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POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

Revisión sistemática y filogenética molecular del género *Brachypelma* Simon, 1891 (Mygalomorphae: Theraphosidae: Theraphosinae).

TESIS

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DOCTOR EN CIENCIAS BIOLÓGICAS

PRESENTA:

MENDOZA MARROQUÍN JORGE IVÁN

TUTOR PRINCIPAL DE TESIS: DR. OSCAR F. FRANCKE BALLVÉ, INSTITUTO DE BIOLOGÍA, UNAM
MIEMBRO DEL COMITÉ TUTOR: DRA. VIRGINIA LEÓN REGAGNON, INSTITUTO DE BIOLOGÍA, UNAM
MIEMBRO DEL COMITÉ TUTOR: DR JUAN JOSÉ MORRONE LUPI, FACULTAD DE CIENCIAS, UNAM

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COORDINACIÓN

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Asunto: Oficio de Jurado para Examen de Grado.

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

Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, celebrada el día 14 de mayo de 2018, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del alumno **MENDOZA MARROQUÍN JORGE IVÁN** con número de cuenta **99192390** con la tesis titulada: **"REVISIÓN SISTEMÁTICA Y FILOGENÉTICA MOLECULAR DEL GÉNERO *BRACHYPELMA* SIMON, 1891 (MYGALOMORPHAE: THERAPHOSIDAE, THERAPHOSINAE)"**, realizada bajo la dirección del **DR. OSCAR FEDERICO FRANCKE BALLVE:**

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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 15 de junio de 2018.

DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA

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

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Nota Aclaratoria

De acuerdo con el artículo 8 del código internacional de nomenclatura zoológica (CINZ) acerca de lo que constituye una obra no publicada. Es que declaro de manifiesto con lo estipulado por los incisos 8.2 y 8.3 del CINZ, que esta obra no ha sido editada para constituir un registro científico público y permanente o para fines de nomenclatura zoológica. Por lo que de igual manera los nombres o actos nomenclaturales contenidos en esta obra, no tienen una finalidad nomenclatural, por lo que estos nombres y actos no se hallan disponibles para su utilización.

INDICE

Resumen

El género *Brachypelma* incluye una serie de especies coloridas que son consideradas como algunas de las mascotas más deseables, y aunque se crían en cautiverio, continúan siendo sacadas clandestinamente de la naturaleza en grandes cantidades. Se consideran especies prioritarias para la conservación y están incluidas en el Apéndice II de la CITES. Las especies a menudo son difíciles de identificar basándose únicamente en la morfología, se requiere una identificación rápida y precisa para llevar a cabo la aplicación de estrategias de protección adecuadas. En esta tesis se presenta la revisión sistemática del género *Brachypelma* con el uso de caracteres morfológicos y moleculares. Se pone a prueba la monofilia del género y también se explora la aplicabilidad del código de barras de ADN basado en CO1 como una herramienta de identificación complementaria para la conservación. Se realizó un análisis filogenético de *Brachypelma* utilizando caracteres morfológicos basado en parsimonia y del marcador CO1 basado en métodos probabilísticos. Las filogenias tanto molecular como morfológica favorecieron fuertemente la división de *Brachypelma* en dos géneros más pequeños. Se diagnosticaron y redescribieron todas las especies consideradas *Brachypelma sensu stricto*, y se proporcionó una clave de identificación para las mismas. Las especies consideradas como *Brachypelma sensu lato* se transfirieron al nuevo género *Tliltocatl*. Además, se realizó un análisis filogenético bajo métodos probabilísticos utilizando cuatro marcadores moleculares (CO1, EF1G, MID1IP1 y MRPL44) para todas las especies conocidas de *Brachypelma* y *Tliltocatl* e incluyendo nuevos registros y otros grupos posiblemente cercanos. Estos análisis permitieron resolver algunas relaciones internas entre especies que no se resolvieron utilizando sólo CO1. Se encontraron nuevas especies del género *Tliltocatl*. Se discutió la distribución geográfica de *Brachypelma* y *Tliltocatl*.

Los resultados muestran que *Brachypelma* está compuesto por ocho especies, todas fueron redescritas y delimitadas con evidencia morfológica y molecular. El marcador de código de barras CO1 demostró ser suficiente para la identificación de especies y tiene utilidad para prevenir el comercio ilegal de estas arañas. Se ha colaborado en la creación de una biblioteca genética de tarántulas mexicanas como parte del proyecto Códigos de Barras de la Vida Silvestre México, que se utilizará como referencia para las autoridades responsables

de la conservación de especies. Para la identificación de las especies de *Brachypelma* propusimos distancias genéticas de CO1: intraespecífica de 1% e interespecífica entre 7-12%. La morfología demostró ser útil para la identificación. Es bien conocido que las tarántulas presentan gran cantidad de caracteres homoplásticos, dado que sus estructuras son sencillas y muy similares en diferentes géneros; sin embargo, una combinación de diferentes caracteres puede ser utilizada para diagnósticar a la mayoría de las especies. La variación de la coloración en algunas especies de *Brachypelma* ha sido problemática para la identificación, por lo que describimos los diferentes morfotipos de color para cada especie problemática. Estandarizamos las diferencias de color mediante una comparación entre los caparazones, cada segmento de las patas y el abdomen. La filogenia basada en la morfología permitió identificar la combinación de caracteres diagnósticos para distinguir claramente entre *Brachypelma* y *Tliltocatl*. Diez especies fueron transferidas a *Tliltocatl*, sin embargo, tres de ellas carecen de información que permita separarlas de otras especies, por lo que *T. andrewi, T. alvarezi* y *T. aureoceps* fueron consideradas *nomina dubia*. *Brachypelma fossorium* no formo parte de *Brachypelma* o *Tliltocatl* en ninguno de los análisis, por lo que después de la revisión de los tipos fue transferida a *Stichoplastoris*, creando la combinación *S. fossorius*. Nuestros análisis filogenéticos moleculares tuvieron altos valores de soporte, y el realizado con marcadores mitocondriales y nucleares resuelve las interrelaciones dentro de *Tliltocatl* y muestra nuevas especies potenciales. Consideramos que los marcadores moleculares pueden ser una herramienta útil para realizar mejores estrategias de reintroducción de tarántulas en las áreas de distribución correctas. Se hacen sugerencias sobre los cambios actuales y futuros en la conservación de *Brachypelma* y *Tliltocatl*, la implicación de los cambios taxonómicos para la CITES y la Ley mexicana para la protección de la vida silvestre.

Abstract

The genus *Brachypelma* includes a series of colorful species that are considered some of the most desirable pets, and although they are bred in captivity, they continue to be smuggled out of nature in large numbers. They are considered priority species for conservation and are included in Appendix II of CITES. Species are often difficult to identify based solely on morphology; prompt and accurate identification is required to carry out the application of adequate protection strategies. This thesis presents the systematic review of the *Brachypelma* genus with the use of morphological and molecular characters. The monophyly of the genus is tested and the applicability of the DNA barcode based on CO1 is also explored as a complementary identification tool for conservation. A phylogenetic analysis of *Brachypelma* was performed using morphological characters based on parsimony and the CO1 marker based on probabilistic methods. The molecular and morphological phylogenies strongly favored the division of *Brachypelma* into two smaller genera. All species considered *Brachypelma sensu stricto* were diagnosed and redescribed, and an identification key was provided for them. The species considered as *Brachypelma sensu lato* were transferred to the new genus *Tliltocatl*. In addition, a phylogenetic analysis was carried out under probabilistic methods using four molecular markers (CO1, EF1G, MID1IP1 and MRPL44) for all known species of *Brachypelma* and *Tliltocatl* and including new records and other possibly nearby groups. These analyses allowed solving some internal relationships between species that were not resolved using only CO1. New species of the genus *Tliltocatl* were found. The geographical distribution of *Brachypelma* and *Tliltocatl* was discussed.

The results show that *Brachypelma* is composed by eight species, all of them were redescribed and delimited with morphological and molecular evidence. The barcode marker CO1 proved to be sufficient for the identification of species and has utility to prevent the illegal trade of these spiders. We have collaborated in the creation of a genetic library of Mexican tarantulas as part of the Barcode of Wildlife Project Mexico, which will be used as a reference for the authorities responsible for the conservation of species. For the identification of the *Brachypelma* species we proposed genetic distances of CO1: intra-specific 1% and interspecific between 7-12%. The morphology proved useful for

identification. It is well known that tarantulas have a large number of homoplastic characters, given that their structures are simple and very similar in different genera; however, a combination of different characters can be used to diagnose most species. The variation of the coloration in some species of *Brachypelma* has been problematic for the identification, reason why we described the different morphotypes of color for each problematic species. We standardized the color differences by comparing the carapace, each segment of the legs and the abdomen. Morphology based phylogeny allowed to identify the combination of diagnostic characters to distinguish between *Brachypelma* and *Tliltocatl*. Ten species were transferred to *Tliltocatl*, however, three of them lack information that allows them to be separated from other species, so *T. andrewi*, *T. alvarezi* and *T. aureoceps* were considered *nomina dubia*. *Brachypelma fossorium* was not part of *Brachypelma* or *Tliltocatl* in any of the analyzes, so after reviewing the types it was transferred to *Stichoplastoris*, creating the combination *S. fossorius*. Our molecular phylogenetic analyses had high support values, and those carried out with mitochondrial and nuclear markers solves the interrelationships within *Tliltocatl* and shows new potential species. We believe that molecular markers can be a useful tool to perform better tarantula reintroduction strategies into the correct distribution areas. Suggestions are made about current and future changes in the conservation of *Brachypelma* and *Tliltocatl*, the implication of the taxonomic changes for CITES and the Mexican Law for the protection of wildlife.

Introducción General

La familia Theraphosidae, que incluye a las tarántulas, está compuesta por 974 especies, agrupadas en 144 géneros (World Spider Catalog 2018) y once subfamilias (Lüddecke *et al.* 2018), distribuidas por todas las regiones tropicales y algunas subtropicales. La subfamilia Theraphosinae es endémica de América, es la mejor estudiada y más diversa, agrupando aproximadamente a la mitad del total de géneros y especies de la familia (Pérez-Miles *et al.* 1996, Bertani 2001, Perafán & Pérez-Miles 2014). Se han realizado algunos estudios filogenéticos con base en caracteres morfológicos para determinar las relaciones internas de esta subfamilia (Raven 1985, Goloboff 1993, Pérez-Miles *et al.* 1996, Pérez-Miles 2000, Bertani 2001, Bond & Opell 2002, Hedin & Bond 2006, Bond & Hedin 2006, West *et al.* 2008, Hendrixson & Bond 2009, Bond *et al.* 2012, Guadanucci 2014, Hamilton *et al.* 2014, Perafán & Pérez-Miles 2014, Hamilton *et al.* 2016, Fukushima & Bertani 2017, Ortiz & Francke 2016). Sin embargo, las relaciones internas de cada género son aún escasas. Hasta el momento son muy pocos los trabajos publicados que incluyan la utilización de caracteres moleculares en el intento de develar las relaciones intragenéricas en Theraphosinae (Lüddecke *et al.* 2018). Recientemente unos pocos se han realizado para observar relaciones internas entre algunas de las especies de los géneros *Aphonopelma*, *Bonnetina* y *Grammostola* (Hamilton *et al.* 2011, Hendrixson *et al.* 2013, Wilson *et al.* 2013, Hamilton *et al.* 2014, Hendrixson *et al.* 2015, Graham *et al.* 2015, Hamilton *et al.* 2016, Montes de Oca *et al.* 2015, Ortiz & Francke 2016).

En México se conocen actualmente 16 géneros y 106 especies, ocupando así el segundo lugar del mundo en cuanto a diversidad de tarántulas después de Brasil (World Spider Catalog, 2018). Entre las tarántulas que habitan nuestro país se encuentra el género *Brachypelma* Simon 1891, que es uno de los más emblemáticos del mundo, principalmente por su auge y distribución como mascota, siendo preferidas por los coleccionistas debido a su singular belleza, tamaño y relativa docilidad (Smith 1994, West 2005). Por estas características algunas de las especies han sido sometidas a una fuerte presión de captura para el mercado de mascotas. Sin embargo, México también se ha caracterizado por un fuerte movimiento en pro de la conservación de estos animales, que ha buscado vías para combatir el problema de la sobre captura, la pérdida de hábitats y otros fenómenos que

amenazan la supervivencia de las tarántulas. Prueba de ello es la inclusión en 1994 de dos especies del género *Brachypelma* en la NOM-059; tanto *Brachypelma smithi* (F. O. P.-Cambridge, 1897), como *Brachypelma emilia* (White, 1856), fueron colocadas bajo la categoría de Amenazada. Posteriormente, en 1996 *B. smithi* fue la primera tarántula incluida bajo alguna categoría de amenaza por la Unión Internacional para la conservación de la naturaleza (IUCN). Finalmente fue en el año de 1997, en que, tras una petición de los Estados Unidos de América, se incluye a los taxones *Brachypelma* spp. y a todas sus sinonimias en el apéndice II del CITES con motivo de regular más estrictamente su comercio internacional. Recientemente, en marzo de 2018 se llevo a cabo el taller trinacional México, Canadá, Estados Unidos, con el fin de realizar propuestas y encontrar soluciones a la problemática del comercio ilegal de tarántulas prioritarias para la conservación. A su vez, los especialistas de la IUCN participaron en la evaluación de cada una de las especies de *Brachypelma* para darlas de alta en la lista roja con su respectivo estatus de protección.

El género *Brachypelma* cuenta con 16 especies formalmente descritas que se distribuyen desde México hasta Costa Rica, de las cuales 12 habitan exclusivamente en nuestro país. El género se caracteriza por poseer: 1) órgano estridulatorio de setas plumosas en el trocánter de la pata I y en la base del fémur I; 2) embolo del bulbo pedipalpal cóncavo, ancho distalmente y presencia de quillas prolateral superior, apical y retrolateral; 3) espermateca de la hembra fusionada con único receptáculo seminal (excepto *B. albiceps* y *B: klaasi*), espermateca con placas de la base bien esclerosadas y separadas en medio, 5) patelas sin ninguna espinación y 5) combinación de setas urticantes tipo I y III (Smith 1994; Locht *et al.* 1999, Mendoza & Francke 2017, Mendoza & Francke 2018 in rev.). Este género ha sido incluido en los análisis filogenéticos que se han hecho sobre Theraphosinae mostrando afinidad con los géneros *Acanthoscurria* Ausserer 1871, *Pamphobeteus* Pocock 1901, *Megaphobema* Pocock 1901, *Sericopelma* Ausserer 1875 y *Theraphosa* Thorell 1870 (Pérez-Miles *et al.*, 1996; Pérez-Miles, 2000; Bertani, 2000, Perafán & Pérez-Miles 2014). Aunque *Brachypelma* es muy reconocido a nivel mundial, nunca se ha realizado una revisión formal del género. Además, presenta una problemática particular a resolver puesto que 10 especies descritas desde 1992 a 2008 han sido publicadas por aficionados europeos

con base en material para el mercado de mascotas extraído ilegalmente de México y muchas carecen de localidad tipo confiable; realizando unas descripciones morfológicas pobres y sin apego real a un escrutinio científico al ser publicadas en revistas que carecen de revisión por pares.

El bajo conocimiento sobre su filogenia, biogeografía y el inexistente sobre su historia natural, lo convierten en un interesante objeto de estudio taxonómico y filogenético. En esta tesis se presenta la revisión sistemática y filogenia molecular de *Brachypelma*. Se realiza un estudio integral de caracteres morfológicos y moleculares, así como se explora la utilidad de los códigos de barras para la identificación de las especies prioritarias para su conservación. Se prueba el uso de caracteres moleculares mitocondriales y nucleares que permitan ayudar en la delimitación de especies. Se actualiza el conocimiento taxonómico del grupo con base en evidencia morfológica y molecular, mostrando que el grupo no es monofilético, dando esto como resultado la creación de un género nuevo y la transferencia de diez especies al mismo, tres de las cuales son consideradas nomina dubia debido a la falta de caracteres que permitan su correcta identificación. Las especies de *Brachypelma* sensu stricto son rediagnósticadas y sus claves de identificación son elaboradas. No hay evidencia de especies nuevas para el género, sin embargo, para el nuevo género *Tliltocatl* se encuentran cinco potenciales especies no descritas. Se exploran los patrones de distribución de ambos géneros. Se espera que a partir de este trabajo se abrán nuevos campos de investigación sobre este grupo de arañas. Es de esperar que la utilización de caracteres moleculares en la taxonomía de Theraphosidae tenga un impacto relevante en nuestra comprensión de su diversidad y especialmente de sus relaciones, que han resultado ser siempre difíciles de descifrar. El hecho de ser un género comúnmente comerciado tanto legal como ilegalmente, hace indispensable el contar con un mejor conocimiento acerca de las especies que lo conforman y su correcta identificación. Finalmente, conocer las distribuciones geográficas reales y potenciales permitirá la toma de mejores decisiones sobre su conservación *in situ*.

Capítulo 1

Uso de los códigos de barras de ADN en la identificación y conservación de especies incluidas en la CITES.

Sección 1.1.

Artículo requisito publicado

Systematic revision of *Brachypelma* red-kneed tarantulas (Araneae: Theraphosidae), and the use of DNA barcodes to assist in the identification and conservation of CITES-listed species

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Systematic revision of *Brachypelma* red-kneed tarantulas (Araneae : Theraphosidae), and the use of DNA barcodes to assist in the identification and conservation of CITES-listed species

Jorge Mendoza[A,B,C] *and Oscar Francke*[B]

[A]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Av. Universidad 3000, C.P. 04510, Coyoacán, Distrito Federal, Mexico.
[B]Colección Nacional de Arácnidos, Módulo D planta baja, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, 3er circuito exterior, Apto. Postal 70-153, CP 04510, Ciudad Universitaria, Coyoacán, Distrito Federal, Mexico.
[C]Corresponding author. Email: nomeireth@hotmail.com

Abstract. Mexican red-kneed tarantulas of the genus *Brachypelma* are regarded as some of the most desirable invertebrate pets, and although bred in captivity, they continue to be smuggled out of the wild in large numbers. Species are often difficult to identify based solely on morphology, therefore prompt and accurate identification is required for adequate protection. Thus, we explored the applicability of using COI-based DNA barcoding as a complementary identification tool. *Brachypelma smithi* (F. O. Pickard-Cambridge, 1897) and *Brachypelma hamorii* Tesmongt, Cleton & Verdez, 1997 are redescribed, and their morphological differences defined. *Brachypelma annitha* is proposed as a new synonym of *B. smithi*. The current distribution of red-kneed tarantulas shows that the Balsas River basin may act as a geographical barrier. Morphological and molecular evidence are concordant and together provide robust hypotheses for delimiting Mexican red-kneed tarantula species. DNA barcoding of these tarantulas is further shown to be useful for species-level identification and for potentially preventing black market trade in these spiders. As a Convention on International Trade in Endangered Species (CITES) listing does not protect habitat, or control wildlife management or human interactions with organisms, it is important to support environmental conservation activities to provide an alternative income for local communities and to avoid damage to wildlife populations.

Additional keywords: DNA bar-coding, taxonomy, molecular phylogenetics.

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Introduction

Brachypelma smithi (F. O. Pickard-Cambridge, 1897) is commonly known in the pet trade as the Mexican red-kneed tarantula. Because of its bright colouration, docile behaviour, longevity and hardiness in captivity, it has been shipped out of Mexico in large numbers (Reichling 2003). For example, between 1984 and 1991 a key trader shipped 3000 *B. smithi* into the UK and Europe. Similarly, a company based in Mexico City shipped 200 000 tarantulas into the USA over a 10-year period. The same company also admitted that four out of five commercially exploited colonies in the Colima-Guerrero region, containing 30 000–40 000 individuals each (before exploitation), had been stripped of their populations (Smith 1994). Because of these activities, population sizes have been affected and the species was declared threatened. In September 1985, *B. smithi* was officially placed on Appendix II of the Convention on International Trade in Endangered Species (CITES) (Schultz and Schultz 2009).

Tesmoingt *et al.* (1997*a*, 1997*b*) described two new species of red-kneed tarantulas (*B. annitha* and *B. hamorii*) from Mexico based on highly variable external characters such as the colouration of the legs and carapace. The specimens designated as holotypes were obtained from the pet trade, and therefore no exact type localities were provided. West (2005) suggests that *B. annitha* and *B. hamorii* are merely colour morphs of *B. smithi*. Currently, *B. smithi* is thought to have a disjunct geographical distribution with one population in the state of Colima, extending south-eastwards into Michoacán, and another population along the coast of the state of Guerrero (Locht *et al.* 1999).

Species 'classification' allows scientists to determine the relationships between species, and species 'identification' allows for determining which specimens have been collected. However, taxonomic identifications are often misdiagnosed due to phenotypic and genetic variability, and lack of diagnostic

features of certain life stages (Hebert *et al.* 2003). Morphology-based identifications of mygalomorph spiders are time-consuming and problematic for several reasons. In many groups, spiders can vary in appearance depending on life cycle and gender. It is difficult to identify juveniles, which are the most abundant individuals at certain times of the year (Greenstone *et al.* 2005; Blagoev *et al.* 2009). Barrett and Hebert (2005) extended DNA barcoding to arachnids and show that it is possible to identify spider fauna using a short fragment of the mitochondrial gene coding for cytochrome *c* oxidase 1 (COI).

The traditional DNA barcode is generated by amplifying a 650-bp segment of the mitochondrial COI gene, usually by PCR (Sun *et al.* 2012), and has been shown to be useful in identifying spider species from around the world (e.g. Barrett and Hebert 2005; Arnedo and Fernández 2007; Longhorn *et al.* 2007; Petersen *et al.* 2007; Blagoev *et al.* 2009; Kuntner and Agnarsson 2011; Hamilton *et al.* 2011, 2014; Hendrixson *et al.* 2013, 2015; Montes de Oca *et al.* 2016; Blagoev *et al.* 2016; Ortíz and Francke 2016). However, a distinction must be recognised between the potential of DNA barcodes for defining or delimiting species and their potential for identifying species. DNA barcodes should be encouraged as a supplement to species description and diagnoses, but should not replace morphological data (Will and Rubinoff 2004; Prendini 2005). Even if debate remains about the characters and criteria for species delimitation, most taxonomists agree that evaluating several lines of evidence within a formalised framework is the most efficient approach to defining robust species hypotheses (Pante *et al.* 2015; Hamilton *et al.* 2016; Ortíz and Francke 2016). For instance, morphological data, geographic distributional data, ecology, behaviour and/or phylogenetically informative molecular markers can be considered concurrently to construct robust taxonomic hypotheses using an integrative decision-making process (e.g. Bond and Stockman 2008; Hamilton *et al.* 2011, 2014, 2016; Hendrixson *et al.* 2013, 2015).

With increased smuggling and difficulties in halting traffickers, the institutions responsible for species wildlife protection in Mexico have joined forces with the academic community to develop a project using DNA barcoding. The goal is to be able to identify species quickly and efficiently. The scientific division of the Federal Police and environmental protection agencies, in collaboration with the Thematic Network Barcode of Life in Mexico (Mexbol), are creating a genetic library to facilitate the identification of animals and plants by using codes made from segments of DNA (BWPM 2014).

In an attempt to resolve known problems in morphology-based taxonomy, a revision was undertaken of all the *Brachypelma* type specimens of species deposited in European collections and of material deposited in the Colección Nacional de Arácnidos in Mexico. This provided the basis to morphologically differentiate red-kneed tarantula species. DNA barcoding techniques were then used to identify *Brachypelma* red-kneed species and their respective delimitations. Parsimony and Bayesian inference analyses were employed for phylogenetic analysis; results were compared with morphology as a strategy for better delimitation of Mexican *Brachypelma* red-kneed species. The present study resolves historical and taxonomic problems within the Mexican red-kneed tarantulas and clarifies geographical distributions. It is hoped that these findings will help create better regulations to protect and conserve these endangered species.

Methods

Morphology

The general descriptive format used in the present study follows Mendoza (2014), with some modifications. All measurements are in millimetres and were taken using an ocular micrometer on a stereomicroscope Nikon SMZ645 (Nikon Instruments Inc., New York, NY) for smaller structures, and a digital caliper with an error of 0.1 mm for larger structures. Leg and palp measurements were taken along the dorsocentral axis of the left side. Description of tarsal scopulae follows Pérez-Miles (1994). Male palpal bulb keel terminology follows Bertani (2000). Description of spermatheca shape follows the general format used with theraphosids (Pérez-Miles 1989; Bertani 2001). In addition, *Brachypelma* species possess a sclerotised area below the spermatheca, the spermatheca baseplate described and named in this study. For comparative purposes, it is necessary to observe the spermatheca in ventral view (in previous publications, the spermatheca is usually drawn or figured from a dorsal view). The spermatheca baseplate is a sclerotised plate located below the base of the spermatheca. It can be completely fused (e.g. genera *Theraphosa* Thorell, 1870 and *Sericopelma* Ausserer, 1875) or medially divided (e.g. genera *Brachypelma* Simon, 1891 and *Phormictopus* Pocock, 1901). The fused baseplate can be as wide as the base of the spermatheca or only a reduced central area, while each side of the divided plate can be narrower than or equal to half of the spermatheca base width.

The photographs in Figs 3–18, 24–36 and 42–49 were taken with a Nikon Coolpix S10 (Nikon Inc., New York, NY) VR digital camera coupled to a stereomicroscope. Descriptions of colours use the standard names of the 267 colour centroids of the NBS/IBCC Colour System (Mundie 1995) to promote standardisation in colour descriptions of live animals. To avoid differences of perception in colouration caused by calibration of the monitor, we extracted the RGB (Red, Green, Blue) code and Pantone colour from photographs using Photoshop CS Live. The eyedropper tool was clicked over the digital image to obtain the RGB colour code in the Set foreground colour, and cross-checked in the colour libraries for the Pantone solid coat. A standard for illumination was deliberately not established, as fluorescent and incandescent lighting provide different colours for the same sample. Habitat and laboratory images were taken with a Canon G12 digital camera. Habitat shots were taken under natural daylight conditions. Laboratory images were taken with a white background and illumination was provided by one fluorescent 30-W light bulb held ~20 cm from the specimen. After the RGB code was obtained, the real colour of the specimen was inferred using the RGB code of colour centroids. The range of each colour centroid as perceived by the human eye is wide enough to account for errors of observation.

Abbreviations used in the text are as follows. Ocular patterns: ALE, anterior lateral eyes; AME, anterior median eyes; PME, posterior median eyes; PLE, posterior lateral eyes. Legs and palpi: d, dorsal; p, prolateral; r, retrolateral; v, ventral; Rap, retrolateral tibial apophysis; Pap, prolateral tibial apophysis; PL, plumose setae. Palpal bulbs: AK, apical keel; PI, prolateral

inferior keel; PS, prolateral superior keel. Chelicerae: CB, cheliceral band. Spermatheca: Bp, spermathecal baseplate. Spinnerets: PMS, posterior median spinnerets; PLS, posterior lateral spinnerets. In species synonymies, we follow the World Spider Catalog (2016): D, described; *t*, transferred; m, male; f, female. Primers: LCO, low cytochrome oxidase; HCO, heavy cytochrome oxidase. Phylogenetics: L, length; Ri, retention index; Ci, consistency index. Institutions: MNHNP, Museum National d'Histoire Naturelle, Paris; NHM, Natural History Museum, London; SNMF, Senckenberg Naturmuseum, Frankfurt; CNAN, Colección Nacional de Arácnidos, México DF; UNAM, Universidad Nacional Autónoma de México, PROFEPA (acronym in Spanish), Federal Environmental Protection Agency.

Taxa

Specimens were collected throughout the known distribution of the genus *Brachypelma* in Mexico with special attention to the type localities (where possible). Material was fixed in 80% ethanol. The third leg on the right side of each spider was stored in 96% ethanol at −20°C. Tissue samples of 26 specimens were used for DNA extraction, representing eight species of *Brachypelma*, including some identified as *B. annitha*, *B. smithi* and *B. hamorii*. Six tissue samples of *B. hamorii*, five of *B. smithi*, three each of *Brachypelma albiceps* Pocock, 1903, *Brachypelma auratum* Schmidt, 1992, *Brachypelma baumgarteni* Smith, 1993 and *Brachypelma boehmei* Schmidt & Klaas, 1993, two of *Brachypelma klaasi* (Schmidt & Krause, 1994) and one of *B. annitha* were used in this study. Additionally, two sequences were retrieved from GenBank to use as outgroups for phylogenetic analyses: *Psalmopoeus cambridgei* Pocock, 1895 [JQ412455.1] and *Eupalaestrus campestratus* (Simon, 1891) [JQ412446.1], both from the same study (Briscoe *et al.* 2013). We obtained sequences of mitochondrial COI from the 26 samples (Appendix 1). Vouchers were deposited in the CNAN and assigned a unique number (CNAN-Ar00xxxx). All sequences were submitted to GenBank, and accession numbers and specimen information are provided in Table 1. For species diagnoses, molecular autapomorphies were obtained with the function *mucDiag* of the R package SPIDER (Brown *et al.* 2012). Diagnostic nucleotides are coded according to their relative position in the alignment (1–641), as defined in Table 2.

DNA protocols

DNA isolation, PCR amplification and sequencing were performed at the Laboratorio de Sistemática Molecular,

Table 1. GenBank accession codes for tissue samples, deposited in the Laboratorio de Sistemática Molecular (Zoología) at the Instituto de Biología, UNAM, Mexico City, from which DNA was extracted and sequenced for phylogenetic analyses of seven species in the genus *Brachypelma* Simon, 1891

Psalmopoeus cambridgei and *Eupalaestrus campestratus* sequences published by Briscoe *et al.* 2013; COI, cytochrome *c* oxidase 1; juv., juvenile; subad., subadult; Un, unsexed

Species	Specimen	Voucher ID	COI
Psalmopoeus cambridgei	1 juv. Un	447_SC_AB	JQ412455
Eupalaestrus campestratus	1 juv. Un	446_SC_AB	JQ412446
Brachypelma albiceps	1 ♂	CNAN-Ar003412	KT995328
Brachypelma albiceps	1 juv. ♀	CNAN-Ar007839	KT995331
Brachypelma albiceps	1 juv. ♀	CNAN-Ar007850	KT995391
Brachypelma auratum	1 ♂	CNAN-Ar003658	KT995348
Brachypelma auratum	1 juv. ♀	CNAN-Ar007136	KT995371
Brachypelma auratum	1 juv. ♀	CNAN-Ar007164	KT995397
Brachypelma baumgarteni	1 ♀	CNAN-Ar007151	KT995332
Brachypelma baumgarteni	1 juv. ♀	CNAN-Ar007161	KT995395
Brachypelma baumgarteni	1 subad. ♂	CNAN-Ar007835	KT995382
Brachypelma boehmei	1 ♀	CNAN-Ar007185	KT995359
Brachypelma boehmei	1 ♀	CNAN-Ar007186	KT995337
Brachypelma boehmei	1 ♂	CNAN-Ar007833	KT995343
Brachypelma hamorii	1 ♂	CNAN-Ar003614	KT995325
Brachypelma hamorii	1 ♂	CNAN-Ar007163	KT995334
Brachypelma hamorii	1 ♂	CNAN-Ar007168	KT995378
Brachypelma hamorii	1 ♂	CNAN-Ar007826	KT995381
Brachypelma hamorii	1 ♂	CNAN-Ar007827	KT995387
Brachypelma hamorii	1 ♂	CNAN-T0900	KT995401
Brachypelma klaasi	1 ♂	CNAN-Ar007160	KT995340
Brachypelma klaasi	1 ♂	CNAN-Ar007162	KT995346
Brachypelma smithi	1 ♀	CNAN-Ar004131	KT995375
Brachypelma smithi	1 juv. ♂	CNAN-Ar007140	KT995380
Brachypelma smithi	1 ♀	CNAN-Ar007143	KT995400
Brachypelma smithi	1 ♀	CNAN-Ar007144	KT995385
Brachypelma smithi	1 ♂	CNAN-Ar007832	KT995374
Brachypelma smithi (annitha)	1 ♀	CNAN-Ar003611	KT995325

Table 2. Mitochondrial cytochrome *c* oxidase 1 (COI) DNA sequence of *Brachypelma smithi* and *Brachypelma hamorii*, showing the nucleotide numbering system (1–641) used to designate molecular autapomorphies for species diagnosis

COI mtDNA (nucleotides 1–641)
Brachypelma smithi CNAN-Ar007144 (KT995385)
CTTCTATGGTGGGAACTGCGATAAGAGTAATTATTCGGGTTGAGTTGGGGCAGGTGGGTAGTTTGTTGGGAGATGATCATTTGTATAATGTG
ATTGTGACGGCTCATGCTTTGGTCATGATTTTTTTTATGGTAATGCCTATTTTGATTGGTGGGTTTGGGAATTGGATGTTGCCTTTAATGAT
GGGTGCTCCTGATATGGCTTTTCCTCGTATGAATAATTTGAGTTTTGATTGTTGCCTCCTTCTTTGTTTTTGTTGATTTTGTCGTCGTTGAC
TGATGTTGGGGTTGGTGCTGGGTGAACTATTTATCCTCCTTTGTCTTCTTTTGTTGGGCATTCTGGGGGTGGTATGGATTTTGCTATTTTTTC
TTTACATTTGGCAGGGGCTTCTTCTATTATGGGGTCTGTAAATTTTATTAGAACTGTTTTGAATATGCGTAGATCTGGAATAACAATAGAG
CGGGTTCCGTTGTTTGTGTGGTCTGTTCTTATTACGACTATTTTGTTGTTGTTGTCTTTACCTGTTTTGGCTGGGGCTATTACTATGTCATTG
TCTGATCGGAATTTTAATACTTCTTTTTTTGATCCGGCTGGTGGGGGGGAACCCGTTTTG
Brachypelma hamorii CNAN-Ar003614 (KT995325)
CTTCTATAGTAGGAACTGCAATGAGAGTTATTATTCGGGTTGAGTTGGGACAGGTGGGTAGTTTATTGGGGGATGATCATTTATATAATGTT
ATTGTGACGGCTCATGCTTTGGTGATAATTTTTTTTATAGTAATGCCCATTTTGATTGGTGGGTTTGGAAATTGAATGTTGCCTTTAATGAT
GGGTGCTCCTGATATGGCCTTTCCTCGAATGAATAATTTGAGTTTTTGGTTGTTGCCTCCTTCTTTGTTCTTGTTGATTTTGTCGTCGTTAAC
TGATGTTGGAGTTGGTGCTGGATGAACGATTTATCCTCCTTTGTCTTCTTTTGTTGGGCATTCTGGAGGAGGGATGGATTTTGCTATTTTTT
CTTTGCATTTGGCTGGAGCTTCTTCTATTATGGGATCTGTAAATTTTATTAGTACCGTTTTGAATATGCGAAGATCGGGAATAACTATGGA
ACGGGTTCCTTTGTTTGTGTGGTCTGTTCTTATTACAACTATTTTGTTGTTGTTGTCTCTGCCTGTTTTGGCTGGAGCTATTACTATGTTATT
GTCTGATCGTAATTTTAATACTTCTTTTTTTGATCCGGCTGGTGGAAGGGATCCTGTGTTGTTTCAACATTTATTTTG

Instituto de Biología, UNAM. Muscle tissue was extracted from the leg by removing ~20 mg of tissue. Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen Sciences Inc., Maryland, USA), following the manufacturer's protocol. The concentration quality of the extracted DNA was quantified with a spectrophotometer (Nanodrop 2000, Thermo Scientific, Delaware, USA) or visualised via agarose gel electrophoresis. DNA amplification was performed using the PCR for the mtDNA barcoding gene region COI. A single set of primers was used: LCO 1490: 5′-GGTCAACAAATCATAA AGATATTGG-3′, together with HCO 2198: 5′-TAAACTTCAG GGTGACCAAAAAATCA-3′ (Folmer *et al.* 1994). This primer set amplified a 710-bp region of the mitochondrial COI gene.

The PCR reaction (100.8 μL) contained 48 μL of 10× PCR buffer, 24 μL of $MgCl_2$, 7.68 μL of forward and reverse primers, 9.6 μL of dNTPs and 3.84 μL of Taq polymerase, using 1 μL of the DNA template for each sample. The PCR program for COI was as follows: initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 45 s, elongation at 72°C for 2 min 30 s; followed by 7 min of final elongation at 72°C. LCO 1490 and HCO 2198 primers were used for single-stranded sequencing. The accuracy of sequences was verified by independently amplifying and sequencing the complementary strands of all fragments. Primer sequences were removed and complementary strands of DNA assembled into consensus sequences, edited and checked for quality using Geneious R8 (Kearse *et al.* 2012). If complementary strands disagreed (excluding minor mismatches), the sample was amplified and sequenced again to resolve discrepancies.

DNA sequence alignment and phylogenetic analysis

Static alignments of COI gene fragments were generated with MAFFT online ver. 7 (Katoh *et al.* 2002, 2005). The G-INS-I strategy was selected, which performs a global alignment based on a fast Fourier transform (FFT) approximation (Katoh *et al.* 2002). This method is suitable for large datasets comprising sequences with relatively limited variation in length, i.e. few short gaps (Katoh *et al.* 2005). The scoring matrix for nucleotide sequences was set to $1/PAM_K = 2$, gap opening penalty to 1.53 and offset value to 0.

Parsimony analysis of the DNA sequence alignment (641 bp) was conducted with equal weighting using TNT ver. 1.1 (Goloboff *et al.* 2008), with Sectorial Search using five replications as starting points for each hit, Ratchet (2000 iterations) with drifting (100 cycles) and with fusing (five rounds; dumping fused suboptimal trees to prevent clogging). The maximum number of trees retained in memory was 30 000.

We also carried out Bayesian inference using MrBayes ver. 3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The best-fitting model of sequence evolution was selected using jModelTest ver. 1.0.1 (Posada 2008) in accordance with the Akaike information criterion, on the basis of which a GTR + G + I model was applied. The analysis comprised four Markov chain Monte Carlo models, performed for 10 million generations for all DNA sequence alignments. Trees were sampled every 1000 generations and those sampled before stationarity were discarded as burn-in.

The relative support for each node in the topology obtained by the parsimony analysis was calculated in TNT, using 1000 jack-knife pseudoreplicates with heuristic searches consisting of 1000 random addition sequences, followed by ten iterations of tree bisection-reconnection, retaining one tree per iteration. Posterior probabilities are shown for the Bayesian phylogram obtained by the mitochondrial sequence alignments.

Results

Phylogenetic analysis

An alignment containing 641 bp was produced for the COI dataset. Parsimony analyses of these sequences recovered three most-parsimonious trees. The strict consensus tree (L: 440, Ci: 0.646, Ri: 0.804) recovered the monophyly of both *B. hamorii* and *B. smithi* with high jack-knife support; *B. annitha* was found nested in the *B. smithi* clade (Fig. 1). The tree topology recovered *B. hamorii* as a sister species of the clade

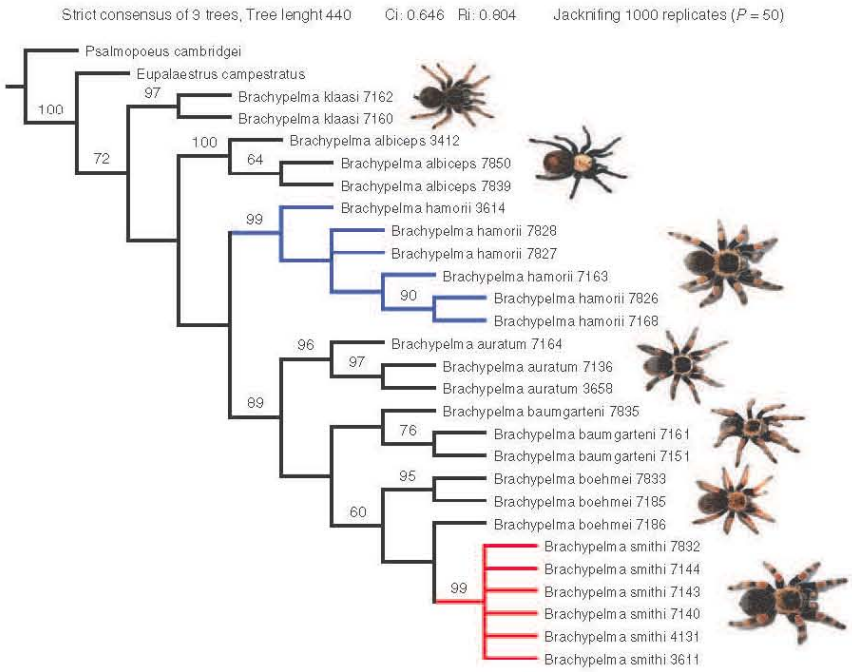


Fig. 1. Strict consensus of the trees obtained by parsimony analysis of 641 aligned nucleotides from barcoding gene cytochrome *c* oxidase 1 (COI) of the mitochondrial genomes of 26 samples from nine *Brachypelma* species, with equal weighting. Jack-knife support with percentages less than 100% indicated above branches.

formed by *B. auratum*, *B. baumgarteni*, *B. boehmei* and *B. smithi*. All *Brachypelma* species analysed except *B. boehmei* were monophyletic, but the interspecific relationships were not as clear, with some nodes lacking support. Despite this, COI has proven useful to identify *Brachypelma* species and is concordant with morphological identification.

Bayesian inference analyses of the COI sequence also recovered the monophyly of both *B. hamorii* and *B. smithi* as separate species, with high posterior probability: 0.996 for the *B. smithi* clade and 1 for *B. hamorii* (Fig. 2). The topology recovered shows *B. hamorii* as a sister species of the clade formed by *B. smithi*, *B. auratum*, *B. baumgarteni* and *B. boehmei*. In this analysis, the three samples available for *B. boehmei* recovered the species as monophyletic. However, no resolution was shown for interspecific relationships in the group comprising *B. boehmei*, *B. baumgarteni* and *B. auratum*.

Despite the differences between the parsimony and Bayesian analyses, *Brachypelma* species can be identified with high accuracy using barcodes. In both cases, *B. hamorii* is the sister species of *B. smithi*, *B. auratum*, *B. baumgarteni* and *B. boehmei*, although the relationships between these species require further resolution.

Taxonomy

Family **THERAPHOSIDAE** Thorell

Subfamily **THERAPHOSINAE** Thorell

Genus ***Brachypelma*** Simon

Brachypelma Simon, 1891: 338.
Type species: Mygale emilia White, 1856, by monotypy.

Diagnosis

Brachypelma differs from all other known theraphosinae genera by having plumose setae on the prolateral face of leg I trochanter and femur and retrolateral face of the palp. Both sexes lack a plumose pad of setae on leg IV femur. Metatarsus IV is 20–35% scopulated distally. All tarsi scopulae are undivided. The femur of leg III is slightly enlarged, but not swollen as in other genera. The male palpal bulb is distally wide and flattened (i.e. spoon-shaped) and comprises prolateral superior and apical keels, and a small or reduced prolateral inferior keel. Females differ by possessing a

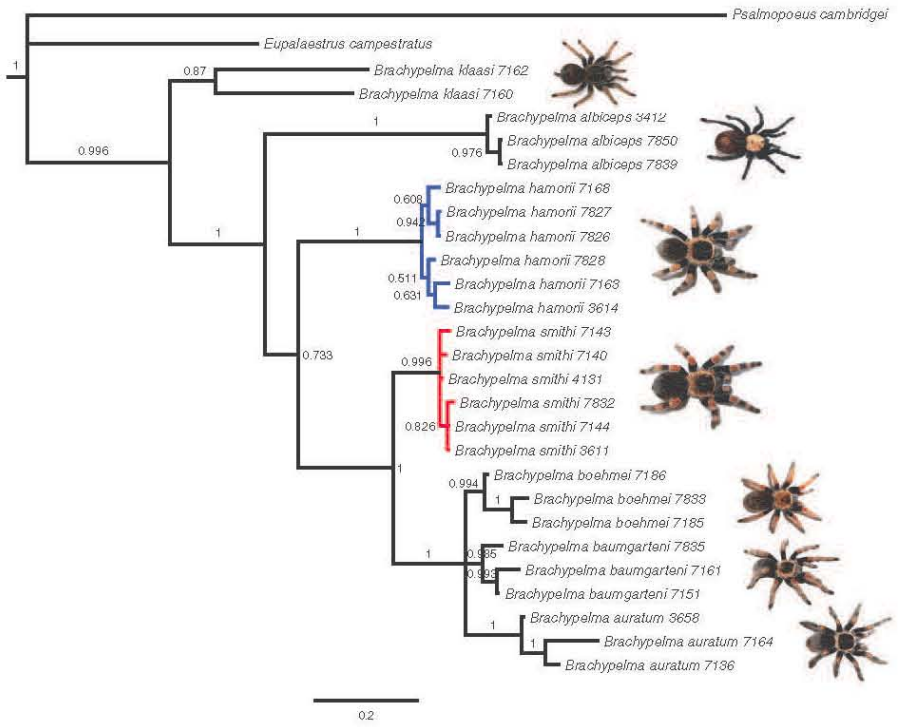


Fig. 2. Bayesian phylogenetic hypothesis of 641 aligned nucleotides from barcoding gene cytochrome *c* oxidase 1 (COI) of the mitochondrial genomes of 26 samples from nine *Brachypelma* species. Posterior probabilities indicated above the branches or close to nodes.

simple undivided/fused spermatheca, apically narrowed. Both sexes possess urticating type I and type III setae; type III located in the dorsoposterior area and type I surrounding these.

Brachypelma smithi (F. O. Pickard-Cambridge)

(Figs 3–23, 42–45, 50, 51, 53–56)

Eurypelma smithi F. O. Pickard-Cambridge, 1897: 20, pl. 1, fig. 4.
Brachypelma smithi (F. O. Pickard-Cambridge, 1897): Pocock, 1903: 103; Locht *et al.*, 1999: 198, fig. 5.
Brachypelma annitha Tesmoingt, Cleton & Verdez, 1997*a*: 9, pls 1 6; Tesmoingt *et al.*, 1997*b*: 2, pls 7, 8, 11; Peters, 2000: 64, figs 205 207; Peters, 2003: 108, figs 428, 435, 436; Teyssié, 2015: 267, fig. 2. Type locality unknown; species described from pet trade material. The type specimens were never deposited in a museum, according to the authors (M. Tesmoingt and F. Cleton, personal communication) and the MNHNP curator Christine Rollard. Thus, they are presumed to be lost.

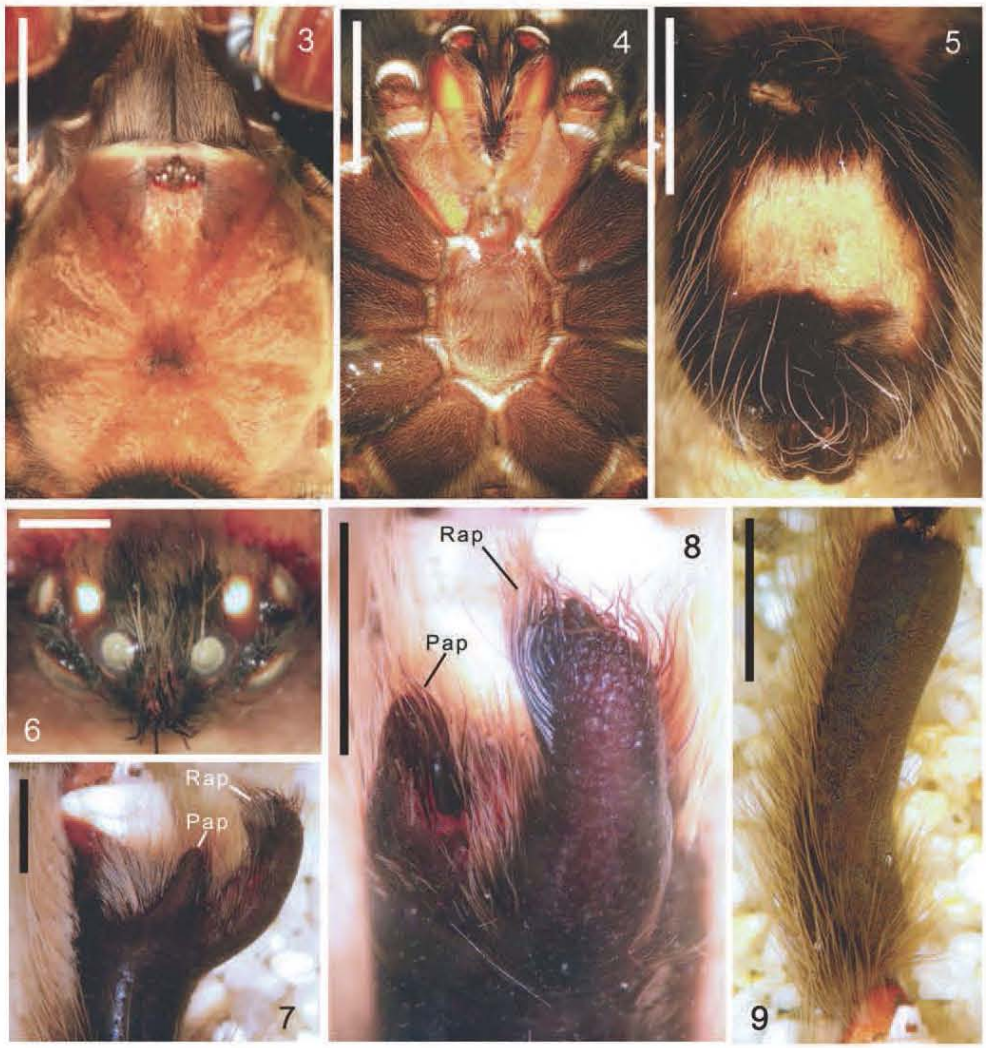
Material examined

Holotype. MEXICO: *Guerrero*: juvenile ♂ (labelled as ♀), Dos Arroyos. H. H. Smith (BMNH 1143; also labelled BM1898.12.24.33).

Other material examined. **MEXICO:** *Guerrero*: 1 ♂, 2 ♀, Mpio Acapulco, Dos Arroyos. 13.xii.2013, J. Mendoza (CNAN-Ar007146, CNAN-Ar007143, CNAN-Ar007144); 1 ♂, Mpio Acapulco, 04.i.1965, E. Rivapalacios (CNAN-Ar003086); 1 ♂, Mpio Atoyac, 27.ii.1984, J. G. Julio (CNAN-Ar003434); 1 ♀, Mpio Acapulco, 10.vii.1979, M. Adams (CNAN-Ar003435); 1 ♂, Mpio Acapulco, without additional data (CNAN-Ar003594); 2 ♀, donation received from private collection of J. Mendoza (identified in pet trade as *B. annitha*) (CNAN-Ar004131, CNAN-Ar003611); 3 ♂, Mpio Coyuca de Benítez, 29.viii.2015, J. Mendoza, R. Ramírez (CNAN-Ar010281, CNAN-Ar010275); 1 ♂, 1 ♀, Mpio Acapulco, El Quemado 14.x.2014, A. Ortega (CNAN-Ar007897, CNAN-Ar007896); 1 ♀, Mpio Josue Azueta, 24.viii.2015, D. Ortiz, J. Baldazo (CNAN-Ar007904).

Diagnosis

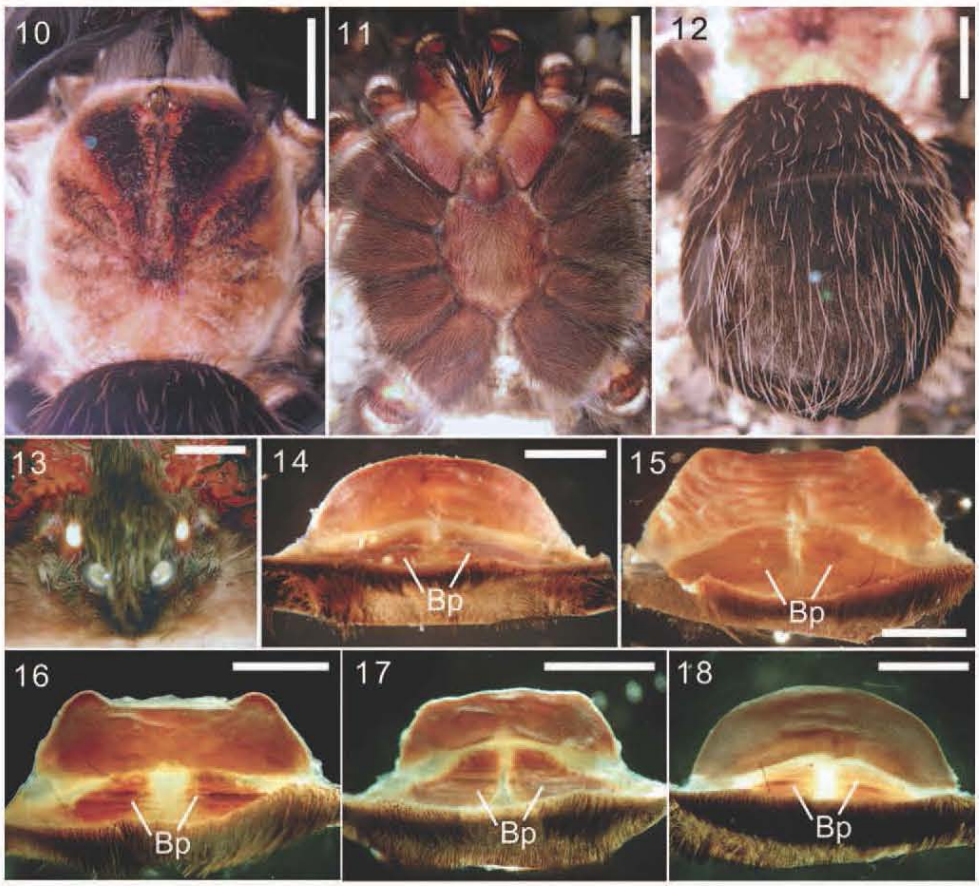
Brachypelma smithi can be distinguished from all other known *Brachypelma* species (except *B. hamorii*) by the colouration of the carapace and legs: red-orange on the patellae, tibiae and metatarsi, and orange-black starburst striations on the carapace. The shape of the genitalia also differs in both sexes with the palpal bulb being straight and broad, and the spermatheca trapezoidal. It differs from *B. hamorii* by the straight palpal bulb having a broad spoon shape, a wider apical keel and the prolateral superior keel being not as elevated. It also differs in the



Figs 3–9. *Brachypelma smithi*, male CNAN-Ar007832. *3*, Carapace, dorsal view; *4*, prosoma, ventral view; *5*, opisthosoma, dorsal view; *6*, ocular tubercle, dorsal view; *7*, tibial apophyses, prolateral view; *8*, tibial apophyses, ventral view; *9*, metatarsus I, prolateral view. Scale bars = 10 mm (*3*, *5*), 8 mm (*4*), 5 mm (*9*), 2 mm (*6*, *7*), 1 mm (*8*).

spermatheca with the spermathecal baseplate being divided and subtriangular. The spermathecal ventral face is also striated, while smooth in *B. hamorii*. Although similar in colouration, *B. smithi* differs from *B. hamorii* by the absence of a cheliceral band. Additionally, the patella flame pattern is more colourful than in *B. hamorii*, with brighter orange setae. The lateral setae are yellowish along the length of the legs, while in *B. hamorii* the same are whitish, providing greater contrast with the darker areas.

This species can also be distinguished from other genotyped *Brachypelma* by the following three diagnostic COI nucleotides



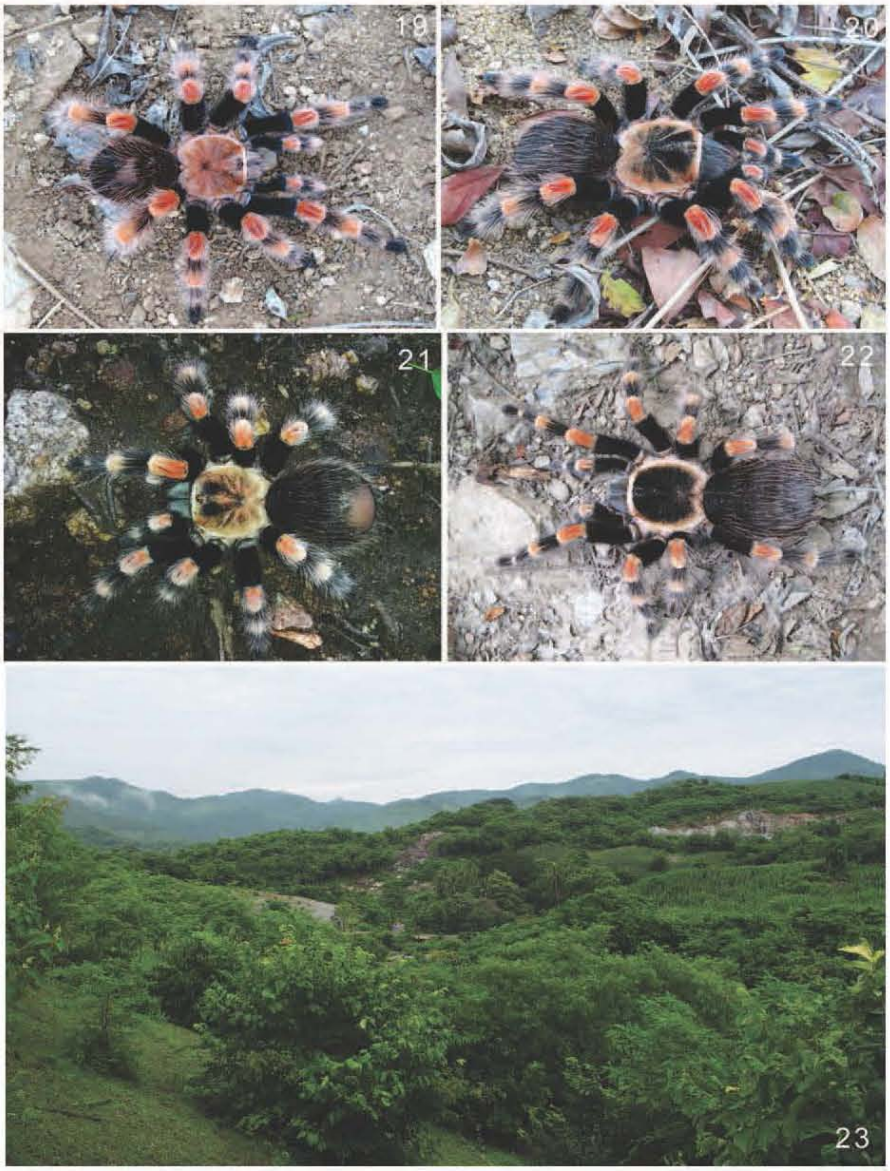
Figs 10–18. *Brachypelma smithi*. *10–14* female CNAN-Ar003611. *10*, Carapace, dorsal view; *11*, prosoma, ventral view; *12*, opisthosoma dorsal view; *13*, ocular tubercle, dorsal view; *14*, spermatheca, ventral view; *15–18*, spermatheca ventral view of: *15*, female CNAN-Ar003611; *16*, female CNAN-Ar004131; *17*, female CNAN-Ar007896; *18*, female CNAN-Ar007904. Scale bars = 10 mm (*10–12*), 2 mm (*14–18*), 1 mm (*13*).

($n = 6$): C (116), A (550), A (563). COI p-distances: interspecific above 4%, intraspecific below 1.5%. *Brachypelma smithi* differs from *B. hamorii* by an 8% COI p-distance.

Brachypelma smithi is identified by possessing the following character combination: male palpal bulb with broad spoon-like embolus; prolateral superior keel normally developed, thin and directed retrolaterally; prolateral inferior keel weakly developed, better seen dorsally; and the apical keel strongly developed, wider in the middle (Figs 42–45). Embolus straight and wide along its length, one and a half times longer than the tegulum (Figs 44, 45). Spermatheca semicircular or trapezoidal; ventral face striated. Spermathecal baseplate divided, subtriangular, four times wider than its height (Figs 14–18). Carapace of adult males orange; in females almost black with orange around the border, with black striated pattern and orange around or almost orange with only black caput area. Legs and palpi have dark reddish patellae, while tibiae and metatarsi have yellowish pink colouration distally (see colour pattern) (Figs 19–22, 50, 51, 53–56).

Description

Male (CNAN-Ar007146) (Figs 3–9, 19, 42–45). Body length 46.17 (not including chelicerae and spinnerets). Carapace length 20.18, width 19.50. Caput not markedly elevated; fovea straight, 4.00 wide (Fig. 3). Eyes: anterior eye row procurved,



Figs 19–23. *19 22*, *Brachypelma smithi*, habitus; *23*, habitat. *19*, Male CNAN-Ar007832 in life; *20*, female CNAN-Ar007144 in life; *21*, female CNAN-Ar007901 in life; *22*, female CNAN-Ar007147; *23*, deciduous forest in type locality of *B. smithi*. Photos: J. Mendoza.

posterior eye row recurved. Eye sizes and interocular distances: AME 0.53, ALE 0.77, PME 0.30, PLE 0.53, AME–AME 0.43, AME–ALE 0.17, PME–PME 1.17, PME–PLE 0.20, ALE–PLE 0.33. Ocular tubercle width 2.77, length 2.33; clypeus length 0.27 (Fig. 6). Labium length 2.50, width 3.40, with 72 cuspules. Maxilla inner corner with ~169 (left) and ~166 (right) cuspules. Cheliceral promargin with 8 (left) and 9 (right) teeth (proximal to distal: first to eighth large, first to third large, fourth to sixth medium, seventh large, eighth to ninth small). Sternum length 8.60. Sigillae oval, first, second and third pairs hardly visible, posterior sigilla one and a half times its length from the margin (Fig. 4). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I 17.18, 9.97, 13.59, 14.21, 9.35, 64.30; II 16.09, 9.16, 12.39, 12.96, 9.07, 59.67; III 14.78, 8.59, 11.66, 14.81, 8.12, 57.96; IV 17.41, 9.11, 14.79, 18.55, 9.84, 69.70. Palp: 11.62, 7.06, 10.12, –, 4.29, 33.09. Spinnerets: PMS 2.07 long, 1.50 apart; PLS 4.00 basal, 2.25 middle, 3.25 distal. Tarsi I–IV entirely scopulated. Metatarsus I densely scopulated, II densely scopulated, III 65% scopulated distally, IV 50% scopulated distally. Tibia I with two tibial apophyses normally developed, which originate from a common base. Prolateral apophysis with one inner conical spine; retrolateral apophysis almost the same width along its length (Figs 7, 8). Metatarsus I curved (Fig. 9). Stridulatory setae: with plumose setae on palp trochanter and femur retrolateral face, leg I trochanter and femur prolateral face. Chaetotaxy (left side): palp femora 1p; patellae none; tibiae I 1p; II 2p, 3v; III 3p, 4v; IV 2p, 3v, 1r; palp 2p, 4v; metatarsi I 2v; II 4v; III 2p, 7v, 2r; IV 1p, 16v. Palp: embolus straight with a broad spoon shape, prolateral superior keel normally developed, thin and directed retrolaterally; prolateral inferior keel weakly developed; apical keel strongly developed, wider in medially; opening of the embolus on the prolateral side, with a concavity just behind the opening that delimits the apical keel boundary from the remaining part of the embolus; embolus apex slightly curved retrolaterally (Figs 42–45). Urticating setae: types I and III arranged in one dorsoposterior patch, black; type III located in an oval dorsomedial area extended towards posterior; type I surrounding the type III area, with intermediates in transition areas between types III and I (Fig. 5). Variations summarised in Table 3.

Colour pattern. In live specimens, adult males with moderate yellowish brown carapace, juveniles and subadults light brown around the border and brownish black dorsomedially; chelicerae dorsally bluish grey; ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with light orange setae, ventrally brownish black. Legs and palpi: femora bluish black, patellae with a proximal dorsomedian dark reddish orange flame-shaped area, distodorsal paramedian light yellowish pink with light orange setae laterally; tibiae proximal half bluish black with light orange setae, distal half pale yellowish pink and metatarsi bluish black with light orange setae and a pinkish white ring at the terminal end; tarsi bluish black (Fig. 19).

Female (CNAN-Ar007143) (Figs 10–18, 20, 51, 53–56). Body length 56.45 (not including chelicerae and spinnerets), carapace length 24.61, width 21.88. Caput not markedly elevated; fovea recurved, 5.00 wide (Fig. 10). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.47, ALE 0.72, PME 0.40, PLE 0.52, AME–AME 0.55, AME–ALE 0.30, PME–PME 1.40, PME–PLE 0.17, ALE–PLE 0.52. Ocular tubercle width 3.13, length 2.57; clypeus length 0.53 (Fig. 13). Labium length 3.35, width 4.25, with 88 cuspules. Maxilla inner corner with ~198 (left) and ~207 (right) cuspules. Cheliceral promargin with 8 (left) and 9 (right) teeth (proximal to distal: first to eighth large; first to fifth large, sixth medium, seventh to ninth large). Sternum length 10.6. Sigillae oval; first, second and third pairs hardly visible; posterior sigilla once its length from the margin (Fig. 11). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I 17.88, 9.95, 13.36, 11.60, 8.22, 61.01; II 15.99, 9.57, 12.09, 12.28, 8.29, 58.22; III 13.74, 9.11, 11.13, 13.60, 8.23, 55.81; IV 17.43, 9.82, 13.40, 16.80, 8.80, 66.25. Palp: 12.45, 7.46, 10.08, –, 8.10, 38.09. Spinnerets: PMS 2.43 long, 2.70 apart; PLS 4.60 basal, 3.10 middle, 4.85 distal. Tarsi I–IV entirely scopulated. Metatarsi I and II entirely scopulated, III 65% scopulated distally, IV 50% scopulated distally. Stridulatory setae: lacking. Plumose setae: on palp

Table 3. ***Brachypelma hamorii*** **and** ***Brachypelma smithi*** **variations of some quantitative characters in the specimens' series analysed**

Measurements in mm; hyphens represents the interval between the lowest and highest value of each character; when left and right side values were taken it was indicated as (left/right)

Specimen measurements	*Brachypelma hamorii*		*Brachypelma smithi*	
	Males (n = 13)	Females (n = 7)	Males (n = 8)	Females (n = 7)
Total length	45.90–51.54	52.57–54.22	44.26–48.40	52.51–58.82
Carapace length	20.50–21.73	22.41–23.81	20.18–21.71	21.06–25.24
Carapace width	19.39–20.44	20.61–21.99	19.47–20.83	19.80–22.55
Sternum length	8.60–9.20	9.80–11.20	8.60–9.10	9.80–10.60
Sternum width	7.90–8.12	9.20–9.30	7.60–7.80	9.20–9.40
Labium length	2.43–2.70	2.67–3.35	2.23–2.50	3.05–3.65
Labium width	2.87–3.70	3.50–4.42	3.10–3.43	3.60–4.45
Chelicaral teeth (left/right)	9–12/10–11	8–9/8–9	8–9/9–10	8–12/8–10
Labial cuspules	109–139	92–132	64–81	88–122
Maxillary cuspules (left/right)	184–203/218–264	277–312/232–272	169–181/155–186	194–265/152–244
Spermatheca length medially	–	1.00–1.25	–	0.97–1.50
Spermatheca base width	–	5.50–6.05	–	4.30–6.30

trochanter and femur retrolateral face, and leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p, II 1p, palp 1p; patellae palp 1p; tibiae II 2p, 5v; III 3p, 3v; IV 4v; palp 2p, 7v, 1r; metatarsi I 3v; II v; III 1p, 6v, 2r; IV 1p, 16v, 1r. Genitalia: fused semicircular spermatheca with a single receptacle strongly sclerotised, ventral face is faintly striated. Four times wider than its height. Spermatheca baseplate divided, subtriangular, almost as high as half the width of its base, narrowing the upper side towards the outer side (Fig. 14). Variation: semicircular or trapezoidal shape with ventral face very or poorly striated. Baseplate division can vary in length (Figs 15–18). Urticating setae: types I and III arranged in one dorsoposterior patch, black; type III located in an oval dorsomedian area extended to posterior; type I setae surround the area of type III setae with intermediates in transition areas between types III and I (Fig. 12). Variations summarised in Table 3.

Colour pattern. In live specimens, adult females with three carapace patterns: (1) greyish yellowish pink around the border and behind the fovea, with starburst bluish black pattern from fovea to caput (Figs 20, 51); (2) light brown around the border and bluish black in dorsomedian (Figs 22, 50); (3) greyish yellowish pink on almost all of carapace except two longitudinal black patches in the caput (Fig. 21), with juveniles or subadults light brown around the border and brownish black dorsomedially; chelicerae dorsally dark greyish blue; ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with light orange setae, ventrally brownish black. Legs and palpi: femora bluish black, patellae with a proximal dorsomedian area dark reddish orange flame-shaped, distodorsal paramedian area light yellowish pink, with light orange setae laterally; tibiae proximal half bluish black with light orange setae, distal half pale yellowish pink and metatarsi bluish black with light orange setae and a pinkish white ring at the terminal end; tarsi bluish black (Figs 53–56).

Distribution and habitat

Brachypelma smithi is known from the Pacific coast of Guerrero. We indicate on the map three localities from which we were not able to review specimens (La Laja, El Guayabito and Tierra Colorada, specimens were observed by D. Ortiz, J. Hinojosa and V. Jiménez respectively, pers. comm.) (Fig. 61). Burrows occur under large rocks or tree roots in dense thickets or vegetation of dry thorn forests and deciduous forests (Fig. 23). There are no traces of silk at the burrow entrance, and the interior is often multi-tunnelled. The breeding season occurs late in the wet season and early in the dry season (September to January). Egg sacs are constructed in the drier winter months with young emerging and dispersing in late spring, just before the onset of the early summer rains.

Remarks

The type material of *B. smithi* was originally identified as female by F. O. Pickard-Cambridge (1897). Schiapelli and Gerschman inspected the specimen in May 1968 and noticed that it was not a female, but an immature male. Smith (1994) confirmed that the holotype was a juvenile male and redescribed the species using an adult female deposited in the NHM with the code BM1898.12.24.33 and with a male from the Hull-Williams collection without a catalogue number. However, when the material deposited in the NHM was cross-examined for this study, we found that the catalogue number BM1898.12.24.33 was in fact assigned to the holotype and no other specimen was found labelled with that number. The adult male Smith used to redescribe the species could not be located. NHM curator Janet Beccaloni indicated that it was not possible to find any registration for those specimens, and that they are presumably lost. Based on the redescription made by Smith (1994), it is apparent that the specimens he used belong to *B. hamorii* and not *B. smithi*.

Brachypelma hamorii Tesmoingt, Cleton & Verdez

(Figs 24–41, 46–49, 52, 57–60)

Brachypelma hamorii Tesmoingt, Cleton & Verdez, 1997*a*: 9, pls 1–6; Tesmoingt *et al.*, 1997*b*: 3, pls 9–11.

Euathlus smithi (F. O. Pickard-Cambridge, 1897): Baxter, 1993: 73, figs 17, 18, pl. A, figs 1–6 (misidentification).

Brachypelma smithi (F. O. Pickard-Cambridge, 1897): Hancock & Hancock, 1989: 44, fig. 39 (misidentification); Schmidt, 1992*a*: 10; Schmidt, 1992*b*: 14, figs 2, 4; Schmidt, 1993: 82, fig. 190; Smith, 1994: 170, figs 940–956; Tesmoingt *et al.*, 1997*a*: 9, pl. 2, fig. 2 (♀); Tesmoingt *et al.*, 1997*b*: 4, pls 10, 11; Schmidt, 1997: 19, fig. 195; Peters, 2000: 72, figs 235, 236; Peters, 2003: 125, figs 510, 512, 513; Schmidt, 2003: 153, figs 283, 284; Teyssié, 2015: 273, fig. 2 (misidentifications).

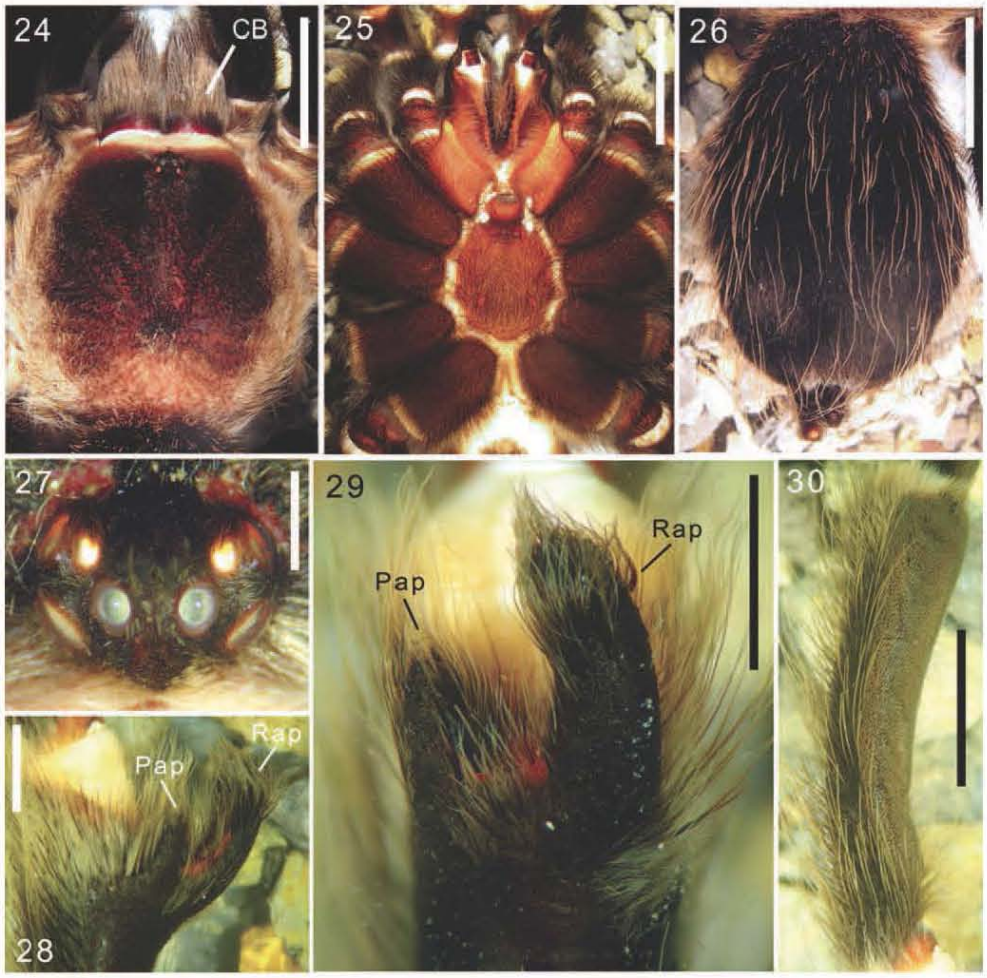
Material examined

Neotype. Mexico: *Colima*: ♂, Mpio. Tecoman, 5.xii.2013, D. Barrales, G. Contreras, D. Ortiz (CNAN-T0900).

Other material examined. **Mexico:** *Colima*: 1 ♂, Mpio Tecoman, 1.xii.2012, E. Goyer, E. Hijmensen, D. Ortiz (CNAN-Ar003614); 1 ♀, Mpio Colima, 30.xi.2012, E. Goyer, E. Hijmensen, D. Ortiz (CNAN-Ar004779); 2 ♂, Mpio Tecoman, 5.xii.2013, D. Ortiz, D. Barrales, G. Contreras (CNAN-Ar007163, CNAN-Ar007826); 1 ♂, Mpio Tecoman, 3.xii.2013, D. Ortiz, D. Barrales, G. Contreras (CNAN-Ar007168); 1 ♂, Mpio Manzanillo, iv.2004, IBT (CNAN-Ar010278); 1 ♂, Mpio Manzanillo, 4.xii.2013, D. Ortiz, D. Barrales, G. Contreras (CNAN-Ar010277); 1 ♂, donation received from private collection of J. Mendoza (CNAN-Ar003616); 1 ♂, Mpio 3.xii.2013, D. Ortiz, D. Barrales, G. Contreras (CNAN-Ar007827); 1 ♂, Mpio Manzanillo, 4.xii.2013, D. Ortiz, D. Barrales, G. Contreras (CNAN-Ar007171); 3 ♀, Mpio 11 km SE de Colima (CNAN-Ar007870, CNAN-Ar007872, CNAN-Ar007873); 1 ♀, Mpio. Tecoman, 2.xii.2012, E. Goyer, E. Hijmensen, D. Ortiz (CNAN-Ar007871); *Jalisco*: 2 ♀, Mpio Pihuamo, 30.xi.2012, E. Goyer, E. Hijmensen, D. Ortiz (CNAN-Ar007874, CNAN-Ar010279); 1 ♂, Carretera entre Colima y Jalisco, carretera Colima-Cd. Victoria, 8.viii.2008, A. Cervantes, M. E. Olsen (CNAN-Ar003425); *Michoacán*: 1 ♂, Mpio Lázaro Cardenás, 8.xi.2011, G. Vila (CNAN-Ar003340); 1 ♂, Michoacán, Mpio Aquila, G. Vila (CNAN-Ar003659).

Diagnosis

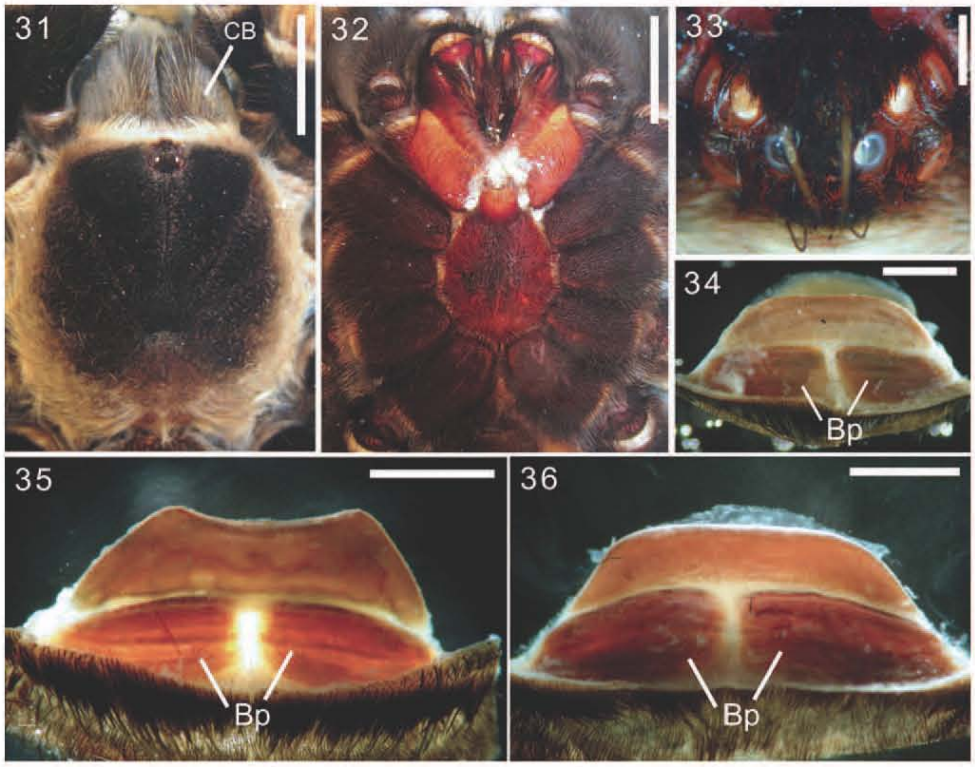
Brachypelma hamorii can be distinguished from all other known *Brachypelma* species (except *B. smithi*) by the colouration of the legs, with yellow-orange patellae, tibiae and metatarsi; in addition, *B. hamorii* differs in the shape of genitalia in both sexes with palpal bulb curved and short, and spermatheca a rounded trapezoid shape. Differs from *B. smithi* by the narrow curved embolus, the prolateral superior keel being shorter and dorsally wider, and the apical keel being slightly developed. Also, *B. hamorii* differs by the spermatheca ventral face being smooth, and the spermathecal baseplate being elliptic. Although



Figs 24–30. *Brachypelma hamorii*, neotype male CNAN-Ar007828. *24*, Carapace, dorsal view; *25*, prosoma, ventral view; *26*, opisthosoma, dorsal view; *27*, ocular tubercle, dorsal view; *28*, tibial apophyses, prolateral view; *29*, tibial apophyses, ventral view; *30*, metatarsus I, prolateral view. Scale bars = 10 mm (*24–26*), 5 mm (*30*), 2 mm (*28*, *29*), 1 mm (*27*).

similar in colouration, *B. hamorii* differs from *B. smithi* by the presence of a cheliceral band (better seen in recently moulted specimens). Patella flame shape not as colourful as in *B. smithi*, with diffuse orange or black setae around the flame-shaped patch. Lateral setae along length of legs whitish, contrasting more with darker areas; the same setae in *B. smithi* are more yellowish. This species can also be distinguished from other genotyped *Brachypelma* by the following eight diagnostic COI nucleotides ($n-6$): A (11), G (116), C (203), C (254), G (305), A (386), C (425), T (470). COI p-distances: interspecific more than 7.8%, intraspecific less than 0.5%. *Brachypelma hamorii* differs from *B. smithi* by a 7.8–8.0% COI p-distance.

Brachypelma hamorii is identified by the following character combination: male palpal bulb with narrow spoon-like embolus; prolateral superior keel shorter than in other species, thin and directed retrolaterally; prolateral inferior keel weakly developed, directed from dorsal to ventral, better seen dorsally; apical

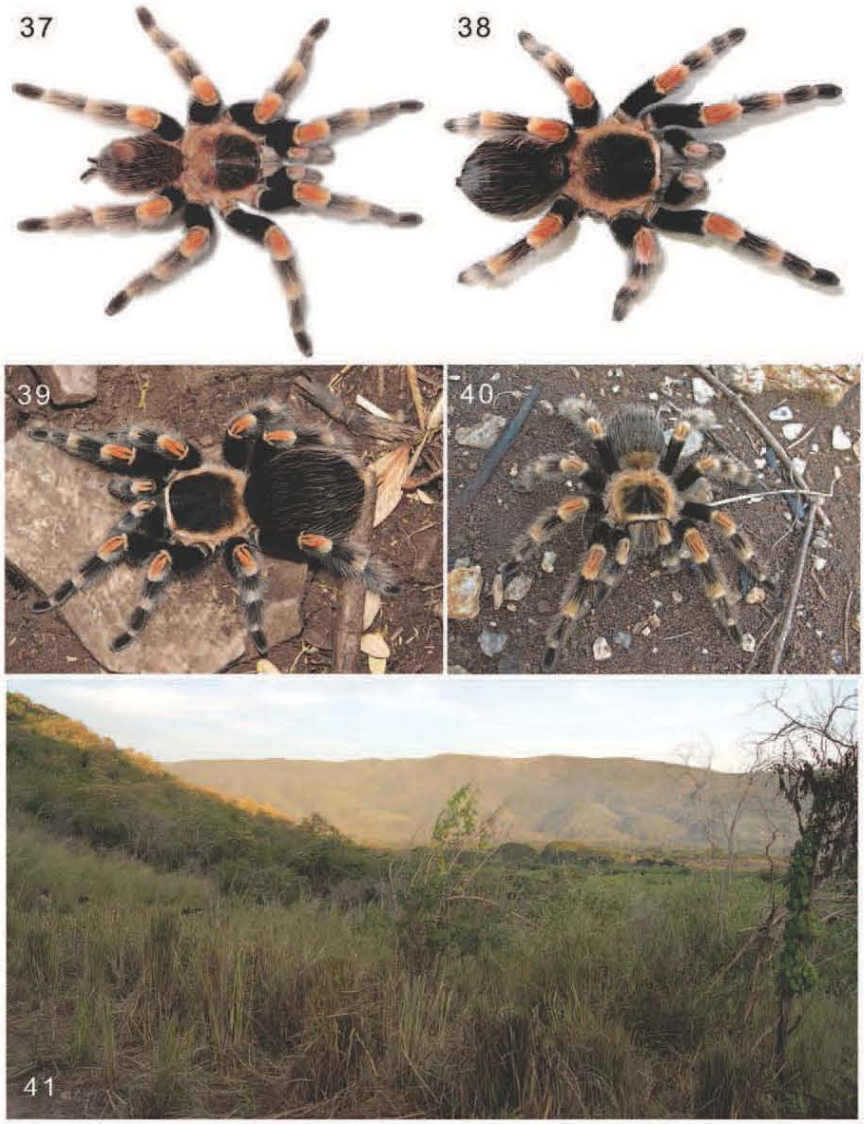


Figs 31–36. *Brachypelma hamorii*. *31–34*, Female CNAN-Ar007874. *31*, Carapace, dorsal view; *32*, prosoma, ventral view; *33*, ocular tubercle, dorsal view; *34*, spermatheca, ventral view; *35*, *36* spermatheca ventral view of: *35*, female CNAN-Ar010279; *36*, female CNAN-Ar010280. Scale bars = 10 mm (*31*, *32*), 2 mm (*34–36*), 1 mm (*33*).

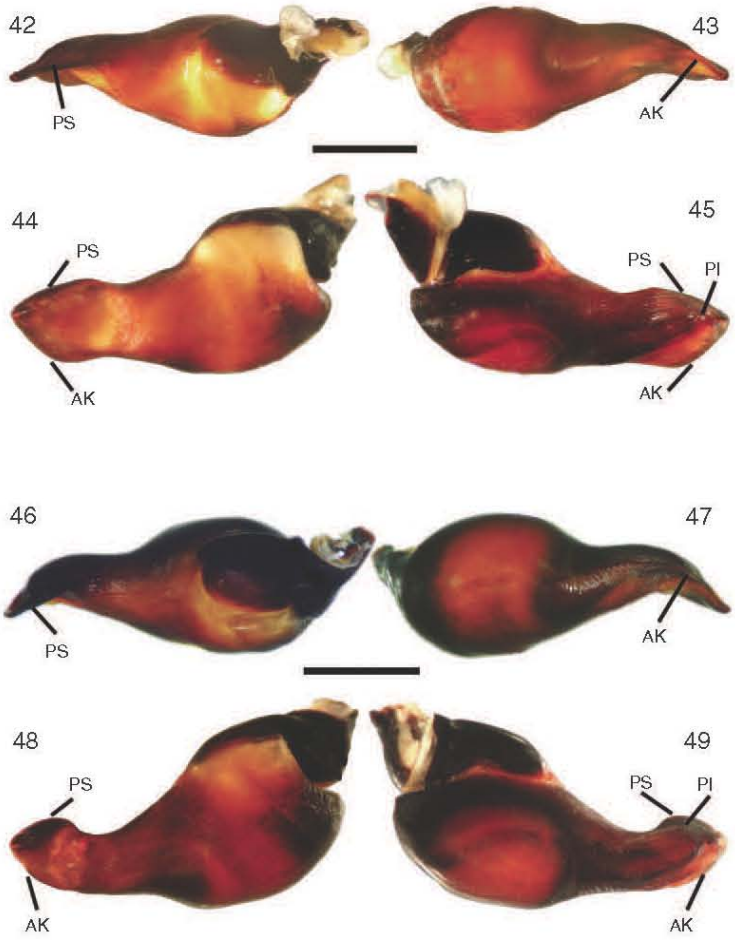
keel smaller than in other species, short and thin (Figs 46–49). Embolus tapered along its length, but widening at the apex, and slightly curved dorsally (Figs 48, 49). Spermatheca rounded trapezoid; ventral face almost smooth. Spermathecal baseplate divided, elliptic; five times wider than its height (Figs 34–36). Carapace in general black with orange setae on border, with cheliceral band in most of specimens (see colour pattern for variation). Legs and palpi with deep orange and pale orange on patellae, tibiae and metatarsi with orange yellow/white setae on distal (Figs 37–40, 52, 57–60).

Description

Male (neotype) (CNAN-T0900) (Figs 24–30, 37, 46–49). Body length 51.54 (not including chelicerae and spinnerets), carapace length 21.73, width 20.36. Caput not markedly elevated; fovea recurved, 4.00 wide (Fig. 24). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.40, ALE 0.70, PME 0.33, PLE 0.67, AME–AME 0.43, AME–ALE 0.17, PME–PME 1.17, PME–PLE 0.13, ALE–PLE 0.30. Ocular tubercle width 2.47, length 2.20; clypeus length 0.27 (Fig. 27). Labium length 2.63, width 3.40; with 139 cuspules. Maxilla inner corner ~184 (left) and ~218 (right) cuspules. Cheliceral promargin with 9 (left) and 11 (right) teeth (left side, proximal to distal: first to third large, fourth small, fifth to ninth large; right side, first to third large, fourth small, fifth to sixth large, seventh small, eighth to eleventh large). Sternum length 9.20. Sigillae oval; first, second and third pairs hardly visible, posterior sigilla once its own length from the margin (Fig. 25). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I 19.13, 9.81, 14.72, 15.72, 11.12, 70.50; II 17.45, 9.38, 13.75, 15.21, 9.83, 65.62; III 15.61, 8.85, 12.03, 15.80, 9.46, 61.75; IV 18.93, 9.58, 15.33, 19.13, 11.66, 74.63. Palp: 12.51, 6.92, 11.21, –, 4.66, 35.30. Spinnerets: PMS 2.37 long, 1.67 apart; PLS 4.50 basal, 3.50 middle, 4.15 distal. Tarsi I–IV entirely scopulated. Metatarsi I and II entirely scopulated, III 65% scopulated distally, IV 35% scopulated distally. Tibia I with two tibial apophyses normally



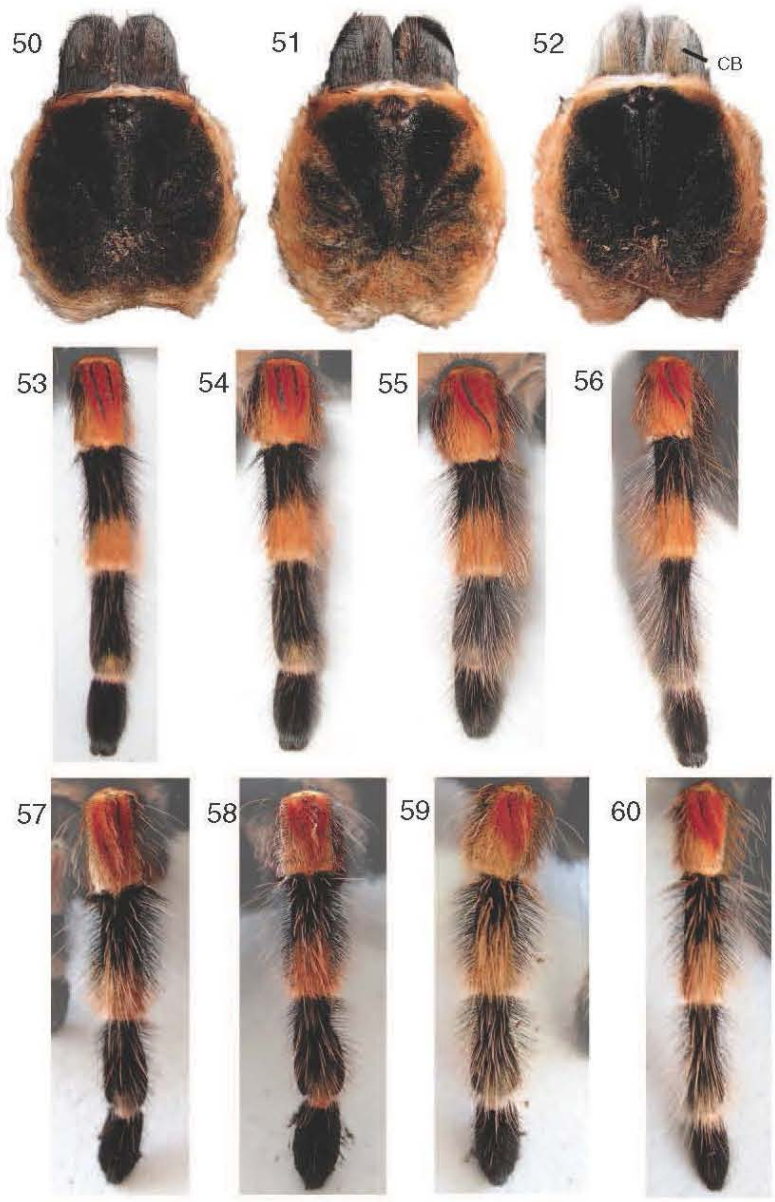
Figs 37–41. *37 40 Brachypelma hamorii*, habitus; *41*, habitat. *37*, Neotype male CNAN-Ar007828, in life; *38*, female CNAN-Ar007874, in life; *39*, female, in habitat (Colima); *40*, female, in habitat (Michoacán); *41*, deciduous forest in Colima, habitat of *B. hamorii*. Photos: J. Mendoza (*37*, *38*), E. Goyer (*39*, *41*), G. Vila (*40*).



Figs 42–49. *42 45 Brachypelma smithi*, male CNAN-Ar007832; *46 49 Brachypelma hamorii*, neotype male CNAN-Ar007828. Left palpal bulb: *42*, *46*, dorsal view; *43*, *47*, ventral view; *44*, *48*, retrolateral view; *45*, *49*, prolateral view. Scale bar=2 mm.

developed that originate from a common base. Prolateral apophysis with inner spine half its length; retrolateral apophysis same width along its length, apex slightly curved to prolateral (Figs 28, 29). Metatarsus I curved (Fig. 30). Stridulatory setae: with plumose setae on palp trochanter and femur retrolateral face, and leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; palp 1p; patellae none; tibiae I 1p; II 1p, 3v; III 2p, 3v, 1r; IV 2p, 3v, 1r; palp 3p, 1v; metatarsi II 1p, 4v; III 2p, 7v, 1r; IV 9v.

Palp. Embolus with narrow spoon-like shape, slightly curved to dorsal, prolateral superior keel short, thin and directed retrolaterally, prolateral inferior keel weakly developed directed from dorsal to ventral, better seen dorsally, apical keel short and thin. Opening of the embolus on the prolateral side, a concavity located just behind the opening delimits the apical keel boundary from the rest of the embolus. Embolus apex strongly curved towards the retrolateral (Figs 46–49). Urticating setae: types I and III arranged in one dorsoposterior patch, black. Type III located in an oval dorsomedian area extended to posterior; type I setae surround the area of type III setae with intermediates in transition areas between type III and I (Fig. 26). Variations summarised in Table 3.



Figs 50–60. *Brachypelma smithi*. *50*, female (CNAN-Ar007147) carapace; *51*, female (CNAN-Ar007143) carapace; female (CNAN-Ar007144). *53*, Leg I; *54*, leg II; *55*, leg III; *56*, leg IV; *Brachypelma hamorii*. Female (CNAN-Ar007874). *52*, Carapace; *57*, leg I; *58*, leg II; *59*, leg III; *60*, leg IV.

Colour pattern. In live specimens, adult males with carapace light greyish red around the border and behind the fovea, reddish black from the fovea to caput; chelicerae dorsally brownish pink; ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with light greyish yellowish brown setae, ventrally brownish black. Legs and palpi: femora black, patellae with a proximal flame-shaped dorsomedian area deep orange, distodorsal paramedian area pale orange yellow, with brownish pink setae laterally; tibiae proximal half reddish black with brownish pink setae, distal half pale orange-yellow with brownish pink setae and metatarsi reddish black with brownish pink setae and a yellowish white ring at the terminal end; tarsi black (Fig. 37).

Female (CNAN-Ar007874) (Figs 31–34, 38, 52, 57–60). Body length 53.70 (not including chelicerae and spinnerets), carapace length 23.81, width 21.78. Caput not markedly elevated; fovea straight, 5.00 wide (Fig. 31). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.47; ALE 0.67; PME 0.47; PLE 0.70; AME–AME 0.63; AME–ALE 0.37; PME–PME 1.40; PME–PLE 0.20; ALE–PLE 0.33. Ocular tubercle width 2.83, length 2.33; clypeus length 0.50 (Fig. 33). Labium length 3.35, width 4.35 with 92 cuspules. Maxilla inner corner with ~241 (left) and ~277 (right) cuspules. Cheliceral promargin with 8 (left) and 8 (right) teeth (proximal to distal: first to seventh medium, seventh large, eighth medium; first medium, second to third large, fourth to eighth medium). Sternum length 11.20. Sigillae oval, second and third pairs hardly visible; posterior sigilla one and half its length from the margin (Fig. 32). Leg formula: IV, I, III, II. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.65, 10.39, 13.22, 12.42, 8.32, 61.00; II: 14.88, 9.42, 11.07, 11.35, 8.50, 55.22; III: 14.09, 8.81, 11.17, 12.41, 8.50, 54.98; IV: 17.28, 9.64, 13.55, 17.23, 9.43, 67.13; palp: 12.40, 7.63, 8.72, – 10.02, 38.77. Spinnerets: PMS, 2.37 long, 2.17 apart; PLS, 5.25 basal, 3.00 middle, 4.60 distal. Tarsi I–IV entirely scopulated. Metatarsi I and II entirely scopulated, III 65% scopulated distally, IV 50% scopulated distally. Stridulatory setae lacking; plumose setae on palp trochanter and femur retrolateral face, and leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora palp 1p; patellae palp 1p; tibiae I 1p; II 2p, 2v; III 3p, 6v; IV 3v; metatarsi I 2v; II 2v; III 2p, 4v, 2r; IV 1p, 8v, 2r. Genitalia: fused rounded trapezoid spermatheca with a single strongly sclerotized receptacle, ventral face almost smooth, five times wider than its height. Spermatheca baseplate divided, elliptic, as high as half the width of its base, outer side slightly smaller than inner (Fig. 34). Variation: semicircular or trapezoidal shape with an incurvature in the upper edge. Ventral face smooth. Baseplate division can vary in length (Figs 35, 36). Urticating setae: types I and III arranged in one dorsoposterior patch, black; type III located in an oval dorsomedian area extended towards posterior; type I surrounding area of type III, with intermediates in transition areas between types III and I. Variations summarised in Table 3.

Colour pattern. In live specimens, adult females with two carapace patterns: (1) brownish pink around the border and black dorsomedially, juveniles and subadults same pattern (Figs 38, 39); (2) pale orange-yellow around the border and behind the fovea, with starburst black pattern from fovea to caput (Fig. 40); chelicerae dorsally light bluish grey with two brownish pink cheliceral bands (not all specimens have clearly visible bands, more readily seen in recently moulted specimens) (Fig. 52); ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with light reddish brown setae, ventrally brownish black. Legs and palpi: femora black, patellae with a proximal dorsomedian deep orange flame-shaped area, distodorsal paramedian area pale orange-yellow (specimens of some populations only present pale orange-yellow on distal half of patella) with brownish pink setae laterally; tibiae proximal half black with brownish pink setae, distal half light orange-yellow with brownish pink setae and metatarsi black with brownish pink setae and a yellowish white ring at the terminal end; tarsi black with a few dorsal brownish pink setae (Figs 57–60).

Distribution and habitat

Brachypelma hamorii is known from the southern Mexican states of Colima, southern Jalisco and the north-western coast of Michoacán (Fig. 61), where it occurs in thorn and deciduous secondary forests (Fig. 41). It is a fossorial species whose modified or self-excavated burrows can be found under fallen logs, large rocks and large tree roots among thorny brush or tall grass thickets. Burrows do not have any silk around the entrance.

Remarks

The type locality of *B. hamorii* is unknown, and the species was described from pet trade material (Tesmoingt *et al*. 1997*a*). The type specimens were never deposited in a museum and, according to the authors (pers. comm.), and to MNHNP curator Christine Rollard, they are lost. Because of the taxonomic confusion between *B. hamorii* and *B. smithi* and the absence of comparative material, we hereby designate a male neotype (CNAN-T0900) to clarify the taxonomic status of *B. hamorii*. Based on our evidence and key features such as the male palpal bulb, this specimen matches the original description of the species.

Schmidt (1992*b*) was the first to notice differences in the morphological features of the male and female sexual organs of what he thought was *B. smithi*. However, due to the lack of information about the precise collection sites of the specimens he had at hand, he mistakenly described the male bulb and spermatheca of another species as those of *B. smithi*, triggering a cascade of subsequent errors. We believe that authors such as Smith (1994), Tesmoingt *et al*. (1997*a*, 1997*b*), Peters (2000, 2003), Schmidt (1992*a*, 1993, 1997, 2003) and Teyssié (2015) incorrectly identified *B. hamorii* specimens as belonging to *B. smithi*.

Discussion

West (2005) mentioned having seen many colour and dorsal pattern variations in *B. smithi*, and considered that *B. annitha* and *B. hamorii* were described based on superficial characters. He also stressed the need for more careful and professional taxonomic studies to solve the question of variants versus what constitutes a valid species in *Brachypelma*.

Tesmoingt *et al*. (1997*b*), in their description of the male palpal bulbs of *B. annitha* and *B. hamorii*, made it possible to readily identify the red-knee tarantula they had determined as *B. smithi*.

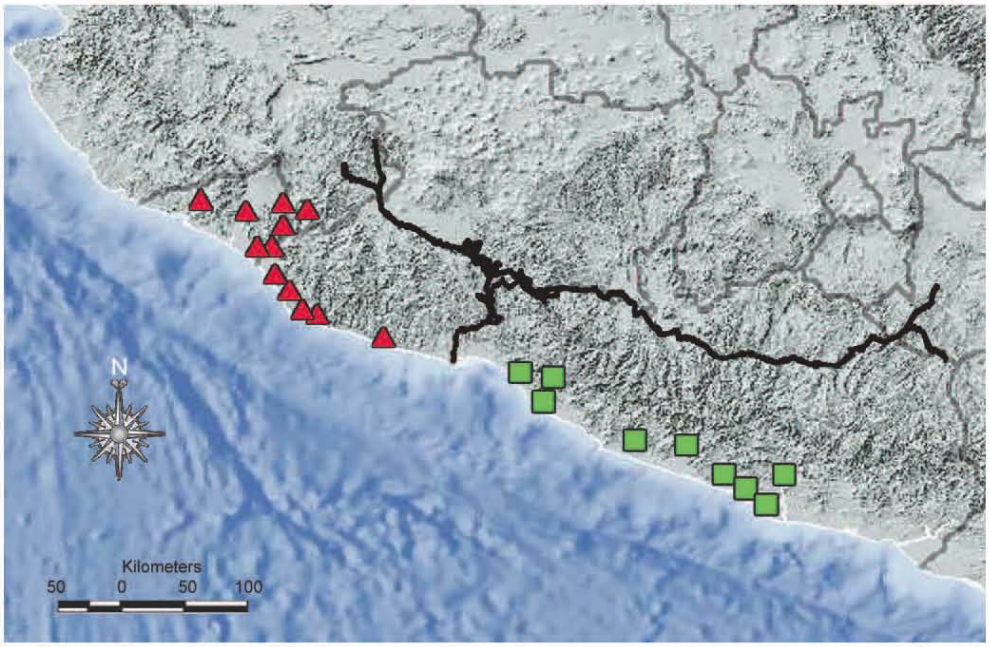


Fig. 61. Map showing records of *Brachypelma* Mexican red-knee tarantulas in Colima, Guerrero and Michoacán states. Triangles, *B. hamorii*; squares, *B. smithi*. The dark black line represents the Balsas River basin.

According to this description, the male palpal bulb of *B. smithi* is curved and tapers towards the embolus. They also mentioned that the shapes of the bulbs of *B. smithi* and *B. hamorii* are fairly similar and without notable morphological distinguishing features. This description fits perfectly with specimens from Colima and Michoacán, but not with those from Guerrero, especially those from the type locality of *B. smithi*. It is thus apparent that *B. smithi* was misidentified and therefore incorrectly referred to. Notwithstanding, the description of *B. annitha* mentions that the male palpal bulb is essentially straight, composed of a pear-shaped bulb narrowing slightly above (or pas:) the embolus. This consists of a much larger bowl-shaped embolus than in what Tesmoingt *et al.* (1997*b*) incorrectly considered to be *B. smithi*. In summary, their description of the male copulatory apparatus of *B. annitha* matches that of the true *B. smithi* from Guerrero. Therefore, our results confirm that the red-knee tarantulas from Guerrero are *B. smithi*, and that *B. annitha* is the same species, which therefore becomes a junior synonym of *B. smithi*. It is also clear that the populations from Jalisco, Colima and Michoacán are *B. hamorii*.

The geographic distribution of *B. smithi* was previously thought to be disjunct by Smith (1994) and Locht *et al.* (1999). Two main populations were reported: one in Colima and Michoacán and the other in Guerrero. Due to similar colouration between specimens from Colima and Michoacán and those from Guerrero, it was assumed that they belonged to the same species. However, these two populations do not come into contact for two distinct reasons: the Balsas River basin (Fig. 61) acts as a geographical barrier separating the species, and populations of *B. baumgarteni* and *B. boehmei* are found between these two areas (Fig. 62). Our phylogenetic analysis utilising mtDNA proved to be a highly useful complement to morphological data and was able to reliably identify *Brachypelma* species. However, the observed phylogenetic relationships and clades may change with the inclusion of nuclear data.

DNA barcoding

Morphological identification of organisms requires experienced taxonomists. Incomplete identification often occurs when important morphological features are damaged as a result of improper specimen handling (Chan *et al.* 2014). Molecular markers, such as COI, may provide species boundary information in certain taxonomic groups and consequently have the potential to be a rapid and efficient means to delineate and identify species (e.g. Chen *et al.* 2011; Hamilton *et al.* 2014). Unfortunately, molecular identification of species is fraught with the same constraints and inconsistencies that plague

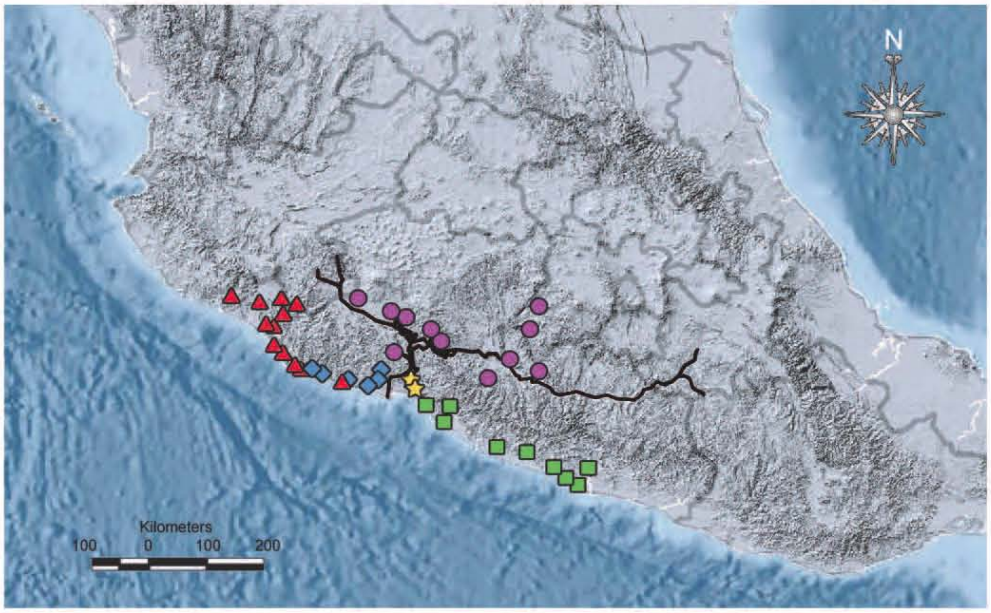


Fig. 62. Map showing records of *Brachypelma* Mexican red-knee, flame-knee and red-legged tarantulas in Colima, Guerrero and Michoacán states. Triangles, *B. hamorii*; squares, *B. smithi*; diamonds, *B. baumgarteni*; stars, *B. boehmei*; circles, *B. auratum*. The dark black line represents the Balsas River basin.

morphological judgments of species boundaries (Ortíz and Francke 2016). However, most taxonomists base their conclusions on a suite of complex morphological characters, rather than relying on part of a single gene (Will and Rubinoff 2004). While it is true that DNA-based methods are not demonstrably more objective, accurate or useful than morphology or other sources of phenotypic data for species identification (Prendini 2005), DNA barcoding is a very useful technique that, together with morphology, field observations and collection, allows for better definition and delimitation of species (Scotland *et al.* 2003; Slowik and Blagoev 2012; Hendrixson *et al.* 2015). Furthermore, in the particular case of tarantulas, molecular markers allow for the correct identification of juveniles and females, which are traded much more frequently than adult males. The use of non-destructive sampling techniques, such as the removal of a single leg and cauterisation of the wound, render this approach quite feasible (Longhorn *et al.* 2007; Hamilton *et al.* 2011; Hendrixson *et al.* 2013).

Our phylogenetic analyses of the COI sequences using parsimony and Bayesian inference corroborated the monophyly of *B. hamorii* and *B. smithi*, with *B. annitha* nested within *B. smithi*, and *B. hamorii* as sister species of all other red-legged *Brachypelma* (Figs 1, 2). This result confirmed the need to redefine the species that form the Mexican red-kneed tarantula group, based on morphological and molecular evidence. The other *Brachypelma* species from the Pacific coast of Mexico (*B. auratum*, *B. baumgarteni* and *B. boehmei*), which are geographically distributed between the *B. hamorii* and *B. smithi* populations, also present concordance with the topologies obtained with the phylogenetic analyses. Although we consider that the morphological evidence is good enough to justify the delimitation of *B. hamorii* and *B. smithi* as distinct species, with *B. annitha* as a junior synonym of the latter, the use of molecular characters of the barcoding gene COI has enabled us to provide a more robust dataset to support this conclusion. Most DNA-based theraphosid species delimitation studies have been primarily done with *Aphonopelma* Pocock, 1901 within the United States (Graham *et al.* 2015; Hamilton *et al.* 2011, 2014, 2016; Hendrixson *et al.* 2013, 2015; Wilson *et al.* 2013). Other DNA-based studies include the South American *Grammostola* Simon, 1892, with which taxonomy has been problematic due to the morphological homogeneity of its species (Montes de Oca *et al.* 2016), the poorly studied group *Bonnetina* Vol, 2000 (Ortíz and Francke 2016), and a single work on the CITES-protected genus *Brachypelma* Simon, 1891 using exuviae to obtain DNA (Petersen *et al.* 2007). These studies show strong differences between the current morphology-based taxonomy, and the evidence from molecular and ecological data: in *Grammostola* and *Bonnetina* there are species that are difficult to separate using only morphological characters, but by using molecular characters can be identified with more certainty (Ortíz and Francke 2016). Hamilton *et al.* (2016) mentions that

the limitations of mtDNA have been documented extensively; specifically, gene tree/species tree incongruence and the haploid, non-recombining nature of the molecule, with mtDNA representing only one particular genealogy out of all possible within a genome. However, Petersen *et al.* (2007) shows that although DNA barcodes do not capture the dynamic process of evolution nor reflect the precise interrelationships within a group, they can provide legislators within a framework when enacting protection laws. This could translate into greater success in prosecuting those involved in the illegal pet trade. It is agreed that mtDNA alone is insufficient for precise species delimitation; however, it can be useful for identification if the findings correspond with morphological evidence. In the present case, we support the use of molecular markers for identification of *Brachypelma* species.

With the taxonomic status now clear and concise delimitations set for the populations of Guerrero and of Jalisco, Colima and Michoacán, further protection strategies can now be implemented. The clear demarcation between the two species of Mexican red-kneed tarantulas has an important significance in helping to establish the correct identification of species that are illegally traded and that are difficult to identify at a glance by PROFEPA officials, who are responsible for the protection of fauna and flora in Mexico. The barcode marker COI for these tarantulas proved to be sufficient for correct species identification. Thus, it may be a useful tool for preventing black market trade and reintroducing tarantulas into the right distribution areas. Because of this, we are also creating a genetic library of Mexican tarantulas as part of the Wildlife Barcode Project in Mexico, to be used as a reference for the authorities responsible for species conservation.

Conservation issues

CITES is the most important international convention dealing with wildlife conservation. The fundamental concept behind CITES is to protect and control the international trade of organisms that may be threatened or endangered in any of the signatory countries (Schultz and Schultz 2009). However, despite increased recognition, these trade measures do not necessarily benefit wild species. Moreover, it is often difficult to assess the consequences of such measures as the criteria used to make listing decisions include little consideration (if any) of whether proposed trade measures will be effective (Hutton and Dickson 2000). While there have been numerous revisions to the listing criteria, they still focus almost exclusively on the biological and trade status of the species and scarcely touch on whether the listing will benefit the conservation status of the species (Dickinson 2002).

At sustainable levels of consumption, both wildlife and people benefit from trade. Granting people an economic stake in wildlife provides the best incentive for careful stewardship of species and habitats (Carey 1999). Nevertheless, wild populations of red-legged tarantulas are now declining. Habitat destruction combined with cultural bias against these spiders and a lack of economic interest in their conservation have led to the current practice of killing tarantulas that are found in fields or houses. Tarantulas are seen as harmful creatures in many local communities. One negative result of the *Brachypelma* CITES listing has been a decrease in the supply of some species, leading to increased desirability and thus demand. This has encouraged the development of a black market, which smuggles protected species both locally and abroad (Reichling 2003). A study on the concept of controlled harvesting in Mexico is necessary as there is currently no provision to enable sustainable exploitation of such a resource (López and Iñigo 2009). Unfortunately, over the past few years, at least 3000 specimens of Mexican tarantulas were reportedly sent to Europe or the United States; most were red-kneed tarantulas (PROFEPA, unpub. data).

Red-kneed tarantulas are among the ten most trafficked animal species in Mexico (PROFEPA 2009). They are collected for profit by people in need to sustain themselves and their families (Schultz and Schultz 2009). Unfortunately, collectors disturb the habitat and do not attempt to maintain sustainable harvesting practices. Traffickers pay the collectors around US$2–3 dollars per tarantula, which can be sold for up to US$200 dollars on the international market (Inecc 2012). Large tracts of land are pitted from excavation of burrows. Habitat is further lost during the rainy season due to landslides. The traffickers have unique methods of facilitating illicit traffic, often hiding tarantula specimens in photographic film containers, small plastic tubes or plastic bags (BWPM 2014). The animals are crammed against each other and many die due to dehydration, insufficient space during moulting or suffocation in their own exuviae (Rojo 2004). Sometimes the orange-red markings on the legs are covered up with black marker in an attempt to pass them off as non-protected species (R. West, personal communication).

Very little is known about the conservation status of Mexican *Brachypelma* species, especially those restricted to areas particularly vulnerable to overexploitation. In general, tarantulas tend to have clumped distributions in small areas with specific soil types, and are 'common' only sporadically across their entire geographic range. These factors leave them vulnerable to natural stochastic events (Reichling 2003). One of the authors observed this during the hurricane season of 2012–13, when the Mexican government reported serious environmental damage and changes to the flow of rivers along the Pacific coast. The coast of Guerrero was one of the most heavily affected areas, with extensive flooding of the Papagayo River nearly causing the complete destruction of two *B. smithi* populations.

Until a thorough assessment is undertaken of the environmental, legal, social and economic factors impacting on wildlife trade in Mexico, there is little that can be done to develop successful management strategies and species conservation initiatives. In addition, it is essential to explore other non-subtractive economic endeavours such as tourism, wildlife observation or sale of captive-bred animals to provide alternative incomes for local communities and to avoid further damage to wild populations. Legal breeders should also be included in the conservation strategies of Mexican red-kneed tarantulas to support their efforts to breed and reintegrate tarantulas in their natural distribution ranges.

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Appendix 1. Terminal taxa, specimens and tissue samples used for phylogenetic analyses of seven species in the genus *Brachypelma*

Material examined is deposited in the Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City

Outgroup

Two theraphosid sequences were retrieved from GenBank for cladistic analyses: *Psalmopoeus cambridgei* Pocock, 1895 447_SC_AB (JQ412455) and *Eupalaestrus campestratus* (Simon, 1891) 446_SC_AB (JQ412446). Both specimens are deposited in the Bangor University collection, United Kingdom.

Ingroup

1. *Brachypelma albiceps* (Pocock, 1903): 1 ♂ (CNAN-Ar003412), MEXICO: Guerrero, Mpio. Copalillo, 14.X.2008, coll. J. Mendoza. 1 juv. ♀ (CNAN-Ar007839), MEXICO: Guerrero, Mpio. Tixtla de Guerrero, 23.IX.2012, coll. J. Mendoza, G. Contreras, J. Lopez, D. Ortiz. 1 juv. ♀ (CNAN-Ar007850), MEXICO: Guerrero, Mpio. Azoyu, VII.2011, coll. A. Alcaraz.
2. *Brachypelma auratum* Schmidt, 1992: 1 ♂ (CNAN-Ar003658), MEXICO: Michoacán, Mpio. Los Reyes, 02.II.2013, coll. J. Mendoza, G. Contreras, D. Ortiz, D. Barrales. 1 juv. ♀ (CNAN-Ar007136), MEXICO: Guerrero, Mpio. Arcelia, 03.II.2013, coll. J. Mendoza, G. Contreras, J. Cruz, D. Ortiz. 1 juv. ♀ (CNAN-Ar007164), MEXICO: Michoacán, Mpio. San Lucas, 02.II.2013, coll. J. Mendoza, G. Contreras, D. Ortiz, D. Barrales.
3. *Brachypelma baumgarteni* Smith, 1993: 1 ♀ (CNAN-Ar007151), MEXICO: Michoacán, Mpio. Lázaro Cárdenas, 10.XII.2013, coll. J. Mendoza. 1 juv. ♀ (CNAN-Ar007161), MEXICO: Michoacán, Mpio. Lázaro Cárdenas, Carr. Neixpa-Manzanillo, 11.XII.2013, coll. J. Mendoza. 1 subad. ♂ (CNAN-Ar007835), MEXICO: Michoacán, Mpio. Lázaro Cárdenas, 11.XII.2013, coll. J. Mendoza.
4. *Brachypelma boehmei* Schmidt & Klaas, 1993: 1 ♀ (CNAN-Ar007185), MEXICO: Guerrero, Mpio. La Unión de Isidoro Montes de Oca, 12.XII.2013, coll. J. Mendoza. 1 ♀ (CNAN-Ar007186), MEXICO: Guerrero, Mpio. La Unión de Isidoro Montes de Oca, 12.XII.2013, coll. J. Mendoza. 1 ♂ (CNAN-Ar007833), MEXICO: Guerrero, Mpio. La Unión de Isidoro Montes de Oca, 12.XII.2013, coll. J. Mendoza.
5. *Brachypelma hamorii* Tesmoingt, Cleton & Verdez, 1997: 1 ♂ (CNAN-Ar003614), MEXICO: Colima, Mpio. Tecomán, 01.XII.2012, coll. E. Goyer, E. Hijmensen, D. Ortiz. 2 ♂ (CNAN-Ar007163, CNAN-Ar007826), MEXICO: Colima, Mpio. Tecomán, 05.XII.2013, coll. D. Ortiz, D. Barrales, G. Contreras. 1 ♂ (CNAN-Ar007168), MEXICO: Colima, Mpio. Tecomán, 03.XII.2013, coll. D. Ortiz, D. Barrales, G. Contreras. 1 ♂ (CNAN-Ar007827), MEXICO: Colima, Mpio. Colima, 03.XII.2013, coll. D. Ortiz, D. Barrales, G. Contreras. 1 ♂ (CNAN-T0900), MEXICO: Colima, Mpio. Tecomán, 05.XII.2013, coll. D. Ortiz, D. Barrales, G. Contreras.
6. *Brachypelma klaasi* (Schmidt & Krause, 1994): 1 ♂ (CNAN-Ar007160), MEXICO: Jalisco, Mpio. Cihuatlán, 04.XII.2013, coll. D. Ortiz, D. Barrales, G. Contreras. 1 ♂ (CNAN-Ar007162), MEXICO: Colima, Mpio. Manzanillo, 04.XII.2013, coll. D. Ortiz, D. Barrales, G. Contreras.
7. *Brachypelma smithi* (F. O. Pickard-Cambridge, 1897): 1 ♀ (CNAN-Ar004131), MEXICO: Guerrero, Mpio. Acapulco, collection of J. Mendoza. 1 juv. ♂ (CNAN-Ar007140), MEXICO: Guerrero, Mpio. Acapulco, 20.IX.2012, coll. J. Mendoza, G. Contreras, J. Cruz, D. Ortiz. 2 ♀ (CNAN-Ar007143, CNAN-Ar007144) and 1 ♂ CNAN-Ar007832, MEXICO: Guerrero, Mpio. Acapulco, 13.XII.2013, coll. J. Mendoza. 1 ♀ (CNAN-Ar003611), MEXICO: Guerrero, Mpio. Acapulco, collection of J. Mendoza (pet trade labelled as *B. annitha*).

Capítulo 2

Taxonomía y Sistemática de *Brachypelma*: nuevas implicaciones para su conservación.

Sección 2.1.

Artículo en revisión enviado a:

Zoological Journal of the Linnean Society

Systematic revision of Mexican threatened tarantulas *Brachypelma* Simon 1891 (Araneae: Theraphosidae), with description of a new genus, and implications on conservation status for its CITES-listed species.

Zoological Journal of the Linnean Society

Systematic revision of Mexican threatened tarantulas Brachypelma Simon 1891 (Araneae, Theraphosidae, Theraphosinae), with description of a new genus, and implications on conservation status for its CITES-listed species.

Journal:	*Zoological Journal of the Linnean Society*
Manuscript ID	Draft
Manuscript Type:	Original Article
Keywords:	new genera < Taxonomy, mitochondrial DNA < Genetics, phylogenetic nomenclature < Phylogenetics, distribution < Geography, genus revision < Taxonomy
Abstract:	Brachypelma includes colorful species that are highly sought for the commercial pet trade. They are included in the CITES Appendix II. We present phylogenetic analyses using molecular and morphological characters to revise Brachypelma. We include all curently known species within the genus. Our results agree with a previous study that shows the non-monophyly of Brachypelma. Both phylogenies strongly favor the división of Brachypelma into two smaller genera. The first clade (Brachypelma sensu stricto) is formed by B. albiceps, B. auratum, B. baumgarteni, B. boehmei, B. emilia, B. hamorii, B. klaasi and B. smithi. The species included in the the second clade (Brachypelma sensu lato) were transferred into their own genus Tliltocatl gen. nov. and its formed by T. albopilosum comb. nov., T. epicureanum comb. nov., T. kahlenbergi comb. nov., T. sabulosum comb. nov., T. schroederi comb. nov., T. vagans comb. nov and T. verdezi comb. nov. Both genera can be differentiated by their coloration and by the shape of genitalia. We considered as nomina dubia: T. alvarezi, T. andrewi and T. aureoceps. We transfer B. fossorium to Stichoplastoris. We discuss the implications of this taxonomical changes for CITES and for the Mexican Law for protection of wildlife.

SYSTEMATIC REVISION OF MEXICAN THREATENED TARANTULAS BRACHYPELMA SIMON 1891 (ARANEAE, THERAPHOSIDAE, THERAPHOSINAE), WITH DESCRIPTION OF A NEW GENUS, AND IMPLICATIONS ON CONSERVATION STATUS FOR IT'S CITES LISTED SPECIES.

Systematic revision of *Brachypelma* within new genus.

JORGE MENDOZA[A,B] & OSCAR FRANCKE[B]

[A]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Av. Universidad 3000, C.P. 04510, Coyoacán, Distrito Federal, México.

[B]Colección Nacional de Arácnidos, Módulo D planta baja, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, 3er circuito exterior, Apto. Postal 70-153, CP 04510, Ciudad Universitaria, Coyoacán, Distrito Federal,
[C]urn:lsid:zoobank.org:author:7BA11142-CBC1-4026-A578-EBAB6D2B6C0C
[D]corresponding author: nomeireth@hotmail.com

Abstract.

Brachypelma includes colorful species that are highly sought for the commercial pet trade. They are included in the CITES Appendix II. We present phylogenetic analyses using molecular and morphological characters to revise *Brachypelma*. We include all curently known species within the genus. Our results agree with a previous study that shows the non-monophyly of *Brachypelma*. Both phylogenies strongly favor the división of *Brachypelma* into two smaller genera. The first clade (*Brachypelma sensu stricto*) is formed by *B. albiceps, B. auratum, B. baumgarteni, B. boehmei, B. emilia, B. hamorii, B. klaasi* and *B. smithi*. The species included in the the second clade (*Brachypelma sensu lato*) were transferred into their own genus *Tliltocatl* gen. nov. and its formed by *T. albopilosum* comb. nov., *T. epicureanum* comb. nov., *T. kahlenbergi* comb. nov., *T. sabulosum* comb. nov., *T. schroederi* comb. nov., *T. vagans* comb. nov and *T. verdezi* comb. nov. Both genera can be differentiated by their coloration and by the shape of genitalia. We considered as *nomina dubia*: *T. alvarezi*, *T. andrewi* and *T. aureoceps*. We transfer *B. fossorium* to *Stichoplastoris*. We discuss the implications of this taxonomical changes for CITES and for the Mexican Law for protection of wildlife.

Key words: *Brachypelma*, new genus, mtDNA, tarantula, conservation, CITES

Introduction

Tarantulas (family Theraphosidae) are the world's largest and heaviest spiders. They can inhabit in almost every terrestrial ecosystem (with exception of polar areas), but they are mainly found in tropical, subtropical, semi-arid and arid regions around the world. They can be found from sea level to 4000 meters of altitude, some even inside caves down to -800 meters deep (Bond *et al.* 2012, Lüddecke *et al.* 2018, Mendoza 2014a). According to the World Spider Catalog (2018), there are currently 967 described species. Many of them are colorful in appearance and docile in captivity, making them attractive pets for collectors (West 2005). Some of the most appreciated by enthusiasts are those of the genus *Brachypelma* due to their longevity, size and nice coloration. Because of the high demand for specimens of *Brachypelma smithi* (F.O. Pickard-Cambridge 1897) in the late 80's, and with the concern that they might be in danger authorities placed this species on Appendix II of the Convention on International Trade and Endangered Species (CITES). Then, in 1994 to prevent the same problem from araising with other *Brachypelma* species, the total inclusión of the genus in CITES Appendix II was proposed (Smith 1994, West 2005). Later, the World Conservation Monitoring Center (1996) evaluated and included *B. smithi* as a near threatened species in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. In recent years, the Commission for Environmental Cooperation has been working with authorithies from Mexico, United States and Canada to generate and implement action plans to promote the legal, sustainable and traceable trade in selected North American species that are listed in Appendix II of CITES. These actions were developed based on the information compiled from consultation with stakeholders. Sixteen tarantula species, comprising one from the genus *Aphonopelma* and 15 from the genus *Brachypelma*, were selected as "priority tarantula species" and are the subject of one of the action plans (CEC 2017).

Currently, there are 18 described species of *Brachypelma* ranging from Mexico to Costa Rica (Smith, 1994, Locht *et al.*, 1999, Gabriel & Longhorn, 2015, WSC 2018). Of these, 13 occur in Mexico and 12 of them are endemic to Mexico. The most northerly species is *Brachypelma emilia* (White, 1856) found west of the Sierra Madre Occidental mountain

range in the states of Sinaloa, Durango and Nayarit. This highly colourful species is a fossorial burrower whose habitat ranges widely from the drier coastal thorn forests and savannahs, through the palm transition forests and wetter inland tropical deciduous forests, up into the higher and cooler elevations of the pineoak forests (West 2005). The southernmost species was *Brachypelma embrithes* (Chamberlin 1925), which is found in Panamá, but Gabriel & Longhorn (2015) transferred this species to the genus *Sericopelma* (Ausserer, 1875). This is the reason why actually the southernmost species in the genus is *Brachypelma albopilosum* (Valerio, 1980) from Costa Rica

Although correct species identification is a fundamental component of many biological investigations, taxonomic expertise is shrinking and is being overwhelmed by demand. The correct identification of a species based on morphological characters can sometimes take too long or can be impossible for certain life stages like immature spiders, or even for members of one sex (Greenstone *et al.* 2005, Blagoev *et al.* 2009). There are also some problems in the identification of species in groups with similar patterns of convergence or morphological conservatism (Hebert *et al.* 2003, Locke *et al.* 2010, Niemiller *et al.* 2011, Hamilton *et al.* 2014, Hamilton *et al.* 2016). Theraphosid spiders are known by having simple genitalia and tend to show relatively low inter-specific and relatively high intra-specific variation, which have revealed widespread patterns of homoplasy among traditional taxonomic characters (Raven 1985, Goloboff 1993, Pérez-Miles *et al.* 1996, Pérez-Miles 2000, Bertani 2001, Bond & Opell 2002, Hedin & Bond 2006, Bond & Hedin 2006, West *et al.* 2008, Hendrixson & Bond 2009, Bond *et al.* 2012, Guadanucci 2014, Hamilton *et al.* 2014, Perafán & Pérez-Miles 2014, Hamilton *et al.* 2016, Fukushima & Bertani 2017, Ortiz & Francke 2016). An alternative for a sustainable identification is the construction of systems that employ DNA sequences as taxon barcodes (Sun *et al.* 2012). Molecular markers, such as COI, may provide species boundary information in certain taxonomic groups and consequently have the potential to be a rapid and efficient means to delineate and identify species (Hebert *et al.* 2003, Barrett & Hebert 2005, Arnedo & Fernandez 2007, Chen *et al.* 2011, Kuntner & Agnarsson 2011, Hamilton *et al.* 2014, Blagoev *et al.* 2016). However, molecular identification is not free of problems and may have restrictions and inconsistencies. So, the use of molecular data together with

morphology is recommended, along with collection data and field observations to allow for a better species delimitation and proposal of phylogenetic relationships (Will & Rubinoff 2004, Prendini 2005, Bond & Stockman 2008, Scotland *et al.* 2003, Slowik & Blagoev 2012, Hendrixson *et al.* 2015, Ortiz & Francke 2016). In the particular case of tarantulas, there are a limited number of studies that have used molecular information for species delimitation, and some of them found moderate to deep inconsistencies in the morphology-based taxonomy due to homoplasy in some characters (Hamilton *et al.* 2011, Hendrixson *et al.* 2013, Wilson *et al.* 2013, Hamilton *et al.* 2014, Hendrixson *et al.* 2015, Graham *et al.* 2015, Hamilton *et al.* 2016, Montes de Oca *et al.* 2015, Ortiz & Francke 2016).

To date no taxonomic revision has been made of the genus *Brachypelma* despite its popularity, being widely exploited for the pet trade, and being a priority group for conservation protected by international conventions such as CITES or the General Law of Wildlife (LGVS) in Mexico. Also, although geographical ranges have been published for *Brachypelma* species of Mexico (Baerg 1958, Valerio 1980, Smith 1994, West 1996, Locht *et al.* 1999, Reichling, 2001, Reichling 2003, Rojo 2004, West 2005, Arisqueta-Chablé *et al.* 2009, Shaw *et al.* 2011, Hijmensen 2012, Longhorn 2014, Mendoza & Francke 2017), these ranges are general in scope or incomplete (Fig 1). Furthermore, studies to determine the susceptibility, exact zoogeographical range and/or genome for each priority tarantula species have not been professionally conducted (Garcia 2016). The geographic distribution of *Brachypelma* shows a clear difference between the “Red Leg” and “Red Rump” species, the first ones mostly distributed through the Mexican Pacific Coast and central Mexico and the second ones distributed along the Southwest of Mexico from the Gulf of Mexico to the Pacific Coast at the south of Guerrero and continuing southward to northern Costa Rica (Fig 2). Most *Brachypelma* studies have focused on a few taxa, as those of population interactions, ethology, morphological variation and genetic diversity in *Brachypelma vagans* (Ausserer 1875) (Reichling 2000, Longhorn 2002, Machkour-M’Rabet et al 2005, Shillington & McEwen 2006, Machkour-M’Rabet *et al.* 2007, Dor *et al.* 2008, Dor & Hénaut 2011, Machkour-M’Rabet *et al.* 2011, Mackour *et al.* 2012, Dor & Hénaut 2012, Dor & Hénaut 2013, Vilchis-Nestor *et al.* 2013, Machkour-M’Rabet *et al.* 2015, Hénaut *et al.* 2015, Machkour-M’Rabet *et al.* 2017); from courtship and habitat preference of *Brachypelma klaasi* (Schmidt & Krause 1994) (Yáñez & Locht 1999, Yáñez & Flotaer

2000) and only a few studies have tested the utility of molecular markers for species delimitation or identification of the priority tarantula species (Longhorn *et al.* 2007, Petersen *et al.* 2007, Machkour-M'Rabet *et al.* 2009, Mendoza & Francke 2017, Turner *et al.* 2018).

In Mexico, wild populations of *Brachypelma* tarantulas are in decline, due to habitat loss, because people usually kill them in their natural distribution sites, and because large numbers are being collected of some of the more colorful species from the Pacific Coast to supply the national pet market. Field caught tarantulas are usually either sold in traditional markets with little or no law enforcement or exported illegally for the commercial pet trade (García 2016, CEC 2017). The sustainable use, conservation and management of native tarantulas in Mexico is regulated via the General Law of Wilflife (LGVS for its spanish acronym) and its by-laws which allow the creation of the called Management Units for the Conservation of Wildlife (UMA for its spanish acronym). The UMA refers to the registered facilities that operate in accordance with an approved management plan; they are intensive and extensive breeding sites where wild flora, fauna and fungi are reproduced and propagated; and products and by-products destined to the different types of use are generated. Their general objective is the conservation of natural habitat, populations and specimens of wild species. They function as: centers of breeding stock, germplasm banks, alternative *ex-situ* conservation and reproduction of key species or that are in some category of risk, environmental education work, research, hunting purposes and production units of species, parts and derivatives of wildlife species that can be incorporated into the different circuits of the legal market for commercialization (SEMARNAT 2000). Under the UMA program, qualified persons may present a request to collect a limited number of wild tarantulas to keep and breed in captivity. The resulting offspring may then be sold domestically or exported, and even used for reintroduction programs. Few hobbyists, breeders or even academics would be experienced enough to identify each species of prioritary tarantula on sight. So that the objectives focused on the protection and conservation of tarantulas supported by international policies and those of Mexico can have better results, it is of great importance to have an adequate description of the priority tarantula species for their conservation, as well as the development of morphological guides

and molecular databases that allow their correct identification. In addition, it is necessary to delimit their natural distribution sites in order to develop better strategies that allow determining the status of their populations, their habitat preferences and the best sites with the potential to be used for *in-situ* repopulation programs. So as starting point, it was the goal of this work to revise the genus *Brachypelma* Simon 1891 and to test its monophyly by the use of morphological and molecular characters. Some species were originally described based solely on one sex or even using only exosqueletons as type specimens, so that specimens of both sexes have been reviewed and illustrated. Some new characters are introduced as a result of comparative morphological studies carried out on these species and their morphological variability. The redescription of the *Brachypelma* species and the relevant taxonomic changes are made based on our results. Distribution maps for all known species are presented and general description of their habitat and lyfe cycle is showed.

Methods

The general descriptive format used in the present study follows Mendoza & Francke (2017). All measurements are in millimeters and were taken using an ocular micrometer on a stereomicroscope Nikon SMZ645 for smaller structures, and a digital caliper with an error of 0.1mm for larger ones. Leg and palp measurements were taken along the dorsal central axis of the left side. Description of tarsal scopulae follows Pérez-Miles (1994). Male palpal bulb keel terminology follows Bertani (2000). Spermatheca shape follows the general description format used with theraphosids (Pérez-Miles 1989, Bertani 2001, Mendoza & Francke 2017).

The photographs of figures (5–7, 9–10, 11C, 12, 14–16, 18–19, 20B, 21, 23–25, 27–29) were taken with a Nikon Coolpix S10 VR digital camera coupled to a stereomicroscope. Descriptions of colors use the standard names of the 267 Color Centroids of the NBS/IBCC Color System (Mundie, 1995). The colors depicted herein are included to promote some standardization in describing coloration of live animals. To avoid differences of perception in coloration because of the calibration of the monitor, we extracted the RGB code and Pantone Color from the photographs using Photoshop CS Live. A digital photograph was opened and the eyedropper tool clicked over the image to obtain the RGB color code in the

Set foreground color and cross-checked in the color libraries for the Pantone solid coat. A standard for illumination was deliberately not established, since fluorescent versus incandescent light provide different colors for the same sample. Both habitat and laboratory images were taken with a Canon G12 Digital camera. The habitat shots were taken under natural daylight conditions. The images with white background were taken in the laboratory; general illumination was provided by one fluorescent 30-watt light bulb held approximately 20cm from the specimen. Once the RGB code was obtained, it was possible to infer the real color of the specimen using the RGB code of Color Centroids. The range of each color centroid as perceived by the human eye is wide enough to account for errors of observation.

Abbreviations used in the text are as follows: *Ocular patterns*: ALE, anterior lateral eyes; AME, anterior median eyes; PME, posterior median eyes; PLE, posterior lateral eyes. *Morphology*: Legs and palpi: d, dorsal; p, prolateral; r, retrolateral; v, ventral; Rap, retrolateral tibial apophysis; Pap, prolateral tibial apophysis; PL, plumose setae; Palpal bulbs: AK, apical keel; PI, prolateral inferior keel; PS, prolateral superior keel. Chelicerae: CB, cheliceral band. Spermatheca: Bp, spermathecal baseplate. Spinnerets: PMS, posterior median spinnerets; PLS, posterior lateral spinnerets. In species synonymies, we follow the World Spider Catalog (2018): D= Described, T= Transferred, m= male, f= female. Institutions: MNHNP= Museum National d´Histoire Naturelle, Paris, NHM= Natural History Museum, London, SNMF= Senckenberg Naturmuseum, Frankfurt, UCR = Museo de Zoología Universidad de Costa Rica, CNAN = Colección Nacional de Arácnidos, México DF; UNAM= Universidad Nacional Autónoma de México, PROFEPA (acronym in Spanish)= Federal Environmental Protection Agency.

Morphological protocol.

Taxon sampling

The cladistic analysis was based on 24 taxa. The ingroup included 16 species of *Brachypelma*. The outgroups were selected based on the cladystic analyses of the subfamily Theraphosinae made by Pérez-Miles *et al.* (1996), Pérez-Miles (2000) and Perafán & Pérez-Miles (2014). The number of external groups used was eight, belonging to the

following taxa: *Acanthoscurria geniculata* (C.L. Koch 1841), *Aphonopelma seemanni* (F. O. P.-Cambridge 1897), *Bonnetina cyaneifemur* Vol 2000, *Megaphobema mesomelas* (O. Pickard-Cambridge 1892), *Psalmopoeus victori* Mendoza 2014b, *Sericopelma melanotarsum* Valerio 1980, *Theraphosa stirmi* Rudloff & Weinmann 2010 and *Xenesthis immanis* (Ausserer 1875). The trees were rooted on *P. victori*, selected because it belongs to the subfamily Psalmopoeinae Schmidt 2010 (formerly Avicullariinae), which makes it useful as outgroup for the subfamily Theraphosinae.

Character matrix and cladistics.

The character matrix comprised 103 characters of adult morphology scored for 24 taxa, 8 characters were non-informative (Table 1, Appendix 1). Multistate characters were optimized as non-additive (Fitch 1971), in the absence of evidence that would support the order in the multistate characters. The heuristic searches were conducted in TNT versión 1.1 (Goloboff *et al.* 2008). To analyze the search method applied was New Technology Search (Goloboff *et al.* 2008) using Sectorial Search, Ratchet (5,000 iterations and 2,000 substitutions), with drifting (100 iterations) and Tree Fusing (10 rounds; dumping fused subobtimal trees to prevent clogging); memory with a maximum of 30,000 trees retained. The analysis was performed using equal weighting, the length (L) of the optimal trees, consistency index (Ci) and retention index (Ri) are reported. Optimization of ambiguous characters is carried out using ACCTRAN (Accelerated Transformation), in order to disadvantage parallels and promote regressions. According to Agnarsson & Miller (2008), there are no grounds for preferring some algorithm to solve ambiguous optimizations. So, the use of ACCTRAN is somewhat arbitrary and is only used for the sole purpose of presenting a hypothesis about a resolution of these ambiguities. The relative support for each node on the preferred hypothesis was calculated with bootstrap resampling (Felsenstein 1985). Bootstrap support was estimated with heuristic searches of 1000 pseudoreplicates, with p= 50. Cladograms obtained by TNT were exported in Tree file, to be properly edited in WinClada Asado Ver 1.7 (Nixon 1999-2004), mapping all the characters on the consensus tree and subsequently edited with Adobe Illustrator CS5.

Molecular protocol.

Taxa

Specimens were collected throughout the known distribution of the genus *Brachypelma* with special attention to the type localities (where possible). Material was fixed in 80% ethanol. The third leg on the right side of each spider was stored in 96% ethanol at -20°C. Tissue samples of 69 specimens were used for DNA extraction, representing 16 species of *Brachypelma*. Five tissue samples of each *Brachypelma albiceps* Pocock 1903, *B. auratum* Schmidt 1992b, *B. baumgarteni* Smith 1993, *B. emilia* (White 1856), *B. epicureanum* (Chamberlin 1925), *B. hamorii* Tesmoingt, Cleton & Verdez 1997, *B. kahlenbergi* Rudloff 2008, *B. klaasi* (Schmidt & Krause 1994), *B. schroederi* Rudloff 2003, *B. smithi* (F. O.-Pickard Cambridge1897); four each of, *B. boehmei* Schmidt & Klaas 1993, *B. verdezi* Schmidt 2003; three each of, *B. sabulosum* (F. O.-Pickard Cambridge1897), *B. vagans* (Ausserer 1875), and one of each, *B. albopilosum* Valerio 1980, *B. fossorium* Valerio 1980, *Aphonopelma seemanni* (F. O.-Pickard Cambridge1897), *Megaphobema mesomelas* (O. Pickard-Cambridge 1892), *Sericopelma melanotarsum* Valerio 1980 were used in this study. Additionally, four sequences were retrieved from GenBank to use as outgroups for phylogenetic analyses: *Psalmopoeus cambridgei* Pocock, 1895 [JQ412455.1], *Eupalaestrus campestratus* (Simon, 1891) [JQ412446.1] (both from Briscoe *et al.* 2013), *Lasiodora parahybana* Mello-Leitao 1917 [JN018128.1] (from Arabi *et al.* 2012), *Xenesthis immanis* (Ausserer 1875) [MG273518] (from Luddecke *et al.* 2017). We obtained sequences of mitochondrial COI from the 69 samples**.** Vouchers were deposited in the CNAN and assigned a unique number (CNAN-Ar00xxxx). All sequences were submitted to GenBank, and accession numbers and specimen information are in Appendix 2.

DNA protocols

DNA isolation, PCR amplification and sequencing were performed at the Laboratorio de Sistemática Molecular, Instituto de Biología, UNAM. Muscle tissue was extracted from the leg by removing ~20 mg of tissue. Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit™, following the manufacturer's protocol. The concentration of the extracted DNA was quantified with a spectrophotometer (Nanodrop 2000 Techno Scientific) or visualized via agarose gel electrophoresis. DNA amplification was performed using the

polymerase chain reaction (PCR) for the mtDNA barcoding gene region COI. A single set of primers was used: LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3', together with HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994). This primer set amplified a 710-bp region of the mitochondrial cytochrome oxidase subunit 1 gene.

PCR reaction (100.8 µl) contained 48 µl 10x PCR-buffer, 24 µl $MgCl_2$, 7.68 µl of forward and reverse primer each, 9.6 µl dNTP's and 3.84 µl *Taq* polymerase, using 1 µl of the DNA template for each sample. PCR program for CO1 followed initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 45 s, elongation at 72°C for 2:30 min; followed by 7 min of final elongation at 72°C. LCO 1490 and HCO 2198 primers used for single-stranded sequencing. The accuracy of sequences was verified by independently amplifying and sequencing the complementary strands of all fragments. Primer sequences were removed and complementary strands of DNA assembled into consensus sequences, edited and checked for quality using Geneious R8 (Kearse *et al.* 2012). If complementary strands disagreed (besides minor mismatches), the sample was amplified and sequenced again to resolve the discrepancies.

DNA sequence alignment and phylogenetic analysis

Static alignments of COI gene fragments were generated with MAFFT online version 7 (Katoh *et al.* 2002, 2005). The G-INS-I strategy, which performs a global alignment based on an FFT approximation**,** was selected (Katoh *et al.* 2002). This method is suitable for large datasets comprising sequences with relatively limited variation in length, i.e. few short gaps (Katoh *et al.* 2005). The scoring matrix for nucleotide sequences was set to 1/PAM $_K$ = 2, gap opening penalty to 1.53, and offset value to 0.

We carried out Maximum Likelihood inference using RAxML-HPC BlackBox on XSEDE (8.2.8) (Stamatakis *et al.* 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller *et al.* 2010) under GTR+gamma+Pinvar model of nucleotid evolution and Bootstrap support obtained by running 1000 pseudo replicates. The relative supports are showed for each clade on the best tree.

Results

Phylogenetuc analysis

Morphological analysis of 103 characters found two most parsimonious trees (MPTs) (L= 433, Ci= 0.443, Ri= 0.541). The genus *Brachypelma* it recovers as non-monophiletic being separated in two different clades. The only difference between the two MPTs was the position of *Tliltocatl verdezi* (formerly *Brachypelma*) as a sister species of *Tliltocatl kahlenbergi*, or as a sister species of a small clade formed by *T. kahlenbergi*, *T. epicureanum*, *T. vagans* and *T. sabulosum*. The strict consensus tree showed same topology as MPTs, except for the collapse of the internal clade that relates to *T. verdezi* and *T. kahlenbergi* due to the change of position of *T. verdezi* (Fig 3). The first clade includes a group within the 'Red Leg' *Brachypelma* (*Brachypelma sensu stricto*) that includes the type species of the genus *B. emilia*. Although this group is not well supported (P= 15). The strict consensus tree showed that *Brachypelma* possesses four synapomorphies formed by the following characters: Tibiae color pattern completely orange (char35), Embolus apical region slightly broad (char78), apical keel developed and slightly extended to backwards (char89) and spermatheca baseplate divided and narrower than base width (char100). The other three characters that support the *Brachypelma sensu stricto* clade are homoplastic but can be used in the diagnosis of the genus: Fovea shallow (char80), embolus retrolateral curvature only in apex (char75) and embolus apical shape very flattened concave/convex appearance with neck in the base (char79) (Fig 3).

The second clade includes a group within the 'Red Rump' *Brachypelma* (*Brachypelma sensu lato*), which is formaly described here as *Tliltocatl* gen. nov. Although this group is not well supported (P= 11). A small clade formed by *Aphonopelma seemanni*, *Xenesthis immanis* and *Sericopelma melanotarsum* appears as its sister group. The strict consensus tree showed that *Tliltocatl* possesses one synapomorphy which is the embolus prolateral inferior keel longitudinal superior and larger than the prolateral superior keel (char86). The other nine characters that support the *Tliltocatl* clade are homoplastic but can be used in the diagnosis of the genus: Fovea in males and females procurved (char6), fovea in males and females pit-like (char7), Distal scopula of metatarsus III with 75% of coverage relative to

its length (char13), femur I prolateral face setae plumose (char29), male femur III slightly incrassate (char33), patella I with one spine (char60), embolus curved to retrolateral (char75), embolus apical region broad (char78), and embolus apical shape very flattened concave/convex appearance with neck in the base (char79) (Fig 3). In the case of the nominal species *Brachypelma fossorium*, it is shown that it does not belong to *Brachypelma* or *Tliltocatl*, although in the strict consensus tree appears to be closely related to *Tliltocatl*. *B. fossorium* lacks important features present in Tliltocatl such as urticating setae type III, prolateral inferior inferior keel longer than prolateral superior keel, femur III incrassate, percentage coverage of distal scopula of metatarsus III is not more than 50%. All these differences are enough to propose the transference *B. fossorium* to another genus (see transferred to another group).

Maximum likelihood inference analysis of the COI sequences also strongly supports the non-monophyly of *Brachypelma* and is congruent with the analysis of morphological characters, final ML optimization score was -5527.883771. The topology recovered shows two clades, the first one formed by species of *Brachypelma* 'Red Leg complex' thet includes the type species of the genus *B. emilia* (*Brachypelma sensu stricto*) and a second one formed by species in the 'Red Rump complex' (*Brachypelma sensu lato*, here transferred formally to *Tliltocatl* gen. nov.) (Fig 4). The *Brachypelma sensu stricto* clade has a bootstrap support value of 78, it is formed by eight species with high bootstrap values. The species *Sericopelma melanotarsum, Aphonopelma seemanni, Xenesthis immanis* and *Brachypelma fossorium* are shown more related to the *Tliltocatl* species. The clade formed by 'Red Rump species' of *Tliltocatl* has a bootstrap support of 92, it is formed by seven species with relative high support, but the internal relationships are not very clear. *T. schroederi* is shown as sister species of the rest but there is no support for the position of *T. verdezi* and *T. kahlenbergi*. After that follows an internal node with *T. epicureanum* as sister species of *T. sabulosum, T. albopilosum* and *T. vagans*. However, no resolution was shown for interspecific relationships for the three-last species. The species *Brachypelma fossorium* is clearly shown as not included in either *Brachypelma* or *Tliltocatl*, being closer related to *Sericopelma* and *Aphonopelma* (Fig 4). Just as in the morphological analysis,

differences are enough to transfer *B. fossorium* to another genus as is proposed in the taxonomical section.

Despite the differences between the parsimony and Maximum likelihood analyses, both topologies indicate that *Brachypelma* as currently recognized is not monophyletic and that it includes two highly divergent lineages. The species of both *Brachypelma* and *Tliltocatl* can be identified with high accuracy using COI as a barcode (probably due to the relatively high mtDNA divergence between species). In both analyses inner relationships of some of these species require further resolution, and in the case of morphology the homoplasy in some of the characters can lead to not strongly supported topologies. On the other hand, the molecular phylogenies based on mtDNA have limitations as gene tree/species tree incongruence, with CO1 representing only one particular genealogy out of all possible within genome.

Species considered nomina dubia

Brachypelma andrewi Schmidt 1992

Schmidt (1992a) described this species based on a single specimen located in the collection of the British Museum of Natural History (currently Natural History Museum). The history of this specimen was mentioned by Smith (1992), the holotype used for *B. andrewi* description comes from the L. Koch collection. Another specimen from the same Koch collection was found in the NHM containing an alleged specimen not described. Initially the jar which contains the specimen of what would later be described as *B. andrewi*, was labelled as the holotype of *Euathlus truculentus* Ausserer 1875. This created a confusion that ended with the genus *Brachypelma* as junior synonym of the genus *Euathlus* Ausserer 1875 as was proposed by Raven (1985). Smith (1992) redescribed the specimen in the NHM labeled as *Euathlus truculentus* holotype believing it to be the one used by Ausserer (1875). He used his description to support Raven's decision that the genus *Brachypelma* was synonymous with *Euathlus*. However, shortly afterwards Schmidt (1992a) observed that the specimen seen by Raven & later by Smith was not the same species as the type of *Euathlus truculentus*, but, was in fact a new species of *Brachypelma*. Schmidt stated as

follows "*Raven had almost come to the conclusion that Brachypelma was a junior synonym of Euathlus, while Smith, after studying the said Brachypelma, was of the opinion that Raven was right with his synonymizing. He had not noticed that only the body type of the Euathlus type, called Ausserer, deviates considerably from those of the alleged 2nd Euathlus truculentus: e.g. In the real type, the body length is 44 mm, while in the erroneously paratype Brachypelma is 55 mm. This Brachypelma is said to originate from Cuba and has not been mentioned so far - I shared the error with Smith in a letter dated 28 May 1992 and confirmed that he was wrong in the matter of synonymisation and was no longer tenable*". After this Schmidt (1992a) named the undescribed *Brachypelma* as *B. andrewi* after Andrew Smith. Unfortunately, because of the lack of a useful locality for this species, no new specimens can be collected for comparison. Also, after our visit to the arachnological collection of the NHM, it was not possible to locate the type specimen of *Brachypelma andrewi*, which is why it is considered lost to date. After reviewing in detail, the redescription of the specimen made by Smith (1992) it is possible to know that *B. andrewi* is a species of the "red rump complex" (*vagans* group) tarantulas (which was transferred to *Tliltocatl* **gen**. **nov**. in this work). So, we propose that this species be transferred as *Tliltocatl andrewi* **comb**. **nov**. However, without being able to make a direct observation of the specimen, it is not possible to correctly identify the species or compare it with those close related already described. Additionally, given the lack of data on its distribution, it is not possible to obtain new material. Therefore, based on the loss of the holotype and in its inability to be identified, we propose that the species *T. andrewi* be considered *nomen dubium.*

Brachypelma aureoceps (Chamberlin 1917)

This species was described by Chamberlin 1917 based on a single female (RVC43 – examined) from Florida, Tortugas. However, there is no evidence of native tarantulas from Florida (Smith 1994, West pers. com.). Smith (1994) mentioned that is higly probable that *B. aureoceps* specimen was accidentaly imported onto the Tortugas island. This specimen have morphological features such as spermatheca without baseplate, highly notched medially, patellae spination and plumose setae on prolateral trochanter and femur of leg I, showing that it belongs to the genus *Tliltocatl* **gen**. **nov**. So, we propose that this species be

transferred as *Tliltocatl aureoceps* **comb**. **nov**. Nevertheless, this species is similar to other valid species distributed in Mexico (e. g., *T. kahlenbergi* (Rudloff 2008) **comb**. **nov**. and *T. vagans* (Ausserer, 1875) **comb**. **nov**.) and cannot be differentiated adequately from them. In such a way that molecular data or accurate morphological features are needed to properly differentiate this species, and without the possibility to collect fresh specimens we consider *T. aureoceps* a *nomen dubium*.

Citharacanthus alvarezi Estrada-Álvarez, Guadarrama & Martínez 2013

This species was described by Estrada-Álvarez *et al.* 2013 based on a single specimen referred to as female (CNAN-T01275 – examined) from an uncertain locality in Tuxtla Gutierrez, Chiapas, that was donated by an unknown collector to the Zoológico Miguel Álvarez del Toro (ZooMAT). After examination of the holotype we were able to determine that it does not belong to the genus *Citharacanthus* Pocock 1901, and it is not a female, but a subadult male belonging to the genus *Tliltocatl* gen. nov. The main evidence of this is the presence of type I and III urticating setae, in combination with stridulatory plumose setae on trochanter and femur I, and femur III slightly thickened. It was evident that the specimen is a subadult male just by the presence of the accessory organs, these gland-like structures can be very prominent in some groups, such as *Tliltocatl*, and are often mistaken for paired spermatheca (as was in this case). The male accessory organs do not have direct connection with the gonopore and neither do they present uterus externus as if observed in a spermatheca. Based on the evidence we proposed to transfer the species as *Tliltocatl alvarezi* comb. nov. Unfortunately, subadult males do not show features that could be useful to identify a species. Due to the lack of morphological and/or molecular data that would allow us to make a comparison with the already described known species of *Tliltocatl*, as well as more precise collection locality, we propose *T. alvarezi* be considered as *nomen dubium*.

Transferred to another group.

Stichoplatoris fossorius (Valerio 1980) **comb. nov.**

Brachypelma fossoria Valerio 1980: 271, fig. 25-28 (D male and female); Smith 1986: 49, fig. 28h (male); Smith 1987: 49, fig. 28h (male).

Brachypelma fossorium Schmidt 1992: 10, fig. 9-12 (transfered male and female from *Euathlus*); Rudloff 2003: 8, fig. 26-29 (male); Schmidt 2003: 153, fig. 278-281 (male and female).

Misidentification: Peters 2000: 70, fig. 228 (male); Peters 2003: 121, fig. 486, 488-489 (male and female). Peters (2000) and Peters (2003) show as *B. fossorium* an unidentified species of *Tliltocatl*, possibly pet-trade material which makes it difficult to identify.

Holotype and paratype of Brachypelma fossorium. COSTA RICA: *Guanacaste:* 1♂, Filadelfia. Col. E. Herrera (UCR-238) examined. 1♀, Liberia, finca Santo Tomas, without more data (UCR-126) not examined.

Remarks: Originally Valerio (1980) placed this species in the genus *Brachypelma* based only in the shape of the male palpal bulb and female genitalia. However, after the revision of the holotype and based in the cladistic analysis we conclude that this species does not belong in *Brachypelma* or even in *Tliltocatl* gen. nov. This species has a very short embolous with presence of prolateral superior, prolateral inferior and apical keels, but with a different configuration from that observed in *Brachypelma* and *Tliltocatl*. The keels arrangement is more similar to *Stichoplastoris*. The urticating setae present in *B. fossorium* are only type I, whereas *Brachypelma* and *Tliltocatl* possesses type I and III. Also *B. fossorium* has the scopula IV divided by a strong band of setae, while *Brachypelma* and *Tliltocatl* have all their scopulae entire. These characteristics fit those found in some species of the genus *Stichoplastoris* (e .g., *S. elusinus* (Valerio 1980), *S. obelix* (Valerio 1980)). The weakest point of the kinship with *Stichoplastoris* would be the general shape of the male palpal bulb that is wide in the apex, and the spermatheca of the female that is unilobular, whereas in *Stichoplastoris* the bulb looks thinner apically and the spermatheca is divided (although in species like *S. obelix* it is wider in its base). Despite this, there are similar examples in genera such as *Brachypelma* in which species such as *B. klaasi* and *B. albiceps* have thinner palpal bulbs and a divided spermatheca, unlike the rest of the species

within the genus that have wider bulbs apically and fused spermatheca. We therefore propose based on the similarities that *B. fossorium* be transferred to the genus *Stichoplastoris* creating the new combination *Stichoplastoris fossorius*.

Taxonomy.

Family **THERAPHOSIDAE** Thorell 1869

Subfamily **THERAPHOSINAE** Thorell 1870

Brachypelma Simon, 1891

(Figs 1–33)

Brachypelma Simon, 1891: 338.

Type species: **Mygale emilia** White, 1856, by original designation.

Diagnosis

Brachypelma can be distinguished from all other known theraphosinae genera (except Tliltocatl gen. nov.) by having plumose stridulatory setae on the prolateral face of leg I trochanter/femur and on the retrolateral face trochanter of the palp. Both sexes lack a plumose pad of setae on leg IV femur. The metatarsus IV is 20-40% scopulated distally. All tarsi scopulae are undivided. The femur of leg III is slightly enlarged but not swollen as in other genera. The male palpal bulb distally wide and flattened (spoon-shaped) and presents prolateral superior and apical keels. Can have a small or reduced prolateral inferior keel, except in B. albiceps, B. baumgarteni, B. emilia and B. klaasi. The apical keel can extend slightly or widely backwards. Females can have a divided spermatheca with each lobe as large as wide (present in B. albiceps and B. klaasi), or a simple undivided/fused spermatheca**,** apically narrowed (present in B. auratum, B. baumgarteni, B. boehmei, B. emilia, B. hamorii and B. smithi). Both sexes possess urticating setae types I and III; type III located in the dorsoposterior area and type I surrounding these. It differs from Tliltocatl by the red/orange coloration pattern on legs and/or carapace (except B. albiceps). The shape of genitalia also differs in both sexes with the male palpal bulb apex shorter than in

Tliltocatl and by the lacking of prolateral inferior keel or restricted to the apex when present, while in Tliltocatl it is posteriorly extended and parallel to prolateral superior keel. It also differs in lacking any spination on patellae of palps or legs. Females differs by the spermatheca apex not as inwardly curved, being generally straight (except B. klaasi and B. albiceps due to divided spermatheca). Brachypelma differs also by having the spermatheca baseplate more developed and sclerotized than Tliltocatl.

Brachypelma albiceps Pocock 1903

(Figs 2, 5–8, 33)

Eurypelma pallidum F. O. Pickard-Cambridge 1897: 21, plate 1, fig. 17 (female misindetified).

Brachypelma albiceps Pocock 1903: 103 (D female); Schmidt 2004: 4, fig. 1 (transferred to synonymized generic name); Locht *et al.* 2005: 108 (transferred from *Aphonopelma*); Estrada-Alvarez 2014: 57, fig. 18 (female); Teyssié 2015: 266-267 (female).

Aphonopelma albiceps Smith 1994: 70, fig. 76-82 (removed female from synonymy of *A. pallidum*); Peters 2000: 23, fig 43 (female); Peters 2003: 32, fig. 107 (female).

Brachypelmides ruhnaui Schmidt 1997a: 205, fig. 1-4 (D male and female); Schmidt 1997b: 19, fig. 199-201 (male and female); Locht, Yáñez & Vázquez 1999: 196, fig. 3 (male); Peters 2000: 76, fig. 250-252 (male and female); Peters 2003: 133, fig. 542, 545-547 (male and female); Schmidt 2003: 137, fig. 207 (male and female).

Material examined

Holotype of Brachypelma albiceps. MEXICO: *Guerrero:* ♀, Venta de Zopilote. No more data (NHM labelled as BM1898.12.24.34-37).

Holotype and paratype of Brachypelmides ruhnaui. MEXICO: *Estado de Mexico:* 1♂, 1♀, Toluca. Col. Mark Ruhnau (SMF39013).

Other material. MEXICO: *Guerrero:* 1 ♀, no more data, source E. Hijmensen, S. Longhorn (RUHZ Longhorn DNA sample 2005); 1♂, 1♀, Mpio. Copalillo, Papalutla,

14/X/2008, J. Mendoza (CNAN-Ar003412); 1 ♀, Mpio. Azoyu, El Carrizo VII/2011, A. Alcaraz (CNAN-Ar007850); 2 ♀, Mpio. Tixtla de Guerrero, Zotoltitlan, 23/IX/2012, J. Mendoza, G. Contreras, J. Cruz, D. Ortiz (CNAN-Ar007839, CNAN-Ar007843); 1 juvenile, Mpio. Chilpancingo de los Bravo, Milpillas, 22/IX/2012, J. Mendoza, G. Contreras, J. Cruz, D. Ortiz (CNAN-Ar007852); 1 ♂, Mpio. Arcelia, Teloloapan, 15/IX/1952, col. L. Vazquez (CNAN-Ar003099); 1 ♂, Mpio. Arcelia, Presa Vicente Guerrero, 05/X/1976, (CNAN-Ar003413); 1 ♂, Mpio. Arcelia, Presa Vicente Guerrero, 23/XI/1976, A. Castillo (CNAN-Ar003437); 1 ♂, Mpio. Eduardo Neri, Ahuelican, 03/X/2008, O. Francke, A. Valdez, T. Lopez (CNAN-Ar004128); 1 ♂, Mpio. Eduardo Neri, Venta Vieja, 26/V/1960, (CNAN-Ar004129); 1 ♂, Mpio. Tepecoacuilco, Ahuehuepan, 30/VIII/2009, O. Francke, A. Valdez, T. Lopez, C. Santibañez (CNAN-Ar004130). *Morelos*: 1 ♀, Mpio. Cuernavaca, 21/IX/1959, (CNAN-Ar003082); 1 ♂, Mpio. Tequesquitengo, 24/IX/1961, Santibañez (CNAN-Ar003113); 1 ♂, Mpio. Amacuzac, Huajintlán, 28/VIII/2009, O. Francke, A. Valdez, C. Santibañez, T. Lopez, (CNAN-Ar010574).

Diagnosis

Brachypelma albiceps can be distinguished from all other known *Brachypelma* species by its coloration, consisting of brown range setae on the carapace and red setae on opistosoma. The shape of the genitalia also differs in both sexes with the palpal bulb being sharp and tapered, and the spermatheca separated and with rounded receptacles.

Brachypelma albiceps is identified by possesing the following character combination: male palpal bulb with sharp embolus curving to dorsal through its length, prolateral superior keel very reduced, apical keel developed, wider behind embolus tip. Embolus tip directed to retrolateral. Embolus similar in length to tegulum (Fig 6A–D). Spermatheca separated with semicircular receptacles. Spermathecal baseplate divided, oval; twice wider than its height (Fig 7E–G). Carapace of both sexes golden yellow (Fig 8A–B).

Description

M*ale* (CNAN-Ar003412) (Fig 5–6): body length 38.45 (not including chelicerae and spinnerets). Carapace 17.64 length, 16.61 width. Caraput not markedly elevated; fovea straight, 3.25 wide (Fig 5A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.45; ALE 0.70; PME 0.35; PLE 0.53; AME-AME 0.38; AME-ALE 0.20; PME-PME 0.98; PME-PLE 0.10; ALE-PLE 0.18. Ocular tubercle width 2.23, length 1.98; clypeus lacking (Fig 5D). Labium length 2.43, width 2.77; with 95 cuspules. Maxilla inner corner (left-right) with approximately 167-194 cuspules. Cheliceral promargin with (left-right) 10-10 teeth. Sternum length 7.90. Sigillae oval, second and third pairs hardly visible, posterior sigilla once its length from the margin (Fig 5B). Leg formula: IV, I, III, II. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.68, 8.70, 13.10, 12.76, 8.05, 59.29. II: 14.37, 7.54, 11.35, 11.54, 8.62, 53.42. III: 14.13, 7.07, 10.32, 13.78, 8.85, 54.15. IV: 16.88, 7.68, 14.29, 18.88, 10.60, 68.33. Palp: 10.51, 5.96, 9.53, -, 3.88, 29.88. Spinnerets: PMS, 1.90 long, 1.00 apart; PLS, 2.90 basal, 1.95 middle, 3.25 distal. Tarsi I-IV entirely scopulated. Metatarsi I densely scopulated, II densely scopulated, III scopulated 75% distally, IV scopulated 50% distally. Tibia I with two tibial apophyses normally developed which originate from a common base. Prolateral apophysis with one inner conical spine; retrolateral apophysis almost the same width throughout its length and curved to dorsal on apex (Fig 5F–G). Metatarsus I curved (Fig 5E). Stridulatory setae: with plumose setae on palp trochanter retrolateral face, and leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora palp 1p; patellae none; tibiae II 2v; III 1r, 2v; IV 1p, 2v; palp 1p, 1v; metatarsi II 2v; III 2p, 6v; IV 2p, 9v, 1r.

Palp. Embolus tapering and with very reduce spoon-like shape at tip. Embolus slightly twisted to retrolateral. Prolateral superior keel very reduced and thin, prolateral inferior keel absent, apical keel extending to posterior and wider at the embolus tip. The opening of the embolus is on the prolateral side, just behind the opening is located a concavity which delimits the apical keel boundary from the remaining part of the embolus. Tegular apophysis rounded. (Fig 6A–D). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I surrounding the type III area, with intermediates between type III and I in transition areas (Fig 5C).

Female (CNAN-Ar003082) (Fig 7A–E): body length 53.61 (not including chelicerae and spinnerets), carapace 23.50 length, 22.40 width. Caput not markedly elevated; fovea procurved, 5.80 wide (Fig 7A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.47; ALE 0.50; PME 0.30; PLE 0.40; AME-AME 0.67; AME-ALE 0.33; PME-PME 1.40; PME-PLE 0.15; ALE-PLE 0.43. Ocular tubercle width 2.80, length 2.43; clypeus lacking (Fig 7C). Labium length 2.95, width 4.15; with 94 cuspules. Maxilla inner corner (left-right) with approximately 129-130 cuspules (Fig 7D). Cheliceral promargin with (left-right) 9-10 teeth (proximal to distal: first-third large, fourth-sixth medium, seventh-eighth large, ninth small ; first-third large, fourth small, fifth medium, sixth-tenth large). Sternum length 11.40. Sigillae oval, third pair hardly visible; posterior sigilla once its length from the margin (Fig 7B). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.52, 10.27, 13.05, 12.26, 8.36, 60.73. II: 15.35, 9.57, 10.94, 10.57, 7.94, 54.37. III: 14.40, 8.49, 9.30, 12.35, 6.92, 51.46. IV: 17.65, 9.00, 12.90, 17.39, 8.79, 65.91. Palp: 12.88, 6.12, 9.16, -, 9.12, 37.28. Spinnerets: PMS, 3.00 long, 3.50 apart; PLS, 4.75 basal, 3.10 middle, 3.65 distal. Tarsus I-IV entirely scopulated. Metatarsi I-II entirely scopulated, III scopulated 75% distally, IV scopulated 50% distally. Stridulatory setae: with plumose setae on leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora palp 1p; patellae none; tibiae I 2v, II 1p, 4v; III 3v, 1r; IV 3v, 1r; palp 5v, 1r; metatarsi I 1v; II 3v; III 2p, 5v, 1r; IV 13v, 2r. Genitalia: Spermatheca separated with semicircular receptacles strongly sclerotized, ventral face smooth. Spermathecal baseplate divided, oval; twice wider than its height. (Fig 7E). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area; with intermediates between type III and I in transition areas.

Color pattern. In live specimens, adult of both sexes has the carapace brown range (pantone 719c) in color (Fig 8A–B), with juveniles or subadults paler in color; chelicerae dorsally dark blue gray; ventral coxae, labium, maxillae and sternum brownish black; abdomen

dorsally black with light orange setae, ventrally brownish black. Legs and palpi: bluish black. (Fig 8A–B).

Distribution and habitat

Brachypelma albiceps is known from the Central Mexico in Morelos, part of Puebla and Estado de Mexico to middle of Guerrero as the southern limit of its distribution. Its burrows occur in dense thickets or vegetation of dry thorn forests under large rocks or tree roots (Fig 2, 8C, 33). There are no traces of silk at the burrow entrance, and the interior can often be multi-tunnelled. The breeding season occurs during the last part of rainy and first part of dry seasons (august to january). Egg-sacs are constructed through the drier winter months with young emerging and dispersing in the late spring, just before the onset of the early summer rains.

Brachypelma auratum Schmidt, 1992

(Figs 2, 9–13, 31A–D, 33)

Brachypelma auratum Schmidt 1992b: 9, fig. 1, 3 (D male and female); **Schmidt, 1993**: 82, fig. 187 (female); **Smith, 1994**: 160, fig. 851-866 (male and female); **Tesmoingt, Cleton & Verdez, 1997a**: 9, plate 2, fig. 5 (female); **Schmidt, 1997b**: 19, fig. 192 (female); **Locht, Yáñez & Vázquez, 1999**: 196, fig. 6 (female); **Peters, 2000**: 65, fig. 210-211 (male and female); **Peters, 2003**: 110, fig. 439-440, 443, 446-447 (male and female); **Schmidt, 2003**: 137, fig. 203 (female).

Material examined

Holotype of Brachypelma *auratum*. MÉXICO: ♂, donated 22/II/1992 by Fritzlen (SMF 38045). Female spermatheca from exuvia mounted in a permanent preparation (SMF 38045).

Other material

MÉXICO: *Michoacán*: 1 ♂, Mpio. Los Reyes, Los Reyes Salgado, 2/II/2013, Col. J. Mendoza, G. Contreras, D. Ortiz, D. Barrales (CNAN-Ar003658); 1 ♀, Mpio. San Lucas, Salguero 2/II/2013, J. Mendoza, G. Contreras, D. Ortiz, D. Barrales (CNAN-Ar007164); 2♀, Mpio. La Huacana, 5km SE of Zicuirán, 16/IX/2015, Col. J. Mendoza (CNAN-Ar007903, CNAN-Ar010284); 1♀, Mpio. La Huacana, 1km SE of Chau (CNAN-Ar010284); *Guerrero:* 1 ♀, Mpio. Arcelia, Desviación a Chacamerito, 3/II/2013, J. Mendoza, G. Contreras, J. Cruz, D. ortiz (CNAN-Ar007136); 1 ♀, Mpio. Chilpancingo de los Bravo, carretera Coyuca de Catalán-Zihuatanejo, cols. A. Zaldivar, M. García, J. Martínez, V. Salinas (CNAN-Ar007878); *Estado de México*: 1 ♂, Mpio. Luvianos, 2/11/2002, E. Gonzalez-Santillan, R. Paredes, C. Durán (CNAN-Ar003592); 2 ♂ and 1♀, donation received from private collection of J. Mendoza (CNAN-Ar003660, CNAN-Ar003676. CNAN-Ar003657).

Diagnosis

Brachypelma auratum can be distinguished from all other known *Brachypelma* species by the coloration of the legs with red-orange flame shape on patellae. Also differs in the shape of genitalia in both sexes with palpal bulb straight, embolus short and broader at apex. The prolateral superior keel normally developed and slightly directed retrolaterally. Apical keel with a great backward development, extending almost the same length of prolateral superior keel. The prolateral inferior keel is joined to prolateral superior keel at the apex and slightly extended to downwards. Also differs by the spermatheca ventral face smooth, with spermathecal baseplate subrectangular.

Brachypelma auratum is identified by possesing the following character combination: male palpal bulb straight with a broad spoon shape, prolateral superior keel normally developed, directed retrolaterally and slightly extended to backward; prolateral inferior keel weakly developed restricted to embolus apex, better seen dorsally, apical keel developed extending widely backwards. Embolus tip slightly directed to retrolateral. Embolus short and wide, similar in length to tegulum (Fig 10A–D). Spermatheca fused semicircular, with a single receptacle strongly sclerotized. Spermathecal baseplate divided, more separated above;

ovate and 2.5 wider than its height (Fig 11C, 12E–F). Both sexes possess an orange flame shape area over the patellae (Fig 13A–C).

Description

Male (CNAN-Ar003658) (Fig 9–10): body length 42.09 (not including chelicerae and spinnerets), carapace 18.16 length, 18.00 width. Caput not markedly elevated; fovea straight, 4.15 wide (Fig 9A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.40; ALE 0.55; PME 0.40; PLE 0.65; AME-AME 0.50; AME-ALE 0.23; PME-PME 1.13; PME-PLE 0.13; ALE-PLE 0.37. Ocular tubercle width 2.60, length 2.13; clypeus absent (Fig 9D). Labium length 2.07, width 2.27; with 113 cuspules. Maxilla inner corner with approximately 270 (left) and 248 (right) cuspules. Cheliceral promargin with 10 (left) and 10 (right) teeth (proximal to distal: first large, second small, third large, fourth-tenth large). Sternum length 9.00. Sigillae oval, first to third pairs hardly visible, posterior pair once its length from the margin (Fig 9B). Leg formula: : IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 17.25, 9.45, 14.08, 15.17, 10.32, 66.27. II: 15.92, 9.32, 12.61, 13.91, 9.06, 60.82. III: 14.49, 8.06, 11.73, 14.15, 8.68, 57.11. IV: 17.61, 8.40, 14.79, 19.44, 9.61, 69.85. Palp: 10.88, 7.08, 10.35, -, 3.55, 31.86. Spinnerets: PMS, 2.10 long, 1.30 apart; PLS, 2.83 basal, 2.20 middle, 3.70 distal. Tarsi I-IV entirely scopulated. Metatarsus I entirely scopulated, II scopulated 75%, III scopulated 50% distally, IV scopulated 25% distally. Tibia I with two tibial apophyses normally developed, which originate from a common base. Prolateral apophysis with inner spine half its length; retrolateral apophysis same width throughout its length, apex slightly curved to prolateral (Fig 9F–G). Metatarsus I curved (Fig 9E). Stridulatory setae: with plumose setae on palp trochanter retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; II 1p; palp 1p; patellae none; tibiae I 2p, 1r; II 2p, 3v; III 3p, 4v, 2r; IV 1p, 4v, 1r; palp 2p, 3v; metatarsi II 1p, 1v; III 3p, 7v, 2r; IV 2p, 13v, 2r.

Palp. Embolus almost straight with a broad spoon shape, prolateral superior keel normally developed, directed retrolaterally and slightly extended to backward; prolateral inferior keel weakly developed restricted to embolus apex, better seen dorsally, apical keel developed extending widely backwards. Opening of the embolus is on the prolateral side, just behind

the opening is located a concavity which delimits the apical keel boundary from the remaining part of the embolus. Embolus apex slightly curved to retrolateral (Fig 10A–D). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area; with intermediates between type III and I in transition areas (Fig 9C).

Color pattern. In live specimens, adult males with carapace peach color (pantone 712c) around the border, some specimens also present this color behind the fovea. Carapace color is black (pantone process black); chelicerae dorsally black; ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with sparse rose color setae (pantone 487c), ventrally brownish black. Legs and palpi: femora black, patellae with a proximal dorsomedian signal orange (pantone 173c) flame shape area, distal ring putty color (pantone 728c), with some brownish pink setae laterally; tibiae proximal three quarters process black with some sparse brownish pink setae, distal quarter brown range and metatarsi process black with brownish pink setae and a warm gray ring at the terminal end; tarsi black (Fig 13A).

Female (CNAN-Ar007878) (Fig 12A–E): body length 61.36 (not including chelicerae and spinnerets), carapace 26.34 length, 24.61 width. Caput not markedly elevated; fovea straight, 5.70 wide (Fig 12A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.47; ALE 0.80; PME 0.37; PLE 0.70; AME-AME 0.67; AME-ALE 0.33; PME-PME 1.57; PME-PLE 0.13; ALE-PLE 0.43. Ocular tubercle width 2.97, length 2.93; clypeus lacking (Fig 12C). Labium length 3.05, width 3.55; with 86 cuspules. Maxilla inner corner with approximately 250 (left) – 276 (right) cuspules (12D). Cheliceral promargin with 10 (left) – 11 (right) teeth. Sternum length 11.39. Sigillae oval, first to third pairs hardly visible; posterior pair once its length from the margin (Fig 12B). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.56, 10.85, 13.73, 13.01, 8.64, 62.79. II: 15.65, 10.60, 11.60, 12.49, 7.59, 57.93. III: 14.59, 9.57, 10.83, 13.77, 8.52, 57.28. IV: 17.94, 10.05, 14.04, 18.78, 9.75, 70.56. Palp: 12.62, 8.57, 8.93, -, 10.35, 40.47. Spinnerets: PMS, 2.50 long, 2.05 apart; PLS, 4.15 basal, 4.15 middle, 4.25 distal. Tarsus I-IV entirely

scopulated. Metatarsi I entirely scopulated, II scopulated 90%, III scopulated 70% distally, IV scopulated 40% distally. Stridulatory setae: with plumose setae on palp trochanter retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora palp 1p; patellae none; tibiae palp 1p, 5v; II 1p, 3v; III 3v; IV 3v; metatarsi II 2v; III 5v, 2r; IV 1p, 8v, 1r. Genitalia: fused semicircular spermatheca, with a single receptacle strongly sclerotized, four times wider than its height. Spermatheca baseplate divided, more separated above, each baseplate ovate 2.5 wider than high, outer side slightly smaller than the inner (Fig 12E). Variation: some specimens have a wider base up to five times greater than height, young or juveniles can present the superior edge slightly inward in middle. Ventral face smooth. Baseplate division can vary in length (Fig 11C, 12F). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas.

Color pattern. In live specimens, adult females with two carapace patterns: (1) light brown (pantone 7414c) around the border and process black in dorsomedian, juveniles or subadults same pattern (Fig 13B); (2) light brown around the border and extended behind the fovea, with process black from the fovea to caput (Fig 13C); chelicerae dorsally oxford blue (pantone 532c); ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with sparse rose color setae (pantone 487c), ventrally brownish black. Legs and palpi: femora black, patellae with a proximal dorsomedian signal orange (pantone 173c) flame shape area, distal ring putty color (pantone 728c), with some brownish pink setae laterally; tibiae proximal three quarters process black with some sparse brownish pink setae, distal quarter brown range and metatarsi process black with brownish pink setae and a warm gray ring at the terminal end; tarsi black (Fig 31A–D).

Distribution and habitat. Brachypelma auratum is known from part of the Neotransvolcanic belt, being found in southwestern Estado de México, northwesternf Guerrero, central Michoacán and northeast border of Jalisco with Michoacán (Fig 2, 33), where it occurs in thorn and deciduous secondary forests (Fig 13D). It is a fossorial species

whose modified or self-excavated burrow**s** can be found under large rocks and large tree roots amongst thorny brush. Burrows do not have any silk around the entrance.

Remarks

The type specimen of *B. auratum* (SMF38045) lacks of palpal bulbs because they were removed possibly when was described (Fig 11A). It was not possible to find them in the holotype jar nor even in the laminated collection at Senckenberg Museum. One mounted spermatheca was found instead of a palpal bulb (Fig 11B–C). Origin of that spermatheca is uncertain. However, the other features like patellae coloration and tibial apophysis in the type specimen are enough for identification of species.

Brachypelma baumgarteni Smith, 1993

(Fig 2, 14–17, 32A–D, 33)

Brachypelma baumgarteni Smith 1993: 15, fig. 1-10 (D male); **Smith, 1994**: 163, fig. 875-883 (male); Teyssié 2015: 269, fig. 1 (female).

Misidentified in Peters 2000: 66, fig. 214-215 (male); Peters 2003: 114, fig. 457, 460 (male), the species showen in the figures is an adult male of *Brachypelma hamorii* Tesmoing, Cleton & Verdez 1997 not *B. baumgarteni*.

Material examined

Holotype of Brachypelma *baumgarteni*. MÉXICO: Michoacán: ♂, Sierra Madre del Sur, M. Baumgarten (BMNH-1999-122).

Other material

MÉXICO: *Michoacán*: 1 ♂, Carretera La Mira-Arteaga, 09/IX/2012, without collector (CNAN-Ar003597); 2 ♀, Mpio. Lázaro Cárdenas, Los Amates 11/XII/2013, J. Mendoza (CNAN-Ar007149) (CNAN-Ar007150); 1 ♀, Mpio. Lázaro Cárdenas, Puente Chuquiapan, 10/XII/2013 J. Mendoza (CNAN-Ar007151); 1 ♂, Mpio. Lázaro Cardenás, Los Coyotes, 8/XII/2016, J. Mendoza, R. Ramírez (CNAN-Ar010588).

Diagnosis

Brachypelma baumgarteni can be distinguished from all other known *Brachypelma* species by the coloration of the legs with deep orange flame shape on patellae with yellowish around, tibia and metatarsus similar yellowish color. Also differs in the shape of genitalia in both sexes with palpal bulb slightly curved to dorsal, embolus shorter than tegulum and broader at apex. The prolateral superior keel broad and short, slightly directed retrolaterally. Apical keel thin, extending more than the length of prolateral superior keel. Also differs by the spermatheca ventral face smooth, with spermathecal baseplate oblong.

Brachypelma baumgarteni is identified by possesing the following character combination: male palpal bulb slightly curved to dorsal with a narrow spoon shape at apex, prolateral superior keel short, thin and directed retrolaterally; apical keel short and thin, larger than prolateral superior keel. Embolus tip slightly directed to retrolateral. Embolus compact and thin, shorter in length than tegulum (Fig 15A–D). Spermatheca fused trapezoidal, with a single receptacle strongly sclerotized. Spermathecal baseplate divided, more separated below; oblong and three times wider than its height (Fig 16E–G). Both sexes possess a yellowish longitudinal line just at metatarsi middle, this is better seen on legs I and IV (Fig 32A–D).

Description

Male (CNAN-Ar010588) (Fig 14, 15, 17A): body length 49.63 (not including chelicerae and spinnerets), carapace 22.59 length, 21.54 width. Caput not markedly elevated; fovea recurved, 3.75 wide (Fig 14A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.40; ALE 0.43; PME 0.33; PLE 0.70; AME-AME 0.40; AME-ALE 0.20; PME-PME 0.97; PME-PLE 0.10; ALE-PLE 0.23. Ocular tubercle width 2.50, length 2.13; clypeus 0.17 (Fig 14D). Labium length 2.57, width 2.90; with 80 cuspules. Maxilla inner corner with approximately 179 (left) and 148 (right) cuspules. Cheliceral promargin with 10 (left) and 10 (right) teeth (proximal to distal: first-third large, fourth-medium, fifth large, sixth small, seventh-tenth large). Sternum length 9.60. Sigillae oval, second and third pairs hardly visible, posterior pair twice its length from the margin (Fig 14B). Leg formula: : IV, I, II, III. Length of legs and palpal segments

(femur, patella, tibia, metatarsus, tarsus, total): I: 19.29, 9.87, 14.70, 16.21, 9.97, 70.04. II: 17.52, 9.79, 12.35, 13.39, 9.91, 62.96. III: 16.15, 9.13, 11.83, 15.25, 9.01, 61.37. IV: 18.92, 9.99, 15.38, 19.99, 11.10, 75.38. Palp: 11.95, 7.32, 11.26, -, 5.20, 35.73. Spinnerets: PMS, 2.90 long, 1.45 apart; PLS, 4.10 basal, 2.95 middle, 4.15 distal. Tarsi I-IV entirely scopulated. Metatarsus I entirely scopulated, II scopulated 75%, III scopulated 60% distally, IV scopulated 30% distally. Tibia I with two tibial apophyses normally developed, which originate from a common base. Prolateral apophysis with inner spine half its length; retrolateral apophysis same width throughout its length, apex slightly curved to prolateral (Fig 14F–G). Metatarsus I curved (Fig 14E). Stridulatory setae: with plumose setae on palp coxa and trochanter retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; II 1p; palp 1p; patellae none; tibiae I 2p; II 3p, 4v; III 3p, 5v, 1r; IV 4v, 1r; palp 2p, 1v; metatarsi I 3v; II 4v; III 3p, 6v, 2r; IV 3p, 12v, 2r.
Palp. Embolus with narrow spoon-shape, slightly curved to dorsal. Embolus short and flat at base. Prolateral superior keel short, thin and directed retrolaterally. Apical keel short and thin, larger than prolateral superior. Both keels fusioned at apex and extended retrolatrally forming a well defined concave area on retrolateral. Opening of the embolus is on the prolateral side, just behind the opening is located a concavity which delimits the apical keel boundary from the remaining part of the embolus. Embolus apex slightly curved to retrolateral (Fig 15A–D). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas (Fig 14C).

Color pattern. In live specimens, adult males with carapace rust color (pantone 167c) with the border clearest. Chelicerae dorsally Del rio color (pantone 4735c); ventral coxae, labium, maxillae and sternum brownish black; abdomen dorsally black with sparse light terra di siena setae (pantone 472c), ventrally brownish black. Legs and palpi: femora black, patellae with a proximal dorsomedian signal tomato red (pantone 485c) flame shape area, distodorsal paramedian tierra di siena setae with some long setae laterally of the same color; tibiae raw sienna (pantone 722c) with some sparse setae from same color and

metatarsi corvette color (pantone 720c) with mandys pink (pantone 473c) longitudinal line just at metatarsi center; tarsi black (Fig 17A).

Female (CNAN-Ar007150) (Fig 16, 17B, 32A–D): body length 65.97 (not including chelicerae and spinnerets), carapace 25.67 length, 23.57 width. Caput not markedly elevated; fovea recurved, 5.10 wide (Fig 16A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.53; ALE 0.60; PME 0.50; PLE 0.53; AME-AME 0.50; AME-ALE 0.20; PME-PME 1.30; PME-PLE 0.13; ALE-PLE 0.27. Ocular tubercle width 2.53, length 2.47; clypeus 0.47 (Fig 16C). Labium length 2.85, width 3.85; with 128 cuspules. Maxilla inner corner with approximately 211 (left) – 225 (right) cuspules (Fig 16D). Cheliceral promargin with 8 (left) – 9 (right) teeth (all big). Sternum length 11.40. Sigillae oval, second and third pairs hardly visible; posterior pair once and half its length from the margin (Fig 16B). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 17.88, 11.25, 14.47, 13.15, 9.50, 66.25. II: 15.64, 10.49, 12.88, 12.58, 10.71, 62.30. III: 14.61, 10.10, 11.01, 15.58, 9.52, 60.82. IV: 18.60. 10.16, 14.66, 19.72, 10.71, 73.85. Palp: 13.08, 8.25, 10.37, -, 10.14, 41.84. Spinnerets: PMS, 2.50 long, 1.65 apart; PLS, 4.05 basal, 3.10 middle, 4.15 distal. Tarsus I-IV entirely scopulated. Metatarsi I and II entirely scopulated, III scopulated 75% distally, IV scopulated 50% distally. Stridulatory setae with plumose setae on palp trochanter and femur retrolateral face; leg I coxa, trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; II 1p; palp 1p; patellae none; tibiae palp 1p, 6v; I 3v; II 4v; III 1p, 4v, 1r; IV 1p, 3v, 1r; metatarsi I 2v; II 4v; III 2p, 5v, 1r; IV 13v, 2r.

Genitalia: fused trapezoidal spermatheca with a single receptacle strongly sclerotized, ventral face looks smooth. Three times wider than its height. Spermatheca baseplate divided, oblong, almost as high as one-third the width of its base. Baseplate division very narrow and poorly sclerotised in the basal half (Fig 16E). Variation: young or juveniles can present the superior edge slightly inward in middle. Baseplate division can vary in length, and some specimens can look more sclerotized (Fig 16F, G). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval

dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas.

Color pattern. In live specimens, adult females with two carapace patterns: (1) provincial pink (pantone 4685c) around the border and black (pantone 426c) in dorsomedian, juveniles or subadults same pattern (Fig 17B); (2) Tuscany (pantone 7522c) around the border and extended behind the fovea, with black pearl (pantone black 6c) from the fovea to caput (Fig 17C); chelicerae dorsally manatee (pantone 5285c) with two oriental pink (pantone 7521c) cheliceral bands; ventral coxae, labium, maxillae and sternum black pearl; abdomen dorsally black pearl (pantone black 6c) with rosy brown (pantone 5005c) setae, ventrally black pearl. Legs and palpi: femora black pearl, patellae with a proximal dorsomedian cinnabar (pantone 7597c) flame shape area, distodorsal paramedian spanish white (pantone 4685c) setae with some long setae laterally of the same color; tibiae eunry (pantone 4735c) with some sparse setae from same color and metatarsi hemp (pantone 8021c) with mandys pink (pantone 473c) longitudinal line just at metatarsi center; tarsi black (Fig 32A–D).

Distribution and habitat. Brachypelma baumgarteni is known only from Michoacán on the Sierra Madre del Sur Region (Fig 2, 33), where it occurs in deciduous forests (Fig 17D). It is a fossorial species whose modified or self-excavated burrows, can be found between large tree roots amongst large trees. Burrows do not have any silk around the entrance.

Brachypelma boehmei Schmidt & Klaas, 1993

(Figs 2, 18–22, 32, 33)

Brachypelma boehmei Schmidt & Klaas 1993: 7, fig. 1-2 (D male and female); **Schmidt, 1993**: 82, fig. 193a (male); Schmidt & Klaas 1994: 8, fig. 1-2 (male and female); **Smith, 1994**: 164, fig. 884-900 (male and female); **Tesmoingt, Cleton & Verdez, 1997a**: 9, plate 2, fig. 1 (female); **Schmidt, 1997b**: 19, fig. 190 (male); **Bertani, 2000: 30, fig. 41-42 (male); Peters, 2000**: 67, fig. 218 (female); **Peters, 2003**: 115-

117, fig. 461-469 (male and female); **Schmidt, 2003**: 137, fig. 201, 273 (male and female).

Material examined

Holotype of Brachypelma *boehmei*. MÉXICO: ♀, no more data, K. Böhme (SMF 40590). *Paratype*. MÉXICO: ♂, no more data, K. Böhme (SMF 38043).

Other material

MÉXICO: *Guerrero*: 1 ♂, 2 ♀, Mpio. La Unión Isidoro Montes de Oca, 3km NE of La Unión, 12/XII/2013, J. Mendoza (CNAN-Ar007185, CNAN-Ar007186, CNAN-Ar007833); 2 ♂, Mpio. La Unión Isidoro Montes de Oca, 1km SW of La Unión, 17/XII/2016, J. Mendoza (CNAN-Ar010589, CNAN-Ar010591); 2 ♀, Mpio. La Unión Isidoro Montes de Oca, Carretera Fed 37D, 16/IX/2015, J. Mendoza (CNAN-Ar007905, CNAN-Ar010285); *Oaxaca:* 1 ♂, Mpio. Salina Cruz, X/1963, E. Martín (CNAN-Ar003426) (Collection data of this specimen are considered wrong due to lack of evidence of the species in Oaxaca and considering the restricted distribution of the species to a single municipality in Guerrero).

Diagnosis

Brachypelma boehmei can be distinguished from all other known *Brachypelma* species by the coloration of the carapace and legs which are orange. Also differs in the shape of genitalia in both sexes with palpal bulb straight, embolus similar in length as tegulum and broader than embolus base at apex. The prolateral superior keel normally developed and slightly directed retrolaterally. Apical keel with a great backward development, extending more than the length of prolateral superior keel. The prolateral inferior keel is joined to prolateral superior keel at the apex and slightly extended to backwards. The prolateral apophysis is almost half the length of the retrolateral apophysis. Also differs by the spermatheca ventral face striated, with spermathecal baseplate lanceolate, notoriously separated and lower than the height of the spermatheca.

Brachypelma boehmei is identified by possesing the following character combination: male palpal bulb straight with a broad spoon shape at apex, prolateral superior keel normally developed and slightly directed retrolaterally; prolateral inferior keel weakly developed restricted to embolus apex; apical keel developed extending to backwards more than the length of the prolateral superior keel. Embolus tip slightly directed to retrolateral. Embolus large and broad, similar in length to tegulum (Fig 19A–D). Spermatheca fused trapezoidal, with a single receptacle strongly sclerotized. Spermathecal baseplate divided, noticeably separated; lanceolate and three times wider than its height (Fig 20B, 21E–G). Carapace and legs of both sexes are orange in general (Fig 22A–C).

Description

Male (CNAN-Ar010589) (Fig 18, 19, 22A): body length 41.39 (not including chelicerae and spinnerets), carapace 19.71 length, 18.82 width. Caput not markedly elevated; fovea recurved, 4.50 wide (Fig 18A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.46; ALE 0.53; PME 0.46; PLE 0.56; AME-AME 0.43; AME-ALE 0.20; PME-PME 1.20; PME-PLE 0.13; ALE-PLE 0.43. Ocular tubercle width 2.47, length 2.23; clypeus absent (Fig 18D). Labium length 2.40, width 3.25; with 85 cuspules. Maxilla inner corner with approximately 248 (left) and 187 (right) cuspules. Cheliceral promargin with 10 (left) and 10 (right) teeth (proximal to distal: first-second large, third medium, fourth large, fifth small, sixth-tenth large). Sternum length 8.70. Sigillae oval, first to third pairs hardly visible, posterior pair once its length from the margin (Fig 18B). Leg formula: : IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.94, 9.01, 13.20, 13.72, 8.47, 61.34. II: 15.56, 8.79, 11.94, 12.60, 8.48, 57.37. III: 14.40, 8.00, 11.29, 13.86, 8.43, 55.98. IV: 17.26, 8.51, 13.06, 18.04, 9.26, 66.13. Palp: 11.02, 6.54, 9.51, -, 4.51, 31.58. Spinnerets: PMS, 2.15 long, 0.95 apart; PLS, 3.80 basal, 2.55 middle, 3.25 distal. Tarsi I-IV entirely scopulated. Metatarsus I entirely scopulated, II scopulated 80%, III scopulated 60% distally, IV scopulated 40% distally. Tibia I with two tibial apophyses normally developed, which originate from a common base. Prolateral apophysis with inner spine half its length; retrolateral apophysis same width throughout its length, apex slightly curved to prolateral (Fig 18F–G). Metatarsus I curved (Fig. 18E). Stridulatory setae: with plumose setae on palp

trochanter and femur retrolateral face; leg I coxa, trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; II 1p; palp 1p; patellae none; tibiae II 3p, 3v, 1r; III 3p, 4v, 2r; IV 1p, 4v, 1r; palp 2p, 2v; metatarsi I 2v, II 1p, 4v; III 2p, 7v, 2r; IV 2p, 15v, 1r. Palp. Embolus straight with a broad spoon shape at apex and similar in length as tegulum. Prolateral superior keel normally developed and slightly directed retrolaterally; prolateral inferior keel weakly developed restricted to embolus apex, better seen dorsally, apical keel developed extending to backwards more than the length of the prolateral superior keel. Opening of the embolus is on the prolateral side, just behind the opening is located a concavity which delimits the apical keel boundary from the remaining part of the embolus. Embolus apex slightly curved to retrolateral (Fig 19A–D). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas (Fig 18C).

Color pattern. In live specimens, adult males with carapace color persimmon (pantone 166c) and tacao (pantone 721c) around the border (Fig 22A); chelicerae dorsally tussock (pantone 729c); ventral coxae, labium, maxillae and sternum seal brown (pantone 440c); abdomen dorsally coffee bean (pantone black 2c) with sparse light raw sienna (pantone 722c) setae, ventrally seal brown. Legs and palpi: femora coffee black, patellae with a proximal dorsomedian persimmon flame shape area, surrounding this and distally is raw sienna, with some setae laterally of same color; tibiae and metatarsi persimmon, with some lateral raw sienna setae; tarsi proximally backer’s chocolate (pantone 732c) and distally coffee bean (Fig 32E–H).

Female (CNAN-Ar007905) (Fig 21A–E, 22B): body length 61.36 (not including chelicerae and spinnerets), carapace 26.34 length, 24.61 width. Caput not markedly elevated; fovea straight, 5.70 wide (Fig 21A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.47; ALE 0.80; PME 0.37; PLE 0.70; AME-AME 0.67; AME-ALE 0.33; PME-PME 1.57; PME-PLE 0.13; ALE-PLE 0.43. Ocular tubercle width 2.97, length 2.93; clypeus lacking (Fig 21C). Labium length 3.05, width 3.55; with 86 cuspules. Maxilla inner corner with approximately 250 (left) – 276

(right) cuspules (Fig 21D). Cheliceral promargin with 10 (left) – 11 (right) teeth. Sternum length 11.39. Sigillae oval, first to third pairs hardly visible; posterior pair once its length from the margin (Fig 21B). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.56, 10.85, 13.73, 13.01, 8.64, 62.79. II: 15.65, 10.60, 11.60, 12.49, 7.59, 57.93. III: 14.59, 9.57, 10.83, 13.77, 8.52, 57.28. IV: 17.94, 10.05, 14.04, 18.78, 9.75, 70.56. Palp: 12.48, 7.51, 9.34, -, 9.32. Spinnerets: PMS, 2.50 long, 2.05 apart; PLS, 4.15 basal, 4.15 middle, 2.55 distal. Tarsus I-IV entirely scopulated. Metatarsi I entirely scopulated, II scopulated 90%, III scopulated 70% distally, IV scopulated 40% distally. Stridulatory setae: with plumose setae on palp trochanter retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p, II 1p; patellae none; tibiae I 1p, 3v; II 3p, 4v; III 3p, 6v, 2r; IV 1p, 4v, 1r; palp 2p, 6v; metatarsi I 3v; II 1p, 3v; III 2p, 8v, 1r; IV 2p, 15v, 1r.

Genitalia: fused trapezoidal spermatheca, with a single receptacle strongly sclerotized, three times wider than its height. Spermatheca baseplate divided, widely separated in middle, each baseplate lanceolate three times wider than high, outer side slightly smaller than the inner (Fig 21E). Variation: some specimens have a wider base up to four times wider than height, young or juveniles can present the superior edge slightly inward in middle. Ventral with variation on striation. Baseplate division can vary in length (Fig 20B, 21F–G). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I.in transition areas

Color pattern. In live specimens, adult females with two carapace patterns: (1) tango (pantone 7412c) in all the carapace with pink flare (pantone 5025c) around the border, juveniles or subadults same pattern (Fig 22B); (2) pink flare around the border and sandy brown (pantone 7411c) in dorsomedian, with two seal brown (pantone 440c) longitudinal patches from the fovea to caput (Fig 22C); chelicerae dorsally beaver color (pantone 4715c); ventral coxae, labium, maxillae and sternum seal brown; abdomen dorsally black (pantone 426c) with putty color (pantone 721c) setae, ventrally black. Legs and palpi: femora coffee black, patellae with a proximal dorsomedian cinnabar (pantone 173c) flame shape area, surrounding this and distally is dark salmon (pantone 472c), with some setae

laterally of wafer color (pantone 4745c); tibiae and metatarsi chardonnay (pantone 1355c), with some lateral wafer setae; tarsi proximally backer's chocolate (pantone 732c) and distally coffee bean (Fig 32E–H).

Distribution and habitat. Brachypelma boehmei is the species with the most restricted distribution being only known from Sierra de Cumbres region, that is characterized by low complex and rolling hills with plains, belonging to the subprovince Costas del Sur in the southwest of Guerrero (Fig 2, 33). It occurs in thorn and deciduous forests (Fig 22D). It is a fossorial species whose burrows can be found under large angular rocks and large tree roots amongst thorny brush. Burrows do not have any silk around the entrance.

Brachypelma emilia (White 1856)

(Figs 2, 26–29, 31E–H, 33)

Mygale emilia White 1856: 185, pl. 43 (D male).

Brachypelma emilia **Simon 1891**: 338, (D male and female); **Smith, 1986**: 49, fig. 27h (Tmf from *Eurypelma=Avicularia*); **Smith, 1987**: 49, plate 2, fig. 27h (male); **Hancock & Hancock, 1989**: 46, fig. 41 (female); **Schmidt, 1992**: 10, (Tmf from *Euathlus* per Raven); **Schmidt, 1993**: 82, fig. 188 (female); **Smith, 1995**: 166, fig. 901-915 (male and female); **Pérez-Miles *et al.*, 1996**: 46, fig. 9-10 (male and female); Tesmoingt, Cleton & Verdez, 1997a: 9, plate 2, fig. 6 (female); Schmidt, 1997: 19, fig. 191, 193 (male and female); Locht, Yáñez & Vazquez, 1999: 196, fig. 7 (female); Peters, 2000: 68, fig. 222 (female); Bertani, 2001: 338, fig. 153-156 (male and female); Peters, 2003: 117, fig. 473-474, 477, 480, 483 (male and female); Schmidt, 2003: 152, fig. 274-277 (male and female); Gabriel & Longhorn, 2015: 100, fig. 13 (female).

Material examined

Neotype of Brachypelma emilia. MÉXICO: *Durango*: ♂, Ciudad, leg Mr. Forrer (BMNH-1898-12-24-32). Paraneotype (labeled as paratype). *Durango*: ♂, Ciudad, leg. Forrer (OUNMH Jar 106).

Other material

MÉXICO: *Nayarit*: 1 ♂ 4 ♀, Mpio. Compostela, 9.xii.2012, E. Goyer, E. Hijmensen, D. Ortiz (CNAN-Ar003599, CNAN-Ar007153, CNAN-Ar007173, CNAN-Ar007178, CNAN-Ar007875); 1 ♂, Mpio. Estación Ruíz, 1.xii.1989, A. Cadena (CNAN-Ar003436); 4 ♂, Mpio. Estación Ruíz, 5.xii.2014, J. Mendoza, G. Contreras (CNAN-Ar007146, CNAN-Ar007894, CNAN-Ar007895, CNAN-Ar007899); *Sinaloa*: 1 ♂, Mpio. Mazatlan, vii.1959, without more data (CNAN-Ar003427); 1 ♂ 1 ♀, Mpio. Mazatlan, 3.xii.2014, J. Mendoza, G. Contreras (CNAN-Ar007898, CNAN-Ar010602); 1 ♂, Mpio. Mazatlan, without more data, Collection E. Simon (MP Ar4871A); 1 ♂, Mpio. Sinaloa de Leyva, 30.i.1965, without more data (CNAN-Ar003590); *Sonora*: 1 ♂, Mpio. Altar, 9.i.1970, W. Lopez Forment (CNAN-Ar003578); *Jalisco*: 1 ♀, Norte del Río Santiago, Godman, Salvin, without more data (BMNH-1962-2-28-1); 1 ♂, donation received from private collection of J. Mendoza (CNAN-Ar003631).

Diagnosis

Brachypelma emilia can be distinguished from all other known *Brachypelma* species by the coloration of the carapace and legs, with carapace orange except in the caput which is black in color; the legs have femora and patellae black, tibiae orange and metatarsi I-III black, IV orange. Also differs in the shape of genitalia in both sexes with palpal bulb slightly curved to dorsal having a small and narrow spoon shape. The prolateral superior keel slightly developed, thin and directed retrolaterally; prolateral inferior keel absent; apical keel normally developed, wide but not shorter than prolateral superior keel. Also differs by the spermatheca ventral face smooth, with a single receptacle strongly sclerotized slightly notched in the middle, spermathecal baseplate oblanceolate.

Brachypelma emilia is identified by possesing the following character combination: male palpal bulb with narrow spoon shape embolus curving slightly to dorsal through its length,

prolateral superior keel slightly developed; apical keel normally developed, wide but not shorter than prolateral superior keel tip. Embolus tip directed to retrolateral. Embolus similar in length than tegulum (Fig 24A–D). Spermatheca fused with single semitrapezoidal receptacle. Spermathecal baseplate divided, oblanceolate; one and half wider than its height (Fig 25E–F). Carapace of both sexes carapace orange with black triangle in the ocular area (Fig 26A–C).

Description

Male (CNAN-Ar003599) (Fig 23, 24, 26A): body length 40.16 (not including chelicerae and spinnerets), carapace 20.23 length, 18.85 width. Caput not markedly elevated; fovea procurved, 3.80 wide (Fig 23A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.43; ALE 0.60; PME 0.33; PLE 0.37; AME-AME 0.70; AME-ALE 0.20; PME-PME 1.23; PME-PLE 0.13; ALE-PLE 0.37. Ocular tubercle width 2.47, length 2.27; clypeus 0.23 (Fig 23D). Labium length 2.60, width 3.40; with 109 cuspules. Maxilla inner corner with approximately 205 (left) and 179 (right) cuspules. Cheliceral promargin with 10 (left) and 9 (right) teeth (proximal to distal: first-third large, fourth small, fifth-seventh medium, eighth-tenth large; first-third large, fourth small, fifth-seventh medium, eighth-ninth large). Sternum length 10.10. Sigillae oval, fourth pair hardly visible, posterior pair one and half its length from the margin (Fig.). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 17.25, 9.39, 11.67, 12.22, 9.28, 59.81. II: 15.93, 9.20, 10.96, 11.89, 8.21, 56.19. III: 14.35, 8.11, 10.00, 12.08, 8.15, 52.69. IV: 16.86, 8.63, 12.81, 16.13, 9.92, 64.35. Palp: 11.83, 7.23, 9.61, -, 4.14, 32.81. Spinnerets: PMS, 2.20 long, 1.00 apart; PLS, 3.10 basal, 2.20 middle, 3.20 distal. Tarsi I-IV entirely scopulated. Metatarsus I entirely scopulated, II scopulated 75%, III scopulated 50% distally, IV scopulated 30% distally. Tibia I with two tibial apophyses normally developed, which originate from a common base. Prolateral apophysis with inner spine third its length; retrolateral apophysis wider in basal half, apex almost straight (Fig 23F, G). Metatarsus I curved (Fig 23E). Stridulatory setae: with plumose setae on palp trochanter retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; II 1p; palp 1p; patellae none; tibiae I 1p,

2v; II 2p, 3v; III 2p, 5v, 1r; IV 1p, 4v, 1p; palp 2p, 1v; metatarsi I 1v; II 2v; III 3p, 7v, 1r; IV 1p, 18v, 1r.

Palp. Embolus slightly curved to dorsal having a small and narrow spoon shape, prolateral superior keel slightly developed, thin and directed retrolaterally; prolateral inferior keel absent; apical keel normally developed, wide but not shorter than prolateral superior keel. Opening of the embolus is on the prolateral side, just behind the opening is located a concavity which delimits the apical keel boundary from the remaining part of the embolus. Embolus apex slightly curved to retrolateral (Fig 24A–D). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas (Fig 23C).

Female (CNAN-Ar010602) (Fig 25A–E): body length 61.31 (not including chelicerae and spinnerets), carapace 26.34 length, 24.33 width. Caput not markedly elevated; fovea straight, 6.40 wide (Fig 25A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.65; ALE 0.70; PME 0.50; PLE 0.70; AME-AME 0.63; AME-ALE 0.33; PME-PME 1.57; PME-PLE 0.07; ALE-PLE 0.40. Ocular tubercle width 3.25, length 2.83; clypeus 0.27 (Fig 25D). Labium length 3.05, width 3.85; with 94 cuspules. Maxilla inner corner with approximately 187 (left) – 208 (right) cuspules. Cheliceral promargin with 9 (left) – 9 (right) teeth. Sternum length 11.60. Sigillae oval, fourth pair hardly visible; posterior pair twice its length from the margin (Fig 25B). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 17.87, 10.79, 13.69, 13.40, 8.75, 64.50. II: 16.35, 10.18, 12.05, 11.77, 8.94, 59.29. III: 15.69, 9.40, 10.97, 12.79, 8.50, 57.35. IV: 18.34, 10.20, 14.20, 17.91, 9.98, 70.63. Palp: 13.20, 8.35, 10.12, -, 9.92, 41.59. Spinnerets: PMS, 2.90 long, 3.00 apart; PLS, 4.30 basal, 2.90 middle, 4.35 distal. Tarsus I-IV entirely scopulated. Metatarsi I entirely scopulated, II scopulated 90%, III scopulated 50% distally, IV scopulated 40% distally. Stridulatory setae: with plumose setae on palp trochanter and femur retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora I 1p; palp 1p; patellae none; tibiae I 1p, 3v; II 1p, 4v; III 1p, 5v, 1r; IV 1p, 6v, 1r; palp 2p, 5v; metatarsi I 4v; II 4v; III 1p, 8v, 2r; IV 2p, 14v, 1r. Genitalia: fused semicircular

spermatheca, with a single receptacle strongly sclerotized slightly notched in the middle, four times wider than its height. Spermatheca baseplate divided, widely separated above, each baseplate oblanceolate 3.5 wider than high, inner side smaller than the outer (Fig 25E). Variation: some specimens have a wider base up to five times greater than height, young or juveniles can present the superior edge slightly inward in middle. Ventral face smooth. Baseplate division can vary in length (Fig 25F). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas (Fig 25C).

Color pattern. In live specimens, adults of both sexes have the carapace orange (pantone 7412c) on almost all the carapace except in the caput, which is Pantone Process Black in color, also has a longitudinal line of beaver color (pantone 4715c) that goes from back of the eyes to the fovea (Figs 26A–C); chelicerae dorsally blue whale color (pantone 533c); ventral coxae, labium, maxillae and sternum black pearl color (pantone black 6c); abdomen dorsally black with Christine color setae (pantone 7583c), ventrally black pearl color. Legs and palpi: femora and patellae black pearl; tibiae orange (pantone 157c); metatarsi I-III black (pantone black 7c) with proximal third rope color (pantone 876c), IV black (pantone black 7c) with large brandy punch color (pantone 722c) covering it almost completely; tarsi black (pantone black c) (Fig 31E–H). With juveniles of same pattern but paler in color.

Distribution and habitat. Brachypelma emilia is known from the north of the Pacific Coast region on the western side of Sierra Madre Occidental, being found in southern of Sonora, Sinaloa, Nayarit and a small area in northwestern of Jalisco, just in the border of Jalisco with Nayarit; could also be possibly found in Durango, but there is no accurate evidence of that (Fig 2, 33). It occurs in in drier coastal thorn, grasslands, palm transition to deciduous forest, and into higher elevations of oak forest (Fig 26D). It is a fossorial species whose modified or self-excavated burrows can be found under large rocks, under dense thorny thickets, large tree roots or burrows on the leafy ground cover, in both forested and moderately disturbed areas. Some can be also found close to some houses or human structures, but this is most likely because the spiders lived there before the constructions.

Burrows do not have any silk around the entrance to indicate there is a spider inside. This species is sympatric (overlapping distributions) with a small population of *Brachypelma klaasi* in the southwest area of Nayarit.

Brachypelma klaasi (Schmidt & Krausse 1994)

(Figs 2, 27–30, 31I–L, 33)

Brachypelmides klaasi Schmidt & Krause, 1994: 7, fig. 1-2 (D male and female); **Schmidt, 1997b**: 19, fig. 198, 202 (male and female); **Locht, Yáñez & Vázquez, 1999**: 196, fig. 4, 9 (male and female); Vol, 1999: 11, fig. A (female); **Peters, 2000**: 75, fig. 244-247 (male and female); **Peters, 2003**: 131, fig. 533-535, 537 (male and female); **Schmidt, 2003**: 137, fig. 204, 208 (male and female).

Brachypelma klaasi **Smith, 1994**: 169, fig. 926-939 (male and female).

Material examined

Holotype of Brachypelmides klaasi (by original designation). MÉXICO: *Nayarit*: ♀, close to Tepic, K. Böhme (SMF 40599) (only microscope slide with spermatheca).

Other material

MÉXICO: 1 ♀, without more data (SMF 58101-84) (only microscope slide with spermatheca); 1 ♂, without more data, Böhme (SMF 38044); *Jalisco*: 2 ♂, 1 ♀, Mpio. Tomatlán, 6/XII/2012, D. Ortiz , E. Goyer, E. Hijmensen (CNAN-Ar003333, CNAN-Ar003341, CNAN-Ar007831); 1 ♂, Mpio. Cihuatlán, 4/XII/2013, D. Ortíz, D. Barrales, G. Contreras (CNAN-Ar007160); 1 ♀, Mpio. La Huerta, Reserva Chamela, 6/XII/2013, D. Ortíz, E. Goyer, E. Hijmensen (CNAN-Ar007879); 1 ♂, Mpio. La Huerta, Reserva Chamela, 8-20/XI/2014, W. Maddison (CNAN-Ar007857); 1 ♂, Mpio. La Huerta, Reserva Chamela, 18/V/1981, A. Pescador (CNAN-Ar003432); 1 ♂, donation received from private collection of J. Mendoza (CNAN-Ar003344); *Colima:* 1 ♂, Mpio. Manzanillo, 4/XII/2013,

D. Ortiz, D. Barrales, G. Contreras (CNAN-Ar007162); 1 ♂, Mpio. Colima, 30/XI/2012, D. Ortiz, E. Goyer, E. Hijmensen (CNAN-Ar007845).

Diagnosis

Brachypelma klaasi can be distinguished from all other known *Brachypelma* species by the coloration of the legs with pinkish color on tibiae and metatarsi. Also differs in the shape of genitalia in both sexes with palpal bulb almost straight, tapering and lacking the typical spoon shape. The prolateral superior keel reduced, directed retrolaterally and slightly extended to backward. Apical keel thin and reduced, shorter than prolateral superior keel. Lacking prolateral inferior keel. Also differs by the divided spermatheca widely separated, with spermathecal baseplate oblong. Ventral face smooth.

Brachypelma klaasi is identified by possesing the following character combination: male palpal bulb with embolus almost straight, tapering and without typycal spoon shape due to the reduced apical keel. Prolateral superior keel reduced, apical keel thin and reduced, shorter than prolateral superior keel. Embolus tip directed to retrolateral. Embolus similar in length to tegulum (Fig 28A–D). Spermatheca separated with semitriangular receptacles. Spermathecal baseplate divided, oblong; twice wider than its height (Fig 29D–F). Carapace of both sexes black (Fig 30A–C).

Description

Male (CNAN-Ar003333) (Figs 27, 28. 30A): body length 48.60 (not including chelicerae and spinnerets), carapace 22.82 length, 22.17 width. Caput not markedly elevated; fovea straight, 4.15 wide (Fig 27A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.55; ALE 0.80; PME 0.40; PLE 0.70; AME-AME 0.55; AME-ALE 0.20; PME-PME 1.20; PME-PLE 0.05; ALE-PLE 0.23. Ocular tubercle width 2.80, length 2.55; clypeus 0.25 (Fig 27D). Labium length 3.05, width 3.55; with 98 cuspules. Maxilla inner corner with approximately 175 (left) and 171 (right) cuspules. Cheliceral promargin with 11 (left) and 12 (right) teeth (proximal to distal: first-second large, third medium, fourth large, fifth small, sixth-eleventh large; first large, second small, third large, fourth small, fifth large, sixth small, seventh-twelve large).

Sternum length 10.8. Sigillae oval, second to third pairs hardly visible, posterior pair once its length from the margin (Fig 27B). Leg formula: : IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 18.95, 10.29, 14.84, 15.15, 10.72, 69.95. II: 17.25, 9.79, 13.15, 14.09, 10.07, 64.35. III: 16.11, 8.27, 11.83, 15.10, 10.28, 61.59. IV: 18.89, 9.38, 15.53, 18.32, 10.63, 72.75. Palp: 12.28, 7.47, 11.92, -, 5.71, 37.38. Spinnerets: PMS, 2.40 long, 1.15 apart; PLS, 4.05 basal, 2.35 middle, 3.60 distal. Tarsi I-IV entirely scopulated. Metatarsus I entirely scopulated, II scopulated 90%, III scopulated 60% distally, IV scopulated 40% distally. Tibia I with two tibial apophyses normally developed, which originate from a common base. Prolateral apophysis with inner spine half its length; retrolateral apophysis tapering throughout its length, apex slightly curved to prolateral (Fig 27F, G). Metatarsus I curved (Fig 27E). Stridulatory setae: with plumose setae on palp trochanter and femur retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora none; patellae none; tibiae II 1v; III 3v; IV 2v; palp 1v; metatarsi I 1v; II 3v; III 1p, 5v; IV 9v.

Palp. Embolus almost straight, tapering and lacking the typical spoon shape due to the reduced apical keel. Prolateral superior keel reduced, directed retrolaterally and slightly extended to backward; prolateral inferior keel absent. Apical keel thin and reduce, shorter than prolateral superior keel. Opening of the embolus is on the prolateral side, just behind the opening is located a concavity which delimits the apical keel boundary from the remaining part of the embolus. Embolus apex slightly curved to retrolateral. Embolus similar in length as tegulum (Fig 28A–D). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas (Fig 27C).

Female (CNAN-Ar007831) (Fig 29, 30B): body length 63.26 (not including chelicerae and spinnerets), carapace 27.65 length, 24.27 width. Caput not markedly elevated; fovea straight, 6.40 wide (Fig 29A). Eyes: anterior eye row procurved, posterior eye row recurved. Eye sizes and interocular distances: AME 0.57; ALE 0.63; PME 0.43; PLE 0.67; AME-AME 0.50; AME-ALE 0.33; PME-PME 1.33; PME-PLE 0.07; ALE-PLE 0.07. Ocular tubercle width 2.80, length 2.33; clypeus 0.47. Labium length 3.65, width 5.25; with

94 cuspules. Maxilla inner corner with approximately 68 (left) – 58 (right) cuspules. Cheliceral promargin with 10 (left) – 11 (right) teeth. Sternum length 11.60. Sigillae oval, first to third pairs hardly visible; posterior pair once its length from the margin (Fig 29B). Leg formula: IV, I, II, III. Length of legs and palpal segments (femur, patella, tibia, metatarsus, tarsus, total): I: 16.86, 10.39, 12.94, 12.74, 8.92, 61.85. II: 15.61, 10.06, 11.28, 12.49, 9.04, 58.48. III: 14.82, 9.59, 10.07, 13.65, 9.09, 57.22. IV: 17.26, 9.68, 12.32, 18.29, 9.53, 67.08. Palp: 12.55, 8.02, 9.09, -, 9.29, 38.95. Spinnerets: PMS, 2.80 long, 3.65 apart; PLS, 5.50 basal, 4.15 middle, 4.75 distal. Tarsus I-IV entirely scopulated. Metatarsi I entirely scopulated, II scopulated 80%, III scopulated 70% distally, IV scopulated 50% distally. Stridulatory setae: with plumose setae on palp trochanter and femur retrolateral face; leg I trochanter and femur prolateral face. Chaetotaxy (left side): femora none; patellae none; tibiae I 2v; II 2v; III 3v; IV 2v; palp 1p, 5v; metatarsi I 3v; II 4v; III 5v; IV 1p, 14v, 1r. Genitalia: Spermatheca separated with subtriangular receptacles strongly sclerotized, each receptacle almost the same height as width. Spermatheca baseplate divided and widely separated, each baseplate oblong twice wider than high (Fig 29D). Baseplate division can vary in length (Fig 29E, F). Urticating setae: types I and III arranged in one dorso-posterior patch, black in color. Type III located in an oval dorsomedian area extended to posterior. Type I are surrounding the type III area, with intermediates between type III and I in transition areas (Fig 29C).

Color pattern. In live specimens, adults of both sexes have the carapace black (pantone process black c) with zinnwaldite color (pantone 489c) around the border, despite the black carapace the radiating thoracic sulci are clearly seen (Fig 30A–C); chelicerae dorsally brown color (pantone 7533c) with some large setae French beige color (pantone 4715c); ventral coxae, labium, maxillae and sternum black pearl color (pantone black 6c); abdomen dorsally black with corvette color setae (pantone 720c), ventrally black pearl color. Legs: femora black pearl; patellae black pearl with scattered setae shilo color (pantone 488c); tibiae and metatarsi mandys pink color (pantone 473c); tarsi black (pantone black c); palpi: femora black pearl; patellae, tibia and tarsi black pearl with scattered setae shilo color (Fig 31I–L). With juveniles of same pattern but paler in color.

Distribution and habitat. Brachypelma klaasi is known from the Pacific Coast region of Jalisco on the western side of Sierra Madre Occidental, with small populations in Colima and southern Nayarit (Fig 2, 33). It occurs in in drier coastal thorn, deciduous forest and higher elevations of oak forest (Fig 30D). It is a fossorial species whose modified or self-excavated burrow**s,** can be found under large rocks, under thorny thickets, tree roots or burrows on hillsides, in both forested and moderately disturbed areas. Burrows do not have any silk around the entrance to indicate there is a spider inside. This species is sympatric (overlapping in range) with *Brachypelma emilia* in the southwestern area of Nayarit, and with *Brachypelma hamorii* in Colima.

Identification key for Species of *Brachypelma* Simon 1891.

Adult males

1. With any other color besides black in any segment(s) of the legs (generally orange or reddish) (Fig 31, 32)……2
   With all segments of the legs black in color, carapace golden yellowish (Fig 8A)……B. albiceps
2. All patellae with any other color besides black (generally pink or reddish) (Fig 31A-D, 31I-L, 32)……..3
   All patellae totally black in color, with orange coloration in all tibiae and metatarsus IV, male palpal bulb with narrow spoon shape, prolateral superior keel slightly developed (Fig 24, 26A, 31E-H)……..B. emilia
3. All patellae with a central flame pattern orange or reddish, male palpal bulb with a distinct spoon shape, apical keel curved outward (Fig 10, 15, 32A-H, 32)……4
   All patellae without central flame pattern, with pinkish color on patellae, tibiae and metatarsi, male palpal bulb without distinct spoon shape, apical keel curved inward (Fig 28, 30A)……B. klaasi
4. Embolus equal or slightly larger than tegulum and broad at apex with large spoon shape, prolateral superior keel narrow and widely extended backwards (Fig 10)……..5

Embolus shorter than tegulum and broad at apex with short spoon shape, prolateral superior keel wide and short, not extended widely to backwards (Fig 15)…….7

5. All patellae orange or yellowish with central flame shape area reddish in color, without distal white rings on patellae or tibiae; large embolus base and slightly narrower than the apical spoon shape (Fig 19, 22A)……6
   All patellae black with a central flame shape area reddish or orange, with a distal white ring on patellae, tibiae and metatarsi; short embolus base and similar in width to the apical spoon shape (Fig 10, 13A)……B. auratum
6. Carapace orange or black, patellae orange with central flame shape reddish area, tibiae with orange in distal half, metatarsi with yellowish ring distally; ventral apophysis wide through its length, apex slightly curved to dorsal; apical keel very wide and larger than prolateral superior keel (see Mendoza & Francke 2017 figs 3, 7, 8, 19, 42-45)……B. smithi
   Carapace always orange, patellae, tibiae and metatarsi completely orange in color; ventral apophysis wide in base and tapering, apex straight; apical keel wide and similar in length or slightly larger than prolateral superior keel (Fig 18F, 19, 22A)……..B. boehmei
7. Carapace always orange, patellae yellowish with central flame shape reddish area, tibiae and metatarsi yellowish, with a distinctive diagonal yellow line on metatarsi; tegulum swollen, embolus base very short, apical keel wide and longer than prolateral superior keel (Fig 15, 17A)……B. baumgarteni
   Carapace black or yellowish with only ocular area with some black, yellowish color around the border, patellae dorsally orange slightly expanded laterally, with central flame shape reddish area, tibiae distal half yellowish, metatarsi with distal white ring; tegulum globose but not swollen, embolus base short and thin, apical keel slightly wide and similar in legth to prolateral superior, apex very curved to retrolateral (see Mendoza & Francke 2017 figs 24, 28,29, 46-49, 37)……B. hamorii

Adult females

1. With any other color besides black in any segment(s) of the legs (generally orange or reddish) (Fig 31, 32)……2
   With all segments of the legs black in color, carapace golden yellowish; spermatheca separated and with rounded receptacles (Fig 8B, 7E-G).B. albiceps
2. All patellae with any other color besides black (generally rose or reddish) (Fig 31A-D, 31I-L, 32)…….3
   All patellae totally black in color, with orange coloration in all tibiae and metatarsus IV, spermatheca with a single receptacle strongly sclerotized slightly notched in the middle, spermathecal baseplate oblanceolate (Fig 25E, F, 26B, C, 31E-H)……B. emilia
3. All patellae with a central flame pattern orange or reddish, spermatheca fused with single receptacle (Fig 12E, F, 21E-G, 32A-H)…….4
   All patellae without central flame pattern, with pinkish color on patellae, tibiae and metatarsi, spermatheca separated with semitriangular receptacles, spermathecal baseplate oblong (Fig 29D-F, 30B, C, 31-I-L)……B. klaasi
4. Spermathecal baseplate lower than the seminal receptacle, spermatheca ventral face striated (Fig 12E-F, 21E-G)……...5
   Spermathecal baseplate higher than the seminal receptacle, spermatheca ventral face smooth (Fig 16E-G)……...7
5. All patellae orange or yellowish with central flame shape area reddish in color, without distal white rings on patellae or tibiae; spermatheca ventral face with clearly defined striatation (Fig 21E-G, 22B, C, 32E-H)……6
   All patellae black with a central flame shape area reddish or orange, with a distal white ring in patella, tibiae and metatarsi; spermatheca ventral face with slight striatation, spermatheca baseplate more separated above, each baseplate ovate (Fig 12E, F, 13B, C, 31A-D)……B. auratum
6. Carapace could be yellowish around the border and behind the fovea with starburst black pattern from the fovea to caput; or yellowish around the border and black in dorsomedian; or yellowish pink in almost all carapace except by two longitudinal black areas in the caput; all patellae orange with central flame shape reddish area, tibiae with orange in distal half, metatarsi with yellowish ring distally; spermatheca

baseplate subtriangular, decreasing the upper side toward the outer side (see Mendoza & Francke 2017 figs 14-18, 20-22, 50, 51, 53-56)……..B. smithi

Carapace could be completely orange or orange in almost all carapace except by two longitudinal black areas in the caput; patellae, tibiae and metatarsi completely orange in color; spermatheca baseplate widely separated in middle, each baseplate lanceolate, outer side slightly smaller than the inner (Fig 20B, 21E-G, 22B, C)…….B. boehmei

7. Carapace could be light orange around the border and black in dorsomedian or light orange around the border with orange extended behind the fovea and black from the fovea to caput; patellae yellowish with central flame shape reddish area, tibiae and metatarsi yellowish, with a distinctive diagonal yellow line on metatarsi; spermatheca baseplate oblong, baseplate division narrow, baseplate poorly sclerotized in the basal inner corner (Fig 16E-G, 17B, C)..B. baumgarteni

   Carapace could be yellowish around the border and black in dorsomedian or pale orange around the border and behind the fovea with starburst black pattern from the fovea to caput; patellae dorsally orange slightly expanded laterally, with central flame shape reddish area, tibiae distal half yellowish, metatarsi with distal white ring; spermatheca baseplate elliptic, outer side slightly smaller than the inner (See Mendoza & Francke 2017 figs 34-36, 38-40, 52, 57-60)……..B. hamorii

Tliltocatl gen nov

(Fig 34)

Type species ***Eurypelma vagans*** (Ausserer 1875), by original designation.

Species included: Tliltocatl albopilosum (Valerio 1980) **comb. nov.**, Tliltocatl epicureanum (Chamberlin 1925) **comb. nov.**, Tliltocatl kahlenbergi (Rudloff 2008) **comb. nov.**, Tliltocatl sabulosum (F. O. Pickard-Cambridge 1897) **comb. nov.**, Tliltocatl schroederi

(Rudloff 2003) **comb. nov.**, Tliltocatl vagans (Ausserer 1875) **comb. nov.**, Tliltocatl verdezi (Schmidt 2003) **comb. nov.**

Diagnosis

Tliltocatl gen nov. can be distinguished from all other known theraphosinae genera (except Brachypelma) by having plumose stridulatory setae on the prolateral face of leg I trochanter/femur and retrolateral face trochanter of the palp. Both sexes lack a plumose pad of setae on leg IV femur. The metatarsus IV is 20-40% distally scopulated. All tarsi scopulae are undivided. The femur of leg III is slightly enlarged but not swollen as in other genera. The male palpal bulb distally wide and flattened (spoon-shaped) and has prolateral superior and apical keels united at the apex. Prolateral superior and prolateral inferior keels are at the same height, joined at their distal end and widely separating towards the embolus base (better seen in dorsal position). The prolateral inferior keel is longer than the prolateral superior. The apical keel can extend widely to backwards just as the prolateral inferior keel, and usually is broader on its distal half. Females have a single fused spermatheca**,** apically narrowed. Both sexes possess urticating setae type I and type III; type III located in the dorsoposterior area and type I surrounding these. Most of the species are black with long red setae on the opisthosoma (except T. albopilosum, T. schroederi and T. verdezi). It differs from Brachypelma by the coloration of legs which are black or have long whitish setae (as T. albopilosum) in combination with a dark carapace and long red setae on abdomen. The shape of genitalia also differs in both sexes with the male palpal bulb apex larger than in Brachypelma and by the presence of prolateral inferior keel well developed and posteriorly extended. The apical keel is also larger than in Brachypelma and wider on distal half. Embolus is regularly similar in length or longer than the tegulum wheres in Brachypelma it is shorter. It also differs, by having spination on the patellae of palps and legs. Females differs by the spermatheca apex inwardly curved and by lacking spermathecal baseplate or, poorly sclerotized and widely separated when present (such as T. albopilosum).

Remarks.

Tliltocatl gen. nov. can be confused by unexperienced people with *Sericopelma* spp. due to the similar coloration. But, they can be easily differentiated one from the other because adult males of *Sericopelma* lack tibial apophysis, the female spermatheca has not notched apical edge, also the spermatheca is distinctly swollen on the apex showing a P-shape. In addition, *Sericopelma* has a distinct radiating sulcus on the carapace.

Etymology

The genus gender is masculine. The name is a noun in apposition comprising the Nahuatl words *Tlil*, which means "black", and tocatl, which means "spider". Generic name refers to the black coloration of the species in the genus.

Distribution

Tliltocatl is located in Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua and Costa Rica. The genus lives in deciduous forest, rainforest and grasslands along the Pacific coast, Mexican Gulf and Atlantic Coast. Specimens live inside burrows under flat rocks, fallen logs, sidehills, tree roots and even some species build burrows in farmlands, houses' backyards or close to flood lands (Fig 34).

Discussion

Non-monophyly of Brachypelma and the use of Barcodes.

Simon (1891) described several diagnostic characters for *Brachypelma* such as: femur IV lacking inner scopula (no dense pad of plumoise setae), presence of a distinct scopula on the metatarsus IV, palpal bulb narrowly piriform, but with the apex wide and attenuated, very much compressed and obtuse. Pocock (1903) also distinguished the plumose setae on the prolateral face of leg I trochanter/femur and retrolateral face of the palp. These key features have been supported by subsequent authors as diagnostic for *Brachypelma*, as also are, not tarsal división by strong setae, the male palpal bulb distally wide and flat (commonly mentioned as spoon-shaped), two unequal apophyses on male tibia I, and the spermatheca regularly fused, semifused or with two separated wide lobes (Schmidt 1992a,1992b, Smith 1993, Smith 1994, Pérez Miles *et al.* 1996, Locht *et al.* 1999, Gabriel & Longhorn 2015, Mendoza & Francke 2017). Smith (1994) adequately explains the

taxonomic history of *Brachypelma* which has been complicated and which multiples taxonomic changes (Simon 1891, F. Pickard-Cambridge 1897, Simon 1903, Roewer 1942, Valerio 1980, Raven 1985, Schmidt 1992). Then in earlier 90's new *Brachypelma* species were described based mainly in material collected for hobby (*B. auratum*, *B. baumgarteni*, *B. boehmei*, *B. klaasi*, *B. hamorii*, *B. annitha*). Although there was evident the color differences between the species from the Mexican Pacific Coast and Mexico Southwest-Central America, no study had been done in depth to prove the monophyly of the genus. Rudloff (2003) was the first in proposed a designation of complex of species for *Brachypelma* based on color characteristics and with no intention of systematic claim. He postulated a called "vagans" group formed by the species with dark to black base color on them (*B. albopilosum*, *B. angustum*, *B. aureoceps*, *B. embrithes*, *B. epicureanum*, *B. fossorium*, *B. sabulosum*, *B. schroederi* and *B. vagans*); and the named "emilia" group formed by the species with red/orange banded legs (*B. annitha*, *B. auratum*, *B. baumgarteni*, *B. boehmei*, *B. emilia*, *B. klaasi*, *B. smithi*). He also said that the species *B. ruhnaui* (currently *B. albiceps*) cannot be classified in this scheme and its possible closer related to *Aphonopelma* than *Brachypelma*.

Petersen *et al.* (2007) developed a method to obtain mtDNA from *Brachypelma* spp. using exuvia. Their results show a phylogeny based on a Cytochrome oxidase 1 (COI) gene frament trimmed to 205bp where two *Brachypelma* subgroups corresponding to the "Red Rump vagans group" and the "Red leg emilia group" were recovered but were simply (mis)-grouped together because no other related genera were used for comparison. Mendoza & Francke (2017) used molecular data for a phylogenetic analysis with COI data to clarify some relationships within redknee species and revised nomenclature, but only included some species from the "Red leg emilia group" and not including the type species *B. emilia*, leaving the monophyly of the genus unquestioned. Recently, Turner *et al.* (2017) presented a mtDNA gene tree of tarantula spiders based on the mitocondrial 16S-tRNA (leu)-NDI gene región as a initial hypothesis to clarify some taxonomic relationships of the subfamily Theraphosinae. Their recovered phylogeny in both Bayeasian and Maximum Likelihood analyses strongly supported the non-monophyly of *Brachypelma* and *Aphonopelma*, indicathing that neither of both genera, as currently recognized, are

monophyletic. This result is totally congruent with our results based on COI, so it is another strong support for the non-monophyly of *Brachypelma*. We agree with the results of Turner *et al.* (2017) and proposed the "Red Led group" which contains the type species *B. emilia* as *Brachypelma sensu stricto*, while the "Red rump group" is considered as a different genus here described as *Tliltocatl gen. nov*. with designation of *T. vagans* as type species. We also performed a morphological character-based analysis of the genus *Brachypelma* using parsimony to test the monophyly of the genus and to reconcile morphological and molecular characters. Our best tree is based on the strict consensus of two most parsimonious tres obtained from the parsimony analysis of 103 characters. In this phylogeny the "Red Leg group" and the "Red Rump group" were also recovered as separated genera giving additional evidence for the non-monophyly of *Brachypelma*. However, morphological data in Theraphosidae tend to be homoplastic due to the similarities, simplicity in sexual structures and conserved characters (Raven 1985, Goloboff 1993, Pérez-Miles *et al.* 1996, Pérez-Miles 2000, Bertani 2001, Bond & Opell 2002, Hedin & Bond 2006, Bond & Hedin 2006, West *et al.* 2008, Hendrixson & Bond 2009, Bond *et al.* 2012, Guadanucci 2014, Hamilton *et al.* 2014, Perafán & Pérez-Miles 2014, Hamilton *et al.* 2016, Fukushima & Bertani 2017, Ortiz & Francke 2016). Despite this, both *Brachypelma* and *Tliltocatl* have diagnostic characteristics and synapomorphies that differentiate them from each other, the most evident in addition to their differences in coloration, are the prolateral inferior keel absent or slightly developed and restricted to the bulb apical, the spermatheca baseplate well developed and well sclerotized in *Brachypelma*; wheres in *Tliltocatl* the prolateral inferior keel is always present and is widely extended backwards, the spermatheca do not have baseplate or only present a small area slightly sclerotized. *Brachypelma fossorium* is a species which is not grouped with either *Brachypelma* or *Tliltocatl* in both the morphological and molecular phylogenies (Fig 3, 4). This species was originally described by Valerio in 1980 as *Brachypelma* based solely in the shape of the male palpal bulb and female genitalia. However, as is showed in our results we conclude that this species does not belong to *Brachypelma* or even to *Tliltocatl*. Despite the similar shape of the genitalia, this species does not possess the diagnostic features of *Brachypelma* or *Tliltocatl*, it has a very short embolous with different arrangement of keels from that observed in *Brachypelma* and *Tliltocatl*. Also, the urticating setae present in *B.*

fossorium are only type I, wheres *Brachypelma* and *Tliltocatl* possess types I and III. *B. fossorium* has the scopula IV divided by a strong band of setae, and *Brachypelma* and *Tliltocatl* have all their scopulae entire. So, based on this evidence and other arguments exposed in our morphological revision of the species above, we are sure that this species does not belong in either *Brachypelma* or *Tliltocatl*.

Mendoza & Francke (2017) concluded that barcode marker CO1 in *Brachypelma* proved to be sufficient for correct species identification. They consider it also as useful tool in preventing black market trade and in providing better strategies to reintroduce tarantulas into the correct distribution area**s**. DNA barcoding is a very useful technique that together with morphology, field observations and museum collections allow for better definition and delimitation of species (Scotland *et al.* 2003, Chen *et al.* 2011, Slowik and Blagoev 2012, Chan *et al.* 2014, Hendrixson *et al.* 2014, Pante *et al.* 2015). However, its use to solve phylogenies is limited, Hamilton *et al.* (2016) mentioned that the limitations of mtDNA used for phylogeny are gene tree/species tree incongruence and the haploid, non-recombining nature of the molecule, with CO1representing only one particular genealogy out of all possible within a genome. These limitations are shown in our ML tree, with the strong support for the confidence of each genus, but with low resolution on some inner clades in both *Brachypelma* and *Tliltocatl* (Fig 4). In the case of *Brachypelma* the most problematic is the relationship of *B. baumgarteni*, *B. boehmei* and *B. auratum*, just as is seen also in Mendoza & Francke (2017) where all these species were well supported but the clade which includes them was collapsed. In our phylogeny something similar happens with *B. auratum* and *B. baumgarteni* as sister species but with low support and with poorly resolution within *B. boehmei* (Fig 4). For *Tliltocatl* the ML tree shows no resolution in the position of *T. verdezi* and *T. kahlenbergi*, both as sister of a clade formed by *T. epicureanum*, *T. albopilosum*, *T. sabulosum* and *T. vagans*. In this clade *T. epicureanum* appears as a sister species of the other three; although the clade which shows *T. vagans* as sister of *T. sabulosum* and *T. albopilosum* has no support (Fig 4). The morphology-based tree has some similarities with the one obtained with ML for CO1, showing *B. emilia* as the sister species of all other *Brachypelma*, followed by *B. klaasi* and *B. albiceps* which are supported by a combination of five homoplastic characters but not by bootstrap value.

Three of these characters are related to the spermatheca, which have similarities because they are the only species with divided spermatheca. So, this is the main reason why the three species are grouped as sister species in the morphological phylogeny, unlike the molecular where they are separated (Fig 3, 4). The same occurs in the case of the group formed by *B. baumgarteni* and *B. hamorii* since these species are supported by two synapomorphies and six homoplastic characrers, the synapomorphies are the coloration of the chelicerae and the similar coloration on the tibiae. Three of the six homoplastic characters are related to the male palpal bulb since both species have a similar shape with the apical keel silightly developed and the prolateral superior keel wide and short (Fig 3). In contrast, in the ML tree *B. baumgarteni* and *B. hamorii* are genetically distant, with *B. hamorii* followed by *B. smithi* and this followed by a group formed by *B. boehmei*, *B. baumgarteni* and *B. auratum* (Fig 4). For the genus *Tliltocatl* there are differences between both topologies. Where in the morphological tree *T. albopilosum* and *T. schroederi* are shown as sister especies supported by only three homoplastic characters and no bootstrap support. All the characters are related to the number of spines in patellae II and III, and metatarsus III (Fig 3). The ML tree shows *T. schroederi* as the sister species of all other within the genus; whereas *T. albopilosum* is in an unresolved group with *T. sabulosum* and *T. vagans*. The other species resolution is more similar in both topologies, with *T. verdezi* and *T. kahlenbergi* with no resolution and as sister species of an inner clade formed by *T. epicureanum*, *T. vagans* and *T. sabulosum* in the morphology tree, and with the inclusion of *T. albopilosum* in the molecular as was shown above (Fig 3, 4). These discrepancies between morphological and genetic phylogenies are apparently given by the grouping of some species based mainly on the similarity of their reproductive organs. Also, is possible that the inclusion of other outgroups can help to resolve better the morphological hypothesis. We suggest that future studies must explore the use of different molecular markers such as nuclear ones which are more conservative and could help to resolve the inner relationships within the species. Also, the use of next generation sequencing methods can help to resolve phylogenetic problems, evolutionary concerns or even to help in conservation strategies (Turner *et al.* 2017). Further, the Inter Simple Sequence Repeats (ISSR) used by Machkour-M'Rabet *et al.* (2009) as molecular marker for the study of genetic diversity in populations of tarantulas and prooved to be a useful tool to know the

intraspecific variation in these organisms, which can be a valuable resource to evaluate the population structure of prioritary tarantulas such as *Brachypelma* and *Tliltocatl*.

Conservation issues

As was mentioned by Turner *et al.* (2017) the non-monophyly of *Brachypelma* with some species being transferred to another genus has immediate implications for conservation. The main consternation for those authors lies in the state of conservation that could be affected after the removal of the "Red Rump" species complex from *Brachypelma*. This is because as was observed we still know very little about their diversity, distribution, ecological characteristics, habitat preferences, reproductive success and how much they are affected by habitat loss and predation (Reichling 2000, Longhorn 2002, Machkour-M'Rabet et al 2005, Shillington & McEwen 2006, Machkour-M'Rabet *et al.* 2007, Dor *et al.* 2008, Dor & Hénaut 2011, Machkour-M'Rabet *et al.* 2011, Machkour-M'Rabet *et al.* 2012, Dor & Hénaut 2012, Dor & Hénaut 2013, Vilchis-Nestor *et al.* 2013, Machkour-M'Rabet *et al.* 2015, Hénaut *et al.* 2015, Machkour-M'Rabet *et al.* 2017). Nevertheless, CITES (2017) is aware of possible changes in nomenclature within the taxonomic groups included for its regulation and in its resolution Conf. 12.11 revised in the COP17 held in Johannesburg, is mentioned that "*whenever a change in the name of a taxon included in the Appendices is proposed, the Secretariat, in consultation with the Animals or Plants Committee, determine whether this change would alter the scope of protection for fauna or flora under the Convention*". This makes reference that in the case where the scope of a taxon is redefined, the Animals or Plants Committee shall evaluate whether acceptance of the taxonomic change would cause additional species to be included in the Appendices or listed species to be deleted from the Appendices and, if that is the case, the Depositary Government should be requested to submit a proposal to amend the Appendices in accordance with the recommendation of the Animals or Plants Committee, so that the original intent of the listing is retained. This means that even if the species that belong to the "Red Rump" group are transferred from *Brachypelma* into another genus *Tliltocatl*, these species will not lose their protective status given by CITES appendix II. However, this is only concerning international trade, but it is equally important to know if the taxonomic change of these species will affect their protection status in Mexico. In the

country, it is the LGVS which regulates the sustainable use, conservation, and management of native wild animals and plants. It regulates the protection of species or populations that are at risk, including both terrestrial and aquatic species (SEMARNAT 2000). The LGVS establishes the national policy for wildlife protection and sustainable use via the SUMA program and the Mexican Official Standard NOM059-SEMARNAT-2010 (NOM-059) on Mexican species at risk. In addition, the LGVS regulates the creation of UMAs. To change the protection status of the species protected by law it is necessary to submit a petition for evaluation with information about trade, conservation and population status, so despite the changes, all the species formerly known as *Brachypelma* will remain protected in Mexico also. In fact, a particular interest has recently emerged from the Mexican government to protect tarantulas and regulate their legal trade.

In 2015, the governments of Canada, Mexico and the United States initiated a collaborative project through the Commission for Environmental Cooperation (CEC) to strengthen the conservation and sustainable trade of the 16 tarantulas that are included in Appendix II of the CITES. The result of this was the creation of an action plan for the sustainable trade in tarantulas. This action plan includes information on 16 prioritary tarantula species, comprising one from the genus *Aphonopelma* and 15 from the genus *Brachypelma*. The information was compiled for the species as a group, and included: the impact of trade on conservation and livelihoods, and identification challenges for CITES enforcement. Currently, there are insufficient population data available for Mexican tarantulas, especially those restricted to small geographical areas particularly vulnerable to overexploitation (Reichling 2003). Without such information, it would be very difficult to make a study that the export of adult or sub-adult specimens was not detrimental to the species survival in the wild, unless there was compelling information available to show that the specimens in question were captive-bred. Preliminary field studies indicate that *B. baumgarteni*, *B. boehmei* and *B. hamorii* have small zoogeographical ranges and are sensitive to habitat disruption. Therefore, at least these three species should not be considered for direct capture and export until more research has been conducted on their viability (CEC 2017). At sustainable levels of consumption, both wildlife and people can benefit from legal trade.

Granting local people an economic stake in wildlife provides the best incentive for careful stewardship of species and habitats (Carey 1999, Dickinson 2002).

Although, DNA barcodes are not good enough to neither capture the dynamic process of evolution nor reflect the precise interrelationships within a group, they could provide legislators with a framework when enacting protection laws. This could translate into greater success in prosecuting those involved in the illegal pet-trade. It is agreed that mtDNA alone is insufficient for precise species delimitation. However, it can be useful for identification if the findings correspond with morphological evidence (Petersen *et al.* 2007). Because of this, we create a genetic library of priority tarantulas (*Brachypelma* and *Tliltocatl*) as part of the Wildlife Barcode Project in Mexico (BWPM 2014), to be used as a reference for the authorities responsible for species conservation. It is essential to explore other non-subtractive economic endeavors such as tourism, wildlife observation or sale of captive-bred animals to provide alternative income**s** for local communities and to avoid further damage to wildlife populations.

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Illustration Legends

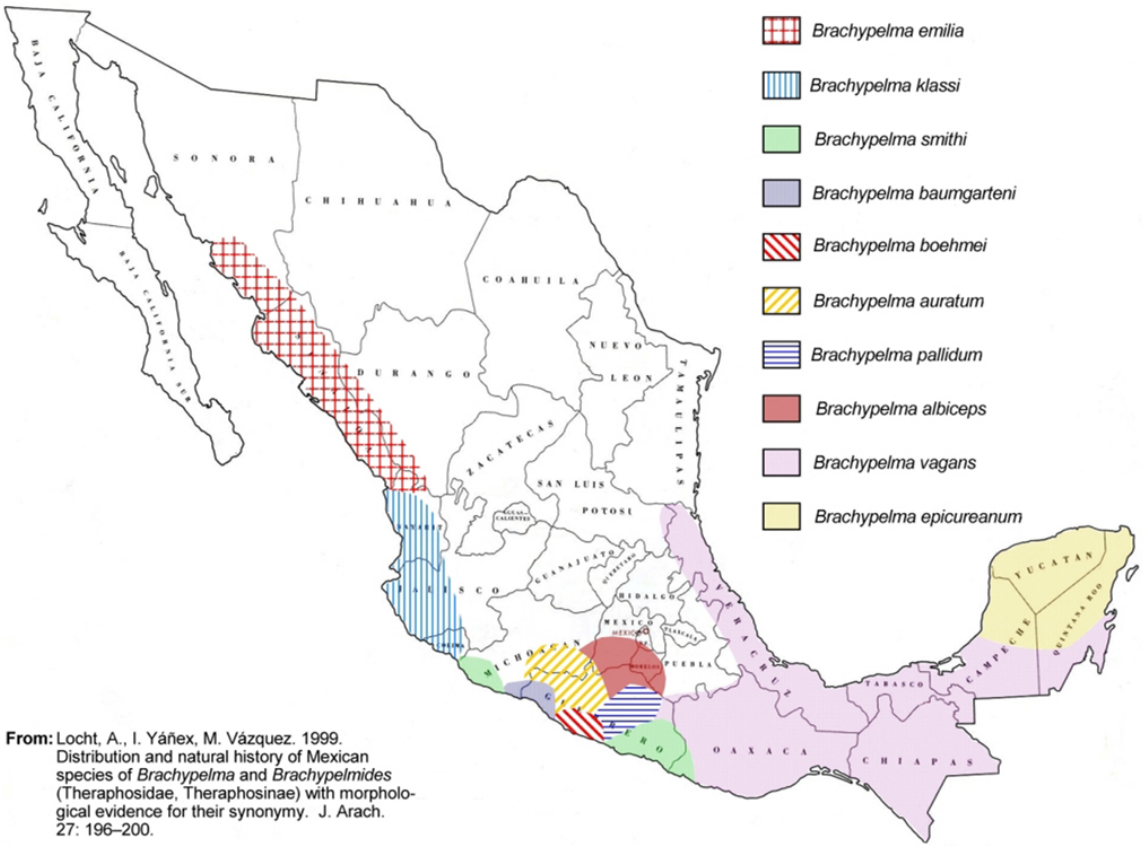


Fig 1. Previous distribution map of Mexican species of *Brachypelma*, from Locht *et al.* (1999). Note: *Brachypelma pallidum* as was labelled originally belong to *Brachypelma verdezi*, species which here is transferred to *Tliltocatl* gen. nov.

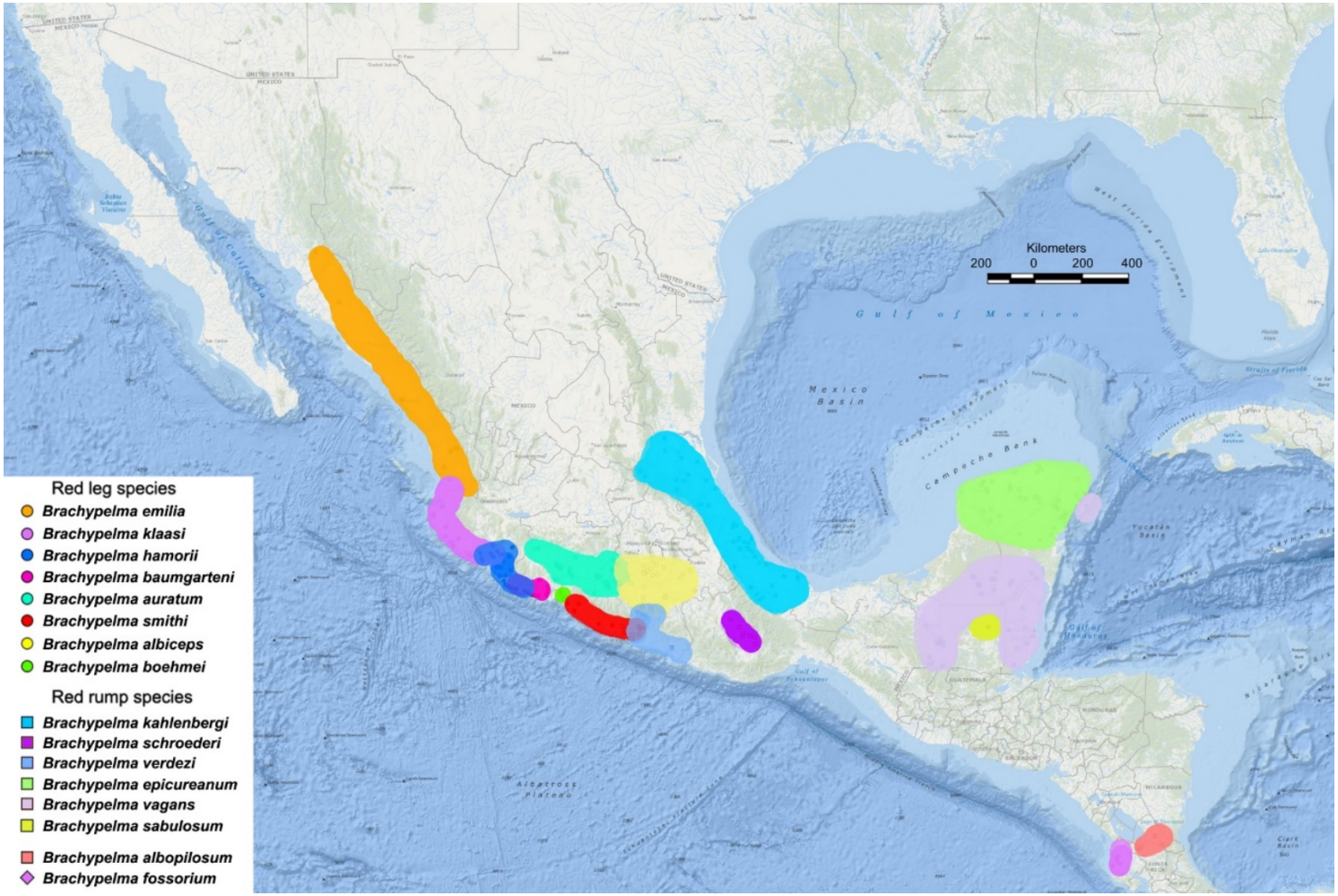


Fig 2. Distribution map of formerly known *Brachypelma* redleg and redrump species complex with more accurate distribution areas based on museum specimens. Circles = Redleg complex; squares = redrump complex; diamond= *Brachypelma fossorium* which actually does not belong to any of the mentioned groups.

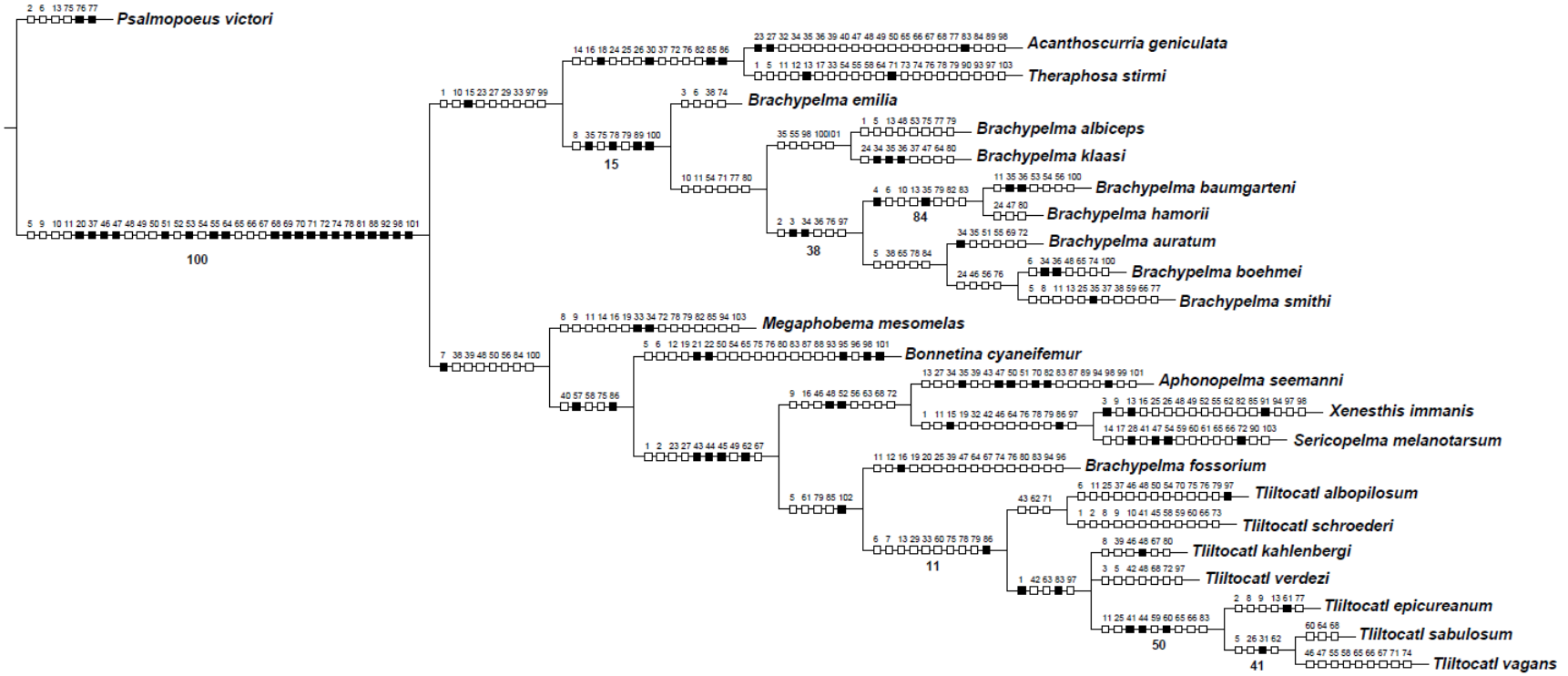


Fig 3. Strict consensus of the two trees obtained by parsimony analysis of 103 morphological characters for 24 taxa with equal weighting. Tree shows the non-monophyly of *Brachypelma* with the species which remain in the genus and the ones transferred to *Tliltocatl* gen. nov. Bootstrap support with percentages less than 100 indicated above branches. Synapomorphies were mapped on the tree. Tree length 443, Ci: 0.443, Ri: 0.541.

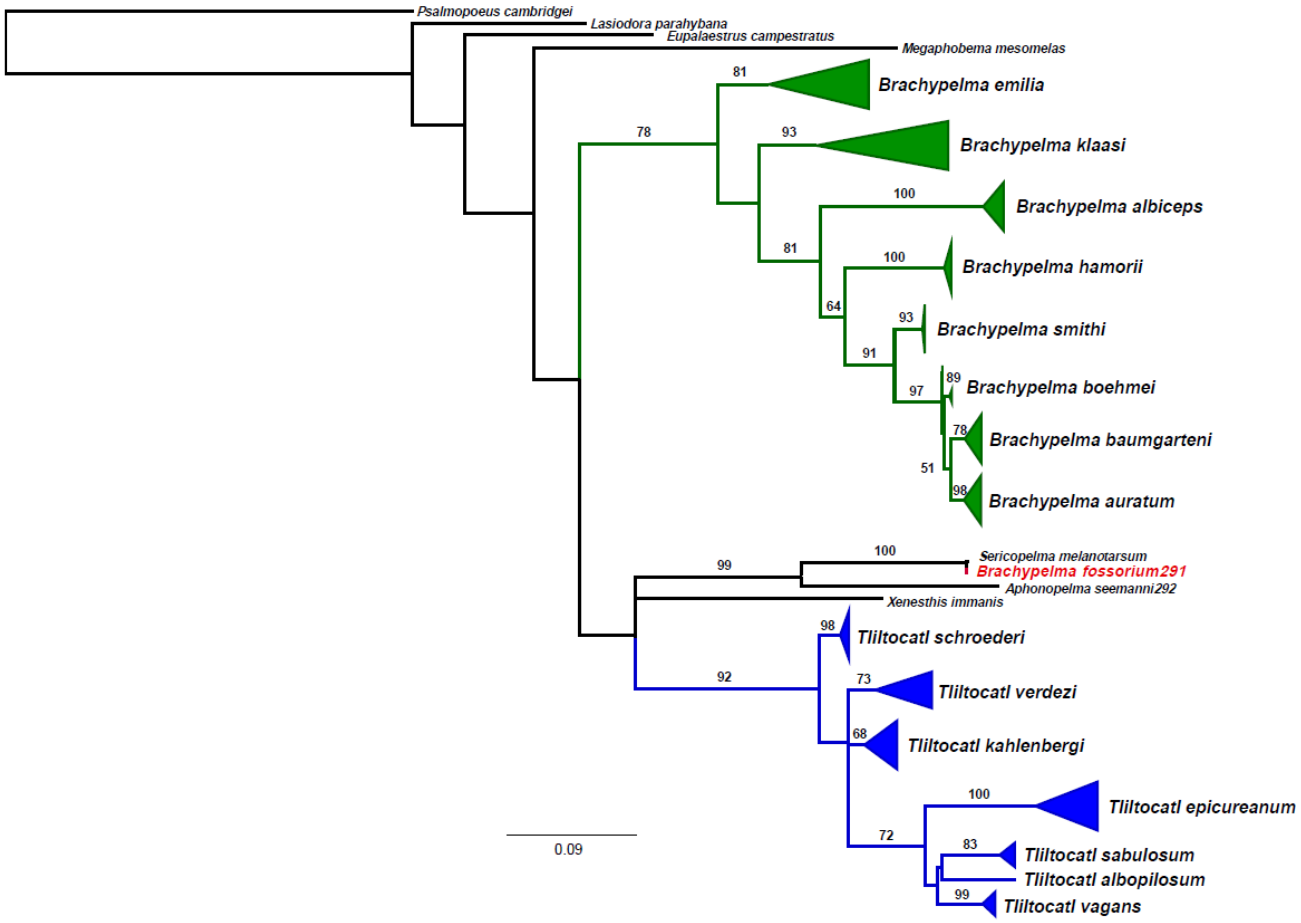


Fig 4. Maximum likelihood phylogenetic hypothesis of 658 aligned nucleotides from barcoding gene CO1 of the mitochondrial genomes of 66 samples from 8 *Brachypelma* and 7 *Tliltocatl* gen. nov. species. Nodal support shows maximum likelihood bootstrap. Tree shows the non-monophyly of *Brachypelma* with the species which remain in the genus and the ones transferred to *Tliltocatl* gen. nov.

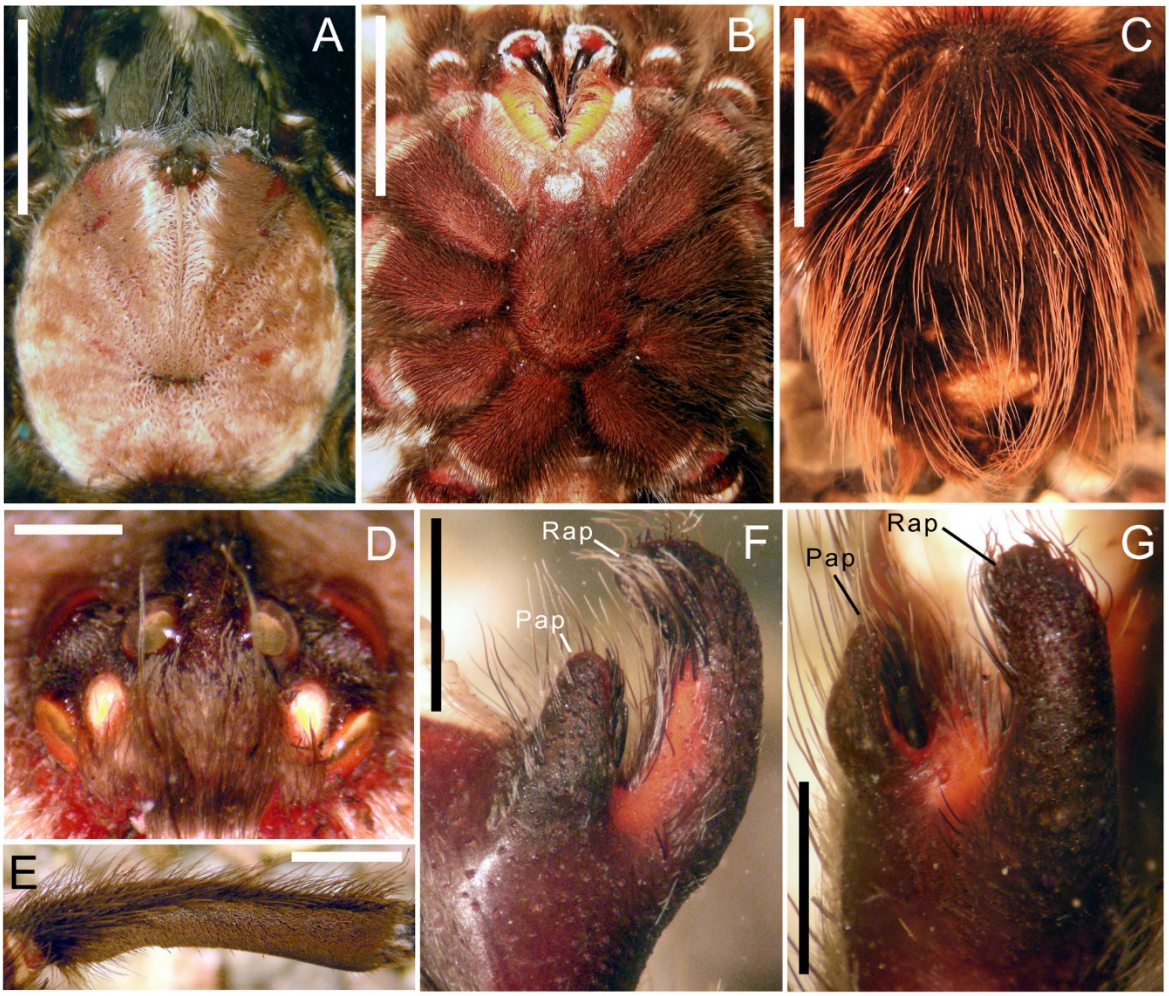


Fig 5. *Brachypelma albiceps*, male CNAN-Ar003412. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, metatarsus I, prolateral view; F, tibial apophyses, prolateral view; G, tibial apophyses, ventral view. Scale = 10mm (A–C), 8mm (E), 4mm (F, G), 1mm (D).

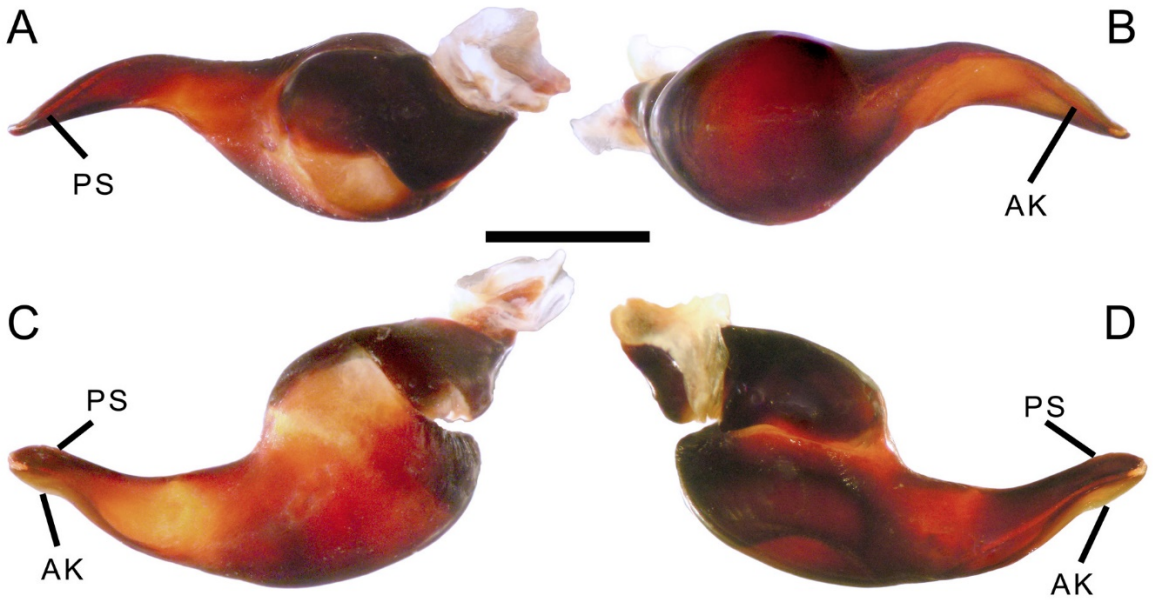


Fig 6. *Brachypelma albiceps*, male CNAN-Ar003412. Left palpal bulb: A, dorsal view; B, ventral view; C, retrolateral view; D, prolateral view. Scale = 2mm.

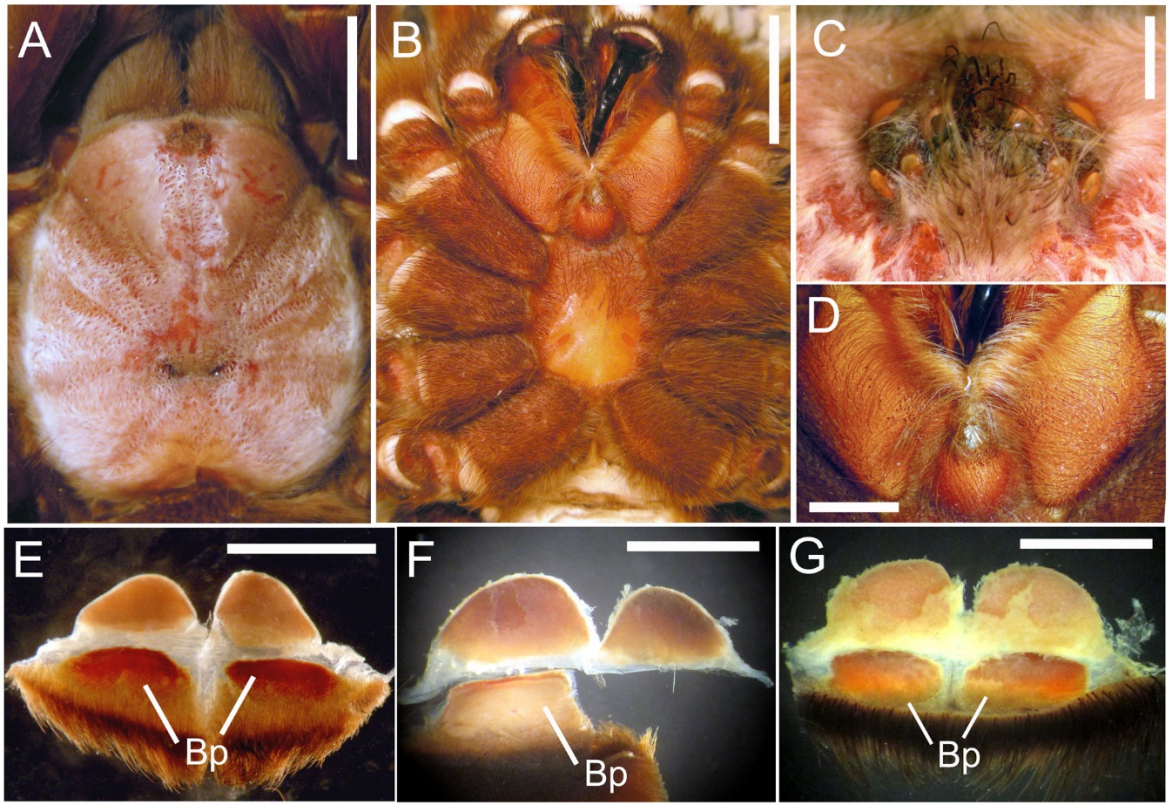


Fig 7. *Brachypelma albiceps*. A─E female CNAN-Ar003082. A, carapace, dorsal view; B, prosoma, ventral view; C, ocular tubercle, dorsal view; D, labial and maxillary cuspules; E, spermatheca, ventral view; F, G, spermatheca ventral view of: F, female BMNH1898.12.24.34-37; G, female BMNH. Scale = 10mm (A─B), 2mm (D), 1mm (C, E─G).

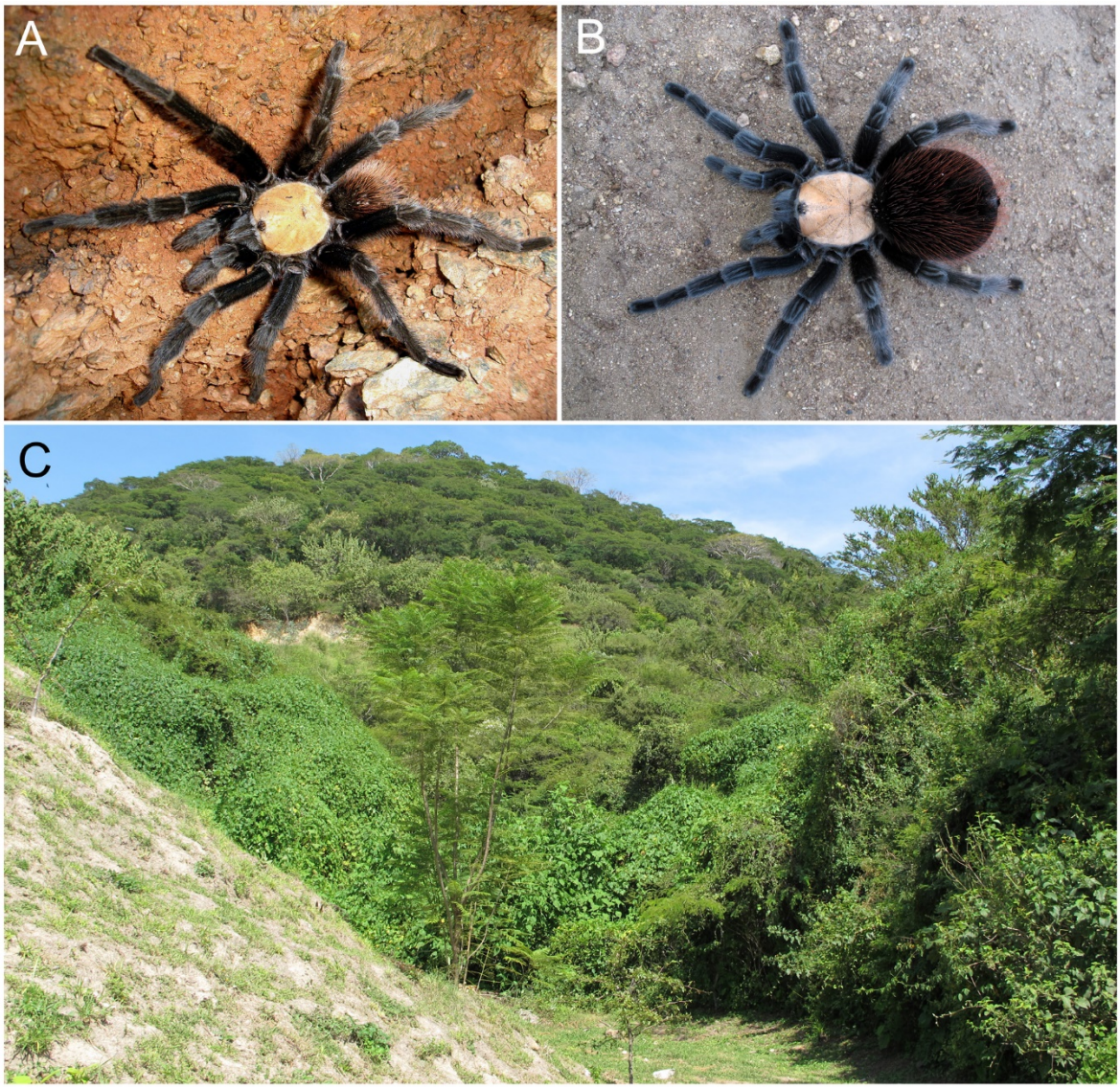


Fig 8. A─B *Brachypelma albiceps*, habitus; C, habitat. A, male; B, female, C, deciduous forest in the habitat of *B. albiceps*. Photos: J. Mendoza.

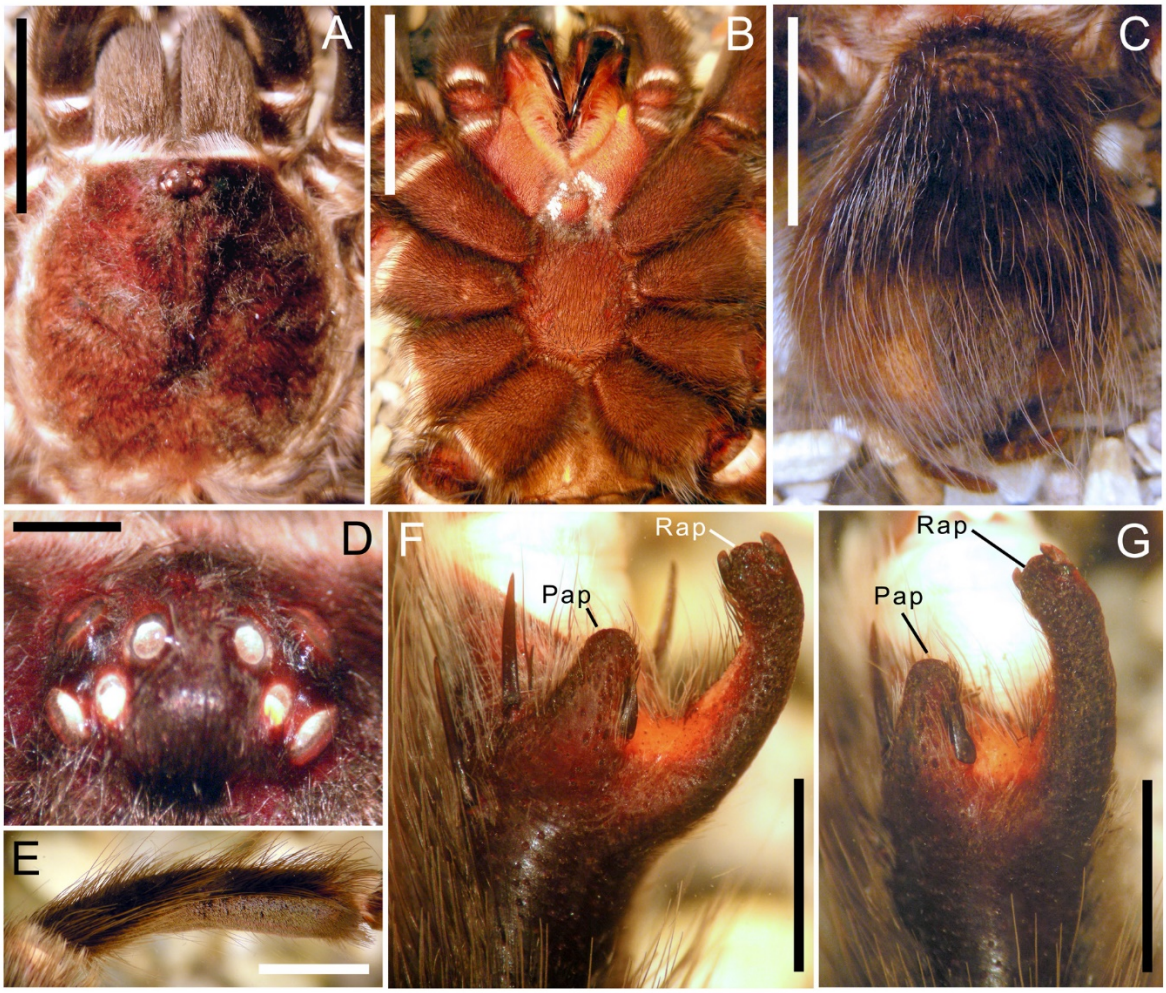


Fig 9. *Brachypelma auratum*, male CNAN-Ar003658. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, metatarsus I, prolateral view; F, tibial apophyses, prolateral view; G, tibial apophyses, ventral view. Scale = 12mm (A–C), 8mm (E), 4mm (F, G), 1mm (D).

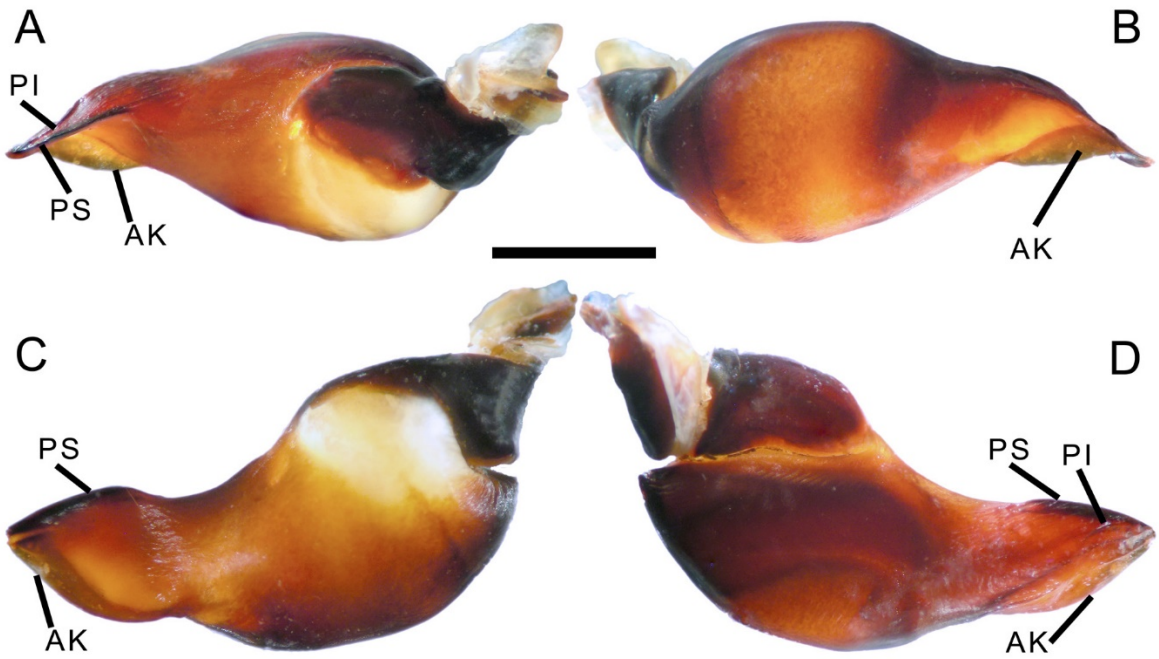


Fig 10. *Brachypelma auratum*, male CNAN-Ar003658. Left palpal bulb: A, dorsal view; B, ventral view; C, retrolateral view; D, prolateral view. Scale = 2mm.

Fig 11. *Brachypelma auratum*. A─C holotype male SMF-38045. A, habitus, dorsal view; B, laminated spermatheca; C, spermatheca, ventral view.

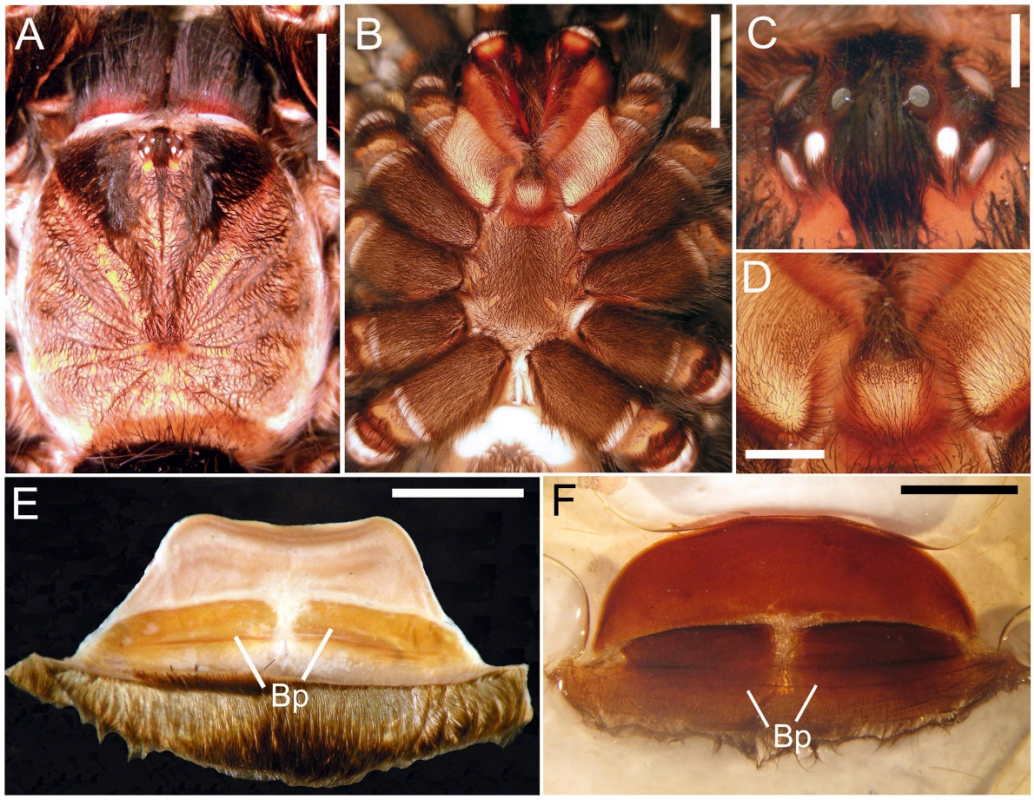


Fig 12. *Brachypelma auratum*. A─E female CNAN-Ar007878. A, carapace, dorsal view; B, prosoma, ventral view; C, ocular tubercle, dorsal view; D, labial and maxillary cuspules; E, spermatheca, ventral view; F, spermatheca ventral view of: F, female SMF-58207. Scale = 10mm (A─B), 2mm (D), 1mm (C, E, F).

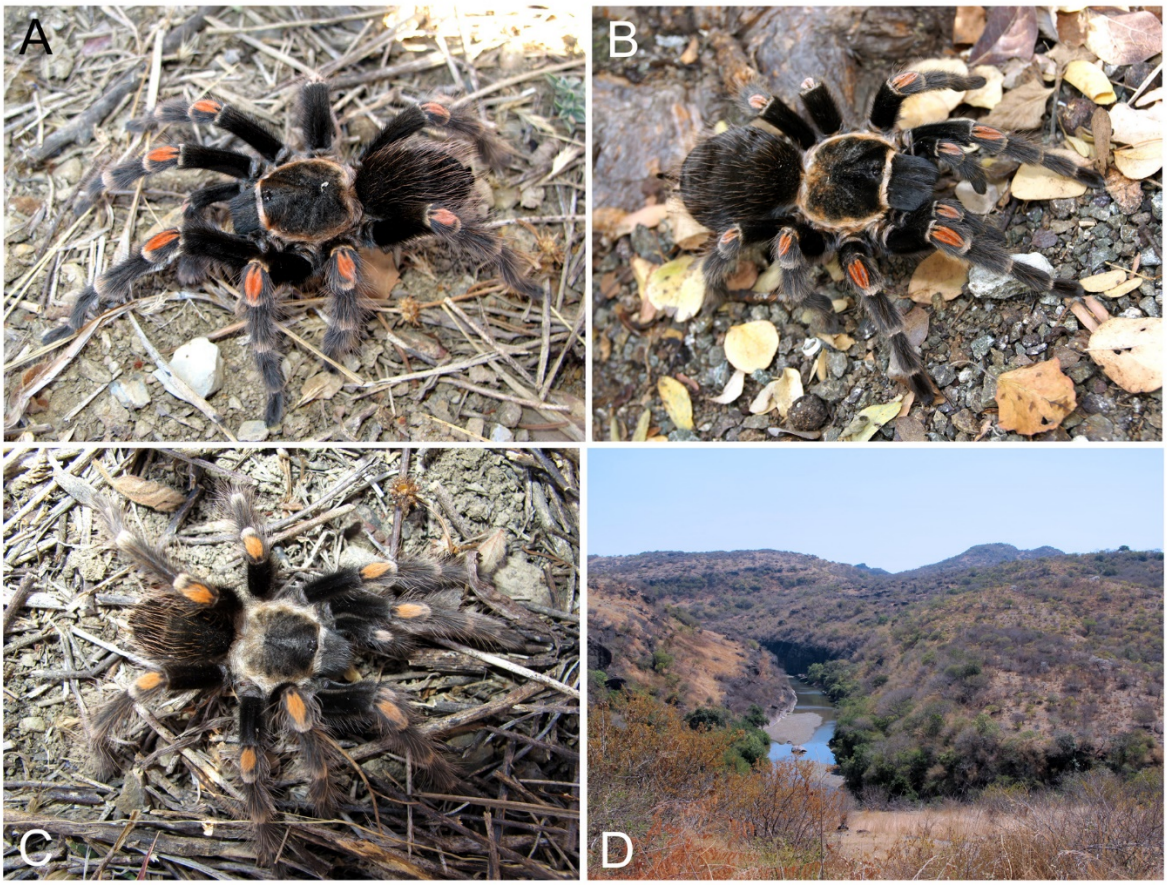


Fig 13. A─C, *Brachypelma auratum*, habitus; D, habitat. A, male; B, female; C, juvenile; D, deciduous forest and shrubland in the habitat of *B. auratum*, C. Photos: J. Mendoza.

Fig 14. *Brachypelma baumgarteni*, male CNAN-Ar010588. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, metatarsus I, prolateral view; F, tibial apophyses, ventral view; G, tibial apophyses, prolateral view. Scale = 12mm (A–C), 8mm (E), 4mm (F, G), 1mm (D).

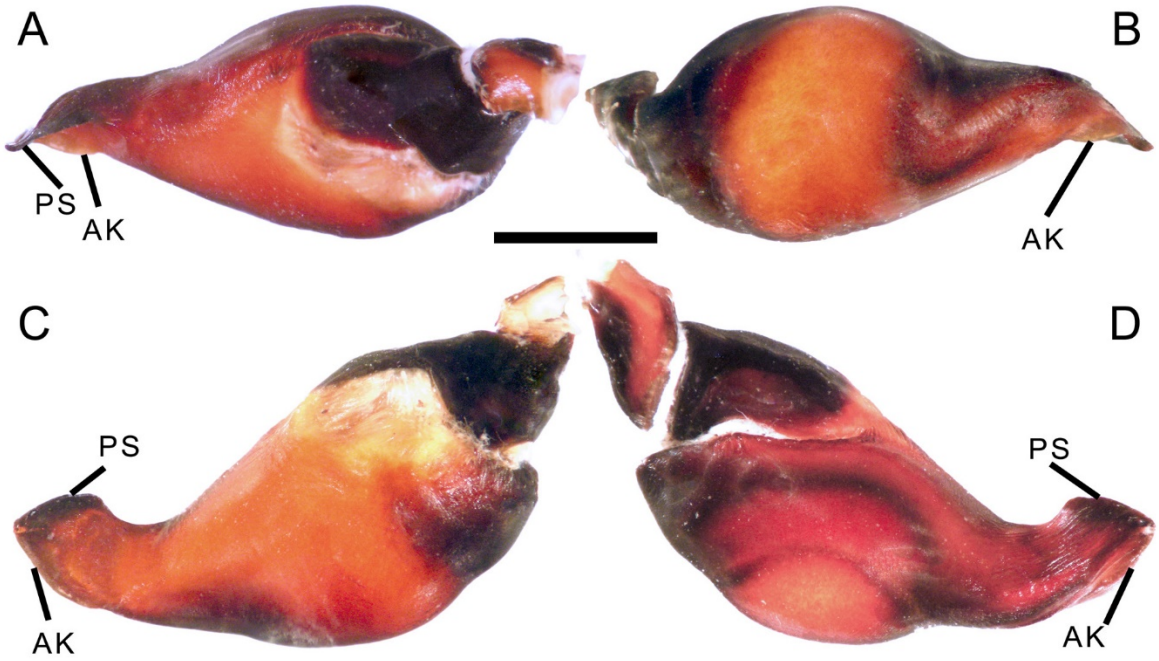


Fig 15. *Brachypelma baumgarteni*, male CNAN-Ar010588. Left palpal bulb: A, dorsal view; B, ventral view; C, retrolateral view; D, prolateral view. Scale = 2mm.

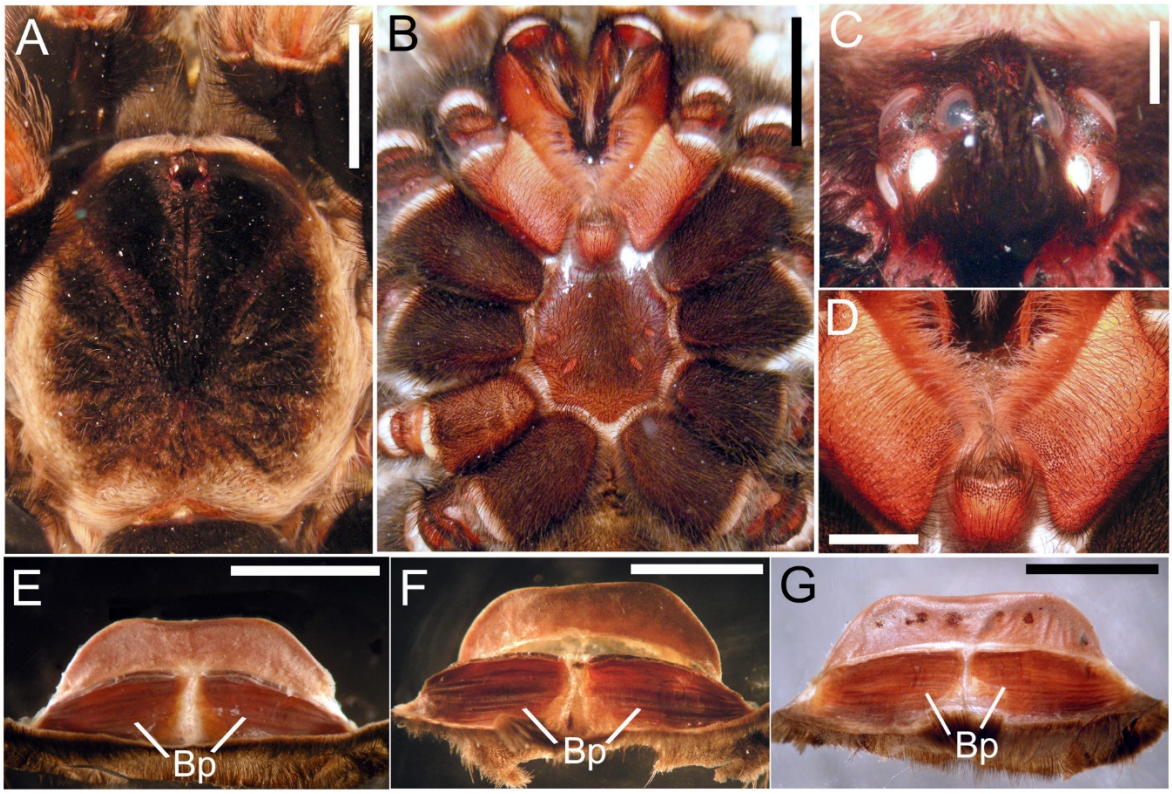


Fig 16. *Brachypelma baumgarteni*. A─E female CNAN-Ar007150. A, carapace, dorsal view; B, prosoma, ventral view; C, ocular tubercle, dorsal view; D, labial and maxillary cuspules; E, spermatheca, ventral view; F, G, spermatheca ventral view of: F, female CNAN-Ar007149; G, female CNAN-Ar007151. Scale = 10mm (A─B), 3mm (D), 1mm (C, E─G).

Fig 17. A─C, *Brachypelma baumgarteni*, habitus; D, habitat. A, male; B, female dark carapace; C, female clear carapace; D, deciduous forest in the habitat of *B. baumgarteni*. Photos: J. Mendoza.

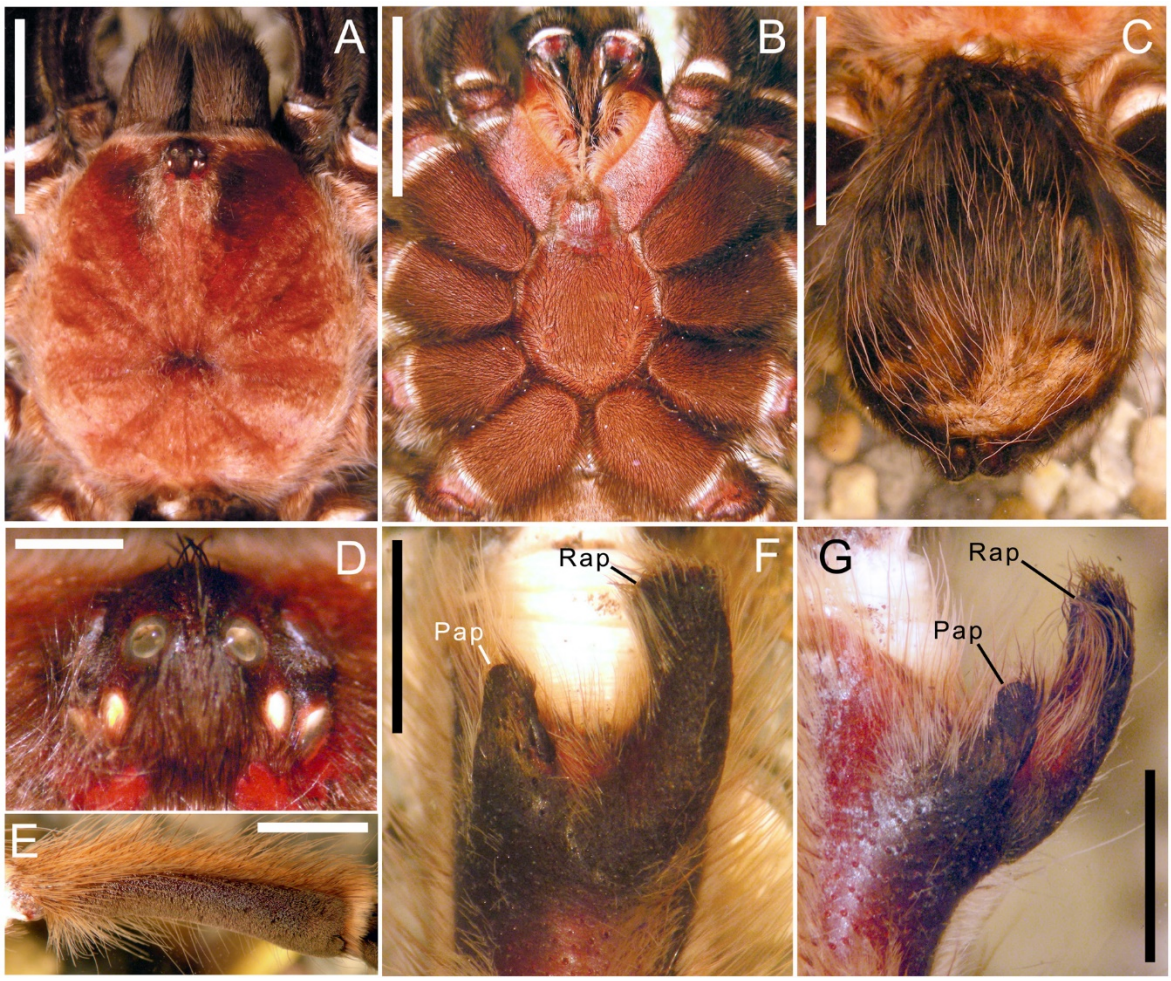


Fig 18. *Brachypelma boehmei*, male CNAN-Ar010589. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, metatarsus I, prolateral view; F, tibial apophyses, ventral view; G, tibial apophyses, prolateral view. Scale = 12mm (A–C), 8mm (E), 4mm (F, G), 1mm (D).

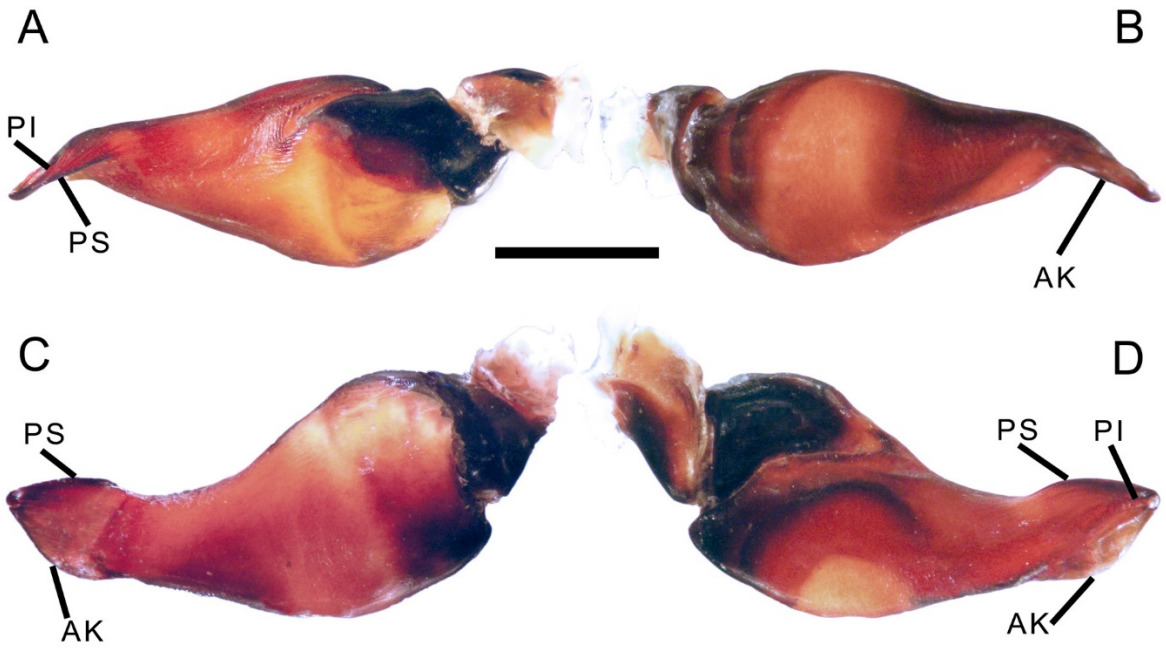


Fig 19. *Brachypelma boehmei*, male CNAN-Ar010589. Left palpal bulb: A, dorsal view; B, ventral view; C, retrolateral view; D, prolateral view. Scale = 2mm.

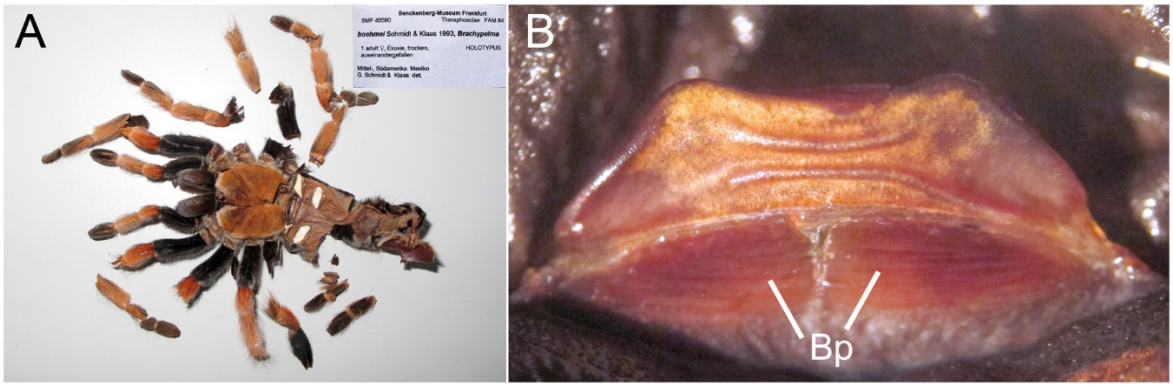


Fig 20. *Brachypelma boehmei*. A─B holotype female SMF-40590. A, exuvia used as holotypus; B, spermatheca, ventral view (was not removed from the exuvia).

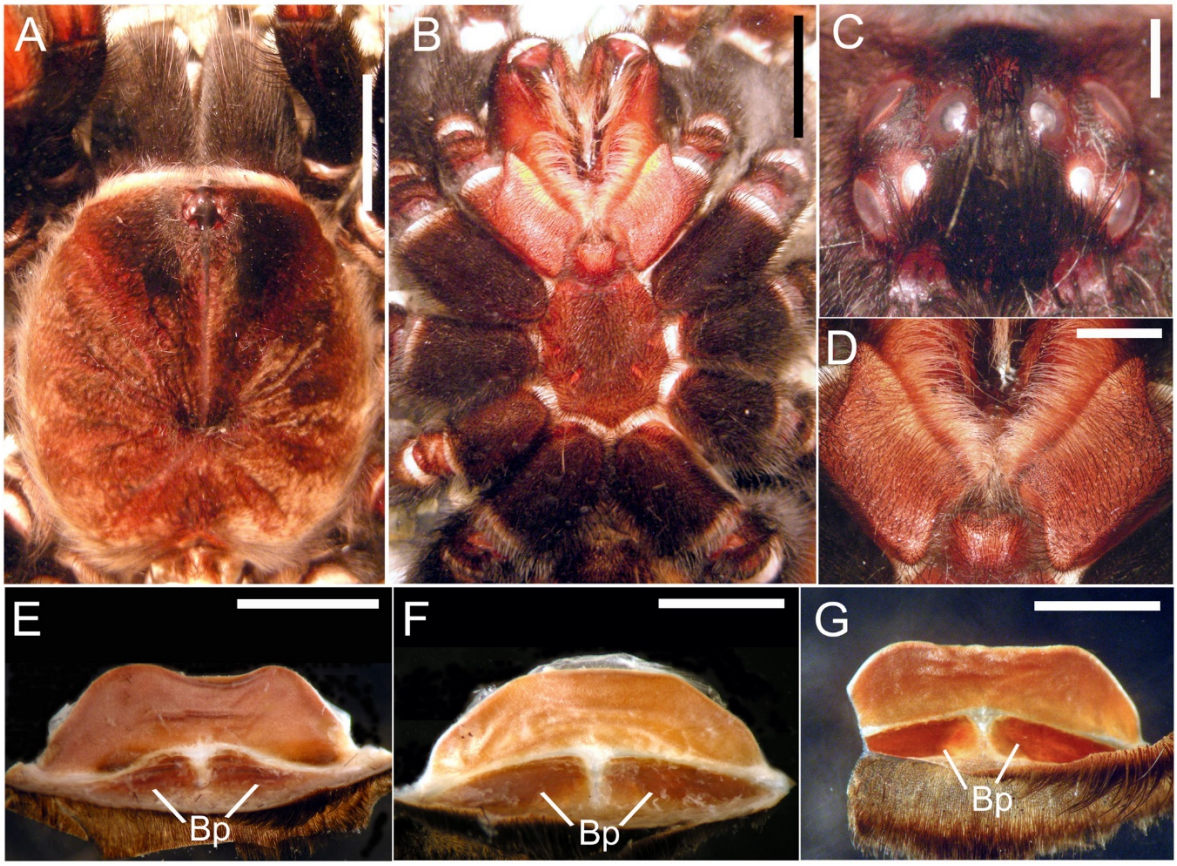


Fig 21. *Brachypelma boehmei*. A─E female CNAN-Ar007905. A, carapace, dorsal view; B, prosoma, ventral view; C, ocular tubercle, dorsal view; D, labial and maxillary cuspules; E, spermatheca, ventral view; F, G; spermatheca ventral view of: F, female CNAN-Ar007185; G, female CNAN-Ar007833. Scale = 10mm (A─B), 3mm (D), 1mm (C, E─G).

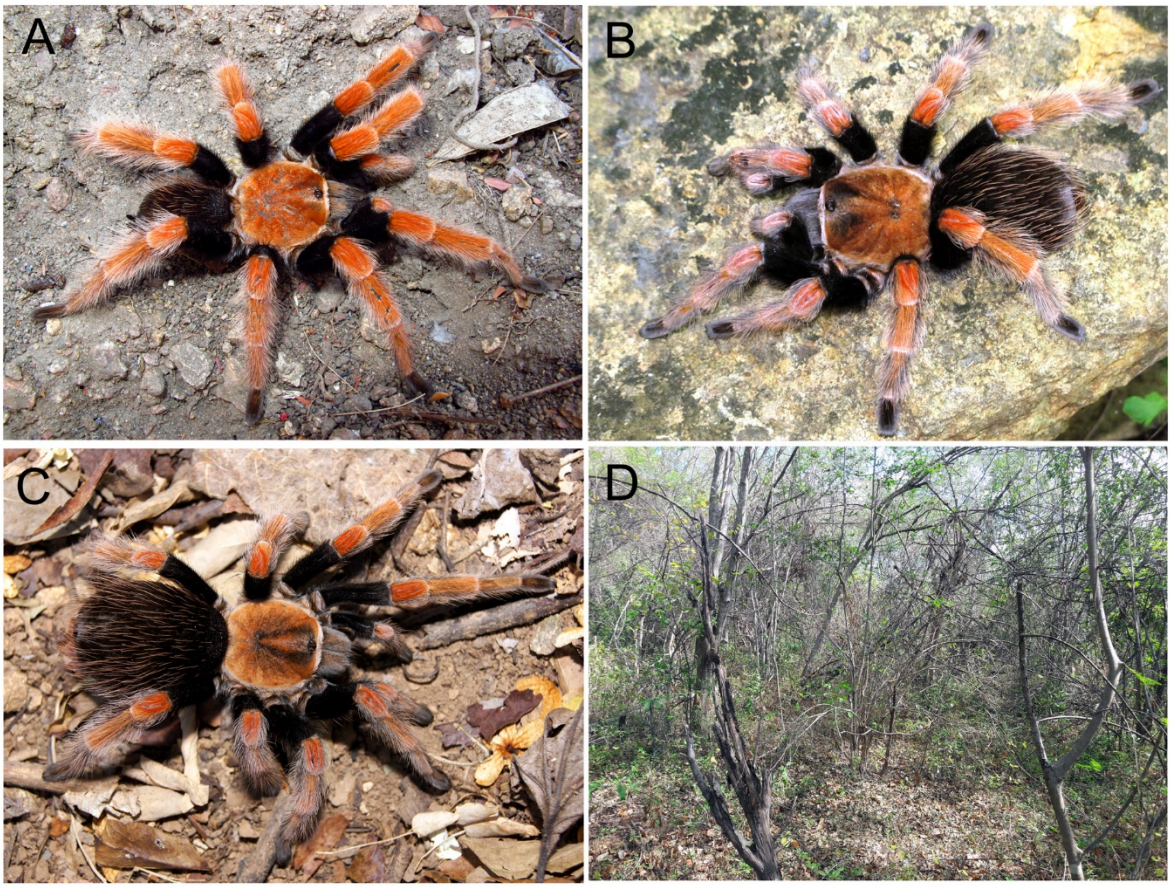


Fig 22. A─C, *Brachypelma boehmei*, habitus; D, habitat. A, male; B, female with black around ocular area; C, femalewith black lateral areas on ocular regions; D, deciduous forest and shrubland in the habitat of *B. boehmei*. Photos: A, B, D, J. Mendoza; C, E. Goyer.

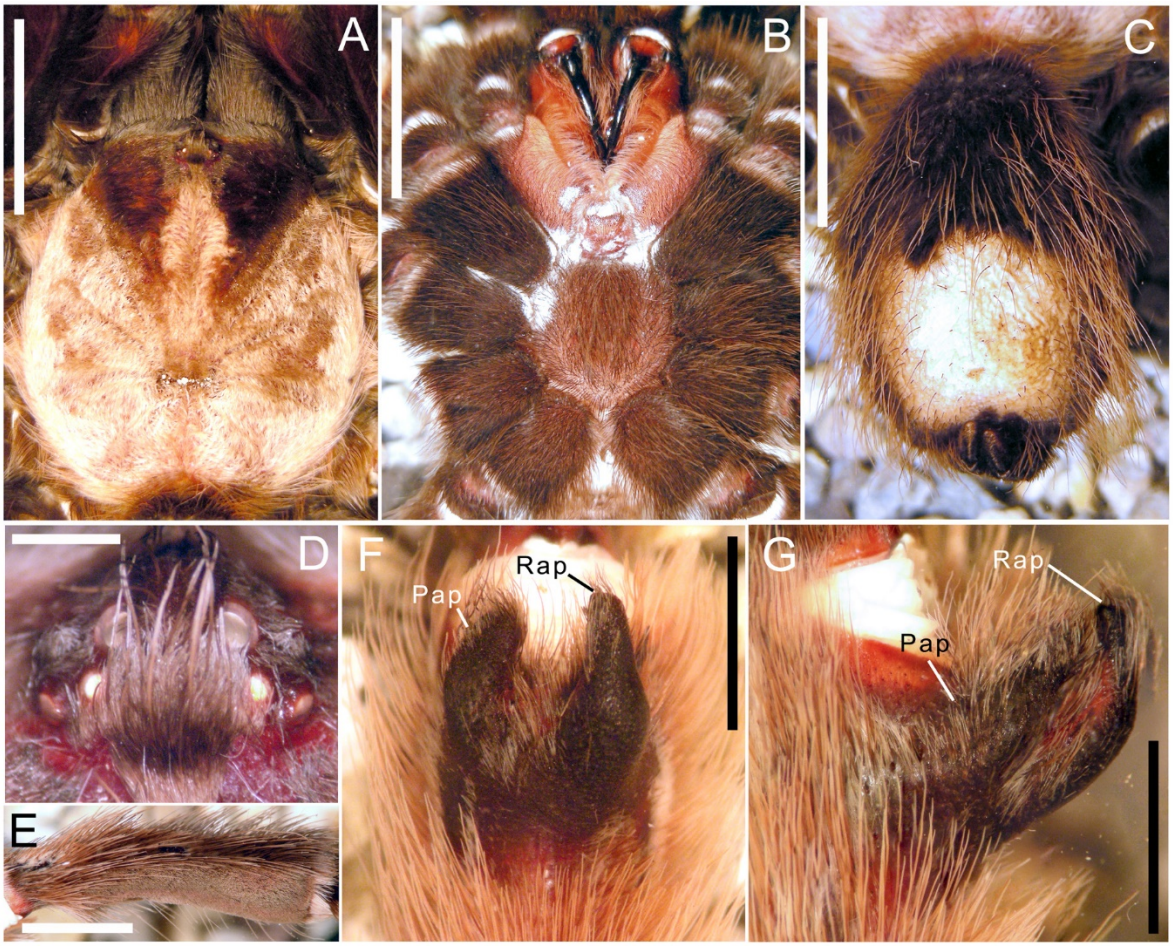


Fig 23. *Brachypelma emilia*, male CNAN-Ar003599. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, metatarsus I, prolateral view; F, tibial apophyses, ventral view; G, tibial apophyses, prolateral view. Scale = 12mm (A–C), 8mm (E), 4mm (F, G), 1mm (D).

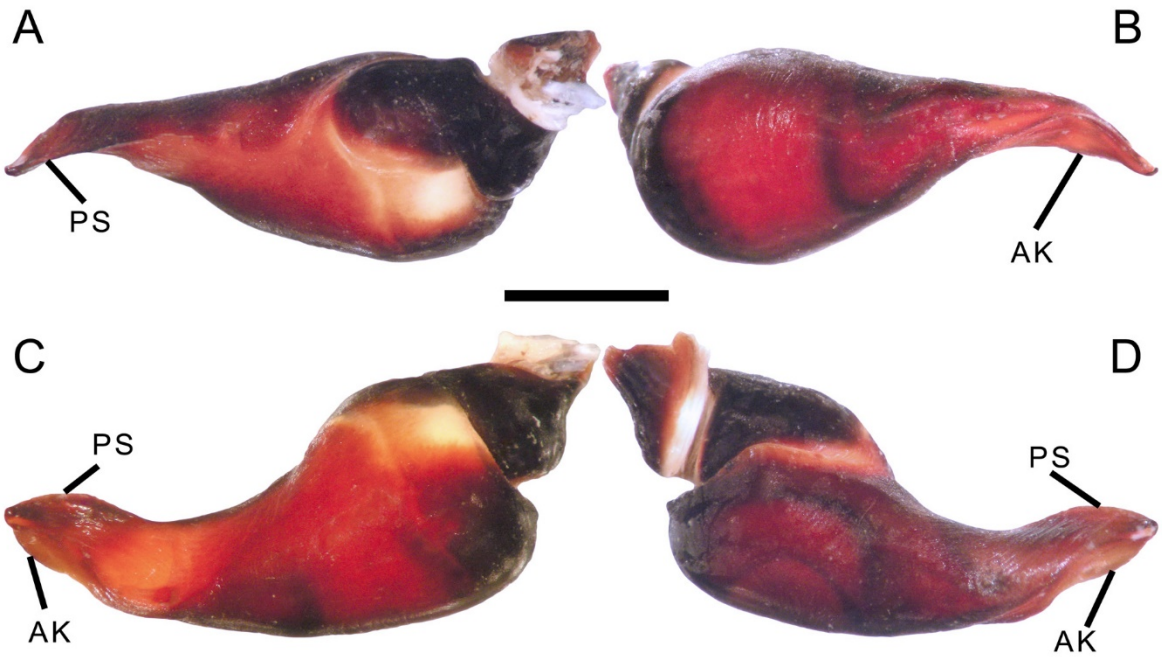


Fig 24. *Brachypelma emilia*, male CNAN-Ar003599. Left palpal bulb: A, dorsal view; B, ventral view; C, retrolateral view; D, prolateral view. Scale = 2mm.

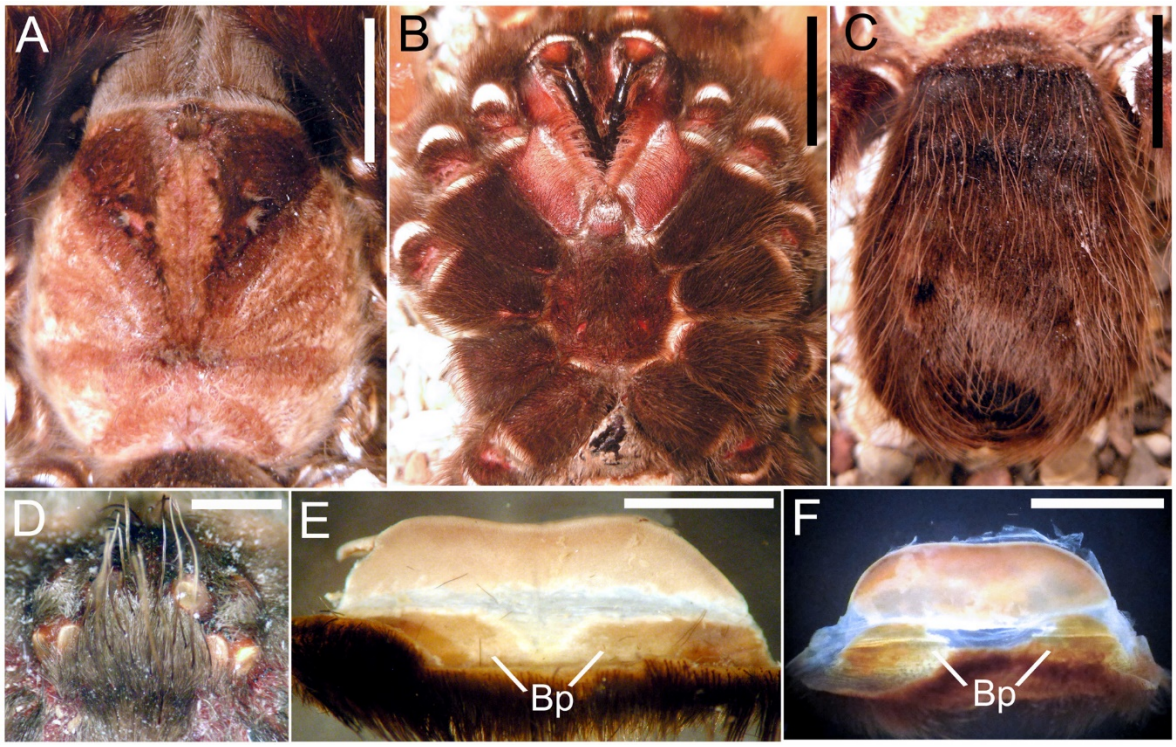


Fig 25. *Brachypelma emilia*. A─E female CNAN-Ar010602. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, spermatheca, ventral view; F, spermatheca ventral view of: F, female BMNH1962.2.28.1. Scale = 10mm (A─C), 1mm (D ─F).

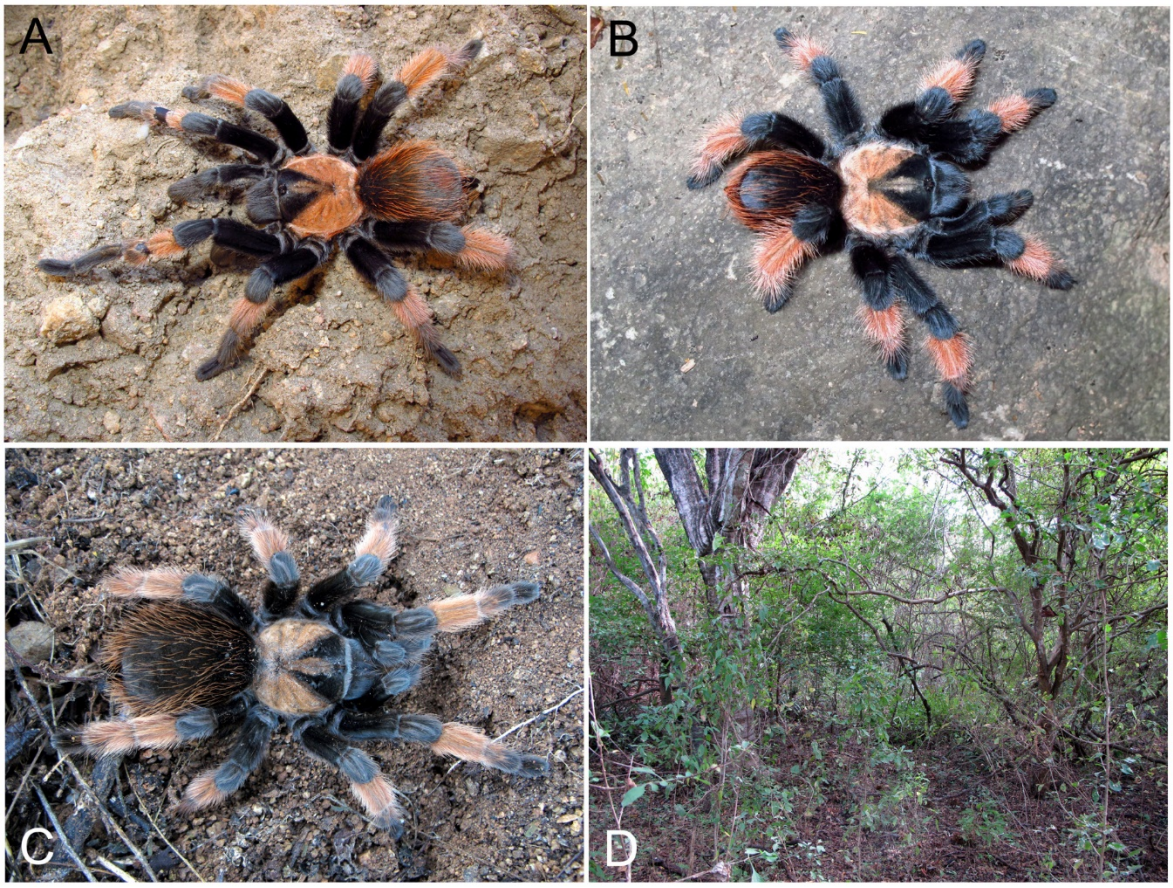


Fig 26. A─C, *Brachypelma emilia*, habitus; D, habitat. A, male; B, juvenile female; C, adult female; D, deciduous forest in the habitat of *B. emilia*. Photos: J. Mendoza.

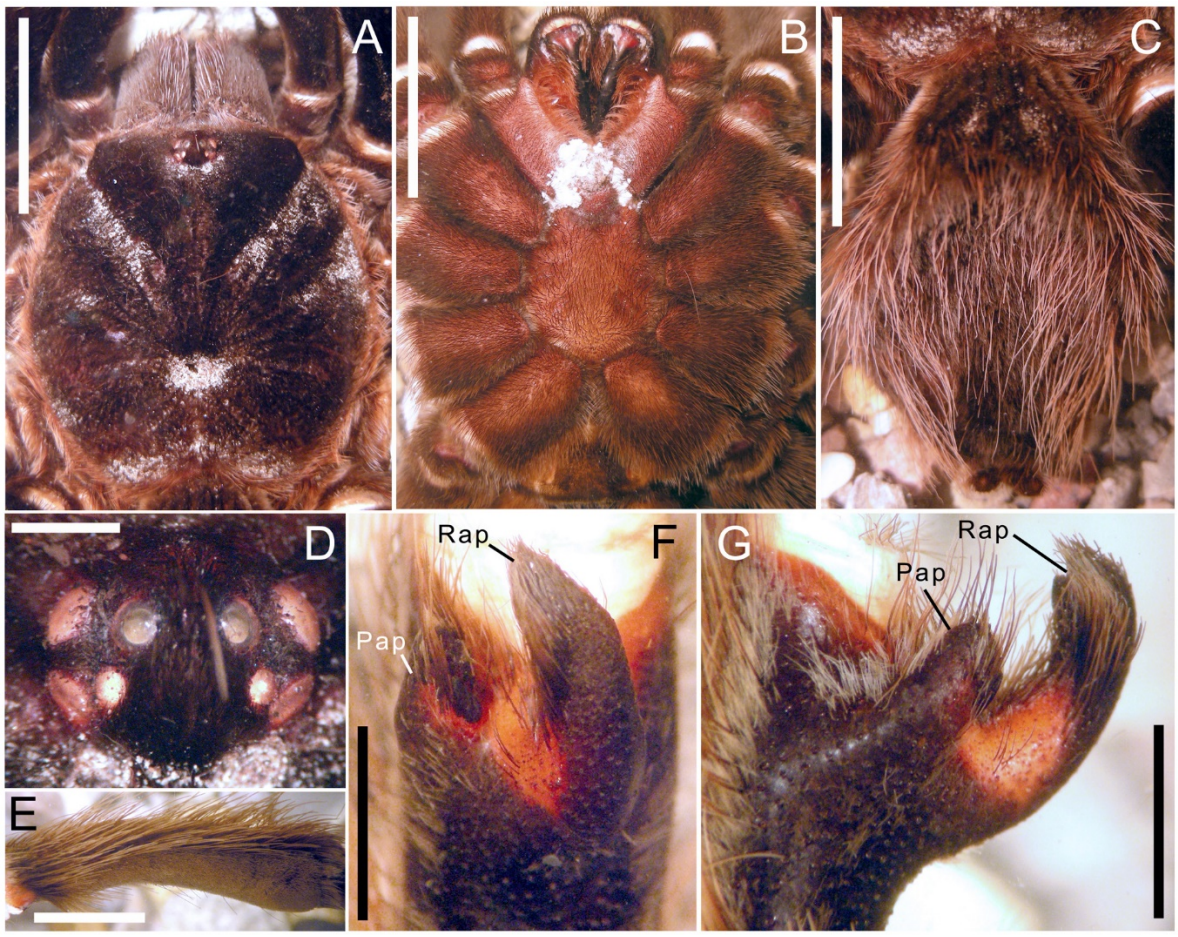


Fig 27. *Brachypelma klaasi*, male CNAN-Ar003333. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, ocular tubercle, dorsal view; E, metatarsus I, prolateral view; F, tibial apophyses, ventral view; G, tibial apophyses, prolateral view. Scale = 12mm (A–C), 8mm (E), 4mm (F, G), 1mm (D).

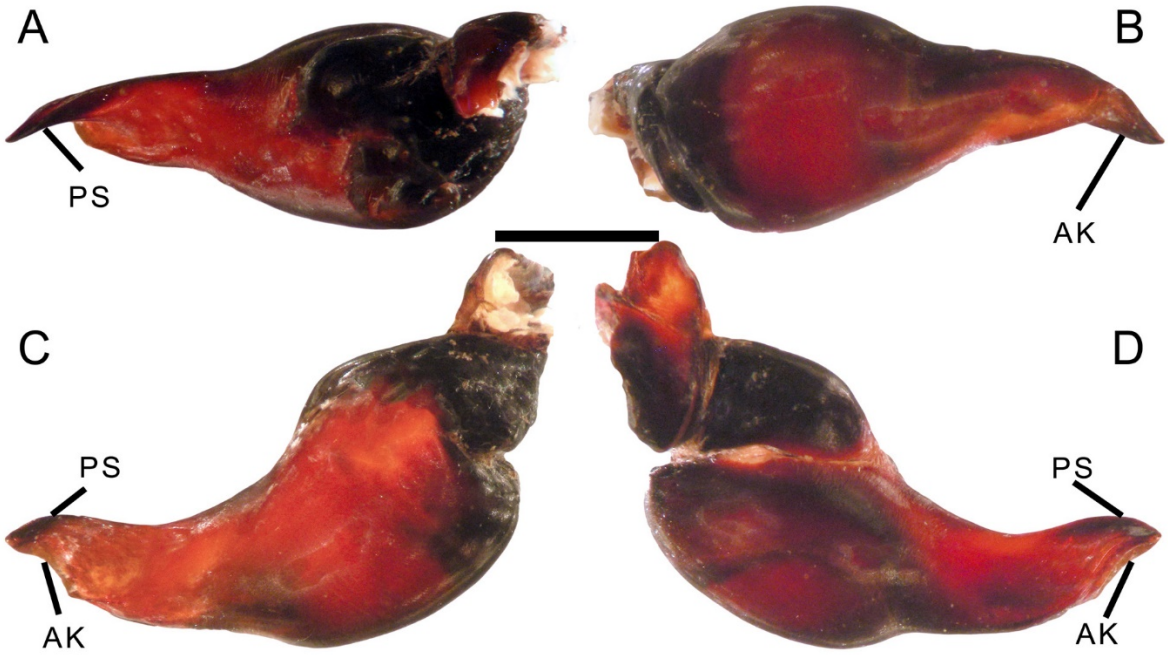


Fig 28. *Brachypelma klaasi*, male CNAN-Ar003333. Left palpal bulb: A, dorsal view; B, ventral view; C, retrolateral view; D, prolateral view. Scale = 2mm.

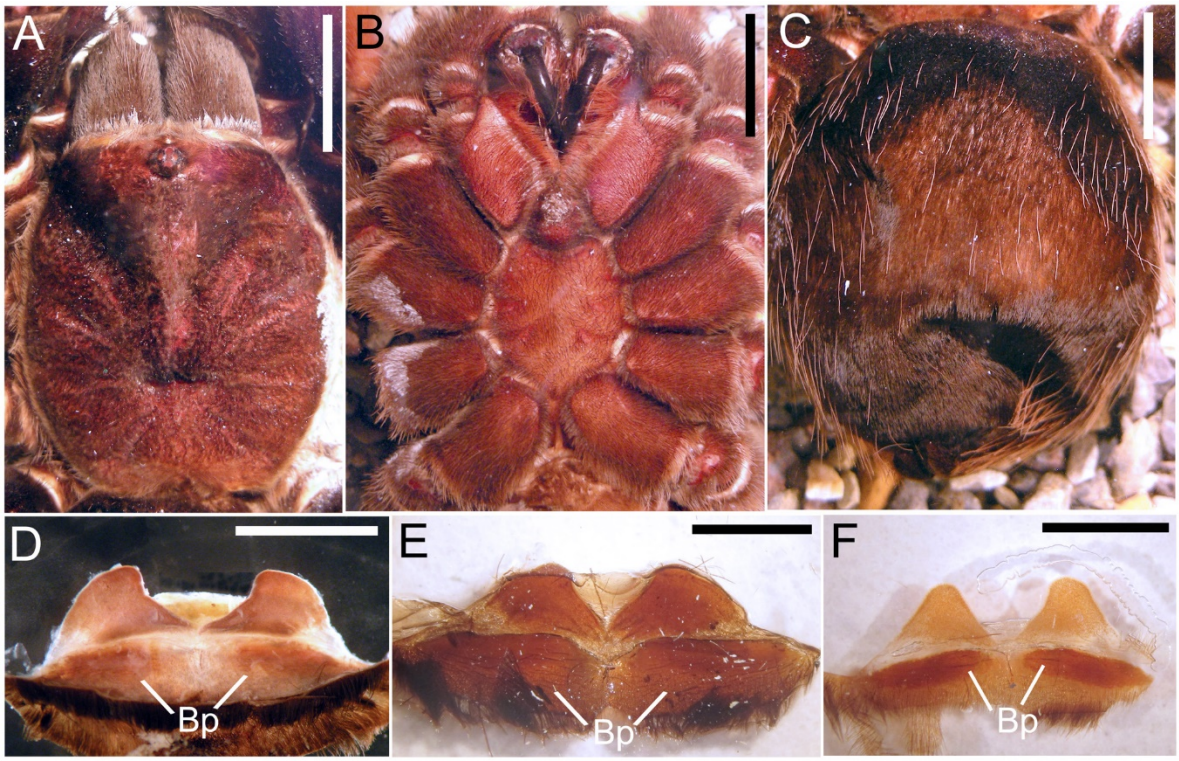


Fig 29. *Brachypelma klaasi*. A─D female CNAN-Ar007831. A, carapace, dorsal view; B, prosoma, ventral view; C, opisthosoma, dorsal view; D, spermatheca, ventral view; E─F, spermatheca ventral view of: E, female SMF-40599; F, female SMF-58101. Scale = 10mm (A─C), 1mm (D ─F).

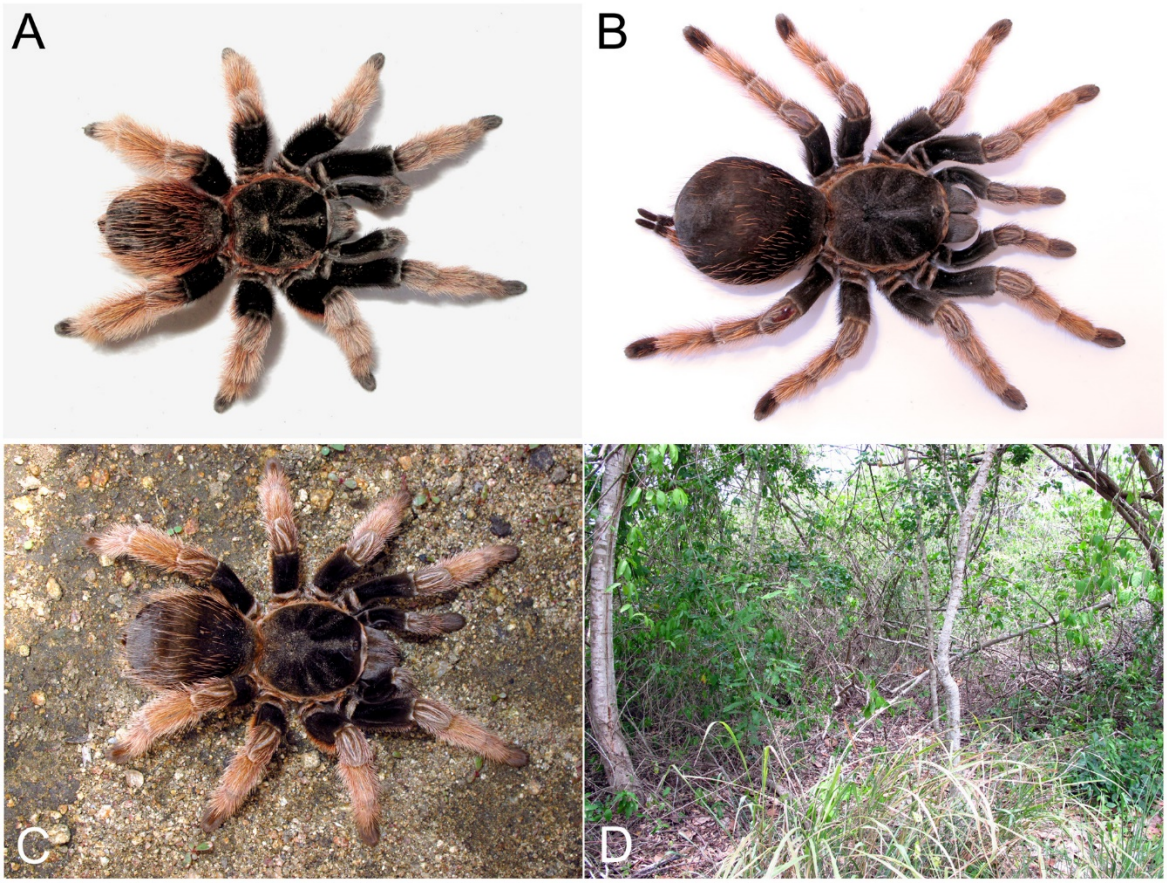
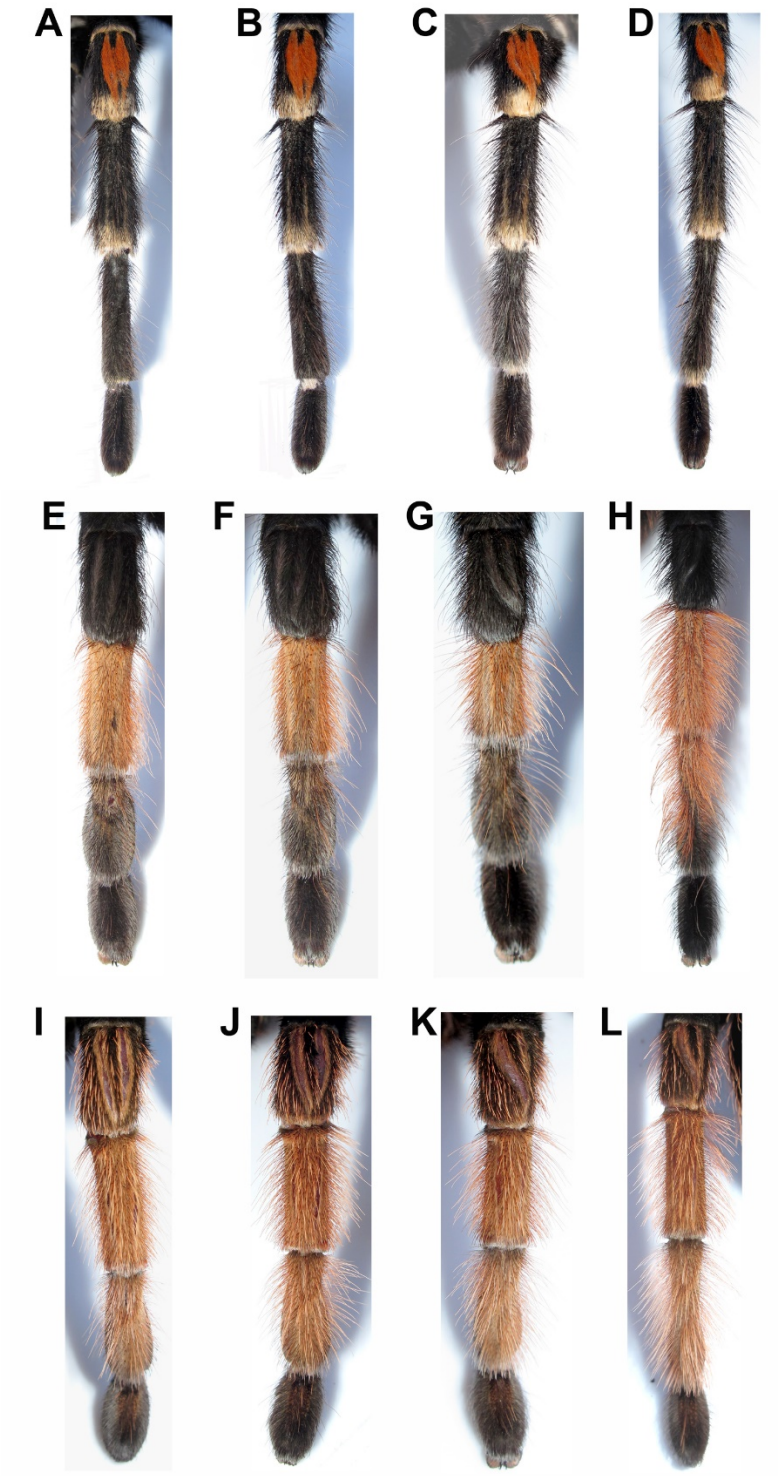


Fig 30. A─C, *Brachypelma klaasi*, habitus; D, habitat. A, male; B, female from coast line; C, female from inland; D, deciduous forest in the habitat of *B. klaasi*. Photos: J. Mendoza.



Figs 31. A–D, *Brachypelma auratum*, female (CNAN-Ar007878) A, leg I; B, leg II; C, leg III; D, leg IV; E–H, *Brachypelma emilia*, female (CNAN-Ar010602) E, leg I; F, leg II; G, leg III; H, leg IV. I–L, *Brachypelma klaasi*, female (CNAN-Ar007831) I, leg I; J, leg II; K, leg III; L, leg IV.

Figs 32. A─D, *Brachypelma baumgarteni*, female (CNAN-Ar007150) A, leg I; B, leg II; C, leg III; D, leg IV; E─H, *Brachypelma boehmei*, female (CNAN-Ar007905) E, leg I; F, leg II; G, leg III; H, leg IV.

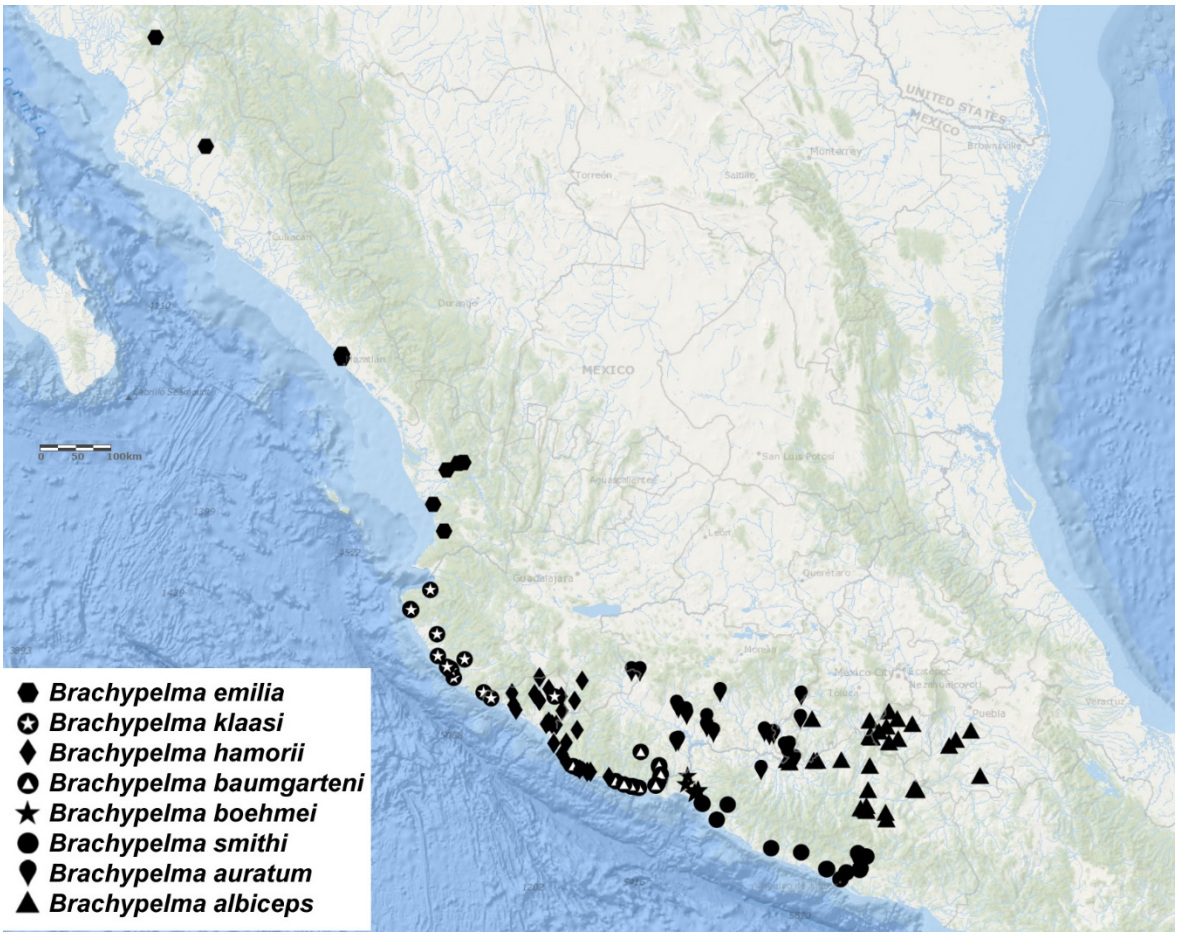


Fig. 33. Geographic distribution of the genus *Brachypelma* from published records and specimens collected or examined for this study.

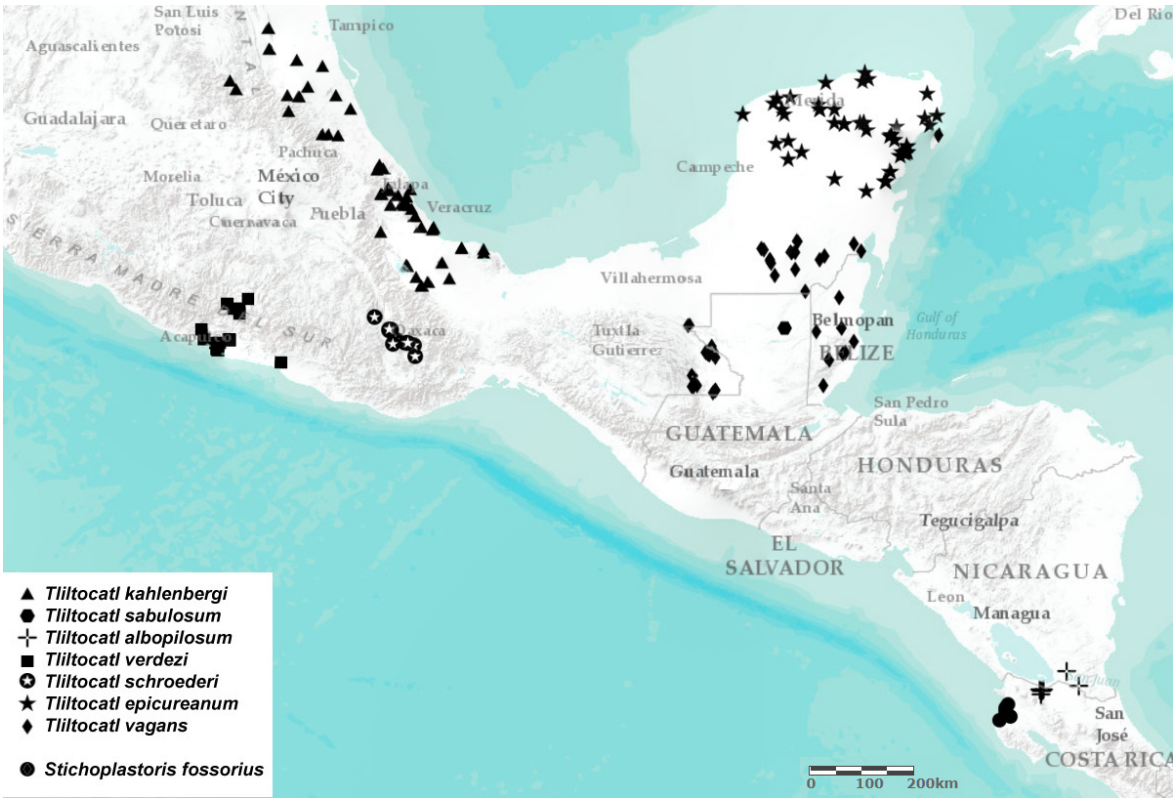


Fig. 34. Geographic distribution of the described species of the genus *Tliltocatl* from published records and specimens collected or examined for this study from Mexico to Costa Rica. Also, is recorded de distribution of *Stichoplastoris fossorius* in Costa Rica.

Table 1. Characters matrix used in cladistic analysis of *Brachypelma* sensu lato and sensu stricto. (–) inapplicable (?) unknown; treated as missing data in the analysis.

	1	2	3	4	5	6	7	8	9	10
	0	0	0	0	0	0	0	0	0	0
Psalmopoeus victori	0100021100002101101000000000000000000000000000000000000000000000000000000000000000000103330000--0---0--00----000-1-0									
Acanthoscurria geniculata	10001011122010-011???02111201101156100110000012222111210000000022222112211000100120120010001110012102-0									
Bonnetina cyaneifemur	0000210111211101100111000000000000001111000001121111111111000002211111211122110111110110-0010111040-0-0									
Xenesthis immanis	0110100111103121100100101110000100001111011112121211200110001131122112211101330124114012011120022O-2-0									
Megaphobema mesomelas	00001000213010-0102100000000000026001110000001121211121100000002111112211011330124113012001120003O-201									
Sericopelma melanotarsum	01001001011010-00????0100011000100001111111112442212131011211213222211231110133011410401210111001З0-201									
Aphonopelma seemanni	1100100101202100101100100000000005701121000110342302121011000212112212221111110010110111000110000113l-0									
Theraphosa stirmi	00002011123100-00????01111101100000000000000011111111100010000011111113200021330124O---1210101000310201									
Brachypelma albiceps	0000001011102111101100100010100010001000000001121111010000000002111111111121221111З0---11001110010131-0									
Brachypelma albopilosum	1100021111102101101100101010100010000111000121121111111111011102112110111112133011411201200111003З0-210									
Brachypelma auratum	1130001011101111101100100010100011611100000001111101110000000000201110110113003211141030110011100231220O									
Brachypelma baumgarteni	1131121012302111101100100010100012421000000001111111021100000002111111111130021112З0---11001110023132OO									
Bracnhypelma boehmei	1130021011101111101100110010100013331100000002121111111100000002111111111031032111410301100111002313200									
Brachypelma emilia	1020111012201111101100100010100010301100000001111111121000000002111111211031122011З0---11001110013122OO									
Brachypelma epicureanum	2000011021101101101100101010100010001111211211122211121111122212222111211121232011411201200111001З0-210									
Brachypelma fossorium	1100000111111102102000101010000000001121001111222211121111001203111111211010111113111012001100103O-210									
Brachypelma hamorii	1131121012102111101100110010100012211000000001011111111000000002111111111130021012З0---11001110023122OO									
Brachypelma kahlenbergi	2100011011202101101100100010100010001121011112132211121111011212111112111211321112112012001110013O-210									
Brachypelma klaasi	1000101011101111101100110010100014540000000001011111110000000001111111111131022011З0---11001110010131-0									
Brachypelma sabulosum	2100111111102101101100101110101010001111211211122211121111111111222211211121132011411201200111001З0-210									
Brachypelma schroederi	0000011022202101101100100010100010001111100101122211121110201102122111110121132011411201200111000З0-210									
Brachypelma smithi	1130101111202111101100111010100012110000000002111111111100100002021111111131132111410301100111002312200									
Brachypelma vagans	2100111111102101101100101110101010001111211212222211120110121111211111111102113201141120120011100130-210									
Brachypelma verdezi	2120111111202101101100100010100010001111001111112211121111011212112211221121132011211201200111002З0-210									

Appendix 1

List of Characters:

PROSOMA

Dorsal

1. Carapace of adult females, edges setae coloration: same as the carapace (0); clearest than the carapace (1); both can be present in the species (2).
2. Carapace of adult males and females, color dimorphism: absent (0); present (1).
3. Carapace of adult females, dorsal color pattern: single color (0); with dark stripes (1); with dark cephalic area (2); two or more patterns (3).
4. Coloration over chelicera of males and females: without (0); with (1), clear stripes.
5. Clypeus in males and females: absent (0); narrow (1); wide (2).
6. Fovea in males and females, curvature: straight (0); procurved (1); recurved (2).
7. Fovea in males and females, closure: slit-like (close) (0); pit-like (open) (1).
8. Fovea in males and females, depth: shallow (0); deep (1).

Ventral

9. Sternum, posterior sigillae, shape: oval enlarged (0); oval (1); circular (2).
10. Sternum, female posterior sigillae, its own distance from the margin: half (0); once (1); once and half (2).
11. Sternum, male posterior sigillae, its own distance from the margin: half (0); once (1); once and half (2); twice (3).
12. Tarsus IV, scopula: undivided (0); divided (1), by strong band of setae.
13. Metatarsus III, distal scopula, coverage percentage relative to the length of the metatarsus: 25% (0); 50% (1); 75% (2); 90% (3).
14. Metatarsus IV, distal scopula: absent (0); present (1).
15. Metatarsus IV, distal scopula, coverage percentage relative to the length of the metatarsus: 25% (0); 40% (1); 80% (2).
16. Male metatarsus I: straight (0); curved at base (1); curved at middle (2).
17. Male tibia I, tibial apophyses, absent (0); present (1).
18. Male tibia I, number of tibial apophysis: two (0); one (1).
19. Male metatarsus I, closes: between the two apophyses (0); over Rap (1); on retrolateral face of the apophyses (2).
20. Male tibia I, tibial apophyses, base: separated (0); fused (1).
21. Male tibia I, tibial apophyses: convergent (0); divergent (1).

Palpi

22. Male palpal tibia: without (0); with (1), retrolateral process.
23. Trochanter, retrolateral face setae: normal (0); plumose (1); claviform (2).
24. Femur, retrolateral face setae: normal (0); plumose (1).

Legs

25. Coxa I, prolateral face setae: normal (0); plumose (1).
26. Coxa I, retrolateral face setae: normal (0); plumose (1).
27. Trochanter I, prolateral face setae: normal (0); plumose (1); claviform (2).
28. Trochanter I, retrolateral face setae: normal (0); plumose (1).

29. Femur I, prolateral face setae: normal (0); plumose (1).
30. Coxa II, prolateral face setae: normal (0); plumose (1).
31. Trochanter II, prolateral face setae: normal (0); plumose (1).
32. Femur IV, retrolateral face setae: normal (0); plumose (1).
33. Male, femur III: not incrassate (0); slightly incrassate (1); swollen (2).
34. Males and females, patellae dorsal color pattern: none (usually black) (0); only central reddish (flame-shape) (1); central reddish with yellowish around (2); overall reddish (3); large reddish setae (4); white stripes (5); orange stripes (6).
35. Males and females, tibiae color pattern: none (usually black) (0); distal half reddish (1); distal half yellowish (2); completely orange (3); completely yellowish (4); large reddish setae (5); distal whitish (6); white stripes (7).
36. Males and females, metatarsus color pattern: none (usually black) (0); whitish on distal fourth (1); completely yellowish with one stripe (2); completely orange (3); large reddish setae (4).

Male spination

37. Femur I spines: none (0); 1 to 3 (1); 4 to 7 (2).
38. Femur II spines: none (0); 1 to 3 (1); 4 to 7 (2).
39. Femur III spines: none (0); 1 to 5 (1); 6 to 10 (2); 11 to 15 (3).
40. Femur IV spines: one (0); 1 to 5 (1); 6 to 10 (2); 11 to 15 (3).
41. Palp patella spines: none (0); one (1); two (2).
42. Patella I spines: none (0); 1 to 2 (1).
43. Patella II spines: none (0); 1 to 2 (1).
44. Patella III spines: none (0); 1 to 2 (1); 3 to 4 (2).
45. Patella IV spines: none (0); 1 to 2 (1); 3 to 4 (2).
46. Palp tibia spines: none (0); 1 to 5 (1); 6 to 10 (2); 11 to 15 (3).
47. Tibia I spines: none (0); 1 to 5 (1); 6 to 10 (2); 11 to 15 (3); 16 to 20 (4).
48. Tibia II spines: none (0); 1 to 5 (1); 6 to 10 (2); 11 to 15 (3); 16 to 20 (4).
49. Tibia III spines: 1 to 9 (0); 10 to 19 (1).
50. Tibia IV spines: 1 to 9 (0); 10 to 19 (1); 20 to 29 (2).
51. Metatarsus I spines: none (0); 1 to 5 (1).
52. Metatarsus II spines: none (0); 1 to 5 (1); 6 to 9 (2).
53. Metatarsus III spines: 1 to 9 (0); 10 to 19 (1).
54. Metatarsus IV spines: 1 to 9 (0); 10 to 19 (1); 20 to 29 (2); 30 or more (3).

Female spination

55. Femur I spines: none (0); 1 to 3 (1).
56. Femur II spines: none (0); 1 to 3 (1).
57. Femur III spines: none (0); 1 to 5 (1).
58. Femur IV spines: none (0); 1 to 3 (1).
59. Palp patella spines: none (0); one (1); two (2).
60. Patella I spines: none (0); one (1); two (2).
61. Patella II spines: none (0); one (1); two (2).
62. Patella III spines: none (0); one (1); two (2).
63. Patella IV spines: none (0); one (1).
64. Palp tibia spines: none (0); 1 to 5 (1); 6 to 10 (2); 11 to 15 (3).
65. Tibia I spines: none (0); 1 to 5 (1); 6 to 10 (2).

66. Tibia II spines: none (0); 1 to 5 (1); 6 to 10 (2).
67. Tibia III spines: none (0); 1 to 9 (1); 10 to 19 (2).
68. Tibia IV spines: none (0); 1 to 9 (1); 10 to 19 (2).
69. Metatarsus I spines: none (0); 1 to 5 (1).
70. Metatarsus II spines: none (0); 1 to 5 (1); 6 to 9 (2).
71. Metatarsus III spines: none (0); 1 to 9 (0); 10 to 19 (1); 20 or more (2).
72. Metatarsus IV spines: 1 to 9 (0); 10 to 19 (1); 20 to 29 (2); 30 or more (3).

Male palpal bulb.

73. Subtegulum, length relative to width: equal (0); longer (1).
74. Tegulum, length relative to width: equal (0); 1.5x longer (1).
75. Embolus, retrolateral curvature: not curved (0); slightly curved (1); curved (2); curved only in apex (3).
76. Embolus, length relative to tegulum: shorter (0); equal (1); 1.5x longer (2); 2x or more longer (3).
77. Embolus, length relative to width of embolus base: 1.5x (0); 2x (1); 2.5x (2); 3x or more (3).
78. Embolus, apical region: long and slender (0); short and tapering (1); slightly broad (2); broad (3).
79. Embolus apical shape: Conical, retrolateral side slightly convex (0); laterally flattened, retrolateral slightly concave (1); very flattened, concave/convex appearance with neck (2); very flattened, concave/convex appearance without neck (3).
80. Tegular apophysis: absent (0); present (1).
81. Embolus, prolateral superior keel: absent (0); present (1).
82. Prolateral superior keel development: slightly developed (0); narrow (1); broad (2).
83. Embolus, prolateral superior keel position: transversal (0); longitudinal prolateral (1); longitudinal superior larger than apical keel (2); longitudinal superior shorter than apical keel (3); longitudinal superior equal than apical keel (4).
84. Embolus, prolateral inferior keel: absent (0); present (1).
85. Embolus, prolateral inferior keel development: slightly developed (0); narrow (1); broad (2).
86. Embolus, prolateral inferior keel position: transversal (0); longitudinal prolateral (1); longitudinal superior larger than prolateral superior keel (2); longitudinal superior shorter than prolateral superior keel (3); longitudinal superior equal than prolateral superior keel (4).
87. Embolus prolateral inferior keel: not denticulate (0); denticulate (1).
88. Embolus, apical keel: absent (0); present (1).
89. Embolus, apical keel development: slightly, restricted to embolus apex (0); developed, slightly extended backwards (1), widely extended backwards (2).
90. Embolus, prolateral superior keel and apical keel fusion at apex: not fused (0); fused (1).
91. Embolus, retrolateral keel: absent (0); present (1).

OPISTHOSOMA

Dorsal

92. Urticating setae: absent (0); present (1).

93. Urticating setae, type I: absent (0); present (1).
94. Urticating setae, type III: absent (0); present in both sexes (1); present in only one sex (2).
95. Urticating setae, arranged in patch: one dorso-lateral posterior (0); one dorso-median (1).
96. Urticating setae, borders of patch: poorly defined (0); well defined (1).
97. Opisthosoma, pattern coloration: large setae of same color (0); with large reddish setae (1); with large yellowish setae (2); with large whitish setae (3).

Ventral

98. Spermathecae fusion: completely separated (0); partially fused at base (1); widely fused at base, with the middle sclerotized (2); completely fused, there is no trace of the two spermatheca (3); fused single thin receptacle.
99. Spermatheca baseplate: absent (0); present (1).
100. Spermatheca, baseplate shape: fused, as wide as base width (0); fused, reduced to central base (1); divided, as wide as base width (2); divided, narrower than base width (3).
101. Spermatheca, length relative to width: larger (0); equal (1); shorter (2).
102. Fused spermatheca, superior edge: straight (0); curved medially inward (1).
103. Fused spermatheca, apical cross-section: swollen (0); flat (1).

Appendix 2. Genbank accession codes for tissue samples, deposited in the Laboratorio de Sistemática Molecular (Zoología) at the Instituto de Biología, UNAM, Mexico City, from which DNA was extracted and sequenced for phylogenetic analyses of 8 species in the genus *Brachypelma* Simon 1891 and 7 species in the *Tliltocatl* genus. *P. cambridgei*, *E. campestratus*, *Lasiodora parahybana* and *Xenesthis immanis* are found in GenBank.

Species	Specimen	Voucher ID	COI
Psalmopoeus cambridgei	1 juv. Un	447_SC_AB	JQ412455
Eupalaestrus campestratus	1 juv. Un	446_SC_AB	JQ412446
Lasiodora parahybana	unk	MNHN-JAC99	JN018128
Megaphobema mesomelas	1 juv. ♀	CNAN-DNA-Ara0284	unreleased
Sericopelma melanotarsum	1 juv. ♀	CNAN-DNA-Ara0293	unreleased
Aphonopelma seemanni	1 juv. ♀	CNAN-DNA-Ara0292	unreleased
Xenesthis immanis	1 juv. Un	none	MG273518
Stichoplastoris fossorius	1 juv. ♀	CNAN-DNA-Ara0285	unreleased
Brachypelma albiceps	1 ♂	CNAN-Ar007843	KT995398
Brachypelma albiceps	1 juv. ♀	CNAN-Ar007839	KT995331
Brachypelma albiceps	1 juv. ♀	CNAN-Ar007850	KT995391
Brachypelma albiceps	1 ♀	CNAN-Ar007852	KT995384
Brachypelma albiceps	1 ♀	CNAN-Ar007865	KT995354
Brachypelma auratum	1 ♀	CNAN-Ar003657	KT995338
Brachypelma auratum	1 ♂	CNAN-Ar007878	KT995353
Brachypelma auratum	1 ♂	CNAN-Ar003658	KT995348
Brachypelma auratum	1 juv. ♀	CNAN-Ar007136	KT995371
Brachypelma auratum	1 juv. ♀	CNAN-Ar007164	KT995397
Brachypelma baumgarteni	1 ♂	CNAN-Ar007149	KT995386
Brachypelma baumgarteni	1 ♀	CNAN-Ar007150	KT995402
Brachypelma baumgarteni	1 ♀	CNAN-Ar007151	KT995332
Brachypelma baumgarteni	1 juv. ♀	CNAN-Ar007161	KT995395
Brachypelma baumgarteni	1 subad. ♂	CNAN-Ar007835	KT995382
Brachypelma boehmei		CNAN-Ar007905	unreleased
Brachypelma boehmei	1 ♀	CNAN-Ar007185	KT995359
Brachypelma boehmei	1 ♀	CNAN-Ar007186	KT995337
Brachypelma boehmei	1 ♂	CNAN-Ar007833	KT995343
Brachypelma emilia	1 ♀	CNAN-Ar007898	unreleased
Brachypelma emilia	1 ♂	CNAN-Ar007899	unreleased
Brachypelma emilia	1 ♂	CNAN-Ar007173	KT995365
Brachypelma emilia	1 ♂	CNAN-Ar003599	KT995326
Brachypelma emilia	1 ♀	CNAN-Ar007153	KT995350
Brachypelma hamorii	1 ♂	CNAN-Ar003614	KT995325
Brachypelma hamorii	1 ♂	CNAN-Ar007163	KT995334

Brachypelma hamorii	1 ♂	CNAN-Ar007168	KT995378
Brachypelma hamorii	1 ♂	CNAN-Ar007826	KT995381
Brachypelma hamorii	1 ♂	CNAN-Ar007827	KT995387
Brachypelma klaasi	1 ♀	CNAN-Ar003333	KT995330
Brachypelma klaasi	1 ♂	CNAN-Ar003341	KT995329
Brachypelma klaasi	1 ♀	CNAN-Ar007845	KT995349
Brachypelma klaasi	1 ♂	CNAN-Ar007160	KT995340
Brachypelma klaasi	1 ♂	CNAN-Ar007162	KT995346
Brachypelma smithi	1 ♀	CNAN-Ar004131	KT995375
Brachypelma smithi	1 juv. ♂	CNAN-Ar007140	KT995380
Brachypelma smithi	1 ♀	CNAN-Ar007143	KT995400
Brachypelma smithi	1 ♀	CNAN-Ar007144	KT995385
Brachypelma smithi	1 ♂	CNAN-Ar007832	KT995374
Tliltocatl albopilosum	1 juv. ♂	CNAN-DNA-Ara0290	unreleased
Tliltocatl epicureanum	1 ♀	CNAN-Ar007886	unreleased
Tliltocatl epicureanum	1 ♀	CNAN-Ar007881	unreleased
Tliltocatl epicureanum	1 ♀	CNAN-Ar007880	unreleased
Tliltocatl epicureanum	1 ♀	CNAN-Ar007884	unreleased
Tliltocatl epicureanum	1 ♀	CNAN-Ar007885	unreleased
Tliltocatl kahlenbergi	1 ♀	CNAN-Ar003424	KT995356
Tliltocatl kahlenbergi	1 ♂	CNAN-Ar007166	KT995397
Tliltocatl kahlenbergi	1 ♂	CNAN-Ar003598	KT995358
Tliltocatl kahlenbergi	1 ♀	CNAN-Ar007113	KT995361
Tliltocatl kahlenbergi	1 ♂	CNAN-Ar007117	KT995388
Tliltocatl sabulosum	1 ♂	CNAN-Ar007169	KT995373
Tliltocatl sabulosum	1 ♂	CNAN-Ar003326	KT995404
Tliltocatl sabulosum	1 ♀	CNAN-Ar003343	KT995403
Tliltocatl schroederi	1 juv. ♂	CNAN-Ar007139	KT995399
Tliltocatl schroederi	1 ♀	CNAN-Ar007868	KT995376
Tliltocatl schroederi	1 ♀	CNAN-Ar007892	KT995333
Tliltocatl schroederi	1 juv. ♂	CNAN-Ar003414	KT995345
Tliltocatl schroederi	1 ♀	CNAN-Ar007893	KT995390
Tliltocatl vagans	1 juv. ♂	CNAN-Ar003327	unreleased
Tliltocatl vagans	1 ♀	CNAN-Ar003670	KT995342
Tliltocatl vagans	1 ♀	CNAN-Ar007167	unreleased
Tliltocatl verdezi	1 ♀	CNAN-Ar007844	KT995352
Tliltocatl verdezi	1 ♀	CNAN-Ar007848	KT995392
Tliltocatl verdezi	1 juv. ♂	CNAN-Ar007849	KT995379
Tliltocatl verdezi	1 ♀	CNAN-Ar007853	KT995369

Capítulo 3

Utilidad de las secuencias de genes mitocondriales y nucleares en especies prioritarias para la conservación.

Sección 3.1.

Artículo en preparación

THE USE OF MOLECULAR MARKERS WITH VULNERABLE TARANTULAS (ARANEAE, THERAPHOSIDAE, THERAPHOSINAE) RESOLVES THEIRPHYLOGENETIC RELANTIONSHIPS AND REVEALS HIDDEN DIVERSITY

THE USE OF MOLECULAR MARKERS WITH VULNERABLE TARANTULAS (ARANEAE, THERAPHOSIDAE, THERAPHOSINAE) RESOLVES THEIRPHYLOGENETIC RELANTIONSHIPS AND REVEALS HIDDEN DIVERSITY

JORGE I. MENDOZA MARROQUIN[A,B] & OSCAR F. FRANCKE[B]

[A]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Av. Universidad 3000, C.P. 04510, Coyoacán, Distrito Federal, México.

[B]Colección Nacional de Arácnidos, Módulo D planta baja, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, 3er circuito exterior, Apto. Postal 70-153, CP 04510, Ciudad Universitaria, Coyoacán, Distrito Federal,
[C]urn:lsid:zoobank.org:author:7BA11142-CBC1-4026-A578-EBAB6D2B6C0C
[D]corresponding author: nomeireth@hotmail.com

Abstract.

The use of molecular markers for tarantula taxonomy had been limited until recently, with most previous studies relying on morphological methods. Approaches based on molecular data have the potential to provide better resolution with robust support in situations where morphological characters have proven problematic. We present a multilocus phylogenetic analysis of the genera *Brachypelma* and *Tliltocatl*, employing one mitochondrial (COI) and three nuclear (EF1G, MID1IP1 and MRPL44) loci. We included all currently valid *Brachypelma* and *Tliltocatl* species, as well as 14 additional species and 9other undetermined species. Phylogenetic inference was performed using Maximum Likelihood and Bayesian methods. Both analyses recovered *Brachypelma* and *Tliltocatl* as monophyletic with high support. Internal relantionships within *Brachypelma* species were resolved, and undescribed species of *Tliltocatl* was found. *Brachypelma* is shown to be related with South American genera, wheres *Tliltocatl* is more closely related to the Central American ones. *Brachypelma* is restricted to three biogeographic areas along Pacific Coast of Mexico; and *Tliltocatl* is distributed in six biogeographic regions from southeastern Mexico to Costa Rica. The use of molecular markers for conservation of these priority tarantula species is discussed.

Key words: nuclear genes, barcode, tarantulas, conservation, distribution

Introduction

In 2015, the governments of Canada, Mexico and the United States initiated a collaborative project through the Commission for Environmental Cooperation (CEC) to strengthen the conservation and sustainable trade of 56 North American taxa that are included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). These 56 taxa were organized into five groups: parrots, sharks, tarantulas, timber species (specific cacti and tropical hardwoods), and turtles and tortoises. Later, a stakeholder consultation was held in Mexico City to gather information and recommendations for actions to promote sustainable trade and conservation of the priority tarantula species (CEC 2017). This action plan includes information on sixteen priority tarantula species, comprising one from the genus *Aphonopelma* and 15 from the genus *Brachypelma*. In Mexico, wild populations of *Brachypelma* tarantulas are in decline, due to both habitat loss and to large numbers being collected to being either sold in traditional local markets or exported illegally for the commercial pet trade (Smith 1994, West 2005). Species of the priority tarantulas vary in their adult coloration and markings. For example, some species have dark-colored legs, whereas others have legs that display distinct bands of colors (Hijmensen 2012). These morphological differences are used to identify adult specimens. The differences between species can, however, be subtle and vary between specimens of the same species. Identification of those species that do not exhibit bright colors or bold patterns may be more difficult and requires expertise (Mendoza & Francke 2017, Mendoza & Francke 2018 in rev.).

In most groups, traditional taxonomic research is based on morphological characters. This can result in many, sometimes intractable, problems, especially in groups that have great homoplasy or cryptic and polymorphic species (Xiao *et al.* 2010). With modern day extinction rates estimated at 1000–10,000 times higher than the background rate (Barnosky *et al.* 2011), effective approaches are desperately needed to accelerate species discovery and identification. New species discovery and associated diversity (e.g., molecular, morphological, ecological) is paramount to understanding evolutionary patterns and processes. As is commonplace in arachnological taxonomy (Coddington 2005; Ramírez

2014), the systematics of theraphosid spiders is largely based on analysis of morphological characters. Using those, Raven (1985) formulated a first evolutionary hypothesis on intrafamilial relationships, but later recognized the limits of this morphology-based approach by referring to Theraphosidae as a "taxonomic nightmare" (Raven 1990) The group is ancient (Penney & Selden 2011, Selden & Gall 1992), morphologically homogeneous (Bond & Hedin 2006), and prone to genetic structuring at a microgeographical scale (first demonstrated by Bond *et al.* 2001); mygalomorph spiders possess life-history traits that markedly differ from their sister lineage (the infraorder Araneomorphae) and most other arthropod groups. They typically have limited dispersal abilities, display site and habitat fidelity, require a long time to reach sexual maturity (4–7 years), and have long life spans (15–30 years) (Bond *et al.* 2001, Hendrixson & Bond 2005, Arnedo & Fernández 2007, Hendrixson & Bond 2007, Starrett & Hedin 2007, Stockman & Bond 2007, Bond & Stockman 2008, Cooper *et al.* 2011, Hedin & Carlson 2011, Satler *et al.*, 2011, Hedin *et al.* 2013). Due to this suite of unique life-history traits, these taxa are often vulnerable to stochastic processes and therefore provide ideal candidates for evolutionary, biogeographical, and conservation studies (Raven 1980, Hedin & Bond 2006, Hendrixson & Bond 2007, Hamilton *et al.* 2011, Bond *et al.* 2012, Bond 2012, Hendrixson *et al.* 2013, Opatova *et al.* 2013). Taken together, these traits have needed more integrative approaches to species delimitation that employ molecular, geographical, and ecological data (Bond *et al.* 2006, Arnedo & Fernández 2007, Hendrixson & Bond 2007, Starrett & Hedin 2007, Stockman & Bond 2007, Bond & Stockman 2008, Cooper *et al.*, 2011, Hamilton *et al.* 2011, Satler *et al.* 2011, 2013, Hedin *et al.* 2013, Hendrixson *et al.*, 2013).

In recent years, several molecular-based studies focused on Theraphosidae species delimitation, identification and species-level phylogeny have been made. Most of them have dealt with the United States species of *Aphonopelma* Pocock 1901 (Graham *et al.*, 2015; Hamilton *et al.*, 2016, 2014, 2011, Hendrixson *et al.*, 2015, 2013; Wilson *et al.*, 2013), Uruguayan *Grammostola* Simon 1892 (Montes de Oca *et al.*, 2015), and some studies have focused on Mexican *Brachypelma* Simon 1891 (Longhorn *et al.* 2007, Petersen *et al.* 2007, Machkour-M'Rabet *et al.* 2009, Mendoza & Francke 2017, Mendoza & Francke 2018 in rev., Turner *et al.* 2018). The usefulness of COI barcode marker to identify accurately priority tarantula species of *Brachypelma* and *Tliltocatl* was showed by

Mendoza and Francke (2017) and Mendoza & Francke 2018 in rev. However, its use to solve phylogenies is limited, as Hamilton *et al.* (2016) mentioned that the limitations of mtDNA used for phylogenetic studies are gene tree/species tree incongruence and the haploid, non-recombining nature of the molecule, with CO1representing only one particular genealogy out of all possible within a genome. These limitations are exposed in the recent phylogenies for *Brachypelma* and *Tliltocatl*, with strong support for the monophyly of each genus, but with low resolution on some inner clades between different species (Mendoza & Francke 2017, Mendoza & Francke 2018 in rev.). The objectives of this study are threefold: (1) to evaluate the efficiency of molecular-based species phylogenetic relationships based on nuclear and mitocondrial markers; (2) identify the biogeographic distribution of *Brachypelma* and *Tliltocatl* to understand better the relationships between species; and (3) to integrate methodologies so that an efficient, consistent, and more effective DNA barcoding strategy can be employed for delimiting species of priority tarantula species for their conservation.

Methods

Taxon sampling

Specimens were collected throughout the known distribution of the genera *Brachypelma* and *Tliltocatl* with special attention to the type localities (where possible). Material was fixed in 80% ethanol. The third leg on the right side of each spider was stored in 96% ethanol at -20°C. Tissue samples of 68 specimens were used for DNA extraction, representing 8 species of *Brachypelma*, 7 species of *Tliltocatl* with ten undetermined species and sixteen specimens from other genera used as outgroup. We used in general three sequences for each known species of both *Brachypelma* and *Tliltocalt*, representing the northern, central and southern limits of the distribution of each species. We included a wide representation of taxa to assess *Brachypelma* and *Tliltocatl* relationships, due mainly to the uncertainty with some *Tliltocatl* species. We included species from Central and North American tarantula lineages that share habitat and similarities with *Brachypelma* and *Tliltocatl*: *Cardiopelma mascatum* Vol 1999, *Sphaerobothria hoffmanni* Karsch 1879, *Aphonopelma seemanni* (F. O.-Pickard Cambridge1897), *Aphonopelma chalcodes* Chamberlin 1940, *Hemirrhagus coztic* Pérez-Miles & Locht 2003, *Hemirrhagus pernix* (Ausserer 1875),*Megaphobema mesomelas* (O. Pickard-Cambridge 1892), *Sericopelma*

melanotarsum Valerio 1980, *Psalmopoeus victori* Mendoza 2014, *Eupalaestrus campestratus* (Simon, 1891), *Lasiodora parahybana* Mello-Leitao 1917, *Xenesthis immanis* (Ausserer 1875) were used in this study. Additionally, one sequence for COI was retrieved from GenBank to use as outgroup for phylogenetic analyses: *Bonnetina tenuiverpis* Ortiz & Francke 2015 [KC807369.1]. We also included sequences of unidentified taxa from the genera: *Cotztetlana* Mendoza 2012 and *Aphonopelma* Pocock 1901**.** Vouchers were deposited in the Coleccion Nacional de Aracnidos (CNAN), Instituto de Biologia, UNAM, Mexico and assigned a unique number (CNAN-Ar00xxxx). All sequences were submitted to GenBank, and accession numbers and specimen information are in Table 1. *Brachypelma* specimens were identified from previous integrative studies (Mendoza & Francke 2017, Mendoza & Francke 2018 in rev.). Individuals with doubtful species assignment were tagged by the collection locality names or unpublished names (e.g. *Tliltocatl sp.* Lagartero). When species delimitation was necessary in absence of a solid framework, we used the confidence thresholds proposed by Mendoza & Francke (2018 in rev.): 2% for maximum COI intra-specific variability and 4% for minimum inter-specific variability.

DNA protocols

DNA isolation, PCR amplification and sequencing were performed at the Laboratorio de Sistemática Molecular, Instituto de Biología, UNAM. Muscle tissue was extracted from the leg by removing ~20 mg of tissue. Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit™, following the manufacturer's protocol. The concentration quality of the extracted DNA was quantified with a spectrophotometer (Nanodrop 2000 Techno Scientific) or visualized via agarose gel electrophoresis. Three nuclear and one mitochondrial genetic markers were used. The fast evolving mitochondrial Cytochrome c Oxydase subunit I (COI) was chosen for its potential to solve the shallow to middle branches of the phylogeny. To resolve the middle to deepest branches, we employed the slower-evolving Elongation Factor I subunit Gamma (EF1G), MID1 Interacting Protein 1 (MID1IP1) and Mitochondrial Ribosomal Protein L44 (MRPL44). COI sequences were mostly taken from previous studies (Mendoza & Francke 2017, Mendoza & Francke 2018 in rev.), or were amplified as indicated there. We followed the protocols and primers to amplify EF1G, MID1IP1 and MRPL44 (Table 2). All three markers were amplified using

nested PCR to maximize the specificity of the amplification and the quality of the reads (Ortiz & Francke 2018 in rev.). DNA amplification was performed using the polymerase chain reaction (PCR) for the four genes as follows: COI (100.8 µl) contained 48 µl 10x PCR-buffer, 24 µl $MgCl_2$, 7.68 µl of forward and reverse primer each, 9.6 µl dNTP's and 3.84 µl *Taq* polymerase, using 1 µl of the DNA template for each sample. PCR program for CO1 followed initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 45 s, elongation at 72°C for 2:30 min; followed by 7 min of final elongation at 72°C. LCO 1490 and HCO 2198 primers used for single-stranded sequencing. External PCR reactions for nuclear genes were performed at 12.5 µl using mostly reagents from the TaKaRa ExTaq DNA Polymerase kit (Takara Bio Inc: Shiga, Japan) and contained: 7.7 µl of water, 1.25 µl of 10X PCR Buffer, 1 µl of 20mM MgCl2, 1 µl of 2.5 mM dNTP Mix, 0.0625 µl (0.32 U) of Taq DNA Polymerase, 0.25 µl of each 10 pmol primer, and 1 µl of DNA. All external reactions PCR protocols included touchdown procedures. *EF1G* was amplified as described by Bond *et al.* (2012). *MID1IP1* was amplified using 26 cycles of denaturation at 94ºC for 30 s, initial annealing at 60ºC for 30 s (-0.5ºC per cycle), and elongation at 72ºC for 1 min; this was followed by 14 cycles of denaturation at 94ºC for 30 s, annealing at 48ºC for 30 s, and elongation at 72ºC for 1 min. *MRPL44* was amplified using 26 cycles of denaturation at 94ºC for 30 s, initial annealing at 63ºC for 30 s (-0.5ºC per cycle), and elongation at 72ºC for 1 min; this was followed by 14 cycles of denaturation at 94ºC for 30 s, annealing at 50ºC for 30 s, and elongation at 72ºC for 1 min. Internal PCR reactions were performed identically for EF1G, MID1IP1 and MRPL44, using the GoTaq Green Master Mix kit (Promega: Fitchburg, WI, USA). 25 µl reactions contained 9.05 µl of water, 12.5 µl of GoTaq Green Master Mix, 1.25 µl of 10 µg/µl bovine serum albumin, 0.6 µl of 10 pmol primer, and 1 µl of external PCR product after being diluted in 100 µl of water. The thermal protocol was: 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, and elongation at 72ºC for 1 min. In case of failure, a second attempt was made using 1 µl of undiluted external PCR product. The products obtained from the internal PCR reaction were purified using Amicon Ultra-0.5 centrifugal filters (Millipore: Billerica, MA, USA) or ExoSAP-IT (Affymetrix: Santa Clara, CA, USA). The accuracy of sequences was verified by independently amplifying and sequencing the complementary strands of all fragments. Primer sequences were removed

and complementary strands of DNA assembled into consensus sequences, edited and checked for quality using Geneious R8 (Kearse *et al.* 2012). If complementary strands disagreed (besides minor mismatches), the sample was amplified and sequenced again to resolve the discrepancies.

DNA sequence alignment and phylogenetic analysis

Static alignments of gene fragments were generated with MAFFT online version 7 (Katoh *et al.* 2002, 2005). The G-INS-I strategy, which performs a global alignment based on an FFT approximation**,** was selected (Katoh *et al.* 2002). This method is suitable for large datasets comprising sequences with relatively limited variation in length, i.e. few short gaps (Katoh *et al.* 2005). The scoring matrix for nucleotide sequences was set to 1/PAM $_K$ = 2, gap opening penalty to 1.53, and offset value to 0.

We carried out Maximum Likelihood inference using RAxML-HPC BlackBox on XSEDE (8.2.8) (Stamatakis *et al.* 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller *et al.* 2010) under GTR+gamma+Pinvar model of nucleotid evolution and Bootstrap support obtained by running 1000 pseudo replicates. The relative supports are shown for each clade on the best tree. We also carried out Bayesian inference using MrBayes ver 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The best fitting model of sequence evolution was selected using jModelTest ver 1.0.1 (Posada 2008), in accordance with the Akaike information criterion, based on which GTR + G + I model was applied. The analysis comprised four Markov chain Monte Carlo models, performed for 10 million generations for all concatenated DNA sequence alignments. Trees were sampled every 1000 generations, those sampled before stationarity were discarded using the *burnin* command.

As extra evidence to test the congruence in the species relationships we used the historical geology of the biogeographic provinces where they are distributed. Based on the biogeographic provinces proposed by Morrone (2005), it was established that *Brachypelma* is distributed in three provinces: 1) Costa del Pacífico (CPA), Sierra Madre Sur (SMS) and Depresión del Balsas (DBA) (Fig 3), whereas *Tliltocatl* is distributed in six prvovinces: 1)

Costa del Pacífico, (CPA), 2) Sierra Madre Sur (SMS), 3) Golfo de México (GM), 4) Yucatán (YUC) and 5) Petén (PET) and 6) Central America (CAM) (Fig 4).

Results

Phylogenetic analyses

Sequences of thirty-eight species were obtained, from these twenty-nine belong to formally described species and the remaining nine are from undescribed species. Most molecular protocols successfully amplified individuals across all main lineages, except for that of MRPL44, which failed to yield results with three specimens of the species that have only type I urticating setae (*Aphonopelma seemanni* CNAN-DNA-Ara0282, *Aphonopelma sp* Compostela and *Sphaerobothria hoffmanni*).

Maximum likelihood inference and MrBayes analyses of the sequences are congruent and mostly resolved and strongly supported (Fig 1, 2). Final ML optimization score was -5416.771443. The ML topology recovered shows two main clades, the first one formed by species of *Tliltocatl* with a bootstrap value of 96 and showing 6 undescribed species. Most of the currently recognized species of the genus have high bootstrap values. The second one formed by the eight recognized *Brachypelma* species (Fig 1). The *Brachypelma* clade has a bootstrap support value of 88, most of the species and the interspecific relantionships have high bootstrap values; lowest botstrap values and shorter distances are for *B. boehmei, B. baumgarteni* and *B. auratum*, a result is consistent with that obtained by Mendoza & Francke (2017, 2018 in rev.). The lineage of *Brachypelma* is closely related to some South American lineages, including the Brazilian and Colombian species used in the analyses; however, the inner relationships have low support values, possibly due to the lack of additional samples and the genetic distances between these genera. The genus *Tliltocatl* is shown more closely related to Central American lineages. Distances within *Tliltocatl* are short, so this could indicate a very recent radiation of its species, and this can also explain why some inner relationships cannot be easily resolved. In *Brachypelma* most distances are large and consistent, except for the species distributed in the area of the Balsas river and it is possible that the most recent species radiation has been throughout this area. This in turn could explain why species of this zone have very similar coloration and morphology.

Bayesian inference analyses also recovered the same two clades as in ML, one including all *Brachypelma* species and the second one including all *Tliltocatl* species, both genera with high posterior probability, 0.96 for *Brachypelma* and 1 for *Tliltocatl* (Fig 2). The topology and inner relationships between species in both clades is very similar to that on the ML analyses; the six undescribed taxa of *Tliltocatl* are also recovered, and the inner relationships within *Brachypelma* are resolved with high posterior probability values (Fig 2).

Molecular results were compared with the morphospecies for the discrimination of species. When one molecular marker identifies molecular species consistent with observed morphospecies, we consider that this marker identifies the individuals into true species. We are also aware of the possibility that molecular data further split morphospecies into more cryptic species as was observed with specimens previously identified by morphology as *Tliltocatl kahlenbergi* and *Tliltocatl sabulosum*. Although a further morphological revision on *Tliltocatl* is needed for confirmatory identification. However, the identification results within *Brachypelma* match the morphospecies very well, so nuclear genes aside with mitochondrial sequences provide powerful tools for species identification, especially for highly polymorphic species.

Discussion.

Molecular markers, like CO1, may possess effective species boundary information within certain taxonomic groups and consequently have the potential to be a rapid and efficient means to delineate and identify species. However, taken alone it has limited use in species delineation, and serves for species identification once a comprehensive taxonomy has been produced). The molecular phylogenies based on mtDNA have limitations as gene tree/species tree incongruence, with CO1 representing only one particular genealogy out of all possible within genome. Thus, here we present a more inclusive molecular approach using nuclear and mitochondrial markers to resolve the inner relantionships in the genera of prioritary tarantulas for conservation. Both ML and Bayesian analysis strongly recovered *Brachypelma* and *Tliltocatl* as monophyletic genera being congruent with previous studies (Mendoza & Francke 2017, 2018 in rev.). Two main lineages are shown, with

Brachypelma more closely related to South American taxa, whereas *Tliltocatl* is more close related to Central American genera than it is to *Brachypelma*. These results are congruent with the one obtained by Turner *et al.* (2017) where the redrump *Tliltocatl* species are clustered within Central American genera, whereas the redleg *Brachypelma* is clustered with the South American *Xenesthis* and *Pamphobeteus* as sister groups. Also, our results show the genus *Aphonopelma* as polyphyletic with at least three different lineages following a similar pattern as *Brachypelma* and *Tliltocatl*, with two groups clustered with Central American lineages and one clustered with South American ones. With the combination of mitochondrial and molecular characters the inner relationships within *Brachypelma* was resolved with high support values in both analyses. The analyses only with CO1used in previous studies, had failed to clarify the relationships between *B. boehmei*, *B. baumgarteni* and *B. auratum*, but in the present analyses *B. boehmei* is shown as the sister species of the other two. However, in *Tliltocatl* most of the species are strongly supported and new taxa were determined, but the inner relationships are not totally clear, with only a small clade of clustered species resolved. An explanation for this is possibly found in the work of Machkour *et al.* 2009 where they tested the use of molecular markers to study genetic diversity in tarantulas. Their study was done with *Tliltocatl vagans* and revealed a high level of polymorphism in this species; they mentioned that the species has a great genetic diversity, which is congruent with a species having open populations and wide distribution with high gene-flow probabilities (Roux *et al.* 2007, Bouzid *et al.* 2008). In addition, the distances shown in our phylogenies suggest that the radiation of *Tliltocatl* could be very recent, and this could be a factor of why despite conducting a multilocus phylogenetic analysis our study is still unable to entirely resolve the stem relationships of the genus. Further sampling and the use of different molecular markers or even an analysis combining molecular and morphological characters could help to resolve *Tliltocatl* relationships.

Our results support the usefullness of middle-rate evolving EF1G, MID1IP1 and MRPL44 loci for phylogenetic reconstruction at the generic level in Theraphosinae and show that they are a useful complement for the usual mitochondrial barcode. Even though each marker by itself did not provide a lot of information, together they were decisive to recover

and support several branches in the phylogeny. They also have a potential used for conservation issues of tarantulas; using nuclear markers for species identities in control border revisions has the advantage that conclusions can be verified by examining a number of independent loci. This ability is particularly important in cases where PCR contamination is a possibility, or when hybridization between species is known to occur. Because mtDNA is inherited maternally the results of nuclear markers can be compared with the mitochondrial ones to be sure of the identification. This is helpful in the case of tarantulas that are confiscated and intended to be reintegrated into their environment. Because if the origin of those specimens is uncertain, it is necessary to assure that they are a natural species and not hybrids from pettrade. Spiderlings could also be identified to species via DNA analysis. This, however, would be problematic for a border control officer faced with a shipment of hundreds of spiderlings, possibly including multiple species. DNA analysis is not cost-effective or practical for routine inspections, and testing would cause unacceptable delays and likely result in the death of specimens within the shipment. DNA testing would also require housing and caring for the specimens for an extended period. Furthermore, spiderlings are very fragile, and manipulating them to get a DNA sample could result in the death of the sampled specimen

We must be aware that barcoding is not the panacea to the species problem; its shortcomings often mirror those that rely strictly on morphological identifications (lack of informative characters, homoplasy, etc.). As the new age of genomics continues to evolve and vast amounts of whole genome data become available, one is left wondering if single marker DNA barcoding analyses will remain relevant. However, the ease with which these data can be collected and analyzed for hundreds or thousands of individuals supports the notion that there is still value in utilizing such an approach if it is effective for species delimitation; that is, the larger genomic data sets may be unnecessary for particular questions if species can be effectively delineated. DNA barcoding can be effective if it is not wielded as a blunt instrument, but instead in an informed manner that considers the group’s taxonomy, ecology, biogeography, and population genetics in an integrative decision making process.

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Legends of illustrations.

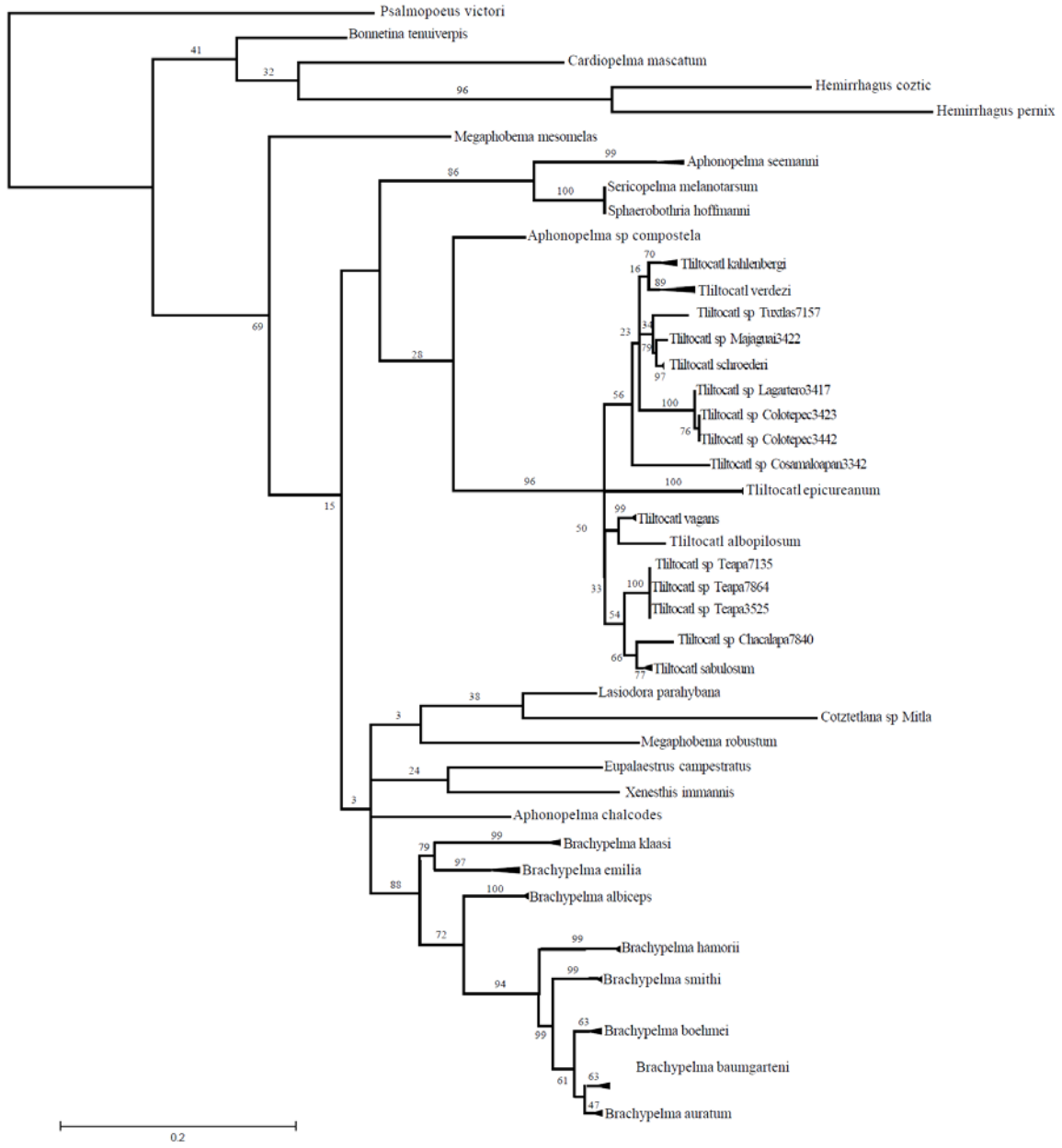

Fig. 1 Maximum likelihood phylogenetic hypothesis of CO1, EF1G, MID1IP1 and MRPL44 of the mitochondrial and nuclear genomes of 72 samples from 8 *Brachypelma*, 7 *Tliltocatl* species and 22 taxa of different genera. Nodal support shows maximum likelihood bootstrap. Tree shows the monophyly of *Brachypelma*, resolution of inner relantionships within the genus, and the monophyly of *Tliltocatl* with six undescribed species.

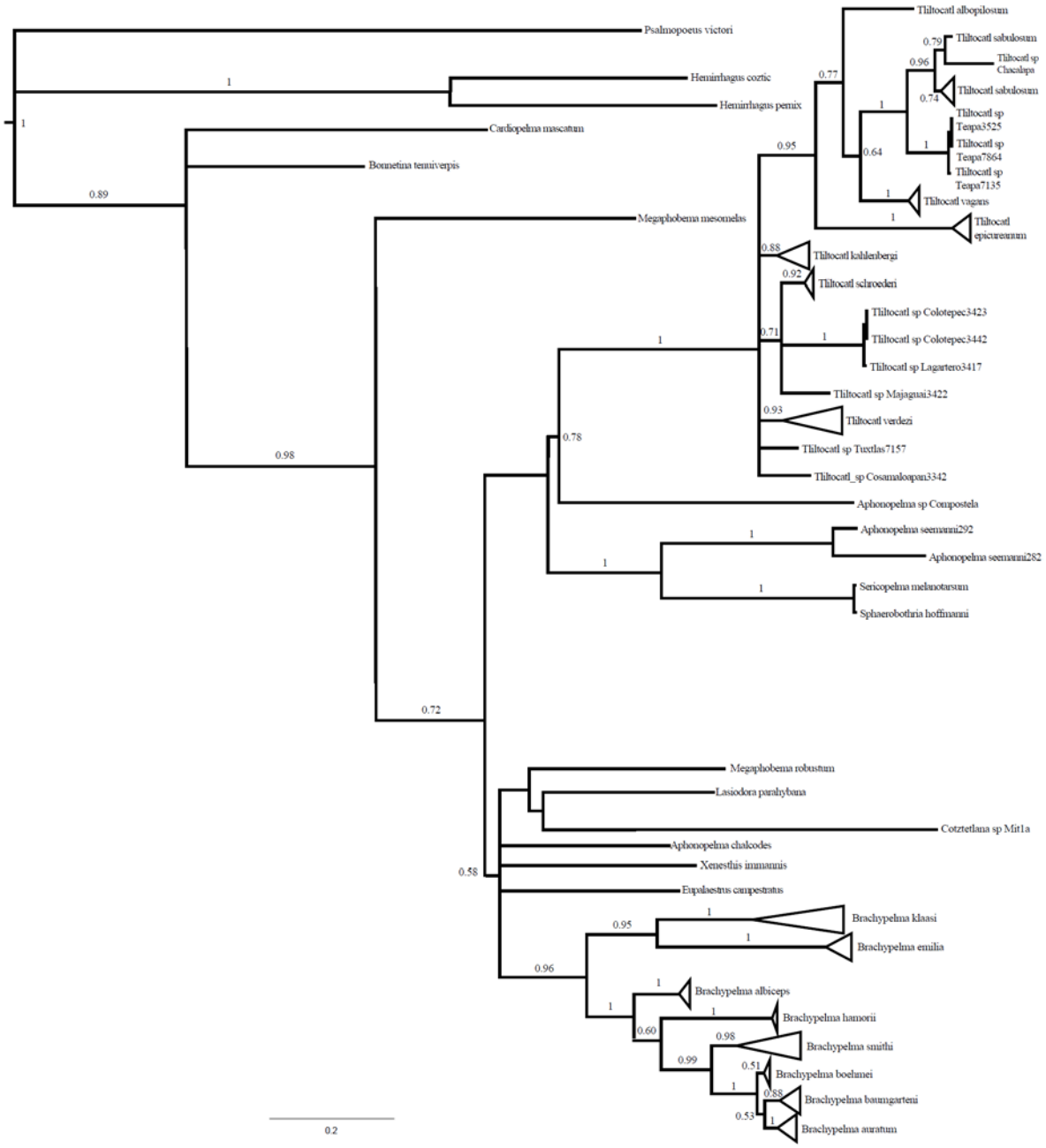

Fig. 2 Bayesian phylogenetic hypothesis of barcoding gene cytochrome c oxidase 1 (COI) and nuclear genes (EF1G, MID1IP1, MRPL44) of 72 samples from from 8 *Brachypelma*, 7 *Tliltocatl* species and 22 taxa of different genera. Posterior probabilities indicated above the branches or close to nodes.

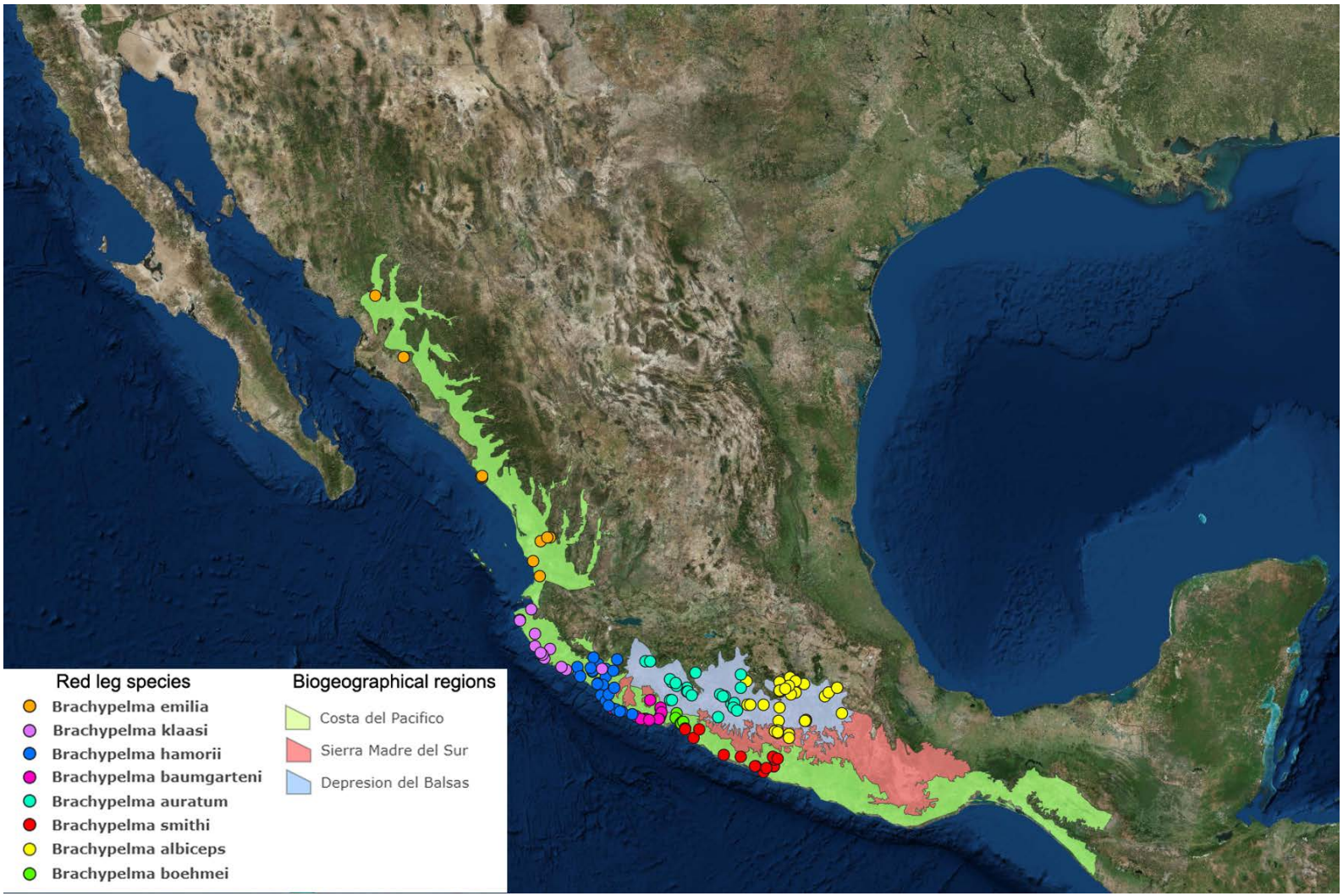

Fig. 3. Geographic distribution of the genus *Brachypelma* from published records and specimens analized in this study. The biogeographic regions where each species occurs is indicated.

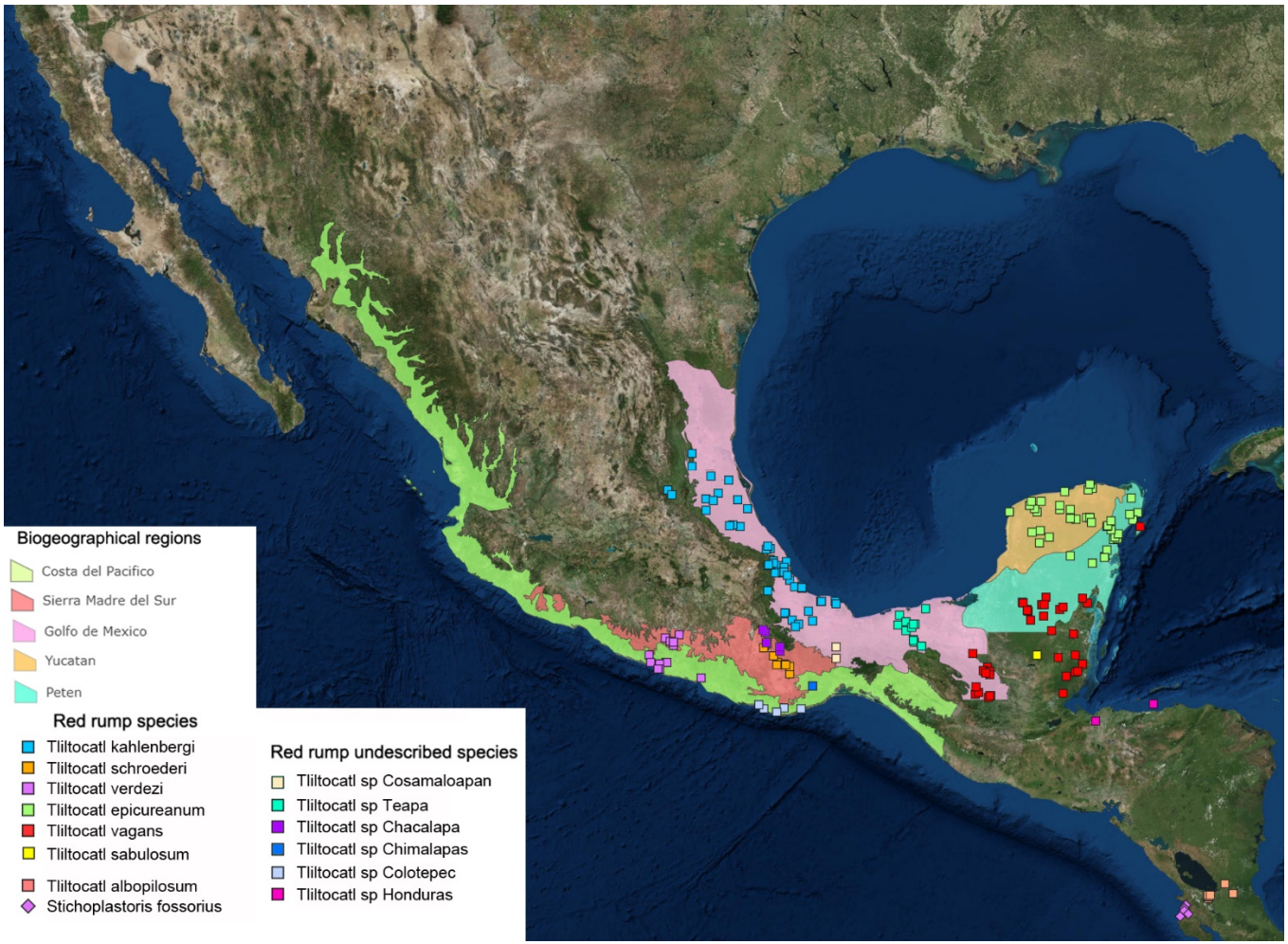

Fig. 4. Geographic distribution of the described species of the genus *Tliltocatl* from published records and specimens analized in this study from Mexico to Costa Rica. Unidentified or undescribed taxa from new records are shown the distribution of *Stichoplastoris fossorius* in Costa Rica is recorded. The biogeographic regions where each species occurs is indicated.

Table 1. Genbank accession codes for tissue samples, deposited in the Laboratorio de Sistemática Molecular (Zoología) at the Instituto de Biología, UNAM, Mexico City, from which DNA was extracted and sequenced for phylogenetic analyses of 8 species in the genus *Brachypelma* Simon 1891 and 7 species in the *Tliltocatl* genus, 10 undetermined specimens of *Tliltocatl*. Outgroup formed by 16 specimens of 15 species.

Species	Specimen	Voucher ID	COI	EF1G	MID1IP1	MRPL44
Psalmopoeus victori	1 juv. Un	CNAN-Ar010271	Unreleased	New	New	New
Eupalaestrus campestratus	1 juv. Un	CNAN-Ar010272	Unreleased	New	New	New
Lasiodora parahybana	Unk	CNAN-Ar010320	Unreleased	New	New	New
Megaphobema mesomelas	1 juv. ♀	CNAN-DNA-Ara0284	New	New	New	New
Sericopelma melanotarsum	1 juv. ♀	CNAN-DNA-Ara0293	New	New	New	New
Aphonopelma seemanni	1 ♂	CNAN-DNA-Ara0282	New	New	New	
Aphonopelma seemanni	1 juv. ♀	CNAN-DNA-Ara0292	Unreleased	New	New	New
Xenesthis immanis	1 juv. Un	CNAN-DNA-Ara0293	New	New	New	New
Aphonopelma chalcodes	1 ♀	CNAN-Ar10308	New	New	New	New
Aphonopelma sp Compostela	1 ♀	CNAN- Ar10305	New	New	New	
Bonnetina tenuiverpis	1 ♂	CNAN-T0755	KC807369.1	New	New	New
Cotztelana sp Mitla	1 ♀	CNAN-Ar10300	New	New	New	New
Cardiopelma mascatum	1 ♂	CNAN-Ar10320	New	New	New	New
Hemirrhagus coztic	1 ♂	CNAN-Ar10294	New	New	New	New
Hemirrhagus pernix	1 ♂	CNAN-Ar10290	New	New	New	New
Sphaerobothria hoffmanni	1 juv. ♀	CNAN-DNA-Ara0286	New	New	New	
Brachypelma albiceps	1 juv. ♀	CNAN-Ar007839	KT995331	New	New	New
Brachypelma albiceps	1 juv. ♀	CNAN-Ar007850	KT995391	New	New	New
Brachypelma albiceps	1 ♀	CNAN-Ar007865	KT995354	New	New	New
Brachypelma auratum	1 ♂	CNAN-Ar007878	KT995353	New	New	New
Brachypelma auratum	1 juv. ♀	CNAN-Ar007136	KT995371	New	New	New
Brachypelma auratum	1 juv. ♀	CNAN-Ar007164	KT995397	New	New	New
Brachypelma baumgarteni	1 ♀	CNAN-Ar007151	KT995332	New	New	New
Brachypelma baumgarteni	1 juv. ♀	CNAN-Ar007161	KT995395	New	New	New

Brachypelma baumgarteni	1 subad. ♂	CNAN-Ar007835	KT995382	New	New	New
Brachypelma boehmei	1 ♀	CNAN-Ar007905	unreleased	New	New	New
Brachypelma boehmei	1 ♂	CNAN-Ar007833	KT995343	New	New	New
Brachypelma emilia	1 ♀	CNAN-Ar007898	unreleased	New	New	New
Brachypelma emilia	1 ♂	CNAN-Ar007899	unreleased	New	New	New
Brachypelma emilia	1 ♂	CNAN-Ar003599	KT995326	New	New	New
Brachypelma hamorii	1 ♂	CNAN-Ar003614	KT995325	New	New	New
Brachypelma hamorii	1 ♂	CNAN-Ar007826	KT995381	New	New	New
Brachypelma hamorii	1 ♂	CNAN-Ar007827	KT995387	New	New	New
Brachypelma klaasi	1 ♂	CNAN-Ar003341	KT995329	New	New	New
Brachypelma klaasi	1 ♀	CNAN-Ar007845	KT995349	New	New	New
Brachypelma klaasi	1 ♂	CNAN-Ar007160	KT995340	New	New	New
Brachypelma smithi	1 juv. ♂	CNAN-Ar007140	KT995380	New	New	New
Brachypelma smithi	1 ♀	CNAN-Ar007143	KT995400	New	New	New
Brachypelma smithi	1 ♂	CNAN-Ar010286	unreleased	New	New	New
Tliltocatl albopilosum	1 juv. ♂	CNAN-DNA-Ara0290	unreleased	New	New	New
Tliltocatl epicureanum	1 ♀	CNAN-Ar007881	unreleased	New	New	New
Tliltocatl epicureanum	1 ♀	CNAN-Ar007880	unreleased	New	New	New
Tliltocatl epicureanum	1 ♀	CNAN-Ar007884	unreleased	New	New	New
Tliltocatl kahlenbergi	1 ♀	CNAN-Ar003424	KT995356	New	New	New
Tliltocatl kahlenbergi	1 ♀	CNAN-Ar007113	KT995361	New	New	New
Tliltocatl kahlenbergi	1 ♂	CNAN-Ar007117	KT995388	New	New	New
Tliltocatl sabulosum	1 ♂	CNAN-Ar007169	KT995373	New	New	New
Tliltocatl sabulosum	1 ♂	CNAN-Ar003326	KT995404	New	New	New
Tliltocatl sabulosum	1 ♀	CNAN-Ar003343	KT995403	New	New	New
Tliltocatl schroederi	1 juv. ♂	CNAN-Ar007139	KT995399	New	New	New
Tliltocatl schroederi	1 ♀	CNAN-Ar007868	KT995376	New	New	New
Tliltocatl schroederi	1 juv. ♂	CNAN-Ar003414	KT995345	New	New	New
Tliltocatl vagans	1 juv. ♂	CNAN-Ar003327	unreleased	New	New	New
Tliltocatl vagans	1 ♀	CNAN-Ar003670	KT995342	New	New	New

Tliltocatl vagans	1 ♀	CNAN-Ar007167	Unreleased	New	New	New
Tliltocatl verdezi	1 ♀	CNAN-Ar007844	KT995352	New	New	New
Tliltocatl verdezi	1 ♀	CNAN-Ar007848	KT995392	New	New	New
Tliltocatl verdezi	1 juv. ♂	CNAN-Ar007849	KT995379	New	New	New
Tliltocatl sp Colotepec	1 juv. ♂	CNAN-Ar003423	Unreleased	New	New	New
Tliltocatl sp Colotepec	1 ♀	CNAN-Ar003442	Unreleased	New	New	New
Tliltocatl sp Lagartero	1 ♀	CNAN-Ar003417	Unreleased	New	New	New
Tliltocatl sp Majaguai	1 ♀	CNAN-Ar003422	Unreleased	New	New	New
Tliltocatl sp Teapa	1 juv. ♂	CNAN-Ar003525	Unreleased	New	New	New
Tliltocatl sp Teapa	1 ♀	CNAN-Ar007840	Unreleased	New	New	New
Tliltocatl sp Teapa	1 ♀	CNAN-Ar007135	Unreleased	New	New	New
Tliltocatl sp Tuxtla	1 ♀	CNAN-Ar007157	Unreleased	New	New	New
Tliltocatl sp Chacalapa	1 ♀	CNAN-Ar007864	Unreleased	New	New	New
Tliltocatl sp Cosamalopan	1 ♀	CNAN-Ar003342	Unreleased	New	New	New

Table 2. PCR Primers Used.

Marker	Primer Name	Primer sequence (5'-3')	Reference
CO1	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. 1994
	HCO2198	TGATTTTTTGGTCACCCTGAAGTTTA	
EF1G (external reaction)	EF1Gf78	ATTGCBGCNCAGTAYAGYGG	Ayoub *et al*. 2007
	Ef1Gr1258	CCTTGRTTGAAYTTCTTTCC	
EF1G (internal reaction)	EF1G-TaF1	CAGTGGATTAGCTTTGCTG	Ortiz & Francke in prep.
	EF1G-TaR1	CGCATTTTATCTAGCCTCTG	
MID1IP1 (external reaction)	Mid1-InterF	AGGCGATTGTCKTATGCAGGATTGG	Ortiz & Francke in prep.
	Mid1-InterR	ATGGTGTGCAAGCCTTGCAAATGATG	
MID1IP1 (external reaction)	Mid-TaF	GCATCATTCCTTTGGTGGTG	Ortiz & Francke in prep.
	Mid-TaR	CAGATGAGAACCCGCAGAAG	
MRPL44 (external reaction)	MRPL44-Ta18	TATGGCGGTTTGTTAGGTTTGTTAC	Ortiz & Francke in prep.
	MRPL44-Ta937	TGGAAGTGGCCTATTATCTAAATTAAG	
MRPL44 (external reaction)	MRPL-Ta38	GTTACTAGGTGTTTGAACTTAATGC	Ortiz & Francke in prep.
	MRPL44-Ta905	CAAACTTCTTAAAGCATCAAATGCTG	

Discusión General.

En este trabajo se realizó un estudio integral de la sistemática del género *Brachypelma* y se ofrece, asimismo, un panorama general de su distribución y acciones que ayuden en su conservación. Se ha demostrado la utilidad de los códigos de barras para la rápida y precisa identificación de las especies dentro del género, como una alternativa para ayudar a las autoridades en contra del tráfico ilegal de estas especies prioritarias. Utilizando evidencia morfológica y molecular para la delimitación de sus especies se observo que el género *Brachypelma* no era monofilético y estaba compuesto por dos linajes. *Brachypelma sensu stricto* se conforma por las especies del complejo de patas rojas, puesto que en el se encuentra la especie tipo *B. emilia*, mientras que el grupo considerado *Brachypelma sensu lato* se conforma por las especies del complejo de cadera roja. Se ha redescrito *Brachypelma* y sus características diagnósticas fueron determinadas. Se describió el nuevo género *Tliltocatl* para albergar a las especies del complejo de cadera roja. Finalmente se ha realizado la filogenia de *Brachypelma* y *Tliltocatl* con el objetivo de resolver las relaciones interespecifícas dentro de cada género y las relaciones filogenéticas con los grupos cercanos de Centro y Sudamérica. Se pudieron determinar taxones no descritos del género *Tliltocatl*.

Si bien se ha observado que en algunos grupos el ADN mitocondrial por sí sólo es insuficiente para la delimitación precisa de especies, en el caso de *Brachypelma* demostró ser suficiente en la correcta identificación de especies. También considero que será una herramienta útil para prevenir el comercio en el mercado negro y proporcionar mejores estrategias para reintroducir tarántulas en las áreas de distribución adecuadas. Mientras que para resolver cuestiones filogenéticas y evolutivas los marcadores nucleares pueden usarse en grupos problemáticos como es el caso de *Tliltocatl*. Los resultados obtenidos evidencian la utilidad de combinar la taxonomía y filogenia morfológica tradicional con la información almacenada en el DNA, en estudios sistemáticos de tarántulas, un grupo que ha sido muy difícil de descifrar bajo un esquema tradicional debido a la limitada variación en sus caracteres morfológicos. Este estudio se suma a otros realizados en años recientes en terafósidas de los géneros *Aphonopelma* (Graham *et al.*, 2015; Hamilton *et al.*, 2011, 2014,

2016, Hendrixson *et al.*, 2013, 2015; Wilson *et al.*, 2013), *Grammostola* (Montes de Oca *et al.*, 2015) y *Bonnetina* (Ortiz & Francke 2016) que han llegado a conclusiones similares.

Durante este trabajo se logro determinar el área de distribución de las especies de *Brachypelma*, encontrar las zonas dónde algunas especies son simpátricas y el estado de conservación de algunas poblaciones. Todos estos datos eran desconocidos y son muy valiosos para poder llevar a cabo propuestas adecuadas para la conservación y aprovechamiento sustentable de las diferentes especies. Para el caso de *Tliltocatl* nuevos sitios de muestreo revelaron la presencia de especies no descritas que fueron identificadas gracias a las filogenias moleculares y los altos valores de soporte que mostraron. Es muy probable que *Tliltocatl* aún posea más taxones no descritos aparte de los encontrados durante el desarrollo de este trabajo, dado que no fue posible realizar muestreos en la mayoría de los países en Centroamerica, por lo que es esperable que en lo concerniente a este grupo estemos teniendo una visión parcial de su diversidad.

Las arañas terafósidas son conocidas por tener estructuras reproductoras simples y tienden a mostrar una variación intraespecífica relativamente alta y una variación interespecífica relativamente baja. que han revelado patrones generalizados de homoplasia entre los caracteres taxonómicos tradicionales (Raven 1985, Goloboff 1993, Pérez-Miles *et al.* 1996, Pérez-Miles 2000, Bertani 2001, Bond & Opell 2002, Hedin & Bond 2006, Bond & Hedin 2006, West *et al.* 2008, Hendrixson & Bond 2009, Bond *et al.* 2012, Guadanucci 2014, Hamilton *et al.* 2014, Perafán & Pérez-Miles 2014, Hamilton *et al.* 2016, Fukushima & Bertani 2017, Ortiz & Francke 2016). Por lo que estas son proclives a poseer una fuerte estructuración genética, posiblemente debido a su bajo poder de dispersión, dando lugar a una rica diversidad y a muchas especies con distribución restringida. Sin embargo, nuestros resultados y los de otros trabajos (Hamilton *et al.*, 2011, 2014, 2016; Montes de Oca *et al.*, 2015; Wilson *et al.*, 2013) sugieren que las terafósidas, especialmente las de gran tamaño, tienen un potencial mayor de dispersión y confirman que la variación intraespecífica puede ser alta en el grupo. Por lo tanto, utilizar criterios biogeográficos y diferencias morfológicas sutiles en ausencia de un amplio muestreo de ejemplares para delimitar especies de terafósidas, puede llevar a resultados erróneos.

Finalmente, durante el desarrollo de este trabajo se colaboró en la creación de una biblioteca genética de tarántulas mexicanas prioritarias para su conservación como parte del Proyecto de Código de Barras de Vida Silvestre en México, que se utilizará como referencia para las autoridades responsables de la conservación de especies. Hasta que se realice una evaluación seria de los factores ambientales, legales, sociales y económicos que afectan el comercio de vida silvestre en México, es muy poco lo que se puede hacer para desarrollar estrategias de gestión exitosas e iniciativas de conservación de especies. Además, es esencial explorar otras actividades económicas no extractivas, como el turismo, la observación de la vida silvestre o la venta de animales criados en cautiverio para proporcionar ingresos alternativos a las comunidades locales y evitar daños adicionales a las poblaciones de vida silvestre.

Conclusiones

- COI demostró ser un buen marcador de código de barras para la identificación de tarántulas del género *Brachypelma*, aunque en el caso de *Tliltocatl* puede ser susceptible de error con algunas especies cercanamente relacionadas.

- Para la identificación molecular de especies de *Brachypelma* utilizando COI, proponemos umbrales de variación intraespecífica e interespecífica de 1% y 7-12% respectivamente.

- La utilidad de los códigos de barras de ADN en el caso de especies vulnerables reside en poder identificar con cierta precisión especies de manera rápida, incluyendo ejemplares juveniles, proporcionando a los oficiales encargados de la detección de organismos traficados una herramienta para acelerar los procesos de investigación y detención de presuntos traficantes.

- Ejemplares adultos de las 8 especies reconocidas y formalmente descritas de *Brachypelma* son claramente diferenciables morfológicamente.

- El género *Brachypelma* tras su revisión en esta tesis resulto estar compuesto por dos linajes diferentes, dando como resultado la creación de *Tliltocatl* para albergar a las especies de cadera roja. Mientras que las del complejo de patas rojas permanecieron en *Brachypelma*.

- Las 7 especies reconocidas y formalmente descritas de *Tliltocatl* son claramente diferenciables morfológicamente observando las estructuras reproductoras de machos y hembras. Mientras que los ejemplares jóvenes son díficiles de diferenciar entre las diferentes especies dada la similitud en su coloración.

- Tras los resultados de esta tesis se determino que *Brachypelma* tiene distribución únicamente en el territorio mexicano, estando presente en 10 estados y tres

provincias biogeográficas: Cuenca del Balsas, Sierra Madre del Sur y Costa del Pacífico.

- *Brachypelma* se diagnóstica principalmente por la forma de cuchará del bulbo del macho, una quilla prolateral inferior muy reducida o ausente, carece de cualquier clase de espinación en las patelas, la espermateca de las hembras con una placa basal dividida y que es útil para la identificación de las especies y una coloración naranja o amarillenta en el caparazón o algunos segmentos de las patas.

- La utilización de caracteres moleculares permitió desarrollar las diagnosis de las especies de *Brachypelma*.

- El género *Brachypelma* mostro estar relacionado con linajes sudamericanos, mientras que *Tliltocatl* lo esta con los Centroamericanos.

- Tanto *Brachypelma* como *Tliltocatl* son recuperados como monofiléticos con un alto soporte.

- Los marcadores nucleares utilizados en esta tesis (EF1G, MID1IP1 y MRPL44) fueron muy útiles para resolver las relaciones internas dentro de *Brachypelma*.

- De acuerdo con los resultados obtenidos es probable que *Brachypelma* y *Tliltocatl* provengan de dos eventos separados de colonización, pues que al menos uno de ellos compartío un ancestro común procedente de Sudamérica.

- *Brachypelma* y sus grupos cercanos poseen un alto nivel de homoplasia morfológica, que cuestiona la validez de estos caracteres para estimar sus relaciones filogenéticas.

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