Universidad Autónoma de Tlaxcala

Posgrado en Ciencias Biológicas

Sistemática filogenética molecular, diversificación y datación de linajes del género de arañas *Physocyclus* Simon, 1893 (Araneae: Pholcidae)

T E S I S

Doctorado en Ciencias Biológicas

P r e s e n t a

Samuel Nolasco Garduño

Director: Dr. Alejandro Valdez Mondragón

Tlaxcala, Tlax. Agosto, 2023

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Dr. Alejandro Valdez Mondragón
Dr. Aníbal Díaz de la Vega Pérez
Dr. José Martín García Varela

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A

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Sirva este medio para describir el proceso de revisión de la tesis realizada por el estudiante **Samuel Nolasco Garduño** titulada "**Sistemática filogenética molecular, diversificación y datación de linajes del género de arañas *Physocyclus* Simon, 1893 (Araneae: Pholcidae)**" para optar por el grado de **Doctor en Ciencias Biológicas**.

El documento de la tesis de **Samuel Nolasco Garduño** fue revisado por mí como Director de tesis antes de presentarse en cada examen tutoral y, posteriormente a los exámenes tutorales, los miembros de su comité tutoral realizaron también sus respectivas observaciones. De esta manera, el documento llevó un proceso de revisión por varios profesores expertos en el tema. En el mes de julio, el manuscrito final fue procesado con el programa Copy-Spider, marcando poco texto con similitudes del **6.3%** (valores admitidos por el programa). Examinando los detalles de la búsqueda, las partes del documento detectadas como similitudes están relaciones con nombres de instituciones, autores y de organismos. Así mismo, algunas similitudes se correlacionan con la metodología, correspondiendo principalmente a tecnicismos, nombres de programas especializados y métodos que son de uso común. Por lo anterior, confirmo que **el estudiante no incurrió en ninguna práctica no deseable** en la escritura de la tesis, la cual es un documento original.

Sin más por el momento, reciban atentos saludos.

CORDIALMENTE

La Paz, Baja California Sur, México; a 13 de julio del 2023

Dr. Alejandro Valdez Mondragón
Director de Tesis
Investigador Titular "A".
Colección Aracnológica (CARCIB),
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COORDINACIÓN DOCTORADO EN CIENCIAS BIOLÓGICAS
UNIVERSIDAD AUTÓNOMA DE TLAXCALA
P R E S E N T E

Los abajo firmantes, miembros del jurado evaluador del proyecto de tesis que **Samuel Nolasco Garduño** realiza para la obtención del grado de **Doctor en Ciencias Biológicas**, expresamos que, habiendo revisado la versión final del documento de tesis, damos la aprobación para que ésta sea impresa y defendida en el examen correspondiente. El título que llevará es **"Sistemática filogenética molecular, diversificación y datación de linajes del género de arañas *Physocyclus Simon*, 1893 (Araneae: Pholcidae*)*.**

Sin otro particular, aprovechamos para enviarle un cordial saludo.

A T E N T A M E N T E
TLAXCALA, TLAX., A 14 DE AGOSTO DEL 2023

DR. ANÍBAL HELIOS DÍAZ DE LA VEGA PÉREZ

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"Generalmente, cuando más sabes acerca de una cosa, menos miedo le tienes… y más peligroso te vuelves"

RESUMEN

El género de arañas *Physocyclus* Simon, 1893 está clasificado dentro de la familia Pholcidae y está conformado por 37 especies que se distribuyen principalmente en ambientes áridos y tropicales caducifolios de México. Este estudio tuvo como objetivo conocer las relaciones filogenéticas del género *Physocyclus* utilizando evidencia combinada (datos morfológicos, moleculares y biogeográficos), determinando sus patrones de diversificación a través de la delimitación de especies y datación de linajes. Los análisis de distancias genéticas se realizaron con base en el criterio de Neighbor-Joining bajo el umbral del 2%, mientras que, para los análisis de delimitación de especies, se utilizaron métodos basados en el código de barras de ADN (ABGD), distancias genéticas (ASAP), coalescencia (GMYC) y aquellos basados en árboles (bPTP). Se realizaron análisis filogenéticos con 54 caracteres morfológicos en combinación con dos marcadores moleculares nucleares (ITS2 y 28S) y uno mitocondrial (CO1). Se realizaron análisis de datación de linajes y reconstrucción de áreas ancestrales. Los métodos de delimitación de especies muestran alta congruencia entre ellos, sin embargo, el método bPTP tiende a sobreestimar el número de especies, en algunos casos. Adicionalmente, se detectaron posibles complejos de especies dentro de *Physocyclus*. Los análisis filogenéticos con evidencia morfológica, molecular y combinada (morfología + molecular), mostraron que los grupos de especies *globosus* y *dugesi* que conforman al género *Physocyclus*, son monofiléticos, con una morfología distintiva y patrones biogeográficos característicos para cada grupo de especies. De acuerdo con la datación de linajes y reconstrucción de áreas ancestrales, ambos grupos de especies divergieron hace aproximadamente 15 millones de años. Esta divergencia está estrechamente relacionada con la compleja orografía del país y con la formación de las diferentes cadenas montañosas, que influyeron de manera diferente en la diversificación de ambos grupos, siendo el Cinturón Volcánico Transmexicano el de mayor influencia en el proceso de diversificación. Los procesos de especiación ocurrieron principalmente por vicarianza durante el Mioceno Medio al Plioceno, seguido de posteriores eventos de dispersión durante el Plioceno al Pleistoceno. Con la evidencia aquí recopilada, se propone al grupo de especies *dugesi*, como un género de arañas nuevo: *Mictlanus* **gen. nov.**, dentro de la familia Pholcidae, por lo siguiente: 1) Características morfológicas diagnósticas para cada género; 2) ambos son grupos monofiléticos bajo diferentes tipos de evidencia (morfológica, molecular y evidencia

combinada); 3) ambos tienen diferentes patrones de distribución; 4) tiempos de divergencia similares, pero procesos evolutivos independientes, y, 5) diferentes provincias biogeográficas y áreas ancestrales que explican sus patrones de diversificación.

ÍNDICE

1. INTRODUCCIÓN

La familia de arañas Pholcidae C. L. Koch, 1850 está conformada actualmente por un total de 97 géneros y 1919 especies (WSC 2023), es una de las más diversas del orden Araneae y la más diversa dentro del clado Synspermiata (Wheeler y cols. 2016). Está familia se subdivide en cinco subfamilias: Arteminae Simon, 1893, Modisiminae Simon, 1893, Ninetinae Simon, 1890, Pholcinae C.L. Koch 1850 y Smeringopinae Simon, 1893 (Huber 2011). Dentro de la subfamilia Arteminae, el género *Physocyclus* Simon, 1893 es el más diverso en Norteamérica, con 37 especies que se distribuyen principalmente en México, desde el sur de los Estados Unidos hasta Centroamérica (Valdez-Mondragón 2010, 2013, 2014; Nolasco y Valdez-Mondragón 2020, 2022a, b; WSC 2023). Este género habita principalmente en ambientes de bosques tropicales caducifolios, aunque también se han colectado especies en climas áridos y semiáridos, por debajo de los 1900 metros sobre el nivel del mar (Valdez-Mondragón 2010, 2013, 2014; Jiménez y Palacios-Cardiel 2013; Nolasco y Valdez-Mondragón 2020, 2022a, b). Sin embargo, este género cuenta también con especies asociadas a viviendas o hábitats humanos (especies sinantrópicas), tal es el caso de *Physocyclus dugesi* Simon, 1893 y *Physocyclus globosus* (Taczanowski, 1874), siendo esta última especie cosmopolita e introducida en varios países de Sudamérica, África y Asia por actividades antropogénicas (Valdez-Mondragón 2010; Nolasco y Valdez-Mondragón 2022a). Aunado a esto, *Physocyclus* presenta hábitos troglófilos, ya que algunas especies son comúnmente encontradas en las entradas e interiores de las cuevas, lo que es común para varios géneros de la familia, siendo Pholcidae la familia de arañas que tiene el mayor número de especies asociadas a cuevas en todo el mundo (Gertsch 1971; Valdez-Mondragón 2013; Huber y cols. 2018). A pesar de esta gran diversidad de especies asociadas a cuevas, para el género *Physocyclus* no se han reportado especies troglobias o con troglomorfismos marcados hasta el momento.

Morfológicamente, el género *Physocyclus* se caracteriza por presentar coloraciones que van del beige al marrón y suelen mostrar una serie de patrones dorsales de coloración oscura en el caparazón. Presentan abdomen globular con manchas irregulares en tonos grises oscuros y claros. Sus patas son largas con relación al cuerpo, el cual oscila entre los 3 a 5 mm y muestran una ornamentación con forma de anillos oscuros en la parte distal de las tibias

y fémures. La coloración del cuerpo y algunas estructuras somáticas son similares dentro de las especies del género, lo que indica que presentan un patrón morfológico muy conservado (Valdez-Mondragón 2010, 2013, 2014; Nolasco y Valdez-Mondragón 2020, 2022a).

Basado solamente en caracteres morfológicos, *Physocyclus* es un género monofilético, sin embargo, las relaciones filogenéticas al interior del género no se recuperan del todo resueltas. Esto es algo común en las filogenias morfológicas con distintos géneros de Pholcidae, debido a su morfología conservada (Huber 2005, 2013, 2017; Huber y cols. 2015). A su vez, el género *Physocyclus* está conformado por dos grupos de especies: el grupo *globosus*, con 15 especies y el grupo *dugesi* con 22 especies (Valdez-Mondragón 2013, 2014; Nolasco y Valdez-Mondragón 2020, 2022a, b). Ambos grupos de especies son monofiléticos, tales monofilias están soportadas por sinapomorfías asociadas principalmente a caracteres sexuales primarios y secundarios de machos y hembras. Los caracteres sexuales son importantes para la correcta identificación a nivel de género y especie, ya que estos evolucionan y presentan tasas de cambio más rápidas que los caracteres somáticos (Huber 2003; Huber y cols. 2018; Valdez-Mondragón 2020). Existen casos donde las hembras de algunas especies de *Physocyclus* (ej: *P. enaulus*) presentan diferentes morfotipos en la forma del epiginio, mientras que, en algunos machos (ej: *P. enaulus*, *P. merus* y *P. sprousei*) los palpos y sus caracteres particulares son similares entre sí (Valdez-Mondragón 2010). Esto imposibilita, en algunos casos, identificar correctamente a las especies y establecer los límites entre ellas, ya que podrían tratarse de complejos de especies. Lo anterior, vislumbra la necesidad de utilizar otro tipo de evidencia, como datos morfológicos más detallados, marcadores moleculares o evidencia biogeográfica, para reconocer a las especies, así como la delimitación de las mismas (Eguiarte y cols. 2007; Valdez-Mondragón y Francke 2015; Huber y cols. 2018).

De acuerdo con su biogeografía, el grupo de especies *globosus* presenta un patrón de distribución en los componentes bióticos Mesoamericano y Mexicano de Montañas (Valdez-Mondragón 2013, 2014; Nolasco y Valdez-Mondragón 2022a). El grupo de especies *dugesi*, tiene un patrón de distribución orientado hacia los componentes bióticos Mesoamericano y Neártico Continental (Valdez-Mondragón 2013, 2014; Nolasco y Valdez-Mondragón 2022a). Este patrón de distribución ligado principalmente a la Zona de Transición Mexicana (ZTM), permite pensar que el origen y diversificación del género *Physocyclus*, así como de

ambos grupos de especies, están correlacionados con la compleja historia biogeográfica de la región (Nolasco y Valdez-Mondragón 2022b; Morrone 2023). Esto debido a que la ZTM presenta características ambientales y componentes bióticos de las regiones Neártica y Neotropical, actuando como barreras, limitando la continuidad de las poblaciones o sirviendo como corredor biológico, promoviendo la dispersión y el entrecruzamiento genético de muchos grupos biológicos (Mastretta-Yanes y cols. 2015; Morrone 2023). La ZTM es la responsable de la diversificación por vicarianza y posteriores procesos de dispersión, originados por actividad volcánica, periodos interglaciares durante el Pleistoceno, la aparición y fragmentación de diferentes ecosistemas, para algunos grupos de vertebrados e invertebrados, como saltamontes (Pedraza-Lara y cols. 2015), serpientes de cascabel (Bryson y cols. 2011), ranas (Mulcahy y Mendelson III 2000), iguanas (Zarza y cols. 2008) y arañas (Valdez-Mondragón y Francke 2015). Sin embargo, la falta de evidencia genética para el género *Physocyclus*, impide realizar cualquier aproximación, contextualizada dentro de un marco biogeográfico histórico, sobre la evolución y diversificación de este grupo de arañas.

El uso de marcadores moleculares de diferente origen (nucleares y mitocondriales), en conjunto con los datos morfológicos, provee un marco de información amplio y eficaz para la elaboración de análisis filogenéticos más robustos y con mayor resolución (Eguiarte y cols. 2007; Dimitrov y cols. 2013; Huber y cols. 2018). Aunado a esto, el uso de evidencia molecular permite responder preguntas tanto macro como micro evolutivas, a partir del estudio de las variaciones genéticas entre las poblaciones y especies (Valdez-Mondragón 2020; Nolasco y Valdez-Mondragón 2022b). De esta manera, el objetivo principal de este trabajo es poner a prueba la monofília de *Physocyclus* bajo un contexto integrador, con evidencia morfológica, molecular y biogeográfica, y vislumbrar con mayor resolución las relaciones filogenéticas entre las especies. Simultáneamente, es posible pensar que los grupos de especies antes mencionados sean, en realidad, dos géneros distintos, pues cada clado es soportado por caracteres sinapomórficos exclusivos (Valdez-Mondragón 2013, 2014), además de tener patrones biogeográficos diferentes. Adicionalmente, se incluyeron análisis de delimitación de especies y datación de linajes, todo esto bajo un contexto de taxonomía integradora, y bajo un contexto biogeográfico histórico. Esto nos permitió conocer el origen y diversificación de *Physocyclus*, y contrastarlo con los eventos biogeográficos y geológicos históricos del país. Del mismo modo, los métodos de delimitación de especies nos

permitieron identificar y reconocer los distintos linajes, además de descubrir posibles complejos de especies y especies nuevas (DeSalle y cols. 2005).

2. ANTECEDENTES

Respecto a la diversidad del género *Physocyclus*, las primeras 10 especies se describieron como parte de diferentes estudios aranofaunísticos (Taczanowski 1874; Simon 1893; Banks 1898; Chamberlin 1921, 1924; Chamberlin y Gertsch 1929; Mello-Leitão 1940; Gertsch y Davis 1942). Gertsch (1971) realizó un estudio sobre la diversidad de arañas en diversas cuevas a lo largo del territorio mexicano, donde describió siete especies más para el género. Huber (1998) describe *Physocyclus guanacaste*, de Costa Rica, siendo esta una de las dos especies que no se encuentran en México y la más austral, en el continente americano. La otra especie es *Physocyclus viridis* Mello-Leitão, 1940, descrita de Brasil, sin embargo, hoy en día no se considera una especie del género (*nomen dubium*). Hasta el año 2010 se tenían descritas 18 especies, sin embargo, en la revisión taxonómica del género y filogenias morfológicas realizadas por Valdez-Mondragón (2010, 2013, 2014), se redescriben 12 especies y se describen 13 especies nuevas, además se proporciona la primera clave de identificación del género. Las últimas seis especies del género *Physocyclus* descritas hasta el momento son *Physocyclus palmarus* Jiménez y Palacios-Cardiel 2013, *Physocyclus xerophilus* Nolasco y Valdez-Mondragón, 2020 y *Physocyclus pocamadre* Nolasco y Valdez-Mondragón, 2022, las tres provenientes de Baja California Sur, *Physocyclus lyncis* Nolasco y Valdez-Mondragón, 2022 y *Physocyclus mariachi* Nolasco y Valdez-Mondragón, 2022, ambas recolectadas en el estado de Jalisco *y Physocyclus sikuapu* Valdez-Mondragón y Nolasco, 2022, descrita del estado de Michoacán. Nolasco y Valdez-Mondragón (2022a), además de describir las cuatro últimas especies para el género, también actualizan las claves taxonómicas de identificación para el género. De acuerdo con Nolasco y Valdez-Mondragón (2022a), la Depresión del Balsas, es la provincia con la mayor diversidad de especies de *Physocyclus*, la cual, está influenciada por elementos tropicales Mesoamericanos. Los eventos de vicarianza asociados con la evolución biótica de esta zona de transición, pueden estar relacionados con grandes cambios orográficos, tales como el desarrollo de la Sierra Madre Occidental, la Sierra Madre del Sur y el Cinturón Volcánico Transmexicano. Además, la Depresión del Balsas está rodeada por tres puntos de intersección que pueden ser

considerados como nodos panbiogeográficos, (áreas con alta diversidad debido al contacto entre diferentes elementos bióticos) lo que explica por qué esta región biogeográfica es una de las más biodiversas del país y a nivel mundial (Morrone 2005, 2023; Mastretta-Yanes y cols. 2015).

Respecto a las relaciones filogenéticas entre las especies, Valdez-Mondragón (2013, 2014) pone a prueba la monofília del género *Physocyclus*, con 54 caracteres morfológicos (44 binarios y 10 multiestado), recuperando el grupo como natural o monofilético. Las sinapomorfías que soportan la monofília del género son tres: 1) la apófisis ventral pareada en la parte anterior del epiginio de la hembra, 2) las constricciones laterales en la parte media del epiginio de la hembra y 3) el arco esclerotizado del útero, con una sola proyección en la parte anterior. Aunado a esto, se proponen dos grupos de especies dentro de *Physocyclus*, el grupo *globosus* y el grupo *dugesi*, los cuales tienen patrones biogeográficos distintos (Valdez-Mondragón 2010). El grupo de especies *dugesi* presenta una serie de conos esclerosados (>30) en la parte frontal de los quelíceros de los machos, mientras que el epiginio de las hembras presenta una forma de campana, debido a las constricciones laterales bastante marcadas, y presenta unas apófisis ventrales largas y cónicas en el epiginio. En contraste, el grupo de especies *globosus* no exhibe conos esclerosados en las láminas frontales de los quelíceros de los machos y si los llega a presentar, son escasos (<5). El epiginio de las hembras muestra una forma triangular, con unas constricciones laterales apenas visibles o incluso ausentes y las apófisis ventrales del epiginio son cortas, cónicas y en algunas especies, están ausentes.

Como ya se mencionó, la familia Pholcidae se caracteriza por tener una morfología muy conservada entre los diferentes géneros, por lo que generalmente las filogenias basadas exclusivamente en datos morfológicos se caracterizan por tener pocos caracteres y no recuperan de forma clara las relaciones filogenéticas entre las especies. De manera general, la topología de estas filogenias, tiene una resolución no significativa con bajos valores de soporte de ramas (Huber 2005; 2013; 2017). El caso de *Physocyclus* no es la excepción, las propuestas filogenéticas de Valdez-Mondragón (2013, 2014) con evidencia morfológica, aunque demuestran la monofília del género, no resuelven totalmente las relaciones filogenéticas internas entre las especies. Esto impide establecer, por un lado, dichas

relaciones y por otro, la interpretación de diversificación en un contexto biogeográfico histórico, ya que no se usaron datos genéticos para estudiar dicha diversificación.

El uso de marcadores moleculares ha sido ampliamente utilizado para conocer las relaciones filogenéticas en diversos grupos biológicos incluidas las arañas. Dentro de la familia de arañas Pholcidae, diferentes marcadores moleculares, tanto mitocondriales (CO1, 16S, 12S) como nucleares (ITS2, 18S, 28S, H3), se han utilizado ampliamente para poner a prueba diferentes hipótesis filogenéticas, principalmente sobre las relaciones de las cinco subfamilias pero además, trabajos sobre reconstrucciones filogenéticas a nivel genérico (Bruvo-Madaric y cols. 2005; Huber 2011; Dimitrov y cols. 2013; Valdez-Mondragón y Francke 2015; Eberle y cols. 2018; Huber y cols. 2018). Aunado a esto, se han elaborado estudios filogenéticos donde no solo se integran datos moleculares, sino que también se incluye evidencia morfológica, lo que se conoce como evidencia combinada. Estos análisis filogenéticos integradores, han generado hipótesis más robustas, en comparación con hipótesis que utilizan evidencias de manera independiente (Vink y Paterson 2003; Bruvo-Madaric y cols. 2005; Dimitrov y Hormiga 2011; Valdez-Mondragón y Francke 2015).

Por otra parte, aunado al uso de marcadores moleculares para reconstrucciones filogenéticas de los grupos, los marcadores mitocondriales (principalmente CO1 e ITS2), se han utilizado en diferentes grupos de arácnidos, con diversos métodos moleculares para la delimitación de especies y detección de complejos de especies crípticas o pseudo crípticas (especies que son morfológicamente similares pero diferentes genéticamente), con resultados efectivos (Astrin y cols. 2006; Agnarsson y cols. 2010; Hamilton y cols. 2011; Ortiz y Francke 2016; Candia-Ramírez y Francke 2020; Navarro-Rodríguez y Valdez-Mondragón 2020; Valdez-Mondragón 2020).

Los diferentes métodos moleculares de delimitación de especies, surgen bajo la necesidad de identificar, reconocer y clasificar con mayor eficiencia a las especies, que, en muchos grupos, su diversidad aún está subestimada, sobre todo en grupos de artrópodos tropicales (DeSalle y cols. 2005; Carstens y cols. 2013; Rannala y Yang 2020). Son una herramienta adicional para identificar y reconocer especies en grupos donde la morfología suele ser muy conservada o donde existen complejos de especies, como en algunos arácnidos (Hamilton y cols. 2011; Valdez-Mondragón 2020). Entre los métodos de delimitación de especies más utilizados actualmente, se encuentran aquellos basados en el barcoding de ADN

(Hebert y cols. 2003), como ABGD (Automatic Barcode Gap Discovey) (Puillandre y cols. 2012), los métodos basados en distancias genéticas, como ASAP (Assembly Species by Automatic Partitioning) (Puillandre y cols. 2021), los métodos basados en coalescencia, como GMYC (General Mixed Yule Coalescent) (Pons y cols. 2006) y aquellos basados en el uso de un árbol generado *a priori*, como bPTP (Bayesian Poisson Tree Processes), con base en lo métodos de Máxima Verosimilitud o Inferencia Bayesiana (Rannala y Yang 2020). El uso de diferentes métodos moleculares de delimitación de especies, se basa en el criterio de integración por congruencia, pues entre más métodos se usen y los resultados sean similares o congruentes entre sí, las hipótesis e interpretaciones serán más robustas (Carstens y cols. 2013).

Los marcadores moleculares (mitocondriales y nucleares) también han sido utilizados para conocer la edad y divergencia de los linajes en arácnidos y proponer hipótesis sobre la historia biogeográfica de los grupos (Dimitrov y cols. 2013; Valdez-Mondragón y Francke 2015; Cruz-López y cols. 2019). Del mismo modo, la reconstrucción de las posibles áreas de distribución ancestral permite conocer cuáles son los principales factores que promovieron la diversidad de un grupo (en términos de dispersión, extinción y vicarianza), así como reconocer cuáles son las áreas geográficas que tienen más influencia en la diversificación de las especies (Kornilios y cols. 2016; Schramm y cols. 2021).

Debido a lo anterior, en este proyecto, el uso de evidencia molecular (marcadores mitocondriales y nucleares) en conjunto con la evidencia morfológica (sistemática integradora), nos permitió establecer de manera más precisa las relaciones filogenéticas entre las especies del género. Además, fue posible reconocer si los grupos de especies propuestos (*globosus* y *dugesi*) son en realidad dos géneros distintos, así como conocer su diversificación dentro de un contexto biogeográfico histórico y establecer los límites entre las especies que conforman al género *Physocyclus*.

3. HIPÓTESIS

Los grupos de especies *globosus* y *dugesi* pertenecientes al género *Physocyclus*, son dos géneros distintos, con patrones de distribución e historias evolutivas diferentes, relacionadas con la complejidad orográfica en la Zona de Transición Mexicana y con una diversificación predominantemente en la parte central del país.

3. 1 PREDICCIONES

1. Los grupos de especies *globosus* y *dugesi* consistirán en géneros distintos dentro de la subfamilia Arteminae, estableciendo una clasificación más robusta de las especies.
2. Dentro de un contexto biogeográfico, relacionado con la complejidad orográfica de México, la vicarianza y la dispersión serán los posibles factores que más influyeron en la diversificación de especies dentro del género *Physocyclus*.
3. Las distancias genéticas promedio entre las especies del género *Physocyclus* serán similares a las de la familia Pholcidae y serán congruentes con los métodos de delimitación de especies y con la evidencia morfológica tradicional, estableciendo así los límites entre las especies, además de reconocer la posible existencia de complejos de especies dentro del género.

4. OBJETIVOS

4. 1 Objetivo general

Conocer las relaciones filogenéticas entre las especies del género *Physocyclus* utilizando evidencia combinada (datos moleculares, morfológicos y biogeográficos), y determinar sus patrones de diversificación a través de la datación de linajes y la delimitación de especies.

4. 2 Objetivos específicos

1. Establecer las relaciones filogenéticas entre las especies del género utilizando evidencia combinada de caracteres morfológicos, moleculares y biogeográficos.
2. Poner a prueba la monofília de los grupos de especies *globosus* y *dugesi* a través de sus relaciones filogenéticas y datación de dichos grupos en un contexto biogeográfico.
3. Conocer la diversificación del género *Physocyclus* en un contexto biogeográfico histórico, determinando los posibles factores extrínsecos que promovieron la diversificación del grupo en un contexto biogeográfico histórico.
4. Identificar y delimitar a las especies de arañas de este género, recurriendo a diferentes métodos moleculares de delimitación de especies y corroborándolos con evidencia morfológica (taxonomía tradicional).
5. Describir especies nuevas encontradas bajo un contexto de taxonomía integradora.

5. MATERIALES Y MÉTODOS

El proyecto consiste en cuatro partes esenciales: 1) Revisión morfológica de los ejemplares de *Physocyclus*, 2) obtención de las secuencias genéticas mitocondriales y nucleares para los análisis moleculares, 3) realización de análisis filogenéticos, datación de linajes, reconstrucción de áreas ancestrales y delimitación de especies con datos moleculares y morfológicos (taxonomía integradora), y 4) descripción de especies nuevas (taxonomía alfa).

A continuación, se describe de manera resumida los materiales y métodos utilizados para los análisis morfológicos y los estudios moleculares, ya que, en cada capítulo de esta tesis, se describen de manera detallada y concisa.

5.1 Material biológico

5.1.1 Colecciones científicas

Se revisaron en total 2787 ejemplares, pertenecientes a 30 especies del género *Physocyclus*, entre machos, hembras y juveniles depositados en las siguientes colecciones biológicas: Laboratorio de Aracnología (LATLAX), Instituto de Biología, UNAM-Tlaxcala, Tlaxcala, Colección Nacional de Arácnidos (CNAN), Instituto de Biología, UNAM, Ciudad Universitaria, Ciudad de México, Colección de Aracnología (CARCIB), Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Baja California Sur, y la Colección Aracnológica de la Facultad de Biología de la Universidad Michoacana de San Nicolás de Hidalgo (CAFBUM), Morelia, Michoacán.

5.2 Trabajo de campo

Se realizaron varios muestreos en campo cuyo objetivo fue colectar ejemplares del género *Physocyclus* para los estudios morfológicos y moleculares. Estas colectas se centraron en cinco expediciones. La primera se realizó en los estados de Baja California Norte y Baja California Sur, durante los meses de julio y agosto del 2019, abarcando un total de 80 localidades. La segunda expedición se efectuó en los estados de Coahuila, Colima, Durango, Guerrero, Hidalgo, Jalisco, Michoacán, Nayarit, Nuevo León, Oaxaca, Puebla, San Luis Potosí, Tamaulipas y Veracruz durante los meses de septiembre y octubre del año 2019, contemplando un total de 73 localidades. La tercera salida a campo se llevó a cabo en la costa del Pacífico de México y zonas aledañas, muestreando en los estados de Colima, Guerrero,

Hidalgo, Jalisco, Michoacán, Morelos y Nayarit durante el mes de noviembre del 2020, donde se recolectaron ejemplares en 65 localidades. El cuarto muestreo de campo se realizó durante el mes de octubre del 2021, en el Bajío mexicano, principalmente en los estados de Querétaro, Guanajuato y San Luis Potosí, visitando un total de 43 localidades. El último muestreo se realizó en partes de la Zona Pacífico-Sur del país, en los estados de Chiapas, Oaxaca y Puebla, durante el mes de septiembre del año 2022. En esta expedición se visitaron 29 localidades.

Los ejemplares se colectaron de forma manual y se depositaron en frascos con alcohol etílico al 80%. Cada frasco se rotuló con los datos de colecta (localidad, fecha, vegetación, coordenadas, colectores). Durante estas cinco expediciones, se recolectaron un total de 921 ejemplares, de los cuales 112 no se pudieron identificar debido a su estadio juvenil, pues al no poseer estructuras sexuales desarrolladas, no se pudo corroborar su identidad a nivel específico. Estos ejemplares no identificados se excluyeron de los posteriores análisis.

6. ANÁLISIS MORFOLÓGICOS

La identificación del material se realizó en el Laboratorio de Aracnología Tlaxcala (LATLAX), del Laboratorio Regional de Biodiversidad y Cultivo de Tejidos Vegetales (LBCTV), Instituto de Biología (IBUNAM), Tlaxcala. Se identificaron un total de 30 especies, lo que representa un 88% del total de especies del género (Cuadro 1). De los 2787 ejemplares revisados, 507 no pudieron ser identificados debido a dos problemas principales: 1) el material se encontraba demasiado maltratado como para ser identificado, o 2) solo contenía ejemplares juveniles, lo cual imposibilitaba el análisis de las estructuras sexuales, importantes para la correcta identificación de las especies. Los ejemplares no identificados se excluyeron de cualquier tipo de análisis morfológico. Del mismo modo, se detectaron en total cinco nuevas especies, de las cuales de tres (*P. mariachi*, *P. sikuapu* y *P. xerophilus*), se obtuvieron ejemplares machos y hembras, del resto de especies (*P. lyncis* y *P. pocamadre*), solo se obtuvieron hembras (Cuadro 1). Se utilizaron 34 especies para los análisis morfológicos. Asimismo, algunas especies (*P. huacana, P. montanoi, P. pedregosus, P. peribaniensis, P. platnicki, P. sarae* y *P. sprousei*) no se pudieron recolectar por dos razones principales: la inseguridad latente en las localidades tipo de las especies o por las restricciones sanitarias impuestas a causa de la pandemia global (Cuadro 1), sin embargo, en

algunos casos, los holotipos fueron revisados para realizar los análisis morfológicos correspondientes.

Las observaciones de los ejemplares fueron realizadas con un microscopio estereoscópico marca Zeiss Discovery Stereoscope V.8. Los ejemplares fueron depositados en alcohol etílico al 80% dentro de un contenedor con arena, esto para su observación y facilitar su manipulación. Se realizó la disección del palpo derecho de los machos y del epiginio de las hembras, los cuales fueron colocados en KOH (10%) por unos minutos, para digerir el tejido blando y observar estructuras internas. Para la identificación, se ocupó la clave taxonómica de Valdez-Mondragón (2010) para el género *Physocyclus*. Las medidas de las estructuras se obtuvieron con ayuda de una reglilla incorporada al microscopio, haciendo una conversión de medidas a milímetros (mm). Las fotografías para las placas utilizadas en los capítulos taxonómicos se tomaron con un microscopio marca Zeiss Axio Zoom V.16, y los softwares de digitalización Axio Zoom Zen y Zen Pro. Dichas fotografías, se editaron con el programa Photoshop CS6 v.1.30x32.

Cuadro 1. Material examinado para los análisis morfológicos por cada especie del género *Physocyclus*.

Especie	No. Ejemplares	Estados de México (*otro país)
P. bicornis	54	Guerrero
P. brevicornus	157	Colima, Guanajuato, Jalisco, Michoacán, San Luis Potosí
P. cornutus	7	Baja California Sur
P. darwini	24	Guerrero
P. dugesi	1101	Ciudad de México, Edo. México, Guanajuato, Guerrero, Jalisco, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, Tlaxcala, Veracruz,
P. enaulus	18	Chihuahua, Coahuila, Durango
P. franckei	21	Hidalgo
P. gertschi	35	Guerrero
P. globosus	408	Baja California Sur, Chiapas, Colima, Guanajuato, Guerrero, Jalisco, Michoacán, Nayarit, Oaxaca, Puebla, Quintana Roo, Sinaloa, Veracruz

P. guanacaste	1	Honduras*
P. hoogstraali	34	Coahuila, Nuevo León
P. huacana	1	Michoacán
P. lautus	101	Colima, Jalisco, Michoacán
P. lyncis	6	Jalisco
P. marialuisae	1	Baja California Sur
P. mariachi	5	Jalisco
P. merus	25	Coahuila, San Luis Potosí
P. michoacanus	34	Edo México, Guerrero, Michoacán, Oaxaca
P. modestus	178	Guerrero, Jalisco, Morelos, Nuevo León, Oaxaca, Puebla
P. montanoi	1	Michoacán
P. mysticus	21	Baja California, Baja California Sur
P. palmarus	1	Baja California Sur
P. paredesi	16	Oaxaca
P. pedregosus	1	Coahuila
P. peribaniensis	1	Michoacán
P. pocamadre	4	Baja California Sur
P. reddelli	228	Hidalgo, Querétaro, San Luis Potosí
P. rothi	30	Baja California Sur
P. sarae	1	Michoacán
P. sikuapu	14	Michoacán
P. sprousei	1	Chihuahua
P. tanneri	8	Sonora
P. validus	254	Colima, Guerrero, Morelos, Sinaloa, Puebla
P. xerophilus	9	Baja California Sur
TOTAL	**2787**	**30 estados (2 países: México y Honduras)**

7. ANÁLISIS MOLECULARES

La separación del tejido, las extracciones de ADN, amplificaciones por reacciones en cadena de la polimerasa (PCR) y purificación se realizaron en el Laboratorio de Biología Molecular del Laboratorio de Biodiversidad y Cultivo de Tejidos Vegetales.

7.1 Separación de tejido

El principal criterio de selección de material biológico fue que no tuviera una antigüedad mayor a cinco años de colecta. La cantidad de tejido seleccionado para ejemplares adultos, fue de una a tres patas, mientras que, en juveniles, se contemplaron de cuatro a cinco patas o incluso el ejemplar completo (debido a su menor tamaño y dependiendo del caso). Posteriormente, se almacenó el tejido en etanol (96%) y en refrigeración a -20°C, hasta su uso para la extracción de ADN. Los datos del material fueron capturados en la base de datos del LATLAX.

7.2 Extracción y cuantificación de ADN

La extracción de ADN total se realizó utilizando el kit de extracción Qiagen DNeasy Tissue, con las modificaciones realizadas por Valdez-Mondragón y Francke (2015). Después del aislamiento del material genético, se realizó su cuantificación, con ayuda de un espectrofotómetro Colibri Microvolume Spectometer-Titertek Berthold, colocando 1 µL de muestra de ADN y se efectuó una lectura entre 260 y 280 nm. Los valores aceptados de cuantificación y pureza del ADN oscilan entre 1.8 – 2.2 unidades de A260/280, ya que valores menores indican de manera regular la presencia de proteínas y baja pureza del ADN.

7.3 Reacción en cadena de la Polimerasa (PCR) y geles de electroforesis

La elaboración de la PCR se llevó a cabo para los marcadores moleculares CO1, ITS2 y 28S (los oligonucleótidos y protocolos específicos utilizados se detallan en los capítulos uno y dos). Después de cada PCR, se elaboró un gel de agarosa al 0.5% para verificar si se obtuvieron las regiones genéticas de interés. Este gel fue elaborado con TBE (50 ml) y agarosa (0.5 gr). El buffer de carga Loading Dye fue mezclado con 1 µL de GelRed (Loading Dye + GelRed), para teñir cada muestra y posteriormente visualizar los fragmentos obtenidos en un fotodocumentador BioDoc-It2 Imager 315 Imaging System LMS-20 Transilluminator.

Se colocó dentro de un pozo del gel, un marcador de peso molecular 100 pb Perfect DNA 100 bp Ladder Novage, para comparar el tamaño de los amplicones. Del mismo modo, se utilizó un control negativo para detectar cualquier tipo de contaminación en la PCR. En los pozos restantes, se colocó una mezcla consistente de 1.75 µL del producto de PCR más 1 µL de buffer de carga Loading Dye + GelRed. Estos geles se corrieron en cámaras de electroforesis marca Thermo Scientific durante un periodo de 30 minutos a 100 volts.

7.4 Purificación y secuenciación de ADN

Para eliminar cualquier residuo de compuestos que no fuera ADN, además de concentrar y mejorar la calidad de los fragmentos de ADN, cada producto de PCR se purificó con ayuda de un kit de purificación QIAquick QIAGEN, siguiendo el protocolo del fabricante. Las muestras purificadas se procesaron en un secuenciador Genetic Analyzer 3730xL a cargo de la M. en C. Laura Margarita Márquez Valdelamar, Técnico Académico Titular B, responsable del Laboratorio de Biología Molecular y de la Salud del Instituto de Biología de la UNAM, Ciudad Universitaria, Ciudad de México.

7.5 Edición de secuencias

7.5.1 Identidad y ensamble de secuencias

Las secuencias fueron recibidas en archivos digitales en formato ".abi". Cada secuencia (forward y reverse) fue revisada mediante un análisis con el programa BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) en la plataforma GenBank-NCBI, con el propósito de confirmar la identidad de los ejemplares y detectar posibles contaminaciones. Se descartaron aquellas secuencias cuya calidad era baja (>30% HQ-High Quality). Para el ensamble de las secuencias forward y reverse de cada una de las muestras generadas en este estudio, se ocupó el programa bioinformático Geneious v.8.1.9 (Rozen y Skaletsky 2000), con el cual se obtuvo una secuencia consenso para cada par de secuencias (forward y reverse) por muestra, esto para realizar los análisis posteriores. La parte inicial y final de cada secuencia se eliminó utilizando el algoritmo "Trim" del programa Geneious v.8.1.9, ya que son partes de baja calidad de las secuencias, originadas por el proceso de secuenciación.

7.5.2 Alineamiento múltiple de secuencias

Se elaboró un alineamiento final con el programa MAFFT v.7 (Katoh y Toh 2008) mediante su plataforma en línea (https://mafft.cbrc.jp/alignment/server/) usando la siguiente estrategia de alineamiento: Auto (FFT-NS-2, FFTNS-i o L-INS-i; "dependiendo del tamaño de la matriz"). En algunos casos, donde la calidad de la secuencia no era lo suficientemente alta (31–65 %), el alineamiento resultante se editó manualmente con el programa BioEdit v.7.0.5.3 (Hall 1999), tomando como guía, las lecturas de los electroferogramas de cada secuencia. Los tres genes (CO1+ITS2+28S) se concatenaron en una sola matriz de datos moleculares, aunado a una concatenación posterior con datos morfológicos (Morfología+CO1+ITS2+28S), esto a partir del programa Geneious v.8.1.9.

8. ESTRUCTURACIÓN POR CAPÍTULOS

Para facilitar la organización de los análisis de la presente investigación, tanto morfológicos como moleculares, esta tesis está organizada en cuatro capítulos, que conforman los artículos científicos, resultados de este proyecto. En cada capítulo se especifican a detalle los materiales y métodos.

8.1 Capítulo 1.

Artículo de delimitación de especies: **"To be or not to be… Integrative taxonomy and species delimitation in the daddy long-legs spiders of the genus *Physocyclus* (Araneae, Pholcidae) using DNA barcoding and morphology"**.

Este artículo se publicó en la revista *Zookeys*. Este capítulo trata sobre el uso de los marcadores mitocondriales CO1 e ITS2 y el gen nuclear 28S para los análisis de delimitación de especies del género *Physocyclus*. Los métodos de delimitación de especies utilizados en este capítulo son: 1) Automatic Barcode Gap Discovery (ABGD), 2) Assembly Species by Automatic Partitioning (ASAP), 3) General Mixed Yule Coalescent (GMYC) y 4) Bayesian Poisson Tree Proceses (bPTP). También se realizó un análisis de distancia genéticas (distancias-*p* corregidas) bajo el criterio de Neighbor Joining (NJ).

Cita completa del artículo:

Nolasco, S. & Valdez-Mondragón, A. 2022. To be or not to be… Integrative taxonomy and species delimitation in the daddy long-legs spiders of the genus *Physocyclus* (Araneae, Pholcidae) using DNA barcoding and morphology. *ZooKeys* 1135: 93–118. https://doi.org/10.3897/zookeys.1135.94628

8.2 Capítulo 2.

Artículo sobre Sistemática Filogenética: **"One or more genera? Phylogenetics and diversification of the spider genus *Physocyclus* Simon (Araneae: Pholcidae) based on morphological and molecular evidence, with the description of a new genus: *Mictlanus* gen. nov."**

Este capítulo se centra en la reconstrucción filogenética del género, con base en los marcadores moleculares CO1, ITS2 y 28S, aunado a caracteres morfológicos. Se integran a los análisis filogenéticos morfológicos las especies *Physocyclus mariachi*, *P. sikuapu* y *P. xerophilus*¸ ya que no se incluyeron en los análisis filogenéticos morfológicos de Valdez-Mondragón (2013, 2014). La matriz morfológica utilizada consta de 54 caracteres (44 binarios + 10 multiestado), la matriz molecular concatenada está conformada por 2048 caracteres (644 pb para CO1, 501 pb para ITS2 y 903 pb para 28S), mientras que la matriz concatenada con evidencia combinada (Morfología+CO1+ITS2+28S) es de 2102 caracteres. Los análisis filogenéticos realizados en este capítulo son de Parsimonia, Máxima Verosimilitud e Inferencias Bayesianas. También se muestran los resultados sobre los análisis de datación de linajes y sobre la reconstrucción de áreas ancestrales potenciales, así como de los procesos que influyeron en la diversificación del género. Este capítulo actualmente se encuentra en revisión en la revista *Molecular Phylogenetics and Evolution*.

Cita completa del artículo:

Valdez-Mondragón, A & Nolasco, S. (*in press*). One or more genera? Phylogenetics and diversification of the spider genus *Physocyclus* Simon (Araneae: Pholcidae) based on morphological and molecular evidence, with the description of a new genus: *Mictlanus* gen. nov. *Molecular Phylogenetics and Evolution.*

8.3 Capítulo 3.

Artículo sobre la descripción de cuatro nuevas especies del género *Physocyclus*, en México: **"Four new species of the spider genus *Physocyclus* Simon, 1893 (Araneae: Pholcidae) from Mexico, with updated taxonomic identification keys"**.

Este artículo se publicó en el *European Journal of Taxonomy*. En este trabajo se presentan las ultimas descripciones taxonómicas del género. Se describen cuatro especies nuevas de *Physocyclus*, encontradas en México (*P. lyncis* sp. nov., *P. mariachi* sp. nov., *P. pocamadre* sp. nov. y *P. sikuapu* sp. nov.), y se realiza una actualización de la clave de identificación taxonómica tanto para machos como hembras, donde se integran siete especies adicionales. La diversidad de especies del género *Physocyclus* aumenta a 37 especies descritas hasta el momento.

Cita completa del artículo:

Nolasco, G. S. & Valdez-Mondragón, A. 2022. Four new species of the spider genus *Physocyclus* Simon, 1893 (Araneae: Pholcidae) from Mexico, with updated taxonomic identification keys. *European Journal of Taxonomy*. 813: 173–206. https://doi.org/10.5852/ejt.2022.813.1739

8.4 Capítulo 4.

Artículo sobre la descripción de una nueva especie del género *Physocyclus*, en el estado de Baja California Sur: **"On the daddy long-legs spiders of the genus *Physocyclus* (Araneae: Pholcidae) from Mexico: description of a new species from the Baja California Peninsula"**.

Este artículo fue publicado en la *Revista Mexicana de Biodiversidad*. En este trabajo taxonómico, se describe *Physocyclus xerophilus*, encontrada en matorrales xerófilos cercanos en la costa del Golfo de California, en el estado de Baja California Sur.

Cita completa del artículo:

Nolasco, S. & Valdez-Mondragón, A. 2020. On the daddy long-legs spiders of the genus *Physocyclus* (Araneae: Pholcidae) from Mexico: description of a new species from the Baja California Peninsula. *Revista Mexicana de Biodiversidad* 91 pags.7. https://doi.org/10.22201/ib.20078706e.2020.91.3316

9. Capítulo 1

Artículo Delimitación de Especies

To be or not to be… Integrative taxonomy and species delimitation in the daddy long-legs spiders of the genus *Physocyclus* (Araneae, Pholcidae) using DNA barcoding and morphology

Samuel Nolasco – Alejandro Valdez-Mondragón

Ser o no ser… Taxonomía integradora y delimitación de especies en las arañas patonas del género *Physocyclus* (Araneae, Pholcidae) usando código de barras de ADN y morfología

Samuel Nolasco – Alejandro Valdez-Mondragón

Nolasco, S. & Valdez-Mondragón, A. 2022. To be or not to be… Integrative taxonomy and species delimitation in the daddy long-legs spiders of the genus *Physocyclus* (Araneae, Pholcidae) using DNA barcoding and morphology. *ZooKeys* 1135: 93–118. https://doi.org/10.3897/zookeys.1135.94628

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To be or not to be… Integrative taxonomy and species delimitation in the daddy long-legs spiders of the genus *Physocyclus* (Araneae, Pholcidae) using DNA barcoding and morphology

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Abstract

Integrative taxonomy is crucial for discovery, recognition, and species delimitation, especially in underestimated species complex or cryptic species, by incorporating different sources of evidence to construct rigorous species hypotheses. The spider genus *Physocyclus* Simon, 1893 (Pholcidae, Arteminae) is composed of 37 species, mainly from North America. In this study, traditional morphology was compared with three DNA barcoding markers regarding their utility in species delimitation within the genus: 1) Cytochrome c Oxidase subunit 1 (CO1), 2) Internal Transcribed Spacer 2 (ITS2), and 3) Ribosomal large subunit (28S). The molecular species delimitation analyses were carried out using four methods under the corrected *p*-distances Neighbor-Joining (NJ) criteria: 1) Automatic Barcode Gap Discovery (ABGD), 2) Assemble Species by Automatic Partitioning (ASAP), 3) General Mixed Yule Coalescent model (GMYC), and 4) Bayesian Poisson Tree Processes (bPTP). The analyses incorporated 75 terminals from 22 putative species of *Physocyclus*. The average intraspecific genetic distance (*p*-distance) was found to be < 2%, whereas the average interspecific genetic distance was 20.6%. The ABGD, ASAP, and GMYC methods were the most congruent, delimiting 26 or 27 species, while the bPTP method delimited 33 species. The use of

traditional morphology for species delimitation was congruent with most molecular methods, with the male palp, male chelicerae, and female genitalia shown to be robust characters that support species-level identification. The barcoding with CO1 and 28S had better resolution for species delimitation in comparison with ITS2. The concatenated matrix and traditional morphology were found to be more robust and informative for species delimitation within *Physocyclus*.

Keywords
Arteminae, cellar spiders, molecular markers, molecular methods, North America

Introduction

Species delimitation is the act of identifying species-level biological diversity (Carstens et al. 2013). It arises from the need to classify, identify, and establish the limits between species (DeSalle et al. 2005; Carstens et al. 2013; Rannala and Yang 2020). The assignment of individual organisms into pre-existing species or higher-level categories (e.g., genus, family, order, etc.) and the designation of new species with proper diagnoses to distinguish them were, for a long-time, roles performed by taxonomist using only traditional morphology and/or somatic characters (Jörger and Schrödl 2013; Rannala and Yang 2020). However, the presence of plastic characters (i.e., characters with high morphological variation), relatively few distinctive morphological traits among species, and morphological stasis due to environmental selection in some groups of organisms (Bickford et al. 2007; Carstens et al. 2013), often makes species delimitation using only morphological evidence extremely difficult or impossible (DeSalle et al. 2005; Rannala and Yang 2020). Spiders are no exception, and due to the complications of using only morphology to identify and delimit species in some groups of araneomorphs and mygalomorphs spiders, different molecular methods have been applied to delimit spider species (Huber et al. 2005; Galtier et al. 2009; Hamilton et al. 2011; Ortiz and Francke 2016; Candia-Ramírez and Francke 2020; Navarro-Rodríguez and Valdez-Mondragón 2020; Valdez-Mondragón 2020; Hazzi and Hormiga 2021). Studies using DNA and RNA have been successful in classifying difficult groups and uncovering underestimated biodiversity (Fox et al. 1977; Wayne et al. 1987; Wilson 1995; Bond 2004).

The spiders of the family Pholcidae C. L. Koch, commonly known as cellar spiders or daddy long-legs spiders, is currently composed of 1,896 species in 97 genera (WSC 2022). Pholcidae is the ninth largest spider family in the World and the most diverse within the Synspermiata clade. The family is composed of five subfamilies: Arteminae Simon, 1893, Modisiminae Simon, 1893, Ninetinae Simon, 1890, Pholcinae C. L. Koch, 1850, and Smeringopinae Simon, 1893 (Huber 2011; Dimitrov et al. 2013; Eberle et al. 2018; Huber and Carvalho 2019). Arteminae includes 107 species distributed in nine genera: *Artema* Walckenaer, 1837, *Aucana* Huber, 2000, *Chisosa* Huber, 2000, *Holocneminus* Berland, 1942, *Nita* Huber & El-Hennawy, 2007, *Pholcitrichocyclus* Ceccolini & Cianferoni, 2022, *Physocyclus* Simon, 1893, *Tibetia* Zhang, Zhu & Song, 2006, and *Wugigarra* Huber, 2001 (WSC 2022).

Physocyclus comprises 37 described species, classified into two species groups proposed by Valdez-Mondragón (2013, 2014). To date, the *globosus*-group includes 15 species, while 22 species are recognized in the *dugesi*-group (Valdez-Mondragón 2013, 2014; Jiménez and Palacios-Cardiel 2013; Nolasco and Valdez-Mondragón 2020, 2022). Recently, five species from Mexico were described by Nolasco and Valdez-Mondragón (2020, 2022) based only on traditional morphology, four from the *globosus*-group and one of the *dugesi*-group. The genus is distributed mainly in arid and semiarid ecosystems, as well as tropical dry forests, mostly in North America, with some species in Central America (Valdez-Mondragón 2010, 2013, 2014; Jiménez and Palacios-Cardiel 2013; Nolasco and Valdez-Mondragón 2020, 2022). Most species in the genus are found under 1900 m a.s.l., with the exception of *Physocyclus dugesi* Simon 1893 and *Physocyclus globosus* (Taczanowski, 1874), whose distributions are influenced by synanthropic activities that have allowed them to occupy higher elevations in association with human dwellings (Valdez-Mondragón 2010). *Physocyclus globosus* is considered a cosmopolitan species, with records from North and Central America, the Caribbean Islands, the Pacific Islands, South America, Asia, Africa, and Oceania (Valdez-Mondragón 2010; WSC 2022).

The most recent taxonomic revisions and morphological phylogenetic analyses (Valdez-Mondragón 2010, 2013, 2014), as well as new species descriptions using traditional morphology (Jiménez and Palacios-Cardiel, 2013; Nolasco and Valdez-Mondragón 2020, 2022) have revealed 20 new species from Mexico, which represents 54% of the known species diversity in *Physocyclus*.

The general morphology among the different genera of pholcid spiders is conservative, with slight differences in somatic structures. However, primary sexual structures such as male palps and female genitalia, as well as secondary sexual structures such as male chelicerae, are important features used for identification and species diagnosis due to the fact that genitalia evolve more rapidly than non-genital morphological features (Huber 2003; Huber et al. 2018; Valdez-Mondragón 2020). Speciation in arthropods is associated with marked changes in genital morphology, which explains the usefulness of genitalia in distinguishing closely related species (Huber et al. 2005). Sexual structures have different intraspecific and interspecific variation rates in spiders (Eberhard 1985; Eberhard et al. 1998). However, some groups show an overlapping of characters due to minimal morphological variation, making the identification and delimitation of species difficult (Hamilton et al. 2011; Zhang and Li 2014; Ortiz and Francke 2016; Tyagi et al. 2019; Valdez-Mondragón et al. 2019; Candia-Ramírez and Francke 2020; Navarro-Rodríguez and Valdez-Mondragón 2020; Hazzi and Hormiga 2021). Additionally, speciation without changes in genital shape has been recorded in some pholcid spiders, as demonstrated by Huber et al. (2005) with two species of "*Psilochorus*" from South America that showed the same genitalia shape but extreme differences in size and coloration pattern.

Spiders with generally simple genitalia, such as mygalomorphs and some araneomorphs (Synspermiata), are complicated cases for species delimitation and identification using morphology (Huber et al. 2005; Hamilton et al. 2011; Huber and Dimitrov

2014; Valdez-Mondragón 2013, 2014, 2020; Nolasco and Valdez-Mondragón 2022). Additionally, intrasexual polymorphism has been described among females in some pholcids species. Huber & Pérez-González (2001a, b) described two different epigyne morphotypes in females of *Ciboneya antraia*. In the same way, Valdez-Mondragón (2010) described discontinued interspecific variation in the epigyne shape in females of *Physocyclus enaulus* Crosby, 1926, with three distinct morphotypes.

Due to the poor morphological variation and simple genitalia in some taxonomic groups, approximations based on DNA barcoding using mitochondrial data are often used to establish limits between species, detect species complexes, and/or discover new species in different spider groups (Barrett and Hebert 2005; DeSalle et al. 2005; Bickford et al. 2007; Galtier et al. 2009; Carstens et al. 2013; Navarro-Rodríguez and Valdez-Mondragón 2020; Rannala and Yang 2020; Valdez-Mondragón 2020). Barcoding based on cytochrome c oxidase subunit 1 (CO1) is the common standard in animal Barcoding (including spiders), and its effectivity and resolution has been tested in many studies (Astrin et al. 2006; Planas and Ribera 2015; Ortiz and Francke 2016; Valdez-Mondragón et al. 2019; Navarro-Rodríguez and Valdez-Mondragón 2020; Valdez-Mondragón 2020). While the mitochondrial marker CO1 seems to be suitable for DNA barcoding, it is susceptible to over- and underestimating the diversity in some cases (Astrin et al. 2006; Ortiz and Francke 2016). As such, it is preferable to complement the use of the CO1 marker with other informative mitochondrial (16S) or nuclear (ITS1 or ITS2) markers, as well as morphological evidence, to obtain better resolution (Astrin et al. 2006; Agnarsson 2010; Planas and Ribera 2015; Ortiz and Francke 2016; Valdez-Mondragón et al. 2019; Navarro-Rodríguez and Valdez-Mondragón 2020). The nuclear molecular marker 28S has been used mainly in phylogenetic analyses, providing high resolution for basal clades (Bruvo-Madarić et al. 2005; Álvarez-Padilla et al. 2009; Arnedo et al. 2009; Dimitrov and Hormiga 2011; Dimitrov et al. 2013). Due to its low substitution rate, 28S is useful for estimating phylogenetic hypotheses of taxa with very old divergence times (Hillis and Dixon 1991). However, this marker has never been tested in species delimitation analyses within the family Pholcidae.

In modern systematics, integrative taxonomy studies that combine different sources of evidence, such as molecular markers and morphological data, are commonly implemented to help delimit and diagnose new species or even identify cryptic species complexes (Astrin and Stueben 2008; Álvarez-Padilla et al. 2009; Correa-Ramírez et al. 2010; Hamilton et al. 2011; Montes de Oca et al. 2015; Planas and Ribera 2015; Valdez-Mondragón and Francke 2015; Cao et al. 2016; Valdez-Mondragón et al. 2019; Newton et al. 2020; Valdez-Mondragón and Cortez-Roldán 2021). Recent publications on spider taxonomy have additionally used other sources of information, such as Ecological Niche Modeling (ENM), lineal morphology, and even geometric morphology to characterize species (Valdez-Mondragón et al. 2019; Navarro-Rodríguez and Valdez-Mondragón 2020; Solís-Catalán 2020).

From the first proposal for a DNA barcoding initiative using a single locus (the CO1 mitochondrial gene for animals) as a diagnostic for assigning species (Hebert et al. 2003), the field of species delimitation has been refined and improved, incorporating different theories (e.g., coalescence) and methods (DeSalle et al. 2005; Hickerson et

al. 2006; Pons et al. 2006; Puillandre et al. 2012, 2021; Carstens et al. 2013; Rannala and Yang 2020). Species delimitation analyses using DNA Barcoding, initially with only a single locus (CO1), is limited as a diagnostic for assigning species based on the fact that intraspecific and interspecific sequence distances may be similar in large populations (Hebert et al. 2003; Rannala and Yang 2020). Therefore, many studies have opted for a multi-locus approach, combining CO1 with other molecular markers to give additional robust evidence for species delimitation (Astrin et al. 2006; Agnarsson 2010; Planas and Ribera 2015; Ortiz and Francke 2016; Ballesteros and Hormiga 2018; Navarro-Rodríguez and Valdez-Mondragón 2020; Hazzi and Hormiga 2021).

The aim of this study is to carry out different species delimitation methods within the spider genus *Physocyclus* under an integrative taxonomic approach. To carry out this, we use a combination of molecular markers (CO1, ITS2, and 28S) and traditional morphology of diagnostical features (e.g., male palps, male chelicerae, and female epigynes) to test the validation of the currently recognized species within the genus.

Materials and methods

Biological material

Specimens were provided by the Laboratory of Arachnology (**LATLAX**) **IB-UNAM**, Tlaxcala, Mexico; the Colección Nacional de Arácnidos (**CNAN**), Institute of Biology, Universidad Nacional Autónoma de México (**IB-UNAM**), Mexico City; Centro de Investigaciones Biológicas de Noreste (**CIBNOR**), La Paz, Baja California Sur, Mexico; and Colección Aracnológica de la Facultad de Biología de la Universidad Michoacana de San Nicolás de Hidalgo (**CAFBUM**), Michoacan, Mexico. Specimens were preserved in 80% ethanol for morphological studies and 96% ethanol for molecular studies. Female sexual structures (epigyne) were dissected in 80% ethanol and cleaned with potassium hydroxide (10% KOH). This to remove all soft tissue and observe with clarity the taxonomically important internal structures, such as the pore plates. The left male palps were dissected and observed in 80% ethanol. Structures were photographed submerged in commercial gel alcohol to hold them in the appropriate position, while the preparation was done with structures completely covered with 80% ethanol. Specimen observations and identifications were carried out using a Zeiss Discovery V8 stereo microscope. A Zeiss Axiocam 506 color camera attached to a Zeiss AXIO Zoom V16 stereo microscope was used to photograph focal structures (male palps, female epigynes, and male chelicerae). Digital images of morphological structures were edited in Adobe Photoshop CS6.

Taxon sampling

The molecular analyses were based on a total of 194 sequences of 23 putative species. Species used in the molecular analyses are listed in Table 1. The ingroup includes 188 sequences of 22 species of *Physocyclus* previously described by Valdez-Mondragón (2010, 2013, 2014) and Nolasco and Valdez-Mondragón (2020, 2022). The CO1 se-

Table 1. Specimens sequenced for each species of *Physocyclus*, DNA voucher numbers, localities, and GenBank accession numbers for CO1, ITS2, and 28S. Mexican state abbreviations: BC, Baja California; BCS, Baja California Sur; COL, Colima; GRO, Guerrero; HGO, Hidalgo; JAL, Jalisco; MICH, Michoacán, OAX, Oaxaca; PUE, Puebla. **Non-Mexican localities.

Species	DNA Code LATLAX	Locality (Mexico)	CO1	ITS2	28S
P. bicornis	Ara0394	GRO: Copala	OP293157	OP296540	OP295410
P. bicornis	Ara0396	GRO: Quechultenango	OP293158	OP296538	OP295411
P. bicornis	Ara0398	GRO: Coyuca	OP293159	OP296539	
P. bicornis	Ara0445	GRO: Quechultenango	OP293160	OP296541	OP295412
P. brevicornus	Ara0515	JAL: Cocula	OP293161	OP296542	OP295413
P. brevicornus	Ara0516	MICH: Morelia	OP293162		
P. brevicornus	Ara0518	MICH: Morelia	OP293163	OP296543	OP295414
P. cornutus	Ara0405	BCS: Los Cabos	OP293164		OP295415
P. cornutus	Ara0406	BCS: Los Cabos	OP293165		OP295416
P. dugesi	Ara0597	HGO: Tula	OP293166	OP296544	OP295417
P. dugesi		Costa Rica**	AY560787		AY560750
P. enaulus	Ara0391	COA: Saltillo	OP293167	OP296545	OP295418
P. enaulus	Ara0392	COA: Saltillo	OP293168	OP296546	OP295419
P. enaulus	Ara0393	COA: Saltillo	OP293169	OP296547	OP295420
P. enaulus		U.S.A.**	MG268722		
P. franckei	Ara0378	HGO: Tolantongo	OP293170	OP296548	
P. franckei	Ara0379	HGO: Cárdenas	OP293171	OP296549	OP295421
P. franckei	Ara0381	HGO: Cardonal	OP293172	OP296550	
P. franckei	Ara0382	HGO: Cardonal	OP293173	OP296551	
P. gertschi	Ara0575	GRO: José Azueta	OP293174	OP296552	OP295422
P. gertschi	Ara0576	GRO: José Azueta	OP293175	OP296553	OP295423
P. gertschi	Ara0577	GRO: José Azueta	OP293176	OP296554	OP295424
P. globosus	Ara0473	COL: Coquimatlán	OP293177	OP296555	OP295425
P. globosus	Ara0533	BCS: Comundú	OP293178		
P. globosus	Ara0535	GRO: Tĕcpan	OP293179	OP296556	OP295426
P. globosus		Quintana Roo	MT888253		
P. globosus		Cuba**	AY560788		AY560751
P. lautus	Ara0459	MICH: Cárdenas	OP293180	OP296557	OP295427
P. lautus	Ara0579	MICH: Coahuayana	OP293181	OP296558	OP295428
P. lautus	Ara0583	JAL: La Huerta	OP293182		OP295429
P. lyncis	Ara0437	JAL: Zapopan	OP293183	OP296559	OP295430
P. lyncis	Ara0754	JAL: Zapopan	OP293184	OP296560	OP295431
P. mariachi	Ara0745	JAL: Hostotipaquillo	OP293185	OP296561	OP295432
P. mariachi	Ara0746	JAL: Hostotipaquillo	OP293186	OP296562	OP295433
P. mariachi	Ara0748	JAL: Plan de Barrancas	OP293187	OP296564	OP295434
P. merus	Ara0898	SLP: Villa de Reyes	OP293188	OP296565	OP295435
P. merus	Ara0915	SLP: Villa de Reyes	OP293189		OP295436
P. merus	Ara0916	SLP: Villa de Reyes	OP293190	OP296566	OP295437
P. merus	Ara0917	SLP: Villa de Reyes	OP293191	OP296567	OP295438
P. merus	Ara0918	SLP: Villa de Reyes	OP293192		OP295438
P. michoacanus	Ara0585	MICH: Tzitzio	OP293193	OP296568	OP295440
P. michoacanus	Ara0586	MICH: Tzitzio	OP293194	OP296569	OP295441
P. michoacanus	Ara0598	JAL: Jilotlán	OP293195		OP295442
P. modestus	Ara0467	PUE: Miahuatlán	OP293196	OP296570	OP295443
P. modestus	Ara0469	GRO: Tepecoacuilco	OP293197	OP296571	OP295444
P. modestus	Ara0480	GRO: Escudero	OP293198		OP295445
P. modestus	Ara0482	GRO: Quechultenango	OP293199		
P. mysticus	Ara0450	BC: Ensenada	OP293200	OP296572	
P. mysticus	Ara0451	BC: Ensenada	OP293201	OP296573	
P. mysticus	Ara0452	BCS: Mulegé	OP293202		OP295446

Species	DNA Code LATLAX	Locality (Mexico)	CO1	ITS2	28S
P. mysticus	Ara0453	BCS: Mulegé	OP293203		OP295447
P. mysticus	Ara0524	BC: Ensenada	OP293204		
P. paredesi	Ara0483	OAX: Tadela	OP293205	OP296574	OP295448
P. paredesi	Ara0484	OAX: Tadela	OP293206	OP296575	OP295449
P. paredesi	Ara0485	OAX: Totolapa	OP293207	OP296576	OP295450
P. paredesi	Ara0486	OAX: Totolapa	OP293208		OP295451
P. pocamadre	Ara0371	BCS: Mulegé	OP293209		
P. reddelli	Ara0487	HGO: Araya	OP293210	OP296577	OP295452
P. reddelli	Ara0488	HGO: Araya	OP293211	OP296578	
P. rothi	Ara0383	BCS: Comundú	OP293212	OP296579	OP295453
P. rothi	Ara0384	BCS: Comundú	OP293213	OP296580	OP295456
P. rothi	Ara0386	BCS: La Paz	OP293214		OP295454
P. rothi	Ara0387	BCS: La Paz	OP293215		OP295455
P. sikuapu	Ara0749	MICH: Costa Aquila	OP293216	OP296581	OP295457
P. sikuapu	Ara0750	MICH: Costa Aquila	OP293217	OP296582	OP295458
P. sikuapu	Ara0751	MICH: Costa Aquila	OP293218	OP296583	OP295459
P. sikuapu	Ara0752	MICH: Costa Aquila	OP293219	OP296584	OP295460
P. validus	Ara0502	COL: Coquimatlán	OP293220	OP296585	
P. validus	Ara0503	GRO: Eduardo Neri	OP293221	OP296586	OP295461
P. xerophilus	Ara0372	BCS: Mulegé	OP293222	OP296587	OP295464
P. xerophilus	Ara0373	BCS: Mulegé	OP293223	OP296588	OP295462
P. xerophilus	Ara0374	BCS: Mulegé	OP293224	OP296589	OP295465
P. xerophilus	Ara0375	BCS: Mulegé	OP293225	OP296590	
P. xerophilus	Ara0376	BCS: Mulegé	OP293226	OP296591	OP295463
P. xerophilus	Ara0377	BCS: Mulegé	OP293227	OP296592	
Chisosa sp.	Ara0454	PUE: Miahuatlán	OP293228	OP296593	OP295466
Chisosa sp.	Ara0455	PUE: Miahuatlán	OP293229	OP296594	OP295467

quence matrix is composed of 75 sequences (22 species), ITS2 with 55 sequences (20 species), and 28S with 58 sequences (21 species). Four different partitions were used in the analyses: 1) CO1: 642 bp, 2) ITS2: 505 bp, 3) 28S: 891 bp, and 4) the concatenated matrix CO1+ITS2+28S: 2038 bp. Since this study is focused solely on species delimitation within *Physocyclus* and not on the molecular dating and phylogenetic relationships within Arteminae, only *Chisosa* sp. (Arteminae) was used as an outgroup, to root the trees in the various analyses.

DNA extraction, amplification, and sequencing

For DNA extraction, we used the Qiagen DNeasy extraction kit, following the modifications suggested by Valdez-Mondragón and Francke (2015) and Valdez-Mondragón (2020). Three legs from each specimen were used for DNA extraction, using males, females, and juveniles, depending on the available specimens per species. The criterion for selecting tissues was based on tissue antiquity, considering only specimens collected in the last five years in order to extract high DNA quality. Amplification of the CO1 locus was carried out using two different primer sets: LCO1490/HCO2198 and LCO-JJ/HCO-JJ; for ITS2, the primer set 5.8S and CAS28SB1d was used; and for 28S, 28S-B1 and 28S-B2 (Table 2). Polymerase chain reactions (PCR) were carried out in a Verity-Applied Biosystems 96 Well Thermal Cycler. The final volume of

Table 2. Primer sets used in this study for PCR amplification.

Molecular marker	Primer	Sequence (5'-3')	Author
CO1	HCO2198	TAAACTTCAGGGTGACCAAAAAATC	Folmer et al. (1994)
	LCO1490	GGTCAACAAATCATAAAGATATTGG	
	HCO-JJ	AWACTTCVGGRTGCVCAAARAATCA	Astrin and Stueben (2008)
	LCO-JJ	CHACWAAYCATAAAGATATYGG	
ITS2	5.8S	CGCCTGTTTATCAAAAACAT	Ji et al. (2003); Planas and Ribera (2014)
	CAS28sB1d	TTC TTT TCC TCC SCT TAY TRA TAT GCT TAA	
28S	28S-B1	GACCGATAGCAAACAAGTACCG	Bruvo-Mađarić et al. (2005)
	28S-B2	CACGGGTCGATGAAGAACGC	

each PCR tube was 20 µl: 2.3 µl injectable H_2O, 2.0 µl Q-solution, 10 µl Multiplex-Mix PCR, 1.6 µl of each primer (forward and reverse), and 2.5 µl of extracted DNA sample. The cycles and optimal temperatures for CO1 and ITS2 amplification were as follows: Initial heating phase of 15 min at 95 °C, 35 amplification cycles of 35 s at 94 °C (denaturing), 1 min 30 s at 40 °C (alignment), and 1 min 30 s at 72 °C (elongation), with a final elongation of 10 min at 72 °C. Two different protocols were used to amplify the 28S region. The first followed Eberle et al. (2018): initial heating phase of 15 min at 95 °C, 35 amplification cycles of 35 s at 95 °C (denaturing), 1 min at 51 °C (alignment), and 1 min at 72 °C (elongation), with a final elongation of 10 min at 72 °C. The second protocol is a modification of Eberle et al. (2018): initial heating phase of 15 min at 95 °C, 35 amplification cycles of 35s at 94 °C (denaturing), 1 min 30 s at 59 °C (alignment), and 1 min 30 s at 72 °C (elongation), with a final elongation of 10 min at 72 °C. Gel electrophoresis was carried out with 0.5% agarose using the molecular weight marker Perfect DNA 100 bp Ladder Novage to calculate fragment size of amplifications. Gels were visualized in a photodoc BioDoc-It2 Imager 315 Imaging System LMS-20 Transilluminator. PCR products were purified using a QIAquick Qiagen purification kit. Tissue selection, DNA extraction, amplification, and purification were performed at the Laboratory of Molecular Biology at Laboratorio Regional de Biodiversidad y Cultivo de Tejidos Vegetales (**LBCTV**), IB-UNAM, Tlaxcala City. Sanger sequencing was done at the Laboratory of Molecular Biology and Health, IB-UNAM, Mexico City.

DNA sequence alignment and editing

Both forward and reverse DNA strands were sequenced. DNA sequences were edited in Geneious v. 8.1.9 (Rozen and Skaletsky 2000). Multiple alignment of sequences was implemented using MAFFT v. 7 (Katoh and Toh 2008) through online platform (https://mafft.cbrc.jp/alignment/server/), with the following commands: Auto (FFT-NS-2, FFTNS-I or L-INS-I, depending on data size). In some cases, alignment was done manually and edited with BioEdit v. 7.0.5.3 (Hall 1999). The concatenated matrix (COI+ITS2+28S) was built in Geneious v. 8.1.9. The aligned matrices were subsequently used in the molecular analyses.

Molecular analysis and species delimitation

Four different molecular delimitation methods were used under the corrected *p*-distances Neighbor-Joining (NJ) criteria: 1) ABGD (Automatic Barcode Gap discovery) (Puillandre et al. 2012), 2) ASAP (Assemble Species by Automatic Partitioning) (Puillandre et al. 2021), 3) GMYC (General Mixed Yule Coalescent) (Pons et al. 2006), and 4) bPTP (Bayesian Poisson Tree Process) (Zhang et al. 2013; Kapli et al. 2017).

p-distances under Neighbor-Joining (NJ)

The genetic distance tree was reconstructed with MEGA v. 10.1.7 (Kumar et al. 2016) under the following parameters: Number of replicates = 1000, Bootstrap support values = 1000 (significant values ≥ 50%), Substitution type = nucleotide, Model = *p*-distance, Substitution to include = d: Transitions + Transversions, Rates among sites = Gamma distributed with invariant sites (G+I), Missing data treatment = Pairwise deletion.

Automatic Barcode Gap Discovery (ABGD)

This method aims to find gaps in genetic divergence, considering that intraspecific genetic variation is theoretically smaller than interspecific divergences. It first generates a prior partition of the data into putative species (initial partitions, IP). Then, these initial partitions are recursively partitioned until there is no further partitioning of the data (recursive partitions, RP). ABGD analyses were carried out on the online platform (https://bioinfo.mnhn.fr/abi/public/abgd/) using the following options: K2P distances non-corrected, P_{min} = 0.001, P_{max} = 0.1, Steps = 10, Relative gap width (X) = 1, Nb bins = 20.

Assemble Species by Automatic Partitioning (ASAP)

This is an ascending hierarchical clustering method. Sequences are merged into groups that are successively merged further until all sequences form a single group. Partitions are equivalent to each sequence merge step. The software analyzes all partitions and scores the most probable groups into a tree (Puillandre et al. 2021). ASAP analyzes were run on the online platform (https://bioinfo.mnhn.fr/abi/public/asap/) using Kimura (K80) distance matrices and configured under following parameters: Substitution model = *p*-distances, Probability = 0.01, Best scores = 10, Fixed seed value = -.

General Mixed Yule Coalescent (GMYC)

This approach applies single (Pons et al. 2006) or multiple (Monaghan et al. 2009) time thresholds to delimit species in a Maximum Likelihood context, using ultrametric trees as input (Ortiz and Francke 2016). Ultrametric trees were generated with phylogenetic analyses in the BEAUti and BEAST v. 1.10.4 software (Drum-

mond et al. 2002–2018) using a coalescent (constant population) tree prior. An independent log normal uncorrelated clock was applied to each partition with their respective evolution model and substitution rates (CO1: GTR + I + G; ITS2: K2P; 28S: GTR + I + G). Five independent analyses were run, each with 40 million iterations. Tracer 1.6 (Rambaut and Drummond 2003–2013) was used to evaluate convergence values, with the ESS (Effective Sample Size) > 200. Tree Annotator 2.6.0 (a BEAST package) was used to construct maximum credibility of clades trees, after discarding the first 25% of each independent run as burn-in. Finally, the GMYC method was implemented in the web platform (https://species.h-its.org/gmyc/), which uses the original R implementation of the GMYC model (Fujisawa and Barraclough 2013).

Bayesian Poisson Tree Processes (bPTP)

This method is similar to GMYC, but does not require an ultrametric tree as input because the models of speciation rate are implemented directly using the number of substitutions calculated from branch lengths. The Bayesian and Maximum Likelihood variants were carried out on the online platform (https://species.h-its.org/ptp/), with the following options: Rooted tree, MCMC = 1000000, Thinning = 100, Burnin = 0.1, Seed = 123. The resulting trees were edited with the iTOL online version (https://itol.embl.de/) (Letunic and Bork 2021) and Photoshop CS6. To delimit different species, we used the congruence integration criteria. It is based on the correspondence among the different molecular methods to generate a highly supported species hypothesis (DeSalle et al. 2005; Hamilton et al. 2011; Navarro-Rodríguez and Valdez-Mondragón 2020; Valdez-Mondragón 2020).

Results

Molecular analyses of genetic distances

The corrected *p*-distances under NJ using the CO1 matrix recovered 22 species of *Physocyclus*. This is concordant with the morphology analysis of features commonly used to identify and diagnose at the species level (Valdez-Mondragón 2013, 2020; Valdez-Mondragón and Francke 2015) (Fig. 1). Both the morphology and all genetic distance analyses using CO1 recovered groups that correspond with a described species. The NJ analyses using the ITS2 marker recovered 20 species (Fig. 2), whereas analyses with 28S recovered 21 species (Fig. 3). The average genetic *p*-distances among *Physocyclus* species were 16.4% (min: 14.89%, max: 17.96%) for CO1, 29.4% (min: 25.37%, max: 38.59%) for ITS2, and 14.4% (min: 14.15%, max: 17.87%) for 28S (Figs 1–3, Table 3). The average intraspecific distances using CO1 were below 2% for most species (18/22). However, four species (*P. enaulus* Crosby, 1926, *P. modestus* Gertsch, 1971, *P. mysticus* Chamberlin, 1924, and

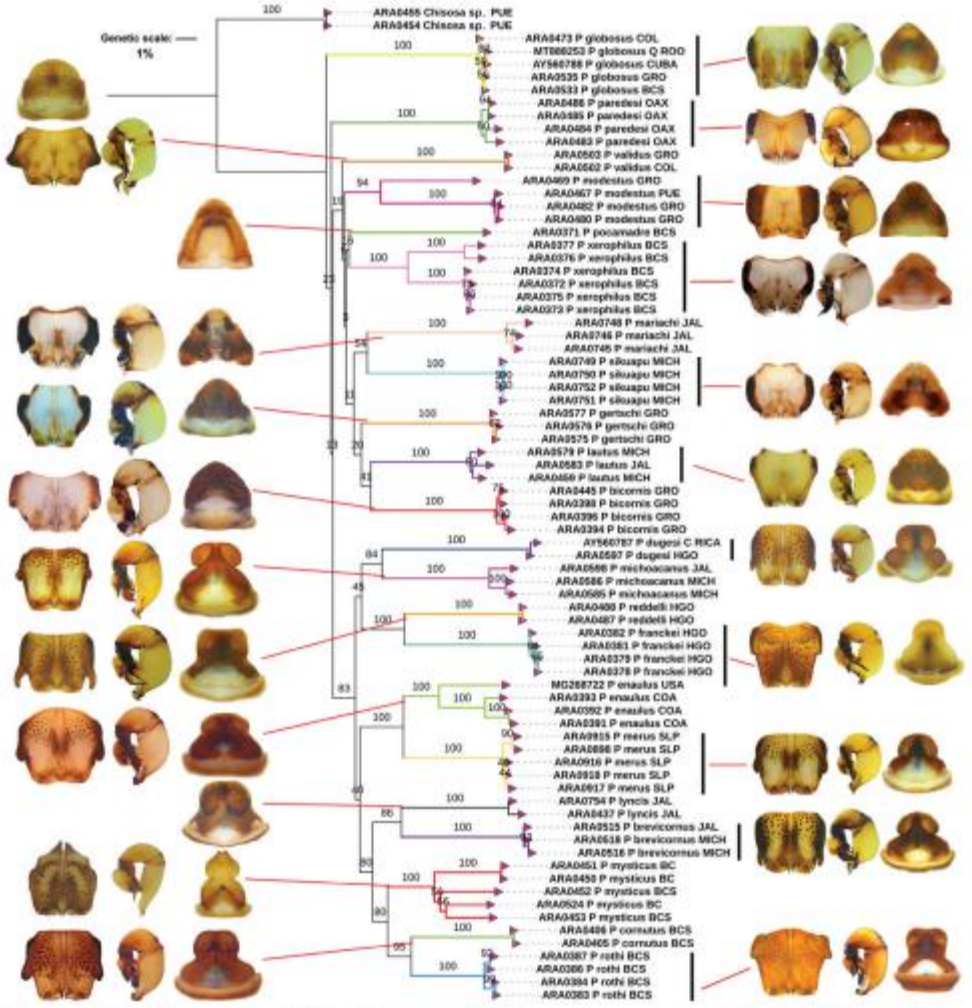

Figure 1. Neighbor-Joining (NJ) with corrected *p*-distances tree constructed with CO1 barcode sequences from different species of *Physocyclus*. Branch colors indicate putative species. Male chelicerae, male palps, and female epigynes are shown for each species. Numbers above branches represent significant Bootstrap support values (> 50%).

P. xerophilus Nolasco & Valdez-Mondragón, 2020) showed average intraspecific genetic distances between 4–6% (Table 3), with high Bootstrap support in each case (> 94%) (Fig 1). The Bootstrap support values for all species were high (100%) (Fig. 1). The *globosus* and *dugesi* species groups were not recovered in the CO1 topology. In the ITS2 tree, six species (*P. bicornis* Gertsch, 1971, *P. lautus* Gertsch, 1971, *P. lyncis* Nolasco & Valdez-Mondragón, 2022, *P. modestus*, *P. mysticus*, and *P. validus* Gertsch, 1971) had average intraspecific genetic distances below 2%, while the rest showed average intraspecific genetic distances over 2% (Fig. 2). Bootstrap

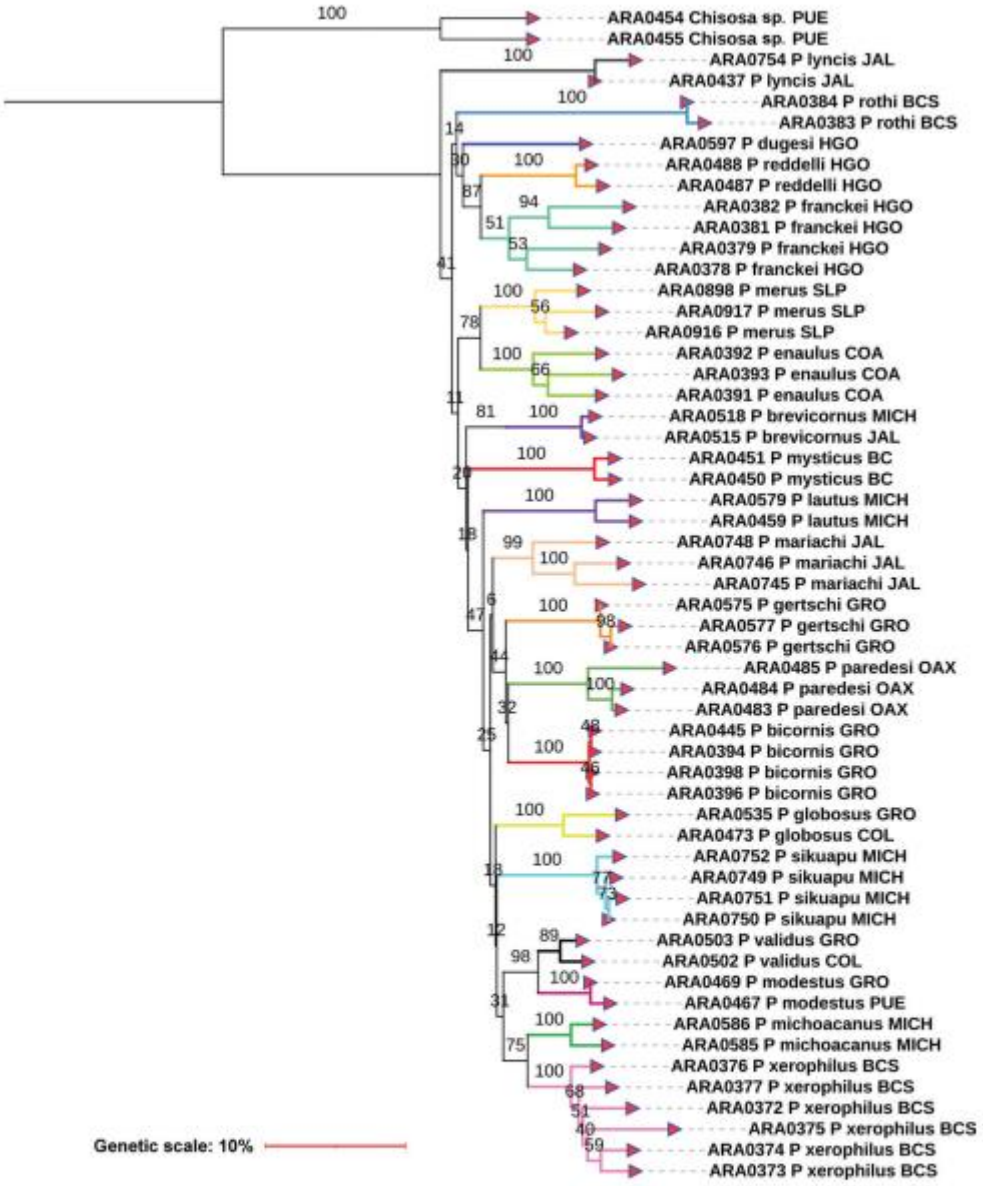

Figure 2. Neighbor-Joining (NJ) with corrected *p*-distances tree constructed with ITS2 barcode sequences from different specimens and species of *Physocyclus*. Branch colors indicate putative species. Numbers above branches represent significant Bootstrap support values (> 50%).

support values for species in the ITS2 topology were significant (over 89%), except for *P. franckei* (51%). Using the 28S marker, average intraspecific genetic distances for all species were below 2% (Fig. 3) with high Bootstrap support values (> 95%).

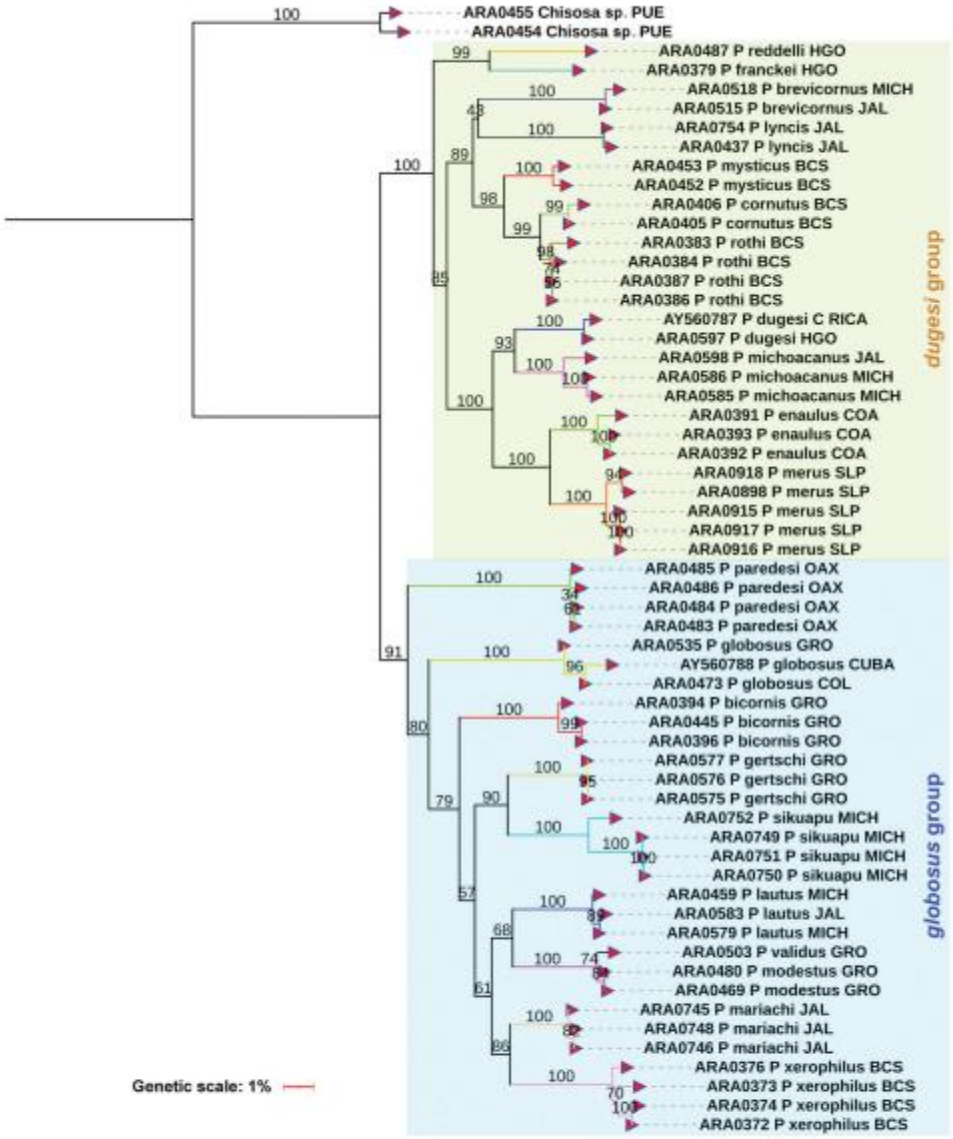

Figure 3. Neighbor-Joining (NJ) with corrected *p*-distances tree constructed with 28S barcode sequences from different specimens and species of *Physocyclus*. Branch colors indicate putative species. Numbers above branches represent significant Bootstrap support values (> 50%).

The 28S tree was the only one to recover both the *globosus* and *dugesi* species groups with high Bootstrap support values (100% for the *dugesi* group and 91% for the *globosus* group) (Fig. 3).

Table 3. Average CO1 genetic distances (*p*-distances) among *Physocyclus* species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. ***P. bicornis***	0.7																					
2. ***P. brevicornus***	18.4	0.3																				
3. ***P. cornutus***	16.4	14.1	0.2																			
4. ***P. dugesi***	20.3	17.1	18.5	0.5																		
5. ***P. enaulus***	17.6	17.3	15.3	17.6	4.5																	
6. ***P. franckei***	20.3	17.7	17.9	18.2	17.4	0.3																
7. ***P. gertschi***	13.8	18.3	17.1	20.1	19.7	19.4	0.2															
8. ***P. globosus***	18.3	19.5	18.4	18.4	16.5	18.9	16.8	0.4														
9. ***P. lautus***	12.9	17.4	15.9	17.8	16.9	18.4	14.3	17.1	1.4													
10. ***P. lyncis***	18.0	12.7	15.8	17.3	16.2	19.1	18.3	18.3	17.3	1.1												
11. ***P. mariachi***	16.4	20.1	20.1	18.4	20.0	19.2	16.5	19.6	15.6	18.3	1.5											
12. ***P. merus***	17.6	17.2	15.8	17.9	11.0	19.7	19.1	17.9	16.9	15.5	19.4	0.9										
13. ***P. michoacanus***	18.3	17.0	17.2	14.7	15.0	16.6	17.4	16.7	15.3	16.3	20.3	17.0	1.9									
14. ***P. modestus***	15.6	19.1	17.6	18.8	16.4	18.5	16.1	15.8	14.1	18.9	17.9	17.9	17.1	5.8								
15. ***P. mysticus***	18.2	14.3	12.8	16.0	15.0	16.9	17.4	16.9	16.2	15.3	17.8	14.6	15.8	16.9	5.7							
16. ***P. paredesi***	18.0	19.5	19.2	18.5	17.0	19.4	17.0	17.5	16.3	18.0	18.9	18.2	19.8	17.8	17.6	1.3						
17. ***P. pocamadre***	15.8	18.7	16.5	19.6	16.6	19.8	16.3	16.9	13.8	16.1	15.8	16.4	17.9	15.1	15.4	16.4	0.0					
18. ***P. reddelli***	19.2	17.5	18.5	17.1	16.6	13.0	19.2	19.4	17.5	16.8	18.6	17.0	17.1	18.4	15.8	17.7	16.9	0.0				
19. ***P. rothi***	16.0	13.0	9.7	16.4	14.3	14.6	14.2	17.0	15.6	14.6	17.3	15.5	14.9	16.6	11.0	17.8	16.5	17.5	0.8			
20. ***P. sikuapu***	16.6	17.9	18.5	18.3	18.2	19.1	14.5	18.3	15.7	17.1	15.1	18.1	19.0	16.0	16.8	16.8	15.6	16.6	16.6	0.0		
21. ***P. validus***	16.7	20.0	19.5	20.4	18.9	19.5	16.1	17.4	16.9	18.8	19.0	18.3	17.8	16.8	18.9	17.6	16.2	18.9	19.5	16.8	0.0	
22. ***P. xerophilus***	15.9	18.6	17.7	19.3	17.1	17.9	14.3	15.8	13.9	18.2	15.7	16.4	18.1	14.8	16.9	15.8	13.7	16.7	17.4	15.3	15.2	4.0

Molecular methods for species delimitation

The Maximum Likelihood (ML) tree of the concatenated matrix (CO1+ITS2+28S) (Fig. 4) found congruence among the four different molecular species delimitation methods and using traditional morphology. This was found for 15 species: *Physocyclus bicornis*, *P. brevicornus*, *P. cornutus*, *P. dugesi*, *P. gertschi*, *P. globosus*, *P. lautus*, *P. lyncis*,

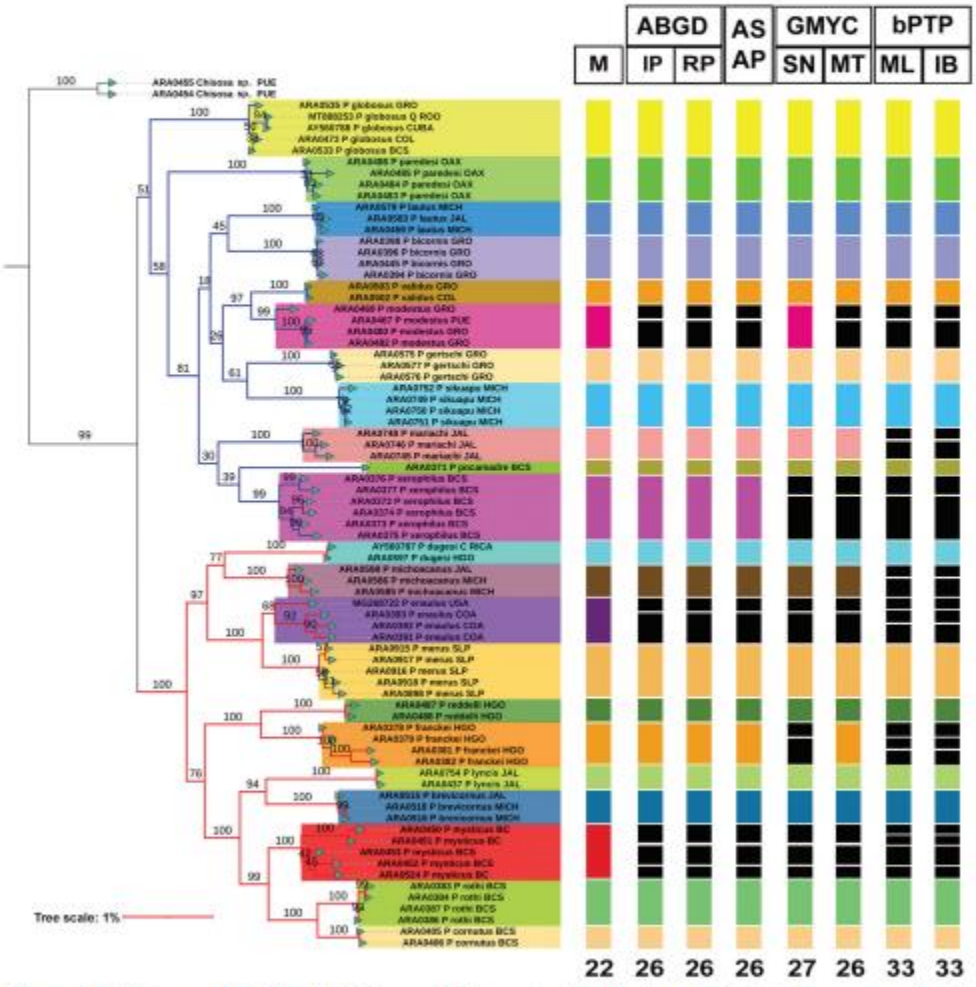

Figure 4. Maximum Likelihood (ML) tree of *Physocyclus* (log likelihood: -3749.87) constructed with the concatenated matrix (CO1+ITS2+28S). Bar colors represent putative species in the tree and in the columns, which represent the different species delimitation methods analyzed. Branch colors represent species groups: *globosus* (blue) and *dugesi* (red). Numbers below the columns represent the species recovered in each species delimitation method (not considering *Chisosa* sp.). Numbers above branches represent Bootstrap support values for ML (> 50% significant). Column abbreviations: Morphology (M); ABGD with initial (IP) and recursive (RP) partitions; ASAP; GMYC with single (SN) and multi (MT) thresholds; bPTP with Maximum Likelihood (ML) and Bayesian Inference (IB) variants.

P. merus, *P. paredesi*, *P. pocamadre*, *P. reddelli*, *P. rothi*, *P. sikuapu*, and *P. validus* (Fig. 4). *Physocyclus mariachi* and *P. michoacanus* are recovered as distinct species by most methods except for bPTP, whereas *P. franckei* is not recovered under the bPTP and GMYC methods in the single threshold (SN) (Fig. 4). *Physocyclus enaulus*, *P. modestus*, *P. mysticus*, and *P. xerophilus* were found to contain more than two species (2–4) by most of the species delimitation methods (Fig. 4).

The most congruent methods with morphology were the barcoding method ABGD, ASAP, and GMYC, which delimited 26 (ABGD IP and RP, ASAP, and GMYC MT) and 27 (GMYC SN) putative species, respectively. The most incongruent result of the analyses was bPTP, which delimited 33 putative species under ML and IB variants (Fig. 4).

Both species groups (*globosus* and *dugesi*) were recovered in the ML analysis using the concatenated matrix (CO1+ITS2+28S) (Fig. 4). The *dugesi* group was recovered with significant Bootstrap support (100%), whereas the *globosus* group had Bootstrap support value of 51%.

Discussion

Two different approaches (DNA taxonomy and DNA barcoding) were proposed by DeSalle et al. (2005) to overcome several weaknesses of traditional morphology-based taxonomic systematics, and to resolve the crucial need for accurate and rapid species identification tools (Hebert et al. 2003; Tautz et al. 2003). DNA barcoding is useful for recognizing cryptic species (two or more distinct species that are erroneously classified as the same species due to similar morphology) (Bickford et al. 2007). Even only with CO1, DNA barcoding is helpful in species diagnosis due to the fact that sequence divergences are ordinarily much lower among individuals of the same species than between closely related species (Hebert et al. 2004). For some groups of arachnids, traditional morphology fails to recognize and delineate species boundaries. Also, identify sister or cryptic species requires other types or evidence such as molecular data, ecological niche modeling, morphometric morphology, haplotype networks, and biogeographical approximations (Hebert et al. 2003, 2004; Hamilton et al. 2011, 2014; Montes de Oca et al. 2015; Ortiz and Francke 2016; Cruz-López et al. 2019; Valdez-Mondragón et al. 2019; Newton et al. 2020; Valdez-Mondragón and Cortez-Roldán 2021). However, as demonstrated herein, spiders of the genus *Physocyclus* have robust morphology for diagnosis and identification at the species level, mainly of primary and secondary sexual characters, such as chelicerae and palps (males) or epigynes (females).

In the genetic distance analyses performed with independent matrices of CO1, ITS2, and 28S, all the species terminals were recovered. The genetic intraspecific distances for ITS2 were found to be relatively high in the majority of species (over 2%). However, the average intraspecific genetic distances using the CO1 and 28S markers were lower in the majority of species (< 2%). Agnarsson (2010), Valdez-Mondragón et al. (2019), and Navarro-Rodríguez and Valdez-Mondragón (2020) mention that ITS2 is inadequate for resolving relationships between closely related species of spiders. Our

data corroborate this, showing the unreliability of this gene for genetic distance (and species delimitation) analyzes when used on its own. However, the use of a concatenated matrix of nuclear (28S and ITS2) and mitochondrial (CO1) markers provides better results with more robust evidence for delimiting species based on molecular data (Astrin et al. 2006; Agnarsson 2010; Planas and Ribera 2015; Ortiz and Francke 2016; Navarro-Rodríguez and Valdez-Mondragón 2020).

When looking at CO1, Pholcid spiders generally show high genetic divergences among species (Astrin et al. 2006). In contrast to reports for spiders in general that show values of 14.4% (Hebert et al. 2003) and 16.4% (Barrett and Hebert 2005), Pholcids' average interspecific genetic distance for CO1 is 19.8% (Astrin et al. 2006). Our results found a value of average interspecific distance at the CO1 marker observed in *Physocyclus* of 16.4% (Fig. 1, Table 3). This value fits within the average limits of other spiders; however, this tendency in the Pholcidae family is not always the case. The average interspecific genetic distance for CO1 in other genera such as *Ixchela* Huber, 2000 (Modisiminae) were found to be lower, 12% (Valdez-Mondragón 2020). Although most species were included in the analyses (22 of 37), missing species might have an effect and overestimate the average interspecific distances, because perhaps the sister species of each species is not found in the data set. However, the results and topologies were consistent along the analyzes.

With regards to molecular delimitation methods, ABGD used to be sensitive to sampling effect and tended to moderately over-split, as demonstrated in the mygalomorph genera *Aphonopelma* Pocock, 1901 by Hamilton et al. (2014) and *Bonnetina* Vol, 2000 by Ortiz and Francke (2016) and Candia-Ramírez and Francke (2020). Similar results were observed in *Loxosceles* Heineken & Lowe, 1832 (Valdez-Mondragón et al. 2019; Navarro-Rodríguez and Valdez-Mondragón 2020), where the ABGD method generated an inflated number of delimited species. However, within the pholcid genus *Ixchela*, it was observed by Valdez-Mondragón (2020) that the most incongruent method was bPTP (both ML and IB variants), as was also found in the molecular analyses herein with the genus *Physocyclus*.

The most congruent methods that delimited a similar number of species in this study were ABGD, ASAP, and GMYC, which was corroborated by traditional morphology (Fig. 4). The bPTP method delimited a higher number of species in comparison with morphology. These results contrast with those found by Ortiz and Francke (2016), Valdez-Mondragón et al. (2019), and Navarro-Rodríguez and Valdez-Mondragón (2020), in which the bPTP and GMYC analyses were the most congruent methods with the morphology in spiders. This may be due to the inclusion of the 28S marker, which might be causing tree-based analyzes (bPTP) to generate an overestimation in the number of putative species recovered. According to Luo et al. (2018), GMYC and bPTP are negatively influenced by gene flow and are sensitive to the ratio of population size to divergence time, reflecting the important impact of incomplete lineage sorting on species delimitation.

In such cases of incongruency between the molecular methods and morphology, as in *Physocyclus enaulus*, *P. modestus*, *P. mysticus*, and *P. xerophilus*, no significant morphological

differences were found within individuals of each species. However, *P. enaulus* was the exception, where Valdez-Mondragón (2010) recorded three different morphotypes of epigyne shape. Unfortunately, we could not to get sequences of all the morphotypes. As Valdez-Mondragón (2010, 2014) mentions, the morphology of somatic and sexual characters in the genus *Physocyclus* is usually highly conserved. Similarly, morphological changes might not be correlated with species boundaries, or may not be useful for delimiting species if interspecific recognition occurs in non-visual signs (chemical or mating calls), or even biogeographical traits (microhabitats). Furthermore, it is possible that the species may be under stabilizing selection that promotes morphological stasis (Bickford et al. 2007).

According with Hamilton et al. (2011), species delimitation based only on molecular data can rarely be achieved, and additional types of evidence such as biogeographical information is needed. In the case of the multiple species inferred within *P. mysticus*, no significative morphological or habitat differences are apparent. All specimens are from xerophytic scrub and present similar microhabitats of living under big boulders on the ground. Sympatric speciation may be possibly, as other species of the genus *Physocyclus* have been collected in the same locality (Valdez-Mondragón 2010, 2013). *Physocyclus modestus* presents a similar case, being one widespread species from Guerrero, Morelos, Oaxaca, and Puebla. However, habitat differences exist among the different populations of this species. Some specimens are from xerophytic scrub, while others are found in lowland forest. Microhabitat likely has a direct influence on the diversification of pholcids spiders, as was demonstrated by Eberle et al. (2018). Therefore, the morphological and molecular evidence suggest that *P. modestus* might be a species complex, as observed in *P. mysticus*. However, more male and female specimens from different populations are needed to confirm this assumption.

As Carstens et al. (2013) suggested, probing different methods or lines of evidence is necessary for properly implementing species delimitation. When the information and results are incongruent, it is better to be conservative about assumptions of species delimitation. In the case of some species in this study (e.g., *P. enaulus* and *P. xerophilus*), more detailed analyses of the morphological structures are necessary. Maybe including ultra-morphology, lineal morphology, or geometric morphometry in somatic features such as length legs, carapace shape, or even in sexual structures (Valdez-Mondragón et al. 2019). Lineal and geometric morphology has provided strong evidence for splitting species in cases where traditional morphology fails to delimit species. This has been demonstrated in araneomorph spiders (Planas and Ribera 2015; Valdez-Mondragón et al. 2019) and in brown recluse spiders of the genus *Loxosceles* (Solís-Catalán 2020). In this works, significant differences were found in carapace length, male palp shape, and length leg I among different species from the Canary Islands and central Mexico. *Physocyclus enaulus* is a widespread species from northern Mexico and southern United States. Valdez-Mondragón (2010) reported three different types of ventral apophyses in the female epigyne, which suggests that it might comprise a species complex rather than wide intraspecific morphological variation.

Although the number of described species in the genus *Physocyclus* has doubled in the last decade (Valdez-Mondragón 2010, 2013, 2014; Jiménez and Palacios-Cardiel 2013; Nolasco and Valdez-Mondragón 2020, 2022), the diversity of this genus in Mexico is still poorly known. Provinces such as the Sonoran and Chihuahuan Deserts have been poorly sampled, despite their arid and semiarid ecosystems being common habitats for the genus *Physocyclus*. Furthermore, cave habitats have been virtually unexplored in this genus, and will likely produce new troglophilic species (Valdez-Mondragón 2010, 2020; Huber et al. 2018).

In regard to the molecular methods used herein, each one presents its advantages and disadvantages. Barcoding methods (ABGD) can distinguish within-population differences caused by species divergences, analyzing the gaps of a data set and using it as a barcode to recognize different species (Puillandre et al. 2012; Rannala 2015; Rannala and Yang 2020). However, this method does not consider the rates of intra- and interspecific variation as an initial parameter, causing barcode values to vary among species groups and, in some cases, generating over splitting (Hickerson et al. 2006; Rannala 2015; Rannala and Yang 2020). The hierarchical integration of the ASAP method allows for many possible clusterings of terminals to be tested (Puillandre et al. 2021). However, it can offer different delimitation species hypothesis with similar asap-scores. In the case of *Physocyclus* spiders here, both methods offered a high congruence between themselves and the morphological evidence.

The coalescence method (GMYC) is robust because it uses an a priori ultrametric species tree, taking into account the groups formed in the topology. However, this method assumes that the lineages in each population coalesce before any speciation event occurs, implying the absence of incomplete lineage sorting and ignoring the coalescent process within populations of ancestral species (Rannala and Yang 2020). The bPTP method accommodates the use of large data sets with thousands of species and considers the rates of intra- and interspecific genetic variation. However, it does not take into account the stochastic fluctuations in the coalescent process among the different loci in large multi-locus analyses (Rannala and Yang 2020). The coalescent analyses for species delimitation (GMYC, bPTP) do not require reciprocal monophyly to delimit species (Knowles and Carstens 2007) and can incorporate statistical uncertainty in gene trees. They are based on Maximum Likelihood and Bayesian Inferences and not only on corrected genetic distances.

In conclusion, CO1 and 28S provide robust evidence for species-level delimitation in the genus *Physocyclus*, with high congruence among all methods. The genetic variability of ITS2 makes it an unreliable molecular marker for species delimitation on its own, however, it provides good information when used in combination with others mitochondrial and nuclear markers. Sexual morphological characters (male palps, male chelicerae, and female epigyne) are robust features for identifying and diagnosing *Physocyclus* species. However, in some cases, morphology alone is not enough to detect sister species, cryptic species, or even species complexes.

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10. Capítulo 2

Artículo Sistemática Filogenética

One or more genera? Phylogenetics and diversification of the spider genus *Physocyclus* Simon (Araneae: Pholcidae) based on morphological and molecular evidence, with the description of a new genus: *Mictlanus* gen. nov.

Alejandro Valdez-Mondragón – Samuel Nolasco

(*In review***)**

¿Uno o más géneros? Filogenia y diversificación del género de arañas *Physocyclus* Simon (Araneae: Pholcidae) basada en evidencia morfológica y molecular, con la descripción de un nuevo género: *Mictlanus* gen. nov.

Alejandro Valdez Mondragón – Samuel Nolasco

(En revisión)

Valdez-Mondragón, A & Nolasco, S. (*in press*). One or more genera? Phylogenetics and diversification of the spider genus *Physocyclus* Simon (Araneae: Pholcidae) based on morphological and molecular evidence, with the description of a new genus: *Mictlanus* gen. nov. *Molecular Phylogenetics and Evolution.*

One or more genera? Phylogenetics and diversification of the spider genus *Physocyclus* Simon (Araneae: Pholcidae) based on morphological and molecular evidence, with the description of a new genus: *Mictlanus* gen. nov.

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Abstract

Mexico is characterized by complex biogeographical and geological patterns, which are the result of the overlapping of the Neartic and Neotropical biotic regions. This region, better known as the Mexican Transition Zone, represent a high-biodiversity region worldwide. The spider genus *Physocyclus* Simon, 1893 is composed by 37 species, mainly distributed in Mexico. We tested the monophyly and investigate the relationships among the species using morphological and molecular evidence (CO1, ITS2, 28S) of the species groups *globosus* and *dugesi*. Parsimony (PA) analyses with 54 morphological characters (10 multistate and 44

binary); Maximum Likelihood (ML) and Bayesian Inferences (BI) for CO1+ITS2+28S (2048 characters) and with combined evidence (Morphology+CO1+ITS2+28S) (2102 characters) recovered both species groups as monophyletic, one of them (group *dugesi*) proposed herein as a new genus: *Mictlanus* **gen. nov.** *Physocyclus* and *Mictlanus* **gen. nov.** appeared approximately 15–14 million of years ago (Mya) and the species diversification occurred during the Middle Miocene to Pleistocene. The potential ancestral areas that influenced the diversification of both genera were the Trans-Mexican Volcanic Belt, Chihuahuan Desert, Baja California, Pacific Lowlands and the Sierra Madre Oriental biogeographical provinces. 22 species were transferred (new combination) from *Physocyclus* to *Mictlanus* **gen. nov.** whereas *Physocyclus* is composed by 15 species. We proposed that *Physocyclus* and *Mictlanus* are different genera for the next: 1) diagnostic morphological features are different for each genus; 2) both are monophyletic under different sources of evidence (morphological, molecular and combined evidence); 3) different biogeographical distribution pattern; 4) similar evolutionary divergence times but evolving independently, and, 5) difference of biogeographical provinces and ancestral areas that explain their diversification patterns.

Keywords. Integrative systematics, Arteminae, Mexican Transition Zone, Pleistocene, new combinations.

1. Introduction

The spider family Pholcidae C. L. Koch commonly known as “daddy-long-legs spiders” or “cellar spider”, is the ninth most diverse family in the order Araneae and the most diverse in the Synspermiata clade, with 1909 species classified in 97 genera (WSC, 2023). Pholcidae belongs to Lost Tracheae Clade sensu Wheeler et al. (2016), and their sister group is the clade (Tetrablemmidae + Plecturidae + Diguetidae + Pacullidae). The family Pholcidae is currently composed by five subfamilies: Arteminae Simon, 1893; Modisiminae Simon, 1893; Ninetinae Simon, 1890; Smeringopinae Simon, 1893 and Pholcinae C. L. Koch, 1850 (Huber, 2011a). Huber (2000) proposed the first phylogenetic analysis for the Pholcid family based only in morphological characters, where are included the genera of the Americas. However, the phylogenetic relationships were not clear for most of the genera and the current

five subfamilies are not recovered. Bruvo-Madaric et al. (2005) for the very first time carried out a phylogenetic analysis using combined evidence of morphological and molecular data (12S, 16S, CO1, 28S). This analysis recovers partially four of the five current subfamilies, with high support values. The use of combined evidence including morphological and genetic data in the phylogenetic reconstructions, gave a major resolution and better supported hypothesis in comparison with the analyses carried out with separated evidence.

Huber (2011a) proposed the actual classification of the five subfamilies based on cladistic analyses of morphological and molecular data and on qualitative character assessment. Dimitrov et al. (2013), carried out a phylogenetic analysis for Pholcidae with emphasis in its biogeography and evolution. Their suggest that family Pholcidae appeared and diversified during the early Mesozoic, 207 million of years ago (Mya), before the rupture of the supercontinent Pangea. In the same way, it is suggested that subfamily Arteminae is monophyletic and that appear during the median Mesozoic (175 Mya), which explains why the genera of the subfamily are widespread in different continents (Eberle et al., 2018; Huber et al., 2018; WSC, 2023). Eberle et al. (2018) based on mitochondrial and nuclear markers implemented the first phylogenetic analyses including 600 species representing more than 85% of the currently described pholcid genera (the most complete phylogeny of the family so far), combined with information of the microhabitat and recovering the five subfamilies suggested by Huber (2011a). However, in the case of the subfamily Arteminae, there was a discordance in the phylogenetic relationships of the genus *Artema* Walckeaner, 1837, being the sister group of the subfamily Ninetinae and not into the subfamily Arteminae (Eberle et al., 2018).

Posteriorly, Huber et al. (2018) realize a re-evaluation of phylogenetic relationships of the family Pholcidae, however, the position of genus *Artema* still dubious, because it is out of the subfamily Arteminae, being the genus that gives the name to the subfamily. Despite the evidence, the authors do not propose any taxonomic change and the internal relationships of the genera of Arteminae still dubious. The subfamily Arteminae is composed by 104 species, classified in nine genera: *Artema*, *Aucana* Huber, 2000, *Chisosa* Huber, 2000, *Holocneminus* Berland, 1942, *Nita*, Huber and El-Hennawy, 2007, *Pholcitrichocyclus* Ceccolini and Cianferoni, 2022, *Physocyclus* Simon, 1893 *Tibetia* Zhang, Zhu and Song, 2006, and *Wugigarra* Huber, 2001 (WSC, 2023).

Previous to this work, the most diverse genus of the subfamily Arteminae was *Physocyclus* with 37 described species, distributed mainly in Mexico (Taczanowski, 1874; Simon, 1893; Banks, 1898, Chamberlin, 1921, 1924; Chamberlin and Gertsch, 1929; Mello-Leitão, 1940; Gertsch and Davis, 1942; Gertsch, 1971; Huber, 1998; Jiménez and Palacios-Cardiel, 2013; Valdez-Mondragón, 2010, 2013, 2014; Nolasco and Valdez-Mondragón 2020, 2022a). The genus *Physocyclus* habits mainly in arid zones and in lowland forests, below of 1900 m.a.s.l. (Valdez-Mondragón, 2010; Jiménez and Palacios-Cardiel, 2013; Nolasco and Valdez-Mondragón, 2020, 2022a). The species *Physocyclus dugesi* Simon, 1893 and *Physocyclus globosus* (Taczanowski, 1874), which give the names to each species group previously to this work, have a cosmopolitan distribution influenced by synanthropic activities (Valdez-Mondragón, 2010).

According with this distribution pattern in North America, it is possible to think that the origin and diversification of the genus is correlated with the complex biogeographic history of the region (Nolasco and Valdez-Mondragón, 2022a, b). The Mexican Transition Zone (MTZ) is located between the Neartic and Neotropical regions, and is conformed mainly by Trans-Mexican Volcanic Belt, Sierra Madre Oriental, Sierra Madre Occidental Sierra Madre del Sur and Chiapas Highlands (Morrone, 2005, 2006, 2023). The MTZ share environmental characteristics and biotic components of both Neartical and Neotropical regions, acting as barriers, limiting the continuity of populations, or serving as biological corridor, promoting dispersal and genetic interbreeding in many biological groups (Morrone, 2023). This biogeographic pattern has been previously found in other pholcids spiders from North America. Valdez-Mondragón and Francke (2015) proposed that Trans-Mexican Volcanic Belt (TMV) might be considered as the diversification zone of the genus *Ixchela* Huber, 2000 which distributes in the Mexican Mountains biotic component. In the same way, based in phylogenetic reconstructions and lineages dating, the origin of the genus *Ixchela*, occurred during the late Miocene, and their diversification is during the late Pliocene. According with Mastretta-Yanes et al. (2015), the events of major speciation in the mountain chains in Mexico occurred during Pleistocene, where the climate change contributed to repeated glaciations that derivate in the existence of Pleistocene refuges, generating a major diversification in several group of arthropods. However, not only the diversification during the Pleistocene in temperate regions has been reported, also the diversification in arid regions

and dry tropical forests from North America has been reported for other arachnids. Graham et al. (2013) studied the genetic structure of the hairy scorpion *Hadrurus arizonensis* Ewing, 1928 from North America and they found that the main clades diverged between the late Pliocene and early Pleistocene, whereas subsequent divergences between lineages occurred in the middle and late Pleistocene. Schramm et al. (2021) demonstrated how the tropical deciduous forests served as a conduit for dispersal with their disappearance imposing barriers in the whip spider *Acanthophrynus coronatus* (Butler, 1873) during the Pliocene–Pleistocene glacial/interglacial cycles, and the impact of the TMV as driver of diversification in the Mexican Neotropics.

According with the internal phylogenetic relationships of *Physocyclus*, Valdez-Mondragón (2013, 2014) based in morphological characters recovered *Physocyclus* as a monophyletic group and recovering two species groups: *globosus* and *dugesi*. However, each species groups have different morphology of primary and secondary sexual characters and particular biogeographic distribution pattern as was demonstrated in the phylogenetic reconstructions by Valdez-Mondragón (2013, 2014). Following to Valdez-Mondragón (2013, 2014), the species group *globosus* is distributed mainly to Mexican Mountain and Mesoamerican biotic components, whereas the species group *dugesi* is distributed mainly in Mesoamerican and Continental Nearctic biotic components. However, both works were based only in morphology and represented the first phylogenetic approaching to stablish the internal phylogenetic relationships of the genus, but non biogeographical history reconstruction and diversification hypothesis was proposed.

Although the morphological data suggest that *Physocyclus* is a monophyletic group, it is possible it might be two different genera with distinct morphology, genetic variation and different evolutive and biogeographic history in North America. To corroborate this, the aim of this work is to carry out phylogenetic analyses under an integrative approaching, including morphological and molecular data. Also, the lineage dating analyses and reconstruction of ancestral areas was implemented to provide information about the hypothesis of diversification under a biogeographical context.

2. Material and methods

2.1. Biological material

The material examined were provided by the following biological collections: Laboratory of Arachnology (**LATLAX**), Institute of Biology, Universidad Nacional Autónoma de México (**IB-UNAM)**, Tlaxcala, Mexico; Colección Nacional de Arácnidos (**CNAN**), **IB-UNAM**, Mexico City; Centro de Investigaciones Biológicas del Noreste (**CIBNOR**), La Paz, Baja California Sur, Mexico; and Colección Aracnológica de la Facultad de Biología de la Universidad Michoacana de San Nicolás de Hidalgo (**CAFBUM**), Michoacan, Mexico. The specimens were preserved in 80% ethanol for morphological studies and identification and 96% ethanol for molecular studies. The male palps and female epigyna were dissected in 80% ethanol for species identification. The epigyna were cleaned with KOH (10%) to eliminate all soft tissue and observe with clarity the internal structures, such as the pore plates. The species identification was done following Valdez-Mondragón (2010, 2014) and Nolasco and Valdez-Mondragón (2020, 2022a). The observations were made with a stereoscope Zeiss Discovery v.8. Palps, epigyna and male chelicerae were photographed submerged in commercial gel ethanol (70%) to hold them in the appropriate position, posteriorly covered with 80% liquid ethanol to avoid light diffraction. The photographs were made with a microscope Zeiss Axio Zoom v.16 and digital software Axio Zoom Zen and Zen Pro v.2. The maps were made with the program Q-GIS v.3.10. The photographs and maps were edited with the program Photoshop CS6 v.1.30x32.

2.2. Taxon sampling

Based on previous phylogenetic works by Valdez-Mondragón (2013, 2014), the morphological phylogenetic analysis was made with specimens of 38 species including the outgroups (OG). The molecular phylogenetic analyses were made with 209 sequences of 34 species including OG. For the molecular analyses, 164 sequences of 22 species of the ingroup were obtained (Valdez-Mondragón, 2010, 2013, 2014; Nolasco and Valdez-Mondragón, 2020, 2022a). Four sequences of three species (*P. dugesi*, *P. enaulus* and *P. globosus*) were downloaded from GenBank (Table 1). The CO1 matrix is composed by 75 sequences of 22 species (644 bp), ITS2 with 54 sequences of 20 species (501 bp) and 28S with 58 sequences of 21 species (903 bp). Concatenated molecular matrix (CO1+ITS2+28S) was composed by

187 sequences of 22 species (2048 bp). A combined matrix (Morphology+CO1+ITS2+28S) (2102 characters) of 22 species was used for the analyses under total evidence (Table 1).

The OG used in the morphological analysis were: *Artema atlanta* Walckenaer, 1837, *Pholcitrichocyclus nigropunctatus* (Simon, 1908), *Pholcitrichocyclus nullarbor* (Huber, 2001) and *Priscula binghamae* (Chamberlin, 1916), being these last species used to root the tree and the others to test the monophyly. The OG used in the molecular analyses were the 22 remaining sequences, belong to 12 species: *Artema atlanta*; *Artema bunkpurugu* Huber and Kwapong, 2013; *Chisosa* sp.; *Chisosa diluta* (Gertsch and Mulaik, 1940); *Modisimus macaya* Huber and Fischer, 2010; *Modisimus seguin* Huber and Fischer, 2010; *Holocneminus* sp.; *Nita elsaff* Huber and El-Hennawy, 2007; *Pholcitrichocyclus* sp.; *Pholcitrichocyclus balladong* Ceccolini and Cianferoni 2022; Huber, 2001; *Pholcophora americana* Banks, 1896; *Wugigarra yawai* Huber, 2001 (Table 1). These sequences were downloaded of GenBank (https://www.ncbi.nlm.nih.gov/genbank/). *Artema atlanta* and *A. bunkpurugu* were used to root the trees. Only *Artema atlanta* was used like OG in both analyses (morphological and molecular). The sequences of *Modisimus macaya*, *M. seguin* and *Pholcophora americana* were used only to the calibration of the lineage dating analyses.

2.3. Morphological data

The morphological matrix carried out by Valdez-Mondragón (2013, 2014) was used for the morphological analyses. This matrix is composed by 54 morphological characters (44 binary and 10 multistate) and 34 terminals as the ingroup. *Physocyclus mariachi* Nolasco and Valdez-Mondragón, *Physocyclus sikuapu* Nolasco and Valdez-Mondragón, and *Physocyclus xerophilus* Nolasco and Valdez-Mondragón were added and codified to the phylogenetic analyses. A new multi-state character (character state 5) was codified for the character 38: Male: Shape of dorsal embolic sclerites in the embolus: (0) long and wide, occupying almost the total length of embolus (Valdez-Mondragón, 2010; fig. 65); (1) small, occupying almost the total length of embolus, without notch in middle part; (2) long and oval distally, localized in basal part of embolus (Valdez-Mondragón, 2010; fig. 149); (3) small, with notch in middle part (Valdez-Mondragón, 2010; fig. 79); (4) small, projected beyond of total length of embolus (Valdez-Mondragón, 2010; fig. 156); (5) small, with semilunar inverted shape,

localized in the base of embolus (Nolasco and Valdez-Mondragón, 2020; fig. 8) (Appendix). See Valdez-Mondragón (2013, 2014) for the complete list of characters.

2.4. DNA extraction, amplification and sequencing.

For DNA extraction, an extraction kit Qiagen DNeasy® was used, following the modifications of Valdez-Mondragón and Francke (2015) and Valdez-Mondragón (2020). DNA extraction was realized with 1–3 legs of males, females or complete juvenile specimens. The criteria to select tissue for DNA extraction was specimens with collecting data no longer than five years old. The CO1 amplifications was realized with two set primers: LCO1490, HCO2198 and LCO-JJ, HCO-JJ; for ITS2: 5.8SF, CAS28SB1d; and for 28S: 28S-B1, 28S-B2 (Table 2). The polymerase chain reactions (PCR) were carried out in a thermal cycler Verity (Applied Biosystems 96 Well Thermal Cycler). The final volume of each PCR tube was 20 µl: 2.3 µl nuclease free water, 2.0 µl Q-solution, 10 µl Multiplex-Mix PCR, 1.6 µl each primer (forward and reverse) and 2.5 µl sample DNA. The cycles and optimal temperatures for amplification of CO1 and ITS2 were the following: Initial phase 15 min at 95°C, amplification x 35 cycles of 35s at 94°C (denaturing), 1 min 30s at 40° C (alignment) and 1 min 30s at 72°C (amplification), with a final elongation of 10 min at 72°C. Two different protocols were used to amplify the 28S region. The first one followed Eberle et al. (2018): initial heating phase of 15 min at 95°C, 35 amplification cycles of 35s at 95°C (denaturing), 1 min at 51°C (alignment), and 1 min at 72°C (elongation), with a final elongation of 10 min at 72°C. The second protocol was followed Nolasco and Valdez-Mondragón (2022b), that is a modification of Eberle et al. (2018): initial heating phase of 15 min at 95°C, 35 amplification cycles of 35s at 94°C (denaturing), 1 min 30s at 59°C (alignment), and 1 min 30s at 72°C (elongation), with a final elongation of 10 min at 72°C. Gel electrophoresis was carried out with 0.5% agarose using the molecular weight marker Perfect DNA 100 bp Ladder Novage to calculate fragment size of amplifications. Gels were visualized in a photodoc BioDoc-It2 Imager 315 Imaging System LMS-20 Transilluminator. PCR products were purified using a QIAquick Qiagen purification kit. Tissue selection, DNA extraction, amplification and purification of genetic sequences, were performed at the Laboratory of Molecular Biology at Laboratorio Regional de Biodiversidad y Cultivo de

Tejidos Vegetales (**LBCTV**), **IB-UNAM**, Tlaxcala City. Sanger sequencing was done at the Laboratory of Molecular Biology and Health, **IB-UNAM**, Mexico City.

2.5. Editing and DNA sequencing alignment

Edition sequences was done using the program Geneious v.8.1.9 (Rozen and Skaletsky, 2000) and Seaview v.4 (Gouy et al., 2010). A final multiple sequences alignment was elaborated with MAFFT v.7 (Katoh and Toh, 2008) through the online version with the next parameters: Auto (FFT-NS-2, FFTNS-i or LNS-i "depending on data size". In some cases, the alignments were edited manually with BioEdit v.7.0.5.3 program (Hall, 1999). The concatenated matrix (CO1+ITS2+28S) was constructed using Geneious program.

Table 1. Specimens sequenced per species of *Physocyclus* and *Mictlanus* gen. nov. DNA voucher numbers, localities, and GenBank accession numbers for CO1, ITS2, and 28S. Mexican states abbreviations: BC, Baja California; BCS, Baja California Sur; COL, Colima; GRO, Guerrero; HGO, Hidalgo; JAL, Jalisco; MICH, Michoacán, OAX, Oaxaca; PUE, Puebla. **Other countries.

Species	**DNA Code LATLAX**	**Locality (Mexico)**	**CO1**	**ITS2**	**28S**
Physocyclus bicornis	Ara0394	GRO: Copala	OP293157	OP296540	OP295410
P. bicornis	Ara0396	GRO: Quechultenango	OP293158	OP296538	OP295411
P. bicornis	Ara0398	GRO: Coyuca	OP293159	OP296539	-
P. bicornis	Ara0445	GRO: Quechultenango	OP293160	OP296541	OP295412
P. gertschi	Ara0575	GRO: José Azueta	OP293174	OP296552	OP295422
P. gertschi	Ara0576	GRO: José Azueta	OP293175	OP296553	OP295423
P. gertschi	Ara0577	GRO: José Azueta	OP293176	OP296554	OP295424
P. globosus	Ara0473	COL: Coquimatlán	OP293177	OP296555	OP295425
P. globosus	Ara0533	BCS: Comundú	OP293178	-	-
P. globosus	Ara0535	GRO: Técpan	OP293179	OP296556	OP295426
P. globosus	-	Quintana Roo	MT888253	-	-
P. globosus	-	Cuba**	AY560788	-	AY560751

P. lautus	Ara0459	MICH: Cárdenas	OP293180	OP296557	OP295427
P. lautus	Ara0579	MICH: Coahuayana	OP293181	OP296558	OP295428
P. lautus	Ara0583	JAL: La Huerta	OP293182	-	OP295429
P. mariachi	Ara0745	JAL: Hostotipaquillo	OP293185	OP296561	OP295432
P. mariachi	Ara0746	JAL: Hostotipaquillo	OP293186	OP296562	OP295433
P. mariachi	Ara0748	JAL: Plan de Barrancas	OP293187	OP296564	OP295434
P. modestus	Ara0467	PUE: Miahuatlán	OP293196	OP296570	OP295443
P. modestus	Ara0469	GRO: Tepecoacuilco	OP293197	OP296571	OP295444
P. modestus	Ara0480	GRO: Escudero	OP293198	-	OP295445
P. modestus	Ara0482	GRO: Quechultenango	OP293199	-	-
P. paredesi	Ara0483	OAX: Tadela	OP293205	OP296574	OP295448
P. paredesi	Ara0484	OAX: Tadela	OP293206	OP296575	OP295449
P. paredesi	Ara0485	OAX: Totolapa	OP293207	OP296576	OP295450
P. paredesi	Ara0486	OAX: Totolapa	OP293208	-	OP295451
P. pocamadre	Ara0371	BCS: Mulegé	OP293209	-	-
P. sikuapu	Ara0749	MICH: Costa Aquila	OP293216	OP296581	OP295457
P. sikuapu	Ara0750	MICH: Costa Aquila	OP293217	OP296582	OP295458
P. sikuapu	Ara0751	MICH: Costa Aquila	OP293218	OP296583	OP295459
P. sikuapu	Ara0752	MICH: Costa Aquila	OP293219	OP296584	OP295460
P. validus	Ara0502	COL: Coquimatlán	OP293220	OP296585	-
P. validus	Ara0503	GRO: Eduardo Neri	OP293221	OP296586	OP295461
P. xerophilus	Ara0372	BCS: Mulegé	OP293222	OP296587	OP295464
P. xerophilus	Ara0373	BCS: Mulegé	OP293223	OP296588	OP295462
P. xerophilus	Ara0374	BCS: Mulegé	OP293224	OP296589	OP295465
P. xerophilus	Ara0375	BCS: Mulegé	OP293225	OP296590	-
P. xerophilus	Ara0376	BCS: Mulegé	OP293226	OP296591	OP295463
P. xerophilus	Ara0377	BCS: Mulegé	OP293227	OP296592	-
Mictlanus brevicornus	Ara0515	JAL: Cocula	OP293161	OP296542	OP295413
M. brevicornus	Ara0516	MICH: Morelia	OP293162	-	-
M. brevicornus	Ara0518	MICH: Morelia	OP293163	OP296543	OP295414
M. cornutus	Ara0405	BCS: Los Cabos	OP293164	-	OP295415

M. cornutus	Ara0406	BCS: Los Cabos	OP293165	-	OP295416
M. dugesi	Ara0597	HGO: Tula	OP293166	OP296544	OP295417
M. dugesi	-	Costa Rica**	AY560787	-	AY560750
M. enaulus	Ara0391	COA: Saltillo	OP293167	OP296545	OP295418
M. enaulus	Ara0392	COA: Saltillo	OP293168	OP296546	OP295419
M. enaulus	Ara0393	COA: Saltillo	OP293169	OP296547	OP295420
M. enaulus	-	U.S.A.**	MG268722	-	-
M. franckei	Ara0378	HGO: Tolantongo	OP293170	OP296548	-
M. franckei	Ara0379	HGO: Cárdenas	OP293171	OP296549	OP295421
M. franckei	Ara0381	HGO: Cardonal	OP293172	OP296550	-
M. franckei	Ara0382	HGO: Cardonal	OP293173	OP296551	-
M. lyncis	Ara0437	JAL: Zapopan	OP293183	OP296559	OP295430
M. lyncis	Ara0754	JAL: Zapopan	OP293184	OP296560	OP295431
M. merus	Ara0898	SLP: Villa de Reyes	OP293188	OP296565	OP295435
M. merus	Ara0915	SLP: Villa de Reyes	OP293189	-	OP295436
M. merus	Ara0916	SLP: Villa de Reyes	OP293190	OP296566	OP295437
M. merus	Ara0917	SLP: Villa de Reyes	OP293191	OP296567	OP295438
M. merus	Ara0918	SLP: Villa de Reyes	OP293192	-	OP295438
M. michoacanus	Ara0585	MICH: Tzitzio	OP293193	OP296568	OP295440
M. michoacanus	Ara0586	MICH: Tzitzio	OP293194	OP296569	OP295441
M. michoacanus	Ara0598	JAL: Jilotlán	OP293195	-	OP295442
M. mysticus	Ara0450	BC: Ensenada	OP293200	OP296572	-
M. mysticus	Ara0451	BC: Ensenada	OP293201	OP296573	-
M. mysticus	Ara0452	BCS: Mulegé	OP293202	-	OP295446
M. mysticus	Ara0453	BCS: Mulegé	OP293203	-	OP295447
M. mysticus	Ara0524	BC: Ensenada	OP293204	-	-
M. reddelli	Ara0487	HGO: Araya	OP293210	OP296577	OP295452
M. reddelli	Ara0488	HGO: Araya	OP293211	OP296578	-
M. rothi	Ara0383	BCS: Comundú	OP293212	OP296579	OP295453
M. rothi	Ara0384	BCS: Comundú	OP293213	OP296580	OP295456
M. rothi	Ara0386	BCS: La Paz	OP293214	-	OP295454
M. rothi	Ara0387	BCS: La Paz	OP293215	-	OP295455
Artema atlanta	-	U.S.A.**	AY560771	-	-
A. bunkpurugu	-	-	MG268734	-	-

Chisosa diluta	S430	U.S.A.**	MG268626	-	MG268556
Chisosa sp.	Ara0454	PUE: Miahuatlán	OP293228	OP296593	OP295466
Chisosa sp.	Ara0455	PUE: Miahuatlán	OP293229	OP296594	OP295467
Chisosa sp.	Ara0456	PUE: Miahuatlán	OR106010		OR101945
Chisosa sp.	Ara0457	PUE: Miahuatlán	OR106011	-	-
Holoceneminus sp.	-	-	MG268714	-	-
Holoceneminus sp.	-	-	MG268712	-	-
Holoceneminus sp.	-	Philippines**	KX038750	-	-
Modisimus macaya	-	Haiti**	MG268671	-	-
M. seguin	-	Haiti**	FJ228026	-	-
Nita elsaff	-	Egypt**	JX023601	-	-
Pholcitrichocyclus balladong	-	-	MG268635	-	-
P. balladong	-	Australia**	AY560773	-	-
Pholcitrichocyclus sp.	-	Australia**	AY560772	-	-
Pholcophora americana	-	Canada**	JF887156	-	-
Wugigarra yawai	-	Australia**	MG268749	-	MG268401

Table 2. Primer sets used in this study for PCR amplification.

Molecular marker	Primer	Sequence (5'-3')	Author
CO1	HCO2198	TAAACTTCAGGGTGACCAAAAAATC	Folmer et al. (1994)
	LCO1490	GGTCAACAAATCATAAAGATATTGG	
	HCO-JJ	AWACTTCVGGRTGCVCAAARAATCA	Astrin and Stueben (2008)
	LCO-JJ	CHACWAAYCATAAAGATATYGG	
ITS2	5.8S	CGCCTGTTTATCAAAAACAT	Ji et al. (2003); Planas and Ribera (2014)
	CAS28sB1d	TTC TTT TCC TCC SCT TAY TRA TAT GCT TAA	
28S	28S-B1	GACCGATAGCAAACAAGTACCG	Bruvo-Madarić et al. (2005)
	28S-B2	CACGGGTCGATGAAGAACGC	

3. Phylogenetic analyses

3.1. Parsimony analysis

The morphological matrix was constructed using the program Winclada-Asado v.1.7 (Nixon, 2004). The cladistic analysis with equal weighting was carried out under the program TNT (Goloboff et al., 2008) using Traditional Search, with following parameters: Wagner Trees: Random Seed = 1000; Replications = 10,000; Swapping algorithm = Tree Bisection and Reconnection (TBR); trees to save per replication = 1000. The support branches were calculated using Jackknife (Farris et al., 1996) with 1000 replicates, with a probability of 36%, using a traditional search. Additionally, Bremer support values (Bremer, 1988), with a retention of suboptimal trees by five steps were calculated. A strict consensus tree of the most parsimonious trees found in the phylogenetic analysis was calculated. The optimization of ambiguous characters was resolved using accelerated transformations (ACCTRAN) (Farris, 1970; Swofford and Maddison, 1987; Agnarsson and Miller, 2008). The non-informative characters were deactivated to avoid the underestimation of consistence index and the most parsimonious length tree. Multi-state characters were treated like non-additives (Fitch, 1971). The strict consensus tree was edited in Winclada-Asado and Adobe Photoshop CS6.

4. Phylogenetic molecular analyses

4.1. Maximum Likelihood Analysis (ML)

The phylogenetic analyses were carried out in the CIPRES Science Gateway website (https://www.phylo.org/) (Miller et al., 2010), using the concatenated matrix (CO1+ITS2+28S). The evolution models for molecular analyses were selected using IQTree v2.1.3 (Posada and Buckley, 2004), under the Akaike information criteria (AIC). The selected model for each partition were the next: CO1 = GTR+I+G (1st and 2nd codon positions) and GTR+G (3rd codon position), ITS2 = TVM+G and 28S = GTR+I+G. The ML analysis was conducted with 1000 replicates of Bootstrap, with a statistical support value of 64%. The phylogenetic tree generated was edited using the program ITOL v.6.7.3 (Letunic and Bork, 2021) and Adobe Photoshop CS6.

4.2. Bayesian Inferences Analysis (BI)

The phylogenetic analysis was conducted with the CIPRES Science Gateway website, using the concatenated molecular matrix (CO1+ITS2+28S) and combined evidence (Morphology+CO1+ITS2+28S). The combined evidence matrix was concatenated with the program Mesquite v.2.75 (Maddison and Maddison, 2011). The evolution models were selected with IQTree program under the Bayesian Information Criteria (BIC). The selected model for each gene were the next: CO1 = GTR+I+G (1st and 2nd codon positions) and GTR+G (3rd codon position), ITS2 = K3P+G, 28S = TIM3+I+G. The morphology was coded under G distribution. Both analyses, under molecular and combined evidence, were realized with the following commands: MCMC (Monte Carlo Markov Chain) generations = 10 000 000, print frequency = 1000, sampling frequency = 1000, number of runs = 2, number of chains = 4, MCMC burnin = 2500, sumt burnin = 2500, sump burnin = 2500. The associated statistic with the sampled trees in the MCMC, with the EES (Effective Sample Size) >200 was evaluated with the program Tracer v.1.6 (Rambaut and Drummond 2003–2013). The 95% majority consensus tree was edited in ITOL.

4.3. Molecular dating

For lineage dating, JModelTest (Posada, 2008) program was used to test the strict molecular clock to dating divergence time among *Physocyclus* and *Mictlanus* **gen. nov.** The model of a strict molecular clock was rejected by the likelihood ratio test (LTR) (Huelsenbeck and Crandall, 1997). Therefore, was implemented a lognormal relaxed molecular clock, using BEAUti (Bayesian Evolutionary Analysis Utility) v.1.7.5 (Drummond et al., 2012), and BEAST (Bayesian Evolutionary Analysis Sampling Trees) v.1.7.5 (Drummond et al., 2012). The interval of divergence time of lineages for both genera and their internal relationships was established calculating 95% of the highest interval of posterior divergence (HPD) for each *tmrca* (time of the most recent common ancestor) in each node. For the calibration, two alternatives were used: the fossil record and the substitution rate of CO1. Because there is no fossil record of the subfamily Arteminae, two calibration points using fossil of other subfamilies were implemented. The first fossil with 23 Mya age belongs to the subfamily Modisiminae (*Modisimus chiapanecus*), described from Simojovel amber, Chiapas, Mexico by García-Villafuerte and Valdez-Mondragón (2020). This genus was represented by the species *M. macaya* and *M. seguin*, both from Haiti. The second fossil belongs to the subfamily Ninetinae (*Pholcophora*), with 15 Mya and described from the Dominican amber by Wunderlich (1988). The genus was represented by *Pholcophora americana*, which is distributed in North America. The value of 23 and 15 Mya was used like two different calibration points in *prior* section of BEAUti, using a normal distribution of 1. Also, as an additional calibration point in the molecular dating, the estimated substitution rate of CO1 is 0.0178 was included in the analysis (Papadopoulou et al., 2010). We used the concatenated matrix (CO1+ITS2+28S) to calculate the divergence times. The values for the molecular clock were analyzed posteriorly using Tracer v.1.6 to evaluate the parameters and the ESS >200% of the MCMC. The parameters used in BEAUti for the analysis in BEAST were the next: Model = Lognormal uncorrelated relaxed clock, estimate; Tree Prior = Speciations: Yule Process, with random starting tree; Substitution and Site Heterogeneity model = GTR+I+G; Base frequencies = Empirical; Number of gamma categories = 4; MCMC length of chain = 40 000 000.

4.4. Ancestral Areas Reconstruction

The reconstruction of ancestral areas, vicariance and dispersal events, were carried out with the program RASP4 v.4.2 (Reconstruction Ancestral States in Phylogenies) (Yu et al., 2015, 2019, 2020). The analyses were done including 14 biogeograpical provinces proposed by Morrone et al. (2017) and Morrone (2019) for North America. The maximum range size of the ancestral areas reconstructed includes these areas. The RASP4 implemented R-package Biogeobears (Matzke, 2013, 2014), used to reconstruct ancestral geographical distributions: DEC; DIVALIKE; BAYAREALIKE; and the corresponding models including jump dispersal ("+J"). The models selection strongly supported DIVALIKE+J (DIVA-J) had the best fit to the data.

5. Results

5.1. Morphological phylogenetic analyses

The phylogenetic morphological analyses found 18 most parsimonious trees. The consensus tree is showed in the figure 1 (L= 135, CI= 0.64, RI= 0.84). The monophyly of *Physocyclus* (ex-*globosus* group) was supported with high Jackknife and Bremer values of 60% and 2 respectively, and by five synapomorphies (Appendix, characters 3, 4, 10, 30 and 37; Fig. 2): 3) The presence of posterior dorsal sclerotized protuberance of female carapace (Fig. 2 A, red arrow), 4) the presence of sclerotized patch, on dorsal anterior part of female opisthosoma (Fig. 2 A, blue arrow), 10) the pore plates (PP) short, wide, oval-shaped (Fig. 2 J), 30) the spine dorso-distal in the procursus (Fig. 2 D, E, red arrows), and 37) the embolic sclerites (ES) dorsally in the embolus (Fig. 2 D, E).

The monophyly of *Mictlanus* gen. nov. (ex-*dugesi* group) was supported with high Jackknife and Bremer values of 70% and 3 respectively, and by five synapomorphies (Appendix, characters 9, 18, 39, 43 and 45; Fig 3): 9) The lateral constrains in the middle part of female epigynum, with bell-shaped (Fig. 3 G, H, red arrows), 18) the lateral apophysis (LAC) of the male chelicerae, wide and projected toward front (Fig. 3 A–C), 39) embolus long, with "J"-shape, strongly sclerotized (Fig. 3 D, E), 43) the presence of sclerites on retrolateral part of the bulb (SB) (Fig. 3 D, E), and 45) the notch between the SB and the embolus (E) (Fig. 3 D, E).

Based on morphological evidence, the phylogenetic relationships among species of *Physocyclus* are not resolved because there is a polytomy and just some clades into the genus were recovered, which had low branch Jackknife support values (Fig. 1). Only two clades were recovered for this genus (Fig. 1). The first composed by *P. montanoi* (*P. modestus* (*P. sarae* + *P. sikuapu*) and the second clade composed by *P. paredesi* (*P. lautus* (*P. bicornis* + *P. gertschi*) (Fig. 1). Otherwise, the phylogenetic relationships into *Mictlanus* gen. nov. were totally resolved or dichotomous, however, there were low Jackknife support values on internal clades (Fig. 1).

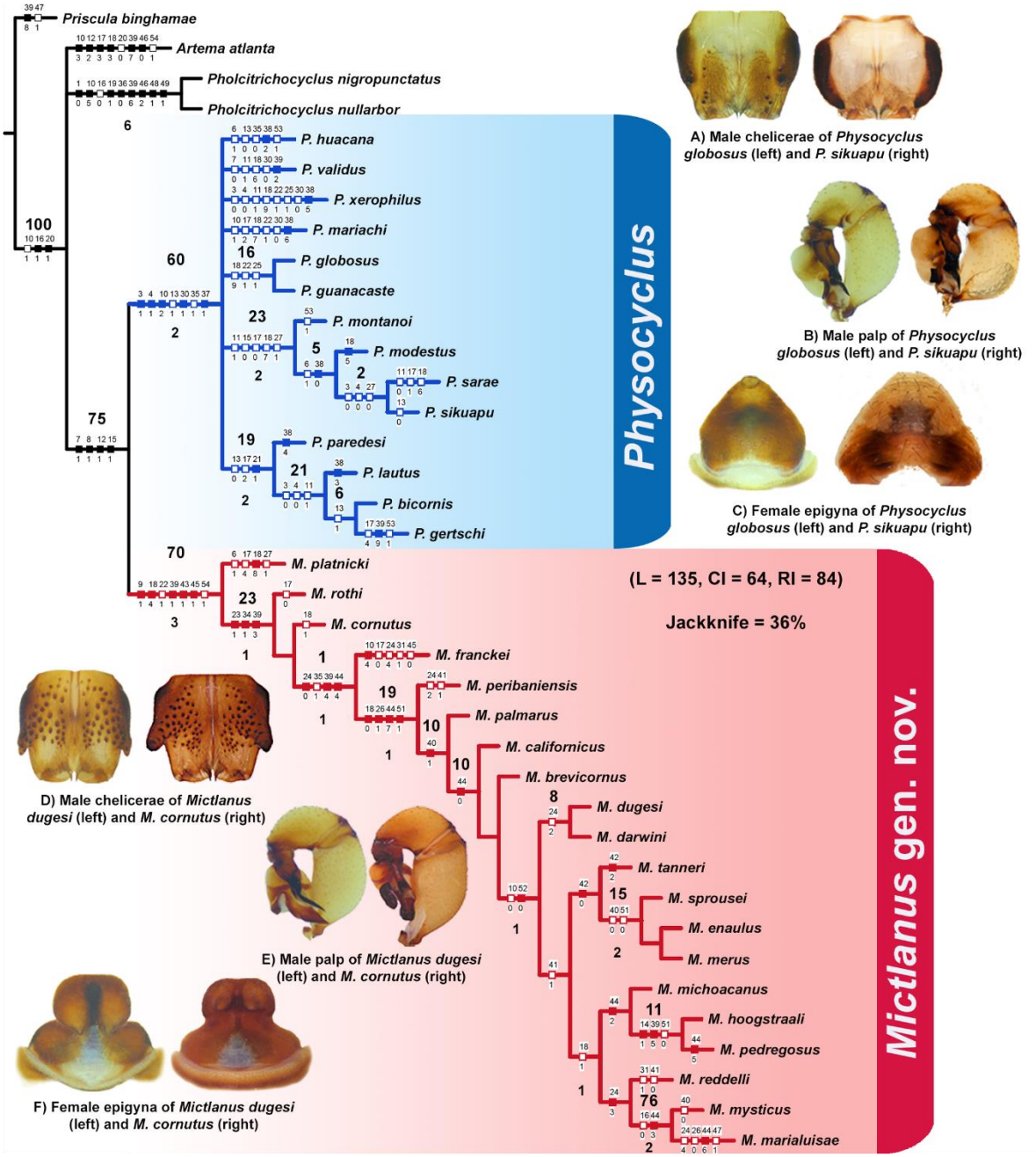

Figure 1. Strict consensus tree of 18 most parsimonious trees found by morphological cladistic analysis under equal weighting of characters of *Physocyclus* and *Mictlanus* gen. nov. Filled squares on branches indicate synapomorphic states, white squares indicate homoplastic characters. Small numbers above bars indicate character number, small numbers below bars indicate character state. Large numbers above branches indicate Jackknife support values (>64%). Large numbers below branches indicate Bremer support values. L= Length Tree, CI= Consistency Index, RI= Retention Index.

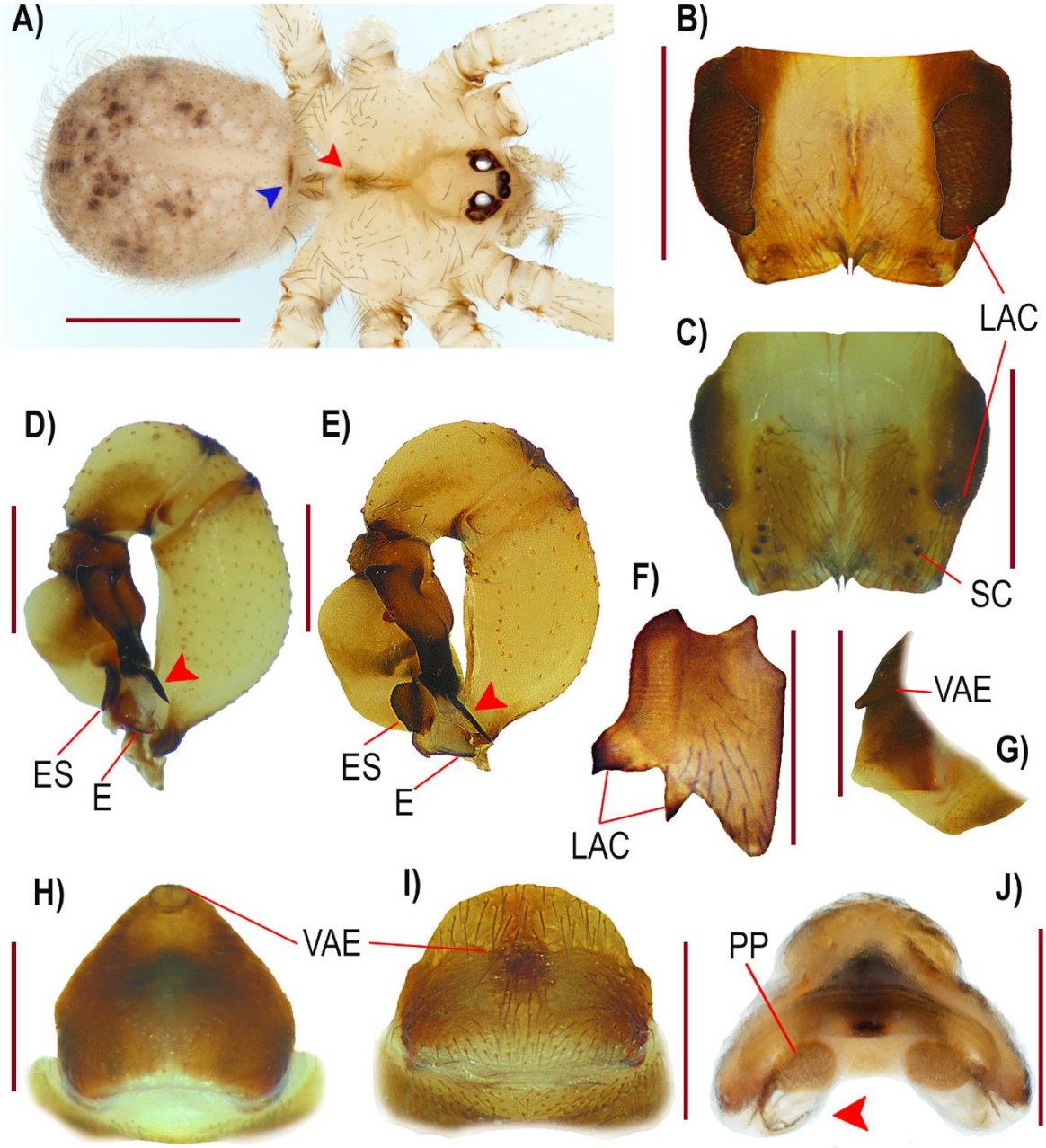

Figure 2. Morphological features of *Physocyclus*, explained in the text. A) Female habitus, dorsal view (*P. pocamadre*). B-C) Male chelicerae, frontal view (*P. modestus* and *P. globosus*, respectively). D-E) Male palps, retrolateral view (*P. globosus* and *P. lautus*, respectively). F) Male chelicera, lateral view (*P. bicornis*). G) Female epigynum, lateral view (*P. modestus*). H-I) Female epigynum, ventral view (*P. globosus* and *P. validus*, respectively). J) Female epigynum, dorsal view (*P. xerophilus*). Scales: A = 1 mm; B-J = 0.5 mm. Abbreviations: E, embolus; ES, embolic sclerites; LAC, lateral

apophyses of chelicerae; PP, pore plates; SC, sclerotized cones; SF, stridulatory files of chelicerae; VAE, ventral apophyses of epigynum. Arrows indicate important structures described in the text.

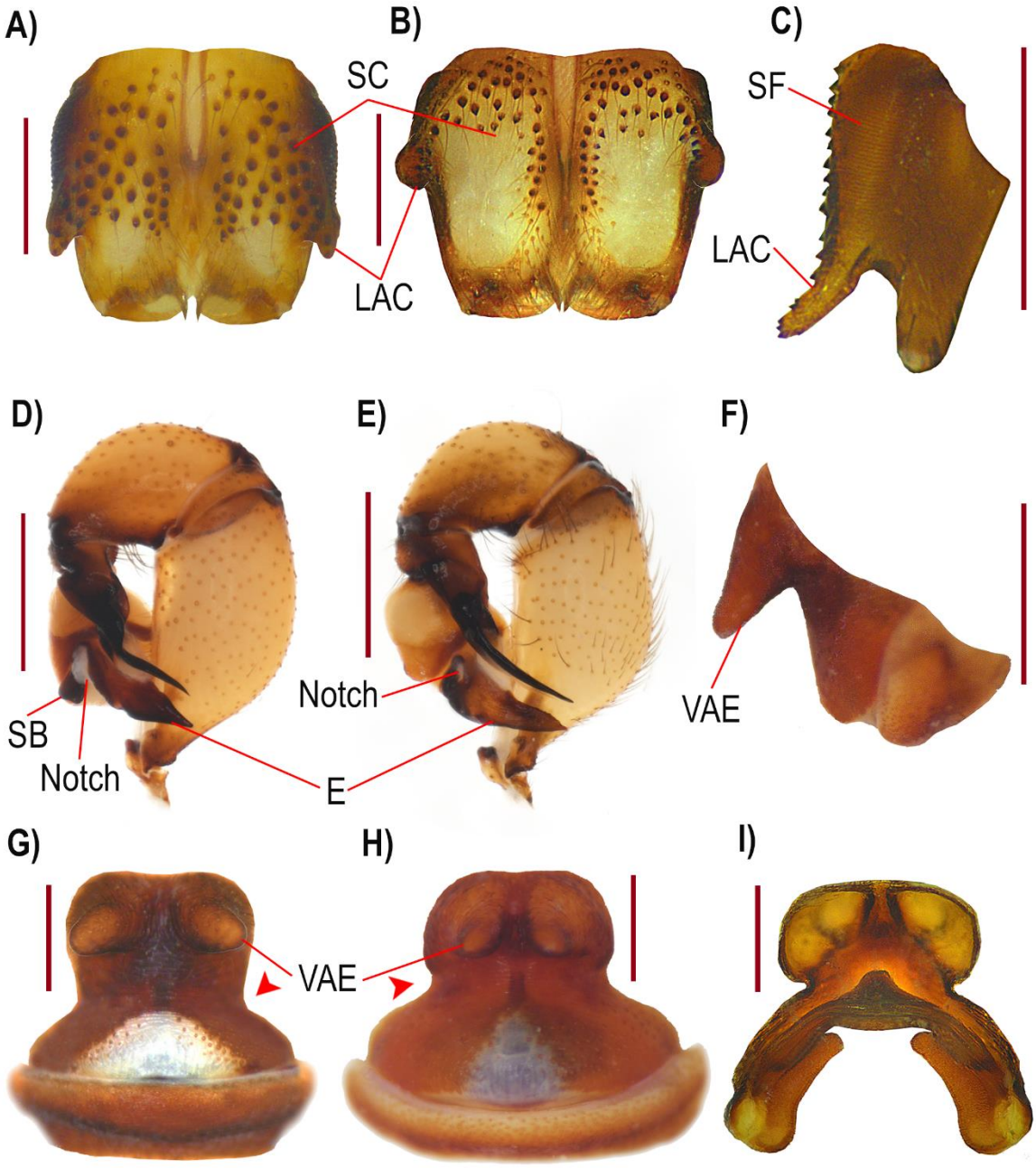

Figure 3. Morphological features of *Mictlanus* gen. nov. explained in the text. A-B) Male chelicerae, frontal view (*M. dugesi* and *M. michoacanus*, respectively). C) Male chelicera, lateral view (*M. reddelli*). D-E) Male palps, retrolateral views (*M. dugesi* and *M. brevicornus*, respectively). F) Female epigynum, lateral view (*M. cornutus*). G-H) Female epigynum, ventral view (*M. rothi* and *M. cornutus*, respectively). I) Female epigynum, dorsal view (*M. michoacanus*). Scales: 0.5 mm.

Abbreviations: E, embolus; LAC, lateral apophyses of chelicerae; SB, sclerites on bulb; SC, sclerotized cones; SF, stridulatory files of chelicerae; VAE, ventral apophyses of epigynum. Arrows indicate important structures described in the text.

5.2. Molecular phylogenetic analyses

According with the molecular phylogenetic analyses with concatenated matrix (CO1+IT2+28S) under ML and BI (Figs. 4, 5 respectively), both topologies were the same, so they are described in the same way. Both genera *Physocyclus* (ex-*globosus* group) and *Mictlanus* gen. nov. (ex-*dugesi* group) are recovered as a monophyletics groups. *Mictlanus* gen. nov. with high branch support values of 100% of Bootstrap and Posterior Probabilities (PP) (Figs. 4, 5). Also, the support values of *Physocyclus* are significant, with Bootstrap 70% and PP 85% respectively (Figs. 4, 5).

In both analyses (ML and BI), into *Mictlanus* gen. nov, there were five sub-clades (Figs. 4, 5). The first clade (A) includes *M. dugesi* + *M. michoacanus* as sister species, with significant branch support values (Bootstrap 86% and PP 99%). The clade B, includes *M. enaulus* + *M. merus* as sister species with high branch support values (Bootstrap 100% and PP 100%). The clade C includes *M. reddelli* + *M. franckei* as sister species with significant branch support (100% for Bootstrap and PP). The clade D is composed by *M. lyncis* + *M. brevicornus* as sister species with high support values (Bootstrap 97% and PP 100%). The last clade (E) is composed by *M. mysticus* (*M. cornutus* + *M. rothi*) with high branch support values (Bootstrap 99% and PP 100%) (Figs. 4, 5).

For *Physocyclus*, four sub-clades were conformed (Figs. 4, 5). *Physocyclus globosus* is the sister species of all the species of *Physocyclus*, meanwhile *P. paredesi* is the sister species of the remains species of *Physocyclus* (except of *P. globosus*). Both species has high branch support in both analyses ML and BI (Bootstrap 100% and PP %100). The clade F is composed by *P. mariachi* (*P. pocamadre* + *P. xerophilus*) with significant support values (Bootstrap 50% and PP 72%). The clade G is integrated by *P. lautus* + *P. bicornis* as sister species with significant branch support values (Bootstrap 52% and PP 79%). The clade H correspond to *P. validus* + *P. modestus* as sister species with robust branch support values (Bootstrap 98% and PP 100%). The last clade I includes *P. gertschi* + *P. sikuapu* as sister species with significant branch support values (Bootstrap 58% and PP 80%) (Figs. 4, 5).

The phylogenetic analysis conducted with BI and using total combined evidence (Morphology+CO1+ITS2+28S) (Fig. 6), recovered *Mictlanus* gen. nov. and *Physocyclus* as monophyletic, with high branch support values (PP 100% and 87% Bootstrap, respectively) (Fig. 6). The phylogenetic relationships inside *Mictlanus* gen. nov. is the same as was found in the ML and IB analyses based only in molecular data (Figs. 4, 5). However, the internal relationships recovered in *Physocyclus* were different. *Physocyclus pocamadre* appear as the sister species of *P. paredesi*, forming a clade (J) with significant PP support value (83%) (Fig. 6). The remain of the phylogenetic relationships into *Physocyclus* were the same (Fig. 6).

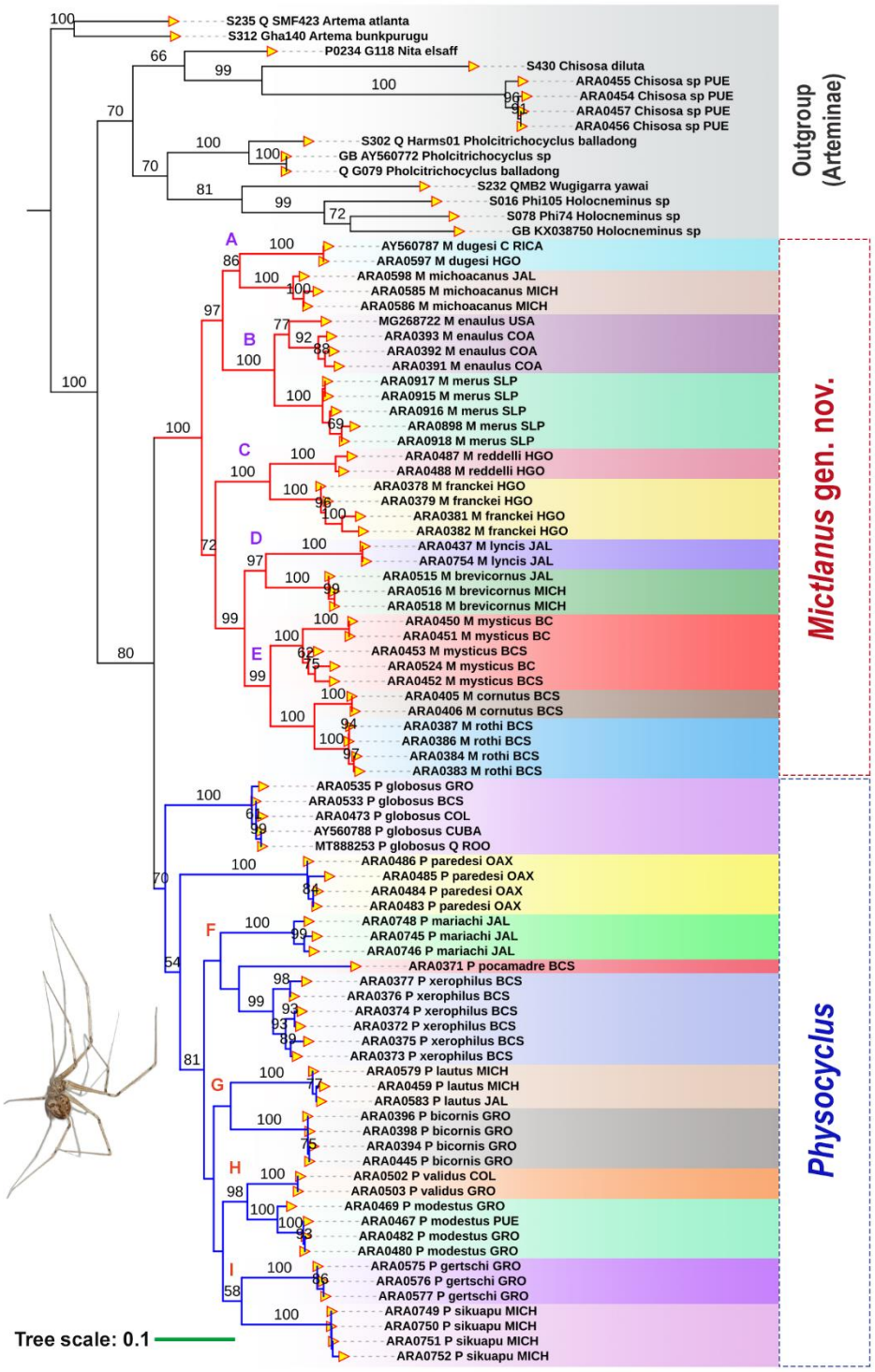

Figure 4. Phylogenetic reconstruction under Maximum Likelihood (ML) with a molecular concatenated matrix (CO1+ITS2+28S) for *Mictlanus* gen. nov. and *Physocyclus*. Bootstrap support values above the branches. Letters above branches indicates the clades described in the text. Color bars on the terminals indicate species.

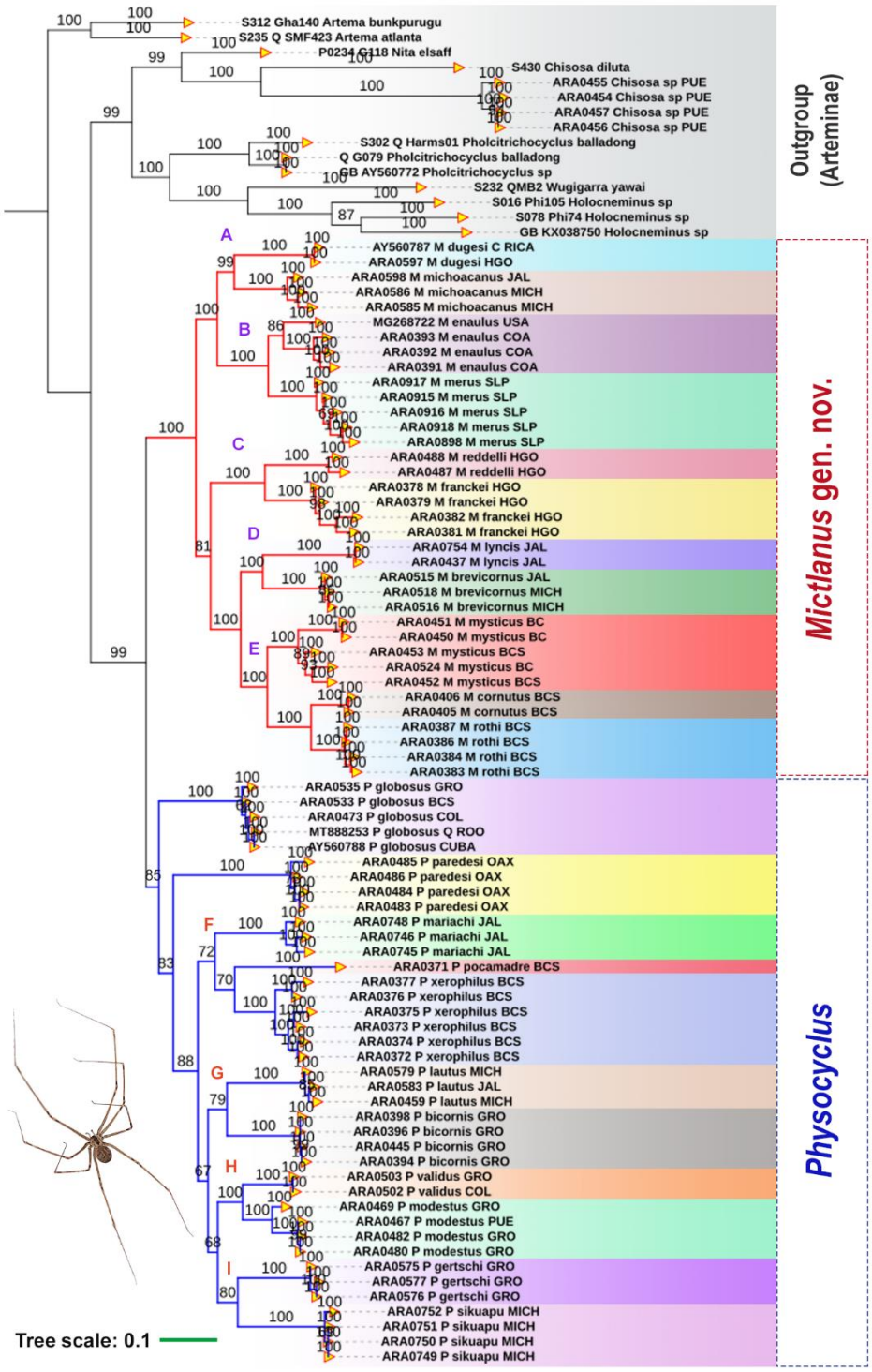

Figure 5. Phylogenetic reconstruction under Bayesian Inferences (BI) with a molecular concatenated matrix (CO1+ITS2+28S) for *Mictlanus* gen. nov. and *Physocyclus*. Posterior Probabilities support values above the branches. Letters above branches indicates the clades described in the text. Color bars on the terminals indicate species.

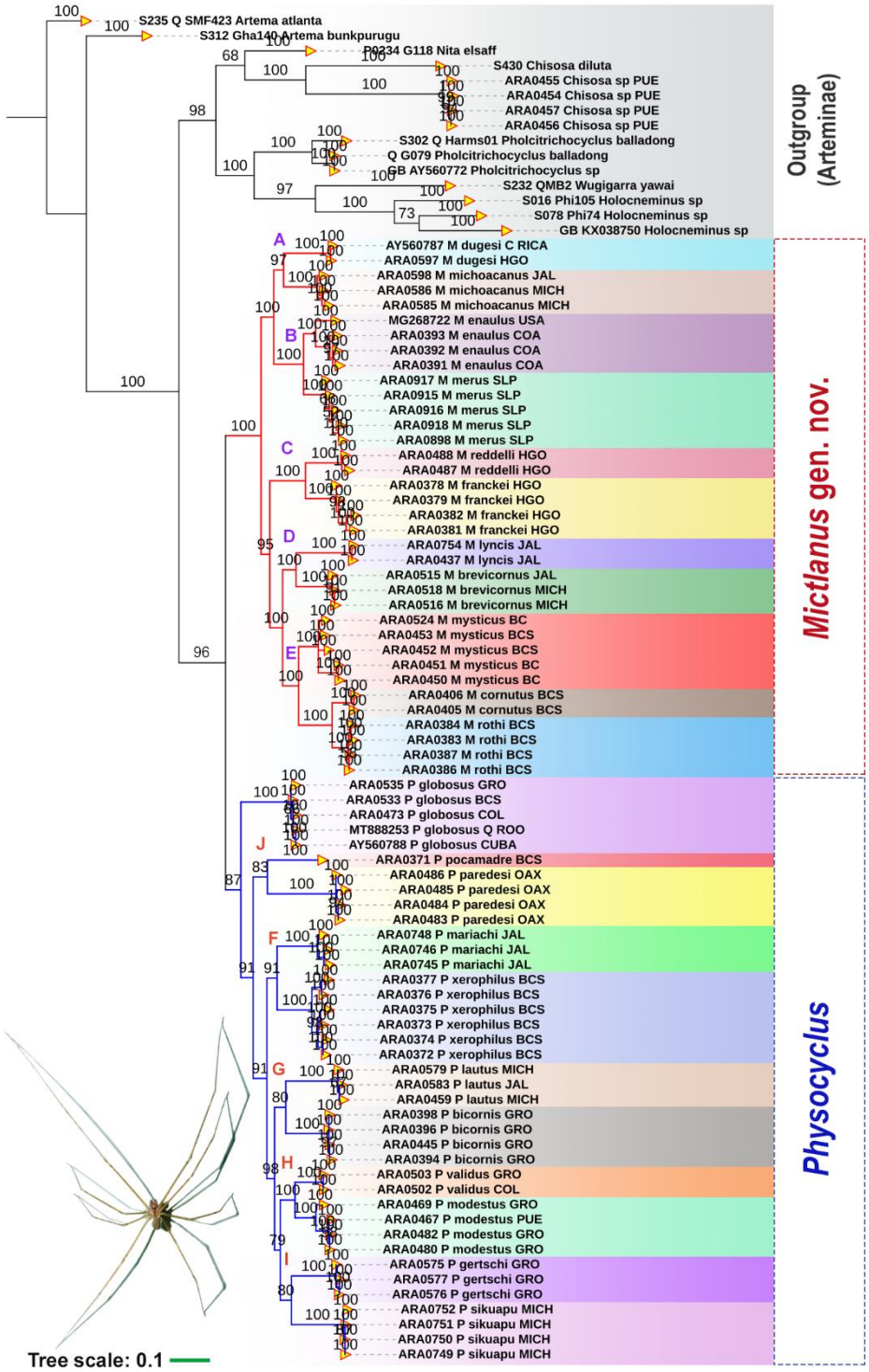

Figure 6. Phylogenetic reconstruction under Bayesian Inferences (BI), with the combined concatenated matrix (Morphology+CO1+ITS2+28S) for *Mictlanus* gen. nov. and *Physocyclus*. Posterior Probabilities support values above the branches. Letters above branches indicate the clades described in the text. Color bars on the terminal indicate species.

5.3. Molecular dating and reconstruction of ancestral areas

Based on the calibration point of Dominican amber (15 Mya), the Simojovel amber (23 Mya), and the substitution rate of CO1 (0.0178), the divergence age of both genera *Mictlanus* gen. nov. (ex-*dugesi* group) and *Physocyclus* (ex-*globosus* group) was 18.9 Mya (96% HPD) (Fig. 7) during the Middle Miocene. The first divergence event within the genus *Mictlanus* gen nov. was 14.6 Mya (95% HPD: 17.2 – 11.4 Mya), forming two different clades with several species each one (Fig. 7). Most of the diversification and speciation events within *Mictlanus* gen. nov. seems to have occurred mainly in Late Miocene (9 – 5 Mya), with few speciation events such as *M. cornutus* + *M. rothi* at the beginning of the Pliocene (3.5 Mya).

The first divergence event within the genus *Physocyclus*, between *P. globosus* and the rest of the species, occurred in the Middle Miocene, 14.8 Mya (95% HPD: 17.7–11.6 Mya) (Fig. 7). The second divergence event between *P. paredesi* and the rest of the species (except *P. globosus*), occurred also in the Middle Miocene, 13.2 Mya (95% HPD: 16.2– 10.1 Mya) (Fig. 7). Most of the diversification and speciation events within the genus *Physocyclus* seems to have occurred mainly Late Miocene (11 – 5 Mya), with two speciation events such as *P. validus* + *P. modestus* (3.7 Mya) and *P. pocamadre* + *P. xerophilus* (4.7 Mya) during the Middle Pliocene (5.3–2.3 Mya) (Fig. 7).

The reconstruction of ancestral areas (AA) shows five main biogeographical provinces that influenced in the diversification of *Mictlanus* gen. nov. and *Physocyclus* (Fig. 8): 1) Sierra Madre Oriental, 2) the Trans-Mexican Volcanic Belt, 3) the Chihuahua Desert, 4) the province of Baja California and 5) the Pacific Lowlands.

The divergence between *Mictlanus* gen. nov. and *Physocyclus* seems to have occurred under vicariance, being the Trans-Mexican Volcanic Belt and the Pacific Lowlands the ancestral areas for their diversification (Fig. 8). For the genus *Mictlanus* gen. nov., the diversification seems to be occurred also due to several vicariance events, with the Trans-Mexican Volcanic Belt as an ancestral area that drove the diversification (Fig. 8). Also, the Sierra Madre Oriental and Trans-Mexican Volcanic Belt were recovered as ancestral areas for different internal nodes of *Mictlanus* gen. nov. (Fig. 8). The diversification of *M. dugesi* + *M.* michoacanus was influenced mainly by the Trans-Mexican Volcanic Belt whereas the Balsas Basin had minimum influence in such as diversification (Fig. 8). The Chihuahuan

Desert influences directly in the diversification of *M. enaulus* + *M. merus*. The clade (*M. dugesi* + *M. michoacanus*) + (*M. enaulus* + *M. merus*) had an ancestral area shared by the Trans-Mexican Volcanic Belt and Chihuahuan Desert. The speciation between *M. redelli* + *M. franckei* was directly influenced by the Sierra Madre Oriental (Fig. 8). In the same way, the speciation for clade composed by *M. lyncis* + *M. brevicornus* was determined mainly by the Trans-Mexican Volcanic Belt. The province of Baja California had an important influence in the diversification of clade composed by the species *M. mysticus* (*M. cornutus* + *M. rothi*) (Fig. 8). The clade (*M. reddelli* + *M. franckei*) + (*M. lyncis* + *M. brevicornus*) + *M. mysticus* (*M. cornutus* + *M. rothi*) has an ancestral area influenced by Trans-Mexican Volcanic Belt, Sierra Madre Oriental and Baja California province (Fig. 8).

For the genus *Physocyclus*, the diversification seems to be occurred also by vicariance events, influenced by the Trans-Mexican Volcanic Belt and the Pacific Lowlands mainly, as ancestral areas (Fig. 8). *Physocyclus globosus* and the rest of the species, has an ancestral area influenced mainly by Trans-Mexican Volcanic Belt and Pacific Lowlands (Fig. 8). *Physocyclus paredesi* and the remains species (except *P. globosus*) share an ancestral area conformed by the Pacific Lowlands and, in a minor rate, by Trans-Mexican Volcanic Belt. The diversification of *P. lautus* + *P. bicornis* and *P. gertschi* + *P. sikuapu* was influenced directly by the Pacific Lowlands. The diversification of *P. validus* + *P. modestus* was determined by the Pacific Lowlands and in a minor grade by Trans-Mexican Volcanic Belt. The diversification of *Physocyclus mariachi* was directly influenced by the Trans-Mexican Volcanic Belt, whereas the diversification of *P. pocamadre* + *P. xerophilus* was drived by the Baja California province. Finally, the diversification of the clade *P. mariachi* (*P. pocamadre* + *P. xerophilus*) was driven by the Trans Mexican Volcanic Belt and Baja California Province (Fig. 8).

Although seems to be that important vicariance events drove mainly the diversification for both genera, also posterior dispersal event influenced the species diversification, between Miocene and Pliocene (~5 Mya) (Fig. 8, green vertical line).

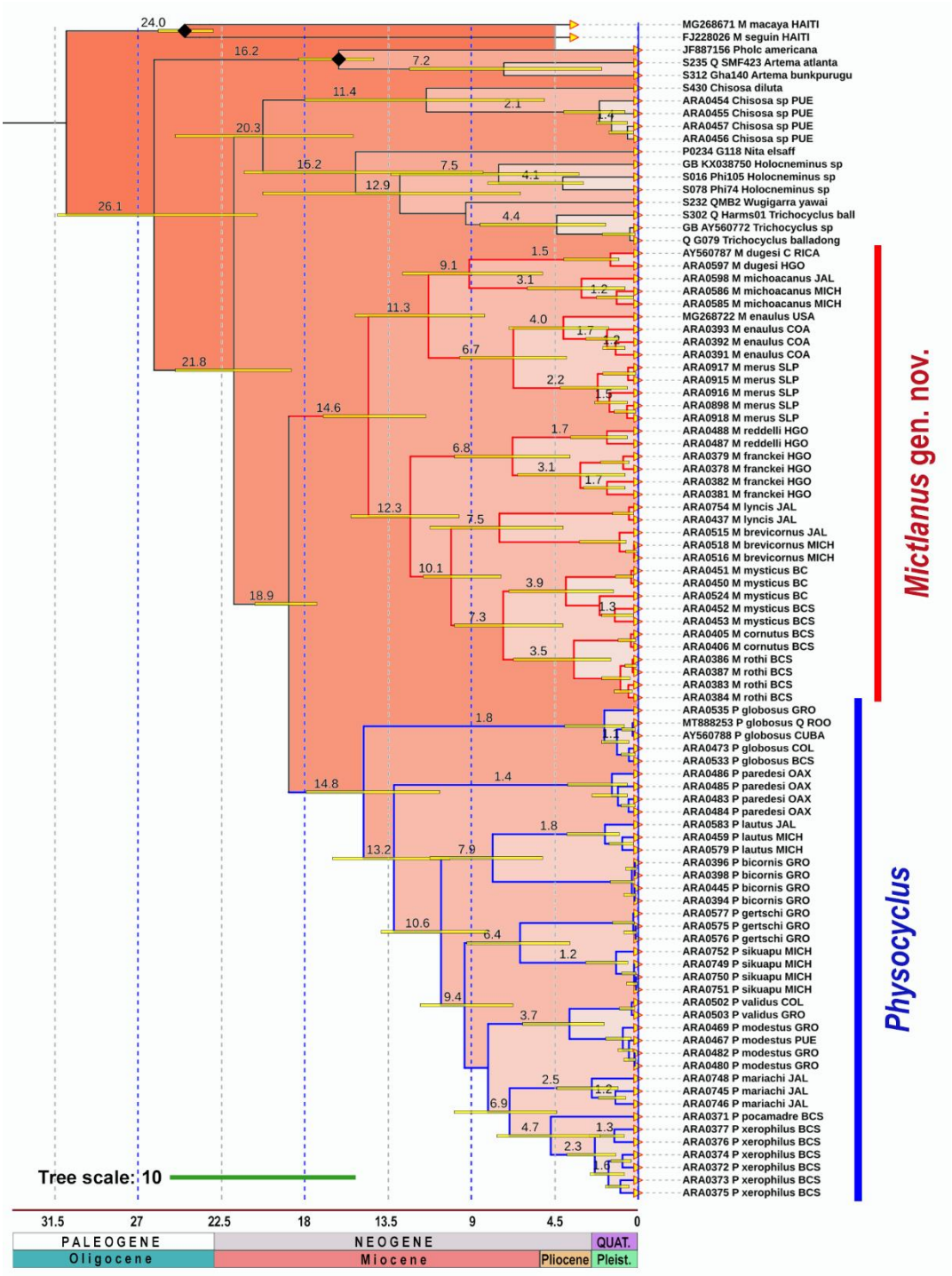

Figure 7. Chronogram with the molecular dating for *Mictlanus* gen. nov. and *Physocyclus* using the lognormal uncorrelated relaxed clock model. Bars on the nodes indicates the 95% highest posterior density interval (HPD) to each *tmrca* (time of the most recent common ancestor). Numbers above bars indicate the average age for each node in millions of years (Mya). Gradient of colors indicates

ages of nodes, form the older nodes (darker red) to recent nodes (lighter red). Black diamonds indicate calibration points.

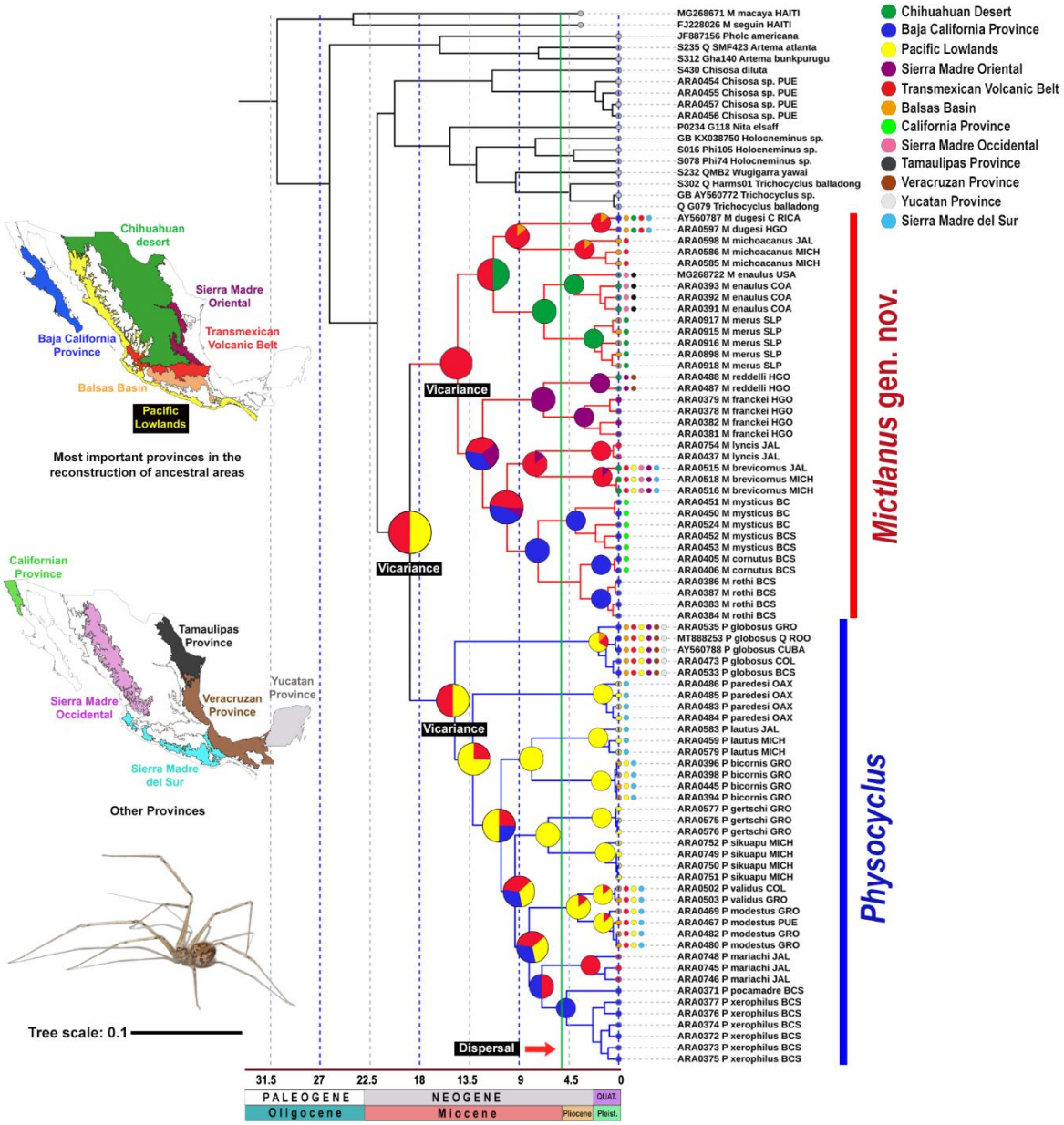

Figure 8. Divergence dating and ancestral biogeographic areas reconstruction for *Mictlanus* gen. nov. and *Physocyclus*. Circles in color on the nodes indicate the ancestral areas corresponding to the biogeographical provinces (BP). Colors in the maps indicate the BP where the species are distributed. Green vertical line represents dispersal events. Scale numbers on the bar below indicate the time expressed millions of years (Mya) and geological eras.

6. TAXONOMY

Pholcidae C.L. Koch 1850

Physocyclus Simon 1893

Physocyclus Simon 1893:1(2), 257–488.

Type species: *Pholcus globosus* Taczanowski 1874:105

Species included: *Physocyclus bicornis* Gertsch, 1971, *P. gertschi* Valdez-Mondragón, 2010, *P. globosus*, *P. guanacaste* Huber, 1998, *P. huacana* Valdez-Mondragón, 2010, *P. lautus* Gertsch, 1971, *P. mariachi* Nolasco and Valdez-Mondragón, 2022, *P. modestus* Gertsch, 1971, *P. montanoi* Valdez-Mondragón, 2010, *P. paredesi* Valdez-Mondragón, 2010, *P. pocamadre* Nolasco and Valdez-Mondragón, 2022, *P. sarae* Valdez-Mondragón, 2010, *P. sikuapu* Nolasco and Valdez-Mondragón, 2022, *P. validus* Gertsch, 1971 and *P. xerophilus* Nolasco and Valdez-Mondragón, 2020. See the taxonomic revision by Valdez-Mondragón (2010) for complete diagnosis and description per each species.

Diagnosis: The genus *Physocyclus* can be distinguished from all another known genera of the subfamily Arteminae by the following combination of characters: Males: 1) lateral apophyses of chelicerae short and the frontal lamina of chelicerae without sclerotized cones or if they present, <5 in each chelicera (e.g., *P. globosus*, *P. guanacaste*) (Figs. 1 A; 2 B, C); 2) dorso-distal spine on the embolus; and 3) sclerites dorsally on the embolus (Figs. 1 B; 2 D, E). Females: 1) epigynum with lateral constrictions barely visible or absent (Figs. 1 C; 2 H, I); 2) ventral apophyses of epigynum short and conical, in some cases, absent (Figs. 1 C; 2 G–I); 3) pore plates short, wide, oval-shaped (Fig. 2 J); 4) the presence of posterior dorsal sclerotized protuberance of carapace, and the sclerotized patch on dorsal anterior part of opisthosoma in some species (e.g., *P. pocamadre*) (Fig. 2 A, red and blue arrows, respectively).

Description: Following Valdez-Mondragón (2010, 2013, 2014): Medium sized spiders (total length 3–7 mm). *Prosoma.* Carapace light beige to brown (with variation color among

species), with marginal dorsal spots (Nolasco and Valdez-Mondragón, 2022a; figs 14, 28). Fovea with irregular pattern around it, light gray to brown coloration (Fig. 2 A). Carapace with a dorsal notch in the posterior dorsal part. Ocular region with darker coloration than carapace (Fig. 2 A). Eight eyes in a region slightly elevated (Valdez-Mondragón, 2010, 2014; figs 4, 5, 21, 22; Nolasco and Valdez-Mondragón, 2020; fig. 4). Clypeus wide, gray to dark brown (Nolasco and Valdez-Mondragón, 2020; fig. 4; Nolasco and Valdez-Mondragón, 2022a; figs 17, 41). Male chelicerae without sclerotized cones in frontal lamina, in the most of the species, and if it presents (e.g., *P. globosus* and. *P. guanacaste*), less than five cones on each chelicera (Fig. 2 B, C). Chelicerae with small, short and conical lateral apophyses. Stridulatory files present. Sternum and labium wider than long, gray to brown. Endites long (Nolasco and Valdez-Mondragón, 2022a: figs 16, 30, 40). *Legs.* Male legs longer than the female, with distal rings in femora and tibiae (in *Physocyclus paredesi* with dark spots spread along). Leg formula for most of the species is 1–4–3–2. Legs without spines, setae curved and long in male tibiae and metatarsi. *Opisthosoma.* Globular, wider than long, larger in females than males, with irregular spots gray, brown or white. (Fig. 2 A). Lateral anterior spinnerets conical, posterior lateral spinnerets almost flat, with marginal thick setae, posterior median spinnerets cylindrical. *Palps*. Males. Massive, bigger than the body, femur wide, tibia ending in conical shape. Procursus long, dark, sclerotized (Figs. 2 D, E). Embolic sclerites small, short and wide. Spermatic operculum in distal part of embolus (Nolasco and Valdez-Mondragón, 2020; fig. 6). Palps in females are simple, thin and short, without ornamentations.

Species *nomina dubia* and *incertae sedis*: *Physocyclus rotundus* O. P. Cambridge, 1898 (*nomen dubium*): In the original description is described the female, however, the diagnostic characters do not correspond with *Physocyclus*, but they might be a female of the genus *Coryssocnemis* Simon 1893, or even *Ixchela* Huber, 2000. F. O. P. Cambridge (1902) provided a brief description of the female, however, in the figures *Physocyclus globosus* which has a high number of synonymies is cited. The holotype is lost, so they identity cannot be confirmed so far.

Physocyclus viridis Mello-Leitao, 1940 (*incertae sedis*): The original description is based on adult male, and it is mentioned that the chelicerae present an anterior apophyses

"*Queliceras com um dente anterior*". This limited description difficult to classify the species on *Physocyclus* or another genus. No figures or schemes are provided and the holotype is probably lost.

Distribution: The genus *Physocyclus* is native from North and Central America. They are distributed mainly to Mexican Mountain and Mesoamerican biotic components (Morrone, et al., 2017; Morrone, 2019; Valdez-Mondragón 2010, 2013, 2014). Most of the species are distributed in the Pacific lowlands and in Balsas Basin, mainly in the states of Colima, Guerrero, Jalisco, Michoacán, Morelos, Nayarit, Oaxaca and Sinaloa (Fig. 9). *Physocyclus pocamadre* and *P. xerophilus* are distributed in the biogeographic province of Baja California, specifically in Baja California Sur state (Nolasco and Valdez-Mondragón 2020, 2022a) (Fig. 9). *Physocyclus modestus* in Puebla state corresponds to the species with the wide spread distribution in the central region of Mexico (Valdez-Mondragón, 2010). *Physocyclus globosus* has cosmopolitan distribution and can be found it in different regions in the country to those mentioned before (Valdez-Mondragón, 2010). This is the reason why those records of *P. globosus* were not shown in the distribution map. In other hand, *Physocyclus guanacaste* is only recorded from Costa Rica (Guanacaste, type locality) being the species with the southest distribution for the genus (Fig. 9).

Natural history: *Physocyclus* habits mainly in deciduous forests, characterized by the dominance of trees with spread canopy (average height 7–8 m) and arid ecosystems, recognized by the low levels of annual precipitation. These spiders can be found between big boulders, in bark tree, holes in the ground, among the exposed roots of big trees, inside caves and in their fissures of the walls. All species are located below of 1900 m.a.s.l. (Valdez-Mondragón 2010; Jiménez and Palacios-Cardiel, 2013; Nolasco and Valdez-Mondragón, 2020, 2022a), except by *Physocyclus globosus* (cosmopolitan), which can be found in higher elevations, always close to synanthropic habits. Due to their troglophile habits, some species habits into caves in karstic zones, mainly in the entrances of the caves and close to the bats populations where they feed of insect and other arthropods that lives on the guano. In some species, the abundance and density use to be high, found a great number of males, females and juveniles in a short distance (6 spiders/m^2) (Valdez-Mondragón, 2010).

Mictlanus GEN. NOV.

Type species: *Mictlanus dugesi* (Simon, 1893), herein designated by principle of priority.

Species included: *Mictlanus brevicornus* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus californicus* (Chamberlin and Gertsch, 1929), comb. nov., *Mictlanus cornutus* (Banks, 1898), comb. nov., *Mictlanus darwini* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus dugesi* (Simon, 1893), comb. nov., *Mictlanus enaulus* (Crosby, 1926), comb. nov., *Mictlanus franckei* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus hoogstraali* (Gertsch and Davis, 1942), comb. nov., *Mictlanus lyncis* (Nolasco and Valdez-Mondragón, 2022), comb. nov., *Mictlanus marialuisae* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus merus* (Gertsch, 1971), comb. nov., *Mictlanus mexicanus* (Banks, 1898), comb. nov., *Mictlanus michoacanus* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus mysticus* (Chamberlin, 1924), comb. nov., *Mictlanus palmarus* (Jiménez and Palacios-Cardiel, 2013), comb. nov., *Mictlanus pedregosus* (Gertsch, 1971), comb. nov., *Mictlanus peribanensis* (Valdez-Mondragón, 2014), comb. nov., *Mictlanus platnicki* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus reddelli* (Gertsch, 1971), comb. nov., *Mictlanus rothi* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus sprousei* (Valdez-Mondragón, 2010), comb. nov., *Mictlanus tanneri* (Chamberlin, 1921), comb. nov.

Etymology: The genus name is a noun in apposition and masculine, which refers to the "*Mictlán"*, the underworld or place of the death in the Mexica (Aztec) mythology. Mictlán is believed to be ruled by the king Mictlantecuhtli ("Lord of the underworld").

Diagnosis: *Mictlanus* can be distinguished from other genera in the subfamily Arteminae by the following combination of characters: Males: 1) chelicera with >30 sclerotized cones in each one (except *M. platnicki*, where they are absent), usually with different location pattern in the different species (Figs. 1 D; 3 A, B); 2) lateral apophysis of male chelicerae usually long and projected toward front (Fig. 3 A–C); 3) embolus long, with "J"-shape, strongly chitinized (Fig. 3 D, E); 4) sclerites on retrolateral part of the bulb (SB) (Figs. 1 E; 3 D, E); and 5) notch between SB and embolus (Figs. 1 E; 3 D, E). Females: 1) lateral constrains in

the middle part of female epigynum (quite visible) (Figs. 1 F; 3 G, H, red arrows); 2) epigynum with bell-shaped (Figs. 1 F; 3 G, H); and 3) the ventral apophyses of epigynum usually long, curved, wide and conical (Figs. 1 F; 3 F–H).

Description: *Prosoma*. Carapace color and shape similar among species (Nolasco and Valdez-Mondragón 2022a; Valdez-Mondragón 2010, 2014). Carapace color varies among light yellow to brownish. The most of the species present marks with different shape ("Y" or "V"-shaped) around the fovea, with a coloration that oscillates among light beige to dark gray (Nolasco and Valdez-Mondragón 2022a; fig. 58; Valdez-Mondragón, 2014; figs 1, 12, 18, 28). This mark extends towards posterior part of ocular region. Almost all species presents three irregular spots on the dorsal part of carapace, with different tones of coloration between light gray to dark brown (Valdez-Mondragón, 2010; Nolasco and Valdez-Mondragón 2022a; fig. 58; Valdez-Mondragón, 2014; figs 1, 12, 18, 28). Clypeus wide, some species with two brown, gray or orange lines, darker in males (Valdez-Mondragón, 2014; figs 4, 21). Chelicerae slightly darker than carapace (Fig. 3 A, B). In some species, stridulatory files present on males (Fig. 3 C) (Nolasco and Valdez-Mondragón 2022a; Valdez-Mondragón, 2014; figs 6, 7, 23, 24). Sternum regularly darker than carapace, with different marks with different coloration (light gray to dark gray) (Nolasco and Valdez-Mondragón 2022a; fig. 60; Valdez-Mondragón, 2014; figs 3, 14, 20, 30). Labium darker than carapace, wider than long. Endites with same color of labium, longer than wide (Nolasco and Valdez-Mondragón 2022a; fig. 60; Valdez-Mondragón, 2010, 2014; figs 3, 14, 20, 30). *Legs.* Longer in males than females, with distal rings in femora and in basal and distal part in tibiae. Coloration varies among species and corresponds with the carapace color; however, the coloration of the ringed ornamentation is darker. Leg formula for most of the species is 1–4–3–2. Legs without spines. Coxae with prolateral groove visible. Trochanter with prolateral notch. *Opisthosoma*. Globular, longer than wide and higher than long, bigger in females (Nolasco and Valdez-Mondragón, 2022a: figs 58–60). Dorsal and laterally with different irregular marks white, gray, beige, marron or pale yellow. Lateral anterior spinnerets conical, posterior lateral spinnerets almost flat, posterior median spinnerets cylindrical, with lower size than the others. *Palps.* Massive in comparison with the size of the body. Femur wide, tibia ending in conical form, procursus long, sclerotized and conical, with different shape

among species. Spermatic operculum in distal part of embolus. In females, the palps are simple, thin and small. *Epigynum*. Usually wider than long, but in a few species is longer than wide (e.g., *M. hoogstraali* and *M. michoacanus*). Two VAE ever present, in anterior part of epigynum, with different shape and position, except in *M. marialuisae*, that has two anterior and two posterior VAE (Valdez-Mondragón, 2010; figs 165, 167). PP regularly long, with different shape and position (Fig. 3 I).

Distribution: *Mictlanus* **gen. nov.** occurs mainly in Mexico, in Mesoamerican and Continental Nearctic biotic components (Morrone et al., 2017; Morrone, 2019; Valdez-Mondragón 2010, 2013, 2014) (Fig. 9). This genus is distributed from the south of the U.S.A. to south of Mexico. The most of the species are distributed mainly in part of the biogeographical provinces of Pacific Lowlands, Sierra Madre Oriental, Sierra Madre Occidental, Baja California, Chihuahuan Desert (Mexican Altiplano) and the Trans-Mexican Volcanic Belt. *Mictlanus dugesi* has a wide spread distribution along the Mexican territory by anthropochory (introduced species), ever associated to synanthropic habits. This is not a natural distribution pattern and they records are not showed in the distribution map. *Mictlanus enaulus*, *M. californicus* and *M. tanneri* occur in the south of the U.S.A. (Fig. 9). Different species have been recorded in the states of Baja California, Baja California Sur, Chihuahua, Coahuila, Colima, Durango, Estado de Mexico, Guanajuato, Guerrero, Hidalgo, Jalisco, Mexico City, Michoacan, Nayarit, Nuevo León, Puebla, Querétaro, San Luis Potosí, Sinaloa, Sonora, Tamaulipas and Tlaxcala (Fig. 9).

Natural history: The species have similar habitat that *Physocyclus*, being mainly in deciduous forest and arid ecosystems. However, *Mictlanus* **gen. nov.** is most diverse in the arid, dry, and semiarid regions, found species even close to oasis in deserts (Jiménez and Palacios-Cardiel, 2013). The microhabitat is the same than *Physocyclus*, but also, some species have been collected inside of dead cacti and agaves, and in road cuts.

***Mictlanus dugesi* (Simon, 1893) comb. nov**.

(Valdez-Mondragón, 2010; figs 22–28)

Type material: Mexico: *Guanajuato*: 1 ♂. Around 300 km to NW of Mexico City. AMNH, material revised.

New records: Mexico: *Estado de Mexico*: 1♀ (LATLAX Ara-0702) (06/08/2016). Tulipanes St., suburb Vista Hermosa, municipality of Ecatepec (lat: 19.6050°, long -99.07520°, 2320 m.a.s.l.). Cols. M. Cortez; R. Matías. *Guanajuato*: 3♀ (LATLAX Ara-0934) (14/10/2021). "Las letras", 3 km to N of Pénjamo, municipality of Pénjamo (lat: 20.45801°, long: -101.72265°, 2041 m.a.sl.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. *Hidalgo*: 1♂, 6♀, 6 juveniles (LATLAX Ara-0780) (20/11/2020). Country zip lines "Las Cascadas", municipality of Tula de Allende (lat: 20.03440°, long: -99.44326°, 2280 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. *Jalisco*: 3♂, 7♀, 15 juveniles (LATLAX Ara-0794) (08/11/2020). "Cueva del lince" inside of Ecotourist Park, "Bosque de la Primavera", municipality of Zapopan (lat: 20.71200°, long: -103.57000° 1605 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. *Morelos*: 2♂ (LATLAX Ara-0828) (24/11/2020). 1.5 km to W of Quilamula, municipality of Tlalquiltenango (lat: 18.51510°, long: -99.03210°, 1124 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. *Oaxaca*: 2♀, 1 juvenil (LATLAX Ara-0687) (06/07/2017). Recreative center "Piedra del agua", municipality of Villa de Tamazulapan (lat: 17.68956°, long -97.57685°, 1970 m.a.s.l.). Cols. A. Valdez; M. Cortez; A. Juárez; J. Valerdi. 1♀ (LATLAX Ara-0689) (08/07/2017). 1 km to S of archaeological zone "El Yagul", Municipality of Tlacolula de Morelos (lat: 16.9459°, long: -96.45016°, 1632 m.a.s.l.). Cols. A. Valdez; M. Cortez; A. Juárez; J. Valerdi. 2♀, 1 juvenil (LATLAX Ara-1016) (20/09/2022). 400 m to S of archaeological zone "El Yagul", Municipality of Tlacolula de Morelos (lat: 16.94544°, long: -96.45351°, 1640 m.a.s.l.). Cols. A. Valdez; S. Nolasco; A. Juárez. 3♂, 3♀, 12 juveniles (LATLAX Ara-1002) (16/09/2022). 3.5 km to SE of Chilapa de Díaz, municipality of Vila de Chilapa de Díaz (lat: 17.56232°, long: -97.61321°, 1832 m.a.s.l.). Cols. A. Valdez; S. Nolasco; A. Juárez. *Puebla*: 2♂, 2♀, 2 juveniles (LATLAX Ara-0686) (05/07/2017). Insurgentes St. Section San Juan, San Pablo Anciano, municipality of Acatlán de Osorio (lat: 18.44469°, long: -98.08665°, 1139 m.a.s.l.). Cols. A. Valdez; M. Cortez; A. Juárez; J. Valerdi. 1♀ (LATLA Ara-0683) (05/07/2017).

Road to Tepenene, municipality of Izúcar de Matamoros (lat: 18.49335°, long: -98.39623°, 1300 m.a.s.l.). Cols. A. Valdez; M. Cortez; A. Juárez; J. Valerdi. 1♂, 7♀, 8 juveniles (LATLAX Ara-0994) (14/09/2022). Botanic garden "Helia Bravo Hollis", Biosphere Reserve Tehuacán-Cuicatlán, municipality of Zapotitlán Salinas (lat: 18.33246°, long: -97.47779°, 1482 m.a.s.l.). Cols. A. Valdez; S. Nolasco; A. Juárez. *Tlaxcala*: 3♂, 3♀, 3 juveniles (LATLAX Ara-0762) (31/10/2019). Apatlahuaya section, Santa Elena St., municipality of Apizaco (lat: 19.39680°, long: -98.13559°, 2433 m.a.s.l.). Col. S. Nolasco. 3♀, 9 juveniles (LATLAX Ara-0711) (03/07/2018). Roberto Covarrubias St., Santa Cruz section, municipality of Huamantla (lat: 19.31710°, long: -97.92970°, 2524 m.a.s.l.). Cols. A. Valdez; I. Navarro; P. Solís; M. Cortez; A, Juárez; M. Islas. 1♂, 1 juvenil (LATLAX Ara-0712) (14/09/2016). Juárez Sur Av., Centro section, municipality of Huamantla (lat: 19.31002°, long: -97.92193°, 2511 m.a.s.l.). Cols. A. Valdez; I. Navarro; P. Solís; M. Cortez; A, Juárez; M. Islas. 2♂, 5♀, 3 juveniles (LATLAX Ara-0962) (14/09/2016). Nicolás Bravo St., Neighborhood of Xaxala, municipality of Santa Ana Chiautempan (lat: 19.30437°, long: -98.19154°, 2321 m.a.s.l.). Cols. A. Valdez; M. Cortez; A. Juárez; J. Valerdi. 2♂, 6♀, 5 juveniles (LATLAX Ara-0958) (17/06/2021). Santiago Tlacochcalco, municipality of Tepeyanco (lat: 19.269171°, long: -98.22333°, 2280 m.a.s.l.). Cols. A. Valdez, A. Juárez, I. Navarro, L. Cabrera, S. Nolasco. 10♂, 5♀, 3 juveniles (LATLAX Ara-0988) (10/10/2020). Ignacio Carranza Av., municipality of Tepetitla de Lardizabal (lat: 19.26627°, long: -98.38114°, 2230 m.a.s.l.). Col. S. Nolasco. 1♂ (LATLAX Ara-0665) (20/12/2016). Vista Hermosa Av., Centro section, municipality of Tlaxcala de Xicohténcatl (lat: 19.31380°, long: -98.23630°, 2230 m.a.s.l.). Col. A. Valdez. 6♂, 5♀, 3 juveniles (LATLAX Ara-0761) (03/2020). Cedros St., San Buenaventura Atempan, municipality of Tlaxcala de Xicohténcatl (lat: 19.32410°, long: -98.21670°, 2257 m.a.s.l.). Col. A. Valdez. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus brevicornus* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 133–139)

Type material: Mexico: *Colima*: 1 ♂ (CNAN-T0350) (22/01/2008), Recreative center "El Salto", municipality of Minatitlán (lat: 19.36878, long: -104.08535, 636 m.a.s.l.). Cols. A. Valdez; O. Francke: H. Montaño; N. Pérez. Paratypes: 1♀ (CNAN-T0351), 1♂ (CNAN-

T0352), 1♀ (CNAN-T0353), 1♂, 8♀, 5 juveniles (CNAN-T0354), same data as for holotype. Material revised.

New records: Mexico: *Colima*: 2♂, 1♀, 1 juvenile (LATLAX Ara-0756) (26/06/2019), Recreative center "El Salto", municipality of Minatitlán (lat: 19.36878°, long: -104.08535°, 650 m.a.s.l.). Cols. A. Valdez; I. Navarro; A. Cabrera; J. Flores. *Guanajuato*: 3♂, 3♀, 6 juveniles (LATLAX Ara-0933) (13/10/2021). 3.4 km to N of Hacienda de Arriba, municipality of León (lat: 21.2625°, long: -101.70123°, 2045 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 4♂, 10♀, 10 juveniles (LATLAX Ara-0935) (14/10/2021). "Las letras", 3 km to N of Pénjamo, municipality of Pénjamo (lat: 20.45801°, long: -101.72265°, 2041 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 1♀, 3 juveniles (LATLAX Ara-0937) (15/10/2021). 800 m to E of San Antonio Eménguaro, municipality of Salvatierra (lat: 20.14311°, long: -100.88402°, 1812 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. *Jalisco*: 7♂, 8♀, 8 juveniles (LATLAX Ara-0745) (21/06/2019). September 16th St., Camichines, municipality of Cocula (lat: 20.49920°, long: -103.80112°, 1272, m.a.s.l.). Cols. A. Valdez; I. Navarro; A. Cabrera; J. Flores. 3♂, 3♀, 5 juveniles (LATLAX Ara-0796) (08/11/2020). 3 km to SE of Hostotipaquillo, municipality of Hostotipaquillo (lat: 21.03140°, long: -104.06680°, 1336 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 2♀, 2 juveniles (LATLAX Ara-0798) (09/11/2020). 2.5 km to SE of Plan de Barrancas, municipality of Plan de Barrancas (lat: 21.02390°, long: -104.19070°, 915 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 1♂ (LATLAX Ara-0792) (06/11/2020). 6 km to E of Tonaya, municipality of Tonaya (lat: 19.75320°, long: -103.92640°, 905 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 1♀, 5 juveniles (LATLAX Ara-0793) (07/11/2021). 6 km to SW of Unión de Tula, municipality of Unión de Tula (lat: 19.91570°, long: -104.30890°, 1333 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 1♂, 1♀ (LATLAX Ara-0795) (08/11/2020). "Cueva del lince" inside of Ecotourist Park, "Bosque de la Primavera", municipality of Zapopan (lat: 20.71200°, long: -103.57000°, 1605 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 2♀, 2 juveniles (LATLAX Ara-0787) (05/11/2020). 4 km to S of Agua Bendita, municipality of Jilotlán de los Dolores (lat: 19.46340°, long: -102.51120°, 1406 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. *Michoacán*: 1♀ (LATLAX Ara-0790) (06/11/2020). 6 km to

E of Cotija de la Paz, municipality of Cotija (lat: 19.79150°, long: -102.64960°, 1673 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 3♂, 5♀, 13 juveniles (LATLAX Ara-0791) (06/11/2020). Road Luis G. Urbina, municipality of Cotija (lat: 19.80870°, long: -102.68840°, 1630). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. *San Luis Potosí:* 2♂, 9♀, 5 juveniles (LATLAX Ara-0928) (09/10/2021). 3 km to E of Lagunillas, municipality of Lagunillas (lat: 21.61119°, long: -99.55437°, 982 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus californicus* (Chamberlin and Gertsch, 1929) comb. nov.**

(Valdez-Mondragón, 2010; figs 8–14)

Type material: United States: *California*: 1 ♂ holotype. Redlands (lat 34.055567°, long -117.18167°). AMNH, material revised. Paratypes: 3 ♂, 4♀, same data as for holotype. See Valdez-Mondragón (2010) for complete diagnosis and description.

***Mictlanus cornutus* (Banks, 1898) comb. nov.**

(Valdez-Mondragón, 2010; figs 15–21)

Type material: Mexico: *Baja California Sur*: 1 ♂. Los Cabos. Without more data. AMNH, material revised. Paratypes: 1 ♂, 2♀, same data as for holotype.

New records: Mexico: *Baja California Sur:* 1♀ (LATLAX Ara-0645) (06/08/2019). 5 km to SW of San Antonio de la Sierra, municipality of La Paz (lat: 23.73211°, long: -110.02494°, 557 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 2♀, 2 juveniles (LATLAX Ara-0642) (03/08/2019). 5 km to N of Santa Anita, municipality of Los Cabos (lat: 23.22072°, long: -109.72770°, 162 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 1♀ (LATLAX Ara-0643) (04/08/2019). Ejido Boca de la Sierra, 5.5 km to NW of Miraflores, municipality of Los Cabos (lat: 23.37591°, long: -109.81299°, 293 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus darwini* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 176–182)

Type material: Mexico: *Guerrero*: 1♂ (CNAN-T0406) (15/01/2007). Plan de Liebres, municipality of Zumpango (lat: 17.75013°, long: -99.55874°, 768 m.a.s.l.). Cols. O. Francke; J. Ponce; H. Montaño; M. Córdova; A. Ballesteros. Paratypes: 1♀ (CNAN-T0407), 7 ♂ (CNAN-T0408), 5♀ (CNAN-T0409), 17 juveniles (CNAN-T0410), same data as for holotype. Material revised.

New records: Mexico: *Guerrero:* 8♂, 2♀, 2 juveniles (LATLAX Ara-0824) (23/11/2020). Plan de Liebres, municipality of Zumpango (lat: 17.75050°, long: -99.55951°, 778 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus enaulus* (Crosby, 1926) comb. nov.**

(Valdez-Mondragón, 2010; figs 33–41)

Type material: United States: *Nuevo Mexico*: 1♀. Carlsbad Cave (lat 32.4205°, long -104.22833°). AMNH, material revised. Paratypes: 1♀, same data as for holotype.

New records: Mexico: *Coahuila*: 1♂, 1♀, 1 juvenile (LATLAX Ara-0733) (12/10/2019). Km 37 in federal highway 40, 3 km to NW of tollbooth "Plan de Ayala" (lat: 25.46396°, long: -101.31840°, 1831 m.a.s.l.). Cols. A. Valdez; B. Huber; L. Cabrera. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus franckei* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 119–125)

Type material: Mexico: *Hidalgo*: 1♂ (CNAN-T0334) (27/04/2006). Jiliapa, municipality of Metztitlán (lat: 20.546383°, long: -98.71475°, 1293 m.a.s.l.). Cols. A. Valdez; O. Francke: R. Paredes; G. Villegas. Paratypes: 1♀ (CNAN-T0335), 2♂, 1♀, 2 juveniles (CNAN-T0336), same data as for holotype. Material revised.

New records: Mexico: *Hidalgo*: 1♂, 4♀, 2 juveniles (LATLAX Ara-0651) (24/01/2019). Grutas de Tolantongo, road to "El Paraíso", municipality of Cardonal (lat: 20.65028°, long: -99.00029°, 1312 m.a.s.l.). Cols. A. Valdez; P. Solís; M. Cortez; D. Montiel; J. Flores. 5♂, 4♀, 2 juveniles (LATLAX Ara-0653) (22/05/2018). Grutas de Tolantongo, municipality of Cardonal (lat: 20.65039°, long: -99.00047°, 1315 m.a.s.l.). Cols. A. Valdez; P. Solís; I. Navarro; J. Valerdi; L. Cabrera. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus hoogstraali* (Gertsch and Davis, 1942) comb. nov.**

(Valdez-Mondragón, 2010; figs 56–62)

Type material: Mexico: *Nuevo León:* 1♀ (14/06/1940). "Cueva del diablo" (Bat Cave), Sabinas Hidalgo (lat: 26.5°, long -100.16667°). AMNH, material revised. Paratypes: 2♀, 1♀ subadult, 1♂ subadult, same data as for holotype.

New records: Mexico: *Nuevo León:* 1♀, 4 juveniles (LATLAX Ara-0705) (30/09/2005). "Cueva de La Perra", municipality of Aramberri. Col. C. Kennedy. 1♀ (LATLAX Ara-0713) (17/11/2015). Santa Catarina, municipality of Santa Catarina. Col. D. Barrales. 5♂, 7♀, 1 juvenile (LATLAX Ara-0735) (15/10/2019). "Cueva la boca" ("Cueva de los murciélagos"), municipality of Santiago (lat: 25.43360°, long: -100.11390°, 409 m.a.s.l.). Cols. A. Valdez; B. Huber; L. Cabrera. 1♂, 5♀, 5 juveniles (LATLAX Ara-0734) (14/10/2019). Grutas de San Bartolo (lat: 25.58095°, long: -100.44714°). Cols. A. Valdez; B. Huber; L. Cabrera. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus lyncis* (Nolasco and Valdez-Mondragón, 2022) comb. nov.**

(Nolasco and Valdez-Mondragón, 2022a; figs 58–63)

Type material: Mexico: *Jalisco*: 1♀ (CNAN-T01476) (08/11/2020). "Cueva del Lince" inside of Ecotourist Park "Bosque de La Primavera", Municipality of Zapopan (lat: 20.6892°, long: -103.5820°, 1605 m.a.s.l.). Cols. A. Valdez; I. Navarro; A. Juárez; S. Nolasco. Paratypes: 1♀ (CNAN-T01477) same data as for holotype. 2♀ (CNAN-T01478) (30/03/2012). Ecotourist Park "Bosque de La Primavera" (lat: 20.6892°, long: -103.5820°, 1645 m.a.s.l.) Cols. L. Olguín; J. Mendoza; G. Contreras; C. Santibañez; D. Ortiz. Material

revised. See Nolasco and Valdez-Mondragón (2022a) for complete diagnosis and description.

***Mictlanus marialuisae* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 161–167)

Type material: Mexico: *Baja California Sur:* 1♂, (CIBNOR-CARCIB 0006) (21/04/1988). Sierra de "La Laguna", municipality of La Ventana. Material revised. Paratypes: 1♀, same data as for holotype. See Valdez-Mondragón (2010) for complete diagnosis and description.

***Mictlanus merus* (Gertsch, 1971) comb. nov.**

(Valdez-Mondragón, 2010; figs 70–76)

Type material: Mexico: *San Luis Potosí:* 1♂ (03/08/1966). Sumidero de Matehuala, 3 km to E of Matehuala (lat: 23.65°, long -100.65°). AMNH, material revised. Paratypes: 1♂, 2♀, same data as for holotype.

New records: Mexico: *San Luis Potosí:* 10♂, 10♀. 5 juveniles (LATLAX Ara-0931) (12/10/2021). 1 km to N of Pueblo Rodrigo, Highway San Luis Potosí – Villa de Arteaga, muncipality of Villa de Reyes (lat: 21.89928°, long: -100.96056°, 1890 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus mexicanus* (Banks, 1898) comb. nov.**

Type material: Mexico: *Nayarit:* 1♀. CAS, material no examined. Without more data.

***Mictlanus michoacanus* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 126–132)

Type material: Mexico: *Michoacán:* 1♂ (CNAN-T0338) (23/11/1985). Arroyo Frío, 3 km of Pedernales, municipality of Tacámbaro (lat: 19.164444°, long -101.46306°, 1200 m.a.s.l.). Paratypes: 1♂ (CNAN-T0340) (26/10/1985), same data as for holotype. 1♀ (CNAN-T0339), 1♂ (CNAN-T0341), the rest of the data as the same as for holotype. Material revised.

New records: Mexico: *Michoacán*: 1♀ (LATLAX Ara-0786) (05/11/2020). Agua Bendita, municipality of Jilotlán de los Dolores (lat: 19.46890°, long: -102.54600°, 1163 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. 3♂, 2 juveniles (LATLAX Ara-0781) (03/11/2020). 2 km to W of Tzitzio, municipality of Tzitzio (lat: 19.57200°, long: -100.92350°, 1466 m.a.s.l.). Cols. A. Valdez; I. Navarro. A. Juárez. S. Nolasco. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus mysticus* (Chamberlin. 1924) comb. nov.**

(Valdez-Mondragón, 2010; figs 84–90)

Type material: Mexico: *Baja Caliornia Sur:* 1♂ (22/06/1921). Isla Tortuga, Gulf of California. Paratypes: 1♂ (10/05/1921). San Francisquito Bay. 1♀ (14/06/1921). Puerto Escondido (lat: 31.7225°, long: -116.69611°). 1♀ (09/06/1921). Isla Ballena. CAS, material no examined.

New records: Mexico: *Baja Caliornia:* 1♂, 11♀ (LATLAX Ara-0632) (23/07/2019). Beach "Punta San Francisquito, San Francisquito Bay, municipality of Ensenada (lat: 28.40898°, long: -11285815°, 4 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 1♂ (LATLAX Ara-0633) (26/07/2019). 63 km to SW of San Francisquito Bay, municipality of Ensenada (lat: 28.13715°, long: -113.30584°, 473 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 1♀, 1 juvenile (LATLAX Ara-0634) (26/07/2019). 3.5 km to N of Río Grande, municipality of Ensenada (lat: 28.27974°, long: -113.79730°, 128 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. *Baja Caliornia Sur:* 1♂ (LATLAX Ara-0640) (31/07/2019). Village Oasis "El Pilar", municipality of La Paz (lat: 24.46951°, long: -111.00297°, 117 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 1♂ (LATLAX Ara-0631) (27/07/2019). 3 km to E of Palo Verde, municipality of Mulegé (lat: 27.02665°, long: -112.06029°, 15 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 2♀ (LATLAX, Ara-0635) (27/07/2019). 28 km to E of Bahía Tortugas, municipality of Mulegé (lat: 27.66356°, long: -114.66098°, 256 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 1♀. 1 juvenile (LATLAX Ara-0636) (27/07/2019). 1 km to SE of Marasal, municipality of Mulegé (lat: 27.49621°, long: -113.30904°, 76 m.a.s.l.). Cols. A. Valdez; P.

Solis; D. Montiel; A. Cabrera. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus palmarus* (Jiménez and Palacios-Cardiel, 2013) comb. nov.**

(Jiménez and Palacios-Cardiel, 2013; figs 4–13)

Type material: Mexico: *Baja California Sur*: 1♂ (CARCIB 013) (04/03/2013). San Pedro Oasis, municipality of La Paz (lat: 23.2322°, long: -110.1230°, 6 m.a.s.l.). Cols. C. Palacios; M. Jiménez. 1♀ (CARCIB 086), same data as for holotype. Material revised. See Jiménez and Palacios Cardiel (2013) for complete diagnosis and description.

***Mictlanus pedregosus* (Gertsch, 1971) comb. nov.**

(Valdez-Mondragón, 2010; figs 91–97)

Type material: Mexico: *Coahuila:* 1♂ (30/12/1967). Cueva Circular Pedregosa, 32km to No of Cuatro Ciénegas (lat: 26.74995°, long: -101.87356°). Deposited in AMNH, material revised. Paratypes: 5♀, same data as for holotype. See Valdez-Mondragón (2010) for complete diagnosis and description.

***Mictlanus peribaniensis* (Valdez-Mondragón, 2014) comb. nov.**

(Valdez-Mondragón, 2014; figs 1–17)

Type material: Mexico: *Michoacán*: 1♂ (CNAN-T0823) (01/06/2010). 8.4 km on the road Copetir-Jalpa, municipality of Peribán (lat: 19.4677°, long: -102.5308°, 1277 m.a.s.l.). Cols. J. Ponce; A. Quijano; V. Guzmán. 1♀ (CNAN-T0824), same data as for holotype. Material revised. See Valdez-Mondragón (2014) for complete diagnosis and description.

***Mictlanus platnicki* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 197–203)

Type material: Mexico: *Sinaloa:* 1♂ (22/07/1954). 9.6 km to E of Culiacán, municipality of Culiacán (lat 24.8°, long: -107.48333°). AMNH, material revised. Paratypes: 1♂, 5♀, 3 juveniles, same data as for holotype. See Valdez-Mondragón (2010) for complete diagnosis and description.

***Mictlanus reddelli* (Gertsch, 1971) comb. nov.**

(Valdez-Mondragón, 2010; figs 98–104)

Type material: Mexico: *Hidalgo:* 1♂ (19/08/1965). 5.6 km to N of Lagunillas, Grutas de Xoxafí, municipality of Santiago de Anaya (lat: 20.40945°, lon: -99.020283°). AMNH, material revised. Paratypes: 1♂, 1♀, 5 juveniles, same data as for holotype.

New records: Mexico: *Guanajuato:* 9♂, 3♀, 3 juveniles (LATLAX Ara-0938) (16/10/2021). Cerro "La Morita", 3 km to N of Comonfort, municipality of Comonfort (lat: 20.74153°, long: -100.7368°, 1812 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 2♂, 1♀, 4 juveniles (LATLAX Ara-0936) (14/10/2021). 7.5 km to SE of Cortazar, municipality of Cortazar (lat: 20.4253°, long: -100.91446°, 2160 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 4♂, 3♀, 1 juvenile (LATLAX Ara-0932) (12/10/2021). Outside of Cueva "La Lonja", 11 km to E of San Felipe, municipality of San Felipe (lat: 21.46916°, long: -101.10182°, 2146 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 3♂, 7♀, 7 juveniles (LATLAX Ara-0939) (16/10/2021). 200 m of San Luis Rey section, 3 km to N of San Miguel de Allende, municipality of San Miguel de Allende (lat: 20.93802°, long: -100.73125°, 1997 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 1♂, 2♀, 1 juvenile (LATLAX Ara-0940) (16/10/2021). 1 km to NE of Las Cañas, municipality of San Miguel de Allende (lat: 21.06334°, long: -100.72108°, 2003). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 1♂, 2♀, 6 juveniles (LATLAX Ara-0909) (06/10/2021). 6 km to NW of Santa Catarina, minucipality of Santa Catarina (lat: 21.13297°, long: -100.12818°, 1712 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. *Hidalgo*: 1♀, 1 juvenile (LATLAX Ara-0681) (23/05/2018). Grutas de Tolantongo, municipality of Cardonal (lat: 20.65039°, long: -99.00047°, 1315 m.a.s.l.). Cols. A. Valdez; P. Solís; I. Navarro; J. Valerdi; L. Cabrera. 1♀, 3 juveniles (LATLAX Ara-0738) (22/10/2019). Grutas de Xoxafí, municipality of Santiago de Anaya (lat: 20.38960°, long: -99.02720°, 2003 m.a.s.l.). Cols. A. Valdez; B. Huber; L. Cabrera. 1♂, 4♀ (LATLAX Ara-0663) (25/01/2019). Inside of Grutas de Xoxafí, municipality of Santiago de Anaya (lat: 20.38937°, long: -99.02693°, 2008 m.a.s.l.). Cols. A. Valdez; P. Solís; M. Cortez; D. Montiel; J. Sánchez. 1♂ (LATLAX Ara-0701) (19/03/2017). 8 km to S of Zimapán, municipality of Zimapán (lat: 20.70957°, long: -99.34367°, 1828 m.a.s.l.). Cols. A. Valdez; E. Briones; A. Juárez; J. Valerdi; M. Sánchez.

Querétaro: 1♀, 1 juvenile (LATLAX Ara-0906) (05/10/2021). 1.4 km to S of Tepozán, municipality of Arroyo Seco (lat: 20.88261°, long: -99.65736°, 2443 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 2♂, 3♀, 9 juveniles (LATLAX Ara-0925) (08/10/2021). 2.5 km to SE of Puerto Ayutla, municipality of Arroyo Seco (lat: 21.35724°, long: -99.5892°, 997 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 4♂, 6♀, 6 juveniles (LATLAX Ara-0927) (08/10/2021). Camping Resort "Las Brisas", municipality of Arroyo Seco (lat: 21.39403°, long: -99.58328°, 519 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 1♀ (LATLAX Ara-0903) (04/10/2021). 8 km to N of Cadereyta, municipality of Cadereyta de Montes (lat: 20.76602°, long: -99.9728°, 2142 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 2♀ (LATLAX Ara-0904) (04/10/2021). 7 km to E of Acueducto II, Water Treatment Plant "Las Tuzas", municipality of Cadereyta de Montes (lat: 20.73266°, long: -99.63705°, 2020 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 1♀, 5 juveniles (LATLAX Ara-0908) (06/10/2021). 3.5 km to E of Peñamiller, Antenna RMO Motoshi, municipality of Peñamiller (lat: 21.0562°, long: -99.7481°, 1448). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 1♀, 2 juveniles (LATLAX Ara-0921) (07/10/2021). 2 km to N of Health Center "Los Encinos", municipality of Peñamiller (lat: 21.15348°, long: -99.75838°, 1829 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 3♀, 2 juveniles (LATLAX Ara-0922) (07/10/2021). 2 km to N of Río Blanco, municipality of Peñamiller (lat: 21.22292°, long: -99.74277°, 1560). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 8♂, 8♀, 6 juveniles (LATLAX Ara-0924) (07/10/2021). Camping Resort "Las Trancas", municipality of Pinal de Amoles (lat: 21.09062°, long: -99.61317°, 1648 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 3♂, 4 juveniles (LATLAX Ara-0943) (18/10/2021). 8 km to SE of Industrial Park "Querétaro", municipality of Querétaro (lat: 20.79506°, long: -100.38002°, 2055 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 12♂, 8♀, 15 juveniles (LATLAX Ara-0905) (05/10/2021). 2.5 km to W of CALCIMEXICANA S.A de C.V. (lat: 20.84991°, long: -99.73593°, 1887 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. 6♂, 6♀, 7 juveniles (LATLAX Ara-0907) (05/10/2021). 3 km to W of La Culata, municipality of Vizarrón de Montes (lat: 20.905°, long: -99.72617°, 1680 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. *San Luis Potosí:* 3♀ (LATLAX Ara-0930) (10/10/2021). 10 km to NW of Mojarras de Arriba, municipality of Ciudad Fernández (lat: 22.12056°, long: -

100.33778°, 1213 m.a.s.l.). 11♂, 11♀, 2 juveniles (LATLAX Ara-0929) (10/10/2021). 15 km to S of Río Verde, municipality of Río Verde (lat: 21.80461°, long: -99.95197°, 972 m.a.s.l.). Cols. A. Valdez; A. Juárez; L. Cabrera; S. Nolasco. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus rothi* (Valdez-Mondragón, 2010) comb. nov.**

(Valdez-Mondragón, 2010; figs 183–189)

Holotype: Mexico: *Baja California Sur:* 1♂ (19/04/1985). 32.2 km to N of La Paz, municipality of La Paz. AMNH, material. Paratypes: 1♀, same data as for holotype.

New records: Mexico: *Baja California Sur:* 1♀ (LATLAX Ara-0639) (31/07/2019). 22 km road to Comundú, municipality of Comundú (lat: 24.61199°, long: -111.70070°, 19 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 2♀ (LATLAX Ara-0641) (02/08/2019). 1 km to NE of El Pescadero (lat: 23.39453°, long: -110.09918°, 206 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 2♀ (LATLAX Ara-0638) (29/07/2019). Resort "La Purísima", municipality of Comundú (lat: 26.18978°, long: -112.07323°, 103 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 3♂, 9♀, 5 juveniles (LATLAX Ara-0649) (30/07/2019). Around to the Oasis of Misión San Luis Gonzaga Chiriyaqui, municipality of Comundú (lat: 24.90943°, long: -111.28940°, 155 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. 2♂, 3♀, 1 juvenile (LATLAX Ara-0650) (31/07/2019). Village Oasis "El Pilar", municipality of La Paz (lat: 24.46951°, long: -111.00297° 117 m.a.s.l.). Cols. A. Valdez; P. Solís; D. Montiel; A. Cabrera. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

***Mictlanus sprousei* (Valdez-Mondragón, 2010) comb. nov**.

(Valdez-Mondragón, 2010; figs 168–175)

Type material: Mexico: *Chihuahua*: 1♂ (CNAN-T0402) (11/06/2008). Jiménez, muncipality of El Hundido (lat: 27.08886°, long: -103.98221°). Paratypes: 1♀ (CNAN-T0403), same data as for holotype. Material revised See Valdez-Mondragón (2010) for complete diagnosis and description.

***Mictlanus tanneri* (Chamberlin, 1921) comb. nov.**

(Valdez-Mondragón, 2010; figs 105–111)

Type material: Unites States: *Utah:* 1♂. St. George (lat: 37.095277°, long -113.57833°). Material no examined, it is not known where is deposited the material type, but Huber (2010) mention that probably is deposited in MCZ.

New records: Mexico: *Sonora:* 1♀, 7 juveniles (LATLAX Ara-1033). Cuitaca, municipality of Cananea (lat: 31.00093°, long: -110.29263°, 1255 m.a.s.l.). Cols. D. Barrales. 1♀, 7 juveniles (LATLAX Ara-0708) (27/02/2006). Cueva del Tigre, municipality of Carbó (483 m.a.s.l.). Cols. J. Krejca; P. Sprouse. See Valdez-Mondragón (2010) for complete diagnosis, description and list of localities.

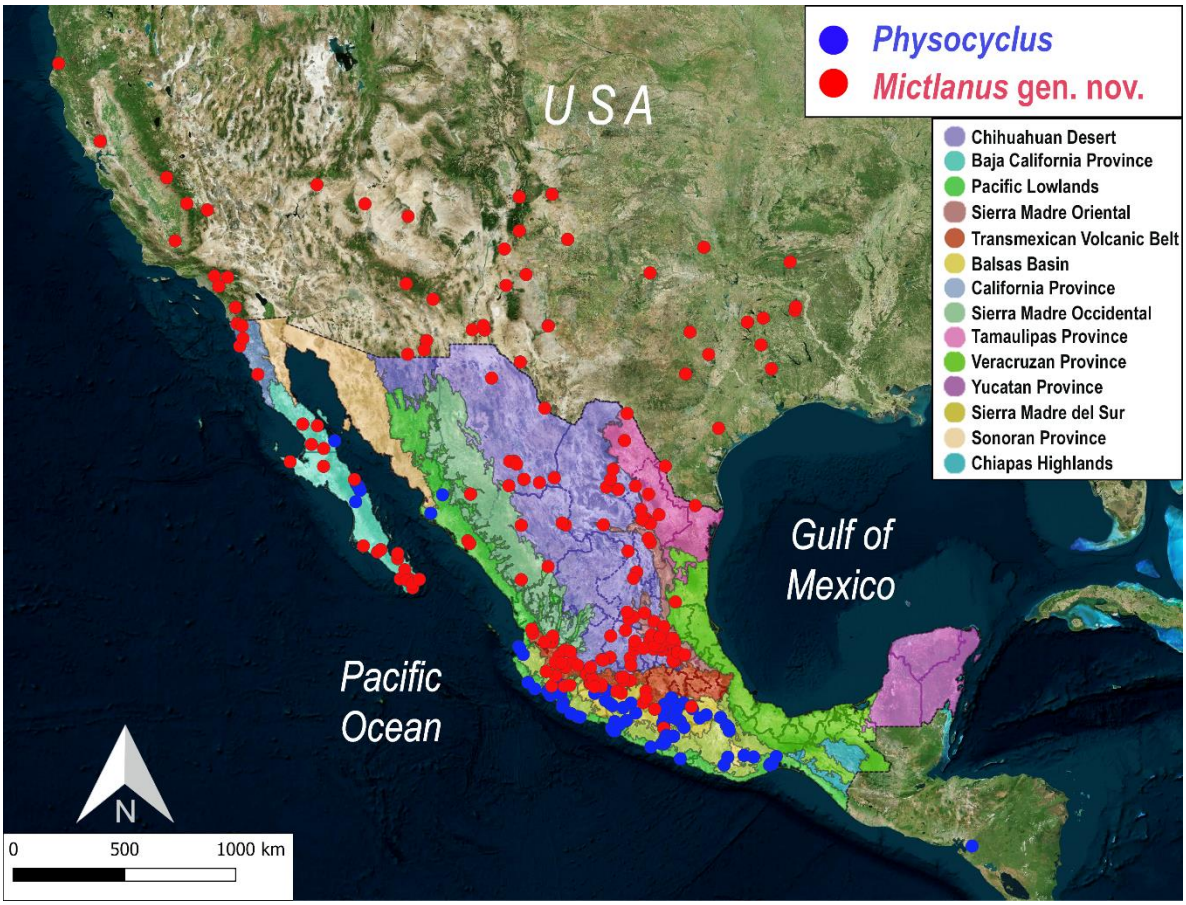

Figure 9. Natural distribution patterns and records for *Physocyclus* and *Mictlanus* gen. nov. Colors in the map represent the biogeographical provinces.

7. Discussion

7.1 Phylogenetic analyses

In modern systematics, to corroborate a phylogenetic hypothesis is necessary to use different data sets of different sources, analyzed independently and/or in combination (Kluge, 1989; Valdez-Mondragón and Francke, 2015). Although we are in the genomic era, the morphology still being a robust, easy, and cheap source of evidence for phylogenetic analyses nowadays. For this reason, the use of morphological evidence altogether with molecular data, substantially improves the relationships among the species of a phylogeny, mainly under a biogeographic context. This has been previously carried out in the spider family Pholcidae, where Bruvo-Madaric et al. (2005), using combined evidence (morphology and molecular) provided a better resolution in the phylogenetic relationships into the family. At genus level with North American pholcids, a similar situation has been found with the genus *Ixchela* where the internal phylogenetic relationships based only with morphological data were dubious (Valdez-Mondragón and Francke, 2015). However, in the phylogenetic analyzes conducted based on combined evidence (morphology and molecular), the internal phylogenetic relationships were clear and totally resolved (Valdez-Mondragón and Francke, 2015).

In pholcid spiders, the overall morphology remains with few changes, nevertheless, structures such as primary (male palps and female epigyna) and secondary (male chelicerae) sexual features, provides robust information to identify and diagnosis at genus and species level (Huber 2003; Huber et al. 2018; Nolasco and Valdez-Mondragón, 2022b). The sexual traits are important features used for identification due to the fact that genitalia evolve more rapidly than non-genital morphological features (Huber 2003; Huber et al. 2018; Valdez-Mondragón 2020). In previous phylogenetic analyses of the genus *Physocyclus* by Valdez-Mondragón (2013, 2014) based on morphological characters, although the internal phylogenetic relationships were not clear, the monophyly of the species groups *globosus* (*Physocyclus*) and *dugesi* (*Mictlanus* gen. nov.) was recovered. However, the phylogenetic relationships into each species groups were unclear and not resolved completely. According with our phylogenetical morphological analysis, the somatic characters between both genera are quite similar, however, the synapomorphies that support them are associated to the primary and sexual traits in males and females. These synapomorphies support the

monophyly of both genera and facilitates their identification and diagnosis as different genera. In *Mictlanus* gen. nov., the presence and the number (>30) of sclerotized cones in frontal lamina of males, is a synapomorphy associated to a secondary sexual trait, and it is present in all species, except in *M. platnicki*. According with the morphological phylogeny, *M. platnicki* is the sister species of the rest of species of the genus *Mictlanus* gen. nov. and their absence of sclerotized cones is considered a reversion to ancestral state. In *Physocyclus*, despite two species (*P. globosus* and *P. guanacaste*) present sclerotized cones in frontal lamina of males, always are less than five cones and allow them to be differentiated from *Mictlanus* gen. nov. In the rest of species of *Physocyclus*, the sclerotized cones in frontal lamina are absent. For *Physocyclus*, have been described different morphotypes for the epigyna of females of *P. enaulus* (Valdez-Mondragón, 2010). However, despite this morphological variation in some species, the females of *Physocylus* can be distinguish from the females of *Mictlanus* gen. nov. by the shape and size of the VAE, of the epigynum and of lateral constrictions of epigynum. Also, the general shape of the male embolus in the new genus is quite different from those in *Physocyclus*, being usually markedly quitinized and with sclerites on the bulb, whereas the embolus in the species of *Physocyclus* is otherwise and having sclerites on the embolic but never on the bulb (Figs. 2 D, E; 3 D, E).

Generally, the concept of species group refers to a work category or practical use, and not to a formal and natural classification (Dubois, 2007; Laurin, 2010). However, the species groups are commonly composed by closely related species, which belong to the same evolutionary lineage and share many morphological traits, ecological functions or distribution patterns. Despite this, the species groups tend to have low support in phylogenetic analyses, which makes it difficult to raise them to a higher and formal taxonomical category (e.g., genus, subgenus) (Laurin, 2010; Huber, 2011b; Huber, 2014; Zhao et al. 2023). Although the use of species groups is commonly used nowadays in the taxonomy for those groups where the taxonomic position or phylogenetic relationships are uncertain, also provide the idea that those groups could have different evolutionary pattern and even to correspond to new lineages or genera (Huber et al., 2014).

In other pholcids, Huber et al. (2016) have been mentioned the existence of species groups into the genus *Pholcus* Wlackenaer, 1805, with a very particular morphology. However, in some cases, due to a lack of robust phylogenetic analyses, these species groups

have not been proposed or elevated to genus rank. The genus *Leptopholcus* Simon, 1893 was composed by the species group of the Caribbean and South America (New World), and the African species group (Old World). However, after of a combined analysis using morphological and molecular data, the species groups from the Americas were transferred to the genus *Micropholcus* Deeleman-Reinhold and Prinsen, 1987 by Huber et al. (2014). The authors mention that due to their similar morphology of these groups, the inclusion of the molecular data (combined analyzes) was the main way to resolve their phylogenetic position and posterior classification. In our study, the phylogenetic analyses using morphology, molecular data, and combined evidence (morphology and molecular) showed that both species groups were also monophyletic with significative branch support, particular morphological characters, different biogeographic and evolutionary history, and can be recognized as independent lineages, rising both groups at genus level.

7.2 Molecular dating and reconstruction of ancestral areas

The lineage dating is the act to know the estimated age of a divergence event in a clade under molecular evidence. It is necessary the use of a molecular clock, to assume that molecular evolution rate is constant through time, based in the accumulation of molecular changes, that corresponds with mutations (Zuckerland and Pauling, 1965; Bromham and Woolfit, 2004). Using this molecular clock, it is possible to measure time in arbitrary units and then calibrate it using the fossil record or geological events, to known the estimated minimum age of a lineage (Sanderson, 1998; Benton and Donoghue, 2007). These estimated ages can help to explain if a specific observed pattern is supported by the hypothesis of processes like dispersal events or vicariance (Morrone and Escalante, 2018).

According with the lineage dating, Dimitrov et al. (2013) suggest that the genus *Physocyclus,* has approximately 80 million of years ago (Mya), however, in that phylogenetic study of the family Pholcidae, only two species of *Physocyclus* were included, therefore this approximation can be overestimated. Contrasting with the lineage dating realized herein, *Physocyclus* and *Mictlanus* gen. nov. appear during the Miocene, characterized by the high volcanic activity in the central region of Mexico (Mastretta-Yanes et al., 2015). In the Miocene rise the Trans-Mexican Volcanic Belt (TMV), which is part of the Mexican Transition Zone (MTZ), resulting from the overlapping of the Neotropical and Neartic

regions (Morrone, 2014, 2023). In this complex transition zone, the interaction of mountains and different climatic factors produces high environmental heterogeneity, resulting in cradles of biodiversity, refugia, bridges and barriers for dispersal of species (Morrone, 2023). The MTZ was the result from different orogenic events and climatic oscillation in the past, is a high-biodiversity region, with biota of Neotropical and Neartic regions, and cenocrons associated to specific elevation and types of vegetation (Brooks, 2005; Morrone, 2004, Morrone, 2023). The TMV is the main mountain chain complex that drove the diversification of *Physocyclus* and *Mictlanus* gen. nov. by many vicariance events mainly. This diversification pattern has been observed in different endemic lizards and snakes of the genus *Barisia* Gray, 1838 and *Crotalus* L. respectively, where the Neogene formation of the TMV appears responsible for structuring geographic diversity among major lineages (Bryson and Riddle, 2011a; Bryson et al., 2011b). In arthropods, such as endemic grasshoppers of the genus *Sphenarium* Charpentier (Orthoptera), the divergence times suggest late Miocene to Pliocene, for origin and diversification in the genus, influenced by the formation of the TMV (Pedraza-Lara et al., 2015). In pholcids spiders such as the genus *Ixchela*, the divergence times are main during the Late Pliocene and Pleistocene strongly influenced by the TMV and the glacial/interglacial cycles (Valdez-Mondragón and Francke, 2015).

The genera *Physocyclus* and *Mictlanus* gen. nov. have important diversification events during the Middle Miocene to Pliocene (14.8 – 5 Mya), because in this period appear the most of speciation events. The divergence ages for these groups are congruent with the formation of the TMV. The rise of this mountain chain promoted different orographic changes, that function like a biogeographic barrier, segregating the populations, that promoted the subsequent cladogenesis through Pleistocene glaciations and the glacial and interglacial periods (Zarza, et al., 2008; Pedraza-Lara et al., 2015). In other taxa with similar distribution, such as grasshoppers (Pedraza-Lara et al., 2015), rattlesnakes (Bryson et al., 2011) toads (Mulcahy and Mendelson III, 2000) or iguanas (Zarza et al., 2008) the diversification pattern is similar, influenced by the geographic barriers caused by the rise of the TMV, the formation of sky islands, that are high and isolated regions in the mountains (Dodge, 1943), and the interglacial periods during Pleistocene. Also, in *Physocyclus* and *Mictlanus* gen. nov. the diversification process might had given in the low altitudes (because does not habit in altitudes higher than 1900 m.a.s.l.), like the coasts, deserts or tropical

deciduous forest (main habitat). This diversification could be promoted by the population segregation caused by different barriers, such as habitat fragmentation, the rise of mountains, the formation of rivers and the apparition of the different ecosystems during the interglacial periods.

Following the reconstruction of ancestral areas, the TMV is one of the most important biogeographical regions that drove for the diversification in both genera, *Physocyclus* and *Mictlanus* gen. nov. (Fig. 9). This mountain chain functions like a biogeographical barrier in the vicariance process, due to the times of divergence and formation are similar, approximately in 18–15 mya (Ferrari et al., 2012; Mastretta-Yanes et al., 2015). However, the speciation by dispersal played an important role in the diversification process of both genera. Generally, the species of both genera have a low rate of dispersal, the main dispersal mechanism is by walking and not by *ballooning* like other spiders which use to have wide distribution patterns. For both genera, primary divergence events were caused by vicariance process, during the Middle Miocene and Pliocene (19–5 Mya), influenced by geographic barriers associated to the rise of the TMV. Posteriorly, dispersal events might drove the diversification for both genera, through different types of ecosystems such as tropical deciduous forests, deserts, and xerophytic scrubs during the Pliocene and Pleistocene (5–0.5 Mya). In other groups of vertebrates and arthropods, it has been observed that diversification patterns and speciation caused by dispersal of populations using different types of ecosystems, posterior to events of allopatric speciation caused by large vicariance events (Bryson et al. 2011; Schramm et al. 2021).

Other important biogeographical regions that drove the diversification of both genera were the Pacific Lowlands and the province of Baja California, where are distributed many species of both genera. The distribution of *Physocyclus* is restricted by the TMV, to the Nearctic region of the country (Valdez-Mondragón, 2013, 2014; Morrone, 2019; Nolasco and Valdez-Mondragón, 2022a), however, some species are distributed also in the Baja California Province. The divergence times of the species from the Baja California Peninsula correspond to separation of this region from the mainland, and subsequent incursion of Gulf of California, hypothesized in 12-5 Mya across multiple marine incursions (McDougall et al., 1999; Oskin and Stock, 2003; Crews and Hedin, 2005). These species were probably distributed in that region before the Peninsula separated from the mainland. The distribution

pattern of *Physocyclus*, continues along the Pacific Lowlands, important biogeographical province that drove the diversification of the genus during the Middle-Late Miocene, probably by fragmentation of populations, caused by the apparition of new habitats.

Finally, the Balsas Basin is the most diverse province in species number of *Physocyclus* and *Mictlanus* gen. nov., although our results suggest that this region did not drove significatively the diversification of both genera. However, the high diversity of the region may be explained because is located between important chain mountains (Trans-Mexican Volcanic Belt and the Sierra Madre del Sur), and surrounded by three intersect points that can be considered as panbiogeographic nodes, which are areas with high diversity due to contact between different biotic elements (Craw et al., 1999; Morrone and Márquez, 2008).

8. Conclusion

The present study addressed the evolutionary history and diversification of two North American genera of spiders of the Arteminae subfamily: *Physocyclus* and *Mictlanus* gen. nov. The results showed that morphological and molecular evidence are robust to explain the evolutionary and diversification history of both genera. The diversification may primarily be a product of several vicariance events and the possible appearance and disappearance of barriers along the complex landscape. The complex history of major barriers such as the TMV drove the diversification, but also the dispersal capacity and ecological features may be important. We conclude that both species groups (*globosus* and *dugesi*) are two different genera, due to the following evidence: 1) diagnostic morphological features are different in each genera and makes it possible to differentiate one from to each other; 2) both genera are monophyletic having significant support values in the different analyses under different source of evidence (morphological, molecular and combined evidence); 3) different distribution pattern for each genus (Nolasco and Valdez-Mondragón, 2022; Valdez-Mondragón, 2013, 2014), being the Mexican Transition Zone where the distribution pattern of both genera is interrupted (Fig. 9); 4) both genera present similar evolutionary divergence times, but being lineages that evolve independently, and, 5) different biogeographical provinces and ancestral areas explain the diversification patterns for each genus.

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Appendix. Morphological matrix used in the phylogenetic analysis for *Physocyclus* and *Mictlanus* gen. nov.

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Priscula binghamae	1	1	0	0	0	0	0	0	-	0	0	0	0	0	0	0	-	-	0	0	0
Artema atlanta	1	0	0	0	0	0	0	0	-	3	0	2	0	0	0	1	3	3	0	0	0
Trichocyclus nigropunctatus	0	1	0	0	0	0	0	0	-	5	0	0	0	0	0	0	-	-	1	1	0
Trichocyclus nullarbor	0	1	0	0	0	0	0	0	-	5	0	0	0	0	0	0	-	-	1	1	0
P. huacana	1	1	1	1	0	1	1	1	0	2	0	1	0	0	1	1	1	2	0	1	0
P. montanoi	1	1	1	1	0	0	1	1	0	2	0	1	0	0	1	1	2	2	0	1	1
P. modestus	1	1	0	0	0	0	1	1	0	2	1	1	0	0	1	1	2	2	0	1	1
P. sarae	1	1	0	0	0	0	1	1	0	2	1	1	1	0	1	1	2	2	0	1	1
P. globosus	1	1	1	1	0	0	1	1	0	2	0	1	1	0	1	1	1	9	0	1	0
P. guanacaste	1	1	1	1	0	0	1	1	0	2	0	1	1	0	?	1	1	9	0	1	0
P. validus	1	1	1	1	0	0	0	1	0	2	1	1	1	0	1	1	1	6	0	1	0
P. paredesi	1	1	1	1	0	0	1	1	0	2	1	1	1	0	0	1	0	7	0	1	0
P. lautus	1	1	1	1	0	1	1	1	0	2	1	1	1	0	?	1	0	5	0	1	0
P. bicornis	1	1	0	0	0	1	1	1	0	2	0	1	1	0	0	1	1	6	0	1	0
P. gertschi	1	1	0	0	0	1	1	1	0	2	1	1	0	0	?	1	0	7	0	1	0
M. platnicki	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1	4	8	0	1	0
M. cornutus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	1	0	1	0
M. rothi	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	0	4	0	1	0
M. franckei	1	1	0	0	0	0	1	1	1	4	0	1	0	0	1	1	0	4	0	1	0
M. californicus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0
M. enaulus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0
M. merus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0
M. tanneri	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0
M. brevicornus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0
M. sprousei	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0
M. dugesi	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0
M. darwini	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0
M. reddelli	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	0
M. mysticus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	-	-	0	1	0
M. marialuisae	1	1	0	0	1	0	1	1	1	0	0	1	0	0	1	0	-	-	0	1	0
M. michoacanus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	0
M. hoogstraali	1	1	0	0	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1	0
M. pedregosus	1	1	0	0	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1	0
M. peribaniensis	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0
M. palmarus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0
P. xerophilus	1	1	0	0	0	0	1	1	0	2	1	1	1	0	?	1	1	9	0	1	0
P. mariachi	1	1	1	1	0	0	1	1	0	1	0	1	1	0	1	1	2	7	0	1	0
P. sikuapu	1	1	0	0	0	0	1	1	0	2	1	1	1	0	1	1	4	2	0	1	1

Appendix. Continuation.

Species	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**
Priscula binghamae	0	-	-	0	0	0	1	0	0	0	0	0	0	0	1	0	-	8	-	-	-
Artema atlanta	0	-	-	0	0	0	0	1	0	0	0	0	0	0	1	0	-	7	-	-	-
Trichocyclus nigropunctatus	0	-	-	0	0	0	0	1	0	0	0	0	0	0	0	0	-	6	-	-	-
Trichocyclus nullarbor	0	-	-	0	0	0	0	1	0	0	0	0	0	0	0	0	-	6	-	-	-
P. huacana	0	-	-	0	0	0	0	1	1	0	1	0	0	0	1	1	2	0	-	-	-
P. montanoi	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	4	0	-	-	-
P. modestus	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	3	0	-	-	-
P. sarae	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-
P. globosus	1	0	-	1	0	0	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-
P. guanacaste	1	0	-	1	0	0	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-
P. validus	0	-	-	0	0	0	0	1	0	0	0	0	0	1	1	1	1	2	-	-	-
P. paredesi	0	-	-	0	0	1	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-
P. lautus	0	-	-	0	0	1	0	1	1	0	0	0	0	1	1	1	0	0	-	-	-
P. bicornis	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	-	-	-
P. gertschi	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	-	-	-
M. platnicki	1	0	-	0	0	1	0	1	0	0	0	0	0	0	1	0	-	1	-	-	-
M. cornutus	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	-	3	-	-	-
M. rothi	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	-	3	-	-	-
M. franckei	1	1	4	0	0	0	0	1	0	1	0	0	1	1	1	0	-	4	0	0	-
M. californicus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-
M. enaulus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	0
M. merus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	0
M. tanneri	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	1	2
M. brevicornus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-
M. sprousei	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	0
M. dugesi	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-
M. darwini	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-
M. reddelli	1	1	3	0	1	0	0	1	0	1	0	0	1	1	1	0	-	4	1	0	-
M. mysticus	1	1	3	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	1
M. marialuisae	1	1	4	0	0	0	0	1	0	0	0	0	1	1	1	0	-	4	1	1	1
M. michoacanus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	1	1
M. hoogstraali	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	5	-	-	-
M. pedregosus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	5	-	-	-
M. peribaniensis	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	1
M. palmarus	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-
P. xerophilus	1	0	-	1	0	0	0	1	0	0	0	0	0	1	1	1	5	0	-	-	-
P. mariachi	1	0	-	0	0	0	0	1	0	0	0	0	0	1	1	1	6	0	-	-	-
P. sikuapu	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	1	9	-	-	-

Appendix 1. Continuation.

Species	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**
Priscula binghamae	0	-	-	1	1	0	0	1	0	-	0	0
Artema atlanta	0	-	0	0	0	0	0	0	0	-	0	1
Trichocyclus nigropunctatus	0	-	0	2	0	1	1	0	0	-	0	0
Trichocyclus nullarbor	0	-	0	2	0	1	1	0	0	-	0	0
P. huacana	0	-	0	1	0	0	0	0	0	-	1	0
P. montanoi	0	-	0	1	0	0	0	0	0	-	0	0
P. modestus	0	-	0	1	0	0	0	0	0	-	0	0
P. sarae	0	-	0	1	0	0	0	0	0	-	0	0
P. globosus	0	-	0	1	0	0	0	0	0	-	0	0
P. guanacaste	0	-	0	1	0	0	0	0	0	-	0	0
P. validus	0	-	0	1	0	0	0	0	0	-	0	0
P. paredesi	0	-	0	1	0	0	0	0	0	-	1	0
P. lautus	0	-	0	1	0	0	0	0	0	-	0	0
P. bicornis	0	-	0	1	0	0	0	0	0	-	0	0
P. gertschi	0	-	0	1	0	0	0	0	0	-	0	0
M. platnicki	1	1	1	1	0	0	0	0	0	-	0	1
M. cornutus	1	1	1	1	0	0	0	0	0	-	0	1
M. rothi	1	1	1	1	0	0	0	0	0	-	0	1
M. franckei	1	4	0	1	0	0	0	0	0	-	0	1
M. californicus	1	0	1	1	0	0	0	0	1	1	0	1
M. enaulus	1	0	1	1	0	0	0	0	0	-	0	1
M. merus	1	0	1	1	0	0	0	0	0	-	0	1
M. tanneri	1	0	1	1	0	0	0	0	1	0	0	1
M. brevicornus	1	0	1	1	0	0	0	0	1	1	0	1
M. sprousei	1	0	1	1	0	0	0	0	0	-	0	1
M. dugesi	1	0	1	1	0	0	0	0	1	0	0	1
M. darwini	1	0	1	1	0	0	0	0	1	0	0	1
M. reddelli	1	0	1	1	0	0	0	0	1	0	0	1
M. mysticus	1	3	1	1	0	0	0	0	1	0	0	1
M. marialuisae	1	6	1	1	1	0	0	0	1	0	0	1
M. michoacanus	1	2	1	1	0	0	0	0	1	0	0	1
M. hoogstraali	1	2	1	1	0	0	0	0	0	-	0	1
M. pedregosus	1	5	1	1	0	0	0	0	0	-	0	1
M. peribaniensis	1	7	1	1	0	0	0	0	1	1	0	1
M. palmarus	1	7	1	1	0	0	0	0	1	1	0	1
P. xerophilus	0	-	0	1	0	0	0	0	0	-	0	0
P. mariachi	0	-	0	1	0	0	0	0	0	-	0	0
P. sikuapu	0	-	0	1	0	0	0	0	0	-	1	0

11. Capítulo 3

Artículo Taxonómico

Four new species of the spider genus *Physocyclus* Simon, 1893 (Araneae: Pholcidae) from Mexico, with updated taxonomic identification key

Samuel Nolasco – Alejandro Valdez Mondragón

Cuatro nuevas especies del género *Physocyclus* Simon, 1893 (Araneae: Pholcidae) de México, con una actualización de la clave de identificación taxonómica

Samuel Nolasco – Alejandro Valdez Mondragón

Nolasco, S. & Valdez-Mondragón, A. 2022. Four new species of the spider genus *Physocyclus* Simon, 1893 (Araneae: Pholcidae) from Mexico, with updated taxonomic identification keys. *European Journal of Taxono*my 813: 173–206. https://doi.org/10.5852/ejt.2022.813.1739

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Four new species of the spider genus *Physocyclus* Simon, 1893 (Araneae: Pholcidae) from Mexico, with updated taxonomic identification keys

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Abstract. Four new species of the spider genus *Physocyclus* Simon, 1893 are described from Mexico. Two species are described based on male and female adult specimens: *Physocyclus mariachi* sp. nov. and *P. sikuapu* sp. nov. Two species are described only with female adult specimens: *P. lyncis* sp. nov. and *P. pocamadre* sp. nov. The biogeographical province with the highest diversity of species is the Balsas Depression, located in the Mexican Neotropic, with 12 species. *Physocyclus lyncis* sp. nov. belongs to the *dugesi* species group, whereas the other three new species belong to the *globosus* species group. The total number of species of *Physocyclus* is increased to 37, distributed in North America (mainly Mexico) and Central America, with one cosmopolitan species: *P. globosus*. Updated taxonomic identification keys for males and females are provided.

Keywords. Taxonomy, species groups, Arteminae, Jalisco, Michoacan, Baja California Sur.

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Introduction

The spider family Pholcidae C.L. Koch, 1850 is the ninth largest family in the order Araneae, currently composed by 96 genera and 1854 species, including the species herein described (WSC 2022). The family is subdivided into five subfamilies: Arteminae Simon, 1893 Modisiminae Simon, 1893, Ninetinae Simon, 1890, Pholcinae C.L. Koch, 1850, and Smeringopinae Simon, 1893 (Huber 2011; Dimitrov *et al.* 2013; Eberle *et al.* 2018). The spider genus *Physocyclus* Simon, 1893, is classified in the subfamily Arteminae, which is the most diverse of the subfamily, with 34 species not considering the species described herein. In the last phylogenetic proposal by Eberle *et al.* (2018), the phylogenetic relationships of *Physocyclus* are not clear yet the genus might be related to the genus *Nita*, but also closely related to: ("*Wugigarra*" Indonesia (*Trichocyclus* (*Wugigarra* Australia + *Holocneminus* exc. *huangdi*))).

The genus *Physocyclus* is a common pholcid spider in Mexico (Valdez-Mondragón 2010, 2013, 2014; Jiménez & Palacios-Cardiel 2013), currently with 32 species distributed throughout the Mexican territory. *Physocyclus* is distributed mainly in North and Central America, from the south of the United States to Costa Rica (Valdez-Mondragón 2010, 2013; Jiménez & Palacios-Cardiel 2013). The habitat of these spiders is mainly dry and semiarid ecosystems, xeric shrub zones, deserts, and tropical deciduous forest (Valdez-Mondragón 2010, 2013, 2014; Nolasco & Valdez-Mondragón 2020). Some species are found in caves and holes in walls, other species among big boulders or tree bark on the ground. All the species of the genus are considered troglophilic, and so far no troglobitic species have been found. Also, is it common to find sympatric species of *Physocyclus* in the same locality, such as some regions of western Mexico where *P. brevicornus* Valdez-Mondragón, 2010, *P. lautus* Gertsch, 1971, and *P. globosus* (Taczanowski, 1874) have been collected in the same locality (Valdez-Mondragón 2010). There are two synanthropic and common species, *P dugesi* Simon, 1893 and *P. globosus*, the latter with a cosmopolitan distribution influenced by human activities (Beatty *et al.* 2008; Valdez-Mondragón 2010; Huber & Kwapong 2013) (Figs 1–7). Species associated with human habitation are found in places like roof corners in bedrooms, basements and bathrooms, under sinks, tables and benches, under electrical facilities, under stored objects and furniture, and under street drains, in dark warm places with few drafts and little disturbance.

The morphology of the genus comprises a small body ranging from 3 to 5 mm, and long legs with dark rings on the distal part of the femora and tibiae. Male chelicerae are wide and complex, with lateral apophyses variable in size, shape, position, and number. The coloration of the body varies between beige and pale brown, with irregular dorsal grey or brown patterns on the carapace. However, the general shape and coloration is similar among species, differing mainly in the shape of the reproductive structures (male palps and female genitalia) (Valdez-Mondragón 2010, 2014).

According to Valdez-Mondragón (2013, 2014), *Physocyclus* is a monophyletic genus based in morphological phylogenetic analyses, constituted by two species groups, *globosus* and *dugesi*, with 15 and 22 species respectively, including the new species described herein. The distribution pattern of these species groups is the Mesoamerican and Mexican Mountain biotic components for the *globosus* group, and the Mesoamerican and Continental Nearctic biotic components for the *dugesi* group (Valdez-Mondragón 2013, 2014).

In this work, we describe four new species from the states of Jalisco, Michoacan and Baja California Sur, Mexico. With this contribution, the number of described species of the genus increases to 37, described species, with Mexico being the country that holds the highest diversity of this genus. Additionally, updated taxonomic identification keys for males and females are provided.

Material and methods

Specimens were collected manually, preserved and observed in 80% ethanol. The type material is deposited at the Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional

Autónoma de Mexico, Mexico City. The additional examined material is deposited at the Laboratory of Arachnology (LATLAX), Laboratorio Regional de Biodiversidad y Cultivo de Tejidos Vegetales (LBCTV), IBUNAM, Tlaxcala City, Mexico; and the American Museum of Natural History (AMNH), New York City, United States. The specimens were measured and examined with a Zeiss Discovery Stereoscope V.8, and the photographs were done using a Zeiss Axio Zoom V.16 microscope and Axio Zoom Zen and Zen Pro digital software. The map was made with QGIS ver. 3.10. The photographs and map were edited in Photoshop CS6 ver. 130x32. The measurements of all specimens are in millimeters (mm). The male palps and female epigyna were dissected and observed in ethanol (80%). Photography was conducted with specimens and structures submerged in commercial-use gel alcohol (to hold them in the appropriate position), and the preparation completely covered with 80% ethanol. A dissecting microscope (Zeiss Discovery Stereoscope V.8) fitted with a camera lucida was used to make the drawings. The female genitalia were cleaned in potassium hydroxide (KOH 10%) for 10 minutes, to clean the soft tissues around the pore plates. Morphological terminology and descriptions follow Valdez-Mondragón & Francke (2015) and Nolasco & Valdez-Mondragón (2020).

Abbreviations

ALE	=	anterior lateral eyes
AME	=	anterior median eyes
BU	=	bulb of palp
DAP	=	dorsal apophyses of procursus
E	=	embolus
ES	=	embolic sclerites
LAC	=	lateral apophyses of chelicerae
L/d	=	length/diameter
PLE	=	posterior lateral eyes
PME	=	posterior median eyes
PP	=	pore plates
PR	=	procursus
SF	=	stridulatory files of chelicerae
SO	=	spermatic operculum
VAE	=	ventral apophyses of epigynum

Results

Class Arachnida Cuvier, 1812
Order Araneae Clerck, 1757
Family Pholcidae Koch, 1850
Subfamily Arteminae Simon, 1893
Genus *Physocyclus* Simon, 1893

Type species

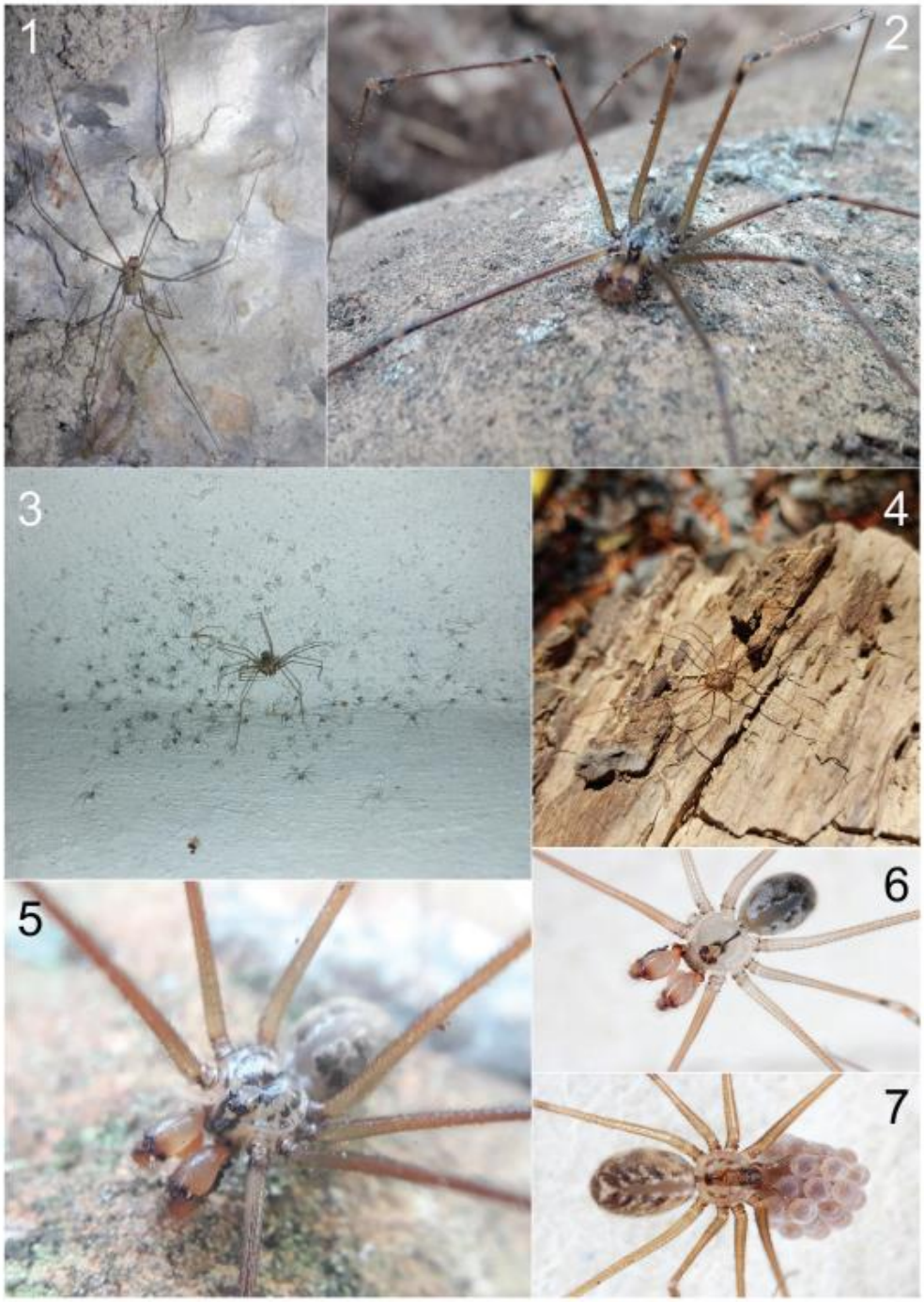
Pholcus globosus Taczanowski, 1874.

Diagnosis

See Valdez-Mondragón (2010, 2013, 2014).

Distribution

The genus *Physocyclus* is native and endemic in North and Central America (Valdez-Mondragón 2010), with the exception of *P. globosus*, which has a cosmopolitan distribution due to human activities (Valdez-Mondragón 2010, 2013, 2014). Huber & Villarreal (2020) mentioned that Caporiacco's (1955) records



Figs 1–7. Live specimens of the spider genus *Physocyclus*. **1**. Male of *Physocyclus darwini* Valdez-Mondragón, 2010. **2**, **5**. Males of *P. michoacanus* Valdez-Mondragón, 2010. **3**. Female and juveniles of *P. dugesi* Simon, 1893. **4**. Male of *P. dugesi*. **6**. Male of *P. reddelli* Gertsch, 1971. **7**. Female of *P. globosus* (Taczanowski, 1874), holding the ovisac. Photos 6 and 7 by Bernhard A. Huber (2019).

of *P. dugesi* Simon, 1893 from Miranda (1 ♀) and Caracas (1 juv.) are dubious and presumably based on specimens of *P. globosus* or a species of *Priscula* Simon, 1893. The natural distribution of *Physocylus* is mainly in arid and dry ecosystems (Figs 9–10, 13, 76, 77), such as xerophilous scrubs or deserts, although some species occur in zones with temperate and subtropical climates, such as deciduous forest (Figs 8, 12, 70, 72). The specific habitat of these spiders is between big boulders, in tree bark, or inside any hollow that provides protection (Figs 11, 71). In karstic zones, due to their trogophilic habits, they are commonly found inside caves, on the walls or between fissures (Figs 11, 73, 75) (Valdez-Mondragón 2010).

Composition

The genus *Physocyclus* is composed of two species groups: *globosus* and *dugesi*. The *globosus* group includes 15 species: *P. bicornis* Gertsch, 1971, *P. gertschi* Valdez-Mondragón, 2010, *P. globosus*, *P. guanacaste* Huber, 1998, *P. huacana* Valdez-Mondragón, 2010, *P. lautus* Gertsch, 1971, *P. mariachi* sp. nov., *P. modestus* Gertsch, 1971, *P. montanoi* Valdez-Mondragón, 2010, *P. paredesi* Valdez-Mondragón, 2010, *P. pocamadre* sp. nov., *P. sarae* Valdez-Mondragón, 2010, *P. sikuapu* sp. nov., *P. validus* Gertsch, 1971, and *P. xerophilus* Nolasco & Valdez-Mondragón, 2020.

The *dugesi* group includes 22 species: *P. brevicornus* Valdez-Mondragón, 2010, *P. californicus* Chamberlin & Gertsch, 1929, *P. cornutus* Banks, 1898, *P. darwini*, *P. dugesi*, *P. enaulus* Crosby, 1926, *P. franckei* Valdez-Mondragón, 2010, *P. hoogstraali* Gertsch & Davis, 1942, *P. lyncis* sp. nov., *P. marialuisae* Valdez-Mondragón, 2010, *P. merus* Gertsch, 1971, *P. mexicanus* Banks, 1898, *P. michoacanus*, *P. mysticus* Chamberlin, 1924, *P. palmarus* Jiménez & Palacios-Cardel, 2013, *P. pedregosus* Gertsch, 1971, *P. peribanensis* Valdez-Mondragón, 2014, *P. platnicki* Valdez-Mondragón, 2010, *P. reddelli*, *P. rothi* Valdez-Mondragón, 2010, *P. sprousei* Valdez-Mondragón, 2010, and *P. tanneri* Chamberlin, 1921.

The identification key of species of *Physocyclus* Simon, 1893 is updated from Valdez-Mondragón 2010 (hereafter VM 2010), using the same abbreviations.

Identification keys

Males

1. Sclerotized cones on frontal lamina of chelicerae present (VM 2010: figs 15, 22) 2
– Sclerotized cones on frontal lamina of chelicerae absent (VM 2010: figs 63, 77) 22

2. Few sclerotized cones (<10) on each frontal lamina (VM 2010: figs 42, 49) 3
– Numerous sclerotized cones (>10) on each frontal lamina (VM 2010: figs 105, 119) 5

3. Procursus pointing to front of palp, with basal half dark brown and distal half dark, with thin and long spine distally; sclerites on bulb, with oval shape in prolateral view (Nolasco & Valdez-Mondragón 2020: figs 6–8) ..... ***P. xerophilus*** Nolasco & Valdez-Mondragón, 2020
– Procursus pointing to base of palp (VM 2010: figs 44, 51) 4

4. Embolic sclerites half-moon shaped in retrolateral view; without projection directed to base of embolus; procursus with long ventral notch and ventral protuberance on median part (VM 2010: figs 44–45)…… ..... ***P. globosus*** (Taczanowski, 1874)
– Embolic sclerites oval-shaped in retrolateral view; with conical projection directed to base of embolus; procursus with short ventral notch and without ventral protuberance on median part (VM 2010: figs 51–52)…….. ..... ***P. guanacaste*** Huber, 1998

5. Palp femur with a ventral conical apophysis in the middle (VM 2010: figs 86, 121) 6
– Palp femur without ventral conical apophysis in the middle (VM 2010: figs 128, 135) 8

6. Palp femur thin, with long conical apophyses; embolus short, curved and thin; lateral apophyses of chelicerae absent; chelicerae with big cones on basal and prolateral parts of frontal lamina, except on anterior half of region with upside-down U-shape (VM 2010: figs 84–86) ..... ***P. mysticus*** Chamberlin, 1924
– Palp femur wide, with small conical apophyses; embolus large and wide; lateral apophyses of chelicerae present; chelicerae with small cones scattered (VM 2010: figs 98–100) 7

7. Chelicerae with large and curved lateral apophyses, without oval protuberance on basal part of frontal lamina; sclerotized cones on prolateral part of frontal lamina and toward prolateral part of lateral apophyses of chelicerae; procursus with short and thin apical spine; embolic sclerites small, oval apically; embolus rounded in dorsal part (VM 2010: figs 98–100) .....***P. reddelli*** Gertsch, 1971
– Chelicerae with small and trapezoidal lateral apophyses, with oval protuberance on basal part of frontal lamina; sclerotized cones along frontal lamina and on frontal protuberance of chelicerae; procursus with large and wide apical spine; embolic sclerites large, triangular apically; embolus straight and triangular (VM 2010: figs 119–121) .....***P. franckei*** Valdez-Mondragón, 2010

8. Embolus pointing in perpendicular position to longitudinal axis of palp femur (VM 2010: figs 10, 135) 9
– Embolus pointing to base of palp femur (VM 2010: figs 31, 107) 14

9. In retrolateral view, palp with inconspicuous notch between embolic sclerites and embolus; embolus partially covering embolic sclerites; dorsal protuberance present on bulb; chelicerae with lateral apophyses slightly curved; sclerotized cones of chelicerae on half basal, on prolateral part of frontal lamina, and prolateral part of lateral apophyses (VM 2010: figs 133–136) ..... ***P. brevicornus*** Valdez-Mondragón, 2010
– In retrolateral view, palp with conspicuous notch between embolic sclerites and embolus; embolus not covering embolic sclerites; dorsal protuberance absent on bulb; chelicerae with lateral apophyses straight; sclerotized cones of chelicerae in other position (VM 2010: figs 8, 10, 22, 24) 10

10. Embolic sclerites thin; circular notch between embolic sclerites and embolus; embolus with circular dorsal part; sclerotized cones of chelicerae on prolateral part of frontal lamina and prolateral part of lateral apophyses (VM 2010: figs 8–11) ..... ***P. californicus*** Chamberlin & Gertsch, 1929
– Embolic sclerites wide; oval notch between embolic sclerites and embolus; embolus with curved dorsal part; sclerotized cones of chelicerae scattered (VM 2010: figs 24–25, 178–179) 11

11. Chelicerae with sclerotized cones on basal half of frontal lamina and few scattered cones on distal part; lateral apophyses of chelicerae long; procursus pale in basal half and dark in distal half, with a short apical spine; embolus wide, oval basally (Jiménez & Palacios-Cardiel 2013: figs 5–8) ..... ***P. palmarus*** Jiménez & Palacios-Cardiel, 2013
– Chelicerae with sclerotized cones on ¾ of total length of frontal lamina and prolateral part of lateral apophyses; lateral apophyses of chelicerae short; procursus totally dark, with a long apical spine; embolus with other shape (VM 2010: figs 22, 24, 176, 178) 12

12. Chelicerae without sclerotized cones on small central region of frontal lamina; in retrolateral view, procursus with long ventral notch, starting at level of dorsal apophyses of procursus (VM 2010: figs 176–178) .....***P. darwini*** Valdez-Mondragón, 2010
– Chelicerae with sclerotized cones totally covering ¾ of length of frontal lamina; in retrolateral view, procursus with small ventral notch at middle (VM 2010: figs 22) 13

13. Chelicerae with long cones on frontal lamina; procursus shorter than in *P. peribanenis*; distal spine slightly wider than in *P. peribanensis*; in retrolateral view, procursus with conspicuous

notch between embolic sclerites and embolus; embolus wide and straight (VM 2010: figs 22–24) ..... ***P. dugesi*** Simon, 1893

– Chelicerae with small cones on frontal lamina; procursus longer than in *P. dugesi*; with distal spine thinner than in *P. dugesi*; procursus with inconspicuous notch between embolic sclerites and embolus; embolus wide and sinuous (Valdez-Mondragón 2014: figs 6, 8) ..... ***P. peribanensis*** Valdez-Mondragón, 2014

14. Embolus with distal concavity, ending in a sharp tip (VM 2010: figs 31–32) 15
– Embolus without distal concavity, ending in a rounded or curved tip (VM 2010: figs 17–18) 19

15. Embolus with dorsal part slightly curved, almost straight, and with small distal ventral concavity (VM 2010: figs 72–73) 16
– Embolus with dorsal part strongly curved, distal ventral concavity large (VM 2010: figs 163–164) 17

16. In retrolateral view, lateral apophyses of chelicerae short and conical, in frontal view without protruding laterally of chelicerae; embolus straight and long (VM 2010: figs 29–31) ..... ***P. enaulus*** Crosby, 1926
– In retrolateral view, lateral apophyses of chelicerae wide and rounded apically, in frontal view without protruding laterally of chelicerae; embolus straight and shorter than in *P. enaulus* and *P. sprousei* (VM 2010: figs 70–72) ..... ***P. merus*** Gertsch, 1971
– In retrolateral view, lateral apophyses of chelicerae strongly wider than in *P. enaulus* and *P. merus*, in frontal view protruding laterally of chelicerae; embolus long and sinuous (VM 2010: figs 168, 170) ..... ***P. sprousei*** Valdez-Mondragón, 2010

17. Sclerotized cones of chelicerae scattered along frontal lamina; embolic sclerites partially covered by embolus; in dorsal view, embolic sclerites sigmoid; lateral apophyses of chelicerae absent; chelicerae with basal-frontal protuberance; palp femur with ventral-distal conical apophysis, rounded apically (VM 2010: figs 161–163) ..... ***P. marialuisae*** Valdez-Mondragón, 2010
– Sclerotized cones of chelicerae not scattered along frontal lamina; embolic sclerites not covered by embolus; in dorsal view, embolic sclerites straight; lateral apophyses of chelicerae present; chelicerae without basal-frontal protuberance; palp femur without ventral-distal conical apophysis (VM 2010: fig. 105) 18

18. Embolic sclerites short; embolus with claw-shape apically, with deep distal-ventral concavity; lateral apophyses of chelicerae short and straight, ending in rounded tip; sclerotized cones of chelicerae on prolateral part and basal half of frontal lamina (VM 2010: figs 105–108) ..... ***P. tanneri*** Chamberlin, 1921
– Embolic sclerites long, spine-shaped; embolus wide, with shallow distal ventral concavity; lateral apophyses of chelicerae long and straight, ending in a sharp tip; sclerotized cones of chelicerae on prolateral part of frontal lamina, first basal ⅓ of chelicerae, and on prolateral part of lateral apophyses (VM 2010: figs 126–129) ..... ***P. michoacanus*** Valdez-Mondragón, 2010

19. In retrolateral view, embolus apically ending in a rounded or curved shape; procursus thin and short, with inconspicuous distal spine; sclerotized cones of chelicerae scattered along frontal lamina (VM 2010: figs 15, 17, 183, 185) 20
– In retrolateral view, embolus apically ending in a rounded shape in ventral part, and dorsal part ending in a sharp tip; procursus wide and long, with conspicuous distal spine; sclerotized cones of chelicerae on prolateral-basal part of frontal lamina and prolateral part of lateral apophyses (VM 2010: figs 56, 58, 91, 93) 21

20. Embolus apically ending in a rounded shape; embolic sclerites long and wider than in *P. rothi*, without distal notch dorsally; dorsal apophysis of procursus shorter than in *P. rothi*; sclerotized cones of chelicerae along of frontal lamina and on prolateral part of lateral apophyses; lateral apophyses of chelicerae wide and long, in lateral view conical and curved (VM 2010: figs 15–17) ..... ***P. cornutus*** Banks, 1898
– Embolus apically ending in a curved shape; embolic sclerites long and thinner than in *P. cornutus*, with small distal notch dorsally; dorsal apophysis of procursus larger than in *P. cornutus*; sclerotized cones distributed until ⅔ part of length of frontal lamina and on lateral apophyses; lateral apophyses of chelicerae wide and short, in lateral view conical, ending in two tips with different sizes (VM 2010: figs 183–185) ..... ***P. rothi*** Valdez-Mondragón, 2010

21. Embolic sclerites long and wide, rounded apically; embolus without apical notch; procursus with apical spine thinner than in *P. hoogstraali*; sclerotized cones on basal half, prolateral part of frontal lamina and prolateral part of lateral apophyses; lateral apophyses of chelicerae shorter than in *P. hoogstraali*, projected at almost same length of chelicerae (VM 2010: figs 91–94) ..... ***P. pedregosus*** Gertsch, 1971
– Embolic sclerites long and thin, spine-shaped apically; embolus with apical notch; procursus with apical spine wider than in *P. pedregosus*; sclerotized cones on basal and prolateral part of frontal lamina of chelicerae but missing on lateral apophyses; lateral apophyses of chelicerae longer than in *P. pedregosus*, projected at same length of chelicerae (VM 2010: figs 56–59) ..... ***P. hoogstraali*** Gertsch & Davis, 1942

22. Two lateral apophyses in each chelicera (Figs 17–19) 23
– One single apophyses in each chelicera (VM 2010: figs 77–78) 27

23. Chelicerae with both lateral apophyses close to each other, in parallel position, pointing downwards; lateral apophyses of chelicerae with 3-4 sclerotized cones (Figs 17–19) 24
– Chelicerae with lateral apophyses pointing in perpendicular position, forming different angles; lateral apophyses of chelicerae without sclerotized cones (VM 2010: figs 1–2) 25

24. In frontal view, longest lateral apophyses of chelicerae wide and dark, with stridulatory ridges on it, without sclerotized cones; frontal-distal apophyses of chelicerae conical, with 3-4 sclerotized cones; procursus straight, with ventral notch at median part, with a long spine distally; in retrolateral view, embolic sclerites short; in dorsal view, embolus thin basally and becoming wider distally and in retrolateral view ending in a rounded tip (Figs 17–19, 21–22, 24–27) ..... ***P. mariachi*** sp. nov.
– In frontal view, longest lateral apophyses of chelicerae short and pale, with 3-4 apical sclerotized cones; frontal-distal apophyses of chelicerae conical, without sclerotized cones; procursus curved, with ventral notch at basal part, without distal spine; in retrolateral view, embolic sclerites long and wider than in *P. mariachi* sp. nov.; in dorsal view, embolus straight and in retrolateral view slightly curved, ending in "J" shape (VM 2010: figs 197, 200) ..... ***P. platnicki*** Valdez-Mondragón, 2010

25. Chelicerae with basal-lateral apophyses short, ending in small rounded tip; chelicerae with distal-frontal conical apophyses, sclerotized, protruding from the distal margin of chelicerae; procursus with long and thin distal spine; embolic sclerites rounded dorsally; embolus square in retrolateral view, with a small spine sub-distally on dorsal view (VM 2010: figs 63–66) ..... ***P. lautus*** Gertschi, 1971
– Chelicerae with paired lateral apophyses, conical, ending in sclerotized and sharp tips; chelicerae with small distal-frontal conical apophyses or lateral-distal apophyses; procursus with short distal spine or long and wide; embolic sclerites curved or square-shaped dorsally (VM 2010: figs 1–3; Valdez-Mondragón 2014: figs 23–25) 26

26. In retrolateral view, chelicerae with one lateral wide apophysis and the other small and triangular, forming a 90° angle between them; procursus conical, with rounded ventral notch in retrolateral view; embolic sclerites wide and square; embolus without distal spine (VM 2010: figs 1–4) ..... ***P. bicornis*** Gertschi, 1971
– In retrolateral view, chelicerae with one lateral apophysis wide and ax-shaped and the other thin and conical, forming a <90° angle between them; procursus straight, with oval ventral notch in retrolateral view; embolic sclerites short, slightly oval; embolus with a wide distal spine (Valdez-Mondragón 2014: figs 21, 23–26) ..... ***P. paredesi*** Valdez-Mondragón, 2010

27. Stridulatory ridges occupying full length of lateral apophyses of chelicerae (VM 2010: figs 112–113) 28
– Stridulatory ridges occupying part of total length of lateral apophyses of chelicerae (VM 2010: figs 140–141) 32

28. Procursus wide and straight, ending in a short spine (Fig. 44; VM 2010: fig. 79) 29
– Procursus thin and conical, ending in a long spine (VM 2010: figs 156, 192) 30

29. Procursus wider in distal half than basal half, with curved projection ventrally; embolic sclerites with square notch in median part; embolus ending in a rounded small ventral projection, without ventral projections and without small spine dorsal-apically but with a conspicuous triangular projection in dorsal view (VM 2010: figs 79–80) ..... ***P. modestus*** Gertsch, 1971
– Procursus wider in basal half than distal half, without curved projection ventrally; embolic sclerites square-shaped and without square notch in median part; embolus ending in square shape, with a small ventral projection pointing to base of femur and with small spine dorsal-apically (Figs 44–47, 50–51) ..... ***P. sikuapu*** sp. nov.

30. Palp femur straight ventrally; lateral apophysis of chelicerae wide, shield-shaped; embolic sclerites slightly curved in retrolateral view; embolus wide, with apical wide and curved concavity (VM 2010: figs 190–192) ..... ***P. sarae*** Valdez-Mondragon, 2010
– Palp femur curved ventrally (VM 2010: fig. 114) 31

31. Palp femur thinner than in *P. montanoi*; lateral apophysis of chelicerae wide and triangular-shaped in frontal view, with half length of chelicerae; embolic sclerites short, not protruding embolus; embolus long and inverted S-shaped (VM 2010: figs 112–114) ..... ***P. validus*** Gertsch, 1971
– Palp femur wider than in *P. validus*; lateral apophysis of chelicerae wide and shield-shaped in frontal view, almost with same length as chelicerae; embolic sclerites long, with an S-shape, protruding embolus; embolus small and square (VM 2010: figs 154–156) ..... ***P. montanoi*** Valdez-Mondragón, 2010

32. In lateral view, chelicerae with lateral-basal apophysis projected toward frontal part, and thin lateral-distal apophysis, ending in serrated tip; procursus curved, with curved ventral notch and a rounded distal-dorsal projection; embolic sclerites wide and conspicuous, with small rounded distal notch; in retrolateral view, embolus wider than in *P. huacana*, without notch between bulb and embolus (VM 2010: figs 140–143) ..... ***P. gertschi*** Valdez-Mondragón, 2010
– In lateral view, chelicerae with only one lateral apophysis wide and square-shaped; procursus conical, with ventral notch "V"-shaped; embolic sclerites conical and slightly curved, without small rounded distal notch; in retrolateral view, embolus thinner than in *P. gertschi*, with rounded notch between bulb and embolus (VM 2010: figs 147–150) ..... ***P. huacana*** Valdez-Mondragón, 2010

Females

1. Epigynum in ventral view, bell-shaped, with lateral constrictions strongly marked in median part, with anterior half smaller than posterior half (VM 2010: figs 19, 26) 2
– Epigynum in ventral view, with other shape, without constrictions in median part, or if present, barely visible (VM 2010: figs 60, 67) 19

2. Ventral apophyses of epigynum long and conical or long and flat (VM 2010: figs 19, 21, 130, 132) . 3
– Ventral apophyses of epigynum small, with other shape (VM 2010: figs 95, 97, 137, 139) 15

3. Pore plates long and thin (VM 2010: figs 39–41, 75) 4
– Pore plates with other shape (VM 2010: figs 20, 27) 9

4. Epigynum with light ventral region, rounded, close to epigastric furrow (VM 2010: figs 35, 74) 5
– Epigynum with light ventral region, triangular, close to epigastric furrow (VM 2010: figs 109, 130) 8

5. Epigynum with ventral apophyses long or flat (Fig. 61; VM 2010: fig. 74) 6
– Epigynum with ventral apophyses short, conical and rounded distally (VM 2010: figs 33–38) 7

6. Epigynum with ventral apophyses long and flat, rounded distally; epigynum rounded in lateral view; wide light region in median part triangular; pore plates wide and oval (Figs 61–63) ..... ***P. lyncis*** sp. nov.
– Epigynum with ventral apophyses thin and sharp apically, slightly curved in lateral view; epigynum slightly curved in lateral view; wide light region in median part circular; pore plates thin and long (VM 2010: figs 74–76) ..... ***P. merus*** Gertsch, 1971

7. Epigynum with ventral apophyses rounded apically, with upside-down chair shape (type I), or with ventral apophyses long and conical, ending in a rounded tip (type II); in frontal view, anterior rounded protuberances on ventral apophysis absent; pore plates shorter than in *P. sprousei* (VM 2010: figs 33–41) ..... ***P. enaulus*** Crosby, 1926
– Epigynum with ventral apophyses wide and rounded basally, ending in a conical tip; in frontal view, anterior rounded protuberances on each ventral apophysis; pore plates longer than in *P. enaulus* (VM 2010: figs 172–175) ..... ***P. sprousei*** Valdez-Mondragón, 2010

8. Epigynum with ventral apophyses straight in ventral view, short and conical in lateral view; epigynum with light ventral region close to epigastric furrow shorter than in *P. michoacanus*; pore plates short and thin, straight, slightly wider in posterior part (VM 2010: figs 109–111) ..... ***P. tanneri*** Chamberlin, 1921
– Epigynum with ventral apophyses wide and curved in ventral view, wide and curved in lateral view; epigynum with light ventral region close to epigastric furrow longer than in *P. tanneri*; pore plates long, slightly curved, wider in anterior part (VM 2010: figs 130–132) ..... ***P. michoacanus*** Valdez-Mondragón, 2010

9. Epigynum with ventral apophyses wide in basal part, with small tips (VM 2010: figs 26, 180) 10
– Epigynum with ventral apophyses long and conical, with wide tips (VM 2010: figs 19, 165) 11

10. Epigynum with tips of ventral apophyses separated from each other, tips longer than in *P. darwini*; pore plates thin, curved, "V"-shaped; light region close to epigastric furrow smaller than in *P. darwini* (VM 2010: figs 26–28) ..... ***P. dugesi*** Simon, 1893
– Epigynum with tips of ventral apophyses very close to each other, tips smaller than in *P. dugesi*; pore plates wide and straight; light region close to epigastric furrow longer than in *P. dugesi* (VM 2010: figs 180–182) ..... ***P. darwini*** Valdez-Mondragón, 2010

11. Epigynum with ventral and conical paired projections on posterior margin, ventral apophyses conical and slightly curved, separated by a notch on anterior margin; pore plates wide and curved (VM 2010: figs 165–167) ..... ***P. marialuisae*** Valdez-Mondragón, 2010
– Epigynum without ventral and conical paired projections on posterior margin (VM 2010: fig. 19) 12

12. Epigynum with ventral apophyses thin and long, with a sharp tip; epigynum with wide and light region on median part; epigastric furrow triangular-shaped; pore plates with half-circle shape (Jiménez & Palacios-Cardiel 2013: figs 10–13) ..... ***P. palmarus*** Jiménez & Palacios-Cardel, 2013
– Epigynum with ventral apophyses wide and long, with a rounded tip (VM 2010: figs 19–21) 13

13. Epigynum with ventral apophyses close to each other; epigynum with thin and triangular light region in median part; pore plates wide and oval, slightly curved, close to each other (VM 2010: figs 19–20) ..... ***P. cornutus*** Banks, 1898
– Epigynum with ventral apophyses widely separated from each other; epigynum with wide light region in median part (VM 2010: figs 187, 189) 14

14. Epigynum with ventral apophyses pointing downward; light region in median part with rhomboid shape; pore plates thinner than in *P. rothi*, forming a 90° angle (Valdez-Mondragón, 2014: figs 15–17) ..... ***P. peribaniensis*** Valdez-Mondragón, 2014
– Epigynum with ventral apophyses pointing toward front; light region in median part oval; pore plates wider than in *P. peribanensis*, forming a >90° angle (VM 2010: figs 187–189) ..... ***P. rothi*** Valdez-Mondragón, 2010

15. Chelicerae with stridulatory ridges; epigynum with ventral apophyses wide and flat; pore plates wide, oval-shaped (VM 2010: figs 12–14, 137–139) 16
– Chelicerae without stridulatory ridges; epigynum with ventral apophyses small and conical; pore plates with other shape (VM 2010: figs 95–97, 123–125) 17

16. Epigynum with ventral apophyses flat and rhomboid-shaped, with porosities; epigastric furrow forming a >90° angle; pore plates wide and oval, without a sclerotized region in anterior margin (VM 2010: figs 12–14) ..... ***P. californicus*** Chamberlin & Gertsch, 1929
– Epigynum with ventral apophyses rounded, without porosities; epigastric furrow curved; pore plates wide and oval, with a sclerotized region in anterior margin (VM 2010: figs 137–139) ..... ***P. brevicornus*** Valdez-Mondragón, 2010

17. Anterior part of epigynum circular; ventral apophyses of epigynum inconspicuous; epigynum with dark central region forming upside-down "T" shape; pore plates small and triangular (VM 2010: figs 123–125) ..... ***P. franckei*** Valdez-Mondragón, 2010
– Anterior part of epigynum square; ventral apophyses of epigynum conspicuous; epigynum without dark central region with upside-down "T" shape; pore plates elongated (VM 2010 figs 95–97) 18

18. Ventral apophyses of epigynum close to each other, smaller than in *P. reddelli*; epigynum with conspicuous triangular dark region in median part; pore plates large and oval, with a small constriction in middle, without sclerotized region in anterior margin (VM 2010: figs 95–97) ..... ***P. pedregosus*** Gertschi, 1971
– Ventral apophyses of epigynum separated from each other, longer than in *P. pedregosus*; epigynum without triangular dark region in median part; pore plates markedly curved at posterior part, with sclerotized region in anterior margin (VM 2010: figs 102–104) ..... ***P. reddelli*** Gertschi, 1971

19. Epigynum longer than wide (VM 2010: figs 60, 88) 20
– Epigynum wider than long (VM 2010: figs 46, 53) 22

20. Epigynum with two ventral apophyses on anterior margin, small and cylindrical; a third large and conical apophysis located on basal ⅓ part, ending in a rounded tip; pore plates markedly thin and long, almost in parallel position to each other (VM 2010: figs 88–90) ..... ***P. mysticus*** Chamberlin, 1924
– Epigynum without two ventral apophyses on anterior margin; epigynum without sharp and conical apophyses, and without a third large and conical apophysis ventrally (VM 2010: fig. 60; Figs 67–68) 21

21. Epigynum with two anterior, circular, wide apophyses, short and circular in ventral view; two ventral protuberances, flat and curved on middle part; epigynum without long and light bell-shaped region in median part; pore plates thin and long, wider in posterior half (VM 2010: figs 60–62) ..... ***P. hoogstraali*** Gertsch & Davis, 1942
– Epigynum with two small anterior apophyses, oval in ventral view; without two ventral protuberances on middle part; epigynum with long and light bell-shaped region in median part; pore plates markedly thinner and longer than in *P. hoogstaali* (Figs 67–69) ..... ***P. pocamadre*** sp. nov.

22. Epigynum with ventral paired concavities on median part (VM 2010: figs 67, 151) 23
– Epigynum without ventral concavities on median part (VM 2010: figs 46, 81) 29

23. Epigynum with oval and large concavities on median part (VM 2010: figs 5, 151) 24
– Epigynum with small concavities on median part, "U"-shaped, close to epigastric furrow (VM 2010: figs 67, 144) 26

24. Chelicerae with stridulatory ridges; ventral apophyses of epigynum wide, forming a "T" shape with median region between central concavities; pore plates wide, slightly curved, pointing to each other (VM 2010: figs 151–153) ..... ***P. huacana*** Valdez-Mondragón, 2010
– Chelicerae without stridulatory ridges; ventral apophyses of epigynum thin, not forming a "T" shape with median region between central concavities (VM 2010: figs 5–7, 194–196) 25

25. Ventral apophyses of epigynum wider than in *P. sarae*, located on anterior part; in ventral view, apophyses pointing to each other; pore plates wide and oval, without oval translucid structures below them (VM 2010: figs 5–7) ..... ***P. bicornis*** Gertsch, 1971
– Ventral apophyses of epigynum thinner than in *P. bicornis*, located on central part; in ventral view, apophyses pointing downwards; pore plates small, slightly curved in posterior part, located above two oval translucid structures (VM 2010: figs 194–196) ..... ***P. sarae*** Valdez-Mondragón, 2010

26. Ventral concavities of epigynum close to each other (VM 2010: fig. 67) 27
– Ventral concavities of epigynum separated from each other (VM 2010: fig. 144) 28

27. Epigynum with ventral apophyses small and triangular in ventral view; ventral concavities on posterior part, with "W" shape; pore plates oval, smaller than in *P. paredesi*, above translucid structures (VM 2010: figs 67–69) ..... ***P. lautus*** Gertsch, 1971
– Epigynum with apophyses small and conical in ventral view; ventral concavities large and oval, on central part; pore plates semicircular, longer than in *P. lautus*, without oval translucid structures below (Valdez-Mondragón 2014: figs 31–33) ..... ***P. paredesi*** Valdez-Mondragón, 2010

28. Epigynum with ventral apophyses wide in ventral view, close to each other, ending in a rounded tip, located on median part; in ventral view, two deep and elongated anterior concavities absent; pore plates small and oval, above oval translucid structures (VM 2010: figs 144–146) ..... ***P. gertschi*** Valdez-Mondragón, 2010
– Epigynum with ventral tiny apophyses, widely separated from each other, ending in a small sharp tip, located on anterior margin of epigynum; in ventral view, two deep and elongated anterior concavities

present; pore plates wide and oval, with a small contraction in middle, without oval translucid structures below (VM 2010: figs 201–203) ..... ***P. platnicki*** Valdez-Mondragón, 2010

29. Epigynum with ventral apophyses small, conical, on median part (VM 2010: figs: 81, 83) 30
– Epigynum with ventral apophyses with different size, shape and position, or even absent (VM 2010: figs 116, 118) 33

30. Chelicerae without stridulatory ridges; epigynum corrugated (Figs 55–56) 31
– Chelicerae with stridulatory ridges; epigynum not corrugated (VM 2010: fig. 81) 32

31. Pore plates circular, small, widely separated from each other, without translucid structures below them; in lateral view, epigynum without curved concavity (VM 2010: figs 158–160) ..... ***P. montanoi*** Valdez-Mondragón, 2010
– Pore plates oval, longer than in *P. montanoi*, close to each other, with translucid oval structures below; in lateral view, epigynum with curved concavity (Figs 55–57) ..... ***P. sikuapu*** sp. nov.

32. Epigynum with ventral apophyses small and conical in ventral view, thin and conical in lateral view; pore plates elongated, above oval translucid structures (VM 2010: figs 81–83) ..... ***P. modestus*** Gertsch, 1971
– Epigynum with ventral apophyses small and slightly elongated in ventral view, wide and conical in lateral view; pore plates oval, above oval translucid structures (Nolasco & Valdez-Mondragón 2020: figs 14–16) ..... ***P. xerophilus*** Nolasco & Valdez-Mondragón, 2020

33. Epigynum with rounded shape, without ventral apophyses; with dark central spot; pore plates short and triangular, above oval translucid structures (VM 2010: figs 116–119) ..... ***P. validus*** Gertsch, 1971
– Epigynum with bell shape, with ventral apophyses; without dark central spot; pore plates oval or elongated, without oval translucid structures below (Figs 31–33) 34

34. Pore plates elongated, wider distally; conical apophyses located on two dark regions; epigynum with paired concavities close to epigastric furrow (Figs 31–33) ..... ***P. mariachi*** sp. nov.
– Pore plates oval; conical apophyses not located on two dark regions; epigynum without paired concavities close to epigastric furrow (VM 2010: figs 46–47, 53–54) 35

35. Epigynum with one long and curved apophysis, slightly bifurcated at tip; pore plates with half oval shape, close to each other (VM 2010: figs 46–48) ..... ***P. globosus*** (Taczanowski, 1874)
– Epigynum with three ventral apophyses, one on anterior part and two in median, not bifurcated distally; pore plates oval, separated by their own width (VM 2010: figs 53–55) ..... ***P. guanacaste*** Huber, 1998

Physocyclus mariachi sp. nov. Nolasco & Valdez-Mondragón
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Figs 14–33

Differential diagnosis

Males of *Physocyclus mariachi* sp. nov. resemble *P. paredesi* in the shape of LAC, and by absence of sclerotized cones in frontal part of chelicerae (Figs 17–18, 24; VM 2010: figs: 204, 205). In *P. mariachi* sp. nov. (1) two longer and more sclerotized LAC on each male chelicera (Figs 17–19, 24–25; VM 2010: figs 204–205; Valdez-Mondragón 2014: figs 21, 23–24); (2) LAC wider, more curved (Figs 17–18, 24); (3) distal-frontal apophyses of chelicerae longer, with some sclerotized ones (Figs 17–18, 24); (4) in retrolateral view, PR of male palp darker, straight (Figs 21, 26); (5) distal

spine of PR harpoon-shaped; (6) in dorsal view, ES wide, sigmoid-shaped (Figs 22–23, 27); (7) in retrolateral view, E thinner but longer, with distal concavity between two small sclerotized spines (Figs 21–23, 26–27). Females of *P. mariachi* sp. nov. with (1) triangular epigynum (Fig. 31); (2) with small and conical apophyses on anterior part, located on two dark regions (Figs 31–32); (3) in dorsal view, PP long, thin, in convergent position to each other (Fig. 33); PP oval, almost circular in *P. paredesi* (Valdez-Mondragón 2014: fig. 33).

Figs 8–13. Typical habitats of the spider genus *Physocyclus* Simon, 1893 from Mexico. **8**. Deciduous forests (Nayarit). **9–10**. Oasis in desert (Baja California Sur). **11**. Cave entrance located in a deciduous forest (Guerrero). **12**. Thorny scrub with columnar cacti (Guanajuato). **13**. Xerophilous scrub (Baja California Sur).

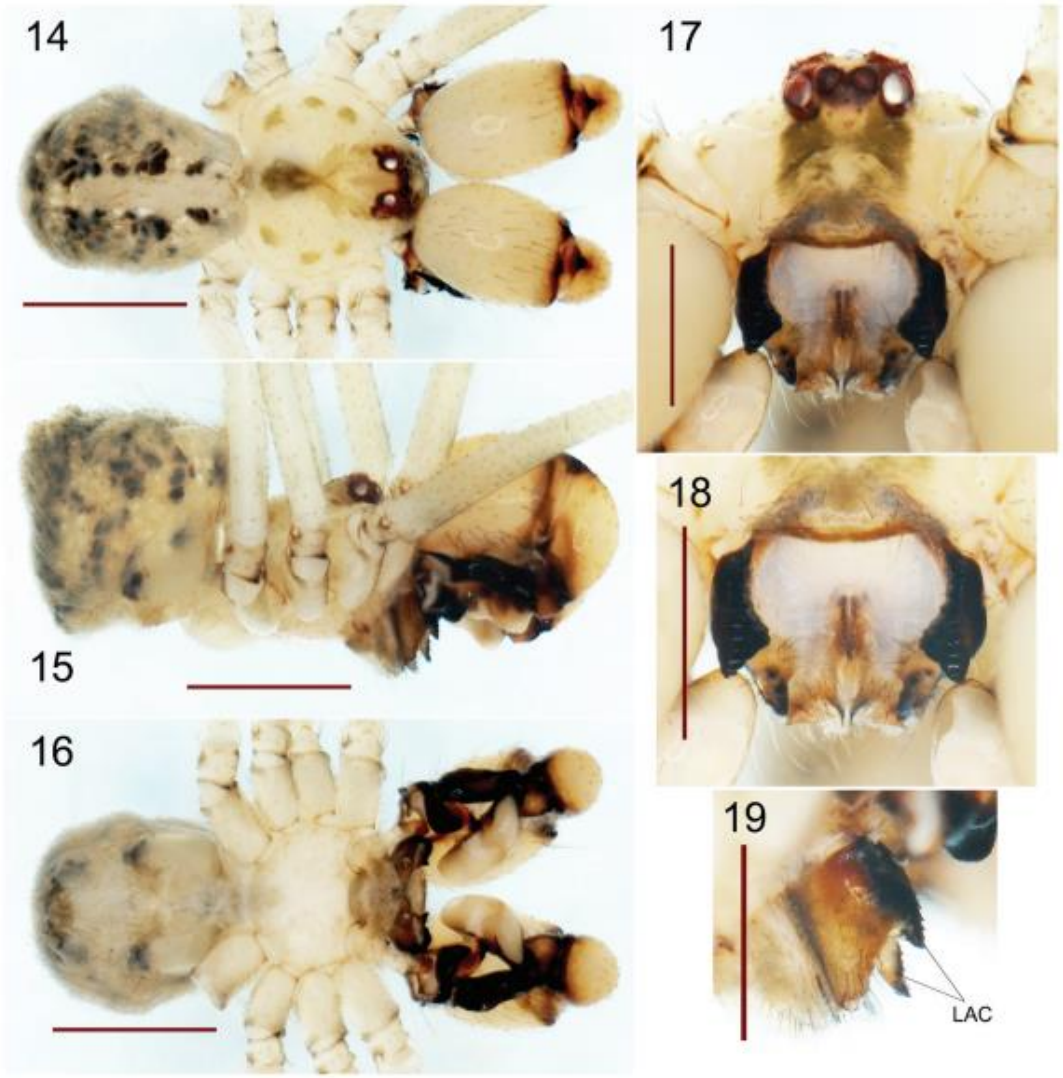
Etymology

The species name is a noun in apposition, and it refers to a genre of regional and popular Mexican music commonly known as "Mariachi", from the state of Jalisco where the type locality of the species is located.

Material examined

Holotype

MEXICO • ♂; Jalisco, Municipality of Hostotipaquillo, 2 km SW of Hostotipaquillo; 21.0879° N, 104.0685° W; 1355 m a.s.l.; 9 Nov. 2020; A. Valdez, I. Navarro, A. Juárez and S. Nolasco leg.; daytime collection; CNAN-T01471.



Figs 14–19. *Physocyclus mariachi* sp. nov. Male (holotype). **14–16**. Habitus in dorsal, lateral and ventral views, respectively. **17**. Carapace, frontal view. **18**, **19**. Chelicerae in frontal and lateral views, respectively. Scale bars: 14–16 = 1 mm; 17–19 = 0.5 mm.

Paratypes

MEXICO • 1 ♀; same collection data as for holotype; CNAN-T01472 • 1 ♀; Municipality of Plan de Barrancas, 2.5 km SE of Plan de Barrancas; 21.0239° N, 104.1907° W; 915 m a.s.l.; 9 Nov. 2020; A. Valdez, I. Navarro, A. Juárez and S. Nolasco leg.; daytime collection; CNAN-T01473.

Other material

MEXICO • 1 ♂, 1 ♀; Nayarit, Municipality of El Nayar, Arroyo Santiago; AMNH • 1 immature; same collection data as for holotype; LATLAX • 1 immature; same collection data as for second paratype; LATLAX.

Description

Male (holotype, CNAN-T01471)

Measurements. Total length 2.5. Carapace 1.11 long, 1.3 wide. Clypeus 0.5 long. Diameter AME 0.08, ALE 0.12, PME 0.10, PLE 0.12. Distance ALE-PME 0.06, PME-PME 0.13. Leg lengths: I (total 23.5): femur 6.4/patella 0.5/tibia 6.7/metatarsus 8.5/tarsus 1.3; tibia II: 4.5; tibia III: 3.1; tibia IV: 5; tibia I L/d 40.5.

Prosoma. Carapace light beige with Y-shaped light gray pattern around fovea and posterior part of carapace. Fovea longitudinal. Carapace with three irregular spots on each side (Figs 14–15). Clypeus wide, with dark brown irregular mark lengthwise (Fig. 17). Chelicerae pale, with two lateral apophyses on each side, with triangular tip and pointing down (Figs 17–19, 24–25). Anterior apophyses of chelicerae wide, dark, sclerotized, with small cones, in lateral view looks like a sawn surface. Posterior apophyses of chelicera small and thinner than anterior apophyses, with 4–5 sclerotized cones on each side (Figs 17–19, 24–25). Chelicerae with stridulatory files. Sternum light beige, with small and inconspicuous light brown spots. Labium brown, wider than long. Endites brown prolaterally, beige retrolaterally, longer than wide (Fig. 16).

Legs. All segments with beige coloration. Tibiae and femora with brown rings distally. Trochanters light brown (Figs 14–16).

Opisthosoma. Globular, longer than wide, high, with numerous white, gray and dark spots (Figs 14–15). Spinnerets dark brown, with short seta around (Fig. 16).

Palp. DAP wide and dark (Fig. 21). PR entirely dark, with notch in middle part, ending in long thin spine (Figs 21–23, 26). ES small and short, with oval shape in prolateral and retrolateral views (Figs 20–21, 26), wide, long and sinuous in dorsal view (Figs 22–23, 27). E small and short in retrolateral view, ending in a small tip. SO positioned in distal part of E (Fig. 26).

Female (paratype, CNAN-T01472)

Similar to the male, differences:

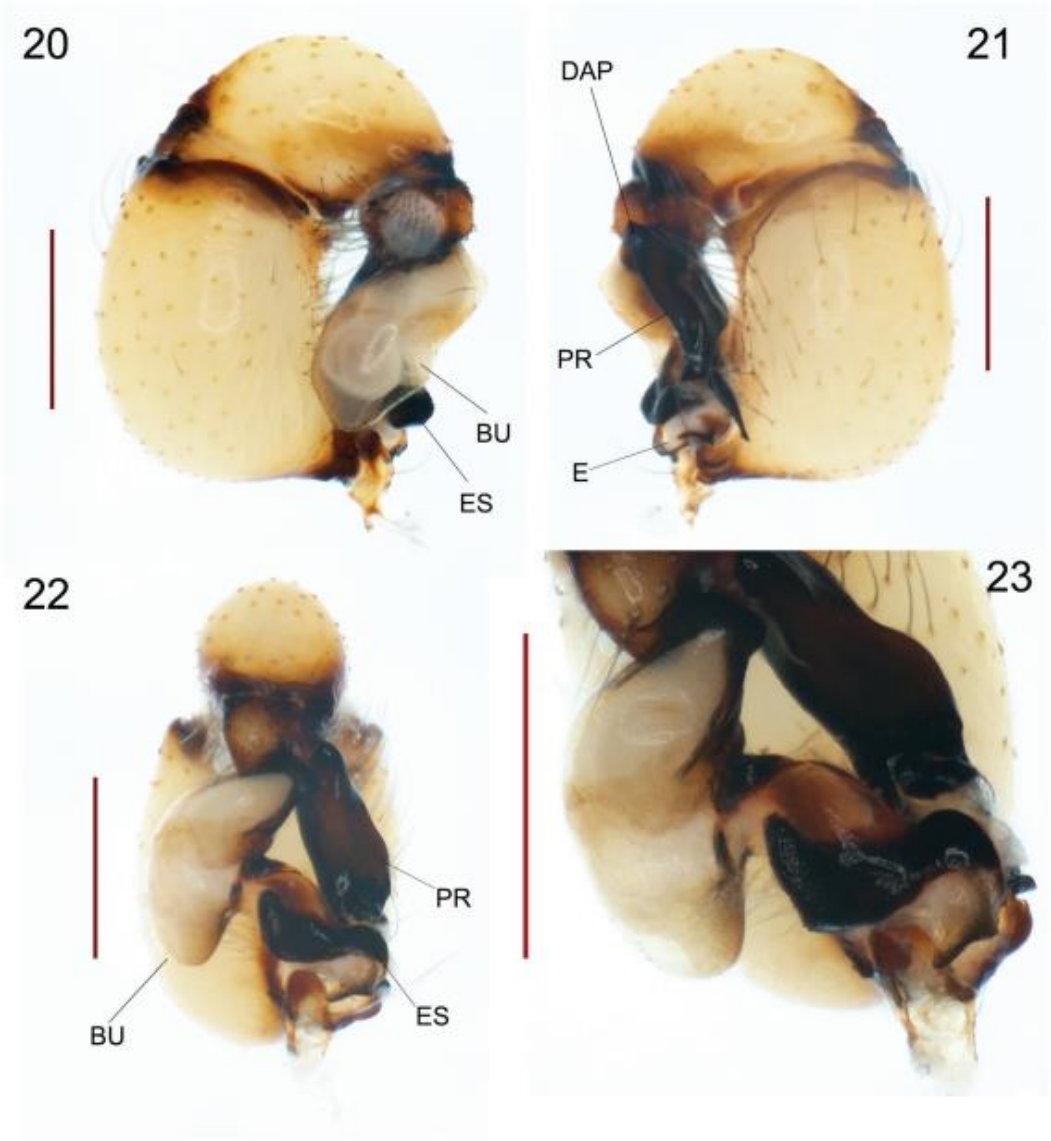
Prosoma. Carapace clearer, almost white, with darker dorsal pattern (Fig. 28). Carapace with small protuberance on posterior part (Fig. 29, red arrow). Sternum brown, with beige region in middle part (Fig. 30). Clypeus darker than in male, beige region in central part. Chelicerae with brown coloration, without LAC or sclerotized cones (Fig. 29).

Opisthosoma. With dorsal patch in anterior part (Fig. 28, red arrow). This structure might be a functional complex (with the protuberance of prosoma).

Legs. Femora and tibiae with darker rings distally (Figs 28–30).

EPIGYNUM. Wider than long, bell-shaped, with VAE small, conical, pointing forward, surrounded by dark spot, with clear stripe across epigynum longitudinally (Figs 31–32). PP small and elongated, oval-shaped, located in perpendicular position (Fig. 33).

MEASUREMENTS. Total length 3.7. Carapace 1.2 long, 1.2 wide. Clypeus 0.4 long. Diameter AME 0.08, ALE 0.12, PME 0.11, PLE 0.11. Distance ALE-PME 0.06, PME-PME 0.12. Leg lengths: I (total 16.8): femur 4.4/patella 0.5/tibia 4.6/metatarsus 6.1/tarsus 1.1; tibia II: 3.3; tibia III: 2.4; tibia IV: 3.6; tibia I L/d: 24.6.



Figs 20–23. *Physocyclus mariachi* sp. nov. Male (holotype). **20–22**. Left palp, prolateral, retrolateral and dorsal views, respectively. **23**. Bulb of male palp, dorsal view. Scale bars: 0.5 mm.

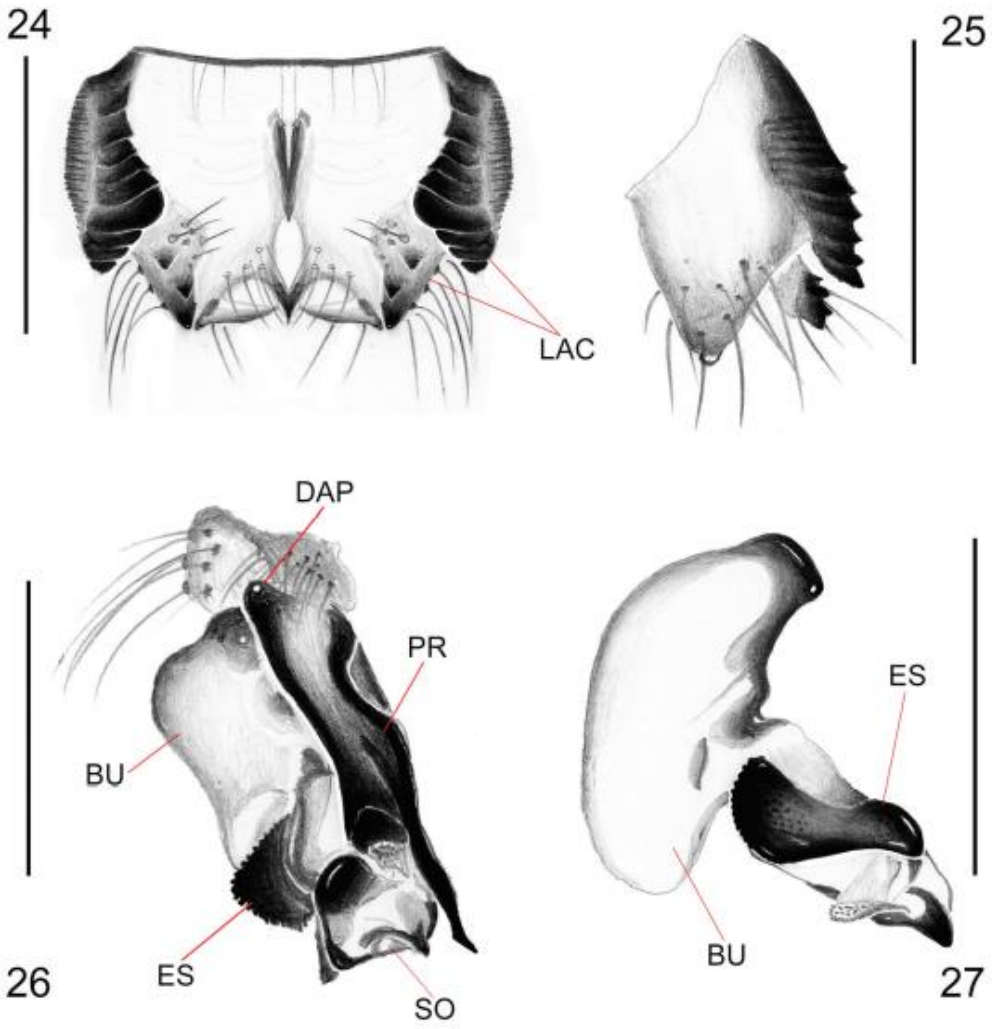
Variation

Females

Female collected in Plan de Barrancas is bigger than female collected at type locality. With an ocher coloration. The coloration of the dorsal pattern of the carapace and chelicerae is darker in this female. (*N*=1): Tibia I: 5.7; tibia II: 3.9; tibia III: 2.9; tibia IV: 4.4; tibia I L/d: 26.2.

Distribution

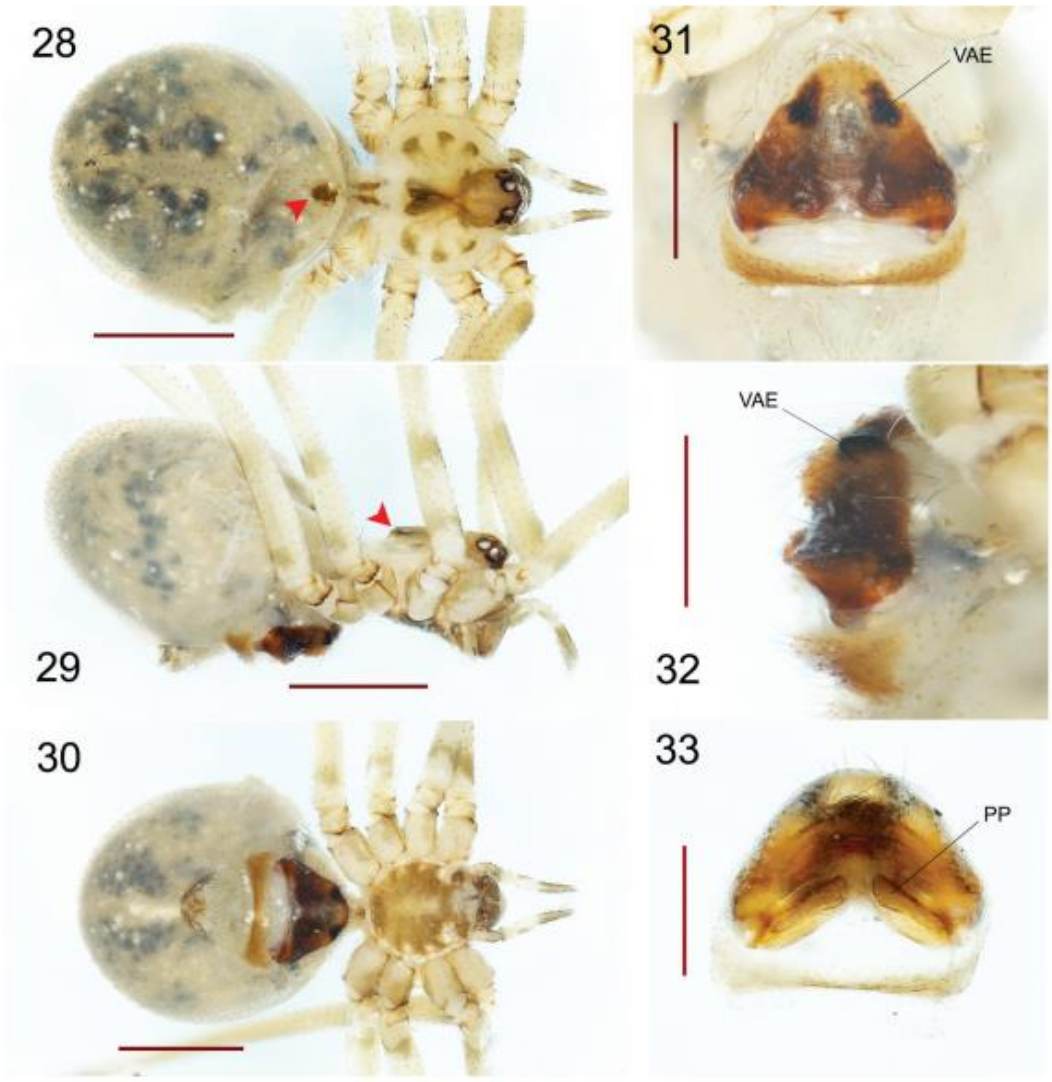
Mexico: Jalisco (Fig. 78).



Figs 24–27. *Physocyclus mariachi* sp. nov. Male (holotype). **24–25**. Detail of chelicerae, frontal and lateral views, respectively. **26**. Details of procursus, embolus and embolic sclerites of left palp, retrolateral view. **27**. Detail of bulb, dorsal view. Scale bars: 0.5 mm.

Remarks

This new species was previously recorded by W. J. Gertsch from Arroyo Santiago, Nayarit, Mexico, who tentatively named the species as *Physocyclus* "*nayaritus*". The species was never described, however, Gertsch drew some sketches consulted by A. Valdez-Mondragón at the American Museum of Natural History (AMNH) (year 2008). Those sketches are shown herein (Figs 34–37).



Figs 28–33. *Physocyclus mariachi* sp. nov. Female (paratype). **28–30**. Habitus in dorsal, lateral and ventral views, respectively; red arrows indicate a dorsal patch in the opisthosoma (28) and the dorsal protuberance in carapace (29). **31–33**. Epigynum, ventral, lateral and dorsal views, respectively. Scale bars: 28–30 = 1 mm; 31–33 = 0.5 mm.

Natural history

The specimens were collected among boulders on the ground. The male type and one female were collected close together during copulation. The vegetation of the type locality was disturbed deciduous forest (Figs 70–71).

Physocyclus sikuapu sp. nov. Valdez-Mondragón & Nolasco
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Figs 38–57

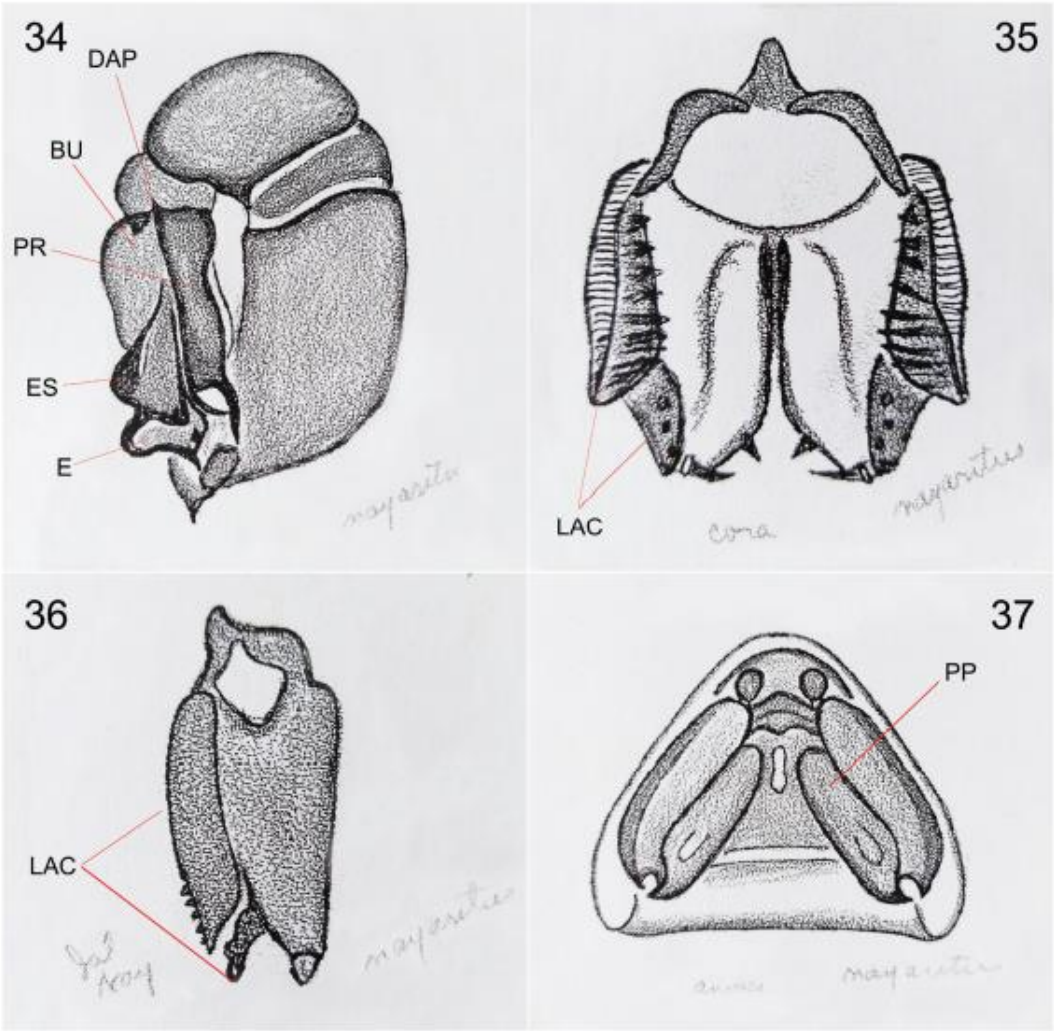
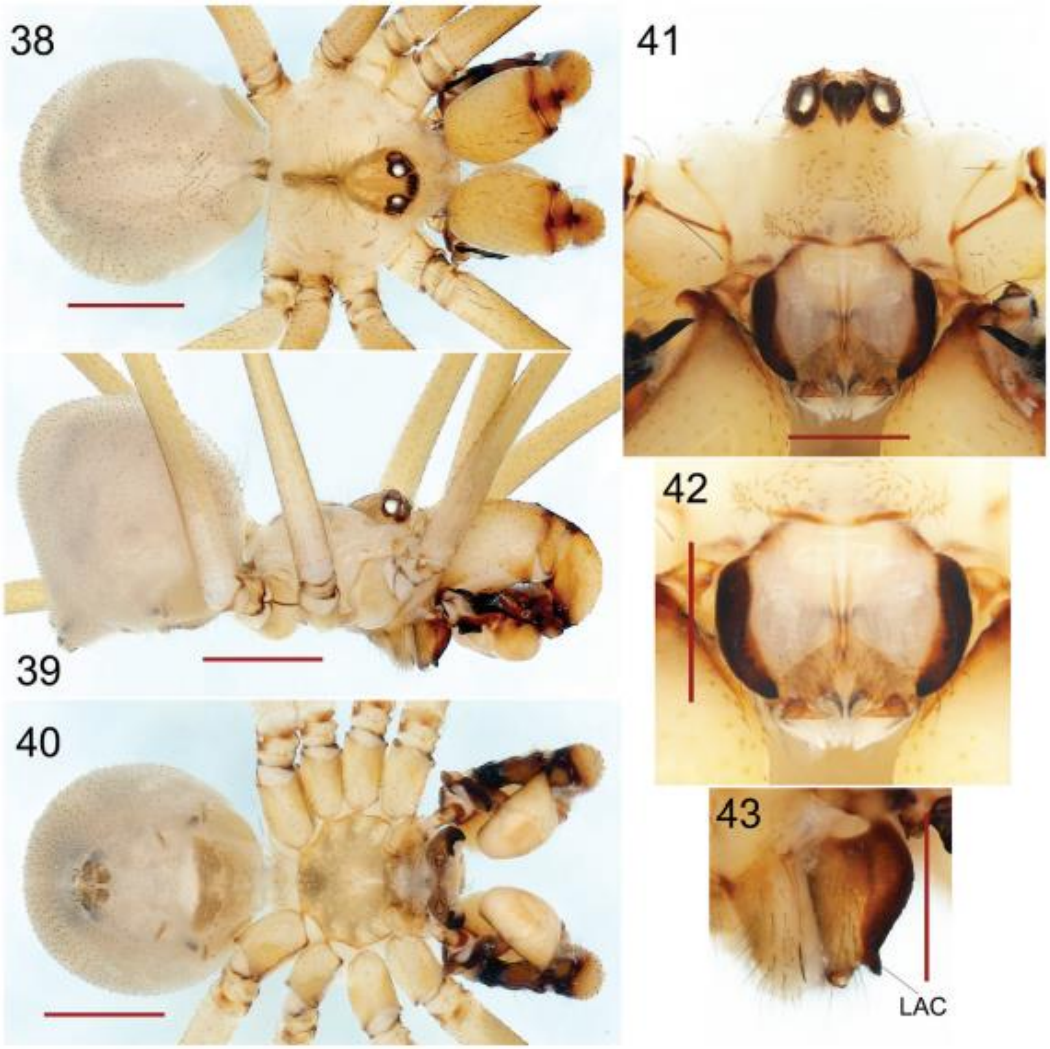


Fig. 34–37. Unpublished drawings of *P. mariachi* sp. nov. (= *P.* "*nayaritus*") made by W.J. Gertsch, archived at AMNH. **34**. Male palp in retrolateral view. **35–36**. Left male chelicerae in frontal and retrolateral views, respectively. **37**. Female epigynum, dorsal view.

Differential diagnosis

Males of *Physocyclus sikuapu* sp. nov. resemble *P. globosus* in having LAC short, by shape of distal spine of PR, pointing to the base of palp femora, and by the half-moon shape of the EE (Figs 42–44, 48–49; VM 2010: figs 42–44). In *P. sikuapu* sp. nov. (1) chelicerae completely flat, without sclerotized cones, with uniform beige coloration (Figs 42–43, 48–49); (2) LAC short, conical, pointing down, with SF occupying entire length of chelicerae (Figs 41–43, 48–49); (3) ES wider, shorter (Figs 44, 46–47, 50–51). Females of *P. sikuapu* sp. nov. with (1) bell-shaped epigynum in frontal view, with stretch marks transversally in posterior part (Figs 55–56); (2) VAE small, slightly curved in lateral view (Fig. 56); (3) in dorsal view, PP long, oval, with two bag-shaped structures below them (Fig. 57, red arrow).



Figs 38–43. *Physocyclus sikuapu* sp. nov. Male (holotype). **38–40**. Habitus in dorsal, lateral and ventral views, respectively. **41**. Carapace in frontal view. **42–43**. Chelicerae in frontal and lateral views, respectively. Scale bars: 38–40 = 1 mm; 41–43 = 0.5 mm.

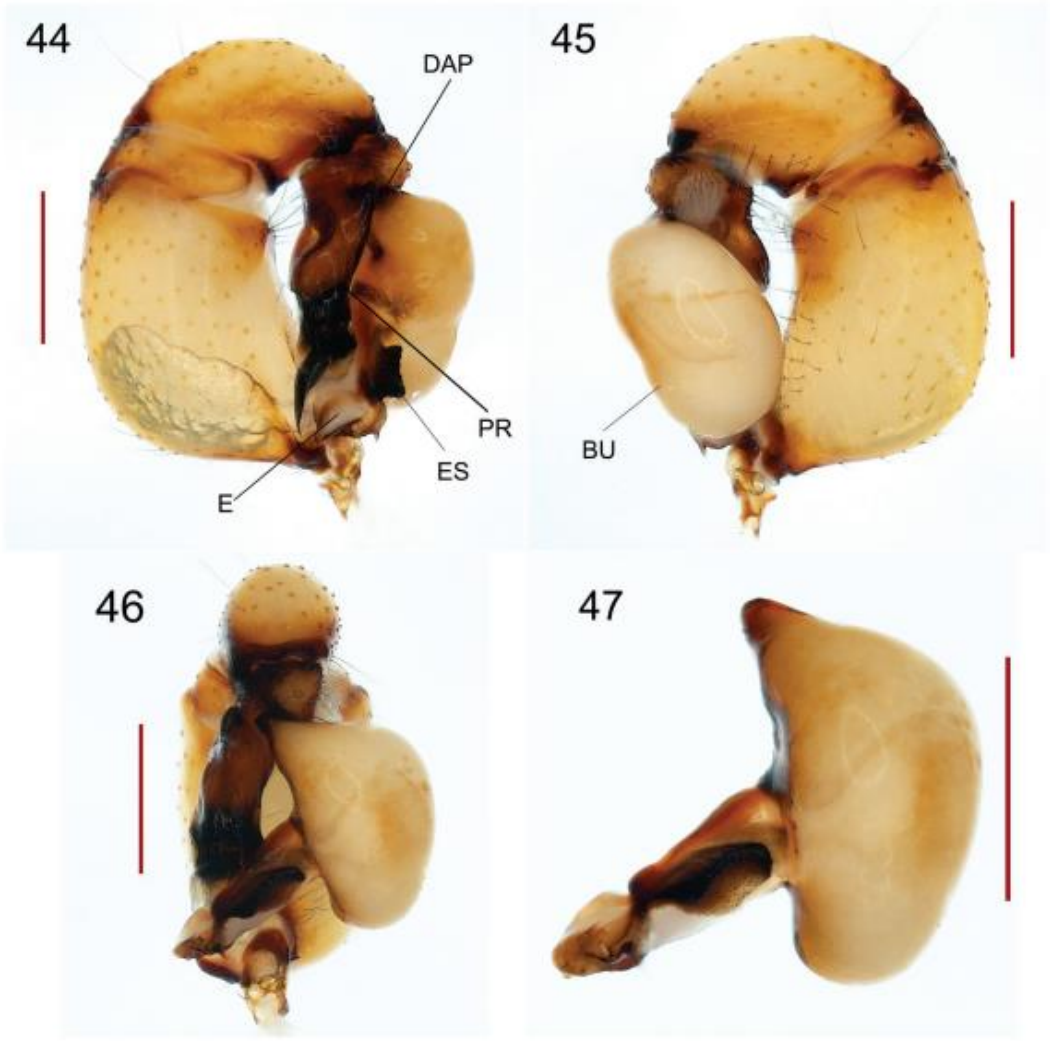
Etymology

The species name is a noun in apposition that means spider (sīkuapu = *spider*) in the Purépecha language from the pre-Columbian Purépecha state, which existed from the early 14th century until 1530, and today is known as the state of Michoacan, where the type locality is found.

Material examined

Holotype

MEXICO • ♂; Michoacan, Municipality of Aquila, cave at the viewpoint of Costa Aquila; 18.5634° N, 103.6471° W; 160 m.a.s.l.; 19 Nov 2020; A. Valdez, I. Navarro, A. Juárez and S. Nolasco leg; daytime collection; CNAN-T01474.



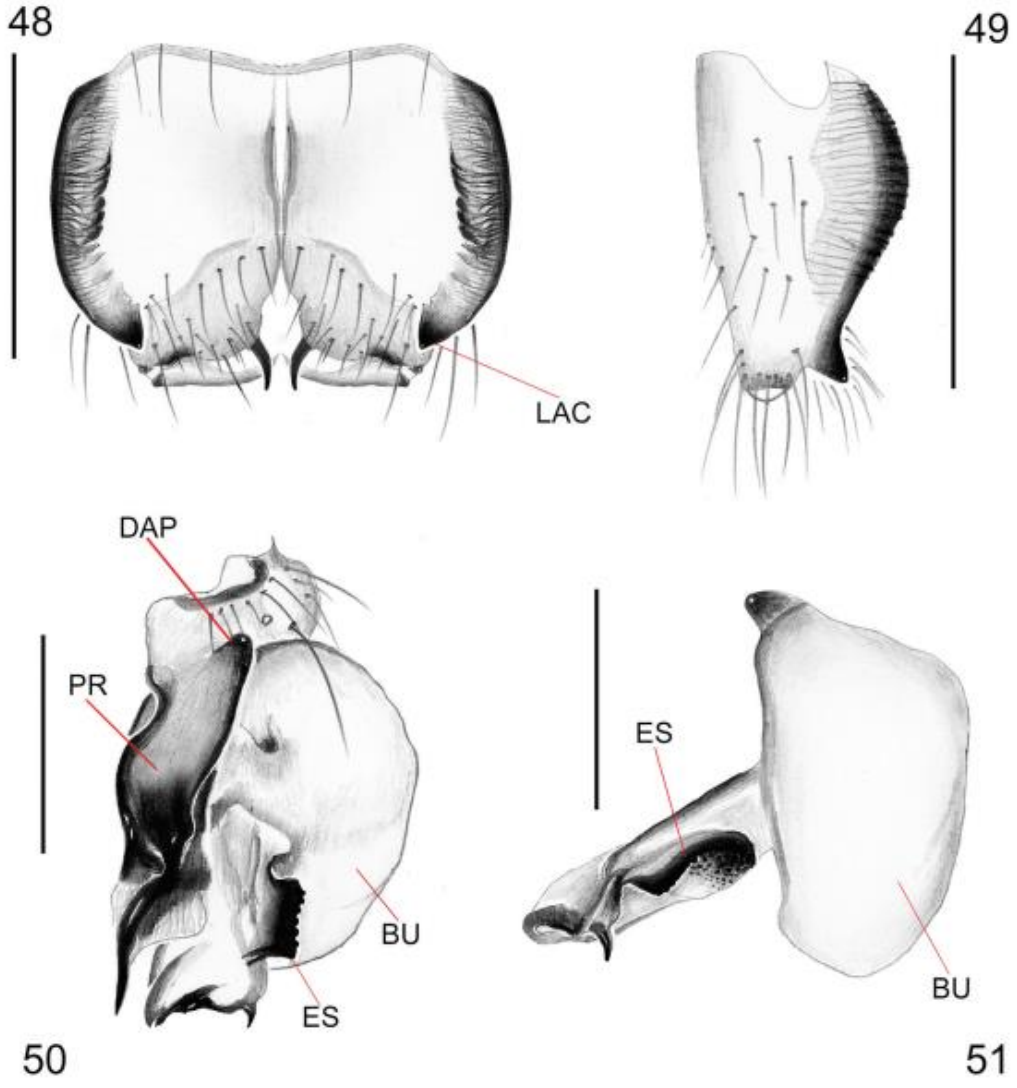
Figs 44–47. *Physocyclus sikuapu* sp. nov. Male (holotype). **44–46**. Right palp, prolateral, retrolateral and dorsal views, respectively. **47**. Bulb of male palp, dorsal view. Scale bars= 0.5 mm.

Paratypes

MEXICO • 1 ♂; same collection data as for holotype; CNAN-T01474 • 2 ♀♀; same collection data as for holotype; CNAN-T01475.

Other material

MEXICO • 8 immatures; same collection data as for holotype; LATLAX.

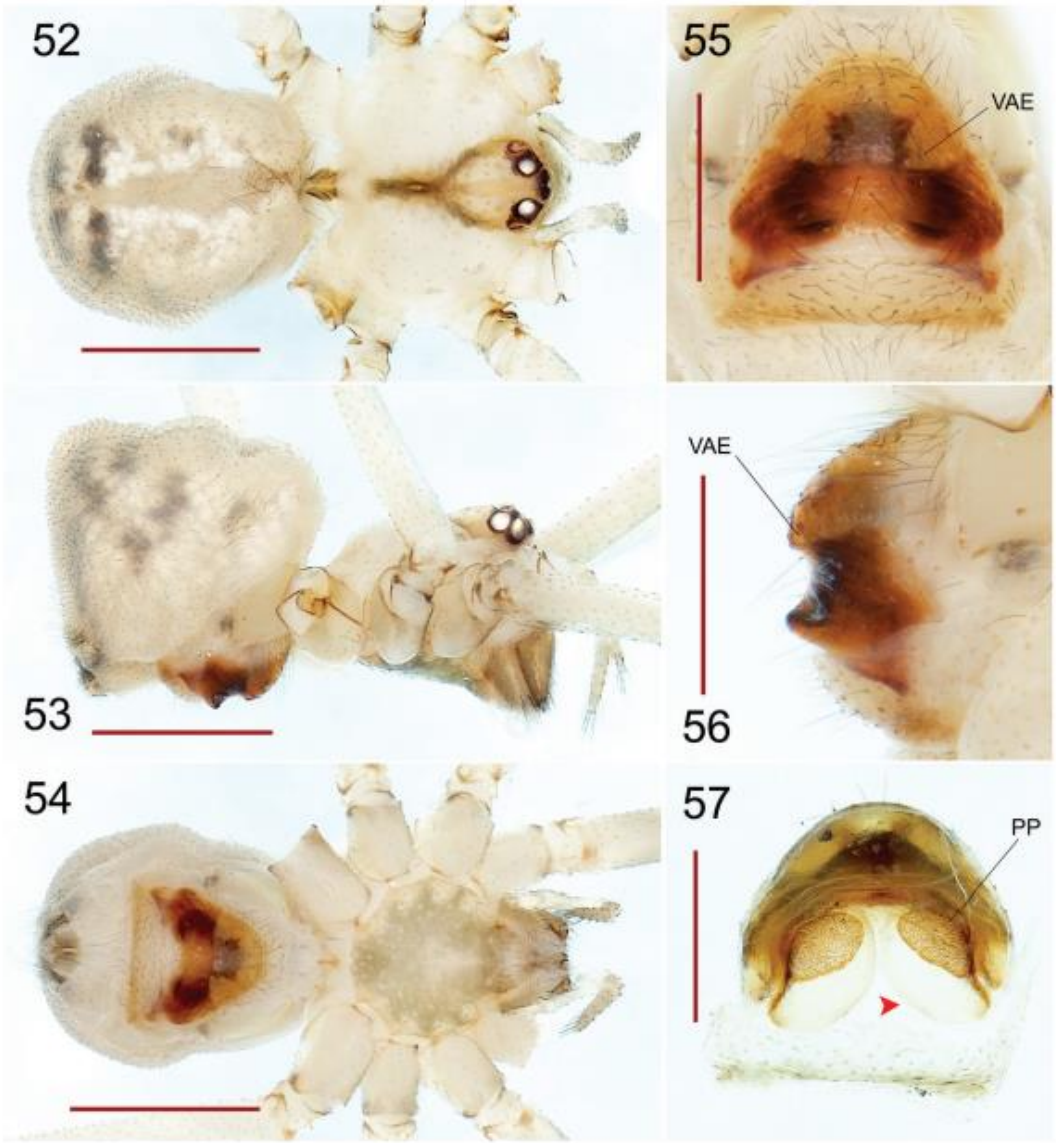


Figs 48–51. *Physocyclus sikuapu* sp. nov. Male (holotype). **48–49**. Detail of chelicerae, frontal and lateral views, respectively. **50**. Details of procursus, embolus and embolic sclerites of right palp, in retrolateral view. **51**. Detail of bulb, dorsal view. Scale bars= 0.5 mm.

Description

Male (holotype, CNAN-T01474)

MEASUREMENTS. Total length 3.8. Carapace 1.7 long, 1.6 wide. Clypeus 0.6 long. Diameter AME 0.08, ALE 0.12, PME 0.11, PLE 0.12. Distance ALE-PME 0.06, PME-PME 0.13. Leg lengths: I (total 38.7): femur 9.8/patella 0.6/tibia 10.6/metatarsus 15.9/tarsus 1.7; tibia II: 7.5; tibia III: 5.3; tibia IV: 7.4. tibia I L/d: 61.2.



Figs 52–57. *Physocyclus sikuapu* sp. nov. Female (paratype). **52–54**. Habitus in dorsal, lateral and ventral views, respectively. **55–57**. Epigynum, ventral, lateral and dorsal views, respectively (red arrow indicates translucid bag-shaped structure). Scale bars: 52–54 = 1 mm; 55–57 = 0.5 mm.

Prosoma. Carapace light brown, with a Y-shaped mark around fovea and posterior part of ocular region. Fovea longitudinal. Carapace with three conspicuous irregular spots, barely visible on each side (Fig. 38). Clypeus wide, with same color of carapace (Fig. 41). Chelicerae light beige, without sclerotized cones, with short and conical lateral apophyses, pointing down (Figs 42, 48). SF laterally occupying total length of chelicerae (Figs 42–43, 48–49). Sternum pale grey, with four pale marks on each side. Labium brown, wider than long. Endites brown, longer than wide (Fig. 40).

Legs. All segments with light beige coloration. Trochanters light brown, darker than in female. Femora and tibiae with barely visible ring-shaped marks distally.

Opisthosoma. Globular, longer than wide, with inconspicuous irregular marks. Spinnerets light beige (Figs 38–40).

Palp. DAP thin and conical. In retrolateral view, PR slightly sinuous, with basal half light brown and distal half dark, ending in a long, curved, distal spine (Figs 44, 50). PR with a notch in basal half. In retrolateral view, ES short, square-shaped (Figs 44, 50); in dorsal view curved, C-shaped (Figs 46–47, 51). E short, square-shaped distally in prolateral view (Figs 44, 20), long and semi-conical in dorsal view (Figs 46–47, 51).

Female (paratype, CNAN-T01475)
Similar to the male, differences:

Prosoma. Carapace with pale coloration (Fig. 52). With a Y-shaped mark darker than in male. Clypeus slightly darker than in male. Chelicerae brown, without sclerotized cones or lateral apophyses (Fig. 53).

Opisthosoma. With numerous and irregular grey, white and brown marks (Figs 52–54).

Epigynum. Wider than long, bell-shaped, VAE small and slightly curved in lateral view (Figs 55–56). Anterior part of epigynum has a central gray spot, between the VAE (Fig. 55). Posterior part of epigynum darker than anterior part and seems to have stretch marks transversally (Figs 55–56). PP oval, with a pair of bag-shaped structures below them, which have an oval shape (Fig. 57).

Measurements. Total length: 3. Carapace 1.3 long, 1.3 wide. Clypeus: 0.5. Diameter AME 0.06, ALE 0.11, PME 0.08, PLE 0.12. Distance ALE-PME 0.08, PME-PME 0.12. Leg lengths: I (total 27.7): femur 6.8/patella 0.6/tibia 7.8/metatarsus 11/tarsus 1.6; tibia II: 5.3; tibia III: 3.8; tibia IV:5.5; tibia I L/d: 52.3.

Variation

Males

Male paratype collected at the same locality as male holotype is smaller, with a light beige coloration. Sternum with same color as carapace, with irregular darker marks in the periphery. The marks in the opisthosoma are slightly darker. (*N*=1): tibia I: missing; tibia II: 7.6; tibia III: 5.4; tibia IV: 7.6.

Females

The other female paratype is a little bigger, with the VAE coloration clearer. (*N*=1): tibia I: 8.6; tibia II: 6; tibia III: 4.5; tibia IV: 6.3.

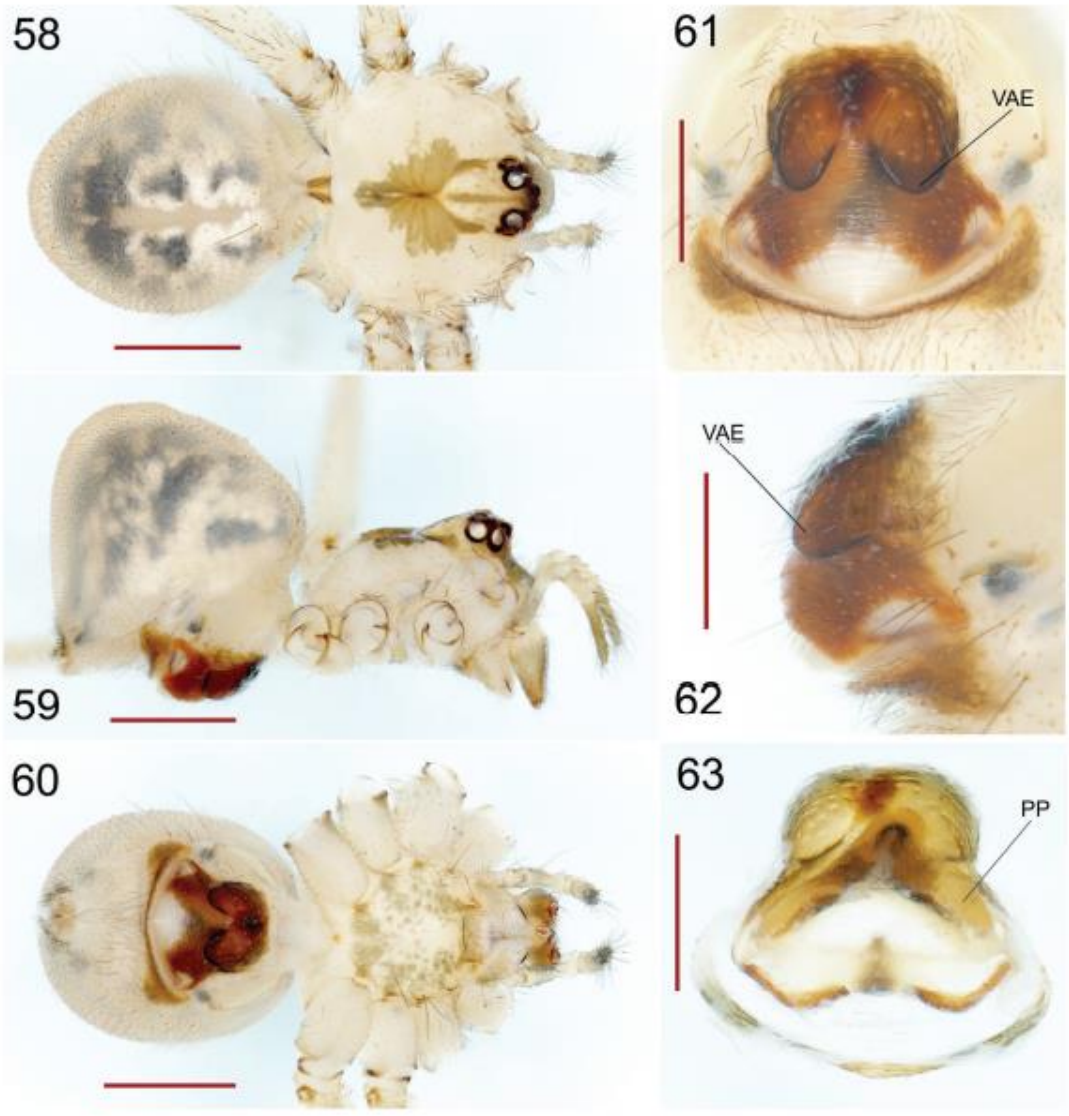
Natural history

The specimens were collected among big boulders in a disturbed tropical deciduous forest, and inside of a small karstic cave on walls and in the ground (Figs 72–73).

Distribution

Mexico: Michoacan (Fig. 78).

Physocyclus lyncis sp. nov. Nolasco & Valdez-Mondragón
urn:lsid:zoobank.org:act:F0F73C91-0BF2-4841-88E6-56B9AE3D1BE5
Figs 58–63



Figs 58–63. *Physocyclus lyncis* sp. nov. Female (holotype). **58–60**. Habitus in dorsal, lateral and ventral views, respectively. **61–63**. Epigynum in ventral, lateral and dorsal views, respectively. Scale bars: 58–60 = 1 mm; 61–63 = 0.5 mm.

Differential diagnosis

Females of *Physocyclus lyncis* sp. nov. resemble *P. californicus* by shape of epigynum, with semisquare shape in anterior part; VAE with a flat, wide shape in frontal and lateral views (Fig. 61; VM 2010: figs 12, 14). In *P. lyncis* sp. nov. (1) VAE long, wide, almost flat, ending in rounded tip, with dark region between them (Figs 61–62); (2) VAE closer to each other; (3) PP long, elongated, slightly curved (Fig. 63); (4) sclerotized arc of uterus pointing toward right side; (5) body coloration light beige, with a light brown inverted triangle pattern in carapace (Fig. 58); (6) irregular pale gray marks spread on sternum (Fig. 60).

Etymology

The species name is a noun in apposition and refers to the type locality where the species was collected: Cueva del Lince, Ejido La Primavera, Ecotourist Park, Jalisco, Mexico.

Material examined

Holotype

MEXICO • ♀; Jalisco, Municipality of Zapopan, Cueva del Lince, Ejido La Primavera, Ecotourist Park; 20.7120° N, 103.5700° W; 1605 m a.s.l.; 8 Nov. 2020; A. Valdez, I. Navarro, A. Juárez and S. Nolasco leg.; daytime collection; CNAN-T01476.

Paratypes

MEXICO • 1 ♀; same collection data as for holotype; CNAN-T01477 • 2 ♀♀; Municipality of Zapopan, Ejido La Primavera, Ecotourist Park; 20.6892° N, 103.5820° W; 1645 m a.s.l.; 30 Mar. 2012; L. Olguín, J. Mendoza, G. Contreras, C. Santibañez and D. Ortiz leg.; daytime collection; CNAN-T01478.

Other material

MEXICO • 2 immatures; same collection data as for holotype; LATLAX.

Description

Female (holotype, CNAN-T01476)

Measurements. Total length: 4.5. Carapace 1.6 long, 1.9 wide. Clypeus 0.5. Diameter AME 0.10, ALE 0.12, PME 0.12, PLE 0.15. Distance ALE-PME 0.11, PME-PME 0.18. Leg lengths: I missing; tibia II: missing; tibia III: 4.8; tibia IV: 6.4.

Prosoma. Carapace light beige with light brown irregular pattern, triangular-shaped around fovea and posterior part of ocular region (Fig. 58). Fovea longitudinal. Clypeus entirely light gray, without marks. Chelicerae light gray, without stridulatory files (Fig. 59). Sternum with light beige coloration, with spread and irregular pale gray spots. Endites and labium longer than wide, with light brown coloration (Fig. 60).

Legs. All segments with same coloration as carapace. Tibiae and femora with light gray rings distally. Trochanter light gray retrolaterally (Fig. 60).

Opisthosoma. Globose, longer than wide, with big dark marks, gray and white (Figs 58–59). Spinnerets light brown (Fig. 60).

Epigynum. Wider than long, with semisquare shape in anterior part; VAE long, wide, semicircular distally and almost flat, pointing downwards (Figs 61–62). Pale region close to epigastric furrow (Fig. 61). PP wide, sclerotized arc of uterus (Fig. 63).

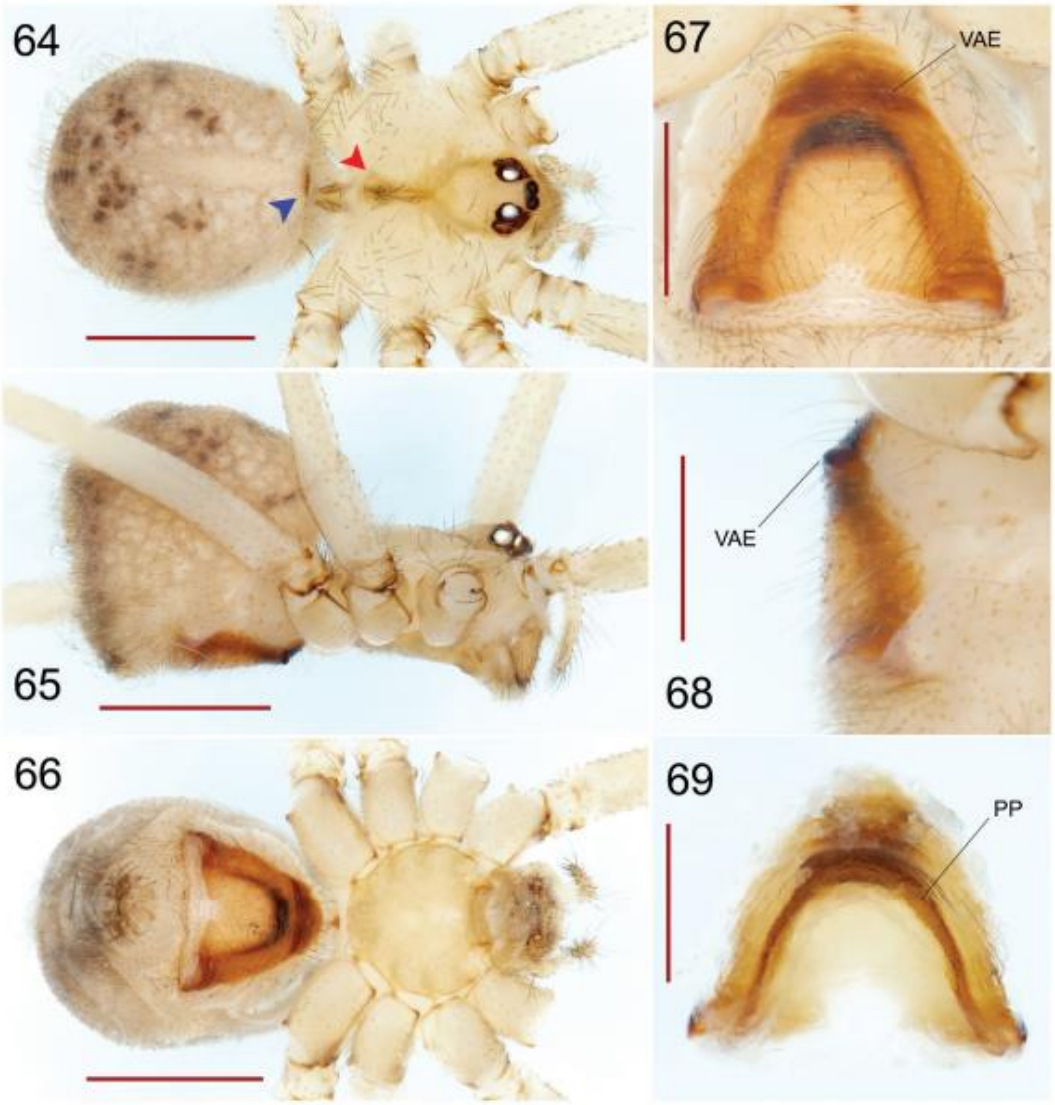
Male

Unknown.

Variation

Females

Female paratype collected from the type locality smaller than female holotype. The body coloration and its patterns are lighter. The marks on the ophistosoma are only dark. (*N*=1): tibia I: missing; tibia II: missing; tibia III: missing; tibia IV: 5.3. Females collected in 2012 are bigger than female holotype. Body coloration is ocher and the marks are dark brown. Dark region between the VAE is more marked



Figs 64–69. *Physocyclus pocamadre* sp. nov. Female (holotype). **64–66**. Habitus in dorsal, lateral and ventral views, respectively (red and blue arrows indicate the dorsal protuberance on carapace and dorsal patch on opisthosoma). **67–69**. Epigynum in ventral, lateral and dorsal views, respectively. Scale bars: 64–66 = 1 mm; 67–69 = 0.5 mm.

Differential diagnosis

Females of *Physocyclus pocamadre* sp. nov. resemble those of *P. xerophilus* by shape and size of VAE. In *P. pocamadre* sp. nov. (1) VAE slightly elongated, in parallel position (Fig. 67–68); (2) epigynum longer than wide (Fig. 67); (3) lateral constrictions of epigynum absent; (4) PP elongated, thin, without bag-shaped structures below (Fig. 69).

Etymology

The species name is a noun in apposition, from the Mexican slang words "poca madre", which mean *something cool* or *something nice*.

Material examined

Holotype

MEXICO • ♀; Baja California Sur, Municipality of La Paz, cave close to the beach "El Tecolote", highway La Paz – El Tecolote; 24.3330° N, −110.3127° W; 4 m a.s.l.; 7 Aug. 2019; A. Valdez, P. Solís, A. Cabrera and D. Montiel leg.; daytime collection; CNAN-T01479.

Figs 74–77. Habitat and microhabitat of *Physocyclys lyncis* sp. nov. (74–75) and *Physocyclus pocamadre* sp. nov. (76–77). **74**. Pine-oak forest, predominant vegetation in the Ejido La Primavera, Ecotourist Park, Zapopan, Jalisco, Mexico. **75**. "Cueva del Lince" (type locality), inside of the Ejido la Primavera, Ecotourist Park, Zapopan, Jalisco, Mexico. **76**. Habitat close to the beach "El Tecolote", La Paz, Baja California Sur. **77**. Xerophytic scrub, close to the beach "Punta San Francisquito" (type locality), Baja California.

Paratypes

MEXICO • 2 ♀♀; same collection data as for holotype; CNAN-T01480. • 1 ♀; Baja California, Municipality of Ensenada, beach "Punta San Francisquito", San Francisquito Bay; 28.4089° N, 112.8581° W; 4 m a.s.l.; 23 Jul. 2019; A. Valdez, P. Solís, A. Cabrera and D. Montiel leg.; nighttime collection; CNAN-T01481.

Description

Female (holotype, CNAN-T01479)

MEASUREMENTS. Total length 3.2. Carapace 1.4 long, 1.5 wide. Clypeus 0.3. Diameter AME 0.07, ALE 0.11, PME 0.11, PLE 0.12. Distance ALE-PME 0.05, PME-PME 0.13. Leg lengths: I (total 18.2): femur 5.4/patella 0.6/tibia 5.8/metatarsus 5.2/tarsus 1.2; tibia II: 4.3; tibia III: 3.3; tibia IV: 4.4; tibia I L/d: 41.1.

PROSOMA. Carapace beige color, with a Y-shaped pale brown pattern around fovea and posterior part of ocular region (Figs 64–65). Carapace with a dorsal protuberance in posterior part (red arrow on Fig. 64). Fovea longitudinal. Clypeus without marks, entirely pale beige. Chelicerae with light brown coloration, without stridulatory files (Fig. 65). Sternum pale brown. Labium and endites dark brown, both wider than long (Fig. 66).

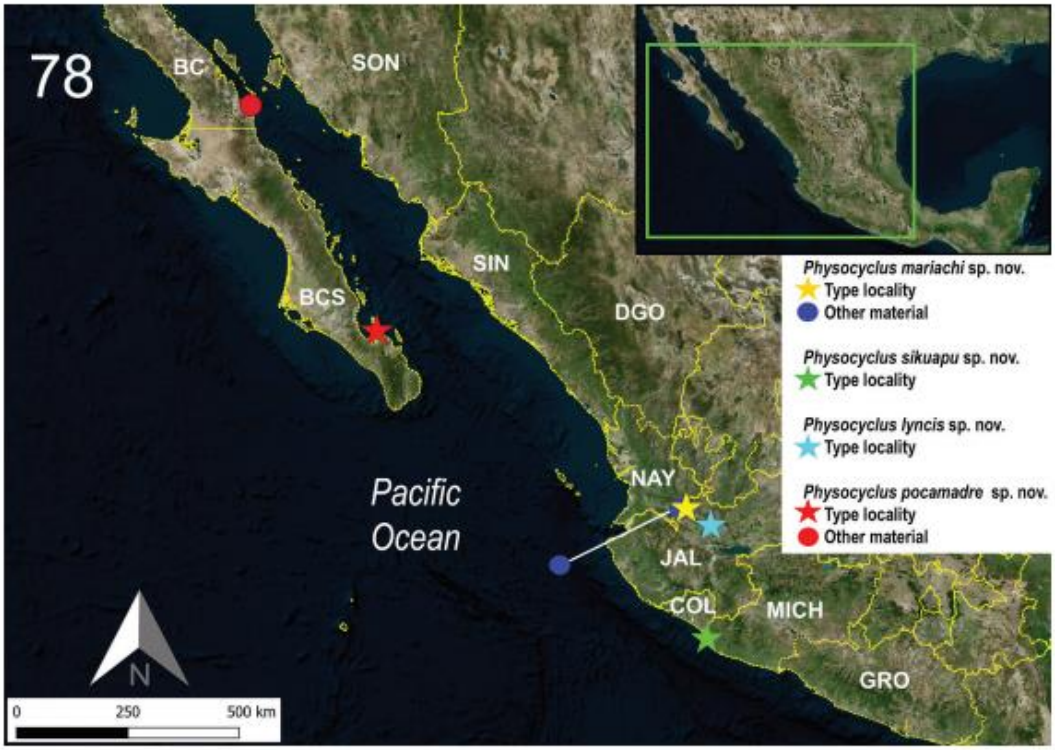


Fig. 78. Known records of *Physocyclus mariachi* sp. nov., *P. sikuapu* sp. nov., *P. lyncis* sp. nov., and *P. pocamadre* sp. nov. from Western Mexico. Abbreviations: BC = Baja California; BCS = Baja California Sur; COL = Colima; DGO = Durango; GRO = Guerrero; JAL = Jalisco; MICH = Michoacan; NAY = Nayarit; SIN = Sinaloa; SON = Sonora.

Legs. All segments with beige coloration. Tibiae and femora with light gray rings distally. Trochanters light beige, with a notch distally (Figs 64–66).

Opisthosoma. Longer than wide, with a dorsal patch located in anterior part (blue arrow on Fig. 64). This structure might be functional, together with dorsal protuberance of carapace (Fig. 64).

Epigynum. Longer than wide, with VAE small, semi-conical, pointing forward (Figs 67–68). VAE darker than rest of epigynum (Fig. 67). PP thin and markedly long (Fig. 69). Sclerotized arc of uterus thin (Fig. 69).

Male

Unknown.

Variation

Females

Females paratypes smaller than female holotype. One of them has a pale beige coloration and the pattern of the body has a light gray coloration, whereas the other female has a similar color as the female holotype. (*N*=2): female 1: tibia I: 5.3; tibia II: 3.9; tibia III: 2.9; tibia IV: 3.9. Female 2: tibia I: missing; tibia II: 3.6; tibia III: 2.7; tibia IV: 3.8. The ring coloration of tibiae and femora are light beige, barely visible. The pattern on carapace is slightly dark. (*N*=1): tibia I: 7.8; tibia II: 5.6; tibia III: 3.8; tibia IV: 5.4.

Natural history

The specimens were collected on their sheet webs among and under large boulders on the ground at both localities, close to the beaches "El Tecolote" and "Punta San Francisquito" (Figs 76–77). The vegetation type of both localities is dry xerophilous scrub, with cacti and scrubby vegetation (Figs 76–77). The specimens collected close to the beach "El Tecolote" were collected on their sheet webs among and under large boulders on the ground, and on walls of the cave, which is a concavity on a big wall.

Distribution

Mexico: Baja California and Baja California Sur (Fig. 78).

Discussion

The distribution pattern of the species groups is the Mesoamerican and Mexican Mountain biotic components for the *globosus* group, and the Mesoamerican and Continental Neartic biotic components for the *dugesi* group (Valdez-Mondragón 2013, 2014). Recent molecular phylogenetic studies under mitochondrial and nuclear markers support the monophyly of both species groups (in prep.).

Mexico is by far the richest country in terms of troglomorphic species of pholcid spiders, although the apparent dominance of species richness may be partly due to collectors' and taxonomists' biases (Huber 2018). Although the species of the genus *Physocyclus* are commonly found in caves and grottos in Mexico, no troglomorphic species has been collected so far, and all the species of the genus are considered troglophilic.

The total number of species of *Physocyclus* increases to 37, with 36 distributed along the Mexican territory. However, despite the growing knowledge of the genus *Physocyclus* since previous revisions by Valdez-Mondragón (2010) and the morphological phylogeny by Valdez-Mondragón (2013, 2014), the diversity of this genus in Mexico is still poorly known. The biogeography of Mexico is extremely complex; there were several dispersal and vicariance events because the Nearctic and Neotropical biotic

elements, known as the Mexican Transition Zone, overlap in Mexico (Morrone 2005). Provinces such as the Pacific Coast, Baja California, the Southern Altiplano, the Sonorense Province and long regions of tropical deciduous forest in the Pacific region (Valdez-Mondragón & Francke 2015: figs 22–23) are poorly sampled. The province of the Balsas Depression (BD), which has the highest diversity of species of *Physocyclus*, is the one that exhibits a greater influence of the Tropical Mesoamerican element. The vicariant events associated with the biotic evolution of the Transitional component would be related to the development of the Sierras Madre and the volcanism of the Trans-Mexican Volcanic Belt, which explain why the BD is one of the most biodiverse regions in the country (Morrone 2005).

Intensive sampling is needed to uncover the diversity of these spiders mainly in such semiarid ecosystems, xeric shrub zones, deserts, and tropical deciduous forest. Additionally, caves from tropical deciduous forest, a typical ambient for some genera of pholcids from Mexico, could be a significant source of new species (Valdez-Mondragón 2010).

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12. Capítulo 4

Artículo Taxonómico

On the daddy long-legs spiders of the genus *Physocyclus* (Araneae: Pholcidae) from Mexico: description of a new species from the Baja California Peninsula

Samuel Nolasco – Alejandro Valdez Mondragón

En las arañas patonas del género *Physocyclus* (Araneae: Pholcidae) de México: descripción de una especie nueva de la península de Baja California

Samuel Nolasco – Alejandro Valdez Mondragón

Nolasco, S. & Valdez-Mondragón, A. 2020. On the daddy long-legs spiders of the genus *Physocyclus* (Araneae: Pholcidae) from Mexico: description of a new species from the Baja California Peninsula. *Revista Mexicana de Biodiversidad* 91. https://doi.org/10.22201/ib.20078706e.2020.91.3316

Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 91 (2020): e913316

Taxonomy and systematics

On the daddy long-legs spiders of the genus *Physocyclus* (Araneae: Pholcidae) from Mexico: description of a new species from the Baja California Peninsula

Sobre las arañas patonas del género Physocyclus *(Araneae: Pholcidae) de México: descripción de una especie nueva de la península de Baja California*

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http://zoobank.org/urn:lsid:zoobank.org:pub:23829591-817F-4AFE-BF3D-A3012229D9E2

Abstract

A new species of daddy long-legs spiders of the genus *Physocyclus* Simon, 1893 of the *globosus* species group is described from the Baja California Peninsula desert, in the state of Baja California Sur, Mexico. The species is described based on adult males and females: *Physocyclus xerophilus* sp. nov. This is the sixth species described from Baja California Sur. The total number of species of *Physocyclus* increases to 34, including the new species described herein. Thirty-two of 34 species are distributed in Mexico, with one cosmopolitan: *Physocyclus globosus*. The states of Baja California Sur, Guerrero and Michoacán have the highest diversity of species in the country, with 8 species each.

Keywords: Arteminae; Taxonomy; Xerophytic scrub; Baja California Sur

https://doi.org/10.22201/ib.20078706e.2020.91.3316

Resumen

Se describe una especie nueva de araña patona del género *Physocyclus* Simon, 1893 del grupo de especies *globosus* del desierto de la península de Baja California, en el estado de Baja California Sur, México. *Physocyclus xerophilus* sp. nov. se describe con base en machos y hembras adultos y es la sexta especie descrita para este estado. El número total de especies del género *Physocyclus* aumenta a 34, incluyendo la especie nueva descrita aquí; 32 de 34 especies están distribuidas en México, con una cosmopolita: *Physocyclus globosus*. Baja California Sur, Guerrero y Michoacán tienen la mayor diversidad de especies en el país, con 8 especies cada uno.

Palabras clave: Arteminae; Taxonomía; Matorral xerófilo; Baja California Sur

Introduction

The spider family Pholcidae Koch, 1850 is the ninth largest in the order Araneae, with 1,743 species including the new species described herein, distributed in 94 genera (WSC, 2020). The family is subdivided into 5 subfamilies: Arteminae Simon, 1893, Modisiminae Simon, 1893, Ninetinae Simon, 1890, Pholcinae C.L. Koch, 1850 and Smeringopinae Simon, 1893 (Dimitrov et al., 2013; Eberle et al., 2018; Huber, 2011). The spider genus *Physocyclus* Simon, 1893 is classified in the subfamily Arteminae, which currently has 103 species in 9 genera.

Physocyclus is one of the most diversified and common pholcid spiders in Mexico, which currently includes 33 species (Jiménez & Palacios-Cardiel, 2013; Valdez-Mondragón 2010, 2013, 2014; WSC, 2020). The genus is distributed mainly in North and Central America, with most of the species from Mexico, including the synanthropic species *Physocyclus dugesi* Simon and *Physocyclus globosus* (Taczanowski), the latter with a cosmopolitan distribution influenced by human activities (Beatty et al., 2008; Huber & Kwapong, 2013; Valdez-Mondragón, 2010). Although some species are synanthropic, the common habitat is in dry and semi-arid climates, and most of the species have been collected in dry tropical deciduous forests and deserts, as the new species described here. In karstic zones, some species are found frequently in their sheet webs inside caves, on walls, holes in walls, amid large rocks on the ground, or among the karst formations (stalagmites, stalactites and columns) (Valdez-Mondragón, 2010, 2014).

Morphologically, their legs are long with relation to the body, which ranges from 3 to 5 mm, and show a ringed coloration on tibiae and femora. The body coloration varies between white and pale yellow, with an irregular brown dorsal pattern on the carapace, usually with 3 symmetrical spots on each side. However, the general overall shape and coloration is similar among the species, which indicating a conserved morphological pattern (Valdez-Mondragón, 2010, 2014).

Physocyclus is a monophyletic group based on morphological characters currently constituted by 2 species groups, *globosus* and *dugesi*, with 12 and 20 species respectively, including the new species (Valdez-Mondragón, 2013, 2014). The *globosus* group has a biogeographical distribution pattern in the Mesoamerican and Mexican Mountain biotic components, whereas the *dugesi* group has a biogeographical distribution in the Mesoamerican and Continental Nearctic biotic components (Valdez-Mondragón, 2013, 2014).

In this contribution we describe a new species of daddy long-legs spiders of the genus *Physocyclus* belonging to the *globosus* species group, based on adult males and females from the desert of the Baja California Peninsula in the state of Baja California Sur, Mexico.

Material and methods

The specimens used for this study are preserved in ethanol (80%), and for future molecular studies in ethanol (96%), deposited at the Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (curator: Dr. Oscar F. Francke Ballvé). The specimens were collected under the Scientific Collector Permit FAUT-0309 from the Ministry of Environment and Natural Resources (Semarnat) granted to Dr. Alejandro Valdez Mondragón. All the specimens were measured and examined with a Zeiss Discovery Stereoscope V.8. Digital photographs were made using a Zeiss Axio Zoom V.16 microscope, and Axio Zoom Zen and Zen Pro software. All measurements are in millimeters (mm). The male palp and female epigynum were dissected and observed in ethanol (80%), and the epigynum was cleaned in potassium hydroxide (KOH, 10%) for a few minutes to remove all soft tissues around the pore plates. The map was done with QGIS v.3.10. The digital photographs and map were edited in Photoshop CS6 v.1.30x32. Morphological terminology follows Valdez-Mondragón and Francke (2015). Abbreviations: ALE, anterior lateral eyes; AME, anterior median eyes; PLE, posterior lateral eyes; PME, posterior median eyes; P, procursus; E, embolus; DAP, dorsal apophyses of procursus; SO, spermatic operculum; ES, embolic sclerites; BU, bulb of palp; SF,stridulatoy files of

cheliceare; LAC, lateral apophyses of chelicerae; VAE, ventral apophysis of epigynum; PP, pore plates; L/d, tibia I lenght/diamater.

Description

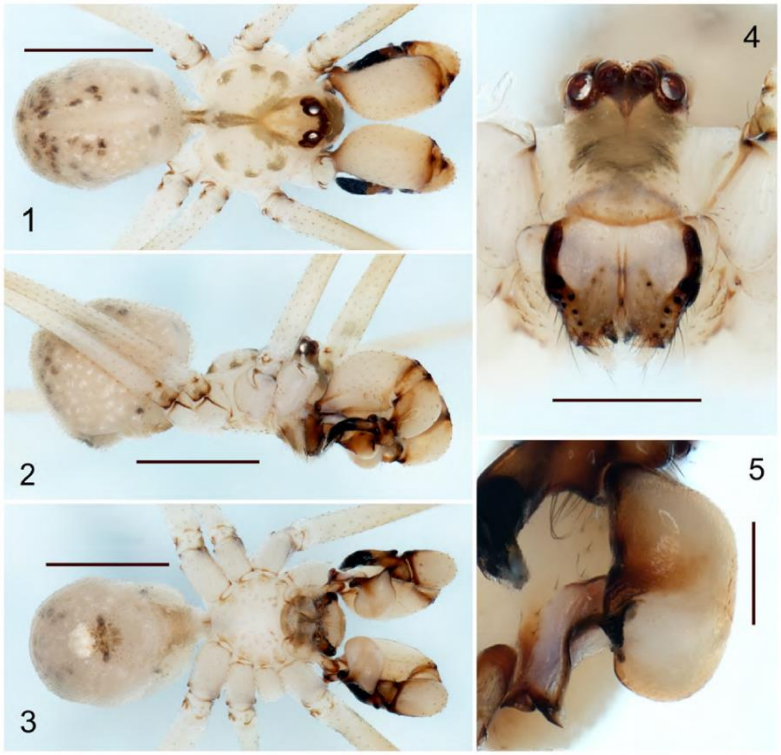
Family Pholcidae C. L. Koch, 1850
Genus *Physocyclus* Simon, 1893
Type species. *Physocyclus globosus* (Taczanowski, 1874)
Physocyclus xerophilus sp. nov.
Figs. 1-16
http://zoobank.org/urn:lsid:zoobank.org:act:18DBFAA3-D914-4DDC-9F27-7821C3E1246F

Diagnosis. Males of *Physocyclus xerophilus* sp. nov. resemble *Physocyclus globosus* in the number of sclerotized cones on the frontal part of the chelicerae (4-5 cones on each chelicerae) (Figs. 9, 10; Valdez-Mondragón, 2010: figs. 42-43). However, in the new species the lateral conical apophyses of the chelicerae are longer and sharper than *P. globosus* (Fig. 10; Valdez-Mondragón, 2010: Fig. 43). In retrolateral view, the procursus in *P. xerophilus* sp. nov. is thinner and curved, with a thin distal spine (Fig. 6); whereas in *P. globosus* the procursus is wider and conical, with a small, wide spine distally (Valdez-Mondragón, 2010: Fig. 44). The embolic sclerites (ES) in the male palp are on the bulb in *P. xerophilus* (Fig. 5), whereas the ES in *P. globosus* are located on the embolus (E) (Valdez-Mondragón, 2010: Fig. 45). In dorsal view, the embolus in *P. xerophilus* sp. nov. is wider and curved (Fig. 45), whereas the embolus in *P. globosus* is thinner and almost straight (Valdez-Mondragón, 2010: Fig. 45). Females of *P. xerophilus* sp. nov. resemble *Physocyclus lautus* Gertsch, 1971 in the shape of the epigynum (Figs. 14-16; Valdez-Mondragón, 2010: Figs. 67-69). However, in the new species the ventral apophyses of the epigynum (VAE) are shorter and in oblique position (Figs 14, 15), whereas in *P. lautus* the VAE are longer and conical (Valdez-Mondragón, 2010: Figs. 67, 69). The epigynum of *P. lautus* has paired W-shaped concavities in the middle (Valdez-Mondragón, 2010: Fig. 67), whereas in *P. xerophilus* sp. nov. those concavities are absent (Fig. 14). In dorsal view, the pore plates (PP) of the epigynum of *P. xerophilus* sp. nov. are almost circular (Fig. 16), whereas in *P. lautus* the PP are longer and oval (Valdez-Mondragón, 2010: Fig. 68). The PP in the new species have bag-shaped structures posteriorly, which are oval and smaller (arrow, Fig. 16), whereas in *P. lautus* they are longer and circular (Valdez-Mondragón, 2010: Fig. 68).

Male (holotype) (CNAN-T01365). Prosoma. Carapace light beige, with a Y-shaped light brown pattern around fovea, which extends towards the posterior part of carapace. Fovea longitudinal. Carapace with 3 irregular spots on each side (Fig. 1). Clypeus with a wide light brown mark lengthwise. Each chelicera with 4-5 sclerotized cones frontally (Fig. 4). Chelicerae with stridulatory files laterally (Figs. 4, 9, 10). Chelicerae with a lateral conical apophysis (Fig. 10). Sternum light beige, with small and inconspicuous light brown spots. Labium brown, wider than long, merged to the sternum. Endites brown prolaterally, beige retrolaterally, longer than wide (Fig, 3). Legs. All segments with beige coloration. Trochanters light brown retrolaterally with a notch distally (Figs. 2, 3). Femora and tibiae with brown rings distally. Opisthosoma. Globular, longer than wide and high, with numerous white and dark brown spots (Figs. 1-3). Spinnerets brown, with a short brown seta around (Fig. 3). Palp. Dorsal apophyses of procursus (DAP) conical (Fig. 6). Procursus (P) thin, with basal half dark brown and distal half dark, ending in a thin and long spine with a brush of pseudotrichia distally (Figs. 6-8). Femur without apophyses (Figs. 6, 8). In prolateral view, ES with oval shape located on the bulb (Figs. 5, 7, 8). E wide, slighly curved in retrolateral view, strongly curved on dorsal view (Figs. 5, 6). E with V-shaped apically (Fig. 5). Spermatic operculum (SO) on apical part of E (Fig, 6). Measurements. Total lenght 2.7. Carapace 1.18 long, 1.23 wide. Clypeus 0.6 long. Diameter AME 0.08, ALE 0.12, PME 0.10, PLE 0.11. Distance ALE-PME 0.05, PME-PME 0.15. Leg I: 27.19 (7.04+0.47+7.68+10.6+1.4). Tibia II: 5.18, tibia III: 3.32, tibia IV: 5.12; tibia I L/d: 51.2.

Female (paratype) (CNAN-T01366). Similar to the male, differences: Prosoma. Dorsal pattern of carapace darker brown than male (Fig. 11). Sternum brown, with a small white region on anterior part (Fig. 13). Labium and endites brown, darker than male (Fig. 13). Clypeus darker than male. Chelicerae without sclerotized cones nor lateral apophyses. Legs. Trochanters light brown retrolaterally, darker than male. Femora and tibiae with darker brown rings distally. Epigynum. Wider than long, bell-shaped, VAE small and conical (Figs. 14, 15). PP small, oval, with bag-shaped structures below them (Fig. 16). Measurements. Total lenght 3.04. Carapace 1.20 long, 1.23 wide. Clypeus 0.4 long. Diameter AME 0.08, ALE 0.11, PME 0.10, PLE 0.10. Distance ALE-PME 0.05, PME-PME 0.14. Leg I: 19.49 (5.18+0.46+5.50+7.25+1.). Tibia II: 3.60, tibia III: 2.28, tibia IV: 4.00; tibia I L/d: 30.5.

Variation. Male paratype smaller than male holotype, with lighter coloration. Chelicerae of the male paratype has darker coloration on basal part. Females with small variation in size. Female collected on Santipac Beach is smaller and with darker coloration in the epigynum than the females from the type locality. Females from Cocos Beach, Misión Mulegé, and the road to San Isidro-San José



Figures 1-5. *Physocyclus xerophilus* sp. nov. Male (holotype): 1-3, Habitus, dorsal, lateral and ventral views respectively; 4, carapace and chelicerae, frontal view; 5, bulb of male palp, dorsal ventral view. Scale bars: 0.2 mm (Fig. 5), 0.5 mm (Fig. 4) and 1 mm (Figs. 1-3).

Comundú with small variaton in size. Males: (N = 2), tibia I: 6.64, 7.68. Females: same locality as holotype (N = 2), tibia I: 4.50, 5.81. 800 SE of Misión Mulegé (N = 1), tibia I: 5.75. Santipac Beach (N = 1), tibia 1: 4.55. Cocos Beach (N = 1), tibia I: 5.37. Comundú (N = 1), tibia I: missing.

Taxonomic summary

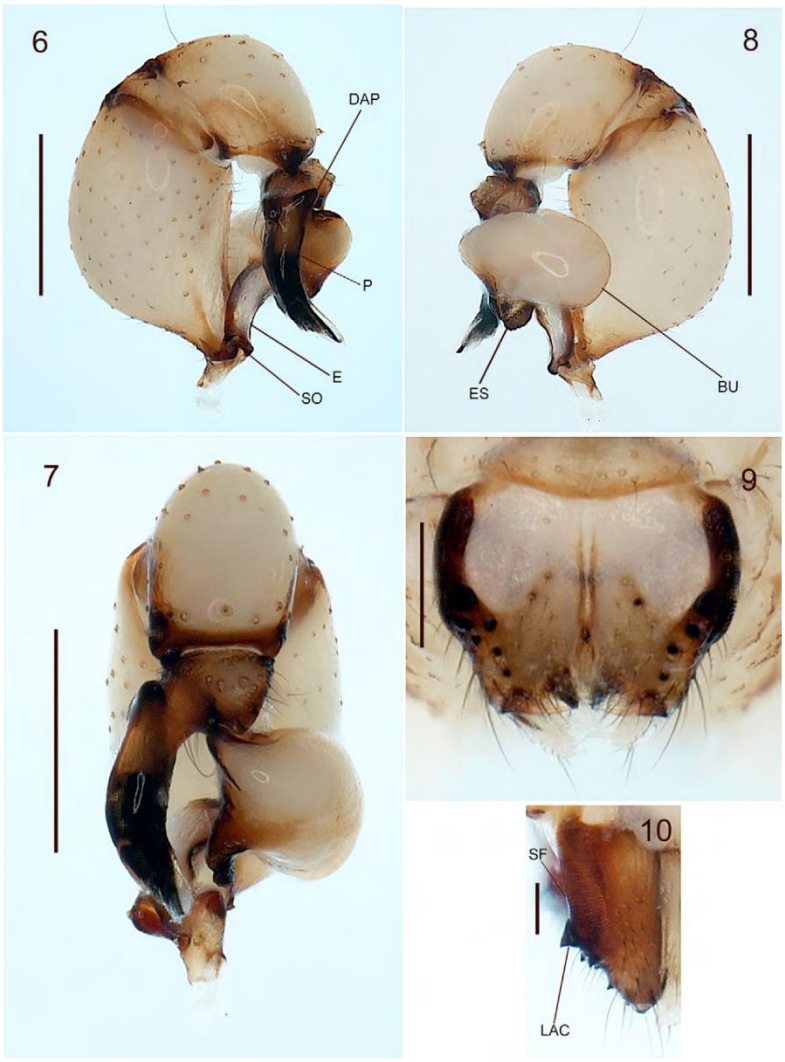
Type material. Mexico: Baja California Sur: 1 male holotype (CNAN-T01365) [21 July 2019; A. Valdez; P. Solís; D. Montiel; A. Cabrera Cols.], from close to "El Requesón" Beach (26.63539° N, -111.83173° W; 6 m), Mulegé Municipality. Paratypes: 1 female (CNAN-T01366); 1 male, 2 females (CNAN-T01367), same data as holotype.

Other material examined. Mexico: Baja California Sur*:* 2 females (Ara-0541) [21 July 2019; A. Valdez, P. Solís, D. Montiel, A. Cabrera Cols.], 800 m SE from Misión Mulegé (lat 26.88183°, lon -111.99310°; 9 m), Mulegé Municipality. 1 female (Ara-0542) [21 July 2019; A. Valdez, P. Solís, D. Montiel, A. Cabrera Cols.], Santipac Beach, Loreto-Santa Rosalía road (26.76198° N, -111.87842° W; 2 m), Mulegé Municipality. 1 female (Ara-0543) [29 July 2019; A. Valdez, P. Solís, D. Montiel, A. Cabrera Cols.], Cocos Beach (26.74279° N, -111.90151° W; 1 m), Mulegé Municipality. 1 female (Ara-0544) [30 July 2019; A. Valdez, P. Solís, D. Montiel, A. Cabrera Cols.], Km 1 San Isidro-San José Comundú road (26.22135° N, -112.01165° W; 137 m), Comundú Municipality.

Etymology. The specific name is a noun in apposition and refers to the vegetation type where the type locality of the species is: *xerophilous* scrub or desert.

Natural history. The specimens were collected on their sheet webs among and under large boulders on the ground in the different localities. The vegetation type of the zone is dry xerophilous scrub, with cacti and scrubby vegetation (Figs. 17, 18).

Distribution. Mexico: Baja California Sur (Fig. 19).



Figures 6-10. *Physocyclus xerophilus* sp. nov. Male (holotype): 6-8, Right palp, retrolateral, ventral and prolateral views respectively; 9, chelicerae, frontal view; 10, chelicerae, lateral view. Scale bars: 0.1 mm (Fig. 10), 0.2 mm (Fig. 9), 0.5 mm (Figs. 6–8). Abbreviations: DAP, dorsal apophysis of procursus; P, procursus; E, embolus; SO, spermatic operculum; ES, embolic esclerites; BU, bulb of palp; SF, stridulatory files; LAC, lateral apophysis of chelicerae.

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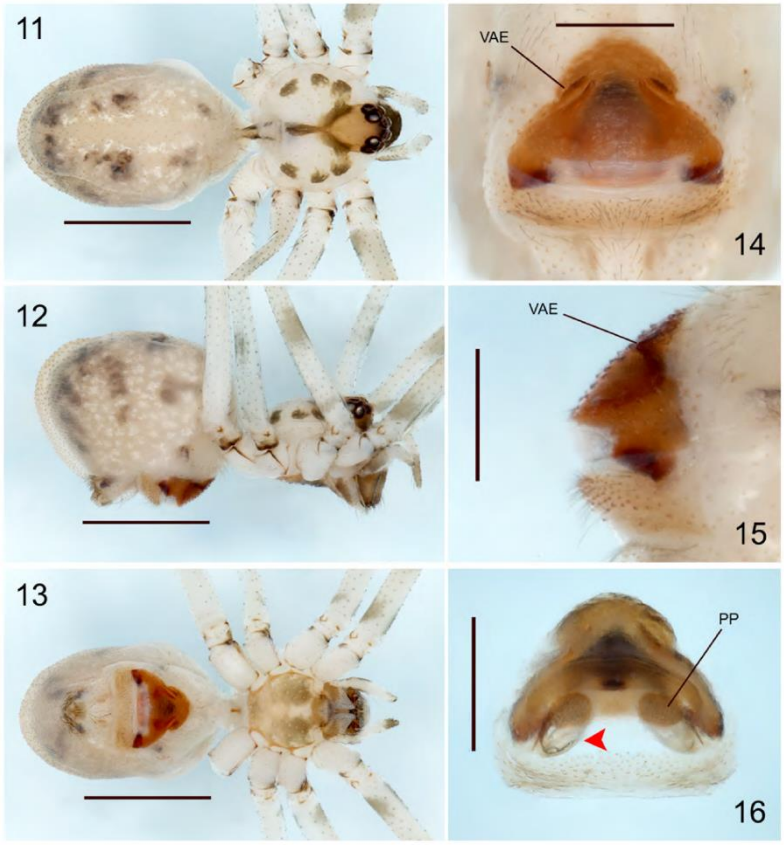

Figures 11-16. *Physocyclus xerophilus* sp. nov. Female (paratype): 11-13, Habitus, dorsal, lateral and ventral views respectively; 14-16, epigynum, ventral, lateral and dorsal views respectively (arrow indicates the bag-shaped structures below the pore plates). Scale bars: 0.5 mm (Figs. 14-16), 1 mm (Figs. 11-13). Abbreviations: VAE, ventral apophysis of epigynum; PP, pore plates.

Figures 17-18. Habitat and microhabitat of *Physocyclus xerophilus* sp. nov. 17, Xerophilous scrub close to the El Requesón Beach (type locality) at Baja California Sur, Mexico; 18, rocks on the ground where the specimens of the new species can be collected (arrows indicate the specific microhabitat).

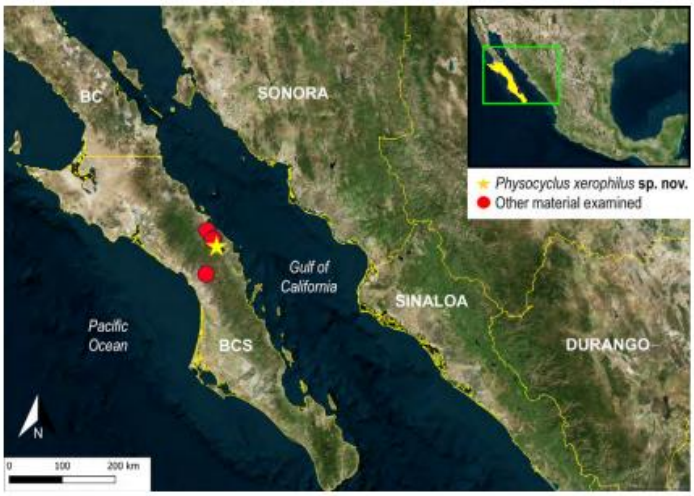

Figure 19. Known records of *Physocyclus xerophilus* sp. nov. from Baja California Sur, Mexico. Star (type locality). Abbreviation: BC, Baja California.

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13. DISCUSIÓN GENERAL

Según el Catálogo Mundial de Arañas (WSC, 2023), la diversidad de arañas de la familia Pholcidae está conformada actualmente por 1919 especies clasificadas en 97 géneros. Para el género *Physocyclus*, hasta el 2010, se tenían descritas 18 especies, mientras que, en los últimos 13 años, el número de especies descritas incrementó a 37 especies, lo que representa un aumento de más del 50% de la diversidad de especies para este género. En este trabajo, se describieron cinco nuevas especies provenientes principalmente de climas áridos y tropicales caducifolios. Este tipo de ambientes representan los hábitats principales para el género *Physocyclus*, los cuáles son abundantes en territorio mexicano. Esta combinación de factores sugiere que la diversidad de arañas de este género sigue sin ser conocida o al menos, estar bien representada, ya que existen muchas zonas del país que están pobremente estudiadas o inexploradas. Aunado a esto, las cuevas han mostrado ser ecosistemas idóneos para el desarrollo de estas arañas, por lo cual, representan una fuente importante para la exploración y conocimiento de la diversidad de las arañas del género *Physocyclus.*

En relación a los análisis de distancias genéticas y delimitación de especies, la evidencia corroboró la validez de las especies del género *Physocyclus*, previamente identificadas morfológicamente. La variación genética intraespecífica promedio para CO1 y 28S, está por debajo del 2%, lo cual es evidencia de su utilidad en análisis de distancias genéticas, a nivel de especie. Por otro lado, ITS2 resultó ser inadecuado a nivel de especie, pues su variación intraespecífica promedio, está por encima del umbral de Neighbor Joining (NJ) del 2%. Solo el marcador 28S, recuperó ambos grupos de especies (*globosus* y *dugesi*) en el análisis fenético de NJ. Los diferentes métodos de delimitación de especies (ABGD, ASAP y GMYC) fueron congruentes entre sí, delimitando un número de especies similar, sin embargo, el método que tuvo mayor discrepancia fue bPTP, pues delimitó un número mayor de especies. En cada caso, todos los métodos fueron corroborados con la morfología, confirmando como válidas a la mayoría de las especies, salvo en algunos casos (*P. enaulus*, *P. modestus*, *P. mysticus* y *P. xerophilus*) donde se podría tratar de casos de complejos de especies. En estos casos particulares, los cambios morfológicos pueden no estar correlacionados con los límites entre especies, lo que dificulta su uso para la apropiada la delimitación de especies. Es posible que el reconocimiento interespecífico ocurra por señales

no visuales, como la señalización química, aspectos conductuales (etológicos) o incluso por rasgos biogeográficos (microhábitats). Del mismo modo, las especies pueden estar bajo una selección estabilizadora que promueva la estasis morfológica. En cualquier caso, se recomienda ampliamente utilizar los tres marcadores moleculares (CO1, ITS2 y 28S) en conjunto para los análisis de delimitación de especies.

Los análisis filogenéticos morfológicos, moleculares y con evidencia combinada, corroboraron la monofília de los grupos de especies *globosus* y *dugesi*, con valores de soporte significativos. Los análisis filogenéticos morfológicos mostraron que ambos grupos de especies ostentan una morfología particular, que permite identificarlos correctamente, cuyos caracteres sinapomórficos principales, están asociados a los caracteres sexuales primarios y secundarios. Asimismo, con los análisis de delimitación de especies, se comprobó que estos caracteres morfológicos proveen información robusta para la identificación a nivel de especie. Los análisis filogenéticos moleculares de Máxima Verosimilitud (ML) e Inferencias Bayesianas (IB) realizados con la matriz concatenada (CO1+ITS2+28S) mostraron que ambos grupos de especies son linajes independientes, aunque estrechamente relacionados, reconocidos como grupos naturales o monofiléticos. Con el uso de esta evidencia, las relaciones filogenéticas de ambos grupos de especies están completamente resueltas, en contraste con los análisis filogenéticos morfológicos, donde existe poca resolución, en el clado del grupo de especies *globosus*. Al mismo tiempo, existe una alta correspondencia entre los diferentes sub-clados de cada grupo de especies, con la evidencia morfológica y biogeográfica, ya que todos los sub-clados se agrupan en función de sus características morfológicas o su patrón de distribución. En cuanto a los análisis filogenéticos de IB con evidencia combinada (Morfología+CO1+ITS2+28S), se encontró una topología similar a la encontrada utilizando solo evidencia molecular. El único cambio ocurre en la especie *P. pocamadre*, que aparece como la especie hermana de *P. paredesi*. Este cambio puede deberse a la falta de información morfológica para la especie *P. pocamadre*, ya que fue una especie descrita solo con la hembra, el macho es desconocido hasta el momento. La posición filogenética de *P. pocamadre* necesita ser corroborada incluyendo la información morfológica faltante.

Respecto a la datación de linajes y reconstrucción de áreas ancestrales, ambos grupos de especies (*globosus* y *dugesi*) aparecieron hace aproximadamente 15 millones de años

(Mya), durante el Mioceno, época caracterizada por la alta actividad volcánica en la región central de México. Durante este periodo, apareció el Cinturón Volcánico Transmexicano (CVT), provincia biogeográfica reconocida como el área ancestral más importante que influyó significativamente en la diversificación por vicarianza en ambos grupos de especies. La diversificación de ambos grupos de especies ocurrió durante el Mioceno Medio al Plioceno (14.8 – 5 Mya), ya que ocurrieron la mayoría de eventos de especiación. La aparición del CVT ocasionó diferentes cambios orográficos que funcionaron como barreras geográficas, promoviendo la segregación de poblaciones e interrumpiendo el intercambio y flujo génico. Esto conllevó a importantes procesos de cladogénesis influenciados por las diferentes barreras geográficas generadas por el alzamiento del CVT, como la formación de "islas del cielo", que fueron regiones aisladas en las montañas que sirvieron como refugios durante los periodos interglaciares durante el Pleistoceno. Aunado a esto, la dispersión jugó un papel significativo en el proceso de diversificación, a través de la aparición y fragmentación de diferentes ecosistemas (como desiertos, matorrales xerófilos o bosques tropicales caducifolios) durante el Plioceno al Pleistoceno (5 – 0.5 Mya). Otras provincias que influyeron significativamente en el proceso de diversificación de ambos grupos de especies son la Provincia de Baja California y las Tierras Bajas del Pacífico, las cuáles promovieron la especiación por dispersión, posterior a distintos eventos de vicarianza. Los tiempos de divergencia de las especies de la Península de Baja California corresponden con la separación de esta región con el continente y la subsecuente incursión del Golfo de California (12 – 5 Mya) a través de múltiples incursiones marinas. Estas especies probablemente se distribuían en la región antes de que la Península se separara del continente. El patrón de distribución de *Physocyclus* continúa a lo largo de las Tierras Bajas del Pacífico, una provincia biogeográfica importante que condujo la diversificación del género durante el Mioceno Medio-Tardío, probablemente por la fragmentación de poblaciones, causada por la aparición de nuevos hábitats. Cabe mencionar que estos patrones evolutivos han sido registrados en diferentes grupos de vertebrados (serpientes de cascabel, ranas, iguanas, etc.) e invertebrados (saltamontes y otros arácnidos). Asimismo, la Depresión del Balsas, aunque no condujo significativamente la diversificación de estos grupos de especies, es la provincia con la mayor diversidad de arañas de lo que actualmente es considerado como *Physocyclus*. Esto se debe a que se encuentra entre dos provincias biogeográficas importantes en la

diversificación de *Physocyclus* (CVT y la Sierra Madre del Sur), además de estar rodeada de tres puntos de intersección considerados como nodos panbiogeográficos.

Finalmente, dada la evidencia morfológica, molecular y biogeográfica, se propone elevar al grupo de especies *dugesi* a la categoría de género: *Mictlanus* **gen. nov.**, mientras que, por principio de prioridad, el grupo de especies *globosus*, se mantiene como el género *Physocyclus* siendo *P. globosus* la especie tipo del género, mientras que para *Mictlanus*, bajo el principio de prioridad *M. dugesi* representa la especie tipo. Lo anterior, se sustenta con la evidencia recopilada en este estudio: 1) ambos géneros presentan una morfología particular que permite identificarlos apropiadamente, 2) ambos géneros son monofiléticos con distintos tipos de evidencia, analizada de manera independiente y en combinación, con valores de soporte significativos en cada caso, 3) presentan un patrón de distribución actual diferente, 4) tiempos de divergencia, aunque similares, son independientes, con procesos evolutivos particulares para cada uno y 5) la influencia de áreas ancestrales en la evolución de cada género es diferente, así como su efecto en el proceso de diversificación.

Los resultados aquí recopilados corroboran la hipótesis de trabajo, pues se demuestra con diferentes líneas de evidencia, que ambos grupos de especies (*globosus* y *dugesi*) son dos géneros distintos con una historia evolutiva independiente, relacionada con la complejidad orográfica de la Zona de Transición Mexicana. Las áreas ancestrales, al influir de diferente manera en los procesos de diversificación en ambos géneros, conllevó a que estos tengan un patrón de distribución diferente, delimitado por las diferentes provincias biogeográficas actuales. Asimismo, la predicción sobre los factores que promovieron la divergencia en ambos géneros fue correcta, pues el factor extrínseco más importante fue la vicarianza, tal y somo se ha observado en otros grupos de organismos, seguido de la divergencia generada por la dispersión. La predicción sobre los límites de las especies, también se corroboró correctamente, pues las distancias genéticas intra e interespecíficas promedio son similares a lo reportado para la familia Pholcidae y otros grupos de arañas. Del mismo modo, existe una alta congruencia entre los diferentes métodos moleculares de delimitación de especies y la morfología particular de cada una de las especies.

14. CONCLUSIONES

- Los grupos de especies *globosus* y *dugesi* son dos géneros de arañas diferentes: *Physocyclus* y *Mictlanus* **gen. nov.** respectivamente.
- El Cinturón Volcánico Transmexicano fue la provincia que impulsó significativamente la diversificación de ambos géneros mediante vicarianza.
- Las áreas ancestrales y provincias biogeográficas actuales influyeron en diferente magnitud en el proceso de diversificación de ambos géneros.
- Ambos géneros poseen un patrón de distribución y una morfología distintiva.
- La dispersión jugó un papel secundario en la diversificación de ambos géneros.
- La Depresión del Balsas, las Tierras Bajas del Pacífico y el Cinturón Volcánico Transmexicano son las provincias biogeográficas de mayor diversidad de ambos géneros.
- Los genes CO1 y 28S proveen información robusta para diferenciar a nivel de género y especie.
- La región ITS2 es muy variable, no provee buena información para diferenciar a nivel de especie, pero en una matriz concatenada provee mejores resultados, tanto en delimitación de especies, como en aproximaciones filogenéticas.
- El método bPTP sobreestima el número de especies delimitadas, posiblemente debido a la inclusión del gen nuclear 28S.
- *Physocyclus enaulus*, *P. modestus*, *P. xerophilus* y *Mictlanus mysticus* pueden estar conformados por dos o más especies lo que podría tratarse de complejos de especies, de acuerdo con el consenso estricto y por mayoría de los métodos utilizados.
- Los caracteres sexuales primarios (palpos de los machos y epiginios de las hembras) y secundarios (quelíceros de los machos), proveen información robusta para diferenciar a nivel de género y especie.
- La riqueza de ambos géneros está subestimada para Norteamérica, sobre todo para la parte Norte de México, por lo que es necesario realizar más estudios sistemáticos.

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16. TRABAJOS EN PARALELO

En esta sección, se muestran los trabajos en paralelo desarrollados a la par que el proyecto general. Estos trabajos, no conforman la estructura principal de la tesis, por lo que sólo se mencionan de manera breve y concisa.

Artículo de taxonomía integradora: **"On the subfamily Arteminae: A new species of *Physocyclus* Simon (Araneae: Pholcidae) from northwest Mexico based on CO1 mtDNA barcoding and morphology"**

Este artículo se encuentra en revisión en la revista *Zootaxa*. Este trabajo se centra en la descripción de una especie nueva del género *Physocyclus*, encontrada en ecosistemas áridos, en el estado de Sonora. Se realizaron análisis morfológicos de la especie (taxonomía alfa), además de integrar estudios de distancias genéticas de Neighbor-Joining (NJ), con el gen CO1, bajo el umbral del 2%. Los caracteres sexuales primarios y secundarios de machos y hembras de esta nueva especie son distintivos y diagnósticos, lo cual, permite identificarla y diferenciarla de las demás especies del género. La distancia genética promedio de esta nueva especie es del 16.5%, con respecto a las demás especies del género *Physocyclus*. Los análisis morfológicos y de distancias genéticas, son congruentes entre sí, por lo que no queda duda de que esta especie es una entidad diferente y válida, con respecto al resto de especies actualmente descritas.

Cita completa del artículo:

Nolasco, S. & Valdez-Mondragón, A. (*in press*). On the subfamily Arteminae: A new species of *Physocyclus* Simon (Araneae: Pholcidae) from northwest Mexico based on CO1 mtDNA barcoding and morphology. *Zootaxa*.

Nota importante: No se adjunta evidencia del envío del trabajo a la revista, porque no tienen una plataforma para subir el artículo, todo se hace a través de correo electrónico, por lo que no existe un comprobante como tal.

Artículo de reconstrucción de áreas ancestrales: **"Dispersal or vicariance? Pliocene-Pleistocene and their effects on the diversification of the spider genus *Ixchela* Huber (Araneae: Pholcidae) with the description of a new species from Mexico"**

Este artículo se encuentra en revisión en el *Jorunal of Arachnology*. Este trabajo trata sobre la historia evolutiva del género de arañas *Ixchela* y la descripción de una especie nueva. Se realizaron análisis de distancias genéticas de Neighbor-Joining (NJ), bajo el umbral del 2%. También se realizaron análisis de datación de linajes, utilizando dos puntos de calibración fósil y la tasa de sustitución del gen CO1. Además, se incorporó un análisis de reconstrucción de áreas ancestrales. Los caracteres sexuales primarios y secundarios de machos y hembras, así como algunos caracteres somáticos son distintivos para esta nueva especie. El valor de la distancia genética de esta nueva especie es de 3.5% (NJ), con respecto a la especie más correlacionada: *I. azteca*. De acuerdo con la datación de linajes y reconstrucción de áreas ancestrales, el género *Ixchela,* apareció aproximadamente 6.9 millones de años (mya), con una diversificación primordial durante el Pleistoceno (>2.5 mya). Las áreas ancestrales que potencialmente influyeron en el proceso diversificación son: Cinturón Volcánico Transmexicano, Sierra Madre del Sur, Sierra Madre Oriental y el Desierto Chihuahuense. El proceso principal de diversificación fue a través de la vicarianza, con posteriores eventos de dispersión.

Cita completa del artículo:

Valdez-Mondragón, A. & Nolasco, S. (*in press*). Dispersal or vicariance? Pliocene-Pleistocene and their effects on the diversification of the spider genus *Ixchela* Huber (Araneae: Pholcidae) with the description of a new species from Mexico. *Journal of Arachnology*.

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VALDEZ-MONDRAGÓN & NOLASCO— DIVERSIFICATION OF THE GENUS IXCHELA

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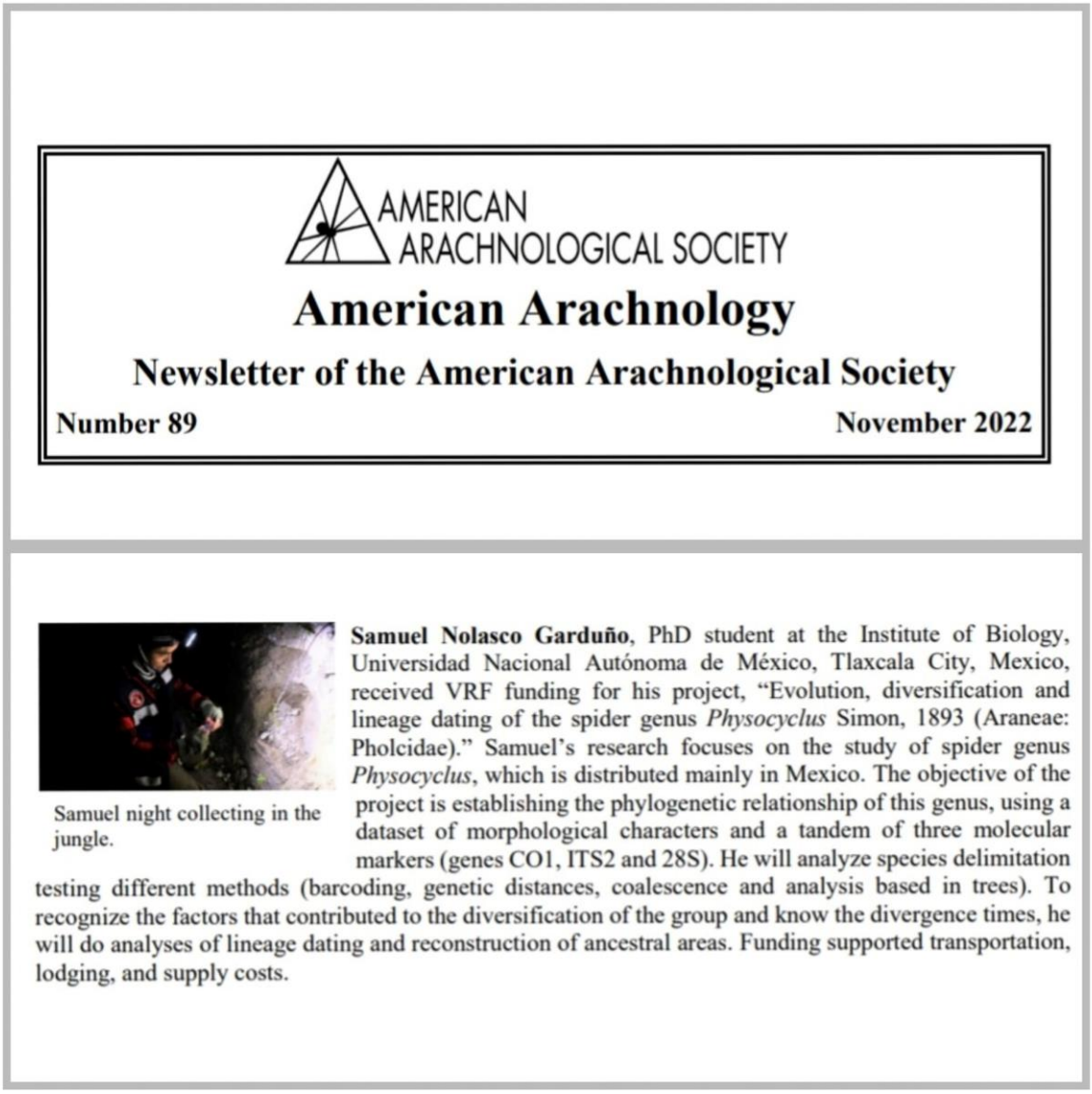

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Samuel night collecting in the jungle.

Samuel Nolasco Garduño, PhD student at the Institute of Biology, Universidad Nacional Autónoma de México, Tlaxcala City, Mexico, received VRF funding for his project, “Evolution, diversification and lineage dating of the spider genus *Physocyclus* Simon, 1893 (Araneae: Pholcidae).” Samuel’s research focuses on the study of spider genus *Physocyclus*, which is distributed mainly in Mexico. The objective of the project is establishing the phylogenetic relationship of this genus, using a dataset of morphological characters and a tandem of three molecular markers (genes CO1, ITS2 and 28S). He will analyze species delimitation testing different methods (barcoding, genetic distances, coalescence and analysis based in trees). To recognize the factors that contributed to the diversification of the group and know the divergence times, he will do analyses of lineage dating and reconstruction of ancestral areas. Funding supported transportation, lodging, and supply costs.

Link de descarga: https://www.americanarachnology.org/news-projects/aas-newsletter/