eventos históricos vicariantes del pasado han jugado un papel importante en la diversificación del género *Sphenarium*. No obstante, la baja movilidad de estos chapulines univoltinos y ápteros, más la combinación de presiones de selección sexual y natural intensas sobre el tamaño corporal e historias de vida asociadas a compleja heterogeneidad ambiental de México, pudieron favorecer también el aislamiento y especiación de estos chapulines neotropicales.

Direcciones futuras

Esta investigación sienta las bases para un estudio más detallado de los procesos y mecanismos asociados a la especiación del género *Sphenarium* que podría ayudar a comprender y identificar patrones de diversificación comunes en otros insectos neotropicales.

En primera instancia, la evidencia filogeográfica y temporal de este estudio sugiere que la mayor diversificación del género ocurrió durante las fluctuaciones climáticas del Cuaternario. Sin embargo, comprender y comprobar el efecto de estos eventos climáticos requiere probar explícitamente sus expectativas demográficas (e.g Knowles 2000; Waltari 2007). Por ejemplo, se ha propuesto que las poblaciones ancestrales contrajeron sus tamaños y distribución durante los ciclos glaciales y se expandieron durante los ciclos interglaciales (Hewitt 1996). Así, mediante un análisis detallado de la variación genética interpoblacional y de modelos de distribución paleoclimática de las especies de origen más reciente se pretende probar si la evidencia demográfica histórica es congruente con las expectativas propuestas bajo el modelo de especiación cuaternaria. La evidencia genética preliminar obtenida en poblaciones de *S. purpurascens* y *S. rugosum* dan soporte a estas hipótesis (Sanabria-Urbán 2010). Simultáneamente, un estudio genético interpoblacional más amplio, con un mayor número de loci independientes, podría ayudar a evaluar más adecuadamente el papel relativo del flujo génico y deriva génica en la evolución del género.

La clina climática en el tamaño corporal de las especies de *Sphenarium* puede ser resultado de variación genética adaptativa y/o plasticidad fenotípica en sus atributos de historias de vida. Por lo tanto se pretende analizar los niveles variación genética y plasticidad fenotípica en el tamaño corporal y tiempos de maduración dentro y entre las especies de *Sphenarium*, con la finalidad de comprobar el efecto de la selección natural sobre atributos sus historias en respuesta a la heterogeneidad climática del país (e.g. Berner et al. 2004). Tal vez las especies con distribución más amplia muestren los niveles más altos de plasticidad fenotípica.

Los patrones de divergencia genética y de las estructuras genitales masculinas de *Sphenarium* brindan la oportunidad de probar el impacto relativo de selección sexual sobre la diversificación del género. Así, se pretende realizar un estudio comparativo sobre la divergencia y evolución de los genitalia de machos de estas especies. Si la selección sexual ha sido responsable de esta diferenciación se esperaría una diferenciación morfológica mayor entre las especies geográfica y filogenéticamente más cercanas. Finalmente, la notable variación intraespecífica observada dentro de algunas especies brinda la oportunidad estudiar procesos de adaptación local y especiación insipiente.

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